Modern Variety Breeding for Present and Future Needs

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Jaime Prohens and María Luisa Badenes

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Part 1

Conservation of Genetic Resources and Prebreeding

Eds. J. Prohens & M.L. Badenes
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Plant genetic resources for future breeding

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ABSTRACT: Recognizing the danger due to a permanent risk of loss of the genetic variability of cultivated plants and their wild relatives in response to changing environmental conditions and cultural practices, plant ex situ genebank collections were created since the beginning of the last century. World-wide more than 6 million accessions have been accumulated including the German ex situ genebank in Gatersleben, one of the four largest global collections, housing 150,000 accessions belonging to 890 genera and 3,032 species. This paper summarises the ex situ plant genetic resources conservation behaviour with special emphasis on German activities. Strategies of maintenance and management germplasm collections are discussed beside general aspects on utilisation for future breeding.

Keywords: *ex situ* collections, germplasm, long term seed storage, modern breeding.

Introduction

The global number of higher plant species (angiosperms and gymnosperms) is thought to in the range 300,000 to 500,000, of which approximately 250,000 have been identified or described (Wilson, 1988). Of these, some 30,000 are edible and 7,000 have been collected or cultivated at one time or another by humans for food (Wilson, 1992). However, just 30 species have been identified as those which 'feed the world' (Mooney, 1983), providing 95% of dietary energy (calories) or protein. Wheat, rice and maize on their own contribute more than 50% of the world's plant-derived energy intake. A further 25% is provided by the six species sorghum, millet, potato, soybean, sugar cane and sugar beet (FAO, 1991). Given the importance of a relatively small number of crop species for global food security, it is particularly important that their genetic diversity is conserved effectively and managed wisely.

Recognizing the risk of loss of the genetic variability of cultivated plants in response to changing environmental conditions and cultural practices, plant specialists in several countries initiated collection missions from the 1920's in order to accumulate and store genetic recourses in *ex situ* seedbanks. Vavilov was among the first to collect material systematically from all climatic zones within and beyond the USSR (Breznev, 1970). Stimulated by his ideas, many genebank collections have been created during the last century. Based on FAO estimates made in 1998, existing *ex situ* plant collections contain about 6 million accessions

(FAO, 1998), and the number has probably increased further since then. Over 40% of all accessions in genebanks are cereals. A list of the ten largest germplasm collections by crop is given in Table 1.

The German *ex situ* genebank is one of the four largest global collections, maintaining about 150,000 accessions belonging to 890 genera and 3,032 species. Larger collections are hosted by the Institute of Crop Germplasm, Beijing, China, the National Seed Storage Laboratory, Beltsville, USA and the N. I. Vavilov Research Institute, St. Petersburg, Russia (FAO, 1998).

Maintenance and management of plant genetic resources

Plant *ex situ* genebank collections comprise seed genebanks, field genebanks and *in vitro* collections. Species whose seed can be dried, without damage, down to low moisture contents, can be stored in seed banks. Such "orthodox" seeds can be expected to maintain a high level of vigour and viability at the least between the time of harvest and the following growing season. Field genebanks and *in vitro* storage are used primarily for species which are either vegetatively propagated or which have non-orthodox seeds that cannot be dried and stored for long periods. In addition, perennial species, for example certain forage species, which produce small quantities of seed, and long-lived plants (in particular, trees) are also maintained this way. It is estimated that worldwide, less than 10% of genebank holdings are stored *in vivo* in the field, and less than 1% are conserved *in vitro* (FAO, 1998). Clearly, seed storage is the predominant mode of plant genetic resources conservation, accounting for about 90% of the total *ex situ* accessions.

Table 1. List of the ten largest world-wide germplasm collections by crop (FAO, 1998).

Crop	*Genus*	Accessions
Wheat	*Triticum*	788,654
Barley	*Hordeum*	486,724
Rice	*Oryza*	420,341
Maize	*Zea*	261,584
Bean	*Phaseolus*	268,369
Oat	*Avena*	223,287
Soybean	*Glycine*	176,400
Mustard	*Brassica*	106,923
Sorghum	*Sorghum*	168,550
Apple	*Malus*	97,543

At the IPK Gatersleben, seed storage is managed in five large cold chambers, two maintained at 0°C and three at -15°C. Seeds are kept in glass jars, covered with bags containing silica gel. Germination rate is assessed before storage, and then at regular intervals during storage. More than 190,000 germination tests have been conducted since the cold store was established in

1976. Monitoring for viability is based on these data (Specht et al., 1997; 1998). Species differ in their longevity, however, in addition considerable genetic variation within species, determined by the genotype was determined (Landjeva and Börner 2008).

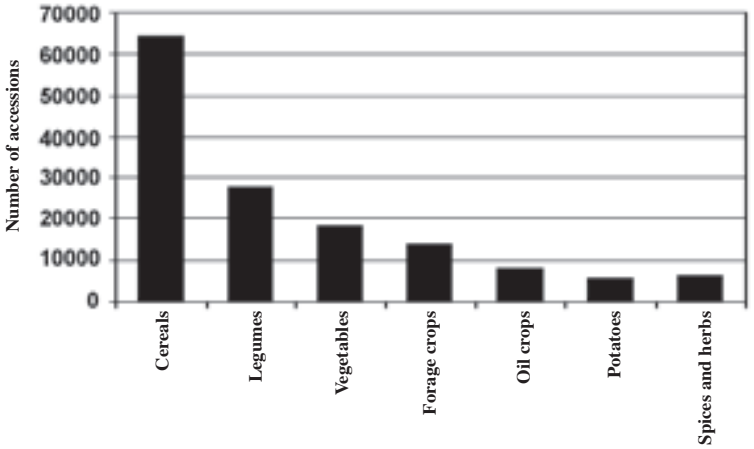

Figure 1. Inventory of the Gatersleben *ex situ* collection.

The maintenance of genebank accessions requires the long term storage as well as its regeneration. Each year between 8 and 10% of the collection is grown either in the field or in the glasshouse. Regeneration becomes necessary when: (1) the quantity of stored seed has dropped below a pre-set threshold, due to supply to users, (2) viability falls below a pre-set threshold, (3) phenotypic evaluations of the accessions need to be conducted or (4) new accessions, which require multiplication and characterization, enter the collection. Regeneration is carried out locally to ensure genetic integrity and to minimize genetic erosion. Voucher specimens, photographs and written documentation are used to monitor the identity of the material. Special attention has to be given to out-pollinating species, which are either multiplied in a small glasshouse or in isolation plots in the field. Depending on the species, the separation distance between isolation plots varies between 100m and 150m. In some cases, flowers are bagged to enforce self-pollination.

About 1,500 accessions (1% of the collection) are maintained permanently in the field. These are mainly perennial species including many medicinal herbs and herbs used as spices and condiments. However, reliance on field collections for preservation is risky, as germplasm can be easily lost due to pests, diseases and adverse weather conditions.

Therefore, *in vitro* conservation methods have been developed and are used at IPK for potato, the vegetatively maintained part of the *Allium* collection and other genera such as *Mentha*, *Brassica* and *Dioscorea*. In total 3,008 accessions are maintained *in vitro*, amongst which are 2,320 accessions of potato stored as microtubers. The maintenance of *in vitro* collections is still labour-intensive, and there is always the risk of losing accessions due to fungal/bacterial contamination, human error or somaclonal variation. Hence freeze-preservation or cryo-preservation at an ultra-low temperature (-196°C, i.e. the boiling point of liquid nitrogen) is an alternative for the long term conservation of *in vitro* tissues/organs or recalcitrant seeds (Keller et al., 2006; Senula et al. 2007). Of the Gatersleben genebank collection, 1.088 potato accessions are cryo-preserved beside smaller collections of garlic (38 accessions) and mint (22 accessions).

Utilisation of plant germplasm

Each year between 10,000 and 15,000 seed samples are supplied to a heterogeneous group of users. Approximately half of the material is distributed within Germany. Since 1953 more than 730,000 samples have been shipped. More than 50% of requests originate from research institutes, including IPK departments and universities. Additional users are not only other genebanks or botanical gardens but mainly plant breeding companies.

The aim of modern breeders is the same as that of early farmers - to produce superior crops. Conventional breeding, relying on the application of classic genetic principles applied to the phenotype of the organism concerned, has been very successful in introducing desirable traits into crop cultivars. However, nowadays plant breeding is adopting new approaches for developing improved varieties. New genetic tools based on molecular marker-technologies have evolved during the last two decades, which provided an opportunity to increase the selection efficiency through indirect following the inheritance of agronomically valuable traits in many crops. Employing such technologies a great number of loci controlling morphology, development, adaptability, plant disease resistance, etc. have been mapped to specific chromosomes, chromosome arms or regions as demonstrated for wheat (Landjeva et al. 2007). They include major genes as well as loci for complex traits (quantitative trait loci). Examples for the availability of molecular markers for genes determining disease resistance (leaf rust) in wheat are given in table 2. Especially sources from alien species maintained in genebanks were identified to be used for the improvement of our cultivated crops.

Finally it should be stated, that the concentration on certain crops (species) and/or cultivars (genotypes) lead to a reduction in genetic diversity, but at the same time there is a continued need of modern breeding programs for new characteristics and genes in these species. *Ex situ* genebanks play a substantial role in preserving the wild relatives of crop plants, as well as local varieties providing the basic input to plant breeding programmes.

Table 2. Examples of reported molecular markers for genes determining leaf rust resistance in wheat (Landjeva et al. 2007).

Gene	Chromosome	Donor
Lr1	5DS	T. aestivum
Lr9	6BL	*Ae. umbellulata*
Lr10	1AS	*T. aestivum*
Lr13	2BS	*T. aestivum*
Lr19	7D	*Ae. elongatum*
Lr23	2BS	*T. turgidum*
Lr24	3DL	*Ae. elongatum*
Lr25	4BL	*S. cereale*
Lr27	3BS	*T. aestivum*
Lr29	7DS	*Ae. elongatum*
Lr32	3DS	*Ae. tauschii*
Lr34	7DS	*T. aestivum*
Lr35	2B	*Ae. speltoides*
Lr39	2DS	*Ae. tauschii*
Lr47	7A	*Ae. speltoides*
LrTr	4BS	*Ae. triuncialis*
LrTt1	2A, 2B	*T. timopheevii*

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Statistical tools for genomic prediction of breeding values on modern varieties

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ABSTRACT: The accuracy of predicting breeding values affects the rate of genetic improvement of plant breeding populations for complex quantitative traits. Also, breeders and geneticists have been using genetic markers to study basic questions concerning QTL, e.g. number, modes and sizes of action, and to map QTL onto the genome to facilitate their manipulation for breeding purposes. We put forward a flexible Bayesian approach (Bink et al., 2008) that allows simultaneously modeling multiple genetic components (QTL, major genes, genetic background). The approach was successfully applied to an apple dataset comprising 27 related mapping populations generated within the EU-HiDRAS project (http://users.unimi.it/hidras/). The data held phenotypic records on 1450 genotypes for the quantitative trait firmness after two months of cold storage. The marker map contained 86 SSR loci covering 1102 centiMorgan. The approach estimated the genome-wide number of QTL directly and produced posterior probabilities on QTL regions and estimates for QTL contributions to the trait firmness. Subsequently, these inferences were used for genomic prediction of breeding values. The results indicated that low marker density and polymorphism level in several genomic regions affected accurate estimation and localization of multiple QTL. However, the genomic prediction of breeding values seemed more robust to variability in marker information along the genome. Scenarios for genomic prediction of breeding values in case many thousands of single-nucleotide polymorphisms (SNPs) become available across the genome of modern plant varieties will be discussed.

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Ecotypes of Italian ryegrass from Swiss permanent grassland outperform current recommended cultivars

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ABSTRACT: Plot trials with 20 ecotype populations were conducted 2005 to 2007 at three locations, supplemented by row trials at two additional locations, and including four currently recommended cultivars as controls. Dry matter yield, vigour and disease scores varied significantly among the ecotypes. Performance in terms of yield, vigour and resistance to snow mold of ecotypes was superior to the cultivars mean on average, and between 8 and 17 of the 20 ecotypes performed significantly better than the cultivars mean, depending on the characteristic. However, resistance against crown rust of the ecotypes was significantly poorer than that of the cultivars. Correlation and regression analysis showed that more eastern and more northern collecting sites harboured better performing ecotypes, whereas a high Calcium content of the soil and a high species richness of the vegetation often had a negative influence on the performance of collected ecotypes. However, the most consistent site related factor favouring performance of the ecotypes was geographical closeness to the test sites. It is concluded that new collections of ecotypes of Italian ryegrass are highly promising for use in breeding, but ways must be sought to efficiently reduce their high susceptibility to crown rust.

Keywords: Italian ryegrss, ecotypes, cultivars, genetic resources

Introduction

Italian ryegrass (*Lolium multiflorum* ssp. *italicum* Volkart *ex* Schinz *et* Keller) thrives well in permanent grassland in mild and moist regions of Switzerland. Ecotypes from such grasslands have successfully been used to breed cultivars like AXIS and ORYX (Boller *et al*. 2002). A collection made in 1996 at 6 sites in Switzerland yielded ecotypes with a fair yielding potential and promising resistance against bacterial wilt (Boller *et al*. 2005). However, rust resistance of this material was clearly insufficient. Fostered by the National Plan of Action for the conservation and sustainable use of plant genetic resources, a more comprehensive collection of 30 Italian ryegrass ecotype populations was carried out in 2003. Analysis of molecular markers showed that within a subset of 12 populations, there was little differentiation among the populations and their genetic structure was not influenced by site related factors (Peter-Schmid *et al*. 2008). We report on agronomic performance of 20 populations, including those

of the above mentioned molecular study. Performance was related to collection site factors to define criteria for targeted conservation and utilisation.

Material and methods

Ecotype populations were collected in different regions of Switzerland in early summer of 2003. Details of collecting procedure and sites are reported by Peter-Schmid *et al*. (2008). Seed was increased in small plots isolated with at least 15 m of a rye crop as barrier. Plot trials (6 x 1.5 m plots) with 20 ecotype populations and four recommended cultivars (Suter et al. 2002) were established in spring of 2005 on the experimental farms of ART at Ellighausen and Oensingen. The same entries were sown in row trials (3 m rows 0.5 m apart) at Watt near Regensdorf and Gibswil (1000 m a.s.l.). A reduced set of 10 ecotype populations and two cultivars was investigated in a plot trial at Zürich-Reckenholz. Dry matter yield was determined in the plot trials for each of five cuts in 2006 (first full harvest year) and 2007 (second full harvest year). Disease and vigour scores were rated on a 1 to 9 scale (9=best). All data were analyzed using procedures GLM, CORR and STEPWISE of SAS software. Least squares means of performance characteristics are reported.

Results and discussion

The ecotype populations varied significantly in all performance characteristics investigated (Table 1). Eight ecotypes produced significantly higher yields than the average of the cultivars in the first full harvest year (YTOTH1), and even 12 ecotypes outyielded the cultivars significantly in the second full harvest year (YTOTH2). The highest yielding ecotype outyielded the highest yielding cultivar in both years, the difference being significant in the second full harvest year. First cut yield (YCT1) of the ecotypes was particularly high, with 16 ecotypes producing a significantly higher yield than the cultivars mean. The yields of the summer cuts (YCT23) of some ecotypes were significantly below those of the cultivars and this was related to insufficient resistance to bacterial wilt (XANT). However, on average, resistance of the ecotypes against bacterial wilt was only slightly inferior to the cultivars and 6 ecotypes had even significantly superior resistance. Vigour scores generally reflected the differences in yield except that the ecotypes were scored relatively best in summer (VIG23) where 9 ecotypes performed significantly better than the best cultivar. Ecotypes showed a remarkable resistance against snow mold (SNOM), with 17 out of the 20 ecotypes being significantly more resistant than the cultivars mean. Conversely, they were less resistant against leaf diseases than the cultivars. This was particularly striking for crown rust (RUST). Without exception, the ecotypes were significantly more susceptible than the cultivars mean, and 6 ecotypes were scored significantly poorer than the most susceptible cultivar.

Table 1. Performance characteristics of 20 ecotype populations of Italian ryegrass, compared to currently recommended cultivars.

	Yields (dt/ha)[2]				Vigour (9=best, 1=poorest)[3]						Disease resistance (9=best)[4]			
Accession	YTOTH1	YTOTH2	YCT1	YCT23	VIG1	VIG23	VGEND	VIGH1	VIGH2	VGALT	SNOM	RUST	XANT	LSPOT
Bazenheid	125.3 b	163.9 b	38.8 b	62.1 b	6.78 b	7.72 a	7.33 b	7.20 b	7.36 b	3.75	6.14 b	5.24 c	7.32 b	6.29 c
Doppleschwand	122.7	158.5 b	39.5 b	59.5	7.24 b	7.59 a	6.61	6.51	6.72	4.50	6.12 b	5.35 c	6.23	6.43 c
Egg	124.9 b	170.0 a	39.6 b	63.2 b	7.22 b	7.69 a	7.67 b	7.29 b	7.53 b	5.88 b	6.09 b	6.03 c	6.38	6.76
Egliswil	124.6	165.2 b	37.2	61.8	6.41	6.93 b	8.28 b	6.78	7.82 a	2.75	5.81 b	5.20 c	7.38 b	7.05
Gachnang	119.9	155.8	37.7 b	56.0	6.28	7.14 b	6.50	7.20 b	7.16 b	1.25 c	5.87 b	6.59 c	5.38 c	7.05
Gommiswald	126.0 b	151.5	38.2 b	58.8	6.99 b	6.93 b	6.78	7.14 b	6.42	6.25 b	6.00 b	4.38 d	7.00	5.91 c
Huetten	125.9 b	154.8	37.1 b	60.0 b	6.56	6.97 b	5.89	7.02 b	6.38	5.00 b	5.73 b	4.59 d	6.96 b	6.54
Huettlingen	122.8	162.2 b	37.9 b	60.8 b	6.11	7.00 b	7.56 b	6.98	7.36 b	2.75	5.91 b	6.24 c	6.80	6.93
Laenzen	126.3 b	162.5 b	39.3 b	60.4 b	7.44 b	7.92 a	8.11 b	7.91 a	7.64 b	6.50 b	6.64 a	5.54 c	7.11 b	6.51 c
Latterbach	115.9	146.0	36.8	54.0 c	5.66	6.76	6.28	6.08	6.45	3.50	5.81 b	5.20 c	5.77	6.43
Littau	124.5	163.9 b	39.1 b	60.7 b	7.32 b	7.47 a	7.11 b	7.34 b	7.34 b	4.88 b	6.31 b	4.76 d	7.15 b	6.84
Niederurnen	124.6 b	159.1 b	38.1 b	61.3 b	7.39 b	7.83 a	7.33 b	7.51 b	7.47 b	5.75 b	6.00 b	4.89 d	7.48 b	6.37 c
Oberehrendingen	121.7	155.2	37.8 b	56.0	6.41	7.18 b	6.53	6.68	7.12 b	2.63	6.18 b	6.68 c	5.69	7.22 b
Pfisterboden[1]	116.4	143.9	34.9	54.5 c	5.41	6.39	5.69	6.08	6.02 c	4.25	5.37	6.38 c	5.61	6.77
Reichenbach	112.1 c	128.3 d	32.5	50.2 d	5.72	5.89 c	5.28	6.02	5.11 d	2.63	5.09	5.66 c	5.11 c	5.61 d
Root	124.2	165.4 b	38.7 b	60.2 b	7.32 b	7.59 a	7.78 b	7.24 b	8.00 a	2.50	6.37 b	6.41 c	6.31	7.15
Tuerlen	123.7	159.6 b	37.8 b	59.8 b	5.89	6.64	7.17 b	6.31	6.91 b	3.63	5.59	6.40 c	6.01	6.88
Weiningen	125.5 b	161.5 b	37.4 b	59.5	6.07	7.34 a	7.53 b	7.24 b	7.70 b	2.00	5.93 b	5.82 c	6.08	7.05
Wernetshausen	126.4 b	159.9 b	40.0 b	60.0 b	7.11 b	7.94 a	7.11	7.22 b	7.49 b	6.00 b	6.37 b	4.78 d	6.59	6.78
Wolhusen	120.9	146.1	38.1 b	55.5	6.41	7.18 b	5.69	6.61	6.17	2.63	6.06 b	5.00 d	5.61	6.19 c
Ecotypes mean	**122.7**	**156.7**	**37.8**	**58.7**	**7.60**	**7.20**	**6.91**	**6.92**	**7.01**	**3.95**	**5.97**	**5.56**	**6.40**	**6.64**
ABERCOMO	115.7	157.1	31.8 c	61.1 b	5.32 c	6.09	7.61 b	5.94	7.09	2.50	4.50 c	6.62	4.92 c	6.36 c
AXIS	114.8	147.0	31.6 c	57.5	5.17 c	5.86 c	5.89	6.58	6.07 c	1.38 c	5.14	8.59 b	7.32	7.10
BARLIZZY	125.3 b	147.2	38.1 b	55.6	7.07 b	6.64	6.11	7.01 b	6.35	4.88 b	6.12 b	5.62	6.84	6.98
ORYX	122.4	149.4	37.3 b	55.4	6.61	6.81	6.17	6.87	6.67	3.50	5.77	8.13 b	6.22	6.90
Cultivars mean	**119.5**	**150.2**	**34.7**	**57.4**	**7.61**	**6.35**	**6.44**	**6.60**	**6.54**	**3.06**	**5.38**	**7.24**	**6.33**	**6.84**

[1] Ecotype Pfisterboden was identified as being tetraploid and was excluded from further analysis

[2] Yields: YTOTH1, YTOTH2: total 1st, 2nd full harvest year; YCT1, YCT23: 1st cut, 2nd+3rd cut; means 1st and 2nd full harvest year

[3] Vigour scores: VIG1, VIG23 1st cut, 2nd+3rd cut, means 1st and 2nd full harvest year; VGEND at end of 2nd full harvest year; VIGH1, VIGH2 means 1st, 2nd full harvest year; VGALT at high altitude (1000 m a.s.l.)

[4] Resistances: SNOM snow mold (different fungi); RUST crown rust (*Puccinia coronata*); XANT bacterial wilt (*Xanthomonas translucens* pv. *graminis*); LSPOT *Drechslera* leaf spots

Values of individual accessions followed by these letters are: a: significantly superior to best cultivar; b: significantly superior to cultivars mean; c: significantly inferior to cultivars mean; d: significantly inferior to poorest cultivar

These comprehensive results, based on multilocal trials, confirm previous single location observations (Boller *et al*. 2005) and show a high potential for agronomic performance of Italian ryegrass ecotypes. The best ecotypes, such as Laenzen and Niederurnen, significantly outperformed the cultivars mean in all yield and vigour related characteristics as well as in snow mold and bacterial wilt resistance, and would have had a fair chance of being recommended for cultivation except for their extremely high susceptibility to crown rust.

Table 2. Relationship between selected performance characteristics (see Table 1 for explanation) and collection site data for 19 ecotypes of Italian ryegrass from Swiss permanent grassland: Pearson correlation coefficients[1)] and (*in Italics)* sign and ranking of significant variables resulting from stepwise regression analysis[2)].

Collection site data	Yields (dt/ha)		Vigour (9=best, 1=poorest)			Disease resistance (9=best)		
	YTOTH1	YTOTH2	VIGH1	VIGH2	VIGALT	SNOM	RUST	XANTH
Geographical data								
Average distance to test site	-0.77	-0.73 *-1*	-0.59	-0.64 *-1*	-0.44 *-1*	-0.45		
Longitude	0.63	0.51	0.70 *+1*					0.52 *+1*
Latitude	0.54	0.56	0.48	0.57	*-2*			
Altitude					0.50			
Slope			*-2*	*-2*				
Soil data								
pH	-0.47				-0.60			
P content						*+2*		
Ca content	-0.84 *-1*	-0.66	-0.70 *-3*	-0.56		-0.61 *-1*		
Mg content							0.55 *+1*	
Organic carbon content	-0.57							
Clay content			*-4*					
Vegetation characteristics								
Percentage Italian ryegrass								0.58 *+2*
Species richness		-0.51 *-2*	-0.48	-0.59 *-3*				-0.60

[1)] Only correlation coefficients significantly ($p<0.05$) different from 0 are shown

[2)] Stepwise regression was calculated for accession means, using parameters $p=0.15$ for entry and $p=0.05$ for removal of variables. Sign and ranking of remaining significant variables according to partial r^2 are shown for each performance characteristic.

In order to define criteria for targeted *in situ* or *ex situ* conservation of genetic resources of Italian ryegrass, as well as to optimize sampling strategies for use in breeding, collection site factors were related to performance characteristics using two approaches (Table 2). First, Pearson correlation coefficients were calculated between site variables and performance characteristics. Second, a stepwise regression model was applied to each performance characteristic, including all measured site factors as potential explicatory variables. The site factors average distance to test site, longitude, Ca content and species richness showed a consistent relationship with at least two performance characteristics, which was always in the same direction: soils with a low Calcium content, located east and close to the test

sites, and having a low species richness harboured the best performing ecotypes. However, the effects of distance to test site, longitude and low Calcium content were correlated with each other (data not shown) and therefore, it remains unclear which of these factors was the primary determinant of the observed relationship. This is not true of the observed negative relationship between species richness on performance of the Italian ryegrass ecotypes. There was no correlation between species richness and geographical location, and nevertheless ecotypes from more species rich grassland performed less well in terms of yield, vigour and *Xanthomonas* resistance. This latter observation points to a possible conflict between conserving species rich grassland as a contribution to biodiversity at the species and ecosystem level, and conserving valuable plant genetic resources for food and agriculture. A similar conflict was identified by Peter-Schmid *et al*. (2008) regarding reduced occurrence of rare alleles in *Festuca pratensis* ecotype populations from extensively managed habitats. Altitude of the collection site was positively correlated with performance at high altitude; however, in stepwise regression analysis, this effect was overridden by that of closeness to the testing site.

In conclusion, ecotypes of Italian ryegrass from Swiss permanent grassland exhibit very promising agronomic performance characteristics, justifying there further use to aliment breeding programmes. Populations from north eastern Switzerland growing on soils with a low Calcium content and in habitats with low plant species diversity appear to be the most valuable. However, the analysis also showed that the closer to the testing sites the ecotypes were collected, the better was there performance. High susceptibility to crown rust was the major drawback of the ecotypes. Efficient ways to introgress powerful rust resistance genes would greatly help exploiting their high performance potential.

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Wild *Lactuca* germplasm for lettuce breeding: recent status, gaps and challenges

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ABSTRACT: Recent status, gaps and challenges regarding to wild *Lactuca* L. germplasm, their taxonomy, bio-geography, biology and ecology, gene pools, field explorations and collecting activities, germplasm collections, development of descriptors, characterization and evaluation, lettuce breeding and enhancement of *Lactuca* spp. are summarized and critically analysed. Future research and activities related to wild *Lactuca* germplasm are considered.

Keywords: biogeography, breeding, collecting, gene pools, genetic diversity, lettuce.

Introduction

Genetic resources of wild *Lactuca* species are an integral part of world plant heritage as conserved in gene bank system, and they play important role in recent lettuce breeding (Lebeda et al., 2007c; Mou, 2008). An enormous progress in theoretical research and practical applications of *Lactuca* germplasm was achieved during last 25 years (Lebeda et al., 2007c). Persisting gaps, problems and confusions in this area, with recent progress in their solving, are highlighted in this paper.

Taxonomy of *Lactuca* L.

Taxonomic and phylogenetic studies range the genus *Lactuca* L. to the Compositae (Asteraceae) (Funk et al., 2005), one of the largest plant families; subfamily Cichorioideae, tribe Lactuceae. Detailed search of available literature confirmed about 100 wild *Lactuca* spp. with the highest number of autochtonous species and species richness in Asia (51 species) and Africa (43 species) (Lebeda et al., 2004b). Latest molecular data on phylogenetic relationships among *Lactuca* species (Koopman et al., 1998) confirmed, with some modifications, a previously elaborated broader generic concept of this genus (summarized by Lebeda et al., 2007c). However, formal classification and the subgeneric division (Lebeda et al., 2007c) need critical reconsideration, further development and clarification.

Geographic distribution and hot-spots of diversity

Of late years the potential value of genes from wild species has for many times been stressed (Gass and Frese, 1999). Regarding the lettuce, the reasons are firstly the lost of genetic diversity and limited number of those species in current *ex-situ* germplasm collections (Lebeda et al., 2004a, 2007c). A primary objective is to concentrate the lettuce progenitors and/or wild relatives to world's collections from the areas of probable lettuce origin and those where the highest genetic diversity of *Lactuca* species occur (Lebeda et al., 2004c). The high levels of diversity of *Lactuca* species confined to the Mediterranean basin and southwest Asia indicate that those regions may be seriously considered as hot-spots for lettuce conservation priorities (Beharav et al., 2008; Lebeda et al., 2001). Future eco-geographic studies and collecting strategy must be more intensively oriented to the hot-spots in the Central and South Africa and North America as well (Lebeda et al., 2007c).

Biology and ecology

To the genus *Lactuca* belong annual, biennial or perennial herbs, rarely shrubs with abundant latex. In the sections Phoenixopus, Mulgedium, Lactucopsis, Tuberosae, Micranthae and Sororiae there are included mostly biennial or perennial species (Lebeda and Astley, 1999). The division of section Lactuca into two subsections Lactuca and Cyanicae is based on life cycle of their representatives (Feráková, 1977). Subsection Lactuca comprises annual, winter annual or biennial herbs; perennial species belong into subsection Cyanicae. The autochtonous North American species are biennial. The African species are annual or perennial herbs or sub-shrubs, rarely scandent. The genus *Lactuca* L. comprises species with wholly different ecological requirements occupying various habitats. The species of the lettuce genepool (those of the main breeder's interest) *L. serriola*, *L. saligna* and *L. virosa* are weedy and occur on waste places and ruderal habitats – mainly along roads, highways and ditches (Lebeda et al., 2001, 2004b, 2007a). The majority of *Lactuca* spp. like *L. perennis*, *L. viminea*, *L. graeca*, *L. tenerrima* are calciphilous plants and settle limestone and dolomite areas, mostly rocky slopes. Endemic lianalike species are found in rain forests of East Africa. A comprehensive survey regarding biology and ecology of European *Lactuca* species was done by Feráková (1977) and Lebeda et al. (2004b) who summarized available information about hundred *Lactuca* spp. from current world literature. However, basic data about biology and ecology in most of *Lactuca* spp. (esp. African and Asian origin) are still missing.

Gene pools and genetic diversity

Recent available knowledge concerning to exploitation of wild relatives in commercial lettuce breeding was specified by Lebeda et al. (2007c). In general, the primary gene pool comprises numerous cultivars and landraces of *L. sativa* and the wild ancestor *L. serriola*. The wild *serriola*-like species from Southwest Asia (*L. aculeata*, *L. altaica*, *L. azerbaijanica*, *L. georgica*, *L. scarioloides*) and African *L. dregeana*, which indicate very similar interfertility with the crop, belong to the primary gene pool as well (Lebeda et al., 2007c). Although

L. saligna and *L. virosa* have been intensively studied by plant evolutionists and breeders, the categorization to secondary and tertiary gene pools has remained open to question. A view completely different from the previous conception was proposed by Koopman et al. (1998) who suggested that section Lactuca subsection Lactuca comprises the primary and secondary gene pool, while the sections Phaenixopus, Mulgedium and Lactucopsis include the tertiary gene pool. However, categorization of many *Lactuca* spp. into gene pools is still questionable.

Germplasm collections - recent status and problems

Collections, their structure and gaps

Detailed information about wild *Lactuca* germplasm collections around the world was published by Lebeda et al. (2007c). From this survey it is evident that there are only few important collections in Europe (ca 5) and USA (ca 3). In the centres of high species richness and diversity there are no germplasm collections with local accessions. Analysis of ILDB (International Lactuca Database) showed that over 90% of the collections are represented by only three *Lactuca* species (*L. serriola, L. saligna, L. virosa*), mostly of European origin. The autochtonous species from other continents (Asia, Africa, America), which form ca 83% of known *Lactuca* species richness (Lebeda et al., 2004b), are represented in collections by ca 3% of accessions (Lebeda et al., 2004a). Broadening of the collecting activities in natural habitats of these areas is considered as a crucial point for future development of *Lactuca* spp. collections (Lebeda et al., 2007c).

Taxonomic status of accessions and duplicates

Correct taxonomic ranging of accessions is a base of all operations with plant material in gene banks, to prevent any genetic pollution and lost, to reduce redundancy within and among collections by eliminations of duplicates, and for correct interpretation of results of research work with germplasm material. Insufficient or incorrect passport data complicate evaluation of accessions morphologically, and by using biochemical and molecular markers (Hintum and Boukema, 1999).

Basic errors in taxonomic status of accessions as declared by gene banks were found during recent studies. When evaluating a set of 49 accessions of 24 wild *Lactuca* species by the mean of morphological characters, chromosome number, relative DNA content and isozyme polymorphism, a total of 17 accessions were reclassified and/or their taxonomic status was discussed (Doležalová et al., 2004).

Within a set of 95 *Lactuca* spp. accessions provided by gene banks in Czech Republic, Germany, Netherlands, UK and the USA, and represented by 12 *Lactuca* species (*L. aculeata, L. altaica, L. dentata, L. dregeana, L. indica, L. livida, L. perennis, L. quercina, L. saligna, L. serriola, L. tatarica* and *L. virosa*), morphologic assessment of plants confirmed taxonomic status declared only for 50 accessions. Other accessions were taxonomically re-determined

(31 acc.); represented by mixtures of *L. serriola* forms, and/or different *Lactuca* species or interspecific hybrids of *Lactuca* (14 acc.) (Doležalová et al., 2007a).

Duplication of accessions is important problem of efficient genebanking. Comparison of passport data of four large collections (CGN, WRPIS, IPK and HRI) showed that 60% of the accessions are duplicated once, twice or in all studied collections (Hintum and Boukema, 1999). Morphologic assessment of the above mentioned set of 95 *Lactuca* spp. accessions forming 34 duplicate groups on the base of passport data, showed that 69 accessions can be considered as morphologic duplicates (Doležalová et al., 2007a).

Field studies and collecting activities

The increasing interest in sampling and study of geographic distribution of wild *Lactuca* populations in natural habitats resulted in collecting missions which have effectively been initiated in the early nineties at the Department of Botany, Palacký University in Olomouc (Czech Republic). In the years 1995-2008, expeditions were conducted in fourteen European countries (Austria, Croatia, the Czech Republic, France, Hungary, Germany, Greece, Italy, the Netherlands, Slovakia, Slovenia, Sweden, Switzerland, the United Kingdom), nine states of USA (Arizona, California, Idaho, Iowa, North Carolina, South Dakota, Utah, Wisconsin, Wyoming) and Canada. Field studies and collecting of *Lactuca* spp. germplasm were also made in Turkey, Israel, Jordan, South Korea and New Zealand. Almost 1300 seed samples of twelve wild *Lactuca* species were collected (Doležalová et al., 2001; Křístková and Lebeda, 1999; Křístková et al., 2001; Lebeda et al., 2001a; Lebeda, unpubl.).

Collecting expeditions of *L. serriola* germplasm in four European countries were conducted within the framework of the EU-funded project "GENE-MINE" (Lebeda et al., 2007a). The seed material (800 accessions originating from 50 locations) was used for regeneration, inclusion into the National Germplasm Collections of individual countries and for research purposes in follow-up studies (e.g. Lebeda and Petrželová, 2004).

In cooperation with the Institute of Evolution (Haifa University in Israel) searching and collecting trips of the wild species *L. saligna* (least lettuce, willow-leaf lettuce) were conducted in 2004-2007 to protect Israel and world lettuce crop (Beharav et al., 2008; Lebeda, unpubl.). In all cases the seed samples were harvested from individual plants following the international standards for germplasm acquisition (Guarino et al., 1995) and in order to avoid collection of duplicated material (Hintum and Boukema, 1999; Lebeda et al., 2004a). Basic passport data specifying the locality (sample identification, geographic coordinates, information on sampling, unique terms as an occurrence of diseases and pests, etc.) were recorded and are available in author's databases.

Descriptors development

An exact, detailed and distinct description of genetic resources serves as a tool for their correct taxonomic determination and a definition of both interspecific and intraspecific variations (Lebeda et al., 2007b). There are national descriptors elaborated for characterization of each

national collections, e.g. those from the Centre for Genetic Resources (CGN, Wageningen, The Netherlands) (Boukema et al., 1990); the Western Regional Plant Introduction Station Pullman, Washington (USA) (McGuire et al., 1993).

An international minimum descriptor lists were elaborated for genetic resources of *L. sativa* and wild *Lactuca* spp. (*L. serriola* and related species from the primary gene pool) by the community of the representatives of European gene banks within a framework of the activities of the European Cooperative Programme (ECP/GR), Working Group of Leafy Vegetables (Lebeda and Boukema, 2005).

The descriptors and codes for genetic resources of cultivated lettuce (*Lactuca sativa* L.) (Křístková et al., 2008) and wild *Lactuca* spp. (English-Czech version) (Doležalová et al., 2002a) were developed as a basic rule for documentation of the characterization and evaluation of the Czech collection of lettuce accessions within the National Programme of Conservation and Utilization of Plant Genetic Resources of the Czech Republic. International list of the most important morphological characters of wild *Lactuca* species was created within the framework of the EU-funded project "GENE-MINE" (Fifth Framework Programme of the European Commission) (Doležalová et al., 2003).

Characterization and evaluation of wild *Lactuca* germplasm

Morphology

Morphologic assessment of each accession is performed during their regeneration by using descriptor lists (Doležalová et al., 2002a, 2003; Křístková et al., 2008). Detailed study of morphologic variation was performed for *L. serriola* and *L. saligna*. Fifty *L. serriola* populations collected in four European countries (Czech Republic, Germany, The Netherlands, United Kingdom) (Lebeda et al., 2007a) were cultivated in a greenhouse under controlled conditions. Assessment included 26 quantitative and qualitative characters of the stem (e.g. stem length), rosette and cauline leaves (e.g. depth of incisions), the inflorescence and flower (e.g. anthocyanin coloration on bracts) (summarized in Lebeda et al., 2007b) including the fruit morphology (e.g. length and width of achene body, length of achene beak and number of ribs) (Doležalová et al., 2007b). Similar morphologic assessment was performed for about 70 populations of *L. saligna* originated from Czech Republic, France, Italy, Portugal, Israel, Jordan and Turkey (Beharav et al., 2008; Křístková et al., 2007a).

Phenology

The genus *Lactuca* is extremely variable also from the phenological viewpoint. Among the developmental characteristics, substantial differences in the time of flowering were recorded between accessions originating from individual countries (Doležalová et al., 2005; Lebeda et al., 2007b, c). Substantial differences in developmental stages (beginnings of bolting and flowering) were recorded among 89 *L. serriola* samples originating from different eco-geographic conditions of Europe (Austria, Czech Republic, France, Germany, Italy, Slovakia, Switzerland, the Netherlands, the United Kingdom) when grown under unified conditions in

a greenhouse. Developmental stages of plants, as being influenced (formed) by original eco-geographic conditions of samples (Lebeda et al., 2001), persist when plants are cultivated in unified environmental conditions and are fixed genetically (Křístková et al., 2007b).

Karyology and DNA content

Wild *Lactuca* species can be divided into three main groups, according to base chromosome number (Feráková, 1977). First group is relatively small and contains perennial species of Europe and the Himalayas with haploid chromosome number n=8. The haploid chromosome number n=9, characterizes the majority of European and Mediterranean species, as well as species from the Middle East, Africa and India in the second group. The third group, containing of North American species distributed from Canada to Florida, is marked by a haploid chromosome number of n=17. It is of amphidiploid origin and somewhat geographically and genetically isolated. Our understanding of this genus remains incomplete, because the chromosome numbers of numerous *Lactuca* species are not known (Lebeda and Astley, 1999) or especially chromosome numbers of North American species may differ from the reported data (Doležalová et al., 2002b).

Until now, analyses of variation in nuclear DNA content have been performed on only a limited number of *Lactuca* species (*L. sativa, L. serriola, L. saligna, L. virosa*) (Bennett and Leitch, 1995; Koopman and De Jong, 1996; Koopman, 1999, 2000). Karyotype analysis and relative DNA content were used for characterization of *L. sativa, L. serriola, L. saligna,* and *L. virosa* and their evolutionary relationships (Koopman and De Jong, 1996). Flow cytometry was tested for reliability as a tool to distinguish some *Lactuca* species (Koopman, 1999, 2000). Doležalová et al. (2002b) analyzed fifty accessions of 25 *Lactuca* species (including hybrid *L. serriola* × *L. sativa* and *Mycelis muralis*) for chromosome number and relative DNA content variation. Later, Koopman (2002) showed that five *Lactuca* species (*L. viminea, L. virosa, L. serriola, L. sativa* and *L. sibirica*) have significant intraspecific variation in DNA content, but it was concluded that only the variation within *L. virosa* seems to have evolutionary significance. Recently, the studies are focused on intraspecific differences in DNA content in *L. serriola* germplasm, originating from the twelve European countries (Lebeda et al., 2004c, 2007c).

Resistance to diseases and pests

Recent advancement in research and breeding of lettuce for resistance to diseases and pests was summarized elsewhere (Lebeda et al., 2007c; Mou, 2008). Traditionally, *Bremia lactucae* is considered as the most important disease of cultivated lettuce. Limited availability of durable sources of resistance stimulates an increasing interest of breeders for new sources of resistance from wild *Lactuca* spp. (Lebeda et al., 2002). Current studies (Beharav et al., 2006; Lebeda et al., 2008; Lebeda and Zinkernagel, 2003; Petrželová et al., 2007) demonstrated that wild *Lactuca* (e.g. *L. saligna, L. serriola*) germplasm have enormous potential from this viewpoint. Their more intensive exploitation in resistance research and breeding is primarily

based on increasing number of wild *Lactuca* accessions from various eco-geographical areas (Lebeda et al., 2004a, b).

Biochemical features (chemical compounds)
Current research of *Lactuca* germplasm is also focusing on detection and characterization of some chemical compounds (e.g. sesquiterpene lactones, phenolics and glucosides) of pharmacological importance (Kisiel and Barszcz, 1998; Kisiel and Zielinska, 2000). This aspect has been underestimated till now however that there is increasing potential for utilization, at least of some *Lactuca* germplasm, in further medicinal research and pharmacological purposes (Chen et al., 2007; Kim et al., 2007).

Protein and molecular polymorphism
The status of characterization of *Lactuca* spp. germplasm by protein and molecular markers was summarized by Dziechciarková et al. (2004a) and Lebeda et al. (2007b). Various methods and approaches have been applied for this purpose, however, only relatively limited number of species and accessions have been analysed (mostly same sets of accessions from a few germplasm collections) (e.g. Jansen et al., 2006). More detailed population studies are needed to define various relationships between eco-geographical distribution of *Lactuca* spp. and their genetic polymorphism. Recently some of these studies (e.g. for *L. saligna, L. serriola*) are in progress (Dziechciarková et al., 2004b; Kitner et al., 2008; Kuang et al., 2008).

Conclusions and future prospects
Despite enormous progress in research of wild *Lactuca* germplasm, the recent review demonstrated many gaps in our knowledge and understanding of various aspects related to this topic. As the most crucial points could be considered:

1) Complex taxonomic and phylogenetic treatment of the genus *Lactuca* L.;
2) Detailed floristic, bio-geographic and ecologic delimitation of distribution of known *Lactuca* spp.;
3) Clarification of the structure of *Lactuca* gene pools;
4) Reconsideration of germplasm collections structure from the viewpoint of quality and quantity;
5) Collecting and exploration missions, esp. to the areas of high species richness and diversity (e.g. South Africa and Asia);
6) Enlargement of activities focused on complex characterization and evaluation with importance not only for management of wild *Lactuca* gene bank collections, however also for their efficient utilization in lettuce breeding;
7) Broad international cooperation of different institutions, incl. Bioversity International.

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The role of crop/pollinator relationship understanding in breeding for pollinator-friendly legume varieties; from a breeding perspective

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ABSTRACT: Following the reports of the decline in solitary and social bees, breeders are encouraged to develop a breeding approach that strives to integrate food production into the healthy functioning of the agro-ecosystems. In the particular case of legumes, this approach should preserve bee fauna by providing suitable floral resources within the crops themselves (Allen-Wardell et al., 1998; Maria-Klein et al., 2007). In parallel, legume breeding for sustainable agriculture demands the development of non-food services such as the environmental services (Helenius and Stoddard, 2007). Legumes are visited by a great number of bees; bumble bees, honey bees; and wild bees. Foraging places and nesting sites for solitary and social bees are some of the ecological services provided for legumes to a sustainable agriculture. Consequently, we face a situation where the development of pollinator-friendly varieties is needed. Such a situation may require a re-thinking of crop breeding strategies and objectives, and production practices. The role of bee pollinators as agents of pollination and agents of hybridisation needs to be considered. Crops with insect floral attractiveness and reward can be used to potentially maximise pollinators conservation as well as crop yield and yield stability. Optimising the crop/pollinator relationship (CPR) would be a key to the establishment of improvement breeding strategies that increase the yield and yield stability. We need to understand the role of the solitary and domestic bees; thereby facilitating the development of "pollinator friendly" cultivars. The benefits of approaching legume improvement by understanding and maximizing CPR are both direct (seed yield and stability increase) and indirect (conservation of biodiversity, beneficial insects). Our contribution will analyze how understanding CPR can contribute to underpinning the production of high-yielding and pollinator-friendly varieties by examining: 1) The status of knowledge on grain legume mating systems; 2) The status of knowledge on floral traits for improving CPR: their role and genetic control; 3) The contribution of CPR understanding to plant breeding: hybrid seed production and open population improvement.

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High throughput genotyping for characterization of barley germplasm

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ABSTRACT: Barley (*Hordeum vulgare* L.) is the second most important crop plant in Latvia by production (298,100 tons in 2006) and by sowing area (149,800 hectares in 2006) with extensive breeding traditions going back to early 20th century. Modern requirements for safe food and feed in combination with high yields and defined quality features mandates plant breeders to employ novel tools, such as molecular marker-assisted selection (MAS), for breeding new varieties. However, MAS will only be effective, if appropriate and well-characterized germplasm is available for specific breeding purposes. Exotic barley genetic resources are useful sources of novel disease and abiotic stress resistance genes, but it is also essential to characterize the adapted germplasm that will carry the introgressed alleles. Recently, high throughput DArT and Illumina SNP genotyping platforms have been developed for barley (Rostoks et al. 2006; Wenzl et al. 2004) facilitating studies of genetic diversity, as well as linkage and association mapping. Twenty three Latvian barley varieties and 72 breeding lines were genotyped at 1536 SNP loci using Illumina barley OPA1 (BOPA1). In parallel, the same 23 Latvian barley varieties and 19 European parental varieties were genotyped at over 1100 loci using DArT markers. Principal Coordinate Analysis (PCoA), phylogenetic trees and population structure analysis indicated that Latvian barley varieties cluster with other European varieties as could be expected based on the known pedigrees. Germplasm was partitioned in several subsets by PCoA and population structure analyses, however, there were no obvious major features differentiating the germplasm groups. Genotyping data will be used in association genetics studies for mapping agronomic and quality traits and for development of molecular markers for barley breeding in Latvia.

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Use of wheat genetic resources at CIMMYT: toward new germplasm with enhanced genetic diversity

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ABSTRACT: Wheat domestication and modern breeding led to a loss of potentially beneficial alleles that increases the risk of genetic vulnerability. Exploiting the untapped genetic diversity of related species is consequently a major challenge (Warburton et al., 2006). CIMMYT is facing this challenge through a multi-step process including i) establishment of large collections and accurate documentation of genetic resources, ii) analysis of genetic diversity and precise phenotyping for major traits of interest, and iii) extensive development of pre-breeding material. The genebank of the International Maize and Wheat Improvement Center (CIMMYT) contains the largest wheat collection in the world including 150,000 unique samples of wheat and its wild relatives from more than 100 countries. Information is however lacking for most wheat relatives, and efforts are systematically made for their taxonomic validation and better documentation. The use of molecular markers permitted to find useful diversity in the collections and to better define target species and accessions to be used. For example, higher genetic diversity was identified in cultivated emmer than in durum wheat and it was confirmed that emmer based synthetic wheat and back-cross lines represent new sources for drought tolerance diversity. The analysis of genetic diversity within large collections has also led to the establishment of sets of representative samples, facilitating phenotyping and identification of useful accessions. In the development of pre-breeding material, molecular markers are used to detect introgressions of alien chromosomes into cultivated background (Zaharieva et al., 2003) and localize translocation breakpoints in recombinant chromosomes. Finally, analyzing the relationship between genotyping and phenotyping data allow mapping and dissecting complex traits. Examples are provided for these different steps and main achievements are highlighted.

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Melokhia (*Corchorus olitorius* L.) a leafy vegetable with good export potential

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ABSTRACT: In the genus *Corchorus* are an annual herbaceous plant species mainly known for its fibre product (jute). However, several species of *Corchorus* are used as a leafy vegetable of which *C. olitorius* is the most frequently cultivated one and an important green leafy vegetable in many tropical and subtropical areas in Asia and Africa. In Egypt, the genus *Corchorus* is represented by four species among them *C. capsularis* is cultivated for its fibres and *C. olitorius* "Jew's mallow", commonly known as Melokhia, is cultivated for its leaves. Melokhia is a very important green leafy vegetable. The area under cultivation is about 4.000 ha with fresh matter yields of up to 22 t/ha. It has been proven that Melokhia has many benefits for health. It is a complete nutrition meal rich with vitamins A and B, mineral salts, carbohydrates and fibres. It contains a high level of essential amino acids, and protein content. Research proved that it is a strong sexual enhancer increasing the excretion of sexual hormones. Despite its richness, adaptation to a low input agriculture, and widespread use by the people, Melokhia is still neglected and underutilized species. Insufficient effort has been made to ensure the conservation of biodiversity. Therefore, it is necessary to protect and prevent the local germplasm sources of *C. olitorius* from genetic erosion and to conserve its diversity for sustainable development and adaptation to a changing environment.

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Breeding value of germplasm of grain amaranths (*Amaranthus cruentus* L.)

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ABSTRACT: Since ancient times, grain amaranths have been cultivated by the natives cultures in central and south states of Mexico. There are two species cultivated for grain: *Amaranthus hypochondriacus* and *Amaranthus cruentus*. *A. cruentus* is more adapted to warm conditions and it is possible to harvest it twice during a year. Eleven landraces of *A. cruentus* collected in Guerrero, Morelos and Puebla were evaluated in Marín N.L. under field conditions. It was possible to obtain grain from seven landraces and their breeding value was evaluated using genetic parameters.There were landraces from highland regions of Guerrero that were affected by the photoperiod when sown in the lowlands of the north of Mexico. According to the breeding values obtained in this study, landraces from Puebla and Morelos, which are located in the central states of the country, do not have any problem when cultivated in lowlands of the north of Mexico and are not affected by the photoperiod. Landraces of Morelos and Puebla showed better response to selection and were not affected by environmental conditions. These landraces may be selected for setting up a breeding programme for this species in the lowlands of the north of the country.

Keywords: *Amaranthus cruentus*, landraces, response to selection, heritability.

Introduction

Grain amaranths (*Amaranthus* spp.) are a native crop from México, It has been cultivated since 5000 years (Sauer, 1993). Ancient cultures in Mexico grew this crop, and the Aztec Empire used this grain as food and also for ceremonial purposes. During the discovery of the New World many native crops were substituted by European species and cultivation of grain amaranth declined (Williams, 1995; Paredes, 1994). There are two species cultivated for grain in Mexico: *Amaranthus cruentus* and *A. hypochondriacus,* each of which has different requirements for climatic conditions. *A. hypochondriacus* grows well in template climate of highlands in a range of 1700 to 2500 masl. *Amaranthus cruentus* is well adapted to warm regions in a range of 200 to 1500 masl (Alejandre and Gispert, 2004). Most of the areas where this grain is cultivated are located in the central and south states of México.

There exists a great variability in the landraces of these two species, such as plant height, seed colour, stem colour, panicle colour, leaf colour (Grubben and Sloten, 1981). Both species have different agronomic characteristics and are used for human consumption.

Within landraces of both species there is also great variability, and this is a handicap for introducing this crop to another regions of mechanized agriculture. Nevertheless, this great variability may be utilized in breeding programmes. Grain amaranths have received little attention regarding to breeding landraces. There are few varieties developed mainly for *A. hypochondriacus* in Mexico. In the case of *A. cruentus,* up to date there are not improved varieties available in Mexico.

The aim of this work was to assess the agronomic potential, genetic parameters and response to individual and family selection in the landraces of grain amaranths; collected where this crop is stillcultivated under low income agriculture and for indigenous groups in several regions of Mexico

Material and methods

The basic material for the study comprised eleven landraces and ten families for each one.

Eleven experiments were evaluated at the Agricultural Experimental Station of Faculty Agronomy, University of Nuevo León, Marín, N.L. The experimental site is located at 25^0 56' north latitude and 100^0 3´ west longitude, and at an altitude of 350 m. Eleven land races were evaluated under field conditions.. The landraces were collected in the states of Guerrero, Morelos and Puebla. Each experiment was performed in a randomized block design with two replications. Parameters were calculated using expected mean square of treatments and square mean of experimental error (Steel and Torrie, 1980) model II, total genetic variance was estimated as: $Vt=Vg=(\sum MST-\sum MSE)/ro$, where $ro=(\sum ri.-\sum r^2/\sum ri)1/t-1$, heritabilily in broad sense as $H^2=Vg/SMT$ (Cornide et al., 1985). It was assumed that each family of each landrace corresponds to half sib maternal family, and response to individual selection was estimated as $ISR=iH^2Vf$ (Falconer, 1981), and response to family selection as $FSR=1.4(Vg)/\sqrt{MST}$. Response to selection for grain yield per plant was assessed considering differential selection for every landraces. This differential selection was calculated as the mean of two selected families minus mean of every landraces $R=DH^2$ (Marquez, 1985).

Results and discussion

Grain amaranths landraces had different response in the study area. Three landraces were sensitive to photoperiod; this plant material was collected in highlands of the state of Guerrero, localized in the south of Mexico, and two landraces were affected by diseases in the field. Landrace (1) Santiago Xochistlahuaca had the lowest value for heritability in broad sense and was affected by environmental factors; it has small seed respect to others and the crop cycle was 80 days to harvest. For the rest of landraces, the crop cycle was 110-120 days to harvest. All landraces may be sown twice during the year. Results are according to many authors (Kulakow and Jain, 1986; Grubben and Sloten, 1981) indicate that warm climate conditions are suitable conditions for growing *A. cruentus*. Landraces 3, 2 and 7 showed highest values for heritability in broad sense. These seeds were collected in the highlands of the states of Morelos and Puebla. Also these landraces were not affected by environmental conditions.

Table 1 shows the values of heritabilities for landraces 3, 2 and 7, which were the highest values for this parameter. These landraces may be chosen for a short term breeding program. These results are partially in agreement to those of many authors that found low values for heritability (Vaidya and Jain, 1987; Joshi 1986; Hauptli and Jain,1984).
Landrace 1 Santiago Xochistlahuaca, from State of Guerrero had the lowest value for heritability in broad sense. This means that little response to selection is expected. Also, this landrace is affected by environmental factors and it is possible to exploit the genetic variability.

Due to the fact that grain amaranths are mainly considered as autogamous, but also have a high level of outcrossing rate (Kulakow and Jain, 1986; Vaidya and Jain, 1987; Lehman et al., 1991) it was convenient calculate the mean between response to individual selection and family selection, and to obtain an average value for the response to selection and to try to determine which are the best landraces of potential breeding value (Table 2). In this case, the best landrace was Santiago Xochistlahuaca from Guerrero State (South Mexico) with the highest value of yield of grain per plant and also the best mean of both individual selection and family selection (Table 2).

Table 1. Variances: phenotypic (V_P), genetic (V_G), environmental (V_E), heritability in broad sense (H^2) and response to individual selection (RIS) per yield per plant.

Landrace	V_P	V_G	V_E	H^2 %
3	1032.5	159.8	17.6	15.4
2	228.7	34.5	29.8	15.0
7	297.3	43.0	31.0	14.4
6	89.4	10.6	15.8	11.8
4	143.1	16.6	12.5	11.6
5	281.7	30.5	42.0	10.8
1	2875.7	201.3	526.1	7.0

Table 2. Mean of yield of grain in g·plant^{-1} (M_{yg}), expected response selection to individual selection (RIS) and family selection (RFS)

Landrace	M_{yg} obtained	RIS	RFS	mean RIS and RFS	M_{yg} expected
1	51.00	5.25	5.25	5.25	56.25
5	38.40	2.34	2.55	2.34	40.74
3	28.90	6.74	6.96	6.85	35.75
6	32.50	1.45	1.56	1.50	34.00
7	26.00	3.37	3.49	3.43	29.43
4	24.00	1.84	1.94	1.89	25.89
2	21.00	3.17	3.19	3.18	24.18

Table 3. Response to selection estimation within the landraces with 2 families selected from 10 families (p = 20%).

Landraces	M_{yg} SF1	M_{yg} SF 2	M_{yg} Sel. Fam	M_{yg} landrace	D	H^2	R
3	61.5	35.8	48.7	28.9	19.8	0.154	3.04
7	38.2	36.5	37.4	26.0	11.4	0.144	1.64
1	82.5	65.8	74.2	51.0	23.2	0.070	1.62
2	33.9	29.5	31.7	21.0	10.7	0.150	1.60
5	48.5	48.3	48.4	38.4	10.0	0.100	1.00
4	29.6	28.6	29.1	24.0	5.1	0.110	0.56
6	37.2	35.8	36.5	32.5	4.0	0.118	0.47

M_{yg} SF1 and M_{yg} SF2 = mean of yield grain g. plant^{-1} of selected families 1 and 2 respectively, M_{yg} landrace. = Mean landrace grain yield, D = differential of selection, H^2 = heretability in broad sense and R=response to selection.

The best landraces for a short term breeding program in the lowlands of north of Mexico are landraces 3 and 1 considering results shown in the Table 2 and Table 3. The best response to selection was for landraces 3, 7 and 1, but in the table 2 landraces 1 and landrace 3 were the best for grain yield. The great variability found in the landraces of *A. cruentus* from the states of Guerrero, Morelos and Puebla, may be useful for setting up a breeding program with this specie.

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The morpho-physiological analysis of biodiversity of pepper germplasm cultivated in Romania

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ABSTRACT: Romania represents a peculiar case when comparing with other European and world countries in what concern the market exigencies for the pepper fruit's colour, shape and taste. The most important difference about the pepper fruits consumed in Romania is the colour of the fruit that is yellow instead of dark green - as it is required by the consumers in other countries. This is the reason why the Romanian genotypes are somehow different from the other ones normally cultivated in other countries. Due to this peculiar situation of Romanian market, the authors analysed the pepper germplasm that fit better to this requirements. The present paper focus on the characterisation from morphological and physiological point of view of 26 cultivars of *Capsicum annum* L. var. *grossum* Sendt. (sweet and round pepper), 8 cultivars of *C. annum* L. var. *longum* Sendt. (long pepper) and 4 cultivars of *C. annum* L. var. *accuminatum* Irish (hot pepper). The purpose of this study was an hierarchic differentiation of the best Romanian pepper genotypes having as selection criteria their quantitative and qualitative characteristics.

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Prospecting almond germplasm in Arribes del Duero

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ABSTRACT: Arribes del Duero is a relativedy small area situated among North-west of Salamanca and South-west of Zamora, where the Duero River serves as natural border between Spain and Portugal. Due to proximity of the river, temperatures are warmer in winter than typical continental climate of Castilla y León, and high in summer, allowing the presence of typical Mediterranean crops such as citrus and almonds. The aim of the project was the identification, characterization and collection of almond local varieties of Castilla y León in order to avoid the loss of these genetic materials and to determinate their potential for future breeding programmes. This survey has been carried out during 2006 and 2007. During this time more than one hundred trees have been geolocalized and characterized morphologically by IBPGR (1985) and UPOV (1978) descriptors. Traditional varieties from Spain (Marcona, Desmayo Rojo, Largueta, Esperanza) and from the region of Tras-os-Montes in Portugal like Pestañeta, Verdeal or Verdinal were found. Also, French varieties (Ferragnes, Ferraduel), and lots of native varieties belong to the area were found too. Seventy seven trees were analyzed. On the basis of leaf, fruit and kernel traits data it has been possible to describe a wide variability between landraces. Furthermore, the results have led to detect the presence of homonyms and synonyms in local varieties and a large confusion between them about the names used, showing disregard and neglect of the crop in this area, and the necessity and urgency for preservation of this genetic material which could become useful for solving future problems, like a reserve of genes, in plant breeding.

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Nuclear and cytoplasmic polymorphism in fig (*Ficus carica* L.) : evidence of microsatellites and *trnL-trnF* intergenic spacer

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ABSTRACT: Tunisian fig (*Ficus carica* L.) resources are threatened by severe genetic erosion due to biotic and abiotic stresses. Therefore, the establishment of a strategy aimed at the preservation of these phytogenetic resources, has become imperative. For this purpose, molecular markers have been designed in fig. This study shows the achievement of cultivar fingerprints using nuclear SSRs and sequence variation of the chloroplastic DNA *trnL-trnF* spacer. Tunisian fig cultivars have been screened with six SSR primer pairs. The revealed genotypes have been unequivocally distinguished and the statistical analyses show a continuous genetic diversity among the studied varieties. Moreover, sequence variations of the intergenic spacer showed that the length of this region varied from 430 to 464 bases. The relatively high A+T values (63.7 % - 64.4 %) of *trnL-trnF* intergenic spacer may explain the high proportion of transversions found (ti/tv = 0.9). Fourteen haplotypes were detected among yielding a haplotypes diversity of 0.983 and a high level of nucleotide diversity (0.0100). In fact, relationships inferred from the cp DNA analysis suggest no geographical structure. The observed variation pattern of cp DNA provides evidence that the fig germplasm has undergone rapid expansion in recent history. The nuclear and the cytoplasmic variability indicate a narrow genetic base in cultivated common fig. Data are discussed in relation with a sustainable management of the local germplasm and to promote biodiversity conservation.

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Tunisian fig (*Ficus carica* L.) genetic diversity: evidence of Amplified Fragment Length Polymorphism (AFLP) markers

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ABSTRACT: Common fig, (*Ficus carica* L.; 2n = 26) is one of the most ancient cultivated Mediterranean fruit. In Tunisia, fig cultivars are highly diversified and provide a large number of genotypes which are facing genetic erosion. In order to characterize the local germplasm, we become interested to the development of the amplified fragment length polymorphism (AFLP) markers. Starting from a set of three primers combinations, the resultant amplified banding profiles have allowed 166 molecular markers. The high percentage of polymorphic bands of 95 and the resolving power (*R*p) collective rate value of 69.05 were scored. The UPGMA dendrogram exhibits the unstructured variability in this crop and the observed diversity was typically continuous. As result, data permitted to evidence high polymorphisms and proved that AFLP is an attractive and powerful procedure to survey fig cultivars genetic diversity. The opportunity of the designed method is discussed in terms of the molecular characterization of genotypes and to study the genetic diversity and its structuration in order to enhance the conservation and the improvement the local germplasm.

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Collecting genetic diversity in natural populations of wild plant species: Case study on *Lactuca saligna* and *L. aculeata* in Israel

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ABSTRACT: Genetic diversity at the species level, i.e., genetic variability or differences among populations of a single species and among individuals within a population, is the basic component of ecosystem biodiversity. Results from natural populations of various plant species indicated notable differences in dispersion of important alleles (e.g., for resistance to biotic or abiotic stresses) harbored by natural populations of the progenitors of cultivated species. The results support sampling strategies which emphasize the importance of increasing the number of sample sites based on the largest ecological spectrum available. Only in case of close accordance of trait with the ecological conditions (e.g. Beharav and Nevo, 2004) is sampling strategy emphasized on sample sites with the appropriate ecological condition available. Based on the above approach, seed samples from 562 plants that morphologically seemed to be *Lactuca saligna* L. (least lettuce), as well as from 151 plants that looked like *L. aculeata* Boiss & Ky., were collected on search and collection trips in September-October of 2004-2007 throughout Israel. *L. saligna* was recorded throughout Israel except for desert areas (e.g., Negev and Judean desert) and in extreme environmental/soil conditions (e.g., Dead Sea area). The accessions of *L. saligna* were collected from 41 localities, representing different climatic and edaphic environments throughout Israel. In all, the taxonomic status of 214 of the accessions were morphologically validated as *L. saligna* with multiplication of 220 accessions in the greenhouse. In rosette formation and leaf morphology, different morphotypes of *L. saligna* were distinguished in the territory of Israel. Individual populations varied in size and morphological uniformity/heterogeneity. The accessions of *L. aculeata* were collected from 11 localities, nine of them representing different regions across the Golan Heights, while two populations with high density were, suprisingly, observed on the Hula Plain (first report of this species in this region). The recent collections of both species are larger and unique in comparison as with those reported in International *Lactuca* database (Lebeda et al., 2004). Accessions of *L. saligna* from the new collection (113 altogether), as well as 33 accessions from previous collections (in Israel, France, Jordan, and Turkey), were screened for *Bremia lactucae* response (Petrželová et al., 2007). A recent study as well as our previous results (Beharav et al., 2006) showed that *L. saligna* is generally highly resistant to most *B. lactucae* races; however it may not be an absolute non-host species for *B. lactucae*, contrary to what has been assumed for approximately the last 30 years. This resistance mechanism is still not clearly understood; this situation could be corrected by our unique approach testing more detailed populations and by other studies.

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Genetics resources of Tunisian plum (*Prunus domestica L.*): morphological and molecular (RAPD) diversity

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ABSTRACT: Plum *Prunus domestica* L. (*Prunus*, Rosaceae), is an hexaploid species (2n=46), native to Europe. In Tunisia, the *Prunus* germplasm is characterized by a great number of varieties. However, many of them are disappearing due to biotic and abiotic stresses. Therefore, the establishment of a strategy aimed at the preservation of these plant genetic resources, has become imperative. For this purpose and as a part of our work, we became interest for the study of genetic diversity of this fruit crop. Several prospections have been conducted in regions of north Tunisia. Morphological and RAPD analysis was used to study the genetic diversity of 14 Tunisian plums. Twenty five morphological traits were studied based on tree and fruit parameters. Analysis of variance and multivariate analysis showed a significant morphological variation within and among cultivars. A set of 5 arbitrary primers that were tested generated a total of 69 polymorphic bands. The scored markers are used either to assess the genetic diversity or to detect cases of mislabelling in the local resources. The Rp value has a collective rate of 35.36, the highest Rp rate of 9.07 was estimated for OPA_2 primer. We assume that this primer mainly contribute to elucidate genetic diversity in this crop. The Genetic distances values ranging from 0.17 to 0.70 with a mean of 0.44. As a result, considerable genetic diversity was detected among the studied Plum accessions. In addition, the UPGMA dendrogram shows two groups of varieties. The derived clustering has proved that the RAPD method is suitable to describe genetic diversity, thus discrimination between varieties has been achieved. The observed variation suggests the presence of a common genetic basis that characterizes the cultivars analysed due to intercrossing. Data analyses using the two kinds of markers at the intra and inter cultivars levels showed that there is an importance genetic diversity. The implication of these results for management and conservation of plum germplasm will be discussed.

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Evaluation of apricot genotypes in a collection of genetic resources

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ABSTRACT: The low adaptability of the apricot species to Slovakian conditions requires the development of breeding programs for creating varieties adapted to the specific condition of the Slovak production area. The breeding program of apricot genetic improvement began in 1962 at the RIPP Piestany and has continued since 1978 on the RBS Vesele. This program has been carried out according to several objectives: the most important were resistance to frost, fruit quality and resistance to diseases (*PPV, Monilinia spp*., *Gnomonia*). Up to now 10 apricot cultivars and 2 rootstocks have been registered. The most promising are Veharda and Vemina with resistance to PPV, 'Veharda' and 'Vegama' with frost resistance, 'Vesna' with tolerance to *Gnomonia erythrostoma,* 'Veselka' with attractive appearance, 'Vesprima' and 'Vemina' with firm flesh. The collections of apricot genotypes are placed on the two localities and contain 314 genotypes originated from all eco-geographic groups. The trees are evaluated by phenotypic-agronomic characters on the one hand and we are going to evaluation for the molecular markers on the other hand. Simple sequence repeats (SSRs or microsatellites) have become the genetic markers of choice in many plant species because they are PCR-based, polymorphic and generally codominant in plant genomes. In many cases, DNA sequences flanking SSRs are conserved between taxa. Twenty-one SSR primer combinations, developed from peach SSR-enriched genomic libraries of peach (*Prunus persica* (L.) Batsch.), were tested with 45 apricot (*Prunus armeniaca* L.) germplasm accessions. All data are included into databases of the National program for conservation PGR in Slovakia.

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AFLP analysis used for molecular characterization of *Ginkgo biloba* L. species

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ABSTRACT: Recently, AFLP (Amplified Fragment Length Polymorphism) technique (Vos et al., 1995) was used to analyze genetic relationships in some of the ornamental cultivars (Wang et al., 2006) which were considered to be an important germplasm source for *Ginkgo biloba* breeding. The aim of our study was to reveal the polymorphism among the DNA genomic samples isolated from trees grown in different sites in Romania and Denmark. In our study, out of the 144 primer combinations, EcoRI/ Mst I, Pst/ Mst I were used and the combinations Ecs-AGG+MAC, Ecs-AGG+MCG, Ecs-AGG+MGA; Ecs-ACC+MCT, Ecs-ACC+MAC, Ecs-ACC+MCT, Ecs-ACC+MGC have revealed the greatest number of polymorphic bands among samples of *Ginkgo biloba* originated in Romania and Denmark. In DNA samples originated in *Ginkgo biloba* provenances from Romania there have been registered few polymorphic bands, with band size comprised between 240-380 bp, while in those originated in Danish *G. biloba* provenances the number of polymorphic bands has been much greater (more than 100), with band size comprised between 240-500 bp. We consider that a through analysis of these polymorphic bands, including statistical ones, could render possible the identification of subspecies or clines within different provenances of *G. biloba*. The UPGMA built dendrogram shows the genetic relationships among individuals grown in different sites in Romania and Denmark (Botanical Garden Cluj-Napoca-Romania, Botanical Garden Craiova-Romania, KVL Arboretum Horsholm-Denmark, Botanical Garden Copenhagen-Denmark, KVL Garden-Denmark and Private Garden Roskilde-Denmark). These results could be explained by the rather common and unknown origin of Romanian *Ginkgo biloba* samples while the Danish *G. biloba* provenances are known to have different origin (Japan and China).

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A sample of biodiversity from Turkey: common bean (*Phaseolus vulgaris* L.) landraces from Artvin

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ABSTRACT: Turkey has rich genetic diversity. Artvin province located in north-east region of Turkey is small province but has rich plant diversity due to its different geographical and ecological formation. Significant part of this province has been flooded by the dams built. The common bean is a very important crop for Artvin's farmers. This study was carried out with the aim of determining and preserving the characteristics of domestic bean varieties grown in Artvin Common bean. A total of 279 samples were collected in 7 districts of 74 villages which will be flooded in the future. These seeds were allocated to 1034 sample groups according to their shapes and colours. With the aim of determining the variability, seed sizes and size indices and their frequency distributions were determined. 11 monocoloured and 21 dicoloured or policoloured seed groups were determined. Majority of the seed were subcompressus type seeds. The samples in 1st group were ranked first in terms of size index. Landrace bean varieties grown in Artvin are a rich genetic source and are irreplaceable sources that can be used in future research.

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In vitro propagation of *Hibiscus rosa-sinensis* L.

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ABSTRACT: *Hibiscus rosa-sinensis* L. (Hrs) is a woody ornamental shrub belonging to the Malvaceae family, cultivated throughout tropicals and subtropicals regions. This species appears to have innumerable variations in the morphological characters: variability concerns size and shape of leaves, colour and petal arrangement in flowers. The very huge number of cultivars (more than 5000) shows large differences in the agronomical traits regarding adaptation to temperate climate, calendar of flowering or sensibility to the several pests. *Hibiscus rosa-sinensis* are mainly propagated by cuttings, layering or grafting in spring (The tropical Hibiscus handbook, 2003). Nevertheless this technique does not ensure healthy and disease-free plants and is strictly dependent on season or climatic condition. Moreover a huge number of varieties are recalcitrant to conventional propagation methods thus, in vitro multiplication may overcome the above constraints. In a recent work, the influence of iron, calcium and BAP on establishment and *in vitro* multiplication of two cultivars of HRS was investigated. (Christensen *et al*., 2008). In this report, seeds deriving from the cross between the commercial varieties Coloumbine x Dark Casino were surface sterilized and germinated *in vitro* onto Brooks and Hough medium (Brooks and Hough, 1958). Nodal cuttings were excised from thirty-day-old aseptically germinated seeds and were cultured on half strength MS (Murashige and Skoog) basal medium supplemented with 2.68 μM NAA (1-naphthaleneacetic acid). After eighty days, multiple shoot clumps were separated and transferred to shoot elongation and rooting medium (half strength MS basal salts with 1.14 μM IAA 3–indoleacetic acid). *In vitro* rooted plantlets were acclimatized in a greenhouse under mist conditions and then grown to maturity. The *in vitro* propagation system described in this work has the potential for efficient multiplication of *H. rosa-sinensis* L. and can be extended to the most recalcitrant commercial variety.

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Evaluation of the native sour cherry genotypes, *"ex situ"* collected in the Romanian National Germplasm

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ABSTRACT: The Romanian territory is located in the extended area limits of the geographic genetic center for cherry, which grows wild or weedy, and presents a high genetic diversity, all over the country. In the past, sour cherry has been propagated to a large extend by seeds and suckers, resulting a wide range of variability. Subsequently, by selection and clonally propagation of valuable individuals within seedling population from different growing areas, many local cultivars were obtained. Additionally, as a result of breeding programs started more than 50 years ago, 18 new varieties were released. Some of them are preserved in cherry collections which include 174 sour cherry (from which 54 are autochthonous biotypes, breeder's lines, advanced old or new cultivars) accessions held in duplicate in two different locations. To give new opportunities for conservation of sour cherry biodiversity and sustainable use of genetic resources, all native genotypes have been evaluated for some morphological and biological characteristics as well as agronomic traits according to the numerical scale of RESGEN descriptors. Obtained results, as level of numerical scale for descriptors evaluation, range from 3 (Marculesti 33/20, Mari timpurii, Timpurii de Cluj, Timpurii de Marculesti cvs.) to 9 (Pitic cv.) for "blooming period", from 3 (Mari timpurii clones, Tarina, Timpurii de Marculesti, Timpurii de Osoi, Timpurii de Pitesti cvs.) to 9 (Pitic cv.) for "harvest maturity", from flat round to elongate for "fruit shape", from 3 (Drobeta, Marculesti 33/13, Marculesti 33/21, Marculesti 4 vie, Pitic, Timpurii de Marculesti cvs.) to 9 (Crisana clones, Leordeni, Locale de Bistrita, Tg. Jiu 401, Tg. Jiu 505 cvs.) for "fruit size", from red to dark red for "fruit skin color", from pink to black red for "juice color", from 3 (Dropia cv.) to 8 (Timpurii de Pitesti, Timpurii de Marculesti, Marculesti 33/20 cvs.) for "juicyness" and from 1 (Pitic cv.) to 7 (Leordeni, Locale de Bistrita, Tg. Jiu 401, Tg. Jiu 505 cvs.) for "tree vigor". Low susceptibility to *Monilia laxa* (Aderh et. Ruhl) was showed by De Botoşani, HV 47/11, HV 45/90, HV 43/32, Mari timpurii cl. 1, Mari timpurii cl.2 and P1 Vie genotypes. Observations made on Băneasa 44/7, Bizigheşti, HV 45/40, HV 47/11, HV 43/32, Mari timpurii cl. 1, Mari timpurii cl. 2, P1 Vie, Suraia and Topoloveni 6 accessions lead to the same level of evaluation concerning the susceptibility to *Blumeriella jaapi* (Rehm) Arx. Collected data offer new possibilities to select valuable genotypes useful by their characteristics for breeding program and also to register other interesting local landraces with fruit of quite importance to domestic market.

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Genetic resources from Mediterranean pasturelands: native species for energetic, environmental and health needs

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ABSTRACT: The valorization of plant species from local flora can be useful to develop the production of new biomass for energetic purposes; for phytoremediation activities to enhance the reduction of the impact of human activities on the environment; for the isolation of natural extracts of potential nutraceutic and phytotherapic interest also to increase animal welfare and quality of animal productions in organic farming. The preliminary results about the characterization for productive and multiple uses of a collection of genetic resources from Sardinian Mediterranean pasturelands are reported here. *Silybum marianum* Gaertner (milk thistle) is a herbaceous thorny species reputed as a dangerous weed in cropping areas for its aggressive vegetative growth and high competition ability. Its seeds contain a complex substance called silymarin well appreciated for the treatment of human liver diseases and animal wellbeing. Due to its rusticity and high growth rates a research was started in Sardinia to evaluate the potential of a *S. marianum* population for bioenergy purposes. First results evidenced that *S. marianum* has an interesting potential for biomass yield with a satisfactory efficiency in energy conversion and productivity. This species can be grown under low inputs and without irrigation, avoiding competition with other important crops, and its annual cycle allows a high degree of flexibility, compared to perennial species (Sulas et al., 2008). A screening of natural populations of species collected from Sardinian heavy metal polluted areas was performed, in order to characterise genetic resources from contaminated areas (Bullitta et al., 2007) and bring them into cultivation to increase the availability of germplasm useful for phytoremediation purposes. Among the examined species, *Chrysanthemum coronarium* was found suitable for further research in order to select fast growing and tolerant genotypes able to colonise polluted areas. Phenolic compounds are of considerable interest due to their antioxidant properties (Surveswaran et al., 2007). The antioxidant capacity (ABTS and DPPH assays) and phenolics content (Folin Ciocalteau method) of 22 species from Sardinian pasturelands have been investigated. The highest antioxidant capacity and phenolics content was detected in *Cistus creticus* (111 mmol/100 g DM and 114 g/kg DM), *Pistacia lentiscus* (131 mmol/100 g DM and 148 g/kg DM) and *Myrtus communis* (133 mmol/100 g DM and 139 g/kg DM). The characterization of several native species from Mediterranean pasturelands has shown that they can be useful resources to accomplish the current requirements of multifunctional agriculture for productive, energetic, environmental and health purposes.

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The biodiversity in the potato land races of west Romania

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ABSTRACT: During 20 years we have been involved in collecting, making and inventory, evaluation and preservation the potato land races from Western part of Romania. Comparison between two big areas of potato growing: Apuseni Mountains (20,000 km^2) and the Maramures County (6,215 km^2) were made. In Apuseni Mountains, on the platforms arranged in terraces potato land races are cultivated. In Maramures County situated in the North West, potato land races are grown far among hills on small plots. For the inhabitants the potato represents the main food and feed around whole year. In both areas the potato plots looks wonderful. The identification, evaluation and conservation of potato biodiversity were the main objectives. At the collection, we gathered up information about history of cultivation, vegetation and maturity, disease resistance, quality and utilization. Among multiple reasons to be kept by the cultivators "interesting peculiarities", "fun", e.g. blue colored plants and tubers or sickle tubers were observed. *Ex situ* land-races preservation was carried out by *in vivo* (2001 – 2008) and *in vitro* techniques. In the small "gene bank" on USAB Timisoara the potato land-races were constantly preserved via unselected tubers. In successive *ex situ* cultivations, the UPOV standard evaluation were followed. Large variations of morphological characters, resistance to diseases and pest, yielding and yield structure as well as quality and molecular structure were followed. In this sense the potato land-races from the Apuseni Mountains emphasized a larger biodiversity. The Maramures land-races had less variation. In *ex situ* as well as *in situ* cultivation among the land races, the same rank of tuber weight was emphasized ($p>0.1\%$). One explanation can be the high stability for tuber weight genes. The total protein content was high in Bacaia-18 land-race (17.8%) with significant differences against Cristian and Runica control varieties ($d = 3.58 \pm 1.63$ and $d = 1.28 \pm 2.16$ respectively). The other landraces showed lower protein content than Bacaia 18 but comparable with the control varieties. The starch content was more uniform between both Apuseni and Maramures landraces (16-17.5%). The data obtained by RAPD analysis (10 primers) revealed a high polymorphism rate among the landraces. Only 50% of them generated fragments. Bacaia-18, Repedea-100, Salistea de Sus-1600 land-races displayed the largest amount of amplified fragments. The molecular analysis of land-races indicated a high polymorphic structure in each case, for example: Galbina-51 had two extremely low fragments and Almasul Mic de Munte-*49* had three fragments in the lower range of molecular weight. The 5'TGCCGAGCTG3' RAPD primer amplified the largest number of fragments in 40% of the cases. In the same area, the polymorphic profile differentiated the potato land races. The Galbina - 51 displayed the larger number of amplified fragments while no response was observed in Curechiu - 90. The large variation of landraces phenotype sis

the result of the micro-climate interaction with the anthropic specific activities and time and source of their origin. As an “outsider plant”, the potato was introduced into our area in 1780 in Tohanul Nou and Zarnesti (Brasov*). During ongoing dispersion cultivators tried to keep their own valuable materials. Our data suggests primary centers of introduction as Transylvania and Banat. The presumption is supported by custom to cultivate varieties obtained from land races (i.e. Roz de Belint). The Apuseni Mountains, Maramures County and other areas are secondary centers where the diversification by adaptation in new anthropic ecosystems took place.

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Neglected and underutilized wheat crop as genetic resources for improvement of the quality

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ABSTRACT: Modern agronomic practices have reduced the genetic variability of cultivated wheats, which has given great importance in the search for species that could be useful in contributing genes for wheat improvement. Between these species, the hulled wheats such as einkorn (2n=2x=14, AA; *Triticum monococcum* ssp. *monococcum* A. et D. Löve), emmer (2n=4x=28, AABB; *T. turgidum* ssp. *dicoccum* L. em. Thell.) or spelt (2n=6x=42, AABBDD; *T. aestivum* ssp. *spelta* L. em. Thell.) have proved to be rich-sources of useful genes. Unfortunately, because of the progressive neglect of these crops by other more-economic profit, most of the genetic resources for these species are only present in Germplasm Banks. Furthermore, some hulled wheats survives in marginal farming areas of Asturias (North of Spain), where is named as "escanda" or "Asturias fisga" and the farmer grow it by traditional farming systems for home consumption. Our group is developing studies about the genetic diversity existing in the collection of durum and bread wheat stored in the Banks. The same work is being carried out with the wheat still grown in Asturias fields. Because of this HMW and LMW glutenin and gliadin's electrophoretic studies were realized. The plants with interesting protein patterns were recovered and collection studies in Asturias were done. The use of a low-polyacrylamide-concentration gel permits a best discrimination at the level of the genetic diversity existing in conserved collection of *Triticum aestivum* ssp. *spelta*. This variability has been confirmed with the use of the urea-SDS-PAGE gel, which showed the presence of some subunits more clearly than did the normal SDS-PAGE gel. This way two and four alleles for the Glu-B1 and Glu-D1 loci respectively that have not been described previously were found, which were included in the Catalogue of Wheat Gene. Spanish escanda accessions stored in the Germplasm Banks presented a high genetic diversity level for the Glu-1 and Glu-3 loci and gliadins. Some allelic variants were dominant while others were only presented in one accession. The same diversity pattern is shown in tetraploid wheat (*Triticum dicoccum* and *T. turgidum*). A collecting mission was carried out with the objective to collect hulled wheats in all places in Asturias where these crops are still cultivated. The genetic diversity found in the collected materials was high but lower than in the accessions stored in Germplasm Bank. In the other hand, *Triticum dicoccum* is not grown currently being considerate a weed. The genetic diversity for the morphological traits in the collected material in Asturias was lower than the plants recovered from seed of Germplasm Banks. The Spanish wheat collections conserved in the Germplasm Banks as well as the crops still

cultivated in Asturias could play an important role as source of genetic diversity for wheat plant breeding and to promote the crop of escanda. Evaluation and characterization of these materials is urgent in order to avoid the lost of genetic diversity that is remained in these landraces.

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Genetic diversity of woad (*Isatis tinctoria* L.) populations based on molecular markers

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ABSTRACT: Woad, also known as Dyer's Woad (*Isatis tinctoria* L.) was cultivated as the source of a blue dyestuff for over 2000 years in Europe, and was only superseded around 50 years ago by indigo. The glucoside indican in the leaves is indoxyl, the chemical precursor to the dye Indigo blue. When the dead leaves ferment, indican is hydrolysed to indoxyl which is readily oxidised by air to indigo. In the beginning of twentieth century, the interest of textile industry for the natural dyes almost disappear due to the advent of synthetic dyes (Bechtold et al., 2003). As the synthetic dyes are responsible for high pollution and as some of them are toxic when in contact with skin (Anliker et al., 1988), the interest of textile industry in the natural dyes has increased, in present years, not only in Europe, but also in U.S.A. and in Japan. The aim of this study was to evaluate the genetic diversity between populations of woad from different European countries with two molecular markers (RAPD and ISSR). Eleven random amplified polymorphic DNA (RAPD) and ten inter-simple sequence repeat (ISSR) markers were used. The results showed that there are variability between populations.

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Signature of selection in pedigrees of modern bread wheat varieties

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ABSTRACT: Bread wheat (*Triticum aestivum* L.) was first domesticated in Neolithic age. Until the 1800s, only landraces were cultivated, and then modern selection took place in the middle of the 19th century. It is known that regions of the genomes conferring a positive selective value present a reduced allelic diversity and a longer range of local linkage disequilibrium (LD) pattern due to hitch-hicking effects. Based on these facts, we described haplotypic variability within pedigrees of elite breeding lines to detect signature of "modern" selection. Pedigrees of a selected set of seven recent, widely grown French varieties have been reconstructed back to the landraces whenever possible. In a first approach, 65 SSR markers of chromosome 3B of bread wheat were genotyped, then multi-locus haplotype diversity and linkage disequilibrium patterns were used to compare ancient and modern genitors. Three regions present a relative loss of haplotype diversity along the chromosome, one of them showing a longer range of LD. Interestingly, haplotype diversity profiles of ancient and modern genitors were identical in the third region, which suggests the presence of an imprint of domestication. Comparison with the B-genome ancestor, *Triticum turgidum* ssp. *dicoccoides* will be discussed. Finally, a genome-wide multilocus screen was carried out with Diversity Array Technology (DArT) in order to identify across the whole genome other regions suspected to be under selection.

Keywords: Genetic diversity, genetic drift, linkage disequilibrium, chromosome scan, haplotype.

Introduction

The aim of this study was to detect signatures of « modern » selection, which took place in XIXth century and lead to improved cultivars, which progressively replaced old landraces. Thanks to breeders and research institutes, many landraces, but also old cultivars released since 1850 have been conserved in gene banks and are still available for genetic studies. In the neighbourhood of a gene which has a selective value, several parameters are expected to change as a consequence of so called "hitch-hiking effect" (Rafalski and Morgante, 2004) : 1) genetic or haplotypic diversity is expected to decrease due to the selection of the "best" allele(s); 2) the range of local linkage disequilibrium should be longer and 3) the pattern of allele frequency change is expected to be more pronounced than expected by chance (i.e. from genetic drift only). In the present study, rather than comparing independent sets of old vs. modern accessions (as in Roussel et al., 2005), we tried to recover most ancestor and

intermediate lines in the pedigree of a limited number of "successful" French cultivars. This should further allow us to exploit pedigree relationships for simulating "neutral" evolution, and test whether selection did occur by comparing actual patterns to simulated ones. However in this presentation we give only illustrative results from the scan of one chromosome and did not perform such tests yet.

Material and methods

Plant material

Seven French cultivars were chosen, taken from the most recently cultivated ones in France (here considered as a measure of evolutionary success), and for which most lines of their pedigree can be found in gene banks. The total number of independent genitors found in the (registered) pedigrees (http://genbank.vurv.cz/wheat/pedigree/) was 359, among which 235 could be recovered, i.e. 66%. The oldest lines date back to 1830, thus on average 10-12 generation of selection have occurred along the pedigrees. Analyses have been realised on 7 elit cultivars and 235 genitors, 242 lines in totally.

Molecular markers

Seventy-four SSR markers, quite evenly distributed along chromosome 3B, were genotyped in the 242 lines in this pilot study. Forty-nine markers giving reliable results with few missing data were used in further analyses. Total number of alleles, allele frequencies per locus, number of rare alleles (with $f<0.05$) per locus were calculated using the GENETIX software (Belkhir et al., 2004). Polymorphic Information Content (PIC) values of markers were calculated according to Nei (1973). In order to smooth the signal from variation in polymorphism level from one marker to another, we rather estimated "haplotypic" diversity indices ($H=1-\Sigma p_i^2$, Nei, 1973) by considering n successive markers in a sliding window along the chromosome. More stable results were obtained with n=4. LD patterns were estimated using TASSEL software (Zhang et al., 2006) and variance effective size (Ne) were estimated from temporal change of allele frequency according to Waples (1989) using software NeEstimator (Peel et al., 2004).

Results and discussion

Allelic diversity

The total number of alleles found in the set of 49 SSR was 378, i.e. an average of 7.71 alleles/locus. However allelic diversity was found to vary according to the period of release.

When comparing haplotypic diversity (n=4) variation between « old » and « modern » lines along chromosome 3B, three windows show dramatic decrease below the steady level. The first window is located in deletion bin 3BS7-3BS9 (spanning 15 cM), between WMC418(1)-GPW7452-CFT3260-WMC540 (window 1). The second window is located in deletion bin 3BL7, between markers CFT53-CFT3063-GPW8064-CFT3059 spanning 5 cM.

These differences in diversity level between old and new lines may suggest that selection has acted on gene(s) in these regions. In a third window, between markers WMM1966-WMM1758-GPW5007(1)-CFT3366 in deletion bin 3BL7 (21 cM), both old and recent lines show a dramatic decrease in haplotype diversity. This might also suggest an effect of selection, but which occurred much before recent selection, perhaps at the time of wheat domestication. A comparison with wild wheat such as *Triticum turgidum* ssp. *diccocoides* would be needed to test this hypothesis.

Table1. Mean allele number, rare alleles and PIC values in old, semi-modern and modern cultivars.

	Landraces	Before world war II	Modern cultivars
Allele/locus	5.38	6.73	5.79
Rare allele (f<5%)	2.14 (30.67%)	3.48 (38.93%)	2.61 (35.65%)
Average PIC ($1-\Sigma p_i^2$)	0.5116	0.5270	0.4993

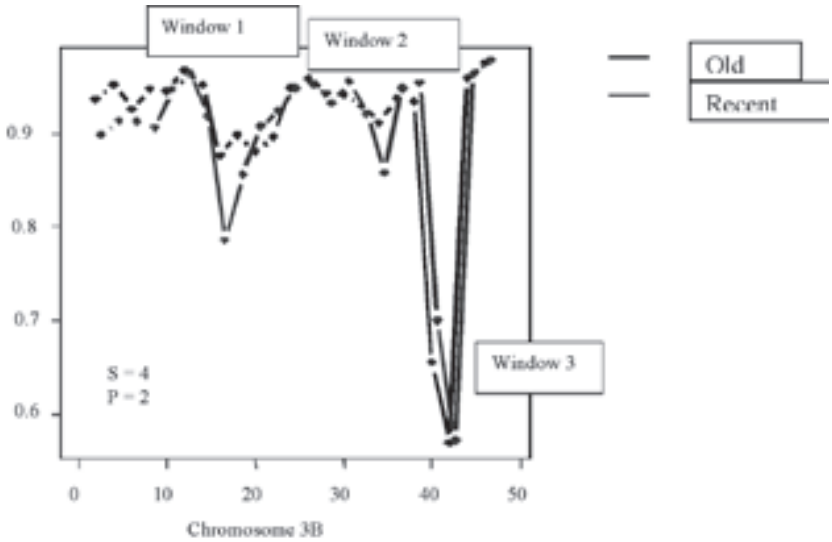

Figure 1. Haplotype diversity of old and recent varieties along chromosome 3B.

These preliminary results were confirmed, for windows 2 and 3, but not for windows 1, by using two other parameters: the range of local LD (not shown), and temporal change of allele frequency (Fc: Figure 2).

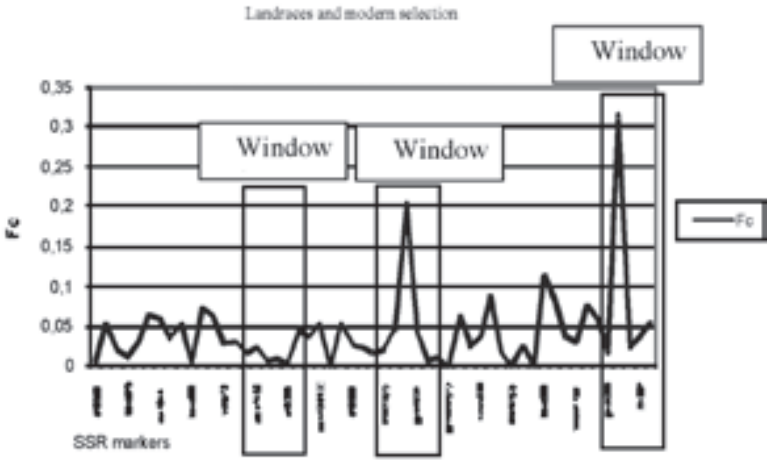

Figure 2. Temporal change of SSR allele frequency between old and recent lines.

A genomewide study using DArT markers is currently carried out to check for selection imprinting on other chromosomes. Results of such studies may help geneticist to confirm QTL region and breeders to focus on searching new sources of alleles in those regions which have already proved to have a positive selective value.

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Quantitative analysis of spontaneous *Sulla spinosissima* populations based on AFLP markers

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ABSTRACT: Population structure and genetic diversity of four spontaneous populations of *S. spinosissima* were analyzed by AFLP markers. Four AFLP primer combinations were used and generated a total of 240 polymorphic bands. The calculation of Shannon index has revealed a high diversity within the analysed populations. Principal Component Analysis revealed the existence of an important diversity within analysed populations with the Feriana population presenting the lowest genetic variability. In addition, Gabes, Jerba and Feriana populations overlapped, suggesting a common genetic base for these three accessions; whereas, Sousse population diverged from the other populations. On the whole, spontaneous populations of *S. spinosissima* were related to their bioclimatic origins especially arid and semi arid areas.

Keywords: AFLPs markers, genetic variability, phylogeography, *Sulla spinosissima*

Introduction

Sulla genus, belonging to the Fabaceae family and the Hedysarea tribe, is an annual and economically important plant. It is exploited either for forage or for soil protection. This genus is geographically widely distributed in the Mediterranean countries and easily adapts to different ecological conditions (Trifi-Farah et al., 2002). It comprises six species, among which the autogamous *S. spinosissima* which is mainly distributed in the arid and semi arid areas (Choi and Ohashi, 2003). This species constitutes a wild resource containing many excellent properties and specific genes, especially many arid-tolerant and drought-resistant genes (Baatout et al., 1985). Thus it is necessary to carry out genetic diversity research of *Sulla spinosissima* for effective protection and utilization of this precious resource.

In recent years, a number of molecular markers, such as RAPD, ISSR, SSR and AFLP have been widely used to detect genetic diversity in several plants. Some of these markers showed a high degree of variability within species, in particular, AFLP markers (Vos et al., 1995). While previous studies focused on identification and genetic variation among different species of *Sulla,* little is known about the genetic variation in *S. spinosissima* spontaneous populations. In the present study, the AFLP technique was applied to examine four populations of *Sulla spinosissima* to evaluate her population-level genetic structure.

Material and Methods

Plant Materials

Ten seedlings per population of four *S. spinosissima* wild accessions, were raised to assess intra- and inter-population variation. These were: Jerba [Jr], Feriana [Fr], Sousse [Ss] and Gabes [Gs]. All seeds were scarified, soaked in water for 24 hours, and then germinated on moist blotter paper. Total genomic DNA was extracted individually from fresh obtained seedlings using the adapted extraction procedure described by Dellaporta et al. (1983).

AFLP analysis

The amplified fragment length polymorphism (AFLP) protocol followed that described by Vos et al. (1995) and was carried out using primers and adaptors from AFLP Analysis System I and AFLP Starter Primer Kit (Invitrogen) according to the manufacturer' instructions. The products were loaded on 6% denaturing polyacrylamide gel in 1 X TBE buffer at a constant power (50 - 55 W) until the xylene cyanol was about two-thirds down the length of the gel. Subsequently, AFLP products were silver stained and were overnight dried prior to visually scoring polymorphic bands (Bassam et al., 1991, Chalhoub et al., 1997).

Data analysis

Only distinct, reproducible and well resolved fragments were scored as present (1) or absent (0) for each of the AFLP markers. Genetic variations among and within the populations were analyzed by the calculation involving the Shannon index (Bussel, 1999, Lynn and Chaal, 1989). The binary data was also subjected to Principal Component Analysis (PCA) (Gower, 1966) and scores for the first, and the second components were plotted by Statistica 6.0 (by StatSoft, Inc.) analysis software.

Results and discussion

In order to assess genetic variability in *Sulla spinosissima* populations, four AFLP primer combinations were used (Table 1). A total of 240 polymorphic bands were obtained, corresponding to an average of 60 markers per primer. This result indicates that AFLPs markers are powerful tools in population genetic studies of *Sulla spinosissima*.

The analyses of the data obtained shows that the intra-population variability, as measured by the Shannon index, varied from 0.0554 in Feriana population to 0.0695 in Sousse (Table 1). The total variability (H_{sp}) was 0.0629 for all individuals accross populations and the average intra-population variability (H_{pop}) was 0.0627. Therefore, the proportion of genetic diversity between populations (G_{st}) was 0.1792, indicating that the inter-population variation contributed only 17.92% to the total genetic diversity, while 82.08 % of genetic variation was due to variation between populations.

Table 1. Genetic diversity (Shannon's index) in *S. spinosissima* and participation of the genetic diversity within and between populations based on AFLPs data

	Jerba	Feriana	Gabes	Sousse	H_{pop}	H_{sp}	H_{pop}/H_{sp}	G_{st}
E_{AAC}/M_{CAT}	0.1035	0.0863	0.0928	0.0982	0.0952	0.1093	0.8706	0.1295
E_{AAC}/M_{CAG}	0.0332	0.0308	0.0324	0.0335	0.0325	0.0378	0.8592	0.1408
E_{AGC}/M_{CAA}	0.0798	0.0366	0.0643	0.0731	0.0634	0.0786	0.8075	0.1925
E_{AGG}/M_{CAA}	0.0308	0.0680	0.0662	0.0733	0.0596	0.0798	0.7460	0.2540
H_0	0.0618	0.0554	0.0639	0.0695	0.0627	0.0629	0.8208	0.1792

In addition, Principal Component Analysis (PCA) was applied to the data matrix. The percentage of variability revealed that the first three principal components absorbed 64.94% of the total inertia (Table 2). The first axis explained 29.76% of inertia and was defined mainly by AFLPs markers generated by E_{AGC}/M_{CAA} and E_{AAC}/M_{CAG} primer combinations. The second axis absorbed 20.17% of total inertia and was defined by AFLPs corresponding to E_{AAC}/M_{CAT} primer combinations. The third axis explained 15.01% of inertia and was defined by E_{AGG}/M_{CAA} AFLPs (Table 2).

Table 2. Definition and percentage of inertia absorbed associated with the three principal components of the PCA.

Principal component	Absorbed inertia (%)	Cumulated inertia (%)	Contribution of AFLPs
1	29.76	29.76	E_{AGC}/M_{CAA}, E_{AAC}/M_{CAG}
2	20.17	49.93	E_{AAC}/M_{CAT}
3	15.01	64.94	E_{AGG}/M_{CAA}

The graphic representation of the dispersion of the individual genotypes in the plan generated by the first two axes (Figure 1) showed the existence of an important diversity within analysed populations The Feriana population, originating from the arid area, presented the lowest genetic variability.

In addition, three populations overlapped to a great extent, suggesting a common genetic base between them. The Sousse population formed a distinct cluster. This last population also had the highest value of intra-population variability (Table 1). In fact, Sousse is placed in the middle of Tunisia, and may constitute a crossroads of diversity between the Northern and the Southern populations. Moreover, the plot shows a convergence of Jerba [Jr] and Gabes [Gs] populations which originate from the lower-arid bioclimatic area. On the whole, we found that PCA grouped populations according to bioclimatic origins. This could be a starting point for programs of forage improvement and identification of QTLs controlling the adaptation to the arid conditions.

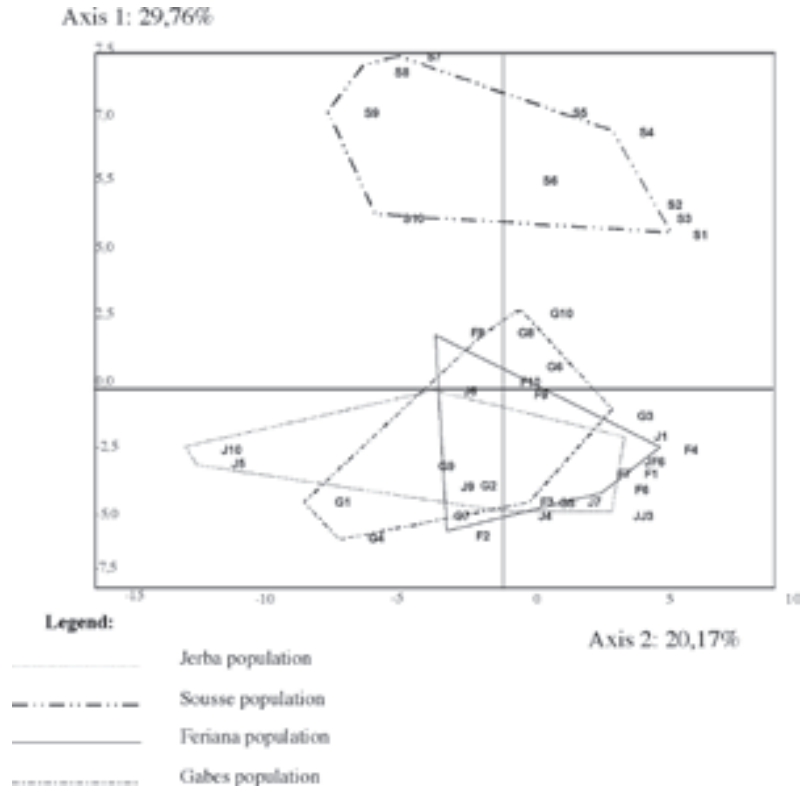

Figure 1: PCA plot according to engendered by the two first components of analysed species based on AFLPs markers.

In conclusion, the above results show that AFLPs can be successfully employed to assess the population-level of polymorphism and diversity in *Sulla spinosissima*. Therefore, this study has contributed to the analyses of population structure, which was previously poorly known but important for forage breeding and conservation.

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Molecular characterization of Tunisian tall fescue (*Festuca arundinacea* Schreb)

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ABSTRACT: In Tunisia, genetic conservation, evaluation and development of perennial grass species, such as tall fescue, have priority for pasture sustainability. *Festuca arundinacea* Schreb is the most important perennial forage species of the genus *Festuca*. Samples of five spontaneous populations were collected in the North of Tunisia ranging from the humid to the semiarid bioclimatic areas. The inter simple sequence repeats (ISSR) technique was used to examine the molecular polymorphisms in Tunisian tall fescue. Four 3' anchored ISSR primers generated a total 90 polymorphic DNA bands with a mean of 22.5 polymorphic bands per primer. Levels of diversity within populations were estimated and compared using Shannon's Index, and diversity was partitioned into within and between population components. The majority of variation (G_{ST}= 57.6%) occurred between, rather than within, populations. The results obtained have important implications for the conservation and genetic improvement of this species.

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Molecular and cytological characterization of F_1 (*Allium cepa* × *Allium roylei*) plants

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ABSTRACT: Onion (*Allium cepa*), 2n=2x=16 is one of the most important edible *Allium* crops. Unfortunately, onion is completely susceptible to downy mildew (*Peronospora destructor*). Only *Allium roylei* (2n=2x=16), a wild species that origins from northwest India, is completely resistant to the infection of the pathogen. The investigations on introgression of downy mildew resistance to the bulb onion have been carried out from last century (Kik 2002). The aim of this study, carried out in the years 2004-2006 was to obtain and characterize interspecific (*Allium cepa* × *Allium roylei*) plants. The F_1 (*A. cepa* × *A. roylei*) plants were obtained through *in vitro* cultures, *via embryo rescue* technique. Verification of the hybrid character of almost thousand of the obtained F_1 plants was based on molecular techniques, using PCR reaction. ACS-F/ ACS-R primers were designed for *A. cepa* anthocyanidin synthase gene. For all maternal, paternal and *A. cepa* × *A. roylei* plants, separation of PCR products on 1% agarose gel, revealed a monomorphic 750 bp product. After restriction digestion of the annealed DNA with Hin1II enzyme and resolving the products on polyacrylamide gel, polymorphisms differentiate *A. cepa* and *A. roylei* plants were obtained. Fragments, specific both for maternal and paternal plants were found in 97% of the obtained plants (Chuda and Adamus, 2005). Genomic *in situ* hybridization (GISH) was conducted on a selected population of F1 (*A. cepa* × *A. roylei*) hybrids (2n=2x=16). To conduct GISH, young roots of the hybrids were collected from each plant individually. Root tips were pretreated for one hour with aqueous solution of 8-hydroxyquinoline and then incubated in mix containing two enzymes: cellulase and pectolyase. Macerated root tips were spread on glass slides by dissecting and squashing in a drop of fixative solution. Total genomic DNA, isolated from *A. roylei* was used as a probe DNA. After being autoclaved for 4 min, the probe DNA was labeled by nick translation, using DIG-Nick Translation Mix. Blocking DNA was total genomic DNA, isolated from *A. cepa* and autoclaved for 12 min. In the hybridization mixture the probe : block ratio varied between 1 : 50 and 1 : 100. After hybridization and detection, chromosomes of F_1 (*A. cepa* × *A. roylei*) hybrids were visualized by using an appropriate filter in a fluorescence microscope. Eight chromosomes of each analysed hybrid plant hybridized with *A. roylei* DNA, while other eight did not.

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The establishment of the Spanish bread wheat (*Triticum aestivum* L.) core collection

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ABSTRACT: According to the Convention on Biodiversity formulated in Rio de Janeiro in 1992, it is a committment from the countries to conserve the genetic resources of the most cultivated species. The national genebanks have created collections of genetic resources of cultivated crops for their long-term conservation and to facilitate their utilization for breeding and research purposes. In fact, now, the problem of many collections of germoplasma is their great size and organization. One of the most important objectives in the management of these collections has been to develop procedures to reduce their size and do them more manageable and accessible. Core collection was defined as a representative sample of the whole collection with the minimum repetitiveness and maximum genetic diversity of a crop species (Brown, 1989). This concept was proposed by Frankel (1984). The Spanish Plant Genetic Resources Centre (CRF-INIA) maintains the collection of cultivated bread wheat (*Triticum aestivum* L), made up 1700 accessions, most of which are Spanish landraces. The aim of this work is the establishment of the Spanish Bread Wheat Core Collection to facilitate their use by the plant breeder's, researchers and other users. Five researcher institutions collaborate in this project. During the first year, 570 accessions have been selected for being integrated into the core collection. These entries have been distributed in equal sets and sown in five locations. During this cropping cycle, several agromorphological traits, according with IPGRI descriptors, will be taken. This information will be used for choose the core entries. The core should consist of about 10% of the collection (Brown, 1989).

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Recent advances in eggplant microspore culture for production of androgenic doubled haploids

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ABSTRACT: Doubled haploid production through androgenic microspore cultures is a convenient technique to obtain pure lines in a short period of time. However, in eggplant this technique is still far from optimized. One of the main drawbacks is the fact that doubled haploids are regenerated from calli, instead of embryos. In this work, we apply this technique to three eggplant cultivars, being able to regenerate doubled haploid plants, and demonstrating that immediately after induction, the microspore does not develop into callus, but directly into embryo. However, later embryogenesis seems to be impaired and embryos enter into a callus phase. These results open up new ways to improve the efficiency of microspore cultures in eggplant.

Keywords: androgenesis, doubled haploid, haploid, microspore embryogenesis, *Solanum melongena*.

Introduction

Doubled haploid (DH) production through androgenesis is a promising alternative to classic breeding procedures to obtain homozygous, pure lines with considerable reductions in costs, time and resources (Forster et al., 2007; Seguí-Simarro and Nuez, 2008b). Androgenesis induction is currently being applied in breeding programs of several agronomically interesting crops, but unfortunately, in eggplant (*Solanum melongena*) this technology is still poorly developed. From the different methods to obtain androgenic DHs, only the culture of anthers works in some eggplant cultivars (Kashyap et al., 2003). On the contrary, the most efficient method, culture of isolated microspores, is still at its infancy and only one study has been published reporting the regeneration of DH plants from isolated microspores (Miyoshi, 1996). In this work we have applied the microspore isolation and culture methodology to several commercial cultivars of eggplant. We have analyzed microscopically the process of androgenesis induction, focusing on the first stages of this developmental switch. Using flow cytometry, we have studied the variations in ploidy level of the regenerants obtained from microspores. Our results provide important insights on the morphogenic routes followed by the reprogrammed microspore in the specific case of eggplant, contributing to a better understanding of the particularities of androgenesis in this species.

Material and methods

We have used three commercial hybrids of interest for the breeding programs currently carried out by our Institute: Bandera, Ecavi and ANS-3. Flower buds at the appropriate

stage of development were excised and surface sterilized. Their anthers were dissected and microspores were isolated, plated, pretreated with heat (35ºC) and starvation, and cultured in liquid NLN medium at 25ºC according to Miyoshi, 1996. Induced calli were rescued and transferred to solid MS medium (Miyoshi, 1996) where they developed and regenerated full plantlets or apical shoots. Calli were subcultured every 15 days, and those developing rootless shoots were additionally transferred to V3 medium (Dumas de Vaulx and Chambonnet, 1982) for improved rooting. Full plantlets were acclimated and transferred to the greenhouse. The first stages of microspore development were followed with conventional and inverted light microscopes, both equipped with phase contrast and DIC optics. Microcallus and subsequent stages were observed and recorded through a dissection microscope.

For flow cytometry, small pieces of young leaves from regenerants and donor plants were processed using the CyStain UV Precise P kit (Partec). Samples were chopped at 4ºC with a razor blade in 400μl of nuclei extraction buffer (NEB). After a 1 minute incubation in NEB, 1.6ml of DAPI-based staining buffer was added and incubated for 10 minutes. The extracted nuclei preparation was filtered through 30μm CellTricks filters (Partec) and immediately analyzed in a Partec PA-I Ploidy Analyzer.

Results

Establishment of microspore cultures for the Bandera, Ecavi and ANS-3 cultivars

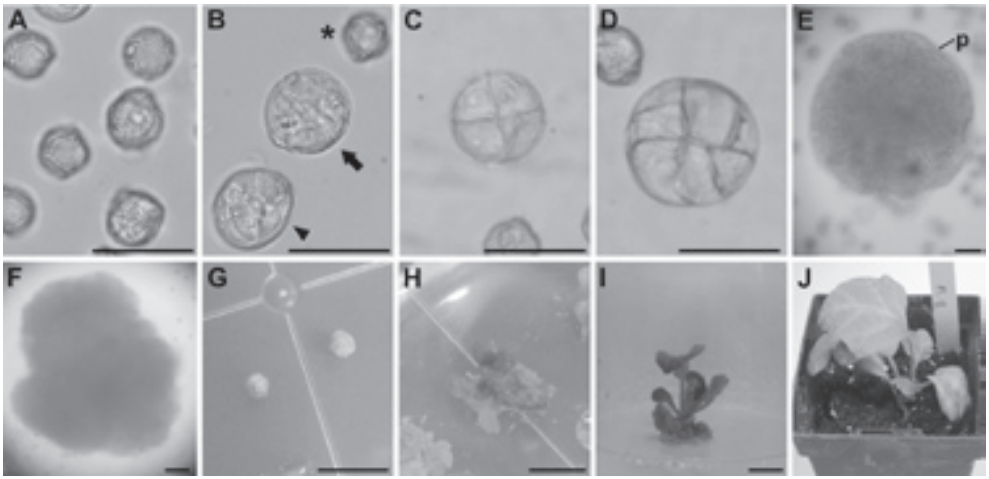

Figure 1. Microspore cultures of eggplant. A: Freshly isolated microspores. B: Just induced cultures with a sporophytic microspore (arrow), a gametophytic-like microspore (arrowhead) and an arrested microspore (asterisk). C: Four-celled microspore. D: Multicellular microspore. E: late globular embryo with developing protoderm (p). F: Microcallus in liquid culture. G: Isolated calli in solid culture. H: Organogenic callus with emerging buds. I: Excised, individualized developing shoot. J: Fully regenerated and acclimated eggplant plantlet. Bars in A-F: 50 μm. G-I: 1 cm.

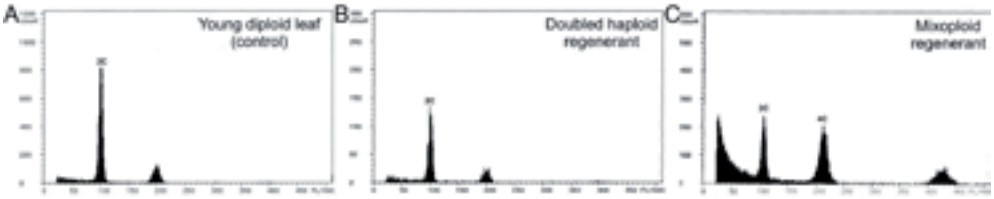

Figure 2. Flow cytometric analysis of eggplant androgenic regenerants. A: Diploid control, young leaf from the donor plants. B: Doubled haploid regenerant. C: Mixoploid (2n+4n) regenerant.

Microspore cultures at the moment of plating consisted mainly of vacuolate microspores and young bicellular pollen (Figure 1A). Five days after the pretreatment, some microspores were observed to divide symmetrically (Figure 1B), indicating the onset of the sporophytic route. Some others were observed to follow a gametophytic-like development towards mature pollen, and many of them arrested and did not follow any type of development. Within the first week of 25ºC culture four-celled microspores were identified (Figure 1C), and at about ten days, multicellular structures were formed, as evidence by the presence of multiple cell walls (Figure 1D). During the first four weeks, these multicellular structures developed into early globular embryos (data not shown), and by the end of the first month in culture, late, large globular embryos could be evidenced by the incipient presence of a differentiating protoderm (Figure 1E). In parallel loosely arranged, irregular callus-like structures (microcalli) began to be observed (Figure 1F). Soon they became abundant, whereas the presence of globular embryos progressively decreased and embryos at later stages were never observed, suggesting that microcalli originate from late globular embryos. When 6-week old, microcalli were isolated from the liquid culture and transferred to solid medium, they increased in size and after two months their diameter reached few millimeters and became eye-visible (Figure 1G). Some of them developed into hard, non-organogenic calli (data not shown), and some others into pale green, friable and organogenic calli. In three-month old friable calli the first buds were observed to arise from the callus surface (Figure 1H). Buds rapidly gave rise to shoots that rooted either spontaneously or after transference to V3 medium (Figure 1I). Upon acclimatization, fully regenerated plantlets were obtained (Figure 1J), five months after microspore isolation.

Assessment of the ploidy of the regenerants by flow cytometry

In order to check the ploidy of the regenerated plants, we analyzed leaf samples of the regenerants by flow cytometry (Figure 2). We also used a leaf sample from a donor plant as a control reference for 2C DNA content (Figure 2A). From the regenerants analyzed, ~65% presented a DNA content equivalent to the diploid control (Figure 2B), which indicates that they were DHs. However, we also observed other ploidies such as 2C+4C mixoploids (Figure 2C) in ~20% of the cases, and ~15% of tetraploids (4C; data not shown).

Discussion

In this work we have documented the regeneration of plants in three commercial cultivars of eggplant through isolated micropore cultures. In this culture technique, only the two stages more responsive to induction of androgenesis (vacuolate microspores and young pollen grains; Seguí-Simarro and Nuez, 2008b) are used, and any other cell types are removed or filtered away. This implies that regenerants will be haploid, DH, or sometimes polyploid but always homozygous for all of their genes (Seguí-Simarro and Nuez, 2008b), since the possibility of appearance of somatic diploids coming from the anther walls is totally excluded. This is why it is not necessary to perform any additional test to claim that the regenerants obtained by this procedure have a haploid origin.

Once androgenesis is induced, the cells may remain haploid or alternatively, may undergo rounds of genome doubling to become DH (Seguí-Simarro and Nuez, 2008a). Since the interesting individuals for breeding purposes are only the DH, it is convenient to check for the percentage of haploids and DHs, in order to increase, if needed, the yield of DHs by adding a step of induction of genome doubling. In our varieties, we could not find any haploid plant, which indicates that all of the obtained regenerants duplicate their genome with no need for specific induction of doubling. The observation of some higher ploidies might suggests a strong effect of the *in vitro* culture conditions, particularly hormones (Seguí-Simarro and Nuez, 2008a), and opens up the possibility of testing different hormone types and concentrations to reduce the observed ploidies.

In the literature there is only one previous report (Miyoshi, 1996) describing an achievement in eggplant similar to that hereby reported. In that work, Miyoshi described the exclusive formation of callus from isolated microspores. This situation strikingly contrasts with that of most of the androgenic systems known to date, where microspores are induced to produce embryos instead of calli (Seguí-Simarro and Nuez, 2008b). Even in eggplant, microspores in anther cultures clearly give rise to embryos, and callus is mostly originated from anther somatic tissue (our unpublished results). Since this latter option is excluded in microspore cultures, we can conclude that in our system, microspores are also the origin of calli.

So the question arises as to why eggplant microspores behave so differently? In order to shed light into this question, we examined under the light microscope the first stages of microspores induction to androgenesis. As seen in Figures 1A-E, the first stages of microspore development are fairly similar to those reported for most of the dicot androgenic systems (Seguí-Simarro and Nuez, 2008b), where a phase of indifferentiated, proliferative and loosely patterned growth ends up with the formation of a radially symmetric globular embryo (Figure 1E). This is a critical stage for embryogenesis, since a transition from radial to bilateral symmetry marks the onset of the elongation and differentiation phases (West and Harada, 1993). However, the situation in eggplant seems to be largely different. In Figures 1F-H it is observed that globular embryos transform into microcallus which, after subculture,

regenerate plantlets through organogenesis. This developmental sequence suggests that androgenic globular embryos of eggplant, under the described experimental conditions, are not capable of undergo the symmetry transition. In other words, the embryogenic program arrests at the globular stage, and cells adopt a proliferative callus growth. Evidence of this embryogenic arrest has been also observed in eggplant anther cultures when embryos are not rescued on time from the anther, and a reversion to callus is observed (our unpublished results). It can be speculated with several reasons for the observed embryogenic arrest, but the most likely reason is that the experimental conditions are not well suited to promote later embryogenic development. In anther cultures, the presence of anther tissues may compensate for this, but in the anther-free medium used here, the lack of embryo-promoting factors may appear critical. It is likely that future research on medium optimization will overcome this problem and reveal that eggplant microspore embryogenesis follows the general pattern described for most of the androgenic systems.

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In vitro morphogenetic responses of tomatoes (*Lycopersicon esculentum* Mill.) explants for the regeneration of true-to-type plants

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ABSTRACT: In Romania, as well as in other countries, tomato is a major vegetable crop that has achieved tremendous popularity, being present in almost each country of the world. This situation is due to its importance in the human consumption and to the possibility of different canning methods. The development of protocols for *in vitro* selection can provide new advances for the production of new cultivars with importance in the classical breeding. The aim of the present study was the screening of the "*in vitro*" morphogenetic responses of different explants for the establishment of the best explant to be used for the production of true-to-type plants. We used plant regeneration capacity to produce individuals genetically identical to the explant's donors and to themselves. Although, some information is available on the morphogenesis of tomato, the techniques have not been developed to a level at which they can be utilized in the multiplication of different commercially important cultivars. Different combinations of growth hormons were tested in order to achieve regeneration structures from young tissues of tomatoes. Kinetin (KIN) and α-naphthaleneacetic, kinetin (KIN) and indolilacetic acid (IAA), or benzilaminopurine (BAP) and NAA or IAA, zeatine were added to basic medium composed of full strength MS salts, MS vitamins, 3% sucrose and 0,8% agar-agar. Young explants (apexes, hypocotyls, leaves, roots) derived from aseptic germination of seeds were cultivated on these media. We succeeded in obtaining plants, especially on media containing zeatine alone and in combination with IAA. The morphogenetic response is highly dependent on the initial explants, hormons used in the media, genotype, and environmental conditions.

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Powdery mildew resistance in *Hordeum spontaneum* populations

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ABSTRACT: Since 1960s fungicide treatment against *Blumeria graminis* f. sp. *hordei* was used as common practice to reduce the severity of powdery mildew on the field. However, pathotypes of *B. graminis* f. sp. *hordei* that are resistant to commonly used fungicides now have been identified. Also, fungicide cost and environmental concerns regarding pesticide use lead to a gradual limitation of their use for control of powdery mildew. An alternative approach to control of powdery mildew is breeding for resistance. The incorporation of new genes for resistance to powdery mildew into barley cultivars has been useful in combating powdery mildew (Czembor, 1996, 2005). Resistance of populations of wild barley (*Hordeum spontaneum*) to powdery mildew was investigated. The purpose of this study was creation of lines, which will be used as an initial material for breeding of barley cultivars resistant to powdery mildew. Ninety eight populations of *H. spontaneum* were used. These populations were collected in the Mediterranean Region. Based on preliminary resistance tests 32 lines from 20 populations were selected. These lines were tested using 20 differential isolates of powdery mildew. Disease reaction was observed on 3 cm leaf segments from the middle part of the primary leaf of the 10 - 14 day old seedlings. These segments were laid on a 7 g/l water agar medium with 0.03 g/l of benzimidazole. This experiment was done in 3 replications and leaf segments of susceptible variety Manchuria were used as a control. Based on obtained results it may be concluded that tested lines possess high level of powdery mildew resistance. The most frequently observed resistance eaction was immunity (score 0). Powdery mildew resistant lines of *H. spontaneum* will be used in barley breeding programme.

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Characterization of genetic resources in kiwifruit breeding

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ABSTRACT: Kiwifruit (*Actinidia*) are a relatively new fruit crop, introduced into cultivation early last century. The world market is currently based on a limited number of cultivars, mostly selections of *A. chinensis* and *A. deliciosa*, selected or developed from a narrow range of the available genetic resources. The two species *A. chinensis* and *A. deliciosa* are currently treated as separate species based on floral, vegetative and fruit morphology, although sometimes they are treated as variants. There is the potential to develop new kiwifruit cultivars to increase market size: a better characterisation of relationships between *Actinidia* species, and populations, would assist breeding programmes. The genus *Actinidia* has a wide geographic distribution in eastern Asia, with most taxa in south central and southwest China. *Actinidia* consists of 76 species, many of which contain character states that are absent from *A. deliciosa* and *A. chinensis* (Huang and Ferguson, 2006). They can be diploid, tetraploid, hexaploid or octaploid depending on the species or even particular genotypes within a species. The estimation of genetic diversity among plant populations that differ in ploidy levels is difficult, as most of the statistical methods are inappropriate for polyploid species. A set of 336 genotypes from 106 accessions of 23 species was sampled from HortResearch's *Actinidia* germplasm collection. Individuals were genotyped for 20 microsatellite loci. Seven primer pairs amplified more than the expected number of loci in some individuals and were discarded from the data set. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) trees were generated based on Nei's genetic distances (Nei, 1972) calculated using the remaining 13 markers. Microsatellite alleles were scored either as present or as missing data. The resulting UPGMA tree of genetic distance in *Actinidia* strongly suggests that the current infrageneric subdivision of *Actinidia* is not natural and that substantial revision is needed. Although the Leiocarpae appears to be monophyletic, the other three traditional sections, Maculatae, Stellatae, Strigosae, seem artificial. Within *A. chinensis*, four sub-populations are suggested, three diploid and one tetraploid, these sub-populations corresponding to four geographic locations. A comparison among *Actinidia* populations of differing ploidy levels is problematic and the allele frequency-based analysis we have used here will be biased because of the co-dominant nature of microsatellite markers. In this analysis, the frequency of alleles present in more than one copy in an individual will be underrepresented compared with rare alleles. Alternative methods of analysis, by first estimating allele frequencies from observed marker phenotypes (De Silva et al., 2005), are discussed.

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Pollen size mutants in *Arabidopsis thaliana*

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ABSTRACT: Evidence for ancient or so-called paleoploidization can be found in many species, suggesting that there is a slow but positive drive to select for this phenomenon. Polyploidization has been proposed to result from fertilization events involving unreduced (2n) gametes (Harlan and deWet, 1975; Ramsey and Schemske, 1998). We know little about the occurrence and inheritance of unreduced gamete formation apart from that it is environmentally controlled and depends on only few genetic factors. A tight correlation was found between pollen size and DNA content (ploidy) using diameters and cross-section surface area of pollen derived from Arabidopsis diploid, tetraploid, and octaploid plants. Based on pollen size measurements, we developed a screening method to identify plants producing unusually large pollen, which is indicative for the presence of high chromosome numbers. EMS mutagenized Arabidopsis individuals (>3000) were screened for giant pollen with a size threshold of 500 μm^2 surface area, comparable to pollen from tetraploid lines. About 3% of the analyzed plants produced giant pollen with frequencies varying from 0,5 to 90,3%. One of these mutants carried tetraploid DNA levels in somatic tissue, suggesting that it had spontaneously ploidisized. We are currently characterizing these mutants using microscopy of pollen meiosis, fluorescence assisted cell sorting, and by genetic mapping.

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A study on the presence of self compatibility alleles in Iranian wild almonds (*Amygdalus* spp.) using specific allele amplification

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ABSTRACT: There is a rich almond (*Amygdalus* spp.) germplasm in Iran which includes over 800 cultivated genotypes and different wild species growing in different climatic regions. Some of these species, such as *A.elaegnifolia*, *A.lycioides*, *A.haussknechtii*, etc. are growing in Iran exclusively. This wild germplasm includes many desirable traits such as pest and disease resistance, which can be incorporated into cultivated ones in breeding programs. Also in the case of almond, one of the most important traits which is found in a wild species is selfcompatibility. This study was carried out to evaluate the possible presence of S*f* alleles in different Iranian wild almond species. Plant materials (seeds), collected from the most important almond growing regions of Iran, include *A.elaegnifolia*, *A.lycioides*, *A.haussknechtii*, *A.orientalis*, *A.scoparia*, *A.communis* and *Prunus dulcis* (some cultivated genotypes). Seeds were stratified and prepared for germination. Young leaf samples used to extract the genomic DNA (Kadkhodaei et al., 2005) and then the presence of S*f* alleles evaluated through PCR amplification using the specific primers (Channuntapipat et al., 2003; Ma and Oliveira, 2001). The results showed that the species have not Selfcompatibility alleles.

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Genetic diversity analysis of *Festuca arundinacea* Schreb. in Tunisia

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ABSTRACT: Tall fescue [*Festuca arundinacea* Schreb.var. *arundinacea* Schreb.] is commonly grown as forage in the temperate regions of Tunisia, where it is the most important perennial forage species of the genus Festuca. Few information is available on genetic diversity within tall fescue in Tunisia. Accordingly, the amplified fragment length polymorphism (AFLP) method was used to detect genetic diversity and relatedness among spontaneous accessions. Three primers combination have generated a total of 133 polymorphic bands out of 134. An important level of polymorphism was detected since the percentage of polymorphic markers is 99.25%. These results concur with those describing the application of AFLP technique to access large number of polymorphic markers. The resultant genetic distance matrix according to the Nei and Li's formula was analysed to show the relationships among the tested accessions using UPGMA method. The accessions were grouped into two major clusters and subclusters independently of their geographic origins. In fact, such spontaneous accessions provide a source of genetic variation for tall fescue breeding programs. Results are discussed in terms of adaptation of local and introduced cultivars in comparison with wild populations.

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Autonomous self-pollination in Fabaceae-Papilionoideae in Northwestern Argentina

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ABSTRACT: Breeding systems largely determine the genetic characteristics that form the basic material for future selection. As a first step in the characterization of ten simpatric species of Papilionoideae, in this work we determined the reproductive advantages provided by autonomous self-pollination. Three pollination treatments were carried out: (a) autonomous self-pollination (ASP), (b) hand self-pollination (HSP) and (c) natural pollination (NP). All the studied species presented mechanisms of autonomous selfing as an alternative way to set fruits and seeds without the intervention of pollinators. Considering all the species, we found that seed set within pollinator exclosures was 85 % of the seed set in open-pollinated flowers (ASP= 7.03 ± 1.23 *vs*. NP= 9.81 ± 2.81 seeds / fruit).

Keywords: autonomous self-pollination, breeding system, Fabaceae, Papilionoideae, pollination, self-compatibility.

Introduction

Breeding systems largely determine the genetic characteristics that form the basic material for future selection. Furthermore, breeding systems are under genetic control and themselves subject to natural selection. Consequently, it is crucial to have an accurate account of mating processes. A plant's mating system affect how we should collect optimally, conserve effectively, and use efficiently the genetic resources of domesticated plants and their wild relatives (Brown, 1990).

Wild species that are related to crops are important resources for crop improvement, and often are useful species in their own right (Richards, 2001). The Fabaceae, one of the largest families of the Angiosperms, are widely distributed in both hemispheres, from wet tropics to temperate zones (Heywood, 1993). This is one of the most important plant groups for humanity. Many of them are characteristic of disturbed places, demonstrating a good adaptation to grow and to reproduce under unfavorable conditions, *e.g*., soils poor in nitrogen, given their capacity to fix the atmospheric nitrogen by symbiosis with species of *Rhizobium* (Harborne, 1981). The Fabaceae is divided in three subfamilies: Papilionoideae, Cesalpinioideae and Mimosoideae.

The Papilionoideae represents 70% of the family. Most of the cultivated legume species belongs to this subfamily, e.g., soya (*Glycine max* (L.) Merril), common bean (*Phaseolus vulgaris* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), peanut (*Arachis hipogaea* L.). This group occupies the second place of food source, after cereals (Duke 1981). The seeds of the Fabaceae, compared to the cereals, accumulate proteins which are highly nutritious (Harborne, 1994). In addition to the crops used as food, the Papilionoideae are used also as forage, ornamentals, medicine, etc. (Burkart ,1943; Marzocca, 1993).

Many native Papilonoideae of northwest Argentina have been mentioned for their forage value for livestock, given their high protein content, *e.g*., *Desmodium* Desv., *Zornia* Gmel., *Stylosanthes* Sw., *Arachis* L., *Aeschynomene* L., *Centrosema* (DC.) Benth. *Crotalaria* L., *Vigna* Savi, *Lupinus* L., *Trifolium* L., *Adesmia* D. C., *Macroptilium* and *Phaseolus* L. (Burkart, 1943). Studies of this group are of great interest because they would offer an alternative to introduced forage species because they are native, and, therefore, they are adapted to local conditions. Also, the knowledge of the dependence among the native Papilionoideae, that represent one of the predominant groups in the Lerma Valley, and their pollinators will be indispensable for the protection and conservation of the biodiversity of the Lerma Valley.

As a first step in the characterization of ten sympatric species of Papilionoideae, in this work we determined their capacity for autonomous self-pollination.

Material and methods

Study site

The present study was carried out in the Lerma Valley (Salta, Argentina), where the Papilionoideae represents one of the major groups, distributed mostly in the Selva de Transición district and in the Chaco Serrano district (Cabrera 1976). In the Chaco district, the Papilionoideae are mainly distributed in the community of the Pastizales Serranos, which is composed of herbaceous species. Its composition is modified by degradation due to constant grazing pressure by domestic cattle. In the Selva de Transición, the Papilionoideae frequently grows along river beds, forming aggregations that are removed by temporary floods.

Three pollination treatments were carried out to determine the breeding system of each species. Unopened flower buds on an average of 10 individuals (range: 4-14) were bagged just before flower opening. A set of flowers in each individual was tagged and assigned to one of the following treatments: (a) autonomous self-pollination (ASP), in which buds were kept untouched throughout their flowering period; (b) hand self-pollination (HSP), in which flowers were hand self-pollinated immediately after flower opening, and (c) natural pollination (NP), in which flowers were exposed to the natural agents of pollination. The number of seeds per fruit was recorded after a variable period according to species. Previous data showed that all the studied species are self-compatible (Alemán et al., 2007), so, an index of automatic self-pollination (IAS) was obtained by comparing seed set following

autonomous selfing and controlled selfing (HSP/ASP) (Ruiz and Arroyo, 1978). Voucher specimens are deposited in the MCNS.

Results and Discussion

All the studied species presented mechanisms of autonomous selfing as an alternative way to set fruits and seeds without the intervention of pollinators (Table 1). In general, the automatic selfing indices exceeded 0.8, except *Crotalaria megapotamica*, which present a value of 0.35. *Crotalaria pumila*, *Macroptilium erythroloma, M. fraternum,* and *Zornia contorta* are highly autogamous, with IAS >1. In these species, the seed set following hand-self pollination were lower than the seed set via autonomous selfing. These results are probably related to short pollen viability.

Table 1. Seed set per fruit following hand self-pollination autogamous self-pollination and natural pollination; Automatic Selfing Index (IAS) in ten species of Fabaceae-Papilionoideae growing in Northwestern Argentina; mean ± standard error; between braquets = number of fruits.

Species	Hand self-pollination (HSP)	Autogamous self-pollination (ASP)	Natural pollination (NP)	Automatic selfing index (IAS)
Centrosema virginianum (L.) Benth.	16.31 ± 0.59(13)	15.20 ± 1.01(15)	18.54 ± 2.27(22)	0.93
Cologania ovalifolia H. B. K.	9.71 ± 1.27 (7)	8.84 ± 0.88 (13)	7.42 ± 0.86 (19)	0.91
Crotalaria megapotamica Burk.	19.4 ± 1.92(27)	6.77 ± 0.79 (50)	34.61 ± 1.72(28)	0.35
Crotalaria pumila Ort.	4.83 ± 0.91(12)	6.12 ± 0.32 (32)	5.96 ± 0.35 (24)	1.27
Desmodium incanum D.C.	2.77± 0.27 (22)	3.45 ± 0.13 (34)	3.77± 0.25 (35)	1.24
Galactia latisiliqua Desv.	7.60 ± 0.55 (15)	7.25 ± 0.25 (56)	7.95 ± 0.25 (20)	0.95
Macroptilium erythroloma (Benth.) Urban	10.40 ± 1.72 (5)	10.82± 0.77 (22)	10.65 ± 2.25 (48)	1.04
Macroptilium fraternum (Piper) Juárez y Pérez	4.75 ± 0.33 (24)	5.29±0.27 (77)	5.67 ± 0.24 (48)	1.11
Rynchosia edulis Griseb.	2.11 ± 0.09 (26)	1.77± 0.08 (30)	1.97 ± 0.03 (31)	0.84
Zornia contorta Mohl.	3.85 ± 0.49 (13)	4.41 ± 0.28 (27)	4.15 ± 0.47 (26)	1.14

Considering all the species, we found that there are not significant differences among the pollination treatments (Table 2). Seed set within pollinator exclosures was 72 % of the seed set in open-pollinated flowers (7.03 vs. 9.81 seeds/fruit). The mechanisms of autonomous selfing appear to be common among self-compatible species. In several cultivated legumes, Frankel y Galun (1977) reported that self-pollination before flower opening ("bud pollination") is

common, although their flowers are of conspicuous entomophilous type, e.g., *Vicia sativa* (vetches), *Phaseolus vulgaris* (common bean), *Pisum sativum* (garden peas) and *Glycine max* (soybean). Anthers dehisce in the bud, although fertilization may be delayed until after flower opening because of protandry. Previous studies of this species showed that the maximum stigmatic receptivity occurs some hours after flower opening (e.g. *Macroptilium lathyroides*: Etcheverry et al. 1998; *M. erythroloma*: Etcheverry et al., 1999, *Crotalaria micans*: Etcheverry et al., 2003). Besides, in all cases 1- to five pollinators (mean 2.82 ± 0.43, Etcheverry et al. 2007) visited the studied populations, activating the pollination mechanisms and taking rewards (nectar or pollen and nectar) Thus, species with these characteristics are likely to be classified as mixed-mating species. In the common bean, Ibarra-Pérez et al. (1996) demonstrated the occurrence of mixed paternity using genetic markers. They reported that all the sired pods produced both, nonhybrid and hybrid seeds and as many as two successful fathers per pod were identified, the maternal parent and at least one outcrossed parent.

Table 2. Comparison of seed set in three pollination treatments of Fabaceae-Papilionoideae in Northwestern Argentina (mean ± standard error).

Pollination treatment	Seed set	Statistic
Hand self-pollination	8.24 ± 2.07	
Natural pollination	9.81 ± 2.81	
Autogamous self-pollination	7.03 ± 1.23	$H = 0.165$, $P = 0.9$

The mixed mating system depends, among other factors, on temperature, humidity, and the biotic environment such as pollinators responsible for pollen carryover and on the number of available flowers that would serve as pollen sources and pollen recipients. Therefore, it is reasonable to expect variations between plant populations and between years.

The reproductive assurance hypothesis have been widely accepted as an explanation to the evolution and maintenance of selfing and mixed mating systems (Jain 1976, Fausto et al. 2001, Etcheverry 2005).

With respect to the autonomous self-pollination, it is proposed to occur when biotic pollination is insufficient for full pollination of the ovules, because pollinators or mates are scarce or absent (Wyatt, 1983; Goodwillie, 2001; Etcheverry, 2005). Our data suggest that the reproductive assurance that autonomous selfing can provide is important in this group of species.

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Evaluation of genetic variation of bermudagrass (*Cynodon dactylon*) using morphological characteristics and ISSR markers

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ABSTRACT: The genetic diversity of 23 bermudagrass accessions and 4 hybrid cultivars *(Cynodon dactylon* × *Cynodon transvaalensis*) were assessed using morphological traits and ISSR markers. Fourteen morphological traits were measured in a field trial using a randomized complete block design with three replications. Data were analysed using SAS software based on Euclidean distance. Cluster analysis revealed that genotypes were categorized in three groups differing in plant height. After DNA extraction and setting up PCR conditions, 14 primers were used to study genetic diversity of genotypes by ISSR markers . Total of 336 fragments were produced which 305 of them were polymorphic. Data were analyzed using NTSYS V. 2.02. Mantel correspondence test were conducted to choose the best similarity coefficient. A dendrogram constructed using the Jaccard's coefficient with unweighted pair group mathematical average (UPGMA). Cluster classified genotypes in four distinct groups. The results showed that triploid cultivars and diploid accessions belonged to the same group , but were separated from tetraploid accessions. This study showed that ploidy levels are partly distinguishable by ISSR markers. Results showed that there was no significant correlation (r=0.21) between similarity matrices of morphological and ISSR markers. This lack of correlation may be due to environmental effects on morphological markers, or by the amplification of non-coding regions by molecular markers.

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The use of treated sewage water for afforestation and conservation of the diversity and forest tree genetic resources

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ABSTRACT: The promotion of a diversity and of a greener environment is an area where the government of Egypt has been very actively involved lately through a number of relevant ministries and component organisations both governmental and non-governmental. Such activities aim at reducing the negative impacts of pollution and associated health hazards. Thus, greening efforts, in which the use of diversity and conservation of forest tree genetic resources, have been carried out in number of cities and governorates. And since the development of the necessary infrastructure for irrigation and water reuse combined with an appropriate use of diversity is considered essential for ensuring the sustainability of any greening activity in the target areas, continued efforts are being exerted in development of new innovative ideas for irrigation systems, as well as for the use and conservation of native genetic resources of forest tree species. Within this context Egypt is currently witnessing a wide range of new projects aiming at expanding the green stretch in the desert by establishing forest plantations (Man-made Forests), making use of the treated sewage water to produce timber tress of high economic value. This contributes to satisfying Egypt's local needs of wood and help in recovering this vital resource. It also plays an important role in fixing sand dunes, combating drought and desertification, plus protecting the soil from possible erosion as it acts as windbreaks and shelterbelts. Furthermore, it contributes to the conservation of other natural resources, namely; air, water, soil and biodiversity in the entire ecosystem. In this context, an adequate use of the genetic resources and materials used in the afforestation is essential for the success of such activities and can be useful for conserving endangered local genetic resources of tree species.

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Molecular marker assisted broadening of heterotic groups in rye with exotic germplasm

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ABSTRACT: A major concern for rye breeders is the narrowing of the current elite germplasm. The objective of our study was to investigate the potential use of molecular markers for a systematic broadening of the existing European heterotic pattern through introgression of exotic rye populations. In total, 610 S_0-clones from the two European heterotic groups A and B as well as from five exotic populations from Eastern Europe were fingerprinted with 30 SSR markers. In addition, testcrosses of the five exotic populations were produced crossing the S_0-clones with testers from both heterotic groups. The test crosses were evaluated for grain yield in four environments in Germany. The molecular results from a model based cluster analysis clearly showed that the exotic populations are more closely related to heterotic group A than to B. This is in accordance with the observation that the five exotic populations showed higher F_1 performance when crossed with the tester from heterotic group B compared to testcrosses with heterotic group A. We conclude that molecular markers are a powerful tool for a systematic introgression of exotic germplasm.

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Preliminary data on evaluation of a *Triticale* genetic map

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ABSTRACT: Triticale (*x Triticosecale* Wittmack) is an important cereal crop in Europe. There is an increasing interest in its cultivation due to better adaptation to adverse environmental conditions and efficient utilization of nutrients as compared to wheat or barley. These properties favor triticale for low input agriculture systems. In order to locate genes involved in control of adaptive traits, saturated molecular linkage maps are preferentially needed. Although such maps exist for rye and wheat, they are still missing for triticale. Development of genetic maps is of great utility for any molecular breeding program. The aim of our study was to evaluate limited genetic maps of triticale using microsatellite and AFLP markers. Three F2 mapping populations using in experiments were obtained by crossing aluminum sensitive and tolerant double haploid plants cv. Bogo. Ten AFLP selective primer combinations, 53 rye (Khlestkina et al., 2004; Matos et al., 2007) and 50 wheat (Röder et al., 1998) microsatellite primers were tested on those populations. The obtained data concerning the evaluated maps will be presented on our poster.

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Latvian genetic resources – conservation and utilisation

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ABSTRACT: The conservation of genetic resources in Latvia is guaranteed through the "Programme on long-term preservation and utilization of plant, animal, forest and fish genetic resources for agriculture and food" developed by the Ministry of Agriculture. To coordinate this program, the Genetic Resource Centre was established in 2006, which incorporates the Latvian Gene Bank of cultivated plants, the central database, and a DNA marker laboratory. To preserve genetic diversity and to maintain high-viability accessions for long periods the Latvian Gene Bank of Cultivated Plants (LGB) was founded in 1998 within the framework of the Nordic-Baltic project. The LGB preserves genetic resources mainly of Latvian origin; varieties, most valuable breeding lines, genetic stocks and wild relatives of cultivated plants collected in Latvia. The main functions of the LGB are: conservation of plant genetic resources which include seed preparation and storage and periodic seed testing; seed distribution and genetic resource documentation. Altogether, 72 species from 38 genera are represented. Approximately 10% of the collection comprises of wild relatives of forage grasses collected within Latvia. Currently, seed accessions of the majority of Latvian crop cultivars are represented in the LGB, and are available for distribution. Seeds of Latvian –bred cultivars are also held in a safety duplication collection at the Nordic Gene Bank. In addition to the seed collections, Latvian genetic resources are held in field collections, and in the case of potato varieties, *in vitro*. The field collections are partially duplicated at breeding institutes and collection holders within Latvia. To promote utilization of germplasm, evaluation and characterization of plant genetic resources using plant descriptors and DNA fingerprinting has been started by experts from various plant breeding institutions in Latvia. Evaluation according to established descriptor protocols is already underway, and descriptors are being established or adapted for the remaining species. DNA markers are being used to characterise and evaluate genetic resource collections, as well as being incorporated into breeding programs. The plant genetic resources central database has been built using the SESTO documentation system developed and hosted by the Nordic Gene Bank. For crop species, the main passport data, germination and storage information are available through the SESTO database. A platform for entering and maintaining the plant descriptor data has been established. Each major genetic resource area (plant, animal, forest and fish), has established or is developing a database platform. All of these databases and other information are accessible through the Latvian Genetic Resources internet site (www.genres.lv).

