Molecular Tools for Genebank Management and Evaluation

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Abstract: Molecular markers were developed for many species and enabled us to use them for the characterisation of genebank collections. We used the marker technology (microsatellites) for studying the genetic integrity of the self pollinating species wheat (*Triticum aestivum* L.) and the open pollinating species rye (*Secale cereale* L.). The study became possible, because at IPK both the *ex situ* collection, consisting of seeds from the most recent regeneration and a herbarium collection is maintained. In the herbarium collection from each accession samples of grains and complete spikes are deposited as vouchers when they are grown initially. For the wheat accessions investigated the comparison of the DNA fingerprints showed a high degree of idendity. No contamination due to foreign pollen or incorrect handling during the multiplication cycles was discovered. For the open pollinating species rye, however, major changes in allele frequencies were detected. Overall, nearly 50% of the alleles discovered in the original sample were not found in the material present in the *ex situ* collection now. In some cases alleles were detected in the most recently propagated subpopulations that were not observed in the investigated plants of the original one. In addition to the integrity studies we are in process of utilizing molecular markers for a marker assisted screening of genebank collections. Salt tolerance of barley has been shown as a case study in the present article.

Keywords: genetic integrity; evaluation; self pollinating crops; open pollinating crops; abiotic stress; molecular markers

It is estimated that world-wide existing *ex situ* collections contain approximately 6 million accessions of plant genetic resources. In the mean time, this number may have been increased even further. Over 40% of all accessions in genebanks are cereals, followed by food legumes constituting about 15%. Vegetables, roots and tubers, fruits and forages each account for less than 10% (FAO 1998). In the Gatersleben genebank about 150 000 accessions, including cereals, legumes, vegetables, oil and fibre plants, medicinal herbs, spice plants, forages and tubers (potatoes), are maintained. An overview on accession numbers of selected crops maintained in Gatersleben as well as in seedbanks world-wide are given in Table 1.

Depending on the storage conditions and the frequency of providing genebank material to users regeneration becomes necessary. For that different procedures have to be applied regarding to the pollination systems of the particular crops. Especially open pollinating species need extended efforts in order to maintain the genetic integrity of the germplasm accessions. However, a contamination by foreign pollen or incorrect handling during multiplication may affect the genetic identity of self pollinating species as well.

In the first part of this publication, we are presenting data about the integrity of germplasm maintained in the Gatersleben collection. Randomly selected accessions of one self pollinating (*Triticum aestivum* L.) and one open pollinating species (*Secale cereale* L.) were investigated by employing molecular markers. We compared seed samples stored in the cold storage and originated from the most recent regeneration, with samples of grains maintained as reference (herbarium) collection, deposited as vouchers when the accessions are grown initially. Although the samples are stored at room temperature

Table 1. Germplasm accessions of selected crops stored in genebanks world-wide (FAO 1998) and at the IPK Gatersleben (Annual Report IPK 2004). Crops are divided into self and open pollinators regarding to the dominating pollination system

Crop	Genus	Accession numbers	
		Global	IPK Gatersleben
Self pollinating crops			
Wheat	*Triticum*	788 654	28 191
Barley	*Hordeum*	486 724	21 244
Bean	*Phaseolus*	268 369	8 640
Tomato	*Lycopersicum*	78 376	4 043
Pea	*Pisum*	75 288	5 633
Pepper	*Capsicum*	53 558	1 525
Flax	*Linum*	24 879	2 338
Open pollinating crops			
Maize	*Zea*	261 584	1 660
Cabbage	*Brassica*	106 923	4 718
Faba bean	*Vicia*	31 831	3 392
Rye	*Secale*	