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Microsatellite fingerprinting of commercial apple cultivars and land varieties

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ABSTRACT: The accessibility of reliable genetic markers is essential for variety identification and distinction, for development of efficient breeding methods to create new apple cultivars selected for disease resistance, fruit quality or tree growth characteristics and other traits.
Microsatellites (SSR-Simple Sequence Repeats) are the most widely used and may be the best molecular markers in these fields, since they show high polymorphism, co-dominant inheritance and good reproducibility. In this study, altogether 106 apple varieties were screened (66 commercial cultivars /Galli et al., 2005/ and 40 old – mainly – Hungarian land varieties / Wichmann et al., 2007/) with six previously described microsatellite markers (Liebhard et al., 2002). A total of 82 polymorphic alleles were detected (average 13.7 alleles/locus) and the heterozygosity of markers averaged very high (0.8). SSR database was generated from the results what is available from our Institute's home page: http://www.mkk.szie.hu/dep/gent/. The genetic variability among the genotypes proved to be such remarkable that as few as four markers from the applied six were enough to distinguish the 106 cultivars/varieties, except somatic mutants, which are proved to be indistinguishable from each other and their progenitors with these microsatellite markers. The probability that two different varieties displayed the same genotype at all investigated loci (Probability of Identity) was calculated to be 1.79×10^{-4} in case of commercial cultivars and 2.53×10^{-5} in case of land varieties. This confirms the high potential of SSR's for differentiation of apple genotypes and the huge genetic variation within land varieties, as well. This high genetic variation also demonstrates the usefulness of germplasm collections which are excellent sources for efficient breeding methods to create new apple cultivars with increased disease resistance, better fruit quality, or other traits. Developed SSR profiles can be very useful in practice, e.g. for selection of distant parents to obtain higher genetic variation in progenies, to identify outcrosses or self-pollinated individuals which do not belong to the progeny of the applied cross, for nursery control and protection of breeders' rights, etc.

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Molecular characterization of Tunisian perennial ryegrass germplasm using SSR and ISSR markers

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ABSTRACT: Four Tunisian spontaneous perennial ryegrass and one introduced cultivar has been studied with SSR and ISSR markers. To carry out this work, 6 specific microsatellite (SSR) loci were targeted and provided a total of 28 alleles generating 43 genotypes. A large number of polymorphic ISSR markers were generated using 10 appropriate primers complementary to simple sequence repeats. Markers were considered to estimate the genetic distance among the studied accessions and to examine their genetic relationships. The SSRs and ISSRs markers are sufficiently abundant and sufficiently polymorphic to be useful genetic markers in Tunisian perennial ryegrass. However, it was shown that geographical origins did not correspond with population clustering.

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Improvement of the European Central Cucurbits Database (ECCUDB) by including characterization and evaluation data

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ABSTRACT: The proposal to establish a formal ECP/GR WG on Cucurbits as part of the Vegetables Network was approved by the ECPGR Steering Committee in October 2003. The main objectives of the CWG are in accordance with the ECPGR priorities, one of them being the Documentation and Information. Until now, two meetings of the CWG have taken place, in Adana (Turkey) and Plovdiv (Bulgaria), respectively (Díez et al., 2002, 2008). One of the first tasks undertaken in the group was the establishment of the European Central Cucurbit Database (ECCUDB) at the Institute for the Conservation and Improvement of Agrodiversity. Passport data from 39 European institutions and belonging to 22 genus and 90 species are included in the database. Currently, the database holds information of passport data of 24899 accessions. The number of accessions belonging to the main cultivated species correspond to *Cucumis melo* (3449), *Cucumis sativus* (6398), *Citrullus lanatus* (5794), *Cucurbita maxima* (1904), *C. moschata* (884), *C. pepo* (3731), *C. argyrosperma* (21), and *C. ficifolia* (106). The database also included information about taxonomy on cucurbits, links related to cucurbits information, and the core collection of *C. pepo* constructed with the COMAV´s collection. Until present, characterization data belonging to COMAV´s *C. lanatus* and *C. sativus* collections have been uploaded into the database and are available online for consultation. The technical data of the database are the following: a) database structure: the database uses Microsoft Access because of its easy development and data introduction facilities, and the characterization and evaluation data are stored using a relational database management system (RDBMS); b) database queries: the database queries are being developed using Active Server Pages (ASP) as a programming language and is on line via ECCUDB web site (http://www.comav.upv.es/eccudb.html), and the query results are also downloadable in csv format.

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Obtaining of double haploid lines for Latvian breeding programs: barley, wheat and triticale anther culture optimization

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ABSTRACT: Production of double haploid (DH) cereal plants by the anther culture method is an important biotechnological approach, which enable shortening significantly the breeding process. In the same time use of this technology is often limited due to relatively low yield of pollen-derived embryos, high frequency of albino and low number of green plants-regenerants. Various improvements of the method have been made, including elaborating of effective pretreatment methodology, optimizing induction medium composition (Xynias et al., 2001, Wojnarowiez et al., 2002), plant regeneration in culture, plant rooting in soil etc. Nevertheless, obtaining of large number of DHs by anther culture method for cereal breeding programs is still problematic. Results of anther culture are highly depended on used genotypes (Zamani et al., 2003). The goal of the current work was to find out the best protocols for DH lines produced by the anther culture from barley, wheat (both spring and winter) and triticale hybrids from parents with unknown androgenic response. As initial material breeder's F_1, F_2 and F_3 hybrids were used. The cold (+4 ºC, 14 days) pre-treatment was applied for all used crops. For barley the pre-treatment by mannitol (62 g/L) with and without addition of $CuSO_4$ x $5H_2O$ (2.5 mg/L) was used for 4 days. Barley spikes were sterilized 4 min by 70% ethanol, wheat and triticale spikes – 17 min by 50% solution of bleach with shaking. Barley anthers were cultivated either on the solid or liquid (C_3, FHG with or without addition of 2.5 mg/l $CuSO_4$ x $5H_2O$) induction mediums, wheat and triticale anthers either on the 190-0, AMC with or without addition of 2.5 mg/l $CuSO_4$ x $5H_2O$ induction mediums. After 3-6 weeks wheat and triticale embryos and calli were transferred into 190-1 regeneration medium, barely embryos and calli were transferred on M_1 regeneration medium. Green plants-regenerants of barely were obtained only if pre-treatment by mannitol were used. The best for obtaining wheat and triticale embryos was AMC medium, but higher frequency of green plant regeneration was observed if AMC medium with Cu was used. Produced DH plants were transferred to appropriate Latvian breeding institutions for testing in the framework of corresponding breeding programs.

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Conservation of genetic resources of medicinal and aromatic plants in Croatia

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ABSTRACT Natural aromatic and medicinal plant populations in Croatia show great biodiversity in morphological, biochemical and genetic level. The main aim of the management of plant genetic resources is conservation, analyzing and documentation of the existing genetic biodiversity. For the purpose of conservation of these valuable genetic resources, The Collection of Medicinal and Aromatic Plants (MAP) has been established at the Faculty of Agriculture, University of Zagreb. Currently, the MAP Collection covers 1740 accessions of 266 MAP species. The collected accessions are held at classical *ex situ* maintenance facilities (+4°C) (Grdiša et.al., 2007). Most accessions (80%) represent wild material from Croatia. The most represented species are basils (*Ocimum* sp. mainly *O. basilicum* L.), St. John's wort (*Hypericum perforatum* L.), Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trev./ Schultz Bip.), Dalmatian sage (*Salvia officinalis* L.), marshmallow (*Althea officinalis* L.) and oregano (*Origanum vulgare* L.). Standardized MAP multicrop form was used during the collecting mission to obtain data on accession identification, collecting site information and assessment of genetic erosion. In 2006, the first phase of The Croatian Plant Genetic Resources Database (CPGRD) project has been successfully carried out for the purpose of establishment of national documentation system. The passport data, based on EURISCO descriptors and collecting data based on MAP multicrop collecting form are currently available. In the future the database structure will also include characterization and management data. All collected plant material is freely available for future use in scientific research and breeding programs (Šatović et.al., 2007).

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Establishment of a TILLING platform for sugar beet

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ABSTRACT: TILLING is a new, effective, tool of reverse genetics to produce and identify loss or gain of function mutants. The strategy is based on the production of an EMS mutagenised population and rapid systematic identification of allelic series of EMS-induced point mutations in specific target genes with a high throughput screening technique. The project uses the structural and functional genomic tools from *Arabidopsis* to identify mutations in orthologs of sugar beet genes related to resistance gene analogs, defense response, vernalization requirement, flowering induction and seed germination. An EMS (ethylmethanesulfonate) mutant collection was produced to identify loss-of-function mutants in a bolting sugar beet genotype. The M0 plants have been treated for different times (4, 6, 8, 12 and 14 h) and concentrations in EMS (0.5% and 1%) to cover a wide range of mutagen treatments. For the TILLING project, approximately 12000 M2 plants have been grown and selfed in the field. DNA extraction from 2700 plants representing 672 M2-families has been performed using a 96-well format plant DNA extraction kit. A TILLING platform for sugar beet will be established including an efficient pooling strategy of DNA and an optimized protocol for heteroduplex detection. Within the German genome project, GABI-TILL, a database for primer design, submission of screening requests, and documentation of results is constructed.

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Soybean and tobacco floral nectaries differ developmentally and functionally to produce nectar

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ABSTRACT: Pollinator attraction and pollination of flowers are mediated by characters such as flower color (visual cue); flower shape (accommodation); flower opening and pollen dehiscence (timing); volatiles production (smell); and nectary gland and nectar secretion (reward). This gland and its complex secretory product represent two important aspects of a larger, basic-biological study dealing with production of hybrid soybean seed at Iowa State University. The cultivated soybean, *Glycine max*, is highly self-pollinated. Floral nectaries are diverse secretory glands that vary widely in their flower location, size, and ability to produce sufficient nectar to serve as a reward, and possibly as an attractant to a variety of pollinators. The pollinator vector involvement is vitally important in establishing cross-pollination, to produce hybrid seed for agronomic performance studies. *Arabidopsis* flower nectaries were not studied because they are very small structures that are essentially non-functional. However, very little comparative work has been done to assess differences between nectaries of self-pollinated and cross-pollinated species. To capitalize on this disparity, we are studying an ornamental tobacco (*Nicotiana* LxS8) that produces copious amounts of nectar and cross pollinates (Carter et al. 2007; Horner et al. 2007). The annual soybean cultivars (Horner et al. 2003) that have relatively poor (Clark) and good (Raiden) pollinator attraction, and the perennial *G. tomentella* that outcrosses better than 50 percent of the time, represent prime subjects for our study which focuses on developmental and comparative anatomical results of nectaries of these taxa using histochemical, *in situ* hybridization, and immunocytochemical procedures. Our ongoing work with tobacco and the *Glycine* species demonstrates varying levels of nectary starch buildup, starch loss at a time of nectar production and secretion, expression of two tobacco nectary-specific genes (*NEC 1* and *NOX 1*), and expression of a protein (NEC 1) associated with *NEC 1* gene. Our hypothesis is nectar quantity and composition are of great importance to successful insect pollinator hybridization, especially in soybean.

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Use of genetic resources in molecular breeding of barley and wheat for disease resistance and quality

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ABSTRACT: The genetic resources are often used in plant breeding as donors of interesting genes for requested traits. Molecular markers closely linked to these genes enable early, proper and fast detection of individuals with desired allele during backcross breeding, what can make plant breeding faster and cheaper. We are focused on molecular breeding of barley and wheat lines for disease resistance and some important quality traits. As acceptors of interesting genes we use especially elite Slovak and Czech cultivars and lines. After five backcross generations with the help of MAS new created lines carrying markers linked to desired genes undergo resistance, agronomic and technological tests. In breeding of winter barley for resistance to BaYMV/BaMMV viruses we use co-dominant STS and SSR markers linked to *rym4* and *rym11* resistance genes. Cultivar Romanze has been used as a donor of *rym4* gene and landrace Russia57 as gene *rym11* donor. In spring barley we are focused on transfer of *Yd2* gene from landraces Shannon and Sutter resistant to BYDV by use of dominant ASPCR marker. Plants with the presence of marker linked to *Yd2* gene were tested to powdery mildew resistance (*Blumeria graminis* f. sp. *hordei*) and only plants resistant to both fungal and viral disease were selected. We are also working on transfer of effective leaf rust resistance genes *Lr19*, *Lr24* derived from *Thinopyrum ponticum* and gene *Lr35* from *Aegilops speltoides* into hexaploid wheat by use of dominant STS and SCAR markers. Near isogenic lines with these genes are used in gene pyramiding to develop a single line with all three genes. By use of protein markers we develop near isogenic wheat lines for higher sedimentation values, higher dough strength and better bread making quality. These are lines with new combination of high molecular weight glutenin subunits (21*, 7+8, 5+10) as well as wheat lines with new unknown HMW-GS and with new HMW-GS pair, which were detected in lines of landraces Eritrospermum 917 and Kotte. These near isogenic lines will be valuable for future assessment of the effects of 21*, 6.2+8.3 subunits on agronomic performance and end-use quality.

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Morphological and cytological variability in interspecific hybrids *Trifolium pratense* x *T. medium*

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ABSTRACT: *Trifolium pratense* is a high yielding, high quality fodder crop. However, it shows rather low persistency, which could be overcome by hybridisation with a species creating rhizomes. Hybrids between *T. pratense* and *T. medium* have been previously obtained by embryo rescue and the number of chromosomes was evaluated by flow cytometry. The DNA content converted into number of chromosomes ranges from 27 to 57 in individual plants. This work aimed at the evaluation of twelve morphological traits (stem weight/length, internode number, length/width of the central leaflet of the uppermost triple leaf below the top head, length/ width of the central leaflet of the triple leaf on the 4th internode, stem thickness on the 4th internode, stem and head number per plants, intensity of white marks and average leaf area) in ten populations (550 plants) derived from F_1 hybrids and in the parental species. The significance of morphological differences was determined by ANOVA test. Nearly all examined traits were intermediate in hybrids; they reached higher values than in *T. medium* and lower values than in *T. pratense*. The stem number was significantly higher in ten hybrid populations compared with both parental species.

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Comparison of SDS PAGE and chip electrophoresis in *Amaranthus* species assesment

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ABSTRACT: The seed protein profiles of 15 *Amaranthus* accessions from the Czech Gene Bank were studied. These profiles were evaluated to elucidate the taxonomic classification inside of the genus. SDS – PAGE and chip electrophoretic profiles of *Amaranthus* proteins were compared. The higher sensibility and wider range of bands were detected in chip electrophoresis. The confirmation of classification to the species were done.

Keywords: *Amaranthus* sp., chip capillary electrophoresis, SDS PAGE electrophoresis.

Introduction

The genus *Amaranthus* L. consists of about 70 species (Costea et al., 2001) some of them are the world´s most stubborn weeds (Horak&Loughin, 2000); others are cultivated as cereals, vegetables or ornamentals (Brenner et al., 2000). The genus is considered difficult by taxonomists, weed scientists, and horticulturists because its taxa are extremely polymorphic and often not easy to identify; additionally the genus has many nomenclatural problems (Costea&Tardif, 2003). The cultivated grain species are distinguished by characteristics of the female flowers and inflorescences according to the taxonomic key developed by Sauer (1967).

For the Gene Bank, we need simple methods for identification or confirmation of the species preferably according to the quality of the seeds. Capillary electrophoresis of the seed proteins offers good distinguishing capability and also greater speed, plus the possibility of automatic interpretation of results. The Lab-on-a-chip system provides compact equipment plus enhanced speed (Siriamornpun et al., 2005).

Material and Methods

The seeds samples of *Amaranthus* sp. used in this study were obtained from the amarant collection of the Gene Bank at the Crop Research Institute in Prague, Czech Republic. Table 1 shows 15 evaluated samples of the *Amaranthus* genus.

Table 1. List of evaluated accessions.

	ECN	Origin		ECN	Origin		ECN	Origin
1	01Z5200006	unknown	6	01Z5200045	India	11	01Z5200123	CR
2	01Z5200021	unknown	7	01Z5200071	USA	12	01Z5200134	Hungary
3	01Z5200035	USA	8	01Z5200075	Peru	13	01Z5200143	USA
4	01Z5200043	India	9	01Z5200112	unknown	14	sample 1	unknown
5	01Z5200044	India	10	01Z5200121	unknown	15	sample 2	unknown

SDS-PAGE Electrophoresis

Total protein from single seeds were extracted in 18 µl solution of 0.0625 M Tris-HCl, 5% BME, 2% SDS, 5% glycerol, 0.01% BPB.

Tubes were kept at a temperature 4 °C for 4 hours. Then the tubes were centrifuged at 12000 x g for 15 min. The supernatant was heated in a boiling water bath for 2 min as well.

The *Amaranthus* seed storage protein extracts were separated in conditions of discontinuous electrophresis (SDS-PAGE) according to Laemmli (1970). The electrophoresis was performed at a constant voltage 350 V and 45 mA per 1.5 mm thick gel.

Chip Electrophoresis

In analysis, we used the Experion™ Pro260 Analysis Kit (Bio-Rad Laboratories, USA) for the quantitative determination of proteins in *Amaranthus* species. The Experion automated electrophoresis system utilizes LabChip microfluidic separation technology and fluorescent sample detection (Caliper Life Sciences) to perform automated analysis of 10 protein samples per chip. The electrophoretograms of the assessed varieties were scanned and managed in Adobe PhotoShop 7.0 and then evaluated by specialised software Bioprofil 1D++ (Vilber Lourmat, Paris, France). It enabled the calculation of the similarity matrix and construction of dendrograms using Nei-Li coefficient and construction method UPGMA.

Results and Discussion

In this study, 15 samples of *Amaranthus* genus were evaluated. Our results confirmed that SDS-PAGE is a suitable tool for identification of the *Amaranthus* genus (Drzewiecki, 2001). The protein profile was especially abundant in the lower part of the gel. SDS-PAGE showed a higher concentration of protein bands in two positions on the gel with a molecular weight of 70 - 55 kDa and 37 - 10 kDa contrary to that of Rocio et al. (2007), who noted bands in the ranges 64 – 49.2 kDa and 36.8 – 32.8 kDa. Chip electrophoresis detected the protein bands with high sensibility in the broader range of 6.1-96.4 kDa (Figure 1). In the sample ´01Z5200134´ the protein band with molecular weight 68.934 kDa was identified, but in SDS-PAGE was not found. However, in both types of electrophoresis used in this study the

highest protein band polymorphism was found in the range of 70-55 kDa in contrast to that of Drzewiecki (2001), who published the most significant interspecific variation in zones between 36 and 66 kDa.. Four major polymorphic protein bands were identified in protein profile of chip electrophoresis. These bands were designated with letters **a** (57.090 kDa), **b** (61.325 kDa), **c** (63.235 kDa) and **d** (68.934 kDa) (Figure 1).

A similarity analysis based on the protein profile of the seed was carried out. The constructed dendrogram displayed a tree of all tested samples and four major clusters were obtained (Figure 2). First cluster A includes samples of *Amaranthus caudatus* with protein band in positions 63.235 kDa (**c**). The genotype ´01Z5200021´created the single cluster B with 70% of similarity to cluster A. In the genotype ´01Z5200021´ two protein bands, **c** and **d,** were detected. Protein band **d** was not described in any other *Amaranthu*s samples used in this study. In five *Amaranthus* samples, protein band **b** was found in the gel position with 61.325 kDa. This protein band is characteristic for *A. cruentus* samples. In this study *A. cruentus* was clearly distinguishable from *A. caudatus*, Drzewiecki (2001) obtained similar protein profiles of both species. Cluster D represented with six samples of *A. hypochodriacus* with typical protein band **a** was clearly distinguished from other species.

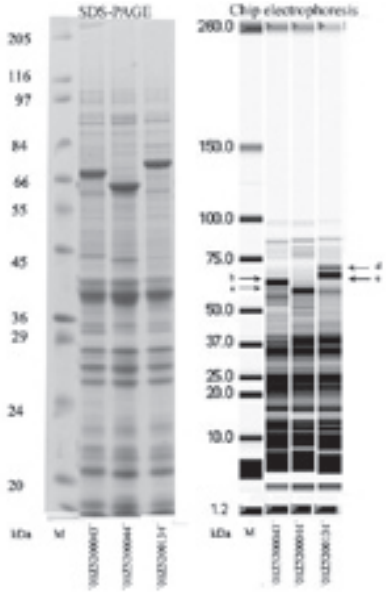

Figure 1. Comparison of protein patterns in SDS-PAGE and Chip electrophoresis with defined polymorphic protein bands.

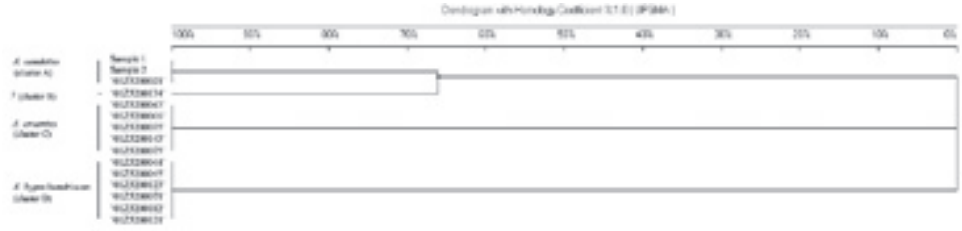

Figure 2. Similarity dendrogeam of tested *Amaranthus* samples.

Conclusion

The chip electrophoresis can provide the detailed information about protein polymorphism of *Amaranthus* sp. The chip electrophoresis can clarify the positions of protein bands with high sensitivity and relationships among *Amaranthus* species including weedy and wild species.

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Wild populations of red clover and their agro-morphological characteristics in Latvia climate condition

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ABSTRACT: Since 2000, each year scientific expeditions have been organized for collecting forage grasses accessions from different natural habitats in Latvia and neighbouring countries with aim to gather material and information about biodiversity of forages. Every year about 10-20 accessions of red clover are gathered from the wild. Collected accessions are gradually sown for evaluation, description, multiplication and regeneration, and most valuable accessions are consigned to the Latvian Gene Bank for long term storage. Evaluation of our genetic resources of forage grasses has shown that there are good opportunities to search for genetic resources in Latvia and in other countries with similar climatic conditions since the wild ecotypes collected in various districts are distinguished by a great diversity of morphological characters and agronomic properties within the genera (Kanapeckas *et.al.,* 2002). In our field trials we found some wild ecotypes with interesting characters. Accessions Nº36 and Nº37 showed rather high seed production capacity and good winterhardiness, while accession Nº6 was extremely early for Latvia climate condition and it had an uncommon color of flowers.

Keywords: red clover, wild populations, morphological characteristics.

Introduction

Latvia is situated in the bank of the Baltic sea, in temperate climate area, therefore forage grasses and legumes are the most important plants in the fields, as well as widely spread in natural meadows, pastures and other habitats. Red clover is a regular component of numerous associations in wild flora. Since 2000, 14 research expeditions there have been organized with aim to collect forage grasses and legumes from Latvia nature (Jansone *et al*., 2007). One of the most important tasks was to collect wild or semi-natural ecotypes of forages.

The goal of our research expeditions was to study the diversity of agromorphological traits of red clover, collected from different locations in Latvia and in neighbouring countries. In field trials we investigated winterhardiness, growing intensity, earliness, number of internodes, seed production and other characteristics.

Material and methods

Wild ecotypes of red clover were collected in different geographic locations and districts in different soil and climate conditions. The location of the habitat was precisely identified using a GPS system. Accessions were collected by seeds.

During 2006 – 2007 year in Latvia Research Institute of Agriculture a row trial was established with the aim to evaluate accessions collected from nature. The soil characteristic were: sandy loam with organic matter content 2,3%, P_2O_5 – 154 mg kg^{-1}, K_2O – 121 mg kg^{-1}, pH_{KCl} – 6,2. Ten accessions of red clover from different collecting places were sown in May. The early flowering variety 'Skriveru agrais' was chosen as a standard. A field trial with a randomised complete block design with 3 replications was established, and each accession was sown in double rows of 2 m length, with 60 cm between rows.

In the sowing year we estimated tendency to form inflorescences in autumn of sowing year.

In the 1st year of use there we estimated among other: winterhardiness, spring growth and regrowth after cutting, form of central leaflet, frequency of plants with white marks, length and thickness of stems, density of hairs, determined date of flowering and other phenological phases, color of flowers, and etc. (Barnes 2003). In autumn of the 1st year of use after evaluation all accessions were separately harvested for estimation of seed production.

Results and discussion

In the natural habitats, a lot of different red clover ecotypes were found. During the expeditions, accessions were collected from the Central highland of Vidzeme, which is hilly, located more than 200 m above see level, from river banks, flood-meadows, as well as from the Lithuania with loamy carbonate soils and warmer climate. Differences between red clover wild accessions for agromorphological characteristics were highly significant (Vilčinskas et al., 2007).

In the autumn of sowing year all accessions were well developed and left in good condition for wintering. In spring we observed large differences among the accessions. Winter hardiness as expressed by % survival varied from 30% to 85% (Fig. 1). All tested accessions showed poorer winterhardiness than the standard variety 'Skriveru agrais'.

Best winterhardiness (75-80%) was found for wild populations collected from habitats with severe climate - Central highland of Vidzeme (Latvia). Wild populations from Lithuania suffered considerably more winter damage (30-60%). These accessions were characterized by small, narrow leaves, thin stems, and a semi-erect bush-like growth habit. The seed yield of these accessions was not high. However, the red clover ecotype material is quite variable and will be useful for breeding work in future.

Large diversity among red clover ecotyps was obtained in flowering time. Very early flowering for Latvia was observed for accession Nº6 (June 2) and Nº27 (June 5), but some accessions were later than the standard variety (Fig.2). The most variable trait was seed yield (Table 1). There were adverse weather conditions for seed formation of red clover because of a lot of rainy days at flowering time. Nevertheless, two accessions considerably exceeded the standard variety in seed yield nearly 2 times. This is a very important parameter especially in Latvia, where the seed production for forage legumes often is limited by variable climatic conditions.

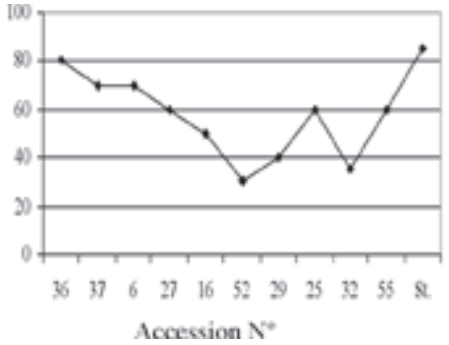

Figure 1. Winterhardiness of red clover accessions

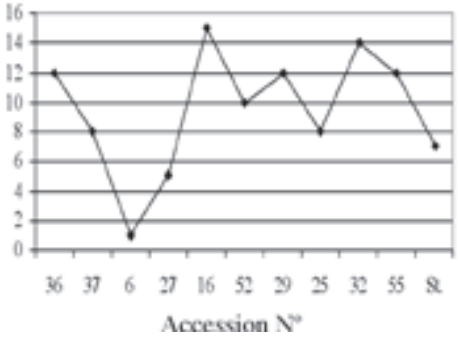

Figure 2. Diversity of flowering time-data of beginning florescence

Red clover accessions collected at the expeditions differed by many characteristics. The accession Nº6, which we found at the castle hill of Aizkraukle, is distinguished by it's earliness. That accession is also characterized by bright purple red color of flowers and thin violet- brown stems. These plants were not tall but they were homogeneous and had decorative traits. The accession Nº36 collected from a meadow in the Central hills of Vidzeme is characterized by good winterhardiness and highest seed yield, exceeding the standard variety by 90%.

Table 1. Results of morphological traits analyses of red clover accessions.

Accession	Length of plants, cm	Inflorescences, number per plant	Number of internodes	Seeds, g per plot	Color of seeds, %	
					yellow	violet
Nº36	62	28,4*	7,2	83,0*	100	
Nº37	65	18,6	8,0*	70,5*	50	50
Nº6	56*	21,2	6,4	20,4*	95	5
Nº27	67	18,2	6,2	47,6	100	
Nº16	65	15,4	7,8*	27,6*	90	10
Nº52	60	14,0	6,8	11,7*	30	70
Nº29	63	13,0*	6,0	17,2*	65	35
Nº25	67	9,6*	8,2*	7,0*	50	50
Nº32	69	14,4	7,8*	25,8*	40	60
Nº55	64	14,0	5,6*	27,6*	50	50
Standard 'Skriveru')	67	19,0	6,8	43,6	75	25
$LSD_{0,05}$	*7,7*	*5,9*	*1,0*	*14,3*		

*- significantly different from standard with P<0.05

Rather large differences were observed fo the number of inflorescences per plant, ranging from 9,6 for accession No25 to 28,4 for Nº36. For some accessions seeds were uniformly yellow, for many the violet color accounted for 5 to 50%, and for two accessions (Nº52 and Nº32) violet color of seeds was predominant (Table 1). Wild populations of red clover are a rich source of variability for creation of new varieties for different purposes.

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Study of molecular polymorphism of Tunisian pomegranate (*Punica granatum* L.) cultivars using SSR and AFLP markers

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ABSTRACT: Microsatellite markers (SSRs) were used to molecularly characterize 18 Tunisian pomegranate cultivars. Using four pre-selected SSR primers, 13 alleles have been revealed and permitted to fingerprint all the cultivars studied. For AFLPs analysis, four primers' combinations were tested for their ability to generate markers. A total of 192 bands were scored. A genetic distance matrix according to Nei and Li's formula was established and permitted to construct an UPGMA phenogram. The derived typology is similar to the UPGMA dendrogram based on Cavalli-Sforza and Edwards genetic distances that generated from SSR data. These results proved that a high level of molecular polymorphism characterizes the local pomegranate germplasm. In addition, correlation between distance matrixes was assessed using the Mantel test. Therefore, AFLP and SSR data are not significantly correlated ($r = 0.03$, p-value>0.05) suggesting an evident difference via the type of targeted markers. Studied cultivars constitute an important source of genetic diversity usable in future breeding programs. Data are discussed in relation with the efficiency of each marker in the surveying and/or in the differentiation between cultivars.

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ABRIISTAT 30: molecular data analysis software for genetic diversity studies

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ABSTRACT: Generally, in genetic diversity studies there are both lots of data for analysis and also different parameters must be considered according to the objectives. Thus, it may leads to miscomputing (Peakall and Smouse, 2006). Nowadays, computer softwares allow precise and quick analysis as much as possible. But almost it can not be found software which completely covers all of the analysis and genetic parameters. So, researcher needs to use several softwares to study required parameters. This software programmed in Microsoft Excel 2007, and simply can be applied. This version of the software in which different mathematical, conditional, reference, search functions, etc. and also mostly combination of the functions have been used, includes five parts: (A) Raw data entry; bands sizes obtained for each genotype, (B) 0, 1; transformed data in part A into 0 and 1, (C) Allelic genotype; indicates alleles of each genotype as alphabetic letters (for codominant markers including SSRs), (D) Alleles; indicates alleles of each genotype as their sizes, and (E) Results; compute some parameters including Ho, He (PIC=1-∑fi^2), Discrimination Power (1-∑pi^2), fixation index, index genotypes and etc. using the four previous parts. Validation of the results was made by comparing other softwares such as POPGENE and NTSys. Some of the advantages of software over other similar softwares already available, includes: (A) possible specific alleles for genotypes can be identified using this software simply, (B) It is easy to convert raw data as alleles, size of the bands and 0/1 to exploit them in other softwares according their import data format. Some specific studies used this software (Kadkhodaei et al., 2007).

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Development of a molecular identification key for the most important Iranian almond cultivars using DNA fingerprinting methods

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ABSTRACT: In order to obtain a rapid and precise discrimination of almond cultivars, Multiplex PCR was used to analyse several markers in a single gel lane, based on the allele size range of marker loci (Narvel et al., 2000). After basic experiments on 169 almond cultivars and identification of combinable primers (Testolin et al., 2004), the best primer set selected was [UDA009/BV102482, UDA015/BV102474, UDA023/BV102477]. This primer set was selected due to the relative similarity of Tm and consequently Ta, lack of primer dimers, indicating appropriate polymorphism among the 169 almond cultivars, lack of duplicate DNA fingerprints among them and different allele size range. Using this primer set, all of the almond genotypes were discriminated and showed a distinct DNA fingerprint, in particular for the most important Iranian almond cultivars, and which led to the development of a molecular identification key. The best method for genotyping (molecular coding) among the suggested coding methods in the paper, regarding to the universality and simplicity considered as a two part code, is an international code (gene bank no. or SSR loci) related to the amplified loci in the first part, and to the amplified allele sizes in the second part.

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Microsatellite loci inheritance in a Citrus interspecific somatic hybrid

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ABSTRACT: Polyploidy plays an important role in plant evolution. Many works were made to investigate the establishment, formation, genome organisation and evolution of polyploids. Allelic diversity and heterozygosity in polyploids may provide a genetic buffer against inbreeding depression. Many strategies have been developed for triploid citrus breeding. One of these strategies consists in sexual hybridization between diploids and allotetraploid somatic hybrids. Genetic structure of gametes of allotetraploids depends on the mode of chromosome association at meiosis. Microsatellite markers can provide many informations on the structure and genetic studies. In the present work, the allelic segregation is studied on a mandarin (*Citrus reticulata* Blanco) + lemon (*Citrus limon* L.) interspecific citrus somatic hybrid. A progeny derived from crossing pollen of allotetraploid hybrid (MC+EUR) with *Citrus maxima* (Burm.) *Merr* (CH) (2n=2x=18) was used in the present work for genetic analyses. 17 polymorphic markers have been selected among 200 microsatellites markers. Chi-square goodness-of-fit analysis test (χ^2) was carried to determine inheritance mode and which hypotheses described best the obtained frequencies. These results support a tetrasomic inheritance on a Citrus interspecific somatic hybrid. That means that chromosomes pairing on a Citrus interspecific somatic hybrid is the same as on autotetraploid. The allelic inheritance mode of this somatic hybrid provided valuable information for their utilization in a citrus breeding program.

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Genetic resources of fruit crop in Belarus: preservation and utilization

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ABSTRACT: The beginning of collection and preservation of fruit genetic resources was in 1925 for a special purpose in Belarus. It was attending to preservation, evaluation and collection of the accessions during all time before our days. Some of the accessions are using in our own research also for genetic resources exchange between the institutions. The Fruit Breeding Department of Institute for Fruit Growing of National Academy of Sciences in Republic of Belarus has the biggest fruit collections in our country – 2222 accessions. There are small collections in Belarusian State Agricultural Academy, Gorki, Mogilev reg. (124 accessions), Brest regional experimental station (clone collection of 127 accessions); Grodno Regional Institute of Plant Growing (142 accessions). At present our gene bank of *Malus* comprises 1146 accessions including 17 autochthonous species (*M. sieboldii, M. sieversii, M. baccata, M. sargentii, M.* x *prunifolia etc.)* and interspecific hybrids, *Pyrus* – 503 (6 species and interspecific hybrids: *P. communis , P. salicifolia*, *P. pyrifolia, P. ussuriensis, etc*.), *Prunus* – 248 (6 species: *P. cerasifera, P. americana, P. spinosa, P. pissardii, etc*.), *Cerasus* – 325 (6 species: *C. avium*, *C. vulgaris, C. fruticosa, C. vulgaris* x *Padus maakii etc*.). Institute for Fruit Growing is a centre for fruit crop research and breeding. Many of the accessions are results of our breeding program. The most active work is in apple. About 60 cultivars and 476 of the advanced selections are originating in our country. We have recently re-propagated approx 400 mainly foreign cultivars and advanced selections to make a new gene bank for research and breeding at Institute for Fruit Growing. This selection was based on preservation of maximum diversity and also a good assortment of commercially important cultivars, as well as cultivars with resistance genes. Last year after the revision more than 210 accession were discarded. We use seedling rootstocks (virus free) and Russian dwarf rootstock 62-396 for about 400 newly propagated cultivars. All the other varieties are older trees on seedling rootstocks. 5-6 trees of each cultivar are grown. Plant protection is carried out according to standard Belarusian regulations, no irrigation. We are used DNA-based markers: SSR (14 loci) have been applied to 110 cultivars of local and foreign selection. Researchers at our Institute have analyzed about 100 mainly foreign cvs for e.g. freezing tolerance of trees, have analyzed approx 200 cultivars for scab susceptibility. Results are usually published in international journals. Major funding comes from the State Program 'Fruit growing', supplementary funding – from the Program 'Preservation of Genetic resources'.

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Genetic diversity of 'heritage peas' collected throughout Sweden as revealed by molecular markers

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ABSTRACT: During the years 2002-2004 altogether 60 accessions of hitherto unknown 'heritage peas' (*Pisum sativum* L.), still in cultivation, were collected from all parts of Sweden. The genetic diversity of 34 of these cultivars was analysed using SSR markers and compared with 46 previously collected pea accessions currently in storage at the Nordic Genetic Resource Centre (NGRC) in Alnarp, Sweden. Initially five SSR primer pairs were used to screen the 80 pea accessions. Later, 12 of the accessions were reanalysed using eight additional primer pairs in an attempt to obtain better genotype separation. We found genetic variation both within and between accessions kept at the NGRC, as well as in the 'heritage pea' accessions. Of the 80 accessions analysed, 61 genetically different types were found. Seventeen of these were shown to be non-homogenous (i.e. a single accession contained several different genotypes). Fifty-one accessions were found be genetically unique and thus lacking duplicates. The remaining 29 accessions shared alleles with at least one other accession. Twenty-one of the 'heritage peas' were genetically unique, while the remaining 13 were genetically similar to other heritage or NGRC accessions. However, morphological markers indicate that some of the duplicates can be separated further. Our findings have shown that even a 'well mapped' country such as Sweden may contain unknown crop genetic diversity.

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Towards the construction of chromosome maps in *Lilium* based on recombination points analysed with GISH

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Longiflorum (L), Asiatic (A) and Oriental (O) lilies belong to section Leucolirion, Sinomartagon and Archelrion of genus *Lilium* respectively. Both LA and OA hybrids are promising in lily breeding for various agronomical traits. 2*n* and *n* gametes producing LA and 2*n* gametes producing OA hybrids have been used to develop progenies in a backcross (BC) breeding program. Genomic *in situ* hybridization (GISH) was used to discriminate the parental genomes and sites of recombination in these hybrids. It was found that F1 LA hybrids produced both *n* and 2*n* gametes resulted in the formation of both diploid (2n=2x=24) as well as triploid (2n=3x=36) LAA genotypes. However, most of the OA hybrids had only triploid AOA genotypes resulted from functional 2*n* pollen. In all cases, it was possible to identify the homoeologous recombinant chromosomes as well as accurately count the number of recombination breakpoints. Recombination breakpoints were estimated in the BC progenies of both LA and OA hybrids. These recombination breakpoints were cytologically localized on 12 different chromosomes of each genome in BC progenies of LA and OA hybrids. Cytological maps were constructed on the basis of the percentages of distances (in μm) of the recombination breakpoints from the centromeres. As a result, four complete maps were constructed for three genomes from BC progenies and indicated as Longiflorum (A), Asiatic (L), Oriental (A) and Asiatic (O). It was found that the recombination breakpoints were unevenly distributed among different chromosome of all three genomes. As with GISH it is possible to identify these recombination breakpoints, these can be used for assigning molecular markers or desirable genes to chromosomes of *Lilium*.

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Leafy Veg: a GENRES project to establish a network active in conservation and utilization of plant genetic resources of the most important European leafy vegetables

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ABSTRACT: Only in 2003 a working group on leafy vegetables in the framework of the European Community Programme for Genetic Resources (ECPGR) was established (Maggioni et al., 2005). The main objective of this working group is to manage, conserve and utilize leafy vegetable genetic resources for a sustainable European agriculture. To further strengthen their relationships the working group applied for an EU-GENRES grant which was obtained and since January 2007 the project has started. In total thirteen partners from ten EU countries are active in the project. The project duration is four years and the budget involved is around 1.1 M€ in total. More information on the project can be found at http://documents.plant.wur.nl/cgn/pgr/leafyveg/. An overview will be given of the first years' project activities which were focussed on leafy vegetable database development, regeneration, characterization & evaluation and utilization.

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Identification and frequency of maintainers for Ogura male sterile radish (*Raphanus sativus* L.)

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ABSTRACT: Research has been carried out on the identification of maintainers for cytoplasmic-genetic male sterile lines in European radish accessions. Male sterile plants of 20 different populations of 'Ogura radish' were crossed with male fertile plants of 15 radish cultivars and 27 Polish breeding lines. Out of 174 F_1s, nineteen produced 100% male sterile (MS) progeny, which indicates the presence of maintainer gene(s) in the populations tested. Other combinations tested produced either 100% fertile progeny or segregating progeny with a low frequency of MS plants. In BC_1s, the segregation of 100% MS plants was detected in 21 cross combinations. The male components were derived from 5 different accessions representing different genetic backgrounds and each of them can be used for maintainer breeding. The results indicate that the plants which are homozygous for the maintainer gen(s) are present in the tested populations rather seldom.

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Morphological variation within and between lettuce accessions of cv. ´Atrakce´ in the Czech national germplasm collection

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ABSTRACT: Czech national germplasm collection of leafy vegetables held by the Gene Bank Department (Research Institute of Crop Production) in Olomouc consists of almost 900 accessions of cultivated lettuce (*Lactuca sativa* L.) (http://www.vurv.cz; database EVIGEZ). Within a group of old lettuce cultivars, there are several accessions with the same name. Their detailed morphologic assessment is a crucial point for determination of duplicates, detection of potential genetic shifts during their commercial cultivation and regeneration in the gene banks. Cv. ´Atrakce´ is a type of butterhead lettuce, known since 1890 and cultivated in Czech Lands, Europe and in America under different names (Moravec et al., 1999; Rodenburg, 1960). In the Czech collection it is represented by 12 accessions (CSK09H57: 00015, 00125, 00126, 00127, 00128, 00129, 00130, 00131, 00132, 00133, 00134, 00135) (www.vurv.cz; database EVIGEZ). The first accessions were included to the collection in the year 1955. In two-year (2003-2004) field experiments, accessions were assessed for 22 qualitative and quantitative morphologic, phenologic and economic traits (Křístková et al., 2008) with aim to evaluate variation within and between accessions and to compare with original description of the cv. ´Atrakce´. Four main groups were recognized: 1) type of iceberg lettuce (00134); 2) type with dark green leaves differing substantially from other accessions and to the description of cv. ´Atrakce´ (00126), 3) plants with light green leaves and strong blistering (00125, 00127 and 00132), 4) accessions. with medium green leaves and slight blistering. Within the group 4) several subgroups were recognised: a) heterogeneity in morphologic traits (00130); b) development of long outer leaves different to description of cv. ´Atrakce´ (00133); c) plants with a shorter outer leaves (00015, 00128, 00129, 00131 and 00135). The most similar to the original description of cv. ´Atrakce´ were only few accessions (00015, 00131 and 00135). The results confirmed the genetic shift during cultivation and regeneration of accessions. Potential morphological duplicates should be confirmed by using molecular and protein markers.

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Genetic and morpho-agronomic evaluation of a word collection of *Triticum turanicum* Jakubz

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ABSTRACT: An agronomic, morphological, biochemical, technological (e.g. bread-making attitude) and molecular characterization of the oriental wheat (*Triticum turanicum* Jakubz.) is being conducted at the Institute of Plant Genetics (IGV) in Bari. As for agronomic evaluation, several experimental field trials have demonstrated a good adaptation of this rare cereal to climatic and soil conditions of southern Italy. As a result of this activity, 15 accessions out of the 73, constituting the word collection for this species, were selected for their higher yield performance and production stability. All characterization data are also in the course of publication in a fully illustrated catalogue edited by the IGV. In order to better describe the collection and to clarify the phenotypical relationships among the accessions, a cluster analysis approach was undertaken considering morpho-agronomic traits recorded over three years in a randomized block scheme with three repetitions per year. The *T. turanicum* collection was also analysed using AFLP molecular markers. After testing a number of primer combinations, four were selected because they were highly reproducible and detected a conspicuous number of polymorphic fluorescent fragments. A total of 350 fragments were scored as presence or absence in the range 60-600 bp and 98% were polymorphic. A similarity matrix based on Jaccard index was obtained and a UPGMA dendrogram was constructed. The cluster analysis showed that the analysed samples were mainly grouped, apart from some exceptions, in two big clusters on the basis of their western or eastern origin.

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Morphological diversity among accessions of pepper (*Capsicum annuum* L.) collected from Tunisia

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ABSTRACT: In Tunisia, plant of pepper (Capsicum annuum L.) was considered as one of the most appreciated vegetable and spice grown and consumed by Tunisian people. Although being an introduced crop by Spanish travels, it has adapted very well to hard environmental conditions including soil salinity (Van der Beek and Ltifi, 1991) and fungus attack (Allagui, 1993) and it has being cultivated throughout the country as a spice as well as a vegetable crop. Today, pepper production is based on commercial and improved varieties of Capsicum annuum which have replaced the most of local and cultivated landraces. In Tunisia, there is a little genetic diversity known among local germplasm and studies on vegetative and reproductive characters have not been considered in details. In this work, we have performed a morphological and agronomical characterization of 11 landraces of pepper grown in Tunisia. Accessions were collected from different parts of Tunisia and sixteen morphological characters (8 vegetative and 8 reproductive) were determined during vegetative and reproductive stages. Data collected were pooled for univariate and multivariate statistical analysis. A wide range of variability was observed among accessions and reproductive characters showed the highest variation among characters analysed. Based on Principal component analysis, three components were identified explaining 56% of total variance. Axis 1 accounted for 26.54% of the total variation and had placenta weight, number of seeds as the traits with largest positive coefficients. Comparatively, cross height and number of branches were highly correlated with the second component. Using a hierarchical clustering method, five clusters of morphological and agronomical homogenous behaviour were identified. The level of variation recorded in evaluated accessions indicates that they must be considered as a reservoir of genes that should be conserved in-situ and ex-situ in order to safeguard germplasm pool against the erosion and to make an efficient breeding program.

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Role of climatic parameters on blooming times of apple cultivars in apple gene bank plantation

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ABSTRACT: The climatic parameters influence on the dynamic of blooming of apple cultivars and so their productivity. The study aims to investigate the climatic factors and their influence on blooming times of apple cultivars. The measurements were carried out on 586 apple varieties in the plantation of an assortment (gene bank). The subsequent periods of development of tree named as 'phenophases' such as the beginning of bloom, main bloom and the end of bloom were observed in each of the varieties between 1984 and 2001. During this period the meteorological data-base keeps 7 meteorological variables for analysis. The findings revealed that the hibernal maxima were the most active factor among the meteorological variables of the former autumnal and hibernal periods which influence on the start of bloom of trees in the subsequent spring. The mean differences of daytime and night summer temperature of the last year seemed to be effective on the start of bloom.

Keywords: apple, blooming varieties, phenophases, precipitation, temperature.

Introduction

The life cycle of plants are manifest as their genetic controlled whereas different environmental conditions may change the performance of the varieties. The rhythm, speed and intensity of phenophases are attributed to the varieties of a species can be changed by plant breeding and modified by environmental factors (Bubán 1998). The subsequent phenophases of related varieties may coincide, overlap each other or move away (Brózik and Nyéki, 1974). The life processes of fruit trees are undisturbed within only the limits of temperature. Increasing temperature accelerates the speed of chemical reactions by a rate of two fold per an increase

of 10 °C wich is restricted to a narrow interval concerning on the phenological processes. (Szász and Tőkei,1997).

The dynamic of blooming is important in the blooming process of the apple cultivars (Orosz-Kovács, 2000). The whole blooming process could be estimated by the rate of flowers opened on the first three days. Temperatures above 10 °C registered one or two days before the start of bloom is very closely correlated with the rate of flowers opened at the first day (r=0.78) (Nyéki et al. 2002). Excessively high temperatures made the length of blooming period shorten during bloom. This time the pollens are quickly released however the adherence of pollen grains on the pistil and fertilization is low because of drying out of the stigmatic fluid. The chance of flowers being pollinated and fertilized is low at high temperatures during bloom (Brózik and Nyéki, 1975, Szabó, 1997). The study aims to investigate the climatic factors and their influence on blooming times of apple cultivars.

Material and methods

The apple varieties are grown in the plantation of an assortment (gene bank) of Fruit Research and Extension Institute at Újfehértó in Eastern Hungary. Each of the 586 varieties is represented by two trees. From 1984 to 2001 the subsequent periods of development of tree named as 'phenophases' such as the beginning of bloom C1, main bloom C2 and the end of bloom C3 were observed in each of the varieties. Precipitation, number of sunny hours and mean temperature are recorded during the whole blooming period. The variation has been calculated around the respective sums (precipitation, sunny hours) or means (temperature) thus six categories of the seasons has been performed. The time series fluctuation was analyzed by the dispersion D and the different weather characteristics were determined by weather in spring as follows:

Warm season $f(x) \geq \bar{x} + D$

Cold season $f(x) \leq \bar{x} - D$

Where 'D' is the dispersion of time series and '' is the average of pattern The average of differing from the average for his square the variance, and we receive the scattering after the square root extracted from this. 'D' well represents the changeability of meteorological time fluctuation. In meteorological point of view, there is big uncertainty between the average values, so we cannot use a simple average. Above or under the dispersion give a reasonable result. The sunny cloudy, overcast rainy and dry seasons can be classified by the same method. The applied software for correlation and regression analysis was SPSS 11.0 statistical software.

Results and discussion

The frequency of starting bloom in varieties with different blooming time

As the data of varieties of different blooming dates over 18 years have been evaluated. The early blooming varieties start blooming between 10 and 21 of April. The varieties of intermediate blooming start it at the interval 20 April to 3 May whereas the late blooming

group start at 2–10 May. If we know the starting date of blooming time (days after Jan 1).we can calculate the distribution curve of beginning of blooming. The distribution of beginning of bloom, C1 at different blooming varieties well represent the dynamics of blooming. (Figure 1). The results clearly prove that the probability of starting bloom increases with the time elapsed. The group of intermediate blooming time shows a normal distribution in start of bloom, but there are regressions along this course. That anomaly is attributed to the inconstancy of spring weather. The distribution curve of start of bloom in late blooming varieties is a zigzag line. The steep peeks are characteristic. The amplitude of the distribution is the narrowest in this group.

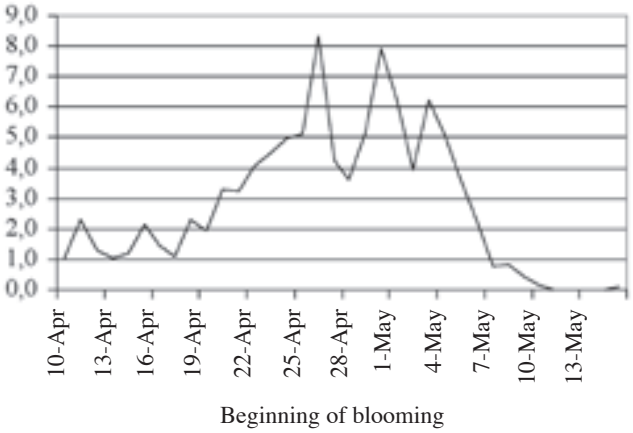

Figure 1. The distribution of beginning of bloom, C1 at different blooming varieties (Újfehértó 1984-2001)

The distribution of starting bloom in different weather characteristics

In warm years the bloom started earlier by 4-5 days than in cold ones. However, the dynamic of flowering was slower in warm years by 2-3 days (Figure 2).

The relations of the start of bloom and the different components of the meteorological environment

Out of the meteorological variables the spring temperature maxima, the mean temperature of the spring as well as the difference between the mean day-time and night–time temperatures produced a significant correlation with the start of bloom (at $P<0.01$). We have also found significant correlation between minimum temperature and beginning of bloom (($R^2= 0,57$).

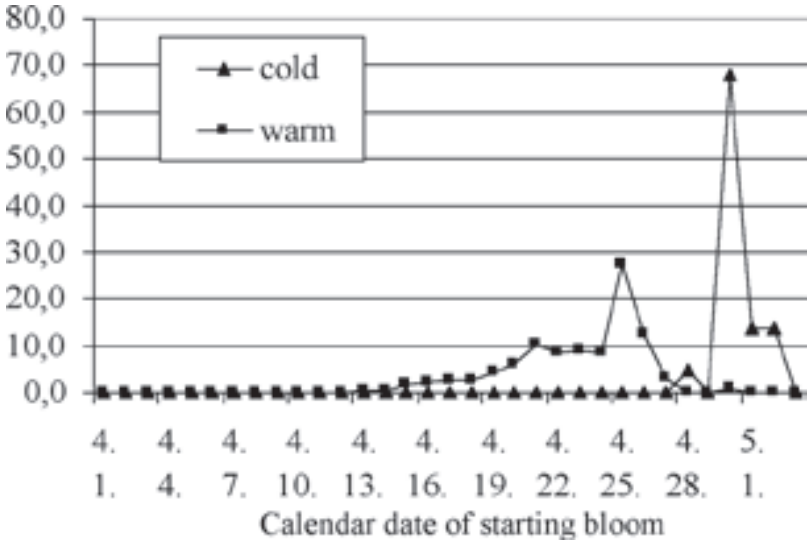

Figure 2. Distribution of the beginning of blooming time in cold and warm years (Újfehértó 1984-2001)

There was a significant effect of the autumnal precipitation, the mean winter temperature and winter minima, the autumnal and hibernal maxima on the start of bloom on late blooming varieties at $P<0.01$ level. The detected of the meteorological parameters of the former year was no significant effect on blooming data of the varieties with early and intermediate blooming. All the three groups of varieties reflected to the hibernal sunshine with blooming dates, significantly. The varieties of a longer ripening period manifested the effects of the mean differences between daytime and night hibernal temperatures, significantly.

The hibernal maxima among the meteorological variables of the former autumnal and hibernal periods were the most active factor influencing the start of bloom in the subsequent spring. In the group of early blooming varieties, there was a significant correlation (at $P<0.01$) on the one hand between the mean temperature, the minima and maxima of the former spring and the mean temperatures of the summer on the other hand the start of bloom in the current spring. The start of bloom of varieties with intermediate blooming reflected significantly the effects of the summer precipitation, the mean differences of daytime and night temperatures and the spring minima. The late blooming varieties did not reflect by their start of bloom the effects of any meteorological parameter measured in the former year.

Among the meteorological parameters the mean differences of daytime and night time summer temperatures of the last year seemed to be effective on the start of bloom. By the Fisher and Yates test, we have found significant correlation between average night and day temperature differences and length of blooming period (Figure 3).

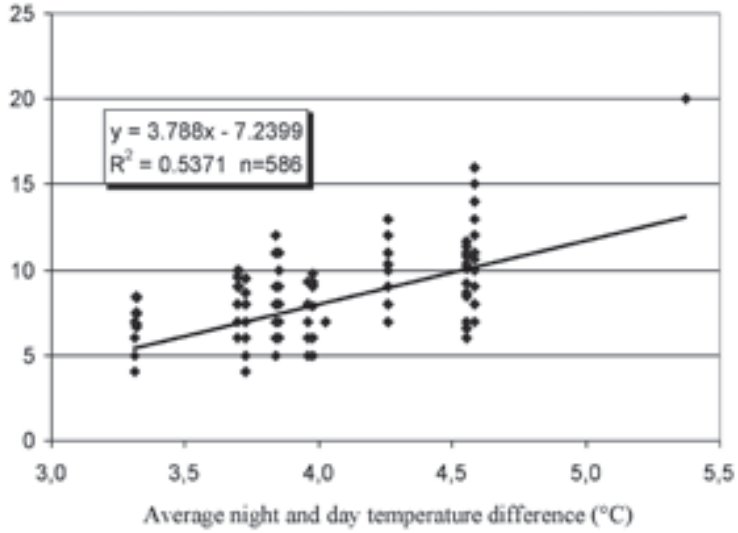

Figure 3. Relationship between the length of blooming and the average night and day temperature difference in Spring Újfehértó, 1984-2001

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Genetic variability of seed longevity in wheat and its implications for biodiversity preservation

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ABSTRACT: Knowledge on the extent of genetic variation in seed longevity is beneficial for the development of strategies for long-term preservation of plant genetic resources. We studied the genetic variability in seed germinability, vigour and deterioration, and seedling growth traits after long-term natural ageing in 75 wheat (*Triticum* sp.) accessions stored for up to 15 years at 0ºC and ambient room temperature (RT) in the seed genebank at IPK, Gatersleben, Germany. Germination, vigour (electrical conductivity, EC, and accelerated ageing, AA) and seedling growth tests were performed on all or selected accessions. The study revealed a considerable genetic variation in seed longevity, determined by the genotype, duration and temperature of storage. Correlation between EC and germinability observed for accessions after 5 and 7 years in storage was not preserved with ageing. The subjection of 0ºC-seed lots to AA resulted in deterioration effects, which were comparable to those caused by 10 years in storage at RT. The AA tests provided additional tools to differentiate between high and lower vigour accessions among high-germinating ones.

Keywords: accelerated ageing, electrical conductivity, seed longevity, seed vigour, variability, wheat.

Introduction

Plant genetic resources (PGR) comprise diversity of landraces, traditional and modern cultivars, and related wild species. Currently, the majority of plant germplasm is maintained as seed in genebanks under specified conditions. The imperative demands to preserve the natural and introduced genetic variation prompted worldwide actions of the national seed genebanks. A substantial part of their programmes alongside collection and conservation is the comprehensive characterization and evaluation of the PGR (Börner, 2006). The identification of genetic variation within germplasm is indispensable to the effective management and rational utilization of genetic resources. Seed longevity is a function of both storage environment and the genetically imposed seed vigour. The existence of genetic variability for seed longevity and its identification is related to the regeneration frequency, integrity maintenance, avoidance of genetic shifts and contamination, and, therefore, has an

impact on both resources preservation strategies and genebank management. Here we present results of a study on genetic variation of seed longevity in wheat, expressed as variation in seed germinability, vigour and deterioration, and seedling growth after long-term ageing at 0ºC and ambient room temperature (RT).

Material and methods

The study was conducted in 2007 on 75 wheat (predominantly *T. aestivum*) accessions originating from 23 countries. These included advanced cultivars (55), landraces (15) and wild relatives (5). Seeds of identical accessions were stored at 0ºC and ambient RT for 5, 7, 10, 12 and 15 years in the seed genebank at IPK, Gatersleben, Germany. From each of the five storage periods 15 accessions were studied. Standard germination and vigour tests (electrical conductivity, EC, and accelerated ageing, AA) were performed according to ISTA rules. Germination % was determined on the basis of normal seedlings on first (day 4) and final (day 8) counts in 4 replications. AA was applied for 72 h at 41.5ºC. Seedling vigour tests were carried out to evaluate the seedling growth retention after long-term storage. For the latter, measurements of the basic growth parameters were obtained after 8 days incubation of seeds at 20ºC, under 12 h photoperiod. Germination and EC tests were performed on all accessions, whereas only 28 selected accessions of high germinability were subjected to AA and seedling growth tests. Statistical analysis of the data was performed with the software package XLSTAT, version 2008.

Results and discussion

Genotype, duration and storage conditions effects

Seed germinability, vigour and seedling growth were determined by the genotype, temperature and duration of storage (Table 1). The genotype was the most powerful factor affecting EC of seed leachate, whereas temperature of storage was the main factor to influence germinability and most of the seedling growth characteristics. The model Temperature x Genotype was responsible for almost the entire phenotypic variation of germinability, vigour, and root, shoot and seedling dry weight. The effect of the storage duration was significant for most of the traits, though the relative contribution to the overall variation was lower compared to the storage conditions and genetic factor. The effect of genotype on germinability and vigour was highly significant for each of the five periods of storage and each of the two storage temperature conditions. These conclusions agree with earlier results by Ruiz et al. (1999) who found that cultivar variation and temperature were the most influential factors relating to seed storability in the three major cereals, barley, oat and wheat, after 10 years in storage.

The average germinability of RT-seed lots decreased rapidly (on day 4) or gradually (on day 8) and was practically nil after 15 years in storage (Fig. 1). The 0ºC-seed lots of the same accessions preserved high germinability (median: 90% on day 4, and 99% on day 8). The highest average germinability was recorded for 12- and 15-years-old accessions. The lower

Table 1. ANOVA results for seed germinability (before and after accelerated ageing, AA), electrical conductivity (EC) of seed leachate, and seedling growth traits in wheat accessions after storage for 5 to 15 years at 0ºC and room temperature. Effect of genotype, duration and temperature of storage.

Trait	Source of variation							
	Duration		Temperature (T)		Genotype (G)		T x G	
	R^2	F	R^2	F	R^2	F	R^2	F
Germ % (day 4)	0.06	9.72***	0.55	725.05***	0.20	1.80**	0.96	35.06***
Germ % (day 8)	0.07	11.10***	0.50	587.40***	0.23	2.06***	0.99	105.24***
AA-Germ % (day 4)	0.08	6.99***	0.45	133.72***	0.24	3.51***	0.95	23.58***
AA-Germ % (day 8)	0.03	2.36***	0.33	80.78***	0.26	4.02***	0.97	55.34***
EC of seed leachate	0.16	13.95***	0.02	6.55***	0.93	41.04***	0.99	4.38***
Root number	0.02	17.19***	0.01	15.74***	0.12	11.50***	0.13	1.53ns
Root length	0.03	22.65***	0.18	485.50***	0.16	16.14***	0.40	11.78***
Coleoptile length	0.05	41.66***	0.09	226.14***	0.22	24.30***	0.37	11.81***
Shoot length	0.08	60.16***	0.11	261.65***	0.17	18.09***	0.34	11.53***
Seedling length	0.05	39.30***	0.16	415.73***	0.16	16.31***	0.38	12.37***
Root dry weight	0.01	0.49 ns	0.49	123.88***	0.31	2.14**	0.93	10.86***
Shoot dry weight	0.01	0.26 ns	0.48	119.11***	0.33	2.28**	0.91	8.17***
Seedling dry weight	0.01	0.35 ns	0.49	126.90***	0.32	2.16**	0.93	10.10***

, * - significant at P<0.01 and 0.0001, resp.

average values in accessions after 5, 7 and 10 years in storage could be explained by genotypic effects, or it could be associated with low initial germinability due to inferior seed quality and vigour, which in turn, depend on the environmental conditions during seed development. The preservation of seed dormancy status for the first few years in storage at 0ºC could contribute to the lower germinability, as well (Stefani et al., 2000). No clear trend of vigour loss, expressed as an increase in the EC of seed leachate, with increasing the duration of storage was revealed (Fig. 1). This was not surprising since this trait was shown to be mostly genotype-dependent and the relative contribution of storage duration was low. The EC of seed leachate was lower in accessions stored at 0ºC compared to storage under RT, which complies with the higher germinability of the 0ºC-stored accessions. Significant negative correlation was established between EC of seed leachate and germination % (day 8) in 5- and 7-years-old accessions (r=-0.663 and -0.797, resp., on average over the two storage conditions). The Pearson r-values were even higher when seeds were stored at 0ºC. However, no correlation was observed between the two traits with seed ageing.

a b

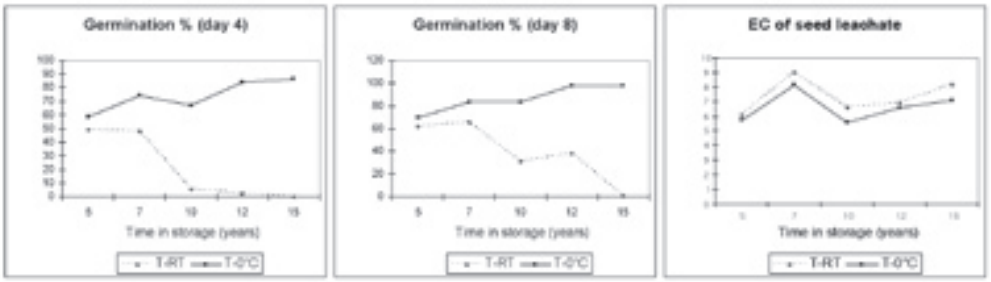

Figure 1. Average germination % on day 4 and day 8 (**a**) and electrical conductivity of seed leachate (**b**) in wheat accessions after prolonged storage at 0ºC and ambient RT plotted against time in storage.

Seed longevity means not only preservation of germinability, but also ability to retain good seedling performance after long-term storage of seeds. The average values of traits characterizing seedling growth vigour decreased with ageing. The difference between the average values of the corresponding traits of identical accessions stored at 0ºC and RT was increasing with the increase in storage duration (data not shown). This observation corresponds well with the data from the germination tests.

Accelerated ageing (AA) tests. Comparisons with the effects of the natural seed ageing

From each of the five storage periods 4 to 6 accessions keeping high germinability were selected and 0ºC-stored seeds were subjected to AA, followed by standard germination and EC tests. The selected accessions within each period were classified as having high vigour (preserving high germinability when stored at both 0ºC and RT), or lower vigour (retaining high germinability only when stored at 0ºC). The subjection of 0ºC-stored seed lots to high temperature and humidity caused significant decrease in germination % both on day 4 and day 8 ($P<0.0001$) (Fig. 2).

The comparison between the natural ageing at RT and AA showed that their effects on germinability were comparable only in the case of 10-years-old accessions. In the group of younger accessions, AA caused greater reduction in germinability than storage at RT, whereas in older accessions the effects of natural ageing at RT were significantly more pronounced.

Whereas the standard germination tests were not able to differentiate between the high vigour and the lower vigour accessions, the two groups differed significantly with respect to their germinability after AA, which was similar (statistically non-significant) to the germination % of the naturally aged seeds at RT (Fig. 3).

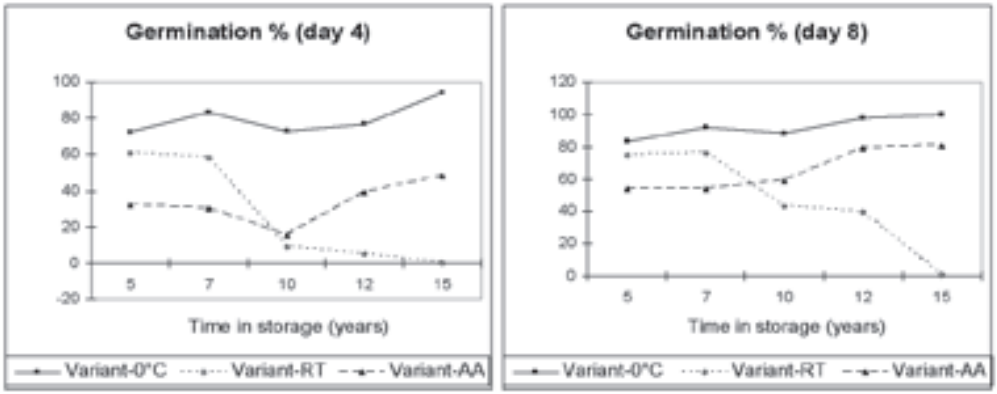

Figure 2. Comparison of the average germination % on day 4 and day 8 of identical accessions naturally aged at 0 ºC and RT, and artificially aged at 41.5 ºC for 72 h (AA).

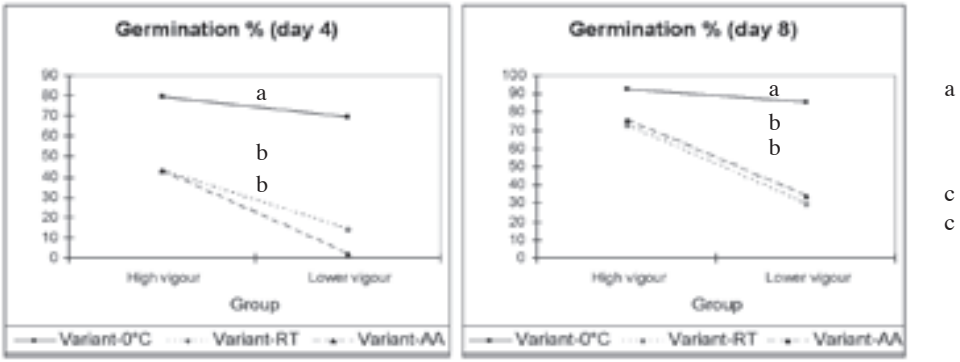

Figure 3. Germinability of naturally aged 0ºC- and RT-stored seeds, and artificially aged (AA) 0ºC-stored seeds. Comparison between high- and lower-vigour high-germinating accessions. Various letters denote significant differences ($P<0.001$) according to Tukey (HSD) analysis of the differences.

In conclusion, the current study revealed considerable genetic variation within wheat germplasm related to seed longevity characteristics. The AA tests provided additional tools to assess seed deterioration after long-term storage and to differentiate high-germinating accessions on the basis of their vigour status. This information is useful for determination of the minimum and maximum storage periods under particular storage conditions. It is also of importance to the control of genetic erosion during storage, and thereby to the rationalisation of the genebanks operation. The discrimination between low and high vigour accessions

is essential for further genetic studies to identify chromosome regions responsible for seed vigour and longevity. Molecular mapping of genomic regions associated with longevity would have important implications for development of strategies to preserve the existing genepool for the needs of future breeding, research, and mankind feeding.

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Information system for the management of the French Genetic Resource Centre of *Brassica oleracea*, and *Brassica napus*

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ABSTRACT: The management of genetic resources requires an user-friendly system of information. The French Brassica Genetic Resource Centre (GRC) was created in 2005 by fusion of both GRC 43 (Oilseed rape, *Brassica napus*) and GRC 49 (Cole crops, *B. oleracea*). The management system of genetic resources is based on a Web-based Information System which follows the workflow and the life cycle of accessions. This system is entirely and highly adjustable (1) to manage locally seed stocks , taxonomy tree with accessions, and ontology descriptors, (2) to integrate a privileged management of users and to preserve the required accession privacy. The concept of this tool is based on a system managing containers or contents. This version was entirely developed in open-source tools with Postgresql as RDBMS and PHP as language. Network and national collections of *Brassica oleracea* and *Brassica napus,* which represent more than 1500 accessions, are present in the Information System. The external access will be available through a Web Portal « www.brassica.fr », which will permit to get information about description of genetic resources available and conditions of seed release. An online demonstration will be soon available on our website on « www.brassica.fr ».

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Evaluation of conditions for plant regeneration and *Agrobacterium* - mediated transformation from tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT: Tunisian commercial culture of tomato (*Lycopersicon esculentum* Mill.) is seriously affected by many viruses such as the TSWV, the PVMV or the TYLCV causing significant yield losses. Therefore, resistant varieties are used to solve this problem. An improve protocol for *Agrobacterium*-mediated transformation of tomato was developed by examining the effects of different factors on the efficiency of transformation. Type of explants (leaf, cotyledon and hypocotyl), the antibiotic used and the frequency of transfer to fresh selective medium were tested. The four accessions tested (Riogrande, Justar, Nemador and Agata) showed a differences in both their aptitude to regeneration and transformation. High success rate was obtained for the first genotype in every stage of the process. Riogrande had the higher organogenic potential (>80%). Shoots were formed on the regeneration medium containing Zeatin/IAA combination. When leaf, cotyledon or hypocotyl explants were cultured on a basal medium with 1.0mgl^{-1} Zeatin plus 0.1mgl^{-1} IAA and supplemented with timentin (500mgl^{-1}), higher regeneration frequency and a greater number of shoots were obtained. The use of timentin is a good alternative to eliminate *Agrobacterium* infection in inoculated explants and increase the morphogenesis of *in vitro* tomato explants. The concentration of 500 mg/l of timentin showed no toxicity to tomato tissue and significantly promotes regeneration. After transformation, shoots were formed on the regeneration medium containing 100 mg/l of kanamycin through direct organogenesis. After two or three subcultures on the same fresh selective medium, elongated shoot buds were excised and rooted on selective medium (with 100 and 50 mgl^{-1} of kanamycin). Root formation reached 96%. Transgenic plants grew to maturity and produced fruits. The integration of the transgene was confirmed by PCR, dot blot, and Southern blot analysis. The collection of T1, T2 and T3 generation plants will be a useful tool in experiments for agronomic evaluation.

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Characterisation and evaluation of the German parsley assortment as a basis for breeding purposes

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ABSTRACT: Parsley (*Petroselinum crispum* (Mill.) Nyman, Apiaceae) is a very old crop plant. It has been cultivated for more than 2,500 years. The ancient Greeks held parsley sacred, using it not only to adorn victors of athletic contests but also for decorating tombs. In the Middle Age parsley was used for medicinal purposes. In Germany it is the most important spice plant today, cultivated on more than 1,500 ha annually. The complete assortment of parsley genotypes from the German genebank (201 accessions) and 19 genotypes from the JKI were grown at the two locations Gatersleben and Quedlinburg in Germany. It contains cultivars and landraces. Both morphological types, leaf parsley and root parsley, are under study to look for the variability within the assortment. They will be characterized morphologically as well as for resistance traits, aromatic components, taste, flavour and ploidy level. In addition, molecular studies shall clarify the intraspecific relationships. The parsley assortment was described morphologically with the help of a descriptor developed especially for cultivars and landraces. Phytopathogens as well as diseases of the accessions could be characterized. The molecular studies show three clusters, one for the leaf parsley with smooth leaves, one for the leaf parsley with crisped leaves and the third one contains all root parsleys. This fits very well with the targeted analysis of the essential oil content and compounds. High concentration of two monoterpenes, myrcene and β-phellandrene, can be correlated with root parsley and leaf parsley, respectively. For the volatile compounds two groups could be defined, one for all leaf parsleys without any difference of the leaf type and one for the root parsleys. In further investigations taste and flavour will be compared with the data from the phytochemical analyses. All available information can be provided in the database from the German genebank. This well described and characterized parsley assortment is a good basis for breeding purposes.

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Morphological and cytometric analyses in doubled haploids of *Triticale*

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ABSTRACT: The major advantage of doubled haploids regeneration is the immediate achievement of complete homozygosity. However, tissue culture manipulation may induce somaclonal variation. Changes induced in culture may affect phenotype of the regenerated plants or induce some mutations at the DNA level. It is often suggested that the method used for plant regeneration, explant source, tissue culture ingredients may be responsible for most of the mentioned changes. In order to study the influence of the tissue culture induced variation a set of regenerants obtained via somatic embryogenesis from immature embryos was used.Androgenesis in anther cultures on solid and on liquid medium were evaluated from double haploid donor plants. Morhological variation among regenerants revealed that 48% of them were albinos and in two instances changes in leaf color pattern were observed. Two hundred and seventy-one (30%) albino plants were observed on solid media and 164 (18%) on liquid media. However, no albino plants were found among regenerants from somatic embryos. Cytometric analisis demonstrated that in 261 out of 771 plants chromosomes were spontaneously doubled. The regenerants will be used for the evaluation of the generative progeny via selfing of regenerants to study inheritance of the changes both at the morphological and DNA levels. Putative DNA variation and changes in DNA methylation pattern is to be evaluated in forthcoming experiments using metAFLP quantitative approach.

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Genetic similarities among Iranian populations of *Festuca*, *Lolium*, *Bromus* and *Agropyron*, using AFLP markers

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ABSTRACT: The study of the degree and distribution of turfgrass genetic variation is essential for the efficient selection of superior plant material, for the adequate management of genetic resources, and for the effective preservation of biodiversity. In Iran, despite wide geographical distribution no report is available on genetic diversity of turfgrass populations and their similarity with accessions of other countries. Amplified Fragment Length Polymorphism (AFLP) was used to detect genetic diversity and relationships of 42 wild populations of *Festuca arundinacea*, *F. pratensis, F. rubra, F. ovina, Lolium perenne, L. rigidum, Bromus tomentellus* and *Agropyron cristatum*. The number of amplified products ranged from 11 to 78 per primer combination and a total of 497 markers were scored. Jaccard genetic similarity coefficient between populations ranged from 0.15 (between *A. cristatum* and *L. rigidum*) to 0.88 (between two populations of *F. arundinacea*) showing high levels of inter and intraspecific genetic diversity. Both the Unweighted Pair Group Method with Arithmetic average (UPGMA) dendrogram and Principal Component Analysis (PCA) clearly demonstrated differences in the degree of similarity between taxonomic units and separated species into distinct groups. Results indicated that there is a broad genetic diversity among Iranian turfgrass populations that can be exploited in breeding programs.

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SSR markers assessment of genetic diversity in sugar beet lines

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ABSTRACT: Genetic diversity of sugar beet (*Beta vulgaris* L.) is a concern due to certain limitations which took place during its breeding process and the reverse correlation between root yield and sucrose content. Production of lines both by biotechnological methods and traditional inbreeding are in need to increase it and to develop heterotic hybrids. Microsatellite are the markers of choice in various genetic studies including evaluation of biodiversity. The aim of this study was to assess the genetic diversity of sugar beet lines (obtained by gynogenesisand by traditional breeding methods) by SSR markers. Plant material comprised 29 accessions. Among them, 17 lines were developed using *in vitro* gynogenesis technique (Svirshchevskaya and Dolezel, 2000), 10 lines were supplied by sugar beet breeding stations and 2 accessions represented 'Belorusskaya 69' and 'Belotserkovskaya 40' diploid cultivars common to Belarus. Microsatellite polymorphism detected by using of ALFexpress II automated sequencer was found to be relatively low. In total, 55 alleles were observed with a set of 15 microsatellite markers (Cureton et al. 2002; Richards et al. 2004) ranging from 2 (Gcc1) to 8 (Bmb4) alleles and an average of 3.7 alleles per marker. The average PIC value was 0.526 ranging from 0.123 (Bmb2) to 0.834 (Bmb4). Neighbor Joining method of clustering revealed 3 main groups of accessions. First cluster comprised 4 haploid cultured *in vitro* gynogenetic lines induced from different unfertilized ovules of a single diploid 'Belorusskaya 69' donor plant. Second cluster was formed by a sample of 'Belotserkovskaya 40' cultivar and homozygous lines of gynogenetic origin from 'Belotserkovskaya 40' and 'Janasz' donor plants after few seed reproduction cycles. Five homozygous lines irradiated with 30Gy and 300Gy doses (IAEA laboratories, Seibersdorf, Austria) were clustered in the same group with non-irradiated ones. Third cluster comprised 'Belorusskaya 69' cultivar and 12 lines of different (*2x* and *4x*) ploidy level. Four out of five tetraploid sugar beet lines and 5 diploid lines with male sterility independently of the different geographical origin were found in the same group. Moreover, these 9 lines were traditionally bred ones in contrast to those of gynogenetic origin representing two other clusters. Thus, the application of 15 microsatellite markers gave us a clear clustering picture of analyzed sugar beet lines.

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Assessment of inter-specific diversity of *Hedysarum* genus in Tunisia

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ABSTRACT: In Tunisia, many grassland and pasture areas are considered to be threatened by genetic erosion. Thus, we were interested in *Hedysarum species* which constitute a very important phytogenetic patrimony able to produce forage and revalorize destroyed pasture land especially in arid and semi-arid areas. In order to elaborate fodder improvement, we investigated the genetic relationships among *Hedysarum* species using IGS (Intergenic Spacer) polymorphism. Appropriate conserved oligonucleotides flanking the rDNA IGS were used to amplify total cellular DNA extracted from eight *Hedysarum* species. Our data show that IGS amplification constitutes an efficient tool to examine the genetic diversity within species in *Hedysarum* genus. Results exhibited a high degree of polymorphism between species and permitted establishing the genetic relationships among species. Our data provide evidence of a nuclear lineage between the species included in the study. In spite of their classification as sub-species of *H. spinosissimum* and their distinctness by agronomic characters, mating systems and geographical distribution, *H. capitatum* and *H. spinosissimum* are characterized by great similarities of the IGS sequence. Both sub-species, closely related to *H. coronarium,* may assist plant improvement programmes.

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Sweet chestnut in Andalusia: A case of threatened "on farm" conservation

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ABSTRACT: Sweet chestnut (*Castanea sativa* Mill.), the only native species of the genus *Castanea* in Europe, occupies over 12.000 ha in Andalusia (South of Spain). In the year 2000, a collecting mission was carried out in this region to evaluate the genetic variability of the species. Two different types of chestnut groves were detected: one of clonal nature (varieties obtained by grafting) and another of open pollination (trees from seed). By means of morphological and molecular analysis we observed a high degree of genetic variability. A total of 38 varieties were identified: 12 in Huelva and 26 in Malaga regions. Moreover we reported data about the presence of synonymies and homonymies. Our results indicate that chestnut production in this region is a traditional system that uses varieties developed "in situ", constituting a genuine system of "on farm" conservation. Nevertheless, their preservation is seriously threatened in most zones due to the varietal substitution, the low profit or the abandonment, which determines the urgent necessity to safeguard these genetic resources.

Keywords: *Castanea sativa*, genetic resources, "on farm" conservation.

Introduction

"On farm" conservation is being object of increasing attention and international initiatives have been encouraged to revalorize and preserve the genetic diversity present in the traditional varieties (FAO, 1996; Esquinas-Alcazar, 2005). In Andalusia (South of Spain), chestnut occupies over 12.000 ha, appearing the principal orchards in the Natural Park of Aracena y Picos de Aroche, in Huelva and in the Genal Valley region, in Malaga. In both areas, chestnut is cultivated for fruit in plantations with traditional varieties grafted onto seedling rootstocks, and represents approximately the 70 percent of the surface in the region. There are also chestnut stands in Granada, Seville, Almeria, Jaen and Cordoba, as well as in other enclaves of Malaga region where currently this species presents a marginal use.

The available information on genetic identity of chestnut traditional varieties is, in general, scarce and doubtful, being their cataloguing very difficult. Morphological characterisation continues to be the initial main step for the description and classification of varieties, but the development of DNA markers has provided a direct study of the genotype, that enables the identification of chestnut cultivars (Mulcahy Bergamini et al., 1996; Botta et al., 1999; Goulão et al., 2001). The possibility of combining the genotypic profile with morphological and phenological traits might implement the identification and description of chestnut varieties (Gobbin et al., 2007). The main objectives of our study were: 1) to describe the state of chestnut stands and their possible genetic composition; and 2) to establish a method for the identification and characterization of chestnut varieties, and apply it to identify the main chestnut traditional varieties in Andalusia.

Materials and methods

When we started our work (2000) there was no reference in the scientific literature on chestnut in Andalusia, so our data collection was based on the search in local publications and on expeditions to the area to gather information and catalogue trees. The samples were collected in the two main cultivated regions of Andalusia 1) Genal river Valley (Malaga) district and 2) the Natural Park of 'Sierra de Aracena y Picos de Aroche' (Huelva).

In the case of grafted chestnuts for fruit production, the names of local varieties were compiled and trees of each one were catalogued. This consisted of their location by geographic coordinates (GPS) together with a local scheme. In the case of chestnuts from seed, groups of trees representative of the distribution area were chosen and identified by their coordinates and schemes. During expeditions, a list of traditional names was compiled in function of the information provided by the farmers.

One hundred grafted chestnuts belonging to 34 local names were selected from this collection for the varietal identification.

Ten morphological and phenological traits as leaf type, stamen, type of male catkin ripening period, fruit shape, fruit glossiness and colour (at harvest), presence and type of stripes, presence of hair on torch in fruit, and contrast of *hilum* to pericarp, were employed to categorise each accession (UPOV, 1989). For molecular analyses, a set of seven polymorphic microsatellites (CsCAT1, CsCAT2, CsCAT3, CsCAT6, CsCAT16, EMCs25 and EMCs38) developed in *C. sativa* (Marinoni et al., 2003; Buck et al., 2003) were tested.

Results and discussion

In both Malaga and Huelva regions, up to 156 trees were identified and catalogued (Martin et al., 2007).

We found a different situation in the two studied areas. In Malaga, chestnut is a crop in expansion, where one variety called 'Pilonga' and its derived denominations represent the 80 percent of the surface. In this zone, it is possible to find centuries old trees, orchards established in the last fifty years and trees planted recently. In Huelva there is not a dominant

variety and the low yield of chestnut has become critical. In this region, most of the orchards are represented by old trees, being the presence of modern orchards and young trees very scarce.

The three of the ten measured phenotypic traits (presence of stripes, glossiness, and contrast of *hilum* to pericarp) were monomorphic for all accessions, while the remaining seven traits have allowed to assign the accessions to different classes.

The microsatellite analysis showed the presence of 35 genotypes in both regions. The accessions from Huelva and Malaga never showed the same genetic pattern, since 23 alleles exclusive from Malaga and 8 alleles from Huelva were detected.

In Huelva, the combined use of morphological traits and SSR markers allowed to identify at least 12 varieties of clonal nature, three of them corresponding to the most popular in the region: 'Planta Alajar', 'Helechal' and 'Comisaria'. Among the remaining nine varieties, six corresponded to new varietal names, which were not reported in the literature before our work.

Following with the same criteria, 24 varieties of clonal nature were identified in Malaga. It was established the existence of at least 8 clones that responded to "pilonga" denomination or its derived. These clones showed different SSR patterns and significant morphological differences. This indicated that they did not represent a polyclonal variety, but a homonymy. Indeed, for farmers "pilonga" is referred to trees with large nuts that are easily peeled, characters both of 'Pilonga', whose clonal nature has been clearly established in our work.

The relationships between varieties could be facilitated by the system that farmers use to the replacement of trees. When a nut falls on ground, the resulting chestnut tree having good fruit characteristics, it is left and used to graft other trees. This may also be the reason why, in addition to the above mentioned clones, it has been found that trees catalogued under "pilonga bravía" denomination showed specific genotypes. In this zone, the term "bravia" is used to designate trees that come from seeds. Therefore, this name is applied to trees from seeds and with "pilonga" fruit traits. We think that these trees may be considered as a population variety. Thus, while the information available before our work suggested one variety called 'Pilonga', dominant in the area, we found that there are other varieties of clonal nature which correspond to 'Pilonga of Parauta', 'Pilonga of Jubrique' and 'Pilonga of Igualeja'.

'Tomasa' variety should be of clonal nature. Other fifteen identified varieties, developed in the region, were represented each of them by few trees.

The trees belonging to "portuguesa" denomination showed an identical SSR pattern but some morphological differences. Moreover, this variety exhibited sterile catkins and four alleles not shared with any other variety. Considering that sterile catkins are associated with interspecific hybrids, mainly between *C. sativa* × *C. crenata*, it is very probable that 'Portuguesa' trees are interspecific hybrids and could constitute a polyclonal variety.

In general, our studies of the Andalusia area indicate that chestnut stands, with the exception of the fruit production zones, can be classified as high forest. However, it is possible to find some coppice stands in Cordoba and Seville provinces, which are used for timber production. In most cases, it was not possible to establish with absolute certainty if the trees were grafted or not. However, the absence of the graft scars, the presence of stumps with numerous sprouts and the low size of the nuts, in most of the trees, suggested that trees were derived from seeds. Up to six traditional names were previously given on the basis of their fruit characteristics, but no information was available on their genetic nature. In most cases, these chestnut groves were abandoned and their conservation status is critical, due to drought or to the effect of the ink disease which was found in Andalusia (Trapero et al. 2003).

Although the biodiversity described in these chestnut zones is high, it is seriously threatened by the varietal substitution in Malaga, the low profit in Huelva and the progressive abandonment in the rest of the regions. Moreover, it was detected the introduction of exotic material, such as 'Portuguesa', without previous evaluations of its agronomic traits or its adaptation to the region. This study highlighted that this system is at risk of genetic erosion. For all this, it is necessary the establishment of conservation strategies, which should involve the Administration, producers associations and small farmers. These last ones represent, in many cases, examples of biodiversity defenders and propagators. However, the age of most of them, suggest the urgency of these strategies that permit the safeguard of this genetic variability.

We believe that there are Spanish and European regulations that would allow protecting some of these chestnut enclaves in Andalusia, considering their role as a genetic diversity reservoir. This is the case of Directive 75/268/EEC, and their development in the Royal Decree 708/2002, and the EC Regulation 870/2004.

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Application of biotechnology tools in the conservation and characterization of critically endangered Moroccan species *Argania spinosa* (Sapotaceae)

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ABSTRACT: Argan tree [*Argania spinosa* (L.) Skeel] is an endemic species from Southwest Morocco being the unique representative of the tropical Sapotaceae in this area. This species plays a great socio-economical and ecological role in these arid zones due to the prised oil from its kernels and its foliage to feed cheep and goat herds. However, a great zoo-anthropologic action led to the absence of natural regeneration of this species. In this work, the application of recent biotechnology tools for conservation and characterization of these rare critically endangered species is exposed. Promising propagation methods include *in-vitro* protocols well adapted to this rare species that allow the introduction, micropropagation and rooting of plant material, have been developed. On the other hand, a standard karyotyping protocol has been described as preliminary tool to start molecular (DNA) studies. In addition, different protocols for DNA isolation and quantification have been assayed in argan. Molecular markers based on PCR amplification of the DNA have also become an essential tool for the characterization and conservation of these species. Regarding this PCR amplification of the DNA, two main strategies, RAPD (because DNA sequence of this species is unknown) and SSR markers (using sequences from related species) have been assayed. These molecular markers have been applied in the genetic characterization of this germplasm, the establishment of genetic relationships between morphotypes, and the future construction of genetic maps of this rare woody species.

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Enhancement and diversity of primitive cotton, *Gossypium hirsutum* L., accessions

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ABSTRACT: Cotton, *Gossypium hirsutum* L., is an important cultivated crop that is grown throughout the world. Improvements in agronomic performance and fiber quality are needed to ensure its economic viability. Primitive accessions of cotton offer a wealth of genetic variability; however, since most of these accessions are photoperiodic they are not readily useable. This study involved 114 day-neutral derived lines that were crossed with two cultivars, Stoneville 474 and Sure-Grow 747. Day-neutral lines, cultivars, and F_2-bulks were grown in field plots for two years and agronomic and fiber traits were determined. The yield for most of the F_2-bulks was not greater than that of the high yield cultivars. All day-neutral lines had lint percentages that were significantly lower than the cultivars. The F_2-bulks had better micronaire and fiber strength than the cultivars. Genotypic effects made significant contributions to phenotypic variation indicating diversity. Dominance effects were the primary genetic effects. No consistent patterns of collection location or taxonomic designation with genetic diversity were identified. Derived day-neutral lines provide a new source of genes for improving and expanding the genetic base of cotton.

Keywords**:** Cotton, genetic diversity, *Gossypium hirsutum,* primitive Accessions.

Introduction

Cotton, *Gossypium* spp., is cultivated in warmer climates throughout the world for its fiber and seed. The spinnable fibers are important raw material which the textile industry use to produce yarn and fabrics. Seeds are processed into oil, meal, hulls and linters. Cultivars with improved yield, fiber quality, and stability are needed to meet the demands of producers, and fiber and seed processors. Improved fiber quality is needed due to changes in fiber processing technology, especially increases in spinning speed. To reduce yarn breakage stronger fiber is desired. Seed processors are seeking improvements in oil and protein. To make improvements, genetic resources with significant variabiltiy must be identified and evaluated.

Diverse genetic resources are essential for development of improved cultivars. Genetic diversity can reduce the risk associated with a narrow genetic base such as the outbreak or

spread of disease epidemics. To broaden the genetic base and maintain genetic diversity, new and unrelated sources of germplasm should be incorporated in cotton breeding programs.

The predominate species grown worldwide is *Gossypium hirsutum* L. This tetraploid species is native to Mexico and Central America. Collection trips to these areas and the Caribbean Islands, particular during the mid and latter part of the last century have resulted in about 2500 primitive accessions in the U. S. Cotton Germplasm Collection (Percival 1987; Anonymous 1997). Some accessions have also been contributed from other world collections. Primitive accessions have been shown to contain useful genetic variance for cultivar improvement with extensive genetic diversity reported for pest resistance, agronomic, morphological, and fiber traits (Percival, 1987; McCarty and Percy, 2001). Their use in breeding programs has been limited because a large proportion of the accessions require short days to initiate flowers; thus harvestable fruit is not set in the US cotton belt. A breeding program has been used to move genes for day-neutrality into the primitive accessions (McCarty et al., 1979). Day-neutral selections derived from this program can be evaluated under field conditions for desirable traits and readily crossed in breeding nurseries.

Research has shown that day-neutral derived primitive accessions have a wide range of variability that can be used in cotton breeding programs (McCarty and Jenkins 1993; McCarty et al., 1995, 1998a, 1998b, 2003, 2005, 2006, 2007, Basal et al., 2003, 2005; and Ragsdale and Smith, 2007). Different backcross generations for 16 day-neutral accessions were evaluated for several agronomic and fiber traits and results indicated useful genetic variability existed (McCarty et al., 1995, 1998a, and 1998b). McCarty et al., 2003 reported that 14 day-neutral derived lines from primitive accessions had fiber strength exceeding commonly grown commercial cultivars.

The objectives of this report are to summarize data assessing the diversity and breeding merit of 114 day-neutral accessions that were crossed with two commercial cultivars. This report will support the use of primitive accessions in cotton improvement programs.

Material and methods

Plant material and field design

Day-neutral germplasm was developed from photoperiodic accessions by crossing with Deltapine 61, a cultivar, followed by the selection of day-neutral plants in the F_2 generation. Seeds from several day-neutral plants within each F_2 were bulked and increased. The F_4 or later generation day-neutral derived lines were crossed to Stoneville 474 and Sure-Grow 747, conventional commercial cultivars. Cultivars were used as female parents with 114 day-neutral derived primitive accession lines. The cultivars are mid to full season types with high yield potential that are adapted to the southern USA growing environments.

The 114 day-neutral lines represent a broad group of photoperiodic accessions. The day-neutral lines were derived from 59 accessions collected from Mexico, 16 from the Caribbean Islands, 11 from Guatemala, 10 from Paraguay, 4 Brazil and 14 from 8 other countries. Fifty-seven of the photoperiodic accessions have been classified to race and include 19 *latifolium*,

9 *marie-galante*, 11 *morrilli*, 3 *palmeri*, 9 *punctatum*, 4 *richmondi*, and 2 y*ucatanense*, with the other 57 being unclassified. Hutchinson (1951) grew a large number of accessions and proposed a classification of seven geopraphic races for *G. hirsutum*. Even though 'race' is not recognized as a formal taxonomic category (Fryxell, 1976) the names are useful in discussuing various groups of noncultivated accessions. Morphological and fruiting habit differences were apparent during visual observations in the breeding nursery.

Crosses and field evaluations were conducted at the Plant Science Research Center, Mississippi State, MS, USA (33.4 N, 88.8 W). The 114 day-neutral derived lines, the two cultivars and their resulting 228 F_2-bulks were grown and evaluated in field plots for two years. Due to the large number of entries they were grouped into 19 field experiments. Each consisted of 6 day-neutral lines, 12 F_2-bulks and the 2 cultivars. The design for each test was a randomized complete block with six replicates. Plot size was a single row 12 meters long with row spacing of 0.97 meters and the planting was a two-planted, one-skip row pattern.

Prior to machine picking for yield determination, a 25-boll hand-harvested sample was collected from each plot. These samples were weighed and ginned to determine boll weight and lint percentage. Fiber samples were measured for determination of micronaire, elongation, fiber strength, and fiber span length 2.5% and 50%. Fiber strength was measured with the 3.2 mm gauge stelometer and length was measured on a digital fibrograph.

Data analyses

SAS proc GLM version 8.0 was used for data analyses (SAS Institute, 1999). In the overall analysis sources of variation were partitioned into year, field experiment within year, block within year and field experiment, entry, entry by year interaction, and random error effects. Additional data analyses were conducted for individual field experiment using ANOVA and LSD values were calculated for comparison of entry mean. A modified AD genetic model was used to estimate variance components for agronomic and fiber traits for the day-neutral derived lines. Genotypic correlation coefficients among traits were also calculated. Correlation and cluster analyses were conducted to determine if relationships existed between collection location and trait performance. The F_2-bulk data were evaluated by the additive-dominance genetic model.

Results and discussion

Seven of 114 day-neutral derived lines produced more seed cotton than Stoneville 474; however the majority were not significantly lower in seed cotton yield than the mean of the two cultivars. The day-neutral lines had significantly lower lint percentages compared to the cultivars. As a result when lint yields were calculated most day-neutral lines were significantly lower than the cultivars. Forty-one and 12 of the 114 lines produced heavier bolls than Stoneville 474 and Sure-Grow 747, respectively; however 33 and 58 had lighter bolls compared to these cultivars.

The fiber properties varied depending on trait. Only one day-neutral line had a micronaire reading that was significantly higher than the cultivars and over one-half had micronaire values significantly lower than the two cultivars. Most of the day-neutral lines had fiber length that was equal to or longer than the cultivars and many produced fibers stronger than the cultivars.

All 228 F_2-bulks had lint percentages that were significantly lower than Stoneville 474 and Sure-Grow 747 which resulted in lint yields being lower than the cultivars. Boll weight of F_2-bulks ranged from 4.16 to 6.41g while cultivars were 5.25 and 5.50g. Since most day-neutral lines had lower micronaire values than the cultivar parents a large proportion of the resulting F_2-bulks produced fiber with lower micronaire values than the cultivar parents. Few F_2-bulks had micronaire values higher than the cultivars. Many of the F_2-bulks had fiber length equal to or longer than the cultivars. More than one-third of the 228 F_2-bulks had fibers stronger than Stoneville 474 and Sure-Grow 747 and only 10 were weaker than Stoneville 474, the stronger cultivar. Results suggested that Sure-Grow 747 was a better general combiner for heavier boll size, increased yield, longer 2.5% span length and higher percent elongation, while Stoneville 474 was a better general combiner for increasing fiber strength. Results suggested that wide selection of day-neutral derived lines can de used as good general combiners with these cultivars for improving fiber quality while maintaining high yields.

Variance component analysis for the day-neutral lines showed that genotypic effects made significant contributions to the phenotypic variation for yield and fiber traits. This is an indication of genetic variation or diversity among these lines. Although we found some strong positive genotypic correlations among some traits, weak genotypic correlations were found between yield and two important fiber traits, 2.5% span length and strength. Analyses using an extended additive-dominance model revealed that dominances effects were the primary genetic effects controlling agronomic and fiber traits, thus strong heterosis would be expected for most traits in some hybrid combinations.

Based on cluster and discrimination analyses for this data set of 114 primitive accessions no consistent effect of collection location or race designation on the traits measured was determined. This indicated that no specific region or races were more important for desirable genetic resources for improving yield and fiber data. The derived day-neutral lines provide gene resources for improving fiber quality while expanding the genetic base of cotton; however, their low lint percentages must be considered when they are used in breeding programs.

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Evaluation of genetic resources for use in breeding programmes at the Agricultural Institute of Slovenia

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ABSTRACT: During the last decade the Agricultural Institute of Slovenia put the emphasis on collecting activities, broadening of the collection with autochtonous material, and central crop database activities. In addition to the ex situ conservation, the material is used in breeding programmes and potentially for use within the National Rural Development Programme as reintroduction of old cultivars or populations cultivation. Early projects to collect Slovenian autochthonous populations, ecotypes and landraces of agricultural species were initiated about 50 years ago. Phytogeographic and historical background has supported the development of the national programme and through that conservation of plant genetic resources in Slovenia. The primary goal of the Slovene Plant Genetic Resources Programme for Food and Agriculture, is to maintain, evaluate, regenerate and preserve Slovenian autochtonous species, ecotypes, populations and landraces of agricultural, medicinal and aromatic plants. Germplasm collection at the Agricultural Institute of Slovenia houses among other, more than 3000 accessions of grain legumes, *Allium* sp., *Solanum tuberosum* L., *Triticum* sp., *Brassica* sp., *Lactuca* sp., forage crops, *Rubus* sp. and *Vitis* sp. The gene bank at the AIS holds an extensive *ex situ* collection of more than 1000 bean (*Phaseolus vulgaris* L.) accessions collected from various parts of Slovenia. In a more comprehensive analysis the genetic variation and relationships among and within accessions were described using molecular (AFLP, SSR, RAPD), biochemical (seed proteins) and morphological markers (Šuštar Vozlič et al, 2006, Maras et al. 2007). In a similar way collection of lettuce (AFLP, SSR, morphological), corn landraces (MITE, IEF, morphological), cabbage (SSR) and potato (SSR) was evaluated and described. The *Phleum pratense* L. and *Trifolium pratense* L. germplasm collections were evaluated using morphological and chemical characteristics and assessement of the net lactation energy and yield. Data obtained from various studies mentioned above, are included in crop specific breeding programmes at the Institute. Along with that we are working on reintroduction of old lettuce and common bean varieties and populations to the farm production in line with the National Rural Development Programme.

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Factors shaping on farm genetic resources of sorghum (*Sorghum bicolor* (L.). Moench) in the centre of diversity, Ethiopia

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ABSTRACT: Ethiopia is cited as one of the centres of sorghum diversity. In order to assess the on farm genetic resources management of sorghum various research methodologies were employed. These were focus group interviews with 360 farmers, key informant interviews with 60 farmers and development agents and semi-structured interviews with 250 farmers. Besides, a diversity fair was done with over 1200 farmers. For quantifying on farm diversity, direct on farm monitoring and participation with 120 farmers were made. Quantification of varietal diversity per farm was counted by a participatory zigzag sampling in the diagonal direction of the plot with the farmer and all encountered varieties were counted. Soil samples were taken from 120 farms and were subjected to analyses of soil pH, P, available nitrogen, organic matter, and exchangeable potassium. Altitude and other related climatic data were collected. The number of varieties conserved by farmers ranged from one to twenty per farm and this is affected by socio-economic and biophysical factors. The mean number of 8.3 and 6.3 varieties were grown by *Oromo* and *Amhara* farmers, respectively. The minimum and maximum range did not vary for both ethnic groups. There was no significant difference in the number of varieties held by various wealth groups. With respect to farm size as explained by the quadratic model, it significantly accounted and predicted for the variation in the number of varieties. The role of soil pH, P, available nitrogen, organic matter, and exchangeable potassium on farm genetic diversity is described. P was a positive limiting factor for varietal diversity. As to the effect of crop ecology, there were more number of varieties in the intermediate altitudes than in the lowland and highland. Both the quadratic and linear equation expressed that distance from the house and town showed non-significant relationship to the number of varieties planted per farm. Varietal mixture is one of the strategies used by the farmers for improved on farm genetic diversity management. Farmers' underlying principles for conserving genetic diversity is described. Three models developed, namely; Bioecogeographic genetic diversity model, Farmer induced genetic diversity model and Farmer-cum-bioecogeographic genetic diversity model are explaining the processes shaping on farm genetic diversity of sorghum in Ethiopia.

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Diversity of words denoting traditional annual legumes in modern European languages

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ABSTRACT: Common vocabularies related to diverse annual legumes to both languages within one and between different linguistic families prove that these crops have been well-known to the ancestors of nearly all modern European nations from time immemorial. The words denoting chickpea in majority of the modern European languages owe their origin to the Proto-Indo-European root **kek-*, denoting pea. The words denoting faba bean in most of the Indo-European languages, as well as in the Finno-Ugric languages that borrowed them, have a common ancestor in the Proto-Indo-European **bhabh-*, denoting the same. The Proto-Indo-European **lent-*, denoting lentil, gave origin to the words denoting the same in nearly all its descending branches and languages, together with neighbouring languages of other linguistic families. The words denoting pea track back their origin in various Proto-Indo-European roots, such as **erəg^{w}(h)-*, denoting a kernel of leguminous plant, **ghArs-*, denoting a leguminous plant, and **pis-*, meaning *to thresh*.

Keywords: annual legumes, etymology, Europe, language, linguistic family.

Introduction

The European continent may be considered extremely rich in a linguistic sense: it is estimated that it has been home to at least three hundred living and extinct languages (Price, 1988). By all means, the most abundant linguistic family of Europe is Indo-European, while another rich European linguistic family is Uralic, with its Finno-Ugric branch. Apart from these two, there are also Altaic, Caucasian, Kartvelian and Afro-Asiatic linguistic families. The Basque language is regarded as a language isolate, with no demonstrable relationship with other languages. Majority of annual legumes traditionally cultivated in Europe originate from the Central Asian, Mediterranean and Near Eastern centres of diversity. At the same time, various archaeological evidence places pea (*Pisum sativum* L.), bitter vetch (*Vicia ervilia* (L.) Willd.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.) among the first domesticated plants in the entire Old World (Zohary and Hopf, 2000). The fact that Europe with Near East and Northern Africa was home to various ethnic groups throughout the history, as well as that there are common vocabularies of diverse annual legumes to both languages within one and

between different families, prove that these crops have been well-known to the ancestors of nearly all modern European nations from time immemorial (Mikić *et al*., 2007).

Chickpea

The derivations of the Proto-Indo-European **erəg^{w}(h)-*, denoting a kernel of leguminous plant, are numerous. One of them is the Greek *erévinthos*, denoting chickpea, that was transferred into Spanish as *garbanzo*, denoting the same. The words denoting chickpea in majority of the modern European languages, such as the Norwegian *kikert*, the Finnish *kikherne* or the Basque *txitxirio*, owe their origin to the Latin *cicer*, denoting the same, that was, in its turn, derived from the Proto-Indo-European root **kek-*, denoting pea. Certain European languages, such as Russian with *nut¸* Romanian with *năut* or Turkish with *nohut*, imported their words denoting chickpea from Persian, where *nuχūd* had the same meaning.

Faba bean

The words denoting faba bean in most of the Indo-European languages, such as the Latvian *pupas*, the Irish *pónaire* or the Bulgarian *bob*, have a common ancestor in the Proto-Indo-European **bhabh-*, denoting the same (Table 1). The same root is present in the languages of other families that borrowed both its form and meaning, such as the Finno-Ugric, with the Estonian *uba* and the Finnish *papu*, and possibly Basque, with *baba*. The Proto-Indo-European **leb-*, denoting blade, gave the Old Greek *lobos*, denoting pod: it is most likely that it has survived in the modern words denoting faba bean in Armenian, with *lobi*, and Georgian, with *lobio*. The Turkic words denoting faba bean, such as the Azeri *bağla* and the Turkish *bakla*, are borrowings of the Arabic *baql*, denoting the same. The Proto-Caucasian **qŏr'ā*, denoting pea, gave the words denoting both lentil and faba bean in its descendants, such as Ingush, with *qeŝ*, and Chechen, with *qö¯*(Nikolaev and Starostin, 1994). Due to a fact that the words that used to denote faba bean in the Germanic languages today primarily denote *Phaseolus* beans, the modern words denoting faba in these languages bean require a closer description, such as the Danish *hestebønne*, the Dutch *tuinboon* and the Swedish *åkerböna*.

Lentil

The Proto-Indo-European **lent-*, denoting lentil, gave origin to the words denoting the same in nearly all its descending branches and languages, such as Norwegian, with *linse*, French, with *lentille*, or Slovenian, with *leča* (Table 1). From neighbouring Indo-European languages, it was borrowed into the Finno-Ugric, with evidence in the Finnish *linssi* and the Hungarian *lencse*. Most of the Slavic languages have another word denoting lentil, derived from the Proto-Slavic **sočevica* of the same meaning. It is curious that the words denoting lentil and bean in Ossetian, *qædur* and *qædur*, have the same form that differs only in gender, the first being feminine and the second masculine. The Azeri and the Turkish words denoting lentil, *mərcimək* and *mercimek*, as well as the variations denoting vetchlings in the latter, such as *mürdümük*, are borrowings of the Persian *marcumak*, denoting lentil. Together with other Semitic languages, such as Arab

Table 1. Words denoting lentil, pea and faba bean in modern European languages.

Family	Branch	Language	*Lens culinaris*	*Pisum sativum*	*Vicia faba*
Afro-Asiatic	Semitic	Maltese	*għads*	*pizella*	*fula*
Altaic	Turkic	Azeri	*mərcimək*	*noxud*	*bağla*
		Turkish	*mercimek*	*bezelye*	*bakla*
	Basque		*dilista*	*ilar*	*baba*
Caucasian	Northeast	Ingush	*qe*	*gerga qeŝ*	*qeŝ*
Indo-European		Albanian	*thjerrëz*	*bizele*	*bathë*
		Armenian	*osp*	*olor*	*lobi*
	Baltic	Latvian	*lēca*	*zirņi*	*pupas*
		Lithuanian	*lęšis*	*žirnis*	*pupa*
	Celtic	Breton	*pizenn rous*	*piz*	*fav*
		Irish	*lintile*	*pis*	*pónaire*
		Welsh	*corbysen*	*pysen*	*ffa*
	Germanic	Danish	*linse*	*ært*	*bønne*
		Dutch	*linze*	*erwt*	*boon*
		English	*lentil*	*pea*	*bean*
		German	*Linse*	*Erbse*	*Bohne*
		Icelandic	*linsa*	*baun*	*erta*
		Norwegian	*linse*	*ert*	*bønne*
		Swedish	*lins*	*ärt*	*böna*
	Hellenic	Greek	*fakí*	*bizéli*	*kukiá*
	Indo-Iranian	Ossetian	*qædur*	*tymbylqædur*	*qædur*
	Italic	Catalan	*llentia*	*pèsol*	*fava*
		Corsican	*lentichja*	*pisu*	*fava*
		French	*lentille*	*pois*	*fève*
		Galician	*lentella*	*ervelha*	*faba*
		Italian	*lenticchia*	*pisello*	*fava*
		Occitan	*mendilh*	*pòis*	*fava*
		Portuguese	*lentilha*	*ervilha*	*fava*
		Romanian	*linte*	*mazăre*	*bob*
		Sardinian	*lentígia*	*pisu*	*fàba*
		Spanish	*lenteja*	*guisante*	*haba*
	Slavic	Belarusian	*sačavica*	*garoh*	*bob*
		Bulgarian	*leshta*	*grah*	*bob*
		Croatian	*leća*	*grašak*	*bob*
		Czech	*čočka*	*hrách*	*bob*
		Polish	*soczewica*	*groch*	*bób*
		Russian	*chechevitsa*	*gorokh*	*bob*
		Serbian	*sočivo; leća*	*grašak*	*bob*
		Slovak	*šošovica*	*hrach*	*bôb*
		Slovenian	*leča*	*grah*	*bob*
		Ukrainian	*sochevitsia*	*gorokh*	*bib*
		Upper Sorbian	*sok*	*hroch*	*bob*
Kartvelian	Georgian	Georgian	*osp'i*	*barda*	*lobio*
Uralic	Finno-Ugric	Estonian	*lääts*	*hernes*	*uba*
		Finnish	*linssi*	*herne*	*papu*
		Hungarian	*lencse*	*borsó*	*bab*

with *'adas* and Hebrew with *adashim*, Maltese owe its word denoting lentil, *ghads*, to the Proto-Semitic **'adaš*-, denoting the same.

Lupins

It is the Latin *lupinus*, a diminutive form of *lupus*, denoting wolf, and with a meaning that they can be grown on poor soils, that gave the words denoting lupins (*Lupinus* spp.) in numerous European languages, such as the Italian *lupino*, the Lithuanian *lubinas* and the Polish *łubin*. The Greek *thérmos*, denoting white lupin (*Lupinus albus* L.), was derived from the Proto-Indo-European **(s)ter(ə)p*-, denoting end, and survived with both form and meaning in the Spanish *altramuz*, the Turkish *tirmis* and the Arabic and Hebrew *turmus*.

Pea

Among the derivations of the Proto-Indo-European **erəg^{w}(h)*- are also the Proto-Germanic **arwait* and the Latin *ervum*. The first one denotes pea and gave similar forms with the same meaning to its modern descendants, such as Icelandic, with its *erta*, while another, denoting bitter vetch, gave some modern forms where it denotes pea, such as the the Portuguese *ervilha* (Table 1). Through the Proto-Baltic word denoting pea, **žirn-i*, the words that denote the same crop in the Baltic languages track back its origin from the Proto-Indo-European **g'^{e}r(a)n*-, denoting grain. The Baltic languages transferred both forms and meanings of the words denoting pea into Finno-Ugric languages, witnessed by the Finnish *herne*. All major Slavic languages derive their words denoting pea from the Proto-Slavic **gorxŭ*, denoting the same, and the Proto-Indo-European **ghArs*-, denoting a leguminous plant (Vasmer, 1953). Numerous European languages belonging to different families, such as Welsh, with *pysen*, Sardinian, with *pisu*, or Maltese, with *pizella*, owe their words denoting pea to the Latin *pisum*, that, through *pīnsere*, meaning *to thresh*, was derived from the Proto-Indo-European **pis*-, meaning the same. The Latin *volva*, denoting envelope, gave words with various meanings: one of them began to denote both pod, such as the French *gousse*, and pea, such as the Spanish *guisante*. The Romanian word denoting pea, *mazăre*, seems to be of pre-Roman, that is, of Dacian origin and is related to the Albanian *modhë*, its alternative word that denotes pea. The Proto-Turkic **burčak*, denoting both faba bean (*Vicia faba* L.) and pea (Starostin *et al*., 2003), was borrowed into Hungarian, where in the form of *borsó* it denotes pea.

Vetches and vetchlings

The Proto-Indo-European **weik*-, meaning *to avoid*, initially gave the Latin *vincīre*, meaning *to bind*, and then *vicia*, denoting vetches (*Vicia* spp.). It is the last one that, in one or more steps, produced the words denoting vetches in most European languages, such as the Breton *gweg*, German *Wicke* and the Hungarian *bükköny* (Mikić-Vragolić *et al*., 2007). At the same time, the words denoting vetches in many European languages are derived from the words denoting pea, such in Ukrainian with *goroshok*, Romanian with *măzăriche* or Georgian with

tsertsvela, derived from *tsertsvi*. The Proto-Indo-European *$*erəg^w(h)$- also gave the Greek *órovos*, denoting bitter vetch, that was borrowed with its meaning into Serbian as *urov*. It seems that vetchlings (*Lathyrus* spp.) were rather often and in many languages considered similar to pea as well, with the names such as the Armenian *tapoloŕ*, the Czech *hrachor* or Estonian *seahernes*. On the other hand, there are names that primarily denote grass pea (*Lathyrus sativus*), such as the French *gesse*, derived from the Latin *faba ægyptia*, meaning *bean from Egypt*, and the Russian *china* and the Serbian *sastrica*, both of obscure origin.

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Genetic variability and agronomic value of a Serbian lucerne core collection

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ABSTRACT: Evaluation of germplasm collections under field conditions is recognized as a general method to estimate genetic diversity. Agronomic traits and forage qualitative parameters were studied between 2001 and 2003 over a total of seven cuts. The differences among lucerne accessions for agronomic and forage quality traits were highly significant. Analyses of the core collection revealed highly significant differences among the accessions. The variability of both agronomic and forage quality characters encourage the use of evaluated accessions as genetic material in research and breeding programs.

Keywords: accessions, breeding, core collection, lucerne, variability.

Introduction

Lucerne (*Medicago sativa* L.) is one of the most significant open pollinated forage crops. It is an entomophilous species that produces high biomass yields of excellent nutritive value. According to Michaud *et al.,* (l988), lucerne is grown on over 32,000,000 ha worldwide. The wide geographic distribution of lucerne is a result of this species' great adaptability to different climatic and soil conditions, pests and diseases (Julier *et al.,* 1995). Lucerne breeding is a complex endeavour due to the genetic nature of the species, most notably due to it having an autotetraploid genome and allogamy as the dominant method of pollination (Brummer, 2004), which contributes to the increase of the existing variability of lucerne gene pool (Gherardi *et al.,* 1998). Katic *et al.,* (2005) indicate the importance of geographic origin for the level of variability of quantitative traits in lucerne varieties, while Julier *et al.,* (1995) discuss the impact of geographic origin on green forage yields of this crop. Julier *et al.,* (2000) obtained high inter- and intrapopulation variability of green forage yield in their study.

The development of higher-yielding lucerne varieties requires that genetically divergent and variable germplasm be used in the selection process. Lucerne populations are very heterogenous and consist of heterozygous individuals with very high levels of genetic variability both among and within populations (Julier *et al.,* 2000).

The existence of genetic diversity in lucerne varieties and populations and their availability for selection (good combining abilities) are of utmost importance for the advancement of lucerne breeding (Crochemore *et. al.,* 1998; Maureira *et al.,* 2004). Study

of the divergence and agronomic value of local lucerne ecotypes and populations is highly important for the breeding of this crop (Annicchiarico, 2006).

The objective of this study was to investigate the divergence and agronomic value of accessions forming part of the Institute's core collection of lucerne accessions with the aim to make the selection of starting material for breeding easier and more efficient, resulting in a more efficient use of breeding methods and the development of higher-yielding, higher-quality lucerne varieties.

Material and methods

The paper studied 30 lucerne accessions of different geographic origin that are part of the core collection of the Novi Sad Institute (Table 1.). All the accessions studied had been derived from varieties. 60 spaced plants per accession were established in rows in the spring of 2001 with a row-to-row spacing of 0.8 m and a plant-to-plant spacing of 0.5 m. There were 20 plants in each row and a randomized block design with three replicates was used. Traits were analyzed over a total of seven cuts: two in the first year and four in the second plus the first cut of the third year. The values of all the traits were calculated as average values per plant over the seven cuts. The following traits were studied: 1. green forage yield (g/plant); 2. dry matter yield (g/plant); 3. number of shoots per plant; and 4. percentage contribution of leaves to yield. All the traits were subjected to single-factor ANOVA for randomized block design. Coefficients of variation were calculated for all the traits and differences among accessions were tested using the LSD test.

Results and discussion

The lucerne accessions differed significantly with respect to the traits concerned (Tab. 1). The highest average yields of green forage and hay and the largest plants were found in the accession MED 1355 (446.2 and 113.3 g/plant), originating from Romania, and the accession MED 1349 from Iran (444.6 and 108.8 g/plant). The lowest yields of green forage and dry matter per plant and the smallest plants were recorded in the accessions MED 1368 and MED 1353 (291.6 g), originating respectively from France and Britain, followed by MED 1351, MED 1363 and MED 1331, accessions that originated in Northern and Western Europe (Tab. 1). The high yields of green forage and dry matter found in the accession MED 1349 from Iran indicate that this accession is well adapted to Serbian environmental conditions, while the similar forage and dry matter yields of the accessions from Romania and Serbia are indicative of their common origin.

According to our present results, which are in agreement with those of other authors (Julier *et al.,* 1995; Lamb *et al*., 2006), green forage and dry matter yields of the alfalfa accessions vary significantly. The extent of the variability may have been affected by the low stand density. Growing lucerne plants in a dense stand reduces the variability of yield (Rotili *et al*., 1999).

The accessions studied in the present paper differed significantly in the number of shoots per plant, resulting in a coefficient of variation among accessions of 11.3 % (Tab. 1). Shoot

Table 1. Green forage and hay yields, shoot number and leaf proportion per plant of lucerne accessions (mean value of seven cuts during 2001-2003).

Accessions	Origin	Green forage g/plant	Hay g/plant	Shoot number	Proportion of leaves %
MED 1341	Serbia	343.7	90.5	30.9	52
MED 1324		365.1	95.9	30.7	52
MED 1326		353.1	92.1	29.6	52
MED 1335		382.9	99.1	32.0	52
MED 1325		340.8	92.7	32.3	52
MED 1350		357.6	95.2	29.9	53
MED 1380	Romania	329.9	85.2	32.2	53
MED 1355		446.2	113.3	39.8	53
MED 1362		348.5	92.5	29.5	54
MED 1337	Czech Republic	347.3	91.5	32.1	52
MED 1329		372.7	98.3	35.4	51
MED 1340		322.3	85.0	29.4	51
MED 1332	France	325.5	85.6	32.4	55
MED 1347		338.9	90.3	32.0	56
MED 1361		311.6	79.1	30.4	54
MED 1328		303.5	80.8	26.2	53
MED 1368		267.8	72.0	27.9	54
MED 1351		295.7	78.0	27.3	53
MED 1347	Germany	328.9	78.0	32.3	54
MED 1331		297.5	79.2	31.6	57
MED 1353	UK	291.6	75.2	27.2	56
MED 1363	Netherlands	297.2	80.4	29.0	56
MED 1327		312.9	84.0	30.4	51
MED 1334		317.9	85.7	29.1	53
MED 1345	Turkey	342.5	89.0	30.4	52
MED 1356		379.6	96.0	35.8	51
MED 1349	Iran	444.6	108.8	40.8	50
MED 1367	USA	323.8	81.1	35.8	54
MED 1338		336.4	89.1	34.0	54
MED 1360		336.8	91.7	33.9	55
Average		339.8	88.9	31.7	53
CV %		14.9	14.8	11.3	3.4
LSD 0.05		82.6	21.4	5.8	2.7
LSD 0.01		109.4	28.4	7.7	3.9

number per plant is a trait which depends on stand density, soil fertility, and the genetic characteristics of the lucerne variety grown (Rotili *et al.*1999). Plants with more shoots per plant produce higher yields. The highest-yielding accessions in the present study had the highest number of shoots per plant as well: MED 1349, the Iranian accession, had 40.8, while the Romanian accession MED 1355 had 39.8. Significantly lower shoot numbers per plant were found in the accessions MED 1328 (26.2) and MED 1353 (27.2) originating from Northern and Western Europe, followed by the accessions of French origin, MED 1351 and MED 1368 (Tab. 1). Rotili *et al.,* (1999) reported that the effects of shoot number per plant were greater if individual plants were used as opposed to plots with a high seeding rate. Shoot number per lucerne plant is highly correlated with dry matter yield, as confirmed by Jafari and Ghamari (2005).

Percentage contribution of leaves to yield is an indirect indicator of lucerne quality. It is negatively correlated with green forage and hay yields of this crop (Julier *et al.,* 1997). In the present study, the relative contribution of leaves to yield was significantly above the average mean value in the case of MED 1331 (57 %). The percentage contribution found in the Iranian accession MED 1349 (50 %) was significantly below average. These results are in agreement with the findings of previous studies, as the accession MED 1349, which had the lowest proportion of leaves (50 %), was also one of the highest yielding genotypes. The average contribution of leaves to yield was 53 %, with a variation coefficient of 3.4 % (Tab. 1.).

The results of the present study have shown that there are large amounts of variability and divergence among the studied accessions and that some of them are of high agronomic value. The highest degrees of variability among the accessions were found for green forage yield (CV= 14.9 %) and dry matter yield (CV= 14.8 %), and the lowest for leaf proportion in yield (CV= 3.4 %). The highest yields of green forage and hay and the highest number of stems per plant (plant size) were found in two accessions of Serbian origin, MED 1335 and MED 1324, the Romanian accession MED 1355, and MED 1349 from Iran, so these accessions should be utilized in breeding programs as donors of genes for major agronomic traits. The accessions MED 1331, MED 1363, MED 1353 and MED 1347 originating from northwestern Europe have been found to be characterized by a high percentage contribution of leaves to yield and should be used in lucerne breeding for improved quality.

Accessions originating from arid (Iran) and semiarid climates (Pannonian Plain) have larger-sized plants with more shoots and produce higher yields of green forage and dry matter. Accessions originated from Central Europe have medium-sized plants, average yields of dry matter and green forage, and average leaf contributions to yield. Accessions coming from the humid climates of Northern and Western Europe have smaller-sized plants, a smaller number of shoots, and a higher proportion of leaves in the yield.

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Diversity evolution during 20 years of mass selection on 'Fandango', a Portuguese maize synthetic with some degree of fasciation

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ABSTRACT: In 1984, Pêgo started, with the CIMMYT support, an on-farm participatory maize breeding project at the Portuguese Sousa Valley region (VASO). A synthetic population, 'Fandango', was generated, using local populations, and subsequently submitted to 20 years of mass selection. Morphological data evaluation (e.g. yield gain, ear length, fasciation level) was conducted in Portugal (3 locations in 2 years) and in the USA (4 locations in one year) using seven different mass selection cycles. ANOVA comparisons and regression analyses on the rate of direct response to selection were performed. Selection for big size ears (larger and wider ears) revealed that length and kernels per row decreased significantly and at the same time, ear diameter, kernel row number and thousand kernel weights significantly increased so as the fasciation trait.

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Morphological and molecular diversity in eggplants of the "black" varietal group

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ABSTRACT: The "black" varietal group of eggplant (*Solanum melongena*), and in particular the F_1 hybrids of this group, dominates the commercial production of eggplant in Western Europe. Many "black" local varieties exist, and these varieties represent a source of variation of great interest for the improvement of this group (Daunay, 2008). We have performed a morphological and molecular (AFLPs and SSRs) characterization in 38 accessions (26 local varieties and 12 commercial hybrids) of the "black" type. Characterizations were based on 33 morphological and agronomic traits, 3 combinations of AFLP primers, and 14 SSRs. We have found that for most of the morphological traits, local varieties displayed a greater range of variation than commercial hybrids, and that some of the local varieties were competitive in yield with commercial hybrids under open field conditions. A mean of 144 AFLP fragments was scored, of which 41 (28.5%) were polymorphic. Thirteen out of the 14 SSRs tested were also polymorphic. Both the AFLP and SSR analyses showed that local varieties contain much more genetic variation than commercial hybrids. These F_1 hybrids, despite originating from four different seed companies, grouped together in the UPGMA dendrograms, indicating that commercial breeding programmes use a common genepool. The results obtained suggest that the introduction of local varieties of the "black" type in the current breeding programmes could contribute to increase the genetic base of the cultivated materials (Prohens et al., 2005), and also to exploit the heterosis for yield resulting from crossing materials situated at a large genetic distance (Rodríguez-Burruezo et al., 2008). The fact that some of the local varieties present traits of relevance for the development of new cultivars of the "black" type, like intense black colour, lack of prickles in the calyx and leaves, and high yield, should facilitate the introduction of these materials into the commercial breeding programmes.

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Genetic diversity among Iranian sunflower restorer and CMS lines

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ABSTRACT: Like other out-crossing species, sunflower breeding has oriented toward hybrid variety production. In this way, a large number of B-lines (together with their respective CMS lines) and fertility restorer lines are produced and assessed for their general and specific combining abilities. To attain high performance hybrids, lines with different genetic backgrounds should be crossed, therefore knowledge of genetic distance would help breeders to prioritize among their large number of possible crosses. In this respect, the concept of heterotic groups has been proposed and seems to be promising in some crops like maize (Charcosset et al., 1991; Goksoy et al., 2002). In order to assess the genetic diversity among the sunflower inbred lines developed at the Seed and Plant Improvement Institute, Karaj, Iran, 18 R-lines and 31 B-lines were assessed in the field for their morphological traits using the sunflower descriptor (UPOV), using a simple lattice arrangement. Analysis of variance revealed significant differences among both male sterile and fertility restorer lines, as well as between the two groups. Factor analysis provided two important factors for biplot analysis, which successfully separated male sterile and fertility restorer lines. Cluster analysis also separated the two groups. These results are in agreement with those of Cheres et al. (1999). Modifying the cluster analysis slightly, four groups were resulted; three of them included both fertile and sterile lines, while the last group included only restorer lines. Based on this grouping, seven fertility restorer and eight CMS lines were proposed for factorial crosses.

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Cutting immersion as a tool to increase frequency of doubled haploid lines in rapeseed (*Brassica napus* L.)

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ABSTRACT: A high frequency of haploid plants derived from microspore culture is still a major problem in obtaining sufficient numbers of doubled haploid lines for rapeseed breeding purposes. Fifty-one selected lines derived from the BC_4 generation of a cross between 'Express' as recurrent parent and 'RS239' as donor parent were chosen as donor plants for microspore culture in order to develop a set of intervarietal substitution lines. A treatment with 0.01% colchicine solution in the first 24 hours of microspore incubation only yielded 28% of fertile plants. An experiment was conducted to double the chromosome set of haploid plants derived from microspore culture with colchicine re-treatment at different growth stages, length of treatment, and protocols. With about 48%, the colchicine immersion of cuttings for 3 hours showed the highest frequency of chromosome doubling compared to other treatments.

Keywords: Chromosome doubling, colchicine treatment, cutting immersion method and microspore culture.

Introduction

Doubled haploid plants have been used for many purposes in plant breeding programs. In rapeseed, microspore culture is the prominent method for the production of these homozygous lines. However the high frequency of haploid plants derived is a major problem in production of doubled haploid plants. In *Brassica napus,* 70-90% of the microspore-derived plants can be haploid (Lichter, 1982, Chen and Beversdorf, 1992).

The application of chromosome-doubling agents like colchicine at early microspore culture stages could allow for the rapid generation of doubled haploid lines, depending on the genotype used and other conditions of microspore culture. Möllers et al (1994) achieved high rates of diploidization in *Brassica napus* by using 50 mg/l colchicine in the first 24 h of microspore incubation leading to 83-91% diploid embryoids. On the other hand, Weber et al. (2005) using a similar protocol obtained a large variation in diploidization rate depending on the genotypes used, between 31% and 95%. Therefore it is important to have an easy applicable method for chromosome doubling of haploid plants from microspore culture.

Root immersion in colchicine solution (Gland, 1981), injecting colchicine solution into secondary buds (Lichter et al., 1988) and applying a cotton swab soaked in colchicine solution

to the buds (Gland, 1981), or in-vitro colchicine treatment of whole plantlets (Mathias and Röbbelen, 1991) are alternative methods that can be applied to double the chromosome set of haploid plants. However, all these methods are uneasily applicable in practice. In the present experiment we improved the cutting immersion method as an alternative to increase the number of fertile plants that is easier to perform to large number of plants.

Materials and Methods

Plant material, microspore culture and ploidy analysis

Fifty-one selected plants from the backcross four (BC_4) generation of the cross between the Resynthesized line 'RS239' and 'Express' were used as donor plants for microspore culture. These selected plants carry one or two genomic segments of the donor parent 'RS239' in the genetic background of the recurrent parent 'Express'. The microspore culture protocol was based on the procedure of Lichter (1982) and Möllers et al. (1994) with modifications. A 0.01% colchicine solution (w/v) was applied in the first 24 h of microspore incubation. The ploidy status of the plants was analyzed after 3 weeks in acclimatization stage with flow cytometry.

Acclimatization and vernalization

Plantlets that already had well-developed root system were transferred for acclimatization from in-vitro culture to a controlled-climate room. After one month in acclimatization, plants were vernalized at 4°C for 2 months. Then plants were transferred to the green house.

Colchicine re-treatment

Root immersion method

The root immersion method used was based on Fletcher et al. (1998). The roots of haploid plants were washed and immersed with 0.34% (w/v) colchicine solution for 3 hours. Plants were placed under strong light during immersion. After treatment, the treated-roots were rinsed with water and the plants were re-potted. The efficiency of the method was tested at three developmental stages of the plants: after acclimatization, after vernalization and with adult plants. For adult plants, 3 different duration of immersion were evaluated 3 hours, 6 hours and overnight (more than 12 hours).

Cutting immersion method

Shoots of haploid plants with 2 leaves were obliquely cut, immersed in 0.34% (w/v) colchicine solution under strong light. Two shoots were cut from each haploid plant for two different time immersion, 3 hours and overnight. After treatment, cuttings were washed and then the wounded stem surface was treated with root hormone powder (Rhizopon B) and planted in soaked soil in multipot plates. Cuttings were not watered for 1-2 weeks and potted after the root system was well formed.

Results

Ploidy level of microspore derived plants and effect of colchicine treatment at microspore stage

There were 1200 microspore-derived plants regenerated from the 51 BC_4 donor plants. According to flow cytometry 32% of them were diploid, 49% haploid, 3% tetraploid, and 15% had an unknown status because their peaks different from the checks with known ploidy status.

About 28% from the 1200 microspore derived plants produced seeds after colchicine treatment at microspore stage. This number comprised 261 plants that were diploid, 20 that were tetraploid and 51 of unknown ploidy status according to flow cytometry.

Effect of colchicine re-treatment

The success of diploidization due to colchicine re-treatment was based on the ability of the plants to produce seeds. Colchicine re-treatment by root immersion at a young stage after determination of ploidy level at the end of the acclimatization period revealed a higher diploidization rate than at other stages after vernalization and adult plants with the same method and duration of colchicine re-treatment (Table 1.).

Different durations of treatment lead to different frequencies of doubled haploid plants. At the adult plant stage, 3 hours of root immersion was insufficient to induce diploidization. The extension of the immersion period to 6 hours produced 5% fertile plants, and the extension to overnight decreased the percentage of fertile plants. Cutting immersion with 3 hours of immersion resulted in a highest diploidization rate. And it decreased drastically when the immersion was extended from 3 hours to overnight.

Table 1. Effect of different colchicine re-treatments at different developmental stages of the plants.

Treatment	Developmental Stage	Duration of immersion	No. of plants treated	% of plants produced seeds
Root immersion	After aclimatization [a]	3 h	98	13.3
Root immersion	After vernalization [a]	3 h	135	6.7
Root immersion	Adult plants [a]	3 h	35	0.0
Root immersion	Adult plants [b]	6 h	40	5.0
Root immersion	Adult plants [c]	overnight	152	4.6
Cutting immersion	Adult plants[a]	3 h	96	47.9
Cutting immersion	Adult plants [c]	overnight	96	14.6

The number of plants generated from microspore culture and the number of fertile plants obtained from the 51 microspore donor plants is shown in Figure 1. Fertile plants shown in the figure were those, which produced seeds due to colchicine treatment at microspore stage and colchicine re-treatment at other stages. The genotype played an important role in

the number of doubled haploid plants obtained. In this experiment the rate of diploidization varied from 6.25% (microspore donor plants number 4.1.11) with only 1 fertile plant from 16 plants examined, to 93.33% (microspore donor plants number 4.12.13) with 42 fertile plants derived from 45 plants examined.

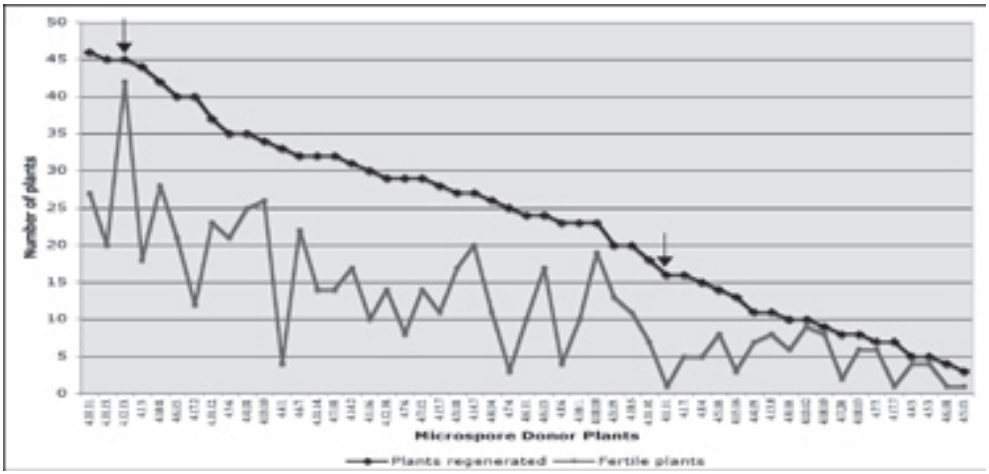

Figure 1. Variation of number of regenerated and fertile plants obtained among 52 lines of microspore donor plants. Frequency of fertile plants varied from 6,25% (line 4.1.11) to 93.33% (line 4.12.13) (arrows).

Discussion

Flow cytometry can be very helpful for an early determination of ploidy level. The flower chraracteristic of the plants observed in the green house showed a correlation to flow cytometry results. All plants tetraploid according to flow cytometry had bigger size of flowers and buds compared to diploid plants. About 79% of the plants, which were diploid according to flow cytometry were fertile. No haploid plant according to flow cytometry was later fertile. Weber et al. (2005) also found a high correlation ($R^2 = 0.97$) between diploidization rates as determined by flow cytometry and flower morphology.

The haploid plants can be treated with colchicine by root immersion after ploidy levels were analysed by flow cytometry, which will lead to a higher diploidization rate than at later developmental stages (Table 1). Foisset et al. (1997) applied colchicine treatment at the four-to-five-leaf stage of haploid plants before vernalization with three hours immersion of roots and achieved a higher diploidization rate compared to present results with 57% of fertile plants.

The cutting immersion method can be used as one alternative to increase the number of homozygous lines. By immersing cuttings in 0.3% of colchicine for 3 hours the diploidization

rate of haploid plants could be increased to 48% (Table 1). The high rate of diploidization obtained with this method might be because the absorption of the colchicine is more efficient through the wounded stem surface than by the root system. Another possible reason is because the size of the explants used is smaller and the colchicine can more easily reach all of the cells. An added advantage of this method is its easy applicability. The shoot with 2 or 3 leaves was simply cut compared to washing the root of the plant for root immersion. Also, more than one explants can be taken per plant for producing more seeds in one step.

The number of plants regenerated and fertile plants was very different among the donor plants (Fig. 1). Large variation caused by genotypes was reported earlier (Zamani et al., 2000; Zhou et al., 2002). However, the 51 donor plants used in the present study have a very similar genetic background and differ only in a small segment introgessed. Whether the presences of different 'RS239' segments in each microspore donor plant contribute to the different diploidization rates is still unclear. Different dates of treatment at a different age and length of donor plants under stress environment in controlled climate room could also be a possible reason.

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Using microsatellites to estimate introgressions of *Solanum galapagense* in *Solanum lycopersicum* breeding lines

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ABSTRACT: A breeding program about soluble solids content in commercial tomato was developed by crossing the commercial cultivar of *S. lycopersicum* 'UC204' with the accession of *S. galapagense* 'LA-530' that has higher content of soluble solids (Gragera, 2006). Here, we investigate the power of several microsatellite loci (He *et al*., 2003; Villalta *et al*., 2005) for measuring accessions differentiation and their suitability for detecting introgressions from *S. galapagense* in genotypes of an advanced generation of this breeding program. The genetic structure and allelic frequency at 48 microsatellite loci in the *S. galapagense* accesion was compared with the *S. lycopersicum* cultivar. High levels of polymorphism were detected for several of the markers used. This will allow to use these polymorphic markers in breeding lines of the breeding program and to study the possible relationship between the DNA introgressions and the agronomic and quality characters previously evaluated.

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Different approaches to analyse biodiversity in different species

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ABSTRACT: The present paper presents different activities carried out at the Department of Agrobiology and Agrochemistry at the University of Tuscia, Italy, which focuses on the genetic diversity of different cultivated crops. In particular, three different methodologies are discussed: a) the use of general molecular markers (AFLP, ISSR) on landraces of 'Romanesco' globe artichoke (*Cynara cardunculus* var. *sativa*); b) identification of selective sweeps analysing the variability of several SSR markers along chromosome 4A of durum wheat (*Triticum turgidum* var. *durum*); c) real time PCR assay to estimate copy number of retrotransposons in landraces of hulled wheat belonging to three ploidy levels: diploid einkorn (*Triticum monococcum*), tetraploid emmer (*T. dicoccum*) and hexaploid spelt (*T. spelta*).

Keywords: Molecular markers, genetic diversity, real time PCR, Selective Sweep.

Introduction

Nowadays DNA marker technology is widely used for the analysis of population genetics and for germplasm characterization (Karp et al. 1998; Spooner et al. 2005). In fact, an understanding of the extent and distribution of genetic variation is key when designing rational strategies for germplasm exploitation and preservation. Molecular analyses are also an important tool for developing effective management strategies for endangered and/or invasive species (Hedrick, 2001). Molecular markers are also essential for plant and animal breeding programmes all over the world to assist breeders in their work of selection and isolation of positive genes, such as the well known as MAS (Marker Assistant Selection).

Molecular markers differ in terms of information provided and in terms of costs for their development and for their utilization (Karp et al, 1997). Dominant markers, such as AFLP and ISSR, require no *a priori* DNA sequence information and genome organization. The more informative SSR markers, with their codominant and highly polymorphic nature, have increasingly become the marker of choice for genetic population analyses with the drawback that the *de novo* development of SSRs is a costly and time-consuming endeavour (Zane et al., 2002; Squirrell et al., 2003). The development of real time PCR has made possible the direct monitoring of the amplification reaction, with its progress now visible. This allows the detection of not only the presence/absence of a particular marker but also its quantitative information.

Clearly, the research is at different stages in the different species. In the globe artichoke (*Cynara cardunculus* var. *sativa*), due to its economic importance, research has focused mainly on its uses, primarily on the consumption of the edible immature flower heads eaten as a fresh, frozen or canned delicacy (Bianco, 2005). Studies have also been undertaken on the extraction of inulin or other secondary metabolites (such as cynarine, luteolin, chlorogenic acid and cynaroside) as well as for the production of alcoholic beverages (Bianco, 2007), where there are very few molecular markers developed as reviewed by Pagnotta et al. (2006). By comparison, in species as durum wheat (*Triticum turgidum* var. *durum*) several markers have been developed thanks also to its similarity with other *Triticeae*, and molecular genetic maps have been obtained through inter- (Blanco et al., 1998) or intra-specific crosses (Nachit et al., 2001). In those species, SSR markers available are numerous and their chromosome localization is well known. It is, therefore, possible to: select several loci along a chromosome and to detect their variability distribution; determine the amount of diversity present in diverse populations and undertake selective sweeps. The principle that selective sweeps are based on is that if a locus is under selection pressure its variability is reduced due to the fixation of the positive allele.

This paper reports on different approaches used at the University of Tuscia, Italy, to analyse biodiversity. In particular, three different methodologies are discussed: **a**) general molecular markers (AFLP, ISSR) used to assess diversity within the landrace of the 'Romanesco' globe artichoke (*Cynara cardunculus* var. *sativa*); **b**) identification of selective sweeps analysing the variability of several SSR markers along chromosome 4A of durum wheat (*Triticum turgidum* var. *durum*); **c**) real-time PCR approach to estimate copy number of retrotransposons in landraces of hulled wheat belonging to three ploidy levels diploid einkorn (*Triticum monococcum*), tetraploid emmer (*T. dicoccum*) and hexaploid spelt (*T. spelta*).

Material and methods

Plant material

a) general markers - Artichoke landraces of the "Romanesco" type were collected from 3 agricultural farms, 30 plants per farm.

b) selective sweeps - 234 single plants of *T. durum* from three populations for each of the three Ethiopian regions: Tigray, Gonder and Shewa.

c) real time PCR - five accessions of each of three hulled wheats species were analysed: einkorn (*T. monococcum*), emmer (*T. turgidum*) and spelt (*T. spelta*).

Markers used

a) general markers - AFLP, with *Mse*I and *Pst*I enzymes, zero plus two amplifications and total of 3 primer combinations: MstAC-PstCT, MstTT-PstCA, and MstGC-PstAC; ISSR primers 841 and 856 (British Columbia University). The data of both markers typology were scored as a matrix of 0 and 1 (0 = absence; 1= presence of the band). The statistical analyses

to assess variation (within and between populations), expected heterozygosity and genetic distances between the different populations were performed by GDA software.

b) selective sweeps - DNA was amplified with 19 SSR primers (WMC516, BARC106, GPW2283, WMS610, GPW1010, GPW2140, GPW2138, GPW2279, WMS269, BARC343, WMC468, GPW2244, CFD257, WMS637, GPW2228, CFD88, GPW356, BARC78, BARC153) located along chromosome 4A. The amplifications, using annealing temperatures specific to each primer, were run on the ABI 3130xl sequencer. Statistical analyses were performed utilizing GDA software to compute the amount of variation detected at each locus in each population.

c) real time PCR - WIS 2-1A (5'-AAGAAAGGTTGT ATGTGATA-3', 5'-GTCAACAACAT ATACTCATC-3') primers were used. Reactions were made up to a final volume of 10µl, containing 10ng template DNA, 0.6µM of each primer, 100µM dNTP, 5mM $MgCl_2$ and 0.5U *Taq* DNA polymerase. The amplification regime consisted of an initial denaturation step of 95°C/2min, followed by 45 cycles of 94°C/1min, 52°C/2min and 72°C/2min, ending with an extension step of 72°C/10min.

Results and discussion

a) general markers

Levels of polymorphism, expected heterozygosity and variance (between and within population) were obtained using different markers and combinations: (i) AFLPs and ISSR; (ii) only AFLP markers; or, (iii) only ISSR markers. In all cases a great amount of variation within population (from 73% to 85%) was found, the differences between plants from different farms account for only 27 to 15% of the total variation. The percent of polymorphic loci is on average 77%. The results obtained with the different markers typologies was slightly different and might be explained by different genome regions detected by the different markers. The expected heterozygosity (H_e) ranged from 0 to 0.5 and provides an indication of whether a polymorphic locus has an equal presence of both alleles (H_e close to 0.5) or whether one an allele is common and the other rare (H_e close to 0). The H_e found indicated that, in general, the high polymorphism revealed was determined by loci with both alleles well represented. The results revealed the existence of great variation within farmers' collections of landraces.

b) selective sweeps

A hitchhiking map for three populations of the Shewa region of Ethiopia was drawn up (Fig. 1). The variability along chromosome 4 revealed the presence of selective sweeps at about 0, 45, 70, 90-95 and 195 cM. Two of main selective sweeps correspond to QTL regions for drought tolerance. The selective sweep detected at the 70 cM was present in all the populations while the selective sweep detected at about 90 cM, is clearly visible in the populations from Shewa, and to a lesser extent, from Tigray populations, which are the drier regions. There were also points of the hitchhiking maps which were consistent among the populations within a region, while others were not, possibly due to selection for other types of stresses.

Table 1. Polymorphism (P) with 0.95, expected Hetreozyosity (He) and variance component within population (s-G) and between populations (s-P) in global artichoke from 3 farmers.

242 loci (191 AFLP and 51 ISSR)					
Population	N	s-P	s-I	P (0.95)	He
Latina1	27		58.85	0.63	0.22
Latina2	28		79.03	0.87	0.33
Latina3	26		72.35	0.81	0.30
Mean	27.0	14.79	68.57	0.77	0.28

191 loci AFLP					
Population	N	s-P	s-I	P (0.95)	He
Latina1	30		37.54	0.57	0.20
Latina2	30		62.82	0.87	0.33
Latina3	29		59.13	0.82	0.31
Mean	29.6	9.08	53.15	0.67	0.26

51 loci ISSR					
Population	n	s-P	s-I	P (0.95)	He
Latina1	16		16.31	0.88	0.32
Latina2	21		16.21	0.86	0.32
Latina3	15		13.22	0.76	0.26
Mean	17.2	5.70	15.42	0.80	0.29

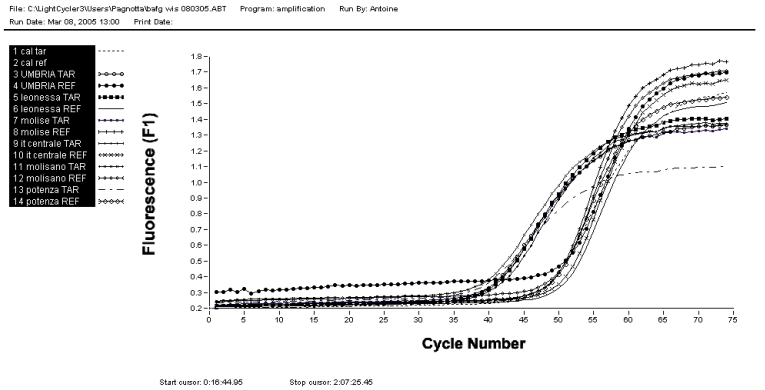

Figure 1. Generation of amplification curves obtained by using WIS2-1A primers (for target gene) and Ay-Vt oligonucleotides primers (for reference gene).

c) real time PCR

Significant differences were observed in the WIS 2-1A copy number both between and within species. WIS 2-1A retrotransposon in einkorn varied from 2-10 per ng of template, from 11-28 in emmer and from 19-27 in spelt. As expected, einkorn, which has the lowest ploidy level, had the lowest WIS 2-1A copy number. Emmer and spelt wheats had similar copy number, in line with the results reported by Moore et al. (1991). Real time PCR represents a useful tool for quantifying intra- and inter-specific retrotransposon copy numbers to assess variation and evolutionary prospectives.

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Genetic variability and combining abilities of popping maize (*Zea mays* L. *everta*) inbred lines

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ABSTRACT: The classification of germplasm of popping maize into heterotic groups, as well as, evaluation of genetic diversity among and within existing heterotic groups are of importance for a successful popping maize breeding programme (Ziegler, 2001). Combining abilities of six popping maize inbred lines were tested in this study by the method of a diallel analysis. The mode of inheritance of grain yield, kernel number in 10 g and popping volume was determined. The rank correlation was estimated for the two most important traits, i.e. for grain yield and popping volume, in order to reveal the concurrence intensity among obtained ranks of parental inbreds for these traits in two tested location. The relatedness and classification of observed popping maize inbred lines were evaluated by the application of protein and molecular genetic markers (RAPD method) (Melchinger et al., 2002). The objective of this study was to determine whether there was and to what extend there was a congruence of data obtained by the diallel analysis and results obtained by the analysis of genome polymorphism of observed popping maize inbred lines and whether combining abilities and heterosis could be anticipated on the basis of polymorphism of biochemical and molecular markers.

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Differentiating a collection of *Capsicum annuum* L. cultivars by microsatellite molecular markers

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ABSTRACT: Microsatellite molecular markers (SSRs) have been used with the aim of differentiating a large number of pepper cultivars (*Capsicum annuum* L.), which represent practically the whole variability to be found on current markets. Twenty-eight polymorphic markers were developed for a subgroup of 25 cultivars. In this first study, 80 different alleles were identified, with an average PIC value (*Polymorphism Information Content*) of 0.3; the genotypic information revealed low values of genetic distance (D_A) between the different genotypes, indicating that there is low genetic diversity between the cultivars. The current work reports on the results obtained from the analysis of applying 14 markers to a total of 222 cultivars. In order to do this, the *PCR multiplex* method has been set up using primers labelled with four different fluorophores for subsequent study. The *PCR multiplex* analysis has been carried out using the Applied Biosystems 3730 sequencer and the software ABI PRISM GeneMapper v 3.0 (Applied Biosystems), and the allele coding with the SoftGenetics GeneMarker v1.50 program. (SoftGenetics LLC). Once the genotype matrix had been established, different diversity parameters were calculated for each of the microsatellite markers analysed: Genetic Diversity or Allelic Diversity, Heterozygosity, PIC and Genotypic Diversity. To gain further insight into the genetic proximity existing between the varieties, the genetic distance matrices have been calculated (D_A) and a dendrogram has been constructed using the *Neighbor-Joining* algorithm, carrying out a *bootstrap* analysis for 1000 replicates; all this via the program *PowerMarker V3.0*. An allelic pattern has been obtained that is clearly identifiable for all the microsatellite loci. The number of alleles per locus varied between 2 and 12, together obtaining a total of 82 different alleles. Eleven loci displayed alleles that were exclusive to the variety. The combination of the 14 markers for each one of the plants gave rise to a total of 277 different genotypic profiles. The average value for distance found between the varieties was 0.24, with a range of values between 0.10 and 0.64. Few associations were established between cultivars with a level of significance, which demonstrates the adequate approach with which many of the varieties have been defined using these genetic markers.

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Morphological and genetic diversity of chili (*Capsicum* spp.) from Tabasco, México

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ABSTRACT: The chili (*Capsicum* spp.) has been studied only scarcely in Mexico, especially in the case of the Tabasco State. In this work the *in situ* morphological and genetic diversity of forty Tabasco´s native and introduced populations of *Capsicum* are described. The samples were classified into twelve different racial types, of which nine belong to *C. annuum*, two to *C. frutescens* and another one to *C. chinense*. *C. annuum* and *C. frutescens* are present in all the explored sites and *C. chinense* was just observed in Nacajuca. The populations were classified: twenty as *C. annuum*, nineteen as *C. frutescens* and one *C. chinense*. The wild forms of *C. annuum* var. *glabriusculum* were found scattered in all the explored sites. In Cardenas the germplasm has originated by *Capsicum* interspecific crosses and by *C. annuum* intraspecific and inter-racial crosses; whereas in Huimaguillo there was an intraspecific and intravarietal population. The eleven qualitative characteristics measured showed from two to five phenotypical classes. The germplasm was grouped in three main conglomerates on the basis of morphological data: one included the 'amashito', 'piquin', 'ojo de cangrejo' and 'desconocido' morphotypes; another one the 'pico paloma', 'garbanzo' and 'muela' morphotypes and the third group included the commercial morphotypes ('serrano', 'morron', 'blanco', 'dulce' and 'habanero'). The AFLP genetic data included 1222 analyzed bands, which did not clearly separate to germplasm on the basis of morphotype, especially in endemic ones of Tabasco, which can be due to the common local agricultural practice to mix morphotypes in parcels by which it is probable that natural random recombination and genetic flow happens. This practice has resulted in the loss of well defined genetic groups at interspecific level. The grouping at species level also showed overlapping, especially between *C. annuum* and *C. frutescens*; on the other hand the wild morphotypes of *C. annuum* var. *glabriusculum* were genetically different from *C. frutescens*. The morphoagronomic characters grouped the germplasm into three main conglomerates on the basis of morphotypes separating clearly Tabasco endemic germplasm from the commercial introductions. The grouping did not show a relation with geographic originof the germplasm.

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Structure and genetic diversity in a core collection established from the main Spanish bean genebank, revealed by molecular markers

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ABSTRACT: Common bean (*Phaseolus vulgaris* L.) is traditionally grown in many Spanish regions, the Iberian Peninsula being considered as a secondary center of diversity for this species. Two gene pools, Mesoamerican and Andean, have been described in wild and cultivated common beans. Variation in the phaseolin, the major seed protein, has contributed to distinguish these two gene pools. The main Spanish bean collection is located at the CRF (Plant Genetic Resources Center, INIA, Madrid), where duplications of most accessions included in the network of the Spanish seed genebanks are maintained. Considering seed traits and geographical criteria, a core collection of 200 accessions was established in order to represent the genetic diversity of the CRF collection. The objective of the present study was to investigate the genetic structure and genetic diversity included in this core collection using the phaseolin protein and one molecular marker per linkage group. Seven well-know common bean cultivars (Michelite, TU, AB136, G2333, Sanilac, classified in Mesoamerican gene pool; MDRK and Tendergreen, classified in Andean gene pool) and two wild accessions (G13004 and G23415) were also included in this study as reference materials. The results revealed, in all the investigated levels, a wide genetic diversity in this core collection. Phaseolin determination showed the presence of the five variants, C (88 accessions), T (59 accessions), S (43 accessions), A (8 accessions) and H (3 accessions). Eighteen accessions presented a mixture of phaseolin variants suggesting that these accessions are constituted by at least two lines. The genetic structure was investigated using eleven molecular markers: two SCAR markers (SAP6, SW12), and nine microsatellite markers (BMd17, BM184, BM151, BMd45, BM210, BM170, BM141, BM175 and BM172). A total of 73 polymorphic bands were scored. An average of 6 bands per marker and PIC values between 0.47 and 0.80, were found with this set of molecular markers in the analyzed material. Principal component analysis from molecular marker data was performed and variation along the two principal components accounted for 25.65% and 11.22% of the total variation, respectively. The graph obtained from the two principal components grouped the accessions in two main groups corresponding to Mesoamerican gene pool and Andean gene pool. However, twenty accessions showed an intermediate position between the two groups. Cluster analysis based on Jaccard distance also showed these two main groups. The two main groups established in the cluster analysis (Andean and Mesoamerican origin) diverged significantly in the relative frequency of the different variants of phaseolin and the eleven DNA markers analyzed. Extensive knowledge of this core collection will contribute to improve the use and the conservation of local plant genetic resources.

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Molecular characterization of *Castanea* and *Corylus* accessions for conservation in gene banks

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ABSTRACT: The conservation of biodiversity, hence genetic resources, represents a priority worldwide. Romania has a rich gene pool of tree species, but lately, a decrease of the valuable accessions number was seen. Genetic erosion is more intense in some species and it creates the possibility of losing some valuable genes. This is the reason why the molecular evaluation followed by vegetative regeneration of the gene pool of *Castanea* and *Corylus* genus from S.C.D.P. Vâlcea constitutes an objective necessity in Romania. In this context, the scientific community became more interested in the rescue, collection and exploitation of endangered germplasm. The *Castanea* and *Corylus* accessions held at S.C.D.P. Valcea come from local populations of natural origin, and the genetic relations between them are unknown. In this context, in order to preserve the most valuable specimens, a molecular study has been carried out in 10 *Castanea* and 24 *Corylus* accessions with the purpose of determining the genetic relatedness of the above. Traditional methods of accession identification rely on morphological characteristics, which can be influenced by environmental conditions, leading to erroneous characterization, so the choice for DNA markers providing plenty of polymorphism has enabled the rapid identification of phenotypically extremely similar accessions. Furthermore, molecular identification techniques can be used at any stage of plant development and they are not affected by environmental factors. Among molecular markers used for identification of the chestnut and hazelnut accessions, RAPD markers are the most common. We used thirteen decamer primers, most of which yielded scorable amplification patterns. These primers generated polymorphic bands among the genotypes studied. Some of the primers produced no amplification or unreadable gel smears. Dendrograms were built using neighbour joining analysis of Jaccard's coefficient of similarity. The accessions clustered into multiple groups and the values of genetic distances between analyzed data showed that there were some genetic differences. In order to certify these differences, further studies using ISSR or SSR markers will take place. RAPD is therefore a reliable technique for distinguishing among *Castanea* and *Corylus* accessions cultivated at S.C.D.P. Vâlcea, assessing the genetic similarity among different genotypes useful in fruit breeding selection programs and also for understanding the genetic diversity of germplasm collections. The use of RAPD technique for molecular characterization of valuable genotypes represents a new approach in Romania. The resultes presented in this paper have been obtained following the experiments performed at the Biotechnology Department from the

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Towards the construction of an integrated linkage map of *Cynara cardunculus*

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ABSTRACT: *Cynara cardunculus* (*Compositae*, 2n=2x=34) is an allogamous species which includes three taxa: globe artichoke (var. *scolymus*), cultivated cardoon (var. *altilis*) and their progenitor wild cardoon (var. *sylvestris*). Globe artichoke represents an important component of the Mediterranean agricultural economy with an annual production of about 750Mt (more than 60% of global production) from over 80kha of cultivated land and Italy is the leading world producer (FAO data 2007: http://faostat.fao.org/). Cultivated cardoon is of regional importance in Italy, Spain and southern France. Globe artichoke commercial production is primarily based on the cultivation of perennial, vegetatively propagated clones, which guarantee high yields of marketable product. In recent years a considerable number of new seed propagated cultivars has been developed, and these are gaining in popularity. Despite their wide cultivation and consumption, artichoke and cardoon breeding activities have been, to date, rather limited. To move towards a modern breeding it is compulsory to generate genetic maps for identifying the genetic bases of key commercial and agronomic traits. Due to the high level of heterozigosity of the species, we applied the two-way pseudo-testcross strategy. We produced three F_1 progenies obtained by crossing an artichoke clone of 'Romanesco C3', as female parent, with three pollen sources: a genotype of artichoke 'Spinoso di Palermo' (progeny A); one of cultivated cardoon (progeny B) and one of wild cardoon (progeny C). Recently we generated the first genetic maps of globe artichoke (Lanteri et al. 2006) by genotyping progeny A, applying AFLP, M-AFLP, retrotransposon based SSAP (Acquadro et al. 2006) and SSR markers (Acquadro et al. 2005). Here we report on the development of a new consensus map based on progeny B, by means of AFLP and microsatellite markers. Another map, based on progeny C, is currently under construction and markers suitable for mapping in the three F1 progenies will be used as point of reference for map integration and comparative genetics analysis. Since *C. cardunculus* is easily vegetatively propagated, the mapping populations are immortalised, and thus will be grown in contrasting environments to investigate genotype x environment interactions, thus making the identification of quantitative trait loci more efficient and reliable.

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Differences in prolamin alleles between the North and South regions and convars. *durum* and *turgidum* in durum wheat landraces from Spain

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ABSTRACT: The allelic variation at seven prolamin loci involved in durum wheat quality was studied in a set of landraces from different Spanish provinces where durum wheat was traditionally cultivated. Protein patterns for the North and South of Spain and for convars. *durum* and *turgidum* were found for the loci with significant influence on quality. Wider variability was observed in the North than in the South, and in convar. *turgidum* than in convar. *durum*, mainly for the loci on chromosome B. Also, convar. *turgidum* showed to be a valuable source for new alleles coding for B-LMW glutenin subunits. Wheats from the South were, however, more diverse for prolamins encoded at *Glu-A3*.

Keywords: durum wheat, geographic origin, landraces, prolamins.

Introduction

The diversity of old varieties preserved in Gene Banks is an important source of genetic variability, and consequently, of valuable traits for wheat breeding. In durum wheat, quality differences between cultivars are strongly dependent upon their allelic composition for endosperm storage proteins, gliadins and glutenins (Carrillo et al., 1991; Turchetta et al., 1995; Porceddu et al., 1998). Genetic studies have revealed that these proteins (prolamins), encoded at several highly polymorphic loci, are useful molecular markers to quantify genetic diversity in wheat collections (Aguiriano et al., 2006; Moragues et al., 2006). In this paper, allelic variation at seven prolamin loci was studied in a set of durum wheat landraces from Spain. Allelic frequencies were used to analyse the presence of protein patterns for the North and South of Spain and for convars. *durum* and *turgidum*.

Material and methods

The sample included 52 durum wheat landraces from Spain maintained at the National Plant Genetic Resources Centre (CRF-INIA). Glutenin alleles were designated according to Payne and Lawrence (1983) for the HMW, and to Nieto-Taladriz et al. (1997) for the LMW subunits. Electrophoresis of prolamins was described in Carrillo et al. (1991).

Results and discussion

Analysis of the North and South geographical areas

The accessions were classified in two groups according to their geographical origin: the North (latitude > 41º 5´ 16´´ N) and the South of Spain (latitude ≤41º 5´16´´ N). These two wide geographical areas have significant environmental differences mainly in solar radiation and temperature.

Among HMW glutenin alleles, *Glu-A1c* was more frequent in the South, whereas *a* and *b* were more common in the North. At *Glu-B1*, some alleles were present in one set but not in the other, the North accessions having greater diversity. For LMW glutenins, the *Glu-A3* locus showed greater variability in the wheats from the South and *Glu-B3* in those from the North. The alleles *Glu-A3a* and *e* were the most frequent in both groups. The most common *Glu-B3* alleles were *a* in the South (45.8%) and *new-1* in the North. At *Glu-B2*, allele *b* was more frequent than *a* in the South, whereas they appeared almost equally distributed in the North accessions.

In respect to gliadin alleles, *Gli-A1b* was the most common in both zones but *Gli-A1e* and *Gli-B1c* were more typical in the South and *Gli-A1c* and *Gli-B1new-1* in the North. The latter group displayed larger variability at *Gli-B1*.

Differences between convars. durum and turgidum

These differences often coincided with those between North and South areas. In fact, 77% of varieties from the North belonged to convar. *turgidum*, while 83% from the South belonged to convar. *durum*. This result is in agreement with the fact that convar. *durum*, more resistant to dry, was traditionally grown in the South and convar. *turgidum*, more resistant to cold, in the North.

The most important allelic differences were at *Glu-A1*, *Glu-B1*, *Gli-B1* and *Glu-B3*. Alleles *Glu-A1c* and *Glu-B1e* only present in convar. *durum* have shown negative effects on quality, while *Glu-A1b* and *a*, very frequent in convar. *turgidum* have shown positive effects (Turchetta et al., 1995). The genotype *Glu-B3new-1* (LMW –2*) - *Gli-B1new-1* (γ-44) was very common in convar. *turgidum* and the North, and *Glu-B3a* (LMW-2)- *Gli-B1c* (γ-45), associated with high quality, in convar. *durum* and the South. This result is consistent with the better quality, in general, of convar. *durum* when compared to *turgidum* taking into account that *Glu-B3* alleles show the most significant effect on gluten quality (Carrillo et al., 1991; Porceddu et al., 1998). The most frequent alleles in other collections (*Glu-A1c*, *Glu-B1d* and *e*, *Glu-B3a* and *Gli-B1c*) were typical of convar. *durum* more habitually analysed (Brites et al., 1996; Moragues et al., 2006; Nieto-Taladriz et al., 1997).

In general, wider variability was observed in convar. *turgidum* and in the North zone, mainly for the loci on chromosome B. Also, convar. *turgidum* showed to be an important source for new alleles coding for LMW glutenin subunits. Wheats from the South were, however, more diverse for *Glu-A3*.

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Application and development of DNA markers for characterising Latvian plant genetic resources and incorporation into breeding programs

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ABSTRACT: The Genetic Resource Centre has been established in Latvia to coordinate the conservation and description of Latvian genetic resources, funded by the Ministry of Agriculture. The Genetic Resource Centre incorporates the Latvian Gene Bank of cultivated plants, the central data base and a DNA marker laboratory. The genetic resource conservation and utilisation program includes cultivated crops, fruit trees and berries, aromatic plants, as well as forest tree species and commercial fish and livestock species. The Genetic Resource Centre is the central genotyping facility in Latvia for genetic resource fingerprinting and marker integration into breeding programs. We are in the process of establishing protocols and genetically fingerprinting the species included in the genetic resources program, and we have fingerprint data for the majority of crop varieties in our collection. We have predominantly used Simple Sequence Repeat (SSR) markers for fingerprinting, due to their ease of use, high information content and availability for major species. This initial phase of genetic fingerprinting has allowed us to rationalise our collections, especially for clonal species. We have also utilised the SSR fingerprinting data to examine changes in genetic diversity between locally maintained and repatriated varieties. Further to the genetic fingerprinting program, we have also incorporated DNA markers into various breeding programs. SSR marker data has been used to assess the success of inbreeding and homogenisation in re-established Latvian melon (*Cucumis melo* L.) lines. SSR markers are being used in the spruce (*Picea abies* (L.) Karst.) and pine (*Pinus sylvestris* L.) breeding programs to characterise seed orchard clones and to assess the level of genetic diversity in the breeding material. We are using Single Nucleotide Polymorphisms (SNPs) to develop species specific markers between black and gray alder (*Alnus glutinosa* and *A. incana*). Naturally occurring hybrids are difficult to identify, and the use of DNA markers to identify hybrids will allow assessment of these individuals and potential inclusion into breeding programs. We have also used DNA markers to assist in the very important task in sweet cherry (*Prunus avium* L.) breeding of overcoming of self-incompatibility, ensured by the multi-allelic *S*-gene. Forty-four varieties of the Latvia State Institute of Fruit-Growing collection were screened for the self-incompatibility (*S*) S_1 to S_6 alleles, using PCR based typing. Compared to the *S*-allele frequencies published for over 250 sweet cherry cultivars from Western and Southern Europe, the Latvian plant material appeared to have a high frequency of the S_6 allele, and a relatively high frequency of the S_5. Our collection contains a high proportion of accessions adapted to north and central European growing conditions, not typical for the majority of the documented sweet cherries, which explains differences in certain *S*-allele occurrence.

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Genetic diversity of *Capsicum baccatum* revealed by AFLP

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ABSTRACT: *Capsicum baccatum* is one of the five cultivated pepper species. In South America *C. baccatum* is known to be the most commonly grown pepper species especially in Peru, Bolivia, Ecuador and Chile. Along with *C. praetermissum C. baccatum* var. *baccatum* (wild varieties) and *C. baccatum* var. *pendulum* (cultivated varieties) represent *baccatum genetic complex* (Pickersgil, 1997; Bosland and Votava, 2000). The work was aimed to investigate genetic variation within *C. baccatum* by using AFLP analysis. Twentysix *C. baccatum* accessions from CGN (the Netherlands) and VNIISSOK (Russia) genebank collections and four accessions morphologically similar to *C. baccatum* attributed as *C. flexuosum, C. microcarpum, C. conicum*, have been taken into the analysis. *C. praetermissum C. chacoense, C. tovarii, C. annuum* were chosen as an outgroup species. One accession (CGN21513) has been represented by 11 individual plants in order to assess intra-accession variability. In total 435 polymorphic bands have been identified by using three AFLP primer/enzyme combinations. AFLP data revealed genetic dissimilarity (GD) values ranged from 0.00 (among CGN21513 individual plants) to 0.45 (between a *C. baccatum* and *C. tovarii* accessions). GD values among the analyzed *C. baccatum* accessions (0.00-0.14) were less then in *C. frutescens* (0.01-0.27) or *C. chinense* (0.01-0.29) accessions of CGN collection (Ryzhova, unpublished date). The cladistic analysis and PCA resulted in grouping of the majority of *C. baccatum* accessions in one well supported clade (100% bootstrap values) with several subclades discrimination. CGN21515 and CGN22786 were the most distant from other *C. baccatum*. GD values between some accessions (e.g. CGN17241-CGN21528, CGN19202-CGN22141) were extremely low and comparable with intra-accession diversity values (0.00-0.05). Those accessions were supposed to be duplicates. Based on GD values and tree topology accessions attributed as *C. flexuosum, C. microcarpum, C. conicum* are strongly fall into *C. baccatum* clade, without forming separate branches. So the revision of the taxonomic status of these accessions has been recommended. Molecular characterization of the *C. baccatum* genetic diversity levels between accessions may help to identify potential new sources of genetic diversity useful in the breeding and pepper cultivars improvement.

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Genetic diversity among the accessions of wild species, *Carthamus oxyacantha* and its relationship with safflower using ISSR markers

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ABSTRACT: Wild species *Carthamus oxyacantha* Bieb. is highly crossable with cultivated safflower, C. tinctorius L. (Ashri and Knowles, 1960) and therefore could be exploited to broaden safflower gene pool in breeding programs for genetic resistance to biotic and abiotic stress conditions. In this study genetic diversity among nineteen accessions of *C. oxyacantha* and their relationship with five genotypes of cultivated safflower were studied using 12 polymorphic inter-simple sequence repeats (ISSR) primers. A total of 182 bands were amplified and 169 bands (92.86%) were polymorphic. The results showed that a range of 9 to 21 polymorphic bands were amplified by each primer, with an average of 14 polymorphic bands per each. Some primers produced a band common to the wild accessions that could be assigned as wild safflower specific band. Cluster analysis based on ISSR data separated the wild accessions from the cultivated genotypes and divided the wild accessions into three distinct groups. It seems that the ISSR is an effective and promising marker system for detecting genetic diversity in cultivated genotypes (Yang et al., 2007) and wild species of safflower and may give some useful information on their genetic relationships. The accessions of C. oxyacantha which had more genetic similarity with safflower could be used for interspecific crosses to exploit their desirable genes in breeding programs of safflower.

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Evaluation of specified omega-gliadins DNA markers (STS marker) for *D*-genome of wheat and examination of old and modern Iranian bread wheat

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ABSTRACT: This paper is based on the most extensive study on gliadins in Iran. It covers all the cultivars so far cultivated in Iran. This paper mainly is focused to study the absences or present and size of ω-gliadin gene in 95 old and modern bread wheat varieties grown in Iran. Published DNA sequences from clones ω-F20b and ω-G3 gene (Hassani, *et al*., 2007) were used to design oligonucleotide primers. These primers were based on nucleotide differences in the conserved regions of the two ω-gliadin gene sequences. Using this approach, a large number of primers including several degenerate primers were evaluated. Among these, combination of forward primers G3f5, G3f5, F20r4 and reverse primers WF20bR, G3r3 and wF20Br successfully amplified the target DNA. In next step, these primers were tested for specificity to *D*-genome of wheat. In this stage, to test these primers we used 25 cultivar of durum wheat (AABB), 25 association of *T.tauschii* (DD), and all of Iranian bread wheats (AABBDD). The results showed an extensive polymorphism among ω-gliadin genes, the studied cultivars had very difference in size and absences/present of bands. The result also showed that these primers were specific for *D*-genome of wheat because these primers don't identify any region in genome B and A (in durum wheats).

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Microsatellite fingerprinting of an indigenous grapevine collection

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ABSTRACT: We report our study on genetic diversity and relationships in an endangered grape germplasm collection using polymorphism at 30 microsatellite loci. A germplasm safeguard programme was set up of grapevine varieties considered as indigenous to North-Eastern Italy. In Italy indeed, as in other Mediterranean countries, grapevine (*Vitis vinifera* L.) used to be characterized by the diffuse presence of indigenous cultivars, originated through centuries of natural and human selection (Martin et al. 2003, Snoussi et al. 2004). To ensure that allele size of SSRs was properly scored, the SSR marker system relied on a *Vitis vinifera* microsatellite genotype database. For this purpose we utilized the GENRES#081 (European Network for Grapevine Genetic Resources Conservation and Characterization; This et al. 2004). The genetic profiles of 19 grapevine indigenous varieties, together with seven international ones taken as reference, were searched for possible parentage relationships and several cases of suspected synonyms have been investigated. In the indigenous varieties the number of alleles per locus ranged from two at UDV109, VVIQ61, VVIU37 and VVIV16 loci, to ten at VVMD7 locus, with an average of 4.6 alleles per locus. The native germplasm displayed a greater diversity with a higher proportion of unique alleles compared to international cultivars. This is probably due to a past contribution of wild grapevine to the cultivated genepool. Furthermore all native varieties clustered in the same branch of the UPGMA phenogram thus confirming their local origin. In this research the majority of local cultivars were demonstrated to constitute an independent source of genetic variation, and therefore a possible valuable resource of interesting genetic traits for breeders.

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Molecular characterization of an Argentinean peach breeding program progeny

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ABSTRACT: Peach [*Prunus persica* (L.) Batsch] is a self-fertile and naturally self-pollinating fruit species with a very low genetic variability. Hybridization in *Prunus* breeding programs is commonly performed by hand pollination of emasculated flowers. This practice tends to avoid self-pollination and make flowers unattractive to pollinator insects, thus decreasing accidental pollination. However, bagging emasculated flowers is considered a safer practice to avoid unwanted pollination. Nevertheless, an accuracy hybrid status determination is a critical task in *Prunus* breeding programs. Microsatellites or Simple Sequence Repeats (SSR) are widely used in paternity analyses in most plant species due to their high polymorphism, codominance and reproducibility. In peach, several SSR markers were reported so far (Cipriani et al., 1999; Dirlewanger et al., 2002; Mnejja et al., 2005). In this work, we have analyzed the hybrid status on 39 cross progeny of INTA San Pedro peach breeding program by SSR markers. Emasculated flowers were hand pollinated and bagged. Peach cultivars Dixiland and Flavorcrest were used as parental on reciprocal crosses. Each parental was self-pollinated as control. A total of 27 peach SSR markers reported on bibliography were successfully amplified in parental plants. PCR products were separated onto 6% denaturating polyacrilamide gels. Three polymorphic markers were obtained (BPPCT007, BPPCT015 and BPPCT033) and used to analyze the cross progeny and self-fertilized control plants. SSR analysis indicated that 95% (37:39) of the progeny were hybrids and 5% (2:39) were self-fertilized. No accidental pollination was observed. SSR molecular markers applied to hybrids identification has been a useful tool in our breeding program.

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Genetic relationships and likely origins of the grapevine varieties cultivated in Castilla y León (Spain)

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ABSTRACT: Castilla y León is the largest region of Spain, consisting of an elevated plateau limited by mountain ranges, and crossed by the Duero river and its tributaries. Due to its large surface, it is able to contain quite different environments. Besides, there is an ancient tradition of viticultural practices in the region, likely previous to Roman colonization. Nowadays viticulture is the most important economic agricultural activity of the area, which hosts some of the best-known wines and wineries of the country, essentially placed in the "Ribera del Duero" AOC. These environmental, cultural and economic factors together make this region potentially suitable to contain a high varietal diversity of grapevines. Nevertheless, our cultivar variability has alarmingly decreased during the last century, because of the incidence of diseases like phylloxera, the abandonment of rural areas, and especially due to the progressive substitution of the local varieties by more productive and known ones. Forty samples of grapevines from the Germplasm Bank of Castilla y León and 381 from fields of the viticole areas of the region were collected. DNA was extracted and analysed with six standard microsatellites in order to confirm their identity and to discard redundant genotypes. The genetic profiles of the analysed samples were checked against the available databases. The non-redundant genotypes were subsequently analysed with an additional set of 16 nuclear microsatellites to check genetic relationships between the authoctonous accesions. We also added 9 microsatellite loci from the chloroplast genome to our study, in order to make an attempt of allocation of the unknown accesions to geographical areas related to established haplotypes (Arroyo-García et al., 2006). Genetic distances between the non-redundant genotypes were calculated by Dc measure. A dendrogram based in those distances and representing the genetic similarities between the profiles was constructed using the Neighbor-Joining method and used to check genetic relationships between the analysed accesions. The closest relationships were tested for possible parentage using IDENTITY. Likelihood ratios for the proposed kinship relationships were calculated as previously described (Bowers and Meredith, 1997), using the relative allele frequencies at the 22 nuclear SSR loci. We discriminated 131 different genotypes out of 421 samples, 47 of them resulting unknown so far after comparison to available databases. Those genotypes belong to minor accesions, and presented in most cases rare alleles, which encouraged us to consider them as likely autochthonous varieties, deserving conservation. The discriminated genotypes showed a high value of gene diversity, confirming our presumption of a considerable persisting

variability in Castilla y León. The analysis of genetic relationships proposed some interesting parentages, confirmed by the results of likelihood ratios, and consistent with historical information.

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Conservation and documentation of genetic resources of saffron and *Crocus* spp.

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ABSTRACT: There are approximately 80 species of genus *Crocus* L. at global level, mainly distributed in the Mediterranean region. The species *Crocus sativus* L. (saffron) has been used since the Greek-Minoam civilization for different purposes such as medicinal, aromatic, food, textile dyeing, etc. (Abdullaev and Espinosa, 2004; Turhar *et al*., 2007) and it is considered one of the oldest organic crop and traditional plant, as well as a significant part of the Eurasian cultural inheritance. Saffron stigmas - the edible part of the plant - are commonly considered the most expensive spice in the world. However, land surface dedicated to saffron has decreased rapidly in many European countries, and is extinct in others, with the corresponding genetic erosion and lost of genetic diversity. In addition, saffron is a sterile species exclusively propagated by vegetative corms, and a very limited genetic variation is suspected for this crop. Creation and maintenance of a collection of landraces, ecotypes or simply accessions of *C. sativus* is an urgent task to ensure the future of saffron crop (CrocusBank, 2005). Also, it is very important to create a collection of saffron allies for research purposes and as exploitable sources of resistance and other agronomical interesting traits to be transferred to saffron through appropriate breeding programmes and technological tools (CrocusBank, 2005). In this work we present the world collection of saffron and wild relatives (*Crocus* spp.), which is being created in the Bank of Plant Germplasm of Cuenca (BGV-CU, Spain). At present, 119 saffron accessions from 14 source countries and 44 wild *Crocus* species originating from 20 countries are being preserved and managed in the BGV-CU. Some passport data, statistic of the collection and the effective documentation system (relational database) for the correct management of the collection, accessible through internet, are commented.

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Assessment of genetic relationship among Iranian and some olive cultivars of other origins using AFLP markers

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ABSTRACT: The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA and provides a novel and very powerful DNA fingerprinting technique for DNAs of any origin or complexity. In this study, genetic diversity of 15 olive cultivars from different origins (Iran, Spain, Syria, America and Greece) was evaluated using AFLP markers using *Pst*I and *Mse*I restriction enzymes. Nine primer combinations amplified 695 polymorphic bands and all of them were polymorphic between cultivars and species. The maximum and the minimum number of bands were produced by P-ACT & M-CCA and P-ACT & M-CCT combinations, with 101 and 64 bands respectively. Mantel correspondence test showed that the best similarity coefficient for clustering is the simple matching coefficient, giving a cofenetic coefficient of 92%. Most of the Iranian cultivars clustered in one group which confirmed morphological data and these cultivars were similar to Spanish cultivars, suggesting that the Iranian and Spanish cultivars have the same origin. The PCOA (Principal Coordinate Analysis) in most cases confirmed cluster analysis.

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Microsatellite polymorphism in barley and its informational content: expectations, disappointments and perspectives

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ABSTRACT: Microsatellites (MS) are short single or several nucleotide repeats interspersed throughout genomes and manifesting high levels of polymorphism in different organisms. Usually polymorphic in length, MS containing region is surrounded by the sequences of high intra- and interspecies conservatism that allow developing molecular markers for amplified fragment sizing. For many years MS sizing was intensively and successfully used as molecular markers of high efficiency for basic and applied research in barley. However, presence of the "same" and "inconsistent" alleles in the results (Russell et. al., 2003; Sjakste et al., 2003) belongs to the main disappointments followed by the decrease of the interest of researchers to MS in SNPs favour as markers of choice. The current avalanche of barley polymorphism research data coupled with sequencing of the MS containing genome regions however makes it essential to re-estimate the informational value of MS as molecular markers. Using own and available published data we have shown here that the sequence motif of the MS repeated region linked with SNPs of the MS flanks results in different MS containing region haplotypes and defines interhaplotype MS variability in contrast to the intrahaplotype MS length polymorphism. Both intra- and interhaplotype variability of several barley MS have been evaluated on the eventual functional significance. We conclude that informational content of MS polymorphism as well as its functional significance can be underestimated in some cases when fragment sizing was used as only parameter in the study. Evidence supporting this suggestion will be provided in the presentation.

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Newly developed PCR-based markers for cytoplasm identification in triticale

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ABSTRACT:

Breeding of hybrid triticale cultivars can be facilitated by application of gametocides or by the use of sterility-inducing cytoplasms. In the latter case a marker system allowing fast and easy identification of breeding cytoplasms would be of great practical importance. In the present work we studied two types of sterilizing cytoplasm potentially useful in the hybrid breeding of triticale: 1) the cytoplasm from *Triticum timopheevi* – this plant material was kindly provided by H. Góral from Agricultural University of Krakow, Krakow, Poland (Góral 2002); 2) the Pampa cytoplasm introduced from *Secale cereale* by B. Łapiński from Plant Breeding and Acclimatization Institute in Radzików, Poland (Łapiński 2005).The markers reported here were developed on the basis of mitochondrial DNA sequence data. They were proved to distinguish between the normal (*Triticum aestivum*) cytoplasm present in winter triticale cultivars and both tested sterility-inducing cytoplasms. One of these markers amplifies a PCR product which is specific for Pampa cytoplasm regardless of whether it is present in triticale or rye. This marker extends the marker system for identification of cytoplasms in rye (Stojałowski et al. 2006). The second marker produces a fragment 0.52 kb for triticale plants with normal cytoplasm. The amplified product of this marker was distinctly longer (approx. 0.58 kb) when the *timopheevi* cytoplasm was present in the studied plants. The combined use of both SCAR markers offers a new tool for the management of breeding cytoplasms in triticale.

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Sequence diversity at the loci of nuclear SSRs and ITS1-5.8S-ITS2 of rDNA of 47 melon (*Cucumis melo*) cultivars and an extinct landrace excavated from the 15th century

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ABSTRACT: Sequence diversity at the loci of nuclear SSRs (nSSR) and ITS1-5.8S-ITS2 of rDNA of 47 melon (*Cucumis melo*) cultivars were analyzed. Sequences were compared to an aDNA (ancient DNA) sample isolated from seed remains excavated from a 15th CENT. site (Budapest, Hungary). Sequence analyses of nSSRs at a dinucleotide *Cm*CT170 - (CT)n, and a trinucleotide *Cm*CTT144 - (CTT)n loci revealed several SSR groups of melon based on the changes in the numbers of core nucleotides. The nSSRs of aDNA showed neither the longest nor the shortest sequence length. For genotype reconstruction rDNA was analyzed at the ITS1-5.8S-ITS2 loci. Sequence and phylogeny cluster analysis (MEGA4) revealed that cv. *Muskotály* (#13), cv. *Hógolyó* (#24) and cv. *Hales-Best* (#28) show the closest similarity to medieval melon. For phenotype reconstruction, current melons were compared by 26 quantitative and qualitative traits, which revealed an extreme diversity among accessions; however they were clustered in the three main melon types of *cantalupensis*, *reticulatus* and *inodorus*.

Keywords: ITS, SSR, aDNA, microevolution, SNP, germplasm, dendogram.

Introduction

Family *Cucurbitace* consists of about 119 genera with 825 species including genus melon (*Cucumis*) comprising increasing numbers of species from 9 (Linnaeus, in Kirkbride, 1993; Jeffrey 2005), 13 (Naudin 1859), 32 (Kirkbride 1993) and 34 (Andres 2004; Chen et al. 1997). Of them, the two commercial vegetable crops melon (*C. melo*) and cucumber (*C. sativus*) are economically important (Kirkbride 1993; Dane and Tsuchiya 1976).

Melon (*C. melo*, 2n = 24) is a temperate and warm season crop with vegetable and ornamental use. As a result of auxin regulated shoot apical dominance an auxin-dependent gradient develops along the vine (shoot) from male to female flowers. Male (*staminate*) flowers develop at the bases of first few leaves, followed by a mixture of male and female flowers, and finally female (*pistillate*) flowers close to the tip, which characters results in determinate and indeterminate cultivars. Botanically, the ovary wall is fused with receptacle

tissue to form a hard rind of fruit, which shows great variation in shape, size, color and texture in melon (Stepansky et al. 1999).

The aim of the present study was to characterize morphological and molecular (ITS and SSR) diversity of 47 melon cultivars including 20 old Hungarian landraces with a final aim of reconstruction of an extinct medieval type.

Materials and Methods

Morphological evaluation

Morphological characterization was carried out according to the combined standards of Descriptor Lists of ABI (Institute of Agrobotany, Tápiószele, Hungary) and the National Institute for Quality Control (OMMI, Budapest, Hungary). In total, 26 traits were recorded by visual inspection such as (1) plant growth habit (determinate, semi-determinate or indeterminate), (2) stem pubescence density (rare, medium or dense), (3) stem pubescence types (smooth, medium or rough), (4) leaf shape (round, reniform, heart-shape, triangular or pentagonate), (5) leaf length (<14 cm, 15-18 cm or 18< cm), (6) leaf colour (light-green, green, dark-green or grey), (7) leaf-lobing (full, weak, medium or strong), (8) sepals shape (ficiform, subulate or leaf-shape), (9) stigma length in cm, (10) ovary pubescence (absent, rare or dense), (11) fruit shape (flat, round, oval, spindle-shape, obovate, pyriform, cylindrical, snake-shape or turban-shape), (12) fruit size in cm, (13) fruit (rind) surface (smooth, grooved, warty, cracked, wrinkled or netted), (14) skin (rind) pattern (absent, stripes or dotted), (15) color of fruit texture (yellow, brown, green or grey), (16) fruit colour at ripening (white to green and white to orange), (17) fruit mass (<0.5 kg, 0.5-2 kg, 2.1-4 kg, 4.1-6 kg or 6 kg<), (18) fruit mesocarp (flesh) thickness in cm, (19) fruit mesocarp (flesh) colour (white, cream, green or orange), (20) abscission (easy or hard), (21) placenta at ripening (liquid, gel or dry), (22) taste (insipid, non-sweet, mildly sweet, sweet, very sweet, bitter or sour), (23) seed color (white, cream, yellow or light-brown), (24) maturity group (<70 days, 70-100 days or 100< days), (25) uniformity at ripening time (low, medium or high) and (26) sex types of flowers (monoecious or andromonoecious).

Seed samples

Seeds of 47 current melon cultivars and landraces (Table 1, Fig. 1a) were selected from the germplasm collections of the Institute of Agrobotany Tapiószele, Hungary and sown in compost media in glass houses. Seedlings at two-three leaf stage were transplanted and grown up in 5 m x 5 m field plots in duplicate experiments. Medieval melon seed remains were excavated at the 15th CENT. sites (8th well, Mansion Teleki, King's Palace of Árpád Dinasty, Buda Hill, Budapest; Hungary), determined and pretreated according to Gyulai et al. (2006); Lágler et al. (2005) and Szabó et al. (2005).

SSR analysis
A minimum of two independent DNA preparations of each sample were used for SSR and ITS analysis following the basic protocols of Katzir et al. (1996) and Danin-Poleg et al. (2001) according to Szabó et al. (2005). A negative control, which contained all the necessary PCR components except template DNA was included in PCR runs. Pooled DNA of medieval melon extracted from the non infected seeds was used.

ITS analysis
The nuclear rDNA diversity was analyzed at the ITS1-5.8S-ITS2 locus probed by ITS specific primer of tcg taa caa ggt ttc cgt agg tg and tcc tcc gct tat tga tat gc according to Garcia-Mas et al. (2004) and Hsiao et al. (1995).

DNA Sequencing
Fragments were subjected to automated fluorescent DNA sequencing (ABI PRISM 3100 Genetic Analyzer) according to Gyulai et al. (2006).

Sequence analysis
Sequence alignments were analyzed by BioEdit Sequence Alignment Editor (NCSU, USA) software programs. For BLAST (Basic Local Alignment Search Tool) analysis NCBI (National Center for Biotechnology Information) computer program was used (Altschul et al. 1997). For the creation of phylogeny trees, Mega 4 (*Molecular Evolutionary Genetics Analysis*) computer program was used (Tamura et al. 2007). Radial and rectangular type of dendrograms were created.

Results and Discussion
Morphological analysis
Cluster analysis based on the morphological markers (1 to 26) grouped the 47 varieties into these three subclusters according to melon types of *reticulatus*, *cantaloupensis* and *inodorus*. Cultivars '*Sweet ananas'* and '*Ezüst ananasz'* showed the closest morphological similarity, cv. *Hógolyó* was grouped in one subcluster with cv. *'Túrkeve'* (Fig. 2).

SSR analysis
Eight of the 20 SSR primer pairs tested amplified 40 microsatellite alleles in identical fragment ranges. A total of 485 alleles were detected in the 47 melon cultivars and ancient sample (Szabó et al. 2005). The number of alleles per marker ranged from 2 to 7 with an average of 5.7 including *Cm*CT44 (2 alleles), *Cm*AG59 (5 alleles), *Cm*GA104 (5 alleles), *Cm*CT134 (4 alleles), *Cm*TA134 (6 alleles), *Cm*CTT144 (7 alleles), *Cm*TC168 (6 alleles) and *Cm*CT170 (5 alleles). Sequence analysis of the microsatellite alleles showed different fragment lengths depending on changes in the number of unit of core sequences.

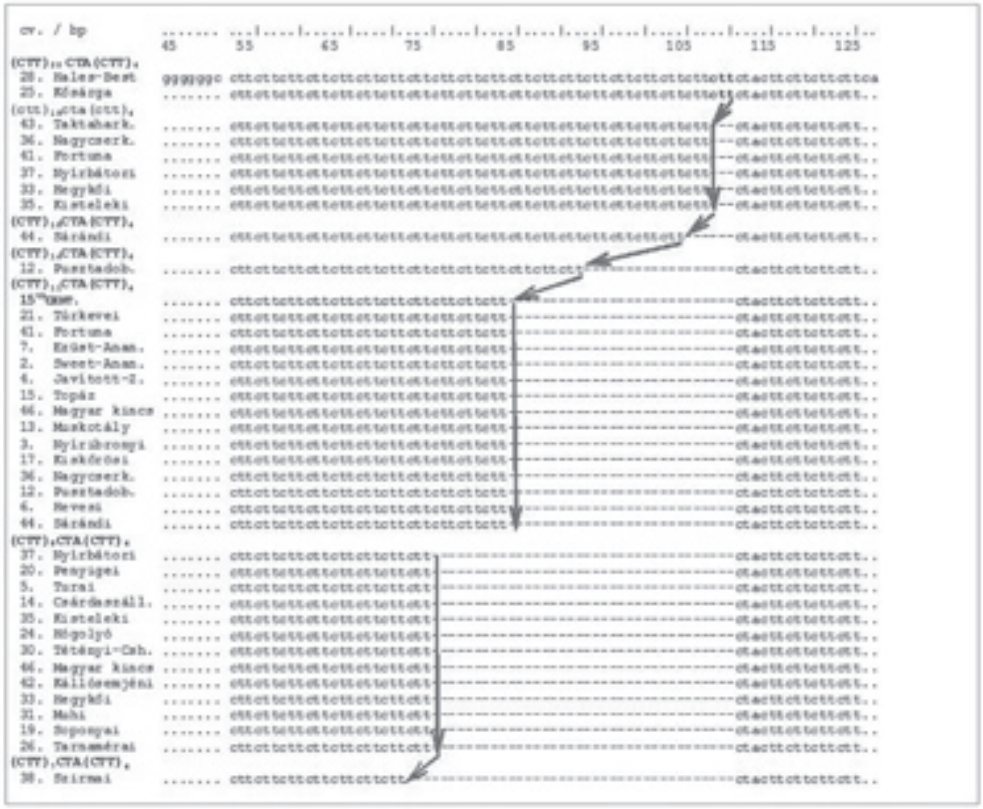

Figure 1. Microsatellite allele diversity at the (CTT)n trinucleotide loci of melon (*Cucumis melo*) cultivars compared to medieval sample (15th CENT.) ('-' indicate deletions).

Sequence analyses of a dinucleotide and a trinucleotide SSR loci (Fig. 1) revealed that medieval melon did not show neither the shortest nor the longest allele sizes, which results indicate that medieval melon did not belong to the oldest melon type. A molecular dendrogram of SSRs revealed that cv. '*Hógolyó*' showed the closest molecular similarity to medieval melon (Szabó et al. 2005). Morphological cluster of the present study revealed that cv. '*Hógolyó*' belongs to *inodorus* melon group (winter melon), which provided insight for reconstruction of the medieval melon phenotype, which might had been an *inodorus* type, winter melons, named 'pocket'(zseb) or 'fist' (ököl) melons after their sizes. As ripen slowly they can be stored in cool, dry, airy places until Christmas or later ('long shelf life melons').

Sequence and cluster (MEGA4) analyses at the ITS1-5.8S-ITS2 locus of nuclear ribosomal DNA revealed that cv. *Muskotály* (#13, *cantalupensis*), cv. *Hógolyó* (#24, *inodorus*) and cv.

Hales-Best (#28, *reticulatus*) and the medieval melon belong to one and the 'most ancient' cluster showing only a few nucleotide substitutions compared to cucumber (*Cucumis sativus*). This result also provides further data for phenotype reconstruction of the medieval melon.

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Genetic and molecular analysis of cauliflower plants carrying *Brassica nigra* cytoplasm

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ABSTRACT: A breeding program carried in the Research Institute of Vegetable Crops in Skierniewice (Poland) resulted in a series of male-sterile cauliflower lines carrying the cytoplasm from *Brassica nigra*. In these cytoplasmic male-sterile (CMS) plants stamens are transformed into petals. When the CMS lines were test-crossed with a collection of male-fertile lines of unknown nuclear genotype, 6 % of the resulting offspring was chimeric – the plants produced shoots bearing either sterile or fertile flowers. This phenotype may result from segregation of plasmotypes within a single plant. In order to identify the mitochondrial DNA (mtDNA) regions associated with the sterilizing effect of the *B. nigra* cytoplasm we performed a series of vectorette PCR experiments. In this analysis also plants with the normal cauliflower cytoplasm and plants with CMS-Ogura were included. Out of 153 polymorphic PCR products 5 were specific to the *nigra* cytoplasm. These DNA fragments were produced with gene-specific primers anchored within 4 distinct mitochondrial sequences. The polymorphic PCR products allowed calculation the genetic distance between the studied cytoplasms. This analysis revealed that the *nigra* and Ogura cytoplasms are more similar to the cauliflower normal cytoplasm than to each other. Within the frame of this project we also designed a series of primers anchored within selected *Brassica* flower organ identity genes. With these primers we were able to amplify cDNAs corresponding to cauliflower homologs of *ap2*, *ap3*, *pi*, *ag* and *se1*. The primers will now be used for a real-time PCR quantitation of the respective mRNAs in the CMS and fertile plants.

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TILLMore, a TILLING platform for mutant discovery in barley

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ABSTRACT: We report on the development and the utilization of a sodium azide-mutagenized population of barley (cv. 'Morex') to identify mutants at target genes using the TILLING (Targeting induced local lesions in genomes; Till et al., 2007)) procedure. Screening for mutations at four agronomically important genes (*HvCO1*, *Rpg1*, *eIF4E* and *NR*) identified a total of 22 new mutant alleles, equivalent to the extrapolated rate of 1 mutation every 374 kb (Talamè et al., 2008). All mutations except one were CG-TA transitions and several (ca. 68%) implied a change in protein amino acid sequence and therefore a possible effect on phenotype. The high rate of mutation detected through TILLING is in keeping with the high frequency (32.7%) of variant phenotypes observed among the M_3 families. Our results indicate the feasibility of using this collection of materials for both reverse- and forward-genetics approaches to investigate gene function in barley and related crops. This TILLING platform is available for collaborations and mutant isolation is offered as a payed-for service on a cost-recovery basis

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Characterization of *Vitis vinifera* L. cv. Žilavka with SSR markers

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ABSTRACT: The grapevine cultivar Žilavka is of undetermined origin, although it has a dominant position in terms of viticulture within the wine growing region of Herzegovina (Bosnia and Herzegovina) and gives high quality white vine. This cultivar is also grown in Dalmatia (Croatia), Former Yugoslav Republic of Macedonia, Serbia and Montenegro. The Žilavka population in Herzegovina is characterized by great heterogeneity in terms of ampleographic and morphometric characteristics. In order to characterize Žilavka, 81 samples from four different localities from the Herzegovina region were analyzed. Samples were screened at 14 SSR loci (VVS2, VVMD5, VVMD6, VVMD24, VVMD25, VVMD27, VrZAG21, VrZAG47, VrZAG62, VrZAG64, VrZAG67, VrZAG79, UCH11 and UCH 29). Four samples showed different allelic patterns (2 to 5 loci out of 14) while the remaining samples had identical profiles at all examined loci. The four samples can be explained by somatic mutation accumulation, the presence of chimera in the meristem structure or the presence of different cultivars due to propagation mistakes. These results provide a starting point for continued investigation of this unexplored cultivar: to standardize the cultivar by genotyping, to look for, find and explore the parents of this cultivar, to analyze the relation to other cultivars grown in the region and further afield, and to determine the geographical origin.

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Genotype (nSSR) and haplotype (cpDNA) identification in watermelons (*Citrullus lanatus* subsp. *lanatus*)

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ABSTRACT: Seed remains of watermelon (*Citrullus lanatus* subsp. *lanatus*) were excavated from two sites of Middle Ages 13th CENT. A.D. (Debrecen) and 15th CENT. A.D. Budapest (Hungary). Seed remains were processed by floatation followed by seed sorting and identification in the laboratory. After seed morphological analysis aDNAs were extracted, analyzed at eleven microsatellite (SSRs) loci with a final aim of sequence recovery and phenotype reconstruction. For comparative analysis a herbarium samples from the 19th CENT. A.D. (Pannonhalma, Hungary) and forty-four current varieties were used. Molecular dendrograms based on microsatellite and cpDNA analyses revealed that middle age samples are close to current varieties with red flesh colour which indicate the preferential cultivation of red flesh – and not yellow flesh- watermelon in the Middle Age Hungary. The 170-yr-old herbarium sample showed close molecular similarity to citron melon (*Citrullus lanatus* var. *citroides*) which also reflects the importance of citron melon as fodder in Hungary. Results of seed morphology highly correlated to molecular data.

Keywords: cpDNA analyses, microsatellite, sequence recovery, watermelon.

Introduction

The aDNA (*a*ncient DNA) recovered from excavated remains of plants and animals supply unique materials not only for the analysis of post-mortem DNA degradation (Brown 1999; Threadgold and Brown 2003), but also for tracing vegetation history and microevolution (Al-Awwam 1158; Gugerli *et al*. 2005). Intact aDNA sequences (Szabó *et al*. 2005; Gyulai *et al*. 2006) or complete genome of the extinct organisms can be reconstructed in the case of optimal preservation conditions. In this study we present the analyses of seed morphology, and nDNA and cpDNA study of 700-, 600- and 170-year-old *Citrullus* samples compared to modern cultivars.

Materials and Methods

Seed samples

Seed remains of watermelon (*C. lanatus* subsp. *lanatus*) from the 13th CENT. were excavated in Debrecen, Hungary (Hajdu Zs. et al., Déri Museum, 2006, Debrecen; http://www.derimuz.

hu/hirek/2006/kutak.html). In total, 95,133 seed remains of 206 plant species were identified. Of them 251 watermelon seeds were determine with the same morphological characters. At the 15th CENT. sites (8th well, Mansion Teleki, King's Palace of Árpád Dinasty, Buda Hill, Budapest; Hungary) 54,415 watermelon seeds were excavated (Gyulai *et al*. 2006). Wet-sieved sediment samples were processed by floatation followed by seed sorting and identification in the laboratory. The 19th CENT. (ca. 1836) seeds were collected from the oldest botanical seed collection of Hungary (Pannonhalma, Hungary) (Vörös 1971), recently exhibited at the Hungarian Agricultural Museum, Budapest (Hungary). For comparative analysis, forty-four modern *Citrullus* species and varieties were included (discussed elsewhere).

DNA extraction

Individual seed were ground in an aseptic mortar with liquid nitrogen in a laminar air flow cabinet according to Gyulai et al. (2006). The quality and quantity of extracted DNA were measured (2 µl) by a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, USA – BioScience, Budapest, Hungary). DNA samples were adjusted to concentration of 30 ng/µl with ddH_2O and subjected to PCR amplification. *PCR primers*. For molecular analysis eleven nSSR (nuclear microsatellite) primer-pairs were used: *Cm*TC51, *Cm*TC168, *Cm*ACC146 (Katzir *et al*. 1996; Danin-Poleg *et al*. 2001); and *bng*l118-2, *phi*118-2, *phi*121, *bng*l339, *Cl*1-06, *Cl*2-23 and *Cl*2-140 (Jarret *et al*. 1997). For cpDNA analysis at the trnVAL locus [tRNA-Val (GAC)] clp-12 primer (agt tcg agc ctg att atc cc, gca tgc cgc cag cgt tca tc) was used (Al-Janabi *et al*. 1994; Demesure *et al*. 1995). *PCR amplification*. Hot Start PCR was combined with Touchdown PCR using AmpliTaq Gold™ Polymerase according to Gyulai et al. (2006). *DNA Sequencing:* Fragments were subjected to automated fluorescent DNA sequencing (ABI PRISM 3100 Genetic Analyzer). Sequence alignments were analyzed by BioEdit Sequence Alignment Editor (NCSU, USA) software programs. For BLAST (Basic Local Alignment Search Tool) analysis NCBI (National Center for Biotechnology Information) computer program was used. *Data analysis*.Cluster analysis was carried out by SPSS-11 computer program was used.

Results and Discussion

The monotypic genus *Citrullus* is comprised of only four diploid (2n = 4x = 22; 4.25 - 4.54 x 10^8 bp; 0.42 pg DNS) species, including the annual watermelon (*C. lanatus*), the perennial colocynth (*syn.*: bitter apple) (*C. colocynthis*), and two wild species growing in Kalahari Desert, Africa as the *C. ecirrhosus* with bitter-tasting fruit, and the annual *C. rehmii* with pink and olive green spotted, mandarin sized, non-edible fruits (Dane and Liu 2006).

Unlike genus *Citrullus*, species watermelon (*C. lanatus*) comprises diverse varieties, subspecies, mutants and feral forms such as the cultivated watermelon (*C. lanatus* subsp. *lanatus*) (syn.: *C. vulgaris*) with its ancient form of citron melon (*syn.*: African tsamma) (*C. lanatus* var. *citroides*); and the Mediterranean seed mutant egusi type watermelon (*C. lanatus*

subsp. *mucospermus*). Watermelon, citron and colocynth have a history of production in Europe (Wasylikowa and Veen 2004).

The oldest, 6,000-yr old watermelon (*C. lanatus* subsp. *lanatus*) seeds were excavated in Helwan (Egypt, Africa), at a site 4.000 B.C. (Barakat 1990). About 5,000-yr old seed remains were excavated in Uan Muhuggiag, Lybia, Africa from a site 3.000 B.C. (Wasylikowa and Veen 2004). Several watermelon seeds were found in Pharaoh's tomb as in Thebes (New Kingdom: 1,550-1,070 B.C.; stored in Agricultural Museum, Dokki, Giza, Egypt) (Warid 1995) and in the pyramid of Tutankhamum ca. 1,330 B.C. (Vartavan and Amorós 1997).

Watermelon seeds excavated at both medieval sites analyzed in the study presented appeared to be extremely well preserved due to anaerobic conditions in the slime of a deep well covered by water, apparently used as dust holes in the Middle Ages (Gyulai *et al*. 2006). The herbarium sample seeds form the 19th CENT. were stored under precise conditions in glass containers (Vörös 1971).

nSSR analysis

Eleven microsatellite probes were used in the study presented for morphological reconstruction of the ancient watermelons. Molecular dendrogram based on SSR allele diversity at eleven nuclear microsatellite (nSSR) loci revealed that middle age samples show close lineages to ancient varieties currently growing in Hungary with red flesh colour. Results of seed morphology correlated strongly to molecular results. The 13th CENT. aDNA (Debrecen) showed close similarity to cv. '*Kecskeméti vöröshéjú*'; the 15th CENT. aDNA (Budapest) showed close similarity to cv. '*Belyj dlinnij*' (# 12). These results also reflect the preferential cultivation of red flesh – and not yellow flesh- watermelon in the Middle Age of Hungary. Red flesh watermelon also appeared in the painting of *Still Life with Melons and Carafe of White Wine* (1603 B.C.) painted by Caravaggio (Janick and Paris 2006). Molecular data obtained might provide further tools for watermelon breeders. The aDNA of 170-yr-old herbarium seed sample (Pannonhalma, Hungary) showed close molecular similarity to citron melon (*C. lanatus* var. *citroides*) cv.'*Újszilvás*' which reflects the importance of citron melon as fodder in the Middle-Age Hungary.

cpDNA analysis

All *Citrullus* sample had a G→T substitution at the 102,029 nt compared to gene bank data of *Cucumis sativus* (AJ970307). Five new haplotypes based on nucleotide substitution at tRNA-Val - *rps*12 locus were identified in *Citrullus* species and varieties. Samples of colocynth (# 1-3), current citron melon (# 4-6) and the 19th CENT. citron melon clustered in a separate group as a result of a G→T substitution at 102,182 nt. Medieval samples and four current watermelons clustered in separate group due to the G→A substitution at the 102,193 nt, which results might indicate unique date for cytoplasm reconstruction of ancient watermelons.

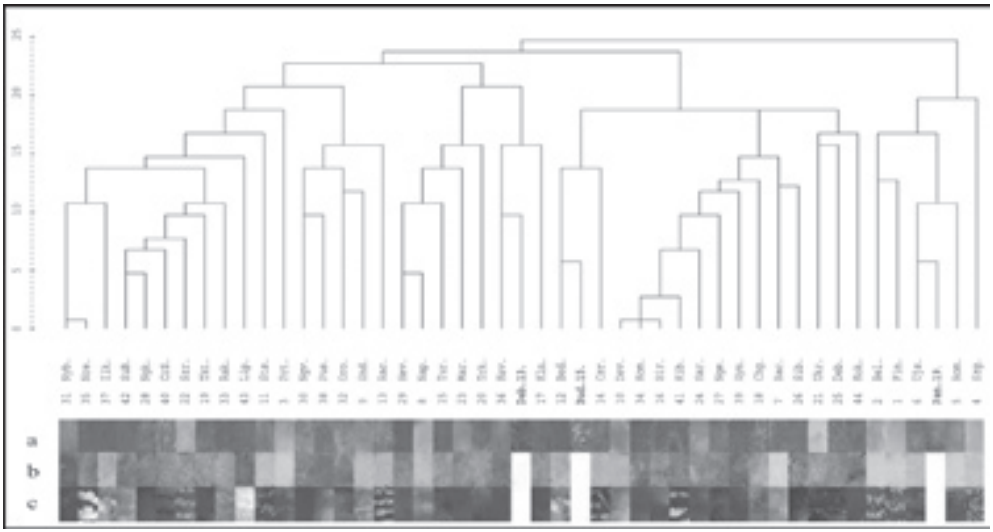

Figure 1. Molecular dendrogram (Rel. Genet. Dist. 0 – 25) of current varieties of colocynth *(Citrullus colocynthis,* **1-3***),* citron melon (*Citrullus lanatus* var. *citroides*, **4-6**) and watermelon (*Citrullus lanatus* subsp. *lanatus,* **7-44**) compared to archaeological watermelon (Debr.13. and Bud.15.) and herbarium citron melon (Pan.19.) samples. Textures of seed coat (**a**), flesh (**b**) and rind (**c**) are indicated.

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A multi-parental (four-way cross) mapping population for QTL discovery in durum wheat

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ABSTRACT: Durum wheat is widely grown in Italy, one of the leading countries in durum and pasta production. DiSTA (University of Bologna) and Società Produttori Sementi have assembled a recombinant inbred mapping population (of ca. 380 lines) developed from a balanced four-way cross. Parental cultivars were Neodur, Claudio, Colosseo and Rascon, characterized by different quality parameters and resistance to powdery mildew, leaf rust and *Fusarium* head blight. This resource should allow for a more efficient analysis of the genetics of complex traits such as yield, adaptation to diverse environment, quality and response to wheat fungal diseases with respect to the use of traditional biparental mapping populations. Additionally, the identification of epistatic interaction effects should be facilitated. As part of the EU-funded project BioExploit, a genetic map is being generated by Keygene N.V. based on AFLP® markers (Keygene N.V.) and anchoring SSR markers (DiSTA) using the single segregating four-way individuals. This preliminary map is being integrated with anchoring SSR markers and further AFLP markers. At Keygene, SNP polymorphisms specific for the four parental cultivars are being identified using the novel Keygene CRoPS ™ Marker Technology (Complexity Reduction of Polymorphic Sequence) assisted by high-throughput sequencing developed by 454 Life Sciences. This map will provide the possibility to assess the effect of multiple alleles at single QTLs and to investigate their interactions.

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Haploid plants regeneration of *Eruca sativa* by in vitro anther culture

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ABSTRACT: During the last decades, rocket (*Eruca sativa* L.) has become an important vegetable crop in Italy. However local cultivars show high variability in plant characters (e.g. size, colour and shape of the leaves, plant growth and habitus) and timing. In this view, at the DOFATA, we studied the possibilities to obtain useful genetic material for breeding programs by using in vitro anther culture. Seeds of an Italian cultivar of rocket were sown in pots and plants were grown in the field together with a cauliflower cultivar, 'Tardivo di Fano', which was used as control. Flower buds (4-5 mm) of both genotypes were collected and excised anthers were plated on solidified Gamborg medium modified by Keller supplemented with silver nitrate, NAA, 2,4 D, and sucrose. After plating, they were exposed to a heat treatment at 35°C for 24 hours prior to incubating them at 25°C in the dark. Embryos of about 4 mm were sub-cultured in the same medium without silver nitrate and supplemented with adenine sulfate. Rootlet apex of regenerated plants were used for cytological analysis to determine ploidy status. The two species did not show any significant differences in terms of embryogenic anthers while, on the other hand, significant differences were observed for the number of embryos obtained. Chromosomes counting of rootlet apex showed the haploid status of 53 % of rocket plants while all cauliflower plants were diploid. Haploid plants had a much slower growth rate than the diploid ones and took about two weeks longer to reach the minimum size for sub-culturing. In the near future the rocket haploid plants will be treated with colchicine to achieve homozygosis. The genotypes developed could represent useful genetic material for breeding programs aiming at improving crop uniformity and help in studying qualitative characters of the edible plant parts.

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Implication of molecular genetic markers for studying biodiversity in Tunisian date-palms, figs and pomegrate.

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ABSTRACT: The use of molecular markers has become prevalent for many purposes such as genetic diversity analyses, management of the biodiversity conservation, crop improvement. In Tunisia, a high biodiversity characterises the fruit germplasm as illustrated by the identified large number of well adapted cultivars selected for their attractive fruit qualities. Nevertheless, this patrimony is seriously threatened by severe genetic erosion due to biotic and abiotic stresses. Thus, elaboration of strategies aiming at the preservation of the local germplasm has become imperative. For this purpose, we have designed the use of the random amplified polymorphic DNA (RAPDs), inter simple sequence repeats (ISSRs), random amplified microsatellite polymorphism (RAMPOs) and simple sequence repeats (SSRs) as suitable techniques to generate molecular markers to assess genetic diversity and relationships between ecotypes. Data proved that among the identified markers, SSRs are the most appropriate to have a deep insight of the genetic diversity organisation and to establish identification key of the cultivars studied. However, the other designed markers (i.e.: RAPDs, ISSRs and RAMPOs) are less informative since they are dominant markers. In addition, the efficiency of the different molecular markers tested to distinguish accessions and to survey genetic diversity organization is compared. The present study portrays the achievement of these methods and their suitability to provide criteria of choice playing an increasing role to precise the biodiversity in the local germplasm.

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The identification of drought tolerant sources from the MRI gene bank

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ABSTRACT: The whole material of the MRI Zemun Polje gene bank of maize, as well as some previous and current commercial inbred lines and hybrids of Maize Research Institute Zemun Polje, were subjected to water stress in Egypt, at the Setz station of the Agricultural Research Centre in 2007. A total of 6,371 accessions were included into the experiment, consisting of 2,217 OP varieties from former Yugoslav territory, 2,254 introduced inbred lines, 1,335 introduced heterozygous accessions (populations, synthetics, land-races, composites), 549 inbred lines from the breeding programme and 16 elite hybrids. The material was sown on August 19, 2007. It was divided into five groups, according to the duration of the vegetation: extra early, early, medium, medium late and late, based on our gained knowledge and experience. There is no precipitation in the vicinity of the Setz station during the vegetative period and, as far as the water supply is concerned, the only uncontrolled factor is the level of underground water. Five groups of the experimental material were sown separately and were irrigated until the appearance of the first tassels (within each group) which was approximately two weeks before flowering of the earliest accessions within groups. After that, no irrigation was applied. Three weeks before harvesting, the material was scored visually, and the best accessions in that stage were recorded. This was done by recording both stay-green (on the 1-5 scale, where 5 was the highest rate), and the total appearance of the accession (on the 1-8 scale, where 8 was the highest rate). Data for pollination and silking of each accession were recorded, and their difference (ASI) was calculated. Harvest occurred on November 8 and 9, 2007. The material was selected on the basis of ASI (3 or less for heterozygous accessions, and 4 or less for homozygous ones), % of plants with seed set (60% for heterozygous, and 50% for homozygous accessions), % of seed set (85% for heterozygous, and 80% for homozygous accessions) and % of grain filling (85% for heterozygous, and 80% for homozygous accessions). The correlation between genotypes with good performances during the vegetation period and those ones selected at harvest was about 56% for the whole material. About 10.5% of the material (a total of 672 accessions) was selected according to reported criteria for further testing in 2008, in three locations (Egypt, Macedonia and Serbia/Zemun Polje). Within this material, there were 167 OP varieties from former Yugoslavia, 201 introduced lines, 204 introduced heterozygous accessions and 100 lines from the breeding programme. This represents a source material for choosing the most drought tolerant sources, which will serve for forming a core-collection for drought tolerance in the MRI gene bank and also for pre-breeding for this trait.

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Molecular evidence of outcrossing rate variability in *Brassica napus*

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ABSTRACT: Assessment of the mating system for a crop is important for the design of adequate breeding and genetic conservation programs. Here, we report the first study on the outcrossing rate in Brassica napus L. cultivated under controlled conditions using a SSR multilocus approach.
Two local populations of leaf rape, MBGBRS0014 (N14) and MBG-BRS0039 (N39), were analyzed. A total of 40 SSRs were screened in 100 plants of each population. Pairs of flowering plants from each population were placed in isolation cages and bumble bees (Bombus sp) were released for facilitating the crosses. A seed sample of the progeny of each plant was analyzed by SSRs that were polymorphic in the parental populations. Outcrossing rates were computed at a family and population level using a multilocus approach. Three SSRs, i.e., Na10-A08 (*A*), Na12-A02 (*B*) and Na14-D07 (*C*) were polymorphic for N39, while one SSR , i.e., Na14-D07 (*C*) was polymorphic for N14. *A* amplified two different and independent loci (*A1* and *A2*). *B* had only one polymorphic locus and presented four alleles. *C* amplified two different loci, although they were not distinguishable since alleles of both loci migrated at the same position. *Brassica napus* is an amphidiploid and, for some markers, an ambiguous relationship between genotype and electrophoretic pattern exists (Becker *et al*., 1992). Two different programs had to be used to compute the outcrossing rate (*t*): MLTR (designed for diploid species) for *A1, A2,* and *B*, and MLTET (designed for tetraploid species) for *C*. The MLTR program produced an estimator of the single outcrossing rate (t_s) at each locus, and the multilocus outcrossing rate (t_m) taking into account all the loci. The values of t_s were very similar for the three loci examined. The t_m value was 0.33 ± 0.10, which is in agreement with those detected for other *B. napus* populations (Becker *et al*., 1992) and does not differ significantly from that detected at the locus *C*. There was variability for t_m values among families, although most of them were between 0.20 and 0.40. The MLTET program could not estimate t_s for the population N14, although the level of outcrossing of this population probably is close to 0.0, since it showed a low level of polymorphism. We found that there is genetic variability among and within *B. napus* populations that could be due to differences in the spatial relationship between stigmas and anthers (Syafaruddin *et al*., 2006) or the presence of auto-incompatible or male-sterile plants (Song *et al*., 2006). Currently we are studying the genetic mechanisms controlling the mating system of this crop, how heterosis and inbreeding depression are affected by the outcrossing rate, and the effect on production.

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Eco-TILLING, a strategy to identify change of function mutations in ecotypes of plants: a candidate gene approach

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ABSTRACT: There is rising interest in the possibility of using naturally occurring ecotypes and association mapping to identify genes responsible for natural variation, particularly those of agronomic and economic importance, in plants. The model plant *Arabidopsis thaliana* is in many ways an ideal candidate for such studies, because it is a selfing hermaphrodite, it exists largely as a collection of ecotypes or accessions, adapted to many climatic regions, and therefore represents wide phenotypic natural variation. The highly inbred accessions need to be genotyped only once and can be phenotyped repeatedly. In our studies, we test the feasibility of genome-wide association mapping in *A. thaliana* by searching for associations with enhanced biomass production and alteration in enzyme and metabolite levels in a sample of 99 accessions. Using a marker set of 864 SNP-markers, association were tested applying three-factorial ANOVA. We found significant associations between metabolic as well as biomass traits and marker alleles. Furthermore, we applied a candidate gene approach using Eco-TILLING for genes correlated with biomass and protein production. We identified five highly significant QTN (quantitative trait nucleotides) in promotor and coding regions responsible for alteration in biomass, protein, starch and sucrose levels. This approach demonstrates the power of Eco-TILLING for identification of natural genetic variation which can be easily adapted to crop plants.

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Genetic introgression in natural and directed interspecific crosses within native Iranian wild almond species

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ABSTRACT: The cultivated almond is thought to have originated in the arid mountainous region of central Asia. In these areas, several wild species are found growing in countries as Iran. Genetic variability is the basic ingredient for breeding. The total genetic diversity of cultivated species and their wild relatives in collectively termed as "genetic resources". Therefore, exploring and describing new genetic diversity in relative species, landraces and old obsolete varieties is of the utmost importance to achieve continuous progress in crop breeding. In this study the genetic introgression in natural and directed crosses within native Iranian wild almond species is studied. Several interspecific populations including two isolated population of *P. scoparia*, two mix population of *P. scoparia* and *P.lycioides* and two interspecific crosses of *P.scoparia* × S5133 (almond selection) and *P.webbii* × S5133 performed by artificial pollination were investigated using 16 nuclear and 5 chloroplast microsatelite markers (SSRs). Results establish the value of SSR markers for distinguishing different genetic lineages and characterize an extensive and largely unexploited inter-species gene pool available to almond breeding programs. Results about the segregation of different crosses also document a rich source of new germplasm for almond improvement programs. The consequencesn for diversity studies and selection process in gene and allele frequency in natural conditions and breeding programs is also discussed. Finally, the genetic variation and evolution of the cultivated almond is discussed.

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Comparison of methods for genetic fingerprinting of polyploid species: a case study with *Solanum tuberosum* L. subsp. *tuberosum*

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ABSTRACT: DNA markers have been broadly applied in plant material identification, genetic diversity evaluation, in gene banks for collection maintenance and in breeding programs. At the Latvian Genetic Resource Centre, we are utilising Simple Sequence Repeat (SSR) markers for the majority of DNA fingerprinting projects, which include such polyploid species as *Trifolium pratense, Triticum aestivum, Avena sativa, Solanum tuberosum,* as well as other species for which SSR markers are not available. The high polymorphism level and co-dominance of SSR markers allow for efficient cultivar characterization. However, several factors must be considered when applying SSR markers such as availability of SSR markers for a particular species, the lack of multiplexing, and the efficiency of co-dominant SSR marker data in polyploid plant species. One of the major problems when using SSR markers in polyploid species is that the allele dosage cannot be directly determined. Another is that the majority of SSR analysis software programs only allow for a maximum of 2 alleles per locus per individual. For polyploids, the SSR data can be recoded as allele presence/absence, which effectively reduces the information content of SSR markers to that of dominant markers. Our aim was to compare DNA marker methods as well as analysis methods, using potato as a model, to determine the most efficient method for genetically fingerprinting polyploid species as well as determining the most effective marker technique for pedigree resolution and cultivar identification. SSR marker data were used for detailed analysis of all potato (*Solanum tuberosum* L. subsp. *tuberosum*) cultivars listed in the Latvian Plant Genetic Resource database. In order to investigate the efficiency and applicability of other DNA marker methods, we also genotyped a subset of these cultivars using other DNA marker methods, including Diversity Arrays Technology (DaRT) (Jaccoud et al., 2001). DArT genotyping data was obtained for 13 of Latvian varieties. Over 500 polymorphic loci were detected with average polymorphism information content (PIC) of 0.37. Currently, we are working on genotyping additional 94 Latvian varieties, breeding lines and progenitors in collaboration with potato DArT consortium. The SSR genotype data was analysed with a variety of methods and data pooling options. The SSRs identified similar levels of polymorphism as reported for other potato collections (Milbourne et al., 1996). Two pairs of tested cultivars were identical in all screened SSR loci and couldn't be discriminated. These are currently being evaluated phenotypically to determine if they can be differentiated. There are no distinct groupings or divisions within Latvian potato cultivars. However, the genetic diversity of potato

cultivars bred after 1978 has increased. This reflects the combination of older, locally adapted material with the increased genetic diversity of modern European potato cultivars.

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Intra- and intergenomic chromosome pairing in Festulolium hybrids

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ABSTRACT: Species within the *Lolium-Festuca* complex, namely Italian ryegrass (*L. multiflorum* Lam.), perennial ryegrass (*L. perenne* L.) and meadow fescue (*F. pratensis* Huds.), offer many desirable and complementary traits, including: rapid establishment, good forage quality and high yield of ryegrasses, and persistency, winter hardiness and freezing tolerance of meadow fescue. These closely-related species of the two genera can form hybrids in which their chromosomes pair and recombine. Intergeneric tetraploid hybrids between *L. multiflorum* or *L. perenne* and *F. pratensis* have been successfully used in breeding programmes to develop Festulolium cultivars, which are being increasingly grown worldwide. In the present work we have used genomic *in situ* hybridization (GISH) to study chromosome pairing at metaphase I of meiosis in F1 hybrids of the allotetraploid *F. pratensis* × *L. perenne* (2n = 4x = 28). This is the first detailed investigation of the specificity of chromosome pairing within and between genomes in tetraploid Festulolium hybrids. The mean chromosome configurations for six hybrids analysed were: 1.13 univalents + 11.51 bivalents + 0.32 trivalents + 0.72 quadrivalents. GISH showed that pairing was predominantly intragenomic, with mean numbers of *Lolium* and *Festuca* bivalents being virtually equal at 5.41 and 5.48 per cell, respectively. Intergenomic pairing between *Lolium* and *Festuca* chromosomes was observed in 33.3% of bivalents (0.62 per cell), 79.7% of trivalents (0.25 per cell), and 98.4% of quadrivalents (0.71 per cell). Approximately 4.0% of total chromosome complements analysed remained as univalents (an average of 0.68 per cell for *Lolium* and 0.45 for *Festuca*). It is evident that in these F1 hybrids there is opportunity for recombination to take place between the two component genomes, albeit at a low level. GISH analysis of chromosome pairing in successive generations of the allotetraploid *F. pratensis* × *L. perenne* is in progress.

Part 2

Breeding for yield and resistance to biotic and abiotic stresses

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Genomics approaches to improve crop tolerance to abiotic stresses

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ABSTRACT: The constant increase in yield potential of crops achieved by conventional breeding has been possible with limited knowledge of the factors curtailing yield under adverse environmental conditions. Crop response to abiotic stresses involves many traits, mostly quantitatively inherited. Genomics-based approaches allow us to identify and characterise the relevant genes and quantitative trait loci (QTLs) underscoring such traits and, upon their isogenization, better characterise their functions and effects on yield. Additionally, QTL mapping enables us to deploy marker-assisted selection to tailor novel genotypes (Varshney and Tuberosa, 2007). This notwithstanding and despite the plethora of QTLs that have been reported in the literature, only in a limited number of cases has the integration of QTL data in breeding programs provided a meaningful contribution for an effective improvement of crop tolerance to abiotic stresses (Collins et al., 2008). Therefore, translating the knowledge gained from QTLs and other molecular studies (e.g. "omics" platforms) into a tangible product for the farmer, particularly in stress-prone areas, is by far one of the most daunting challenges faced by the scientific community (Xu and Crouch, 2008). A number of notable examples will be presented for the main abiotic stresses. Additionally, the constraints that limit the application of genomics-based approaches to improve yield under adverse conditions will be discussed accordingly. It is expected that the increasing availability of sequence data (e.g. deep sequencing of DNA and mRNA) coupled with (i) a more intense exploitation of wild germplasm (e.g. introgression libraries), (ii) novel mapping approaches and genetic materials (e.g. association mapping, nested association mapping, etc.), and (iv) platforms allowing for more affordable high-throughput genotyping and phenotyping will facilitate the identification and cloning of major QTLs for tolerance to abiotic stress. Ultimately, the successful integration of genomics-based approaches into breeding programs aimed at improving tolerance to abiotic stresses will best be achieved within a multidisciplinary context able to provide the operational framework required to correctly link the stress-responsive mechanisms of crops with the functional variation of the relevant molecular networks.

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Prunus genomics resources and their application in translational breeding

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ABSTRACT: Recent efforts by the worldwide Rosaceae research community have produced substantial genomics resources for several key species in the family. These genomics resources are the substrate for experiments to identify and mark regions of the genome controlling characters important to sustainability and improvement of the fruit tree industry. In our programs, we have focused on development of genomic resources for peach as a model *Prunus* genome. These resources include: a complete physical map anchored on the general *Prunus* genetic map, mapped EST resources, large insert libraries of most of the important *Prunus* species, and genomic sequences. All of these are housed in the Genome Database for Rosaceae (GDR) and are being utilized routinely for studies directed at marking of important traits and identification of genes controlling these traits. In this communication, we summarize the status of the *Prunus* genomic resources and present our current research on the application of these resources for characterizing genomic regions in peach and apricot that control, Plum Pox Virus resistance and chilling requirements. Both are important targets for translational breeding and sustainability in the fruit tree industry.

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Dissecting biomass yield in a perennial ryegrass inbred derived population

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ABSTRACT: Seventy-five SSR and AFLP markers were used to construct a perennial ryegrass genetic map based on 360 individuals of an inbred derived F_2 population. Quantitative trait loci (QTL) for biomass heterosis were mapped on this F_2 population. An alpha lattice design was used for the experimental design, both for the greenhouse and field experiments. In the greenhouse three independent replicates were planted as single plants and three harvests were done from the experiment. In the field the genotypes were planted in mini swards in two replicates and four harvests were carried out. Three major QTL for dry weight biomass were identified on linkage groups (LG) 2, 3 and 7, respectively. On LG 2 a QTL with an expected variance of 10% (LOD 4), on LG 3 of 19% (LOD 11) and LG 7 of 10% (LOD 8) were found. Heritablities were reasonably high: greenhouse 68% and field experiment 66%, respectively. Overall at least 39% of the variation for the trait could be explained. All dry weight biomass QTL co-localized with QTL for fresh weight. QTL for biomass is an important agronomic trait and increased biomass is still one of the most important traits in perennial ryegrass breeding programs. The metabolomes of the parental inbred lines and the F1 hybrid were compared across a growth season in a replicated design. The majority of the metabolites which were found to differ most among the inbred lines and the F1 hybrid are unknown compounds. This study gives an indication of the position of biomass QTLs and which metabolites differ among the parental lines.

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Utilization of molecular markers (SSRs and cDNAs) for screening known QTLs for late blight (*Phytophthora infestans*) resistance in potato

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ABSTRACT: A transcriptome map containing around 700 cDNA markers was constructed using the cDNA-AFLP technique. This map was anchored to the bins of a high-density reference map of potato. Subsequently over 200 published QTLs and genes were projected onto this map. The cDNA markers which are co-located with published QTLs for pathogen resistance represent potential candidate genes controlling the trait. Such bands were cloned, sequenced and homology searches were performed. Several interesting homologies were detected with resistance and stress response genes. On the other hand we have screened all known QTL positions on the 12 potato chromosomes *via* linked SSR markers for the presence of a selectable QTL for *Phytophthora* resistance in four different progenies. Progenies descended from different *Solanum* wild species as resistance sources. Leaf and tuber resistances were analysed. In all case studies several selectable QTLs were detected which descended from either parent. Tuber and leaf QTLs varied from progeny to progeny and between leaves and tubers. Resistance levels between tuber and leaf were slightly negative and not significant.

Keywords: transcriptome mapping, cDNA-AFLP, leaf and tuber blight, *Solanum* wild species.

Introduction

Molecular markers are useful to construct linkage maps and to localize monogenic and polygenic traits, allowing the efficient introgression and selection of individuals with specific characteristics already in breeding material. In potato a large amount of genomic resources are being established within the frame of international projects including a potato genome sequencing project (Ritter et al., 2005). A high density reference map of potato is available which contains 10.000 AFLP markers and is cross-referenced by numerous SSR and RFLP to other maps (UHD map; van Os et al., 2006).

Recently a complete transcriptome map of constitutively expressed genes of the potato genome has been constructed using cDNA-AFLP (Ritter et al., 2008). TDFs were anchored to the bins of the UHD map. Numerous QTL analyses have been performed in potato considering resistance and quality traits (Ritter et al. 2005). We have projected these published QTLs onto the UHD reference map and analyzed co-located TDFs.

When starting a new QTL study, usually reduced satellite maps are produced which include markers also present in other maps in order to compare results. However, this approach is laborious, expensive and time consuming. In order to reduce efforts when analysing a trait in a new genetic background, the analyses could be restricted to the screening of all known QTL positions *via* linked SSR markers for the presence of a selectable QTL. We have screened in this way four progenies descending from different resistance sources for selectable QTLs.

Materials and Methods

Plant Materials

Information about parents (SH, RH) and progeny genotypes of the UHD mapping population (SH83-92-488 × RH89-039-16) described by van Os et al. (2006) were considered for the projection of QTLs and markers.

For QTL screening with SSR markers the progenies D: *can 310956.8 x gon703354*, E: *buk210042.5 x phu81*, G: *jam27521.48 x gon703354* and N: *H88-31/34 x rap636* were used. These involve different *Phytophthora* resistance sources descending from *Solanum canasense (can)*, *S. bukakowskii (buk)*, *S. jamesii (jam)* and *S. raphanifolium (rap).*

Bio-Assays

For producing *Phytophthora* infections young plants of the progeny genotypes mentioned above were inoculated with spores of local PI isolate (NE293) and infection levels were evaluated in each genotype according to Trognitz et al. (1995). Potato tubers were inoculated with *P. infestans* following the methodology of Flier et al. (2001).

Molecular methods

Bands corresponding to transcripts co-located with QTLs for *Phytophthora* resistance were isolated, cloned and sequenced applying standard methodology (Sambrook et al., 1989). Sequence homology searches were performed in public sequence databases *via* NCBI using BLAST search algorithms.

SSR analyses were performed according to Milbourne et al. (1998) in parents and progeny genotypes using appropriate PCR conditions. Amplification products were visualized on sequencing gels through PAGE.

Data Analysis

For projection of QTLs and markers numerous publications and relevant databases dealing with QTL studies in potato were consulted. Published QTLs and resistance genes were projected onto the UHD potato reference map based on common marker intervals in different maps as described by Ritter et al (1990).

For QTL screening the detected SSR amplification products were scored for presence or absence. T-Tests were performed to analyse average resistance level differences in the genotypes belonging to the marker classes (presence *vs*. absence) of each SSR allele. Where possible also interval mapping methods for QTLs were applied.

Results

For detecting co-location between QTLs and TDFs a total of 249 published QTLs from 48 publications and different entries of the Sol Genes Database were considered. Published QTLs were projected to 184 loci due to co-location of QTLs from different studies. These loci involve 144 resistance QTLs and 76 loci for quality and other traits. In total 35 *Phytophthora* QTLs and genes were detected which correspond to 27 different locations on all 12 potato chromosomes.

Each QTL locus and TDF marker has a specific bin assignment in the parental linkage maps of the UHD population. For co-location analyses we have scanned the position data of all TDFs and projected QTLs considering distances of +/- two bins between them. In this way we detected 57 TDFs which were linked to Phytophthora QTLs.

We isolated, cloned and sequenced several of these co-located TDFs and performed homology searches. Some interesting homologies with known resistance genes and stress response genes were detected, among them figure LRR and PRP proteins, chitinases and peroxidades.

Co-location analyses between SSR markers and published QTL positions for *Phytophthora* resistance revealed 28 linked SSR markers located on all 12 potato chromosomes. On the other hand sufficient variations in resistance levels were obtained within the progenies of all resistance sources, allowing an efficient QTL analyses.

QTL screening for *Phytophthora* resistance in leaves through linked SSR markers revealed in progeny G (jam x gon) three selectable QTLs on chromosomes III, VI and VII. Significant QT allele effects were observed on chromosome VI for parent P2 (gon) while this was the case for the other parent (jam) at the other two locations. In progeny E (buk x phu) we have detected four selectable QTL s located on chromosomes III, V, VI and X. Only one on chromosome III descended from P1. In progeny D (can x phu) only one QTL was detected on chromosome VIII which descends from *S. phureja*. Some SSR markers did not show segregating SSR alleles in the populations, so that it was not possible to evaluate the corresponding genomic locations.

QTL screening was also performed for tuber infections with *P. infestans* in two populations.

Correlation analyses between leaf and tuber infection levels were slightly negative (-0.17; -0.21) and not significant. This is also reflected in the detected QTLs in each population.

In population G three selectable QTLs were detected. The QTL on chromosome VI descending from P2 (gon) was common for leaf and tuber resistance. The other two QTLs were located on chromosomes V and X and descended from P1 (jam) and P2, respectively. In population D the QTL from P2 (phu) on chromosome VIII was common for tuber and leaf

blight. In addition one QTL was detected on chromosome XI which showed significant QT allele differences for both parents. Moreover, on chromosome VII an additional, significant SSR allele common to both parents was observed.

Discussion

The projection of QTLs and cDNA-AFLP fragments onto the UHD map allowed a co-location analysis between published QTLs for *Phytophthora* resistance and cDNAs. TDF markers have generally a concrete biological meaning since they are derived from mRNA. Therefore, a particular cDNA which is co-located (or closely linked) with a published QTL could represent a potential candidate gene explaining this particular QTL. Although the probability that these TDFs could explain co-localized QTLs seems low based on the resolution of the map, we found in several cases homologies with known resistance genes. Considering that families of resistance genes are frequently organized in clusters (Gebhardt and Valkonen, 2001), the chance of finding a target gene of interest was higher in this case. However, also different, linked genes could be the true loci responsible for the QTL effect. Therefore, it is necessary to perform a complementation assay or silencing experiments in order to verify the function. If the candidate gene should represent a false positive, then it can be used at least as allele-specific marker in marker assisted breeding.

Published QTLs for *P. infestans* resistance have been reported for all 12 potato chromosomes, part of them have been validated by several authors. The SSR map established by Milbourne et al. (1998) allowed us to project linked SSR markers for all loci. Projection of SSR markers and known QTLs allow us to reduce efforts when analysing traits in a new genetic background. All known positions can be screened for the presence of a QTL by analysing only closely linked SSR markers in a new population.

We have screened these published QTL positions for the presence of QT allele differences in four different progenies and detected selectable QTLs for leaf and tuber blight in all experiments. In this way we have established a "genotypic print" of each parent (QTA genotyping) indicating selectable QTL positions and the corresponding SSR markers for marker-assisted selection (MAS) in different genetic backgrounds. These markers can be applied within potato breeding programmes for all crosses which involve the corresponding parental genotypes as resistance sources.

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Molecular mutation breeding: modern variety breeding for present and future needs

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ABSTRACT: Induced crop mutation strategies have, since the seminal article of Stadler (1928), in the past over 50 years played a major role in the development of superior crop varieties. With over 2700 officially released crop mutants in more than 170 plant species, translating into a tremendous economic impact valued in billions of dollars and tens of millions of cultivated hectares. The Joint FAO/IAEA Programme has for over 40 years been promoting the efficient use of mutation techniques as a complementary tool for developing superior crop varieties. The Joint FAO/IAEA Programme through research coordination provides a global platform for scientists to work on common induced crop mutagenesis related themes. Through the Technical Cooperation Project mechanism of the IAEA, direct technical input and guidance have been provided to scientists, especially in the Least Developed Countries (LDC) of the world and have contributed immensely to capacity building and the development of mutant crop varieties that address specific production constraints. The Joint FAO/IAEA Programme has a training, service and research and development (R&D) unit dedicated exclusively to induced crop mutagenesis at the IAEA Laboratories in Seibersdorf, Austria. In addition to the traditional roles of supporting capacity building in LDC member states of both FAO and IAEA, the R&D activities of this laboratory component addresses the enhancement of the efficiency of processes related to induced crop mutagenesis. This paper while presenting an overview of the contributions of induced mutagenesis to sustainable agricultural productivity also posits that the technology has great potentials for adding value to high yielding, stable crop varieties through the development of hardy variants that being adaptable to extreme abiotic stresses are important for addressing the constraints of climate change. Also, through the subtle modification of quality traits in otherwise good varieties, induced crop mutants enrich the crop germplasm useful for mitigating micronutrient deficiencies and meeting the requirements of niche industries. We also present the status of work on the use of a suite of integrated bio-/molecular technologies in enhancing the efficiency levels of induced crop mutagenesis. The paper highlights the central role of cellular biology in the rapid production of histonts as well as the innovative adaptation of the reverse genetics technology, Targeting Induced Local Lesions IN Genomes (TILLING) as a methodology for the high throughput detection of mutation events and hence significant reductions in the sizes of mutant populations for field trials. Reports on TILLING will include progress on the development of platforms for under-researched vegetative crops like banana and cassava. Perspectives for the future direction for the application of induced crop mutagenesis both as crop improvement and functional genomics tools are also provided.

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Synthetic hexaploids and its derivatives as sources of novel germplasm for improving biotic and abiotic stress tolerance in hexaploid wheat

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ABSTRACT: In Central and West Asia and North Africa (CWANA), wheat is the most important crop and the staple food in most countries. Approximately 50 million hectares are cultivated including spring bread wheat, durum wheat and winter/facultative wheats. However, the overall productivity is lower than all other geographic regions of the world, and lower than the world average of 2.5 ton/ha. Agro-ecologies in the CWANA share similar features to those in Australia where wheat production is predominantly rainfed. In both regions, drought and heat stress are the two most important factors limiting wheat productivity. Recently, global warming has exerbated the impact with increasing temperatures and more variable rainfall patterns in timing, duration and intensity. These have led to poor and variable harvests and in some countries within the CWANA region, to food shortages and exorbitant food which lead to concerns about food security. Within the last decade, it has clearly been demonstrated that synthetic wheats can be used to improve the efficiency of wheat germplasm enhancement for biotic stresses and increasingly for abiotic stresses (Ogbonnaya et al., 2005; van Ginkel and Ogbonnaya 2007. We present results from Australia and ICARDA on previous and ongoing efforts in the use of synthetics to mitigate these constraints including development of wheat germplasm with enhanced biotic and abiotic stress tolerance. We also examined the yield of synthetic derived wheats grown as a means of improving yield potential and productivity and found for most agronomic traits investigated that the synthetic-derived wheats have greater range of yield and yield enhancing variation than commercial cultivars under marginal environments in Australia and the CWANA regions.

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Molecular markers in the "real world": identification of resistance genes and marker assisted selection in wheat breeding for leaf rust resistance

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ABSTRACT: The breeding and cultivation of resistant wheat varieties is an effective way of controlling leaf rust (*Puccinia triticina* Eriks.). The use of molecular markers facilitates the identification, incorporation of the major leaf rust resistance genes (Lr genes) into varieties with good agronomic characteristics and the pyramiding of these genes.

Using marker assisted selection (MAS), the resistance genes Lr9, Lr24, Lr25, Lr29 and Lr35 which were not previously utilised in Hungary, have been incorporated into four Martonvásár winter wheat varieties. A backcross programme has begun, aimed at the transfer of effective Lr genes. Wheat varieties susceptible or moderately resistant to leaf rust were crossed with NILs of *Thatcher* each carrying a different Lr gene (Lr9, Lr24, Lr25, Lr29 and Lr35). PCR-based markers were used for MAS. Seven different primer pairs were used for the detection of the four Lr genes. As the result of a backcross programme, the genes were transferred into Martonvásár varieties and various BC generations were produced. Plants in the fifth backcross generation had agronomic traits resembling the recurrent parent. All the primers or primer pairs produced amplification products characteristic of the given Lr gene. By pyramiding several Lr genes in a single variety, the leaf rust resistance of wheat varieties can be made more durable. Work aimed at pyramiding resistance genes is currently underway in Martonvásár, and plants containing the gene combinations Lr9+Lr24, Lr9+Lr25 and Lr9+Lr29 are now available. From the BC_2F_3 generation of the Mv Emma/Tc-Lr9 combination 287 lines were tested for leaf rust resistance in artificially inoculated nursery. DNA samples of the lines were isolated and were sent to Scotland for molecular marker detection. Using a published marker (Gupta et al. 2005), primers were designed to identify both the resistant and the susceptible offsprings. From the 287 lines tested 222 lines proved to be phenotipically resistant to leaf rust. In 52 lines we observed segregation for resistance, while 13 lines were susceptible to the pathogen. The results of the resistance test and the molecular marker detection method developed agreed in most cases. In 282 cases we received correct or acceptable classification with molecular markers.

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Testcross performance of doubled-haploid lines developed from European flint maize landraces

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ABSTRACT: Utilizing the genetic potential of maize (*Zea mays* L.) landraces proved to be very difficult in the past due to deleterious recessive characteristics becoming apparent at high frequency under inbreeding. Landrace-derived doubled-haploid (DH) lines were developed by *in vivo* haploid induction. Materials consisted of 44 DH lines from 'Schindelmeiser', 25 from 'Gelber Badischer Landmais', and 11 from 'Lucq-de- Béarn' along with 3 elite inbred lines and 2 check hybrids. Entries were evaluated for testcross performance under conventional farming, organic farming, and low nitrogen (N) supply in four regions of Germany in 2005. Mean grain yield of DH lines amounted to 76 % compared to that of the 3 elite lines but best DH lines came close to the level of the latter. Yield reduction under low N compared to conventional farming was lower in the DH lines derived from ' Lucq-de-Béarn' than in the elite lines. Moderately strong phenotypic correlations occurred among the 3 farming systems for grain yield but strong ones for dry matter content. Highly significant genetic variance existed in each of the 3 DH-line groups. Coefficients of genetic variation within landraces generally ranged between 5 and 10 % being equal to or larger than most coefficients reported for elite intra-pool F2 populations in the literature. In conclusion the *in vivo* haploid induction technology proved to be a valuable tool for deriving DH lines with a high potential for various utilization purposes, and for broadening the genetic base of elite breeding materials.

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Breeding for stability across increasingly variable environments

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ABSTRACT: Over the last half century, increasingly high wheat yields have come from the integration of breeding and agronomy based on large-scale use of synthetic inputs. These inputs have helped to smooth out variations in the environment towards ideal growing conditions. However, climate change means that the underlying environment is becoming more variable and less predictable. Added to this, the price of oil and other resources is climbing steeply so that alternative ways of buffering against environmental variation are needed. One such is to increase significantly the genetic variation available within crops, for example through 'evolutionary breeding' (Suneson, 1956), based on the idea of cropping segregating populations of inbreeding cereals, rather than monoculture of pure line varieties. Here, we inter-crossed 20 winter wheat varieties in all combinations to produce cross populations based either on high yield or high quality varieties, together with one that included all varieties. A repeat set of these three populations included several male sterile lines. From the F3, they were grown at two conventionally farmed sites and two organic. The results from replicated field trials in the F4 to F6 generations at the four farms will be discussed. The main concern was to determine whether the populations were providing better buffering than their parents against the environmental variation among the trials. However, each character needs to be stable at high levels: stable high yield is much more difficult to achieve than stable low yield. To determine this we used the Genstat GESTABILITY procedure based on Lin and Binns (1988) test for cultivar superiority. For each variety, this is the sum of the squares of the differences between its mean in each environment and the mean of the best variety there, divided by twice the number of environments. The analyses showed that, for a range of important characteristics, the populations produced values that were often above the mean of the relevant parents though not as high as the best single varieties. However, by combining the superiority values across characters, it was then evident that the populations tended to outperform the pure lines in terms of stable high performance. Physical mixtures of the parents also performed well but tended to be less effective than the populations, particularly under organic conditions. There is a clear need to extend the range of test environments to confirm these trends; this is now in progress. The paper will also report on an analysis of a range of molecular markers, unique for each of the parents used. This helps to determine the contributions of the individual parents to population performance and the extent to which this varies across environments. Use of the populations in agriculture will depend on an acceptable process to provide a return for the breeder and to ensure the quality of the material available to the farmer. The DUS system is not relevant for describing dynamic populations, so we are currently proposing a VCU system that depends on

comprehensive traceability of any material released, a minimum seed quantity (to avoid genetic drift) and standard seed quality regulations.

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Reducing the yield penalty of the resistance of wheat to septoria tritici blotch

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ABSTRACT: Septoria tritici blotch (*Mycosphaerella graminicola*) first emerged as a major disease of wheat (*Triticum aestivum*) in Europe in the 1970s. Since then, it has proved difficult, though not impossible, to produce varieties which combine effective resistance to septoria with high yield. Analysis of data from trials in the UK between 1980 and 2000 showed that selection for increased yield has inhibited selection for septoria resistance. Nevertheless, some varieties were considerably less susceptible to septoria than would be predicted by their yield, implying that wheat breeders should be able to separate the two traits and thus produce high-yielding, septoria-resistant varieties. An association genetics analysis of 225 varieties, covering the history of wheat breeding in the UK, predicted several QTLs controlling partial resistance to septoria. Some of these QTLs were associated with reduced yield but others were not. Genetic analysis of septoria resistance and yield in a set of crosses between wheat varieties has confirmed the presence of several of the QTLs postulated by association genetics and has confirmed that resistance to septoria and reduced yield are associated. It has also shown, however, that it should be possible to break that connection because several septoria resistance QTLs are not associated with a yield penalty. By contrast, analysis of the UK trial data detected no association of resistance to powdery mildew with reduced yield. It may be that, because mildew resistance has been selected by breeders for much longer than septoria resistance, genes which associate mildew resistance with reduced yield have been eliminated from UK wheat germplasm.

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Long-distance spread of aggressive wheat yellow rust

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ABSTRACT: Strains of the biotrophic wheat yellow rust fungus, *Puccinia striiformis* f.sp. *tritici* may vary in virulence phenotype ('race') as well as in aggressiveness, i.e., the ability to cause severe disease rapidly on a susceptible wheat cultivar. Both traits have direct implications for the expected level of genetic disease control by host resistance, the former determining the range of major gene (race-specific) resistances that may provide protection against yellow rust at a specific time and location whereas increased aggressiveness may affect any other type of resistance. High mutation rates for virulence and potential spread of infectious uredospores by wind across long distances are other important features of the yellow rust fungus. Here we report an intercontinental spread of two closely related fungal strains characterised by shorter generation time and increased spore production capacity as compared to previous strains found in Europe and North America. We sampled more than 150 representative single-lesion isolates from a large number of wheat cultivars on five continents (Hovmøller et al. 2008). Based on epidemiological observations of yellow rust epidemics in these areas, we demonstrate that the aggressive strains have spread to North America, Australia and Europe in less than three years. They became rapidly widespread across the two former continents, including areas which were previously considered too warm for yellow rust (Milus et al., 2008), while resistant cultivars have so far prevented widespread epidemics in Europe. The strains, which were also observed in West- and Central Asia and East Africa, showed only limited divergence in virulence phenotype. The data also gave evidence for additional intercontinental *P. striiformis* dispersal events in the past. The results stress the importance of a continuous search for sources of resistance, which may contribute to prevent or delay cereal rust epidemics, and the need for international collaboration on research, resistance breeding, and pathogen surveys as well as disease management strategies.

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The EC's sixth framework integrated project BIOEXPLOIT on disease resistance breeding in potato and wheat

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ABSTRACT: In 2005, the European Commission started the funding of a large integrated project on the 'Exploitation of natural plant biodiversity for the pesticide-free production of food (BIOEXPLOIT)'. Sixty-five research groups that are part forty-three universities, institutes, and companies from 12 different countries are involved in BIOEXPLOIT. Groups are working in collaborations towards a wide range of objectives, from fundamental research on molecular mechanisms underlying disease resistance to the application of molecular markers in elite material from small and medium size companies. The activities are divided over eight subprojects that feed into each other.

These subprojects aim at:

- The identification of targets for durable resistance by analyzing fungal effector molecules
- The mapping, isolation and characterization of genes responsible for qualitative and quantitative disease resistance in potato and wheat
- The unraveling of molecular mechanisms underlying innate resistance to plant pathogens
- The exploration of natural biodiversity on genetic loci associated with disease resistance in wheat and potato accessions in genebanks
- The improvement of disease resistance in potato and wheat through marker-assisted breeding
- The improvement of disease resistance in potato and wheat through genetic engineering
- The training the scientists and breeders in molecular disease resistance breeding

The project will last five years with most of the fundamental research (e.g. on fungal effectors and R gene mechanisms) in its first three years, while the technological implementation and validation will happen mostly in the second halve of the project. BIOEXPLOIT is a project that integrates disease breeding research at different levels, including a wide range of academic expertise at fundamental, strategic, and applied levels. But, importantly, it also manages to integrate and to build on various ongoing research and breeding programs that were running independently as national initiatives for some time.

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Molecular marker technologies and resistance gene pyramiding

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ABSTRACT: The aim of the European FP6 "BioExploit Food CT 2005-513959" project is to develop efficient and rational breeding strategies using genomics and post-genomics tools to exploit natural host plant resistance. Keygene N.V. coordinates a subproject with the objectives to increase disease resistance in potato and wheat through MAB by developing and validating high throughput molecular markers technologies for commercial breeding programmes. The development of genotyping platforms able to analyze and score markers at the tetraploid level would greatly enhance the possibility to perform marker assisted breeding in auto-tetraploid species like potato. In collaboration with the University of Wageningen and SCRI, Keygene is developing AFLP® a SNP genotyping platform (SNPWave®) for auto-tetraploid species where the allele-call is based on difference in band intensities. In collaboration with DISTA (University of Bologna) and Societá Produttori Sementi, Keygene N.V. generated a durum wheat genetic map based on AFLP markers analyzing a balanced four-way F_1 population segregating for resistance and quality traits. SNP polymorphisms specific of the four parental cultivars were identified using the novel Keygene CRoPS™ Marker Technology (Complexity Reduction of Polymorphic Sequence) and a subset of SNP were used to integrate in the genetic map. Keygene N.V. is also collaborating with the Plant Breeding and Acclimatization Institute (Radzikow, Poland) and Szelejewo Plant Breeding (Piaski, Poland) using MABC for introgression and pyramiding of resistant genes in wheat.

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Resistance proteins: scouts of the plant innate immune system

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ABSTRACT: With the growing concern for the environment there is a growing demand for production systems that do not rely on the use of pesticides. One way to reduce the need for pesticides is to exploit natural disease resistance sources. Breeders have successfully utilized such natural resistances over the last century and introgressed many, so called, resistance (*R*) genes from wild relatives into their elite lines. To understand the molecular mechanisms underlying *R* gene function, the last decade many research efforts have been focused on cloning of these genes. This resulted in cloning of >50 R genes from model and crop plants. The identified R genes can be divided into two groups. One group contains genes encoding transmembrane proteins carrying an extracellular receptor-like domain and the second group encoding intracellular proteins. The intracellular receptors perceive the presence, or actions, of pathogen-derived proteins secreted inside the host cell, whereas the extracellular ones monitor the presence of such proteins in the extracellular apoplastic spaces. This combination of immune receptors provides a plant with a robust pathogen-sensor system that is hard to evade by an attacking microbe (Tameling et al. 2007). The majority of intracellular resistance proteins are multi-domain proteins containing a central nucleotide binding (NB) domain fused to a leucine rich repeat (LRR) domain. This dual core is often connected with linkers to various N and C-terminal domains. We study the tomato NB-LRR R proteins I-2, mediating resistance to *Fusarium oxysporum*, and Mi-1 conferring resistance to the root-knot nematode *Meloidogyne incognita*. In this talk I will present a structure-function analysis of the different subdomains and report on intra- and intermolecular interactions observed in NB-LRR proteins such as I-2 and Mi-1. This part will be concluded with a model on how R proteins function as molecular switches controlling disease resistance signaling (Takken et al. 2006). To conclude, I present data on *Fusarium* proteins that are recognized by the matching tomato R proteins. Besides activating plant defense, these Fusarium proteins are important for virulence as they enhance colonization of susceptible plants. Unexpectedly, the virulence function of Avr1 turned out to be the suppression of I-2 and I-3 function. This exciting observation provides insight in the complex interactions and ongoing warfare between plants and their pathogens (Houterman et al. 2008).

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Grain yield stability of different genotypes of oat (*Avena sativa* L.)

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ABSTRACT: Genotype × environment interaction and stability for grain yield of nine oat (*Avena sativa* L.) genotypes, including four Canadian cultivars and five breeding lines from Turkey were analyzed. The genotypes were evaluated in 16 environments in 2001 and 2002, using a randomized complete block design with three replications. Highly significant ($P<0.01$) differences were found among the environments and genotypes for days to heading, spikes/m^2, seeds/spike, 1000-seed weight, harvest index and grain yield. Genotype × environment interaction was also significant ($P<0.01$) for 1000-seed weight, seeds/spike and grain yield. Genotypes of Pacer and Boyer from Canada and a breeding line from Turkey had the highest mean of grain yield (6.1, 5.7 and 5.6 t/ha, respectively). Based on the Wricke Ecovalance (Wricke, 1962) and Shukla Stability Variance (Shukla, 1972), classification of genotypes for stability of grain yield was similar and genotypes of Boyer and breeding line No. 36 from Turkey had higher stability. However, based on the method of Muir *et al*. (1992), cultivars of Caliber, Boyer and breeding line No. 36 from Turkey were more stable genotypes. The method of Muir *et al*. was more effective than the other univariate statistical methods to determine the stable oat genotypes.

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Genotype-specific volatile interactions in barley influence resistance to aphids

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ABSTRACT: Volatiles from certain barley genotypes can induce changes in certain other barley genotypes that in turn affect aphid acceptance of those exposed barley plants. When results from resistance screening tests with *Rhopalosiphum padi* were compared with results from volatile-exposed plants, barley genotypes characterized as partially resistant to *R. padi* in screening tests were less accepted by the aphids in tests for volatile-induced resistance. Furthermore, there was a negative correlation between year of cultivar release and reduction of aphid acceptance of barley volatile-exposed plants, i.e. the older the cultivar the less acceptable it became after volatile exposure, in general. One reason for this might be that the seed lots representing older cultivars, up to 100 years old or more, are genetically more heterogeneous. We found a positive correlation between this characteristic based on molecular markers and the strength in aphid avoidance of volatile-exposed plants. Within the group of volatile-treated plants tested together, we also found that plant-to-plant variation in aphid responses was correlated with genetic heterogeneity. We do not know whether the lower frequency of volatile-induction found in combinations of modern compared to older cultivars is due to lower genetic variation within the seed lot, or if the capability to induce and become induced is successively lost with breeding. Crosses have recently been made to test whether the capability to produce inducing volatiles can be combined with the capability to respond to those volatiles, i.e. if a genotype can induce itself. We have found few cases of this, especially in modern cultivars. It should be pointed out that the volatiles in our studies are released by apparently undamaged plants. Efforts are being made to identify the active volatiles in emitting plants and the regulated genes in responding plants. Volatile-induced plant responses may have effects also on other organisms. We have shown that natural enemies of aphids, a ladybird beetle and a parasitoid, can respond with increased attraction, and plant pathogens may also be affected. If so, this may be one reason why cultivar mixtures are often less infected by pathogenic fungi.

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Combining ability analysis of grain yield in maize (*Zea mays L.*) in agro ecological conditions in Kosova

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ABSTRACT: Yields are determined from the genotype, agro ecological and agro-technical factors. A diallel cross between inbred lines of maize *(Zea mays L.*) with medium maturity was carried out and evaluate to estimate genetic trait for yield. The main object was to test and identification reaction of some hybrid combination of F_1 generation for yield. Investigations includes 10 inbred lines that had been in diallel crossing for General Combining Ability (GCA) and Specific Combining Ability (SCA). The component of genetic variance was calculated using Griffing's formula (1956), method 2, mathematic model I. $X_{ij}=\mu+gi+gj+s_{ij}+e$, to detect the relative importance of additive and non additive gene effects. Additive gene effects were more important than non additive since the ratio was 0.14 among GCA and SCA. With maximal yield where heterozygote combination from inbreed lines L_6xL_{10} (xg=14.14 tha^{-1}), while minimal value was combination L_1xL_{10} (7.61t ha^{-1}). The grand mean value μ of F_1 generation was 10.87tha^{-1}. Differences for yield of F_1 generation were +3.34 or 30%, respectively – 3.19 t ha^{-1} or 29%, compared with value μ. The total variability between genotypes was ± 59 % with high significance. ANOVA's test for combination ability of GCA and SCA effects were significant at $p\leq0.05$ and $p\leq0.01$ level of probability, respectively.

Keywords: F_1 generation, combining ability, GCA, SCA, Yield.

Introduction

The Improvement of maize yield depends on the knowledge of the type of the gene action involved in its inheritance and also the genetic control of the related traits such as capacity of production (Rezaei et al., 2004). Also the choice of the most efficient breeding program depends on that information (Liao 1989, Pal & Prodham 1994). The general combining and specific abilities as GCA and SCA effects are important indicators of potential value of inbred lines in hybrid combinations. Differences in GCA effects have been attributed to additive, interaction of additive x additive, and higher-order interactions of additive genetic effects in the base population, while differences in SCA effects have been attributed to non-additive genetic variance (Falconer, 1981). Evaluation of crosses among inbred lines is an important step towards the development of hybrid varieties in maize (Hallauer, 1990). Ideally, this process should be through the evaluation of all possible crosses (diallel crosses), where the merits of each inbred line can be determined. The diallel analysis provides good information

on the genetic identity of genotypes especially on dominance-recessive relations and some other genetic interactions. Diallel crosses have been used in genetic research to determinate the inheritance of a trait among a set of genotypes and to identify superior parents for hybrid or cultivar development (Weikai and Kang, 2003). The objective of our study was to estimate GCA and SCA among these inbred lines for yield in agro ecological condition in Kosova , and consequently, to identify superior genotypes developed from them.

Materials and methods

Plant material used for crosses in this research was 10 selected superior maize inbred lines on code name: L_1, L_2, L_3,..L_{10}, with medium maturity, originating from the Agriculture University of Tirana (AUT)- Albania were used (see table 1). All possible crosses among these inbred lines were made in a diallel crossing block. For three year (2000-2003) period the study was conducting to investigate adaptability of this trait for inbred lines in agro ecological condition of Kosovo, Ferizaj location (580m.a.s.l). After year four (2004), the ten (10) mentioned maize inbreed lines were crossed using diallel system (Griffing, 1956), and at the year five (2005) these genotypes were placed in experimental plots (EP) with F_1 generation (Combination) where study of general (GCA) and specific (SCA) combining ability for yield was conducted. Statistical analysis used mathematical and statistical models "MSM" which involved randomized block design experimental (RBDE) with three replications, 45 combination (C) of F_1 generation x 3 replications (R) = 135 Experimental Plots (EP).The experimental plots was 3m long and 60 cm apart, with plant to plant distance of 30 cm or 55000 plants per ha^{-1}, the active surfaces for repetition was 5.4 m^2/R x 3R = 16.20 m^2, whereas deep planting was 3-5cm, cultivated with intensive agro technique including fertilizer NPK(15:15:15),URE 46%. The number of plants per plot was used as the covariate to adjust plot yield .While yield (t ha^{-1}) was determined from average of 10 ears or plants sampled randomly from each plot with moisture 14% or 10 plants/replication x 3R =30 plants or in total 1350 plants. Genetic interpretations and analyses of similar experiments can be found in numerous papers such as Hayman 1954, Griffing's 1956.The diallel analysis ,as described by Griffing (1956) method 2, model I : $X_{ij} = \mu + gi + gj + s_{ij} + e$. Statistical analysis were calculated with package MSTAT-C (Russel 1996):

Where,

x_{ij} = is the mean of i x j^{th} genotypes, μ = is the population mean,

g_i and g_j = is the GCA effect for parent, S_{ij} = is the SCA effect, and e= is the error.

That formula gives component of genetic variance and genes values .GCA and SCA mean squares were calculated using as follow:

$$gi = \frac{1}{p} + 2(Ti + ii) - \frac{2}{p} GT \quad \text{and} \quad S_{ij} = X_{ij} - \frac{1}{p+2}(Ti + ii) + (Tj + jj) + \frac{2}{(p+1)x(p+2)} GT$$

Where,

g_i = GCA effect of the i^{th} parent ,

g_j = GCA effect of the j^{th} parent ,
s_{ij}= SCA effect of the cross between the i^{th} and j^{th} parents and
p = number of parents involved
Whereas Mid parent heterosis (MPH) was calculated as: $MPH=\frac{F1-MP}{MP} x100$

Where,F_1 is the mean of the F_1 hybrid performance and $MP=\frac{P1+P2}{2}$, in which P_1 and P_2 are the means of the inbred parents, respectively.

Results and discussion

The results showed a significant genotypic effect in the yield with high phenotypes differences. The genotypes average experimental value μ for yield in ours investigations where 10.8 tha^{-1}, that is relatively high that can guarantee a higher yield, when compared with average yields at Kosova was for three (3x) times higher. These hybrid combination showing higher ability of yield in agro ecological conditions in Kosovo region. The hybrid combination of inbred lines L_6xL_{10}, realised maximal average value xg=14.14 t ha^{-1}, these yield comparing with value μ was with differences + 3.34 t ha^{-1} or 30% higher. Minimal average yield has been realised at hybrid combination L_1xL_{10}, xg=7.6tha^{-1}, distinctions with value μ were -3.19 tha^{-1} or 29% lower. Variations between maximal and minimal average value were 6.53 t ha^{-1}, significant at the $p \leq 0.01$.The total differences between F_1 generation compared to their parent lines, was positive values (d=F_1-MP =+7.16), that shows higher heterosis. Value μ for heterosis was 210%. Higher value realised hybrid combination of inbred lines L_2xL_4(326%), compared with value μ distinctions was +116 or 55% higher, while lower heterosis realised combination L_5xL_{10} with value 107% , while differences from μ was -103 or 49%. Many authors as (Tollenar and Lee, 2004) analysed some maize cultivars for heterosis value and yield of F_1 generation that realised average values μ till 167 %.

Table 1. Heterotic effect for grain yield with moisture 14%.

Line	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	L_9	L_{10}	F_1 Mean
L_1	3.0	12.01	11.73	10.45	13.85	12.42	9.0	12.93	11.69	7.61	11.29
L_2		3.4	10.68	11.52	11.82	11.75	10.84	11.06	12.66	9.74	11.25
L_3			3.1	10.40	11.46	9.03	10.90	8.79	11.49	10.90	10.42
L_4				2.10	10.41	11.96	11.51	8.26	9.45	10.97	10.41
L_5					4.50	12.41	10.50	11.65	9.65	8.82	10.60
L_6						4.70	12.52	9.67	12.20	14.14	12.13
L_7							4.80	12.52	12.47	12.05	12.34
L_8								3.50	11.48	10.47	10.97
L_9									3.30	11.04	10.0
L_{10}										4.0	9.5
μ											10.8

$LSD_{0.05}$ = 1.24 ; $LSD_{0.01}$ = 1.64; CV= 7.11 %; SE = 0.69.

ANOVA's test for combining ability was with higher significance for $p \leq 0.01$ and 0.05. Coefficient of value between GCA and SCA was 0.14, these values present that additive gene effects were more important than non additive effects for yield. High estimates of GCA realised inbred lines L_6 (+0.64), while the minimal values realised maize line L_4(-0.720), when comparing with maximal values for GCA (L_6) the variations were ± 0.08 with significance for LSD $_{0.05}$= 0.03 and $_{0.01}$=0.04. The effect of S.E was 0.012. (see Figure 1).

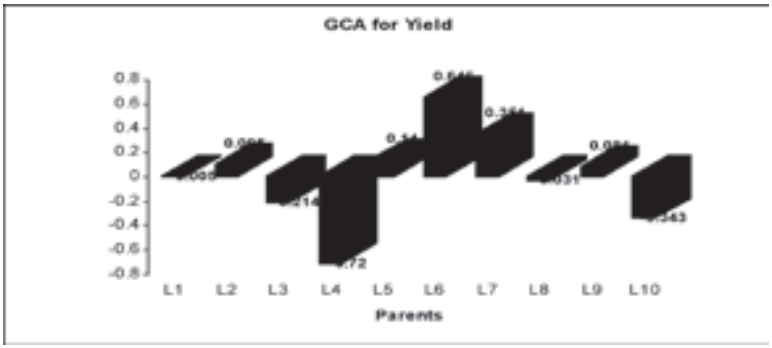

Figure 1. Estimate of GCA effects for maize inbred lines

SCA effects for yield in each parent and parental combination are presented in table 2. The results of our investigations also were highly significant at the $p \leq 0.01$. The best SCA realised hybrid combination L_6xL_{10} (+4.036), these value showed variability between investigative materials and productivity genotypes (Aliu 2003), while the lowest value was obtained at hybrid combination L_1xL_{10} (-1.84). It was not possible to prove the rule that inbreds with good GCA usually had the good SCA . Namely, the inbred L_6 had expressed the investigated trait, but L_{10} of this inbred showed negative value (Aliu 2006).

Table 2. Estimated of SCA effects for grain yield in a diallel among 10 maize inbreds.

Parent	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
P_1	-6.76	2.11	2.15	1.37	3.91	1.98	-1.14	3.16	1.81	-1.84
P_2	2.11	-6.55	1.00	2.34	1.78	1.20	0.59	1.20	2.67	0.19
P_3	2.15	1.00	-6.29	1.53	1.73	-1.20	1.05	2.83	1.81	1.65
P_4	1.37	2.34	1.53	-6.22	1.17	2.23	2.08	-0.79	0.28	2.22
P_5	3.91	1.78	1.73	1.17	-5.61	1.82	0.21	1.74	-0.36	-0.77
P_6	1.98	1.20	-1.20	2.23	1.82	-6.36	1.72	-0.74	1.66	4.036
P_7	-1.14	0.59	1.05	2.08	0.21	1.72	-5.70	2.40	2.24	2.23
P_8	3.16	1.20	2.83	-0.79	1.74	-0.74	2.40	-6.23	1.63	1.04
P_9	1.81	2.67	1.81	0.28	-0.36	1.66	2.24	1.63	-6.62	1.49
P_{10}	-1.84	0.19	1.65	2.22	-0.77	4.03	2.23	1.04	1.49	-5.13

LSD $_{0.05}$= 0.26; LSD $_{0.01}$= 0.34; S.E.(sij) = 0.13.

Conclusions

The diallel combination results obtained from our research for F_1 generation showed a high range of performance of Yield and heterosis for traits investigates and such, they could be exploited further for their heterotic capacities. The parental lines with significant GCA can be used to improve traits for yield .The Inbred line with higher value for GCA was L_6 and L_7. For SCA was combination L_6xL_{10}, while L_1xL_5 ranked with minimal values compared with L6xL10. Considering this, could be stated that strong SCA effects can be advanced for hybrid formula release when other yield stability factors are taken in consideration, also.

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Performance of Chinese vegetable cowpea accessions under dry savanna conditions in North-East Nigeria (Adamawa state)

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ABSTRACT: Despite the importance of vegetable cowpea to both rural and urban communities, the crop is not well known in Nigeria especially in the North-Eastern part of the country where the bulk of the country's cowpea is produced. Ten Chinese accessions of vegetable cowpea, Cultigroup *Sesquipedalis,* were evaluated in two locations in semi-arid conditions in Adamawa State, North-East Nigeria. The objective was to evaluate their performance in the two areas. Significant variability in the performance of the accessions with respect to all traits studied was observed with the exception of days to first flower, days to 50% pod and terminal leaflet length. All the accessions were vigorous growing, flowering earlier and performing very well. TVnu 14869 produced exceptionally long succulent pods measuring 69.76 cm, followed by TVnu 14868 (63.37cm). TVnu 14871 produced the highest fresh pod weight of 4.0 tons per ha, while TVnu 14863 had the highest grain yield (109.3 kg per ha). The accessions also interacted significantly with both the year and the location of production. Because of the good performance of the accessions across different agro-ecological zones, vegetable cowpea can be incorporated into the food security and poverty alleviation strategies of countries especially in semi-arid regions of the developing world.

Keywords: Character, cultivargroup, performance, *Sesquipedalis*, vegetable cowpea.

Introduction

All cultivated cowpea are grouped under *Vigna unguiculata* subspecies (ssp.) *Unguiculata* which is subdivided into cultivar group *Unguiculata*, cultigroup *Biflora*, cultigroup *Sesquipedalis*, and cultigroup *Textilis* (Maréchal et al. (1978a). Faris (1963, 1965) concluded that the cowpea arose from the domestication of *Vigna unguiculata dekindtiana* forms in West Africa. The post-domestication evolution of cultivated *V. unguiculata* has two sequential components, an African followed by an Asiatic. Smartt (1990) noted that the African dimension embraced primary domestication and evolution of *unguiculata* form, and the Asiatic, the subsequent evolution of the cultigroups *Cylindrica* and *Sesquipedalis*. In the African context, the cowpea's role was predominantly that of a pulse although it may be exploited to a minor degree as a leafy vegetable. In Asia, during the process of domestication, the species was subjected to different selection pressures. Steele and Mehra (1980), Smartt (1990) and other workers noted that selection was practiced among the introduced *unguiculata* lines for its long, succulent, and fleshy pod types

which eventually culminated in the *Sesquipedalis* cultivars and landraces. Selection also was practiced for cultigroup *Biflora* for its erect and bushy fodder type.

The crop is grown throughout the tropics and subtropics as a grain legume, green vegetable, forage, cover crop, etc. In Western Europe, for instance, vegetable cowpea is consumed as immature pods. Green mature and immature pods are also grown for the canning and freezing industries. Despite the tremendous importance of vegetable cowpea, however, little is known about it in Nigeria, especially in the North-Eastern part of the country where the bulk of Nigeria's cowpea is produced. The few areas where vegetable cowpea is produced such as in the South East (Uguru, 1998) and in Jos Plateau, the vegetable cowpea grown is that of cultigroup *Unguiculata*. The search for new genetic variability and its utilization by plant breeders for the purpose of improving agricultural productivity has been documented by numerous workers (Aliyu, 2007). The aim of this work was to evaluate the diversity and performance of ten Chinese accessions of vegetable cowpea cultigroup *Sesquipedalis* in Yola and Ganye areas of Adamawa State in North-Eastern Nigeria.

Materials and Methods

Plant material and growing conditions

Seeds of ten accessions of vegetable cowpea (cultigroup *Sesquipedalis*) collected from the World cowpea germplasm maintained at the Gene Bank of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria were used for the study. The accessions were: *Tropical Vigna unguiculata* (TVu) 21, TVu 14861, TVu14863, TVu 14865, TVu 14867, TVu 14868, TVu 14869, TVu14871, TVu 14872, and TVu 14874. TVu 21 collected from The Philippines, had erect growth habit while the remaining nine accessions from China were trailing (climbing). The materials were planted out in a randomised complete design with three replicates for two years in Yola (latitude 9^0 10' N and longitude 12^0 35' E) and Ganye (latitude 8^0 30' N and longitude 11^0 50' E) areas of Adamawa State in North-Eastern part of Nigeria (two locations per year and four locations for two years). Yola, with a mean annual rainfall of 900mm to 1100mm lies in the Northern guinea Savanna Zone while Ganye with a mean annual rainfall of 1100mm to 1600mm is in the Southern Guinea Savanna Zone. The field was measured 37.5 m in length and 27 m in width. This gave a total area of 1012.5 m^2. Each replicate consisted of 10 plots, making a total of 30 plots for each location. Each plot measured 8 m in length and 5 m width. Path ways of 0.5m between plots and I m between replications were provided. The seeds were hand planted at a row spacing of 1m and plant spacing of 1m. Each plot was planted with eight seeds per row in five rows, giving 40 plants per plot. Weeding was done manually with a hoe and staking was done 2-3 weeks after emergence. The pods ripe unevenly, thus five harvests (hand pickings) were conducted.

Characterization

Data were recorded for each of the ten accessions on number of days to first flower, number of days to first pod, number of main branches per plant, number of days to 50% flowers,

number of days to 50% pods, terminal leaflet length (cm), pod length (cm), number of seeds per pod, fresh pod weight (tons/ ha), number of pods per plant (five pickings), 100 seed weight (g), grain yield per plot (kg).

Biometrical procedures

Analysis of variance was performed for each measured trait on individual and across all locations and year basis. Each trait was computed on plot means. The means for the two years and four locations were pooled as described by (Singh and Chaudhary 1985). Means were separated using Duncan's Multiple Range Test (DMRT) described by Duncan (1955) for both individual and combined analysis of variance.

Table 1. Performance (ranges and means) for ten (10) characters of vegetable cowpea.

Character	Range	Mean
Days to fist flower	34.10-42.33	34.76
Days to first pod	46.81-57.21	52.19
Number of branches	4.11-5.59	4.62
Terminal leaflet length (cm)	12.35-15.66	15.34
Pod length (cm)	36.71-69.76	54.77
Number of seeds per pod	16.90-18.76	17.97
Fresh pod weight (t/ha)	1.0-4.0	1.63
Number of pods per plant	10.28-17.81	14.31
100 seed weight	12.07-13.46	12.96
Grain yield (kg/ha)	56.8-109.3	81.74

Results and discussion

Significant differences existed in the mean performance of the ten accessions for all the characters with the exception of days to first flower, days to 50% pod and terminal leaflet length. TVu 14869 which produced the longest pods of 69.76 cm exceeded the average pod length of 54.77 cm for the ten accessions (Table 1). This figure also exceeded the average of 37.23 cm reported by Hazra, *et al*. (1993) from India, 16.2 cm reported by Uguru (1996) from Nigeria and 35.02 cm by IITA characterization, also in Nigeria. Significantly, while, Hazra, *et al*. (1993) conducted their studies in the humid tropics of India, Uguru (1996) conducted his studies in the humid tropics of South-East Nigeria and IITA characterization was done at its headquarters also situated in the humid tropics of South-Western Nigeria, the present evaluation was carried out in the northern and Guinea savannah zones of North-eastern Nigeria. The present study also recorded the earliest flowering time of 35 days after planting to 46.51 days of Hazra, *et al*. (1993) and 42 days of IITA characterization. The results indicate that the accessions would perform better in the hot and dry savannah areas characteristic of Northern Nigeria. The average number of pods (14.31) produced per plant was far less than the 52 pods recorded by Uguru (1996) and 18.86

Table 2. Mean squares for twelve characters of vegetable cowpea measured across two locations for two years.

Source of variation	DF	Days to first flower	Days to first pod	Number of branches	Days to 50% flower	Days to 50% pod	Terminal Leaflet length (cm)	Pod length (cm)	Number of seeds per pod	Fresh pod weight (t/ha)	Number of pods per plant	100 seed weight (g)	Grain yield (kg/ha)
Year	1	1.94	12.74	46.87**	1.87	13.33	1217.30**	464.13**	12.78**	23.49**	48.38**	1.40	2.15
Replication	2	1.72	6.97	0.32	5.83	8.80	203.65	29.26	6.52	0.86	1.97	2.80	1.08
Location	1	491.58*	452.95*	1.36*	880.20*	43.20*	104.16	276.64**	33.70**	18.96	113.68**	15.13	0.133
Genotype	9	216	3.62	5.11**	20.05*	9.04*	147.34*	2150.27**	6.60**	912.86**	15.75**	5.50**	40.85**
Location x year	1	5.34*	4284*	70.53**	0.40*	38.53*	296.41*	10.20*	4.80*	0.66*	24.66**	2.52	16.05*
Genotype x year	9	1.45*	1.93*	0.52*	5.33*	2.79*	147.75*	97.86**	1.76**	19.57**	1.99*	1.04*	3.33*
Location x genotype	9	6.38**	3.5*	3.57**	17.78	22.55**	194.03*	157,55**	4.07*	30.40**	5.44**	1.45	2.74
Genotype x location x year	9	1.65*	2.27*	0.32**	3.94	2.66*	217.97*	29.59*	1.07*	10.70**	3.46*	0.58*	1.80*
Pooled error	80	123	1.39	0.37	6.60	5.04	187.40	8.63	1.12	2.12	1.24	1.20	12.64

*, ** Significant at 0.05 and 0.01 probability, respectively

pods recorded by Hazra, *et al*. (1993). The figure (14.31) of the current evaluation is similar to that of the latter author (18.86 pods) because the materials utilized were from the same cultivar group *Sesquipedalis* which is characterised with the longest pods within the cultigroups. The 52 pods reported by Uguru (1996) is because the materials utilized by the author are from the cultigroup *Unguiculata* which has some characters that are midway between cultigroup *Biflora* and cultigroup *Sesquipedalis*. TVnu 14871 recorded the highest fresh pod weight of 4.0 t/ha. This falls within the upper limit of the yield range of 1.5-6.0 t/ha of vegetable cowpea reported by Tindall (1986).The genotypes (accessions) also interacted significantly with both the year and the location of production (Table 2). This agrees with the fact the two locations in the study are under different agro-ecological zones. Because rural families derive food, animal feed, cash and spill-over benefits to the farmlands, coupled with the fact that the relatively poor urban communities utilise the grains as a cheap nutritious food, vegetable cowpea can be incorporated into the food security and poverty alleviation strategies of the United Nations. This could be particularly so in developing countries especially in the tropics and semi arid lands as indicated by the performance of the Chinese vegetable cowpea accessions in the savannah areas of Adamawa state in the North-Eastern part of Nigeria.

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Citrus tetraploid rootstocks are more tolerant to water deficit than diploid

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ABSTRACT: Citrus are grown in semi arid areas that are exposed to water deficit. In citrus, polyploidy is a natural phenomena and the presence of tetraploid plants among rooostocks seedlings is not rare. Those tetraploid plants originate from the chromosome duplication in nucellar cells (somatic cells) of the diploid apomictic parent. They present a specific anatomy when compared to diploids, like thicker roots and leaves, and change of stomatal density and size, that altogether affect the plant growth rate and seems to make them more adapted abiotic stresses. In the present work we investigated tetraploid tolerance to water deficit by comparing the behaviour of the diploid Rangpur lime, well known for its good tolerance to water deficit stress, and its autotetraploid. Rangpur lime seedlings and Valencia Delta sweet orange grafted on Rangpur lime were also studied along a water deficit. The stomatic conductance, the PSII activity and the water consumption were monitored. At the end of the stress, samplings were harvested for abscisic acid assays and transcriptomic studies. In control conditions, tetraploid Rangpur lime and Valencia Delta grafted on tetraploid Rangpur lime presented a lower stomatic conductance (gs) when compared respectively to diploid Rangpur lime and Valencia Delta grafted on diploid rootstock. This suggests a lower transpiration stream and a lower photosynthesis activity when a tetraploid rootstock is used. This is in agreement with the smaller plant size that is usually observed for tetraploid plants and the reduction of vigour that is observed for varieties grafted on tetraploid rootstock. Under stress condition, gs declined in the same time for diploid and tetraploid rootstock, the combination Valencia Delta / Rangpur lime being less affected by the stress that the non grafted Rangpur lime. The phothosystem II activity was reduced sooner for diploid Rangpur lime and diploid Rangpur lime grafted with Valencia Delta when compared to tetraploid Rangpur lime and tetraploid Rangpur lime grafted with Valencia Delta. Preliminary results suggest that in control condition, tetraploids rootstocks synthesize constitutively more abscisic acid than diploid. Molecular studies are currently performed to try to understand the molecular determinant of the greater tolerance of tetraploid citrus to water deficit. If the higher tolerance of tetraploid rootstocks to drought is confirmed under field conditions, the selection of tetraploids will be a relatively easy way to improve existing rootstocks for citrus.

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Study of adaptation and grain yield stability of wheat genotypes in salt affected regions of Iran

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ABSTRACT: Development of new cultivars with high grain yield, stability and good adaptability under salinity stress is one of the major goals of wheat breeding program for saline areas of Iran. The objectives of this study were the selection of high-yielding genotypes with a stable performance in targeted environments under saline conditions and to compare and evaluate different stability statistics to assess the relative yield stability of new wheat lines by the most appropriate method. Field experiments were conducted with 18 new wheat lines including two salt tolerant controls (Kavir and Bam) in using randomized completely block design with 3 replications at three locations during 2003-2005 cropping seasons under saline conditions. Soil and water salinity (Ec) ranged from 9 to 12 $dsnm^{-1}$ in different locations. Combined analysis of variance on grain yield showed significant differences in locations, and year ×location, genotypes and genotype×location× year interactions. Stability parameters calculated: linear coefficient of regression (b), deviations from regression (S_d^2), coefficient of determination (R^2), environmental variance (S_i^2), coefficient of phenotypic variation (C.V.), Shukla·s stability of variance (σ^2), Wricke·s ecovalance (Wi), Kang's yield-stability statistic (YS_i) , Rank method (non-parametric method) and interaction principal components (IPCA) of additive main effect and multiplicative interaction (AMMI) model. The level of association among the parameters was assessed using Spearman's correlation. Results of rank correlation analysis showed that there were highly significant correlations between many of the stability statistics and mean yields were strongly correlated with many of the stability statistics. Totally, results of combined analysis and different stability parameters showed that genotypes No. 8, 9, 10 and No. 12 have higher mean yield than the other genotypes and were the most stable and well adapted genotypes under saline conditions. The results correlation analysis of stability statistics and yield indicated non-parametric Rank method and YS_i would be useful for simultaneously selecting for high yield and stability. In addition, the results obtained using AMMI analysis indicated that the yield performances of genotypes were under the major environmental effects of genotype by environmental interactions. IPCA1, IPCA2 and IPCA3 were significant and jointly accounted for 86.7% of the genotype × environment (GE) interaction.

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Secondary traits in maize breeding for drought tolerance

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ABSTRACT: Two maize (*Zea mays* L.) genotypes with short and two with long anthesis-silking interval (ASI), as well as A and B parental lines were compared. According to kernel weight, line A was more sensitive than line B, and short ASI genotypes (A1 and B1) produced higher weight in comparison to related long ASI genotypes. In progenies with exotic germplasm embryo proline content was higher than in corresponding lines. The response to polyethylene glycol (PEG) treatment of these genotypes was analyzed in respect to their root morphology, proline content and peroxidase as antioxidative enzyme. Root depth and lateral growth was less developed in all genotypes under PEG treatment. Decrease of peroxidase activities which was demonstrated in all genotypes, was lower in short ASI genotypes (A1 and B1).

Keywords: ASI, drought, peroxidase, proline, *Zea mays*, L.

Introduction

Drought is one of the most important abiotic stresses that seriously decreases final grain yield in maize. Expectations of global climate changes for XXI century are towards higher temperatures, greater evapotranspiration and increased appearance of drought (Hillel and Rosenzweig 2002). Since the occurrence of drought is not predictable, breeders have to produce maize hybrids able to withstand stress and have stable yield under nonstressed conditions. For that breeding strategy identification and measurement of secondary traits that contribute to grain yield under drought e.g. anthesis-silking interval (ASI), kernel number per plant, bareness, stay green, leaf rolling, root architecture and biomass, efficiency of CO_2 fixation, are the most important (Bruce et al. 2002).

Water resources for agriculture are becoming increasingly limited making investigation of root behavior under water stress more important. Root development during water deficit is less inhibited than shoot growth. This important mechanism of plant adaptation to drought conditions is under genetic control (Sharp and Davies 1989).

Dehydration stress, like other stress conditions, induces increased production of reactive oxygen species (ROS), which in excess could be harmful to plant cells. Therefore, it is the balance between production and scavenging of ROS that is critical to the maintenance of the active growth and metabolism of the plant and overall environmental stress tolerance. Complex systems of scavenging ROS exist in plant cells consisting of low-molecular weight antioxidants, as well as antioxidant enzymes (Foyer et al. 1994). Enzymes participating in the

systems of antioxidative protection comprise the enzymes like superoxide dismutase (SOD), which dismutates superoxide to hydrogen peroxide and peroxidases, removing hydrogen peroxide. Maize like other plants responds to dehydration stress by accumulation of a variety of sugars, proline and glycine betaine in addition to changes in protein (Zinselmeier et al. 1999). Proline, which increases proportionally faster than other amino acids in plants under water stress, also plays role in radical detoxification and regulation of cellular redox status (Smirnoff and Cumbes 1989; Hare and Cress, 1997).

Material and methods

Plant material and growing conditions

The study was carried out on the 130 genotypes from the crosses between DTP (Drought Tolerant Population) and two inbred lines from commercial ZP hybrids, A and B. Backcrosses (BC2) were sown in 2007 at two locations Zemun Polje and Skopje. At flowering period in July, average temperatures were 25.6ºC and 27ºC and precipitation 18.9mm and 1.2mm in Zemun Polje and Skopje, respectively. At both locations, there were about 20 days with temperatures over 30 ºC in July. Only two genotypes with short (A1, B1), two with long ASI (A2, B2) from both crosses, as well as A and B lines, were chosen for further analysis in this study. Seeds were germinated for three days and then transferred into plastic pots containing Knopp solution with modified nitrogen content (10.9 mM NO_3^-+7.2 mM NH_4^+). Plants were kept for twelve days in a growth chamber under a 12-h photoperiod at 22/18 ºC and relative humidity of 70%. For the terminal 48 h of growing period, one half of the plants (treatment) were grown on fresh aerated nutrient solution complemented with 4% PEG (Mr 10000, Sigma), while control plants were maintained on the nutrient solution without PEG.

Free proline determination and enzyme assays

Free proline was extracted from embryos and roots of analyzed maize genotypes and its concentration determined by the method of Bates et al. (1973).

For peroxidase (POD) assay the root tissue was homogenised (1:5 fresh weight:buffer volume) with 100 mM K-phosphate buffer, pH 7.5. The homogenate was centrifuged at 20000 g for 15 min and supernatant used for enzyme analysis. The standard guaiacol test (Chance and Maehly 1955) was used for determination of POD activity.

Results and discussion

During pollination and early grain filling period maize is the most sensitive to water stress. Increasing ASI under drought is typical plant response which has negative influence on final grain yield (Edmeades et al., 2000; Bruce et al., 2002). Kernel weight differences, in current study, indicate that line A is the most sensitive to drought stress, with higher variations in this parameter among A, A1 and A2 than in B, B1, B2 genotypes. In chosen short and long ASI genotypes embryo proline content was higher than in corresponding lines (Table 1).

Table 1. Average values of examined traits.

Genotype	ASI (days)	Mass 1000 kernels (g)	Proline in embryo ($\mu g \cdot g^{-1}_{FW}$)
A	6	295	3.238 (100%)
A1	2	420	5.649 (174%)
A2	8	385	5.317 (164%)
B	4	460	1.641 (100%)
B1	2	480	3.313 (202%)
B2	7	400	3.046 (186%)

Root system plays an important role in water acquisition for plants and is a significant component of tolerance to water deficit. An important morphological trait-root depth and lateral growth (Fig. 1) was less developed in analyzed genotypes under PEG treatment. Possible explanation is that inhibition of lateral root development is adaptive response to water stress, which is confirmed in *Arabidopsis* by genetic analysis (Xiong et al., 2006). The other parts of root are often short, tuberized, bulbous and hairless (Vartanian et al., 1994) under stressed conditions. Genotypes A, A1, A2 and B showed similar response, while genotypes B1 and B2 developed short lateral hairs even under PEG treatment. It is known that many other factors are influencing root growth and this morphological trait could be only used for additional characterization of plant response to stress (Xiong et al., 2006).

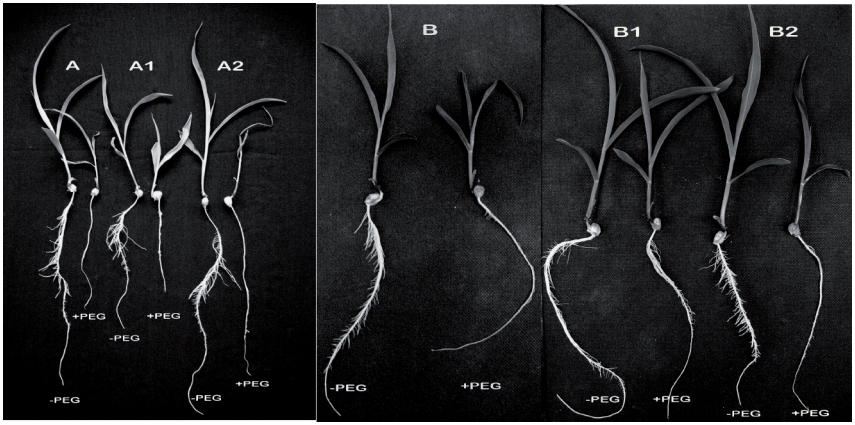

Figure 1. Root morphology and lateral growth under PEG treatment.

Analysis of root antioxidative system revealed differences between lines and corresponding progenies possessing exotic germplasm under PEG treatment (Fig 2). Thus, increase of root

proline content, which was more significant in BC2 crosses than in corresponding lines (Fig 2A), could be related to their increased tolerance to osmotic stress.

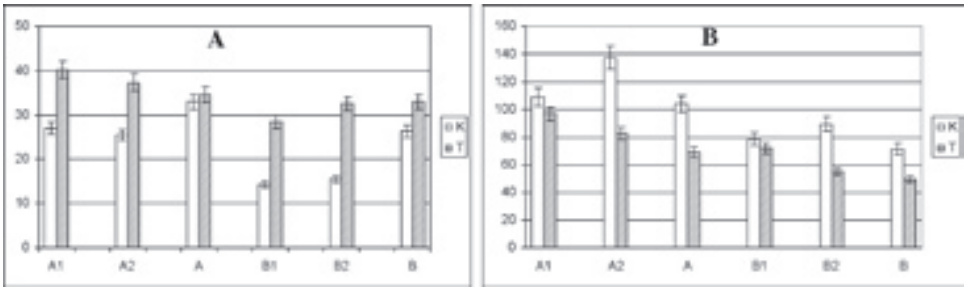

Figure 2. Root (A) proline content (μg g^{-1}FW) and (B) guaicol peroxidase activities (μmol $mg_{prot}^{-1}min^{-1}$)

Peroxidases are the enzymes that play role in growth regulation by producing polymeric products such as lignin and suberin or by cross-linking cell wall polymers. Because H_2O_2 is removed during these reactions, peroxidases are considered to be also antioxidative enzymes, protecting cells from the destructive influence of H_2O_2 and derived oxygen species. In our experiments PEG treatment induced decrease of root peroxidase activity in all genotypes (Fig. 2B). In genotypes with short ASI (A1 and B1) this decrease was less pronounced than in other genotypes. Decrease of peroxidase activities, especially in genotypes with long ASI, indicates lower ROS scavenging ability and thus, higher susceptibility to osmotic stress of these genotypes.

Results of the current study indicate that inbred line B, genotypes B1 and B2 are less sensitive to drought than the other group of analyzed genotypes and could be useful for further breeding programs

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Reaction of triticale cultivars and breeding lines to *Blumeria graminis* sp. in Poland

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ABSTRACT: The incidence of powdery mildew caused by *Blumeria graminis* sp. on triticale was considered for years of negligible in importance. However, since 2002 severe outbreaks of the disease on some of triticale cultivars were observed in Poland. In post-registration field trials conducted by the Center of Variety Testing in Slupia Wielka in 2004-2006, 20 cultivars of winter triticale were evaluated in 6 geographic regions of the country to a natural infection by the pathogen. Disease rating expressed as a percentage of severity varied from 1.6% (resistant) to 91.9 % (susceptible). Differences in resistance were observed among cultivars within a region and between regions in all years of the study. None of the cultivars showed complete resistance. Only three cultivars, i.e. Disco, Fidelio, Moderato, showed close to complete (1.6% - 6.1%) resistance in all regions to the pathogen population. Completely susceptible (81.6% - 91.9%) cultivars were Lamberto, Woltario, Kazo, Kitaro, Prado, Krakowiak, Zorro. Since the problem of powdery mildew outbreaks on triticale with the time being has become aggravating, search for resistance among breeding materials was undertaken. In total, 416 winter triticale breeding lines were tested for resistance to powdery mildew at the seedling and adult plant growth stages. Studies were carried out using the method of artificial inoculation with a mixture of *Blumeria graminis* sp. isolates at the seedling stage in a greenhouse. These isolates used for inoculation were of triticale origin. At the adult plant growth stage in the field, lines were tested under conditions of natural pathogen infection. Only 19% of lines showed high resistance at the seedling stage. At the adult plant growth stage 59% of the lines showed high level of resistance under natural powdery mildew fungus infection in field conditions. Three % of tested breeding lines showed complete susceptibility. The resistance showed by lines at the seedling stage was subsequently repeated at the adult stage. It is inferred that in these lines the same set of genes controls resistance at both growth stages. Therefore, these lines were recommended as a starting material for further breeding. It is known that triticale is attacked by wheat and triticale isolates of *B. graminis* sp. It is not clearly determined whether rye isolates of *B. graminis* sp. also infect triticale. It is also known that hybrid cultures of *Erysiphe graminis* f. sp. *secalis* × *tritici* segregated on wheat without resistance genes, with wheat resistance genes *Pm*1, 3b and 3c and rye resistance genes *Pm*7 and *Pm*8. The present study does not provide a clear evidence of which physiological form of the fungus attacks triticale. This is a subject of further studies.

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Yield stability analysis of hulless barley (*Hordeum vulgare* L.) genotypes in Iran

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ABSTRACT: The interaction of genotype and environment causes that the yield of genotypes be evaluated in a vast scope of environmental condition, until the acquired information increase the efficiency related to their selection and introduction. Perkins and Jinks (1968) determined two parameters including regression coefficient and deviation from regression line as a stable parameters. Also, studies on 5 corn genotypes indicated that the interaction of genotype and environment was significant in all tests (Kang et al., 1991). They also indicated that selecting just based on yield is not sufficient when the interaction of genotype and environment is significant due to testing in different environments. In order to evaluated the stability and determine the highest yielding and adaptability of 20 genotypes of hulless barley in a Randomized Complete Block Design with three replications in six locations (Karaj, Esfahan, Neishaboor, Yazd, Birjand and Zarghan) for 2 years (2002-2004), Iran. Simple and compound analysis of variances indicated significant difference between genotypes. In order to evaluate the interaction and determine the stable genotypes, the stability analysis was done by the use of environmental variance methods, environmental coefficient of variation, Eberhart and Russell's regression method, Finlay and Wilkinson's regression method, Perkins and Jinks's regression method, equivalence of Wrick and stability variance of Shukla. The results were the same. Based on of most of these methods ICN93-328 and Aleli/4/mola2 genotypes were identified as stable genotypes. Gloria genotype was recognized specially for the unpropitious weak areas. In general it can be concluded from this test that Eberhart and Russell methods considering several parameters are regarded as better method of stability analysis.

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Breeding lentil (*Lens culinaris* Medik) for early sowing in Castilla y León (Spain): QTL identification

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ABSTRACT: Early sowing date has been shown as a good strategy for increasing yield in lentil crop in Castilla y Leon Region (Spain). In this environment, flowering time must be adapted to avoid spring frost stress but also drought and heat terminal stresses (Muelhbauer, 2004). But also, it is necessary that the plant can tolerate winter conditions like cold temperatures, frost heaving, water logging, freeze-thaw cycles and winter typical diseases, meaning that genes for winter hardiness must be transferred to adapted germplasm to develop lentils cultivars with improved winter hardiness and sufficient quality for production in Spain. A new research program to identify the lentil ideotype for autumn-winter sowing in our region has being recently implemented. In this contribution we present our preliminary results on field trials and genetic mapping efforts, trying to relate frost tolerance, date of flowering, and other traits with winter yield expression in our regional environments. In this way, we tested 106 lines coming from a $F_{6:7}$ RIL population (WA8649090 x Precoz) in the field with winter and spring sowing dates during 2 different years in two locations. Frost tolerance levels were also studied under environmentally controlled conditions (Caminero, 2004). A genetic map comprising mainly RAPD and AFLP markers is also available (Kahraman, 2004). Three QTLs were defined for winter yield expression, whereas two were found for spring yield, being one (LGIII) common to both environments. Also, one flowering date and three frost tolerance QTLs showed to be related with yield winter expression. The implications of this study in our breeding program are the poster discussed.

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Indirect selection for production through leaf length as agronomic marker in tomato fresh market

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ABSTRACT: The aim of this study was to evaluate 132 accessions of tomato in eight experiments using the path analysis method for total production of fruits (TPF) with the following traits; leaf length (LL), diameter of the central rib (DCR), Stem internode length (SIL), stem internode diameter (SID), number of locules (NL), total number of fruits (TNF), average fruit weight (AFW), percent of non-commercial fruits (%UF), precocity index (PI), total acidity (pH) and total soluble solids content (TSSC). Other estimates were discarded due to multicollinearity problems. It was observed that NTF and AFW had high direct and indirect effects, and correlation (>0.5 or <-0.5). Furthermore, there is no recommendation for indirect selection using this trait, due to difficulty of estimation of TPF. Traits LL as well as SID had a high direct effect and correlation (>0.5 or <-0.5) with TPF. But with the SID there was a low heritability. Thus only LL is recommended for indirect selection for production due to its high heritability (80%) and faster and easier evaluation. The results indicated the possibility of increasing the selection efficiency for production through selection for leaf length.

Keywords: *Solanum lycopersicum*, efficiency of selection, morphological markers.

Introduction

The Vegetable Germplasm Bank, Federal University of Vicosa (BGH-UFV), was created in 1966. Today it holds more than 7.000 accessions (Silva et al., 2001). Tomato represents the 28% of the total number of accessions. More than 200 accessions have been characterized according to the descriptors recommended by the International Plant Genetic Resource Institute (IPGRI). Besides the detection of genes of interest, characterization allows the study of associations between characters. To study the relationships between characteristics is necessary to use an adequate analysis, like the path analysis proposed by Wright (1923). Therefore, the objective of this work was to evaluate the genotypic correlation and its direct and indirect influences on production.

Materials and Methods

Eight experiments were conducted at the Federal University of Viçosa (UFV) to evaluate 132 tomato accessions that represent the variability belonging to the fresh market group of BGH-

UFV. The control genotypes were 'Debora Plus' and 'Santa Clara'. Growing techniques were the usual conducted during evaluation assays. The evaluated descriptors (IPGRI, 1996) were: leaf length (LL), leaf width (LW), diameter from the central rib (DCR), stem internode length (SIL), stem internode diameter (SID), mesocarp thickness (MT), endocarp thickness (ET), height of the main stem (HMS), number of locules (NL), total number of fruits (TNF), average fruit weight (AFW), percent of uncommercial fruits (%UF), precocity index (PI), total acidity (pH), total soluble solids content (TSSC), titratable acidity (TA), and flavor. Each experiment was conducted in randomized block design with three replications and three plants in each.

Analysis of variance was performed in order to obtain the genetic variance and covariance and the matrix of genotypic correlations between the characters. This matrix was used to perform the multicollinearity tests (Montgomery & Peck (1981), the path analysis was done using the software 'GENES' program (Cruz, 2001).

Results and discussion

It was observed that among the primary variables for TPF (Fig.1), AFW and TNF, they possess high direct and indirect magnitude effects (table 1). Larger effects than 1(one) were available since the estimate of path coefficients were based on standardized data. Therefore, the components of correlation had range ≤1 or < -1 of values (Vencovsky and Belly, 1993).

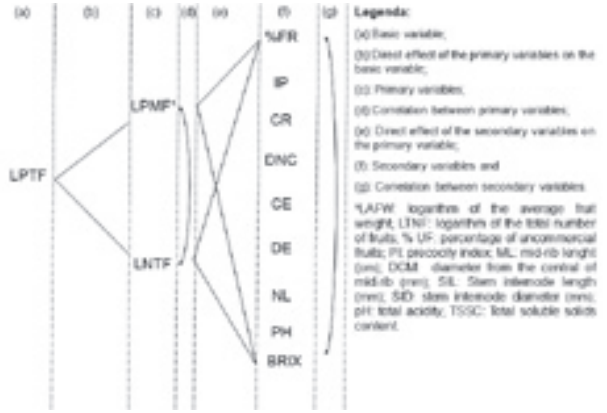

Figure 1. Path diagram of the relationships between the primary, secondary components and the total production of fruits (transformed for the logarithmic scale, TPF).

The indirect effects of LAFW (AFW transformed to logarithmic scale) and LTNF (TNF transformed to logarithmic) on LTPF (TPF transformed to logarithmic scale) were negative.

Due to this result it was observed that there was a decrease of the average weight of fruit with the increase in the number of fruits per plant (Agong et al., 2000). Since the indirect effects were highly negative (less than -0.80) the effectiveness of these components for indirect selection for LTPF is not recommended.

Table 1. Estimation of the direct and indirect effects of primary variables based on LTPF variable (logarithm of the total production of fruits) in tomato cultivar

Primary variables	Effect	Value
LAFW	Direct on LTPF	1.201
	Indirect via LTNF	-0.821
	Total	0.380
LTNF	Direct on TPF	1.230
	Indirect via LAFW	-0.802
	Total	0.428
R^1		0.984
Residual effect		0.128

R^1: coefficient of determination for the used trail model.

According to the secondary descriptors, certain problems with multicollinearity were observed. In consequence only the following descriptors were maintained in the model: non-commercial fruits (% UF), precocity index (PI), leaf length (LL), diameter from the central of mid-rib (DCM), stem internode diameter (SID), stem internode length (CE), number of locules (NL), total acidity (pH), total soluble solids content (TSSC) in addition to the total production of fruits (TPF).

The stem internode diameter (SID) had high positive direct effect on the production (LTPF), which was also observed by Rodriguez et al. (2005). However, its heritability (67.15%) was smaller than the heritability for the production (81.52%) therefore, it wasn't recommended either.

High correlation was observed regarding leaf length (LL) associated to the high direct effect (> 0.5) with LTPF. In addition this trait can be evaluated early and in an easly way (third leaf above the third node), and it has high heritability (83.31%). Thus the leaf length in the indirect selection for the production can be used.

These informations are reliable because these genotypes were evaluated in eight experiments and represent a sample of the germplasm tomato collection of BGH-UFV, which is the second oldest germplasm bank in Brazil, and hence represent fresh market tomato that is grown in Brazil. However, the indirect selection must be employed in the early stages of improvement programme for the selection of genotypes with lower production potential.

Table 2. Estimates of the direct and indirect effects of the secondary variables based on LTPF variable in the culture of the tomato. Vicosa, Minas Gerais. 2006.

Description of the effects	secondary variables								
	%BF[1]	PI	LL	DCM	SIL	SID	NL	pH	TSSC
Direct effect on LTPF	-0,351	-0,200	0,566	-0,321	0,040	0,559	0,242	0,047	-0,220
Effect indirect via:									
%FR		0,148	-0,169	-0,012	-0,046	-0,188	-0,048	-0,113	-0,067
PI	0,084		0,075	-0,011	-0,006	0,139	-0,042	0,102	0,088
LL	0,272	-0,214		0,115	0,212	0,339	-0,010	0,221	0,199
DCM	-0,011	-0,017	-0,065		-0,002	-0,169	0,092	-0,061	-0,050
SIL	0,005	0,001	0,015	0,000		0,001	-0,015	0,004	-0,005
SID	0,299	-0,388	0,334	0,293	0,006		-0,232	0,130	0,233
NL	0,033	0,051	-0,004	-0,069	-0,092	-0,100		0,018	-0,049
pH	0,015	-0,024	0,019	0,009	0,005	0,011	0,004		-0,008
TSSC	-0,042	0,097	-0,077	-0,034	0,029	-0,092	0,045	0,037	
Total	0,305	-0,544	0,694	-0,030	0,146	0,500	0,035	0,385	0,122

[1]%UF: % of uncommercial fruits; PI: precocity index; LL: leaf length (cm); DCM: diameter from the central of rib (mm); SIL: Stem internode length (mm); SID: stem internode diameter (mm); NL: number of locules; pH: total acidity; TSSC: total soluble solids content (Brix).

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Evaluation of transgenic R1 tomato plants against *Tomato yellow leaf curl Sardinia virus* (TYLCSV)

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ABSTRACT: Tomato yellow leaf curl virus (TYLCV) is one of the most serious tomato diseases throughout the Mediterranean region, the Middle East and the tropical regions of Africa and Asia. It is caused by a *Geminivirus* transmitted by the whitefly, *Bemisia tabaci*. In Tunisia, TYLCV has been suspected since the 1980s (Cherif & Russo, 1983). However, first molecular characterizations of Tunisian isolates were carried out later on, showing that they are clustered in the TYLCSV-Sicilian viral group regardless of their host plant origin, tomato, pepper and bean crops (Fekih-Hassen et al., 2003). In plants, post-transcriptional gene silencing (PTGS) acts as an efficient and natural antiviral defence system. In the goal of the development of an antiviral strategy based on this mechanism, a gene silencing construct was developed, harbouring a fragment of the replicase gene double cloned in a vector as inverted repeats separated by an intron. Thus, the construct allows induction of an ihp-RNA which activates the PTGS defense mechanism. Transgenic tomato plants were obtained by transforming a Tunisian variety via *Agrobacterium tumefacien*s using the construction described above. Seeds were collected from the first generation, germinated and submitted to a molecular investigation. Firstly, PCR amplification results gave evidence of the integration of the transgene in all transgenic tomato plants. Then, transgenic plants were evaluated for resistance to the homologous virus by agroinoculation of the TYLCSV infectious clone (Gharsallah-Chouchane et al., 2006) at the 4-leaves stage. Resistance tests were carried from the third week after agroinoculation until the sixth one by PCR targeting the viral coat protein gene. Results showed that, during experiment, none of the transgenic agroinoculated plants gave symptoms like the susceptible non transgenic plant controls did. PCR results allowed us to cluster the transgenic tomato plants into 3 different groups: the first group of plants, for which PCR amplification of the targeted gene was obtained, showed the presence of systemic infections as soon as the third week post virus inoculation and consequently were considered as non resistant plants. The second group was composed of partially resistant tomato plants as far as they presented a delay in virus multiplication. For this group, PCR amplification was obtained starting the sixth week. The last group of tomato plants for which no PCR amplification of the targeted gene was obtained until the end of the experiment did not allow any systemic infections assessing of a resistance behaviour.

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Medicago arborea traits segregating in F2 families of *M. sativa* x *M. arborea* crosses

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ABSTRACT: *Medicago sativa* and *M. arborea* have been hybridized by electrofusion in cell culture (Nenz et al. 1996), and by making large numbers of cross pollinations of *M. arborea* on *M. sativa* male sterile plants selected for the purpose by Bingham (2005) and by Armour et al (2008). Five hybrids produced by Irwin's lab in AU contain part of the *M. arborea* genome based on AFLP markers, anthracnose resistance, and pod coiling. Ten hybrids produced in Wisconsin differ from each other in morphology and fertility possibly due to loss of some chromosomes from both parental genomes during seed development. Most hybrids are male sterile, but three have some pollen and were used in crosses with other hybrids to generate segregating F2 populations. *M. arborea* traits that are segregating and of potential use in *M. sativa* include: 1. large seeds, 2. lodging resistance, 3. frost tolerance, and 4. a heterotic block that we are trying to stabilize and use to increase yield. Improved longevity may be segregating, but will take years to study. Other traits segregating that appear neutral or undesirable for breeding include variation in flower color, erratic flowering and fewer flowers per raceme, fertility, pod shape, contractile seedling growth, root and crown morphology, dormancy, and plant color. Leaf and stem morphology varies dramatically over stages of growth in the field and glasshouse and has not yet been studied. The segregation observed in F2 and F3 generations indicates there is chromosome pairing and recombination involving the two parental genomes, and the extent of the variation indicates that most of the *M. arborea* genome was represented among the original unbalanced hybrids. Results of current crossing and backcrossing efforts will be presented in the poster.

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Relationship between Mahalanobis distances and some breeding characteristics

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ABSTRACT: Relationship between the specific combining ability (SCA), the heterosis effects and the Mahalanobis distances were estimated. In our work the SCA and the heterosis effects were analyzed for nine quantitative traits (diameter of root neck, plant height, number of branches, distance to the first branch, number of pods per plant, number of seeds per plant, seeds weight per plant, 1000 seeds weight and number of seeds per pod) of eight inbred lines and their diallel cross hybrids of spring rape (*Brassica napus* L.). The field experiments (with F_1 and F_2 generations) was carried out in partly balanced square design with four replications. Results for nine analyzed traits show significant relationship between Mahalanobis distance of inbred lines and SCA effects in hybrids. Heterosis effects were statistically significant for plant height, distance to first branch in F_1 and number of branches (in F_2) generation.

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QTL detection in plants under changing environments: a unified mixed model approach for conventional and linkage disequilibrium (LD) mapping

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ABSTRACT: Multi-environment trials are commonly employed in plant breeding. They aim at representing the range of environmental conditions, in terms of biotic and abiotic stresses, that the crop may have to cope with in the future. Breeding for adaptation can benefit from the use of molecular marker technology by identifying QTLs that give consistent advantage (QTL main effect) or specific advantage to certain environments (QTL by environment interaction). For QTL detection two major approaches are available: conventional QTL mapping and linkage disequilibrium (LD) mapping. While conventional QTL mapping makes use of specifically designed crosses (e.g. F2, double haploids, recombinant inbred lines), LD mapping approaches rely on sets of diverse genotypes with possibly heterogeneity of relatedness due to common ancestry. Although undoubtedly each QTL mapping approach has its own particularities, both approaches go after the same goal, that is, to find associations between trait(s) and chromosome regions tagged by markers. Therefore, the statistical approach for either conventional QTL mapping or LD mapping need not be very different. This is something that has not been sufficiently stressed in the literature. In this paper, we present the outline of a QTL mapping framework based on mixed models that can be used in conventional and LD mapping approaches. The motivation for a mixed model framework comes from the necessity to account for the complex genetic variance covariance structure in the data. On the one hand, some degree of genetic correlation between the performances in the different environments is expected because the same set of genotypes is used across the experiments. On the other hand, lack of genetic correlation between environments is also expected because of the ubiquitous and well-known phenomenon of genotype-environment interaction (GxE). In addition, genetic correlations between different genotypes can be observed because of the different degrees of relatedness or kinship between individuals. Mixed models are advantageous in this context as they can allow accounting for all sorts of genetic correlations in the data, improving the tests for the detection of QTLs and the estimates of QTL effects. The mixed-model approach described above will be illustrated with examples in maize and barley, both in conventional QTL mapping and in LD mapping applications.

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Genetic variability of ten Romanian accessions of *Phytophthora infestans*

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ABSTRACT: Revealing of genetic variability of local populations of *Phytophthora infestans* is a crucial step for the development of plant breeding programmes for resistance to this pathogen, as well as for an efficient potato late blight control. Ten accessions of *Phytophtora infestans* were isolated, from different counties of Romania, and grown on rye medium. For characterization of genetic variability of *Phytophtora infestans* we used several types of molecular markers (SSR, RAPD and ISSR) for looking for primary molecular polymorphism or, after digestion with restriction enzymes, for secondary molecular polymorphism (CAPS marker). Genetic distances and phenetic relationships among accessions were established based on RAPD molecular polymorphism,. We tried to increase the RAPD molecular polymorphism by digestion of amplification products with restriction enzymes (Hae III, Rsa I, Not I, Hind III, Hinf 1). Establishing of mating type was done by hybridisation on rye medium of our accessions with A1 and A2 mating types received from Wageningen University, Laboratory of Phytopatology. Metalaxyl susceptibility was determinate by classical methods (Shattock,1988). PCR markers for specific *Phytophtora infestans* genes or for microsatellite DNA, gave no polymorphism among the ten accessions. The amplification products obtained from different accessions by RAPD show a significant polymorphism in relation with the used primer. From the thirteen decamer primers used, two gave the best results (OPC-9 and OPC-20), and the polymorphism obtained in some accessions was obvious and stable. Nevertheless the heterogeneity revealed by dendrogram was quite reduced, and four accessions were monomorphic. By digestion of amplified RAPD products with the five restriction enzymes the heterogeneity increased significantly. The ISSR markers gave similar results to RAPD markers, and the amplification products were more numerous. Analysis of secondary molecular polymorphism at the level of amplified mitochondrial DNA with H4 pair of primers, digested by EcoRI restriction enzyme (CAPS markers), revealed a genetic differentiation among Romanian *Phytophthora infestans* accessions. On this basis it was established that the ten *Phytophthora infestans* accessions belong to two haplotypes, one accession belongs to Ia haplotyp and the other nine belong to IIa haplotype (Griffith and Shaw, 1998). An analysis of our results concerning the characterization of genetic variability by molecular markers, in connection with mating type and susceptibility to metalaxyl of Romanian *Phytophtora infestans* accessions, shows that the resistant or medium resistant *Phytophtora infestans* accessions are polymorphic either in respect with primary molecular polymorphism or in respect with secondary molecular polymorphism. The single A2 mating type identified, polymorphic for OPC-20 primer, and belonging to Ia haplotype (all

the others belongs to IIa haplotype) was medium susceptible to metalaxyl. These results are of interest for plant breeding programmes for resistance to potato late blight.

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Combination of SSH and cDNA microarray to isolate genes affecting tiller development in ryegrass

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ABSTRACT: In order to investigate gene expression during tiller development in perennial ryegrass two PCR-based suppression subtractive hybridization (SSH) libraries were constructed. cDNA isolated from the main/axillary tillers of a perennial ryegrass (*Lolium perenne* L.) mutant with enhanced axillary tillering was used as the driver/tester for forward library production and *vice versa* tester/driver for reverse library production. 384 clones were randomly selected from each library for cDNA fragment amplification. 576 cDNA fragments of sufficient quality were subsequently selected for sequencing and placed onto the cDNA microarray. The average EST sequence length was 249 nt and varied from 30 to 508 nt. Putative function was assigned to 154 ESTs by comparing sequences with publicly available databases of NCBI. Two mRNA samples isolated from the main and axillary tillers and labeled with Cy3 and Cy5, respectively, were used for microarray hybridizations in three replications. In total, 16 ESTs were identified that are either up- or down-regulated compared with the common reference. These differentially expressed genes were categorized as putative novel (9 ESTs), photosynthesis (1 EST), metabolism (4 ESTs) and defense genes (2 ESTs). The large proportion of novel genes isolated in this study may represent important developmental genes controlling tiller production in perennial ryegrass.

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Dissecting the genetic nature of crown rust resistance in perennial ryegrass

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ABSTRACT: Crown rust (*Puccinia coronata* f. sp. *lolii*) is a pathogen of turf and forage grasses, which can result in reduced yield, nutritional quality and palatability. An F_2 mapping population, where the parental material differed in susceptibility to crown rust infection, was evaluated under field conditions. Crown rust infection was scored using a 0-9 scale (10 being a high level of infection) between May and August, 2007. The maternal parent mean disease score ranged between 3.2 and 6.1 depending on scoring date, while the paternal parent ranged between 0.5 and 1.8, making it the more resistant parent. An existing genetic linkage map for the F_2 population was utilized to identify Quantitative Trait Loci (QTL) associated with crown rust susceptibility. This resulted in the consistent detection of a QTL on linkage group two during the evaluation period. The QTL in this region was seen to explain up to 14% of the phenotypic variance for crown rust resistance in this population. When disease pressure was highest in May, the effect of having both alleles from the resistant parent resulted in a mean disease score of 3.9. Having both alleles from the more susceptible parent resulted in a mean disease score of 5.6 and having alleles from both parents resulted in a mean score of 4.6. Scoring of disease resistance for 2008 is ongoing.

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Flowering earliness in wheat inbred breeding lines derived from *T. aestivum* 'Chinese Spring' x *Dasypyrum villosum* hybridization is not related to allelic variation at the vernalization loci *VRN-A1*, *VRN-B1*, and *VRN-D1*

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ABSTRACT: The wheat inbred breeding lines (IBLs) "CSxV58", "CSxV59" and "CSxV60" derived from *T. aestivum* "Chinese Spring" ("CS") x *Dasypyrum villosum* (*"Dv"*) intergeneric hybridization showed several phenotypic differences compared to "CS", including earlier date of flowering. The IBLs have the same "CS" euploid chromosome number and structure, and GISH did not reveal any apparent addition of "*Dv"* chromatin. However, the earlier generation line that gave rise to "CSxV58" had an acentric "*Dv"* chromosome fragment which was lost in the following generations, and the IBLs when compared to "CS" showed differences for about 16% of the 166 AFLP fragments detected in replicated runs of gel electrophoresis. This indicated that substantial criptic chromosome mutations or recombinations or gene mutations have occurred during the earlier generations following the hybridization event and that these rearrangements were transmitted to the plants from which the IBLs derived. One of these mutations might have affected a flowering-promoting gene at a locus different from *VRN-1*, causing (under nonvernalizing condition and 13-14-hour daylength) the IBLs, "CSxV59" x "Salgemma", and "CSxV59" x "Isengrain" F_1 plants to start anthesis in less than 50 days from sowing compared to "CS" and to the two winter bread wheat cultivars "Salgemma" and "Isengrain", which flowered about 150 days later.

Keywords: *Triticum aestivum*, *Dasypyrum villosum*, vernalization, *VRN-1* genes, AFLP.

Introduction

'Chinese Spring' ("CS") as other *T. aestivum* cultivars carries three homoeologous copies of the *VRN-1* gene, one in each of the three genomes, which are designated *VRN-A1*, *VRN-B1*, and *VRN-D1*. *VRN-A1* is the wheat ortholog of the *Arabidopsis* meristem identity gene *APETALA1* (*AP1*). Mutations in the *VRN-1* promoter region (Yan *et al*., 2003) or large deletions within the first intron (Fu *et al*., 2005) produced dominant *Vrn-A1*, *Vrn-B1* and

Vrn-D1 alleles which were sufficient to confer a spring growth habit in diploid and polyploid wheat.

The *VRN2* locus from temperate grasses encode a dominant repressor of flowering down-regulated by both vernalization (Yan et al, 2004) and short-days (Dubcovsky *et al*., 2006; Trevaskis *et al*., 2006). The effect of *VRN2* allelic variation on flowering time is reduced or eliminated by mutations in the promoter or the first intron of the *VRN1* vernalization gene in both wheat and barley (Dubcovsky *et al*., 2005; Fu *et al*., 2005) and in the promoter of *VRN-B3* gene (Yan *et al*., 2006).

The wheat *VRN-B3* locus (previously known as "*E*", *VRN5* and *VRN-B4*) has been mapped on the short arm of wheat chromosome 7B of cv "Hope" and of the 7B-intervarietal-substitution line "CS (Hope7B)" (Law and Worland, 1997). *VRN-B3* is an orthologue of the *Arabidopsis FT* gene. The "CS(Hope7B)" allele associated with early flowering (*Vrn-B3*) has a 5,295-bp repetitive element inserted 591-bp upstream from the start codon. This insertion is absent in the "CS" allele associated with late flowering (*vrn-B3*). Besides the cv "Hope", the *Vrn-B3* allele was found only in 2 out of 266 hexaploid wheat cultivars from China (Zhang *et al.,* 2008).

Substantial criptic chromosome mutations or recombinations or gene mutations have been described in the earlier generations of *Triticum* x *Aegilops* intergeneric hybridization, when the A, B, D, and alien chromosomes were in the same nuclear complement. When a certain chromosome or chromosome fragment from the alien species *A. cylindrica*, *A. triuncialis*, or *A. speltoides* was introgressed in "CS", chromosomal breaks occurred in the gametes and various chromosome mutations were generated, including deletions (Endo and Gill, 1996).

The objective of the present study was to evaluate genetic variability for early responsiveness to flowering in the progenies of [(*T. aestivum* x *D. villosum*), F_1 x *T. aestivum*] hybridization at both cytological and molecular levels in order to select mutants potentially useful in breeding programs.

Material and methods

Three pairs of wheat inbred breeding lines (IBLs), namely "CSxV58", "CSxV59" and "CSxV60", were derived from *T. aestivum* "CS" x *D. villosum (Dv)* interspecific hybridization, followed by one generation of backcross to *T. aestivum* "CS", three generation of selfing (BC_1F_1 S_1 through S_3), five generations of single-spike descent (from S_4 through S_8) and four generations of seed increase (S_{12} IBLs). S_{12} breeding lines traced to the same S_4 plant were considered "sister" IBLs. These lines, showed several phenotypic differences compared to "CS", including earlier date of anthesis (De Pace *et al*., 2007), awnedness, higher grain yield per spike, and enriched prolamin subunits in grain seed storage proteins. F_1 offspring have been obtained after crossing "CSxV59" with the winter bread wheat cultivars ("wbwc") "Salgemma" and "Isengrain". Nonvernalized IBLs, "CS", "wbwc", and F_1 plants were grown under 13-14 hour day-length photoperiod (sowing at the end of July) either in greenhouse or

in the field at the nonvernalizing temperatures above 20°C. Days to heading were recorded during 3 months after sowing in the field and during 5 months after sowing in the greenhouse experiment.

Chromosome counting ad alien chromatin detection was performed using GISH (Minelli *et al*., 2005). AFLP analysis was performed according to Vos *et al*. (1995). Intron 1 deletion at the *VRN-A1*, *VRN-B1*, *VRN-D1* and *VRN-B3* loci were detected using the primer combinations reported by Fu *et al*. (2005).

DNA fragments of the *AP1* gene were amplified from "CS" and "CVxV58", "CVxV59", "CVxV60" genomic DNA by using the primer combinations and PCR conditions described in Yan *et al*. (2003). The obtained amplicons were cloned using the TA cloning kit (Invitrogen). Different clones were sequenced using the ABI Prism 310 Genetic Analyzed (Applied Biosystems). Search for homology were performed against the NCBI databases using the BLAST program and sequences were aligned with the CLUSTAL program.

Results and discussion

The analyzed IBLs have the "CS" euploid chromosome number (2n=42) and structure, and GISH did not reveal any apparent addition of "*Dv"* chromatin. However, (a) metaphase plates prepared from the root meristem cells of a "CSxV58" S_8 plant showed an acentric "*Dv"* chromosome fragment which was lost in the next generations; (b) the proportion of the 166 AFLP bands shared between sister lines was 98% for the "CSxV59" pair, 95% for the "CSxV58" pair, and 92 % for the "CSxV60" pair; (c) the average proportion of AFLP bands shared between anyone of the IBLs and "CS" was 84% (Fig. 1). In principle, the incidence of nonshared bands (about 16%) between one IBL and CS is proportional to the molecular alteration rates around the restriction sites sampled in the IBL (with reference to CS) and detected using the AFLP methodology. The IBLs and the "CSxV59" x "Salgemma", and "CSxV59" x "Isengrain" F_1 plants flowered in about 50 days from sowing, while "CS" and "wbwc" plants grown in the same environmental conditions showed a very late flowering (LF) phenotype. In fact, they did not flower at all up to the end of October (when the field experiment ended) or flowered about 150 days later (in the greenhouse) compared to the flowering time of the IBLs and F_1s.

This indicates that the blockage of flowering (probably due to the activity of the repressor encoded at the *VRN-2* locus) is effective in "CS" or in the "wbwc" parental plants but not in the IBLs or the "CSxV59" x "wbwc" F_1 plants. PCR amplification using primers able to detect the presence/absence of deletions in the first intron of the *VRN-1* gene of the IBLs and "CS", evidenced that both the IBLs and "CS" have the "*vrn-A1*, *vrn-B1*, *Vrn-D1"* genotype at the *VRN-1* homoeoloci (which means absence of first intron deletion in *VRN-A1* and *VRN-B1* and presence of first intron deletion in *VRN-D1*). Since the IBLs and CS have the same genotype at the *VRN-1* homoeoloci and the *Vrn-D1* is a weak flower-promoting allele, it was expected that in the absence of vernalization and long days the IBLs, "CS" and the tested F_1s should have expressed a similar LF phenotype, but this was not the case: IBLs and F_1s were

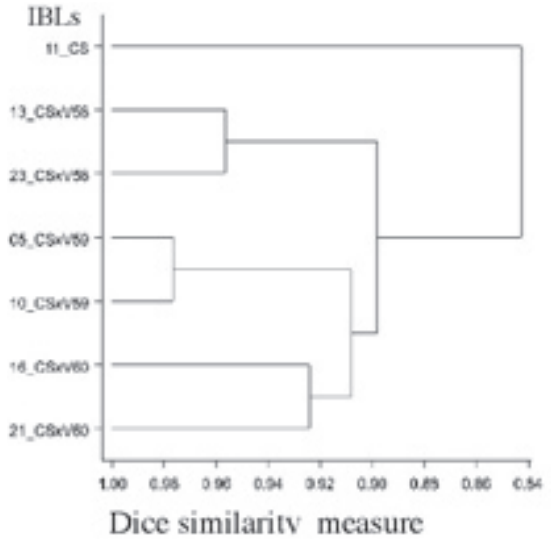

Figure 1. Dendrogram showing the hierarchical similarity relationships between the pairs of "CSxV58", "CSxV59" and "CSxV60" IBLs and "CS". Branches are ordered according to the results of the cluster analysis of the Dice similarity measures. Dice similarity measures were obtained after converting to a 1/0 dichotomous variables, the presence/absence of DNA fragment (band) at given positions in the AFLP electrophoretic patterns of the IBLs and "CS".

much earlier than "CS". Molecular investigation of the *AP1* gene in the *VRN-1* region of the A genome, did not evidence significant alterations of the nucleotide sequence for that gene between the IBLs and "CS". We therefore hypothesize that in the earlier generations following the "CS x Dv" hybridization, a dominant alteration occurred in a key gene (different from *VRN-1* but modulated by the VRN-2 repressor) involved in the flowering pathway of the plant from which the IBLs were selected; the inherited alteration affected a regulatory region (i.e. the promoter) of that gene which, as occurs for *Vrn-1* homoeoalleles, disrupts the downregulation activity of the VRN-2 repressor. This putative gene interaction model is similar to that postulated for the interaction of *VRN-B3* with vernalization by Yan *et al*. (2006). Molecular investigations to test this model is under way.

In summary, this study provides evidence that: (a) inbred selection in progenies from [(*T. aestivum* x *D. villosum*), F_1 x *T. aestivum*] hybridization is a way to recover the euploid *T. aestivum* parent altered by criptic chromosome mutations at several positions scattered in the whole genome, and (b) one such mutation involved a putative key gene (different from those

at the *VRN-1* homoeoloci) causing an earlier flowering phenotype under nonvernalizing and long-day environmental conditions. This inherited variation provides an additional source of genetic variation to the wheat crop primary gene-pool.

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Development of new and diverse elite lines for early-maturing hybrids: Traditional and modern maize breeding

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ABSTRACT: There is a need to increase the useful genetic diversity of maize (*Zea mays* L.) hybrids available in the market with adequate germplasm. Without choosing the right germplasm neither traditional breeding nor modern breeding will be successful. The goals of this research project are to develop early maturing high quality lines for utilization in the northern U.S. Corn Belt and ultimately increase the genetic diversity available in the market. A backcross breeding program was initiated to move U.S. Germplasm Enhancement Maize (GEM) germplasm northward. Five hundred BC1:S1 lines derived from (AR16026:S17-66-1-B x ND2000) x ND2000 were advanced one generation of inbreeding and crossed to industry testers. Experiments including 64 and 81 entries (depending on testers) were arranged in partially balanced lattice designs across 15 North Dakota locations. Preliminary data showed several experimental GEM-Derived lines had better grain yield and test weight performance than popular industry hybrids at similar grain moisture at harvest. This is the first research devoted to germplasm enhancement incorporating tropical and late-temperate genetic materials with <90RM. As a consequence of long-term breeding efforts we currently have early maturing GEM materials adapted to the northern U.S. Corn Belt which are not only sources of useful genetic diversity but also competitive products for industry use.

Keywords: germplasm choice, genetic diversity, *Zea mays* L.

Introduction

The inbred-hybrid concept (East, 1908; Shull, 1909; Hayes, 1963) and the population-hybrid (Carena, 2005; Carena and Wicks III, 2006) concepts were developed in the public sector. The most successful maize germplasm was Iowa Stiff Stalk Synthetic or BSSS (Sprague, 1946), a genetically broad-based population. Its successful derivation, B73 (Russell, 1972), was derived after five cycles of half-sib recurrent selection. BSSS and B73 were diverse products of the public sector and produced billions of dollars to industry. Several factors including the right choice of genetically broad-based germplasm have caused a significant increase in yield performance from the U.S. Civil War (1.9 t ha^{-1}) to 1996 (9.1 t ha^{-1}). A good breeding plan for molecular markers would need to be addressed (Sonino et al., 2006; Barata and Carena, 2006; Hammond and Carena, 2008), if support to complement traditional maize breeding efforts is intended for developing new cultivars.

Maize was the first agricultural crop grown in ND during the 1700s (Olson et al., 1927) challenged by short growing seasons, low precipitation, and limited heat. Native Americans were the first corn breeders in these environments. The corn-breeding program at NDSU has been developing early maturing (65-95RM) maize since 1933 under the advice of H.K. Hayes (Carena, 2007). This program is the most northern public maize breeding program in North America what is unique and essential for the development of new early maturing lines.

Commercial maize hybrids available in ND are mostly bred and developed elsewhere. As a consequence, adaptation is challenging. Hybrids are often late maturing and, therefore, low test weight and starch content are a continuous challenge as well as general poor quality due to state environmental challenges affecting the effective utilization of our state corn into ethanol. Therefore, grain quality, test weight, drought tolerance, cold tolerance, early seedling vigor, uniform emergence in cold soils, fast dry down, and early maturity are very important characteristics (as important as grain yield) and essential to a hybrid in ND.

In 2007, ND farmers planted more than 2.5 million acres of maize due in part to renewable fuel demand. However, the corn-ethanol relationship will only be possible in ND if high yielding early maturing drought tolerant maize quality products are available. The NDSU breeding program has conducted 61 experiments in 2007 including 21,560 plots across 20 ND locations. These experiments included adaptation (e.g. GEM and selection response to earliness trials) and germplasm improvement trials (e.g. intra and inter-population recurrent selection program trials), early and late generation trials with industry testers for inbred line development, and advanced hybrid performance trials. Thousands of maize genotypes have been tested mostly based on incomplete block experimental designs. Our program is one of the few public programs that can still offer a strong emphasis on germplasm improvement, inbred line development, and training of applied plant breeders.

EarlyGEM Efforts

The NDSU corn breeding program has continued to move elite tropical and temperate corn germplasm northward becoming the source of new and diverse elite products. We have created 'EarlyGEM', a continuous effort to incorporate GEM germplasm into the northern U.S. Corn Belt. The EarlyGEM project targets to increase the number of competitive products utilizing useful genetic diversity. The NDSU corn breeding program has become a GEM cooperator in 1999 and since then this program has focused on increasing the genetic diversity of hybrids in the U.S. North Central Region and the need for alternative heterotic patterns (Carena, 2005; Melani and Carena, 2005).

The purpose of this research is to develop early maturing high quality GEM lines for utilization in the northern U.S. Corn Belt. Our specific objectives are to:

1) Incorporate GEM germplasm as donor for high quality and genetic diversity.
2) Adapt GEM germplasm to short-season environments.
3) Develop new early maturing lines competitive for industry use.

Material and methods

Germplasm Development and Inbred Line Development

In 2001, 152 GEM S3 released lines were observed in the Fargo breeding nursery for 15 adaptation traits. The most adapted and top yielding genotypes (based on central U.S. Corn Belt GEM trials) were selected and crossed to ND inbred lines. Only 28 early maturing GEM lines were used to develop new populations. F1 generations were backcrossed to early-maturing lines only once and those with below average agronomic characteristics were discarded. Only nine populations were kept to develop BC1:S1 early maturing lines with approximately 25% GEM germplasm. The source populations consist of three Stiff Stalk (SSS) and six non Stiff Stalk (NSS) populations. Over 3,000 adapted lines were advanced. Stiff Stalk donors (CUBA117:S1520-388-1-B, CHIS775:S1911b-B-B, and AR16026:S17-66-1-B) and non-Stiff Stalk ones (BR52051:N04-70-1, SCR01:N1310-265-1-B-B, FS8B(T):N1802-35-1-B-B, UR13085:N215-11-1-B-B, CH05015:N15-184-1-B-B, and CH05015:N12-123-1-B-B) were advanced to the S3 generation. Only AR16026:S17-66-1-B data is showed (Table 1) since testcrossing for the other population lines is scheduled for 2008. Five hundred BC1:S1 lines derived from (AR16026:S17-66-1-B x ND2000) x ND2000 (Carena and Wanner, 2003) were grown in breeding and disease nurseries at Fargo, Prosper, and Casselton, ND and advanced one generation of inbreeding while crossed to industry testers the same season. First year trials were conducted and top S2 lines were advanced to the S3 generation for both inbreeding and more extensive testing in both winter and summer nurseries (e.g. winter nursery testing of inbreds and hybrids under drought tolerance). Release decisions will be made after testing the lines across 30 to 60 environments.

Evaluation of 2007 Experiments

Experiments including 64 and 81 entries (depending on testers) were arranged in partially balanced lattice designs with two replications per location. Popular known commercial hybrids were included as checks which they were utilized as criteria for different traits and benchmark for advancing pedigrees. Data were collected for grain yield, grain moisture at harvest, root lodging, stalk lodging, test weight, and emergence percentage across environments. A sub-sample of 500 g of kernels was collected from every plot in all environments and used to measure starch content, extractable starch, fermentable starch, oil content, and protein content. Analyses of variance were performed for all traits within and among environments. Genotypes were considered as fixed effects while environments and replications within each environment were considered as random effects. Means adjusted by incomplete blocks and effective errors were utilized. Adjusted or unadjusted means (depending on efficiencies) from each location were used to perform a combined analysis of variance (SAS, 1989). Components of variance and broad-sense heritability were calculated in order to determine the repeatability of genotypes. Fisher protected least significant differences (FLSD) were used to compare among genotype means at $P \leq 0.05$.

Results and discussion

Table 1. shows data generated from top 20 performing hybrids between GEM-derived materials and a Holden Foundation Seed tester. It is important to note that there are not many choices for early maturing industry testers.

Table 1. Means combined over ND environments of six traits for selected entries sorted by yield for GEM-derived maize hybrids.

GEM Pedigree	Hybrid	Grain Yield bu/A	Grain Moisture %	Root Ldg %	Stalk Ldg %	Test Weight lb/bu	Emergence %
	LH176 X ND2002	165.74	19.72	0.88	3.79	56.62	0.70
[(GEM21xND2000)XND2000]-9	LH176XND07-209	164.98	20.34	0.00	0.92	55.77	0.65
[(GEM21xND2000)XND2000]-60	LH176XND07-260	162.19	19.92	0.74	1.86	56.88	0.65
[(GEM21xND2000)XND2000]-28	LH176XND07-228	159.49	20.00	0.00	1.49	56.86	0.75
[(GEM21xND2000)XND2000]-26	ND07-226XLH176	159.33	20.17	0.00	7.28	58.03	0.61
[(GEM21xND2000)XND2000]-7	LH176XND07-207	155.73	20.67	0.00	1.78	55.88	0.63
[(GEM21xND2000)XND2000]-52	LH176XND07-252	155.72	23.80	0.00	5.47	53.59	0.64
[(GEM21xND2000)XND2000]-7	ND07-207XLH176	152.06	21.00	1.63	5.80	55.18	0.65
	TR3026 Bt X TR1957	151.77	20.88	0.00	0.82	57.30	0.70
[(GEM21xND2000)XND2000]-10	ND07-210XLH176	151.66	19.73	0.48	3.78	55.84	0.68
[(GEM21xND2000)XND2000]-55	LH176XND07-255	149.89	19.21	2.61	2.02	56.65	0.64
[(GEM21xND2000)XND2000]-44	LH176XND07-244	149.52	21.46	1.19	0.48	54.53	0.62
[(GEM21xND2000)XND2000]-49	LH176XND07-249	149.32	19.95	0.00	2.77	56.46	0.64
[(GEM21xND2000)XND2000]-5	LH176XND07-205	148.99	19.81	0.00	4.65	55.70	0.63
[(GEM21xND2000)XND2000]-36	LH176XND07-236	148.22	19.83	0.00	3.47	56.41	0.61
[(GEM21xND2000)XND2000]-41	LH176XND07-241	147.97	21.12	0.00	5.11	55.55	0.65
	Pioneer 39D82	146.81	18.14	0.00	2.21	56.12	0.56
[(GEM21xND2000)XND2000]-56	LH176XND07-256	146.65	19.06	0.00	6.53	57.99	0.67
[(GEM21xND2000)XND2000]-18	LH176XND07-218	146.55	20.40	0.00	3.28	56.56	0.62
	TR3621Bt X TR3273	145.11	20.84	0.00	2.44	58.21	0.65
Experiment	Mean	138.12	20.05	0.29	3.79	56.25	0.64
	CV (%)	10.79	4.89	359.79	83.28	2.18	9.28
	LSD(0.05)	22.21	3.34	1.27	5.19	1.39	0.11
Checks (Top Five)	Mean	153.96	19.31	0.18	3.03	57.53	0.64

At least 15 GEM-derived lines showed over 101% of check mean yield and test weight and will be advanced to second year testing. This confirms the finding that inbred lines with 25 to 50% non-Corn Belt germplasm had combining abilities for grain yield greater than the 100%

U.S. Corn Belt inbred lines (Griffing and Lindstrom, 1954) with the advantage that exotic populations carry more genetic diversity (Goodman, 1965). This is the first research devoted to <90RM germplasm enhancement with tropical and late-temperate genetic materials. Winter nurseries with three generations per year could provide new lines in four years.

Future Work

More testing and nutritional evaluation and analyses of hybrids will be continued. Depending on funding we will extend this type of work to the other eight GEM-derived populations that are in need of extensive testing. Benchmarks will increase to 105% of check mean yield at second year testing in 2008. Release decisions will follow. We also plan to develop new early-maturing GEM populations including top lines.

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Optimizing among-and-within-family selection in forage crops

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ABSTRACT: Genetic gains in forage yield lag far behind the gains made in grain yield of cereal crops, partly due to the use of inefficient selection methods that make little use of additive genetic variance within half-sib or full-sib families. Theoretical expected gains show that among-and-within-family (AWF) selection is equal to or better than family selection under all circumstances provided the within-family selection criterion (X or Y) is heritable and has a positive genetic correlation with the desired trait (Y). AWF selection is favored over progeny-test selection by: (1) high heritability on an individual-plant basis (relative to heritability on a family-mean basis), (2) within-family selection intensity greater than among-family selection intensity, and (3) possibly a shorter cycle time (for some species and some breeding programs). Empirical studies on three perennial grasses demonstrated a positive genetic correlation between forage yield (Y) and survivorship (X), resulting in a significant improvement of AWF selection over family selection in two rhizomatous species.

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Differences in barley root system size and nutrient uptake of grain

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ABSTRACT: The issue of the relationship between roots and environment has been studied by many authors. The main topics of the studies have been the effects of abiotic and biotic stresses, spatial exploitation of roots in soil profiles, nutrient acquisition, rhizosphere interactions and hormone and other chemical effects on roots systems. However, one unanswered question remains: how large should the root system be in relation to the environment, yield and quality? Gorny and Larsson (1989) claims, that a larger root system size (RSS) is a yield stabilizing factor because during critical periods, it is able to supply the plant with water and nutrients from deeper soil layers. However, in the optimum environmental conditions, a bigger RSS may consume photosynthetic products. The aim of this study was to establish the relationships among the spring barley yield, the nutrients uptake by grain, and RSS. Root system size (RSS) was measured in six spring barley varieties (*Hordeum vulgare* L., ´Bojos´ [CZ], ´Jersey´ [NL], ´Kompakt´ [SK], ´Malz´ [CZ], ´Sebastian´ [DK] and ´Tolar´ [CZ]) using electrical capacitance (Chloupek, 1977). The evaluations of RSS were taken twice during plant development: 3 tillers detectable (RSS1) and middle of heading (RSS2). The barley varieties were assessed during three years (2005-2007) on the Žabčice (N49°01´ E16°36´) site (CZ). The harvested grain yield was determined, and after mineralization (H_2SO_4 + H_2O_2), the macroelements content was also established, N was assessed according to Kjeldahl, P spectrophotometrically and K, Ca and Mg by AAS. Based on the results, the grain yield (t/ha), nutrients uptake by grain (kg) and their relationships to RSS were evaluated by correlation coefficient by force of general liner regression analysis. On average, a higher yield was achieved by the ´Sebastian´ variety. However, this variety belonged to those with smaller root capacities. High yield was also found in the ´Bojos´ variety that had relatively low RSS1, but it had the highest RSS2. The lowest average yield was established in the ´Jersey´ variety that had always been considered a relatively high RSS. This implies that a large root system of a barley plant presents a competitive sink during the last third of vegetation. Significant differences among the varieties were found in their uptake of basic macroelements. For example, the value of the nitrogen uptake did not always correspond with the root capacity. The ´Bojos´ variety had the highest N uptake and the biggest RSS2. On the contrary, the ´Jersey´ variety showed the second biggest RSS1 and RSS2 values but the lowest N uptake by grain. The variety share in the RSS variability was 9 to 12%. A negative correlation between RSS2 and the yield (r = -0.237**), and between RSS2 and P, K, Ca and Mg uptake (r = -0.272***, -0.229**, -0.197*, -0.322***) were found. A negative dependence of RSS1 on the N uptake by grain (r = -0.202**) was established.

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Occurrence and inheritance of resistance to *Sphaerotheca fusca* in local germplasm of *Cucumis melo*

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ABSTRACT: *Cucumis melo* L. subsp. *melo* conv. *adzhu*r (Pang) Grebensc (Carosello) is a relict of melon cultivars selected for use as immature fruits. It is cultivated specially in Apulia region, where it's possible to find an ample availability of local populations and ecotypes, but due to its valuable organolectic characteristics it is diffused in the others area of Southern Italy. Powdery mildew caused by *Sphaerotheca fusca* is the most widespread and dangerous disease of Carosello. During screenings for resistance towards *S. fusca* on local germplasm of *C. melo* one plant belonging to BA7-2 ecotype resulted healthy. Further, plants obtained by self-fertilization of healthy plants (BA7-2/S progenies) have shown three types of reaction towards pathogen: plant with severe symptom, healthy plants and plant with small chlorotic and necrotic spots covered with thin mycelium and with rare sporulation. From every symptomatological typology, single plants were self-fertilized and progenies were tested. All plants of progenies obtained by self-fertilization of healthy plants resulted healthy; the plants derived by self-fertilization of susceptible plants showed disease symptoms. The progenies derived by self-fertilization of plants showing limited symptoms segregated 39% of healthy plants, 28% of susceptible plants and 33% of plants with limited symptoms. This segregation ratio suggests that the resistance found in the BA7-2 ecotype of *C.melo* L. subsp. *melo* conv. *adzhur* is oligogenic. The Mediterranean basin is confirmed as centre of diversification of *C.melo*, therefore the preservation and the improvement of local germoplasm represents an fundamental aim for important genetic resources.

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Association genetics of UK elite barley

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ABSTRACT: The improvement in the yield potential of UK barley varieties due to plant breeding was estimated at 1% pa up to the 1980s. At that time the varieties Triumph and Igri dominated the spring and winter barley markets respectively and it is now timely to assess the current breeding progress in both crops. The recent development of a SNP based high throughput genome wide assay also means that we can identify genomic regions that are actively being selected in current breeding programmes. Winter and spring barley varieties representing commercial successes and failures spanning the period from 1975 to 2007 were grown in yield trials at a number of sites for harvest years 2006 and 2007. As well as plot yield, all entries were scored for heading date, height, thousand grain weight and grain hardness. The most appropriate spatial model for each trial was identified and then used in a REML meta analysis to estimate the overall means of each genotype. Assessment of genetic progress was assessed by regressing genotype means against the year in which they were first recommended. Genotypic data on the yield trial entries from a high density survey of 1500 SNP loci using Illumina Oligo Pooled Arrays was used in single marker analysis with crop type as a factor to identify genomic regions associated with control of the characters measured. Despite the greater yield potential of the winter crop, there was a significant increase in yield with year of release for spring and winter barley, accounting for over 40% of the variation in yield. Considering the yield components, there has also been a significant increase in TGW over the same period, suggesting that this component was largely responsible for the yield increase. Over the same period, heading data has not altered in both crop types but height and grain hardness have significantly decreased in the spring crop. Over

60% of the SNPs were polymorphic amongst the lines assayed and had minor allele frequencies >0.1 and were therefore used to test for associations with the characters measured. Significant associations were detected in a range of genomic regions, the most frequent being bin 7 on chromosome 2H, 6 on 5H and 7 on 7H.

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Occurrence of fungal pathogens causing leaf spot diseases of wheat in Hungary in 2000-2008

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ABSTRACT: The frequency of infections caused by *Drechslera tritici-repentis*, *Septoria tritici*, *Stagonospora nodorum* and *Bipolaris sorokiniana* significantly increased due to the poor economic situation, increasing use of monocultures and minimum tillage practices in Hungary in the last ten years. Only limited information is available for breeders about the frequency of occurrence of the pathogens and resistance of cultivars. Therefore a new project was started at the Cereal Research Non-profit Company in 2000 to describe the pathogens and the symptoms more precisely. Since 2000, we have collected more than 10 000 leaf samples from 8-13 locations in Hungary four times every year (March, April, May and June) to estimate the occurrence of the above-mentioned necrotrophic pathogens. According to the results of this 8-year survey, the most important pathogens were *D. tritici-repentis* and *S. tritici* in Hungary, occurring in more than 40% of the samples in epidemic years. However, significant differences were observed in their occurrence between different years and locations. The driest years were 2002, 2003 and 2007 when the occurrence of these pathogens was very low. In 2005 and 2006 the dominant pathogen was *S. tritici*, especially in the South part of Hungary. During these routine surveys of wheat-growing areas of Hungary, macro- and micromorphological examinations of single-spore isolates indicated that *Drechslera teres* (teleomorph: *Pyrenophora teres*), a well-known pathogen of barley also occurred frequently on wheat leaves. Species assignment of these isolates was confirmed by sequence analysis of the intergenic transcribed spacer region of these isolates. Pathogenicity tests proved that *D. teres* can cause leaf damage on wheat. *D. teres* was isolated from 0-9 % of spots observed on leaf samples with disease symptoms depending on the location of fields in Hungary. This is the first report on the pathogenicity of *P. teres* to wheat in Central Europe. (Tóth et al., 2008). Another species, *Pithomyces chartarum* (teleomorph: *Leptosphaerulina chartarum*) was also identified during our surveys as a wheat pathogen. The *Pithomyces chartarum* isolates produced a range of secondary metabolites including gregatin, alternariol and alternariol monomethyl ether, but not sporidesmin, a mycotoxin responsible for photosensitisation and liver damage of grazing animals. Pathogenicity tests proved that *Pithomyces chartarum* can cause leaf damage, and these symptoms were strikingly different for different wheat cultivars, which has important implications for breeding for resistance to this disease. This is the first report on the pathogenicity of *Pithomyces chartarum* to wheat in Europe (Tóth et al., 2007).

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Mapping resistance to *Mycosphaerella graminicola* in wheat

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ABSTRACT: Septoria tritici blotch (STB) casused by fungus *Mycosphaerella graminicola* (anamorph *Septoria tritici*) is one of the major disease affecting many wheat growing areas. The recent studies on genetics of resistance to STB have identified both isolate specific and nonspecific resistance. Specific resistance to STB is near-complete, usually oligogenic and follows a gene-for-gene relationship. Isolate nonspecific resistance is polygenic, quantitative and incomplete. The objective of our study is to detect resistance *loci* to STB in Polish Liwilla variety. For mapping, a doubled-haploid (DH) mapping population was established from a cross between the resistant cultivar Liwilla and the susceptible cultivar Begra. The mapping population was evaluated under controlled environment on seedlings with isolate *M. graminicolla* IPO86036. After incubation period the disease was scored as percentage of necrotic leaf area bearing pycnidia. In QTL analysis, we used established earlier molecular map of wheat (Czembor et al., 2007). Using multiple-QTL model (MQM), QTLs associated with resistance (QRLs) were detected on four chromosomes: 3A, 2B, 3B, and 7D. The percentage of phenotypic variance explained by a single QRL ranged from 10,4% (LOD=2,98) to 23,1% (LOD=3,71). QRLs detected on these chromosomes were localized in vicinity to already identified resistance genes to STB, namely *Stb6*, *Stb9*, *Stb2*, *Stb4* and *Stb5*. At this stage of the research it is difficult to infer about their relationships. The QRLs effects detected on seedlings will be verified at adult plant stage under field conditions in near future. The preliminary results indicate on complex and polygenic nature of resistance to STB in cultivar Liwilla.

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Mapping new resistance gene to *Puccinia hordei* Otth. in barley landrace Ph953-2

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ABSTRACT: Leaf rust of barley caused by *Puccinia hordei* Otth., is important disease in many barley growing areas. New virulent isolates as well as combinations of virulent genes are able to overcome resistance expressed by modern barley cultivars. However, only the leaf rust resistance gene *Rph7* is still effective in Europe. Since limited number of effective resistance genes are available it is necessary to identify new sources of resistance. The line Ph953-2 selected from barley landraces originated from ICARDA (International Center for Agricultural Research in the Dry Areas, Aleppo, Syria) carries single resistance gene to leaf rust and it is resistant to isolates virulent on lines containing resistance genes *Rph1* – *Rph6* and *Rph8* – *Rph12*. The allelism test excluded that the resistance is conditioned by gene *Rph7*. Eighty six $F_{2:3}$ families were developed from the cross Ph953-2 × L94 for mapping experiments. Bulked segregant analysis with SSRs revealed linkage of the resisatnce locus with polymorphic microsatellites GMS21, Bmac0213, Bmac0032, Bmag0504 and Bmag0347 – specific to chromosome 1H. Further saturation region of interest with other markers and test of allelism with *Rph4* gene mapped on chromosome 1H are in progress.

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Barley landraces as source of effective resistance against European isolates of powdery mildew

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ABSTRACT: Barley powdery mildew is caused by the pathogen *Blumeria graminis* f. sp. *hordei*. It is one of the most destructive foliar diseases of barley in regions with a maritime climate. This pathogen is showing the high level of pathogenic variability. Consequently, geneticists, plant pathologists, and breeders working with barley are constantly looking for gene pools from which new resistance genes to powdery mildew can be introduced into existing cultivars (Czembor, 1996, 2005). This study was conducted to study resistance to powdery mildew in 196 barley landraces collected from Near East. Seed samples originated from International Center for Agricultural Research in the Dry Areas – ICARDA, Aleppo, Syria. Twenty differential isolates of powdery mildew were used. They originated from the collections in Risø National Laboratory, Roskilde, Denmark; Danish Institute for Plant and Soil Science, Lyngby, Denmark; Edigenossische Technische Hochschule – ETH, Zurich, Switzerland and IHAR Radzików, Poland. The isolates were chosen according to their virulence spectra which were observed on the Pallas isolines differential set provided by Dr. L. Munk (Royal Agricultural and Veterinary University, Copenhagen, Denmark). They were purified by single pustule isolation. Young seedlings of the cultivar Manchuria (CI 2330) were used to maintain and propagate all isolates used. Frequent virulence checks were made to assure the purity of isolates throughout the experiment. Twenty-four single plant lines resistant to powdery mildew were selected. These lines should be used as sources of effective resistance for breeding of barley against powdery mildew.

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Genetic analysis of durable resistance against leaf rust in durum wheat

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ABSTRACT: The Italian durum wheat cultivar Creso possesses a high level of durable resistance to leaf rust based on both hypersensitive and non hypersensitive components. In order to investigate the genetic bases of this resistance, a segregating population composed by 123 recombinant inbred lines (RILs) deriving from the cross Creso x Pedroso, a Spanish susceptible durum wheat variety, was evaluated for disease severity in adult plants under field conditions. Furthermore, the resistance of parents and RILs was evaluated in controlled conditions at two developmental stages (seedling and adult plant) by assessing macroscopically the latency period and microscopically the number and type of pathogen colonies formed following artificial inoculation with a specific isolate. A genetic linkage map for QTL analysis was developed from this cross, consisting of more than 400 molecular markers and spanning more than 1800 cM. One major QTL explaining both reduction of disease severity in the field and increased latency period was found on the long arm of 7B chromosome, and microsatellite and DArT markers strictly associated were identified.

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Simultaneous selection for powdery and downy mildew resistance genes of different origin in grapevine (*Vitis vinifera* L.)

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ABSTRACT: Resistance breeding in viticulture is aimed at producing varieties carrying durable resistance to powdery (PM) and downy (DM) mildews. Durable resistance can be controlled either by one gene, or can be the result of several genes. Since no *Vitis vinifera* cultivars carrying PM resistance genes were found till the mid 1960s, wild *Vitis* species were used as resistance gene sources. *Muscadinia rotundifolia* is an excellent resistance gene source carrying the *RUN*1 dominant PM and the *RPV*1 major DM resistance genes. A BC_4 individual (*M. rotundifolia* x *V. vinifera*) obtained in Hungary in 1996 was used in crosses with *V. vinifera* cultivars. Although the *V. vinifera* cultivars are classified as susceptible to powdery mildew, different cultivars show various levels of susceptibility. On the basis of greenhouse tests, 9 varieties of Georgian, Armenian, Uzbekian and Russian origin were found to be resistant to powdery mildew. 'Kishmish vatkana' is also a PM resistant *V. vinifera* cultivar originating from Uzbekistan, and it was involved first by Kozma et al. (2008) in a resistance breeding program. For pyramiding mildew resistance genes 'Kishmish vatakana' was crossed with the BC_4. The PM resistance gene of 'Kishmish vatkana' was identified previously as *REN*1 (Kozma et al., 2008). While *RUN*1 and *RPV*1 were mapped into the linkage group 12 (Barker et al., 2005), *REN*1 locus was assigned to the linkage group 13 (Hoffmann et al., 2008). Our goal was to apply multiplex PCR for the simultanous screening for *RUN*1/*REN*1/*RPV*1 genotypes in asegregating BC_5 population deriving from the cross of BC_4 x 'Kishmish vatkana'. Marker assisted selection is not only an efficient method for identifying the desirable genotypes but it is indispensable for discriminating the two PM resistance genes (*RUN*1 and *REN*1) having similar phenotypic effects.

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Screening wheat genotypes for germination potential and young seedling growth under water stress

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ABSTRACT: Seed-zone water content is the main factor controlling seedling emergence and survival. Sometimes, cereals in semiarid regions with dryland farming have to germinate with unfavorable seedbed conditions. In such years poor stand establishment is the most important factor affecting cereal grain yields. This study reports on genotypic variation in wheat for germination percentage (GP), coleoptile (CL) and total seedling length (TSL) under normal and water stress conditions. One hundred wheat (*Triticum aestivum* L.) genotypes of worldwide origins, were placed on filter paper moistened with either distilled water or a polyethylene glycol 6000 (PEG) solution at -0.4MPa potential. GP, CL and TSL were measured on three occasions in two consecutive years. Variations of GP, CL and TSL were statistically significant in both treatments. TSL ranged from 11.9 to 119.5 mm in well-watered conditions and from 0.5 to 63.7 mm in PEG. TSL reduction due to PEG ranged from 36 to 97%. Osmotic treatment delayed initiation of seed germination from 1.7 to 10.7 days and reduced GP from 1 to 91%. Thus, considerable genotypic variation exists for germination potential and young seedling growth under droughted conditions. Relationships among the traits were examined by correlation analysis.

Keywords: coleoptile, germination, osmotic stress, seedling growth, *Triticum aestivum*.

Introduction

Water is the predominant resource limiting seedling survival and crop production in semiarid regions. Drought conditions near the soil surface reduce seed germination and subsequent emergence. The critical water potential of the surrounding medium in which seedling of wheat is able to survive, at the stage of germination and emergence were -1.94 and -2.87 Mpa, respectively (Deng et al., 1999). The same authors suggested that plumule elongation of the cell expanding growth was the most sensitive stage to water stress. Dhanda et al. (2004) reported that germination under osmotic stress significantly reduced coleoptile length. The observation of the response of coleoptile length to water stress could be used in plant-breeding applications to identify differences in osmoregulation between genotypes of wheat (Morgan, 1988).

Seed germination and seedling growth can be screened for drought tolerance by using a polyethylene glycol-6000 (PEG) solution as the moisture stress inducing media (Blum et

al., 1980). The present paper reports the results obtained for variation in, and associations among, germination percentage, coleoptile and total seedling growth of wheat under normal and water stress conditions in the laboratory.

Material and methods

Plant material and data collection

One hundred winter wheat (*Triticum aestivum* L.) genotypes of worldwide origin, with the contrasting expression for some traits of agronomic importance, were chosen for evaluation of various traits at the seedling stage under normal and water stress conditions in the laboratory. Fifteen seeds of each genotype were placed on two layers of filter paper under an osmotic potential of 0.0 (T1, distilled water, control) and -0.4MPa (T2) created using a polyethylene glycol solution (PEG-6000) according to the method of Michael and Kaufman (1973) and replicated three times in two consecutive years. The seeds were put on filter paper in transparent plastic bags and placed at random in a growth chamber for 10 (T1) and 25 days (T2), at an average temperature of 21 ± 1 °C under dark incubation. Germination was recorded when radicle reached at least 3 mm in length. Data for coleoptile length and total seedling length (coleoptile + the first leaf from the coleoptile) were obtained from the 10 best seedlings in each replication.

Statistical analysis

The analysis of variance (ANOVA) for a completely randomised design with combined data from the two years was performed. For all statistical analyses, the data of germination percentage were arcsin transformed. Basic statistics such as minimum and maximum values, the arithmetic mean and the coefficient of variation (CV) were determined, and a t-test (Steel et al., 1997) was performed to test the significance of differences between the means. Relationships among the traits at seedling stage were estimated by Pearson correlation analysis.

Results and discussion

Genotypic variation of the traits at seedling stage

Analysis of variance showed the presence of a considerable amount of genetic variability for GP, CL and TSL under both normal (T1) and water stress (T2) conditions (Table 1). The magnitudes of mean squares for CL and TSL in T1 were higher than in T2, and indicated that variability was reduced under water deficit conditions. The reduction of genetic variance under stress is partly a direct result of large environmental (error) variance within the stress environment (Blum, 1988) and partly a result of the suppression of genetic variability under such conditions (Ludlow and Muchow, 1990).

The expression of mean performance for all traits was significantly higher ($P < 0.01$) under normal (T1) than water stress (T2) conditions (Table 1). GP ranged from 91.5 to 100% in well-watered conditions and from 8.9 to 96.7% in PEG. CL ranged from 8.8 to 51.3 mm in well-watered conditions and from 0.0 to 25.8 mm in PEG. TSG ranged from 11.9 to 119.5 mm in

Table 1. Mean squares for genotype (MS), minimum and maximum values, arithmetic mean and coefficient of variation (CV) of 100 wheat genotypes for the traits at seedling stage under normal (T1) and water stress (T2) conditions (averaged across two years).

Trait	Treatment	MS	Min	Max	Mean†	CV (%)
Germination %, GP	T1	75.8**	91.5	100.0	98.1[a]	1.7
	T2	717.6**	8.9	96.7	66.3[b]	25.2
Coleoptile length (mm), CL	T1	4.0**	8.8	51.3	35.7[a]	22.9
	T2	1.9**	0.5	25.8	12.3[b]	46.2
Total seedling growth (mm), TSL	T1	2613.4**	11.9	119.5	79.2[a]	26.4
	T2	1022.3**	0.5	63.7	20.4[b]	64.3

** Significant at P = 0.01.

† Within a trait, means followed by the different letter differ significantly at 1%.

well-watered conditions and from 0.0 to 63.7 mm in PEG. Comparison of average reduction due to T2 condition indicated that TSL was the most sensitive trait (74.9%), followed by CL (65.9%) and GP (32.4%). Dhanda et al. (2004) determined that reduction of germination potential, coleoptile length and shoot length in 30 wheat genotypes due to osmotic stress (-1.0MPa) were 63.3%, 63.9% and 40.2%, respectively. For all traits the coefficient of variation (CV) was higher under T2 than under T1. A wide range of CV under T2 from 25.2 (GP) to 64.3 (TSL) indicating that they were influenced in very different degrees by genotype under water stress (Table 1). Osmotic treatment delayed initiation of seed germination from 1.7 to 10.7 days.

Correlation analysis

Germination percentage under T2 was positively correlated ($P < 0.01$) with CL and TSL under both T1 and T2 (Table 2). On the other hand, GP under T1 showed no significant correlation with CL and TSL under both treatments. Furthermore, no significant correlation ($r = 0.133$) was found between GP under T1 and GP under T2, showing that GP under T1 will not discriminate drought tolerant genotypes at seedling stage. CL showed positive and significant correlation with GP ($r = 0.803$, $P < 0.01$) and TSL ($r = 0.889$, $P < 0.01$) under T2, suggesting that breeding for longer-coleoptile wheats will improve emergence and seedling establishment under water deficit. Dhanda et al. (2004) reported that wheat genotypes with higher coleoptile length showed longer roots under osmotic stress. Rauf et al. (2007) also found that coleoptile was significantly associated with germination percentage and shoot length in wheat.

In conclusion, all the traits, except GP under non-stress conditions, showed considerable genetic variability. Correlation study indicated that germination percentage under osmotic stress can discriminate drought tolerant genotypes at seedling stage.

Table 2. Pearson correlation coefficients (*r*) among the studied traits.

Trait†	GP-T1	GP-T2	CL-T1	CL-T2	TSL-T1
GP-T2	0.133				
CL-T1	0.149	0.450**			
CL-T2	0.048	0.803**	0.565**		
TSL-T1	0.163	0.540**	0.878**	0.559**	
TSL-T2	0.028	0.740**	0.501**	0.889**	0.516**

** Significant at P = 0.01.

† GP = germination %, CL = coleoptile length, TSL = total seedling length, T1 – control variant (0.0 MPa), T2 = PEG stress (-0.4 MPa).

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OsGH3.1 overexpression, a new approach for resistance to fungal pathogen in rice

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ABSTRACT: Plant hormones are implicated in a broad range of plant growth and developmental processes and there is also wide evidence that they mediate plant responses to the environment, including many stresses. The *GH3* gene family is involved in hormonal homeostasis through the conjugation to amino acids of the free form of essential plant growth regulators such as indolacetic and jasmonic acids. A genome wide analysis allowed us to identified all members of this family in rice. Overexpression of one of them, *OsGH3.1*, in rice caused dwarfism and significantly reduced both free auxin content and cell elongation. Transcriptomic profiling revealed that most genes involved in auxin biosynthesis and auxin signaling inhibition were respectively induced and repressed in transgenic plants overexpressing OsGH3.1. Many genes related to cell organization, cell wall loosening and biogenesis were also significantly down-regulated. Genes related to cold response were down regulated but, several genes implicated in pathogenic responses were up-regulated linking pathogenic defense responses and auxin depletion. Infection analysis with the rice pathogen *Magnaporthe grisea* indicated that transgenic plants were more tolerant to fungal attack validating the role of *OsGh3.1* in the regulation of defense mechanisms against a fungal pathogen.

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Genetic analysis of drought tolerance in chickpea (*Cicer arientinum* L.)

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ABSTRACT: Chickpea (*Cicer arientinum* L.) with high protein content and drought tolerance behavior is one of the most edible crops in Iran. Genetic analysis of gene pools and stress tolerance indices are the main steps in crop improvement programs (Singh, 1997; Güler et al. 2001). In order to find out genetic aspect of drought tolerance indices, this research was carried out in Razi University of Kermanshah in 2005. The experiments were set up with 20 chick pea cultivars and land races in a randomized complete block design with three replications in rain fed and irrigated conditions. The following traits were measured and calculated: plant density, pod No. per plant, grain per plant, 1000 grain weight, grain yield, plant height, chlorophyll florescence, relative water content, relative water lost, proline amount. Drought tolerance index, (DTI), mean productivity (MP) , geometric mean productivity (GMP), tolerance (TOL) and susceptibility stress index (SSI). Multivariate statistical procedure such as cluster analysis and principle analysis and other genetic parameters were applied. Analysis of variance revealed significant differences between entries for drought indices at 5% probability levels. Cluster analysis classified all entries to five groups. The greatest genetic distance was between entry No. F2 and Hatam with 59.06 units, and the least genetic distance was between Arman and S1 with 0.027 units. Genetic parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), environmental coefficient of variation (ECV), broad sense heritability (H2b) and genetic advance (GA) were calculated. The greatest amount of GCV belonged to TOL (120.364) and DTI (78.167) and the least amount of GCV belonged CF (0.00). The highest H2b belonged to DTI (0.978) and GMP (0.992). The greatest GA amount belonged to GMP (1118.05) and yield (1196.777). The principle component analysis based on all traits revealed that 86.753 per cent of variance was explained by five PCAs. Yield, MP, DTI and GMP had highest amount of variance portion. These traits revealed the selection direction in breeding program to release improved varieties.

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Two novel whole-plant field phenotyping equations maximize selection efficiency

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ABSTRACT: One of the most crucial goals of a breeding program is the precision of phenotyping. Accurate whole-plant field phenotyping maximizes selection efficiency and speeds up the release of improved cultivars. In addition, accurate phenotyping is of outmost importance to molecular breeding, where obtaining good phenotypic data could be a more limiting factor than obtaining good genotyping data. The objective of this paper is: (i) to discuss the principle factors affecting single plant selection and (ii) to describe two novel whole-plant field phenotyping equations capable of evaluating a large number of individual plants and reducing the time frame to release new and improved cultivars.

Keywords: homeostasis, honeycomb designs, phenotyping equations, selection efficiency.

Introduction

The objective of plant breeding is the development of cultivars combining high and stable productivity with good quality. To accomplish this objective three categories of genes should be incorporated, i.e., those contributing to high productivity, stable productivity, and good quality. Optimal productivity is achieved when all plants in the crop stand yield the same, that is, when plants share the growth resources equally and the plant-to-plant competition is minimized to almost zero (Fasoula and Fasoula, 1997). Zero or minimal competition has two prerequisites, one is genetic and the other agronomic. The genetic prerequisite of zero competition is the utilization of monogenotypic cultivars to erase genetic competition. The agronomic prerequisite refers to the ample and evenly distributed growth resources and to the synchronous germination, growth, and development of the evenly distributed plants across the field.

The need of using monogenotypic cultivars for maximizing productivity imposes as unit of evaluation and selection the individual plant. This in essence means that genes controlling productivity, stability, and quality must be incorporated into a single genotype. To accomplish this crucial goal it is necessary to face or exploit any factor confounding the efficiency of single plant selection. The principle factors affecting single plant selection are: (1) competition, (2) soil heterogeneity, (3) heterozygosity, (4) genotype by environment interaction, and (5) adaptive variation.

Discussion

1. Competition

Competition, defined as the unequal sharing of growth resources among plants, impairs crop yield and selection efficiency in three ways (Fasoula and Fasoula, 2002). First, competition reduces stand uniformity which mirrors crop productivity (Fasoula and Tollenaar, 2005). Furthermore, competition is correlated negatively with the yielding ability and brings about variety degeneration by enhancing the proliferation of high competitors at the expense of the high yielders. Third, competition, by being negatively correlated with yielding ability, favors selection of high competitors at the expense of high yielders, thus, minimizing response to selection (Kyriakou and Fasoulas, 1985; Fasoula, 1990). To minimize the effects of competition on crop yield and selection efficiency, selection is performed at ultra-low plant densities; otherwise selection favors competitive and not yielding ability.

2. Soil heterogeneity

Soil heterogeneity impairs crop yield and selection efficiency by reducing stand uniformity, increasing competition, and restraining selection efficiency through the establishment of non-comparable growing conditions. To minimize the confounding effects of soil heterogeneity on single plant selection, the honeycomb field designs were developed that sample effectively soil heterogeneity and increase response to selection (Fasoulas and Fasoula, 1995). An example of a honeycomb design is given in Figure 1.

The honeycomb arrangement of plants in the field has a number of advantages: (i) It ensures 15% more equidistant plant positions per unit area compared to the squared arrangement; (ii) Each plant occupies the center of a circular complete replicate and is surrounded by plants occurring in the periphery of concentric rings; (iii) Plants of the same line form an equilateral triangular lattice (ETL) pattern which samples effectively soil heterogeneity by placing lines under comparable growing conditions (Figure 1).

To erase the confounding effect of soil heterogeneity on single plant yields, the yield of each plant (x) is expressed as a ratio to the average yield of the surrounding plants included within a ring of a chosen size $(\bar{x}_r)$. Ring sizes can vary from 6 plants to >100 plants. The square of the ratio $x/\bar{x}_r$ is called coefficient of plant yield $CPY = (x/\bar{x}_r)^2$. The ***CPY*** takes values larger than one in the case of superiority over the mean yield of the plants within the ring and smaller than one in the case of inferiority. The ***CPY***, devoid of the masking effect of soil heterogeneity, enables to evaluate the yield of single plants with the same degree of accuracy and apply very high selection pressures, provided that an accurate criterion of stability is also available. Stability is evaluated by another coefficient, called the coefficient of homeostasis which equals $CH = (\bar{x}/s)^2$, where $\bar{x}_r$ and s are the mean yield and the standard deviation, respectively, of the progeny line to which each plant belongs. The coefficient of homeostasis which measures any factor interfering with the stability of performance is estimated with great precision due to the ETL arrangement of the plants within each progeny line.

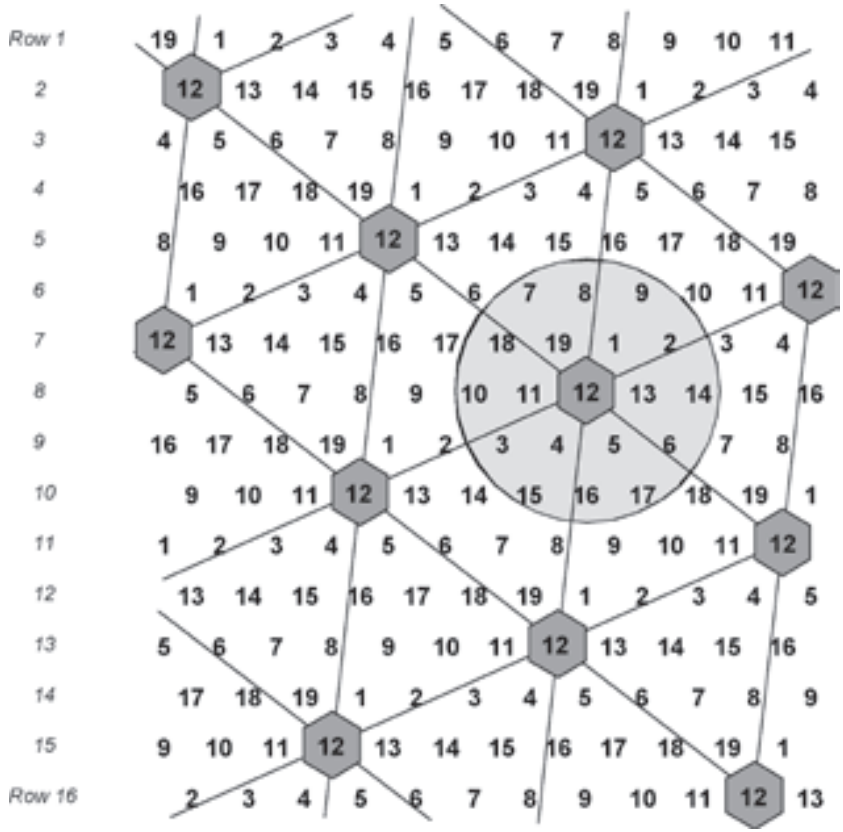

Figure 1. The replicated-19 honeycomb design evaluates plants of 19 progeny lines in ascending order and in horizontal field rows, from left to right. The numbers in the figure represent the position of the plants in the field. Plants of any line form an equilateral triangular lattice pattern (ETL, shown for plants of line 12). Every plant is surrounded by plants occurring in the periphery of concentric rings and its yield is adjusted according to the moving-ring average yield. A ring of 18 plants is illustrated for a plant that belongs to line 12.

The product of the two coefficients forms the following plant yield by homeostasis equation:

$$Plant\ Y \times H = (x/\bar{x}_r)^2 \cdot (\bar{x}/s)^2 \quad \text{[Equation 1]}$$

The plant yield by homeostasis equation is used for efficient selection both among and within progeny lines and allows the application of very high selection pressures. Furthermore,

it renders the yield of the selected lines independent of density, meaning that these lines yield optimally over a wider range of plant densities. This property of the equation explains why good performance under dense stand may be predicted when selecting for high equation values at ultra-low plant densities (Fasoula, D; Lithourgidis, A, this Congress).

When selection is restricted among progeny lines only, another equation may be used which is obtained by replacing the coefficient of plant yield $CPY = (x / \bar{x}_r)^2$ with the coefficient of line yield $CLY = (\bar{x} / \bar{x}_t)^2$; where $\bar{x}$ is the mean yield of the progeny line and is the mean yield of all plants in the trial. In that case, the line yield by homeostasis equation takes the following form:

$$Line\ Y \times H = (\bar{x} / \bar{x}_t)^2 \cdot (\bar{x} / s)^2$$ [Equation 2]

3. Heterozygosity

Heterozygosity exerts a strong confounding effect on selection efficiency since response to selection requires gene fixation which essentially means conversion of heterozygosity to homozygosity. In inbreeders, gene fixation is accomplished automatically and, as expected, the ceiling performance is set by the genotype of a single F_2 foundation plant. Therefore, the accurate evaluation of F_2 plants is essential and it can be accomplished effectively using Equation 1. In outbreeders, the high selection pressures ensured by Equation 1 lead to an enhanced step-by-step fixation of genes belonging to several foundation plants and not just to one as in inbreeders.

4. Genotype by environment interaction

The confounding effect of the G×E interaction on selection efficiency is very strong because when the breeder selects at a single location characterized by specific agroclimatic conditions, the produced cultivar will fail to perform equally well at other sites with different agroclimatic conditions. As a result, the breeder needs to grow the same experiment at several sites and select simultaneously for broad and local adaptation. This is feasible using the honeycomb field designs that, by having as units of evaluation and selection the single plants grown at ultra-low plant densities, ensure large amount of seed per plant that allows growing the same trial at several sites (Fasoula and Fasoula, 2000).

5. Adaptive variation

Adaptive variation is released by the genome in response to environmental stimuli that activate known and unknown sensory genetic and epigenetic mechanisms. This variation is the result of the interplay between the genotype and the environment and is very useful for breeding as it allows selecting genotypes exploiting in the best way the target environments, ranging from favorable to marginal. Since environments are in a constant flux, exploitation of adaptive variation requires constant selection across the target area of adaptation (Fasoula and Boerma, 2005). Nonstop selection enables breeders to remove deleterious mutations, avoid

variety degeneration, and ensure a steady improvement of cultivar agronomic performance across years and different environments (Fasoula and Boerma, 2007).

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Comparison of linkage maps among six citrus species: structural differences and genetic inferences

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ABSTRACT: Two kinds of breeding populations and target traits are managed for citrus improvement, one for the rootstock and another for the scion. Most linkage maps reported in citrus correspond to rootstock breeding populations. The present study shows a comparison of the linkage maps of six species: *Citrus volkameriana*, *C. aurantium*, *Poncirus trifoliata*, *C. reshni*, *C. clementina* (mandarin) and *C. grandis* (pummelo). The linkage maps of the three former species were previously reported (Ruiz and Asins 2003, Asins et al. 2004) while those of the three latter species are new. The *C. reshni* map has been obtained from a progeny of 157 hybrids from the cross *C. reshni* x *P. trifoliata*. The *C. clementina* and *C. grandis* maps have been obtained using a reciprocal cross between two scion cultivars from these true-sexual *Citrus* species that largely differ in *Citrus Tristeza Virus* (CTV) resistance and fruit size, taste and colour. A total of 147 markers (mostly SSRs and IRAPs) and two organelle DNA markers were used to genotype both progenies (174 hybrids). Almost nine percent of the seedlings derived from the pummelo as female parent showed the same organelle markers as those of the mandarin, revealing an exception for their maternal inheritance in citrus. Most segregation distortion affects just the allele frequencies, generally representing differences in pollen fertilization success, as a likely consequence of pollen-pistil interactions. When comparing the pummelo and mandarin linkage maps to those previously reported for rootstock species, structural differences affecting *C. aurantium* linkage groups 3, 7 and 4, and *P. trifoliata* linkage group 4 become evident. The large extension of colinearity found is being helpful to infer the position of orthologous genes and QTLs in citrus species and for a more useful genetic characterization of citrus germplasm collections. Thus, all CTV-resistant species here studied (*P. trifoliata*, *C. aurantium*, *C. grandis*) present a major resistance QTL on linkage group 4 and closely linked markers to this QTL have been used to establish a *C. aurantium* core collection where the CTV resistance response was studied for four years.

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Characteristics of production potential for yield and biomass of new winter wheat (*Triticum aestivum. L*) line developed in Kosova conditions

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ABSTRACT: The basic material, with which new winter wheat lines were developed under the agro ecological conditions of Kosovo, encompassed a wide genetic and geographic diversity. Lines of the F_6 and F_7 generations were evaluated with the aim of distinguishing the best lines. Lines have been evaluated for the following traits: yield, ear shape, biomass and reaction to ecological conditions during years 2006 and 2007. Field design consisted in a randomized blocks design (RBD) with three replications. Differences between genotype lines and treatment were highly significant at P=0.05 and P=0.01. Quantitative parameters of plants, ear and yield within lines showed non significance differences, because breeding and selection increases homozygosis of lines. Average values of lines, for biomass were μ =4.27g/plant with including all its components: stalk, leaves, grain, glumes and awns. The minimal and maximal values for biomass were from 3.27 and 5.13 g/plant. Average yield value μ for gene effects in those lines were 10.27t ha^{-1}. The average yield at L_2 for the first and second year was y_1=13.44 and y_2= 9.42 tha^{-1}, respectively, with significant deviations from μ value, for years: y_1+3.17 and y_2-0.85 tha^{-1} or +0.30% and –0.08%, at P=0.05 and P=0.01. Genetic similarities and dissimilarities of lines were analyzed and presented with dendrogram by Ward's methods.

Keywords: biomass, ear, yield , Winter wheat lines.

Introduction

Food production is the basis of world security and, directly or indirectly, support the livelihoods of every person on Earth. Growth rate of population and the ability of food production are in full correlation. Kosova doesn't have its own wheat cultivars yet. So wheat production and yield in Kosova depends on seed quality imported from neighbors, but also from introduction cultivars form: Croatia, Austria and France and seed multiplication.

Acreage of winter wheat in Kosova its around 100.000 ha (Dushi *et al.*1997), whereas needs for wheat seed per year in are about 10.000-12.000 tones of C_2 certificated seed. Several institutions create and improve new cultivars, in order to achieve higher production, and demands of the population (Vural *et al.*, 2007). Fundamental methods to increase wheat yield are: creating new cultivars with higher genetic potential of production and improvement of existing cultivars. Such reality requires creation of new local wheat cultivars. Research includes testing new lines of wheat, created at Faculty of Agriculture–Prishtina, for ability

production and biomass. Positive results make possible to think over about starting seed production of local cultivars, gradually decreasing seed import.

Material and methods

The planted seed was of six different lines with code name: L_1, L_2, L_3, L_4, L_5, and L_6. Experimental plots were evaluated during 2006 (y_1) and 2007 (y_2) in the didactic farm at Faculty of Agriculture –Prishtina. Density was 450 seed /m^2, while base fertilizing was: N-60, P_2O_5-60 and K_2O- 60 kgha^{-1}. Spring fertilizing was with URE-46% in 92 kg ha^{-1}/N. Herbicide used was: Granstar- WG-75. The experimental design was according to the formula: 6-Lines x 3-Repetitions x 1-Locality x 6-Parameters=108 combinations. The experimental model was a randomized blocks design (RBD). Plots surface was 50 x 1.8 m = 90 m^2. The investigated parameters were: Plant, ear, yield and biomass. For classification was used (Cluster-dendrogram) hierarchical distribution Ward of statistical package SASS-JMP-IN 5.1.2 (2004).

Table 1. Average values during years 2006-2007 for ear, yield and biomass at wheat lines.

Genotype line (GL)						
Line	PBM/g/ plant	PH/cm	NG/S	EL/cm	GW/S/g	Y/ha
L1	4.34	77.87	54.97	9.39	1.72	9.45
L2	4.85	79.05	54.47	9.28	2.03	11.40
L3	4.36	77.5	51.17	9.01	1.96	10.80
L4	3.87	70.8	52.00	8.40	1.81	9.85
L5	4.24	76.75	49.60	7.85	1.70	9.40
L6	3.99	78.55	50.40	8.08	1.92	10.55
μ	4.27	76.75	52.10	8.67	1.86	10.26
P-5%	*0.33*	*3.7*	*0.75*	*0.42*	*0.14*	*0.86*
P-1%	*0.52*	*5.8*	*1.18*	*0.66*	*0.23*	*1.36*

Legend: μ- average value for gene effect, PBM-Biomass of plant, PH-Plant Height, NG/S-Number of grain per spike, EL-Ear Length, GW/S-Grain Weight / spike, Y-Yield.

Results and discussion

In the first year were for biomass production particularly the differences between L_2 and L_4 was 15.92%, while for the second year the differences between L_2 and L_4 was 30.44%. Lines with extreme values, in first year were over μ value, from +4.21 to 20.14%, whereas in second year were +13.58% and -9.36% than value of μ. The genotype and environmental interaction results between y_1L_2-y_2L_6 was 43.55%. Polygene determines Plant Height

(PH), and influences directly stability and yield of lines. Average genes value for PH, was μ =76.78cm, while at first and second year were Xy_1= 80.46 and Xy_2 =73.10cm, with differences 7.36cm or 9.5%. Differences between lines for PH, in first year was $y_1 = L_1 - L_4$ =8.33cm or 10.86%, and second year $y_2 = L_2 - L_4$=10.1cm/μ or 13.15%. Total variability for treatments investigated was: $L_1y_1 - L_4y_2$=17.13cm or 22.31% compared to μ. Interval width for variability was: +8.83% to -13.48%, with significant difference at P=0.05 and P=0.01. In some wheat cultivars average results for PH, average results were 103 cm. (Pagnotta., 2004).

Short stalk genotypes has same number of leaves as the tall ones, and assimilates which would otherwise stay in the stem were translocated intro the spike, resulting most frequently in an increased grain yield, (Evans, 1984). This is why most short –stalk varieties have more fertile spikes than the tall ones (Brooking and Kirby., 1991). Line L_1 for two years had average genotype value for ear length (EL) 9.39 cm. For the two years y_1=9.92cm and y_2=8.86cm differences for L_1 was significant with values 1.06cm or 11.28%. With shorter ear during ours investigations L_6 and L_5, as treatment: y_1L_6=8.43 and y_2L_5 =7.10, with different significance 1.33 or 15.34%. Maximal and minimal value at first and second year compared with μ was significantly different: $L_1y_1 - L_6y_1$=1.49 or 17.18%, and $L_1y_2 - L_5y_2$=1.76 or 20.29%. Distinctions between average additive genotypes values for lines were: $y_1 - y_2$=0.42 or 4.84 % with no significant difference. Number of grain and their weight per spike, in y_1 for L_1 and L_2, had identically number per ear 54.8, but μ value was 52.1. Genotype value of GL_1=54.97, realized more grains/ear. In all cases GL_5 realized minimal grain/ ear, and the variability between them was 5.37 or 10.37%, which makes differences significant. Distinctions between treatment $L_1y_1 - L_5y_1$ and $L_1y_2 - L_5y_2$ were highly significant with values 5.20 or 9.98% and 5.53 or 10.61%. Average value $Xy_1 - Xy_2$=0.35 or 0.67% compared on value μ was non significant.

The final aim of wheat cultivation is weight of grain/ear, while experimental average value μ was 1.86 g/ear. At the first year was distinct: L_2 = 2.36 and L_5 =1.86 g/ plant, with differences of 0.5g/ear or 26.88 %. Similar value for GL had two lines with differences 0.3 or 16.19%, a high significant compared to value μ. The second year was with droughts and minimal differences, but significant only for extreme values: $L_2 - L_4$=0.2 or 10.75% were observed. The highly significant differences, were between years $y_1 - y_2$=0.55g/ear or 29.56%. During the studying of 16 wheat cultivars with different origin in Kosova condition was obtained kernel weight/ear, μ=1.31g (Fetahu *et al*., 2004).

Yield, the main objective of wheat cultivation, depends from many factors as additive and non additive factors and is more variable. Lines in different years realized highly average yield, but with differences y_1=11.82 and y_2=8.7 tha^{-1}, and average gene effect value μ was 10.24 tha^{-1}. For some wheat cultivars yield was over 9.5 tha^{-1} (Sieling *et.al,* 2005). Higher significant genotype differences, were in the first year between lines $L_2 - L_5$ =3.1t/ha or 29.80%, LG=$L_2 - L_5$=2t/ha or 19.53%, while at second year those differences were lower but significant between $L_2 - L_1$ and L_4=1.2t/ha or 11.71%. In the first year line L_2=13.4t/ha was

significantly variable with maximal yield compared to value μ for +30. 85%, while L_5=10.3t/ha, had minimal yield (+0.97%), with non significant differences. Second year L_2 had a difference of –8.20 % and it was non significant differences, compared to value μ, where L_4 with minimal yield had differences of -2.04tha^{-1} or 19.92%. Interaction between genotype and year identified significant differences for treatments: L_2y_1-L_4y_2=13.4-8.2=5.2 tha^{-1} or 50.78%. Production capacity of new lines developed at Agriculture Faculty, compared with average yield in Kosova, X=3.5– 4 tha^{-1}, calculated by using genetic potential of 60%. New cultivars they have possibility to increase yield and production. The Table 1 and Figure 1, show the comparison of lines. Six wheat lines are systematized in two groups: first group with maximal yield (L_2, L_3 and L_6), and second group are lines with lower values: L_1, L_5 and L_4.

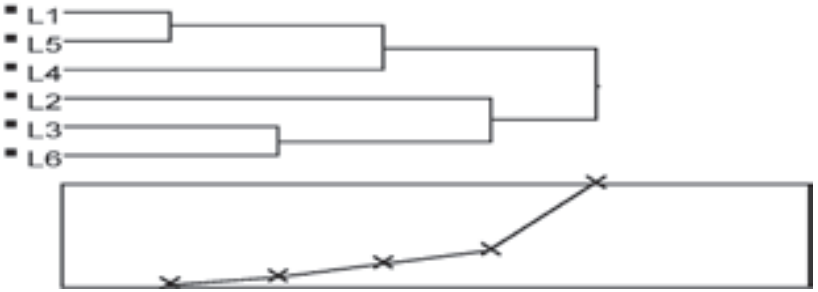

Figure 1. Dendrogram of six winter wheat for yield based in Ward's method.

Conclusions

Line investigated for plant traits including biomass and yield, had with higher potential of production over 10.26t ha^{-1}. Interaction between genotype and agro-ecological, determine the line reaction, especially for production, stability, and minimal variations for yield from +11.11 to -8.38 %. All wheat lines in our research have capability to increase yield average in Kosova, especially lines L_2 and L_3 have good perspective, after evaluated for VCU and DUS- test.

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Natural variation in root structure within *Cucumis melo* L. studied *in vitro*

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ABSTRACT: Natural variation in root systems is very useful in breeding programs against soil biotic and abiotic stresses. However, the underground nature of root systems makes their study difficult. *In vitro* culture has been used successfully for a proper analysis of root development in *Arabidopsis thaliana* (L.) Heynh. Here we use this technique to study the different root system among three genotypes belonging to the two ssp. of *Cucumis melo* L.: *melo* (Piel de Sapo*)* and *agrestis* (PI 161375 parental genotype of the Spanish melon map, and Pat 81 resistant to *Monosporascus* vine decline). The results showed that Piel de Sapo has greater size root systems than PI 161375 and Pat 81 with longer and thicker roots. The fact that Piel de Sapo had a lower root length per cm^2 of root and its laterals had a more superficial distribution suggest its adaptation to high-input growing systems. The two entries of the ssp. *agrestis* showed a smaller root system, with less systematic branching and with a higher predominance of the vertical growth that indicates a priority in reaching deeper layers of the soil with the minimum carbon investment. The variability found between the two exotic accessions suggests the existence of different alleles and/or genes involved in root structure within the subspecies *agrestis*. This gene diversity can be exploited for breeding cultivated melons. We have been able to directly observe the architecture of the root system and distinguish among different root morphologies among the *C. melo* species. This methodology represents a useful tool for performing quick and informative screenings for natural variation in melon root systems.

Keywords: root architecture, abiotic stress resistance.

Introduction

Root architecture has been shown to be linked with resistance to biotic and abiotic stresses. Among crop species, genetic diversity for root architecture has been studied mostly in cereals (rice, maize, wheat, barley and forage grasses) and legumes (common bean, soybean, pea, chickpea, cowpea and lupine) and less extensively in vegetables (lettuce, *Allium* ssp. and melon). In general, wild taxa are useful sources of variation for root architecture, since their roots usually exploit more unpredictable and stressful soil environments than the cultivated taxa (Picó et al. 2007). Melon is no exception to this rule. Most cultivated melons are included in the subspecies *melo*, whereas the subspecies *agrestis* includes wild, semi-wild and exotic germplasm that can be used as the donor of valuable genes for breeding cultivated melons, as both taxa are fully interfertile. In previous studies performed in fields and greenhouses,

we found that the accessions belonging to the two subspecies of *C. melo*: *melo* and *agrestis* (Pitrat 2008) display different root structures (Dias et al. 2004; Fita et al. 2007).

The study of root systems recovered from fields or soil substrate in pots is not an easy task; moreover, usually only sections of the root system can be analyzed. Non-destructive methods such as *in vitro* culture using vertically-oriented agar plates (Devienne-Barret et al. 2006) allow for a temporal *in vivo* monitoring of the root growth and facilitate the digital imaging. This method has been used successfully in *Arabidopsis thaliana* (L.) Heynh for detecting QTLs associated with root length and branching (Loudet et al. 2005). The clear images obtained in the transparent medium allow for a detailed analysis of the length, diameter and branching pattern using specific software specifically developed to analyze root systems (Picó et al. 2007). Therefore, *in vitro* culture can be a powerful tool to screen the natural genetic variability among root systems.

In this study we have used *in vitro* culture to evaluate the root development of three accessions, *C. melo* subsp. *melo* cv. Piel de Sapo (PS), which is the most cultivated type in Spain, *C. melo* subsp. *agrestis* PI161375 (PI) (PSS and PI are the parents used to construct the Spanish genetic map of melon), and *C. melo* subsp. *agrestis* Pat 81, which has been reported as being resistant to *Monosporascus* root rot, the causal agent of melon vine decline (Iglesias et al. 2000; Dias et al. 2004)

Material and methods

Fifteen seeds of the three accessions (PS, PI and Pat81) were surface sterilized for 20 min in 50% bleach with Tween-20, and rinsed 3 times with sterile water. Tubes with standard MS medium including vitamins (Murashige and Skoog, 1962), plus 30 g/l of sucrose and 200 mg/l of cefotaxime to avoid endogenous bacterial contaminations, were prepared (pH adjusted to 5.7) and used to sow the sterilized seeds. After growing in the dark for two days, the germinated seeds were transferred into transparent plates (23x19x1cm) filled with 300 ml of the same medium. The plates were grown in a growth chamber (25 ºC, 16/8 h light/dark). Once a day for 15 days a digital image was taken of each root with a scanner. The digital images were analyzed with the specific software for roots WinRhizo-Pro 2003b (Regent Instruments Inc. Canada). The evaluated traits were: the total root length (the sum of the lengths of all of the roots), L (cm); the root projected area, PA (cm^2); the average root diameter, D (mm); the length of the primary root (cm), the number of laterals emerging from the primary root, NL; and the root width (the maximum horizontal distance between the root tips of the furthest lateral roots), W (cm).

Results and discussion

Piel de Sapo (PS) developed roots with greater projected area (PA) and greater total length (L) than PI 161375 (PI) or Pat 81 (Figure 1). However, a higher PA or L does not necessarily imply an enhanced capacity for soil exploration and water/nutrient absorption. Another parameter such as L/PA could provide more information as it measures the root investment in exploring

more soil volume. L/PA was higher in PI and Pat 81 than in PS (Figure 1). This parameter was negatively correlated to the root diameter (D). PS developed thicker roots than PI and Pat81 (Fig 1). Generally, the absorptive roots are those which are thinner and of a higher order than the structural roots. Therefore, our results indicate that the accessions of the subspecies *agrestis* are more efficient at producing absorptive length per unit of projected area.

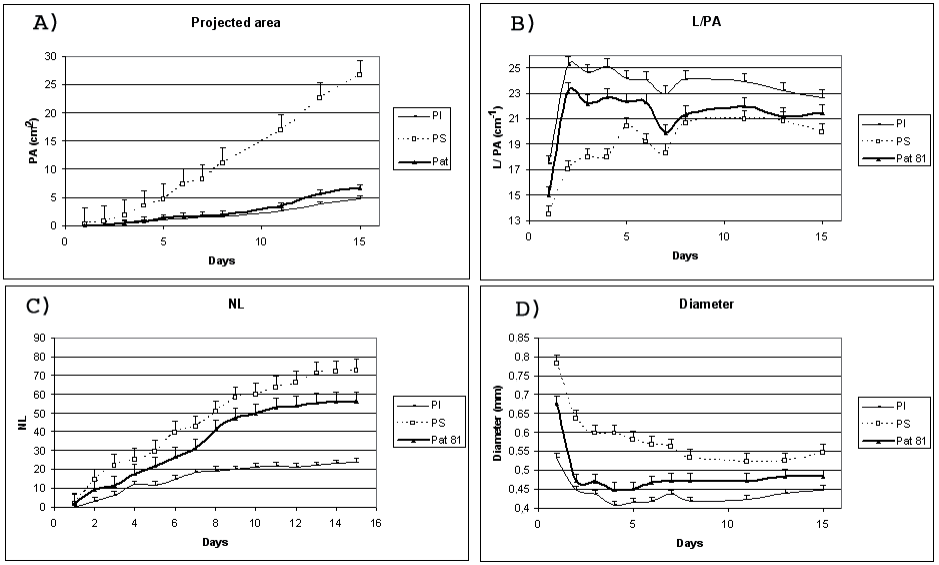

Figure 1. Evolution of the different parameters measured in the roots of *C. melo* subsp. *melo* cv Piel de Sapo (PS), *C. melo* subsp. *agrestis* Pat 81 and *C. melo* subsp. *agrestis* PI 161375 during 15 days after sowing (DAS). A) Root projected area (PA, cm^2), B) root length per unit of projected area (L/PA) (cm^{-1}), C) number of laterals derived from the primary root (NL), D) average diameter of the root (D, mm).

The number of lateral roots (NL) was highest in PS, followed by Pat 81 and PI (Figure 1). In general, the number of lateral roots is restricted by the length of the primary root. In our study, PS showed a higher density of lateral roots (4.8 lateral roots/cm of primary root at 15 days after sowing) in comparison with Pat 81 or PI (3.8 roots/cm and 2.0 roots/cm, respectively). In PS the third order laterals appear quickly (at 4-5 days after sowing) and continuously. On the contrary, the few laterals of Pat 81 and PI do not branch till the 8^{th} day after sowing on average, and the tertiary roots appear scattered on the secondary laterals. This *in vitro* culture assay also provided the opportunity of following the spatial distribution of the roots in the medium. The lateral roots of PS grew more horizontally (W of 14 cm), while

Pat 81 and PI tended to grow more vertically (W of 8.5 and 6.8 cm, respectively). Our results indicate that these wild accessions have priority in penetrating the soil with the minimum carbon investment (few and thin roots), while PS used a larger resource input to explore the topsoil layer rapidly.

One concern of this study is to what extent the differences found in young plants remain in adult plants .When we studied the root systems of these accessions in adult plants grown in different soil environments, we observed a higher length and branching level (number of laterals and root orders) in the Pat 81 in comparison with PS (Dias et al., 2004; Fita et al. 2007). This discrepancy seems to suggest that wild taxa save resources in less stressful conditions, such as the *in vitro* culture in an artificial medium. However, they have a higher plasticity and can react rapidly by modifying their root architecture in stressful soil environments. Due to the fact that plastic responses can change dramatically the appearance of one root system (Lopez-Bucio et al. 2003: Malamy 2005), modifying the medium composition in different layers (creating nutrient depletion zones) could give more information about the ability of the genotypes to react to certain soil stresses.

Pat 81 is a useful source of alleles useful for breeding cultivated melons (Dias et al. 2004; Fita et al. 2006). Recently, *in vitro* culture has been used to study the genetics of root structure using a collection of introgression lines of PI in the genetic background of PS. The detection of several transgressive QTLs for different traits measuring length, and branching level demonstrated the value of PI in the melon root breeding, even when PI has a smaller root system than PS (Fita et al. 2008). In our study, Pat 81 and PI performed similarly in most of the assayed root traits. However, these two accessions differ in relevant characteristics, such as root length and number of laterals, being the phenotype of Pat 81 longer and more branched. These differences indicate the existence of genetic variation for root architecture within the *agrestis* ssp. that can be used trough the accumulation of different favourable alleles in melon varieties. Further studies of the natural variation in melon root systems using rapid phenotyping tools, as the in vitro culture, and genomic tools will help the melon breeding against soil stresses.

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Transcriptional and metabolic profiles in *Lolium perenne* L. genotypes with differential physiological responses to a PEG induced drought stress

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ABSTRACT: Perennial ryegrass is of great economic importance to the leisure industries and Irish agriculture, with perennial ryegrass based pastures accounting for 90% of the agricultural land. One of the objectives of breeding programs is to produce improved cultivars with a response for an increasing range of specific uses/situations. A stepwise approach has been conducted to investigate the transcriptional basis of phenotypic and metabolic plasticity to drought for a set of perennial ryegrass accessions. An ecotype, noted for drought tolerance, was obtained from the USDA GRIN database and was used together with a commercial variety to investigate metabolomic and transcriptional profiles under a PEG induced drought stress. Hydroponics based experiments showed the drought tolerant ecotype to increase root biomass in response to a drought stress and maintain a higher relative water content (RWC) relative to the variety. The use of suppression subtractive hybridisation combined with real-time RT-PCR has enabled the identification of transcripts up-regulated in the drought tolerant ecotype. The use of GC-TOF-MS has facilitated the identification of changes in the metabolome of perennial ryegrass under drought stress in both root and leaf tissue. The outcomes of this research will be discussed.

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Development of hybrids for pepper grafting: evaluation of *Phytophthora capsici* resistance

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ABSTRACT: *Phytophthora capsici* is now one of the most serious threats facing pepper (*Capsicum annuum*) plant production. Although not so limiting as *P. capsici,* root knot nematodes (RKN) is also a damaging pest. Despite breeding efforts, currently, commercial cultivars with resistance to these pathogens are unavailable. Three pepper hybrids, 'Carlo', 'Foc' and 'Charlot' have been obtained by crossing lines that carry several genes for *P. capsici* and RKN resistance to provide rootstocks for control these pathogens. Virulence evaluation in four Spanish *P. capsici* isolates was tested. Isolate Pc8 that damaged the high percentage of *P. capsici* resistant control plants, were selected to assess hybrid *P. capsici* resistance. Irrigation with zoospores to plants at different growing stages was performed. In all these assays, 'Charlot' and 'Foc' hybrids have displayed greater *P. capsici* resistance than the commercial rootstock 'Tresor' and 'Chilcote' and similar or even better resistance than 'Serrano Criollo de Morelos (SCM)', the most promising *P. capsici* resistant source. All commercial hybrids tested in this work, which are commonly used for pepper production ('Almuden', 'Coyote' and 'Quito'), proved highly susceptible to *P. capsici*. Successful grafting was obtained when the commercial hybrids were grafted onto 'Charlot' and 'Foc' rootstocks. Performance of these hybrids for yield production and *M. incognita* resistance are under evaluation.

Keywords: *Capsicum annuum*, *Phythopthora capsici*, *Meloidogyne incognita*.

Introduction

Phytophthora crown rot, caused by the oomycete *Phytophthora capsici* Leon., has become one of the most serious threats to pepper (*Capsicum annuum*) production and constitutes a limiting factor to profitable production of many crops worldwide (Silvar et al., 2006). *P. capsici* can strike pepper plants at any stage of growth and spread quickly and no single method is currently available to provide adequate control. Root-knot nematodes (RKN) are another important pest that can reduce pepper crop yields on a global scale (Nuez et al., 1996; Roberston et al., 2006). Most common pepper varieties are susceptible to the southern RKN *Meloidogyne incognita* (Oka et al., 2004).

Genotypes that exhibit resistance to *Phytophthora* crown rot have been used in breeding programs but to date, no pepper *P. capsici* and RKN resistant cultivars have been commercially released. Probably both, the polygenic nature of *P. capsici* (Pochard et al., 1983: Thabuis et al., 2003) and RKN resistance (Djian-Caporalino et al., 2007) and pathogens diversity (Silvar *et al.,* 2006; Robertston *et al.,* 2006) have limited it.

Grafting onto resistant rootstocks could be an effective control of soil-borne diseases; in fact, there is a common practice in crops such as watermelon. We have produced hybrids from resistant *P. capsici* and/or RKN pepper parents in order to provide rootstocks for effective control of the above mentioned pathogens. Evaluation of *P. capsici* resistance and grafting performance was assayed. Virulence in four *P. capsici* isolates was also tested previously to *P. capsici* resistance evaluation.

Material and methods

Plant material

Genotypes 'SCM 334' (PI636424); 'Chilcote' (PI201234) 'Charleston Hot' (PI640825) and 'Carolina Wonder' (PI632920) were use for hybrid production (UDSA-Plant Genetic Resources Conservation Unit, U.S.A.). Commercial seeds: Cv. 'Padrón' (Semillas Ramiro Arnedo, Spain); 'Coyote'-F1, 'Quito'-F1 and 'Almuden'-F1 (Syngenta Seeds, Holland) and 'Tresor'-F1 (Nunhems Zaden, the Netherlands) were also used in different evaluation experiments:

Fungal material and virulence assay

Four *P. capsici* isolates, were used: Pc7 and Pc8 (from East of Spain) and Pc196 and Pc141 isolates from Northwest Spain. Irrigation of seedling with 5 ml of a solution containing 10^5 or 10^6 zoospores ml^{-1} ($z.ml^{-1}$) of each of four isolates was used for infection. Ten seedlings per treatment were inoculated. This assay was conducted twice. Plants affected by *P. capsici* were visually assessed. A plant was considered dead when it looked irreversibly wilted.

Disease assessment in hybrids

Assays were carried out by inoculating 5 ml of either 10^5 or 10^6 $z.ml^{-1}$ of *P. capsici* Pc8 isolate around the base of the plant. Plants at different development stages were inoculated: seedlings (5 weeks old); semi-adult (with 10-12 developed leaves) and adult plants (a mean of 21 leaves). Twenty plants per genotype were watered previous to inoculation in order to keep the soil wet and facilitate zoospore movement. After inoculation, the pots were watered daily. Mock inoculate plants was used as a control. These assays were repeated twice. Ten adult plants per genotype (grown in pots) were infected and evaluated 90 d post inoculation (dpi).

Graft compatibility experiments

Graft compatibility was assessed in terms of survival percent and plant vigor during the vegetative stage. 'Coyote' and 'Almuden' commercial hybrids were grafted using 'Charlot'

and ‘Foc’ as rootstocks. As controls, ‘Coyote’ and ‘Almuden’ plants were grafted on ‘Coyote’ and ‘Almuden’ plants respectively. Forty plants were grafted per treatment (20 per experiment). The cleft grafting method was employed. Grafted plants were maintained at high humidity (90-95 %) at 25ºC for a week.

Results and discussion

Obtaining hybrids

Three pepper hybrids: ‘Carlo’, ‘Charlot’ and ‘Foc’, were obtained using the accession ‘SCM334’ as pollen parent and ‘Carolina Wonder’, ‘Charleston Hot’ and ‘Chilcote’ as females, respectively. ‘SCM334’ is considered highly resistant to *P. capsici* (Gil Ortega et al., 1991) and has also been described as resistant to *M. incognita* (Djian-Caporalino et al., 2007). Resistance to this RKN has also been described in ‘Carolina Wonder’ (Thies and Fery, 2003), ‘Charleston Hot’ (Oka et al., 2004) and ‘Chilcote’ (Djian-Caporalino et al., 2007) that is also tolerant to *P. capsici* (Walter and Bosland, 1999). Thus, obtained hybrids combine several genes for *P. capsici* and RKN resistance.

P. capsici virulence assay

Four Spanish *P. capsici* isolates were evaluated in pepper genotypes that differed in their degree of susceptibility to the pathogen (cv. ‘Padrón’, susceptible; ‘Chilcote’ tolerant and ‘SCM334’ resistant) and ‘Coyote’, a productive hybrid commonly used in greenhouse production and putative susceptible. Seedling of ‘Padrón’ and ‘Coyote’ inoculated with either 10^5or 10^6 z.ml^{-1} of *P. capsici* resulted wilted within 6 dpi independently of *P. capsici* isolate. In contrast, ‘Chilcote’ and ‘SCM334’ genotypes seemed not to be affected by Pc141 and Pc196. isolates. These genotypes however, were wilted when infected with isolates Pc7 and Pc8 (40-90% of ‘Chilcote’ and 20-40% of ‘SCM334’ inoculated plants with Pc7 and Pc8, respectively).

P. capsici hybrid resistance evaluation

Pc8 the most virulent isolate tested was used to evaluate *P. capsici* resistance in hybrids plants at different developmental stages. Seedlings susceptibility to *P. capsici* is showed in Figure 1. ‘Carolina Wonder’, ‘Charleston Hot’ and ‘Coyote’ seedlings resulted high susceptible and wilted quickly independently of the *P. capsici* dose used for inoculation. With respect to described *P. capsici* resistant materials, ‘SCM334’ displayed higher resistance than ‘Chilcote’ in agreement with that previously reported (Palloix et al., 1988).‘Tresor’ rootstock showed an intermediate tolerance as occurred in Carlo hybrid whereas Foc and Charlot demonstrated similar or even higher *P. capsici* resistance levels than resistant peppers. Root dry weight (DW) of asymptomatic seedlings was compared in non-inoculated vs. inoculated plants and a DW reduction (mg) was observed in all inoculated plants (around 30% in ‘SCM334’ and ‘Charlot’; 45% in ‘Chilcote’ and ‘Foc’; 65% in ‘Tresor’ and ‘Carlo’). Given these results,

'Carlo' hybrid has been excluded from further characterization. The genetic background of female parents seemed to influence *P. capsici* resistance. Thus, although 'Carolina Wonder' and 'Charleston Hot' proved very sensitive to this pathogen; 'Charlot' clearly displayed superior resistance when compared to 'Carlo'.

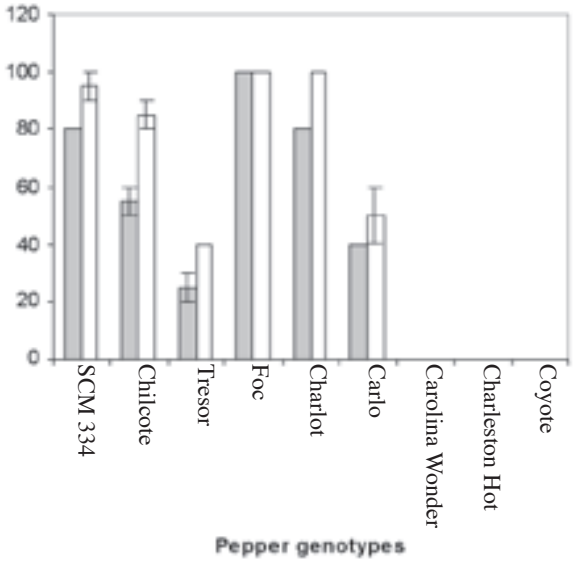

Figure 1. Percentage of asymptomatic seedlings at 5 weeks post-inoculation with 5 ml of either 10^5 (open bars) or 10^6 (dark filled bars) z.ml^{-1} of *P. capsici* Pc8 isolate. Error bars show standard errors of the mean (N=10 seedlings inoculated per dose of inoculums and experiment).

Similar doses of *P. capsici* inoculation in semi-adult and adult plants showed delayed symptoms in susceptible and semi-tolerant plants however, any of them were able to complete the culture period. Genotypes that showed resistance in the previous assay ('Chilcote', 'SCM334', 'Foc' and 'Charlot') did not display external disease symptoms and grew like non inoculated plants with the exception of 'Chilcote' plants that showed a decrease in height (25%) 90 dpi.

Greenhouse compatibility experiments

The present study also evaluated the process of wound healing, which necessary for successful grafting. 'Coyote' and 'Almuden', two commercial hybrids of 'California' and 'Lamuyo' pepper types respectively, were grafted onto 'Charlot' and 'Foc' exhibiting good attachment

and growing like ungrafted plants. Similar percentages of wound healing were obtained when 'Almuden' and 'Coyote' commercial hybrids were grafted on 'Charlot' and 'Foc' produced hybrids or when 'Almuden' and 'Coyote' plants, respectively, were used as rootstocks. On average, 95% of successful grafting was obtained for all treatments.

The results obtained show that the produced hybrids Foc and Charlot exhibited resistance to *P. capsici* at similar or even superior levels than 'SCM334', the most resistant pepper described. Although more work must be undertaken to confirm that these hybrids are feasible as satisfactory rootstock producers, this study provides a preliminary approach to obtain good pepper rootstocks capable of confronting *P. capsici* and putatively RKN.

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Variation in the yield and yield components of winter wheat genotypes

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ABSTRACT: Main goals of winter wheat breeding are increase of grain yield and achieving high quality requirements. Winter wheat yield, as a complex trait, depends of various plant traits and it is influenced by genotype and agroecological conditions (Drezner et al., 2007). Variations in yield and yield components influence breeding work on winter wheat and can hinder selection of high yielding and quality genotypes. Splitting genetic variation of yield and yield components in parts of variations using principal components analysis (PCA) can improve selection of superior winter wheat genotypes. The objective of this paper was to determine variations between examined winter wheat genotypes analyzing yield and six traits important for yield formation: hectoliter weight, thousand kernels weight, plant height, number of ears per m/2, number of spikelets per ear and number of kernels per spikelet. Research work included 25 winter wheat genotypes, 22 of them were new breeding lines and three were recognized varieties Žitarka, Srpanjka and Divana. Žitarka and Srpanjka are high yielding varieties while Divana is high quality variety. The PCA of analyzed traits revealed that 75.41% of the variation could be captured within first three principal components (PC1 to PC3). These components had eigenvalues grater that 1.0. The first principal component had an eigenvalue of 2.27 explaining 32.39% of the whole variation. Number of ears per m/2, number of spikelets per ear, and number of kernels per spikelet contributed strongly to variation for this principal component with eigenvectors above 0.7. Number of spikelets per ear and number of kernels per spikelet were in strong positive correlation while the number of ears per m/2 was in negative correlation with these two traits. The second principal component's eigenvalue was 1.69 and it explained 24.20% of total variation. To the variation of this principal component strongly contributed yield, thousand kernels weight and plant height with eigenvectors above 0.5. These traits were in negative correlation. The third principal component's eigenvalue was 1.32; it explained 18.82% of total variation. Trait that strongly contributed to its variation with eigenvector -0.88 was hectoliter weight. PCA biplot showed that examined genotypes formed three groups around three examined traits; first group was around thousand kernel weight, second around number of spikelet's per ear and third around yield. Varieties Žitarka and Srpanjka grouped close together while Divana was on the opposite side. That was expected since Žitarka and Srpanjka have high yield, number of ears per m/2 and short plant. On the other hand Divana has higher plant, lower yield and number of ears per m/2, but it has high quality parameters.

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Finding variability on leaf green volatiles among varieties of sweet pepper damaged by *Spodoptera exigua* larvae

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ABSTRACT: The release of odorous compounds by plants in response to herbivore attack and the subsequent use of these odorous signals by natural enemies to locate the herbivores is a widespread phenomenon observed in various tritrophic systems, e.g. parasitic wasps and lepidopteran caterpillars on cabbage and cotton. We have just began to study this kind of interaction in a system that comprises sweet pepper (*Capsicum annuum* L.), folivorous caterpillars in the genus *Spodoptera*, and the associated endoparasitic wasps (Degen et al., 2004), looking for variability among sweet pepper genotypes. A breeding program on sweet pepper looking for improvement of biological pest control is needed taking into account the current situation of pesticides regulation of the UE and USA. The need of a method which could allow a great number of samples to be taken and analyzed has carried us to use the SPME (Solid Phase Micro Extraction) (Mena Granero et al., 2005) technique which has proven to be a powerful system to make such type of analysis in a great number of genotypes. We will present here our results obtained so far from five genotypes, including some of the more popular ones cultivated in the zone (for example, Melchor, Zeraim Seeds, Valencia, Spain; Bárdenas, Syngenta Seeds, Vícar, Almería, Spain). Our sampling system consists on a vial with cover which can be perforated, a 65 μm PDMS-DVB fiber and an incubator at 24ºC. Time of extraction can be adjusted to get equilibrium within the vial ('head space' sampling), which uses to be 20 minutes at 24ºC. The number of volatiles analyzed by GC-MS with a DBWAX column (GC System 7890A Agilent Technologies; Mass Spectrometer 5975C, Agilent Technologies, Electronic Impact) has been variable depending on the genotype and treatment of the leaves (control, mechanically damaged with a scalpel or eaten by *Spodoptera exigua* larvae). Among those identified volatiles are worth of been named: 2- Hexanal, 3-Hexen-1-ol, 1-Hexenol, β-Ocimene, which are known to exert attraction ('calling' carnivorous volatiles) toward the parasitoids of *Spodoptera exigua* such as *Cotesia marginiventris*. Statistically significant differences (N= 10; $p<0,05$) on volatiles released from eaten leaves among the genotypes investigated have been found, being the genotypes Melchor and Yellow Wonder those two with a higher amount of 'calling' volatiles per leaf surface.

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Accounting for spatial variation and competition to variety trials of perennial tree crops

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ABSTRACT: The accuracy of field trials used to evaluate varieties of perennial tree crops may be compromised by spatial trends in non-genetic variation, the tendency for individuals in close proximity to experience a similar environment, and competition among individual plants may reduce the accuracy of identifying elite genetic material, particularly if single tree plots are used. In this study, we apply recent developments in statistical methodology to account for these effects to a field trial for evaluation of yield of macadamia varieties planted at a density of 10m x 5m. At six years after planting, the analysis indicated environmental trends along planting rows and particularly across planting rows. By 10 years of age, neighbourhood effects were detected along planting rows, but there was little variation across planting rows. No significant competition effects were detected at six or 10 years of age. This approach allows the effects of spatial heterogeneity in growing environment and competition effects to be quantified and evaluated for the effect on prediction of genetic value.

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Effects of environment on grain weight stability in wheat

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ABSTRACT: The paper studied environmental stability of grain weight (GW) per main spike in 20 wheat varieties of different geographic origin and genetic background. Ten cultivars from Serbia and ten from other countries were tested at the experiment field of the Institute of Field and Vegetable Crops in Novi Sad over a three-year period. The average grain weight per main spike ranged between 0.9 g (the variety Centurk) and 2.45 g (variety NSR-5). Stability parameters were calculated according to Eberhart and Russell. Regression coefficients showed that most of the cultivars had dissatisfactory stability for the studied trait. Analysis of individual genotype responses to environmental changes revealed significant differences. Comparison of regression coefficients and deviation from regression showed that the varieties Zvezda and Mironovska were the most stable, but they had low GW means. Among the cultivars with high GW means, Lasta and Sana were the most stable. Cultivars Centurk and NSR-2 showed the lowest level of stability.

Keywords: grain weight, regression analysis, stability, wheat.

Introduction

In order to improve the genetic potential for wheat yield, it is necessary to have a complete understanding of the individual effects and interrelationships of yield components (Alexander et al., 1984), with grain weight per spike having a direct positive effect on grain yield (Dencic et al., 2000). The real value of a cultivar depends not only on its productivity or the expression of particular traits but also on its ability to realize these traits to a high degree and in different growing conditions (Hristov et al., 2008).

The stability of a given trait is a measure of variability between its genetic potential and realized value in different environments (Heinrich et al., 1983). It may come as a result of genetic divergence of the parental components, compensatory effects among different traits, tolerance of stress, or a combination of all these factors. Grain weight per main spike is a direct reflection of nutrient utilization efficiency and nutrient translocation to generative plant parts (Wardlaw and Willenbrink, 2000). Compared to sources, acceptor of assimilates is a much greater limiting factor in the improvement of genetic potential for wheat yield (Reynolds et al., 2004). Therefore, if conditions are created that allow optimum organic

matter production and distribution by the plant (Mitchell at al., 2006), high and stable yields under different environmental conditions can be expected.

The objective of this paper was to investigate the stability of wheat varieties cultivated under different growing conditions with respect to grain weight per main spike.

Material and methods

In this paper, the material studied were 20 wheat varieties of different origin — 10 from Serbia and 10 from various other parts of the world. Samples for analysis were taken at full maturity (harvesting was done by hand) and consisted of 30 plants (10 per replication).

Over a three-year period (2001-2003), a trial was carried out at the experiment field of the Institute of Field and Vegetable Crops in Novi Sad using a randomized block design with three replications. The size of the experimental unit (basic plot) was $2m^2$, the distance between the rows 10 cm, and the spacing between the basic plots 20 cm.

Experimental data were processed by two-factor ANOVA, while the parameters of stability for grain weight (GW) per main spike were calculated using regression analysis according to Eberhart and Russell (1966). The model uses as stability parameters the mean values (), linear regression coefficient (b_i), and deviation from regression (S^2d_i). These parameters make it possible to perform an analysis of how well adapted a genotype is to environmental conditions. In order to be considered stable, a genotype must have a regression coefficient of one or close to one ($b_i \approx 1$) and as little deviation from regression as posible ($S^2d_i \approx 0$) and its mean value must be at a desired level.

Results and discussion

The wheat varieties tested showed significant phenotypic variability for GW. The mean values of the trait ranged from 1.52 g in 2001 to 1.99 g in 2003 (Tab 1).

In the first year of the study, the lowest mean value of GW was found in the variety Disponent (1.00g) and the highest in the variety Zaporozska (1.95 g). In the second year, GW ranged between 1.17 g (Centurk) and 2.22 g (Nizija), while in the third the minimum and maximum values were 0.90 g (Centurk) and 2.49 g (NSR-5), respectively. The lowest and highest GW means overall were observed in 2003. The lowest three-year GW average was found in the cultivars Disponent and Centurk (1.12 g) and the highest in the variety Sana (2.11 g) (Tab.1). In Serbian growing conditions, GW values of 1.7 to 2.5 g are considered satisfactory when using a low seeding rate (Hristov et al., 2007).

The cultivar Sana did not have the highest GW mean in any of the three study years but produced the highest GW average overall. According to Ozkan et al. (1999), stable genotypes have specific tolerance mechanisms and do not need to have the highest average yield or GW in any particular year for getting satisfactory average results.

Table 1. Mean values (g) and stability parameters for grain weight/main spike of wheat cultivars.

Cultivar (A)	Year (B)			Average	b_i	S^2d_i
	2001	2002	2003			
Jugoslavija*	1.62	1.99	1.91	1.84	0.843	0.014
Rodna*	1.39	1.80	1.99	1.72	1.530	-0.006
KG 56*	1.67	1.72	2.18	1.85	1.161	0.044
Balkan*	1.30	1.70	2.09	1.69	1.949	0.002
Lasta*	1.78	2.07	2.14	2.00	0.939	-0.004
NSR-5*	1.68	2.03	2.49	2.07	1.970	0.013
NSR-2*	1.43	1.89	2.24	1.85	2.017	-0.003
Rana niska*	1.31	1.75	1.92	1.66	1.567	-0.005
Zvezda*	1.46	1.74	1.84	1.46	0.979	-0.005
Nizija*	1.84	2.22	2.07	2.05	0.691	0.029
Sana	1.89	2.13	2.32	2.11	1.094	-0.005
Skopljanka	1.75	1.96	2.36	2.02	1.459	0.016
Yantar	1.88	1.97	2.05	1.97	0.426	-0.006
Martonv. 4	1.35	1.74	1.90	1.67	1.409	-0.005
Disponent	1.00	1.23	1.13	1.12	0.844	-0.006
Mironovska	1.39	1.59	1.79	1.59	0.986	-0.004
Zaporozska	1.95	2.17	2.12	2.08	0.478	0.001
Norin 10	1.21	1.31	1.23	1.25	0.092	-0.001
Centurk	1.29	1.17	0.90	1.12	-0.926	0.005
Fontezuela	1.17	1.27	1.37	1.27	0.493	-0.006
Average	*1,52*	*1,77*	*1,99*	***1,73***		
		A	*B*	*AB*		
LSD	*0.05*	*0.12*	*0.05*	*0.22*		$b_i = 1{,}000$
	0.01	*0.17*	*0.06*	*0.29*		$S_e b_i = 0{,}117$

*varieties originating from Serbia

Analysis of GW variance for the total sample (data not shown) revealed significant differences both among the cultivars and among the years of the study, which is in agreement with Ying Yong et al. (2003). Significant differences among interactions indicate that most varieties have different responses in terms of GW expression under different environmental conditions (Petrovic et al., 2005). In Ishag and Mohamed (1996), the genotype by environment interaction had significant influence on grain weight as the main effect, with the trait most often being influenced by environmental variance, i.e. year (Mladenov et al., 2001).

According to the stability parameters from the present study, the most stable of the varieties having high mean values were Lasta (b_i=0.939 and S^2d_i=-0.004) and Sana (b_i=1.094 and S^2d_i=-0.005). In unfavorable environmental conditions, high GW means were found in the cultivars Zaporozska, Nizija and Yantar. When environmental conditions were favorable, the highest GW means were produced by the varieties Skopljanka and NSR-5, with the latter exhibiting a high level of GW instability as well (Tab.1 and Figure 1).

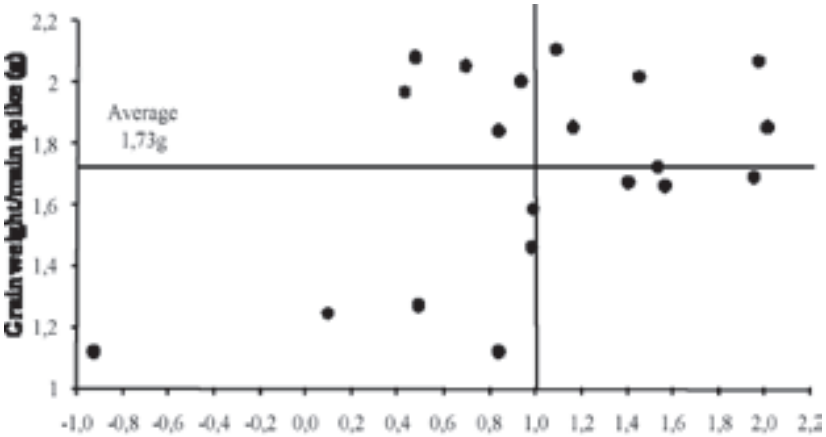

Figure 1. mean value and regression coefficient of grain weight/main spike.

The varieties Zvezda and Mironovska had regression coefficients close to the value of one (b≈1), but their GW means were below average. According to the coefficient of regression and deviation from the same, the cultivars Centurk (b_i=-0.926 and S^2d_i=0.005) and NSR-2 (b_i=2,017 i S^2d_i=-0,003) had the highest amount of instability, i.e. genotype x environment interaction (Tab.1 and Graph1).

The estimated values of genotype stability parameters (Eberhart-Russel, 1966) are not totally independent of each other and have been obtained using the theoretical minimum for the number of years/environments. However, the analysis of two groups of genotypes (10 from Serbia and 10 from various other parts of the world) makes it possible to make a comparison . Almost all of the varieties studied had low values of deviation from regression (S^2d_i), which is indicative of the high degree of reliability of the model used in the study and the findings it produced.

Along with spike number per m^2, GW has a significant influence on yield. Selection for varieties that, regardless of the conditions after anthesis, achieve a stable mean GW by translocating assimilates from the stem to the grain may be a useful objective in cereal breeding programs. Breeders aim to increase cultivar stability for traits (such as GW) that are

greatly dependent on environmental factors. The correct selection of cultivar for a given area may significantly mitigate the effects of these factors, which is of great importance for the attainment of high and stable yields of wheat.

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Genetic strategy for identification of genes involved in citrus salt stress tolerance: status of genome mapping program

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ABSTRACT: Salt stress is one of the most obvious effects of high salinity on *Citrus*, which is classified among the most sensitive tree crops. Genetic and genomic analysis of tolerant and sensitive plants is a prerequisite for breeding programs and the selection of more adapted varieties to high salinity. The combined approach of genetic mapping and localization of candidate genes has been applied in plant genetics in the past decade with the objective of characterizing and cloning quantitative trait loci (QTLs). The segregation of the tolerance/ sensitivity to salt character was initiated on the F2 population resulting from the crossing Cleopatra mandarin X *Poncirus trifoliata*. Citrus linkage map was derived from the segregation analysis of SSR markers from EST or BAC ends. From many polymorphic primers only few makers have Mendelian segregation. The skewed segregation can be due to abnormal meiosis at the intergeneric level. The excess of homozygous locus was observed only for the marker CiBE2626b. No difference was observed between frequency of alleles from Poncirus and alleles from mandarin in homozygous loci. Half of F2 population (61 hybrids) has a percentage of homozygous loci lower than or equal to 20%. Other hybrids seem to have proportions of homozygous and heterozygous loci that follow a normal distribution although there is a slight shift of some homozygote individuals to represent the expected Gaussian curve (~ 0.45 instead of 0.5). We have also studied the segregation and mapped several candidate genes putatively involved in salinity tolerance. We plan to confirm the role played by these genes by gene expression analysis from selected homozygous F2 genotypes under strong salt stress conditions. We will measure the effects of salt stress on physiological traits on the segregating population.

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Genetic variability of S1 families - potentiality for their use in maize drought breeding

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ABSTRACT: Different strategies have been proposed to overcome negative effects of drought on maize. Breeders at Maize Research Institute have created and used adapted drought tolerant population - DTP-A (DTP79xDTP12xDwarf), that was shown to be a good source of drought tolerance for locally adapted germplasm. In this research S1 families from DTP-A x B84 were scored for anthesis-silking interval (ASI) and genotyped with SSR markers using BSA, with the final aim to create a new gene pool for breeding drought tolerant hybrids. Only S1 families with ASI not longer than one day were considered as candidates for participating in the gene pool. Based on the statistical analysis of the data it was shown that the chosen families contained sufficient genetic variability for further exploitation in maize drought breeding. Besides for genetic variability, marker analysis was also performed in order to identify putative genomic segments and allele pattern differences responsible for expression of short ASI. Four SSR loci that could be potentially involved in ASI expression were identified.

Keywords: ASI, BSA, genetic variability, maize, SSR

Introduction

In maize, a major effect of drought stress is a delay in silking, resulting in an increase in the ASI (anthesis-silking interval) which is an important cause of yield failures (Bolanos and Edmeades, 1993; Byrne et al., 1995). Selection for ASI is best achieved under proper drought conditions, which severely limits its use in many breeding programs. At the same time, genetic variability is a fundamental condition for the genetic gain in improvement breeding programs (Vilela et al., 1995). Sustaining genetic variability is of utmost importance because it provides plant adaptation to different environmental conditions.

The objective of this work was to determine genetic variability between S1 families obtained from DTP-A population which will be used for further improvement in maize drought breeding. The final aim of this preliminary research is creating a new gene pool for breeding drought tolerant hybrids. In this early stage of research S1 families were scored for anthesis-silking interval and genotyped with SSR (simple sequence repeats) markers using BSA (bulk segregant analysis) approach.

Material and Methods

ASI was scored for 76 selfed families obtained from DTP-A (DTP79xDTP12xDwarf) x B84 cross. Replicated field trials were conducted in Zemun Polje, in two different years (2005 and 2006), on 20 individual plants per family. ASI was calculated as difference between silking date (the number of days from sowing until 50% of the plants show silks) and anthesis date (the number of days from sowing until 50% of the plants have extruded anthers). Families with no more than 1 day difference were considered as short ASI (sASI), whereas families with no less than 5 days difference as long ASI (lASI). All the other families were treated as „medium"ASI families (mASI).

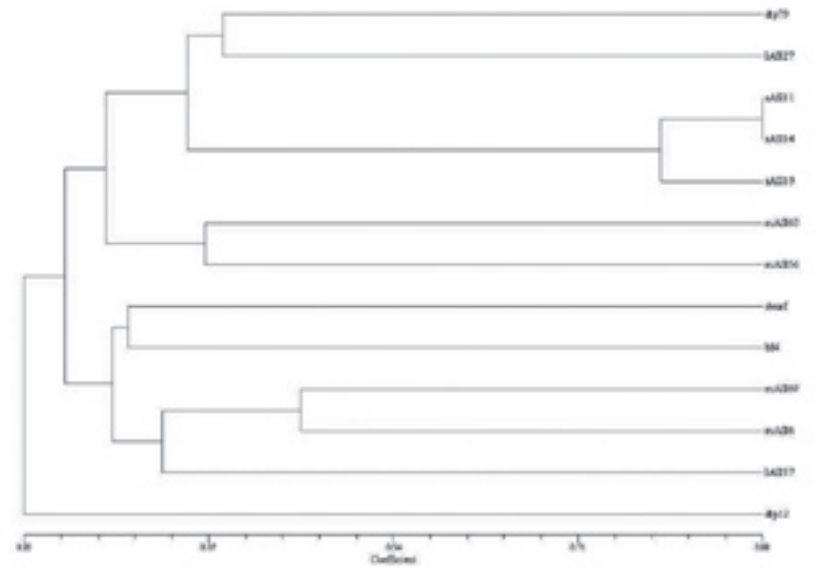
DNA was isolated from leaf samples of parental components and chosen S1 families according to modified method of Sagai-Maroof et al. (1984). BSA was done with 23 SSR markers on short, long and representatives of medium ASI families. SSR analysis was conducted according to slightly modified method of Chin et al. 1996. Products of PCR reaction were separated on 4% MetaPhor agarose gels, stained with ethidium-bromide and photographed. SSR profiles for each primer were scored as presence/absence of individual bands. Cluster analyses were performed using statistical package NTSYSpc (Rohlf, 2000) and UPGMA method based on Jaccard's similarity matrix.

Results and discussion

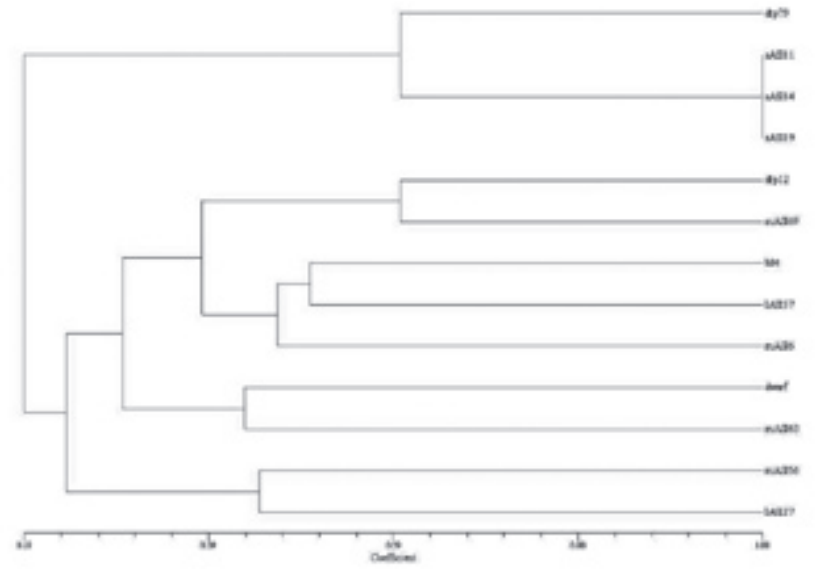
The trait upon which S1 families were assorted was ASI, one of the most important secondary traits for drought tolerance. When ASI is less than -5 or more than 5 days grain yield in maize declines due to poor pollen supply (Bassetti and Westgate, 1994). According to defined criteria three families were identified as sASI (ASI was 0.7, 1 and 1 days) and two as lASI (ASI was 5.5 days for both families). Four families, representatives of mASI (ASI was 2.9, 3.3, 2.9 and 3.9 days) were also analyzed.

All twenty-three markers were multiallelic and a total of 84 bands was recorded. The allelic patterns (number and position of bands) for all the analyzed loci were determined and compared among the analyzed families. Four SSR loci different in allelic structure between sASI families and lASI families, comprising 17 alleles, were identified. The four loci are located on chromosomes 3 and 8. On the same chromosome regions QTL (quantitative trait loci) for ASI were identified (Agrama and Moussa, 1996; Veldboom and Lee, 1996).

Genetic similarity (GS) values were calculated using information from all 23 SSR loci and also from only four loci that were identified as different in allelic structure between sASI and lASI families. The results of the cluster analysis are presented in the form of dendograms (Figure 1a and 1b). In both cases short ASI families grouped together. When all 23 SSRs were analyzed one of the long ASI families (lASI27) grouped with sASI families. This family was separated from sASI group when cluster analysis was done only on four identified loci which potentially influence ASI expression. This alteration in position of lASI27 can be explained by higher level of allele correspondence throughout the genome and at the same time significant differences in chromosome regions influencing ASI expression. Medium ASI families were scattered without any regularities, confirming the assumption of different loci and allele impact on anthesis-silking expression.



1a



1b

Figure 1. Dendogram of 13 S1 families and their parental components based on data of 23 (1a) and 4 (1b) SSR markers.

According to GS calculated for sASI families it can be concluded that genetic variability between s1 families is sufficient for creating a new gene pool for breeding drought tolerant hybrids. Genetic similarities based upon all 23 SSRs for these families were up to 0.74, i.e. genetic distances up to 0.26. When only the four identified SSRs were used, GS were 1, which supports the assumption that these chromosome regions are involved in ASI expression.

These preliminary results indicate that BSA approach with SSR can give a good insight into genetic structure, which is necessary for assortment of S1 families to be used in breeding programs on maize drought tolerance.

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The effect of density, variety, and planting date on yield and yield components of safflower

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ABSTRACT: More than 90 percent of Iranian domestic need for oil is imported. *Carthamus tinctorius* L., one of the native plants in Iran, is tolerant to drought and salinity. Its seeds contain 35% high-quality oil and 15% protein. To study the effect of density, variety, and planting date on yield and yield components of safflower, an experiment was carried out using split-plot design with four replications at Islamic Azad University, Bojnord Branch in fall 2005. Date of planting was the main factor (a1=September 23, a2=October 2, a3= October 12, a4= October 22) and varieties (LRV51.51, Zargsan and 295) and density (4, 9, 12, 15 centimeter) were the sub-factors. The results showed that the best density for seed yield and oil seed was 4 centimeters and the best planting time was September 23. The highest seed yield (with 2679kg/ha) was obtained as the result of 4 centimeter density and date planting of September 23. Among the varieties, LRV51.51 produced the highest amount of oil (721kg/ha). There was a correlation between plant seed yield and oil yield (r=0.89) and the number of seeds per pod (r=0.8).

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Effect of salinity stress on germination indices in seven safflower cultivars (*Carthamus tinctorius* L.)

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ABSTRACT: Environmental stress, especially salinity stress, can play an important role in reduction of the plant growth stage typically germination stage some region in Iran. More than 90% of the Iranian domestic need for oil is imported. Safflower, one of the native and valuable oil seed crops in Iran, is tolerant to salinity. Its seeds contain 35% high-quality oil and 15% protein .In order to study the effects of salinity stress on germination indices in safflower cultivars, an experiment was conducted in factorial form, using a completely randomized design with three replications.In this experiment, seven safflower cultivars (CH353, CH65, Asteria, CH697, Rinconada, Iranian varieties Zarghan and Isfahan) were evaluated in six levels of salinity treatments (distilled water,-3,-6,-9,-12,-15 bar) of NaCl. Results indicated significant differences among cultivars and salt stress levels (P>0.05). In all traits, a significant decrease was observed with increase in stress level. Salinity stress effects on the root length more than -12 bar causes the reduction in root length. Rinconada variety has the longest length of root with 22.78 mm. Isfahan has the longest length of stem. The highest germination percentage belonged to Rinconada (94%). The least germination percentage belonged to CH697 with (66%).The percentage of germination and velocity of germination decreased when drought stress exceeded -12 bar. Rinconada has the shortest germination time. Isfahan and Asteria showed the shortest germination time. Traits in tolerant cultivars did not show a significant decline up to -9 bar. The most tolerant cultivar was Isfahan. Considering all germination indices, CH697 was the most susceptible cultivar.

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Selection of lime-tolerant azaleas based on seed germination responses to pH regimes

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ABSTRACT: In the natural habitat, azaleas grow on acid soils. Depending on the species, the optimal value of pH of the soil ranges between 4.5-6.0. High content of calcium compounds in soil has adverse effect on the development of these plants. Being strongly alkalic, the hydrogen carbonate ions have very toxic influence on the root system and, in consequence, the growth of the plant is inhibited. Thus, the alkalization of rhizosphere constitutes the main factor limiting the possibility of azalea cultivation in calcareous soils. With a long-term goal of selecting genotypes tolerant to pH higher than optimum, seed germination responses of five azalea species (*R. japonicum*, *R. kiusianum*, *R. kaempferi*, *R. macrosepalum*, *R. ripense*) to nine pH regimes (2, 2.5, 3, 3.5, 5, 7, 7.5, 8, 8.5) were investigated. Wild azaleas showed different adaptability to pH conditions, according to the species and habitat environment.

Keywords: abiotic stresses, alkalization, calcium carbonate, chlorosis, rhododendrons, soil.

Introduction

Azaleas growing in their natural habitat are generally found on highly acid soils of low fertility. Azalea cultivars, performing best when the pH of the medium is between 4.5 and 6.0 (acidic), often show strong iron-deficiency chlorosis symptoms if they are grown on calcareous soil. In the beginning, high calcium ion (Ca^{2+}) concentration was assumed to be either directly toxic or to induce deficiencies of other nutrients. Later experiments demonstrated that high calcium supply neither suppresses growth in *Rhododendron* nor causes Fe-deficiency symptoms and that hydrogen carbonate ions (HCO_3^-) were the main factors inducing chlorosis and inhibition of root growth (Preil and Ebbinghaus, 1994). Depending on temperature, water content and concentration of CO_2 in soil, calcium carbonate ($CaCO_3$) decomposes into Ca^{2+} and HCO_3^- that is strongly alkalic. This alkalization of rhizosphere inhibits the growth of root system, making difficult the up-take of nutrients and leading to the atrophy of the all plant (Giel and Bojarczuk, 2002). This stress is one of the most serious difficulties in their cultivation. However, genetic variability, which permits improvement by plant breeding, exists even within species. Wild azaleas showed different adaptability to soil pH, according to the species and habitat environment (Scariot and Kobayashi, 2008).

The aim of the present research was to evaluate seed germination responses of several azalea species to different pH regimes. Results will constitute the starting point for further research on the selection of genotypes tolerant to pH higher than optimum.

Materials and methods

Seeds from five open pollinated species were used for this experiment. The selected species included one deciduous azalea (*R. japonicum*) and four evergreen azaleas (*R. kiusianum*, *R. kaempferi*, *R. macrosepalum*, *R. ripense*).

Thirty-six seeds of each species were treated with 50ppm GA_3 for 1 day and then sowed on polyurethane sponges (Fig. 1a) filled with NaOH or H_2SO_4 solutions to obtain the following pH regimes: 2, 2.5, 3, 3.5, 5, 7, 7.5, 8, 8.5. In order to maintain the pH values stable during the experiment time, solutions were renewed once a week. A constant temperature of 25°C and a daily photoperiod of 16/8 hours were applied.

Results and discussion

Wild azaleas showed different adaptability to pH conditions, according to the species and its natural habitat (Fig. 2). In fact, as previously assessed (Scariot and Kobayashi, 2008), in natural habitat the soil pH from the root zone of *R. kiusianum,* present in active volcanic area, can be very low (3.9). The soil pH of *R. kaempferi* and *R. macrosepalum* populations, growing in edges of secondary forest and hillside, generally ranges between 4.2 and 5.7 while some populations of *R. ripense* located in stony river areas grow at pH 7.6. In this study, however it was not possible to observe a remarkable germination tendency related to pH regimes.

For all the species and conditions, the highest germination rates were observed about 28 days after sowing. Chlorosis and inhibition of shoot and root growth, probably induced by unfavourable pH regimes (Giel and Bojarczuk 2002), were also differently manifested (Fig. 1b). These results support the key role of pH in azalea cultivation. Further research is planned to select genotypes tolerant to pH higher than optimum.

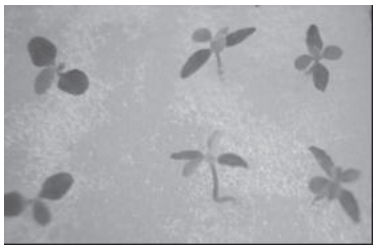
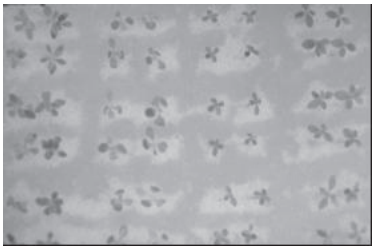
a.   b.

Figure 1. a) Azalea seedlings on polyurethane sponges; b) during pH stress, cotyledons of many seedlings turned yellowish and brownish.

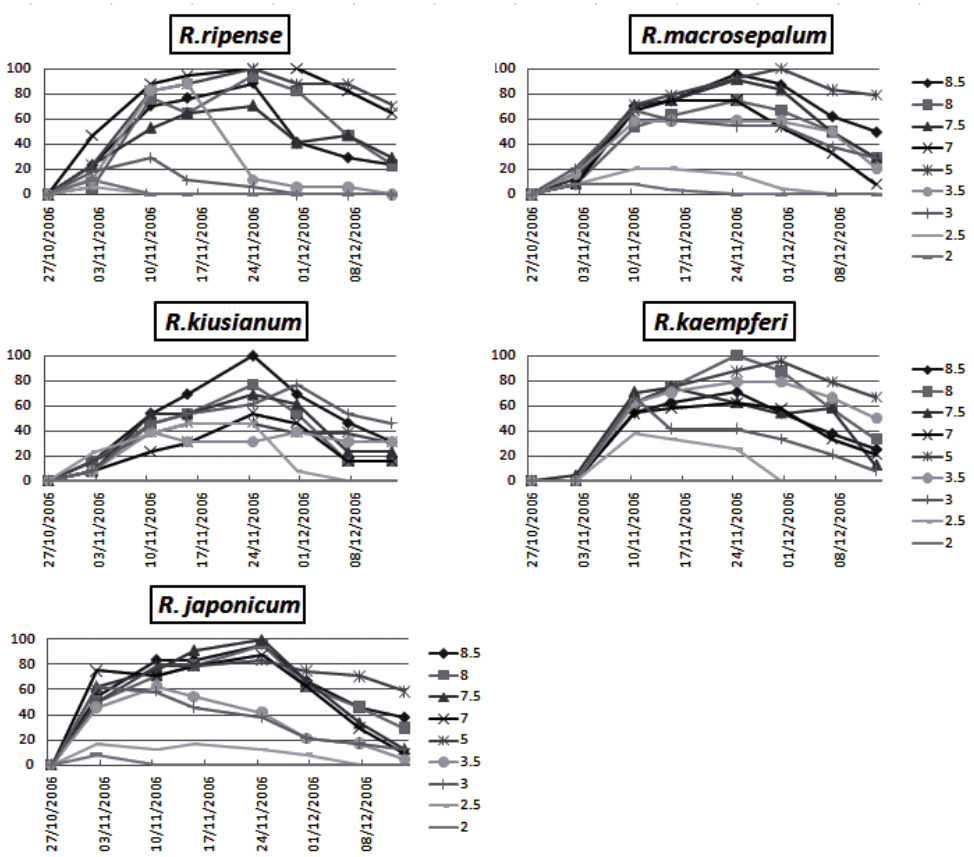


Figure 2. Effect of pH in azalea seed germination.

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In vitro evaluation of drought tolerance in bread wheat (*Triticum aestivum* L.)

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ABSTRACT: The objective of this study was to investigate drought tolerance of ten winter wheat varieties using *in vitro* mature embryo culture. The tolerance was tested on a modified MS medium to which polyethylene glycol (PEG) was added at three concentrations: 5, 10 and 15%. The control group of calluses was cultivated on a PEG-free medium. After two months of cultivation, callus fresh weight and dry matter content were determined. We found significant differences in genotype response to different PEG concentrations. At all PEG concentrations, there was a significant decrease in the fresh callus weight in all genotypes, except in cv. Hays 2. At the lowest concentration (5% PEG), the decrease in fresh weight ranged from 0 in cv. Donska semidwarf to 47.1% in cv. Bankuty 1205, relative to the control. Due to callus dehydration in the presence of PEG, the dry matter content in the calluses of all genotypes increased with increasing PEG concentrations. At the highest PEG concentration (15%) the increase of dry matter content, in relation to the control, ranged from 6.6 (Magnif 41) to 12.2% (Mina). The results indicated that cv. Hays 2 was the most tolerant genotype and cv. Bankuty 1205 was the most sensitive one.

Keywords: drought, *in vitro*, tolerance, *Triticum aestivum* L.

Introduction

Most of the land resource of the world is located in arid and semi-arid regions. The problem of drought is emphasized in developing countries, where 37% of the wheat growing areas are in semi-arid locations, with moisture deficit being the major limiting factor for high yields (Rajaram, 2001).

Plants exposed to stress activate several mechanisms that help them alleviate or tolerate effects of the stress. For breeders, drought tolerance is the ability of a genotype to be more productive than others under suboptimal soil moisture conditions (Osmanzai et al., 1987). Breeding for developing new cultivars or improving stress tolerance within the currently grown cultivars requires a full understanding of the reaction of plant tissues and organs to a particular kind of stress. Accordingly, breeders must cooperate closely with physiologists, phytopathologists, biochemists and molecular biologist in order to develop plants that grow successfully in various environments (Din, 2003).

Conventional breeding brought only partial progress in increasing drought tolerance of cultivated plants. Breeders continue to search for new genetic variability and more effective breeding criteria. Tissue culture methods in combination with genetic engineering offer large possibilities for drought tolerance improvement. Attempts have been made to employ *in vitro* methods in the development of new genotypes of cultivated plants which would maintain high productivity when grown in arid conditions (Wang et al., 1999; Kondic-Spika and Sesek, 2000; Din, 2003; Abd El Ghany et al., 2004).

The objective of this study was to evaluate drought tolerance of winter wheat (*Triticum aestivum* L.) genotypes using *in vitro* culture.

Material and methods

Ten winter wheat cultivars (*Triticum aestivum* L.) were used for isolation of mature embryos. Experimental material was prepared and sterilized according to a previously described procedure (Kondic et al., 1998).

The isolated mature embryos were cultivated on a modified MS (Murashige and Skoog, 1962) medium. Various levels of water, i.e., osmotic stress were induced by adding three concentrations (5, 10 and 15%) of polyethylene glycol (PEG-6000). The control group of calluses was cultivated on a PEG-free medium.

After two months of cultivation, callus fresh weight and dry matter content were determined. The obtained results were processed by the analysis of variance, estimating differences between the treatments and the control by the least significant difference test (LSD).

Results and discussion

All of the tested PEG concentrations inhibited the growth of wheat calluses. At the lowest PEG concentration (5%), the genotypes exhibited significant differences regarding the rate of callus growth. In three wheat cultivars (Donska semidwarf, Hays 2 and Ivanka), this PEG concentration had no significant effect on the growth of fresh callus weight. The PEG concentration of 5% caused a significant reduction ($P<0.05$) of fresh callus weight in two cultivars (Benni multifloret and Mina). In the remaining five cultivars (Bankuty 1205, Capelle Desprez, F 4 4687, Magnif 41 and Mexico 3), this PEG concentration caused a highly significant reduction ($P<0.01$) of fresh callus weight. The higher PEG concentrations (10 and 15%) showed no effect on callus growth only in the cultivar Hays 2. The other cultivars showed various rates of reduction of callus growth in relation to the control (Table 1).

Presence of PEG in the medium caused a significant increase of callus dry matter content in all wheat genotypes (Table 2). The genotypes differed in the rate of callus dehydration, i.e., the rate of dry matter increase. At the lowest PEG concentration (5%), two genotypes (Benni multifloret and Hays 2) had the absolute increase of dry matter content in relation to the control under 2%. In the other genotypes, the rate of increase ranged from 2.5% (Bankuty 1205 and Donska semidwarf) to 6.1% (Ivanka). At the PEG concentration of 10%, the increase in dry

Table 1. Effect of different PEG concentrations on callus fresh weight of wheat genotypes.

No.	Genotype	Callus fresh weight						
		PEG concentration (%)						
		0	5		10		15	
		mg	mg	%	mg	%	mg	%
	Bankuty 1205	81.7	43.2**	52.9	43.1**	52.7	41.9**	51.3
	Benni multifloret	41.3	37.4*	90.5	36.2**	87.6	32.1**	77.7
	Capelle Desprez	65.4	55.9**	85.5	55.8**	85.3	55.6**	85.0
	Donska semidwarf	80.4	81.9	101.8	53.2**	66.2	30.8**	38.3
	F 4 4687	46.1	28.4**	61.6	28.1**	60.9	30.2**	65.5
	Hays 2	20.6	19.5	94.7	19.3	93.7	18.3	88.8
	Ivanka	77.0	75.8	98.4	64.5**	83.8	48.5**	63.0
	Magnif 41	39.6	29.8**	75.2	28.1**	71.0	19.6**	49.5
	Mexico 3	60.4	50.4**	83.4	45.3**	75.0	42.6**	70.5
	Mina	40.7	36.6*	89.9	33.2**	81.6	35.2**	86.5
	LSD 0.05	3.205						
	0.01	4.328						

LSD was calculated only for absolute values of callus fresh weight (mg)
%-ages were calculated in relation to the control (0% PEG)

mater content exceeded 6% in most of the genotypes (60%). The maximum increase was 9.8%, in the cultivar Mina. At the highest PEG concentration (15%), the dry matter content in the calluses ranged from 13.3 (Magnif 41) to 23.8% (Mina), the absolute increase in relation to the control ranging from 6.6 (Magnif 41) to 12.2% (Mina).

The increased dry matter content was not consequence of increased biosynthesis. It was due to callus dehydration caused by the presence of PEG in the medium, which imbalanced the water to dry matter ratio in the calli and made the dry matter content to appear to be increased. The rate of callus dehydration increased with the increase in PEG concentration, the apparent increase in dry matter content becoming more pronounced. These results are in agreement with our previous results (Kondic-Spika and Sesek, 2000), as well as with the results of Mohamed and Tawfik (2007).

Significant differences were observed among the genotypes regarding their tolerance to the osmotic stress caused by the presence of PEG in the medium. The ratio (%) of callus fresh weight at the different PEG concentrations and in the control served as an indicator of genotype tolerance to osmotic stress. According to this criterion, the cultivar Hays 2 was the most tolerant genotype since it showed no significant increase in callus fresh weight regardless of PEG concentration applied. Two more genotypes (Capelle Desprez and Mina)

Table 2. Effect of different PEG concentrations on dry matter content in wheat calluses.

No.	Genotype		Dry matter content (%)			
			PEG concentration			
			0	5	10	15
	Bankuty 1205		10.0	12.5**	14.0**	19.2**
	Benni multifloret		11.5	13.3**	17.5**	20.8**
	Capelle Desprez		10.0	13.6**	16.0**	20.9**
	Donska semidwarf		9.7	12.2**	15.6**	21.1**
	F 4 4687		9.4	14.6**	16.1**	21.1**
	Hays 2		10.8	12.0**	17.4**	21.9**
	Ivanka		7.8	13.9**	16.9**	17.7**
	Magnif 41		6.7	9.7**	12.1**	13.3**
	Mexico 3		8.8	13.2**	16.1**	18.1**
	Mina		11.6	14.7**	21.4**	23.8**
	LSD	0.05	0.4104			
		0.01	0.5541			

also demonstrated very high level of tolerance to osmotic stress caused by 15% PEG, with the ratio values higher than 80%. Two genotypes showed high (70-80%), two genotypes showed medium (60-70%) and three genotypes showed low level of tolerance (<60%) to osmotic stress (Table 1). Three genotypes (Bankuty 1205, Donska semidwarf and Magnif 41) which showed low level of tolerance at the highest PEG concentration (15%), differed significantly at the other two concentrations. Cv. Donska semidwarf had very high tolerance to low level of osmotic stress (5% PEG) and medium tolerance at 10% PEG, while Magnif 41 had high level of tolerance at the both concentrations (5 and 10% PEG). Cv. Bankuty 1205 exhibited low level of tolerance at all PEG concentrations and it was considered as the most sensitive genotype.

This study is another proof that genotypes may be differentiated regarding their drought tolerance in *in vitro* culture, providing that an adequately selective PEG concentration is applied. Many authors had arrived at similar conclusions (Khan et al., 2001; Din, 2003; Abd El Ghany et al., 2004; Bajji et al., 2004; Gawande et al., 2004; Mohamed and Tawfik, 2007).

A broad array of PEG concentrations should be tested in order to determine a most selective concentration for differentiating wheat genotypes regarding drought tolerance. Determining the optimum selective concentration would allow for this method to be used in breeding programs for rapid testing of a large number of genotypes.

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Analysis of *Festuca pratensis* proteome during cold acclimation

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ABSTRACT: The details concerning cold acclimation and resistance to frost are not well identified and understood for *Festuca pratensis*. To recognize some of the mechanisms of cold acclimation the complex proteomic research including two dimensional electrophoresis and mass spectrometry methods was performed on the genotypes of *F. pratensis* distinct in the level of frost tolerance. The proteins that could be crucial in the development of resistance to frost were identified. The selected proteins were shown to be involved mainly in the processes of photosynthesis, cell defense against Reactive Oxygen Species and proper protein folding.

Keywords: cold acclimation, 2-D electrophoresis, *Festuca pratensis*, frost tolerance, MALDI-TOF mass spectrometry, protein profiles.

Introduction

Festuca pratensis (meadow fescue) has the potential of providing genes governing winter hardiness for transfer to closely related *Lolium multiflorum* (Italian ryegrass) and *L. perenne* (perennial ryegrass) both having high productivity and forage quality but rather poor persistence during winter (Kosmala et al., 2006). Winter hardiness is a complex trait that includes the ability to survive the interacting factors of a winter environment changing between years and locations. However, frost tolerance is one of the main components determining levels of winter hardiness. All species which can survive winter (e.g. *F. pratensis*) have the ability to sense low temperature and respond by activating a mechanism which increases frost tolerance; this adaptive process is known as cold acclimation, CA. Unfortunately, the details concerning cold acclimation and resistance to frost are not well identified and understood for *F. pratensis*. To recognize some of the mechanisms of cold acclimation in meadow fescue the complex research on the proteomic level was performed, including: *(i)* the selection of *F. pratensis* genotypes with differentially expressed frost resistance, *(ii)* the comparisons of some protein fractions (extractomes) after different time of cold acclimation between the most and the least frost tolerant genotypes using 2-D electrophoresis, *(iii)* the identification of proteins which are differentially "expressed" between the selected genotypes by the use of a mass spectrometry (MS) approach.

Materials and methods

Physiological analyses – test for freezing tolerance

Forty genotypes of *F. pratensis* cv. Skra were subjected to cold acclimation (3 weeks at +4°C, photoperiod 10/14 h, 200 μmol $m^{-2}s^{-1}$ PPFD, Philips AGRO sodium light source), and freezing tolerance estimated by freezing at –7, –11 and –15°C as described by Rapacz et al. (2004). Frost resistance was scored as the ability of plants to regrow after freezing using the visual scale of Larsen (1978), described in detail by Kosmala et al. (2006), where 0 denoted a completely destroyed plant and 9 a plant without any visible sign of damage. On the selected plants a detailed LT_{50} (lethal temperature) analysis was performed to estimate the level of freezing tolerance after different time of cold acclimation. LT_{50} was defined as the temperature at which there was 50% cell membrane damage determined by the conductivity of the tissue extract derived from leaves frozen at six different freezing temperatures according to Rapacz et al. (2004).

Molecular analyses

Leaf samples were collected after 2, 8 hours, 2, 4, 6, 7, 14 and 21 days of cold acclimation and used to extract proteins for 2-D electrophoresis. Protein samples were prepared as described by Hurkman and Tanaka (1986) and the protein concentration was determined by using the 2-D Quant Kit (GE Healthcare). Aliquots of proteins were used for two-dimensional gel electrophoresis that was performed according to Hochstrasser et al. (1988). In the first dimension – isoelectrofocusing (IEF), a 0.8 ×150 mm capillary was used for polymerization of polyacrylamide column gel with Servalyte pH range 3-10. In the second dimension (SDS-PAGE) the proteins were separated in 13% polyacrylamide slabs (1.5 × 200 × 200 mm). After electrophoresis, the gels were stained in colloidal Coomassie Brilliant Blue (CBB) to recognize both the qualitative (presence or absence of particular spots) and quantitative differences between matched spots on the successive gels. Each extraction procedure and electrophoretic separation was performed twice. Total separated protein spots on the gels were scanned by ImageScanner III (GE Healthcare) and subjected to LabScan 6.0 program (GE Healthcare) processing. Spot detection and image analyses (background subtraction, normalization, spot matching and statistics) were performed by using the Image Master 2-D *Platinum* software (GE Healthcare). The spots were selected for further MS analyses if their "patterns of expression" were different between two genotypes of *F. pratensis* distinct in the level of freezing tolerance. Peptide samples for MS were prepared using the modified method adapted from Shevchenko et al. (1996). Protein spots were excised from the gel, reduced, alkylated and digested with trypsin (Promega). Tryptic-digested peptides were recovered through a series of extraction steps. Peptide masses were measured on a MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time of Flight) MS (Autoflex II, Bruker-Daltonik, Germany) and peptide mass fingerprint data were matched to the MSDB (Model System Database) or SwissProt database using the Mascot program (www.matrixscience.com). The following search parameters were applied. Mass tolerance was set to 0.2 Da, one

incomplete cleavage was allowed, alkylation of cysteine by carbamidomethylation as fixed, and oxidation of methionine as variable modification were set.

Results and discussion

Among 40 *F. pratensis* plants examined for freezing tolerance, on the basis of their ability to regrow after freezing, two genotypes (Fp1 and Fp37) were shown to be the most frost tolerant and two other genotypes (Fp8 and Fp13) the least frost tolerant. The results of the LT_{50} analysis performed on the selected plants are shown on the diagram (Fig. 1). It is clearly visible that the differences in frost tolerance between the selected plants appeared after the 5th day and increased until the 21st day (last day) of cold acclimation.

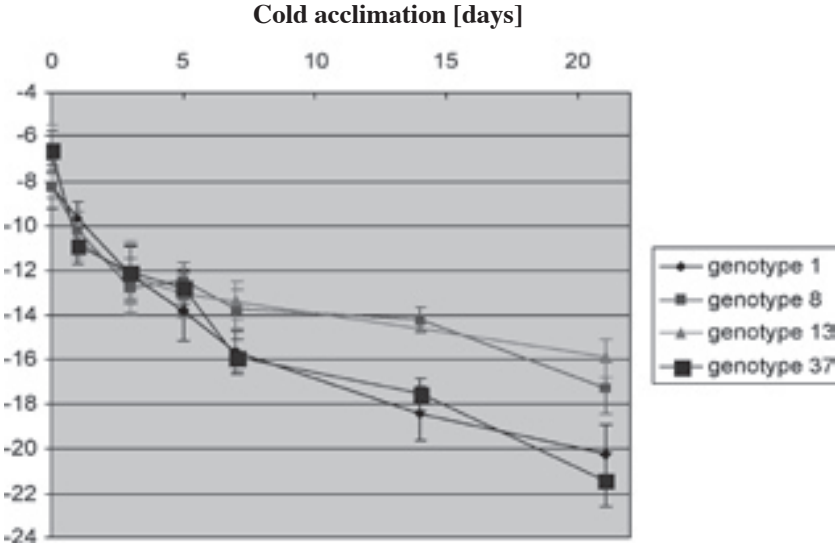

Figure 1. The level of freezing tolerance (LT_{50}) after different time of cold acclimation of the selected *F. pratensis* genotypes (P=0.05).

For further molecular research two *F. pratensis* genotypes with distinct levels of frost tolerance and LT_{50} value were chosen: - Fp8 (LT_{50} after 21 days of CA = -17°C) and Fp37 (LT_{50} after 21 days of CA = -21°C). Protein samples of Hurkman and Tanaka (1986) extractomes for each time point of CA and each genotype were used for electrophoresis. All the 2-D patterns were highly repeatable and after gel analyses detailed 2-D protein maps for each time point of CA for Fp8 and Fp37 plants were obtained.

Table 1. Identification of the selected proteins by the using of MALDI-TOF MS.

Spot no.	Molecular mass (Da)/pI	Protein name
1	71351/5.08	Heat shock protein 70 from *Medicago sativa*
2	75480/5.15	Heat shock protein, chloroplast, 70K from *Cucumis sativus*
3	72607/5.54	Putative FtsH-like protein Pftf. from *Oryza sativa*
4	53721/4.88	SCCPN60 NID (chaperonin) from *Secale cereale*
5	54020/5.38	ATP synthase beta subunit from *Oryza sativa*
6	51381/5.96	Rubisco activase alpha form precursor from *Deschampsia antarctica*
7	47371/7.57	Rubisco activase beta form precursor from *Deschampsia antarctica*
8	35068/6.10	Putative 33kDa oxygen evolving protein of photosystem II from *Oryza sativa*
9	118734/6.15	Sucrose-phosphate synthase from *Beta vulgaris*
10	27964/5.10	Ascorbate peroxidase from *Hordeum vulgare*
11	42124/9.01	Fructose 1,6-bisphosphate aldolase from *Avena sativa*
12	43168/5.60	Glyceraldehyde-3-phosphate dehydrogenase (NADP) (phosphorylating), chloroplast, B precursor from *Arabidopsis thaliana*
13	53688/6.23	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit from *Guzmania monostachia*
14	42122/6.60	Glyceraldehyde-3-phosphate dehydrogenase chloroplast, A precursor from *Nicotiana tabacum*
15	33443/6.20	Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating, cytosolic from *Hordeum vulgare*)
16	42350/9.25	NADPH2 dehydrogenase from *Hordeum vulgare*
17	21919/9.81	Photosystem II chain D precursor from *Hordeum vulgare*
18	40570/8.35	Probable (S)-2-hydroxy-acid oxidase, peroxisomal from *Arabidopsis thaliana*
19	56860/6.34	Catalase from *Festuca arundinacea*
20	31375/4.88	3-phosphoglycerate kinase (fragment) from *Hordeum vulgare*

For the first MALDI-TOF MS analyses 20 abundant spots were selected as their “patterns of expression” were enough clear and different between two genotypes of *F. pratensis* distinct in

the level of freezing tolerance. The results of MS analyses and databases searching are shown in Table 1. The identified proteins are mainly involved in the processes of photosynthesis, cell defense against Reactive Oxygen Species (ROS) and proper protein folding. Further analysis of *F. pratensis* proteome during cold acclimation is in progress.

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Characterization and selection *in vitro* of peas for resistance to necrotrophic fungal (*Fusarium solani, Ascochyta pisi, Phoma pisi*) pathogens – biochemical and morphological approach

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ABSTRACT: Interaction of peas (*Pisum sativum* L.) with necrotrophic fungi (*Fusarium solani* (FS), *Ascochyta pisi* (AP), and *Phoma pisi* (PP)), as well as a response of explant cultures affected with adjusted pathogen-derived agents, were studied in a series of experiments focused on selection *in vitro* for resistance to plant pathogens. Finding of similar biochemical and morphological reactions between both models (i.e. intact plants and *in vitro* cultures) is a basic prerequisite for efficient utilisation of biotechnological approaches in practical breeding of peas for resistance to biotic stress. Organogenic cultures of four peas genotypes (cvs. Adept, Herold, Komet, Menhir) with different degree of susceptibility to three fungal pathogens were treated with culture filtrates of FS, AP and PP in two variants (autoclaved, microfiltrated) and in three different concentrations (0-25-50 %). After the treatment, the samples of plants and organogenic cultures were analysed for total protein content (Bradford 1976), and the activity of three types of peroxidases (POX) – cytosolic, membrane bound, and ionic (Angelini *et al*. 1990). Cytosolic POX activity in explant cultures showed significant decrease as compared to control variant in the most resistant cv. Adept. In cv. Menhir the values were significantly different from Adept, and in average minimally distinct from the controls. Membrane bound POX was not influenced by the filtrate treatment, and ionic form of POX did not imitate the response of resistant and sensitive cultivars, respectively. Further, the enzymatic systems of catalase (CAT) and acid phosphatase (AP) were studied in plants and explant cultures. CAT was proved as inconvenient for the evaluation of the response to filtrates because showed just one uniform band, whereas AP possessed relatively the most distinct bands. Consequently, by histochemical DAB staining (Ruzin 1999), peroxidases were localised in explant surface as well as in vascular tissues of all samples. The signal intensity varied in control and treated variants. Selected morphological traits (length of roots and shoots of regenerating plantlets) in explant cultures and changes in leaf coloration after the treatment with filtrates were precisely evaluated by image analysis LUCIA. From our preliminary data it is evident that at least more detailed study of changes of cytosolic POX in relationship to other traits could be useful for characterization of peas genotypes and selections *in vitro*.

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Assessment of a collection of vegetable *Brassica* crops for *Xanthomonas campestris* pv. *campestris* resistance

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ABSTRACT: Black rot, caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*), is one of the most important diseases affecting *Brassica* crops around the world. Vicente et al. (2001) grouped *Xcc* isolates into six races, being 1 and 4 the most important worldwide. Besides race specific resistance, also race non-specific resistance was described. Two hundred and ninety two accessions from Germplasm Bank in the Misión Biológica de Galicia-CSIC (Spain) belonging to three *Brassica* species (*B. napus*, *B. oleracea* and *B. rapa*), together with several resistant and susceptible controls, were screened for black rot resistance using the methodology described by Lema et al. (2007). Twelve plants per accession were evaluated (rating scale from 1 (resistant) to 9 (susceptible)) and two *Xcc* races (1 and 4) were used. Race 1 was much more virulent on tested materials that race 4. Most of the *B. rapa* and *B. napus* accessions showed resistance to race 4 although a great variability within accessions was found. Of the *B. rapa* accessions, 72% were resistant to race 4, however, most of them showed variable reaction, probably due to a mixture of genotypes. Some level of resistance to both races was found only in one turnip landrace (MBG-BRS0479). In *B. napus* 60% of the accessions were uniformly resistant, 24% were variable to race 4, and MBG-BRS0131 was intermediate for race 1 and variable for race 4. In *B. oleracea,* two commercial cabbage accessions (Quintal de Alsacia and Balon) showed some degree of resistance to both *Xcc* races and one kale landrace (MBG-BRS0070) and one commercial cabbage (Corazón de Buey) were partially resistant to race 4. Noteworthy were data recorded in *B. oleracea* accessions where existing sources of resistance are scarce (Taylor et al., 2002). According these results local material can be used in breeding programs taking in account that mixture of genotypes is frequent in these materials. Intercrossing among varieties, due to the allogamic pollination mechanism, is probably associated with poor isolation produces of highly heterogeneous varieties.

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The effect of mating system for developing combined resistance to chocolate spot and Ascochyta blight in faba bean

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ABSTRACT: Faba bean (*Vicia faba* L.) is one of the most important pulse crops produced through out the world with roughly 46 millions tons grain production. This crop is adversely affected by numerous fungal diseases, which vary in their incidence and severity from one region to another. The most important foliar diseases affecting faba bean in different Mediterranean regions, China, Latin America, Ethiopia and Australia are *Ascochyta fabae* and chocolate spot. Faba bean is a partially allogamous crop with an outcrossing rate ranging from 3 to 85%. It can be developed as self or open pollinated crop. The open pollinated varieties seems to be more resistant to winter stresses than selfed ones (Gasim and Link, 2007). The main objective of this study was to evaluate the effect of biotic stresses under self and open pollinated systems. Four hybrid bulk populations developed at ICARDA during 2002/2003 and 2004/2005 growing seasons to combine early maturity with *Ascochyta* resistance, Botrytis with early maturity and large seed types, cold tolerance with chocolate spot resistance and *Ascochyta* blight with chocolate spot resistance. The populations were exposed to natural and artificial infections with a mixture of eight virulent pathotypes of *Botrytis fabae* at Lattakia and nine virulent *Ascochyta fabae* at Tel Hadya during 2005/2006 and 2006/2007 growing seasons. The progenies of each single plant selection (SPS) were divided equally between experiments under cages at Lattakia and open pollination at Tel Hadya. The single plants from F3 and the F4 progenies of the same mother were selected under both conditions showing high level of resistance for both diseases. The SPS from both selfed and open pollination of the same resistant mothers were planted together in unreplicated design with repetitive susceptible and resistant checks under cages at Lattakia and open field at Tal Hadya. Severity for both diseases in field was scored using a 9 point scale where 1= is highly resistant and 9 = highly susceptible. Evaluations were done twice during the disease development. Spatial analysis was used for data analysis using GenStat model (Singh, 2002). The mean disease severity for open pollinated mothers varied from 2.2 to 3.3 and as compared to the self pollinated mothers which varied from 2.3 to 4.4 for the two years and two different generations. In addition the number of resistant lines selected was higher under open pollinated condition than self pollinated condition. Our results indicate that the mother genotypes obtained under self pollination are more susceptible than those obtained under open pollinated condition. These results showed the possibility to improve higher resistance to biotic stresses in populations rather than in inbred lines.

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Searching for QTLs for adaptation to reduced water and nitrogen inputs in durum wheat

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ABSTRACT: A population of 184 recombinant inbred lines (RILs) was generated from the cross between the two Italian semi-dwarf durum wheat cultivars Meridiano and Claudio. The two parents are both high yielding, medium- to early-heading, elite cultivars widely adapted to the Mediterranean temperate durum wheat growing areas. In 2007, the lines were evaluated at three sites for a total of five field trials: northern Italy (Cadriano), under optimal water and nitrogen conditions, southern Italy in Lucera, Foggia (rainfed Mediterranean environment) and Obregon, Mexico, the CIMMYT's primary yield testing location, under optimal, water-stressed and nitrogen-stressed conditions. The linkage map is presently under construction using both SSR and DArT markers (Mantovani et al., 2008). In a preliminary analysis, 125 evenly spaced SSRs were used to investigate the association (single-marker linear regression) with heading date, plant height, grain yield, grain weight, density of fertile tillers, test weight and grain protein content. The results pointed out that two QTL clusters on chrs. 4B and 5A were primarily involved in the genetic control of most of the investigated traits, including grain yield. Additional markers with highly significant effects on grain yield were found on chrs. 2B, 3B, 5B and 7B, with favourable alleles contributed by both parents, thus accounting for the wide transgressive segregation observed among the RILs and for the presence of lines that significantly out-yielded both parents. Grain yield of the RILs was highly predictable based on the SSR alleles present at four key-chromosome regions. A number of QTLs showed interaction with the water and nitrogen levels.

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Two major QTLs on chr. 2BL and 3BS influence agronomic performance of durum wheat across a broad range of water regimes

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ABSTRACT: Resistance to drought stress is a main objective for durum wheat improvement in drought-prone areas of the Mediterranean basin. Therefore, identification of major quantitative trait loci (QTLs) for yield across a broad range of water regimes would offer the opportunity to deploy marker-assisted selection to improve agronomic performance and, eventually, to clone the sequence underlying the QTL (Tuberosa and Salvi, 2006) In the EU-funded project IDuWUE, 249 RILs (Kofa x Svevo) were evaluated in 16 trials conducted in 2004-2005 in Italy, Spain, Morocco, Tunisia, Syria and Lebanon, under a broad range of water regimes (rainfed and irrigated) and yield potential (from 0.5 to 5.8 t/ha). Two major, epistatic QTLs on chr. 2BL and 3BS influenced yield and related physiological traits, but not heading date, in a broad range of environments (Maccaferri et al., 2008). In both cases, coincidence between the QTLs for grain yield and those for plant height, peduncle length, SPAD, NDVI index and kernel weight was observed. Epistasis favoured the parental genotypes and negatively affected the performance of the recombinant genotypes. On a mean basis, the R^2 values for grain yield of the 2BL and 3BS QTLs were equal to 21.5 and 13.8%, respectively. The effects of these two QTLs were validated in 11 trials conducted in 2006. In view of the relevance and consistency of their effects on grain yield and other agronomically valuable traits, the 2BL and 3BS QTLs are being isogenized to proceed with their fine mapping and, on the basis of their effects on peduncle length, their positional cloning (EU project TriticeaeGenome).

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Study on grain yield stability of winter wheat genotypes using stability indices under normal irrigation and terminal drought stress conditions

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ABSTRACT: The objective of this study was to determine the grain yield (GY) stability, *genotype × environment* interaction and drought tolerance indices in 18 modern winter wheat genotypes. Trials were carried out in four locations (Ardabil, Hamedan, Miandoab and Mashad) under terminal drought stress (TDS) conditions, while trials in 2 stations (Karaj and Ardabil) were conducted under both normal irrigation and TDS conditions during 2005-06 and 2006-07. Under TDS conditions, trials were irrigated just at the booting stage. Stability parametric methods such as variance of genotype across environment (S^2xi), coefficient of variability ($CV_i\%$), Wricke's ecovalance (W_i), Shukla stability variance (σi^2), Eberhart & Russell parameters (S^2di, bi), Pinethus coefficient of determination (R^2), simultaneous selection for yield and stability (ys_i), and non-parametric method of rank were used to determine the GY stability. Analysis of variance showed that differences due to genotypes was significantly different ($P<0.01$). The *location × year* and *genotype × location* interactions were highly significant ($P<0.01$). The *genotype × location× year* interaction was significant ($P<0.01$) just in TDS conditions, while it was not significant under normal irrigation conditions. It is concluded that modern wheat genotypes derived from crosses made between Iranian or Chinese with high yielding cultivars from ICARDA and CIMMYT international centers were the most high yielding genotypes.

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Low-temperature tolerance in cereals: what should we breed for?

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ABSTRACT: An understanding of how adaptive mechanisms such as developmental, structural and regulatory genes operate in cold hardy wheat cultivars will be useful in the design of plant breeding and production systems for cold regions. This paper reports on the role of transition from the vegetative to the reproductive phase, regulated by developmental genes (vernalization, and final leaf), on the expression of LT-tolerance of wheat cultivars including Norstar, MV17, Mirnovoskaya808, Pishtaz, Sardari, Azar2, Backcrossed Roshan, DH42 (doubled haploid42), DH49, DH52, Zagroos, and Koohdasht with a wide range of winter survival that were evaluated under field conditions in cold and temperate regions of Iran from 2002 to 2008. A strong association was found between length of vegetative phase and expression of LT tolerance. Changes in abundance and the appearance of LT-induced proteins during the vegetative/ reproductive transition in proteomics analysis of most cold hardy Norstar and semi- hardy Azar2 grown in controlled conditions are discussed. The paper also reports on the mode of gene action, inheritance and LT tolerance potential of Iranian and alien wheat genotypes with diverse origins that were screened under field conditions in the cold and temperate regions. Morphological, physiological and phenological criteria useful in selecting LT tolerant wheat genotypes for both cold and temperate regions are discussed. Based on observations, breeding strategies are suggested for improvement of LT tolerance in wheat developed for regions with both long and mild winters, like the cold areas of Iran, and a high level of LT stress, like many parts of the northern hemisphere.

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QTL mapping in the context of drought stress trials in maize: a mixed model approach to cope with multiple environments and/or multiple traits

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ABSTRACT: A common experimental setup in plant breeding consists of evaluating the performance of genotypes under different environmental conditions. For example, in breeding for drought stress, genotypes can be observed under different water regimes. Genetic correlation between the performances in the different environments is expected due to a common genetic basis for the phenotypic observations on the same genotypes across the experiments. Conversely, the occurrence of genotype-environment interaction will cause a lack of genetic correlation between environments. If genotypes come from a designed cross (e.g. F2 or RIL population), the data can be used in conventional QTL mapping to detect QTLs and QTLxE (QTL by environment interaction). Mixed models are advantageous in this context as they allow accounting for the genetic correlations in the data, improving the tests for the detection of QTLs and the estimates of QTL effects. Another asset of mixed models is that they can integrate in a natural way the information of different traits (multi-trait analysis). Multi-trait data sets can be used to investigate the causes of genetic correlations between responses, where we can distinguish between correlations caused by pleiotropic QTLs that affect multiple traits and genetic correlations caused by linkage of various QTLs that affect different traits. In analogy to the multi-environment situation, mixed models for multi-trait analyses contain explicit representations of the genetic correlations between traits that result from pleiotropy and/or genetic linkage between QTLs. Furthermore, the multiple environment and multiple trait features can be integrated within the same analysis, leading to multi-trait multi-environment QTL mapping. We will illustrate our mixed-model approach to multi-trait multi-environment QTL mapping with examples in maize based on drought stress trials conducted at CIMMYT.

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Impacts of testing environments and crop density on winter wheat kernel weight

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ABSTRACT: Selection of stabile genotype with broad adaptation to various environments is one of the main goals in winter wheat breeding. Genotype x environment interaction (GEI) hinders selection of the best genotypes due to confounding results for the genetic differences between wheat genotypes. Therefore, attention has been devoted to analyzing genotype by environment interactions (GEI) to improve crop breeding success (Thomason and Philips, 2006). Usually, strategy for selection of winter wheat is that selection should be made on several locations different in climatic and soil conditions. The objectives of this paper were to examine influence of different testing environments and sowing rates on formation of winter wheat thousand kernel weight and, after the stability analysis, to identify most stabile genotypes and locations. Thousand kernel weight is very important trait for yield formation in winter wheat and it is considered to be one of the direct yield component. Research work was conducted during 2006/07 growing season on four test locations with four different soil types: Nova Gradiška – fluvisol; Osijek – eutric cambisol; Požega – pseudogley; Tovarnik – chernozem. Research work included 14 winter wheat genotypes. Genotypes were chosen due to their high yielding performance and good quality. They included recognized cultivars and new breeding lines. The examined genotypes were sowed with two sowing rates: 300 and 600 germinable seeds m^{-2}. Combined analysis of variance showed highly significant ($p \leq 0.01$) influence of genotypes, environments (sowing rate and location) and GEI on thousand kernel weight. AMMI 1 model biplot showed that the location with highest thousand kernel weight in combination with lower sowing rate was Nova Gradiška. The most stabile location in combination with lower sowing rate and high thousand kernel weight was location with the best soil conditions – Tovarnik. Lower sowing rate at all locations showed higher thousand kernel weight than higher sowing rate at the same location. Lower sowing rate at all locations also was more stabile than higher sowing rate. Biplot also showed that locations were spread from locations with lower thousand kernel weight to locations with high kernel weight. That is very important for selection of stabile and adaptable genotypes. Examined genotypes differed in thousand kernel weight and in stability across environments. Breeding line OSK 63/05 has the highest thousand kernel weight, but it was unstable and adapted to higher yielding environments. Stabile genotypes, but with lower kernel weight, were cultivar Srpanjka and breeding line OSK 89/05. Best combination of high kernel weight and good stability was in breeding line OSK 108/04.

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Screening *Pisum* sp. accessions for resistance to bacterial pathogens (*Pseudomonas syringae* pv. *pisi* and *P. syringae* pv. *syringae*)

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ABSTRACT: Pea (*Pisum sativum* L.) is the most important grain legume crop in Europe and Spain. In this country, Castilla y León is the main producer region, where major economics losses due to bacterial diseases caused by *Pseudomonas syringae*, both pv. *pisi* (*Psp*) and pv. *syringae* (*Pss*), have been described (Martín et al., 2007). *Psp* has a well known seven races structure (Bevan et al., 1995), while the information of *Pss* as pea pathogen is very limited. The objectives of this work were: 1) To know the resistance in controlled conditions to the seven races of *Psp* in 242 *Pisum* sp. accessions (commercial cultivars, breeding lines, landraces and wild entries); 2) To check in field environment some of the different resistance sources to *Psp* detected in controlled conditions; 3) To develop a preliminary screening for resistance to *Pss* in *Pisum* sp. under controlled conditions in 52 *Pisum* sp. accessions. Race-nonspecific resistance to *Psp* was identified only in wild accessions (*P. abyssinicum*) and in some Spanish landraces. Race-specific resistance to each race was also identified, but for race 6 just a few Spanish landraces showed this type of resistance. Taking into account that races 2, 4 and 6 are the most frequent in Castilla y León region (Martín et al., 2007), it was found no commercial cultivars or breeding lines resistant to all these races, being only the 5.2% resistant to races 2 and 4 simultaneously. Genotypes with race-specific resistance to race 2 and 4 in controlled conditions behaved also resistant in field inoculations. Race-nonspecific resistance and race-specific resistance to race 6 detected in controlled conditions were not confirmed under field conditions using race 6 inoculation. The lack of joint resistance to races 2, 4 and 6 in commercial genotypes clearly justifies the development of a breeding program. Inoculations performed with *Pss* showed a continuous variation in the infected plant surface. The 13.5% of the entries were considered as resistant, because they showed infection restricted to the inoculation point. These materials comprised some commercial cultivars interesting for Spanish dry lands, being one of these genotypes also resistant to all races of *Psp*, except for the 6 one. At present, some sources of resistance found in this work are being introduced in elite cultivars, and further efforts are undertaking to find more resistant genotypes, mainly to *Psp* race 6 and *Pss*.

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Resistance to leaf rust (*Puccinia triticina*) in a collection of durum wheat cultivars derived from CIMMYT germplasm with adaptation to Spanish conditions

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ABSTRACT: A collection of 16 new cultivars of durum wheat (*Triticum turgidum* L. var. *durum*) derived from CIMMYT (International Maize and Wheat Improvement Center) germplasm were characterized for their resistance against the leaf rust fungus (*Puccinia triticina*). This disease is a major threat to the crop, especially since a new virulent race appeared in 2001. That race came up in different and distant parts of the world such as USA, France and Spain. From that time, the CIMMYT has made a tremendous effort in achieving new resistant cultivars. A sample of them has been selected for their suitability to the Spanish conditions and field trials and several tests in growth chamber have been made. Several cultivars displayed resistance to the new race. In addition, several cultivars showed a fair level of partial resistance (demonstrated in seedling tests) that is considered to be durable. From the parameters assessed in the growth chamber infection type (especially in adult plant) and latency period in adult plant showed the highest correlation with field severity.

Keywords: Durum wheat, leaf rust, partial resistance, severity.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is a crop of great importance in Spain. The favourable environmental conditions for bringing out a good quality product and an increasing demand of pasta from the EU are reasons for more than half million hectares of durum wheat acreage in the country, most of them in Southern Spain (Andalusia).

Leaf rust, caused by *Puccinia triticina* Eriks., is an important disease of wheat worldwide. In durum wheat, it has been particularly severe in recent years, since a new virulent race showed up in 2001 in Mexico (Singh et al. 2004). That race, designated as BBG/BN caused susceptibility of over 80% of the CIMMYT germplasm, including the most popular cultivar, 'Altar 84'. The race spread rapidly to the USA, and it appeared in further places such as France and Spain. Cátedra et al. (2004) reported losses ranging from 20 to 30% of the potential yield in Andalusia (Spain) due to the attack of the new race of leaf rust in practically all the cultivars that rendered susceptible. Since then, CIMMYT has searched for resistance in its germplasm and the first result was the cultivar 'Jupare 2001'.

The aim of this study was to characterize the resistance to leaf rust in a collection of 20 durum wheat cultivars (16 of them new) from CIMMYT origin in field trials and in growth chamber in seedling stage to determine the resistance to several leaf rust isolates and the level of partial resistance.

Material and methods

Three local isolates of leaf rust were used for the tests. They were collected from Conil, Puerto Serrano and Utrera. The first two isolates originated from single pustule whereas the latter was a population. P.t. Puerto Serrano was quite virulent on the most of durum wheat (representant of the new virulent race), and therefore, was used to evaluate the level of horizontal or partial resistance.

For field trials each cultivar was sown in three locations. These were Jerez de la Frontera (Cádiz), Conil de la Frontera (Cádiz) and Écija (Sevilla). In each location three replications per cultivar were sown. Each replication consisted of a 6 m^2 plot (5 x 1.2 m^2). Severity (taken as a global assessment of the percentage of foliar surface covered by the leaf rust) was assessed at the moment the disease reached the highest level (milky grain stage) (73 Zadoks´ scale). Relative severity was referred to the susceptible cultivar 'Don Rafael'. Data were subjected to analysis of variance, and significant differences among treatment means were determined using Duncan´s multiple range test.

Hypersensitive resistance

Cultivars were sown in plastic trays (55x40x9cm) in a growth chamber at 20ºC, with 112 μmol/m^2.sec of light intensity, and a 14 hour photoperiod. Four seedlings per cultivar were raised and fourteen days after sowing inoculations were performed. First leaves were inoculated with a mixture of rust spores and talcum powder (1:15 vol/vol) resulting in a deposition of approximately 200 spores/cm^2. Then, plants were incubated for 12 hours at 20ºC and humidity at saturation. Afterwards, trays were transferred back to the growth chamber. Twelve days after incubation, infection type (IT) was recorded using a 0-9 scale adapted from McNeal *et al*. (1971). ITs 7 or higher were regarded as compatible, and were referred to as high IT. ITs lower than 7 were referred to as low IT, indicative of hypersensitive resistance. Most of the times there was agreement in the IT of the four leaves. Only a few times one leaf presented an IT different from the other three. In such uncommon events the IT of the majority of the leaves were taken as a score.

Partial resistance

Plants were sown in trays in the same way as described above. But at the inoculation, the first leaves were fixed in a horizontal position to allow a more uniform deposition of spores and inoculated with P.t. Puerto Serrano. The trays were incubated and transferred to a growth chamber with the conditions as described above. Five seedlings per accession were evaluated

in three replications. Other three replications were carried out in adult plant stage (fifth leaf, 22 Zadoks´scale). Pustule counts were carried out daily from the beginning of sporulation until the number of pustules no longer increased. Latency period was estimated by interpolation as the time period from the beginning of incubation until 50 % of the total number of pustules appeared (Parlevliet 1975). Latency periods were referred in each tray to the cultivar 'Don Rafael' (=100 %). The infection frequency was determined on the marked area of the leaves. The final number of uredia was used to calculate the number of uredia per cm^2.

Data were subjected to analysis of variance.

Results and discussion

In table 1 the results of field trials are shown. The severity of the susceptible cultivar 'Don Rafael' was the highest in all locations, although some cultivars were almost as susceptible as the susceptible check. The severity of disease in Conil was higher than in the other two locations..

There were eight cultivars completely resistant in all locations. These were: 'Don Patricio', 'Don Jaime', 'Don Juan', 'Don Carlos', 'Don Vicente', 'TDA 833', 'TDA 855' and 'TDA 959'. On the plants of the rest of cultivars uredia were observed. The cultivar 'TDA 845' had a lower severity than the check in all three locations, and the cultivars 'Yavaros' and 'TDA 677' in two out the three locations.

Respect to the inoculation in seedling stage to determine infection types it is important to note that Conil and Puerto Serrano isolates were single pustule origin whereas Utrera isolate was a population, displaying in many cultivars more than one infection type.

Most of the durum wheat were susceptible to Conil and Puerto Serrano isolates, but not against Utrera. It is remarkable that cultivar 'Don Pedro', very susceptible in field trials, displayed seedling resistance against isolate of Conil.

In the tests made in fifth leaf some cultivars changed the infection type ('Don Patricio', 'Don Jaime', 'Don Carlos', 'Don Vicente', 'TDA 833', 'TDA 855' and 'TDA 959').

Regarding partial resistance components (Table 2), latency period of 'Don Rafael' was 140 hours in primary leaf and 172 hours in fifth leaf. The infection frequency was 112 pustules/cm^2 in seedling and 82 in fifth leaf.

Latency period in most of the cultivars were similar to that of the susceptible check. But there were some cultivars with a higher latency period and low infection frequency in the seedling stage ('Don Vicente', 'TDA 677', 'Don Pedro', 'TDA 855', 'Don Carlos' and 'Gallareta'). It must be taken into account that cultivars 'TDA 855' and 'Don Carlos' displayed a moderate infection type that may stretch the latency period and shorten the infection frequency. It is remarkable that for cultivars 'Gallareta' and 'Don Pedro' the relative latency period was greater in seedling than in adult plant. In adult plant (fifth leaf) cultivars 'TDA 845', 'TDA 677', 'Don José' and 'Yavaros' showed a high latency period and a low infection frequency.

Table 1. Relative field severity of leaf rust (*Puccinia triticina*) in a set of durum wheat cultivars in three locations of Andalusia during 2003/04.

	Severity %			Earliness
Cultivar	Jerez	Conil	Écija	
'Don Rafael'	100 a[1]	100 a	100 a	E
	(25)[3]	(70)	(20)	
'Yavaros'	60 b	100 a	60 c	M
'Gallareta'	80 a b	100 a	75 b c	L
'Don Pedro'	100 a	100 a	75 b c	M
'Sula'	100 a	100 a	90 a b	L
'Don Manuel'	100 a	100 a	75 b c	E
'D. Sebastián'	80 a b	100 a	60 c	E
'Don José'	80 a b	100 a	60 c	M
'D. Francisco'	100 a	100 a	60 c	M
'TDA 677'	60 b	100 a	60 c	M
'TDA 845'	60 b	21 b	35 d	L
'TDA 725s'	100 a	100 a	75 b c	L
'Don Patricio'	0 c	0 c	0 e	L
'Don Jaime'	0 c	0 c	0 e	E

	Severity %			Earliness
Cultivar	Jerez	Conil	Écija	
'Don Juan'	0 c	0 c	0 e	E
'Don Carlos'	0 c	0 c	0 e	L
'Don Vicente'	0 c	0 c	0 e	M
'TDA 833'	0 c	0 c	0 e	L
'TDA 855'	0 c	0 c	0 e	L
'TDA 959	0 c	0 c	0 e	M

[1] Figures within a column followed by a letter in common are not significantly different (Duncan, 95%).

[2] Earliness: E-early, M-medium, L-late.

[3] Severity referred to cultivar 'Don Rafael'. Between bracket is the actual severity of leaf rust.

Infection type in seedling and adult plant were highly correlated (coeficient of correlation 0.76). Infection type with isolate of Utrera did not correlated well with the other two, indicating a clear differentiation from them.

Many of the novel cultivars showed resistance against the virulent race present in the Spanish fields. This complete resistance is based on the hypersensitive interaction. Data of these resistant sources are not available. These genes may be different to the known *Lr* genes and specific of durum wheat (Herrera Foessel et al. 2005, Martínez et al. 2007).

Table 2. Macroscopic components of the resistance to *P. triticina* in a collection of durum wheat cultivars.

	Seedling stage					Adult plant stage (fifth leaf)		
Cultivar	RLP[1]	RIF	IT	IT P. t. Utrera	IT P. t. Conil	RLP	RIF	IT
'Don Rafael'	100 e (140)[2]	100 ab (112)	9	2(9)[3]	9	100 cd (172)	100 ab (82)	9
'Yavaros'	100 e	84 bcdef	9	9(7)	9	105 ab	26 g	9
'Gallareta'	109 a	71 efg	9	2	9	100 cd	61 de	9
'Don Pedro'	110 a	69 fg	9	2	1	98 d	117 a	9
'Sula'	100 e	107 a	9	2(9)	9	99 cd	73 cd	9
'Don Manuel'	102 bcde	96 abc	9	2(9)	9	98 d	107 a	9
'Don Sebastián'	101 cde	84 bcdef	9	2(9)	9	103 bc	83 bc	9
'Don José'	102 bcde	84 bcdef	9	5	9	106 ab	59 de	9
'Don Francisco'	100 e	77 cdefg	9	3	9	100 cd	37 fg	9
'TDA 677'	105 bc	80 cdef	9	1(9)	9	106 ab	5 def	9
'TDA 845'	100 e	88 abcde	9	5	9	109 a	36 fg	8
'TDA 725s'	100 de	104 a	9	2(9)	9	97 d	50 ef	9
'Don Patricio'	100 e	91 abcd	8	3	9	-[4]	-	5
'Don Jaime'	100 e	104 a	9	5	9	-	-	4
'Don Juan'	102 bcde	107 a	9	3	9	103 bc	84 bc	8
'Don Carlos'	105 b	75 defg	7	2	8	-	-	3
'Don Vicente'	104 bcd	67 fg	8	2	8	-	-	5
'TDA 833'	100 e	68 fg	9	5	8	-	-	4
'TDA 855'	105 b	68 fg	7	2	9	-	-	3
'TDA 959'	102 bcde	59 g	8	7	9	-	-	6

[1] RLP (relative latency period referred to that of 'Don Rafael') and their actual latency period (in hours) is giving between brackets. RIF (relative infection frequency) is also referred to that of 'Don Rafael' and their actual values (number of pustules per cm^2) is given between brackets.

[2] Figures within a column followed by a letter in common are not significantly different (Duncan, 95%).

[3] Mixtures of infection types. IT between brackets is in lower proportion.

[4] Latency period and infection frequency were assessed with the isolate P.t. Puerto Serrano and they could only be determined when the infection type was high (more than 7).

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Evaluation of *Phaseolus sp.* germplasm response to water deficit

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ABSTRACT: Across all farming systems, biotic and abiotic stresses increasingly represent major constraints on the production and economic yield. They are an important contributor to reduced yields of food legumes. It is important to obtain, by classical breeding or non-conventional approaches, cultivars more resistant to stress, which will at the same time be economically interesting. In order to achieve this, it is necessary to understand the mechanisms of response to stress of different *Phaseolus* species and cultivars. The response to drought stress in *Phaseolus* leaves was analysed using two different approaches. First approach was the identification of genes whose expression is altered under conditions of drought through relative gene expression analysis, while in the second one we looked in the involvement of serine proteases, that like cysteine proteases have been reported to be influenced by drought and are an important first step towards understanding the response of this species. 19 day old plants of eight cultivars grown in a growth chamber and 21 day old plants of four of these cultivars grown in a greenhouse under conditions nearer to those in the field, were subjected to progressive water withdrawal for 6 to 12 days. Relative expression analysis using quantitative real-time PCR was performed for 34 transcripts/genes; which might be involved in the response to drought stress. They were identified previously by differential display RT-PCR or by blast search of drought-inducible genes against *P. vulgaris* EST deposited in the NCBI's dbEST. Nine transcripts were confirmed as up-regulated in drought-stressed plants (in comparison to control plants); and eight transcripts were confirmed as down-regulated. Blast search revealed that up-regulated transcripts/genes belong to various previous reported functional categories characteristic for drought stress: osmoprotectant synthesis, LEA proteins, protein kinases, cellular- and carbohydrate metabolism, aldehyde dehydrogenases and AP2/EREBP transcription factors; while five of eight down-regulated genes belong to the functional category related to photosynthesis (inhibited under drought stress). Expression analysis revealed that 17 statistically confirmed differentially expressed genes respond about the same in all eight *P. vulgaris* genotypes analysed in this study. A wide variety of proteolytic enzymes exist in plants. On their levels depends protein turnover, a fundamental component in plant development and adaptation to environmental conditions. Cysteine proteases have frequently been reported to be influenced by drought, but only a few serine proteases (SP), among them the trypsin-like enzyme and two aminopeptidases from bean leaves. Our starting point was to identify proteolytic activities assigned to SPs that change with drought and then to characterize the corresponding proteases. A quantitative, analytical one-step method was used to separate endopeptidases and

aminopeptidases active against a range of substrates in leaf extracts of plants grown in the field (FC). The influence of drought was determined for those of these activities which were confirmed as SPs, based on their inhibition by specific inhibitors. Under water deficit in plants grown under controlled conditions (CC) their levels changed in different ways. The levels of SP activities in FC plants, observed during a period of relative drought, were similar to those measured in mildly stressed CC plants. Our results point to a number of roles for different SPs in the plant response to water stress, which could range from enhanced protein turnover to limited proteolysis at specific sites.

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Effects of nitrogen levels on grain yield and grain growth in sensitive and tolerant wheat (*T. durum and T. aestivum*) genotypes under post-anthesis heat stress conditions

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ABSTRACT: In order to study the effects of post-anthesis heat stress and nitrogen levels on grain yield and yield components of wheat genotypes, two separate field experiments were conducted in delayed and optimum sowing dates under Ahvaz conditions (2006-2007). The Ahvaz site is located in the south west of Iran (32˚20' N, 40˚20' E) with subtropical climate condition. The experiment site had a moderate winter and dry and hot summer. Plants in delayed sowing dates experienced heat stress at post-anthesis growth stage. Each split- plot experiment had a randomized complete block design with three replications. The application rates of N at three levels (50, 100, and 150 $KgNha^{-1}$) were assigned in main-plots. Sub-plots consisted of six bread and durum wheat genotypes. The results indicated that the grain yield reduction in 50 and 100 $KgNha^{-1}$ compared with 150 $KgNha^{-1}$ treatment was 41% and 21% under optimum and 44% and 26% under heat stress conditions, respectively. In all genotypes, grain yield and 1000-grain weight (TGW) reduction under post-anthesis heat stress conditions was 42% and 33%, respectively. The highest and the lowest grain yield reduction due to heat stress were observed in Star (39%) and Vee/Nac (27%) cultivars. The Grain yield reduction in low nitrogen level treatments and post-anthesis heat stress were due to significant reduction in number of grains.m^{-2} and TGW, respectively. In low nitrogen level treatments grain number per area was reduced due to a reduction in the number of fertile florets/spikelets, spikes.m^{-2}, and spikelets. $spike^{-1}$. Grain growth period average were 23 and 16 days under optimum and post-anthesis heat stress conditions, respectively. Heat stress after anthesis reduced the grain growth rate (12%) and grain growth period (30%) compared with optimum conditions. Further research is recommended for full understanding of the effects of heat stress and N deficiency on yield and yield components of recommended wheat genotypes under agroclimatic conditions of southern Iran such as Ahvaz.

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Study of drought stress on yield and yield components of twenty potato (*Solanum tuberosum*) genotypes

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ABSTRACT: Drought is one of the most important limiting factors for crop production in arid and semi-arid conditions.Twenty potato genotypes were evaluated on randomized block design with three replications under three irrigation regimes in the agricultural research center of Ardabil, Iran. In this experiment the yield, number of tuber per plant,weight of tuber per plant, number of tuber smaller than 35 mm in diameter, weight of tuber smaller than 35 mm in diameter, number of tuber 35 to 55 mm in diameter, weight of tuber 35 to 55 mm in diameter, number of tuber larger than 55 mm in diameter and weight of tuber larger than 55 mm in diameter were calculated. We used the F test and LSD test in analyzing of data and comparing the means of treatment, respectively. Results indicated that Vital and Kaizer genotypes have the most tolerance to drought stress. Results of principal components analysis (PCA) showed that Kaizer, Serenad and Vital genotypes under moderate stress condition and Vital and Kaizer under severe stress conditions, had better yield than other genotypes based on PCA1, PCA2 and PCA3. That water deficit not only reduced tuber yield of potato, but also affected adversely its marketability.

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Developing MAS for the major heritable component of the H3-based resistance to *Globodera pallida* pathotype Pa2/3 in potato and deployment in a commercial breeding programme

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ABSTRACT: The potato cyst nematode *Globodera pallida* pathotype Pa2/3 is one of the most significant soil-borne pests, and developing cultivars expressing high levels of resistance to this pathogen is a major goal of potato breeding programmes targeting the UK and Mediterranean growers market. One excellent source of resistance to this nematode species/pathotype is the polygenic H3 source of resistance originally derived from *Solanum tuberosum* ssp *andigena* accession CPC2802. This resistance source is actively being used in both the SCRI and Oak Park breeding programmes, and a large effect QTL, named Gpa4, has now been mapped to chromosome IV in advanced tetraploid breeding clones called 12601ab1 (from SCRI) and C1992/31 (from Oak Park), which have both been developed from CPC2802 by different routes. The QTL is in the duplex dosage state in 12601ab1, and the simplex dosage state in 1992/31. Developing diagnostic markers for the genes underlying the resistance phenotype is a high priority at both research centres. We have identified SNP markers from the Gpa4 interval by sequencing genetically-anchored BAC clones from this region, and testing these for their diagnostic value for the Gpa4 QTL in a panel of genotypes including both 12601ab1, 1992/31 and other genotypes which are either derived from CPC2802 or not. Based on these results, MAS for the Gpa4 QTL has now been incorporated as a routine activity in the Oak Park potato breeding programme with a focus on efficient deployment of the marker. Two strategies have been adopted; in the commercial variety breeding programme the marker is utilized in the second field generation on material which has been commercially selected visually in the previous generation, the aim being to identify resistant individuals for evaluation as varieties. In the parental breeding programme the marker is used to aid in the multiplexing of Gpa4 in new parents to fix the trait in our breeding populations.

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Genetic analysis of Phytophthora root rot race-specific resistance in chile pepper

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ABSTRACT: *Phytophthora capsici* L. causal agent of phytophthora blight is one of the most devastating pathogens attacking chile pepper (*Capsicum annuum* L.) plants. Production losses up to 100% have been reported in New Mexico (U.S.A.) due to phytophthora root rot, which is one of the most important disease syndromes affecting chile peppers (Sanogo and Carpenter, 2006). Many studies have tried to better understand phytophthora resistance, but the genetic behavior is not completely understood. In order to determine if phytophthora root rot resistance in chile pepper is controlled by multiple alleles at a few loci, or multiple genes at different loci, five Recombinant Inbred Lines (RILs) were evaluated. The resistant accession, 'Criollo de Morelos- 334,'which has always shown the highest level of resistance against phytophthora root rot (Oelke et al., 2003), and the susceptible cultivar, 'Early Jalapeno,' were hybridized to develop multiple RILs. After seven generations of selection using the single seed descent method, four RILs were selected based on their phenotypic response to inoculation by five *P. capsici* isolates. The RILs were hybridized to each other to obtain F_1 and F_2 populations. The F_2 populations were inoculated with single and a mixture of isolates of *P. capsici*. A Chi-square test compared the ratios of resistant to susceptible individuals (R:S) against theoretical mendelian segregation ratios. When the F_2 populations were inoculated with a single race, ratios (3R:1S) were obtained, showing the action of an independent single gene. When the F_2 populations were inoculated with a combination of two races, segregation ratios (15R:1S) were observed. The presence of susceptible individuals in the F_2 population strongly suggests that the resistance genes for the different *P. capsici* races are located at different loci (Boiteux, 1995). None of the F_2 populations evaluated in this study displayed allelism for phytophthora root rot resistance.

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Citrus tetraploid rootstocks are more tolerant to salt stress than diploid

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ABSTRACT: Salt stress has a dramatic impact on the citrus industry by decreasing the growth of trees and fruit yield. We studied at the physiological and molecular level diploid and tetraploid citrus rootstocks when confronted to salt stress. Those tetraploid plants originate from the chromosome duplication in nucellar cells (somatic cells) of the apomictic diploid parent. Diploid and autotetraploid of Poncirus trifoliate (Pomeroy *Poncirus trifoliata*) and Willow-Leaf mandarin (*Citrus deliciosa*) were studied. The allotetraploid somatic hybrid FLHORAG1 which was obtained by electrofusion between Willow-Leaf mandarin and Pomeroy *Poncirus* protoplasts was as well investigated. Poncirus trifoliate is known to be a salt sensitive rootstock. Willow leaf mandarin is not used as a rootstock but was supposed to have a greater property of salt tolerance, similar to other mandarins such as Cleopatra mandarin. The anatomy of the leaf of diploid and tetraploid plants was first investigated. The stomatal area of tetraploid is 1.6 fold higher than the one of diploid plants. Stomatal density was also different between diploid and tetraploid. A 50 mM sodium chloride (NaCl) stress was performed for 9 weeks. Salinity caused leaf injuries and leaf fall in diploid of Poncirus while the tetraploid plants were not damaged. Autotetraploids and the allotetraploid FLHORAG1 acted like salt tolerant when compared to diploid plants. Sodium and chloride accumulation were similar for both diploid and tetraploid genotypes suggesting that root exclusion and/or accumulation of toxic ions in vacuoles is not the only way for salt tolerance. In order to characterize the molecular determinants mediating the salt tolerance of polyploids we investigated the gene expression profiles by using cDNA-AFLP technique. Contrasted gene expression profiles were detected between diploids and tetraploids as well as between control and stressed plants. Transcript Derived Fragments (TDFs) from contrasted profiles pattern were sequenced. 14 genes involved in osmotic adjustment, defense and signal transduction were isolated. The expression of some of those genes such as genes coding for the choline monooxigenase enzyme and the Group 5 late embryogenesis abundant protein (LEA5) and genes involved in the Abscissic Acid biosynthesis pathway are actually monitored by using Real Time PCR.

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Aluminium tolerance genetics in wheat and barley and different facets to improve crop yield on acid soils

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ABSTRACT: The hazardous phytotoxic effect of aluminium (Al) ions in acid soils results in high reduction of cereal crop yield in many parts of the world. The risk is much higher in the densely populated areas of the world where marginal acidic regions should be brought under cultivation using Al tolerant genotypes. Our study focused on the dissection of quantitative trait loci (QTL) controlling Al tolerance in wheat and barley at seedling stage using a nutrient solution culture approach. Analysis of a set of 'Chinese Spring' (CS) / 'Synthetic 6x' single chromosome substitution lines revealed the effect of chromosome 3B and 4D substitutions on Al tolerance. Based on this knowledge, investigations were carried out in wheat using a doubled haploid population derived from 'CS'/ 'CS (Synthetic 3B)' in order to map QTL. As a result, a QTL with major effect explaining 49% of the phenotypic variation was tagged using microsatellite markers. This is a new Al tolerance QTL mapped in 'CS' (*QAlt*$_{CS}$*.ipk-3B*). Another major QTL on chromosome 4DL was identified and mapped using a set of wheat / *Ae. tauschii* introgression lines. This QTL had a major effect and accounted for 31% of the variation. Both QTLs have great potential for increasing the yield of wheat cultivars in highly Al toxic soils. The latter population was also used to map the QTLs for acid (proton) tolerance *per se* which allowed having a good comparison with Al tolerance genetics. Marker validation was performed for the major QTL on chromosome 4DL within a collection of hexaploid wheat accessions to study the utility of the markers for marker assisted selection (MAS). In barley, Al tolerance QTLs were mapped using Oregon Wolfe Barley mapping population with the aid of EST derived SSR markers. In conclusion, the Al tolerance QTLs detected in our studies will have a great impact for the development of Al tolerant cultivars in breeding programs. The information on the genetics of acid (proton) tolerance might contribute to increase the crop yield on acid soils but this needs an extensive investigation.

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Effect of drought stress and protective mechanism in French beans

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ABSTRACT: The responses of French bean varieties based on morphological and physiological changes during the drought stress were investigated in model trial and in phytochamber. A short period of mild drought stress during flowering had no significant effect on the chlorophyll and antioxidant (ACW= antioxidant capacity of water-soluble substances) quantities in the leaves, and thus on plant development. Plants grown for a lengthy period at a temperature of 30/15°C before and during flowering endeavoured to overcome the effects of drought stress by means of morphological changes, such as a reduction in leaf size and mass and an increase in carotene synthesis. Under these conditions there was a slight reduction in the ACW content of the leaves compared with control plants grown at 25/15°C. The results showed that chlorophyll ***b*** was more sensitive to drought stress during flowering (35/25°C) than chlorophyll ***a***. Differences in the adaptability of the varieties to drought stress could be detected as changes in the chlorophyll and carotene contents of the leaves. Further studies will be required to determine the role of antioxidant in the drought stress response of bean varieties and use for improvement of new varieties with drought tolerance.

Keywords: antioxidants, drought stress, French beans.

Introduction

The frequency of dry and marginal conditions due to global climatic changes is one of the most important environmental factors which influences on production of plants. In the course of their development bean plants are able to tolerate high temperature, but water is the major limiting factor for flower and seed formation. Plant water deficiency is indicated when the temperature of the foliage is higher than that of the air (Helyes et al., 2006). The differences in water deficiency tolerance observed between bean varieties could be attributed to differences in the activity of the SOD and catalase enzymes (Türkan, 2005). Numerous, as yet unidentified water-soluble compounds with an antioxidant effect also play a role in plant responses aimed at overcoming environmental stress factors. We showed the different reactions of French bean varieties based on morphological and physiological changes during the drought stress.

Material and methods

The adaptability of yellow podded French bean varieties related to each others was investigated in the model trial. The water supply of the varieties was measured by differences of weight. Another experiment carried out in phytochamber where the plants were grown under water deficiency and heat stresses during flowering in order to investigate the role of leaf pigments and antioxidants for defence of damages. For the first week the plants were kept at a day/night temperature of 25/15°C or at high temperature (30/15°C) with optimum water supplies. After this, some of the pots previously kept at 30/15°C were transferred to the control (25/15°C) chamber, a further group was exposed to mild drought stress at a temperature of 35/25°C, and the third remained at 30/15°C throughout the flowering period. An analysis was made of the chlorophyll and carotene contents of the leaves in mmol/g and the antioxidant capacity of water-soluble substances (ACW) in μm/mg.

Results and discussion

Under water stress conditions the growth of French bean has been slown up and the height of plants depending on varieties was lower of 13 and 25 percentage than irrigated ones. Under this condition the largest decreases were in the weight of root, stem, leaves and pods (Nemeskéri 1990, 2001). The decrease in specific leaf area (SLA) was larger in the varieties being sensitive to water stress under non-irrigated conditions than irrigated ones. Significant correlation was found between the root weight and pod weight in the varieties with tolerance (r=0.64) and with moderate tolerance to drought (r=0.48) and between the root weight and leaves weight (r=0.72 and r=0.48), respectively. These relationships showed that the root is an important regulator in the water circulation of pod and leaves weight under low water content of the soil. The correlations between leaves and pod weight suggested that other controlling mechanisms also contributed to defence of drought.

Table 1. Effects of drought stress on leaves of French beans during flowering.

Code	T* °C	Leaf Average weight (g)	Chlorophyll **a** mmol/g	Chlorophyll **b** mmol/g	Total chlorophyll mmol/g	Carotene mmol/g	ACW *μ*/mg
Co**	25/15	0.37 a	34.13 a	5.19 a	39.31 a	28.23 b	10.29 a
IA	25/15A	0.30 b	35.39 a	5.57 a	40.96 a	29.48 b	6.95 ab
IIA	30/15	0.15 d	35.20 a	5.89 a	41.09 a	38.70 a	7.26 ab
IB	35/25	0.19 cd	31.60 ab	4.57 b	36.18 ab	25.80 b	5.37 b

*Temperature **Control, 25/15°CA transferred from 30/15°C before flowering to the control temperature. Values in a column having different letters are significantly (P<0.05) different at level using Duncan's multiple range test

Table 2. Reactions of French bean varieties for drought stress during flowering.

Varieties		HUNGOLD	MAXIDOR	DEBRECENI SÁRGA
Leaf properties	Temperature °C			
Chlorophyll **a** mol/g	25/15 (Co)	29.22 ab	39.47 a	22.33 b
	25/15 A	31.85 b	23.20 b**	30.00 b
	30/15	41.22 ab	35.69 ab	16.66 c**
	35/25	27.50 ab	24.85 ab	25.55 bc
Chlorophyll **b** mol/g	25/15 (Co)	4.26 c	5.70 ab	3.20 c
	25/15 A	4.79 bc	4.90 bc	4.41 cb
	30/15	6.40 b**	5.62 ab	2.44 d**
	35/25	4.15 c	3.59 c**	3.81 cd
Carotene mmol/g	25/15 (Co)	23.85 cb	31.90 abc	19.70 c
	25/15 A	27.65 b	20.30 c	29.40 bc
	30/15	42.05 a**	42.55 a	22.30 c
	35/25	22.50 cb	21.80 c	22.70 cb
ACW microgram/mg	25/15 (Co)	20.65 a	11.95 b	18.60 a
	25/15 A	8.75 bc**	6.30 c**	10.44 b**
	30/15	10.55 b**	11.06 b	7.81 bc**
	35/25	9.90 b**	7.67 bc	6.76 b**
Leaf average weight (g)	25/15 (Co)	0.36 a	0.36 a	0.29 b
	25/15 A	0.32 a	0.30 a	0.21 bc**
	30/15	0.22 b**	0.20 bc**	0.16 d**
	35/25	0.12 cd**	0.15 c**	0.10 d**

Values in each row and column having different letters are significantly (P<0.05) different level using Duncan's multiple range test. Co= control, 25/15ºCA transferred from 30/15ºC before flowering to the control temperature
**Significantly different from the control at the P<0.05 level.

The average weight of leaves of plants grown in phytochamber at 30/15ºC (day/night) before flowering was remarkably smaller (26.3%) and the chlorophyll and carotene contents were significantly larger than the control plants (25/15ºC) while the level of water-soluble antioxidants (ACW) did not change. The carotene content of leaves in the plants grown at permanently 30/15ºC was significantly increased but decreased with additional increase of temperature. Drought stress (35/25 ºC day/night) resulted a significantly decrease in ACW levels of the leaves compared to the control plants (Table 1). No visible damages in the varieties under flowering if a short period of higher temperature (30/15ºC) before flowering was followed the decrease in temperature to 25/15ºC associated with optimal water supply.

The findings revealed that ***b*** component of the chlorophyll much more sensitive to drought stress (35/25ºC) under flowering than chlorophyll ***a.*** Under this condition a substantial decrease in chlorophyll ***b*** content compared to the control was only recorded for Maxidor variety (Table 2). The *Debreceni sárga* variety had the most sensitive response to a rise in temperature; at 30/15ºC there was a considerable reduction in the chlorophyll ***a*** component and ACW level in the leaves compare to the control. At 30/15ºC temperature combined with water deficiency the high carotene content and lower decrease in ACW level compared to the control in *Hungold* variety proved a better adaptability to drought than in *Debreceni sárga* variety (Table 2).

At a temperature of 30/15ºC, which often occurs under field conditions during flowering, differences in the adaptability of the varieties to drought stress could be detected as changes in the chlorophyll and carotene contents of the leaves. Further studies will be required to determine the role of antioxidants in the drought stress response of bean varieties and use for improvement of new varieties with drought tolerance.

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Resistance gene pyramiding in common wheat as a strategy to control rust diseases

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ABSTRACT: The rust fungi are obligate biotrophic pathogens that depend on living host tissue for their growth. The economic losses caused by rust diseases, the presence of a great number and variability of pathotypes and their wide distribution around the world has made rust fungi the most important wheat pathogens. Due to the limited effectiveness of physical, chemical and biological control methods, the use of genetic resistance is the best management strategy on a medium-long term control. In particular one strategy for increasing the durability of resistance is to pyramidate multiple resistance genes into a single wheat cultivar. Gene pyramiding strategy will force the pathogen to overcome all the resistance genes simultaneously in order to survive on the new genotype. In this work several leaf rust resistance genes (*Lr9*, *Lr10, Lr47* and the clusters *Lr24-Sr24* and *Lr37-Yr17-Sr38*) that have confirmed their efficacy in Italy over a long period, were incorporated in different pyramiding combinations into four susceptible high-quality common wheat cultivars (Bolero, Colfiorito, Bilancia and Spada). The backcross program was carried out by using as resistance donor parents five 'Thatcher' near-isogenic lines (NILs) each possessing one of the above mentioned *Lr* resistance gene. To facilitate gene pyramiding, molecular markers such as STS, CAPS and SCAR, co-inherited with individual resistance genes, were used to select the progenies of five cross combination: *Lr24-Sr24+Lr9, Lr24-Sr24+Lr10, Lr10+Lr47, Lr24-Sr24 +Lr47* and *Lr24-Sr24 +Lr37-Yr15-Sr38,* conferring resistance to *P. triticina* as well as to *P. striiformis* and *P. graminis*. Furthermore screening of *Lr24-Sr24+Lr9* cross combination was simplified and accelerated by the use of high-throughput multiplex PCR system that allows the simultaneous detection of both resistance genes. Reaction conditions, such as annealing temperature, concentrations of primers and type of polymerase were optimized to obtain a robust amplification and reproducible genotype analysis. In addition phytopathological analysis using specific rust pathotypes were performed in order to confirm the absence of suppressing or modifying effects due to the co-presence of the introgressed genes. In conclusion "pyramiding" of relevant resistant genes in an agronomically superior genotype offers real solutions for a longer period of protection and for a shorter breeding-time.

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Construction of a high-density genetic linkage map and detection of QTLs controlling chilling requirement in apricot (*Prunus armeniaca* L.)

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ABSTRACT: The ability of perennial fruit trees for seasonal cycling between vegetative growth, fruit production and dormancy is an adaptive feature highly dependent on environmental conditions. Apricot is a fruit tree particularly sensitive to variability in climatic conditions and is very difficult to introduce for commercial production in geographical regions with relatively hot winters. One of the main problems in such regions is the difficulty of the trees to break dormancy and initiate active growth. Albeit breeding attempts to manipulate chilling requirement (CR), few efforts has been made to identify genes or markers useful in molecular breeding of varieties adaptable to diverse climatic conditions without compromising desirable traits. We took advantage of the availability of apricot cultivars which were selected for low CR for dormancy-break and crossed them with apricot cultivars with much higher CR. The populations established were used for genetic mapping of genes controlling cold response. We report the development of a high-density genetic linkage map and detection of QTLs controlling CR using these populations. A two-way pseudo-testcross population was derived from a cross between two cultivars with high (Perfection) and low (A.1740) CR. High-density male and female parental maps were developed using 473 (18 SSR and 455 AFLP) and 539 (28 SSR and 510 AFLP) markers, spanning 537.4 cM and 402.3 cM with marker intervals of 0.87 cM and 1.01 cM, respectively. Use of a set of common SSR markers from the *Prunus* reference map facilitated alignment of the homoeologous linkage groups from our apricot maps. The two parental maps were highly syntenic as evidenced from alignment of corresponding linkage groups containing 183 common co-dominant AFLP and SSR markers. Phenotyping for CR was conducted on potted trees that were forced to bud-break with cold treatment for 100 to 900 chilling hours. QTL analysis using the two linkage maps with phenotypic trait data of dormancy bud-break resulted in seven QTLs on linkage groups (LG) 1, 2, 3, 5, 6, 7 and 8. The QTL on LG1 mapped to the identical region that harbors the evergrowing loci in Peach. A similar study on CR conducted in peach for a comparative and integrated approach to gene discovery also validated three of these QTLs (on LGs 1, 5 and 7). The scope of future works on positional cloning of the CR QTLs, mapping and cloning of QTLs for other agro-economic traits will be discussed.

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Analysis of gene expression during the fruit set of tomato

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ABSTRACT: The study of tomato fruit set and early fruit development is of up most importance due to their economic implications for crop production. Fruit development is also a process of interest for the study of plant development regulation, and the tomato has been used as a model plant for climacteric fruit development. Fruit development in tomato is divided in four phases: fruit set, cell division stage, cell expansion stage and ripening. In tomato, the fruit set can be defined as the restarting of cell division in the ovary after pollination or fecundation. The development of tomato fruit is independent of embryo development, and the linkage of these processes can be broken. Parthenocarpy, the production of fruits without seeds, is common in this species and can be caused by natural mutations, environmental factors or hormone treatments. In this work, we isolated more than four hundred genes that are differentially expressed during tomato fruit set by using two complementary approaches: suppressive subtractive hybridization (SSH) and genomic comparative analysis. We also detected a high degree of conservation in the expression between *Arabidopsis* and tomato, in spite of the clear differences between the fruits of these species. This conservation between *Arabidopsis* and tomato proves that this orthologue approach can be useful in other biological processes or species, as it is a quick system for identifying candidate genes in species where there are no available microarray facilities. Analysis of the transcriptome of parthenocarpic and non-parthenocarpic tomato lines are in development to identify more pathways and genes implicated in the parthenocarpy in tomato.

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Harmonization of resistance tests to diseases of vegetable crops in EU

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ABSTRACT: Since the sixties, disease resistance has been considered as a character for distinction purposes and the representatives at UPOV (Union pour la Protection des Obtentions Végétales) have made efforts to achieve their recognition at an international level. Three national variety examination offices GEVES (Groupe d'Etude et de Contrôle des Variétés et des Semences) in France, INIA (Instituto Nacional de Investigacion y tecnologia Agraria y Alimentaria) in Spain and Naktuinbouw in The Netherlands proposed to evaluate and harmonize a set of vegetable disease resistance tests. This project of harmonization of resistance tests was co-funded by CPVO (Community Plant Variety Office). The program, realized in 3 years (2004-2006) was managed by GEVES. The aim of this work was to define resistant and susceptible controls, strains and notation scales to validate reliability (reproducibility and repeatability) of tests and to propose robust protocols usable with flexibility in different conditions. This harmonization of protocols will enable to reduce considerably the cost of setting up of experiment and the time spent on ineffective comparisons, to achieve a better characterisation of varieties in test, to obtain a better coherence of results between countries and between the breeder and the official testing offices. The exchanges of strains and their differential hosts will also be facilitated for further characterization and to provide a tool for surveillance of disease evolution in and between the countries. Chosen models are four pathogens of tomato (*Fusarium oxysporum* f. sp. *lycopersici* race 0 and 1, ToMV, *Verticilium dahliae*) and three pathogens of French bean (*Colletotrichum lindemuthianum*, Bean Common mosaic Virus, *Pseudomonas savastanoï* pv. *phaseolicola*). After a first phase of survey of the pathogens and protocols used within the three countries, two ring tests were realized in 2005 and 2006 (Ferreira *et al*., 1998; Taylor. *et al*, 1996; McKern *et al*., 1992) Ring tests were based on conditions of temperature for sowing and test, method of inoculation, concentration of inoculum, stage of inoculation and notation. The first ring test allowed characterizing and comparing the aggressiveness and the virulence of each strain versus standard varieties of each country. The second ring test analysed and compared the results, in relation with the CPVO protocols, in order to define reliable standardised procedures. Strains, resistant and susceptible controls, and notations scales were defined. Seven updated and robust protocols were written. Protocols enabled a range for environmental characters:

strains, resistant and susceptible controls and test conditions which allowed adapting the tests to the various constraints of different laboratories. The updated protocols (reference controls, reference strains and conditions of tests) were accepted by CPVO. The publication by CPVO is planned in spring 2008.

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Identification and selection for drought tolerance in alfalfa (*Medicago sativa* L.)

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ABSTRACT: Although drought reduces the herbage yield of all legumes, alfalfa has the greatest yield potential under drought (Peterson *et al.,* 1992). Progress made in breeding for drought tolerance at alfalfa may be difficult to evaluate when only biomass yield under field conditions is used for germplasm comparison. Wissuwa and Smith (1997) suggested that selection based on multiple traits may be necessary to obtain significant gains in drought tolerance in alfalfa. Patterns of response to hydric stress may be better distinguished when experiments are conducted in a greenhouse where level of drought can be easily controlled. Using this technique, several alfalfa (*Medicago sativa L.*) genotypes from two populations diverging for the multifoliolate character were evaluated. The experiment was carried out in vegetation house in Mitcherlich pots (27 kg capacity) which were filled with a mixture (3:1 of soil and sand). The water stress was imposed starting before flowering by restricting irrigation to 40% of water field capacity. Exposure of plants to drought led to noticeable decreases in shoot lenght, chlorophyll content, leaf water loss by cuticle, with a negative consequence on biomass accumulation. In addition, the drought - stressed plants were shorter and decreased shoots per plant and total plant weights than non - stressed plants. Genotypes reacted differently to water stress. For example, genotype nr. 21 which had the higher cuticular transpiration under no stress, had one of the lowest cuticular transpiration and a very good yield under water stress. On the other hand the genotypes nr. 23, one of the most drought susceptible genotypes concerning fresh matter production, had the highest water loss through cuticle, in both growth conditions. There were significant negative correlations between water loss by cuticular transpiration and biomass both under water stress conditions (r = -0.39*), suggesting that low cuticular water loss could be used in selecting drought tolerant cultivars.

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Genetics of the resistance to CVYV in cucumber

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ABSTRACT: Cucumber vein yellowing virus (CVYV) is one of the most severe viruses that affect cucumber. CVYV is transmitted by the whitefly *Bemisia tabaci* Genn. The virus affects mainly Middle East crops, and recently it has been detected in Spain and Portugal. Control measures are not efficient and the development of resistant varieties is the most promising method of control of this viral disease. Detection methods that are used for resistance screenings are based on symptom evaluation and hybridization techniques with RNA or DNA probes (Martínez-García et al., 2004). Recently, a detection method based in real-time RT-PCR has been developed that allows an early detection and an accurate quantification of viral accumulation (Picó et al., 2005). This method has been used to evaluate resistance found in a Spanish landrace of short cucumber (C.sat-10) that shows a nearly total attenuation of symptoms and a significantly reduced viral accumulation (12 fold reduction compared with susceptible varieties, Picó et al., 2003). In this paper, the response of a segregant F_2 population derived from the cross between the resistant accession C.sat-10 and a susceptible accession (C.sat-22) is evaluated. Disease severity in each plant is assessed through symptom evaluation and viral accumulation determinate by real-time RT-PCR. The combined use of both methods allows a more accurate phenotyping of the response to CVYV. Most of the F_2 plants could be classified as susceptible (with severe symptoms and a high viral accumulation) or resistant (asymptomatic or with mild symptoms and low viral accumulation). The resistant to susceptible ratio at the F_2 fitted to a monogenic control with dominance of resistance, which makes easier the use of this accession in breeding programs.

Keywords: *Cucumis sativus*, monogenic, real-time RT-PCR, resistance.

Introduction

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae family and is grown in temperate, subtropical and tropical areas with more than 40 million tons and 2.5 millions of ha in 2006 (FAOSTAT, 2008). *Cucumber vein yellowing virus* (CVYV) is one of the main viruses that affect cucumber and is transmitted by the whitefly *Bemisia tabaci* Genn. CVYV affects cucumber, watermelon, zucchini and several wild species of the cucurbitaceae family (Jones, 2005). Most common symptoms in cucumber are vein yellowing and chlorosis (Cohen y Nitzany, 1960). Sudden wilt has been also related to this virus in Spain (Janssen y Cuadrado, 2001). CVYV was originally identified in Israel in the 50s (Cohen y Nitzany, 1960) and in the

year 2000 was detected in Almeria (Spain; Cuadrado et al., 2001). Control measures are not effective and the development of resistant varieties is the most promising method of control of this viral disease at medium and long term basis. In the Middle East some tolerant varieties have been used (Mansour y Khlaif, 1998). In Spain resistance was identified in an accession from Andalusia (C.sat-10) held at the Genebank of the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV) at the Universidad Politécnica de Valencia (UPV). It is a Spanish landrace of short cucumber that shows a nearly total attenuation of symptoms and a 12-fold reduction of viral accumulation compared with susceptible varieties (Picó et al., 2003). Additionally to symptom scorings, serological methods, molecular hybridization and conventional PCR are commonly used to detect CVYV and to evaluate disease severity (Rubio et al., 2003; Martínez-García et al., 2004). These methods can show cross hybridization and they do not allow an accurate quantification of the viral content in the sample. Real-time PCR is a method that allows detection and quantification of a chosen fragment by monitoring PCR product accumulation during cycling as indicated by increasing fluorescence. This technique has been used to quantify several vegetable viruses (Beuve et al., 2007) and has been applied in the screening of resistance sources against CVYV (Picó et al., 2005) and also for the detection of CVYV in the insect vector (Gil-Salas et al., 2007). Real-time RT-PCR has many advantages like easy automation, simple interpretation, accuracy and reliability. In this work, a segregant population derived from a cross between a highly resistant accession and a susceptible accession of Spanish cucumber has been accurately phenotyped by combining symptom development and real-time RT-PCR.

Material and Methods

The highly susceptible cucumber landrace C.sat-22 and the resistant landrace C.sat-10 and their progeny (F_1 and F_2 generations) were used. C.sat-22 and C.sat-10 where characterized previously (Picó et al., 2003). A population of 72 plants from the F_2 generation where evaluated against virus infection. Control plants of C.sat-22, C.sat-10, and the F_1 generation were included. Plants were grown in a climatic chamber with controlled light and temperature. Mechanical inoculation was used with an isolate from Almeria (Spain) following the procedure described in Picó et al. (2003). This isolate has been characterized molecularly and has a 95% homology with the isolate of CVYV from Israel (Picó et al., 2003).

Visual evaluation of symptoms was done using a scale from 0 (no symptoms) to 4 (very severe symptoms) as described in Pico et al. (2003) at 9, 11, 16, 21, and 23 days after inoculation (DAI). Ten days after inoculation samples were taken, RNA was extracted and Real-time RT-PCR was conducted using the method described by Picó et al. (2005). Threshold cycles (Ct) and viral accumulation (VA, cDNA molecules (10^3)/ total RNA ng) was determinate for each plant. F_2 plants were classified according to principal component analysis (PCA) and logistic regression of symptoms and VA data. χ^2 analysis was used to contrast the genetic model.

Results and Discussion

Plants of the susceptible landrace C.sat-22 showed severe CVYV symptoms (score 4) from 16 DAI, and accumulated viral amounts from 28.000 to 60.000 cDNA molecules per ng of total RNA in all cases (Figure nº 1). The resistant landrace C.sat-10 showed no or mild symptoms of infection (only in some cases reached score 1 with a mild mosaic that disappeared later) and a viral accumulation from 4.000 to 18.000 cDNA molecules per ng of total RNA. F_1 plants behaved similarly to C.sat 10 like previously described (Picó et al., 2003). F_2 plants showed a variable response with plants without symptoms and others with clear symptoms of CVYV infection. The average viral accumulation in F_2 plants classified on the basis of symptom scoring at 11 DAI is shown in Figure nº 1. F_2 plants with severe symptoms (scores of 4) showed viral accumulation not significantly different to that found in the susceptible parental and F_2 plants with scores from 0 to 3 displayed an average viral accumulation similar to that of the resistant parent and F_1.

To better analyze the main components of the variation a Principal components analysis (PCA) was performed including all the symptoms data and the viral accumulation.

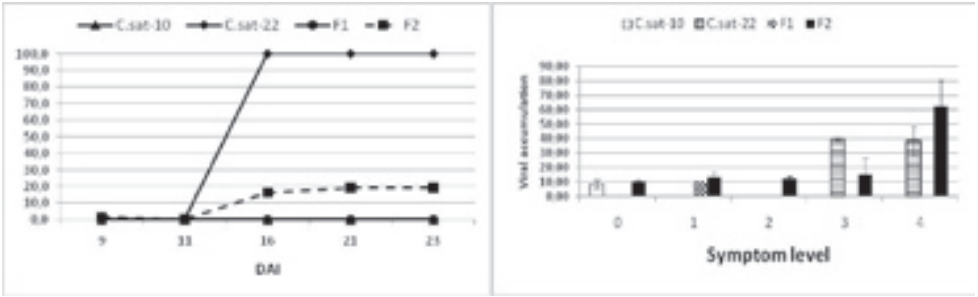

Figure 1. Percentage of plants with CVYV symptoms higher than 2 (left), and average viral accumulation (cDNA molecules (10^3)/ total RNA ng; average ± standard error) in the different generations tested (right).

The first component that explains a 70% of the variation separated those plants that reached severe symptom from 11DAI on from those that at this time remained with symptoms lower than 3, that is all the plants of the susceptible parent and those of the F_2 considered susceptible on the basis of symptom scoring (Figure nº 2). Although, on average all these F_2 plants showed high viral accumulation in 3 cases viral accumulation was lower than that of the resistant parent. This is probably due to a low quality of RNA extraction from very severe damaged plants or also to a reduction of viral accumulation at the end of the infection. In fact, the second component that explains a 15% of the variation mainly separates those plants with severe symptoms according to their viral accumulation. However, plants of the resistant parent, the F_1, and those F_2 with symptoms lower than 2 remain grouped according to the

second component, which indicate a higher uniformity in the viral accumulation in resistant plants, coherent with the better quality of RNA extraction in moderately affected plants. The third component (9% of the variation) separates 3 anomalous plants that showed an early increase on symptoms (along with a high viral accumulation), but a later recovery remaining with mild symptoms at the end of the assay. These plants that would have been classified as resistant on the basis of symptoms were not considered for the genetic studies.

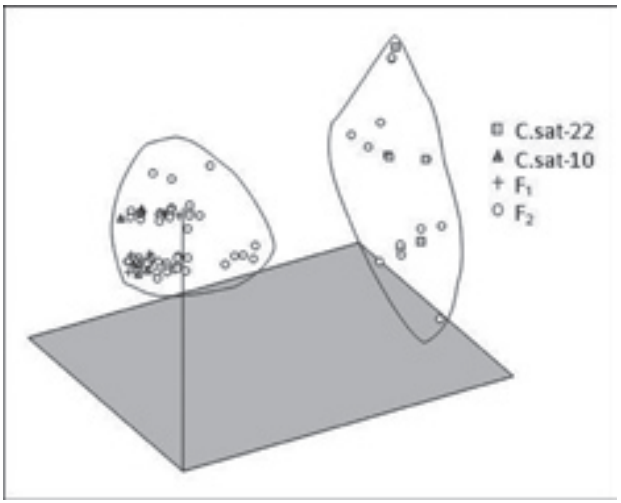

Figure 2. PCA plot indicating relationships among parental, F_1 and F_2 plants tested for resistance to CVYV according to their symptoms and viral accumulation evaluated by Real-Time RT-PCR. Resulting groups of similar samples are graphically circled in the figure.

Symptom and VA from parental and F_1 plants were used to elaborate a logistic regression, suitable for dichotomy events (as it is resistant and susceptible plants), that allowed to make a model that explained a 99.6% of the variation presents and makes an equation to classify F_2 plants in susceptible and resistant categories. Results of both analyses allowed a confidential classification of most F_2 plants: 52 as resistant and 12 as susceptible. These results are adjusted to a monogenic and dominant control of resistance with a χ^2 test (P=0.24 with 1 df.).

In previous works, resistance of C.sat-10 has been described as dominant according to the evaluation of the F_1 generation by symptoms and no quantitative molecular hybridization (Picó et al., 2003). The results that are presented here show a monogenic and dominant control of the resistance. The amount of viral cDNA measured by Real-time RT-PCR is correlated with symptoms and allows a more reliable quantification of VA comparing with other methods (Picó et al., 2003). However, determination of VA should be used in combination with symptoms

scorings as virus titer can decrease in highly damaged plants and RT amplification can fail due to low quality RNA. In plants asymptomatic or with mild symptoms real time RT-PCR is useful to identify susceptible plants in which symptoms are masked, and to select those resistant plants with the lowest viral accumulation. This accurate selection is useful during resistance introgression and for the search of linked molecular markers.

The genetic analysis indicates that C.sat-10 can be easily used in breeding programs to get varieties with a high level of resistance to CVYV. Moreover, this landrace is a Spanish type adapted to Mediterranean conditions and a short breeding period will be needed.

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Structural changes of the photosynthetic apparatus, morphological and cultivation responses in different wheat genotypes under drought stress condition

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ABSTRACT: This project was carried out with 64 different wheat genotypes under both irrigated and drought stress conditions, using simple lattice experimental design with two replications in the research area of the Agricultural Research Station of Ardabil and in the research area and laboratory of the Genetic Resources Institute of Azerbaijan during two growning seasons from 2005 to 2007. Results showed that all characters studied of wheat genotypes had different responses under both irrigated and drought stress conditions. There were significantly differences ($p<0.01$) between studied wheat genotypes in all characters examined. Drought induced a reduction in all characters studied; however, the amount of reductions was different in the genotypes examined. For example highly drought tolerant wheat genotypes showed higher amount of leaf area index, amount of water per dry weight of leaf, and amount of water per wet weight of leaf than other genotypes studied. Drought reduced wheat grain yield by 61.9%, and stress intensity (SI) for wheat grain yield was 0.62. Wheat genotypes studied were classified by genotypic responses of wheat genotypes under both irrigated and drought stress conditions and on the basis of values of SSI and STI indices in three groups: tolerant, moderate tolerance and susceptible. Results of germination capability of wheat genotypes evaluated under 16 atmosphere drought stress of PEG and control (water) treatments showed the same results as field experiments on the basis of the classifications of wheat genotypes for three classes such as tolerant, moderate tolerance and susceptible. Results of changes of amounts of chlorophyll also showed significantly differences ($p<0.1$) between genotypes examined on the basis of amount of chl a, chl b, chl a+b and chl a/b under 20 atmosphere drought stress of PEG and control (water) treatments. Drought, on average, reduced amount of chl a, chl b and chl a+b, by 2.5, 6.6 and 2.5% respectively. However the amounts of reduction in chl a, chl b and chl a+b were different between genotypes studied, so that the amounts of chl a, chl b and chl a+b were increased in drought tolerant wheat genotypes. According to the depression degrees of chl a+b the classifications of wheat genotypes were also same as the classifications of field experiments and germination capability under 16 atmosphere drought stress of PEG and control (water) treatments. The amount of chlorophyll was positively correlated ($r = 0.36^{**}$) with grain yield and with leaf area index ($r = 0.15^{ns}$) of wheat genotypes. Drought reduced genotypic variation and heritability of wheat genotypes in most of examined characters. However, heritability of grain yield and leaf area index was increased in drought stress conditions. Finally according the studied characters the genotypes No. 6, 30, 37, 42, 43 and 44 were found as tolerant genotypes.

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Inheritance of water use-related traits in sugar beet

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ABSTRACT: Improved water use efficiency (WUE, the ratio of dry matter produced to water used) can potentially result in yield improvement in water-limited environments. Genetic variation in WUE can be exploited by carbon isotope discrimination (D) in C_3 species. In order to improve WUE and its associated traits, it is necessary to understand the genetic systems controlling the expression of these traits. A full diallel analysis carried out on sugar beet inbred lines selected from a previous field experiment revealed that D, WUE and specific leaf weight (SLW, the ratio of leaf dry weight to leaf area) had high narrow-sense heritability and were controlled largely by additive gene effects indicating that these traits can be improved by selection in early generations. In contrast, maternal effects had a large influence on phenotypic expressions of total dry matter yield, total water use, chlorophyll content and leaf area suggesting the important role of selection of female parent for improvement of these traits. The parental line R49 was found to be the best general combiner for all of the traits. Genetic variation in SLW was strongly associated with D (R^2 =0.49, $P < 0.01$). This implies that SLW could be used as an inexpensive alternative measure for D to assess genotypes during the early phases of breeding programs.

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Differential expression in pea (*Pisum sativum*) to frost tolerance

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ABSTRACT: Frost tolerance is one of the most studied stresses in plant breeding because its impact on crop yield and quality. Many studies reflect the multiple factors which are involved in the process. As example, at molecular level there have been described several genes, proteins (Iturriaga et al.,1994) and other molecules (Abromeit et al., 1992) which are activated or inactivated during acclimation process at low temperatures and should play important roles in frost tolerance for many crops. In this contribution, a preliminary assay for some different putative genes involved in pea acclimation is presented. We designed primers specific to gene sequences potentially related with acclimation: abscisic acid (ABA-1 and ABA-2), chaperonin and dehydrin genes (Roberton and Chandler, 1992). Four pea lines with different response to cold temperatures were tested: Champagne and Melrose as tolerant; Medora and Terese as non-tolerant. The cold acclimation assay was carried out in environmentally controlled chambers, including replicates and controls. Tissue samplings from each line were taken at different times during a week of acclimation at 4ºC. After RNA extraction, ds-cDNA were synthesized and amplified by conventional PCR. The amplification products were sequenced and new primers were designed for Real-Time PCR assays. The results showed a different expression level for ABA-1 between tolerant and susceptible lines but no difference between treated and control lines was detected. For ABA-2 and the dehydrin gene a different level of expression was shown between treated and non treated lines but not between tolerant and non-tolerant lines. Also, some secondary amplification products were detected using conventional PCR, which should be caused by an alternative splicing or other post-transcriptional processes. This work is an initial study about the molecular processes which are involved in cold tolerance in pea.

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Genetic diversity and heritability of traits in durum wheat genotypes

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ABSTRACT: In order to study the genetic diversity of durum wheat with some morphological traits and also to determine the best genotypes, an experiment was conducted using a simple lattice design with 64 genotypes including 58 exotic and 6 locals' genotypes. This experiment was performed in Tabriz in 2004-2005. Results from combined analysis of data showed a high significant difference among genotypes for all traits, indicating that there is genetic diversity. Comparison of means showed that the genotypes number 62, 33, 55 and 64 had the highest seed yield, being genotypes number 62 and 64 local and genotypes 33 and 55 imported. Although there was no significant difference between the former four genotypes, it showed the yield potential of local genotypes. Estimations of heritability showed that broad sense heritability of traits such as number of seedling time, heading time and internodes length, spike length, plant height, number of kernel in spike and 1000 grain weight were higher than for other characters. Broad sense heritability of grain yield, biomass, harvest index, fertile tiller and leaf area was moderate. General heritability obtained for the traits was as expected, as this experiment was conducted for two consecutive years, and also the evaluation of genotypes was based on genetic variance.

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Identification of resistance genes against powdery mildew in wild barley PI284752 by DNA markers

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ABSTRACT: A newly detected accession of wild barley (*Hordeum vulgare* ssp. *spontaneum*) resistant to powdery mildew caused by *Blumeria graminis* f. sp. *hordei* was studied with the aim of finding the number and identity of genes conferring the resistance. Therefore, F_2 population derived from a cross between the winter variety 'Tiffany' and the wild barley accession PI284752 consisted of 449 plants was established. Use of DNA markers was focused on the identification of individual resistance genes by means of their chromosomal locations. The number of plants in the two phenotypic categories (427 resistant and 22 susceptible) found in F_2 population was compared with a theoretical Mendelian segregation ratio by a chi-square test and a two-locus model of resistance was shown by this genetic analysis (χ^2=1.37). In PI284752, dominant alleles of two independent genes were present; the allelism test indicated that one gene was located at the *Mla* locus. All parental plants of PI284752 showed the highest reaction type RT0; the F_1 generation with RTs ranging between 0 and 1-2 reflected that RT of the other gene was 1-2 and, in parental plants, was overridden by the effect of the first gene. The linkage between a microsatellite DNA marker and particular resistance gene was revealed by means of bulk segregant analysis (BSA) when resistance was dominant over susceptibility. *Bmac0213* on chromosome 1HS and *Bmag0134* on chromosome 2HS were indicated for linkage with the resistance genes. Linkage analysis with the polymorphic markers for which a linkage with individual resistance genes was traced by BSA was carried out with 112 F_2 plants. The *RGH1a* gene sequence from the *Mla* locus was used as source data for development of the cleaved amplified polymorphic sequence (CAPS) marker *RGH1aE2I2* after *Alu*I digestion. An expected position of one resistance gene of the cross 'Tiffany' x PI284752 was established by interval mapping on chromosome 1HS between the markers *Bmac0213* and *RGH1aE2I2*, 6 cM and 1 cM, respectively. The location of the other *R* gene was determined on chromosome 2HS in association with *Bmac0134*. The LRS score between the *RGH1aE2I2* marker and the resistance gene was 166.3. A high significance threshold of 15.7 was determined by a permutation test. Co-segregation between tightly linked markers and the powdery mildew resistance was analyzed by specific DNA fragments associated with each allele of both genes amplified in 112 F_2 plants. For the co-dominant CAPS marker *RGH1aE2I2*, 440 bp fragments were amplified from F_2 plants that exhibited the highest resistance reaction type 0 to powdery mildew; whereas, 395 bp fragments were amplified in susceptible plants. In F_2 plants with RT1 to RT2-3, the resistance was conferred by the *R* gene tightly linked with *Bmac0134* in homozygous or heterozygous constitution.

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Resistance of winter wheat to *Tilletia tritici* (DC.) Tul.: effect of alien translocations

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ABSTRACT: Alien translocations in wheat have been exhaustively investigated relative to various diseases, except for common bunt. During the period 2005-2006 an experiment was carried out at the Lithuanian Institute of Agriculture (LIA) in an artificially inoculated nursery. The test cultivars represent the latest Western European winter wheat genotypes. The total mean of winter wheat infection in the common bunt nursery was 80.9 % and the mean for the selected 36 cultivars was 76.2 %. The high infection level enabled us to screen cultivars for the level of partial resistance. Of the selected 36 cultivars with or without various alien translocations, only 'Quebon' was found to be resistant. The least infected cultivars 'Tommi' and 'Bill', can be classified as medium susceptible, 15.0 and 17.2 %, respectively. The rest of the cultivars were very susceptible, with the mean infection level ranging from 53.5 to 98.8 %. Cultivars with *Aegilops ventricosa* translocation were the least infected of the selected genotypes. The mean infection of the 8 cultivars was 74.9 % and the mean for the rest of the cultivars was 81.2 %. As a result, this translocation could additionally possess some barely effective genes of partial resistance. Complex translocation from *Secale cereale* did not possess effective partial resistance. The mean infection of the cultivars with other translocations was not really different from that of the selected cultivars. Cultivars with translocation from *A. tauschii* were infected more than the mean 87.4 %, whereas cultivars with translocation from *Triticum dicoccum* were infected less than the mean 75.4 %.

Keywords: alien translocations, winter wheat, resistance, common bunt.

Introduction

Alien translocations in wheat have been investigated exhaustively relative to various diseases, except for common bunt (Friebe et al., 2001). Most European winter wheat cultivars possess the translocations described below. Translocation from *Aegilops ventricosa* (*T2A/2AS-2M#1*) is one of the most successful in wheat breeding; it possesses a complex of resistance genes to three rusts and eyespot: *Lr37/Sr38/Yr17/Pch1* (Bartoš et al., 2004). Translocation from *Triticum timopheevii* (*T2B/2G#*1) is very common in the grown wheat cultivars, although resistance genes *Sr36/Pm6* are no longer effective (Bundessortenamt, 2005). Translocation from *Secale cereale* (*T1BL/1RS*) with resistance genes *Pm8/Sr31/Lr26/Yr9* additionally confers a better yielding capacity but lower grain quality (Villareal et al.,1991). Some powdery mildew resistance genes from alien derivatives were introduced alone with shorter

alien chromosome segments: *Pm2* (*5D*) from *A. tauschii*, *Pm4b* (*2AL*) from *T. carthlicum, Pm5a* (*7BL*) from *T. dicoccum* (Hsam and Zeller, 2002).

There is a lack of information about monogenes *(Bt)* or polygenes conferring resistance to this pathogen in mentioned translocations. Considering the situation when genes of partial resistance to common bunt are investigated very poorly (Cao et al., 2001), there are possibilities to find some partial resistance in cultivars with the above mentioned translocations.

Material and Methods

During the period 2005-2006 experiments were carried out at the LIA in an artificially inoculated nursery. The material subjected to bunt resistance tests included cultivars of Western European origin. Inoculation was carried out by shaking seeds with teliospores (5 g spores/1000 g seed) in a flask for 5 min. In October, when the soil temperature had dropped below 7^0C, the inoculated seeds were sown 15 g per genotype per a 3- m- long row at a depth of 10 cm in four replications arranged in different parts of the field. The disease incidence was measured after harvesting 100 heads at medium milk development stage (BBCH 75) as the number of infected ears from the total ears harvested. The following scale was used to estimate varietal resistance: infected ears 0.0 = very resistant, 0.1-5.0 = resistant, 5.1-10.0 = moderately resistant, 10.1-30.0 = moderately susceptible, 30.1-50.0 = susceptible, 50.1-100.0 = very susceptible (Veisz et al., 2003; Bänziger et al., 2003). Statistical analysis consisted of calculation of the mean and its standard devastation (SD), means were compared with Duncan's Multiple Range test at the level of significance p = 0.01.

Table 1. Resistance of winter wheat cultivars with alien translocations to common bunt.

Cultivar	Source of known translocations[a]	Range of infection,%	Average of infection ±SD	Duncan, 01[b]
Quebon	*A. ventricosa, A. tauschii*	2,0-8,0	5,0 ± 2,6	A
Tommi	*A. ventricosa, T. timopheevii*	3,0-25,0	15,0 ± 9,6	A
Bill	*A. ventricosa, T. timopheevii T.carthlicum*	10,3-22,1	17,2 ± 2,5	a
Hermann	*A. ventricosa, T. timopheevii T. dicoccum*	28,0-92,0	53,5 ± 30,2	b
Briliant	*S. cereale*	41,0-93,5	62,1 ± 22,5	bc
Lars	Unknown[c]	49,1-91,0	63,8 ± 18,9	bc
Hattrick	*S. cereale*	53,0-80,8	65,2 ± 12,4	bc
Milvus	*A.ventricosa, T. carthlicum, T. dicoccum, T. timopheevii*	36,0-84,0	65,3 ± 21,0	bc
Privileg	*A.ventricosa, T. timopheevii T. dicoccum, T. carthlicum*	13,8-97,0	68,7 ± 37,4	b-d

Cultivar	Source of known translocations[a]	Range of infection,%	Average of infection ±SD	Duncan, 01[b]
Sobi	*A. ventricosa, A. tauschii, T. carthlicum*	47,0-94,0	69,3 ± 20,1	b-e
Toras	*A. tauschii*	52,0-93,7	70,3 ± 17,5	b-f
Altos	*T. dicoccum*	58,0-84,2	75,0 ± 11,7	b-h
Anthus	*T. timopheevii, T. dicoccum T.carthlicum,*	67,0-82,0	75,8 ± 6,8	b-h
Solitar	*T. timopheevii,*	68,5-82,2	75,9 ± 6,3	c-h
Cetus	*A. ventricosa, T. carthlicum,*	71,0-85,0	76,0 ± 6,2	c-h
Širvinta1	Unknown[c]	51,0-92,0	77,1 ± 19,5	c-h
Cardos	*A. ventricosa*	70,0-90,8	79,2 ± 9,2	c-h
Zentos	Unknown[c]	66,0-88,0	79,8 ± 9,7	c-h
Paroli	Unknown[c]	60,0-97,0	80,6 ± 15,4	c-h
Opus	Unknown[c]	74,0-94,0	83,0 ± 10,0	c-h
SW Topper	Unknown[c]	83,0-86,0	84,8 ± 1,3	c-h
SW Maxi	*T. dicoccum*	76,0-90,0	84,8 ± 6,2	c-h
Tiger	Unknown[c]	85,0-91,0	88,8 ± 2,6	d-h
Tuareg	*T. carthlicum,T. dicoccum, T. timopheevii*	89,0-93,0	91,0 ± 1,8	d-h
Campari	*S. cereale, A. tauschii,*	82,7-97,0	91,2 ± 6,4	d-h
Heroldo	*T. timopheevii*	90,0-95,0	93,0 ± 2,2	e-h
Champion	*S. cereale, A. tauschii, T. carthlicum,*	86,0-97,0	93,0 ± 4,8	e-h
Empire	*A. ventricosa, T. carthlicum, T. timopheevii,*	87,0-99,0	93,3 ± 5,3	f-h
Striker	*A.ventricosa, T. timopheevii*	88,0-97,0	93,5 ± 4,0	f-h
Olivin	*A. tauschii, T. dicoccum*	90,0-98,0	94,0 ± 3,4	f-h
Skater	*T. carthlicum*	91,0-97,0	94,5 ± 2,5	gh
Idol	*A. tauschii, T. dicoccum*	92,0-97,0	95,3 ± 2,2	gh
Marshal	*S. cereale*	90,0-98,0	95,8 ± 3,9	gh
Buteo	*T. timopheevii, T.carthlicum, T. dicoccum*	92,0-98,0	96,0 ± 2,7	gh
Grommit	Unknown[c]	93,0-100,0	97,0 ± 2,9	gh
Alitis	*A. tauschii*	98,0-100,0	98,8 ± 1,0	h

[a] Information about alien translocations taken from the literature.
[b] Means followed by the same letters do not differ at 1 % of significance.
[c] Unknown – possible without translocations.

Results and Discussion

The total mean of winter wheat infection in the common bunt nursery was 80.9 % and the mean for the selected 36 cultivars was 76.2 % (Table 1). This shows that during the experimental period infection was exceptionally high, which is important for testing effectiveness (Liatukas and Ruzgas, 2006). Of the 36 selected cultivars, with or without alien translocations, only 'Quebon' was found to be resistant. The other two, the least infected cultivars 'Tommi' and 'Bill', can be classified as medium susceptible. All the other cultivars were very susceptible: mean infection ranged from 53.5 to 98.8 %. Resistance of the three cultivars mentioned could not be influenced by partial resistance at such a high level (Dumalasova and Bartoš, 2006). This was the effect of resistance to common bunt genes *Bt* (Veisz et al., 2003). We had no possibilities to identify *Bt* genes in these cultivars by comparison of their efficiency or pedigree due to very limited research on the identification of *Bt* genes in European wheat cultivars (Dumalasova and Bartoš, 2006; Bundessortenamt, 2005; Sortsforsøg, 2005). Cultivars with *Aegilops ventricosa* translocation were the least infected of the selected genotypes. Excluding the three most resistant cultivars with *Bt* genes, the mean infection of the rest of the 8 cultivars is 74.9 % and the mean of the selected cultivars is 81.2 %. These cultivars were slightly less infected than the rest, but their mean infection was similar to the total mean (81.2 %) of the 33 cultivars. Therefore, this translocation could additionally possess some barely effective genes of partial resistance. To validate this result we should test the cultivars for another 2 - 3 years and include more cultivars. But it is difficult to obtain a desirable infection level due to the limited possibility to control environmental conditions in the field. Therefore, successful screening of partial resistance to common bunt under field conditions is hardly possible, because it requires precise control of conditions.

The second most promising translocation is from *S. cereale*. It was present in 5 cultivars, and their mean infection was 81.5 %. This result shows that this complex translocation did not possess effective partial resistance genes. Analysis of possible effect of translocations was complicated due to their complex distribution in the cultivars tested. The number of known translocations fluctuated from possibly 0 to 4. The mean infection of cultivars with the other translocations was not really different from the mean of the 33 cultivars. The most similar to the total mean were cultivars with translocations from *T. carthlicum*, *T. timopheevii* and cultivars possibly without translocations, 82.3, 80.6 and 81.9 %, respectively. The cultivars with translocation from *A. tauschii* the rate of infection was higher that average- 87.4 %. Conversely, cultivars with translocation from *T. dicoccum* were infected less than average – 75.4 %. In the case of cultivars with translocation from *A. tauschii* it is clear that this translocation does not possess any effective partial resistance genes. It is likely that translocation from *T. dicoccum,* could induce some slight effect of partial resistance in the cultivars as in the case of translocation from *A. ventricosa*.

Summarizing the obtained effect of alien translocations on winter wheat resistance to common bunt, it is possible to conclude that the studied translocations practically did not contribute to partial resistance.

Accumulation of polygenes which are responsible for disease resistance in winter wheat, for example, to powdery mildew, has been lasting for about 50-60 years. Conversely, in the case of bunt, such accumulation has not been done because there was no need due to the perfect effect of chemical seed dressers. Therefore, detection of some partial resistance is a great achievement. There is some promising research on the other bunt species. Partial resistance was detected in the case of Karnal bunt. Several quantitative trait loci responsible for partial resistance have been identified recently (Singh et al., 2003). The situation could be the same with common bunt if research has been boosted.

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Combining ability and heterosis effects in *Camelina sativa* L. diallel crosses

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ABSTRACT: Gold of pleasure (*Camelina sativa* L.) is one of the oilseed crops cultivated in Europe since the Bronze Age and at present practically forgotten. *Camelina* is low input oilseed crop with potential for food and not food utilization. Gold of pleasure has a unique fatty acid profile with high linolenic acid content (30 – 40%) and eicosenic acid (around 15%). From breeding point of view, understanding genetic control of the desired traits like seed yield and oil contents is necessary along with factors influencing the magnitude of genetic variability associated with them. Objective of the present study was to determine some breeding characteristics (GCA, SCA, heterosis effects). Such information would help in planning comprehensive breeding program aimed to improve seed yield and oil content in *Camelina sativa* L. The plant material consisted of eight cultivars, originated from European collection of this species. These genotypes differing in different quantitative traits were selected from 29 genotypes on the basis of multivariate analysis of variance. The genotypes were crossed in a diallel fashion (type II, Griffing, 1956) to obtain seed of 28 hybrids. The experimental plant material was sown in the field. The seeds of the F_1 with parents were sown in two years experiment in completely randomized block design with three replications. *In vitro* experiment was also performed with parental genotypes and F_1 hybrids. Estimation of GCA, SCA and heterosis effects were calculated for 9 quantitative plant traits, fatty acid profile (6 traits) and 3 traits connected with in vitro culture. In both years of experiment the mean square due to GCA effects were significant for such traits, like number of branches per plant, 1000 seeds weight. The positive heterosis effects were significant in two years only for plant height. Effects GCA, SCA and heterosis depended on the year of experiment.

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Experiences with the use of MAS in practical breeding for FHB resistance

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ABSTRACT: Fusarium head blight (FHB) is one of the most important diseases of wheat nowadays. It causes severe reductions in yied and quality. Content of mycotoxins caused by the infection is hazardous for the health. Therefore there is a strong need for breeding and planting of resistant varieties. Evaluation of FHB resistance is a labourious and depepends on environment conditions, so marker assisted selection (MAS) could be a valuable tool for succesfull breeding. There is several scientific papers describing marker systems for selecting QTLs which should provide significant improvement in FHB resistance. We tested microsatellite marker xbarc133 (Liu et al., 2003). From our experiments we get following results: when a fragment of marker xbarc133 specific for allele of donor Sumai3 is present, there is improvement in symptomatic evaluation of resistance for one point in scale 1-9 (1-susceptible, 9-resistant) in average. That means improvement of disease symptoms for 10% in average. Much more important for breeders is that in population with fragment of marker xbarc133 specific for allele of donor Sumai3 is 40% of individuals with symptomatic evaluation of 7 point (scale 1-9) in average. In contrast in population without fragment of marker xbarc133 specific for allele of donor Sumai3 is only 15% of individuals with symptomatic evaluation of 7 point (scale 1-9) in average. That means very important enrichement of population by the high resistant individuals. To get more effective resistance to FHB pyramiding of more QTLs, eg. 5AS (Buerstmayr et al., 2004) should be effective. We can verify that MAS can be a valuable tool in breeding for FHB resistance.

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Architectural ideotype of pear seedlings in different F_1 hybrid combinations

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ABSTRACT: Low vigour of trees and growth habit remain important characteristics of interest for pear breeders. Spur or compact cultivars are desirable because their trees can be planted more densely, are easier to prune and thus easier to maintain. The segregation for trees habit of F1 pear hybrids belonging from six combinations was analysed. There were studied the seedlings originated in incomplete diallel cross, effectuated with four genitors (Comtesse de Paris, Milenium, Virgiliu Hibernal, Cluj 89-1-81 selection) by direct hybridization, without reciprocal and self-pollination. The seedlings were framed in six classes of growth habit (after UPOV Guidelines), respectively architectural ideotype: 1. Fastigiate; 2. Upright; 3. Semi-upright; 4. Spreading; 5. Drooping; 6. Weeping. The growth and tree habit appreciated by marks was considered as quantitative parameters and was computed by Griffing, respectively Masiukova model. The result for general and specific combining ability (GCA and SCA) suggests that the habit of pear tree is under additive genes control, and the cultivars with upright habit transmit this character genetically, and are expected to produce descendants with spur ideotypes. Based on the values of GCA and SCA, selection of genitors can be very efficient for desirable characters of growth and habit. Because their GCA values, cultivars like Milenium and Virgiliu Hibernal can transmit to its progenies upright, semi-upright (spur) or fastigiate habit.

Keywords: architectural tree, breeding, GCA, habit, hybrids, pear, SCA.

Introduction

Improved productivity and precocity, combined with resistance to critical diseases and tree size control, remained the goals of the pear-breeding program in all over the world. Low vigour of trees and growth habit remained important characteristics of interest to pear breeders. Spur or compact cultivars are desirable because their trees can be planted more densely, are easier to prune and thus easier to maintain (Faust and Zagaja, 1984).

Consequently, tree architecture (tree habit, or architectural ideotypes), is important in terms of production efficiency and pruning aspects, and suited to high-density plantings (Sestras, 2004; Sestras et al., 2007). Since the natural tree habit is under genetic control, and the new pear cultivars with desirable traits can be obtained by cross-pollination, it is

important to select suitable parents in a breeding programme (Ghidra et al., 1998). In addition, according to Gelvonauskis (1998) for apple, it is possible to evaluate combining ability of pear varieties for tree characters in the juvenile period and to use them in breeding for desire habits and other valuable characters.

Material and methods

In a selection field at Fruit Research Station Cluj-Napoca, Romania, the architectural ideotype of seedlings was analysed on F_1 hybrids originated in six crosses between following pear genitors: Comtesse de Paris, Milenium, Virgiliu Hibernal, Cluj 89-1-81. Among the genitors, Comtesse de Paris (with spreading habit) is one of the well-known winter variety cultivated and spread in Romania, Milenium and Virgiliu Hibernal (both upright or semi-upright) are new cultivars created at Fruit Research Station Cluj-Napoca, and Cluj 89-1-81 (spreading) is a selection obtained at FRS Cluj-Napoca, which is in homologation process.

The seedlings analyzed in experiment belonging from incomplete diallel cross, in which were made only direct hybridizations between the genitors, without self-pollination and reciprocal. The hybrids were framed in six classes of growth, respectively architectural ideotype: 1. Fastigiate; 2. Upright; 3. Semi-upright; 4. Spreading; 5. Drooping; 6. Weeping (UPOV Guidelines, 2000). Within each combination, the trait was analysed on 51 hybrids (Comptesse de Paris x Virgiliu Hibernal) to 167 hybrids (Milenium x Virgiliu Hibernal), on their own roots, in the two-four year of vegetation, each hybrid (seedling) being marked by scale from 1 to 6 in accordance with the tree phenotype illustrate in Figure 1.

1. Fastigiate; 2. Upright; 3. Semi-upright; 4. Spreading; 5. Drooping; 6. Weeping

Figure 1. Scale of rating F_1 pear hybrids according to their architectural ideotypes (after UPOV Guidelines, 2000).

Differences among observed hybrids, within each combination, were analysed using "t" test, while general (GCA) and specific (SCA) combining ability of genitors, for the trait, were computed using the mathematical model proposed by Griffing (1956) and Masiukova (1979).

Results and discussion

The average of marks for architectural ideotype of F_1 pear seedlings, computed as mean for each family represented by hybrid combination (Table 1), ranged between 3.00 in Milenium x Virgiliu Hibernal (both cultivars created at FRS Cluj-Napoca) and 3.50 in Comptesse de Paris x Milenium and Milenium x Cluj 89-1-81 combinations. Compared by the mean of experiment, considered as control (3.35), only Milenium x Virgiliu Hibernal combination, with the lowest value, were statistically different.

Table 1. Mean values of the marks for architectural ideotype (scale 1-6) in F_1 seedlings derived from six hybrid combinations, significance values of the Student's *t* statistic and coefficient of variability (*s*%).

Hybrid combination (♀ x ♂)	Mean of marks for ideotype±s_x	±d	*t* value	Signifi-cance	*s*%
1 Comptesse de Paris x Milenium	3.50 ± 0.11	0.15	1.2	-	17.8
2 Comptesse de Paris x Cluj 89-1-81	3.45 ± 0.16	0.11	0.6	-	15.1
3 Comptesse de Paris x Virgiliu Hibernal	3.33 ± 0.21	-0.01	-0.1	-	15.5
4 Milenium x Cluj 89-1-81	3.50 ± 0.15	0.15	0.9	-	19.7
5 Milenium x Virgiliu Hibernal	3.00 ± 0.12	-0.35	-2.6	oo	22.0
6 Cluj 89-1-81 x Virgiliu Hibernal	3.29 ± 0.18	-0.06	-0.3	-	14.9
Mean of experiment (Control)	3.35 ± 0.06	-	-	-	19.5

*, **, ***/o, oo, ooo Significant at P<0.05, 0.01 and 0.001 (positive, respectively negative).

Based on the values of variability coefficients (s%) of the trait, in the studied combinations, the architectural tree of pear seedlings seemed to present a medium variability. The extreme values of variability coefficients were registered in the combination Cluj 89-1-81 x Virgiliu Hibernal (with the lowest level of s% - 14.9), respectively Milenium x Virgiliu Hibernal (s%=22.0). These values of variability suggest that, in the studied descendences, hybrids with different habit of the tree are possible to be identified in each combination. Even that, as results from Figure 2, in all six combinations, there are no extreme ideotypes or are insignificant seedlings with fastigiate or weeping habit. In each combination, the majority of F1 hybrids had spreading habit. The occurrence of high percentages of spreading habit seedlings in all combinations suggests that this ideotype of tree growth is easy to obtain in pear breeding.

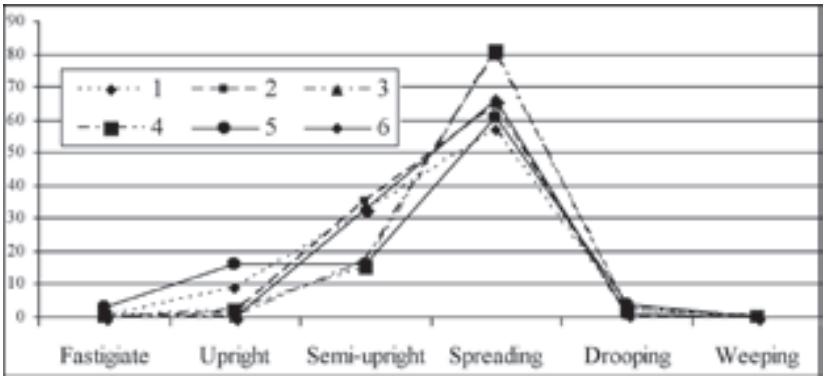

Figure 2. Percent of F_1 hybrids belonging to the six hybrid combinations, framed in six classes for architectural ideotype (1. Fastigiate; 2. Upright; 3. Semi-upright; 4. Spreading; 5. Drooping; 6. Weeping)

General combining ability (GCA) and specific combining ability (SCA) computed for the marks of architectural ideotype of seedlings and the results of genetic analyses of GCA and SCA are presented in table 2.

Values calculated for GCA and SCA in F_1 populations are higher than the theoretical ones for $P_{5\%}$ and $P_{1\%}$, which suggest that, for the genitors used in the analysed combinations, both effects of GCA and SCA are important in the genetic determinism of tree's habit in F_1 hybrids. In spite of that, in the experiment, the analysed trait on F_1 seedlings seemed to be stronger affected by the specific combinability of genitors (SCA effects).

Table 2. Analysis of variance for GCA and SCA of cultivars used as genitors, for architectural ideotype in F_1 pear hybrids, depending on parental combinations.

Source of variance	Sum of square (SS)	Degree of freedom (DF)	Variance (s^2)	"F" Value	
				"F" calculated	"F"(5; 1%) theoretic
GCA	0.1402	3	0.046718	13.583***	2.65; 3.88
SCA	67.2492	2	33.624580	9776.158***	3.04; 4.71
Error	0.3710	294	0.001262	-	-

The experimental results on SCA and GCA effects and the constancy of SCA, for the observed trait of seedlings in analysed combinations, are presented in Table 3.

Among the cultivars which had been used as genitors in the discussed diallel cross, Virgiliu Hibernal showed low and negative value for GCA with statistically assured difference

(very significant) which denote the cultivar inclination to transmit to its progenies upright or semi-upright (respectively spur) growth and habit. Because a valuable type of tree habit for modern plantation is with a spurred growth habit and strong apical control, it can be appreciated that Virgiliu Hibernal can be used as genitor for this trait.

In opposite with Virgiliu Hibernal, Comptesse de Paris and Cluj 89-1-81 selection have a GCA effects that determine a spreading or even drooping habit (both of them with positive and assured values of general combining ability in this direction). The highest percentage of upright and fastigiate habit of seedlings in Milenium and Virgiliu Hibernal crossing is righteous by specific combining ability of these cultivars, with negative and significant value.

Table 3. SCA and GCA effects and SCA constancy and their influence on trunk diameter in F1 pear hybrids, depending on parental combinations.

♀/♂	SCA effect				GCA effect	SCA constancy
	Comptesse de Paris	Milenium	Cluj 89-1-81	Virgiliu Hibernal		
Comptesse de Paris		0.0473	-0.1183	0.0711	0.1255^{***}	0.0089
Milenium			0.0711	-0.1183	-0.0184	0.0089
Cluj 89-1-81				0.0473	0.1017^{**}	0.0089
Virgiliu Hibernal					-0.2089^{ooo}	0.0089

DL for significance of GCA effect: DL 5% = 0.0707; DL 1% = 0.0930; DL 0.1%= 0.1192.
DL for significance of SCA effect: DL 5% = 0.1155; DL 1% = 0.1519; DL 0.1%= 0.1947.

The result suggests that the habit of pear tree is under additive gene control, and the upright varieties, with reduced-size tree, transmit this character genetically, and are expected to produce mainly descendants with this ideotype. Also, in concordance with this hypothesis, selection of parents on the basis of their own phenotypes will result in rapid genetic gains. The others cultivars used as genitor, as Comptesse de Paris and Cluj 89-1-81 selection showed positive and significant values for GCA, thus they can produce descendants with a spreading habit. By the value and positive GCA effect it can be considered that these two genotypes have an obvious tendency of producing descendants with a spreading or even drooping growth and habit.

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Molecular tagging of a novel powdery mildew resistance gene introgressed from *Triticum turgidum* var. *dicoccoides* in durum wheat

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ABSTRACT: Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici*, is one of the most important wheat disease in many regions of the world. *Triticum turgidum var. dicoccoides* (2n = 4x = AABB), the progenitor of cultivated wheats, shows particular promises as a donor of useful genetic variation for several traits, including disease resistances. The wild emmer accession MG29896, resistant to powdery mildew, was backcrossed to the susceptible durum wheat cultivar Latino, and a set of backcross inbred lines (BC_5F_5) was produced. Genetic analysis of F_3 populations from two resistant introgression lines (5BIL-29 x Latino and 5BIL-42 x Latino) indicated that the powdery mildew resistance is controlled by a single dominant gene. Molecular markers and the bulked segregant analysis were used to characterize and map the powdery mildew resistance. Five AFLP markers (*XP43M32*$_{(250)}$, *XP46M31*$_{(410)}$, *XP41M37*$_{(100)}$, *XP41M39*$_{(250)}$, *XP39M32*$_{(120)}$), three genomic SSR markers *(Xcfd07*, *Xwmc75, Xgwm408)* and one EST-derived SSR marker (BJ261635) were found to be linked to the resistance gene in 5BIL-29 and only the BJ261635 marker in 5BIL-42. By means of aneuploid and deletion lines, the polymorphic markers and the resistance gene were assigned to chromosome bin 5BL6-0.29-0.76. These results indicated that the two lines had the same resistance gene and that the introgressed *dicoccoides* chromosome segment was longer (35.5 cM) in 5BIL-29 than that introgressed in 5BIL-42 (less than 1.5 cM). As no powdery mildew resistance gene has been reported on chromosome arm 5BL, the novel resistance gene derived from var. *dicoccoides* was designated *Pm36*. The 244 bp allele of BJ261635 in 5BIL-42 can be used for marker-assisted selection during the wheat resistance breeding process for facilitating gene pyramiding.

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New sources of resistance to *Pepino mosaic virus* (PepMV) in tomato

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ABSTRACT: The disease caused by *Pepino mosaic virus* (PepMV) is currently a serious problem for tomato (*Solanum lycopersicum* L.) crops in several European countries. A collection of accessions from different *Solanum* species was screened in order to find sources of resistance to PepMV. All plants of *S. peruvianum* L. accessions showed a 100% systemic infection rate and apparent symptoms. Some accessions of this species showed low viral accumulation. In two accessions of *S. chilense* (Dunal) Reiche, a variable percentage of plants without systemic infection was observed. The best performance corresponds to *S. lycopersicoides* L. and *S. pseudocapsicum* L. Some accessions of *S. lycopersicoides* showed a variable percentage of plants with systemic infection. All the plants of six accessions of *S. lycopersicoides* kept free of the infection by PepMV. The only *S. pseudocapsicum* tested accession showed no infection in any of the inoculated plants. However, this species has a limited utility for tomato breeding, as it can not be sexually crossed with the cultivated tomato. The identification of resistant accessions in *S. lycopersicoides,* which has been crossed with cultivated tomato, reveals that this species is promising for breeding tomatoes for resistance to PepMV, and may allow the development of commercial varieties of tomato resistant to this virus.

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Vd3, a novel apple scab resistance gene

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ABSTRACT: Apple scab, caused by the fungal pathogen *Venturia inaequalis*, is one of the most devastating diseases for the apple growing in temperate zones with humid springs and summers. Breeding programs around the world have been able to identify several sources of resistance, being the *Vf* from *Malus floribunda* 821 the most frequently used. The appearance in several European countries of two new races of *V. inaequalis* (races 6 and 7) that are able to overcome the resistance of the *Vf* gene put in evidence the necessity of the combination of different resistance genes in the same genotype (pyramiding). Here we report the identification of a new apple scab resistance gene (*Vd3*) from the resistant selection 1980-015-25 of the apple breeding program at Plant Research International. This accession also contains the *Vf* gene. We mapped *Vd3*, using SSR and DArT^{TM} markers (Jaccoud et al., 2001) , on linkage group 1, at a distance of about 4 cM from *Vf* gene, but in repulsion phase to *Vf* . Based on pedigree analysis and resistance tests, it could be deduced that 1980-015-25 had inherited *Vd3* from the founder D3. This gene provides resistance to the highly virulent EU-NL-24 strain of the race 7 of V. inaequalis. This strain has overcome the resistance from both *Vf* and *Vg* (Parisi et al., 2004). However, *Vd3* has been not effective against the majority of other *V. inaequalis* strains we used in our disease tests.

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Environmental effect on common winter wheat productivity

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ABSTRACT: Twelve winter wheat varieties were tested in two locations during four successive years. The aim of the investigation was to determine the nature of the genotype x environment interaction (GEI) and to select the varieties with the most stable productivity. The varieties were grown by the Latin square method in 15 m^2 plots. At full maturity stage plants from each of the 5 replicates were selected on area of ¼ m^2 and the following characters were analyzed: grain yield per ha (GY), number of productive tillers per m^2 (NPT), weight of grain per spike (WGS), thousand kernel weight (TKW) and number of grains per spike (NGS). Variations in the stability of the varieties were registered at different levels of the characters' expression. A significant effect of the GE (year and/or location) was established on the expression of all characters, with the exception of thousand kernel weight. Significantly different among the individual varieties were the used stability parameters, b_i and W_i^2, which allowed specifically comparing them. After precise selection of contrasting conditions, it is difficult but altogether possible to identify genotypes with high production potential and high stability under various combinations of environmental conditions. The varieties Todora and Aglika were determined as relatively universal by productivity and stability in the north-east part of Bulgaria.

Keywords: components of productivity, GE interaction, grain yield, stability, winter wheat.

Introduction

Wheat is a major food crop for the greater part of the world. Wheat yields, in spite of the intensive breeding efforts during the last 50 years, are still insufficient in those places where there are conditions for growing of the crop which vary considerably by years. The stability of the used varieties is of high importance for the yield variations. This stability has been a problem widely researched and disputed in many breeding programs: Tsenov and Penchev (1998); Asif et al (2003); Goncharenko (2005), Aycicek and Yildirim (2006), Tarakanovas and Ruzgas (2006). The growth and development of the wheat crop sometimes changes so much under specific environmental combinations that modern varieties give lower yields, Martin (2004). Therefore the study of the behavior and response of each variety under environments as variable as possible is widely focused on grain yield, (Mohammadi and Amri 2008), grain quality (Williams et al, 2008), and resistance to biotic and abiotic stress (Snape et al, 2007). The researches on yield in this direction are rather concrete and the information reported is

specific. Therefore such investigations on winter wheat should be carried out under specific environments (Ferney et al 2006, Tsenov et al, 2006).

The aim of this study was to find out if there is a significant interaction between genotype and environment and to obtain data on the stability of a group of varieties widely used in wheat production.

Material and methods

Twenty wheat varieties were tested for four successive years during 2004-2007. They were preliminary selected and the group included varieties widely used in production, as well as several varieties approved during the recent years. They were all tested by the method of the Latin square in five replicates at two different locations: DAI, General Toshevo and Obraztsov Chiflik – Rousse. The soil and climatic conditions at these two locations were different with a view of wheat growing. The characters grain yield per ha (GY), number of productive tillers per m^2 (NPT), weight of grain per spike (WGS), 1000 kernel weight (TKW) and number of grains per spike (NGS) were analyzed by selecting plants on ¼ m^2 from each replication. The variance analysis by characters and the figures were worked out through the Stat graphics XV package, and the stability parameters were determined through the specific software GEST98 for analyzing the genotype x environment interaction. To analyze the effect of the environment on productivity and its components, the most informative parameters were selected for each variety from the group – regression coefficient (b_i) according to Finley-Wilkinson (1963), stability ecovalence (W_i), deviation from regression (s_d^2) and the interaction parameter SE, according to Muir et al (1992). The significant differences in the levels of the characters were determined using the descriptive statistics module of Xlstat 2006.

Results and Discussion

The variance analysis of all investigated characters revealed significant interaction between environment (year and location) and the group of varieties, as a factor (Table 1). The only exception in this respect was the character 1000 kernel weight, in which the effect of environment on the genotype was missing. Therefore it was excluded from the discussion and the investigation. In the character number of grains per spike there was only interaction with the year conditions, while locations had no effect on it. Significant heterogeneity of the genotype x environment interaction was observed in all investigated components of productivity, which was an indication of a linear type of response of the individual varieties. This, on its side, allowed analyzing the specific variations in the response of the investigated varieties.

These results contradict to a large extend the investigations of Anwar et al. (2007), who demonstrated under the conditions of Pakistan the presence of significant GEI interaction for the character 1000 kernel weight, while for GY and NPT the interaction was very low and insignificant. These results, however, are similar to and confirm the regularities in the components stability and productivity of the winter wheat type in Turkey, described by Aycicek and Yildirim (2006). Data on the values of parameters b_i, S_d^2 W_i^2 and GE are not

Table 1. Significance of the interaction variances in the productivity components.

Source of variation	Genotype	Environment	GEI [A]		Heterogeneity
			GLI [B]	GYI [C]	
GY	0.000*	0.000*	0.000*	0.000*	0.000*
NPT	0.000*	0.000*	0.000*	0.000*	0.000*
WGS	0.000*	0.000*	0.000*	0.000*	0.000*
TKW	0.000*	0.000*	0.785 ns	0.765 ns	0.000*
NGS	0.000*	0.000*	0.169 ns	0.000*	0.000*

A – (GEI) Genotype by Environment interaction; **B – (GLI)** - Genotype by Location interaction;
C – (GYI) - Genotype by Year interaction

Table 2. Variation in the stability of the varieties with different production potential.

Variety	Grain yield, t/ha		Number of tillers per m^2		Weight of grain per spike, g		Number of kernels per spike	
	b_i	W_1^2	b_i	W_1^2	b_i	W_1^2	b_i	W_1^2
Varieties with significantly high yield								
Todora	2	2	2	1	2	1	3	1
Kristal	3	1	2	3	3	1	2	1
Kristi	3	1	1	3	2	3	3	2
Zlatitsa	3	2	1	3	3	3	2	3
Aglika	2	3	3	2	2	3	2	3
Varieties with medium-high grain yield								
Enola	2	3	1	2	3	1	2	1
Pryaspa	2	3	1	3	1	2	2	3
Milena	2	3	3	1	1	3	3	2
Demetra	2	3	2	3	1	2	3	3
Varieties with significantly low grain yield								
Sadovo 1	2	3	3	1	3	3	2	3
Pobeda	1	3	2	1	3	2	2	1
Zlatina	1	2	2	3	2	3	2	3

presented in this paper due to its large amount. Instead, an attempt was made to express the response of each genotype by the significant variations of their values (table 2). The regression coefficient was used as a measure for the level of stability, and the ecovalence and the deviation from regression - for the variability of stability. To remove any doubt, the approach of Becker and Leon (1988) was used as a model, so the level of stability and the conditions under which it finds expression for each genotype were easily determined. At high stability (bi $\approx$ 1 and low values

of the parameters), the score was (1); at medium stability it was (2) and at low stability – (3). Analogous approach was applied to the absolute values of the characters, in the varieties. The analysis used as a model showed that all types of combinations between the character expression and its stability existed among the varieties selected in the study.

Since it would be very difficult or even practically impossible to identify varieties with high phenotypic expression of the yield components together with the yield itself, only grain yield and grain weigh per spike were chosen for further discussion. The reason for this is the presence of a strong positive correlation between them in this investigation, r=+0.78***. Generally, most valuable with regard to productivity were varieties Todora, Aglika and Pryaspa, which occupy about 25 % of the total wheat area in Bulgaria (table 3). Each of these varieties demonstrated sufficiently high stability in parallel with relatively high productivity. Besides, Varieties Todora and Aglika had stable yields under unfavorable environments (3). Variety Enola also demonstrated stability of both characters under both favorable (1) and unfavorable (3) environments. Variety Milena is worth mentioning too; it demonstrated high stability under unfavorable environments accompanied with medium-high productivity and therefore its share was 10 % of the total area sown with wheat for several years.

Table 3. Varieties with different combinations of stability, GY and GWS.

Variety	Production area of the variety , %	Stability	Level of character	Stable in environments
Todora	3	2*	1	1,3
Aglika	17	2	1-2	3
Pryaspa	5	1-2	1-2	1,3
Milena	10	1-2	2-3	3
Pobeda	2	1,3	2-3	1,3
Enola	22	2,3	1-2	1,3
	***1**	high	high	favorable
	2	medium	medium	all
	3	low	low	unfavorable

Although this investigation was done in two locations only, the behavior of the varieties under other environments in Bulgaria explained to some extend the high share each of these varieties has in production. Variety Pobeda, which is the national standard for high grain quality, showed, however, low stability in combination with low grain yield and as a result its area decreased from 15 to 2 % during the last years.

It may be finally concluded that the information obtained from this investigation is very valuable with regard to grain yield stability of the varieties under the conditions of North-east Bulgaria, where the grain produced annually amounts to about 35 % of the national produce. Besides giving stable yields, Aglika and Milena considerably exceeded the other varieties in the group by

grain and bread quality. In comparison to the standard Pobeda they are a considerable breeding achievement of the recent years with regard both to productivity and its stable expression.

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Attempts to improve the resistance of plant material by treatment with salicylic and *trans*-cinnamic acids using barley *tw* mutants as a model

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ABSTRACT: The barley mutants *tweaky spike* (*tw*) may be successfully used as a test system for evaluating chemical immunoresistance induction. This was shown employing salicylic and *trans*-cinnamic acids. The usefulness of *tw* type barley mutants for the evaluation of the chemical inducer of immunoresistance was especially clear if the frequency of mouldy germinating grains was tested. The effect of TCA was stronger and depended on the quality of seed material growing conditions.

Keywords: chemical immunoresistance evaluation system, salicylic acid, *trans*-cinnamic acid, barley mutants.

Introduction

Ecological agriculture, so emphasised recently, involves several serious problems. One of the most important is the increase of fungal and other infections in plant material if treatment with pesticides is not allowed. In ordert to solve the problem there are two ways. They are both important and may be employed together: creation of very resistant cultivars and chemical induction of immunoresistance by chemical substances which are natural for a plant. Among them, salicylic acid (SA) and its precursor *trans*-cinnamic acid (TCA) may be used (Dixon, 2001, Metraux, 2002, Rajjon et al., 2006). However, direct results with SA and TCA as immunity inducers are contradictory.

The barley mutants *tweaky spike* (*tw*, tw_1 - tw_{11}) are intriguing not only because of their genetic instability and homeotic conversion of lodicules to sexual flower organs (preferably to stamens), but also because of their increased susceptibility to fungal infection, revealed by a higher number of plants affected by *Ustilago nuda*, *Claviceps purpurea* and an increased frequency of mouldy grains when they are planted in Petri dishes (Rančelis, 1993, Rančelis et al., 2004, Vaitkūnienė et al., 2006). The latter peculiarity of *tw* mutants may be used for creating a test system for the evaluation of the effects of SA, TCA and other chemical inducers of immunoresistance.

Materials and methods

All material tested in the work had been cultivated for many years without pesticides at the Botanical Garden of Vilnius University. As a *WT*, the barley cv. Auksiniai II was used, initially obtained from the Lithuanian Institute of Agriculture (Dotnuva). All *tw* mutants come from original sources, induced by chemical mutagens. SA and TCA treatment was combined: grains were soaked in 0; 0.05; 0.25; 0.5 and 1.0 mM solutions for 12 h, and in the field plants were grown without spraying or were sprayed once, twice or three times with the same 0.05 mM concentration of SA or TCA. Experiments were conducted in 2005 – 2007 thus using different seed material. The frequency of plants affected by *Ustilago nuda* (Jens.) Rostr., *Claviceps purpurea* (Fr.: Fr.) Tul., *Drechslera teres* (Sacc.) Shoem. (syn. *Helmintosporium teres* Sacc.), *Puccinia hordei* G.H. Otth, *Erysiphe graminis* D.C. ex Merat. (syn. *Blumeria graminis*) and the extent of leaf injurity by the test pathogens were evaluated in the field conditions by standard methods when plants reached wax ripeness. In each sample, 75 plants and 300 leaves were examined. For evaluation of mouldy germinating grains, all manipulations were made in sterile conditions, and the seed material germinated in Petri dishes in a thermostat at 24ºC in the dark. In each Petri dish, ten grains were placed. In total, 200–600 grains were examined. Methyljasmonate and benzoic acid were also preliminary tested, but the most effective substance was TCA. SA was used for comparison as the best known inducer. Among internal micromycetes, in the barley grains the highest frequency was determined for *Cochliobolus sativus* (*Bipolaris sorokiana*) (Vaitkūnienė et al., 2006). Therefore, a pure culture of *C. sativus* was isolated at the Institute of Botany by R. Mačkinaitė and the action of SA and TCA was determined on *C. sativus*. It was planted on the growth medium with sterilized flour made from grains of *WT* or *tw* mutants. The distribution of internal micromycetes in germinating grains was studied by isolation of a pure culture using the malt extract agar (MEA) medium with streptomycin (250 mg l^{-1}) added. Micromycetes were identified on the basis of morphological and cultural characteristics.

Results and discussion

Despite the fact that the results of SA and TCA action depend significantly on environmental conditions (equally on the year when seed material was grown, and on the air conditions in year of disease evaluation), different effects of SA and TCA on *WT* and *tw* were successfully shown by several tests used in the work.

So, a significant effect of SA and TCA was observed on the frequency of plants affected by *Claviceps purpurea* and *Ustilago nuda* in 2005 when conditions for their development were favourable (Table 1).

WT plants were resistant to *C. purpurea* and *U. nuda*, despite the fact that material has been grown for many years without fungicides, i.e. in conditions of ecological agriculture. The action of SA and TCA on the other fungal diseases was more contradictory, and plant spraying resulted in a more pronounced injury by *Erysiphe graminis*.

Table 1. Frequency of plants (%) affected by *Claviceps purpurea* and *Ustilago nuda* after treatment with SA or TCA.

Conditions of treatment	*WT*			*tw*		
	Number of tested plants	*C. purpurea*	*U. nuda*	Number of tested plants	*C. purpurea*	*U. nuda*
0	349	0	0	305	4.92±1.24	4.26±1.16
SA 1.0[a,1]	291	0	0	273	0.37±0.37	2.56±0.96
SA 1.0[b]	299	0	0	284	2.46±0.92	4.23±1.20
TCA 1.0[a]	285	0	0	236	2.12±0.94	2.97±1.11
TCA 1.0[b]	301	0.33±0.33	0	218	1.38±0.79	1.38±0.79

[1] Only seed treatment with concentration 1.0 mM SA or TCA is shown

[a] Only seed treatment

[b] Seed treatment with 1.0 mM SA or TCA and triplicate spraying with 0.05 mM of test substances

On the other hand, a competitive effect was observed between *E. graminis* and *Drechslera teres*: when one injury increased, the other injury decreased.

The dependence of SA and TCA effect on seed material quality used in the experiment is especially obvious if to compare results of SA or TCA action on seed material from two different years – 2004 and 2005 (Table 2). The year 2005 was more favourable for micromycetes, and the effect of SA and TCA was not so evident.

Table 2. Effect of salicylic (SA) and *trans*-cinnamic (TCA) acid on the frequency of mouldy germinating grains of *WT* and various *tw* alleles.

SA or TCA concentration, mM	Seed material used for treatment							
	2004				2005			
	WT	*tw*	tw_1	tw_2	*WT*	*tw*	tw_1	tw_2
0	8.0±1.9	16.5±2.6	15.0±2.5	18.5±2.8	38.5±3.5	42.5±3.5	41.0±3.5	43.0±3.5
SA 0.5	8.0±1.9	10.5±2.2	8.5±2.0	12.5±2.3	34.0±3.4	36.0±3.4	38.0±3.4	38.0±3.4
SA 1.0	7.0±1.8	10.5±2.2	10.5±2.2	10.0±2.1	30.5±3.3	36.5±3.4	37.6±3.4	39.5±3.5
TCA 0.5	6.0±1.7	11.0±2.2	11.0±2.2	7.5±1.9	31.5±3.3	36.5±3.4	32.0±3.3	37.0±3.4
TCA 1.0	5.0±1.5	9.5±2.1	7.5±1.9	10.0±2.1	26.5±3.2	30.0±3.3	33.5±3.3	33.0±3.4

These observations prompted us to analyse quality of seed material from plants of the previous generation treated with SA or TCA. The spectra of micromycetes in internal grain tissues were analysed, and in Table 3 we show only results for the micromycetes that were most frequent and most abundant. The most frequent was *Cochliobolus sativus*. Its frequency decreased only after treatment with SA and only if seed material from the *tw* mutant was used.

Table 3. Distribution of micromycetes (%) in internal grain tissues of barley *WT* and *tw* mutant after treatment with SA and TCA (treatment in 2005, evaluation in 2006).

Micromycetes	*WT*					*tw*				
	0	SA1	SA2	TCA1	TCA2	0	SA1	SA2	TCA1	TCA2
Alternaria alternata	20	20	26	29	27	13	14	17	15	13
Cochliobolus sativus	42	43	46	55	54	58	27	44	63	48
Fusarium equiseti	51	52	48	39	37	31	57	43	38	30
F. poae	4	7	7	3	2	11	18	15	4	12
Ulocladium oudemansii	9	5	15	6	7	10	2	3	9	10

Table 4. Growth intensity of *Cochliobolus sativus* on medium containing salicylic (SA) or *trans*-cinnamic (TCA) acids and flour from grains of *WT* or *tw* allelic mutants.

SA or TCA concentration		Size of colonies, mm			
		WT	*tw*	tw_1	tw_2
SA	0	87.6±0.4	77.2±0.4	75.4±0.4	78.4±0.8
	0.05	74.6±0.7	74.0±0.6	68.4±1.4	74.8±1.3
	0.25	73.0±1.0	72.2±0.7	65.4±0.4	69.5±1.4
	0.5	70.0±0.6	67.2±1.4	69.8±1.0	69.8±1.1
	1.0	73.8±0.9	71.2±1.4	69.4±0.6	65.2±1.7
TCA	0	87.6±0.4	77.2±0.4	75.4±0.4	78.4±0.8
	0.05	79.2±0.5	78.2±0.8	72.2±1.1	73.4±1.2
	0.25	65.2±1.4	67.6±0.4	68.0±1.2	67.0±3.6
	0.5	62.6±0.8	63.8±1.1	64.0±0.8	63.8±1.2
	1.0	60.8±0.8	60.2±0.2	58.2±0.2	61.8±0.9

In Table 3, only those micromycetes are shown from the 24 fixed in that seed material, which were distributed in the highest frequency. Results are from 100 grain analysis. 0 – without treatment; SA1 and TCA1 – seed treatment with 1mM solutions; SA2 and TCA2 – seed treatment with 1mM solutions and sprayed three times with 0.05 mM solutions of the test substances; SA – salicylic acid; TCA – *trans*-cinnamic acid

In our previous investigation (Vaitkūnienė et al., 2006) we observed a dependence of micromycete spectra on the genotype - *WT* or *tw* mutants. Curiously, but *tw* plants were more resitant to *C. sativus*. For more detail investigation of that phenomenon, action of SA and

TCA was tested on a pure culture of *C. sativus* with addition to the medium of flour made from grains of *WT* or different *tw* allelic mutants (Table 4).

SA and especially TCA depressed noticeably the growth of *C. sativus* colonies, but the effect of SA was slightly stronger if flour from *tw* mutants was added to the nutritive medium.

Conclusions

The results of our experiments confirmed our presumption about the usefulness of *tw* type barley mutants for the evaluation of chemical inducers of immunoresistance.

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Photoperiod and vernalization response of Castilla y León, Spain, landraces and implications for adaptation

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ABSTRACT: In Spain, the varietal structure of bread wheat (*Triticum aestivum* L.) has changed very slowly through time. During the first half of the 20th century the crop was based on local landraces that represented valuable sources of adaptive features, but these were replaced by varieties from modern breeding programs in the second half of the 20th century. The cycle of wheat is determined predominantly by three sets of genes (Worland et al., 1998). Vernalisation and photoperiod genes act in response to the environmental stimuli cold and day length, whilst earliness per se genes act independently of the environment. A large part of the adaptability depends on the variation in photoperiod and vernalization requirement. In this work, twenty seven Castilla y León (north-west Spain) landraces of bread wheat were evaluated in comparison with nine modern improved genotypes. The thirty six lines were evaluated for response to photoperiod and vernalization under four controlled environments in glasshouse to determine the adaptation strategy. Based on the main effects of vernalization and photoperiod, the varieties were grouped into four categories. Four genotypes, all of them modern improved French winter varieties, were highly sensitive to vernalization. The group characterised by absence of sensitivity to vernalization, spring material, is composed by two landraces and four improved material. Most of local cultivars, twenty four, display moderate vernalization response in long photoperiod conditions, lower than the high vernalization requirement of winter European varieties. Unvernalized plants of two old genotypes grow faster in short than in long photoperiod in a pattern similar to winter barleys with *ppd-H2* gene. Thus this collection of landraces could be a source of genes of adaptation to Spanish conditions under-utilised in modern breeding programs.

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Construction of a linkage map spanning the PPV resistance locus region in apricot and identification of first anchored BAC clones

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ABSTRACT: Sharka disease, caused by *Plum Pox Virus* (PPV), produces important economic losses in *Prunus* species such as peach, plum, apricot and cherry. Natural resistance was described in some apricot cultivars (*Prunus armeniaca* L.) from North American origin. Studies of segregation on different intraspecific apricot crosses suggested that PPV resistance is controlled by at least one major dominant locus on the upper part of linkage group 1 (LG1) (Lalli et al., 2008; Soriano et al., 2008). Using microsatellites already mapped in different *Prunus* species we have built a genetic map for an interval of 10 cM comprising this region in apricot. In addition, overgo probes designed from the SSRs are being used to screen an apricot Bacterial Artificial Chromosome (BAC) library. The positive clones identified will be used to construct a BAC contig spanning the apricot genomic region containing the PPV resistance locus using, as a reference, the peach physical map developed by Zhebentyayeva et al. (in press).

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Comparison of tritordeum yield and yield components with triticale and bread wheat under drought conditions in a Mediterranean environment

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ABSTRACT: Four lines of tritordeum were tested in rainfall field conditions in North-Eastern Spain, jointly with two lines of bread wheat and two lines of triticale. Triticale outyielded bread wheat, while tritordeum was the lowest yielding species. Differences between yield components of triticale and wheat were due to a superior number of grains per spike in triticale. Low yield of tritordeum could be explained by a low thousand kernel weight, caused by both a short duration and a low rate of grain filling. Under drought conditions, tritordeum behaves similarly to the first released triticales, and needs breeding improvements, especially in grain size.

Keywords: Breeding, crops, performance, stress.

Introduction

Hexaploid tritordeums are amphiploids derived from the cross between *Hordeum chilense* and durum wheat, with an $AABBH_{(ch)}H_{(ch)}$ genome. Tritordeums are characterized by higher yellow pigment content in their seeds than durum wheat introgressed from *H. chilense* (Atienza et al., 2007). Additionally, it shows a number of disease resistances (Rubiales et al., 1996), good breadmaking quality, and the ability to cross with other Triticeae, which makes this species interesting for breeding (Martin et al, 1999). Tritordeum is a species that needs to be tested under Mediterranean conditions in order to know its potentiality as a new crop. The objective of this study was to test the yield and yield components of tritordeum in contrast with bread wheat and hexaploid triticale, in a dry Mediterranean environment.

Material and methods

Four tritordeum lines (HTC621, HTC1674, THC1939 and HTC2078) were grown in a field experiment jointly with two triticale (Imperioso and Titania) and two bread wheat cultivars (Bancal and Califa Sur). The experiment consisted in 8-row plots of $6m^2$ arranged in a randomized complete block design with three replications, and was located at Gimenells, Lleida (North-eastern Spain), under rainfed conditions during the growing season 2006/2007. The experiment received 218 mm of rainfall during the growing season, mainly during spring.

At anthesis, 30 main spikes having sinchrony in their development were tagged, and five spikes were sampled weekly during grain filling. Six central grains on each sampled spike

were pulled, dried and weighted in order to adjust the logistic curve proposed by Darroch and Baker (1990), and calculate the duration and maximum rate of grain filling, hereafter referred to as "rate of grain filling".

Yield components were determined at maturity on each plot, by pulling the plants included in a 0.5 m-long sample of a central row. On the basis of this sample, the plants per m^2, spikes per plant, grains per spike and thousand kernel weight were determined for each plot. Grain yield per plot was determined by combine-harvesting and after cleaning the schaff in the laboratory.

Analyses of variance were performed and means were compared according to Duncan's Multiple Range test at $P<0.05$ by using the SAS system package (SAS Inst., 2000).

Results and discussion

Significant differences appeared for yield between species, but not within them. Triticale varieties reached the maximum grain yield values, followed by bread wheat genotypes, while tritordeum showed the lowest yields (figure 1).

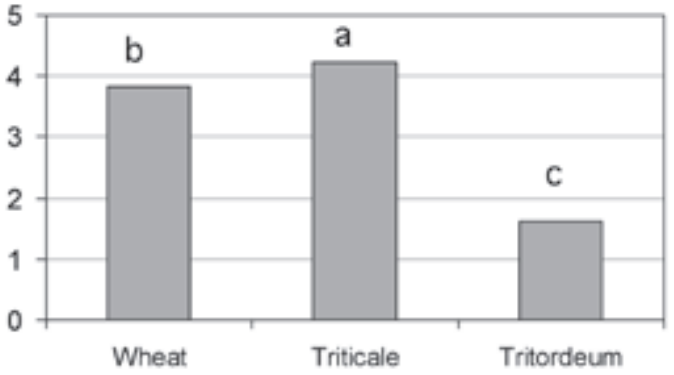

Figure 1. Mean grain yield values obtained for three cereal species grown in a field experiment in Gimenells (Lleida, Spain). Different letters indicate significant differences according to Duncan's criteria at $P<0.05$.

In order to determine the reasons of yield differences, yield components were studied (Table 1). Any difference was found for plant density, while the rest of the components sowed significant differences between species. The advantage of triticale over wheat was due to the number of grains per spike, as the rest of the yield components were not significantly different. Yield advantages of triticale over wheat have already been observed in drought-

prone environments of North Africa (Mergoum and Gómez-Macpherson, 2004). In other studies comparing wheat and triticale, yield components were found different while final grain yield was equivalent for both species (Gul et al., 2004). This indicates that, depending on the conditions, triticale may have or not an advantage over wheat.

Table 1. Yield components for three cereal species grown in a field experiment in Gimenells (Lleida, Spain).

Species	Plants/m^2	Spikes/plant	Grains/spike	Thousand kernel weight (g)
Wheat	304 a	1.55 b	32.7 b	34.9 a
Triticale	296 a	1.42 b	40.9 a	35.2 a
Tritordeum	249 a	1.96 a	34.9 b	21.3 b

Different letters indicate significant differences according to Duncan's test at $P<0.05$.

Tritordeum had significantly more spikes per plant, and a number of grains per spike similar to wheat but these traits could not compensate the yield loss caused by a low thousand kernel weight. Given that the rainfall occurred mainly in the spring, terminal drought is discarded to explain this low kernel size, so it has to be attributed to genotype sensitivity (Sharma and Anderson, 2004).

As thousand kernel weight is dependent on the duration and rate of the grain filling process (Darroch and Baker, 1990), these variables were evaluated in order to focus the main breeding efforts needed. In tritordeum, both the duration and rate of grain filling should be improved as shown in Table 2. In our experiment, anthesis date of tritordeum was about 8 days later than triticale or wheat. To increase grain filling duration, tritordeum lines should have an earlier anthesis date, in order to avoid limiting environmental conditions during grain filling. Grain filling rate is known to depend on the genotype, but is also affected by the environment (Santiveri et al., 2002), and therefore this trait should be improved by selection. It would be expected that with an adequate anthesis time, both the grain filling duration and rate would be improved as grain filling would take place in the optimum temperature conditions (Wiegand and Cuellar, 1981).

Table 2. Rate and duration of grain filling as percentage of triticale values.

Species	Rate	Duration
Wheat	104 a	92 a
Triticale	100 a	100 a
Tritordeum	77 b	78 b

Different letters indicate significant differences according to Duncan's test at $P<0.05$.

The low thousand kernel weight, jointly with advantageous traits of tritordeum, are similar characteristics of the first released cultivars of triticale (Oettler, 2005). Given that the current yield of triticale has been achieved after an important breeding effort, there are reasons to think that tritordeum could become a valuable alternative crop with high nutritive value and appropriate agronomic characteristics, if enough investment is made on it.

In the harsh environment of this experiment, triticale was the species with highest yield, with an advantage over wheat due to the number of grains per spike. Tritordeum lines used in this study should be improved on the thousand kernel weight in order to become a competitive crop in terms of yield under drought conditions.

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Breeding for increased cold hardiness in winter oilseed rape

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ABSTRACT: Breeding materials of *Brassica napus* L. var. *napus* from the Czech breeding companies, advanced doubled haploid lines and several commercial cultivars were evaluated for cold hardiness by field-laboratory method, where plants collected from field are exposed to frost using laboratory freezers. Results of field-laboratory tests were compared with the field winter survival after hard winter in 2002/2003. A high correlation was confirmed between the field-laboratory test and winter field survival in selected breeding materials and cultivars. It was concluded that some foreign cultivars originated from countries with mild winters had lower frost resistance, whereas some domestic perspective advanced breeding materials and doubled haploid lines showed satisfactory level of this character. Incorporation of field-laboratory tests of frost resistance into breeding programs is one of the most important steps for development of cultivars suitable for our specific climatic conditions.

Keywords: *Brassica napus;* breeding; field-laboratory test; frost resistance; oilseed rape.

Introduction

Oilseed rape (*Brassica napus* L.) is the most important oil crop in the Czech Republic with the majority of grown cultivars belonging to winter growth habit. The improvement of the yield as well as the quality of oil and meal are the most important objectives for rapeseed breeders. The breeding for increased tolerance to winter freezing injury in winter oilseed rape has high importance in Central European environmental conditions.

Winter hardiness represents a capability of oilseed rape to resist against all unfavourable conditions during winter. The main component, frost resistance presents resistance to temperatures below zero, and its level is influenced by several factors such as date of sowing, autumn agro technique, diseases and pests attack, type of soil and winter environmental stresses. Frost resistance also depends on the plant developmental stage and internal physiological factors associated with plant metabolism. This character is not stable but depends on the interaction of genotype and suitable induction factors. Thus, one of the important prerequisites for good over-wintering is the capability of the cultivar to harden by a gradual decrease of low temperatures up to the freezing point and to acquire a good frost resistance during the autumn. The capability of plant hardening may decrease in the course of faster growth of plants due to warm autumn or water logging of soil. The prerequisite of a good over-wintering is the development of a ground leaf rose with eight to

ten leaves, well developed root collar and roots. The evaluation of breeding materials in the field conditions is complicated by different course of winter weather in individual years. Therefore, field-laboratory tests and laboratory methods (Prášil *et al*. 2007) have been developed for more reliable selection of resistant genotypes.

The object of our work was to use the field-laboratory method for selection of cultivars and breeding materials of winter oilseed rape with increased frost resistance. For the stabilization of this character, doubled haploid lines have been produced to achieve homozygous initial breeding materials with high winter hardiness appropriate for breeding of new lines and hybrid cultivars.

Material and methods

Breeding materials of *Brassica napus* L. var. *napus* from the Czech breeding companies, advanced doubled haploid lines and several commercial cultivars were evaluated in the field and subjected to field-laboratory freezing tolerance tests under controlled conditions.

Field evaluation of wintering

Winter survival (in %) of breeding materials and cultivars was determined according to a methodology of the Central Institute for Supervising and Testing in Agriculture of the Czech Republic (CISTA).

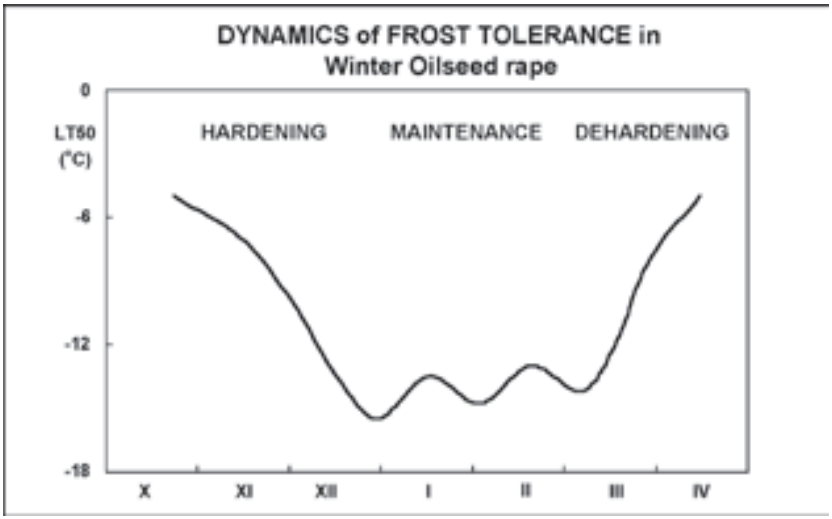

Figure 1. The dynamics of frost resistance (LT-50) of winter oilseed rape during the period from October to April – general scheme.
Abbreviation: LT 50 - temperature lethal to 50% of plants.

Frost tolerance field - laboratory tests performed in the Crop Research Institute in Prague-Ruzyně

The field-laboratory method, where plants collected from field are exposed to frost using laboratory freezers, was applied to test frost tolerance level of oilseed rape samples. The plants were collected at field locations of the Breeding Station Slapy near Tábor from December to January when hardening was supposed (Fig. 1). The plant material from each sample was divided into 5 bundles of 10 plants and then exposed in individual freezers to frost of five different intensities for a period of 24h. The rates of freezing and thawing were at 2°C/h. After thawing, three weeks of regeneration of the plants took place in a greenhouse at 20°C, followed by an evaluation of the percentage of survival at the individual frost temperatures (Fig. 2). The lethal temperature of each sample, LT50 (temperature at which 50% of plants are killed), was then calculated, based on the model of Janáček and Prášil (1991).

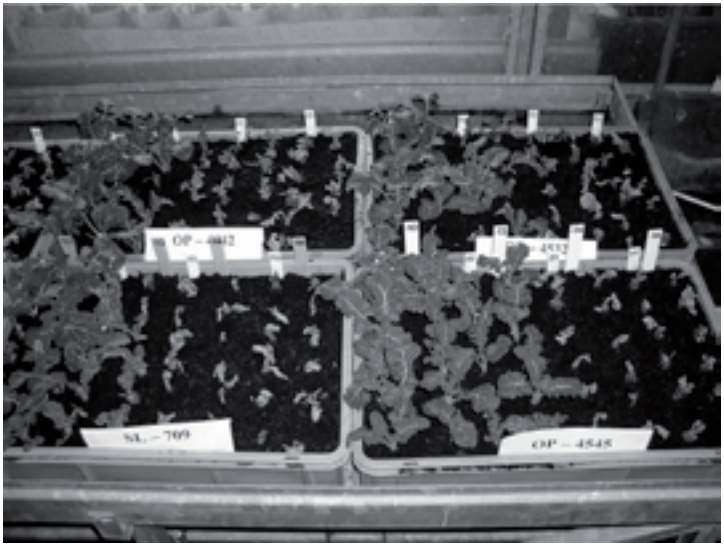

Figure 2. Regeneration of plants in greenhouse conditions after the exposure to a set of different intensities of frost in laboratory tests.

Results and discussion

Results of field-laboratory tests of frost tolerance were compared with the field winter survival after hard winter in 2002/2003. A high correlation was confirmed between the field-laboratory tests and field winter survival in selected breeding materials and cultivars (Table 1). Therefore, the initial breeding materials and selected registered cultivars are evaluated every year in the field for winter survival at nine locations in different parts of the Czech Republic and selected genotypes are subjected to field-laboratory tests. It was found out

that some foreign cultivars had rather lower frost resistance, for example LASER, whereas domestic perspective advanced breeding material OP - BN - 07 and doubled haploid line SL 629 - (DH) showed satisfactory level of this character. With regard to very variable weather conditions in the Czech Republic during autumn and the beginning of winter year by year, annual results of the frost resistance of specific genotypes could be different. Therefore, it is necessary to carry out evaluation of the frost resistance of perspective breeding materials and recommended cultivars repeatedly on several different locations. Consequently, the field – laboratory tests turn out to be more reliable than standard field evaluation.

Incorporation of field-laboratory tests of frost resistance into breeding programs is one of the most important steps for development of cultivars suitable for our climatic conditions.

Table 1. Result of frost resistance test in selected genotypes of winter oilseed rape in comparison to the percentage of winter survival in field conditions after the winter 2002/2003.

Genotype	Exposition temperature (°C)				Mean surviving (%)	Lethal temperature LT 50 [°C]	Significance*	Winter survival (%)
	-10.0	-10.6	-14.1	-15.4				
OP - BN - 07	100	100	50	0	63	-14.1	a	71
SL 629 - (DH)	100	100	40	0	60	-13.9	ab	
RASMUS	100	100	33	0	58	-13.6	abc	64
OPUS	100	100	30	0	58	-13.4	bcd	61
SL 620 - (DH)	100	100	30	0	58	-13.4	bcd	
SL 631 - (DH)	100	100	30	0	58	-13.4	bcd	
SL 630 - (DH)	90	78	50	10	57	-13.3	bcd	
SL 633 - (DH)	100	91	30	0	55	-13.1	cde	
SL 632 - (DH)	100	100	20	0	55	-13.0	cdef	
AVISO	100	90	20	0	53	-12.8	defghi	61
NAVAJO	100	70	30	10	53	-12.6	defgh	67
OPONENT	100	100	0	0	50	-12.4	efghijk	60
APLAUS	100	100	0	0	50	-12.4	efghijk	51
SL 628 - (DH)	89	80	10	0	45	-12.1	ghij	
SL 617- (DH)	75	70	0	0	36	-11.3	klm	
SL 627- (DH)	100	70	0	0	43	-10.8	lmn	
LASER	90	50	0	0	35	-10.6	n	48
SL 626 - DH)	70	30	0	0	25	-10.3	o	

Significance * = homogenous groups for LT 50

Correlation coefficient between field-laboratory test and winter survival in the field in eight genotypes, r = – 0.84476

Abbreviation: DH – doubled haploid, LT 50 - temperature lethal to 50% of plants

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The disease resistance potential of *Aegilops markgrafii* - from introgression to marker validation

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ABSTRACT: In the face of climate change the importance of genetic based resistance of plants against diseases becomes more clear. Higher temperatures and a shift of periods with rainfall are conducive to a successful pathogen development beyond chemical plant protection concepts. Wheat relatives like *Aegilops* species possesses valuable resistances against economically important diseases like powdery mildew, yellow rust and leaf rust. In several pre-breeding programmes introgression lines from a cross of the powdery mildew and leaf rust resistant *Ae. markgrafii* accession 'S740-69' and the susceptible wheat cultivar 'Alcedo' were developed. The introgression lines were either powdery mildew resistant or leaf rust resistant. Regarding to the leaf rust resistant introgression lines chromosome specific SSR markers were applied to detect the location of introgressed segments of *Ae. markgrafii* in the wheat background. In a further step one of the leaf rust resistant introgression lines was crossed with the susceptible cultivar 'Borenos'. The seedling test of the F_2 generation and subsequent linkage analysis indicated the presence of a QTL (*QLr.ipk-2A*) on the short arm of chromosome 2A. The same leaf rust resistant introgression line was used as parent for crosses with introgression lines mentioned above showing different expression of powdery mildew resistance targeted on the combination of both resistances. Seedlings tests of the F_2 generations were carried out on leaf samples with a set of isolates/ races which is officially used to tests new cultivars before release. Segregation analyses with respect to powdery mildew resistance resulted in one dominant gene and some minor factors for three of the families tested. In two families only one recessive gene and in one family two recessive genes were responsible for the resistance reaction. The leaf rust resistance was inherited by two dominant genes except for one family, which was characterised by two recessive genes. In parallel the five powdery mildew resistant introgression lines were crossed with the susceptible wheat cultivar 'Kanzler' to assess the number and location of the resistance genes. The seedling test was performed as described above. Segregation analyses for three F_2 families resulted in an inheritance of the resistance by one dominant gene and some minor factors. The remaining F_2 families contain two and three recessive genes, respectively.
The DNA analyses of the plant material are underway to identify segments of *Ae. markgrafii* in the wheat background. The aim is the validation of the leaf rust resistance QTL (*QLr.ipk-2A*) on chromosome 2AS and the localisation of the powdery mildew resistance genes.

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Mapping genes for ABA and GA synthesis and signaling in rye *(Secale cereale* L.)

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ABSTRACT: ABA and GA are the two phytohormones controlling seed ripening, dormancy, germination and plant growth. ABA and GA-regulated processes are of major importance for yield, quality traits and responsiveness to biotic and abiotic stresses of crop plants. Therefore knowledge on their genetic basis is crucial for breeders developing modern cultivars. One of the first basic steps in gaining knowledge on complex genetic regulatory networks governed by GA and ABA is mapping of individual genes related with these processes. Sequences of many of ABA and GA related genes are available from public databases of Arabidopsis, rice or barley. High level of homology between genetic sequences of plant species opens up possibility to study genes sequences, their polymorphism and chromosomal location in important crops. Rye is a bread cereal predisposed to light soils where wheat production is not efficient. Thus, agronomical, climatic and historical reasons stand beside rye production in Central and Eastern Europe. Rye genome is being studied mainly through development of genetic maps based on molecular markers. Next stage of rye genomic studies is concentrated on mapping genes and QTLs for important traits of agronomic value. This paper presents preliminary results on mapping ABA and GA related genes on rye chromosomes using genetic map of rye developed by means of RAPD, SSR, AFLP and STS molecular markers on the F_7 RILs derived from the 541×Ot1-3 cross. First polymorphisms of GA and ABA related genes within the mapping population were amplified by primers designed from sequences of *ABA2*, *ABF4* and *HvGAMyb* detected in Arabidopsis and barley, available from NCBI database. Primer pairs gave rye polymorphic products of 0.25 kb, 0.7 kb and 0.35 kb for *ABA2*, *ABF4* and *HvGAmyb,* respectively. They were mapped on short arm of chromosome 7R (*ABA2*) in the vicinity of the major QTL for preharvest sprouting and alpha-amylase activity, on the distal part of chromosome 4RL (*HvGAmyb*) and on the distal part of chromosome arm 1RL (*ABF4*) where QTL of medium value for alpha-amylase activity was located. It is supposed that genes for ABA and GA control mapped within or close to QTLs related to sprouting might be of value as candidate genes. Their polymorphism checked on recombinant inbred lines with contrasting PHS phenotypes suggest, that they are promising as molecular markers useful in modern breeding.

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Genetic improvement of nitrogen use efficiency in temperate forage grasses

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ABSTRACT: Breeding forage grasses capable of using nitrogen fertiliser inputs more efficiently offers a clean technology route to increased sustainability of livestock production, via lowering recommended fertilizer rates, reducing the agricultural footprint with respect to pollution and reducing the wider consumption of non-renewable resources. To identify and incorporate new genes associated with nitrogen use efficiency in forage grasses (*Lolium perenne*), we have designed a programme of research that makes use of genetic mapping and marker assisted selection approaches. A mapping population has been developed that segregates for nitrogen uptake and nitrogen utilisation efficiencies. By phenotyping this population under highly controlled optimal and limiting regimes of N supply in flowing solution culture, a number of genomic regions mapping to linkage groups 1, 2, 5 and 7 have been identified that segregates with nitrogen uptake and utilisation efficiencies. This population also segregates for a number of other desirable agronomic traits including flowering time, growth and regrowth after cutting, and forage quality traits such as water soluble carbohydrate content, dry matter digestibility, and concentration of poly unsaturated fatty acid (PUFA) in the forage. An additional advantage of this population stems from the fact it has been developed in the genetic background of an elite agronomic cultivar. The use of such a population not only assists in identifying genes for traits that are not present in elite current cultivars, but also provides a means of fast-tracking their incorporation into future varieties resulting form such gene pool. Mapping results of the traits studied using this population together with new methods and opportunities emerging from advances in genomics will be discussed in the context of improving the efficiency of selection and breeding of complex multiple traits in forage grasses.

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Cloning and characterization of two novel polyamine gene promoters from rice (*Oryza sativa*)

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ABSTRACT: Polyamines are ubiquitous molecules involved in a multitude of developmental, biochemical and physiological processes in plants. These include cell division and growth, morphogenesis and differentiation, tolerance to abiotic stresses, etc (Capell et al, 1998). Arginine decarboxylase (ADC) is the first enzyme in the polyamine biosynthetic pathway. It converts arginine to putrescine via two intermediate steps. For the past several years we have been investigating the mechanism through which polyamines exert their protective effect against abiotic stresses in plants. Our experiments allowed us to put forward a model which explains the role of individual polyamines in abiotic stress tolerance (Capell et al, 2004). In order to develop a more in depth mechanistic understanding of the protective role of polyamines in abiotic stress tolerance at the molecular level, recent studies in our group have focused on endogenous polyamine promoters and their regulation. In this context we report the cloning and characterization of two ADC promoter regions from rice (*Oryza sativa*) corresponding to two different genes, ADC1 and ADC2. Promoter-gusA fusions were introduced into rice by genetic transformation. Marker gene expression was evaluated in vegetative (leaves and roots), reproductive and storage tissues at the mRNA, protein and metabolite levels. Further genetic transformation with constructs encompassing deletion series for the two promoter regions fused to the GUS reporter gene allowed identification of putative TATA boxes prior to the transcriptional start site for both ADC gene promoters. Current experiments focus on the expression profiles of transgenic plants expressing ADC1-gusA and ADC2-gusA under abiotic stress and gaining insights into the transcriptional control of polyamine biosynthetic genes in plants under abiotic stress.

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Application of physiological methods for assessment of drought and high temperature tolerance of wheat (*Triticum aestivum* L.) genotypes

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ABSTRACT: Application of physiological traits in screening process could be helpful for expedition of empirical breeding (Reynolds et al., 2001). Identification of limiting factors can point out to traits useful as indirect selection criteria. For this purpose it is advantageous to identify differences between susceptible and tolerant genotypes using fast and reliable methods; such goals were specified also for our research work with genotypes of winter wheat. In three seasons, vegetation pot trials with seven genotypes of winter wheat (*Triticum aestivum* L.) of different provenance were performed. The water deficit was induced by withholding of irrigation after anthesis. In control and stressed plants several parameters of net photosynthesis, stomatal conductance, fast chlorophyll *a* fluorescence kinetics, leave water status, osmotic adjustment, growth, phenology and yield were determined. We analyzed also mutual relationships among parameters and their correlations with yield. Selected methods were applied also in larger collection of genotypes in field conditions. Drought stress influenced the grain yield of wheat cultivars in all observed years through reduction in number of kernels per spike and grain mass. The phenology was a trait well correlated with yield reduction caused by drought stress. The shift of the most sensitive phenophases of heading and anthesis had impact especially on number of kernels per spike. Stomatal closure was one of the most rapid reactions induced by water deficit. The varieties differed in sensitivity of stomatal closure, similar trend was observed in measurements of net assimilation rate, too. Delayed stomatal closure with higher values of net assimilation rate appeared as a positive property leading to higher performance in drought conditions. Low non-stressed values of stomatal conductance of observed varieties were a suitable character correlated with lover relative yield decrease during drought stress. This trait was associated with leaf transpiration efficiency (TE_L), what was confirmed by gasometry measurements. Observed varieties differed in capacity for osmotic adjustment. We identified groups of genotypes with high and low osmotic adjustment. Higher values of this trait are associated with delayed stomatal closure and higher values of net assimilation rate during drought stress. Fast chlorophyll *a* fluorescence kinetics measurements represented useful tool for monitoring of drought stress effects on photosynthesis (Zivcak et al., 2008). The most sensitive fluorescence parameter recording drought stress effects and genotypic differences in drought susceptibility was Performance Index (PI_{ABS}). The temperature treatment at 40°C allowed distinguishing genotypes upon their thermostability. Maximum quantum yield of photochemistry (Fv/Fm) was a suitable parameter for determination of heat stress effects and genotypic differences.

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Part 3

Breeding for Quality

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Breeding for quality in crop plants by using genetic and genomic tools

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ABSTRACT: In recent years the genetic characterization of quality traits has been taken up. The lack of knowledge about the number of genes involved and their chromosomal location has lead to the realization that careful phenotyping of these traits in well characterized plant material is essential when we want to start breeding for these traits in a directive fashion. What little information there is seems to hint at different types of control for quality traits (depending on the type of the trait) from single to multiple genes or Quantitative Trait Loci (QTL). For many quality traits it is further clear that metabolomics (both targeted and untargeted measurements) in combination with transcriptomics and knowledge of the allelic variation at a given locus is essential to unravel the most important controlling factors for such a trait. Because of the fact that for quality traits, unlike resistance genes against pathogens, no positive direct selection has occurred in the breeding of crops it might well be that the genetic variation in the used plant material is larger then we anticipate. We have been using a variety of combined techniques and approaches in crops such as potato, tomato, brassica, apple and rose to identify, locate, develop markers and in some cases clone the corresponding genes for quality traits. Techniques such as Bulked Segregant Analysis (BSA) in combination with transcript and metabolite profiling have led to the identification of genes conferring quality traits in potato tubers (flesh colour, texture after cooking, chip colour), whereas targeted metabolomics in combination with mapping of candidate genes has lead to the identification of genes important in the production of components beneficial for human health in *Brassica rapa*. In tomato this approach has lead to the identification of candidate genes which are important in taste attributes. The importance of having good characterized plant material (segregating populations, Near Isogenic Lines, Introgression Line libraries and varieties) is apparant from a large number of studies where for instance the natural metabolite variation in a segregating mapping population is combined with the genetic information of the population. We have extended the candidate gene approach, where not only the co-localisation of genes and QTL is considered, but also the co-variation of gene expression, proteins or metabolites in the same genetic material is taken into account. In this way the variation in elements of biosynthethic pathways is associated with variation in traits. Once the involvement of a gene and a trait value is firmly established, the next step is to identify all available alleles for that gene (for example by using single nucleotide polymorphisms or haplotyping). The effect of individual alleles as well as interaction between various alleles on the resulting trait value is not a trivial issue. Once the alleles that really matter can be identified, they can be used in breeding programmes. This approach has lead for instance in apple to identifying those alleles which contribute most to the (oral) allergy of apple. A number of examples will be discussed in more detail highlighting the indicated aspects.

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QTL mapping of alpha acid content and yield in hop

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ABSTRACT: Hop (*Humulus lupulus* L.) is an important ingredient in the beer-brewing process. Slovenia produces mainly traditional European aroma hops, with domestic hop varieties, covering approximately 3% of world hop production. The overall orientation in hop production is towards high alpha varieties (high alpha-acid content) following the demands of the brewing industry. Hop breeding is a lengthy process due to the dioecious nature of hop producing highly heterozygous offspring and only female plants are of commercial interest. Molecular approaches have therefore been developed to support conventional hop breeding programmes.

We studied the effects of quantitative trait loci (QTLs) and determined map locations for alpha-acid content and yield in hop, using amplified fragment length polymorphism (AFLP) and microsatellite markers (SSRs). Genetic linkage maps were constructed from a mapping population consisting of 111 progeny from a double pseudo-testcross. A total of 194 markers (150 AFLPs, 43 SSRs, 1 hypothetical sex marker) were located on the 20 linkage groups (LGs) of the maternal and paternal maps, covering total map lengths of 706 and 616 cM, respectively. Due to the presence of 16 common biparental SSR markers, homology of seven LGs between parental maps could be inferred. The progeny segregated quantitatively for alpha-acid content and yield determined in the years from 2002 - 2006. Thirteen putative QTLs for alpha acid content, 13 for dry cone weight and 18 for harvest index were identified on the two maps. Possible homologies between QTLs for alpha-acid content, dry cone weight and harvest index were detected in different years, as well as in the both maps in at least three chromosomal regions. The results of the study provide a practical tool for the development of a marker-assisted selection programme in hop.

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Genetics of dietary fibre in bread wheat

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ABSTRACT: Arabinoxylans (AX) are major component of cell walls in wheat endosperm. The water-extractable part, WE-AX, is considered as dietary fibres with health promoting effects. AX exhibit large natural variation in their amount but few studies have been carried out on the genetics of WE-AX content and structure in bread wheat. We first carried out a "forward" quantitative genetic approach, using recombinant populations derived from crosses between AX-high and AX-low parents. This allowed us to identify three QTL regions, each explaining 18-32% of phenotypic variation. Then, we focused on key enzymes involved in the biosynthetic pathway of arabinoxylans. About thirty single nucleotide polymorphisms (SNP) were detected in 10 candidate genes by sequencing a set of 46 lines representing a worldwide diversity. Then these SNP were genotyped in a larger subset of 155 lines, most of European origin. Eleven significant associations were found, with either AX content in bran, A/X in bran or flour, but none of them with soluble AX in flour.

Keywords: Arabino-xylan; QTL; candidate gene; association genetics.

Introduction

Cereal grains are the most important renewable resource for food, fodder and industrial raw material. Wheat in particular is one the three major cereal crops that dominate world agriculture (the others being maize and rice). It is grown over a large area with a wide geographical distribution. The major components of the grain are starch (approx. 60-70% of grain, 70-80% of flour) and proteins (approx. 10-15%) with non-starch polysaccharides derived from the cell walls only accounting for about 3-8% of the total. Nevertheless these components have major effects on the use of wheat grain (including milling, baking, animal feed) due to their viscosity in aqueous solution but also to their hydration properties. As dietary fibre, they also have a major impact on the nutritional quality of cereal foods (Fincher and Stone, 1986; Saulnier et al., 2007). In wheat grain, arabinoxylans (AX) are the major polymers of cell walls. The amount and the structure of AX polymers show large differences according to grain tissue but also between wheat cultivars and variation is mainly genetically determined. The development of new wheat cultivars with enhanced nutritional and technological qualities requires a better control of variation in AX and understanding of the cell wall involvement in

the grain development. It is therefore necessary to better understand the genetic bases of cell wall polymer synthesis.

Material and methods

Plant material and growing conditions

Genetic and environment variation for AX amount and composition was explored in a core collection representing the world diversity of bread wheat and was found to be mostly of genetic origin (high heritability, between environment correlations >0.9). Then two recombinant inbred lines (doubled haploids) populations have been developed from crosses between highly contrasted lines for WE-AX content. DH lines were grown in Clermont-Ferrand in 2004 and 2005 and flour samples analyzed for AX composition. DNA from 124 individuals of the valoris x isengrain progeny were used to built a genetic map with 86 SSR markers. Since SSR markers lead to large gaps along the chromosomes, 517 Darts (Diversity Array Technology, TriticarteR) corresponding to 331 loci showing polymorphism between Valoris and Isengrain have been integrated into the SSR genetic map. Overall 655 markers have been computed to create a high resolution VxI genetic map.

In the FP6 programme Healthgrain (HG), a set of 155 wheat accessions, covering most of the worldwide diversity, was grown by Z Bedö and M Rakszegi, Agricultural Research Institute of the HAS Martonvasar, (H), and flour samples analyzed in various laboratories by HG partners. The collection studied was phenotyped by different partners for numerous health traits. The viscosity-related traits used for association studies with polymorphism in candidate genes were provided by Y Delcour (Catholic university of Leuven, L. Saulnier (INRA, Nantes) and D. Boros (IHAR, Poland). They concerned:

1) enzyme activities measured in the flour and in the bran (TAXI and XIP: endoxylanase inhibitor in the flour, TAXI and XIP equivalents (ppm) in the bran; endoxylanase activity (EU/g) in the flour and in the bran;

2) water extractible and total AX contents (WEAXflour), A/X in WE-AX and in total-AX in the flour; such data were obtained in the bran;

3) the beta-glucan content (%) in the whole meal;

4) water extract viscosity

Choice of candidate genes

We focused on key enzymes involved in the biosynthetic pathway of arabinoxylans (Figure 1). As these enzymes belong to multigenic families, many EST contigs are available. Thus we focused on those contigs which were assigned to previously identified QTL regions. About thirty single nucleotide polymorphisms (SNP) were detected in 10 candidate genes by sequencing a set of 46 lines representing a worldwide diversity. Then these SNP were genotyped in a larger subset of 155 lines, most of European origin.

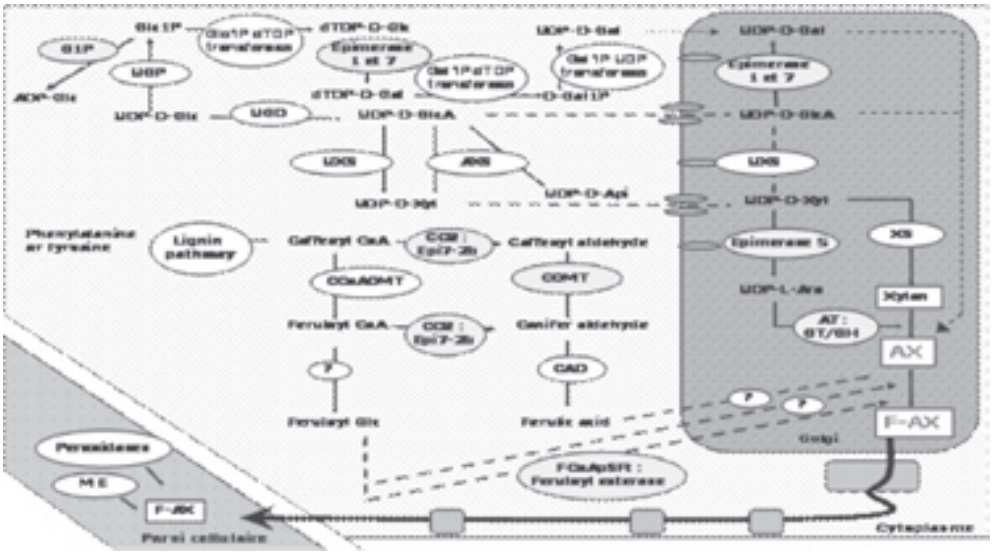

Figure 1. An overview of the pathway of AX synthesis in plant (from saulnier et al 2007). In yellow boxes: enzymes for which a nucleotide sequence was obtained and markers developed in case of nucleotide polymorphism.

The model used to test the associations between polymorphisms at the loci and phenotypic traits took into account the ancestor groups (i.e., the genetic structure among the lines studied to avoid spurious association) as covariate and the polymorphism as tested factor.

Multiple testing increases the false-positive (type I error) rate under nominal significance threshold. To limit that, we performed permutation tests at a global FDR of 5%.

Results and discussion

Quantitative Trait Analysis

AX-traits distribution (e.g. water extract viscosity, data not shown) did not show a bimodal shape, as expected from a single gene being segregating. However, a new, large effect QTL was identified in the Valoris x Isengrain (VxI) population. Using meta-analysis (Goffinet and Gerber 2000) to summarize our data with previously published ones, we found that, overall 3 QTLs have been identified as driving the viscosity trait in wheat on chromosomes 7A (6.3 cM with r^2 of 33.6%), 6B (9.85 cM with r^2 of 39%) and 1B (9.26 cM with r^2 of 29%). This QTL resolution (< 10 cM) have been obtained through a genome wide genotyping approach (with SSR and Darts) as well as fine mapping using COS markers derived from the synteny between wheat and rice. A candidate gene list is available for the 3 loci based on the

annotated gene within the rice orthologous region. Several candidates may be considered as major candidates in regard to the Arabinoxylan biosynthesis knowledge.

Association Genetics

Out of the 34 genes studied, all involved in AX synthesis we discovered 81 SNP and 16 indels in the sequencing panel of 46 diverse lines (Table 1 excluding singletons). We developed primers to genotype 27 markers, 16 of these 27 markers were used in association studies. In our genotyping conditions, we did not obtained good results with 11 of the 27 markers developed. One of these markers showed a rare allele, not suitable for statistical association.

Table 1: Polymorphism found in AX-synthesis candidate genes in the sequencing panel

Genes studied	genome	Assignation	number bp studied	polymorphism SNP	polymorphism InDel	N.B.
Epimerase 1	A	[illegible]	1513	5	1	only V1,RE,CP
	B	[illegible]	2473	36	12	
	D	[illegible]	1416	0	0	only V1,RE,CP,Pin,Rc
Glucose-1-phosphate adenyl transferase	A	[illegible]	1750	0	0	only V1,RE,CP,Pin,Rc
	B	[illegible]	1320	3	0	
	D	[illegible]	1049	0	0	only V1,RE,CP
NAD-dependent epimerase 7-1	A	[illegible]	688	0	0	
NAD-dependent epimerase 7-3	A	[illegible]	566	14	3	
	B	[illegible]	884	0	0	only V1,RE,CP,Pin,Rc
Glycosyl hydrolase family 17	A	[illegible]	1160	8	1	
	B	[illegible]	1661	0	0	only V1,RE,CP,Pin,Rc
	D	[illegible]	1771	0	0	only V1,RE,CP,Pin,Rc
Transparent Testa 1	A	[illegible]	1512	3	1	only V1,RE,CP,Pin,Rc
Caffeic acid O methyltransferase	A	[illegible]	749	4	0	only V1,RE,CP,Pin,Rc
	B	[illegible]	841	0	0	only V1,RE,CP,Pin,Rc
Malate dehydrogenase 1	A	[illegible]	2159	0	2	only V1,RE,CP,Pin,Rc
	B	[illegible]	1500	4	1	
	D	[illegible]	1169	0	0	only V1,RE,CP,Pin,Rc
Cyclophilin	A	[illegible]	1550	2	1	
Epimerase 7	A	[illegible]	2184	5	0	only V1,RE,CP
	D	[illegible]	2080	0	0	only V1,RE,CP
NAD-dependent epimerase 7-2b	A	[illegible]	2381	5	0	only V1,RE,CP
	D	[illegible]	1801	0	0	only V1,RE,CP
Glycosyl hydrolase family 28	A	[illegible]	841	0	0	
Glycosyl transferase family 14	A	[illegible]	in progress			
Glycosyl hydrolase family 16	A	[illegible]	in progress	5	1	
	B	[illegible]	in progress			
NAD-dependent epimerase 7-2a	B	[illegible]	1557	0	0	only V1,RE,CP
	D	[illegible]	2039	0	0	only V1,RE,CP
Epimerase 5	A	[illegible]	377	0	0	
	D	[illegible]	752	1	0	
Feruloyl esterase	A	[illegible]	420	0	0	only anc,rec,cpt,fdl
	B	[illegible]	527	0	0	only anc,rec,cpt,fdl
	D	[illegible]	429	0	0	only anc,rec,cpt,fdl

Several genes show significant association with viscosity-related traits. Among the genes with successfully polymorphisms genotyped, we obtained no association between the traits studied and NAD-dependent epimerase 7-2b and 7-3. This result suggested that these genes are not relevant candidates for the AX biosynthesis. The most significant association with total AX content was found with Glycosyl hydrolase family 16. Moreover, our results highlighted the important role of the A copy of the COMT in viscosity. Since this enzyme is known to be involved in AX- polymerization (through ferrulic acid bounds), it would be interesting to look for COMT-alleles showing a lower expression of this enzyme (or to create them by mutation or gene silencing).

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Molecular breeding for enhanced yield and quality of oilseed crops for Europe

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ABSTRACT: The phenotype of a plant is the product of genotype and environment. Therefore, phenotypic variance can be partitioned into a genetic and a non-genetic component. Since only the genetic variation can be exploited by breeding, it is necessary to have information on the ratio of genetic versus non-genetic variance (heritability) in the breeding material. Since significant heritability is a prerequisite for selection response, it determines the breeding progress for a specific trait of a particular crop plant. Progress in the molecular understanding of major quantitative plant traits such as adaptation, stability and productivity will provide better tools for oil crop improvement by breeding (cf. Snowdon et al., 2004). Major oilseed crops in temperate zones of the world including Europe are rapeseed (*Brassica napus*) and sunflower (*Helianthus annuus*). Whereas oilseed rape is one of the leading agricultural crops in Northern regions of Europe today, sunflower is extensively grown in Southern countries. Other minor oilcrops such as camelina (*Camelina sativa*) and linseed (*Linum usitatissimum*) have a certain potential for specific areas and applications. Whereas the latter two species are selfpollinating, sunflower and rapeseed are predominantly outbreeding. Due to substantial heterosis effects observed for seed yield and yield components in both species, hybrid breeding is efficiently used to develop high-yielding cultivars of sunflower and rapeseed, today. Genetic diversity is a prerequisite of heterosis and breeding progress. Modern molecular tools allow a better characterization and efficient exploitation of such variability (e.g. Friedt et al., 2007). Also, growing knowledge on the genetic control of heterosis will be the basis for further breeding progress regarding seed and oil yield. Since oil plants are grown for their high fat content, the quality of the oil is of particular relevance for its use as food, feed or fuel. Major improvements of oil composition have been achieved in the past, whereas the quality of oilseed meals as by-products of oil extraction deserve improvement. For example, in rapeseed extraction meal a higher protein content and a lower content of anti-nutritive substances (e.g. polyphenolics, glucosinolates) would be deserved for nutritional and feeding purposes. Genetic and breeding progress is expected to promote the development of value-added low-fibre rapeseed varieties, e.g. via yellow-seeded germplasm. Due to the low heritability of quantitative traits such as seed yield and oil content, breeding progress is generally slow. However, the determination of genomic regions (quantitative trait loci, QTL) associated with oil, protein or fibre content and the identification of molecular markers for respective genetic loci allow the application of marker-assisted selection (MAS) enabling enhanced breeding progress. Furthermore, genetic

engineering approaches can be efficiently used to modify and improve the quality of the plant seeds. Recent progress of genetics and breeding in this field will be reported and discussed.

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Genomic approach to breeding for preharvest sprouting resistance in rye

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ABSTRACT: Rye (*Secale cereale* L.) is one of major cereal crops in Central and Eastern Europe. Preharvest sprouting (PHS) and high alpha-amylase activity (AA) negatively affects quality of rye grain as a product for bread making, animal feeding and sawing. Sprouted rye is also not suitable for efficient alcohol production due to low starch content. Late maturity alpha-amylase (LMA), produced in sound grain under specific weather conditions (temperature shock) during ripening is also a problem in rye. Breeding PHS resistant cultivars with low AA is difficult due to a complex not well understood genetic basis of this trait and strong interaction with weather conditions. Ten years ago an extensive study on genetic control of PHS and AA in rye was initiated at the Agricultural University in Szczecin, Poland using two mapping populations and a method of QTL mapping. PHS was measured as a percentage of kernels germinating in spikes at full maturity, which were wetted for 7 days in a moisture chamber. Alpha-amylase activity was determined in harvested grain using a simple method of radial diffusion in agarose gel containing beta-limit dextrin as a specific substrate. Two genetic maps of all seven rye chromosomes aligned with 2-6 common markers per each chromosome were used for QTL mapping. All significant QTLs for alpha-amylase activity and those for preharvest sprouting detected in different vegetation seasons were mapped to construct a consensus map of PHS and AA. Ten to eleven QTLs for AA and five to seven for PHS were found on each map. They were distributed in a non random pattern on all rye chromosomes with one QTL on the short and 1-3 QTLs on the long arm of each chromosome. QTLs common for AA and PHS were identified on chromosome arms 1RS, 2RL, 3RS, 4RL, 5RS, 5RL, 6RS, 6RL and 7RS. Individual QTLs for alpha-amylase activity not coinciding with those for PHS were found on chromosomes 1RL, 2RS, 3RL, 4RS and 5RL and can be assigned to the genetic basis of late maturity amylase (LMA). Separate QTLs for PHS were distributed on chromosomes 1RL, 2RL and 7RL. QTLs varied in their relative effects on the trait. The most effective QTLs for alpha-amylase activity were found on chromosome arms 5RL, 2RS and 3RL. Major QTLs for PHS were detected on chromosomes 5RL, 6RL, 7RS and 7RL. Low value alleles in major QTLs for AA and PHS were found in line Ot1-3 which is a unique source of sprouting resistance. These QTL alleles may be introgressed to rye cultivars by means of marker assisted selection (MAS). However breeding for sprouting resistant rye varieties using molecular markers will be laborious due to a complex system of QTLs demanding pyramiding of valuable alleles in one genotype.

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Marker development and association mapping for malting quality in barley

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ABSTRACT: Barley (*Hordeum vulgare* L.) and its use for malting, brewing and distilling has always been a major factor in crop development besides feed quality. In this study, a number of candidate genes encoding enzymes which are involved in the germination and malting process were selected (Swanston et al., 2002, Fox et al., 2003, Hayes et al., 2003 and others). Our focus is a systematic investigation of barley germplasm for genetic loci determining malting quality and agronomical performance by using a broad association genetics approach which combines phenotypic data with molecular marker data. For obtaining an extensive source of phenotypic data a database called "MetaBrew" was created containing nearly 120 malting and brewing parameters from 170 barley cultivars, collected in the past 20 years from public sources at different experimental sites in Germany. At present, approx. 80 000 records are available. "MetaBrew" provides also tools for screening phenotypic data interesting for any breeding and malting program and statistical analysis. With this data collection it is possible to perform association studies in a large extent in order to predict and select diagnostic markers. In total, 40 SNP- and 8 INDEL-markers could be already developed for 20 candidate genes and large scale genotyping was performed with approx. 500 barley accessions. Up to now, nearly 25 000 marker data records are available. More information about SNP- and haplotype diversity is present for further 20 candidate genes which are currently investigated. Significant associations assuming the "General Linear Model" were found for certain malting and brewing parameters with single SNPs, INDELs or haplotypes, also dependent on population structure in a number of genes. Beneficial alleles could be identified in a set of 141 cultivars, representing the current commercial European germplasm. The significant genetic contribution of some SNPs and INDELs to certain malting and brewing properties could be evaluated for several genes investigated here and some examples for the use of diagnostic markers will be presented, like for Serinecarboxypeptidase I and Flavone-3-hydroxylase. This study provides a complex dataset for performing association genetics in barley. We could reveal an impact of genetic changes in candidate genes on certain malting parameters, which might be interesting not only for breeders but also for the brewing industry.

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Breeding for present and future needs of human health-promoting compounds in vegetables: a case example involving phenolics content in pepper and eggplant

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ABSTRACT: Consumption of vegetables with high levels of antioxidants and other health-promoting compounds is associated to a decreased risk of cancer, cardiovascular, and degenerative diseases, as well as to a delay in ageing. This has led to an increased rise in the demands of vegetables with high levels of these substances. Phenolics constitute one of the groups of compounds present in vegetables with a higher relevance for human health. Phenolics have an important antioxidant activity, and have shown to have free radical and antitumoral activities (Sawa et al., 1998). Among the vegetables, peppers (*Capsicum* spp.) and eggplant (*Solanum melongena*) are important sources of phenolic compounds (Cao et al., 1996), and breeding for higher content in phenolics in these crops can contribute to the development of new cultivars which satisfy the present and future demand for vegetable products with improved nutritional quality (Prohens and Nuez, 2008). We present the results of the works performed in the framework of our breeding programmes to improve the nutritional quality in pepper and eggplant. In common pepper (*C. annuum*) we have evaluated the total phenolics (TP) content in a collection of 30 varietal types from different regions of the world at both green and fully ripe stages (the two stages at which they can be consumed). In addition, we have studied the content in TP in a population of aji (*C. baccatum* var. *pendulum*) and rocoto (*C. pubescens*) from Bolivia, the nuclear centre of *Capsicum* genus. This collection was evaluated under the two main growing systems in Spain, greenhouse and open field. The results of the first experiment showed a wide range of diversity for phenolics content within *C. annuum* in both stages. In general, TP levels were higher at the fully ripe stage, although we also found accessions with high TP levels at the green stage. Several accessions showed high TP levels at its usual stage of consumption (ripe or green), while others did not. On the contrary, many other accessions showed high TP levels at the stage in which they are not usually eaten. These accessions could be useful sources of variation to improve TP levels in other cultivars eaten at this particular ripeness stages. With respect to Bolivian *Capsicum* we also found a high degree of variation in TP. *C. baccatum* accessions showed a range of variation quite similar to those found in *C. annuum* controls. *C. pubescens* showed slightly lower TP levels. Apart from a few exceptions, open field cultivation was found to increase TP levels in most accessions compared to greenhouse cultivation. In eggplant, we have evaluated (including experiments in the open field and in greenhouses) TP in an important collection (in total more than 100 accessions) corresponding to local varieties,

commercial varieties, and related species. Furthermore, we have studied the phenolics content in hybrids obtained by crossing local materials, as well as in interspecific crosses. The results show that within cultivated eggplant there is an important variation for TP, with differences of more than fourfold among materials of the cultivated species. Local varieties had higher mean levels of phenolics than commercial varieties, and the highest values for phenolics content were observed in local varieties. The experiments with intraspecific hybrids show that the narrow-sense heritability value for the content in phenolics is moderate (around 0.50), and that it is possible to obtain hybrids with high phenolics content. Among the related species studied, some accessions of *S. macrocarpon* and *S. incanum* presented levels much higher than those of *S. melongena*. Interspecific hybrids with *S. incanum* and backcross generations to *S. melongena* have been obtained in order to introgress the high content of phenolics of this wild species into *S. melongena*. We are studying the genetic diversity for genes implicated in the pathway of phenolics production in eggplant in order to assess the utility of using these allelic variants as molecular markers in marker assisted selection for high content of phenolics in eggplant. The results obtained of our breeding programmes show that the development of new pepper and eggplant cultivars with higher content in phenolics can contribute to satisfying the demands of vegetables with improved health-promoting properties.

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Modeling patterns of phenotypic and genetic variation across biological organization levels, with an example in tomato quality research

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ABSTRACT: The Center for BioSystems Genomics (CBSG; www.cbsg.nl) is a joint venture in plant genomics of breeding companies, biotech companies, research institutes and universities in the Netherlands. An important part of CBSG concentrates on tomato quality with the objective of developing a better understanding of the biological factors involved in tomato taste, with special attention for the underlying genetics. The basic germplasm for the tomato quality program of CBSG consisted of an association panel of 94 commercial hybrids and a half diallel of six F2 families. The following types of data were collected for the association panel and the half diallel: AFLP markers; morphological and anatomical plant and fruit traits; measurements on soluble fruit content (brix) and fruit firmness; sensory observations on taste attributes scored by a trained panel of observers; metabolic data like gas chromatography-mass spectrometry data, liquid chromatography data for volatile, non volatile and derivatized tomato fruit compounds. With the ultimate goal of modeling various types of phenotypic traits in terms of underlying QTLs, a wide range of statistical techniques was developed and evaluated: a) methodology for studying distribution and variation of individual traits within homogenous sets of traits (all traits of the same type); graphics / descriptive statistics / linear mixed models; b) methodology for studying relations between traits of a particular type (metabolites, sensory data, marker data, etc.); PCA / clustering / networks; c) methodology for studying relations between data sets containing variables of different types; various forms of multivariate regression / networks; d) methodology for simple marker trait association studies: correlation/ regression/ association analysis with correction for population substructure; e) methodology for multiple QTL association studies: various forms of multiple regression. The application of this wide range of statistical methods led to the following main results: description of genetic diversity; identification of main patterns of variation in sensory and metabolic traits; identification of relationships between sensory and metabolic traits; identification of single and multiple markers describing sensory and metabolic trait variation. In the presentation we will give a sketch of the methodology we used to arrive at those results.

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Evaluation of main functional constituents of tomato fruits in *Solanum* section *Lycopersicon* germplasm

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ABSTRACT: In this work we have evaluated thirty-two accessions of tomato and related species for their main functional constituents (lycopene, β-carotene and vitamin C). These compounds have a high positive impact in human health, so the tomato has been pointed as a functional food. The relationships between traits suggest that it is possible to combine higher lycopene, β-carotene and vitamin C contents in a single genotype in future 'more functional' tomatoes through breeding. To carry out this objective, four accessions of *S. pimpinellifolium* (BGV07230, BGV06778, BGV06474 and BGV06333), has been selected as interesting donor parents to start a breeding program.

Keywords: GGE biplot analysis, lycopene, β-carotene, vitamin C, tomato bioactive components.

Introduction

The concept of 'functional food' was born in the 1980s in Japan and was quickly propagated globally. Functional food has not been yet clearly defined by European laws. Generally, it is defined as the food we normally eat as a part of our diet which has bioactive components that confer health benefits and reduced disease risk (Arai, 2005). An example of this type of food is the tomato. Tomatoes contain many bioactive components, including those that act as antioxidants, such as the vitamins C and E, many carotenoids and tocopherols. From these compounds vitamin C, lycopene and β-carotene have been reported to play an important key role against certain tumours and cardiovascular diseases (Higdon and Frei, 2005; Voutilainen *et al*, 2006 and Canene-Adams *et al*, 2005). In this context, the economic demand and health-promoting constituents of the tomato fruit make it an important target for increasing its content of functional constituents. One way to improve the vitamin and carotenoid content in the tomato is the use of high antioxidant *Solanum* germplasm as a donor parent in breeding programmes. In consequence, the aim of the present study is to evaluate the functional characteristics of several accessions of *Solanum* section *Lycopersicon* in order to establish their potential for breeding programmes and functional food development.

Materials and methods

Thirteen accessions of *Solanum lycopersicum* L., nine of *S. lycopersicum* var *cerasiforme* L. and ten of *S. pimpinellifolium* L. from the COMAV genebank (Table 1) were studied. Four controls were selected to have suitable direct references of low-medium, high and very high antioxidants content: two accessions of *S. lycopersicum*, one (LA3538) of which is a high pigment mutant with *hp-1* gene provided by the Tomato Genetics Resource Center of the University of California, the other (BGV012406) is a tomato experimental line with antioxidants content (low-medium) similar to present commercial hybrids; one accession of *S. lycopersicum* var *cerasiforme* (BGV006333) with high content of β-carotene and vitamin C, and one *S. pimpinellifolium* (BGV004587) characterized in previous trials as a very high lycopene content accession.

Twelve plants per accession were grown under greenhouse conditions for suitable production in the Autumn-Winter cycle. Environmental variability was reduced by means of a completely randomized plot design (three plots of four plants for each accession). Fruits were collected from each plant at the red-mature stage (only from the first three trusses). Fruits were homogenized in a laboratory blender. Vitamin C was quantified by Capillary Zone Electrophoresis (Galiana-Balaguer *et al*., 2001). Carotenoid determination was based on spectrophotometric analysis following the method described by Zscheille and Porter (1947) and modified by Rousseaux *et al*. (2005). A graphical multivariate statistical analysis (GGE biplot, Yan and Kang, 2003) was used to obtain and visualize better and deeper relationships between functional constituents and accessions.

Results and discussion

The analytical results for lycopene, β-carotene and vitamin C content show interesting contents of these functional constituents and a high variability between accessions (Table 1). Vitamin C contents were similar to levels observed in other trials (Lenucci *et al*., 2006), whereas lycopene and β-carotene levels are high compared with the commonly reported levels (Kuti and Konuru, 2005, Lenucci *et al*., 2006), but similar to Hanson *et al*. (2004) results. The GGE biplot analyses (Figure 1 to 4) showed a good fit with the data as they explained 77.5% of the variability. In Figure 1, traits and accessions vectors were displayed to examine the relationships between them. The magnitude of the performance is determined by both the cosine of the angle between the vectors and the length of the vectors (greater length and a higher cosine mean better performance). To obtain more functional tomatoes, knowledge of the correlations between traits involved in functional quality is needed. This biplot shows that, for accessions tested, lycopene accumulation was independent of vitamin C content (near right angle between vectors) which agreed with the lack of reported relationship between the proposed biosynthesis pathways of vitamin C (Hancock and Viola, 2005) and carotenoids (Romer and Fraser, 2005). These relationships suggest that it is possible to combine the higher lycopene and vitamin C contents in a single genotype in future 'more functional' tomatoes through breeding programs using appropriate donor parents. This is very interesting

Table 1. Origin, fruit characteristics and lycopene, β-carotene and vitamin C content (mean ± standard deviation) of the accessions evaluated in mg/100g fresh weight.

Sp	Accession	Origin	Fruit characteristics	Lycopene	β-carotene	Vitamin C
1	BGV12406*	Spain	Light red, large	7.3±0.8	0.9±0.2	9.8±3.5
1	LA3538*	USA	Red purple, medium-size	14.4±2.5	2.7±0.2	13.5±2.4
2	BGV04587*	Guatemala	Orange-brownish, small	7.5±2.7	2.4±0.5	16.2±5.8
3	BGV06333*	Ecuador	Deep red, very small	36.2±3.7	1.2±0.3	10.8±3.1
1	BGV06224	Ecuador	Red, large	8.4±3.0	1.0±0.2	16.3±3.1
1	BGV06867	Ecuador	Red, small	6.7±3.2	1.1±0.1	12.2±3.0
1	BGV06881	Ecuador	Deep pink, small	6.5±1.3	1.1±0.3	17.8±5.0
1	BGV06884	Ecuador	Deep red, small	12.1±2.1	1.0±0.1	16.0±5.1
1	BGV12834	Spain	Pink, large	7.7±1.9	0.6±0.1	15.2±4.3
1	BGV12846	Spain	Red, very large	8.2±3.1	0.6±0.2	8.0±1.2
1	BGV12858	Spain	Red, medium-size	8.8±2.2	0.9±0.1	13.5±6.8
1	BGV12867	Nepal	Red, small-medium	12.6±1.5	1.1±0.2	8.0±2.2
1	BGV12871	China	Light red, small	11.1±1.3	1.0±0.3	14.0±4.3
1	BGV13273	Italy	Red, medium-size	9.0±2.5	0.6±0.2	7.8±1.1
1	BGV13274	Italy	Red, large	13.4±3.8	0.9±0.2	14.7±3.2
1	BGV13275	Italy	Red, very large	6.1±1.2	0.5±0.2	6.3±1.2
1	BGV13582	Spain	Pink, very large	7.6±0.9	0.6±0.1	5.6±1.4
2	BGV00251	Angola	Red-orangish, very small	6.2±0.9	1.4±0.2	20.5±7.6
2	BGV00252	Angola	Red, small	16.3±2.4	1.7±0.4	18.7±7.2
2	BGV03105	Spain	Red, small	9.5±0.8	1.1±0.1	11.9±3.1
2	BGV03108	Spain	Red, medium-small	9.2±0.6	1.0±0.2	10.3±3.1
2	BGV03141	Spain	Light red, medium-size	8.0±1.2	1.1±0.9	8.6±3.0
2	BGV04629	USA	Red-pinkish, medium-size	7.9±0.6	1.1±0.3	10.4±2.0
2	BGV06902	Ecuador	Light red, small	16.5±6.6	1.3±0.3	13.7±5.3
2	BGV07642	Bolivia	Pink, small-medium	10.1±2.3	0.6±0.1	9.9±4.2
2	BGV13758	Peru	Red, small	7.3±0.3	2.2±0.1	9.1±3.5
3	BGV04487	Spain	Red, very small	11.8±2.7	2.0±0.5	9.5±1.2
3	BGV04652	USA	Red, small	15.8±0.9	1.9±0.5	13.6±1.8
3	BGV06474	Peru	Red, very small	25.6±0.6	1.9±0.1	11.4±0.4
3	BGV06778	Ecuador	Red, very small	24.2±2.3	1.3±0.4	8.5±3.0
3	BGV07068	Ecuador	Deep red, very small	21.7±2.9	0.9±0.4	19.0±13.8
3	BGV07126	Ecuador	Red, very small	12.2±0.1	1.7±0.1	15.7±0.1
3	BGV07169	Ecuador	Red, very small	11.6±2.6	1.6±0.4	13.7±4.7
3	BGV07183	Ecuador	Deep red, very small	12.1±3.4	2.0±0.7	17.7±1.3
3	BGV07198	Ecuador	Deep red, very small	15.8±3.2	2.7±1.1	12.7±5.3
3	BGV07230	Ecuador	Red, very small	23.8±0.7	1.7±0.1	22.1±0.7

Sp=Species; 1=*Solanum lycopersicum*. 2= *S. lycopersicum* var. *cerasiforme*. 3. *S. pimpinellifolium*. *controls used.

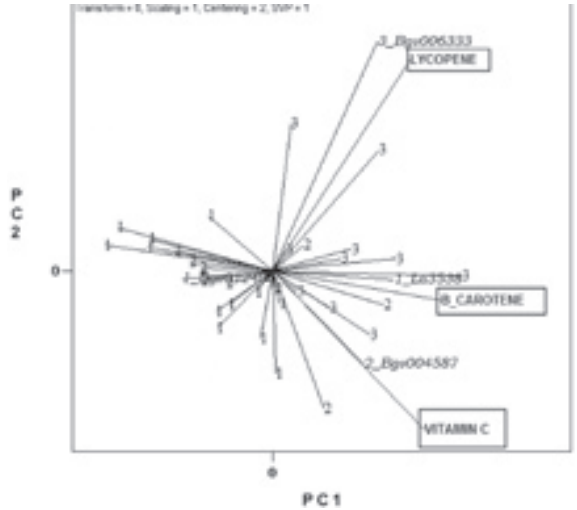

Figure 1. Biplot of the relationship between traits and accessions. Distribution of species (1 *S. lycopersicum*, 2 *S. lycopersicum* var *cerasiforme*, 3 *S. pimpinellifolium*). Controls are indicated.

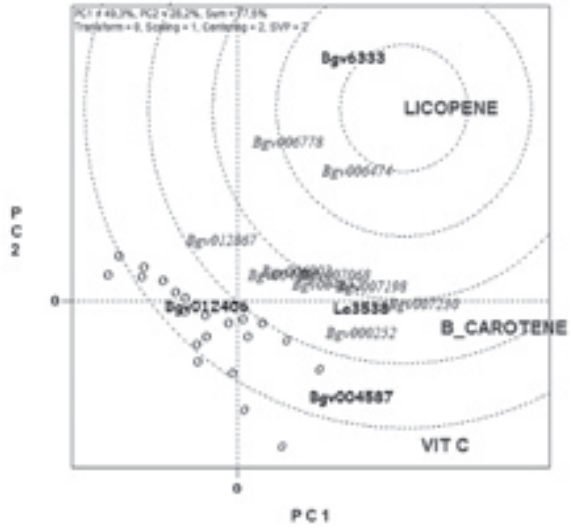

Figure 2. Biplot using lycopene as reference trait. Only the most interesting accessions were represented with the completed code. Controls are represented by black, bold letters.

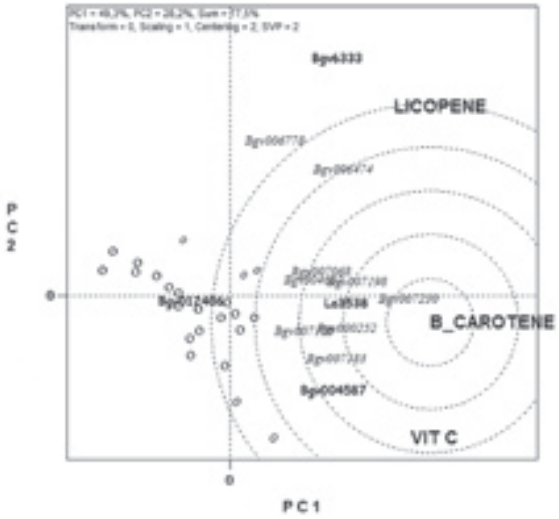

Figure 3. Biplot using β-carotene as reference. Only the most interesting accessions were represented with the completed code. Controls are represented by black, bold letters.

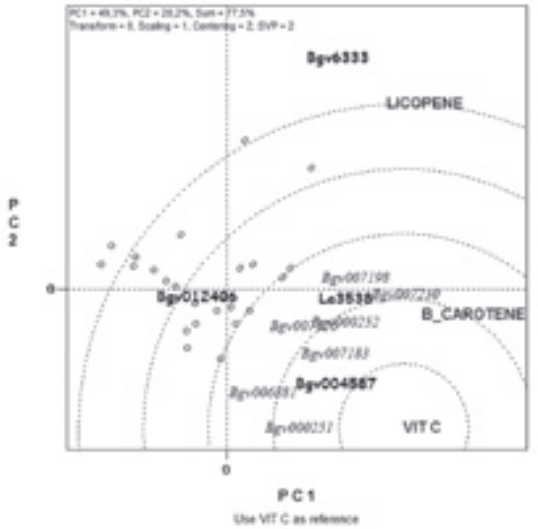

Figure 4. Biplot using vitamin C as reference. Only the most interesting accessions were represented with the completed code. Controls are represented by black, bold letters.

as lycopene and vitamin C represents up to 90% of the functional components studied. In this trial, correlationsbetween lycopene and β-carotene content are low. In tomato carotenoid biosynthetic pathway, lycopene acts as a substrate for β-carotene accumulation (Romer and Fraser, 2005), but in this study we have used a *hp* mutant and several accessions with different fruit color (Table 1) than could have distinct levels of lycopene transformation, so this can explain the low correlation observed. For vitamin C and β-carotene high correlation existed. There are no biosynthetic pathway evidences that justify a linked relation of the characters but, for accessions tested, this is the behaviour observed, so this must be considered when selecting sources of variability for these two characters.

To graphically evaluate the accessions tested we need to identify the controls position in relation to the trait vectors (Figure 1 to 4). BGV012406 (an experimental line with low-medium functional value) was located near the centre of biplot. The other controls were outstanding for each trait, making easier the identification of interesting accessions with high bioactive component content. In this way LA3538 was the control with the highest β-carotene content (3 times the content of BGV012406). BGV004587 control had high vitamin C content (1.65 times the content of BGV012406) and BGV006333 control was the best for lycopene content (5 times the content of BGV012406). With these references we can easily evaluate the functional properties of the rest of accessions comparing their biplot position with the controls. In general, *S. pimpinellifolium* accesssions are located in right hand of biplot between traits vectors (Figure 1), indicating a higher antioxidant content than the accessions located in left hand, so they seem to be good candidates to improve the functionality of cultivated tomato, as pointed out by other studies (Hanson *et al*. 2004). This biplot shows that two *S. lycopersicum* var *cerasiforme* have also interesting functional properties.

In the biplots shown in Figure 2, 3 and 4 each trait has been used as reference to better select the better accessions to be able to start a breeding program. In this study we can select BGV06778, BGV06474 and the control BGV06333 (more than 3 times the content of low-medium control-BGV12406- similar to commercial varieties) for their lycopene content. For β-carotene content the outstanding accesions were BGV007230, BGV000252, BGV007183 and BGV007198 (from 1,9 and 3 times higher than BGV12406) and for vitamin C the best accessions were BGV000252, BGV007183, BGV00251 and BGV07230 (around 2 times the content of BGV12406).

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Heritability of ornamental characters of interest in gillyflowers (*Matthiola incana*) breeding and the genetic correlations among them

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ABSTRACT: Six cultivars (V_1-V_6)of gillyflower (*Matthiola incana*), previously tested for their plant homogeneity of distinctive characters (type of inflorescence, flower color, height of plant, earliness etc.) were used in 2003 in a cyclic crossing system with V_1 as tester parent. This resulted in ten hybrid combinations (direct and reciprocal), each of them containing 40-150 hybrid plants. The F_1 hybrids were tested for their main quantitative characters of interest in the phenotypic expression of plant ornamental value, in 2004 -2006. Heritability, both in wide (H) and narrow (h^2) sense, was computed for seven quantitative characters by means of ANOVA method (for H) and regression of F_1 hybrids to mean of parents (for h^2). Also phenotypic and genotypic correlations were estimated to foresee possible tandem selective characters. There have been found quite a few quantitative traits (plant height, number of simple flowers/ inflorescence, number of composite flowers/inflorescence, begining of blooming, persistence of flowering) in which high values of wide sense heritability (H) were accompanied by medium values of narrow sense heritability (h^2). It is quite reasonable to expect a high efficiency of phenotypic selection for such traits, since additive effects seem to play a major role in their inheritance. Quite a few of the analyzed pairs of characters were found significantly correlated at the phenotypic level, but out of these there were only seven: height of plants with simple flowers and no. of simple inflorescences/plant; no. of composite flowers/plant and the diameter of flower; length of siliqua and size of simple flower; height of plant with simple flowers and persistence of flowering; persistence of flowering and no. of simple flowers/plant; no. of inflorescences with simple flowers and persistence of flowering; and, diameter of simple flower and plant height. It is noticeable that for an efficient tandem selection aiming at improving ornamental value of gillyflower, there are only a few pairs of characters which could really be taken into consideration.

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Genotype x environment interaction for some quality traits of Bulgarian winter wheat varieties

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ABSTRACT: Twenty Bulgarian winter wheat varieties were tested in two locations for four years (2004 – 2007). The aim of this investigation was to determine the nature of the interaction of some quality parameters with the environmental conditions, to define the behavior of the individual varieties, their stability and adaptability. The varieties were grown in the Latin square design, in 5 replications 15 m^2 area each. Grain samples were taken from each variety in standard moisture. The following characters were examined: test weight (TW); wet gluten content (WGC); sedimentation value (SDS); dough resistance (DR); valorimeter value (Val) and loaf volume (Lvol). A high influence of the interaction G x E on test weight, sedimentation and loaf volume was determined. The variety differences for stability and adaptability were defined in details. The quality varieties were specifically adapted to high yielding environments for sedimentation and loaf volume. Varieties Aglika (for sedimentation) and Demetra (for loaf volume) were most stable. Concerning test weight most of the varieties were well adapted to all conditions and variety Enola being most stable.

Keywords: genotype x environment interaction, quality, winter wheat.

Introduction

Common wheat (*Triticum aestivum*) provides the nutrition for the greater part of the world population. The global climatic changes and the increase of the population lead to increasingly stronger emphasis on productivity, as well as on the end-use quality traits of the new wheat varieties. One of the problems in breeding for quality is not only developing new varieties with high level of the indices, but also achieving constant quality through out the years (Johansson et al., 2001; Williams et al., 2008). The grain and flour traits as well as bread making qualities in wheat depend on the genotype, environment and their interaction (Dechev, 2005; Peterson et al., 1992; Stoeva and Penchev, 1999; Tsenov et al., 2004). Some authors note significant G x E influence on wheat quality (Panozzo and Eagles, 2000, Peterson et al., 1998).

The aim of this investigation was to determine the nature of the interaction of some quality traits with the environments, to define the behavior of the individual varieties, their stability and adaptability to the environments.

Material and Methods

Grain samples were taken from 20 Bulgarian winter wheat varieties grown in Latin square design in 5 replications at two locations – DAI and Obraztsov Chiflik - Rouse during the period 2004 - 2007. The quality parameters included in the investigation were determined according to the methods adopted in the DAI laboratory. The standard ISO 9771/2 was used for evaluation of test weight (TW); the method of Pumpyanskii (1971) was used for sedimentation (SDS). The analysis of variances was done by the package Statgraphics XV and the stability parameters were determined by the software GEST98 for studying genotype x environment interaction. The most informative parameters were chosen – the coefficient of regression (b_i) by Finlay and Wilkinson (1963) and the ecovalence of stability by Muir et al. 1992.

Results and Discussion

The analysis of variances (ANOVA) for test weight, sedimentation, loaf volume, dough resistance, valorimetric value and wet gluten content showed that they were all significant at a high level separately for location, year and genotype (data not shown). The interactions among the three factors were significant for the test weight, sedimentation and loaf volume only. Therefore only these three characters were included in the discussion.

Partitioning of the sum of squares (SS) of the components indicated that for test weight 54.1% of the total variation was due to the year, 23.0% due to genotype and 14.5% due to location (table 1). The particular interactions and the residual had a small part in the total variation. For sedimentation genotype portion was 46.2% and the year was second (24.5%). The location and the year x genotype interaction had almost equal effect (10.8% and 9.48%, respectively). The year and the genotype had equal influence for the loaf volume (34.4% and 31.2%, respectively). Fowler et al. (1998), Zhang et al. (2005) found out that the genotype effect is relatively more important for sedimentation than for test weight, while He et al. (1998), Mladenov et al. (1996), Stoeva and Penchev (1999) determined that the environments and the G x E interaction are of greater importance.

Table 1. The factor portion to the total variation

Source	Sum of squares, %		
	TW*	SDS	Lvol
A:Location	14.5	10.8	7.95
B:Year	54.1	24.5	34.4
C:Genotype	23.0	46.2	31.2
AB	1.26	1.92	1.1
AC	1.83	2.66	2.92
BC	3.57	9.48	15.2
Residual	1.73	4.48	7.17

*TW-test weight, kg/hl; SDS – sedimentation, ml; Lvol – loaf volume, ml

According to Finlay and Wilkinson (1963), regression coefficients approximating to 1.0 indicate average stability, but must always be associated and interpreted with the genotype mean of the character. For test weight the varieties Enola, Preslav, Milena, Zlatina were well adapted to all environments, as they had b_i near to 1.0 and high mean value (Table 2). With greater resistance to the environmental changes were varieties Kristi, Zlatitsa, Prelom, but they showed specific adaptation to low yielding environments.

Concerning Wricke's ecovalence analysis (W_i) genotypes with low ecovalence were stable across environments. For test weight these were Kristal, Milena, Albena, Ludogorie, Pliska and Todora.

The group of the high quality wheats included varieties Demetra, Aglika, Progres, Preslav, Albena. Aglika was the most stable for sedimentation and was adapted to all environments. The other varieties were specifically adapted to high yielding environments. With stability above the average but specifically adapted to low yielding environments were the varieties with low or average quality: Yantar, Kristal, Ludogorie, Kristi, Zlatitsa, Boryana. According to the ecovalence value stable were varieties Boryana, Ludogorie, Kristal, Pryaspa, Sadovo 1, Kristi.

The varieties Demetra and Pliska were the most stable and adapted to all environments with high loaf volume. Specifically adapted to high yielding environments but below average stability were Aglika, Zlatina, Progres - these were varieties with good quality. The ecovalence confirmed the regression coefficient and pointed out as most stable the varieties Demetra, Pliska, Yantar, Milena, Sadovo 1.

Variety Todora, which is with low quality, showed stability below average, but it was adapted to high yielding environments. This is an indication that this variety may show good quality under excellent conditions.

The conditions at Dobroudja Agricultural Institute were more favorable for the expression of the characters than these in Obraztsov chiflik (figure 1). A high level of sedimentation and loaf volume was achieved during 2005. The mean sedimentation in the two locations was lower during 2006 and 2007. The variation for test weight was low through the years but in 2005 it was a little bit lower than the others.

The high level of the genotype participation in the total variation for the sedimentation value (46.2%) showed that the selection with high level of the trait in all environments was efficient enough. The other traits were more or less influenced by the environment and the G x E interaction.

The quality varieties were specifically adapted to high yielding environments for sedimentation and loaf volume. Most stable were Aglika (for sedimentation) and Demetra (for loaf volume). Concerning test weight most of the varieties were well adapted to all conditions and variety Enola was the most stable. The varieties with low quality had stability above the average but adapted to low yielding environments. An exception was variety Todora, which was specifically adapted to high yielding environments for sedimentation and loaf volume.

Dobroudja Agricultural Research Institute was a more favorable location for quality breeding. There genotypes showed higher level of the traits regardless of the conditions.

Table 2. Mean and stability parameters of the varieties

Variety	Test weight, kg			Sedimentation, ml			Loaf volume, ml		
	mean	b_i	W_i	mean	b_i	W_i	mean	b_i	W_i
Pliska	78.2	1.132	2.24	36.8	1.198	11.50	683.8	1.019	4.22
Pryaspa	78.6	1.069	3.00	31.4	0.884	5.33	630.6	0.905	10.67
Sadovo 1	80.9	1.297	5.24	36.8	1.211	5.52	660.6	1.303	5.45
Yantar	79.6	0.396	25.80	27.8	0.648	6.46	628.1	0.909	2.72
Kristal	80.4	0.906	1.48	23.3	0.675	5.02	597.5	0.772	12.80
Todora	79.3	1.137	2.34	28.8	1.319	8.28	617.5	1.305	19.42
Enola	81.5	1.008	3.53	35.5	1.150	16.30	643.8	0.598	11.00
Ludogorie	81.4	0.860	1.93	27.1	0.696	4.56	603.1	1.236	12.00
Kristi	78.9	0.614	8.35	28.9	0.754	5.86	638.1	0.789	8.09
Zlatitsa	78.8	0.706	5.54	29.3	0.440	26.70	583.8	0.421	8.02
Prelom	76.1	0.847	3.05	31.5	0.845	9.18	595	0.960	11.77
Boryana	80.5	1.192	3.52	29.6	0.691	2.88	621.9	1.198	8.69
Pobeda	80.6	1.087	2.75	36.9	0.840	8.48	663.1	1.300	7.04
Albena	80.9	0.923	1.85	37.0	1.290	15.40	666.3	0.650	9.67
Preslav	81.1	1.047	2.73	39.5	1.429	8.01	632.5	1.015	10.59
Milena	81.5	1.069	1.54	40.3	0.909	10.70	634.4	0.834	4.56
Aglika	80.6	1.261	6.24	44.5	1.006	18.20	718.8	1.166	12.45
Progres	79.2	1.398	9.48	40.9	1.281	6.39	670.6	1.283	9.32
Zlatina	81.7	1.098	2.40	36.9	1.112	10.20	673.1	1.350	12.36
Demetra	81.1	0.952	3.16	46.0	1.621	22.30	725.6	0.987	2.46
LBM*		0.891	2.34		0.859	7.47		0.878	7.31
UBM*		1.109	7.28		1.141	13.26		1.122	11.02

*Lower bound on mean (95%)

*Upper bound on mean (95%)

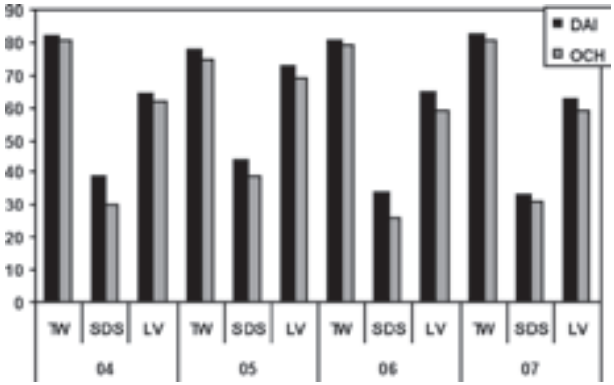

Figure 1. The means for the location through the years of investigation for test weight (TW), sedimentation (SDS) and loaf volume (Lvol)

DAI-Dobroudja Agricultural Institute; OCH-Obraztsov Chiflik

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The comparison of grain hardness in some Polish winter wheat breeding advanced lines

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ABSTRACT: The grain of forty seven of polish wheat breeding lines and three varieties from DANKO breeding stations, all grown at 2 levels of nitrogen fertilization in 2007 were evaluated on NIR apparatus on the basis of protein content, starch content, sedimentation test, wet gluten yield, alveograph W value, and hardness value. Additionally grain hardness of samples was evaluated on one-step Brabender Hardness Tester and expressed as Torque necessary to grind the grain (B.U.) and Wheat Hardness Index (WHI = Torque/% of obtained flour) according to Greenaway (1969). Twenty gram of wheat grain samples were also milled on Qudrumat-Junior Mill and PSI (Particle Size Index) was calculated as a percent of flour passing trough 80 μm sieve openings. It was found, that wheat grain samples grown in mild environmental conditions of Poland are in range of protein content 11.0-16.6%, starch content 63.0-70.2%, wet gluten yield 20.9-36.2%, sedimentation test 24.0-62.6; alveograph W value 189-396 and NIR hardness 18.8-73.3, WHI index 11.6-57.3, PSI index 60.0-40.9 and Torque 310-600 B.U. In that range only few samples were classified as the soft, and few as the hard, most of them were classified as medium hard. To compare, the hardness parameters of commercial durum wheat grain sample (14% moisture) were as follows: Torque 460 B.U.; WHI index = 115.4 ; and PSI index = 30.0. According to results of two way ANOVA all grain hardness parameters are strongly connected with wheat grain line or variety. It was also found the statistically significant effect of nitrogen fertilization during grain growing on grain hardness parameters, but the differences were very small. As the effect of different principles of grain hardness evaluation of all used methods (Obuchowski 1984, Turnbull and Rahman 2002), their results are not strictly comparable. Nevertheless, highly significant coefficient of correlation between grain hardness results from NIR method and WHI = 0.589, NIR and PSI = -0.585, as well as WHI and PSI = -0.583 indicate, that all of the used methods of grain hardness are valuable tool for wheat grain classification also in polish breeding stations, where because of geographical temperate climate wheat grain is not so hard like grain from continental conditions.

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Breeding strategies for improvement of quality and yield of winter oilseeds rape (*Brassica napus* L.) seeds

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ABSTRACT: Oilseed rape is an important source of energy used both for human consumption and feeding livestock as well as for non-edible purposes, first of all for biofuel production. The value and utility of oilseed rape seeds for both nutritional and industrial purposes depend on the quality of seeds: fatty acid composition, content of oil, biological active components in oil and antinutritional compounds. The profitability of oilseed rape products utilization for food and non-food purposes depends on yielding capacity. In this paper novel variability of some quality traits and the strategy of its utilization in marker assisted selection will be presented.

Keywords: *Brassica napus,* molecular markers, quality traits.

Introduction

With the improvement of seed quality through the development of zero erucic and low glucosinolate oilseed rape cultivars this plant has become an important source of energy in human nutrition, as fodder for livestock as well as for non – edible purposes, mainly for biofuel production (Bartkowiak-Broda et al., 2006). However, the value of oilseed rape for different uses can be improved by increasing such traits as: oil, protein and tocopherols content, decreasing of antinutritional compounds, eg. fibre and glucosinolate content and by differentiation of fatty acid composition in oil. All these changes should be accompanied by biological progress in yielding capacity.

In the Department of Breeding and Genetics of Oilseed Crops in PBAI the investigations concerning oilseed rape aim at development for breeding germplasms with desirable traits. The creation of this novel genetic variation is realized using different procedures: conventional breeding approaches (recombinations) (Bartkowiak-Broda et al., 2006), chemical mutagenesis (Spasibionek, 2006), biotechnology – microspore cultures for production of doubled haploids (DH) (Cegielska-Taras et al., 2002). All these methods are combined with biochemical and molecular markers to improve the effectiveness of development of genotypes valuable for breeding.

Fatty acid composition

At present, in Poland as well as in European Union countries double low quality oilseed rape cultivars (without erucic acid and with low glucosinolate content) are in commercial production. The seeds are the source of universal oil which is recognized as perfect oil for edible purposes with very well balanced essential fatty acid composition – linoleic and linolenic and relatively high oleic acid content which allows to use it in technology of biofuel and other biodegradable material production (Scarth and McVetty, 1999). However, the market also demands oil with different fatty acid composition. To obtain more variability in fatty acid composition mutation with the use of ethyl methanesulphonate (EMS) was performed on double low inbred line PN3756/93. It induced mutations in genes *fad2 or fad3 (fatty acid desaturase)*. After selection in several subsequent generations M_2 – M_7, two mutants M-10453 and M-10464 with significantly increased oleic acid content and one mutant M-681 with high linoleic and low linolenic acid content were selected (Table 1) (Spasibionek, 2006). Also high oleic and low linolenic recombinants between these two types of mutants have been obtained.

The high oleic lines containing about 80% of oleic acid have been also selected in intragenus *Brassica napus* recombinants.

Table 1. Characteristics of winter oilseed rape mutants in comparison to wild line PN 3756/93.

Trait	PN 3756/93	M-10453	M-10464	M-681
Palmitic acid	5.0	4.6	3.9	4.7
Stearic acid	1.2	1.2	1.2	1.8**
Oleic acid	65.0	76.1**	76.6**	61.0
Linoleic acid	18.4	8.7**	8.8**	27.5**
Linolenic acid	8.7	7.2**	7.4**	2.7**
ODR – oleic desaturation ratio	29.4	17.3**	17.4**	33.2**
LDR – linoleic desaturation ratio	32.1	45.5**	45.8**	9.1**
Oil content	50.8	48.4**	47.7**	46.6**

Glucosinolates and oil content

Glucosinolates are still the main antinutritive factor in rapeseed meal in spite of their low content in seeds from double low cultivars. Lines with extremely low content of alkenyl glucosinolates, 0.7–1.3 $\mu M \cdot g^{-1}$ of seeds and increased oil content have been obtained through conventional breeding as well as by the development of DH lines (Pietka et al., 2007).

Yellow seeded oilseed rape

The improvement of oilseed rape seeds value in livestock feeding is possible by reducing fiber content. It is linked to the increase in protein and oil content. Fiber reduction is possible

by introducing to oilseed rape breeding yellow seeded genotypes. The origin of oilseed rape with yellow seeds obtained in our Department is a mutant with brighter seeds crossed with spring line of *B.napus* with segregating seed colour (Piotrowska et al., 2003). Several yellow seeded lines with low fiber content have been selected, but their yielding ability should be improved (Table 2).

Table 2. Characteristics of yellow seeded lines.

Line	Colour of seeds	Oil content (%)	Glucosinolate total ($\mu M \cdot g^{-1}$ of seeds)	Fibre content (%)		Protein content (%)	1000 seeds weight (g)
				ADF	NDF		
PNz 022	yellow	46.9	5.7	12.7	16.3	23.0	5.0
PNz 015	yellow	48.8	6.5	13.5	15.3	19.8	4.2
PNz 041	yellow	46.8	6.5	12.3	15.4	21.9	4.6
c.v. Bojan	black	44.7	10.6	20.8	26.6	20.3	3.8
c.v. Lisek	black	45.8	9.0	22.1	28.8	17.1	4.0

ADF – acid detergent fibre; NDF – neutral detergent fibre

Content of tocochromanols

Tocochromanols are an important group of antioxidants with biological activity in vegetable oils. In oilseed rape tocopherols proportions of 65% γ-T, and half as much α-T 35% and amount of β-T and δ-T lower than <1% are commonly found in seed oil. The objective of the research is to find variability in these traits and to increase α-tocopherol content in oilseed rape oil as well as plastochromanol (PC-8) content which is the most effective antioxidant. In 25 DH lines selected according to their different origin and investigated in field trials in two locations variability in tocochromanols content was found which exceeds the content of these compounds in varieties cultivated presently (Table 3).

Table 3. Tocochromanols content in seeds of DH lines of winter oilseed rape in comparison to open pollinated cultivar Bojan (data from trials in two locations).

Field trials	Total T *mg/100 g d.m. seeds*		α-T *% in total T*		γ-T *% in total T*		Ratio α-T/γ-T		PC-8 *mg/100 g d.m. seeds*	
	DHs range	cv. Bojan	DHs range	cv. Bojan	DHs range	cv. Bojan	DHs range	cv. Bojan	DHs range	cv. Bojan
I-B	41.7–79.9	69.4	35.7–51.9	45.1	37.2–63.2	53.0	0.56–1.64	0.85	9.1–13.4	12.1
II-M	43.5–77.8	66.4	38.0–51.7	46.0	37.4–60.3	51.7	0.63–1.63	0.89	7.5–13.2	11.9

T – tocopherol; PC-8 – plastochromanol; I-B – field trial in Borowo; II-M – field trial in Małyszyn

High erucic acid and low glucosinolate genotype of *Brassica napus*

Oil with high erucic acid content can be used in chemical industry. Lines with 57.4–60.4% of erucic acid content and very low glucosinolates level within 2.1–4.1 $\mu M \cdot g^{-1}$ of seeds have been obtained by the development of DH lines from high erucic line (52.1%) (Table 4). In our research these lines are used as phenotypic markers in different projects concerning gene flow between plants of *Brassica* species.

Table 4. Biochemical characteristics of selected high erucic doubled haploid lines of winter oilseed rape.

DH line	Content of:		
	oil (%)	erucic acid (% of total fatty acids)	glucosinolates ($\mu M \cdot g^{-1}$ of seeds)
ER1-18	42.2	57.4	4.1
ER1-223	44.9	60.4	2.7
ER1-265	46.7	56.7	2.0
ER1-280	47.1	58.8	2.1
ER1-317	44.7	57.7	3.6
ER5-7	47.5	57.1	7.9

Molecular markers

Markers for high oleic acid trait (fad2 gene)

In order to use molecular marker assisted selection for breeding of oilseed rape with high oleic acid content in seeds, the mutated M-10453, M-10464 and wild type genes *fad2* were sequenced, making it possible to create the new marker system focused on detection of these specific mutations in breeding populations. These studies were carried out in cooperation with INRA (LeRheu, France) and the results are patented (patent number: FP1862551 A1).

Markers for low linolenic acid trait (fad 3 gene)

Allele-specific SNP markers were developed for the low-linolenic mutant M-681 genotype of winter oilseed rape (Spasibionek, 2006). The markers identify two statistically important point mutations in genes of FAD3 desaturase. One mutation site was identified in the *fad3A* gene, another mutation in the *fad3C* (Mikolajczyk et al., 2007).

Markers for CMS ogura

The new created genotypes with desired quality traits are introduced into CMS *ogura* hybridization system to improve their yielding ability. This process is realized with the use of molecular markers.

The *Rfo* restorer gene for CMS *ogura* was identified with the use of a SCAR marker (Mikolajczyk et al., 2006) developed from the $OPC02_{1150}$ RAPD marker closely linked to the *Rfo* restorer gene (Delourme et al., 1994) (Fig. 1).The presence of the CMS *ogura* is checked

by application of a SCAR marker developed by Sigareva and Earle (1997) for identifying the o*gura* male-sterile cytoplasm in *B. oleracea*, and adapted for *B. napus* (Mikolajczyk et al., 1998) (Fig. 2).

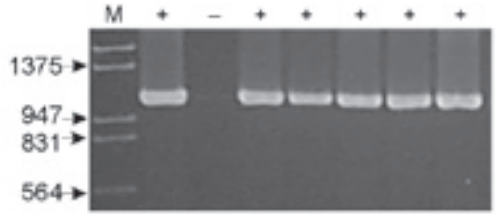

Figure 1. SCAR analysis for the presence (+) or absence (-) of the *Rfo* restorer gene in *B. napus*; bp – base pairs; M – λ Hind III / Eco RI molecular size marker

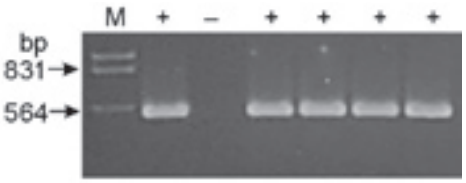

Figure 2. SCAR analysis for the presence (+) or absence (-) of the Ogura male-sterile cytoplasm in *B. napus*; bp – base pairs; M – λ Hind III / Eco RI molecular size marker

Conclusion

Development of new type cultivars of oilseed rape can increase the competitiveness of this plant on the world market of oilseed crops.

The markers for CMS *ogura* hybridization system – for cytoplasm and restorer gene as well as markers for important fatty acids combined with DH lines development are very helpful in breeding of cultivars possessing different quality characters with good yielding ability thanks to the development of F_1 hybrids.

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Evaluation of fruit quality in a lemon cybrid with mandarin mitochondria

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ABSTRACT: Nutritional and organoleptic qualities of fruit are currently very important objectives for plant breeders. Organic acids, sugars and carotenoids were studied by high liquid chromatography on the pulp of a citrus cybrid. This cybrid named 'WLM + EUR' (*Citrus deliciosa* Ten.) + (*Citrus limon* (L.) Burm.), inherited nuclear and chloroplasts genomes of Eureka lemon (*Citrus limon* (L.) Burm.) plus mitochondria from Willow leaf mandarin (*Citrus deliciosa* Ten.). In our work, impact of new mitochondria on fruit quality was studied during the maturity period. We observed that the cybrid was different from willow leaf mandarin and close to lemon parent. Organic acids level is slightly increased in the cybrid fruit pulp compared to Eureka lemon. No significant difference is observed in sugars and carotenoids between the cybrid and the lemon. The results confirm that mains genetic information for sugars, organic acids and carotenoids biosynthesis are contained in the nucleus. Cybridisation should be used in *citrus* as a strategy to breed specific traits associated with mitochondrial genomes such as male sterility without affecting the main organoleptic and nutritional qualities.

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Plant genetic resources of barley for antioxidants

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ABSTRACT: The aim of the study was to determine the content of vitamin E, which together with its isomers (tocopherols and tocotrienols) belong to a group of antioxidants. The antioxidative substances play an important role in the storage of food; they positively affect human health and slow down the aging process. Vitamin E was analyzed in barley green mass of two spring malting varieties (Malz and Sebastian) and one line KM 1910 in two growing phases (DC29, DC31). Material was grown at the locality Kroměříž and Žabčice (CZ) over 2005-2007.

On average, for all the monitored factors (varieties, years, samplings, localities), the variety Sebastian had significantly higher activity of vitamin E (73.06 $mg.kg^{-1}$) compared to the variety Malz (61.84 $mg.kg^{-1}$), while the line KM 1910 (67.81 $mg.kg^{-1}$) did not differ statistically significantly from both varieties. The variety Sebastian (82.61 $mg.kg^{-1}$) together with the line KM1910 (80.72 $mg.kg^{-1}$) had a significantly higher content of total tocols when compared to the variety Malz (73.02 $mg.kg^{-1}$). The variety Sebastian compared to this variety also had a significantly higher content of alpha-tocopherol (71.15 – 59.69 $mg.kg^{-1}$). The line KM1910 was characterized with a significantly higher content of beta+gama-tocopherol (12.67 $mg.kg^{-1}$) and delta-tocopherol (2.42 $mg.kg^{-1}$) in comparison with the other two varieties. Green mass in the first sampling had statistically significantly higher activity of vitamin E, content of total tocols, α- and $\beta+\gamma$-tocopherols (74.53; 85.58; 71.92 and 12.12 $mg.kg^{-1}$, respectively) than the second sampling. In 2007 statistically significantly lower contents of total tocols (62.21 $mg.kg^{-1}$) and activity of vitamin E (52.59 $mg.kg^{-1}$) were observed, which also corresponds to the lowest value of alpha-tocopherol (49.77 $mg.kg^{-1}$) when compared to the 2006 and 2005 years. The highest average values of vitamin E activity (75.19 $mg.kg^{-1}$ d.m.) were assessed in 2006 and content of total tocols in 2005 (87.63 $mg.kg^{-1}$ d.m.). The highest average activity of vitamin E was observed in the first sampling in the variety Sebastian (81.54 $mg.kg^{-1}$). The growing localities did not affect antioxidative activity of vitamin E statistically significantly. Barley green mass did not contain isomers of vitamin E – tocotrienols, which we observed in barley caryopses (Ehrenbergerova et al., 2006).

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Grain quality of hybrid populations in crossings of hulled and hulless barley

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ABSTRACT: The study was carried out at the State Stende Cereals Breeding Institute in 2006-2007. Crossings were done between hulled (HD) and hulless (HS), HS and HD, and HS barley varieties – 3 combinations for each variant. The crude protein, starch and β-glucan contents in hybrid populations of F_3 and F_4 were evaluated. The protein content in hybrid populations had mid-parent value; there was a prevalent parent higher in protein content in all cross combinations HD/HS, HS/HD, HS/HS. The starch content of hulless hybrid populations exceeded that of hulled barley in crosses: HD/HS, HS/HD by 21-24 g kg^{-1} in 2006 and by 5-18 g kg^{-1} in 2007. In both hulled and hulless populations the starch content was lower than that of the corresponding parents. The starch content had mid-parent value or value on the level of parents in crosses HS/HS. In 2006, β-glucan content was lower in hybrid populations compared to parents used in hybridisation.

Keywords: Barley, hulless, hulled, hybrid population, quality.

Introduction

Plant breeding toward improving barley quality corresponding to requirements of different end uses is the main goal in different barley breeding programs. Hulless barley has been recognised for more valuable traits such as higher crude protein, digestible energy and β-glucan content compare to hulled barley (Bhatty, 1999). Both consumers and plant breeders are interested in these traits. The effect of hulless gene *nud* on agronomic traits of barley has been investigated (Choo et al., 2001). More studies are needed to understand better this gene effects on quality traits to develop efficient selection strategies for hulless barley programs. The aim of this investigation was to evaluate effect of hulless gene on grain quality in hybrid populations (F_3 and F_4) in crossings between hulled and hulless barley.

Material and Methods

The field experiments were conducted at the State Stende Cereals Breeding institute (Latvia) in 2006 and 2007. Crossings were done between hulled (Justina, DE, Simba, DK, Gate, LV) and hulless (L-302, LV, Wanubet, USA, Freedom , CA), hulless (Freedom, CA, Wanubet, USA) and hulled (Ansis and Kristaps, both Latvia, Jersey, NL), and hulless (Merlin, McGwire, both CA, KM 2045 and KM 2084, both CZE, SW 1291, SE) barley varieties. Three cross

combinations were estimated for each variant. F_2 hybrid populations were divided in the fractions of hulled and hulless grain. The hulless and hulled hybrid populations were sown separately in plot area 1 m^2 with seed rate 300 germinating seeds. The crude protein (g kg^{-1}), starch (g kg^{-1}) and β-glucan (g kg^{-1}) for hybrid populations of F_3 in 2006 and F_4 in 2007 were examined by Infratec 1241 Grain Analyzer.

During investigation years meteorological conditions differed. The growing season of 2006 characterized with deficit of moisture (only 77% to norm). Very hot and dry weather conditions were in the 1st and 2nd decades of July and also in the 1st decade of August. In 2007, the vegetation period was rich in precipitation (120%), particularly in May and July. It influenced grain filling and quality of grain in both experimental years.

Table 1. Barley grain quality in cross combinations between hulled and hulless barley.

Cross combination	Type of barley	Crude protein content, g kg^{-1}		Starch, g kg^{-1}		β-glucan, g kg^{-1}	
		2006*	2007**	2006*	2007 **	2006*	2007**
♀Justina	Hulled	158	109	604	621	37	31
K 04-9 Justina/ L-302	Hulled	165	155	595	598	25	44
	Hulless	181	173	616	603	39	54
Hulless *vs* hulled		+16	+18	+21	+5	+14	+10
♂L-302	Hulless	206	180	596	596	54	45
♀Simba	Hulled	113	117	649	617	34	32
K 04-10 Simba/ Wanubet	Hulled	170	156	603	603	26	45
	Hulless	182	157	627	621	44	56
Hulless *vs* hulled		+8	+1	+24	+18	+18	+11
♂Wanubet	Hulless	181	156	625	646	57	49
♀Gate	Hulled	161	124	610	616	34	34
K 04-41 Gate/ Freedom	Hulled	163	145	604	600	35	42
	Hulless	171	154	625	616	43	55
Hulless *vs* hulled		+8	+9	+21	+16	+8	+13
Freedom	Hulless	165	155	631	620	56	53

*F_3 **F_4

Results and discussion

Hulled parent varieties characterized with lower crude protein and β-glucan content than hulless varieties (Table 1). The protein content was higher in the dry and hot year 2006 compared to the wet and cool year 2007. In both years the protein content of hybrid populations had mid-parent value and it was closer to parent with higher value of protein. Higher grain protein content (154-182 g kg^{-1}) was observed in hulless populations compare to hulled ones

(145-170 g kg^{-1}). The starch content of hulless hybrid population was exceeded by 21-24 g kg^{-1} in 2006 and by 5-18 g kg^{-1} in 2007 compared with hulled barley. In both of these populations the starch content was lower than for the corresponding parents. The β-glucan in barley grain is a significant component of dietary fibre favourably affecting human health. However, they are not desirable in brewing and feed industries (Newman and Newman, 1992). In our cross combinations, negative transgression was stated regarding to β-glucan content in hulled hybrid populations and mid-parent value in hulless populations in 2006, but it was not observed in 2007. The hulless barley exceeded hulled one in the β-glucan content.

Table 2. Barley grain quality in cross combinations between hulless and hulled barley.

Cross combination	Type of barley	Crude protein content, g kg^{-1}		Starch, g kg^{-1}		β-glucan, g kg^{-1}	
		2006*	2007**	2006*	2007**	2006*	2007**
♀Freedom	Hulless	165	155	631	620	56	53
K 04-6	Hulled	168	136	601	605	32	44
Freedom/Ansis	Hulless	174	144	633	622	52	57
Hulless *vs* hulled		+06	+08	+22	+17	+20	+1.3
♂Ansis	Hulled	128	131	62.6	610	35	34
♀HB 803	Hulless	201	176	621	615	59	46
K 04-32	Hulled	177	153	601	601	38	44
HB 803/Jersey	Hulless	191	148	617	623	44	55
Hulless *vs* hulled		+14	-05	+16	+22	+06	+11
♂Jersey	Hulled	168	130	611	618	35	32
♀Wanubet	Huless	181	153	625	633	57	45
K 04-38	Hulled	159	116	605	621	28	46
Wanubet/Kristaps	Hulless	174	129	623	638	46	58
Hulless *vs* hulled		+15	+13	+18	+17	+18	+12
♂Kristaps	Hulled	172	138	595	604	32	31

*F_3 **F_4

Similar relationships were observed in a cross combination between HS and HD barley as well as in a cross combinations between HD/HS (Table 2). It means that it was not so important which of parent HS or HD was used as female or male parents in crossings.

In cross combinations between hulless barley genotypes the protein content of hybrid populations had mid-parent value (Table 3). Also in these combinations higher crude protein content of parent prevailed over parent with lower crude protein content. The starch content of hybrid populations had mid-parent value if parents differed by these indices (K04-20,

K04-44) or they had value equal to parent if there were no differences between them (K04-43). In our hybrid population, the content of β-glucan differed depending on recombination and influence of weather conditions. Therefore it was possible to obtain hybrid population with a different level of crude protein and starch content.

Table 3. Barley grain quality in cross combinations between hulless barley.

Cross combination	Type of barley	Crude protein content, g kg^{-1}		Starch, g kg^{-1}		β-glucan, g kg^{-1}	
		2006*	2007**	2006*	2007**	2006*	2007**
♀ Merlin	Hulless	186	174	613	617	50	61
K 04-20 Merlin/KM 2045	Hulless	182	164	631	610	52	55
♂KM 2045	Hulless	125	156	662	620	44	41
♀KM 2084	Hulless	184	166	611	608	52	46
K 04-43 KM 2084/SW 1291	Hulless	183	145	608	611	49	56
SW 1291	Hulless	166	156	612	614	57	46
♀Merlin	Hulless	186	174	613	617	50	61
K 04-44 Merlin/ McGwire	Hulless	179	142	629	630	52	60
♂McGwire	Hulless	149	126	634	646	53	49

* F_3, ** F_4

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Detection of QTLs for carotenoid content in durum wheat

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ABSTRACT: Durum wheat is mainly used to produce semolina and pasta. A defining factor for the commercial value of pasta is its colour, which depends on the semolina carotenoid pigment content and on the oxidative enzymatic activity. Among carotenoids controlling yellow colour, the presence of β-carotene is also important as precursors of vitamin A. In order to answer the demands of the market asking for amber-coloured pasta, breeding programs tend to select varieties with a high grain carotenoid content. The aim of the present study was to detect quantitative trait loci (QTL) for carotenoid content in a segregant population of 120 recombinant inbred lines (RILs) derived by crossing the Italian durum wheat cultivars Svevo and Ciccio characterised by low and high values of carotenoid content, respectively. Replicated field trials were conducted at two locations in southern Italy in 2006 and 2007. Grain carotenoid content showed a wide variability among RILs and frequency distribution typical of quantitative traits, proving itself very heritable and not much affected by environmental factors. A negative correlation with 1000 kernel weight was observed. A genetic linkage map was constructed with 285 genomic and EST-derived microsatellite markers. Simple interval mapping (SIM) and composite interval mapping (CIM) for each location and across environments were used for mapping QTLs. Three major QTLs located on 4B, 7A and 7B chromosomes were identified with a LOD>2.9 determined by permutation test. Molecular markers associated with high carotenoid content and not with low kernel weight can be used in marker assisted selection programs.

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Allelic diversity of HMW- and LMW- glutenin subunits wheat varieties (*Triticum aestivum L.)* registered in the Czech Republic

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ABSTRACT: A collection of 86 Czech registered winter wheat varieties was analysed to evaluate the composition of high molecular weight (HMW-GS) and low molecular weight (LMW-GS) glutenin subunits. These proteins were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Intervarietal polymorphism of HMW and LMW glutenin subunits was detected. Twenty one different patterns for HMW- and eighteen for LMW-glutenins were identified. The different alleles encoded at the 6 glutenin loci were determined. At *Glu-A1, Glu- B1* and *Glu-D1,* encoding high HMW-GSs, 3, 6 and 4 alleles were observed respectively. At loci *Glu-A3, Glu- B3* and *Glu-D3* three, eight and three alleles of LMW-GSs were found respectively. Assessed varieties were divided to categories of baking quality and these categories were compared from viewpoint of HMW-GS and LMW-GS alleles.

Keywords: glutenin subunits, characterization, SDS-PAGE, genetic diversity, *Triticum aestivum L.*

Introduction

Glutenin, a major class of wheat storage proteins, is polymeric and consists of high molecular weight and low molecular weight subunits. Glutenin subunits have been separated by SDS-PAGE, with HMW glutenin subunits encoded by G*lu-A1, Glu-B1,* and G*lu-D1* (Payne 1987) and LMW glutenin subunits by G*lu-A3, Glu-B3,* and G*lu-D3* loci (Singh and Shepherd 1988; Pogna et al. 1990). For many years, the high-molecular-weight glutenin subunits (HMW-GSs) have been especially important for quality screening, using the *Glu-l* scoring system. The work of Payne *et al*. (1980) provided evidence of a strong association between the presence of certain alleles coding for HMW-GSs and bread-making quality. Low -molecular-weight glutenin subunit composition (LMW-GSs) is also involved in dough properties hexaploid wheat (Gupta et al., 1994, Eagles et al., 2002).

Material and methods

Plant material

For electrophoretic analyses of HMW-GSs and LMW-GSs standard (etalon) samples of kernels of 86 registered winter wheat varieties were used; the samples were supplied by the Central Institute for Supervising and Testing in Agriculture (CISTA-Czech republic).

Electrophoresis

Glutenins were extracted from single crushed wheat kernels using procedure of Singh et al. (1991) and Bradova (2006). Proteins were fractionated by one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemmli buffer system (Laemmli, 1970). The acrylamide/ bisacrylamide concentration (T) and the cross linker (C) were used as follows, T=10%, and C=2.60%. Electrophoresis was performed at a constant current of 30mA/gel at 10 °C for the time required for the tracking marker dye to migrate off the gel. Protein in the gels were fixed for 1 hour with 10% (*w* /*v*) trichloroacetic acid solution and subsequently stained with 0.5% (*w*/*v*) Coomassie Brilliant Blue R-250 solution, 25% (*v*/*v*) methanol, and 10% *(v/v)* acetic acid. Destaining was carried out with running water.

Nomenclature

The bands of HMW-GSs were read using the nomenclature described by Payne and Lawrence (1983). The nomenclature of Jackson et al. (1996) and Branlard et al. (2003) was used for LMW-GSs.

Table 1. Allele frequencies of *HMW-GSs* and *LMW-GSs* wheat varieties registered in the CR.

		HMW-GSs				LMW-GSs		
Locus	Alelle	HMW-GS	Frequency	%	Locus	Alelle	Frequency	%
Glu-A1	a	1	24	28	*Glu-A3*	a	40	47
	b	2*	1	1		d	25	29
	c	null	61	71		e/f	21	24
Glu-B1	a	7	2	2	*Glu-B3*	a	3	4
	b	7+8	15	17		c	1	1
	c	7+9	34	40		c(d)	2	2
	d	6+8	28	33		d	4	5
	e	20	1	1		f	1	1
	i	17+18	6	7		g	58	67
						j	14	16
						?	3	4
Glu-D1	a	2+12	27	32	*Glu-D3*	a	1	1
	b	3+12	1	1		b	3	4
	c	4+12	1	1		c	82	95
	d	5+10	57	66				

Results and discussion

Polymorphism of HMW-GSs and LMW-GSs

In the studied set of 86 wheat varieties twenty one different patterns for *HMW*- and eighteen for *LMW*- glutenins were identified and a total of 50 different patterns were obtained at

the *Glu-1* and *Glu-3* loci in this collection. 3, 6 and 4 *HMW-GS* alleles were identified at *Glu-A1, Glu-B1* and *Glu-D1* loci respectively. Fourteen alleles encoding *LMW-GSs* were observed in the collection. 3, 8 and 3 alleles corresponded to *Glu-A3, Glu-B3* and *Glu-D3* loci. The highest allelic variability of *HMW-GSs* and *LMW-GSs* was determined at locus *Glu-B1* (Table 1).

Some *LMW-GS* alleles were hard to identify. Mainly the locus *Glu-B3* is characterized by higher variability and the migration of some subunits controlled this locus is similar to the migration of subunits controlled by the locus Glu-*A3*. We used genotypes with the known *LMW-GS* allele composition (Chinese Spring: *Glu-A3a, Glu-B3a, Glu-D3a;* Gabo: *Glu-A3b, Glu-B3b, Glu-D3b;* Orca: *Glu-A3c, Glu-B3c, Glu-D3c*) to characterize Czech wheat varieties (Branlard et al., 2003). Varieties Karolinum, Livia and Rialto are characterised the same allele controlled by the locus *Glu-B3,* but we have not been successful in the identification of this allele.

Allele frequencies

In the Czech Republic registered varieties are grouped to four categories according to their baking quality by the Central Institute for Supervising and Testing in Agriculture (CISTA - Czech Republic), (Table 2).

Table 2. Labelling of baking quality categories wheat varieties registered in the CR (CISTA).

Mark	Category	Quality of wheat varieties
E	elite	very good; improving
A	high-quality	good; separately workable
B	bread-making	additive; used in blend
C	unsuitable	unsuitable for yeast dough production

The evaluated varieties were divided to the baking quality categories and the allele frequencies in the individual categories were observed (Table 3).

The highest frequency in the all baking quality categories (next BQC) was detected in the "null" *Glu-A1c* allele. BQCs labelling "E", "A" and "B" were characterized the highest occurrence alleles *Glu-B1c* (7+9*)* and *Glu-D1d* (5+10) that are known by their favourable effect on dough properties. On the contrary alleles that have a negative effect on dough strength were found in higher representation in BQC labelling "C" (varieties unsuitable for yeast dough production), i.e. *Glu-B1d* (6+8*)* and *Glu-D1a* (2+12). These alleles were present in 54% and 71% of the varieties in the baking quality category "C" respectively.

In point of *LMW-GSs* alleles *Glu-A3a, Glu-A3d* and *Glu-B3g* have a positive effect on dough properties (strength and extensibility) and the allele *Glu-D3e* has been reported to have an unfavorable effect on dough properties (Branlard et al. 2003). And just some

Table 3. Alleles of *HMW-* and *LMW-GSs* with the high occurrence in varieties divided to baking quality categories.

Locus	Allele	Baking quality categories (BQC)							
		E (6 varieties)		A (34 varieties)		B (22 varieties)		C (24 varieties)	
		n	%	n	%	n	%	n	%
Glu-A1	0	5	83	20	59	17	77	19	79
Glu-B1	c (7+9)	3	50	15	44	10	45		
	d (6+8)							13	54
Glu-D1	d (5+10)	6	100	27	79	18	82		
	a (2+12)							17	71
Glu-A3	a			19	56	12	55		
	d	4	67						
	e/f							11	45
Glu-B3	g	4	67	26	76	13	59	15	63
Glu-D3	c	5	83	32	94	22	100	23	96

of these alleles (*Glu-A3a, Glu-B3g* and *Glu-D3c)* were found as the most frequent in our collection of varieties, 47%, 67% and 95% respectively. The allele *Glu-A3d* occurred in the highest frequency in varieties ranked to the category "E" (Table 2). But only 6 varieties were ranked in this category. The allele *Glu-A3e/f* was present in 45% of the varieties ranked to the category "C" (varieties unsuitable for yeast dough production). This allele probably does not have any positive effect on dough properties.

Analysis of glutenins is known to be a powerful tool for evaluation of genetic recourses. The glutenin characteristics of the evaluated varieties can be purposefully used for the creation of new wheat varieties.

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Recuperation of wild edible vegetables for human nutrition: mineral elements profiles

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ABSTRACT: Wild plants are an abundant food resource that is generally predictable. Most species are easy to collect and many fruits and seeds can be easily preserved. They have been essential in the survival of ancient civilizations and also in the few remaining hunter-gatherer communities. However, wild edibles have not been completely neglected by traditional agricultural societies. Until recently, they were used as supplementary foods, playing an important role, especially in times of scarcity and sometimes were also used as medicines (Tardío et al. 2005). Nowadays, there is an increasing interest in wild edible plants, especially caused by their relation to human health, being considered as possible sources of 'nutraceuticals' (Heinrich et al. 2005). Therefore, there is a future possibility for the development of sustainable harvesting or even the domestication of wild vegetables, including their growing, processing and marketing on a more commercial scale. Previous studies have revealed the high vitamin C content of some wild edible plants traditionally consumed in Spain (Cabrera et al., 2008). By studying the nutritional value of some wild plants consumed in a traditional way in Spain, we provide new data for selecting the richest species in micronutrients. They could be included in the diet providing health benefits. This research aims to recover and preserve natural genetic resources for human nutrition, revaluating the consumption of traditional wild plants and promoting their use as sources of new food ingredients. In this work, macro and microelements (Na, K, Ca, Mg, Cu, Fe, Zn, Mn) were analyzed, in order to characterize the mineral micronutrients of leaves from 14 different species of wild plants commonly gathered in Spain. This study seeks to reveal the specific profile of macro and microelements, as a biomarker of nutritional value of these food species. Analytical determinations of ashes and mineral elements were performed on freeze-dried samples, which were brought to dry –ashes by using a microwave method, and then extracted in an acid mixture. Mineral concentrations were determined by Atomic Absorption Spectroscopy. From the analytical results, we can select *Montia fontana* and *Chrondilla juncea* as the species with higher Fe content (1.90 and 1.45 mg/100g); *Silybum marianum, Sonchus oleraceus, Papaver rhoeas, Beta maritima* and *Taraxacum erythrospermum* with important

amount of Ca (210-261 mg/100g), sample *Beta maritima* with values of K of 2.35 g/100g being the rest of the samples superior to 105 mg/100g and finally samples *Chrondilla juncea*, and *Papaver rhoeas* due to its higher Zn content from 1.24 to 2.64 mg/100g. These Ca, K and Zn values are much higher than the data reported in the literature for cultivated species of vegetables.

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Characterization and evaluation of traditional varieties of lettuce grown in organic farming

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ABSTRACT: The present study is aimed at the development of a protocol for the analysis of morphological, agronomical, and chemical characteristics of traditional varieties of lettuce grown in an organic farming system. As a result, a collection of descriptors has been selected, enabling the rapid and efficient identification of varietal types through multivariate analysis. A wide variability has been identified in the collection characterized, which allows the selection of the best accessions in order to re-introduce them in organic farming systems, though it is advisable the application of low selection pressures in order to increase homogeneity in agronomical traits. A complete chemical analysis has shown that it is not necessary to distinguish between internal and external parts of the lettuces. Even though there are significant differences in mineral and antioxidant contents, being generally higher in the external part, the differences among varieties considering only one of the parts represent as well the variability in the other. The variability in nutritional aspects also enables the selection of accessions with an added value within varietal types, as well as encouraging the promotion of certain types.

Introduction

A considerable part of the genetic resources of lettuce, especially those corresponding to the cultivated species, is effectively conserved *ex situ* in seed banks. Nevertheless, the utilization of these resources, which is the final purpose of the conservation, still requires more attention. In this context, an efficient characterization and evaluation should be encouraged. This is the purpose of this study, aimed at the development of a protocol for the analysis of morphological, agronomical, and chemical characteristics of traditional varieties of lettuce grown in an organic farming system. This growing system was selected as its development is tightly linked with the recovery of traditional varieties and represents the best chance to tackle on-farm conservation of these genetic resources.

Materials and methods

A collection of 22 accessions (20 plants/accession) was evaluated, representing romaine, mini romaine, leaf, butterhead, and batavia lettuces. The following traits were measured, including morphoagronomical characteristics and chemical determinations: gross weight, gross diameter (measured and 2/3 height), commercial weight (after discarding external

non commercial leaves), commercial diameter, % marketability, leaf length, maximum leaf width, leaf length/width ratio, proximal leaf midrib width, distal leaf midrib width (measured at 2/3 leaf length), colour (H L,a,b), lettuce section length, lettuce section width, stem length, maximum stem width, distal stem width (measured at 2/3 leaf length), heading habit, texture, head solidity, leaf blade blistering, distal marginal incisions, lateral marginal incisions, leaf division, leaf curling, leaf angle, anthocyanin presence, tipburn sensitivity, downy mildew sensitivity, powdery mildew sensitivity, bolting resistance, dry matter, ash, total polyphenolics content, total nitrate content, proteins, phosphorus, calcium, iron, copper, magnesium, zinc, sodium, potassium, ascorbic acid. Chemical determinations were carried out following widely used and recognized determination methods using 18 of the accessions. Principal component analysis was carried out with morphoagronomical and chemical data independently.

Results and discussion

The principal component analysis of morphoagronomical traits accounted for 52.3% of the variability with the first three components, which were selected using the criterion described by Krzanowski (2000). For the analysis, the variables section length and width, gross weight and diameter, maximum stem width and head solidity were discarded due to high correlation with other traits or high uniformity in the collection assayed. The rest of the variables showed little overlapping when variable weights were represented for each principal component (Figure 1), confirming the good selection of variables. Biplots for components 1-2, 1-3, and 2-3 enabled identification of the main varietal types (leaf lettuce, batavia, butterhead, mini romaine and romaine) and proved to be very useful to establish relations between different accessions in a rapid and easy way. A wide variation was identified, especially in the romaine group, which was represented by a greater number of accessions. In this sense, variability can be found for morphological and agronomical traits such as weight, head solidity, bolting resistance, days to harvest and tipburn sensitivity, and even for homogeneity. This enables the selection of accessions with good agronomic performance in organic farming systems. Nevertheless, considering the variability found in traits such as lettuce commercial weight, diameter and length (table 1), it is advisable the application of low selection pressures in order to increase homogeneity in morphological traits.

In order to promote the recovery of traditional varieties in the European market, besides good agronomic performance it is also important to highlight the nutritive and functional characteristics of these genetic resources. In this context, a complete chemical analysis was carried out. Higher concentrations of Zn, Cu, P, ascorbic acid and proteins were determined on internal samples, while higher concentrations of Fe, Mg, K and nitrates were detected on external samples. These results are coherent with those described earlier in lettuce (Abu-Rayan et al., 2004) and the differences between outer and inner leaves may be explained considering the amount of solar radiation received by each part (Matt et al., 2001). In particular, higher concentrations of Mg and Fe were detected on external leaves, an expected result, as these leaves contain higher amounts of chlorophyll. Fe concentration was negatively correlated

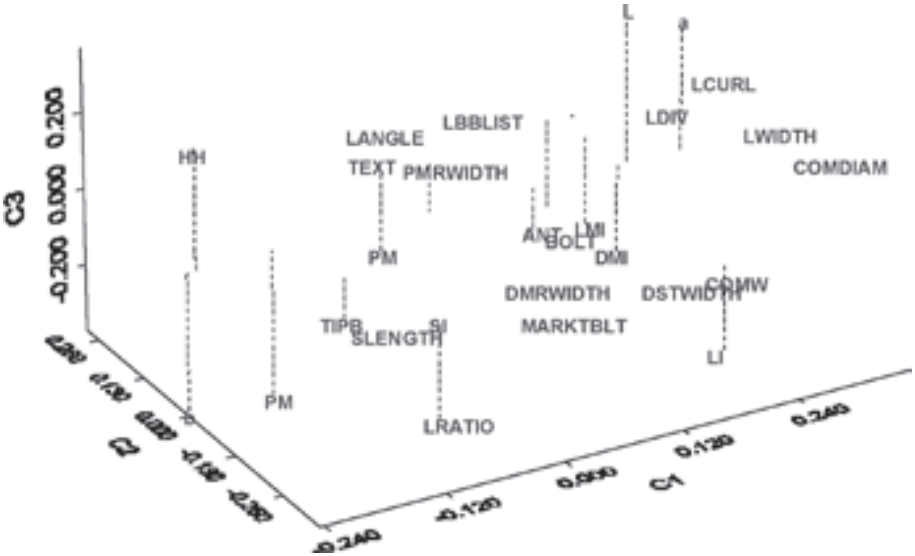

Figure 1. Representation of variable wights for the three first principal components obtained in the PCA of morphoagronomic traits. HH: heading habit; Ll: leaf length; SLENGTH: stem length; LWIDTH: leaf width; DSTWIDTH: distal stem width; ANT: anthocyans; COMDIAM: commercial diameter; MARKTBLT: % marketability, TEXT: texture; LMI: lateral marginal incisions; DMI: distal marginal incisions; DMRWIDTH: distal midrib width; BOLT: bolting; DM: downy mildew; PM: powdery mildew; COMW: commercial weight; LANGLE: leaf angle; PMRWIDTH: proximal midrib width; LRATIO: leaf length/width ratio; LCURL: leaf curling; LBBLIST: leaf blade blistering; LDIV: leaf division; TIPB: tipburn; H, a, b: Hunter L, a, b coordinates.

Table 1. Coefficients of variation for commercial weight, commercial diameter and lettuce length.

	TL1	V-LA-27	CA-LA-11	CA-LA-9	AN-LA-15	Grum	Nav2	TL4	C-LA-26	V-LA-32	AN-LA-25	AN-LA-6	B-LA-3	C-LA-38	Carrus	CL-LA-21	CL-LA-30	TL2	V-LA-10	V-LA-16	V-LA-22	V-LA-8
Cw[1]	0.12	0.28	0.35	0.14	0.44	0.22	0.23	0.21	0.18	0.11	0.18	0.31	0.35	0.21	0.23	0.11	0.14	0.21	0.19	0.16	0.30	0.13
Cd[2]	0.09	0.13	0.18	0.07	0.21	0.11	0.10	0.06	0.07	0.06	0.13	0.18	0.15	0.15	0.12	0.05	0.06	0.09	0.07	0.09	0.11	0.07
L[3]	0.08	0.13	0.09	0.09	0.26	0.09	0.05	0.11	0.21	0.09	0.11	0.11	0.09	0.04	0.15	0.05	0.05	0.11	0.06	0.12	0.05	0.04

[1]Commercial weight ; [2]Commercial diameter; [3]Lettuce height.

with Hunter b coordinate. Leaf lettuce showed the highest values of nutrient accumulation, probably because these lettuces do not head and the light is more uniformly received in all the leaves (Mou and Ryder, 2004).

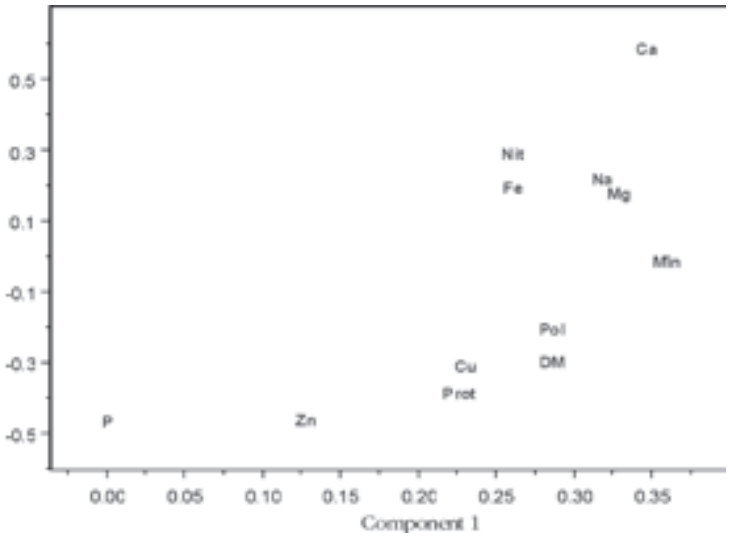

Figure 2. Representation of variable weights for the two first principal components obtained in the PCA of chemical determinations.

High correlations were found between different components in each type of sample, though contents between internal and external samples for each component were not detected. A principal component analysis was carried out for chemical determinations, selecting the two first components following the same criterion already described. These two components explained 86.2% of variation when internal and external samples were considered independently. Most variables were positively correlated with the first component (Figure 2), so that this component reflects higher amounts of nearly all the compounds analyzed. In the biplot of the analysis, samples from outer and inner leaves were clearly grouped and differentiated in the second component (Figure 3). Despite the lack of correlation in components between each type of sample, considering only the external samples or the internal samples, the relative position of each accession was similar in each group, especially considering the first component. This confirms that the accessions that show higher contents in the external sample also have higher concentrations in the internal sample. It can be said, then, that even though there are significant differences in mineral and antioxidant contents, being generally higher in the external part, the differences among varieties the analysis of only one of the parts represent as well the variability in the other, thus enabling the use of only one sample for a rapid an easy preliminary evaluation of accessions. It is recommended the selection of the external sample as it shows higher, and thus limiting, concentration of nitrates.

The variability in nutritional aspects also enables the selection of accessions with an added value within varietal types, as well as encouraging the promotion of certain types.

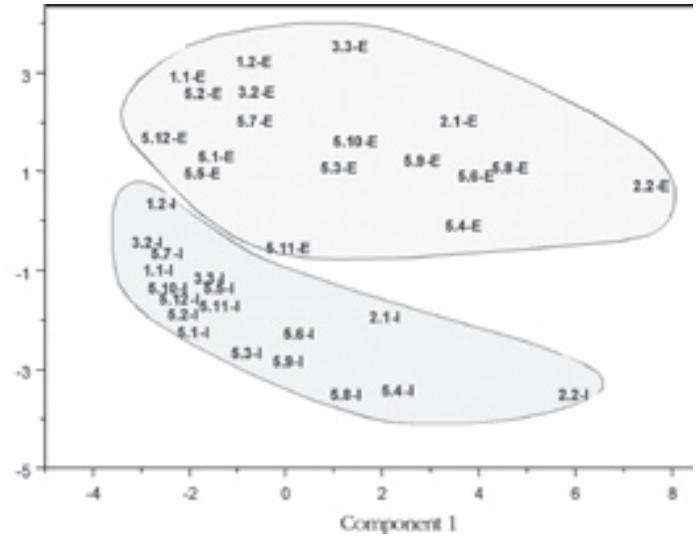

Figure 3. Principal component analysis representation of chemical determinations of internal (-I) and external (-E) samples. 1.1:TL1, 1.2:V-LA-27, 2.1:CA-LA-11, 2.2:CA-LA-9, 3.2:Grum, 3.3:Nav2, 5.1:AN-LA-25, 5.2:B-LA-3, 5.3:C-LA-38, 5.4:Carrus, 5.5:CL-LA-21, 5.6:CL-LA-30, 5.7:TL2, 5.8:V-LA-10, 5.9:V-LA-16, 5.10:V-LA-22, 5.11:V-LA-8, 5.12:AN-LA-6. First number indicates varietal type:1-batavia, 2-leaf lettuce, 3-mini-romaine, 5-romaine.

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Evaluation of tartary buckwheat collection in the Czech Republic

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ABSTRACT: Thirty-two tartary buckwheat accessions were evaluated during two years under conditions of the Czech Republic. Several characters chosen from Descriptors for buckwheat (IPGRI, 1994) were evaluated. The similarity dendrogram was created according to morphological and phenological features. The most numerous group was B in which the varieties from Bhutan and one from Pakistan were situated.

Keywords: Tartary buckwheat collection, plant genetic resources, field evaluation.

Introduction

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is usually put in the shade of common buckwheat *Fagopyrum esculentum* Moench, because of its bitter taste. On the other hand, tartary buckwheat has exceptional protein composition and contains five times more rutin than common buckwheat (Liu et al., 2007). Tartary buckwheat is mainly grown in China, Bhutan, Northern India and Nepal and consumed or traded locally (Zongwen, 2006). In Europe, tartary buckwheat was reported in Poland, grown as a medical plant for rutin, and found in Slovenia (Kreft, 1995) the only one place, where tartary buckwheat is cultivated for human consumption in Europe in the present time, is Islek. It is a small region on the borders of Luxembourg, Germany and Belgium (Zewen et al., 2007).

The use of tartary buckwheat is in a similar way as common buckwheat (Kreft, 1994).

The breeding objective is to find out the new variety with the high yield and high quality, and wide adaptation. A special variery with the high bioflavonoid content and with effective affect to diseases needs to be selected. The elite specimen means wide adaptive ability, strong resistance to seed shattering, pre-eminent resistance to lodging (Gang et al., 2006). In the Czech Republic, there is not any variety of tartary buckwheat registered (Šafaříková, 2007).

Tartary buckwheat breeding is not very developed and there are not almost any experiences with intensive cultivation in the Czech Republic. The basic requirement for tartary buckwheat breeding is the description genetic variability and its characterization.

Material and methods

Plant material

The seeds samples of tartary buckwheat varieties used in this study were obtained from buckwheat collection the Department of Gene Bank, Crop Research Institute in Prague, the Czech Republic. Table 1 shows all 32 evaluated genotypes.

Table 1. Genotypes of tartary buckwheat evaluated in the present study.

	ECN	Origin		ECN	Origin		ECN	Origin		ECN	Origin
1	01Z510001	BTN	9	01Z510013	MEX	17	01Z510027	BTN	25	01Z510039	BTN
2	01Z510004	BTN	10	01Z510014	USA	18	01Z510028	USA	26	Jiujing	CHN
3	01Z510005	BTN	11	01Z510017	unknown	19	01Z510031	BTN	27	Jiianzui	CHN
4	01Z510008	BTN	12	01Z510021	unknown	20	01Z510032	BTN	28	Liu	CHN
5	01Z510009	BTN	13	01Z510023	BTN	21	01Z510034	BTN	29	Qianwei	CHN
6	01Z510010	GER	14	01Z510024	BTN	22	01Z510035	BTN	30	Zhaoqiao	CHN
7	01Z510011	PAK	15	01Z510025	BTN	23	01Z510036	BTN	31	Dianning	CHN
8	01Z510012	CZE	16	01Z510026	BTN	24	01Z510038	BTN	32	Jinqiao	CHN

Field evaluation

The seeds of the tartary buckwheat varieties were sown in the year 2006 all on May 4 and in the year 2007 on April 24, May 4 and May 9. The sowing was done manually in double-line 1.5 m long with 0.25 m distance between rows. During trials, they were evaluated and information was gathered about several morphological traits and phenological phases. The morphological evaluation was done according to the Descriptors for buckwheat created by IPGRI (1994).

Protein determination

The samples were analyzed according to standard Kjeldahl method (ČSN 56 0512-12) in Kjeltec automatic analyzer. The protein factor for tartary buckwheat was set to 6.25.

Another protein analysis was done by FT NIR Antaris II spectrometry analyzer. Results of spectrometry analysis provided by FT NIR Antaris II were processed by MACROS BASIC and OMNIC software. Calibration curve was determined by TQA software

Statistical analysis

Basic statistics were used for calculation of mean x, standard deviation s_x and standard error s_e and coefficient of variation (CV). Analysis of variance (ANOVA) and the Tukey HSD test were used for statistical evaluation, and the dendrogram of distance among tested varieties was calculated with average values by means of cluster analysis (software – Statistica 7.0 CZ)

Results and discussion

Days from emergence to flowering were in 2006 and in 2007 very similar, but the range was wider in 2007 (9-31 days), probably because of the very unstable weather in summer 2007 (Table 2). The tartary buckwheat varieties cultivated on experimental fields reached maturity in the range from 108 to 169 days in 2007 (Table 2) and 125-157 days in 2006. Zongwen (2006) estimated varieties from different provinces of China and got the results ranged between 79 to 119 days. We can concluded that Chinese varieties cultivated in the Czech Republic prolonged their vegetative period because of not so suitable environmental conditions for their growing and showed worse adaptability. The average plant height was higher in year 2007 (93.66 cm) than in 2006 (72.41 cm), what was caused by better distributed precipitation during the year 2007. According to Baniya et al. (1995) the plant height in Nepal varied dramatically from 5 to 102 cm. The value of weight of thousand seeds (WTS) was in the both years very similar and there were not any changes influenced by weather detectable (Table 2). In general, the lowest WTS showed the tartary buckwheat varieties coming from the USA and the highest average WTS had the Chinese tartary buckwheat varieties. This highest measured value of WTS was similar to the lowest WTS measured by Zongwen (2006), what was 17.7 g for tartary buckwheat varieties group from Guinzhou province. The most of the varieties had the achenes color from light brown to brown even to grey (81.25 – 89.38%). Several authors stated in their researches that the differences in protein content among tartary buckwheat varieties could be very wide; Shougui et al. (2006) determined the range even from 7.82 to 18.94%. Czech genotype '01Z510012' had the protein content of 10.9%, what was a bit higher as the content published by Zewen et al. (2007) for tartary buckwheat from Luxembourg (10.3%). None of all investigated Chinese tartary buckwheat varieties reached the newly developed Chinese 'Quianku' variety, which had the crude protein content approximately 13.47% (Chun et al., 2006). Delwiche (1995) compared the crude protein content of different wheat varieties by Kjeldahl method and NIR spectromethry analysis and got similar average results. The dendrogram (Figure 1) calculated according to their morphological and phenological features into 4 groups. In the group A there were 5 Chinese and the Bhutanese variety '01Z510001', which was similar to these varieties. The varieties in group A had in general the longest vegetation periods. The Chinese varieties 'Jinqiao' and 'Dianning' were categorized as a group D, probably because of the exceptionally high WTS and even longer vegetation period than group A and longer period from emergence to flowering. In the group B, there were almost all the varieties from the Bhutan and the variety '01Z510011' from Pakistan. They are characterized by the longer vegetation period. In the group C were the Bhutanese varieties, Mexican variety '01Z510013', Czech variety '01Z510012' and four further varieties: '01Z510017', '01Z510021', '01Z510028', '01Z510014'. These varieties had in general the shortest vegetation period.

Table 2. Mean values of investigated characteristics in the years 2006 and 2007.

	Mean ±SE		Range		CV (%)	
	2006	2007	2006	2007	2006	2007
Days from sowing to emergence (days)	21.38±1.35	21.30±1.29	21-25	9-31	5.4	34.77
Days from emergence to flowering (days)	50.88±1.20	42.85±1.18	40-85	31-54	17.05	16.04
Days from emergence to ripeness (days)	141.17±0.38	138.06±3.24	125-157	108-169	5.45	13.49
1000-seed weight (g)	13.56±2.84	13.x54±0.36	9.13-20.01	9.88-18.22	18.26	15.13
Plant height (cm)	72.59±0.20	93.55±2.22	31-111	67-128	25.08	13.62
Protein in dry matter (%)		11.46±0.11		10.52-12.93		5.2
Protein by Antaris (%)		9.82±0.18		8.58-11.71		8.08

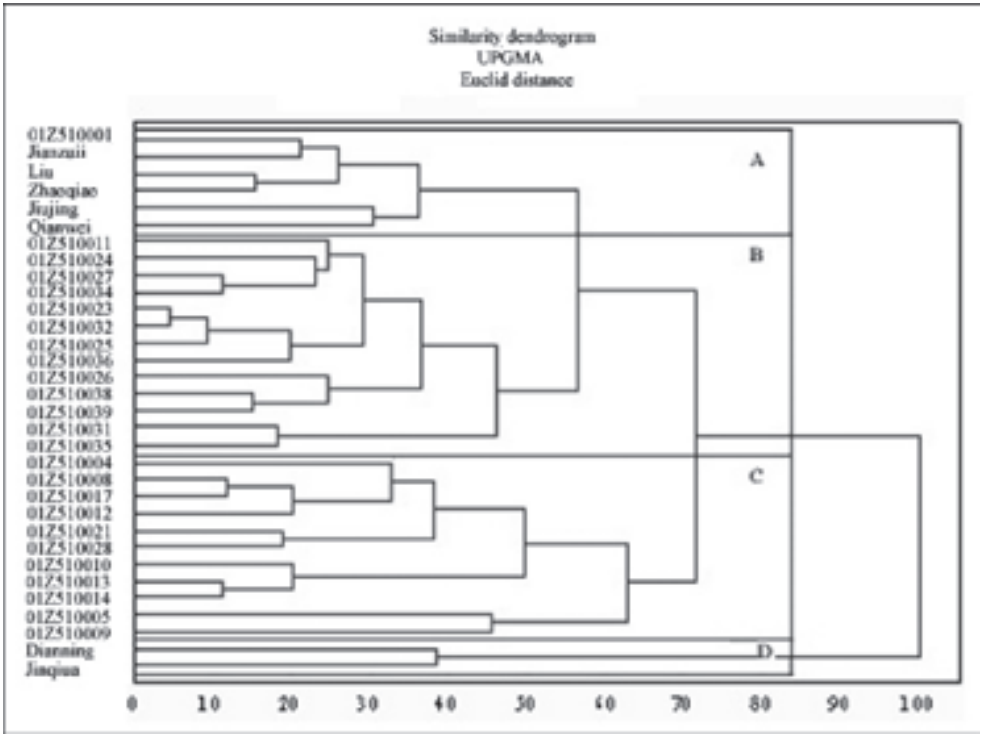

Figure 1. Similarity dendrogram of tested tartary buckwheat genotypes.

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Glucosinolate content and composition in the seeds, stems and leaves of *Brassica napus*

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ABSTRACT: Glucosinolates are secondary plant metabolites, which are believed to have numerous functions in plant-environment interactions. They also have negative effects in animal nutrition and therefore seed glucosinolate content has been reduced by breeding. Much less is known about the glucosinolate content in green biomass, which might be of importance when using the total plant for biogas production. The glucosinolate-microbial interaction during biogas production may inhibit this process. This study examines the glucosinolate content and composition in rapeseed for different genotypes and in different plant organs. HPLC (High Pressure Liquid Chromatography) was used to determine the glucosinolates in the seeds, leaves and stems of 44 winter rapeseed lines. The leaf glucosinolate content varied between 0.28-9.91μmol/g Dry Matter. The maximum glucosinolate content for the leaves was reached for the cultivar "Sollux" with 9.91 μmol/g D.M. The average glucosinolate content in the stems (2.80 μmol/g) was higher as in the leaves (1.48 μmol/g). For glucosinolate content there was no significant correlation between seeds and stems and a relative small significant correlation (r=0.34, p=0.05) between seeds and leaves. This can indicate a different gene action for glucosinolate biosynthesis in the seeds and in the other parts of the plant. In conclusion a considerable variation in glucosinolates content was found, indicating the possibility to develop material with reduced glucosinolate content in total biomass in breeding programs.

Keywords: Glucosinolate, *Brassica napus*, rapeseed, genetic diversity.

Introduction

Glucosinolates are sulphur containing secondary plant components with a low content in the dry matter, varying between 2% in the beginning of the vegetation and 0.1% in its end (Zukalova and Vasák 2002). Glucosinolates have several anti-bacterial and anti-fungal functions (Mithen, 1992). Until now the introduction of glucosinolate low cultivars was limited towards the seeds of rapeseed. This means that when rapeseed is used as a biogas reactor substrate, the presence of occasional higher levels of glucosinolates in the other parts of the plant can inhibit the biogas production process. For this reason aside from a high biomass yield for a sufficient biogas conversion (Ofori and Becker, 2008) also an optimal biomass composition i.e. lowered glucosinolate content in the leaves and stems might be required. The genetic reduction of the glucosinolate contents in the vegetative substances of the plants opens up possibilities for the further development of rapeseed as an energy plant.

This genetic variation can be sought in forage rapeseed and in interspecific crosses between turnip rape and cabbage (Kräling, 1987). This study investigates the genetic variation for the total glucosinolate content and for different glucosinolate types in resynthesized lines (n=31), cultivars (n=5) and doubled haploid lines (n=8) and in the different plant organs (seeds, leaves and stems).

Materials and methods

The material consists of 44 accessions, among them eight doubled haploid lines from the cross between Gaoyou and Sollux together with their parents (Zhao et al., 2005). All other accessions are resynthesized rapeseed lines, except one semi-synthesized line (H111-2), the German oilseed rape cultivar "Express", the German forage rapeseed cultivar "Nikos" and the Turkish cultivar "Eskisehir". This material was sown in August 2006 at Reinshof near Göttingen, Germany, with several replications per genotype. In the spring of 2007 the green material of all 44 accessions was harvested from the field plots at the beginning of flowering. The seeds were harvested from the same plants in the summer. The glucosinolate content and composition in the dry matter was analysed by High Pressure Liquid Chromatography (HPLC) following Thies (1979).

Results

The leaf glucosinolate content among the five cultivars (Gaoyou, Sollux, Express, Eskisehir, Nikos), the 8 doubled haploid lines and the 31 (semi-) resynthesized rapeseed lines varied between 0.36-9.91μmol/g and 0.28-5.74 μmol/g, respectively. The cultivar "Sollux" had the highest leaf glucosinolate content with 9.91 μmol/g, and the resynthesized line "S3" also had a high value of 5.74 μmol/g. The lowest glucosinolate content in the leaves was observed for the resynthesized line "R75" with a value of 0.28 μmol/g, and also the Chinese cultivar "Gaoyou" had a low value with 0.36 μmol/g. To describe the genetic variation for glucosinolate content of the doubled haploid lines and their parents (Sollux, Gaoyou) the total contents of the leaves and the stems are plotted (Figure 1). The figure shows that the contents for the leaf glucosinolates are not transgressive compared to the two parent lines.

The concentration of the glucosinolates in the seeds for the cultivars ranged from 20.8 to 78.9μmol/g; whereas the doubled haploid lines and resynthesized lines ranged from 15.7 to 109.2 μmol/g. The level of glucosinolates in the stems (2.80 μmol/g) is higher than the level of glucosinolates in the leaves (1.43 μmol/g), compared to a mean value of 52.9 μmol/g for the seeds. The concentration of glucosinolates in the stems and the leaves is relatively low compared to the concentration of glucosinolates in the seeds (Figure 2). There was no significant correlation between glucosinolate contents in seeds and stems and a low correlation between seeds and leaves (r=0.34 p=0.05). There is a high correlation between glucosinolates in stems and leaves (r=0.86 p=0.05).

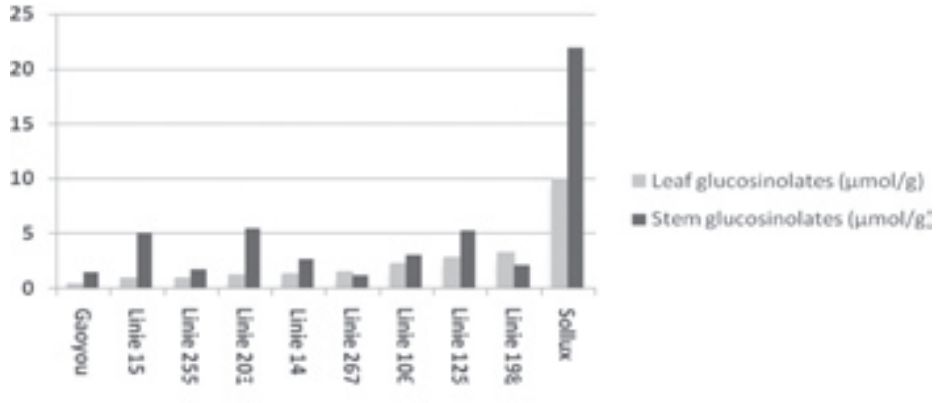

Figure 1. Glucosinolate contents of the doubled haploid lines and the two parental lines.

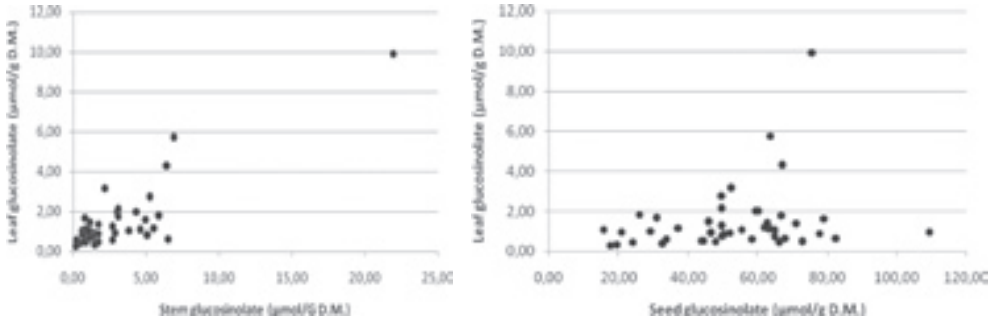

Figure 2. Correlations of the glucosinolates content in the stems, leaves and seeds of all accessions (n=44).

B. napus mainly contained the three typical glucosinolates i.e. GNA (gluconapin), PRO (progoitrin) and GBN (glucobrassicanapin) (Gland et al. 1981); in the seeds a lower content of GBN was recorded. PRO exceeds approx. 50 % of glucosinolates in the respective tissues. Figure 3 shows that the relative concentration of GBN is considerably higher in the green mass than in the seeds, whereas the seeds contain a relative higher amount of SIN (sinigrin). Similar relative mean contents of individual glucosinolates in rapeseed were also observed in other studies (Sang and Salisbury, 1988). Alkenylglucosinolates are the most dominant glucosinolate group in the leaves, stems and seeds (76-89%) followed by indolyl- (8-18%) and phenylglucosinolates (0.5-3%).

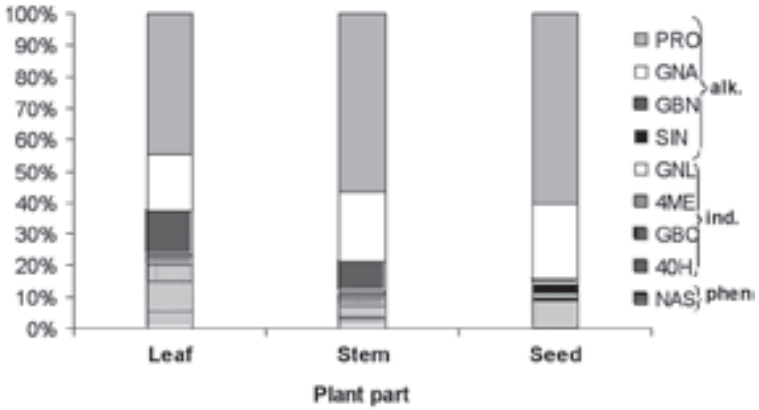

Figure 3. The profile of the glucosinolates in the different plant parts (n=44) (GNL=Gluconapoleiferin, 4ME= 4 Methoxyglucobrassicin, 4OH=4 Hydroxyglucobrassicin, NAS= Nasturtiin, GBC=Glucobrassicin; alk.=alkenylglucosinolates, ind.=indolylglucosinolates, phen.=phenylglucosinolate).

Discussion

Earlier reports on the presence or absence of correlations for the total glucosinolate contents in the different plant organs are rather contradictory (Jürges, 1982). It has even been suggested that weak correlations between seed and leaf glucosinolates content might be caused by the dependence of leaf glucosinolate content on environmental effects and growing stage (Schilling and Friedt, 1991). This means that the concentrations of the glucosinolates in the seeds cannot be used to predict the concentration of the glucosinolates in the leaves. The difference in glucosinolate composition in the seeds (e.g. SIN and GBN) and in the green material can assume a different genetic or enzymatic activity in the different plant organs. The relative high variation among the genotypes might occur for several reasons. Methodological differences (drying temperature) are partly responsible for the variance caused (Stephani, 1985) besides this repeated experiments in multiple environments will be necessary to assess the genotypic variability. In future it is necessary to investigate the influence of glucosinolates on the biogas production process more in detail. If glucosinolates should have an inhibiting influence on the biogas production, a large genetic variation is available and with the help of plant breeding it is possible to reduce the content of glucosinolates in the green material considerably. Another field of interest can be to look for the correlations between glucosinolates and developmental stage. In this way it might be possible to develop cultivars with chemoprotective features in an early developmental stage and ability for biogas production in a later phase.

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Isolation and mapping of cDNAs coding enzymes involved in phenylpropanoid biosynthesis in *Cynara cardunculus* L.

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ABSTRACT: *Cynara cardunculus* L. is a diploid (2n=2x=34) cross pollinated species belonging to the *Compositae* family and native to the Mediterranean basin. The wild taxon (var. *sylvestris*) is considered the ancestor of both globe artichoke (var. *scolymus*) and cultivated cardoon (var. *altilis*) (Lanteri and Portis, 2008). The metabolic composition of *C. cardunculus* has been extensively studied. Chlorogenic acid (CGA) is the major hydroxycinnamoylquinic acid accumulated in leaves and heads. Together with other dicaffeoylquinic acids (i.e.cinarin), which are largely restricted to *Cynara* spp., it belongs to the phenylpropanoids (PP) biosynthesis pathway and has important pharmaceutical properties. At present, the definition of the CGA biosynthetic pathway in plant remains controversial, while the metabolism of dicaffeoylquinic acids is still unknown. Biosynthesis of CGA might occur from: (a) *p*-coumaroyl-quinate, synthetized by HCT (hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase) or HQT (hydroxycinnamoyl-CoA: quinate HCT), and subsequently hydroxylated by *p*-coumarate-3'-hydroxylase (C3'H); (b) caffeoyl-CoA and quinic acid by means of HQT. Our hypothesis is that a related caffeoyl transferase might be the missing link in the pathway to cynarin. We isolated and characterized three genes coding for HCT (Comino *et al*., 2007), HQT and C3'H, enzymes involved in the CGA synthesis, and analysed the allelic variants in two globe artichoke genotypes: 'Romanesco C3' (a late-maturing, non-spiny type) and 'Spinoso di Palermo' (an early-maturing spiny type). The latter are the parents of the F1 segregating population we used for the development of the first genetic linkage maps based on a two-way pseudo-testcross strategy (Lanteri *et al*., 2006). Identification of Single Nucleotide Polymorphisms (SNPs) and linkage analysis of their segregation data enabled us to map the genes. This can be the starting point for reaching a better knowledge of the genetic bases of the PP biosynthesis.

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Breeding high protein content sunflower hybrids for production under organic conditions

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ABSTRACT: Sunflower is produced world-wide for seed oil, which is an important human nutritive and the by-products, originating from processing sunflower seeds, serve also as animal foodstuff. Utilization scales became even larger in the last decades. Demand has arisen for use sunflower oil as source of alternative renewable bio-fuel instead of fossil-based energy. Industrial use has targeted fields unexploited so far for instance extraction of rubber from sunflower. Less attention was paid to the breeding of non-oil type sunflowers and to study rules of protein content inheritance, although sunflower seeds possess significant and valuable protein content in addition to oil content with favourable fatty acid composition. Alimentary-physiological value of the product increases with the higher protein content, keeping the body-mass-index on a convenient level, and the general state of health and reproductive-biological processes are advantageously affected by unsaturated fatty acids and tocopherols of the oil. Seeds of confectionary-type sunflowers are easily dehullable with good hull:kernel ratio. All these characteristics together make non-oil type sunflowers suitable for human consumption and animal food, with special regard to pet animals (birds, rodents) kept under limited mobility. Whereas such types of sunflower are used for consumption directly or after minimal processing, it is important to produce plants under environment-respecting technology, using as few chemicals and fuels as possible. As a consequnce, it is a basic requirement for organic production to use genotypes with genetic resistance to major pathogens and pests. Non-oil type open pollinated variety Iregi Szürke Csíkos (ISzCs) bred and released by our institute has the requested inner content and seed-morphological parameters, but it is susceptible to some pathogens, e.g. downy mildew. Therefore our breeding programme aimes at developing high protein content sunflower hybrids with resistance to the most important diseases, stable productivity likewise open pollinated varieties and adaptability to varying conditions. Breeding of male sterile and maintainer lines began with inbreding of stocks of the variety ISzCs, applying aspects of complex selection. For producing cms analogs, cms convertation was started in S3 generation and in order to accelerate process, back-crossings were carried out in greenhouse in two generations per year. The self-incompatibility problem, typical for this variety, was overcome by development of syntetics, which are still under selection. For breeding of restorer lines, syntetic populations containing restoration allels were assembled on the one hand, and interspecific hybrids, crossing wild sunflower *Helianthus argophyllus* and cms lines of cultivated sunflower were created on the other hand. Progenies with proper seed morphology were selected, besides resistance to *Diaporthe (Phomopsis) helianthi* and

drought was also inherited from the wild species. Pl resistance genes were built into both kind of parent lines using HA 335 and HA 337 public lines as gene-source, respectively. The first series of experimental hybrids were tested in field trials in 2007 giving promising result to continue the work.

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Variation in the mineral composition in the edible parts of borage (*Borago officinalis* L.)

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ABSTRACT: Borage (*Borago officinalis* L.) is a large plant found throughout Europe, North Africa and North America. Borage plants have been used from ancient times as a vegetable as well as in traditional medicine for the treatment of melancholia (*ego borago gaudia semper ago*), sadness and respiratory complaints. Nowadays this species is cultivated in Europe and the USA for the commercial production of seeds as source of gamma linolenic acid. This fatty acid is an intermediate of indispensable compounds in the body, such as prostaglandin E1 and its derivatives. In the north of Spain, mainly in the Ebro Valley, borage is currently used as vegetable, where the petioles, leaves and stems are appreciated and eaten raw in salads and cooked in different types of dishes. The objectives of this work were to evaluate the variability for mineral concentration in a germplasm collection of *Borago officinalis,* and to compare with the mineral composition of the borage variety cultivated and marketed in Spain. Edible parts of borage plants showed a high content in Ca (>200mg/100g fresh weight) and Mg (>45 mg/100g fresh weight), with a moderate concentration in the rest of minerals studied. The range of variability for these components found in the germplasm analysed will allow us to select genotypes with improved nutritional characteristics.

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Effect of crop management and locality on selected grain parameters in selected wheat varieties with different baking quality

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ABSTRACT: The six winter wheat varieties (*Triticum aestivum* L.) different in their declared baking quality (Barroko – A, Cubus – A, Meritto –B, Rheia – B, Florett – C, Biscay – C) were cultivated in two level of crop management (two application level of fertilizers and fungicides and combination of conventional and minimum tillage in two localities of the Czech Republic (Prague-Ruzyně and Chrášťany u Rakovníka) during 2006 and 2007. In the set of wheats was evaluated yield of grain, raw protein content (Kjeldhal method), starch content (Ewers polarimetric method), amylose content (enzymatic method by Megazyme), wet gluten content (WG), gluten index (GI) and Zeleny sedimentation. The aim of the study was to evaluate effect of monitored factors (variety, level of crop management and year) on variability above mentioned grain parameters, including valuation of possible specific aspects of these factors in individual wheat categories with different baking quality. Two-year results, which were evaluated by PCA analysis, confirmed dominant effect of locality and year on variability of all parameters. In spite of the contrast of climatic conditions in both years, the PCA loading scatter testified significant effect of variety on selected grain parameters as well as their belonging to the individual baking category. The high quality category "A", represented by varieties Cubus and Barroko, was significantly separated. Surprisingly, the lower effect on grain parameters was detected in factors of cultivation intensity and soil tillage, which were situated more closely to the centre of the graph. Simplified analysis of variance aimed at effect of factors, baking quality (A, B, C), cultivation intensity and soil tillage, confirmed statistically significant effect of baking quality in all of evaluated grain parameters except amylose content and yield of grain. Statistically significance of cultivation intensity was confirmed only in crude protein and starch content. Effect of conventional and minimum tillage respectively was not separately statistic significant in any tested grain parameters. The average highest content of raw protein was obtained by combination of conventional tillage and higher cultivation intensity in baking category "A", and "B" (14.3%). The highest average starch content was detected in baking category "C" combined with lower cultivation intensity and conventional tillage (67.5%). More significant effect of cultivation intensity on gluten content was visible in wheat category "A". Both technology factors GI and Zeleny sedimentation generally corresponded with relevant category of baking quality. Nevertheless, category "B" even showed lower value of GI (52.7) than substandard category "C" (69.0). The highest values of GI in category "A" (88.3-90.5) were obtained by combination of both soil tillage and lower cultivation intensity. The highest values of Zeleny sedimentation were determined in category "A" combined with both cultivation intensity and minimum tillage (63-64 ml). Amylose content was within evaluated combinations

of crop management high rigid with low effect of monitored factors (26.1-27.6%). In spite of statistical insignificancy of yield differences among baking categories there was possible to register a clear tendency of higher yield in category “C” combined with higher cultivation intensity and both ways of soil tillage (8.3 t/ha).

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The effect of P and K fertilization on saponin contents in some genotypes of alfalfa (*Medicago sativa* L.)

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ABSTRACT: Saponins are a mixture of biologically active compounds found in crops like alfalfa (*Medicago sativa* L.), in levels ranging from trace amounts up to 6% d.m. Contents above 1% d.m. are harmful for ruminants and lethal to monogastric animals, e.g. chickens. At the same time the pharmaceutical, cosmetic as well as other industries search for alfalfa genotypes with high saponin contents for the production of drugs, cosmetics and plant protection agents (fungicides). In the years 2006 – 2007 a field experiment was established using Polish cultivars: Radius, Resis and Boja, and four synthetic populations of alfalfa in order to determine the effect of P and K fertilization on saponin contents. The following doses were applied: P – and K – 0, P – 50 and K – 50, P – 100 and K – 100, P – 150 kg/ha and K – 150 kg/ha, together with different combinations of these doses. Contents of biologically active saponins were determined using a fungus *Trichoderma viride* (Zimmer et al., 1967; Dyba et al., 2004) in aboveground parts of plants before the harvest of green matter. Investigated genotypes differed in terms of saponin contents at fertilization variant of P – 0 and K – 0. Saponin contents changed from 0.25% d.m. to 2.30%, with a mean of 0.95%. The highest saponin content in dry matter was found at a fertilization of P – 150 kg/ha and K – 50 kg/ha, with a mean of 4.98% in dry matter. A statistically significant effect of P and K fertilization was found on saponin content in alfalfa. The application of each P dose resulted in an increase of saponin content. The genotypic response of plants in individual years of the experiment was similar. The effect of P and K on saponin content in alfalfa may be presented in the form of an equation $C = \mu + \beta_1 K + \beta_2 P + \beta_3 P{\times}K$, where C – trait, μ – grand mean, β_1, β_2, β_3, – regression coefficients for K, P and P×K, a3 – coefficient for P and K). No interaction between P and K was found.

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Characterization of anthocyanin-pigmented wheat genotypes

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ABSTRACT: Anthocyanins in the pericarp or aleurone layer are responsible for purple and blue coloured wheat grains. Various health benefits resulting from the antioxidative potential of anthocyanins were reported. Hence, an increased dietary intake of anthocyanins could be possible by the processing of staple food products made from purple or blue wheat. In the present study tetraploid and hexaploid purple pericarp wheats and hexaploid blue aleurone wheats were tested for various agronomic and qualitative traits under organic production in eastern Austria. The anthocyanin-pigmented wheat varieties showed significantly higher contents of phytochemicals, however, and also significantly lower yielding compared to hard red winter wheat check varieties. Yield improvement is a major issue in breeding purple and blue wheat varieties for the production of added-value plant material.

Keywords: Anthocyanins, blue aleurone, phytonutrients, purple pericarp, *Triticum* spp.

Introduction

Anthocyanin pigments are members of the flavonoids, the most important group of phenolic compounds in foods. The health beneficial role of anthocyanins is a well accepted dogma in folk medicine throughout the world. It is only in recent years that specific pharmacological properties of isolated anthocyanins were studied in controlled in vitro, in vivo or clinical research studies. The positive effects of anthocyanins on health could be attributable to their potent antioxidant activity but they may also have other biological activity independent of their antioxidant capacaties. Improved night vision adaptation, reduced cancer cell proliferation, prevention of obesity and diabetes, decreased risk of cardiovascular diseases, prevention of age-related declines in neural function, antimicrobial activity and suppression of inflammatory responses are examples attributed to the broad pharmacological activity of anthocyanins (Stintzing and Carle, 2004). Recently Toufektsian et al. (2008) reported health benefits derived from a chronic dietary intake of anthocyanin rich maize (*Zea mays* L.).

In wheat grains anthocyanins occur either in the pericarp or in the aleurone layer. The origin of purple pericarp wheats can be pinpointed to Ethiopia. Körnicke (1885) and Wittmack (1906) were the first who described purple tetraploid Abyssinian wheats (*Triticum aethiopicum* Jakubz.). Later on the purple seed colour was successfully transferred into hexaploid wheat (*T. aestivum* L.) (Copp, 1965). Since the 1980s hexaploid purple wheats are used in New Zealand

for the production of specialty breads (Lindley and Larsen, 1997). In 2006 the purple seeded wheat variety Indigo was released in Austria for the production of specialty food products. Blue aleurone was transferred to common wheat by alien chromosome substitutions from *T. monococcum* or *T. boeoticum* (Zeller et al., 1991) or *Agropyron elongatum* (Zeven, 1991). Contrary to purple wheats, blue aleurone wheats are so far not commercially exploited for specialty food products. Various sources of purple pericarp and blue aleurone wheats were evaluated for agronomic and qualitative traits in two-year field trials in eastern Austria.

Material and methods

Five purple pericarp *T. aestivum* (Indigo, PWW-1, PWW-2, PSW-1, PSW-2), two blue aleurone *T. aestivum* (T-Bk, SB-3) and four purple pericarp *T. aethiopicum* (Tae258007, Tae258027, Tae258028, Tae258034) genotypes were grown in 2006 and 2007 in organic field trials at Raasdorf (16°35'E, 48°13'N), eastern Austria. For comparison two hard red winter wheat cultivars (Capo, Saturnus) were included in the trials. The material was evaluated for days to heading (HEAD), plant height, lodging, disease resistance, yield (YLD), grain mass (TKW), test weight, grain plumpness (KP25), protein content (PROT), and concentration of phytonutrients, i.e. total phenolics (TPC), total anthocyanins (TAC) and yellow pigments (YP). Determination of total phenolics was carried out by means of the Folin-Ciocalteu reagent (Singleton et al., 1999). Total anthocyanins were analysed modified after Abdel-Aal and Hucl (1999), and the concentration of yellow pigments was done according to ICC Standard Method No. 152. All statistical analyses were carried out using SAS 9.12 software. In the following only the data for HEAD, YLD, TKW, KP25, PROT, TPC, TAC and YP are presented.

Results and discussion

For most traits the main genotypic effect was significant and more important than the main year effect. A higher influence of the year was estimated for HEAD and PROT. The year effect was also essential in regard to TAC. Concerning YLD no anthocyanin-pigmented wheat genotype reached the yield levels of the standard winter wheat cultivars Capo and Saturnus. The released purple wheat Indigo and the breeding line PWW-1 yielded 100 to 200 g m^{-2} less than the mean of the checks (2006: 519 g m^{-2}; 2007: 593 g m^{-2}). If Indigo (alternative growth type) was sown in spring, yield losses were 300 to 450 g m^{-2}. Generally the yield losses for spring wheats were 200 to 320 g m^{-2} in 2006, a year with a normal distribution of spring rainfall, whereas the yield losses increased to 420 to 520 g m^{-2} in 2007, when no rainfall was recorded for six weeks after sowing in mid-March. From this result it becomes obvious that with the currently available varieties the production of purple wheat in the wheat growing regions of eastern Austria is economically comparable to quality wheat only in case of winter wheat with a respectively higher price for the purple grain. No advanced breeding line or genetic resource of either purple pericarp or blue aleurone wheat is at the moment as high yielding as the standard quality wheats. Since the present trials were carried out under

organic conditions it can be supposed that the differences in yield would become even greater under conventional production systems.

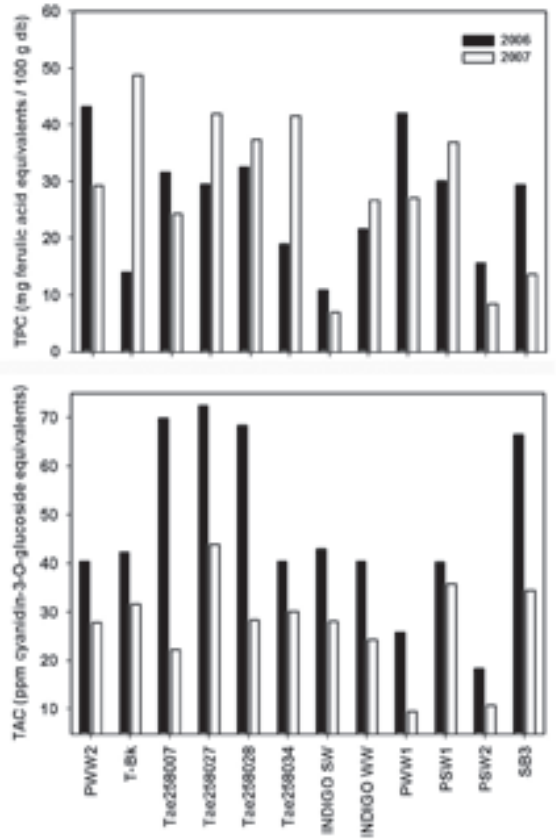

Figure 1. Annual deviations in total phenolics content (TPC) and total anthocyanin content (TAC) of purple pericarp and blue aleurone wheats from the annual means of the check varieties Capo and Saturnus.

With the exception of the blue aleurone wheat T-Bk and the purple seeded Indigo all other genotypes were later in heading compared to the checks. For the winter wheats PWW-1 and PWW-2 this difference was ~5 days, while the spring wheats were ~10 (SB-3, *T. aethiopicum* lines) to ~18 days (Indigo) later. Grain mass measured as 1000-kernel weight was similar (PSW-2) or slightly lower for most genotypes. Significantly lower grain mass compared to the checks was observed for the purple lines PWW-1 and PWW-2 (-6 to -12 g), and for the

blue genotypes T-Bk and SB-3 (-5 to -12 g). Similarly, the percentage of plump kernels >2.5 mm was lower for almost all genotypes. Only the purple seeded spring wheat line PSW-2 performed similarly to the check varieties. Concerning protein content only the tetraploid Ethiopian wheat accessions significantly outperformed the check varieties (+1.5 to +5.7%). All other genotypes yielded protein levels similar to the checks. For the concentration of yellow pigments the lowest values were analysed for the check varieties. Significantly higher contents were observed for the purple line PWW-1, which exhibits levels of yellow pigmentation similar to durum wheats (6-8 ppm).

As expected the purple pericarp and the blue aleurone wheats contained significantly higher amounts of phenolic compounds and anthocyanins. In Figure 1 the annual deviations of the tested genotypes from the annual means of the check varieties are displayed. From the graph it is obvious that higher anthocyanin levels were obtained in the 2006 grains. Considering the climate data it can be concluded that the drought periods in 2007 and the significantly shorter vegetation period led to a lower accumulation of anthocyanins in the grains. In regard to the total phenolics a genotype by year interaction can be observed, especially the tetraploid Ethiopian wheats showed higher TPC values in the dry season of 2007. A generally higher accumulation of phenolic compounds as a reaction of the spring wheats to drought stress can not be observed.

From the obtained results it can be summarized that currently no purple pericarp or blue aleurone wheat variety is available which is as high yielding as the standard quality wheats. The released purple grained variety Indigo is the highest yielding among the tested anthocyanin- pigmented genotypes. However, in regard to the concentration of total phenolics and total anthocyanins and, therefore, antioxidative potential phytonutrients, Indigo is outperformed by the tetraploid purple Ethiopian wheat lines and the blue aleurone genotype SB-3. Since first results revealed that different anthocyanins could be responsible for the purple colouring of the pericarp and the blue colouring of the aleurone layer it should be possible to improve the anthocyanin content of wheat by combining the genes for purple pericarp and blue aleurone. Despite the proven health benefits resulting from a dietary intake of phenolics major improvements in the present material of purple and blue wheats are necessary concerning yield. Otherwise an economic production of functional food products based on anthocyanin-pigmented wheat grains will be difficult and probably only possible for a niche market.

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Concentration of macro- and microelements in a collection of durum wheat cultivars

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ABSTRACT: Most of the inorganic phosphorus (P_i) present in mature cereal seeds (between 40 to 80%) is stored as phytate, an anti-nutritional factor that forms complexes with minerals such as Ca, Mg, Zn and Fe, and reduces the total P bioavailability. The present study was undertaken to determine the variation in P_i and mineral concentration in the whole grains of 93 durum wheat (*Triticum durum* Desf.) cultivars representative of old and modern germplasm adapted to the Mediterranean conditions, and to identify nutritionally superior durum wheat cultivars that possess low phytate content and high concentration of mineral elements in their whole-wheat flour. The cultivars were grown under the same field conditions during 2004-2005 at Foggia, Italy and during 2005-2006 at Foggia and Fiorenzuola d'Arda – Southern and Northern Italy. The phytate of each durum wheat cultivar was estimated indirectly by using the microtitre plate assay evaluating the P_i absorbance at 820 nm, while the Cu, Fe, Mn, Ca, K, Mg, Na and Zn mineral contents were determined by ICP/OES. The results showed a large genotypic variation for all micronutrients evaluated. In particular, the contents of Zn and Fe among the 93 durum wheat cultivars ranged from 23.2 to 58.5 ppm for Zn with an average of 34.0 ppm and from 26.2 to 97.3 ppm for Fe with an average of 43.4 ppm. Regarding the P_i grain content the mean values recorded across the years and the locations ranged from 0.32 mg g^{-1} to 1.09 mg g^{-1} showing a positive correlation to all minerals with the exception of Cu and Zn. These results open the possibility to design a specific breeding program for improving the nutritional value of durum wheat cultivars through the identification of parental lines with low-P_i and high minerals concentration in whole grains.

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General Combining Ability (GCA) and Specific Combining Ability (SCA) in *Actinidia chinensis*: implications for breeding population management and varietal selection

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ABSTRACT: Variances for general combining ability (GCA) and specific combining ability (SCA) and the relationship between mid-parental GCA and SCA effects were estimated for 12 traits from a 6×7 factorial mating experiments in *Actinidia chinensis*, planted at one site at Te Puke, New Zealand and evaluated over three consecutive years. The importance of SCA variance varied, from non-significant to SCA variance accounting for almost half or more the genetic variation among full-sib families for Firmness at 12 weeks, Sweetness and presence of a Hard Core. For those traits with high SCA variance, a positive correlation between mid-parental breeding values and best linear unbiased predictions of the SCA effects was observed. To fully exploit genetic gain from SCA variance in a varietal selection, positive assortative matings are required among the best parents. Additional gain, with significant GCA as well as SCA effects, was achievable relative to gain from GCA only. For a breeding population, selection for breeding values may be sufficient due to positive correlations between breeding values and SCA values. For varietal selection, to capture more SCA genetic gain, it is preferable to make more pair-wise mating for parents with higher breeding values.

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Sensitivity of diploid and tetraploid wheat species to annual influences on the yellow pigment concentration

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ABSTRACT: Tetraploid and diploid wheat species represent an important source of for the processing of food products high in carotenoids concentration. To avoid product losses end-users prefer a constant quality over years and genotypes. In the present study the sensitivity of both released varieties and genebank accessions to annual influences on the yellow pigment concentration was evaluated. Einkorn and durum wheat genotypes with high concentrations of yellow pigments and a low and/or strong response to yearly influences were identified.

Keywords: Carotenoids, phytonutrients, *Triticum durum*, *T. monococcum*, *T. turanicum*

Introduction

Yellow endosperm pigmentation in wheat species is mainly due to the presence of carotenoids. The major component was described as the xanthophyll lutein (Lepage & Sims, 1968; Hentschel et al., 2002). A considerable amount of the yellow pigmentation is yet unidentified (Hentschel et al., 2002). Strong yellow pigmentation is desired in durum wheats (*Triticum durum* Desf.) as the yellow colour is an essential quality trait in pasta. Due to their very efficient antioxidant properties carotenoids may also play a role in disease prevention by impeding oxidative or photooxidative stress. There is increasing evidence from epidemiology, animal studies and in vitro experiments that increased intake of lutein and zeaxanthin – xanthophylls concentrated in unique high levels within the central retina (macula lutea) – is inversely associated with the risk for age-related macular degeneration (AMD) and cataract, major causes of impaired vision or blindness of elderly people (Stahl, 2005). As a staple food, wheats with high yellow pigment concentration could provide a large proportion of daily dietary intake of lutein. High lutein wheat varieties could be a valuable raw material for functional food products at reasonable costs compared to isolated lutein additives and/or supplements (Frégeau-Reid and Abdel-Aal, 2005).

The wide occurrence of genotype by environment interaction (G×E) concerning quality traits hampers both the work of plant breeders and end-users. In case of significant G×E plant

breeders have to cope with reranking of genotypes in different selection environments, end-users permanently have to modify their processing to avoid product loss. A constant quality of raw material would be preferred by the processor. This stability by industry was described by Robert and Denis (1996) as 'economic stability'. This definition corresponds to the static, also called biological concept of stability (Becker and Léon, 1988). The regression analysis of phenotypic values on environmental indices was widely used to describe G×E. Finlay and Wilkinson (1963) proposed the regression coefficient as measure of stability, while Eberhart and Russell (1966) considered the slope as a measure of response to varying environments. In the present study the regression coefficient was used to describe the response (sensitivity) of tetraploid and diploid wheat species to annual influences on the yellow pigment content.

Material and methods

Plant material and chemical analysis

Twenty-five tetraploid (4 *T. aethiopicum* Jakubz.; 10 *T. durum* Desf.; 11 *T. turanicum* Jakubz.) and twenty-three diploid (*T. monococcum* L.) wheat genotypes (released varieties and genebank accessions) were grown from 2003 to 2007 in organic field trials at Raasdorf (16°35'E, 48°13'N), eastern Austria. The yellow pigments were extracted with water saturated *n*-butanol and measured with a spectrophotometer at 440 nm (ICC Standard Method No. 152). The extinction values were transformed into beta-carotene equivalents using the calibration *x (mg/ml) = extinction/262*.

Statistical analysis

The analysis of the multi-year data were carried out as a weighted two-stage analysis. In the first stage genotype by environment means and their standard errors were calculated using the GLM procedure of SAS Vers. 9.1. Subsequently, the means were subjected to a Finlay-Wilkinson regression model using the SAS procedure MIXED and the code as outlined by Piepho (1999). Genotypes were considered as fixed effects, years as random. The reciprocals of the variances of the means (squared standard errors) were used as weights and heterogeneity of the error variances was accounted for by the statements as outlined by Piepho (1999). The statistical analysis was performed separately for the diploid and the tetraploid wheat germplasm.

Results and discussion

Yellow pigment concentration

A wide range of variability for yellow pigment concentration (YP) was found over and within the investigated wheat species. Generally, the highest contents were observed for einkorn (*T. monococcum*) followed by durum (*T. durum*) wheat. Khorasan (*T. turanicum*) and Ethiopian (*T. aethiopicum*) wheat contain significantly lower levels of yellow pigments compared to released Austrian durum varieties. Hence, this germplasm represents no valuable genepool for the improvement of durum endosperm pigmentation, but is interesting concerning other traits like grain weight or composition of phenolics (Grausgruber et al., 2005; Siebenhandl et al., 2007).

Stability statistics

The Finlay-Wilkinson regression coefficients for the diploid and tetraploid wheat species are displayed in Figure 1 and 2, respectively. Genotypes with regression coefficients around zero were insensitive to annual influences, while genotypes with regression coefficients about one showed an average response to changing years. Genotypes with slopes significantly higher than one responded above average to more favourable years. From Figure 1 it is apparent that about half of the tested einkorn accessions always showed a YP >10 ppm. *EI24* and *BVAL211001* were identified as genotypes with high YP and insensitive performance over the years, whereas for *EI18* the highest concentrations of YP and a very sensitive response to annual influences were observed. This genotype would be especially suitable for favourable conditions.

Concerning the tetraploid wheats it is obvious that an insensitive response at a high level of pigmentation was observed only for released durum varieties, which is not really astonishing as these varieties were selected in multi-environment trials among other quality traits for their YP. Astonishing, however, is the reaction of the winter durum varieties Heradur, Superdur and Prowidur which reacted very sensitive to annual influences. YP of winter durum wheat seems to be much more influenced by the annual changes in the distribution of rainfall in the months of late spring and early summer, i.e. the time of grain filling and synthesis of yellow pigments, compared to spring durum wheat.

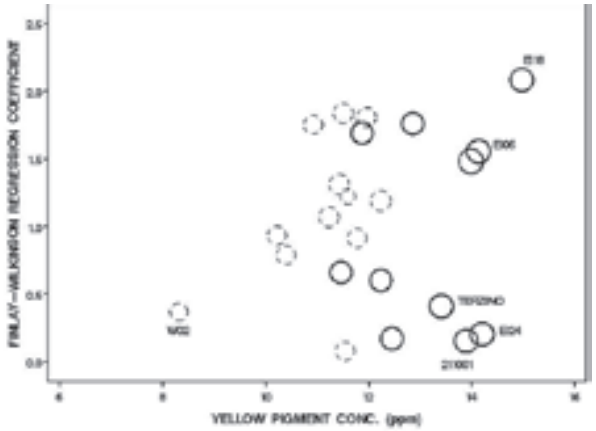

Figure 1. Finlay-Wilkinson regression coefficient plotted against the mean yellow pigment concentration (YP) of einkorn wheat. The bubble size corresponds to the absolute minimum YP value; full bold circles represent genotypes with an absolute minimum value >10 ppm.

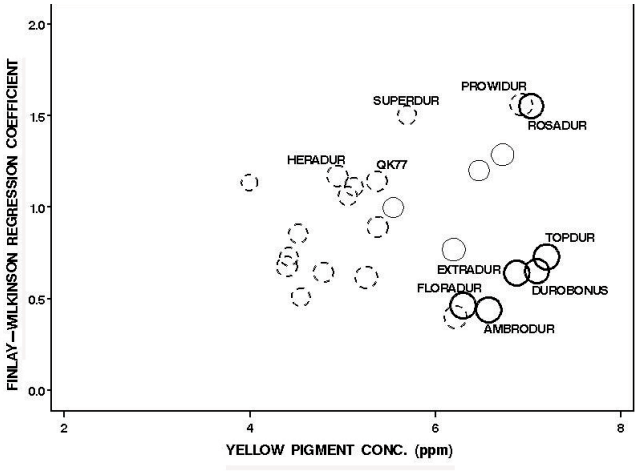

Figure 2. Finlay-Wilkinson regression coefficient plotted against the mean yellow pigment concentration (YP) of durum, Khorasan and Ethiopian wheat. The bubble size corresponds to the absolute minimum YP value; full bold circles represent genotypes with an absolute minimum value >5 ppm; Ethiopian wheats are plotted as fine full circles.

In the present study einkorn wheat accessions and durum wheat cultivars with high levels of yellow pigment concentration and insensitive response to the influence of years were identified. Moreover, genotypes with high yellow pigment contents and sensitive reaction to changing conditions but minimum values above a critical limit were identified. The cultivation of these genotypes in the climatic conditions of the durum growing region of eastern Austria is suggested if a certain level of yellow pigment concentration must be reached for the fulfillment of contracts with the food industry or to receive the premium for quality durum wheat.

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Retama raetama medicinal plant: characterisation, and purification of anti-microbial compounds

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ABSTRACT: Several multiresistant human pathogenic microorganisms have been observed in recent years. Therefore, the search of new antimicrobial substances has been progressed. Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeuticals, natural products still remain as one of the best reservoirs of new structural types. *Retama raetama* (RR) locally named as R'tem is an indigenous plant belonging to the Fabaceae. It is common of our country and in the North and East Mediterranean region. The aim of the present study was to investigate the antimicrobial activity of extracts from RR. The antimicrobial properties of RR methanol extract were investigated. The screening of the antimicrobial activity of extracts from RR was conducted by a disc diffusion test against Gram-positive and Gram-negative organisms .The antimicrobial activity of the extract was stable against heat (120°C,15 min), freezing (-20°C, 16 h) and pH treatment(pH 3-10). The RR extract was separated by reverse-phase HPLC with 20-80% methanol linear gradient to characterize antimicrobial compounds. There was one fraction having antimicrobial activity; the peak had maximum absorbance at 254 nm. The molecular weight of the purified extract will be determined by Mass spectrometric and the identification of the bioactive compound will be done in the further analyses. This is another example on how the use of natural diversity can be used for the benefit of humans.

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Analysis of carotenoids in Bolivian ají (*Capsicum baccatum* var. *pendulum*) and rocoto (*C. pubescens*)

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ABSTRACT: A wide range of colors can be found among the fruits of the cultivated forms of genus *Capsicum,* from pale yellow to deep red. Carotenoids are responsible for such a chromatic diversity. Approximately, thirty of these compounds have been identified in peppers, although a few of them account for 90% of the total carotenoid content (Bosland and Votava, 2000). Capsanthin and, to a lesser extent, capsorrubin, both specific of the genus *Capsicum*, have been reported as the main red carotenoids in peppers, while the predominance of yellow/orange carotenoids depends on the genotype. In general, β-carotene, β-criptoxanthin, and violaxanthin are considered as the most abundant in this fraction. Although there are several studies about the pattern of carotenoids in chile peppers, they have been only performed in fruits from the *annuum* complex (mainly *C. annuum*), while references to the other cultivated forms, *C. baccatum* var. *pendulum* and *C. pubescens*, are nil. At this respect, these species have been traditionally grown in the Andean region for thousands of years, where they are respectively called ajíes and rocotos and represent an essential part of their cookery and cultural inheritance (Nuez et al., 2003). As a part of the COMAV program focused on the genetic improvement of nutritional quality in fruits of *Capsicum*, we report previous results of the first study on the carotenoid pattern in fruits of *C. baccatum* and *C. pubescens*. Fruits from seven accessions belonging to: i) *C. baccatum* (4), ii) *C. pubescens* (1), and iii) *C. annuum* (2) as controls (brown "Pasilla" and Yellow California Wonder: YCW), were analyzed for total and specific carotenoid content by means of HPLC. One g fruit samples were extracted following the protocol of Mínguez-Mosquera and Hornero-Méndez (1993), followed by saponification previously to the HPLC analysis. Among *C. annuum* controls, "Pasilla" showed a high total carotenoid content (TCC), while YCW showed low TCC. Capsanthin and, to a lesser extent, capsorubin were the main red carotenoids in Pasilla, while no red carotenoids were found in YCW. In terms of yellow/orange carotenoids, violaxanthin was predominant in YCW, while high levels ofcapsanthin, capsorubin, cantaxanthin, zeaxanthin, β-criptoxanthin and β-carotene were found in Pasilla fruits. *C. baccatum* accessions showed a wide range of TCC, comprised between the levels of "Pasilla" and YCW. Compared to "Pasilla", red fruited *C. baccatum* accessions showed a low capsanthin and capsorubin content. However, in terms of yellow/orange carotenoids were high and some *C. baccatum* genotypes showed levels similar or higher than Pasilla. Violaxanthin was the main yellow/orange carotenoid, although β-carotene , zeaxanthin, and β-criptoxanthin

were found at low levels. The accession of *C. pubescens* showed a TCC similar to the best *C. baccatum* accessions, with capsanthin and violaxanthin as the main red and yellow/orange carotenoids, respectively.

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Progress toward breeding of waxy (amylose-free) wheats for the Great Plains of North America

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ABSTRACT: In hexaploid wheats, three independent loci (*Wx-A1*, *Wx-B1* and *Wx-D1*) encode production of the granule-bound starch synthase (GBSS) also known as the "waxy" protein. Waxy, or amylose-free wheats, have been developed via combination of null mutations at all three loci (Nakamura et al., 1995). Wheat lines carrying null mutations at either one or two of the Wx loci produce intermediate levels of starch amylose, and have been termed "partial waxy" (Nakamura et al., 1993). Null mutations at the *Wx-A1* and *Wx-B1* loci are fairly common; mutations at the *Wx-D1* locus are rare (Graybosch, 1998). In the late 1990's, breeding efforts were initiated to develop waxy winter wheats for the northern Great Plains of the USA. The breeding program has combined the natural occurring mutations found in the partial waxy wheats Kanto 107 (from Japan), BaiHuo (from China) and Ike (from Kansas, USA) with genes for agronomic adaptation and disease resistance from North American hard and soft winter wheats. In 2003, average grain yields of waxy breeding lines in Nebraska environments were 72% of Millennium, currently the most widely grown wild-type cultivar. By 2007, average grain yields of waxy breeding lines had reached 90% of Millennium. In both 2006 and 2007, average grain yields of NX04Y2107, the highest yielding waxy line, were not significantly different from that of Millennium. In a separate study, grain yields of waxy wheat were compared to those of all 6 possible partial waxy genotypes, and to wild-type, in four genetic populations. Grain yields averaged over two harvest seasons showed no significant differences in grain yield of waxy wheats relative to wild-type cultivars. Identical results were obtained both within all four populations, and across populations. Some differences were noted amongst partial waxy genotypes, but no consistent trends were noted. In addition, no differences in levels of tolerance to pre-harvest sprouting were detected within or across populations. Results suggest there is no grain yield penalty associated with the production of amylose-free endosperm starch, and that waxy wheat cultivars with grain yields equal to those of wild-type wheats will be attainable.

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High oil quality, a priority in sunflower breeding program, at Fundulea Institute, in Romania

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ABSTRACT: The sunflower is a plant (*Helianthus annuus* L.), the seeds of which contain a valuable edible oil that contains more Vitamin E than any other vegetable oil. Most sunflower oil is used in food production. The two most common types of sunflower oil are linoleic and high oleic. There is a third oil type, mid oleic. Linoleic sunflower oil (traditional oil) is a common cooking oil which has high level of fatty acids, called polyunsaturated. This oil contains about 70% polyunsaturated fats, being extremely susceptible to rancidity. Hidrogenation process is used for the linoleic oil to become shelf-stable, this creating trans fatty acids that exert more negative effect for the human health. High oleic sunflower oil has monounsaturated levels of 80% and above (Kinman and Earle, 1964, Vranceanu, 1974). This oil is healthy, shelf-stable and better resistant to oxidation. There is an important genetic variation regarding the fatty acid composition of sunflower oil (Cummins et all., 1967). Solving the issue of the new market requirments for the product safety, it is possible to release new sunflower oil type, with low linoleic acid and high oleic acid content. During the last years, the interest for the non-food utilisation of oil has increased all over the world. The vegetable oils could be turned into bio-fuels by metyl-esterification. For this utilization, the oil with high oleic acid content, obtained from the new sunflower type has an increased potential. In order to incorporate the „high oleic acid content" trait into pure pollen fertility restorer and sterility maintainer lines, existing in the sunflower germplasm collection of Fundulea institute, backcrossing method and selection of individual seeds by oleic acid analysis on half seed, were used. Sunflower lines with superior agronomic traits and improved oil quality, with 80-90% oleic acid content have been obtained by utilization of „donor" sources for „Ol" gene after 4-5 backcross and 1-2 selfpollination generations. Due to embryonic control of gametophyte and involved genes, for the high oleic acid content trait, the obtained hybrids have lower oleic acid content, because the F1 seeds segregate into high, intermediary and low oleic acid levels. By selecting the best hybrid combinations, high oleic hybrids, with 80-85% oleic acid content were obtained, some of them being under SIVTR network testing for registration.

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Identification of candidate alleles for some fruit traits of almond (*Prunus dulcis*) analyzing microsatellite loci

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ABSTRACT: In this study, the association between polymorpohic microsatellite loci and some of the fruit traits of 53 almond genotypes was evaluated. A total of 81 molecular markers derived from 9 microsatellite loci were used. The studied fruit traits included nut and kernel weight, kernel/nut W percentage, double kernel percentage, softness of shell, nut length, width and thickness, kernel length, width and thickness, shell color, kernel color, ease of hulling, nut shape, marking of outer shell, suture opening, shriveling of kernel, kernel pubescence, etc. The stepwise multiple regression analysis (<0.01) was applied for all traits separately to study co-segregation of SSR alleles and morphological traits, and to detect probable informative markers, using SPSS v.16 software. Cluster analysis for each group of data (molecular and morphological) was carried out separately and the results compared. In the preliminary study to determine the suitability of the SSR loci, different parameters were evaluated regarding the polymorphic level derived from molecular data, including mean of heterozygosity (0.8), mean of effective allele number (5.59). The result of stepwise multiple regression analysis showed a total of 34 positive markers (markers with significant correlation) for different traits. There was more than one informative marker for some traits, which in this case had additive effects on the different alleles considered. The results can be effectively exploited in marker assisted selection (MAS) (Oraguzie et al., 2007; Ramezani et al., 2007).

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Evaluation of antioxidant and antiacetylcholinesterase activities of the extracts of *Cymbopogon schoenanthus* L. leaves

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ABSTRACT: *Cymbopogon schoenanthus* L. Spreng, is an aromatic herb used in folk medicine. Due to its pleasant aroma and taste it is used also to prepare an aromatic "tea" that is much appreciated and largely consumed in the north of Africa. In the present study, hexane, dichloromethane, ethyl acetate and methanolic extracts were prepared from leaves of *C. schoenanthus* which were collected during the flowering phase from three locations in Southern Tunisia, and their antioxidant scavenging and antiacetylcholinesterase activities were evaluated. Antioxidant activity was measured by DPPH assay. The best results were obtained with ethyl acetate extract of leaves from the desert region: IC_{50}= 12.57±3.42μg/ml. The antioxidant activity was also assayed using β-carotene-linoleic acid bleaching method. The best result (IC50=0.119±0.01 mg/ml) were obtained by the ethyl acetate extract of leaves from the mountain region. The greatest acetylcholinesterase inhibitory activity (IC50=0.226±0.04 mg/ml) was exhibited by the ethyl acetate extract from the mountain region. The results show that the exploitation of the diversity of wild materials can led to the discovery of materials with improved quality characteristics.

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Molecular and classical base breeding of long shelf life tomato in Belarus

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ABSTRACT: In the last few decades, breeding tools have been considerably improved by the development of molecular biology. The positional isolation of the *ripening-inhibitor* (*rin)*, *non-ripening(nor)*, *Colorless nonripening* (*Cnr*), *Never-ripe* (*Nr*), and *Green-ripe* (*Gr*) genes and the linked molecular markers provide a basis for marker assisted selection (MAS) of a long shelf life trait of tomato fruit (Seymour et al. 2007). The aim of our research was to develop a functional marker assays for the *rin*, *nor* and *alcobaça* genes. Based on GeneBank sequence data for *rin* and *nor* gene (Vrebalov et al., 2002; Giovannoni, 2004) we have designed a set of codominant STS and dCAPS markers detecting functional deletions in these genes. As for *alcobaça* gene, the preliminary sequence data were not available but the comprehensive allele tests, carried out in numerous earlier studies, suggest that the tomato mutations *alc* and *nor* are allelic. To test this hypothesis at the molecular level we have sequenced the entire coding region of the *LeNAC-NOR* gene in the *alc* mutant Mo-950 line background. A single transversion of Thymine-to-Adenine was observed when the *alc* mutant sequence was compared to wild-type plants. This single-base transversion was located to nucleotide 127 of the second exon in the *LeNAC-NOR* gene sequence, and results in the substitution of the valine at position 106 to an asparagine residue. This change eliminates a recognition site for the *Cfr*10I restriction endonuclease presented in the wild-type and in the other currently known tomato mutants at the *LeNAC-NOR* locus. This polymorphism was used to develop a PCR-based DNA marker, which enables an early genotypic selection for breeding long shelf life tomatoes. Thus, the results presented here strongly support the hypothesis that the tomato *alc* (*nor*^{A}) mutation is a novel allele of the tomato of the *LeNAC-NOR* gene. Based on traditional and molecular marker techniques we have initiated Belarusian breeding programmes aimed at producing long shelf life tomato hybrids. A number of promising field grown ('Dubok' x Mo948, 'Dubok' x Mo950) and polyethylene film covered greenhouse grown (B-2-6 x Mo948, Mo950 x №10, №10 x Mo577) hybrid combinations combining a slow ripening with a high productivity and quality traits was developed. In 2006 the first Belarusian long shelf life F_1 hybrid 'Belorusskij lezhkij' was included at State Cultivar Trials.

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Interactions between HMW glutenin subunits and mixographic parametres

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ABSTRACT: Bread-making quality is one of the key factors in wheat improvement. High molecular weight (HMW) glutenin subunits and mixographic evaluation belong to the methods used for the prediction of quality potential in wheat breeding programmes. F_7 progeny derived from the cross between winter wheat varieties Sulamit and Clever having different HMW glutenin alleles was evaluated in this work. PCR based method was used to determination of HMW glutenin subunits coded by genes at the *Glu-A1, Glu-B1*, and *Glu-D1* loci on the group 1 of homeologous chromosomes. Mixing characteristics were obtained from mixographic evaluation performed on ReoMixer instrument. Four of these mixographic parameters from each mixing curve were studied: mixographic index (IHTP), areabelow which describes energy of the dough development, breakdown associated with the weakening of the dough and peakheight expressing the maximum dough development. Multifactor analysis of variance was used to determine effects of HMW alleles. Variability in contribution of different HMW glutenin subunits has been found at the 95.0% confidence level.

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QTL mapping for fruit firmness in apple using a pedigree–based Bayesian approach

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ABSTRACT: Quantitative trait loci (QTL) mapping experiments exploit three sources of information: pedigree structure, phenotypic scores and marker data (genotypes). In plants, populations derived from single crosses of inbred lines (F2, BC, HD, RIL) have predominantly been used in QTL mapping experiments but with such material only a small part of the genetic variability that is actually available is explored. As an alternative, the pedigree-based approach using connected populations have been used in many studies. However, even in these cases, the exploited genetic variability is limited to the scale of one research institute and/or breeding program. To overcome that limitation, the HiDRAS project (High-Quality Disease Resistant Apple for a Sustainable Agriculture) was initiated. The aim of the project was to perform an extended pedigree–based QTL mapping using plant materials which are part of ongoing apple breeding programs in several European countries. The present study concerned quantitative traits loci mapping for apple fruit firmness at four different periods (harvest, two months cold

storage, two months storage plus fifteen days at room temperature and four months cold storage). Plant material consisted of 2207 apple genotypes interconnected through up to seven generation from 132 founders to 26 F1 families of 25 to 100 individuals. Fruit firmness was scored for three year (2003, 2004 and 2005) and all individuals were genotyped by a genome covering set of 83 SSR markers. Due to the complexity of the pedigree, QTL mapping was performed using a Bayesian approach with the FlexQTL^{TM} software (Bink et al., TAG, 2002) which was partly founded by the HiDRAS project. Three QTL identified respectively on linkage groups 1, 6 and 10 remained stable over all storage periods. Other QTL were specific to some storage periods. Fruit firmness involves different factors such as skin, outer and inner parenchyma. So, the dynamic of identified QTL could be useful for further analysis focusing on the identification of the relationship between the QTL and these structures and their contribution to fruit firmness during storage.

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Influences of genotype and soil properties on Ca, Mg, S, Zn, Mn, B, Mo, Sr, Ba and Cd status in maize inbreds

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ABSTRACT: Ten maize inbreds (Bc706-9, Bc273; Bc473E-5; Bc265-1; Bc707-1; Bc31002; Bc837-1; Bc24W; Bc742 and Bc741-23) were grown on two soils (fluvisol and stagnic albeluvisol: pH in KCl = 7.07 and 3.85, respectively; mutually air-distance about 1 km) close to Sesvete (Zagreb County) in the 2001 growing season (four replicates; basic plot 14.0 m^2). Soil influences resulted mainly in considerable differences in nutritional status of maize inbreds. Only leaf S and Zn status were independent on soil. Under conditions of albeluvisol maize inbreds had considerably lower Ca (-25%), Mg (-46%) B (-78%), and Mo (-93%) in leaves compared to growing on fluvisoil. Also, on fluvisol maize inbreds had considerably lower leaf Mn (-77%) and Cd (-92%) compared to growing on albeluvisol. Differences in leaf composition (on dry matter basis) among the genotypes were in the range from 0.53% to 0.94% (calcium), from 0.11% to 0.26% (magnesium) and from 0.18% to 0.38% (sulphur). Microelement differences among the genotypes (mg kg^{-1}) were in the ranges from 26.3 to 51.0 (zinc), from 96 to 193 (manganese), from 2.05 to 5.02 (boron), and from 0.40 to 0.76 (molybdenum) and from 0.04 to 0.98 (cadmium).

Keywords: albeluvisol, alluvial soil, inbred lines, leaf nutritional status, maize.

Introduction

Heredity has important role in uptake of nutrients and harmful elements in maize (Bergmann, 1992; Bahia Filho, 1997; Kovacevic et al., 2004). A combination of soil management practice and growing of tolerant genotypes is a solution for sustainable maize production under suboptimal soil conditions. Aim of this study was testing influences of soil and genotype on status of nine elements (Ca, Mg, S, Zn, Mn, B, Mo, Sr and Cd) in maize leaves, while grain yields and phosphorus (P) and potassium (K) were shown by the earlier study (Havrda, 2005).

Material and methods

The field experiment, sampling and chemical analysis

Ten parents of seed-maize (inbred lines: Bc706-9, Bc273; Bc473E-5; Bc265-1; Bc707-1; Bc31002; Bc837-1; Bc24W; Bc742 and Bc741-23) were grown on two soil types (fluvisol

and stagnic albeluvisol: mutually air-distance about 1 km) near Sesvete (Zagreb County, Croatia) in the 2001 growing season (four replicates; basic plot 14.0 m^2). The total amounts of elements (Ca, Mg, S, Zn, Mn, B, Mo, Sr, Ba and Cd) in maize leaves (the ear-leaf at flowering) were determined using ICP after their microwave digestion by conc. HNO_3+H_2O_2. Soil sampling was made by auger to 30 cm of depth at starting of the experiment. Mobile fraction of individual elements in soil was extracted by NH_4-Acetate +EDTA (pH=4.65).

The soil and weather characteristics

Two soils are very different regarding their chemical properties (Table 1). For example, fluvisol is neutral, while stagnic albeluvisol is strong acid. Mobile fraction of individual elements differences (mg kg^{-1} for fluvissoil and albeluvisol, respectively) are considerable for calcium (25500 and 2300), manganese (328 and 76, zinc (1.61 and 0.53), boron (0.69 and <0.02) and cadmium (0.08 and 0.06).

Weather data in the growing season 2001 were mainly favorable for maize growing. Precipitation and mean air-temperatures for the period May-September (Zagreb-Maksimir Weather Bureau) were 433 mm and 19.0 ^{0}C and similar to long-term means (1961-1990: 436 mm and 17.8 ^{0}C). Less favorable weather conditions for maize growing under conditions of the middle Europe and Croatia are mainly in close relation to drought and high air-temperatures, especially in July and August (Kovacevic et al., 2007).

Table 1. Agrochemical soil characteristics.

	pH		NH_4- Acetate + EDTA extraction (pH=4.65): mg kg^{-1}							
	H_2O	KCl	Ca	Mg	S	Zn	Mn	B	Mo	Cd
A1[a]	7.85	7.07	25500	280	279	1.61	328	0.69	<0.016	0.080
A2[a]	4.93	3.85	2300	279	242	0.53	76	<0.02	<0.016	0.064

[a] Soil properties (0-30 cm): A1= fluvisol; A2= stagnic albeluvisol

Results and discussion

Soil influences resulted mainly by considerable differences in nutritional status of maize (Table 2). Only leaf -S and –Zn status were independent on soil, while in the other tested elements considerable differences were found. For example, under conditions of albeluvisol maize had considerably lower Ca (-25%), Mg (-46%), B (-78%), and Mo (-93%) in leaves compared to growing on fluvisoil. Also, under fluvisoil conditions maize had considerably lower leaf Mn (-77%) and Cd (-92%) compared to growing on albeluvisol.

In our study considerable hereditary influences on nutritional status of maize inbred lines were found (Table 2). Differences in leaf composition (on dry matter basis) among the genotypes were in the range from 0.53% to 0.94% (calcium), from 0.11% to 0.26% (magnesium) and from 0.18% to 0.38% (sulphur). Micronutrient differences among the genotypes (mg kg^{-1}) were in the ranges from

26.3 to 51.0 (zinc), from 96 to 193 (manganese), from 2.05 to 5.02 (boron), and from 0.40 to 0.76 (molybdenum). Harmfullelement cadmium uptake by plants were also under considerable hereditary influences because differences among genotypes were from 0.04 to 0.98 mg kg^{-1}. Specificity of individual element uptake by maize inbred lines (Tables 2 and 3) could be responsible for their different degree of tolerance to mineral stress in soil. For this reason, by choice of more tolerant genotypes is possible improvement of nutrition and sustainable maize status under less favorable environment. Bergmann (1992) listed maize as zinc-intensive plant with high zinc-demand that very positively responds to zinc dressing under low levels of available zinc in soil. The same source listed maize as plant with medium demands for manganese and copper. Although maize and other cereals are moderate B demand crops (Bergman, 1992), using B fertilizers could be useful for seed-maize growing, especially for low leaf B genotypes, because essential role of B in reproductive growth of plants, for pollen tube growth and seed viability (Dell and Huang, 1997).

Testing of the data by correlation coefficients (20 pairs: including the data for both soils), very significant values were found between yield and five leaf composition parameters as follows (Table 4): sinergical effects = Ca:Yield (0.57**), Mg:Yield (0.82**), B:Yield (0.83**), and Mo:Yield (0.90**); antagonistical effects = Mn:Yield (-0.85**). Also, the ratios between some elements were significant and sinergical effects were found in seven cases (Ca:Mg =0.47*, Ca:Mo =0.56*, Mg:B =0.61**, Mg:Mo =0.75**, Zn:Cd =0.51*, Mn:Cd =0.69** and B/Mo (0.91**), while six ratios had antagonistical effects (Ca:Mn = -0.50*, Mg:Mn = -0.69**, Mn:B = -0.84**, Mn:Mo = -0.92**, B:Cd = -0.55* and Mo:Cd = -0.57*).

Table 2. Influences of soil and genotype on nutritional status and yield of maize inbred lines.

Maize	Soil (A)		Mean	Soil (A)		Mean	Soil (A)		Mean
genotype	Calcium (Ca %)			Magnesium (Mg %)			Sulphur (S %)		
(the factor B)	A1[a]	A2[a]	xB	A1	A2	xB	A1	A2	xB
Bc 706 – 9	0.832	0.701	*0.767*	0.246	0.115	*0.181*	0.282	0.271	*0.276*
Bc 273	0.757	0.595	*0.676*	0.224	0.134	*0.179*	0.230	0.202	*0.216*
Bc 473E – 5	0.857	0.593	*0.725*	0.144	0.082	*0.113*	0.221	0.262	*0.241*
Bc 265 – 1	0.549	0.413	*0.481*	0.244	0.096	*0.170*	0.194	0.240	*0.217*
Bc 707 – 1	0.602	0.490	*0.546*	0.302	0.212	*0.262*	0.188	0.198	*0.193*
Bc 31 002	0.944	0.615	*0.779*	0.258	0.157	*0.208*	0.184	0.180	*0.182*
Bc 837 – 1	0.738	0.496	*0.617*	0.226	0.101	*0.163*	0.219	0.264	*0.241*
Bc 24W	0.553	0.511	*0.532*	0.190	0.115	*0.153*	0.340	0.428	*0.384*
Bc 742	0.778	0.595	*0.686*	0.263	0.138	*0.201*	0.189	0.212	*0.200*
Bc 741 - 23	1.099	0.777	*0.938*	0.266	0.108	*0.187*	0.292	0.340	*0.316*
Mean (x) A	*0.771*	*0.577*	0.675	*0.236*	*0.127*	0.182	*0.234*	*0.260*	0.247
LSD-test	A	B	AB	A	B	AB	A	B	AB
LSD 5%	0.028	0.053	0.083	0.008	0.018	0.026	0.011	0.025	0.035
LSD 1%	0.052	0.070	0.123	0.011	0.024	0.034	0.015	0.033	0.047

Maize genotype (the factor B)	Soil (A) Zn (mg kg^{-1}) A1	A2	Mean xB	Soil (A) Mn (mg kg^{-1}) A1	A2	Mean xB	Soil (A) B (mg kg^{-1}) A1	A2	Mean xB
Bc 706 – 9	30.0	30.4	*30.2*	52.0	198.3	*125.1*	7.95	1.87	*4.91*
Bc 273	34.8	29.7	*32.2*	92.5	288.5	*190.5*	3.30	0.80	*2.05*
Bc 473E – 5	27.5	25.2	*26.3*	55.5	279.8	*167.6*	5.76	1.70	*3.73*
Bc 265 – 1	31.9	41.9	*36.9*	45.0	146.8	*95.9*	4.92	1.38	*3.15*
Bc 707 – 1	31.0	45.2	*38.1*	45.0	277.5	*161.3*	5.65	0.96	*3.30*
Bc 31 002	44.9	57.1	*51.0*	54.0	287.3	*170.6*	5.24	0.98	*3.11*
Bc 837 – 1	32.7	37.9	*35.3*	57.3	199.3	*128.3*	6.62	2.19	*4.40*
Bc 24W	41.4	33.9	*37.7*	38.8	198.8	*118.8*	8.10	1.94	*5.02*
Bc 742	37.9	44.4	*41.2*	75.5	311.3	*193.4*	3.34	1.75	*2.55*
Bc 741 - 23	23.3	33.6	*28.4*	30.7	212.0	*121.4*	4.12	1.59	*2.88*
Mean (x) A	*33.5*	*37.9*	35.7	*54.6*	*239.9*	147.3	*5.50*	*1.52*	3.51
LSD-test	A	B	AB	A	B	AB	A	B	AB
LSD 5%	2.7	6.0	8.4	11.1	24.8	35.1	0.24	0.53	0.74
LSD 1%	3.5	7.9	11.2	14.8	33.0	46.7	0.31	0.70	0.99
	Mo (mg kg^{-1}) A1	A2	xB	Cd (mg kg^{-1}) A1	A2	xB	Grain yield (t ha^{-1}) A1	A2	xB
Bc 706 – 9	1.20	0.07	*0.63*	0.05	0.48	*0.26*	5.64	1.91	*3.78*
Bc 273	0.74	0.07	*0.40*	0.12	1.24	*0.68*	4.47	1.73	*3.10*
Bc 473E – 5	1.20	0.07	*0.64*	0.05	0.06	*0.06*	5.11	0.97	*3.04*
Bc 265 – 1	1.24	0.10	*0.67*	0.05	0.06	*0.06*	4.67	1.15	*2.91*
Bc 707 – 1	1.06	0.06	*0.56*	0.13	1.82	*0.98*	6.46	3.37	*4.92*
Bc 31 002	1.07	0.06	*0.57*	0.10	1.46	*0.78*	6.08	1.84	*3.96*
Bc 837 – 1	1.26	0.11	*0.68*	0.18	1.62	*0.90*	8.16	2.51	*5.33*
Bc 24W	1.44	0.09	*0.76*	0.05	0.05	*0.05*	5.17	2.52	*3.84*
Bc 742	0.76	0.09	*0.43*	0.06	1.06	*0.56*	4.44	1.36	*2.90*
Bc 741 - 23	1.45	0.08	*0.76*	0.05	0.04	*0.04*	6.40	2.42	*4.41*
Mean (x) A	*1.14*	*0.08*	0.61	*0.08*	*0.44*	0.44	*5.66*	*1.98*	3.82
LSD-test	A	B	AB	A	B	AB	A	B	AB
LSD 5%	0.05	0.11	0.16	0.07	0.16	0.22	0.41	0.58	0.82
LSD 1%	0.07	0.15	0.21	0.09	0.21	0.29	0.54	0.77	1.09

[a] The ear-leaf of maize (in dry matter): A1=fluvisol; A2=stagnic albeluvisol

Table 3. Description of individual maize genotypes regarding leaf composition.

The genotype	The lower levels in leaf	The higher levels in leaf
Bc 706 – 9	Zn and Mn	Ca, S, B and Mo
Bc 273	B, Mo, S and Zn	Mn
Bc 473E – 5	Zn, Mn and Cd	Ca, Mn, B and Mo
Bc 265 – 1	Ca, Mn, S, Cd and B	Zn and Mo
Bc 707 – 1	Ca, S and B	Mn, Mg and Cd
Bc 31 002	S and B	Zn, Ca, Mg, Mn and Cd
Bc 837 – 1	Ca, Mg and Mn	B, Mo and Cd
Bc 24W	Ca, Mg, Mn and Cd	Zn, S, B and Mo
Bc 742	Zn, Mn and B	S, Ca and Mo
Bc 741 - 23	Cd, Zn, Mn and B	S, Ca and Mo

Table 4. Correlation coefficients.

	The correlation coefficients: the ear-leaf at flowering stage								Grain
	Ca	Mg	S	Zn	Mn	B	Mo	Cd	yield
Ca		0.47*	-0.43	-0.36	-0.50	0.39	0.56*	-0.42	0.57**
Mg			-0.39	-0.05	-0.69**	0.61**	0.75**	-0.26	0.82**
S				-0.29	0.02	0.04	-0.09	-0.32	-0.14
Zn					0.33	-0.25	-0.31	0.51*	-0.25
Mn						-0.84**	-0.92**	0.69**	-0.85**
B							0.91**	-0.55*	0.83**
Mo								-0.57**	0.90**
Cd									-0.42

Conclusion

Based on these results we could recommend growing of the genotypes characterizing affinity for individual elements (the higher leaf-contents) under soil conditions characterizing the higher needs of correspondingly nutrients and lower needs of harmful cadmium. Also, for some inbred lines could be useful foliar spraying with nutrients (especially zinc, boron, molybdenum and magnesium) in accordance with their low leaf nutrient status.

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Assessment of genotypic variation of fruit quality traits in apple breeding families by individual and sensory panel evaluation methods

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ABSTRACT: Internal and external fruit quality is one of the most important breeding aims for the South African Research Council (ARC) apple (*Malus* x *domestica* (Borkh.)) breeding programme. Traditionally, assessment of visual and sensory fruit traits and evaluation for selection purposes are performed by breeders on an individual basis. The aim of this investigation is the identification of key drivers for consumer appeal in apple breeding of preferred traits to increase the efficiency of apple breeding. During this investigation visual and sensory traits of apple breeding families were assessed by means of individual evaluation and panel evaluation. Descriptive sensory analysis using a trained panel as well as individual evaluation of apple parental genotypes and seedlings from breeding families were performed using a 100 mm unstructured line scale. Consumer acceptability was assessed by using 9-point hedonic scale. Multivariate data analysis techniques, such as Principal Component Analysis and Preference Mapping, were performed to project sensory attributes onto the preference dimensions. Consumer preference for apples appeared to segregate into two opposing groups. The first group preferred sweet tasting apples while the second group favoured juicy, crunchy and crispy apples. Mealiness and acidity were opposing and strongly negative attributes that may relate to fruit maturity. There was no clear preference for any particular peel colour. The different quality traits showed considerable variance between genotypes within families. Variation for traits seemed to differ between families.

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Evaluation of a subcollection of spring barley genetic resources with hulless grain

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ABSTRACT: New genetic resources of hulless barley (HB) obtained from collecting expeditions from regions of origin (China - Nepal), international gene banks or breeders were studied. A main advantage of landraces, local or new cultivars and new developed lines of HB is grain quality. New Canadian cultivars of HB are sources of high dietary fibre content. The original resources and cultivars from Asia are distinguished by high content of N-substances and some of them also by required agronomic characters (earliness, short stem, resistance to fungal diseases). In comparison with new genetic resources of HB, developed at the Agricultural Research Institute Kromeriz, Ltd. (Czech Republic), they have lower grain yield as well as starch content, worse levels of main yield components and lower lodging resistance. They will be employed in further research and in the development of resources for breeding cultivars with specific grain quality for food use.

Keywords: breeding, food use, N-substances, agronomic characters.

Introduction

There is an increasing interest in organically managed crops in the Czech Republic as well as in other countries of Central and East Europe, which is a chance for less grown and non-traditional cereal crops, among others, hulless barley (HB) with differentiated grain quality. In the field of human food, beside desirable higher content of total and dietary fibre, the content of N-substances and their amino acid composition are gaining importance (Shewry, 2007). The new barley genetic resources with different ability of protein accumulation can be used in classical breeding processes, which corresponds to the principles of organic farming. They have also to meet requirements related to a level of other important agronomic traits.

Genotypes with both different quality and agronomic traits are maintained within the World Collection of Spring Barley Genetic Resources (WCSBGR) of the Czech Republic, preserved at the Agricultural Research Institute Kromeriz, Ltd. (ARI Kromeriz). Of the total number of 2,830 accessions, the subcollection of HB contains 196 genetic resources, classified into 26 varieties of the species *Hordeum vulgare* L. In comparison with the previous period (Milotova et al. 2003), the number of multirow forms (137) increased *vs*. two-row ones (59).

New donors of the gene *nud* have been investigated with regard to the content of desired nutrients and important agronomic and biological traits and characters.

Materials and Methods

A collection of new genetic resources of HB, obtained from collecting expeditions in China (CHN; Nepal; 24), gene banks (JPN=Japan; 40, USA - 3, ETH=Ethiopia - 2), from breeders (CAN=Canada; 7) and our own new breeding lines (CZE=Czech Republic; 2) was examined in field experiments conducted at Kromeriz in 2004-2006 in comparison with a control cultivar of hulled malting barley, Tolar (std.). The agronomic and biological traits were evaluated according to IPGRI and taxa HORDEUM descriptor lists (1985, 1994). Content of chemical substances in grain (protein, starch in %) were screened in the whole grain using the NIR instrument (INFRAMATIC 9100, PerCon Firm) and standard chemical analyses.

Results and Discussion

The most frequent varieties of multirowed genotypes were *revelatum* Koern and *adisabebae* Vav. et Orl., whereas the dominating variety in two-rowed genotypes is variety *nudum* L. with similar subtaxa (*nigrinudum* Vav. and *bruneinudum* Vav. et Orl.). The examined materials of HB differentiated not only in nutrient content but as well as in a level of important agronomic and biological traits (Table 1). The resources from Japan were the earliest among all accessions (91-98 d), they had the shortest stem (56-95 cm), whereas the original primitive resources from Nepal had the longest vegetative period (100-109 d) and the longest stem (82-102 cm) under our conditions.

Table 1. Characterization of important agronomic traits of HB according to the country of origin (2004-2006).

Origin	N	Veg. period, days		Height, cm		Yield, t/ha		TGW, g		No prod. tillers	
		Mean	SEM[1)]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
JPN	40	94	0.5	69	1.0	4.1	0.11	26.0	0.31	538	11.1
USA	3	95	1.8	96	3.6	4.0	0.39	38.4	1.14	474	40.5
CAN	7	99	1.2	94	2.5	4.5	0.27	37.7	0.79	551	27.9
CHN	24	105	0.6	91	1.3	2.9	0.14	34.8	0.41	469	14.4
CZE	2	102	2.4	80	4.8	8.0	0.52	40.0	1.54	651	54.3
ETH	2	96	2.2	91	4.4	3.9	0.48	45.1	1.40	529	49.6
CZE-std.	1	103	1.7	88	3.4	9.3	0.37	46.2	1.09	682	38.4

[1)] - SEM = standard error of measurement

Regardless of the differences in plant height, both groups were susceptible to lodging even though due to different causes; the materials from Japan lost the resistance mostly due to low stem strength at full maturity.

Besides the differences in lodging resistance, analysis of variance confirmed different susceptibility to powdery mildew (*Blumeria graminis*) in the examined accessions. Both a majority of original resources and Canadian cultivars and our own breeding lines exhibited moderate to higher susceptibility (1-9 scoring scale), however, interesting donors with high field resistance (8.3 - Basan Hadaka Mugi, JPN; 9 - Nudimelanocrithon, ETH) were detected.

Table 2. Biological traits of the hulless barley according to the country of origin (median, 2004-2006).

Origin	N	Lodging	*Blumeria graminis*	*Pyrenophora teres* 9 - 1 [1)]	*Puccinia hordei*
JPN	40	6	4	8	5
USA	3	4	3	7	4
CAN	7	9	4	8	6
CHN	24	5	4	7	5
CZE	2	9	6	7	7
ETH	2	5	7	7	6
CZE-std.	1	9	5	7	7

[1)] - on the scale 9-1, where 9=higher and 1=lower value of traits

Differences in the resistance to other diseases (*Pyrenophora teres*; *Puccinia hordei*) were masked by weather conditions of the crop year, so the differences were significant only between groups according to countries of origin. The highest susceptibility to leaf rust was assessed in gene resources with black grain, obtained from the USA (3-5 scores).

Yielding ability of the investigated materials was affected by both the number of productive tillers and TGW that was on average the lowest in the accessions from Japan (from 19.7 to 36.3 g). Relatively high TGW was assessed in the original 6-rowed materials from China (41.4 g - Hor 14), which surpassed even 2-rowed Canadian HB cultivars on the average of 2004-2006. The highest TGW was produced by original resources with black grain from Ethiopia (Nudimelanocrithon and Nigrinudum). In productive cultivars and new lines of HB, the lower grain weight was compensated for by a number of productive tillers and grains in ear, which affected grain yield. The lines bred at Kromeriz (Vaculova et al., 2004) were superior in grain yield among the other accessions as compared to the control (85.5% of cv. Tolar).

The content of N-substances was high in a set of genotypes of Asian origin (15.1-21.4%) and there were detected also materials with higher N-substances and higher starch content (19.6% and 53.8%, respectively - Yane Hadaka, JPN) or higher N-substances and different content of some essential amino acids (no table). The highest starch content was found in the Czech new lines KM2283 and KM1910 (Fig. 1), followed by the best materials from Japan (Yamato Hadaka and Shirahime with 59.9 and 59.6 %, respectively).

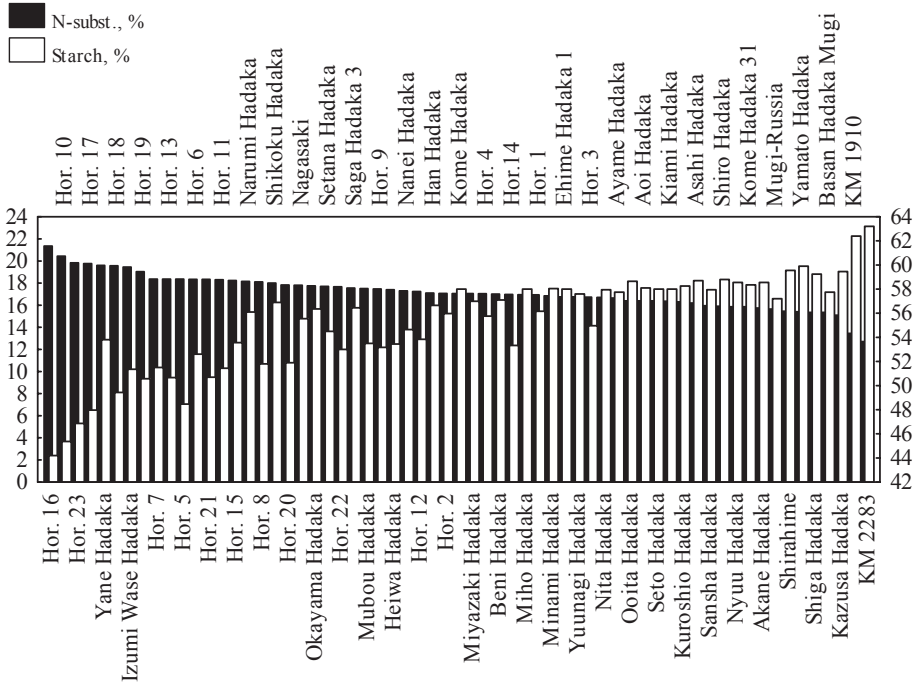

Figure 1. Content of N-substances and starch in dry matter of grain (in %) of selected genetic resources (2004-2006).

In a set of new Canadian cultivars, CDC Alamo, CDC Fibar and CDC Rattan excelled in higher proportion of dietary fibre (particularly beta-glucans) and waxy starch, i.e., a changed ratio of main starch polysaccharides (in favour in amylopectin - see Poster).

Conclusion

A purposeful subcollection of hulless barley within the World Collection of Spring Barley Genetic Resources of the Czech Republic (preserved at the ARI Kromeriz) has been enlarged

due to an increasing interest in the use of HB in organic agriculture and for food production. New genetic resources of spring barley with hulless grain, particularly landraces and local cultivars from Asia, distinguish by high content of N-substances. In comparison with productive new Canadian cultivars and especially own new lines of HB (KM2283 and KM1910), however, they have lower grain yield due to worse levels of main yield components, low starch content and lodging resistance. Due to very high content of N-substances they could be employed in further research for development of usefull breeding resources.

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Development of rapeseed (*Brassica napus* L.) with more than 70% erucic acid in the seed oil

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ABSTRACT: High erucic acid rapeseed (HEAR) oil is of interest for industrial purposes because erucic acid (22:1) and its derivatives are important renewable raw materials for the oleochemical industry. Currently available cultivars contain only about 50% erucic acid in the seed oil. A substantial increase in erucic acid content would significantly reduce processing costs and could increase market prospects of HEAR oil. It has been proposed that erucic acid content in rapeseed is limited because of insufficient fatty acid elongation, lack of insertion of erucic acid into the central *sn*-2 position of the triaclyglycerol backbone and due to competitive desaturation of the precursor oleic acid (18:1) to linoleic acid (18:2). The objective of the present study was to increase erucic content of HEAR winter rapeseed through over expression of the rapeseed fatty acid elongase gene (*fae*1) in combination with expression of the lysophosphatidic acid acyltransferase gene from *Limnanthes douglasii* (*Ld-LPAAT*), which enables insertion of erucic acid into the *sn*-2 glycerol position. Furthermore, mutant alleles for low contents of polyunsaturated fatty acids (18:2+18:3) were combined with the transgenic material. Selected transgenic lines showed up to 63% erucic acid in the seed oil in comparison to a mean of 54% erucic acid of segregating non-transgenic HEAR plants (+9%). Among 220 F2 plants derived from the cross between a transgenic HEAR line and a non-transgenic HEAR line with a low content of polyunsaturated fatty acids, recombinant F2 plants were identified with an erucic acid content of up to 72% and a polyunsaturated fatty acid content as low as 6%. Regression analysis revealed that a reduction of 10% in polyunsaturated fatty acids content led to a 6.5% increase in erucic acid content. Results from selected F_2 plants were confirmed in the next generation by analysing F_4 seeds harvested from five F_3 plants per selected F_2 plant. F_3 lines contained up to 72% 22:1 and as little as 4% polyunsaturated fatty acids content (18:2+18:3) in the seed oil. The 72% erucic acid content of rapeseed oil achieved in the present study represents a major breakthrough in breeding high erucic acid rapeseed.

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Relationship between the apple's cover colour and temperature in apple gene bank plantation

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ABSTRACT: The colouration of fruits is an important quality requirement for the market. The degree of colour coverage has an influence on the saleability particularly in the red coloured cultivars. This study aims to reveal the influence of temperature on the degree of cover colour of apple. We have determined the time change of colouring and estimated it by means of environment variables. The results show significant relationships between the main meteorological variables, such as day and night temperature and the difference between day and night temperature, and the rate of colour cover. The genetic backgrounds of apple varieties ripening in winter have a greater effect on the development of cover colour while the years play larger role on it in the case of apples ripening in summer or autumn. A difference of 4ºC between day and night temperatures resulted in about 79% of cover colour on the fruits, but for differences over 6.2 °C, the extent of cover colour decreased in apples ripening in summer.

Keywords: apple, cover colour, temperature, summer ripening.

Introduction

Cover colour is one of the phenometric variables of fruits but its rate can change year by year. The experienced oscillation can be caused by inappropriate water and nutriment supply, some plant diseases, extremely high or low temperature and setting rate above the average with outstanding fruit density (Racskó et al. 2005). At the end of the 1960s, several studies

published the mutual effect of phenological stage and meteorological parameters (Csöbönyei and Stollár (1969) and the impacts of macro and microclimatic factors on the growth of fruits (Szász 1961). The researches were carried out on evapotranspiration and the water consumption of apples during the fruit growing period (Gergely and Stollár, 1978) and results related to Jonathan apple's ripening period estimated by weather variables (Stollár, 1977). As market requirements for enhanced colour increase growers carry out the management practices designed in order to obtain better fruit colour (Soltész et al. 2004). Fruits with low colouration are prone to diseases and injuries of the skin on the other hand the disadvantageous effects of the latter are more definite during storage due to later harvesting (Racskó et al. 2005).

This study aims to reveal the influence of temperature on the degree of cover colour of apple in a collection of apple germplasm. We have determined the time change of colouring and estimated it by means of environment variables.

Material and methods

The phenological phases and phenometric indicators of 586 of apple varieties were examined in the Fruit Research and Extension Institute at Újfehértó during the years of 1984-2001. The measurements were carried out on 2 trees of all varieties using a repetition system, and the varieties were ranged as (1) ripening in summer (2) ripening in autumn (3) ripening in winter groups. Examining the colouration of these three categories was made using an easy visual assessment: 1. basic colour, 2. transition between basic and cover colour and 3. cover colour. Cover by different colours was expressed as a percentage of the total fruit surface (Holb et al. 2003).The varieties with good cover colour were separated in the sample for analysis in relation to meteorological factors. The effect of average daytime and night temperature on degree of cover colour was measured in different months of the vegetation and 30 days before ripening. During the examining period the air temperature was taken hourly and on daily basis by means of a computer demodulation automatic meteorological measuring station. The night temperature (T_{night}), day temperature (T_{day}) and the difference between day and night temperatures (T_{diff}) were used for calculations. The results were evaluated by average and standard deviation, and regression and correlation analysis.

Results and discussion

The results based on frequency of distribution cover colour on fruits in different ripening groups showed that almost half (49.7%) of the varieties ripening in summer had a good cover colour, while the rate of less well-coloured variety was 25.2% in this group. The apples with notable rate of colouring (64-81%) represented the largest part of the sample (32.1%). It appears the varieties with good cover colour occurred in a significantly larger proportion in the sample from ripening in summer group than varieties with weak cover colour. The apples ripening in autumn showed a fairly stable distribution of colouring. A total of 42.5% of these apples fruits can be characterized as having good cover colour, while 36.4% of that

have weak cover colour. In the sample from this group the number of varieties with good cover colour and weak cover colour seems to be almost equal. The frequency distribution of apples ripening in winter can be described by an U-shape function. This means that there is a predominance of well coloured and weak coloured varieties, respectively and varieties with mid-range cover colour had the smallest proportion.

In total, 34.1 percent of all varieties whose fruits cover colour intensity over the 70 % are considered well colouring varieties (Figure 1). The mid-range colouring varieties represented 41.2% of the sample, and 24.6% of the varieties had a rather low rate of cover colour(10-39%), and therefore belonging to the weak-coloured group.

In the last 20 years the average night temperature of the growing season has increased greatly in the experimental location. The high night temperature has generally an unfavourable effect on the production. Respiration becomes more intense, and therefore daily weight growth decrease. Several quality indicators, e.g. sugar content decrease due to the higher night temperature and the energy needed for an increased respiration is covered by the plant's own reserves. Neither small increasing day temperature nor the same rate of growth in day and night temperature cause disorders in the colouration. Problems can occur if the two examined variables' rate of growth are different.

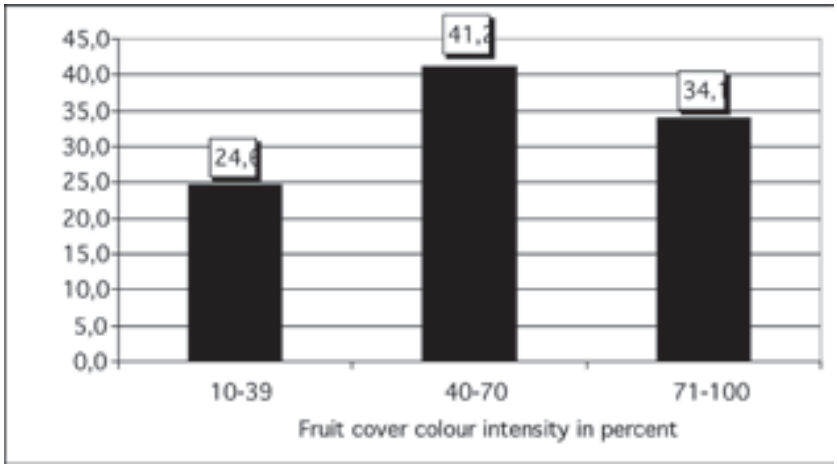

Figure 1. Distribution of apple cover colour at 586 apple cultivars

According to the results, the day temperature rate of growth falls behind the same value of night temperature. There was a significant relation between the night temperature in August and the cover colour of apples ripening in summer. Night temperatures ofup to 5 degrees higher result in a 15-17% lesser rate of cover colour. While high night temperature has an unfavourable effect on the establishment of cover colour of apple, the higher day temperature contributes to spread the cover colour. The relation between the day temperature in October

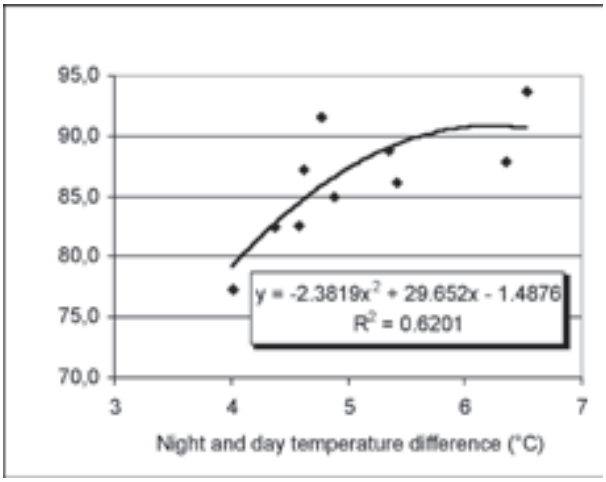

Figure 2. Relationship between day and night temperature difference and apple cover colour in August in summer ripening apple varieties.

and the degree of cover colour is rather good (R^2=0.54) in the apples ripening in autumn. In these apples a 4 °C increase in temperature results in 10-12% more favourable colouring rate.

The difference in day and night temperature has the greatest influence on the cover colour in different ripening groups. The relationships between the difference between average day and night temperature in August and cover colour was significant (R^2=0.62) in summer ripening groups (Figure 2). As seen in Figure 2, a 4ºC difference between day and night temperatures resulted in about 79% of cover colour on the fruits. The larger extent of cover colour can be reached with a difference above 5ºC between day and night temperature, but with differences over 6.2 °C the extent of cover colour of apples ripening in summer starts to decrease. In these cases cooling irrigation can be used for reducing fruit temperature (evaporative cooling) and the development of red colour that significantly improving fruit colour (Nemeskéri 2007).

In conclusion, the good colouration of apple can be estimated by the meteorological variables. The genetic backgrounds of apple varieties ripening in winter have a greater effect on the development of cover colour, while the years play larger role on it in the case of apples ripening in summer or autumn. The difference in day and night temperature has the greatest influence on the cover colour in different ripening groups. The larger extent of cover colour can be reached with a difference above 5ºC between day and night temperature, but with differences over 6.2 °C, the extent of cover colour of apples ripening in summer starts to decrease. In the future it would be useful to analyse the changes of cover colour in the

varieties with green and yellow fruits, since their market value and saleability are greatly influenced by these factors.

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The role of *ACS* genes in fruit softening in pome fruit

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ABSTRACT: The 1-aminocyclopropane-1-carboxylic acid synthase (ACS) enzyme plays an important role in ethylene biosynthesis in many fruit crops. It is encoded by a highly divergent multi-gene family whose members are differentially regulated. Of the 6 members of the *ACS* gene family in apple, *Md-ACS1* is predominantly expressed during fruit ripening (Sunako et al., 1999). The *PpACS1* and *PpACS2* genes are mainly involved in fruit ripening in Asian pears (Itai et al., 2003). *Md-ACS1* segregates in a Mendelian pattern and its allelotypes, *Md-ACS1-1* and *Md-ACS1-2* are linked to both high and low ethylene production, respectively. The epistatic relationship between *PpACS1* and *PpACS2* genes on the other hand, produces genotypes AB/Ab, aB and ab linked to high, medium and low ethylene production, respectively. We conducted studies over a period of 3 years to examine the relationships of *Md-ACS1* in apple or *PpACS1*/ *PpACS2* in pear on fruit softening. Fifty apple cultivars/selections were genotyped with *Md-ACS1* primers and also phenotyped for firmness at intervals of 5, 10, 15, 20, 30 and 40 days following storage at ambient temperatures in 2001. In 2002/2003, 68 apple cultivars/selections were also genotyped while phenotyping for firmness took place at 14 days intervals following cold air storage at 0.5°C. Fifty two pear cultivars/selections belonging to *P. pyrifolia* and *P. communis* were genotyped with cleaved amplified polymorphic sequence (CAPS) primers for *PpACS1* and *PpACS2* genes and phenotyped for firmness at fornightly intervals following cold air storage at 0.5°C in 2002/2003. The apple study showed that late *Md-ACS1-2* genotypes had the slowest rate of softening while early *Md-ACS1-1* cultivars showed the most rapid rate of softening at both ambient temperature and cold air storage. The pear study did not show any significant association between genotype or ethylene production and fruit firmness/ softening partly because only aB and ab genotypes in *P. pyrifolia* were compared while AB/Ab genotypes known for high ethylene production were lacking. Harvest firmness contributed 57% and 46% of the total variation in post-harvest cultivar firmness 50 days after harvest (50DAH) in apple and pear, respectively. These findings suggest that breeders should select for high harvest firmness within genotypes with low ethylene production to increase long term storage potential in pome fruit.

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Expert panel assessment of fruit quality in apple breeding

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ABSTRACT: Consumer acceptance of a new cultivar relies on the fruit being of very high quality in terms of taste, texture and appearance. The HortResearch apple breeding programme, like many others, uses a small number of well experienced assessors, or experts, to organoleptically score the quality of fruit from a large number of genotypes on a daily basis. Although instrumental readings are available for some traits, only those for weight (electronic balance), firmness (penetrometer), soluble solids (refractometer reading) and titratable acidity (autotitrator reading) concentrations are generally considered practical for most breeding purposes, at least in the early stages. However, heritabilities for instrumentally measured traits are typically higher than those for scored traits. This is likely to be partially because the use of scores for sensory attributes introduces a new source of variation, *viz* that attributable to changing perception, which inflates the environmental variance component. We report on a trial, undertaken in the 2003 fruiting season, which was designed to measure the various sources of expert perception error in order to help devise an optimal strategy for fruit quality assessment in apple breeding. Four experts each independently assessed two fruit from each of 126 genotypes taken from 15 crosses which were part of a half diallel originally made to study powdery mildew genetics. Traits assessed were: firmness, acidity, sweetness, juiciness and crispness. Each fruit was assessed by two experts, and was weighed and measured for soluble solids and titratable acidity. The experts did not know the identity of fruit they were assessing, although they were aware of the purpose of the trial. Fruit were presented to the experts in a random order and this order was recorded. We found little evidence of fatigue despite the experts assessing up to 90 fruit a day (34 in a session). However, there were differences between the experts, and evidence of a small influence of the previous fruit on the score of the current fruit for some of the experts. The heritabilities estimated from this study were higher than those reported previously. We discuss potential reasons for this, and the implications for breeding programmes.

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Molecular markers for major QTL underlying alpha-amylase production in rye grain

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ABSTRACT: Rye (*Secale cereale* L.) is an important cereal crop in Central and Eastern Europe. It is cultivated mainly for bread production and animal feeding but also for ethanol production. One of the main quality problems in rye is the high alpha-amylase activity in mature grain, which result in low falling number values and consequently causes economic loses among farmers. A major QTL linked to the alpha-amylase activity QTL (R^2= 0.60) on chromosome arm 5RL has been identified in rye. This chromosome region also contains a QTL for pre harvest sprouting, but located more distally. A dense molecular marker map expanding the region containing both QTLs should clarify their relationship and detect markers tightly linked to the major QTL for alpha-amylase activity which could assist in selection of low amylase genotypes. A total number of 19 molecular markers including RAPD, PCR-RFLP, STS, SSR, ISSR, AS-PCR and SNP were gathered to build a dense map in the distal region of 5RL. The 541xOt1-3 mapping population consisted on 130 F_2 plants.. Preliminary studies on marker polymorphism between parental lines revealed 14 polymorphic markers. Five remaining markers were derived from RFLP probes from wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) syntenic regions. Sequencing of these products from parental lines 541 and Ot1-3 produced 39 SNPs and 5 indels markers used for map construction . Special interest was devoted to the *α-Amy 3* locus located in this region. Studies of its sequence revealed 25 SNPs and major differences in exon II. PCR-RFLP polymorphism detected in *α-Amy3* sequence showed a high correlation with sprouting resistance checked within a group of 40 recombinant inbred lines (RILs) representing opposite extreme values of preharvest sprouting.

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Polymorphism of the storage proteins in Portuguese rye populations

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ABSTRACT: Rye (*Secale cereale* L.), a diploid and open pollinated species belongs to the grass tribe *Triticeae*. Genetic variation and diversity were estimated for four populations of *Secale cereale* from the northern Portugal namely "Montalegre", "Vila Pouca", "Padrela" and "Alvão" rye populations. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) methodology was used to describe allelic diversity in the storage proteins encoded by the corresponding genes located on chromosome 1R, as *Glu-R1* (Sec-3), which controls high molecular weight secalins, *Gli-R1* (*Sec-1*) which encodes some ω and γ secalins, and *Gli-R3* or *Sec-4*, mapped between *Glu-R1* and *Gli-R1* loci. Additionally for *Gli-R2* or *Sec-2* located on the 2RS chromosome 75K γ-secalin proteins were evaluated. The presence or absence of each allele was scored in a binary data matrix. Genotypes were grouped by cluster analysis using the UPGMA (unweighted pair group method with arithmetic average) and NTSYS-pc program, version 2-10a (Rolph, 2000).This material was compared with *Secale vavilovii,* 'Picasso' and 'Marder' varieties. Several alleles were identified by the single electrophoretic mobility patterns. Most of the genetic diversity revealed by secalins was found within the populations and was very small between populations. These results showed that regional rye varieties grown in Portugal exhibit a large genetic diversity. The data also show that this material has a high degree of polymorphism and they are grouped into different clusters related with geographical locations. Knowledge of the diversity of these storage proteins will greatly increase our understanding of the quality between rye varieties, especially taking into account the small number of cultivars of rye grown around the world. These results will allow a more efficient management and conservation of these resources, and better use in breeding programmes.

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Varietal differences of alfalfa (*Medicago sativa* L.) in relation to yield and quality as affected by maturity stages

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ABSTRACT: Little information is available on the consistency of alfalfa (*Medicago sativa* L.) cultivars forage quality and quantity at different maturity stages (Lenssen et al., 1990). This study was conducted to determine the effect of variety and maturity stage on yield and forage quality of alfalfa cultivars. Thirteen cultivars of alfalfa were evaluated in a randomized complete block design with three replications. Plant height, dry matter yield and leaf to stem ratio were recorded at three maturity stages (50% bud, 10% bloom and 50% bloom). Crude protein, Acid Detergent Fiber, Neutral Detergent Fiber and nutrient concentrations were also measured. There were significant differences between varieties and maturity stages on all of the traits. Cody had the highest plant height, dry matter and protein yield on the average of all maturity stages. At 50% bud stage, Cody (18.34 ton/ha) and at 10% and 50% bloom stage, Bagh Malek (22.49 and 26.63 ton/ha, respectively) had the highest dry matter yield. At the 50% bloom stage Cody had the highest percentage of crude protein and Rehnani had the least Acid Detergent Fiber and Neutral Detergent fiber as indicators of higher forage quality. Nutrients concentration decreased with advance in maturity except for boron and phoshorus. Dry matter yield had a positive correlation with leaf yield (r = 0.96**) and stem yield (r = 0.97**) but negative correlation was observed between dry mater yield and leaf to stem ratio (r = -0.43**). Significant differences among the cultivars indicated that selection for higher yield and quality for breeding and production is possible; however cutting should be performed at appropriate stage of the growth (Hall et al., 2000).

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Landraces, a source of variability for sensory traits in common bean (*Phaseolus vulgaris* L.)

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ABSTRACT: In vegetables, sensory traits are a primary objective for present and future breeding, but they generally have a complex genetic base (Casañas and Costell, 2006), so programs aiming to convert productive or resistant populations into elite sensory materials have serious difficulties. Improving agronomic traits in landraces with proven sensory value seems more promising, but little standardized information about the sensory properties of these varieties is available. In a previous study, we recorded sensory and chemical traits in a few Catalan varieties of common beans and in different accessions of one of these varieties. Although great differences were found in chemical traits, only some of them were perceived in the sensory tests (Casañas et al., 2006). The description of Catalan landraces has continued to advance, mainly through a panel trained to objectively describe the sensory characteristics of the full range of Catalan common bean landraces still cultivated. Together with some culinary traits, we recorded seed-coat roughness and seed-coat perceptibility, mealiness of the cotyledon, and intensity of the taste in 15 landraces, using a scale from 0 to 10 . Panelists were previously trained with extreme beans for each recorded trait. We found great variability for all traits: mean seed-coat roughness 4.0±0.4 (range 1.9-7.0); mean seed-coat perceptibility 4.6±0.5 (range 1.3-7.5); mean mealiness 4.8±0.4 (range 2.5-7.6); mean taste intensity 4.6±0.5 (range 2.1-8.4); mean cooking time 73.0'±2.8' (range 56-95'); mean unbroken seeds after cooking 46.9±6.0% (range 17-88%); and mean foam production during cooking 4.4±0.6 (range 0-6.3). Consumers cultural background strongly influences sensory preferences. As this trait has been a component of the landraces selection pressure, the variability found in landraces coming from a small territory forecasts enormous variability in the world set of bean landraces. Exploring this variability, matching cultural preferences with superior landraces, and selecting materials that have both high sensory value and acceptable agronomic behavior for breeding programs, seems a reasonable approach. Therefore, we encourage objective description of sensory properties of the germplasm to be preserved in "in situ" or "ex situ" collections.

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Genetic variability on quality traits of a collection of durum wheat landraces from the Mediterranean Basin

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ABSTRACT: Durum wheat landraces have been recommended as a source of genetic variability for quality improvement (Moragues et al., 2006). A set of 172 landraces from 22 Mediterranean countries was tested for quality traits in order to assess the genetic variability available for breeding purposes. Nineteen modern varieties were also used for comparisons. Grain samples were taken from a field experiment conducted in 2007 in Gimenells (Lleida) under rainfed conditions. The experiment consisted of unreplicated plots ($6m^2$, eight 5-m long rows, spaced 0.15 m apart) arranged according to a modified augmented design with three repeated checks (cv. Simeto, Vitron and Claudio). Seed density was adjusted to 250 germinable seeds per m^2. Protein content (%) was determined with a NIT-equipment, gluten strength (ml) was analyzed by the micro-SDS-test, yellow index was measured with a colorimeter and is expressed as b-index, and test weight (kg hl^{-1}) was measured with a Dickey-John equipment. Raw data were fitted to a linear mixed model with the check cultivars as fixed effects and row number, column number and landrace as random effects. The European quality index (QI described in the Official Journal of the European Union of 24/12/2003) was computed for each landrace in percentage relatively to the mean QI of the three checks that was equaled to 100. Restricted Maximum Likelihood (REML) was used to estimate the variance components and to produce the Best Linear Unbiased Predictors (BLUPs). The analysis was performed using the MIXED procedure of the SAS-STAT statistical package (SAS Institute Inc., 2000). Large genetic variability was found within countries for all the traits. Mean ± SD of landraces across countries were 14.9±0.65 for protein content, 9.0±1 for SDS, 15.9±1.1 for b-index and 79.8±1.7 for test weight, while for the modern varieties the values were 13.5±0.54 for protein content, 10.8±0.80 for SDS, 17.4±0.93 for b-index and 79.7±1.4 for test weight. Mean QI were 99.7 for landraces and 102 for modern varieties. The highest QI was reached by Libyan landraces, whose protein content largely over-passed that of the checks. Greek landraces showed the greatest gluten strength, but the lowest b-index, while landraces from Jordan reached the highest b-index. Principal component analysis allowed grouping countries with landraces showing similar overall quality.

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Variation for fatty acid composition in the accessions of *Carthamus oxyacantha* Bieb. as a potential genetic resource for safflower breeding

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ABSTRACT: Genetic variation for fatty acid composition is essential for genetic improvement of the oil quality and developing new cultivars (Ohlrogge, 1994). Seeds of six safflower (*Carthamus tinctorius* L.) genotypes and 17 accessions of wild safflower species (*C. oxyacantha* Bieb.) were analyzed for oil content and fatty acid composition. Seed oil content of the genotypes ranged from 29.20 to 34.00% and 20.04 to 30.80% in species of *C. tinctorius* and *C. oxyacantha*, respectively. The unsaturated fatty acids of oleic (C18:1) and linoleic (C18:2) along with the saturated fatty acids of palmitic (C16:0) and stearic (C18:0) composed 96-99% and 96-98% of the total fatty acids profile of seed oil in the accessions of wild species and cultivated safflower, respectively. The sum of Myristic (C14:0), palmitoleic (C16:1), arachidic (C20:0) and behenic (C22:0) in seed oil of both species was very low and ranged from 0.43 to 0.53%. The oleic acid content in seed oil of the genotypes in the species of *C. tinctorius* and *C. oxyacantha* ranged from 12.24 to 15.43% and 14.11 to 19.28%, respectively. The corresponding ranges for linoleic acid were 71.05 to 76.12% and 63.90 to 75.43%. The results of this study showed that fatty acids composition of seed oil in safflower and its wild species was not considerably different, indicating the same oil quality of both species. *C. oxyacantha* is easily crossable (Ashri and Knowles, 1960) with cultivated safflower and can be used as a new source of biotic and abiotic stress resistance genes in breeding programs and transfer of these genes may not affect the oil quality of safflower.

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Variation for vitamin C content in traditional and modern eggplant varieties

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ABSTRACT: Eggplant (*Solanum melongena* L.) is one of the most widely consumed vegetables in the world. A wide number of varieties has been used for food and medicinal purposes. Eggplant fruits have a great nutritive potential, due to its high fiber content as well as its high phenolics concentration, which results in a high antioxidant capacity. Nowadays, one of the main goals of the eggplant breeding programs is to obtain eggplant varieties with a high content of vitamin C, which apart from its antioxidant properties, can prevent fruit browning caused by the oxidation of phenolics. The aim of this work is to evaluate the differences on vitamin C composition of modern hybrids (Cristal F_1, Ecavi F_1 and Bandera F_1) and traditional (ALM1, CS16 and IVIA371) eggplant varieties cultivated both on open air and greenhouses. Plants of each one of these varieties were cultivated in COMAV facilities. Samples were analyzed for pH, titratable acidity, dry matter, and vitamin C (both ascorbic acid, AA; and dehydroascorbic acid, DHA fractions) (Sánchez et al., 2000; San José Rodríguez et al., 2006). The results of physicochemical parameters on the six varieties analyzed in both growing condition were quite similar. Main differences were found in eggplants grown in greenhouses, with four times higher vitamin C levels (up to 25mg/100g fresh fruit) than in the open air ones (with a maximum of 6 mg/100g fresh fruit), maintaining in all of them the same proportions between AA and DHA. Results from samples grown in open air are in agreement with previous studies (San José Rodríguez et al., 2006).In the samples analyzed, the DHA form is the predominant, possibly due to the activity of ascorbate-oxydase at pH levels of 5-6, which were found in the samples (Maccarone et al., 1993). The results show that conversely to what occurs for phenolic compounds, good sources of variation for high vitamin C content might be found in modern varieties.

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Gelatinization characteristics of endosperm starch mutants in rice

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ABSTRACT: Physicochemical characteristics of endosperm starches from waxy, dull, floury, and white core rice mutants were evaluated with amylose contents, iodine reaction, disintegration in alkali solution, gelatinization in urea solution, and amylogram properties. Amylose contents of the dull mutant was about half compared to that of the Suweon 472. In the other mutants, the amylose contents were slightly lower than that of the original variety. Endosperm starch samples exhibited distinct colors when stained with the I_2-KI solution. The samples from waxy, dull mutants and Suweon 472 yielded light brown, pale blue-black, and dark blue-black colors, respectively. The alkali digestion value of most of endosperm mutants except white core mutant were low compared to that of the normal counterpart. The initial pasting temperature of most of the mutants except waxy mutant, were increased compared to that of the normal counterpart. Peak, hot and cool viscosities of all of the mutants were less than those of the original variety. These data, togethr with those of former studies (Shin et al., 2004), indicate that endosperm starch granules from the original variety were apparently disintegrated in urea solution while those from the waxy mutants were scarcely degraded. However, the dull, floury and white core mutants were all susceptible to urea.

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Representation of the *Bmy1* intron III haplotypes in covered and naked barley cultivars and landraces

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ABSTRACT: Haplotype variability of the endosperm-specific barley beta-amylase gene (*Bmy1*) intron III was studied by direct sequencing of the corresponding gene portion in a set of 21 covered and 8 naked Latvian and European barley cultivars and breeding lines, as well as in 9 landraces including 5 European and 4 Asian accessions. Data were compared to the previously described allelic variants of the structural gene (Sjakste and Zhuk, 2006). Based on polymorphisms of 59 loci and the specificity of the microsatellite motif, we confirm here our data on the existence of five main haplotypes of the region. The same structural rearrangements turned out to define definite haplotype structure of the intron III sequence in covered and naked, as well as in cultivated barley and landraces. Simultaneously, several previously unknown polymorphisms were revealed in the study. The evolutional aspect as well as the role of breeding process in the *Bmy 1* intron III haplotype stability or dynamics is under discussion.

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Gliadin and HMW-glutenin variations in wheat lines

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ABSTRACT: Eight wheat lines (from cross wheat with *Triticum macha, Triticum polonicum and Triticum dicoccoides*), ten winter wheat and 2 spring wheat with resistance to *Fusarium* head blight were evaluated for the variability of gliadins and HMW-glutenins. A total of nine different HMW-GS were detected; two subunits (1, null) were recognized at *Glu-1A*, five (7+9, 7+8, 6+8, 13+16, 17+18) at *Glu-1B* and two (5+10, 2+12) at *Glu-1D* locus. Among 2 allelic variants detected in this study, subunit "null" at *Glu-1A* was the most frequent variant, represented in 85% of the analysed genotypes. Two wheat lines (P-105-2; SO-107-05) and cultivar Sumai3 carried subunit 1 at this locus. The most frequent subunits at *Glu-1B* were 7+9 (40%), 6+8 (30%), 7+8 (10%), 13+16 (10%) and 17+18 (10%). At the *Glu-1D* locus two pairs of subunits 5+10 and 2+12 were present with a frequency of 65% and 35%, respectively. Three out of the eight wheat lines had identical HMW-glutenin pattern (subunits 0; 6+8; 5+10) and two wheat lines had subunits 0; 7+9; 2+12, and the remaining 3 had not identical subunits. The high Glu-score had two wheat lines (one line had subunits 1, 13+16, 2+12 and second line 1, 6+8, 5+10). The presence of *Gli-1B3* secalin allelic block (bread-making quality inhibitor) is a consequence of translocation of a rye chromosome segment into the wheat genome (*1RS*/*1BL*). The translocation 1BL.1RS was found in four wheat lines carrying 1RS on wheat chromosomes 1B. In wheat lines, P-105-2 had the higher Glu-score and Rye –score, while SO-111-05 had the lowest Glu-score and rye score. The composition of glutenin subunits, Glu-score and Rye-score correlate with wheat flour bread-making quality and can be used for prediction. The results shown that for the improvement of bread-making qualities in wheat lines with resistance to *Fusarium* head blight, it will be necessary to replace some alleles, mainly because alleles at the *Glu-1D* locus (2+12) which occur in wheat lines P-104-2 and *Gli-1B3* secalin allelic block which occur in wheat lines SO-111-07 have a negative impact on wheat quality. In wheat lines P-104-1 a P-109-2 presence of 5+10 glutenin subunits at *Glu-D1*, relative favourable *Glu*-score and *Rye*-score score combined with resistance to *Fusarium* head blight indicates the possibility of using these wheat lines as resources for future breeding program.

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The identification and molecular mapping of major candidate genes (*FAB2*, *FAD2* and *FAD3*) and QTL involved in the fatty acid desaturation pathway in *Brassica napus*

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ABSTRACT: Oilseed rape (*Brassica napus*) is the most important oilseed crop in the UK. The seed-oil is made up of many different fatty acids, and the optimisation of the fatty acid composition of rapeseed oil is a key plant breeding objective. Fatty acid profiles are key determinants of the fitness of rapeseed oil for its various potential end-uses. For example, whilst the presence of polyunsaturated fatty acids (PUFAs) provides health benefits (Ruxton et al., 2004), they also make cooking oil prone to rancidity at high temperatures. The ability to better control the fatty acid composition of rapeseed oil would be aided by a deeper knowledge of the regulation of the biosynthetic pathway involved. In our study, we have focussed upon the regulation of the fatty acid desaturation pathway. Our aims were to identify and position on a linkage map the genes encoding components of the fatty acid desaturation pathway and to identify QTL controlling specific aspects of fatty acid composition of seed oil. We used a *Brassica napus* mapping population, called 'Q', which had been derived from a cross between a resynthesised *Brassica napus* and a cultivar (Tapidor DH). The candidate genes are *FAB2*, *FAD2* and *FAD3,* which encode the desaturase enzymes responsible for most of the conversions in seed oil of: stearic acid to oleic acid (*FAB2*), oleic acid to linoleic acid (*FAD2*), and linoleic acid to linolenic acid (*FAD3*).

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Use of various populations to identify QTL for wheat quality

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ABSTRACT: Improving quality of wheat is an important goal for many soft wheat breeders. Understanding the genetics underlying the many attributes of quality would improve the efficiency of improving quality. We have mapped QTL in six biparental populations derived from crosses among soft wheat parents. Each population has been mapped with >140 markers and has been phenotyped for seven to eight traits associated with quality in soft wheat using grain from at least three environments. Each population showed significant transgressive segregation for each trait and heritability was quite high for each trait. There were 42 trait/population combinations. For these 42 combinations, the largest effect QTL had an $R^2 > 0.14$ in 90% of the combinations, and the largest effect QTL had an $R^2 > 0.20$ in 27% of the combinations. Several QTL had R^2 values exceeding 0.30. Many of the QTL effects were found in only one population, including some with large effects. There were also some QTL that showed some consistency across populations. These included the effect of the rye translocation (chromosome 1BS), a flour protein QTL on chromosome 2B, a starch damage QTL on chromosome 2B, the effect of Glu-B1 (chromosome 1BL) on gluten strength, and the effect of the Glu-B1 region on water absorption traits. In addition to the six biparental populations, we also have data from a related set of full- and half-sibs, and a diverse association analysis population that represents the eastern US soft wheat gene pool. Collectively, these provide an opportunity to assess the ability of different types of population structure and statistical analyses to detect QTL from the same gene pool. We will present results from some family- and population-based association analyses of these populations and compare them to the results from the bi-parental cross analyses.

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Seed storage protein diversity in Slovak wheat cultivars

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ABSTRACT: The quality of wheat-based foods and the processing properties of wheat flour dough are strongly related to the present and properties of very large glutenin protein aggregates. Storage proteins are the first products of gene expression and because of that they are usually used as markers for characterization of technological quality. The objective was to determine the technological quality of Slovak wheat cultivars (*Triticum aestivum L.*) included in the List of Registered Cultivars, registered in the period of 1976-2007. All samples were classificated according to electrophoretical profiles from SDS-PAGE and A-PAGE analyses. We characterized the composition of high-molecular-weight glutenin subunits (HMW-GS) by SDS-PAGE analysis. The most frequent HMW-GS alleles were "null" for Glu-1A, 7+9 for Glu-1B and 5+10 for Glu-1D, respectively. Profile with HMW-GS composition 0, 7+9, 5+10 was the dominating one. The above-mentioned HMW-GS composition was found in 52% of all *Triticum aestivum* L. ssp. *aestivum* genotypes analyzed. There was also used separation of gliadins by A-PAGE for detection of secalin block, which was observed in 12 genotypes. On the basis of composition of HMW-GS and according to the presence of secaline block it is possible to calculate Glu-score which predict the technological quality of wheat cultivars. Until 2007, Axis was the only registered E cultivar which possessed *Glu-1A 2*, Glu-1B 7+8* and *Glu-1D 5+10* composition, with maximum Glu-score and Rye-score 10. On the other hand, the minimum value of Glu-score (4) was observed in cultivar Veldava. Moreover, the frequency of HMW-GS allele *Glu-1B 7+8* is increasing in Slovak wheat cultivars. This *Glu-1B 7+8* allele is present in recently registered cultivars, including E cultivars (Axis, Alacris and Bona Dea), A cultivar (Malvína) and C cultivars (Danubia, Torysa and Markola). For any further improvement of bread-making qualities in wheat cultivars, it will be necessary to replace the null allele with alleles 1* and 2 or with any other known alleles that have not been utilized in crop breeding so far.

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Breeding of Kunitz-free soybean genotypes

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ABSTRACT: The soybean grain is known as an excellent source of protein, but it also contains antinutritive components. Mature grain of conventional soybean varieties requires heat processing to break down trypsin inhibitor's activity before using as food or animal feed. At the same time, protein denaturation and other qualitative changes occur in soybean grain, especially if the temperature of heating is not controlled. About 6% of whole soybean seed proteins belong to Kunitz-trypsin inhibitor (KTI), a predominant antinutritional factor, which makes 30-50% of trypsin inhibitor's activity. The absence of KTI is inherited as a recessive allele to Tia, Tib, and Tic, and has been designated ti. (Orf and Hymowitz, 1979). Due to the lack of KTI, soybean grain can be processed at a lower temperature and for a shorter period of time. By using Kunitz-free genotypes we save energy, and preserve valuable nutritional composition of soybean grain, which is of interest in industrial processing. This trait makes them also suitable for direct feeding in adult non ruminant animals without previous thermal processing. As part of a soybean breeding programme at the Maize Research Institute it was aimed to reduce trypsin inhibitor activity. Variety Kunitz (Bernard et al., 1991.), lacking KTI, has been used, as a parent donor of desirable character, in crosses with high yielding varieties. F2 plants were segregating in a ratio 3:1 (Ti:ti respectively). Identification of Kunitz-free individual plants was done under a few segregating generations. After yield testing, two genotypes were recognized by the Ministry of Agriculture, Forestry and Water Management in the Republic of Serbia.

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Feeding value of wheat lines differing in presence / absence of 1B/1R translocation tested on chicken broilers

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ABSTRACT: In the set of doubled haploid (DH) wheat lines (8 with and 10 without 1B/1R translocation) there were evaluated grain components, technological and bread-making properties, relative viscosity, grain hardiness and their relations to weight gain of chickens. In addition to year and wheat line, the presence of 1B/1R translocation was important factor, which significantly influenced feeding test and increased weight gain of broilers. It is likely, that high ratio of albumins and globulins in grain is significant factor determining higher final feeding value of wheat grain for broilers. The deteriorate effect of relative viscosity on broilers feeding test was not confirmed.

Keywords: chicken broilers, feeding value, protein, wheat.

Introduction

World wheat grain production that reaches annually 600 million tonnes in average (606 million tonnes in 2006) is very important, in addition to human nutrition, also for animal feeding. Wheat grain usually forms important part of feeding mixtures for pigs and poultry. Popularity of chicken meat has increased recently. Consequently, the chicken meat production has doubled since 1990 from 36 to 73 million tonnes in 2006 (FAOSTAT, 2008). In this context quality of wheat grain as a substantial component of feeding mixtures for chicken in North-West and Central Europe plays important role. Differences in feeding value among wheat cultivars described e.g. Shivus and Gullord (2002), Pirgozliev et al. (2003).

The cereal components of pig or poultry feeds may provide three-quarters of the supply of available energy and up to half of the total crude protein supply (Rose, 2003). The protein content and its composition show much higher variability in comparison with variability in content and properties of starch. As criteria of wheat grain feeding value are not uniquely determined (Petr, 2003), we evaluated wheat grain composition, rheological and bread-making properties, viscosity, grain hardiness and their relations to weight gain of chickens as the main objective of this paper. Effect of 1B / 1R translocation has been assessed as well.

Material and Methods

A set of 80 doubled haploid (DH) wheat lines was developed by Ladislav Kučera, from the crossing of wheat cultivar Šárka with advanced line UH 410 (donor of 1B/1R translocation) in the Department of Molecular Biology of CRI Prague. A set of selected 18 DH wheat lines with a higher agronomical potential and according to the presence or absence of allele Gli 1B3 characterising 1B/1R translocation was subsequently divided into two numerically comparable sub-sets (8 lines with and 10 lines without 1B/1R translocation).

The lines together with two check cultivars Šárka and Nela were multiplied in large plot experiments with plot size 0.7 ha in the locality Kralovice for 2 years (2005–2006). Fertilization was applied according to soil analysis in the nitrogen range of 120 – 135 kg N and 50 – 60 kg P_2O_5.

The following grain parameters were tested: content of crude protein – Kjeldahl method (ČSN EN ISO 5983-1); Zeleny sedimentation test (ČSN ISO 5529); wet gluten content (WG) and gluten index (GI) – Glutomatic 2200 (AACC 38-12); protein fractions content (albumins + globulins and their proportion in crude protein; gliadins) – modified Osborne method according to Dvořáček et al. (2001). Content of sum glutenins was calculated as a difference between the content of crude protein and sum of albumins + globulins and gliadins.

Relative viscosity was measured with a micro-viscosimeter Anton Paar according to Saulnier et al. (1994). Grain hardiness as Particle Size Index (PSI) – according to AACC method 55-30.

The other grain chemical analyses were carried out according to Kacerovský et al. (1990): content of crude fat - Soxhlet's extraction; content of crude fiber - two step alkaline and acid hydrolysis on Fibertec; content of ash – mineralization method in 550°C; gross energy (GE) – calorimetric method – burning of grain sample in oxygen atmosphere; content of nitrogen free extract - according to equitation: NFE (%) = 100 – water content – crude nitrogen content – crude fat content – crude fiber content – ash content.

Biological testing (growth experiment) was performed in accredited station of broilers fattening in Krucemburg where each variant of wheat (18 DH lines + 2 check cultivars) was the only different component of feeding mixture BR2. Balanced sets at 200 chickens were used to evaluate each of feeding variants.

The software "Statistica 7.0 CZ" was used to test significant differences by ANOVA/MANOVA, Tukey HSD test, multiple step regression, correlation matrix and PCA (Principle Component Analysis).

Results and Discussions

Two-year average evaluations of line and 1B/1R translocation effect on weight gain of broilers and other tested parameters are illustrated in the table 1. There is evident the high significant influence of year on most of grain characteristics including weight gain of broilers. Only three parameters (content of gliadins, Zeleny sedimentation test and crude protein) didn't show more significant differences between both years. Effect of climatic conditions on changes of

chemical and technological properties in wheat grain is documented in many publications and significant effect on parameters of feeding tests were confirmed by Pirgozliev et al. (2003) or Shivus and Gullord (2002) as well.

Factors of 1B/1R translocation and genotypes influenced variability of tested parameters in much fewer cases. Presence of 1B/1R translocation in wheat lines significantly increased relative viscosity, fractions of gliadins and weight gain of broilers. The significant opposite effect was detected in both technological parameters (Zeleny sedimentation and GI). Increasing trends of other parameters (crude protein, albumins and globulins) were not significant.

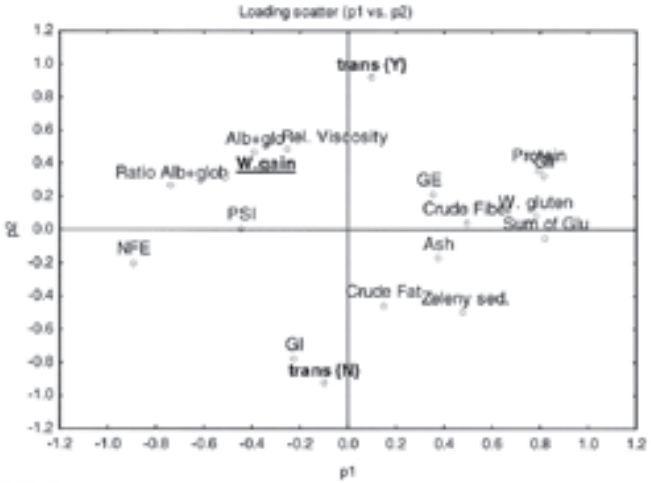

Figure 1. Relationships among weight gain of broilers, tested parameters and factor of 1B/1R translocation after year transformation.

The highest level of wheat line variability was found in several parameters (Zeleny sedimentation, PSI and weight gain of broilers). High number of broilers used for evaluation of each wheat line enabled to obtain precise statistic results confirming several groups of wheat lines with different effect on weight gain of broilers. The highest weight gain was found in translocated wheat line 174 (1660.30g). In the contrast the lowest value of this parameter was obtained in untranslocated line 146 (1466.25g). High values of Zeleny sedimentation were found in wheat lines 144 (59.5ml) and 146 (61.5ml) without 1B/1R translocation. The highest difference among PSI parameters is illustrated by wheat lines 174 (23.99) and 119 (10.64). Significant differences were detected in other grain parameters (Zeleny sedimentation., wet gluten, gluten index, gliadins, sum of glutenins, relative viscosity and ratio of albumins and globulins) as well (tab. 1). These results evoke that 1B/1R translocation is only one of aspects which can significantly influence feeding value.

Table 1. Effect of wheat DH lines on achieved weight gain of broilers and their other chemical and technological characteristics (2005 – 2006).

Factors/trans.	W. gain (g)	GE ($kJ.g^{-1}$)	Crude Fat ($g.kg^{-1}$)	Ash ($g.kg^{-1}$)	Protein (%)	Crude Fiber (g.kg-1)	NFE (g.kg-1)	PSI (%)	Zeleny Sed. (ml)	W. gluten (%)	GI	Alb+glo (%)	Gli (%)	Σ Glu (%)	Rel. Viscosity	Ratio Alb+glo (%)
L110 (Y)	1609.92hijk	18.73a	13.91a	11.94a	13.85a	28.29a	800.97ab	15.94abc	34.00ab	24.25ab	37.76ab	3.92a	4.33ab	5.60ab	2.46b	28.50b
L112 (Y)	1621.92ijk	18.85a	14.37a	15.28a	12.85a	26.92a	808.96ab	15.32abc	30.50a	25.60ab	20.09a	3.86a	3.92ab	5.07a	2.25ab	30.08b
L119 (Y)	1595.52ghij	18.82a	15.37a	17.24a	14.38a	29.26a	787.45ab	10.64a	44.00abc	26.13ab	47.71ab	3.70a	4.68b	6.01ab	2.16ab	25.70ab
L121 (N)	1618.75ijk	18.89a	14.84a	16.56a	13.28a	29.86a	799.96ab	14.92abc	51.50abc	23.48ab	94.89b	3.33a	3.76ab	6.20ab	2.18ab	25.26ab
L126 (Y)	1573.16defhgi	18.75a	16.47a	13.34a	13.78a	26.19a	799.74ab	18.21abc	39.00abc	18.05ab	72.33ab	4.09a	3.96ab	5.74ab	2.40ab	29.68b
L131 (N)	1603.73ghij	18.75a	18.67a	13.36a	11.96a	28.98a	814.00b	21.92bc	38.50abc	12.88ab	92.01b	3.39a	3.10ab	5.48ab	2.31ab	28.46b
L136 (N)	1532.37bcde	18.71a	16.99a	15.82a	13.08a	38.06a	791.86ab	14.33abc	51.00abc	25.53ab	81.64ab	3.40a	3.91ab	5.78ba	2.22ab	25.92ab
L139 (Y)	1487.60ab	18.99a	17.45a	16.28a	14.98a	39.97a	769.33a	13.29abc	43.50abc	33.08b	35.84ab	2.64a	4.94b	7.40b	2.04ab	17.61a
L144 (N)	1553.99cdefg	18.66a	18.05a	18.52a	14.18a	31.33a	783.11ab	12.72ab	59.50bc	29.15ab	72.74ab	3.58a	4.23ab	6.37ab	2.05ab	25.19ab
L146 (N)	1466.25a	18.90a	16.43a	15.70a	13.45a	27.20a	799.91ab	11.74ab	61.50c	25.78ab	91.81b	3.36a	4.08ab	6.01ab	2.21ab	25.03ab
L157 (Y)	1540.37bcdef	18.90a	15.26a	18.36a	13.45a	29.20a	796.31ab	13.93abc	39.50abc	25.55ab	66.33ab	3.38a	4.15ab	5.93ab	2.36ab	25.13ab
L159 (N)	1508.70abc	18.83a	14.70a	14.48a	14.00a	26.16a	798.10ab	16.80abc	45.00abc	26.43ab	87.12b	3.34a	4.30ab	6.36ab	1.77a	23.85ab
L163 (N)	1521.64bcd	19.00a	17.70a	15.73a	14.28a	30.28a	786.78ab	20.17abc	48.50abc	29.25ab	83.13ab	3.09a	4.51ba	6.68ab	2.04ab	21.66ab
L164 (Y)	1605.57ghij	19.08a	14.74a	15.88a	14.99a	30.87a	781.16ab	19.27abc	47.00abc	30.48b	72.86ab	3.63a	4.52ab	6.85ab	2.49b	24.17ab
L167 (N)	1589.92fghi	18.83a	14.79a	15.71a	13.41a	29.16a	799.61ab	21.47abc	42.00abc	25.48ab	82.29ab	3.66a	3.87ab	5.89ab	2.26ab	27.20ab
L171 (N)	1650.67jk	19.01a	17.83a	18.14a	13.75a	28.72a	790.73ab	11.75ab	56.50abc	25.88ab	88.44b	3.31a	4.34ab	6.10ab	2.39ab	24.02ab
L174 (Y)	1660.30k	18.95a	17.35a	17.44a	13.71a	31.85a	790.06ab	23.99c	40.50abc	26.30ab	51.74ab	4.08a	3.82ab	5.81ab	2.27ab	29.89b
L176 (N)	1612.50ijk	18.69a	14.34a	15.53a	12.78a	26.52a	809.88ab	19.08abc	45.50abc	25.23ab	90.30b	3.33a	3.81ab	5.64ab	2.19ab	26.06ab
Nela check v.	1557.75cdefgh	18.83a	16.84a	14.56a	13.38a	28.18a	800.63ba	18.19abc	44.50abc	26.25ab	84.00ab	3.25a	4.20ab	5.94ab	2.09ab	24.43ab
Šárka check v.	1583.56efghi	18.66a	19.68a	17.11a	13.70a	26.04a	794.11ab	18.58abc	50.00abc	24.50ab	79.60ab	3.70a	3.92ab	6.09ab	2.18ab	27.26ab
Trans.-Yes	1586.79b	18.88a	15.61a	15.72a	14.00a	30.32a	791.75a	16.32a	39.75a	26.18a	50.58a	3.66a	4.29b	6.05a	2.30b	26.34a
Trans.- No	1566.65a	18.81a	16.74a	15.93a	13.44a	29.21a	797.39a	16.80a	49.50b	24.98a	85.66b	3.39a	4.00a	6.04a	2.16a	25.36a
Year - 2005	1524.49a	18.20a	15.77a	18.02b	13.44a	32.49b	786.38a	13.72a	45.95a	27.68b	61.06a	3.73b	4.12a	5.59a	1.94a	27.86b
Year - 2006	1624.93b	19.48b	16.81a	13.68a	13.88a	26.81a	803.89b	19.50b	45.25a	23.25a	82.19b	3.27a	4.11a	6.50b	2.49b	23.65a

Values of parameters marked by the different letters are significantly different at $p \leq 0.05$

Table 2. Correlation coefficients among tested parameters in DH lines (2005 – 2006).

	GE ($kJ.g^{-1}$)	Crude Fat ($g.kg^{-1}$)	Ash ($g.kg^{-1}$)	Protein (%)	Crude Fiber (g.kg-1)	NFE (g.kg-1)	PSI (%)	Zeleny Sed. (ml)	W. gluten (%)	GI	Alb+glo (%)	Gli (%)	Σ Glu (%)	Rel. Viscosity	Ratio Alb+glo (%)	W. gain (g)
GE	1.00															
Crude Fat	-0.09	1.00														
Ash	0.26	0.43	1.00													
Protein	0.28	-0.15	0.05	1.00												
Crude Fiber	0.23	0.22	0.38	0.21	1.00											
NFE	-0.33	-0.18	-0.42	-0.85	-0.62	1.00										
PSI	-0.05	0.07	-0.19	-0.26	-0.12	0.28	1.00									
Zeleny Sed.	0.09	0.19	0.31	0.35	0.20	-0.45	-0.33	1.00								
W. gluten	0.26	0.10	0.42	0.57	0.38	-0.67	-0.22	0.29	1.00							
GI	-0.09	0.24	0.01	-0.34	-0.17	0.30	0.13	0.33	-0.34	1.00						
Alb+glo	-0.06	-0.27	-0.12	0.06	-0.14	0.05	0.19	-0.06	-0.27	-0.20	1.00					
Gli	0.21	-0.18	0.04	0.88	0.22	-0.75	-0.46	0.23	0.63	-0.38	-0.12	1.00				
Σ Glu	0.29	0.08	0.12	0.78	0.21	-0.72	-0.18	0.41	0.55	-0.05	-0.49	0.63	1.00			
Rel. Viscosity	0.18	-0.20	-0.09	0.02	-0.10	0.06	0.10	-0.13	-0.28	-0.13	0.37	-0.10	-0.15	1.00		
Ratio Alb+glo	-0.18	-0.16	-0.14	-0.42	-0.23	0.45	0.31	-0.24	-0.51	-0.04	0.88	-0.54	-0.81	0.34	1.00	
W. gain	-0.04	-0.14	-0.04	-0.24	-0.20	0.30	0.23	-0.31	-0.26	-0.12	0.21	-0.30	-0.30	0.32	0.32	1.00

Values written in bold are statistically significant at $p \leq 0.05$

PCA analysis graphically described (loading scatter) mutual relations among tested wheat lines characteristics and effect of 1B/1R translocation (graph 1).

The high effect of both years was eliminated by data transforming when each individual parameter was divided by average value of all lines achieved in corresponding year. The results confirmed positive effect of lines without translocation on technological parameters (Zeleny sedimentation and GI), close relations among crude protein a storage protein fractions (gliadins and sum of glutenins) and their negative relationship to content and ratio of albumins and globulins. The position of weight gain situated to common area with content and ratio albumin and globulin indicates their mutual closer relation. The parameters relative viscosity and PSI seemed to be in positive correlation to weight gain of broilers as well. Our results did not confirmed negative effect higher relative viscosity on poultry gain published by Choct and Annison (1992).

The calculated correlation matrix of tested grain characteristics enabled to analyze their mutual relations (tab. 2). There were confirmed high and statistically significant correlations among crude and storage protein fraction including their high negative correlation to NFE as well. Correlation coefficients among weight gain of broilers and relations of albumins and globulins fraction and relative viscosity were lower (0.32) but statistically significant. Almost same numeric value of negative correlations (-0.30 and -0.30) were detected among weight gain and both storage protein fraction and Zeleny sedimentation test respectively.

Finally, we can conclude that the presence of 1B/1R translocation was important factor (except of year and wheat line), which significantly influenced of feeding test and in contrast to other authors (Rose 2003 and Martinant et. al) increased weight gain of broilers. It is likely, that high ratio of albumins and globulins in grain is significant factor determining higher final feeding value of wheat grain for broilers. The deteriorate effect of relative viscosity on broilers feeding test was not confirmed.

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Fruit quality and disease resistance breeding of cherry and cocktail F1 greenhouse tomato hybrids

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ABSTRACT: Cherry and cocktail tomatoes became very popular in Russia in the early 21st century. The hybrids of cherry type for the protected conditions should meet some requirements, such as high fruit uniformity, adequate fruit shape, color, and taste, high compactness of the infrutescence (i.e, "fish bone" type), attractive green calyx and fruit stalks, high productivity, multiply diseases resistance, and long shelf life. The breeding material derived since 1993 from interspecific crossings of *L.esculentum* (from different geographic areas) with *L.hirsutum, L. hirsutum* f. *glabratum*, *L. pimpinellifolium* Red Currant, *L. cheesmanii* f. *minor*, and *L cerasiforme* were studied under artificial inoculation conditions. The presence of genes such as I_2, $Cf_{9,}$ T_m-2^2 in genotypes is examined by using gene marker techniques. Research on utilization of gene markers for breeding for resistance to *Oidium neolycopericum* was started. Patterns with different types of blossom cluster such as simple short, simple compact, long compact, long very friable ones, and with various fruit shapes and colors were selected. Variation of 4 cluster characters was studied. Breeding line values was determined by estimation of values of F1 hybrids derived from diallel crossings. Sources and donors of valuable characters including good taste, flavor, and good storage ability in cherry and cocktail tomato materials were identified. Heterotic hybrids of cherry type F1 Zimnaya vishnya (T, F_2, C_9, Ol) and F1 Andryushka (T, F_2, C_9, Ol) were created.

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Content and variability of minerals in winter barley and triticale grain

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ABSTRACT: Grain samples of cultivars of winter barley (7 of 6 row and 6 of 2 row) and winter triticale (8), registered for growing in the Czech Republic in 2002-2004, were analyzed for the content of macro- (P, K, Mg, Ca), microelements (Fe, Mn, Zn, Cu) and heavy metals (Hg, Pb), and other significant compounds (phytate, phosphate, crude protein, fibre, starch and ash). Analysis of variance within individual species demonstrated difference in ability of accumulating elements that was influenced both by the examined species and cultivar and by the effect of the crop year. Within the sets of individual species, there were cultivars that significantly differed in the content of more macro- and microelements in grain. Interactions between the content of mineral elements and important nutrients in grain differed between the studied species. The selection of cultivars with higher starch content and lower crude fibre will likely to result in the decrease in the content of some macro- as well as microelements in grain. The assessed negative relationships between the content of phytate, iron, zinc and manganese suggest that it could be possible to develop cultivars with higher content of these elements and simultaneously with lower phytate content in grain.

Keywords: cultivars, interrelationships, macro- and microelements, phytate.

Introduction

The appropriate metabolism, growth, reproduction, health and well-being of human and animal organisms depend, besides water, nutrients and vitamins, on the intake and proper use of a number of minerals (Welch and Graham, 2004). Studies conducted in numerous countries report on the change in the content of minerals in main food and feed crops over the last 30-50 years, which is reflected in nutrition of both humans and farm animals (Ekholm et al., 2007).

In spite of the fact that winter barley (WB) and winter triticale (WT) are grain crops that are used in the Czech Republic exclusively for feeding farm animals, there is currently no detailed information on the variability in the content of mineral elements and their relationships with other nutrients. The assessment of differences in the accumulation of macro- (P, K, Ca and Mg) and microelements (Mn, Fe, Zn and Cu) and heavy metals in the grain of selected WB and WT registered cultivars was part of the research focused on grain nutritional quality of different species and types of cereals for intensive feeding technologies for farm animals, which was conducted in 2003-2007.

Materials and Methods

Grain samples of the cultivars of winter barley (WB; 7 cvs. with 6 row and 6 cvs. with 2 row ear) and winter triticale (WT; 8 cvs.) grown in 2002-2004 in the location of Hradec nad Svitavou (trials of the Central Institute for Supervision and Testing in Agriculture, Czech Republic) were examined. In addition to minerals analyzed according to methodologies of the Czech State Standard, ISO and ICC standards (macroelements: phosphorus-P, potassium-K, calcium-Ca, magnesium-Mg - in $g.kg^{-1}$ of dry weight /DM/; microelements: manganese-Mn, iron-Fe, zinc-Zn and copper-Cu - in $mg.kg^{-1}$ of DM), the content of phytic and phosphoric acids (given below as phytate and phosphate – in $g.kg^{-1}$ of DM, using an instrument Ionosep 2003, Recman) were determined in grain. Standard analytical methods were employed to assess the content of starch, crude protein (CP; N x 6.25), ash and crude fibre (CF); all the compounds were given in $g.kg^{-1}$ of DM.

Results and Discussion

Though cereals are considered as feeds with similar, low content of mineral elements (Weiss and Janssen, 1992), grouping the WB and WT cvs. using a method of cluster analysis based on the content of analyzed macro- (P, K, Ca, Mg), microelements (Mn, Fe, Zn, Cu) and heavy metals (Hg, Pb) demonstrated that not only the two species, but both types of WB (with 6 row and 2 row ear) significantly differed from one another (see Fig in Poster).

The mean content of elements in grain of the examined cereal crops decreased in the following order: K > P > Mg > Ca > Fe > Mn > Zn > Cu. The variability measured by coefficient of variation (V, %) ranged from 4.3% (P-WT) to 43.8% (Mn-WT). The lowest minimum and maximum values of P and K were found for the WB cvs.; however, significant differences between the two species were confirmed only for Ca content, which was on average 43.3% higher in WB (and particularly cultivars with 6 row ear) than in WT cvs. The differences between the species in the content of microelements were larger than in the case of macroelements. On average, WT grain contained more Mn and Cu by 93 and 33%, respectively, than WB grain. In contrast, the latter had higher content of Fe by 27.2%. Both types of WB displayed undesirable ability of accumulating 3 times higher content of Pb in grain in comparison with WT.

The significance of differences between mean values of mineral content in individual cultivars and individual crop years was assessed by analysis of variance (GLM, ANOVA of main effects). The effect of the year was significant for all macro- and microelements and Pb, whereas the effect of the cultivar as a factor of variability was significant for P, Ca, Mg, Mn, Cu, Hg and Pb in the whole set of cereals, in WB for Fe content and in 2 row WB also for Zn content.

The significance of differences between mean values of the content of elements (Fig. 1) calculated for individual cultivars was assessed using a LSD test ($P<0.05$). The number of different homogeneous groups ranged from 2 (for K, where only cvs. Camera-WB and Presto-WT differed from WB cv. Luxor) to 9 (for Ca, where all WT cvs. had considerably lower content than 9 WB cvs.).

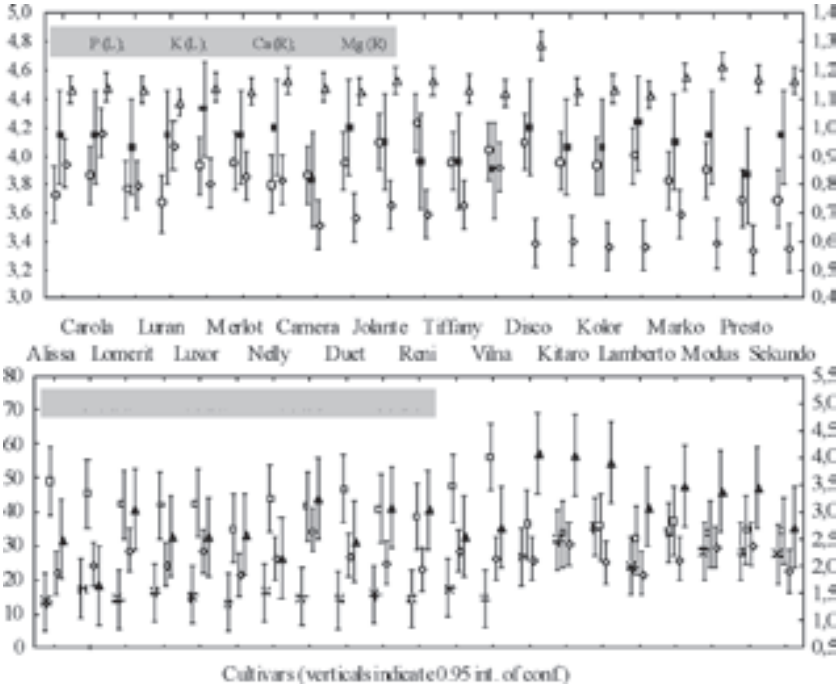

Figure 1. Means and variability of minerals in the grain of winter barley and triticale cultivars (2002-2004).

The graph shows that none of the cultivars produced maximum or minimum content of all or most of the examined elements at the same time. However, some cultivars were more frequent in different homogeneous groups.

In the WB set, cv. Luran ranked among the groups with the lowest content of P, Mg, Zn, however, the highest content of Ca. out of the whole set of cereals, cv. Vilna (2 row WB) produced the highest mean content of Fe in grain (56.2 mg.kg^{-1}), however, in the WB set due to large variability of this element it significantly differed from cvs. Reni and Merlot only. Together with cv. Jolante it ranked among the groups with considerably higher P and Ca contents.

Out of WT cvs., Disco had the highest content of Mg (1.29 g.kg^{-1}) and Cu (4.07 mg.kg^{-1}) in the whole examined set, and belonged to the group of cultivars with higher P and Mn contents. Cv. Marko (WT) was characterized by the highest Ca content when compared with other studied WT cvs., and within the whole set, it also excelled in higher content of Mg, Mn and Cu. In contrast, cv. Lamberto (WT) with the lowest Fe content (31.59 mg.kg^{-1}) also ranked among cultivars with low Mg, Ca and Zn content.

Problems of improving usability of mineral elements (particularly Fe, Zn and Ca) and other nutrients have been solved worldwide in association with a decrease in phytate content in grain as an important natural substance of antinutritional character (Brinch-Pedersen et al., 2007; Raboy, 2001). On average, WT cvs. had insignificantly higher content of phytate and phosphate (12.47 and 2.13 $g.kg^{-1}$, respectively) than WB cvs. In this species, the difference in the content of phytate was found between cultivars with 2 row and 6 row ear (higher than 1% in favour of 6 row cvs.).

The relationships between the content of minerals, phytate and other nutrients in grain were different in both strength and direction (Fig. 2). There was a negative relationship between phytate and Fe, Zn and Mn in both species, while more significant correlations were calculated only for Zn and Mn in 2 row WB cvs. (r=-0.63 and r=-0.72, $P<0.01$, respectively). It indicates a possibility to select cultivars with higher content of microelements together with a lower content of phytate in grain.

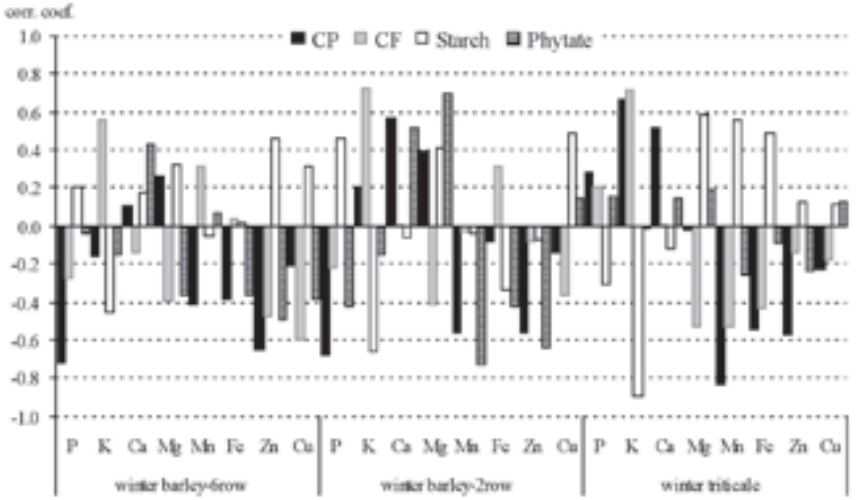

Figure 2. Values of correlations between minerals and selected nutrients in grain of winter barley and triticale.

Interactions between elements and organic compounds in feedstuffs are of a crucial role in mineral nutrition. Selection of cultivars with higher content of CP can result in the increase in K, Ca content (r=0.52-0.67, $P<0.01$) as well as P in WT and Ca, Mg in WB. On the contrary, a significant negative correlation between CP and P in WB (r=-0.67 and r=-0.71, $P<0.01$ for 2 row and 6 row cvs., respectively) and between CP and Mn in WT (r=-0.83, $P<0.01$) indicates an opposite tendency.

Relationships between the content of starch and minerals were in substance a mirror of interactions with the content of CP, which confirmed a known negative correlation between starch and protein in grain. Strong negative relationship between the content of starch and K in WT and WB 2 row cvs. (r=-0.89 and -0.65, $P<0.01$, respectively) is likely to be associated with a role of potassium in the production and transport of sugars and starch synthesis, and together with a negative tendency up to correlation between the starch content and P content in WT cvs. it could be negatively expressed by decreasing their content if productive cultivars with higher starch content in grain were chosen.

Certain reductions in K content in both species and Fe content in barley (mostly with 2 row ear) can also be expected in the development of cultivars with lower content of fibre in grain in relation to its significant relationship to both minerals (r=0.56 to 0.73, $P<0.01$).

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Effect of heat stress on the quantitative and qualitative components of wheat grain

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ABSTRACT: The effect of heat stress was studied under controlled conditions in the Martonvásár phytotron. In order to evaluate heat tolerance, wheat genotypes with different levels of tolerance were examined. The varieties were tested at high temperature in the shooting and grain filling stages of development. The rise in the protein content in response to high temperature during grain filling could be attributed to reductions in the thousand-kernel mass, grain weight and grain size, while the protein increase after treatment at the shooting stage was mainly the result of a drastic reduction in grain number and yield. A very close negative correlation was demonstrated between the yield and protein content of wheat varieties stressed in the shooting stage. In most cases, lower values of UPP% (unextractable polymeric protein) and Glu/Gli (glutenin to gliadin ratio) indicated a decline in grain quality, despite the rise in protein content. Changes indicative of quality deterioration were generally observed in response to treatment at 41°C. Substantial differences were detected between the varieties with respect to changes in quantitative and qualitative parameters in response to stress, suggesting that selection is possible for less sensitive genotypes, leading to the breeding of varieties tolerant of heat stress. The yield and quality results of plants heat-stressed under controlled conditions were similar to those of plants grown in field experiments in the very hot, dry summer of 2007.

Keywords: *Triticum aestivum,* heat stress, grain yield, protein content, glutenin/gliadin ratio.

Introduction

An important precondition for the production of high quality wheat is to grow a variety with satisfactory genetic potential. Within the winter wheat species, quality parameters exhibit very great variation, with protein contents ranging from 10–17%, gluten content from 20–45%, grain hardness from 10–85, falling number from 60–450 and farinograph index from C2 to A1. Differences in quality also depend on the soil, the nutrient supplies, plant protection and many other agronomic factors. The weather of the growing season, particularly the rainfall and temperature, has a significant influence on metabolic processes. A higher mean temperature causes plant development to accelerate, resulting in a shorter crop production period.

A rise in the number of very hot days during the cereal ripening period not only causes substantial yield losses, but also leads to a sharp decline in the breadmaking quality of the flour. High temperatures after heading, during the ripening period, induce stress effects in

wheat plants, resulting in poor grain filling and loss of yield (Wardlaw and Moncur, 1995). Wheat varieties exposed to a short period of high temperature (>35°C) exhibited significant reductions in grain yield and quality (Stone and Nicolas, 1994). A decline in the glutenin-gliadin ratio had a negative effect on flour quality despite the increased protein content (Bencze et al., 2004).

The suitability of wheat for breadmaking depends primarily on the protein composition. The average composition of gluten proteins is 75% gliadin and 25% glutenin. A higher gliadin content leads to soft gluten, while an excess of glutenin makes the gluten too hard. Wheat flour has a mean content of 30–40% wet gluten and 10–14% dry gluten. The gluten proteins, glutenins and gliadins, are responsible for the extensibility and elasticity of the dough. Grain hardness is an extremely important quality trait, determining the products that can be made from various wheat varieties (Singh and MacRitchie, 2001).

Above-average temperatures are frequently experienced during the period after heading, resulting in changes in quality. The present paper discusses the changes observed in the quantitative and qualitative traits of the grain yield in response to heat stress treatments carried out under controlled conditions.

Materials and methods

Plant material and growing conditions

The experiments were carried out under controlled conditions in the Martonvásár phytotron. The following varieties were examined: Plainsman V. (USA), Fatima 2 (H), Mv Mambó (H), Mv Magma (H), Bánkúti 1201 (H), GK Öthalom (H). The experiment consisted of five treatments: control (C), heat stress (30ºC) at shooting in the 8th week (S) and heat stress (35°C, 38°C, 41°C) at ripening (R), 12 days after heading. In all cases the stress was continued for 15 days. The temperature treatments are listed in Table 1.

Table 1. Temperatures applied in the control, and in stress treatments in the shooting and ripening stages.

Control			Heat stress treatments			
Shooting stage (S)		Ripening stage (R)	Shooting stage (S) (Zadoks-32)	Ripening stage (R) (Zadoks-75)		
Day	17ºC	24ºC	30ºC	35ºC	38ºC	41ºC
Night	13ºC	20ºC	20ºC	20ºC	20ºC	20ºC

Characterization

Measurements were made after harvest on the biomass, spike number, number of kernels and grain yield per plant, and these data were used to calculate the thousand-kernel mass per plant and the harvest index.

In order to determine protein content, wholemeal was produced using a Perten Laboratory Mill 3100, after which the protein content was determined for 3×1 g samples using a Kjeltec Auto Sampler System 1035 Analyser (according to the ICC 105/2 standard). The data are presented after conversion with a factor of N × 5.8. The grain hardness was determined using a Single Kernel Characterization System 4100, which calculates the hardness index (HI) from a sample of 300 kernels by measuring grain hardness, moisture content, grain mass and grain size.

The total glutenin, gliadin and albumin+globulin contents of the samples were determined using the SE-HPLC (size exclusion) technique, i.e. by separating the proteins on the basis of size on a Phenomenex BIOSEP-SEC 4000 column, using the modified method of Batey et al. (1991). The proteins were detected at 210 nm. The quantity of unextractable polymeric protein (UPP) was determined using the method reported by Gupta and MacRitchie (1993). Calculations were also made of the glutenin/gliadin ratio and the albumin-globulin %, calculated from the ratio of albumin-globulin to the total polymeric protein.

The data were evaluated using two-factor analysis of variance, where the two factors were the variety and the treatment.

Results and discussion

Determination of the level of heat stress by measuring plant yields for T. aestivum wheat

Significant reductions were recorded in the yield, grain number, thousand-kernel mass, harvest index and biomass of the varieties in response to high temperature (Fig. 1). The only exception was the thousand-kernel mass in the shooting stage treatment, which exhibited an increase of 13.38% after treatment at 30°C. This increase was associated with increases in grain mass and grain diameter. While treatments in the adult stage led to a reduction of 24–35%, averaged over the varieties, the greatest loss of grain yield (-50.47%) was caused by heat stress during shooting. This could be attributed to the fact that heat stress during shooting caused a drastic reduction in grain formation. Among the varieties examined, Plainsman V. proved to be the most resistant. Averaged over the varieties, the decline in grain number (-51.81%), harvest index (-30.02%) and biomass (-30.52%) was also greatest when heat stress occurred in the shooting stage, while these parameters were only reduced by 9–21% when stress treatment was applied during grain filling.

Determination of the level of heat stress by measuring grain yield quality for T. aestivum wheat

The rise in the relative protein content could be attributed to a decline in the thousand-kernel mass, the grain weight and the grain size when high temperature was applied in the grain filling phase (Table 2), while it resulted mainly from the drastic drop in the grain number and yield per plant (together with increases in kernel weight and size and a significant rise in thousand-kernel mass) when treatment was carried out in the shooting stage. Similar conclusions could be drawn from correlation analysis on quality data. A very close negative correlation was revealed between the yield and protein content of wheat varieties stressed in the shooting stage (Fig. 2).

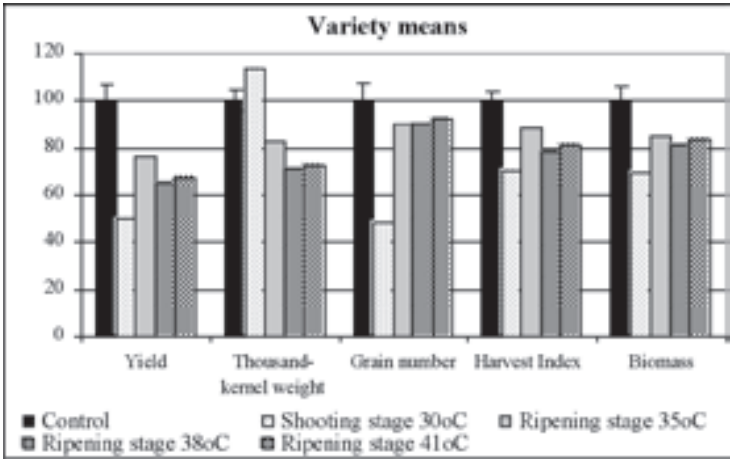

Figure 1. Changes in grain yield, thousand-kernel mass, grain number, harvest index and biomass in response to heat stress, averaged over the varieties.

Table 2. Effect of heat stress on the quality components of the grain yield, averaged over the varieties (↓↑ significant differences between the control and stress-treated plants at the P=5.0% level of significance).

Parameters (averaged over varieties)	*Treatments*					
	Control	Shooting stage 30°C	Ripening stage 35°C	Ripening stage 38°C	Ripening stage 41°C	$LSD_{5\%}$
Protein content (%)	18.41	22.44↑	19.33↑	20.52↑	19.78↑	0.15
Kernel mass (mg)	35.74	38.50	31.89	29.17↓	28.76↓	4.00
Kernel diameter (mm)	2.35	2.47	2.16↓	1.99↓	2.00↓	0.19
Grain hardness	68.22	58.20↓	65.25	61.26	64.94	8.11
UPP%	52.30	52.86	54.67↑	55.67↑	48.62↑	1.15
GLU/GLI ratio	0.77	0.66↓	0.78	0.77	0.73↓	0.02
Albumin-globulin %	9.55	8.76↓	9.23	9.59	11.35↑	0.43

In most cases the lower UPP% and Glu/Gli values demonstrated that the grain quality was poorer in spite of the rise in protein content. Changes indicative of quality deterioration were most frequently observed after treatment at 41°C. This was also true of the glutenin/gliadin ratio in the wholemeal of plants stressed in the shooting stage or at 41°C during grain filling.

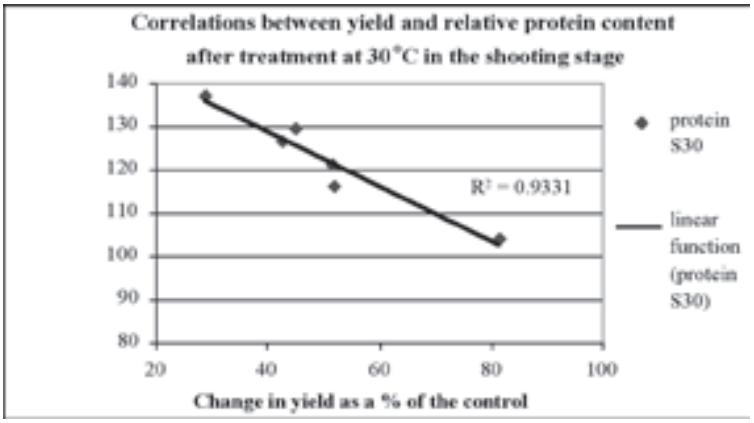

Figure 2. Correlations between yield and relative protein content after treatment at 30°C in the shooting stage (critical r value at P=5%: 0.8114; at P=1%: 0.9172).

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Inheritance of 1000-seed weight and seed protein content in a soybean cross

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ABSTRACT: Seed quality characters of soybean such as 1000-seed weight or seed protein content are important quality features of food grade soybeans grown in Central Europe. Particularly, a high seed protein content is a key requirement for processing of soybean seed. Seed protein content is a quantitative character controlled by multiple genetic factors with small individual effects (Li et al., 2007). Moreover, environmental effects related to soil nitrogen content and biological dinitrogen fixation have considerable influence on seed protein content in soybean (Vollmann et al., 2000). In order to support the development of high quality soybeans, the inheritance of 1000-seed weight and seed protein content were studied in a soybean cross through QTL analysis. A total of 530 F_6-derived lines from a bi-parental soybean cross was phenotyped for seed traits in replicated field trials across four Austrian environments. From a total of over 150 markers used in genotyping, 29 SSR markers were finally applied in QTL mapping. Apart from composite interval mapping (for one chromosome) and one-way ANOVA, a mixed model 2-way ANOVA was used for detection of epistasis; either unadjusted individual plot data or BLUPs (best linear unbiased predictions) were utilized as the phenotype data for QTL analysis. QTL analysis revealed 10 and 8 marker - QTL associations for 1000-seed weight and protein content, respectively. Moreover, 16 and 21 epistatic interactions between loci on different linkage groups were detected for 1000-seed weight and protein content, respectively, which were highly significant statistically. Some of the epistatic QTL effects were stable across environments, while others showed significant epistasis by environment interaction. Loci on soybean molecular linkage group O appeared most important in epistatic interactions. Due to the need for large and homozygous populations, epistasis is rarely considered in QTL mapping and marker assisted selection. However, the present results suggest that epistatic QTL effects are of relevance in controlling soybean seed quality characters. As soybean is a diploidized autotetraploid crop species, part of the epistatic QTL effects detected may be interpreted as relationships between gene loci on homoeologous soybean chromosomes.

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Triticale of improved bread-making quality

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ABSTRACT:

Triticale is widely cultivated in Poland. In 2007, the total area of triticale was 1.3 mln hectares and was doubled in comparison to 2000. Triticale is relatively resistant to many fungal diseases. Moreover, it has good winter hardiness and is tolerant of poor and acid soil conditions. Nevertheless, its end-use is limited mainly to animal feed and as a source of starch to ethanol production. Because of high amylase activity in grains as well as weak gluten structure, triticale flour was not accepted in baking. In those countries, where the wheat-rye bred consumption is the base of human diet, triticale would have been a substitute as it offers ready to use flour, without necessity to mix both wheat and rye flour. In comparison to wheat, in triticale the D genome has been replaced by R genome from rye. Deficiency of gluten proteins encoded on D genome most probably cause poorer triticale bread-makig quality. To create the genetic potential for bread-making quality similar to that of bread wheat, rye chromosome 1R in triticale cv. Presto was cytogenetically engineered to introduce wheat storage protein loci Gli-D1 and Glu-D1 encoding subunits 5+10 (Lukaszewski, 2006). The manipulations were by homoeologous recombination between rye chromosome 1R and 1B or 1D of wheat, followed by homologous recombination of primary recombinants with translocation breakpoints in desired locations. Chromosome Valdy is a three breakpoint translocation with loci Gli-D1, Sec-1 and Glu-D1. Unfortunately Valdy shows a high amylase activity, that considerably reduces the positive effect of the gluten protein composition. In the meantime, in Poland HR Strzelce, Division Borowo was bred triticale variety Pawo, which shows reduced amylase activity and a good HMW-GS composition (Payne et al. 1984): 1 - 7+8, on chromosomes 1A and 1B respectively. The purpose of the work was selection of pedigree obtained from the crosses between Valdy and triticale lines, cv. Pawo mainly, of high yield as well as HMW-GS composition 1 - 7+8 - 5+10 that corresponds to the best bread-making quality wheats. The technique of doubled haploids used in the breeding programme rapidly improves genetic stability and other agronomically important traits such as head length, good baking quality, and yield currently at 108% of the best check cultivars (Moderato, Woltario, Borwo).

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Breeding of oat varieties in Latvia for food industry

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ABSTRACT: According to of *Rigas dzirnavnieks* Ltd (http:www.rigas-dzirnavnieks.lv/main.phl?id=3&sid=29&1=lv), the greatest oat products manufacturer in Latvia, the average values of grain quality indices in oats supplied by local oat breeders are significantly lower than the optimal values, i.e. the average husk content is 270 g kg^{-1}, (optimal < 240 g kg^{-1}), volume weight of grain after cleaning on 2.0 mm sieves is around 520 g l^{-1} (when purchasing, minimal value 480 g l^{-1}, optimal value above 540 g l^{-1}). The presence of double-germ grains, naked and dark grains is unwanted in the mass of grain. Improvement of these grain quality indices is the wish of the oat processing enterprises (Gransmann, 1995). For that reason, a collaboration project has been started to develop recommendations for the local oat growers how to optimize oat growing techniques, which varieties choose when growing oats for food industry. Recent 3-year research results in Stende show that the best grain quality indices were reached with oat varieties Arta'(Latvia), 'Ivory' (Germany), 'Vendela' and 'Margaret' (Sweden) currently grow in Latvia. Unfortunately, the new European oat varieties grow under conditions of Latvia do not fully satisfy demands of food industry, but the productivity level of the oat variety 'Arta' is not corresponding to oat growers' requirements. Testing of 28 promising oat lines developed in Latvia resulted in the choice of four lines: line Nr 29951, line Nr 31534, line Nr 31964 and line Nr 32191. After two observation years the grain volume weight of these oat lines exceeded 500 g l^{-1} (to pass through a 1.8 mm sieve after cleaning), husk content was below 240 g kg^{-1} and the average grain yield exceeded 5.5 t ha^{-1}.

Keywords: oat for food, grain yield, volume weight, husk content.

Introduction

In literature findings oats are mentioned as a valuable and balanced source of indispensable amino acids, unsaturated fatty acids, B-glicane, vitamins and antioxidants (Loskutov, 2007.) The quality of food oats are characterized by several agronomic, morphological and physical traits, such as volume weight, grain size, husk to kernel ratio, colour of the kernel, hardness of grain, etc.(Rhymer et.al. 2005, Doehlet, 2005). Husk content and grain volume weight are most significant indices which influence end-product obtained from 1 kg oat grain. Grain growers, in their turn, evaluate oat varieties mainly by their productivity potential. Oat breeders task is to develop varieties with genetic potential capable of ensuring both high productivity level and grain quality corresponding to requirements of the grain processing enterprises. In 2008, nine varieties of oats have been registered in the Latvian Plant Variety

List and three of them are recommended as oats for food (http:www:vaad.gov.lv/default.aspx?tablID=6&lang=1). Currently 'Laima' (Latvia), 'Freja' and 'Vendela' (Sweden), 'Jumbo' (Germany) and 'Kirovec' (Russia) are the oat varieties most widely bred in Latvia. New oat varieties: 'Magarett'(Sweden), 'Duffy' and 'Ivory' (Germany) are recommended to oat breeders. Frequently oat varieties known and approved for food production in Central and Western Europe, are not capable of ensuring quality indices necessary for food grain production in Latvia (Zute, 2002).

Since 2006, researchers of the State Stende Cereals Breeding institute in collaboration with *Rigas Dzirnavnieks* Ltd are conducting research on the possible improvement of grain quality indices, which are significant for food industry, in Latvia bred oats. One of the research project goals is estimation of the oat varieties currently most widely bred in Latvia and which are recognized as promising ones, as well as estimation of the new promising lines of food oats developed in Latvia, as reported in this article.

Material and methods

The test field trials were established in plant breeding crop rotation of the State Stende Cereals Breeding institute. Eight oat varieties bred in Latvia and 28 promising oat lines of F_6 and F_7 generation developed in Stende were tested. Variants-varieties were arranged with three replications according to standard method. Developed and registered in Latvia, oat varieties 'Laima' and 'Arta' were chosen as control varieties ('Laima' – the oat variety most widely bred in Latvia, 'Arta' – grain quality indices are most corresponding to food industry requirements). Control variety plots were laid down as follows: one after five trial plots. The recorded plot area was 10 m^2. Growing conditions: sod-podzolic soils (WRB – Eutric Podzovisols), well cultivated moraine loamy sand soil. Oat breeding techniques are corresponding to generally accepted practice in Latvia. On plots the harvested grain yield was weighted and expressed at 14% moisture. After first treatment using a cleaning equipment (size of the sieve mesh: 12.0 x 1.9 mm), grains were analysed for agronomic and biochemical properties: husk content (g kg^{-1}), volume weight (g l^{-1}), 1000 grain weight (g), crude protein (g kg^{-1}), crude fat (g kg^{-1}) and other indices at the laboratory of Grain quality and Agricultural chemistry of the Institute. *Ms Excel* ANOVA and correlation analysis was used for data processing.

Results and discussion

Oat variety testing results

Eight oat varieties bred in Latvia, which were recommended or offered by oat breeders for food industry, among them 'Laima', 'Freja', 'Vendela' and 'Jumbo' – most widely cultivated oat varieties, and 'Maragaret', 'Ivory' and 'Jaak'(Estonia) as promising oat varieties under conditions of Latvia, were included in the research. Estimating agronomic indices of these varieties - volume weight, husk quantity, 1000 grain weight, only those of the oat variety 'Arta' corresponded to optimal values (Table 1). Low husk quantity was also recorded for the

oat varieties 'Vendela', 'Margarett', 'Ivory' (< 240 g kg^{-1}), coarse grain - 'Ivoru' and 'Jumbo' (> 40 g), high volume weight - 'Freja' and 'Jaak' (> 510 g l^{-1}). In oat grain, the highest crude protein content was recorded for the varieties 'Arta', 'Jaak', 'Laima' (> 120 g kg^{-1}). The fat content in grain was highest for the oat variety 'Laima' (65.6 g kg^{-1}as the 3-year average). Oat varieties low in grain fat are wanted by oat flour and rolled oats manufacturers to ensure longer storage period of the product. The lowest grain fat content was obtained with the oat varieties 'Margarett' and 'Vendela' (< 35 g kg^{-1}). For the present, representatives of the Latvia food industry do not consider biochemical indices when purchasing grain yet these indices are taken as a basis in accessing dietary value of the produced foodstuffs.

Table 1. Grain quality indices of oat varieties recommended for food industry in Latvia, mean 2005-2007.

Oat breeding lines and varieties	Test weight, g l^{-1}	1000 grain weight, g	Husk, g kg^{-1}	Crude protein, g kg^{-1} (x 5.7)	Fat, g kg^{-1}
Laima	505.5 ±10.88	35.18 ± 2.09	257.9 ± 14.6	122.5 ± 14.6	65.9 ± 3.5
Arta	520.1 ± 8.39	35.30 ± 0.96	209.3 ± 10.5	155.7 ± 28.9	43.6 ± 8.9
Freja	514.8 ± 2.88	36.17 ± 2.86	246.8 ± 6.8	116.9 ± 24.6	56.1 ± 5.4
Jumbo	502.9 ± 27.22	36.50 ± 3.15	252.6 ± 6.7	112.8 ± 19.1	50.7 ± 5.4
Margaret	481.0 ± 28.58	37.22 ± 1.79	229.3 ±15.32	112.8 ± 15.0	30.0 ± 7.1
Vendela	500.8 ± 18.42	37.89 ± 2.20	232.8 ± 15.7	119.7 ± 25.6	34.6 ± 6.4
Ivory	499.2 ± 5.17	41.78 ± 6.63	237.2 ± 20.8	114.3 ± 18.9	52.4 ± 2.5

When estimating productivity potential, control variety 'Laima' was significantly surpassed only by the oat variety 'Ivory', i.e. 6.92 t ha^{-1}, + 0.40 t ha^{-1} to 'Laima' , $LD_{0.05}$= 0.28 t ha^{-1} (Fig.1). Stable grain yields during three test years was obtained with the oat varieties 'Laima', 'Jumbo', 'Freja', 'Arta', 'Ivory' (variation coefficient <5%).

Oat line testing results

Out of 28 oat breeding lines included in the research, four lines: Nr 29951, Nr 31534, Nr 31964 and Nr 32191 showed agronomic indices corresponding to food industry requirements (Table 2). By husk quantity in grain yield and 1000 grain weight, the studied oat lines were of equal worth to the control variety 'Arta', but by test weight and protein content they did not reach the average indices of the variety 'Arta' but corresponded to level of the control variety 'Laima'.

Oat lines Nr. 31964 and Nr. 32191 were the most productive out of the chosen lines, respectively 6.36 and 6.18 t ha^{-1} (control variety 'Laima' – 6.50 t ha^{-1}, $LSD_{0.05}$ = 0.33 t ha^{-1}). The 3-year productivity averages for the oat lines Nr 29951, Nr 31534 are respectively by 15 and 10% lower compare to control variety 'Laima'.

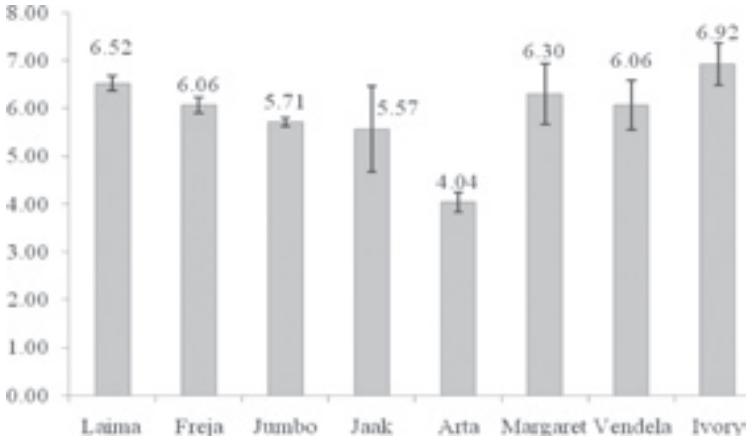

Figure 1. Grain yield of oat varieties recommended for food industry in Latvia, t ha^{-1} ± sd, mean 2005-2007.

Table 2. Grain quality indices of oat lines most suited to food industry as compared to control varieties 'Laima' and 'Arta', mean 2006-2007.

Oat breeding lines and varieties	Test weight, g l^{-1}	1000 grain weight, g	Husk, g kg^{-1}	Crude protein, g kg^{-1} (x 5.7)	Fat, g kg^{-1}
Laima	502.2 ±1.13	35.58 ± 2.80	253.7 ± 7.2	128.5 ± 15.2	66.4 ± 4.8
Arta	519.7 ± 11.18	35.62 ± 1.12	211.5 ± 12.6	163.0 ± 36.7	42.9 ± 12.5
Nr.29951	506.2 ± 8.77	35.65 ± 2.86	229.8 ± 13.5	132.5 ± 31.8	53.4 ± 3.4
Nr.31534	507.8 ± 6.01	36.50 ± 2.25	213.7 ± 7.50	124.7±31.4	47.9 ± 5.5
Nr.31972	504.8 ± 1.06	36.91 ± 1.94	227.7 ±5.02	122.7 ±21.6	59.9 ± 1.1
Nr. 32191	502.5 ± 17.68	34.19 ± 2.63	238.2 ± 7.35	130.3 ± 25.1	56.4 ± 5.1
$LSD_{0.05}$	8.75	1.01	1.22	0.60	0.28

In the process of breeding, it is difficult to select genotypes which concurrently ensure high yield and quality of grain corresponding to food industry demands. Frequently agroclimatic conditions of Latvia are essentially different between years as it is shown in the research by estimated traits' variation between years. Fluctuations in grain volume weight, husk content and values of variation coefficient of the crude protein content reached 15 – 20% in the 3-year research though oats were grow by a uniform method. Within the framework of this project, the promising oat lines will be estimated for two more years paying particular attention also to estimation of growing techniques suited to local conditions.

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Part 4

Evaluation and release of breeding material and new breeding objectives

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Genetic and genomic approach for improving biofuel production from maize

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ABSTRACT: Grasses, which are currently at the basis of cattle feeding, will be in the next future a major source of cell wall carbohydrates for sustainable biofuel production. Association of lignins with other matrix components, together with linkages between cell wall carbohydrates, greatly influences cell wall properties, including the degradability of structural polysaccharide by micro-organisms in animal rumen or industrial fermentors. The improvement of biofuel production from plants is based on the understanding of the cell wall composition and setting up, and on the discovery of genetic and genomic mechanisms involved in each component biosynthesis and their depositions in each lignified tissue. While nearly 40 QTL have been shown for lignin content, only seven locations appeared of greater importance in all investigated genetic resources. Expression studies highlighted that several genes of the lignin pathway are less expressed in lines with higher cell wall degradability. However, only a few lignin pathway genes mapped in QTL positions, and the fully relevant candidates might be genes involved in regulation of lignin pathway genes, or in regulation of lignified tissue setting up.

Keywords: Biofuel, lignin, ferulic acid, *p*-coumaric acid, maize, QTL, transcriptomic

Introduction

Recent economical and environmental contexts have greatly strengthened the necessity of producing in sustainable ways substitutes to fossil fuels. The most important tank for biofuel in future is yet based on the use of ligno-cellulose products. While grasses (*Poaceae*) are currently the major source of nutrients for wild and domesticated herbivores, in the next years, grasses also will be a major source of cell wall carbohydrates for biofuel production. Moreover, maize is likely the plant which is fully relevant as both model and cropped species. The lower energy value of cell wall parts in comparison with grains is related to their more or less high content in phenolic compounds which induce limited and variable carbohydrate degradability by micro-organisms in animal rumen or industrial fermentors. Lignins, which

are important for structural integrity of tissues and impart hydrophobicity to vascular elements, are the main, even only, component in plant cell resistant to bacterial and fungal degradation. Lignin prevents physical access of the rumen microbial enzymes to cell wall carbohydrates and thus strongly limits their valorization. Moreover, their association with other matrix components, together with linkages between cell wall carbohydrates, greatly influences cell wall properties, including the degradability of structural polysaccharides. The improvement of biofuel production from maize stover is therefore based on the understanding of the grass cell wall composition, especially its phenolics composition, structure, interaction between phenolics and carbohydrates, and on the discovery of genetic and molecular mechanisms involved in each component biosynthesis and deposition in each lignified tissue.

Phenolic constituents in the grass cell wall

The lignified grass cell wall is a composite material with cellulose microfibrils, an amorphous matrix consisting predominantly of hemicelluloses (mainly glucurono-arabinoxylans), very little pectins, and phenolics. Phenolics comprise lignins and cell wall-linked *p*-coumaric (*p*CA) and ferulic (FA, along with its array of dehydrodimers) acid derivatives. The participation of *p*-hydroxycinnamates in the cell wall composition and organization of the mature lignified tissues is certainly the most specific trait of grass lignification. Grass lignins comprise of guaiacyl (G) units derived from coniferyl alcohol, syringyl (S) units derived from sinapyl alcohol, and *p*-hydroxyphenyl units (H) derived from *p*-coumaryl alcohol. The H, G and S units of grass lignins are interconnected through labile β-O-4 ether bonds, and through a series of resistant carbon-carbon and biphenyl ether linkages. The low, but significant, amount of H units may significantly impact the properties of grass cell walls as these units increase the frequency of resistant inter-unit bonds. Among cell wall-linked *p*-hydroxycinnamates, *p*-coumarate is mainly esterified to the γ-position of phenylpropanoid side chain of lignin S units. Most *p*-coumarate accretion occurs in tandem with lignification and *p*-coumarate accumulation is thus a relevant indicator of lignin deposition. Ferulic units are primarily esterified to non-cellulosic polysaccharides, such as glucurono-arabinoxylans, while lignins and arabinoxylans are secondarily bridged through FA ether-linkages to the β-position of G units. Moreover, the presence of ferulates linked to arabinosyl sides-chains of arabinoxylans provides a convenient and reliable way of cross-linking these polysaccharide chains. Over 50% of wall ferulates can undergo dehydrodimerization and arabinoxylans are thus extensively cross-linked by ferulate dimerization in mature cell walls (Grabber et al., 2004). Cross-linkages through FA and diFA bridges significantly impede mechanical properties of tissue towards higher stiffness and lower degradability (Grabber, 2005). Free FA could also be polymerized into lignins, in addition to *p*-coumaryl, coniferyl, and sinapyl alcohols (Ralph et al., 2008). While lignins are in fact largely linear, despite they are mostly referred to as branched 3-dimensional polymers, the incorporation of free FA in lignins through bis-8-O-4 cross-coupling provides an extra mechanism for chain branching in the polymer. To

date however, whether this incorporation in grass lignins contributes significantly to their branching is still unknown.

QTL for phenolic components

QTL for lignin content, and to a lesser extent for *p*-hydroxycinnamate contents and cell wall degradability, have been investigated in several maize RIL progenies (review in Barrière et al., 2007). A meta-analysis based on eight RIL progenies has shown that nearly 40 genomic locations are involved in control of lignin content of maize plants, including seven locations in bins 1.07, 2.07/08, 3.02, 4.08, 5.03/04, 6.04, and 9.06 with lignin content QTL originating from at least three RIL families (Barrière et al., 2007). Recently, seven QTL for esterified *p*CA content, four of which explaining individually more than 10 % of the observed genetic variation, and nine QTL for etherified FA contents, three of which explaining individually more than 10 % of the observed genetic variation, have been shown in an early maize RIL progeny (Barrière et al., 2008). In addition, seven and five QTL were also shown for 8-O-4 and 5-5 diFA contents, respectively. QTL were simultaneously shown for H, G, and S lignin monomeric unit release after alkaline nitrobenzene oxidation, with two, four and four QTL that explained individually more than 10 % of the genetic variation for each unit, respectively. Out of seven cell wall degradability QTL (IVNDFD, *in vitro* NDF degradability), colocalizations were shown only four times with lignin content QTL, and especially, no colocalization was observed for the two IVNDFD QTL explaining the greatest part of this trait variation. Complementarily, colocalisations were shown between IVNDFD QTL and esterified *p*CA, etherified FA, and lignin monomeric unit contents. For most of QTL locations, no relevant candidate genes have been validated, or have been still defined, and a lot is expected from the release of the maize sequence (www.maizesequence.org).

Lignin pathway and phenylpropanoid gene expression

Whereas all cytosolic steps of lignin biosynthesis have been presumably identified, little is known for each enzymatic step about the role of each gene member in each multigene family, nor for their possible developmental stage and/or tissue specificity. Moreover, even if the lignin pathway has often been displayed as a metabolic grid, separate pathways leading to G and S units have to be considered. G unit biosynthesis could be preferentially based on caffeoyl-CoA O-methyltransferase (CCoAOMT) activities, while S unit biosynthesis could be preferentially based on a caffeic acid O-methyltransferase (COMT) or more probably on still unknown O-methyltransferase (OMT) activities (review in Barrière et al., 2007). Similarly, the pathway towards the synthesis of FA remains partly hypothetic (Barrière et al., 2007).

Based on a macro-array specifically devoted to maize cell wall genes (Guillaumie et al., 2007a), differential expressions of several genes have been shown between normal plants and their brown-midrib counterparts (Guillaumie et al., 2007b, 2008) or between lines with variable range of lignin content and cell wall degradability (Barrière et al., 2007). Even if

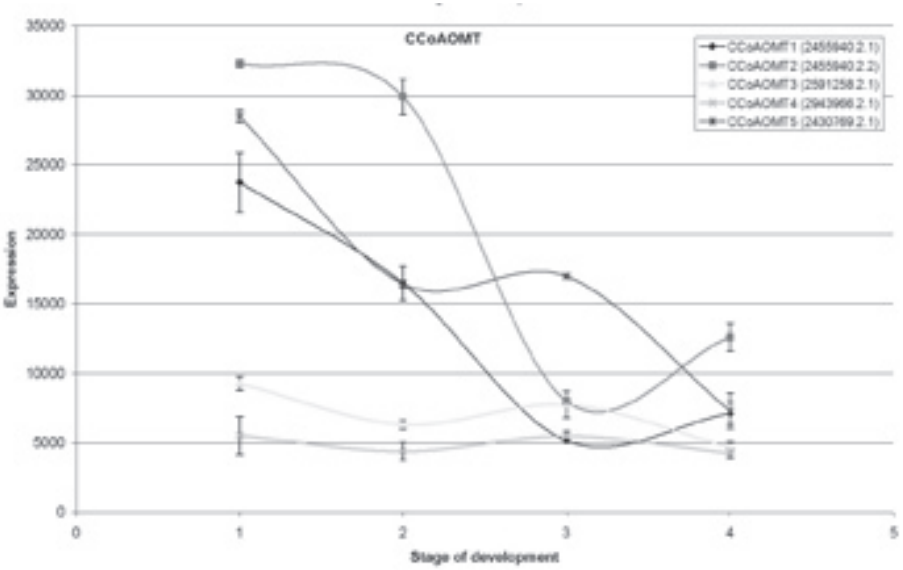

Figure 1. Expression of CCoAOMT genes in the INRA F2 reference line. Expression values are given as the normalized signal hybridization intensity on the MaizeWall macroarray (Guillaumie et al., 2007a) for each CCoAOMT gene in the internode below the ear at 4 stages of plant development [7 days before silking (stage 1), silking stage (stage 2), silking + 8 days (stage 3), and silking + 15 days (stage 4)]. Data from Riboulet et al. (2008).

these results have to be confirmed on a larger number of genotypes, lines with higher cell wall degradability are likely characterized by lower expression of PAL (phenylalanine ammonia lyase) and/or CCoAOMT or COMT (methyltransferase) genes. These investigations are made difficult as phenylpropanoid genes belong to multigene gene families with often close sequences homologies between members. Results obtained for CCoAOMT genes (Riboulet et al., 2008, Figure1) illustrated the variation of each member expression across developmental stages. Moreover, the lower expression of phenylpropanoid genes is probably more related to variation in regulation or transcription factor rather than allelic variation in the considered genes, and this assumption is corroborated by the differential expression of several regulation factors in brown-midrib mutants. In addition, these variations could also be related to variation in extent of lignified tissue setting up, rather than true gene expressions in each tissue, another assumption which is corroborated by the differential expression of auxin-related genes in brown-midrib mutants (Guillaumie et al., 2007b, 2008).

Conclusion

Breeding maize and other grasses for phenolic structures more suitable for energy production (and cattle feeding) could now be considered as a realistic goal by integrating biochemical data, genetic and QTL data, and new genomic-based knowledge on maize lignification and lignified tissue setting up. Investigations should now be preferentially devoted to the understanding of the role of *p*CA, FA and diFA in cell wall structure and degradability, of the major route in lignin pathway and their regulation, and to a major effort in bio-analysis and in silico mapping of cell wall related genes.

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Bud-flowering *Calluna vulgaris* – genetic resources, breeding and genetics

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ABSTRACT: *Calluna vulgaris* L. (Hull.) belongs to the *Ericaceae* family. As an evergreen woody shrub it is common to Europe, the Middle East, North America and South Africa. For the horticultural industry, cultivars of this species are propagated vegetatively. Within the different flower phenotypes of Calluna the so-called bud-flowering types are of highest economic interest. Flowers of this type do never fully open but remain as bud which is the anticipated consequence of the lack of anthers. Over the last decades, breeding of *Calluna* in Europe was focused on the trait bud-flowering. The resulting and protected varieties were all bred by simple phenotype selection using a relatively small pool/amount of genotypes. Thus, juridical variety protection issues become more and more relevant to this species. To improve breeding for new varieties and to overcome challenges induced by the lack of genetic diversity we initiated a diversified study including investigations on the molecular as well as on the phenotype level. By application of RAPD and iSSR fingerprinting using 168 mono- und polymorphisms we analysed the genetic diversity of more than 70 *Calluna* genotypes. Additionally, we recalculated our dataset to introduce a reliable system for identification of EDVs (essentially derived varieties) as proposed by ASSINSEL / Eeuwijk and Law (2004). We successfully validated this system in *Calluna* for selected variety pairs of interest. Breeding for bud-flowering in *Calluna* implies waiting for the flowering season in August. Since costs and time are relevant factors we started the screening for a molecular marker for the trait bud-flowering. Such a marker would remarkably decrease the amount of funds needed for breeding new bud-flowering genotypes since the screening for it would be already possible on the DNA-level of seedlings shortly after sowing. Until now, we succeeded in identifying two RAPD markers linked to the trait of interest. Both markers are target to improvement by SCAR- and CAPS-conversion or SSCP-screening. To look for the basic reason(s) of bud-flowering means to look for the reason(s) of the lack of anthers. Thus, we examined different flower type by histological analysis and started screening for MADS-box genes in young flower buds of *C. vulgaris* using the 3'-RACE PCR technique.

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Improving the yield, processing quality and disease and pest resistance of potatoes by genotypic recurrent selection

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ABSTRACT: In 1991 a breeding programme was started at SCRI to combine quantitative resistances to late blight (*Phytophthora infestans*) and the white potato cyst nematode (*Globodera pallida*) with commercial worth as judged by breeders through a visual assessment of tubers (breeders' preference) (Bradshaw et al., 2003). The parents with resistance to *G. pallida* also had resistance to *G. rostochiensis*, the golden potato cyst nematode. Parents were also included with resistance to *potato leafroll virus*, *potato virus Y* and *potato virus X*, but time and resources did not permit direct selection for virus resistance in each generation (Solomon-Blackburn and Bradshaw, 2007). Such an overall combination of traits was, and still is, lacking in European potato cultivars, despite 50 years of breeding effort. The resistances had been introgressed into the European potato (*Solanum tuberosum*) from the wild and cultivated species of Latin America over many years, starting in the 1930s (Bradshaw and Ramsay, 2005). The breeding programme has involved cycles of crossing, selection between progenies (= full-sib families) and clonal selection within the selected progenies. Progress at the end of the fourth cycle was evaluated during 2007 by comparing the 108 selected clones with the 36 parents used in 1991 and the 15 parents used in 2003. They were assessed for foliage blight resistance in a field test with two replicates, for *G. pallida* resistance in a closed container test with four replicates, and for yield, agronomic and quality traits at Gourdie farm, Dundee. The yield trial had an alpha-design with two complete replicates and incomplete blocks of size 8. It was planted on 20 April, scored for maturity on 20 August, and harvested on 28 September. The assessment confirmed that none of the original 36 parents combined blight and *G. pallida* resistance whereas four of the 15 parents and 43 of the 108 clones did. They had foliage blight scores ≥ 5.5 (≤ 40% necrotic tissue) compared with a score of 2.5 (> 80% necrotic tissue) for susceptible cultivar Maris Piper, 25 days after infectors were placed in the spreader rows of the field trial. They also had *G. pallida* scores of ≤ 3.08 (square root of cyst number) which was the highest score of the nine original parents with resistance. Among the 43 clones were ones with acceptable yield and table or processing quality. The role of genotypic recurrent selection in potato improvement is discussed, along with prospects for introducing molecular marker assisted selection.

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Intellectual property rights and access rules for germplasm: benefit or straitjacket?

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ABSTRACT: Today the free access to germplasm for breeding purposes is becoming more and more limited by two different developments: the patenting of traits and varieties on the one hand and the discussion on Access and Benefit Sharing (ABS) in the Convention on Biological Diversity (CBD) and the International Treaty for Plant Genetic Resources of Food and Agriculture (IT-PGRFA) on the other hand. Patents are generally more restrictive regarding research and breeding than Plant Breeder's Rights (PBR) in which the important notion of the breeder's exemption in the laws based on the UPOV (International Union for the Protection of new Varieties of Plants) Conventions provides free access to commercialized, protected plant varieties for further commercial breeding. This breeder's exemption is limited by the notion of essential derivation: closely bred "essentially derived varieties" (EDV's) need for the purpose of commercialization the permission of the owner of the protected initial variety where the EDV has been derived from. New traits, from natural or artificial sources, can be protected by patents. The plants containing a patented trait do fall under its patent protection. In the USA conventional varieties can also be patented, which is not possible in most other countries in the world. This patent system allows claims on the progenies resulting from crossing patented plants with other plants. As each new variety consists of a unique combination of existing characteristics and crossing this variety with plants of other varieties yield new combinations, the ownership of a particular variety should not extend to the progenies of the crossing parents. The CBD and IT-PGRFA treaties subject the access to germplasm to particular rules for benefit sharing, whereby the terms for access under the CBD are still to be established and the outcome of the present negotiations is very uncertain. On top of these developments private companies use increasingly Material Transfer Agreements and restrictions printed on seed bags to limit the access to commercial varieties for breeding purposes. The question arises whether this tightening straitjacket will benefit or harm the breeding of new varieties in the long run and how the breeders in the field (will) cope with these developments.

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Cisgenesis: a new approach for introgression breeding

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ABSTRACT: Durable resistance to late blight in potato is a problem worldwide and not easily achieved by classical breeding with single *R*-genes or with selection for field resistance. Stacking *R*-genes, originating from *Solanum demissum*, appeared not to be a solution because the pathogen did overcome all these *R*-genes, resulting in complex isolates. However, for resistance breeding, there are three important new developments: 1. cloning of *R*-genes by map based cloning or allele mining from many wild species; 2. the cross reacting *Avr*-genes giving HR (hypersensitivity reaction) are isolated at the same time; 3. marker-gene free transformation is in potato possible allowing cisgenic breeding, with only cloned natural *R*-genes from crossable species. Combination of 1. and 2. is enabling selection of more durable *R*-genes which in combination could bring a high degree of durability. Cisgenic breeding with durable *R*-genes brings new strategies of resistance breeding. It is replacing the long lasting introgression breeding approach with different wild species or accessions for stacking *R*-genes in which linkage drag is a real bottleneck. It can now be made in one step by cisgenesis using existing varieties with a history of safe use. The availability of *Avr*-genes makes testing of many species for presence of cross reacting homologous *R*-genes possible with intriguing results for both classical and cisgenic resistance breeding. The bottleneck of the cisgenic approach is the existence of GM-regulations which have been developed for transgenes but also applied for cisgenes. GM-regulations are based on two principles: 1. the technology or process, and 2. the genetic source. In the EU Directive 2001/18/EC exemptions have been made for induced mutation breeding and for protoplast fusion because of their genetic source being within the breeders' gene pool. The technology used makes them in both cases a GM-plant. For cisgenesis the same situation is found. The transformation technology makes them a GM-plant but the genetic source is within the breeders' gene pool. Therefore, exemption of the GM-regulation is logic, after starting first with derogation for potato GM-field experiments. During these GM-field experiments questions can be answered before exemption of cisgenesis is allowed in a more general way as described in annex 1b of the EU directive 2001/18EC for protoplast fusion between crossable species and for induced mutations. Cisgenic potato, resistant to *Phytophthora infestans* will be presented as a potential business case for SMEs'.

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Viral protein transcomplementation: a neglected risk of virus-resistant transgenic plants

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ABSTRACT: Resistance to specific plant viral pathogens can often be obtained by inserting all or part of the relevant viral genome into plants. The mechanism by which such transgenes confer resistance is probably that expression of the transgene primes the RNA silencing mechanism of the plant to destroy the incoming virus. In the United states, the only country to have approved transgenic virus-resistant cultivars, at least five distinct commercial cultivars have been released and approved, although only two have been widely grown. Each of these approved cultivars contains at least one intact full-length gene derived from a pathogenic viral strain and this has led to the suggestion that these viral genes may have unforeseen or negative consequences. These include recombination to create new strains, human health consequences from ingesting viral proteins and enhanced infection by viral pathogens (also known as transcomplementation). To focus on the last of these we surveyed the scientific literature to assess the extent to which viral plant pathogens can utilise proteins from distinct viruses to enhance their infectious ability (Latham and Wilson, 2007). Analysis of more than 150 publications, each containing one or (often) more reports of transcomplementation by transgenes, successful gene exchanges between viruses, or synergism between viruses, revealed that: (1) diverse viral infection traits can be enhanced by the presence of proteins from phylogenetically distinct viruses. These include the expansion of host range, acquisition of mechanical transmission, enhanced specific infectivity, enhanced cell-to-cell and long-distance movement, elevated or novel vector transmission, elevated viral titre and enhanced seed transmission; (2) transcomplementation and synergism are mediated by many viral proteins including viral inhibitors of gene silencing, viral replicases, coat proteins and movement proteins; (3) although more frequent between closely related viruses, transcomplementation and synergism can occur between viruses that are phylogenetically highly divergent. Our results have significant implications for the risk analysis of virus-resistant transgenic plants but also suggest opportunities to make transgenic virus resistance safer.

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Genetic enhancement for yield and nutritional quality and release of new improved varieties in lentil

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ABSTRACT: Lentil (*Lens culinaris* Medik.) is a staple food legume crop, grown in many countries in the world. Its seed is used for human food and straw is an important source of animal feed. Lentil provides a sustainable cereal-based cropping systems through nitrogen and carbon sequestration in soil, thus improves soil nutrient balance. Its seed is a rich source of protein, essential amino acids, carbohydrates, fibers, vitamins, unsaturated fatty acids, and micronutrients which provides nutritional security to the poor consumers, who can not afford meat products due to high price. The International Center for Agricultural Research in the Dry Areas (ICARDA) is located in the Center of Origin of lentil, and has a worldwide mandate for its improvement. Since its inception, the Center is engaged in genetic enhancement for yield, host-plant resistance to various biotic and abiotic stresses, and improvement in nutritional quality. The national agricultural research systems of the developing world and developed advanced research institutions are the key partners in this mission. The Center has assembled >11,000 cultivated and wild germplasm accessions in its gene bank, the largest collection of *Lens* species in the world, which is the building block of genetic enhancement research for global clients. The value of germplasm collection, characterization, documentation and conservation lies in its potential use to develop improved varieties. Through rigorous evaluation, it has been discovered that considerable variability exists for agro-morphological, phenological, biotic and abiotic stress factors and micronutrient contents among these germplasm. Accessions with desirable yield contributing traits, disease resistance, plant type, maturity period and seed traits have been used directly for cultivar development or utilized in hybridization program to develop improved genetic stocks targeted to various stresses prevailing in specific agro-ecological regions. Improved genetic stocks with combined resistance to rust, Stemphylium blight, and wilt diseases, lodging resistance, high biomass have been developed and shared with national institutions. In this endeavor, >1200 breeding lines have been developed using genes from multiple parents. Varieties with high iron and zinc contents have been identified and are being cultivated in Bangladesh, Nepal, Ethiopia, Morocco, Syria, and Turkey. To date, a total of 105 lentil varieties have been released internationally, which emanated from ICARDA-supplied genetic materials. The improved varieties have made considerable impact in lentil production globally.

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Genetic similarity of some cultivars of *Brassica sp.* tested by sequencing in order to analyze gene flow pollination due to *Apis mellifera*

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ABSTRACT: *Brasicaceae* is a large plant family which includes 338 genera and 3700 species with genuine value, both in culture and in the scientific field; this family also includes model species (*Arabidopsis* and *Brassica*), developing model generic systems (*Boechera*, *Brassica* and *Cardamine*), and many widely cultivated species (Bailey et al., 2006). In this study, 6 improved varieties of *Brassica sp*. and one *Brassica* wild relative - *Brassica rapa campestris* were tested in order to assess genetical differences between them. The results are used as possible markers in horizontal gene flow caused by pollination mediated by *Apis mellifera*. Two primer sets were used to amplify the following genomic markers: ITS 1-5,8 S-ITS 2 region and the region between tRNA-Asp – tRNA-Thr (chloroplastic *trn*D-*trn*T intron). The internal transcribed spacer (ITS) allows us to identify genetic differences between the genera and tribes of *Brasicaceae* (Yang et al., 1999], as does chloroplastic DNA (Warwick and Sauder, 2005). The length of the obtained sequences was between 450-650 bp. To infer the phylogenetic three we used the Kimura 2-parameter model and Minimum Evolution (ME) algorithm (10000, bootstrap value). The phylogenetic analyses were carried out using the MEGA ver. 3.1. software. The phylogenetic analyses based on the two selected markers lead to relatively similar results. The cultivars seem to display statistically non-significant variability among themselves but are statistically different from the wild relative (*Brassica rapa campestris*). Notable exceptions are the detachment of samples 1 and 2 in the tree reconstructed based on the tRNA-Asp-tRNA-Thr chloroplastic region and of sample 4 in the ITS1-5,8S-ITS2 phylogeny. These results may be used as possible markers in the further study of pollination due to *Apis mellifera* and crossings between improved and wild *Brassica* varieties.

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Selection of spontaneous autotetraploid plants from *Citrus* polyembryonic cultivars

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ABSTRACT: Seedlessness is one of the most important characteristics for mandarin fresh fruit market. Mandarin triploid hybrids allow implementing this trait in commercial varieties. Indeed triploids plants are generally sterile and they do not pollinate other varieties. Triploid plants can be recovered by crossing diploid and tetraploid parents. Most citrus genotypes are apomictic. The origin of apomixis is determined by adventitious embryony in polyembryonic seeds (Koltunow, 1996). The adventitious embryos originate from nucellar cells (Kobayashi et. al., 1981). Tetraploid plants are found with variable frequency in seedling populations of polyembryonic citrus genotypes. This allows the recovery of citrus tetraploid genotypes that can be used for triploid breeding. In the framework of the IVIA triploid breeding program (Navarro et. al., 2002), we have searched for tetraploids plants in seedling populations of `Anana´, `Fairchild´, `Kara´, `Page´, `Salteñita´, `Simeto´, `Sunburst´ and `Tardivo di Ciaculli´ mandarins, `Afourer´, `Murcott´ and `Ortanique´ tangors, `Mapo´and `Minneola´ tangelos, `Duncan´ and `Star Ruby´ grapefruits and `Sanguinelli´ orange. Determination of ploidy level was made by flow cytometry and genetic analysis to confirm their genetic origin with 31 SSRs markers. Tetraploid plants were found in all genotypes analyzed, except `Salteñita´ and `Simeto´ mandarins, but the frequency of tetraploids varied with the genotype. `Kinnow´ mandarin produced the larger number of tetraploids plants (9,7%), whereas `Page´ mandarin produced the smaller percentage (0,5%). All the tetraploid plants showed the same ploidy level in all parts of the plant, indicating that they were not chimeras. They presented the same molecular profile as their maternal parents for all *loci* analyzed, thus indicating that these plants originated as a result of the duplication of the chromosome number in nucellar cells. The new tetraploid plants have been included in the collection of tetraploid genotypes of the IVIA Germplasm Bank for their use as male parents in the triploid breeding program.

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Impact analysis of some research results of Seed and Plant Improvement Institute (SPII)

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ABSTRACT: This study was carried out in 2006 in order to assess the effects of research, research achievements, impact and efficiency indices, estimate of cost and benefit of activities and impact of research results of the activities of the Seed and Plant Improvement Institute (SPII). Data on number of released improved cultivars, distributed seeds of these cultivars, cultivated areas of each cultivar and yield increase by improved cultivars during 2000 to 2005 was collected from different research departments at SPII. Data on budget and costs were obtained from Program and Budget office of the Institute. According to the results of the assessments, during the period between 2003 and 2005, impact indices of agronomy recommendations, introduced cultivars, new plants introductions, and presentation of new ideas were 55, 26,16 and 56 cases, respectively. Increasing of staff scientific level and establishment of research facilities were 10310 and 64500 million rials, respectively. Efficiency indices of SPII for articles published in SPII and Scientific Journals were 20 and 157 cases. Presented papers in conferences were 495, edited and translated books were 12, and number of final reports of projects and technical reports were 458 and 92, respectively. Number of scientific cousultations of projects in national and international level was 65. Number of national and international conferences hold by SPII was 44, and sharing or participating in preparation of standards was 19. Present value of gross income increase and costs of activities of research results were estimated to be 24579 and 620 thousand million rials during 2000-2005. Net value of SPII was estimated 23959 thousand million rials in that period. The net present value of activities in Cereal, OilSeeds, Potato and Onion and Maize and Forage crops Research Department were 17941, 3530, 2006 and 756 thousand million rials, respectively. Benefit- cost ratio of activities of SPII during 2000 to 2005 was 39.7. These estimated benefits were only a part of economic benefits of SPII.

Keywords: Research, Results, Impact, Efficiency, Cost and Benefits, SPII.

Introduction and Background

The development of plant breeding research in Iran could be divided into five distinct periods:

The first period (1927 to 1945): Establishment of the Higher School of Agriculture followed by the foundation of the Faculty of Agriculture in 1927, in Karaj, which were the two most prominent initiatives. Many obstacles were experienced during this period, since shifting from traditional and old approaches needed educated and trained man power in new agriculture. Since 1932, seed multiplication and purification were practiced in the experimental field of the Higher School of Agriculture. In 1933, preliminary field experiments and seed multiplication schemes were carried out, for cereals, cotton and sugar beet, in the experimental fields of the Higher School of Agriculture. The first plant breeding company was established in Karaj, in 1935, with the main duty of improvement and multiplication of sugar beet seed. In 1936, in Varamin, one of the main cotton growing areas in Iran, a plant breeding company was established to improve and produce elite seeds of cotton and wheat.

The second period (1945 to 1960): This period commenced with the end of the second world war and continued until the establishment of Seed and Plant Improvement Institute in 1960. Horticultural crops improvement began in 1946, with the establishment of collection gardens of different cold and temperate fruit species, in Karaj, collected from Iran or imported from other countries. In 1948, with the separation of Faculty of Agriculture from the Ministry of Agriculture, all duties, related equipments and facilities for collection, evaluation and experimenting of wheat and barley landraces and local populations were transferred and concentrated in the Karaj Plant Breeding Enterprise. Cotton seed improvement was followed more seriously since 1951, and the initiative of establishing the General Office of Certified Seed Supply was formed and took place in 1956. By organizing the scattered plant breeding research activities in the country, rice breeding research commenced in Lahijan Agricultural Enterprise in 1957 and developed with establishment of Rice Research Field Station of Rasht in 1959. All this endeavors led to the approval of the law of Seed and Plant Improvement Institute by legislative body (Senate Assembly) in 1959, and its establishment in 1960.

The third period (1960 to 1978): Scattered research in different sections of the Ministry of Agriculture, and the then General Office Crop Production with limited organization and facilities could not directly lead the national research system. The Seed and Plant Improvement Institute was established in 1960. To facilitate the activity of these offices the institute also established in Karaj the cereal improvement laboratory, sugar beet seed improvement laboratory, fruits improvement laboratory, as well as the seed improvement laboratory in Varamin, the rice improvement laboratory in Rasht, the date palm improvement laboratory in Ahwaz, and the seed specialized glasshouses in Karaj and Varamin. In addition to the plant breeding centers in Karaj and Varamin, three other main field research stations were also established in Mashhad in Khorasan, Moghan in East Azarbaijan and Darab in Fars. Therefore, the institute went into a change in its organization, as the Oil Seed Crops Research Department was established in 1969 followed by the formation of the Maize Research Department in 1970. In 1971, the general office for horticulture crops research changed into Horticultural Crops Research Department, and the vegetable crops unit was separated to form Vegetable Crops Research Department in 1978.

The forth period (1978 to 1993): In order to proceed with amendment of its organization, the Seed and Plant Improvement Institute followed the strategy of forming new research departments. The Food Legumes Research Department was formed in 1980, and with the development of crop physiology science the Physiology, Biochemistry and Biotechnology Research Department was established in 1983. To enhance the utilization of plant genetic resources in crop breeding programs and increase their efficiency, the Genetic and Plant Genetic Resources Research Department was also formed and established in the second half of 1983. This organization scheme was in place until a dramatic shift occurred in this mother plant breeding and agronomy institute, and new commodity research institutes were separated and formed in early1990s.

The fifth period (1993 to present): This period is known as the period of formation and establishment of commodity research institutes. The first institute that separated from SPII and established as an independent institute in 1992, was the Dryland Agriculture Research Institute (DARI). This was followed by separation and establishment of the Pistachio Research Institute in 1992, the Date Palm and Tropical Fruits Research Institute in 1993, the Rice Research Institute in 1994, the Citrus Research Institute in 1996, the Cotton Research Institute in 1997, the Agricultural Biotechnology Research Institute in 2000 and the Seed and Plant Certification Research Institute in 2004. All these new commodity research institutes are carrying-out breeding and agronomy research for the concerned crops.

Currently, seven research departments conform the skeleton of the Seed and Plant Improvement Institute, and their mandates are, planning, coordinating and conducting research projects and seed/plant increasing programs in the headquarters at Karaj and 35 provincial Agricultural and natural Resources Research Centers and their 80 affiliated field research stations, a very well organized National Research Network across the country. These research departments are: 1-Cereal Research Department ,2-Horticulture Crops Research Department, 3-Maize and Forage Crops Research Department, 4-Oil Seed Crops Research Department 5-Potato, Onion and Irrigated Food Legumes Research Department , 6-Genetics and Plant Genetic Resources Research Department, 7-Vegetable Crops Research Department.

Investment rate of return in research led to the release of Ghods wheat cultivar in Iran by using benefit-cost ratio and internal rate of return was estimated 2.95-23.5 unit and 59.03-66.6%, respectively. Rate of return from wheat research in Fars province in Iran, studying 203 selected farmers by sampling method and production function approach, was estimated to be 43.6%. To assess the economic efficiency of investment in development of wheat varieties in Cereal Research Department, SPII Benefit–Cost Ratio and Internal Rate of Return were used. The total cost and benefit change to present value and economic rate of return with discount rate of 18.5% were estimated. Results showed that: Benefit-Cost ratio of released bread wheat cultivars under research conditions was 25.8. Investment rate of return in newly released irrigated wheat cultivars under research conditions was estimated 77.8%. In addition, it was revealed that investment on wheat research program was economic, because, rate of return was estimated more than the discount rate (18.5%). Furthermore, Benefit-Cost ratio

for Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir, Chamran and Shiroodi bread wheat cultivars under research conditions were estimated as 5.3, 2.5, 10.1, 45.8, 72.2, 2.8, 9.1, 1.5, 13 , 4.4, 18.1 and 9.1, respectively. The investment rate of return for the above cultivars were estimated 61.7, 55.3, 91.5, 137.3, 68.4, 53.1, 70.2,35.6, 62.2,55.5,192.3 and 150.5 percent, respectively .

Materials and Methods

The net present value (NPV) and benefit-cost ratio was used for estimating profitability of research achievements.The model used for estimation is described as follows :

$$Benefit-cost\ ratio=\left[\sum_{t=1}^{n} B_t/(1+i)^t\right]\Big/\left[\sum_{t=0}^{n} C_t/(1+i)^t\right]$$

$$F=P(1-i)^t \qquad Bt=G_t.P_t.A_t.Y_t$$

Where, B_t is annual gross benefits from wheat research in year t, C_t is annual costs from wheat research in year t, F *is* investment value in year t, P is Pr imarily investment, t is year and i was discount rate. A general formulation for the estimation of the gross benefits from a new cultivar (B_t) can be derived as follows. Where P_t is the price crops in year t, g_t is the percentage gain in yield from the breeding program in year t, A_t is the area sown in year t and Y_t is the mean yield of new variety in year t. Required data for this study were; price of crops in different year, research budgets of different crops, distributed seeds of different crops cultivars. average yield of new and old cultivars under research conditions, recommended seed (ha). Data were obtained from different research departments in SPII, and Agricultural Support Services Company. Research costs for 2000-2005 period has been estimated.

Table 1. Number of research projects carried out in SPII during 2000-2005.

Research Departments	2000	2001	2002	2003	2004	2005	Total
Cereal Research Department (including experiments)	595	667	710	677	634	741	4024
Oilseeds Research Department	207	206	233	284	266	271	1467
Potatoes , Onion, Irrigated Food Legumes Research Department	133	133	139	114	99	122	740
Maize and Forage Crops Research Department	145	133	106	101	101	109	695
Horticulture Crops Research Department	176	155	146	115	122	130	844
Plant Genetic Resources Research Department	49	49	48	43	43	56	288

Sources: Research Departmens of Seed and Plant Improvement Institute(SPII), Karaj, Iran, 2006.

Results and Discussion

During 2000-2005, total research projects in Cereal Research Department, including experiments, were 4024. Research projects in Oilseeds Research Department, Potatoes, Onion and Irrigated Food Legumes Research Department, Maize and Forage Research Department, Horticulture Research Department and Plant Genetic Research Department were 1467 , 740, 695 and 844 and 288, respectively .

Research achievements

From the beginning of research activities, the long term research achievements in Cereal Research Department are: irrigated bread wheat (83 cultivars), irrigated durum wheat (4 cultivars), barley (14 cultivars), triticale (2 cultivars) and rye (one cultivar). The main research achievements in Oilseeds Crops Research Department are. sunflower (9 cultivars), soybean (9 cultivars), canola (5 cultivars), sesame (8 cultivars), safflower (4 cultivars) and peanut (one cultivars) during last three decade. The main research achievements in Potatoes, Onion and Food Legumes Research Department are: potatoes (47 cultivars), onion (21 cultivars), and irrigated food legumes including Kidney bean and broad bean (4 cultivars) during 1961-2006. The main research achievements in Maize and Forage Crops Research Department in different decades consist of 42 varieties including maize (21 cultivars), sorghum (4 cultivars) , alfalfa (5 cultivars) , millet (3 cultivars) and Sainfoin and Clover (8 cultivars). The long term research achievements in Horticulture Crops Research Department were 135 varieties including introduction of almond (10 varieties), hazelnut (8 varieties), apple (6 varieties), cherry (13 varieties) , plum (10 varieties), peach (19 varieties), nectarine (12 varieties), olive (6 varieties), pomegranate (5 varieties), grape (7 varieties), and strawberry (6 varieties). The long term research achievements in Plant Genetic Research Department included the evaluation and regreneration of 3473 accessions and the collection of 2447 samples.

During 2000-2005, the main research achievements in the Cereal Research Department for irrigated cereal were 9 varieties including bread wheat (6 varieties), durum wheat (2 varieties), barley (1 variety). The main research achievements in Maize and Forage Crops Research Department were 10 varieties including maize 647, hybrid maize (SC700, SC500 , SC302), sorghum (KFS3) . The main research achievements in Potatoes, Onion and Food Legumes Research Department were 26 varieties including potatoes (21 cultivars), onion (5 cultivars). Also, total genetic resources samples rendered to research and education centers were 41852 samples .

Impact assessment of research achievement

Cost of Research Activities

Based on information obtained from planning and budgeting office in SPII, total finance allocated to different activities in SPII during 2000-2005 were estimated to be 34097, 43016, 42208, 69060, 64300 and 65627 million rials. Total finance for investment was 44330 million rials,

miscelaneous costs of implementation of various projects were about 100536 million rials, staff salaries were 131121 million rials, income and other costs (cost of water, telephone, etc.…) was estimated 42321 million rials. In 2005, the attendant expenditures of different departments from the global finance included: Cereal Research Department 18290 million rials, Oilseeds Crops Research Department 7698 million rial, Maize and Forage Crops Research Department 7089 million rials, Potatoes, Onion and Irrigated Food Legumes Research Department 2426 million rials, Horticulture Crops Research Department 8344 million rials, Plant Genetic Research Department 7709 million rials, and Headquarters 19980 million rials.

Table 2. Expenditure of research activities in SPII during 2000-2005 (million rials).

Expenditures	2000	2001	2002	2003	2004	2005	Total
Investments	2007	2331	11801	10548	9082	8562	44330
Miscelaneous costs of implementation of different projects	15401	18833	9791	21000	19117	16394	100536
Staff salaries	10669	14495	15857	29061	27910	33130	131121
Income and cost of maintenance cost, cost of water , telephone, energy, etc.	6021	7357	4759	8452	8191	7541	42321
Total	34097	43016	42208	69060	64300	65627	318309

Sources: Research Departmens of Seed and Plant Improvement Institute(SPII), Karaj, Iran, 2006.

Table 3. Cost and gross benefit of research achievements in SPII during 2000-2005 (000million rials).

Benefit/Cost	2000	2001	2002	2003	2004	2005	Total
Gross benefit	1265.0	1630.0	2190.3	2743.0	3406.8	3040.3	14275.4
Cost of activities	37.72	47.95	45.64	76.24	71.02	73.33	351.9
Increasing of net income	1227.2	1582.0	2144.3	2666.4	3335.7	2967.0	13922.6

Source: research data.

Table 4. Present value of cost and gross benefit of research achievements in SPII during 2000-2005(000million rial).

Benefit/Cost	2000	2001	2002	2003	2004	2005	Total
Gross benefit	3501.74	3808.2	4320.13	4564.0	4784.0	3602.0	24580.1
Cost of activities	104.53	112.0	89.93	126.78	99.73	86.8	619.77
Increasing of net income	3397.0	3696.2	4230.4	4437.0	4684.0	3515.0	23959.6

Source: research data.

Gross income from substitution of new cultivars

Increasing gross income from substitution of some of the new cultivars during 2000-2005 was estimated 14275 million rials and total costs of research activities led to the development and release of them was estimated 352000 million rials. Net value of activities was estimated 13923000 million rials. During 2000-2005, present value of gross income from substitution of some varieties was estimated 24580000 million rials and present value of costs was estimated 620000 million rials. Net present value of research activities was estimated 23959000 million rials. Benefit –Cost ratio for some of research activities in SPII was estimated 39.7 during 2000-2005 (2).

Conclusions

Present value of gross income increase and costs of research activities research were estimated to be 24579000 and 620000 (million rials) during 2000-2005. Net value of activities was estimated to be 23959000 (million rials) in the concerned period. Net present values of activities in Cereal Research Department, Oil Seed Crops Research Department, Potatoes, Onion and Irrigated Food Legumes Research .Department, Maize and Forage Crops Research Department were estimated to be 17941000, 3530000, 2006000 and 7560000 (million rials), respectively. Benefit- Cost ratio of activities was estimated 39.7 during 2000-2005. Of course estimated benefits are only economical benefits of research activities in SPII. Therefore, the use of new varieties not only prevents reduction of crop yield, it would also minimize the consumption of production inputs (fungicides, herbicides, pesticides etc.), reduces production costs, avoids pollution of surface and ground water and other environmental resources, and increases both short and long-term farm profitability. In conclusion, varieties of good quality reduce crop wastes, and benefits would reach to the consumers and society.

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Activities for exploitation of Sicilian landraces of violet cauliflower

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ABSTRACT: Several landraces of violet cauliflower are widely grown in South of Italy either to supply local markets with fresh product, or for processing in freezing industry. Most of these landraces are related to the traditional type "Violetto di Sicilia", of which a number of variants quite likely derive from broccoli. Over the last decade we started specific activities with the aim to collect, characterize, evaluate and exploit these landraces. Collected genetic material is conserved at the DOFATA (University of Catania) where it has been characterised for the main morpho-biometric descriptors of the plant. Several sib-crossing and double haploid lines were selected and evaluated for agronomical and technological purposes. Particular attention was paid to select lines with high content of antioxidants, such as anthocyanins, carotenoids, ascorbic acid and glucosinolates; for the latter compounds selection work was mainly addressed to high content of glucoraphanin which is hydrolised into sulphorafane, a strong antioxidant effective against several types of cancers. These aspects are particularly appreciated by a new category of consumers who are very interested on healthy traits of produce considering them as functional foods. The lines were also evaluated for volatile compounds of curd tissues, for their resistance to black rot and downy mildew, for freezing, etc. The work carried out to date allowed us to select two cultivars and recently some hybrids of Violetto di Sicilia. Experiments run at the DOFATA led to assist in establishing new food chains devoted to pigmented cauliflower with the aim of diversifying production with new issues which might increase the demand. Moreover, acquired data may offer morpho-biometric and biochemical markers that could be useful, in the near future, to asses a possible PGI certification of this typical Italian vegetable.

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The suitability of oat varieties, mixtures and populations at different positions in an organic rotation

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ABSTRACT: Data from the third year of experiments which aim to study the suitability of new and established conventionally-bred naked and husked oat varieties, variety mixtures and a husked oat population for organic systems is presented. Trials were established as both second and first cereals in the rotation. The average yields of the husked and naked varieties as a second cereal were 97 % and 81 %, respectively, of the first cereal yields. The rankings of the varieties were similar in both rotational positions with the husked varieties Tardis and Mascani, and the naked varieties Expression and Grafton yielding well. Mixtures generally yielded similarly to the means of component varieties but the husked and naked mixtures had 18 % and 12 % less disease, respectively, than the average of the component varieties.

Keywords: husked; naked, oats, organic farming, rotation, varieties, variety mixtures.

Introduction

Oats have many qualities that make them suitable for organic production including high nitrogen use efficiency (Sylvester-Bradley, 1993) and competitiveness against weeds (Seavers and Wright, 1999). The aim of this project is to identify, in existing and novel husked and naked oat varieties, traits that are key for organic farmers. These traits include competitiveness, pest and disease resistance and good combining ability in variety mixtures that may help to overcome variability caused by biotic and abiotic stresses.

This paper discusses the results of the third year (2006/07) of experiments which tests husked and naked varieties, mixtures and populations at different positions in an organic rotation. They are compared with results of the first and second year of trials, details of which can be found in Jones et al. (2006) and Clarke et al. (2007).

Material and Methods

Four trials were established at Sheepdrove Organic Farm, Berkshire on 17th October 2006. The main experiment consisted of two trials (one examining naked and the other husked varieties) and was carried out as the second cereal in the rotation. A secondary experiment repeated the treatments of the main trials, but as the first cereal in the rotation. The naked oat trials contained three UK varieties (Expression, Grafton, Racoon) and their three-way mixture. The husked oat trials tested four UK varieties (Gerald, Tardis, Brochan, Mascani), their four-way mixture and a bulk of IGER lines selected at F2 (‘population’) and grown

at either Sheepdrove Organic Farm (SOF) or Wakelyns Agroforestry, Suffolk (WAF). The experiments were of a replicated split-plot design with 1.45m x 10m split-plots split for either normal (200 kg/ha) or low (150 kg/ha) seed rate. The second cereal and first cereal experiments were harvested on 7th and 8th August 2007, respectively.

Assessments of the main (second cereal in the rotation) experiments included crop emergence and establishment, early crop and weed cover, pests and diseases, crop height, lodging, canopy cover, and grain yield. The first cereal in the rotation experiments were assessed only on yield and quality parameters. A selection of results is shown below.

Results and discussion

Husked oats

The first differences detected among the husked varieties, mixture and populations in the main (second cereal) experiment were amongst early crop cover; there were no differences in the number of plants that emerged or established. Tardis had a significantly ($P = 0.015$) higher level of crop cover than the other varieties and mixture. However, this trend was not repeated later in the season when maximum Leaf Area Index (LAI) was assessed. Unlike in the previous season (2005/06), when Tardis had a significantly higher LAI than the other varieties and went on to have the highest yield, in this season (2006/07) there were no significant differences in LAI among the varieties, the mixture and populations.

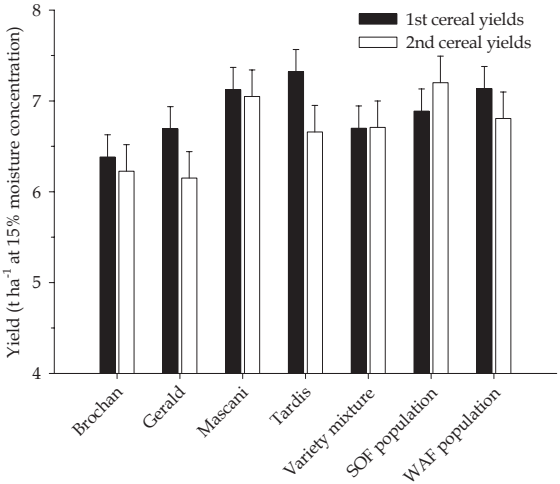

Figure 1. Yields (t ha^{-1} at 15% moisture concentration) of: husked oat varieties Brochan, Gerald, Mascani & Tardis; the variety mixture; & the 'populations' from Sheepdrove (SOF) & Wakelyns (WAF) grown as either the first (black bars) or second (white bars) cereal in the rotation.

Instead, the yields in 2006/07 correlated with disease levels. Unlike 2005/06 which was a low disease year, this season was notable in terms of the large amounts of disease present, especially crown rust. There were significant ($P < 0.001$) differences in total disease levels on the flag leaf among the varieties, mixture and populations. Gerald had significantly higher levels of disease than the other varieties, with Mascani having slightly, but not significantly, lower levels than Brochan and Tardis. These results are reflected in the yields of the varieties with Gerald having the lowest and Mascani the highest yields of the second cereal experiments (Figure 1).

Interestingly, the mixture had 18 % less disease than the average of its component varieties. This is consistent with the results from 2005/06 where the mixture had 25 % less disease than its component varieties and continues to show the effectiveness of mixtures at controlling the spread of disease.

Overall there was only a 3 % reduction in yield from the first to the second cereal experiments. In both experiments, Tardis and Mascani were the higher yielding varieties and Brochan and Gerald slightly lower. However, Tardis and Gerald were notably less productive as second cereals. The mixture performed consistently in both rotational positions and interestingly the SOF population performed better in the second cereal position whereas the WAF population performed better in the first cereal position, which may indicate differential adaptation.

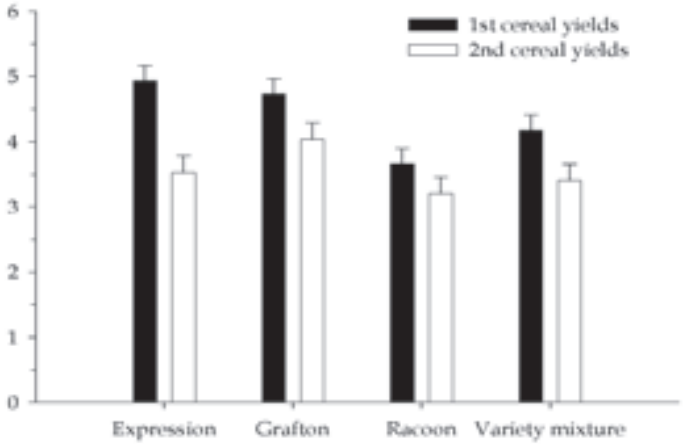

Figure 2. Yields (t ha^{-1} at 15% moisture concentration) of: naked oat varieties Expression, Grafton & Racoon; and the variety mixture grown as either the first (black bars) or second (white bars) cereal in the rotation.

Naked oats

In contrast to the husked oats, there were no differences in disease among the varieties and mixture in the naked oat main (second cereal) experiment. There were, however, significant ($P = 0.023$) differences in yield among the varieties and mixture (Figure 2). Again, when comparing the second and first cereal yields, varieties were relatively consistent in their performance with Expression and Grafton yielding more than Racoon. However, the reduction between the average yields of the first and second cereal experiments was larger at 19% than in the husked experiments (3%). The mixture performed as expected from the average of the component varieties.

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UFO-α, a new series of flat fruit peach varieties complementary of first flat peach UFO series

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ABSTRACT: The interest in flat fruit peach varieties by consumers and fruit growers is becoming greater. Consumers demand this type of fruit because they have the certainty to enjoy a fruit of excellent organoleptic characteristics. The fruit growers are actually planting these varieties due to the high market demand that has so far paid well that product. Just when the first series of nine UFO varieties were introduced on the market, it could seem that they could only be a niche product, while the reality has shown that flat peaches are increasingly gaining an important slice of the peaches market. Spain is actually the country where the flat peaches are mainly planted having reached an area of about 3,000 ha within just a few years. The new five white flesh series of flat UFO-α peach varieties have to be considered complementary of the first nine UFO varieties because they ripen at different times from these first UFO materials (Nicotra and Conte, 2001, 2003). In fact, the earliest UFO-α ripens 5 days before the very early UFO-1, the second in between UFO-3 and UFO-4, while the other three ripen at intervals of 8 -10 days, during the period included between UFO-5 and UFO-7. Thus almost the entire period of peach species maturation is covered by the flat fruit UFO and UFO-α peach varieties.

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Parental heterozygosity and origin of 2n gametes in mandarin: two key parameters for Citrus triploid breeding projects

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ABSTRACT: Citrus are the most important fruit crop in Spain and worldwide. Spain is the fifth producing country and the first in fresh fruit exportation. Seedlesness is a key characteristic for the fresh fruit market. Mandarin varietal structure in Spain causes important commercial problems because of cross-pollination between Clementines and late maturing mandarin hybrids, which produces seeds in both groups of varieties, in spite of their self-incompatibility. Development of triploid hybrids could be a solution for this problem, since they are sterile and they produce seedless fruits, and do not pollinate other varieties (Navarro et al., 2002). Triploid citrus hybrids can be obtained by several strategies (Ollitrault et al., 2008), including hybridization between diploid parents. In the case of Citrus it has been shown that the 2n gametes producing the triploid are of maternal origin. It has been proposed that the origin of 2n gametes is from the second division restitution (SDR) in Clementines and from the first division restitution (FDR) in sweet oranges. No data is available for other genotypes and particularly Fortune, a mandarin hybrid producing very high rate of triploids in 2x x 2x crosses and massively used to create triploid progenies. Mechanism of 2n gametes formation and its implication on parental heterozygosity restitution, as well as the male parent heterozygosity and parental differentiations, are the main parameters determining the genetic and phenotypic structure of the triploid population. Thus, the aim of this work was to evaluate heterozygosity level and differentiation between several male parents and Fortune mandarin and to analyse the mechanism of 2n gamete formation in Fortune. Fifteen parental genotypes used in the IVIA triploid breeding project have been analyzed with 35 codominant molecular markers SSRs (Simple Sequence Repeat), 25 of them being polymorphic for the analyzed genotypes. Heterozygosity levels vary between 37% for Fremont and Ellendale and 54% for Pineapple 4n. Likewise, the same SSR markers were used for analyzing 67 triploid hybrids from the cross between Fortune as female parent and Murcott as male diploid parent. This later was the more genetically distant to Fortune according to information of the previous study (D=0.49). This analysis allows us to study the mechanism of formation of the Fortune 2n gametes. The obtained results are in favour of the SDR hypothesis, rather than FDR. The SDR hypothesis is coherent with the results published in case of the clementine (Luro et al., 2004), which is one of the parents of the Fortune variety.

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Molecular markers as a tool for plant breeding and variety identification

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ABSTRACT: Identification of agricultural and horticultural crop varieties is important during their whole breeding and registration process, seed-production, trade and inspection. So although morphological traits, quality traits and yield characteristics are currently explored for variety protection (ISTA and UPOV directions), new markers are being developed to maintain the efficacy of registration and DUS (Distinctness Uniformity Stability) testing, which guarantees the quality of a new variety for farmers and merchants. Moreover, it is also used to protect intellectual property of the plant breeder (plant breeders' rights), which encourages the continuous development of new cultivars. To complement morphological traits, isozyme analysis and molecular markers have been used for cultivar identification and was included in DUS testing. Molecular markers in general can also be used as potential techniques for variety identification and progressive tool in breeding new varieties (MAS). In comparison with morphological traits, molecular markers have many advantages and relatively high number of different molecular (protein and nucleic acids) techniques and approaches are available for plant genotyping purposes. The marker systems differ in information content, number of scorable polymorphisms, degree of automation, labour, and financial costs. In this contribution we introduce two model applications of molecular markers: a) molecular markers in potato variety identification; b) molecular markers as selectable markers on hybrid breeding in oil seed rape.

Keywords: breeding, molecular markers, oil seed rape, potato, self-incompatibility, variety identification.

Molecular markers in potato variety identification

Cultivated potato (*Solanum tuberosum* L.) is, along with wheat, rice and maize, one of the four most valuable world crops. Potato is an important food crop, as well as being widely used for livestock feeding and industrial processing as feedstock for many industrial and food applications. Currently, there are more than 4,000 different potato varieties which are cultivated in over 100 countries worldwide (Pieterse and Hils, 2007). The improvement and creation of new varieties with new combinations of current features or essentially new features, such as GMO potatoes or conventional varieties with better parameters of quality or resistance to biotic and abiotic factors, is one of the main goals of plant breeding. In many

cases, wild allied species or "old primitive" varieties are used as donors of these features. New breeding approaches based on molecular markers allows for a more efficient use of these donors (Callow et al., 1997). Also the identification of individual varieties of agricultural and horticultural crops is important at every stage of their agri-production: during their breeding, registration process, seed-production, and testing (Görg et al., 1992).

The traditional approach to variety identification is composed of the observation and recording of a range of morphological characters or descriptors. Such an approach is undoubtedly successful for DUS testing. However, it is less suitable when results are required rapidly, such as for the confirmation of tuber material identification. Furthermore, morphological characters are often multigenic, continuously expressed and influenced by environmental interactions, making it difficult to assess them quickly and objectively, and requiring replication of observation. Together with advances in molecular biology, several new molecular and biochemical marker techniques will be adopted. These techniques are a powerful tool for determining genetic distinctness and enable characterization of particular genotypes. Use of molecular markers for variety improvement of agricultural crops has been widely applied in the last decade to exactly determine genetic variation based on DNA analysis (Staub et al., 1996).

In the year 2007 there were one hundred and seventy eight potato varieties present in the Czech List of Registered Potato Varieties and we need powerful tool for variety/tuber identification because the significant law no.110/1997 Sb. requires guarantee of variety declaration in commercial relation for food potato in Czech Republic.

Material and methods

Plant material. Selected set of registered potato varieties was used for analysis respectively Adéla (7), Adora (1), Agria (13), Asterix (18), Colette (2), Dali (8), Desirée (19), Ditta (14), Filea (15), Impala (3), Karin (9), Laura (16), Magda (4), Marabel (10), Rosara (5), Samantana (20), Santana (11), Secura (12), Solara (17) a Velox (6).

The analyses were done according to the standard protocol – Biotechnological centre, Agriculture faculty, University of South Bohemia (http://www.eamos.cz/amos/bc/modules/low/kurz_obsah.php?kod_kurzu=bc_154)

PCR-SSR analysis. SSR analyses were done using primers STM 2005, STM 1102, STM 3012, STM 1106, STM 3015, STWIN 12G and STG BBS.

PCR-ISSR analysis. ISSR were detected using primers P1, P2, P3, P4 a B1.

PCR-RBIP analysis. For PCR-RBIP and IRAP analyses were chosen primer Tst101, Tst103, Tst106.

AFLP analysis. AFLP markers were generated using primer combination EcoRI-ACG / MseI-AGC.

Results

The whole data set obtained after SSR, ISSR, RBIP and AFLP analyses was evaluated using digital image analysis, matrixes of genetic distances were calculated (Nei&Li metrics) and cluster (UPGMA method) and principal coordinates analyses (PCO analysis) were performed.

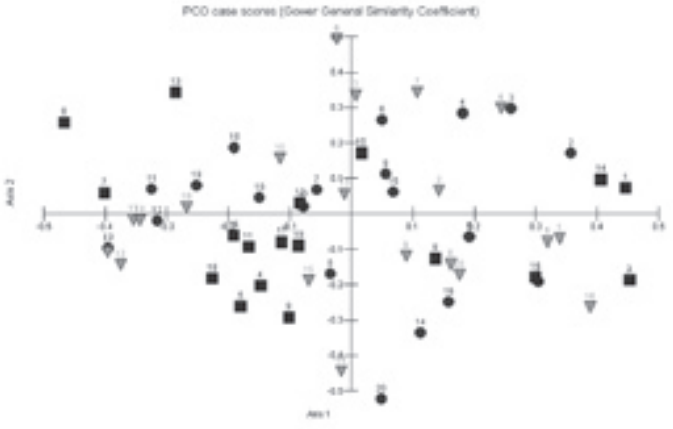

Figure 1. Results of principal coordinates analyses – PCO plot obtained after analyses of 7 SSR markers (squares) and 3 retrotransposon based markers (triangles) and we tested whole set of primers together too (circles).

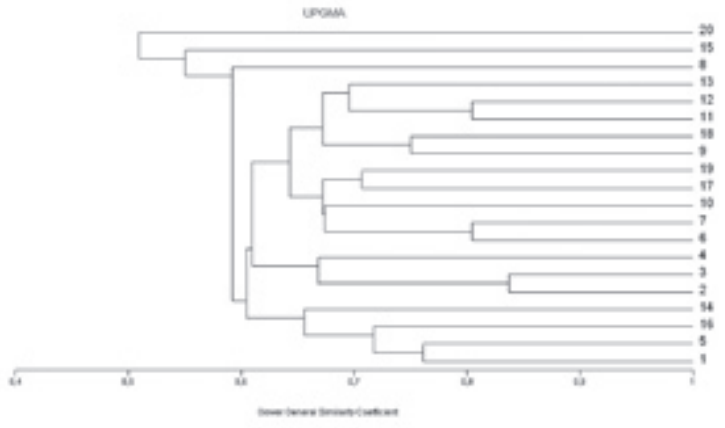

Figure 2. Results of cluster analyses obtained after analyses of set of SSR and retrotransposon based markers.

Conclusion

We obtained patterns of seven SSR and three retrotransposons based markers for the set of twenty selected varieties from the total set of 178 varieties registered in CR in the year 2007. The obtained polymorphism was appraised and the varieties were separated to the categories by the electrophoretical phenotype. Analysis of microsatellites (SSRs) is a suitable method for rating variability and variety identification, however, the low detected polymorphism in SSRs is disadvantage, ISSR analyse afford larger polymorphism, but using this method is connected with relevant disadvantages as is the instability of band pattern depending on age of DNA. Both RBIP and IRAP markers generate sufficient levels of polymorphism and allow distinguish all individual genotypes in the analysed model set of 20 varieties. This approach, utilization of retrotransposon-based markers, is utilizable for screening of large sets of potato samples but has also specific requirements.

Another suitable approach is AFLP analysis, we obtained the significant difference among fifteen potato varieties with the usage of this method. Most of tested methods are utilizable for variety identification. But for wide range identification of variety it seems to be more suitable to use the complex set of molecular and morphological markers and descriptors.

Molecular markers as selectable markers on hybrid breeding in oil seed rape

Self-incompatibility (SI) is a natural mechanism of plants that prevents inbreeding and promotes out-crossing. This system naturally occurs in *Brassica oleracea* and *Brassica campestris*, which are diploid ancestors of amphidiloid *Brassica napus*, but despite of this fact is *Brassica napus* self-compatible (Olsson 1960). In the *Brassica* SI system is controlled by a single polymorphic locus termed S-locus (Bateman 1955). There are three highly polymorphic genes at the S locus: *SRK* (S-locus receptor kinase) (Stein et al. 1991), *SP11*/*SCR* (S-locus protein 11/S-locus cysteine rich protein) (Schopfer et al. 1999, Suzuki et al. 1999), and *SLG* (S-locus glycoprotein) (Nasrallah et al. 1987). S haplotypes have been classified into two classes based on the nucleotide sequence similarity of SLG alleles. Class-I S-haplotypes are known to be generally dominant to class-II S-haplotypes in the pollen (Nasrallah et al. 1991, Nasrallah 1993). SP11s have two classes which is common to the general feature of the S-locus. Class-II SP11s originating from *B. olerecea* and *B. rapa* form a distinct group separated from class-I SP11s (Shiba et al. 2002). In contrast to SLG protein, SCR is supposed as a male determinant with essential function in pollen–stigma recognition (Suzuki et al. 1999, Schopfer et al. 1999). However, SLG seems not to play essential role in pollen-stigma recognition (Okazaki et al. 1999, Suzuki et al. 2000, Suzuki et al. 2000).

Material and methods

A segregating doubled haploid (DH) population of oilseed rape (*Brassica napus*) was derived from four crosses between self-compatible (SC) cultivar 'Lisek' and self-incompatible (SI) line 'AIK 6', SC cultivar 'Rasmus' and SI line 'AIK 6', SC cultivar 'Rasmus' and SI line 'AIK

3', and finally SC line 'OP BN-03' and SI line 'AIK 3'. 'AIK 3' and 'AIK 6' SI lines were derived from SI line 'Tandem' with recessive type of self-incompatibility. This population consisted of 118 plants. Seeds of cultivars and DH lines were obtained directly from the breeding stations Opava and Slapy, Czech Republic. DH populations were regenerated via a microspore embryogenesis procedure from F1 generation after crossing with an objective to fixed SI phenotype and low content of glucosinolates in the Research Institute of Crop Production in Prague.

Genomic DNA was extracted from young leaves of 2-week-old seedlings by the DNeasy Plant Mini kit (QIAGEN).

The PCR reaction was performed with class-I SLG-specific primers PS5 and PS15 (Nishio et al.1996). SCR gene was amplified with class-II SCR-specific oligonucleotide primers designed for functional allele originating from SI line 'Tandem' termed allele 2 (5'-TTGGACTTTGACATATGTTC-3' and 5'-CTCTGAAGTGGGTTTTACAG-3').

Results

Two marker genes were used for SI plants selection. PCR with class-I SLG-specific primers has resulted in approximately 1300 bp fragment together with c. 1000 bp long, probably nonspecific fragment. This fragment was specifically present in plants considered to be self-compatible. This marker gene has been detected in spectrum of naturally self-compatible oilseed rape cultivars whereas in self-incompatible lines not. The second marker system specifically targets allele of class-II SCR gene. This allele was found in self-incompatible lines derived from line 'Tandem'. Amplified fragment of class-II SCR gene allele was 280 bp long and specifically occurred in plants considered to be self-incompatible. The two marker systems segregated in ratio 1:1 as was expected and they exactly correlated each other. On the basis of molecular marker selection, young doubled haploid plantlets of oilseed rape were selected and further subjected to the phenotypical examination.

Conclusion

The two marker genes were used to select self-incompatible plants from segregating doubled haploid populations of oilseed rape. The S-locus specific marker, allele of class-II SCR gene, and the universal marker, class-I SLG gene, exactly correlated with segregation ratio of self-incompatibility in doubled haploid population. Both marker systems would be used for marker-assisted selection in hybrid oilseed rape breeding. Model of utilization of molecular markers in selection of SI plants in hybrid breeding of oil seed rape was proposed.

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Evaluation of Spanish landraces of pepper cultivated in Extremadura (Spain) under organic agriculture techniques

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ABSTRACT: Twenty five landraces of seven different types of pepper and three commercial cultivars, as controls, were assayed in an open field in "La Orden" (Badajoz). The trial was grown using techniques that conform to legislation in organic agriculture. Under these conditions costs were higher than usual costs under conventional systems because fertilizers applied were more expensive, the trial needed weeding out twice, and many treatments against pests and diseases were carried out to ensurea healthy crop. Good red plus green fruit yield, average fruit weight, good red fruit percentage at harvest were scored and a sensory evaluation was made. Average yield ($t \times ha^{-1}$) measured for each type assayed, between 30.1 in *piquillo* and 61.8 in big fruits having a thick pericarp type, was equal to or higher than yields reported by different authors in modern cultivars under conventional techniques. Most of landraces behaved similar, some of them even better, than controls. Taking into account the agronomic and sensory evaluation results, five landraces were selected: 'BGV-005126' big fruit with thick pericarp type, 'MU-CA-8' italian type, 'BGV-000604' morrón type, 'BGV-005137' piquillo type and 'BGV-010474' hot lenghtened type. These selected materials must be additionally assayed several times to confirm their good performance and, in this case, could be advised for commercial use under organic culture conditions.

Keywords: Agronomy, *Capsicum annum*, germplasm, quality, yield, organic.

Introduction

Pepper (*Capsicum annum* L.) is a vegetable that shows a big intraspecific variability (Nuez et al., 1995). In the Spanish market, the types with big fruits having a thick pericarp are commonly used for fresh consumption: green fruits are usually used directly for salads and red fruits are used for roasting; the italian types, with lengthened fruits which have a relatively thin pericarp, are also used for fresh market being mainly consumed fried. Moreover, in Spain there are some types of pepper intensively used for canning or proccesing: *Piquillo* and *morrón* types are usually roasted, peeled and canned; *bola*, also called *ñora*, and *agridulce de la Vera* types are dehydrated and to obtain *paprika*. Less frequent in Spain, but not rare, are the hot types, used as pickles, spice, etc.

In recent years bred cultivars, hybrids or open-pollinated, are extensively grown to the detriment of landraces. The landraces that still are grown, only in some areas and under open field culture conditions, usually belong to less common types (Gutiérrez et al., 2006). Because of this it is necessary to preserve the landraces in germplasm banks (Hawkes et al., 2000).

In developed countries there is a growing interest for food produced using environment friendly techniques (Russo and Taylor, 2006), and also for sensory high quality foods. This interest would encourage the recovery of some Spanish landraces of pepper, that would be economically suitable under organic culture conditions. This work shows yield and quality results obtained from 25 pepper landraces assayed in 2007 in Extremadura under organic agriculture conditions.

Material and methods

The trial was conducted in an open field in "La Orden" farm, located in the Guadiana Valley (Badajoz, Spain), during the spring and the summer of 2007. Twenty five landraces of pepper of seven different types, and three commercial cultivars were assayed (Table 1).

Plants were produced in trays with truncated pyramidal shaped cells in a greenhouse using as potting media a mixture made of natural peat, 70% blond and 30% black, and compost from sheep 3:1 v/v. The seeding was made on March 7th.

The transplant to the field was made on May 21st. The experimental design was a randomized complete block with three replications. Each elementary plot consisted in a double-row bed 4 m length, double rows 0,3 m apart on bed, plants within rows 0,25 m apart. Distance between centers of contiguous double-row beds in each block was 1,5 m. Before transplanting, compost from sheep was applied to the beds as fertilizer, providing 88, 82, and 61 U.F. × ha^{-1} of N, P_2O_5 and K_2O respectively. Beds were covered with a black biodegradable plastic mulch (1200 × 0,015).

Drip irrigation was applied knowing the ETc needs all the time. Organic liquid fertilizer from sheep was applied through the drip system providing 92 and 52 U.F. × ha^{-1} of N and K_2O respectively.

In spite of the black biodegradable plastic mulch, it was necessary to weed out twice because lots of *Cyperus* ssp. plants punctured and passed through this mulch. Pests and diseases were always controlled using treatments that conform to European, Spanish and regional legislation in organic agriculture. Thus, these treatments were carried out: ten *Bacillus thuringiensis* var. *kurstakii* (Berliner) against *Helicoverpa armigera* (Hübner), six copper treatments to prevent several fungal and bacterial diseases and six sulphur treatments to prevent *Oidiopsis* spp. disease and *Tetranychus urticae* (Koch) pest.

The trial was harvested between August 31st and September 5th. At that period of time, it was estimated that the type with big fruits having a thick pericarp cultivars had 40 % red fruit and the remaining cultivars had more than 60 % red fruit. Good red plus green fruit yield (t × ha^{-1}), average fruit weight (g), obtained from a sample of twenty red fruits, and good red fruit yield percentage on good red plus green fruit yield, were measured. Data of these traits were subjected to analysis of variance assuming block and cultivar as fixed factors. Means also were separated using the Student-Newman-Keuls test ($\propto = 0.05$).

At the same time that trial was harvested, samples of twenty red and green good fruits were sent to wellknown cooks from the province of Badajoz for testing the sensory quality. Each of the cooks tested cultivars of only one type excepting one of them, that tested the *bola*, *agridulce* and hot lengthened types.

Results and discussion

There were no differences in yield between type with big fruits having a thick pericarp landraces and control (Table 1). Average fruit weights of 'BGV-005126', 'BGV-005134' and control were very similar. The differences detected in good red fruit percentage showed differences in precocity between cultivars: 'BGV-005126' and 'BGV-001851' landraces were later than control. The 'BGV-005126' landrace had also the best sensory quality, essentially because the thicker pericap and for being peeled easier than the others.

Between italian type landraces, 'BGV-000673' was different from the rest of cultivars, having the highest yield, the highest average fruit weight and being the latest. In sensory evaluation 'MU-CA-8' landrace was the best marked cultivar, specially because of its sweetness and smooth texture.

There were no differences between the *morrón* type cultivars assayed in yield, average fruit weight and good red fruit percentage. However, the 'BGV-000604' landrace stood out in the sensory evaluation because of its big fruits with thick pericarp.

There were only significant differences for average fruit weight between the *piquillo* cultivars. These differences showed that landraces were earlier than control and they could be harvested once without actual losses in yield. The best sensorily evaluated cultivar, because of its perircarp thickness, sweetness and size and shape uniformity, was 'BGV005137'.

The differences detected in yield between hot lengthened type landraces were partially explained by average fruit weight differences. In the sensory evaluation, the landrace 'BGV-004457' showed more sweetness and smoothness and a more spicy taste than the others.

Bola and *agridulce* types were analysed together because they are both used to produce *paprika*. Results suggested that *Agridulce* type bred landraces were earlier, yielded more and showed better fitness for unique harvest than *bola* type landraces. Average fruit weight of the 'BGV-005118' landrace was significantly higher than the one for the 'Jariza' and 'Jeromín' breeded landraces. Further 'BGV-005118' was significantly later than 'Jariza'. In the sensory evaluation of air dried samples of these landraces, 'Jeromín' stood out against the rest because its intense flavour.

These results showed a really good performance of many landraces under organic culture conditions and most of them overcame the yield of modern cultivars growing in open field under conventional techniques (Nuez et al., 1995; Gutiérrez et al., 2006). However, it must be taken into account that those results were measured in an unique place during only one season, 2007, in which temperatures, lower than usual, probably limited pest expansion. Moreover, despite these good results, it must be consider that costs are higher under organic than under conventional conditions (Russo and Taylor, 2006), as can be seen in the material and methods section.

Table 1. Cultivar, seed donor (Proc.), origin, local name (D. local), type and mean±standard deviation of red + green good fruit yield as t × ha^{-1} (Pr.), average fruit weight as g (PF) and good red fruit percentage over good red + green fruit (% R).

Cultivar	Proc.[1]	Origin[2]	D. local	Type[3]	Pr.	PF	% R
BGV-005126	1	Muchamiel (A)	valenciano	1	59.1±17.7	174±22 a[4]	19±10 cd
BGV-001851	1	Maresme (B)	de Reus	1	49.3±4.9	135±37 ab	2±3 d
BGV-005134	1	Alberique (V)	cuatro cantos	1	69.7±17.2	156±12 a	62±22 a
BGV-001868	1	Bages (B)	pebrot llarg	1	74.9±25.6	120±14 ab	51±7 ab
MU-CA-17	2	Murcia	lamuyo	1	57.4±26.1	94±23 b	36±19 abc
Commercial	3	-	lamuyo	1	60.4±9.1	166±20 a	49±3 ab
Type 1 mean:					61.8±17.7	141±34	37±24
BGV-000677	1	Beires (AL)	italiano	2	28.7±5.0 b	44±1 c	90±2 a
BGV-000673	1	Laujar de Andarax (AL)	italiano	2	68.4±12.4 a	89±6 a	27±12 b
MU-CA-8	2	Murcia	italiano	2	44.2±11.6 b	66±0 b	58±11 a
MU-CA-23	2	Murcia	italiano	2	40.3±2.9 b	44±7 c	75±13 a
Type 2 mean:					45.5±17.4	60±21	63±27
BGV-004031	1	Hervás (CC)	Bola de relleno	3	63.3±23.0	109±18	62±10
BGV-000604	1	Alcalá la Real (J)	morrón	3	46.7±4.1	125±1	50±4
MU-CA-12	2	Murcia	morrón conserva	3	47.3±0.1	103±5	58±12
Commercial	3	-	morrón	3	56.0±9.6	110±1	58±11
Type 3 mean:					53.9±13.4	113±12	57±9
MU-CA-6	2	Murcia	italiano	4	38.5±7.3	28±1 b	80±4
BGV-005137	1	Valencia	Piquillo	4	25.3±4.2	37±3 a	78±7
BGV-004449	1	Pamplona	Piquillo	4	30.6±5.3	37±1 a	75±2
BGV-000676	1	Laujar de Andarax (AL)	Piquillo	4	29.4±8.2	35±2 a	74±8
Commercial	3	-	Piquillo	4	29.7±4.1	38±1 a	66±5
Type 4 mean:					30.1±6.3	35±4	74±7
BGV-010474	1	Oyarzun (G)	guindilla	5	28.9±4.9 c	10±1 b	60±22
BGV-005096	1	Giraba (C)	guindilla	5	43.3±3.3 a	24±5 a	60±10
BGV-004457	1	Bilbao	guindilla	5	33.9±6.3 b	25±3 a	84±4
Type 5 mean:					35.4±7.6	20±8	68±17
BGV-005117	1	Rojales (A)	Bola	6	36.0±5.3	29±4 ab	42±18 b
BGV-005118	1	San Fulgencio (A)	americano	6	34.5±3.9	31±0 a	40±8 b
BGV-005119	1	San Fulgencio (A)	Bola tinta	6	35.3±3.2	27±5 ab	48±1 ab
Jaranda	3	bred landrace (X)	guinda	7	43.7±6.3	24±2 ab	72±10 ab
Jariza	3	bred landrace (X)	guinda	7	39.5±3.1	21±2 b	76±6 a
Jeronín	4	bred landrace (X)	guinda	7	36.6±5.3	22±1 b	71±18 ab
Types 6 and 7 mean:					37.6±5.1	26±4	58±19

[1] 1: Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV); 2:Instituto Murciano de Investigación y Desarrollo Agrario (IMIDA); 3:Commercial cultivar; 4:Centro de Investigación Agraria de la Finca "La Orden-Valdesequera".

[2] A:Alicante; AL:Almería; B:Barcelona; C:Castellón; CC:Cáceres; G:Guipúzcoa; J:Jaén; V:Valencia; X: Cáceres, breed at Centro de Investigación Agraria de la Finca "La Orden-Valdesequera".

[3] 1: big fruit having a thick pericarp; 2: Italian; 3:*morrón*; 4: *piquillo*; 5: hot lengthened; 6: *bola*; 7: *agridulce*.

[4] Mean separation between cultivars within rows by Student-Newman-Keuls Test, $P \leq 0.05$, for each pepper type.

Results allowed us to select the 'BGV-005126', 'MU-CA-8', 'BGV-000604', 'BGV-005137' and 'BGV-010474' landraces. These landraces must be assayed several times and, if their suitability would be confirmed, they could be advised for commercial use under organic culture conditions

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Winter wheat variety and mixture superiority analyses in organic and non-organic farming systems

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ABSTRACT: Breeding, with a particular emphasis on cereals, over the last half century has resulted in significant improvements in the productivity of conventional agriculture. The increases in yield have largely been achieved by an improvement in the agronomic conditions, and a corresponding adaptation of varieties to those conditions (e.g. Ceccarelli, 1996). However these developments depend on the increased use of oil-based inputs in agriculture. Unfortunately, organic farmers are largely constrained to the use of these cereal varieties that have been bred for conventional production systems. In organic conditions, the variability of the environment has a greater influence on yield than the choice of variety (Wolfe et al., 2008). This is the result of the limited buffering capacity of a single variety in relation to the the environmental variability across organic farms. Physical mixtures of complementary varieties often provide an improved ability of a crop to buffer variation in soil, climate and disease and weed pressures. Mixtures can also extend the life of a variety that has useful processing characteristics, but has sub-optimal agronomic performance criteria. This variable success with mixtures is likely to relate firstly, to how much environmental variation mixtures of varieties can buffer, and secondly the heterogeneity of the environment. This paper will describe how a 'superiority analysis' can be used to define the performance and stability of performance of 20 wheat varieties and three physical mixtures for three years on conventional and organic farms. This data provides useful information for farmers and informs the debate regarding the breeding of varieties for organic conditions; should there be separate breeding for organic production and, if so, how would the selection process differ between organic and non-organic farming systems?

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Trends in wheat breeding in Iran

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ABSTRACT: Wheat as the most important crop to man, has experienced spectacular improvements in its yield, quality and architecture during the last few decades (Feil 1992, Slafer and Peltonen-Sainio 2001, Heisey *et al*. 2002). In line with international efforts, Seed and Plant Improvement Institute, Iran, has bred and released 84 wheat cultivars for different climatic regions of Iran. In order to trace the trend lines in Iranian wheat breeding, 21 spring wheat varieties recommended for temperate regions during 1942 and 2007 were evaluated in two successive years. These varieties were cultured under a randomized complete block arrangement with 4 replications at the experimental farm of SPII, at Karaj, Iran. Measurements were made on phonological stages, morphological traits and standard quality parameters such as protein content, sedimentation index, percent gluten, SDS sedimentation, falling number, ect. Results revealed that plant breeding has constantly increased the grain yield through the decrease of plant height and the increase of harvest index. Besides, number of grains per spike has been improved through breeding progress. However, protein content and majority of other quality parameters decreased significantly during the breeding process. Cluster analysis divided the cultivars into three main groups of old, medium and modern varieties. Factor analysis suggested that quality parameters and phonological traits accounted for most of the variations among the cultivars, respectively. Among the traits potentially related to wheat grain yield, many didn't show any trends in the Iranian wheat breeding history, and many of them have not been included in our experiment, which necessitates further investigations.

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Economical evaluation of germplasm exchange received from international agricultural research centers on Iran's irrigated cereal research

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ABSTRACT: This study shows the effects of introducing genotypes received from international research centers on irrigated wheat and barley breeding programs. It also presents the results of yield and acceptance levels of irrigated certified wheat varieties and the economic efficiency of some introduced varieties with international origins in Iran. Economic engineering and beneficial indexes were used to determine the economical efficiency of the varieties (Byerlee and Maya, 1993). Since now, 82 varieties of bread wheat, 3 durum wheat varieties and 18 barley varieties have been introduced by the cereal department of Iran Seed and Plant Improvement Institute (Saidi and Chougan, 2000). Out of these, 31 bread wheat varieties (38%), 3 durum wheat varieties (100%) and 14 barley varieties (78%) were directly received from international research centers. In total, 46.6% of all irrigated cereals (bread wheat, durum wheat and barley) introduced by the department of cereals were germplasm and lines directly received from international research centers. During the 2005 – 2006 cropping cycle, among the introduced wheat varieties, Chamran (Attila) possessed the highest area (593653 ha) in Iran. Of the total area of bread and durum wheat, the acceptance level of some major irrigated wheats with international origins was 52.8%. Due to estimations, for each 1 Rials investment on Tajan, Atrak, Nicknejad and Chamran bread wheat varieties, the profits during 1995 – 2000 were 45.8, 10.1, 2.5 and 18.1 Rials respectively and the investment output rate for these varieties was 137.7%, 91.5%, 55.2% and 192.3% respectively (Asadi, 2003). For introduced irrigated barley varieties, for each 1 Rials investment on Makoee, Dasht, Torkaman and Rihan varieties, the profits during 1991 – 2000 was 34.5, 1.3, 10.4 and 5.2 Rials respectively and the investment output rate for these varieties was 94.8%, 23.4%, 66.4% and 58.6% respectively.

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Possibilities for growing new temperate annual legume crops in Serbia

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ABSTRACT: Annual legumes have a long and successful tradition of cultivation for diverse purposes in Serbia for a long time. The most important grain legume crop in Serbia is soyabean (*Glycine max* (L.) Merr.), while the most significant pulse is common bean (*Phaseolus vulgaris* L.). Pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.) are the most intensively grown for utilisation in animal feeding. Species such as faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.) or grass pea (*Lathyrus sativus* L.) are nearly completely forgotten in Serbia nowadays (Mihailović *et al*., 2005). One of the main goals of breeding programmes on annual legumes in the Institute of Field and Vegetable Crops in Novi Sad is the introduction of new crops. Along with several sub-tropical species, such as pigeon pea (*Cajanus cajan* (L.) Millsp.)) and cowpea (*Vigna unguiculata* (L.) Walp.), the most advanced results were obtained with various temperate legumes, especially with white (*Lupinus albus* L.) and blue lupins (*Lupinus angustifolius* L.), chickpea (*Cicer arietinum* L.) and Narbonne vetch (*Vicia narbonensis* L.). All prebreeding activities of the new temperate annual legume crops are carried out in the form of evaluation of their accessions within the Annual Forage Legume Collection of the Institute of Field and Vegetable Crops (AFLCNS). The most important are evaluation of yield, including forage, grain and biomass yields, as well as evaluation of tolerance to abiotic and biotic stresses. There is a large number of white lupin accessions that gave rather encouraging results when grown on alkaline chernozem soils prevailing in the northern parts of Serbia, often with pH value of more than 7.5. Several white lupin landraces of diverse geographic origin gave average three-year grain yields of more than 6000 kg ha^{-1}, distributed in two or three orders (Ćupina *et al*., 2007). On the other hand, blue lupin proved as highly susceptible to the cultivation on alkaline soils, but also as rather promising for pseudogley and other acid soils in central parts of Serbia, with pH values of less than 5.0. Some blue lupin cultivars of German origin produced the average two-year grain yields of more than 2000 kg ha^{-1} (Eickmeyer *et al*., 2007).

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Breeding of wheat varieties in Tajikistan

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ABSTRACT: Tajikistan has been described as one of the centers of origin and diversity of cereals (Vavilov, 1935). A number of wheat landraces are still being grown by small scale farmers in the mountain regions at altitudes up to 3000 masl. During the Soviet Union main breeding resources went into cotton breeding and as a consequence wheat breeding was not well developed. However, since independence in 1991, both wheat acreage and production in Tajikistan has increased drastically from 120,000-130,000 ha and an annual production of 130,000-140,000 tons in 1990 to 320,100 ha and 640,400 tons in 2007.During the period from 1955 to 1990 Tajik wheat breeders have submitted thirty-four wheat lines to official registration trials of which fifteen has been released. However, due to a number of abiotic and biotic constraints, combined with lack of certified seed and modern crop production technologies, the wheat grain yield in Tajikistan is very low averaging 1,5-1,6 t/ha. One of the main diseases affecting grain yield is smut, but recently the rusts, particularly yellow rust, is becoming a very dangerous disease, significantly decreasing the grain yield. The main objective of the National wheat breeding program is to obtain new wheat varieties with high grain yield, resistance to prevalent diseases and good baking quality. Regional and international collaboration has been established with the objectives of strengthening national breeding programs by germplasm exchange and information sharing. Of high importance has been the linkage with the Turkey-CIMMYT-ICARDA International Winter Wheat Improvement Program (IWWIP) located in Turkey, distributing winter and facultative wheat nurseries to Tajikistan. Recently a joint breeding program has been established with Oklahoma State University in which segregating F2 nurseries from Oklahoma Stare University breeding program are distributed to Tajikistan for further selection. Through the National Wheat Breeding and Seed Multiplication Program initiated by a GTZ/CIMMYT program, and later continued through the Sida funded Seed Industry Development Project, several advanced lines selected by the public and private breeding sector are annually tested in multi-location yield trials, and a number of advanced lines and varieties have been identified as high yielding, rust resistant and with good bread making quality; Kauz, Attila, PYN/BAU, CHAM 6/1D13.1/MLT, GRK//ESDA/LIRA, NWT/3/TAST/SPRW//TAW12399.75, NORKAN//TJB406.892/MON, Zander-12, DYBR1982.83/842ABVD C.50, ALMATY POLUKOVILIK, CTY*3/TA2460, TAM200*3/TA2567, VORONA/TR810200,

TAM200/KAUZ, BAYARAKTAR and 1D13.1/MLT//TUI. Through the program six lines were submitted to variety registration trials, and two of them were released in 2007 under the names Norman and Alex.

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Effect of different crop management and locality on starch and bioethanol production in grain of selected winter wheat varieties

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ABSTRACT: Seven registered winter wheat varieties with potentially high production of grain and starch respectively were selected for evaluation: Barroko (A), Biscay (C), Cubus (A), Florett (C), Ilias (A), Meritto (B) and Rheia (B). Varieties were cultivated in two different localities - Prague Ruzyně and Chrášťany u Rakovníka in two years (2006-2007). There was two soil treatments, conventional (CT) and minimum tillage (MT) and two levels of crop management. The different climatic conditions and localities were the most important factors for variability of starch content and bioethanol extraction. The soil tillage was important only in combination with high or low cultivation intensity. the most yielding varieties with the baking quality "C" confirmed the highest yield and extraction of bioethanol. The crucial factor for high extraction of bioethanol seems to be the content of starch. Higher negative correlation (-0.64) between raw protein content and bioethanol extraction could enable effective prediction of suitable wheat materials with high bioethanol production in breeding process.

Keywords: wheat, starch, bioethanol, crop management

Introduction

In the Czech Republic there are cultivating areas in the range 800ths.-1mil.ha, there spring wheat covers only 5% in a recent 5 year span. Aims of the European Union tend to increasing the proportion of wheat for non-food industry especially their utilization for bioethanol production. The EU - directive No. 2003/30/EC from 8 May 2003 imposed a duty on member states (including the Czech Republic) to make another action to increase proportion of bio-fuels in the market. The Czech government accepted this duty by means of the resolution taken on 6 August 2003 within the frame of its programme: Support of production of bioethanol for its addition to gasoline and diesel oil"(Lipavský and Váňa, 2007). From January 2009 this proportion should increase to 4.5% and a year later to 5.75%. Nowadays consumption of bio-fuels in the Czech Republic only accounts for about 0.2%, in adjacent Germany it is about 3.5%. According to EU bio-fuels should form nearly one fifth of consumption of classic fuels in 2020 (Alterová, 2007).

A base on facts mentioned above shows that the interest of wheat cultivation for non-food utilization significantly increases. The advantage of wheat is its high yield stability

and long- term storage ability. Nevertheless, no variety has been registered for production of starch, or bioethanol in the Czech Republic yet and there are no clearly defined breeding criteria for this category of wheat. It is assumed that research and selection varieties and convenient technologies can eminently improve production of starch from winter wheat (Novotný, 2006).

The aim of our study focused on evaluation of bioethanol production in selected modern wheat cultivars cultivated in different localities and by different intensity of agricultural practices.

Materials and Methods

Seven registered winter wheat varieties with potentially high production of grain and starch respectively were selected for evaluation: Barroko (A), Biscay (C), Cubus (A), Florett (C), Ilias (A), Meritto (B) and Rheia (B). The letters behind variety names indicate the level of baking quality.

Varieties were cultivated in two different localities: Prague Ruzyně and Chrášťany u Rakovníka in 2006 and 2007. There was a combination of conventional (CT) and minimal tillage (MT) and two levels of crop management. (Tab.1)

Starch content was measured according to Ewers polarimetric method (ČSN EN ISO 10520). The fermenting test was based on the evaluation of the density of fermenting (?) before and after fermentation (Evans et al., 2003). The content of amylose was detected by enzymatic method (Amylose/Amylopectin assay kit) according to the company protocol (Megazyme, 2006).

The following grain parameters were tested: content of crude protein – Kjeldahl method (ČSN EN ISO 5983-1); Zeleny sedimentation test (ČSN ISO 5529); wet gluten content (WG) and gluten index (GI) – Glutomatic 2200 (AACC 38-12).

The software "Statistica 7.0 CZ" was used to test significant differences by ANOVA/MANOVA, Tukey HSD test, multiple step regression, correlation matrix and PCA (Principle Component Analysis).

Table 1. Characteristics of crop management.

CT = ("conventional tillage" = to 22 cm)
MT = ("minimum tillage" = to 10 cm, part of postharvest residues after winter rape on the surface
„1" = lower cultivation intensity = Ruzyně 100 kg N.ha^{-1} (30 + 40 + 30 kg N.ha^{-1}), Chrášťany 110 kg N.ha^{-1} (30+40+40 kg N.ha^{-1}) without fungicides and growth regulators
„2" = higher cultivation intensity = Ruzyně 150 kg N.ha^{-1} (60 + 60 + 30 kg N.ha^{-1}), Chrášťany 160 kg N.ha^{-1} (60+60+40 kg N.ha^{-1}), fungicides applied twice and growth regulator applied once in both localities

Results and Discussion

Our two-year results confirmed statistical significance of all monitored factors (year, locality, variety and crop management) to variability of yield, starch content, and extraction of bioethanol respectively (Tab.2). PCA loading scatter clearly showed significantly higher effect of year and locality on the tested variability of parameters than the effect of variety and crop management, which are situated in the middle of the graph (Graph 1).

Analysis of variance as well as the PCA loading scatter confirmed significantly higher yield, starch content and extraction of bioethanol in Ruzyně in comparison with drier environment of Chrášťany (Tab.2; Graph 1). as for the year a significantly higher extraction of bioethanol was observed in 2007 in spite of slightly lower content of starch. The lower effectiveness of fermentative process in 2006 could be caused by many factors which negatively influenced physiological state of yeasts (e.g. health condition of grain, fungi, bacteria, mycotoxins et.al) (Dudáš and Pelikán 1989, Charlene and Wolf-Hall 2007). On the other hand the year 2006 confirmed significantly higher yield of grain as well as yield of bioethanol from the experimental field (Tab.2). Within applied crop management it is possible to emphasise the cultivation intensity that combined with both soil tillage (CT, MT), which on the one hand increased the yield of grain but on the other hand decreased the starch content and bioethanol extraction of the grain. Statistically significant higher total yield of bioethanol from the experimental field was achieved by combination of both technologies with higher cultivation intensity (CT2, MT2) in spite of the above mentioned lower extraction of bioethanol from the grain. The variety aspect confirmed the highest yield in varieties with baking quality "C" (Florett – 7.59 $t.ha^{-1}$ and Biscay – 8.01 $t.ha^{-1}$). Thus, the decision of the correct level of crop management will mainly depend on total profitability of the obtained production.

In consequence of the above mentioned high year and locality effect on monitored characteristics a transformation of all data was made by achieved average parameter values within a specific year and locality. It enabled us to evaluate the effect of varieties and technologies and mutual parameter relations more correctly (Graph 2). thus the relationship of both ploughing systems with lower cultivation intensity to higher starch content and extraction of bioethanol, respectively, is obvious. On the contrary both ways of soil tillage with higher cultivation intensity positively influenced the grain yield and thus, the total yield of bioethanol from the experimental field.

In terms of varieties it is possible to emphasize the variety Biscay (C), which (also in this statistical evaluation) confirmed its relation to higher starch content, extraction and yield of bioethanol. While the varieties Rheia (B) and Barroko (A) showed lower suitability for these applications. Correlation analysis confirmed significant effect of starch content on extraction (0.60) and yield (0.39) of bioethanol (Tab. 3). Only a low but statistically significant effect was registered between amylosa content and extraction of bioethanol (-0.22). From other additional characteristics was detected a significant negative correlation between extraction of bioethanol and protein content (-0.64), and Zeleny sedimentation (-0.24) respectively.

Based on multiple regression the following equation with high statistical significance at $p \leq 0.01$, correlation coefficient $r = 0.74$ and error of estimation 0.0111 of bioethanol extraction per 100kg of grain was obtained:

Extraction of Bioethanol = 0.832-0.093***raw protein**+0.274***starch-**0.011***GI**+0.017***Zeleny**+0.017*yield of grain -0.035*amylosa (The parameters with partial statistic significance at $p \leq 0.05$ are boldly emphasized)

Conclusion

The obtained two-year results showed that from the point of view of variability of starch content, bioethanol extraction and yield, variable climatic conditions and locality are the most important factors. The soil tillage was important only in combination with high or low cultivation intensity. The two most yielding varieties with the baking quality "C" confirmed the highest yield and extraction of bioethanol. The crucial factor for high extraction of bioethanol seems to be the content of starch. Higher negative correlation (-0.64) between row protein content and bioethanol extraction could enable effective prediction of suitable wheat materials with high bioethanol production in the breeding process.

Table 2. Two-year averages of obtained grain parameters (2006-2007).

Factors		Yield of grain ($t.ha^{-1}$)	Starch content (%)	Amylosa content (%)	Extraction of bioethanol ($l.100kg^{-1}$ of groats)	Yield of bioethanol ($l.ha^{-1}$)
Variety	Barroko (A)	6.73^{cd}	64.68^{b}	26.61^{a}	41.11^{bc}	2776.03^{c}
Variety	Biscay (C)	8.01^{b}	66.94^{c}	26.19^{a}	42.08^{a}	3378.37^{b}
Variety	Cubus (A)	7.35^{abd}	66.98^{c}	26.99^{a}	41.88^{a}	3091.44^{ab}
Variety	Florett (C)	7.59^{ab}	65.81^{b}	26.75^{a}	41.54^{abc}	3162.92^{ab}
Variety	Ilias (E-A)	7.08^{acd}	65.85^{b}	25.92^{a}	41.54^{abc}	2948.09^{ac}
Variety	Meritto (B)	7.49^{ab}	65.50^{ab}	27.15^{a}	41.59^{ac}	3119.50^{ab}
Variety	Rheia (B)	6.57^{c}	64.91^{ab}	27.03^{a}	40.96^{b}	2700.64^{c}
Locality	Ruzyně	8.75^{b}	66.15^{b}	26.68^{a}	42.25^{b}	3693.78^{b}
Locality	Chrášťany	5.77^{a}	65.47^{a}	26.65^{a}	40.81^{a}	2356.79^{a}
Technology	CT1	6.94^{a}	66.48^{c}	26.30^{a}	41.81^{c}	2917.51^{a}
Technology	CT2	7.35^{ab}	65.12^{a}	26.84^{a}	41.16^{a}	3041.22^{ab}
Technology	MT1	7.03^{a}	66.13^{bc}	26.76^{a}	41.75^{bc}	2946.05^{a}
Technology	MT2	7.71^{b}	65.51^{ab}	26.74^{a}	41.39^{ab}	3196.35^{b}
Year	2006	7.80^{b}	66.84^{b}	24.44^{a}	41.16^{a}	3218.48^{b}
Year	2007	6.71^{a}	64.78^{a}	28.88^{b}	41.90^{b}	2832.09^{a}

Values with different letters are statistically significant at $p \leq 0.05$

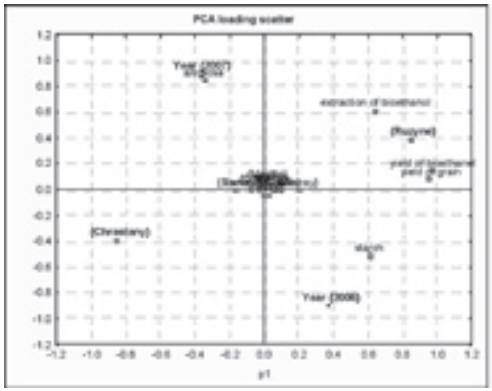

Figure 1. Effect of all factors on evaluated grain characteristics and their mutual relations

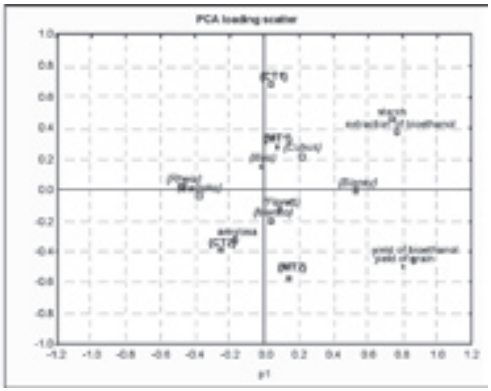

Figure 2. Effect of crop management on evaluated grain characteristics and their mutual relations (transformed data)

Table 3. Correlation matrix of tested grain characteristics after transformation of data.

Grain Parameters	Starch (%)	Amylosa (%)	Bioethanol extraction (l.100kg^{-1} of groats)	Yield of grain (t.ha^{-1})	Yield of bioethanol (l.ha^{-1})	Raw protein (%)	Wet gluten (%)	GI	Zeleny sedimentation (ml)
Starch (%)	1.00								
Amylosa (%)	-0.12	1.00							
Bioethanol extraction (l.100kg^{-1} of groats)	0.60	-0.22	1.00						
Yield of grain (t.ha^{-1})	0.35	0.00	0.41	1.00					
Yield of bioethanol (l.ha^{-1})	0.39	-0.02	0.50	0.99	1.00				
Raw Protein (%)	-0.55	0.21	-0.64	-0.44	-0.48	1.00			
Wet gluten (%)	-0.23	0.01	-0.20	-0.18	-0.19	0.27	1.00		
GI	0.00	0.01	-0.11	-0.02	-0.04	-0.01	-0.40	1.00	
Zeleny sedimentation (ml)	-0.25	0.13	-0.24	-0.24	-0.26	0.37	-0.19	0.63	1.00

Statistically significant values are boldly emphasized

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New cultivars obtained at Cluj-Napoca, Romania: Saruman and Sauron (apple); Arvena (pear)

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ABSTRACT: Apple and pear breeding at Cluj-Napoca, Romania, started in 1953 when Fruit Research Station was founded. The most well-known and spread cultivars of apple created in Cluj were Aromat de vara, Ardelean, Ancuta, Feleac and Rosu de Cluj. In 2005, four new apple cultivars were registered: Auriu de Cluj, Estival, Precoce de Ardeal, Productiv de Cluj. On pear, in addition to Napoca, Doina, Haydeea cultivars, new ones are Ina Estival (1999), Virgiliu Hibernal (2000), Jubileu 50 and Milenium (2003), Rosioara de Cluj (2005). In 2007, the new cultivars Saruman and Sauron (apple) and Arvena (pear) were released, obtained at FRS Cluj-Napoca (Ropan et al., 2002; Sestras et al., 2006, 2007). The apple cultivar Saruman was released by hybridization between Cluj III-VI-5-26 selection (Parmain d'Or, open pollinated) and NJ 46. The trees have a moderate vigour, spreading shape, medium crop yield. The fruits have large size, conic shape and mostly red (purple) coloration; they have white flesh with a sweet, crisp, aromatic flavour and low acidity. The apple cultivar Sauron was identified in open pollinated population of Cluj 3/83 selection [Cluj III-VI-5-26 selection (Parmain d'Or, open pollinated) x NJ 46]. The trees have moderate vigour, upright then spread, with moderate productivity. Fruits are medium to large, usually red, with a portion being greenish or yellow-green, and purple red vertically striped. The fruit has good quality, being soft eating apple due to their lack of crispness. Quality indices include peculiarities as firmness, crispness and excellent flavour. Both Saruman and Sauron fruits become ripe in the two decade of August, and the fruits are proper for dessert and well suited for cooking, applesauce, cider, pies etc. The pear cultivar Arvena was created by selection in F_1 hybrids derived from Triomphe de Vienne, open pollinated. Trees are weak to medium vigour, with moderate-strong branching and semi-upright habit being very productive. Fruit size is medium to large, with soft firmness and juicy flesh; the colour changes from green to yellow attractive upon maturity, copper-coloured. Time of maturity for consumption is the first or second decades of September, when the fresh fruit has a good quality rating. The new cultivars obtained at FRS Cluj-Napoca, Romania, enrich the international germplasm repository, representing useful genetic resources, which could be used for apple and pear breeding programs in the future.

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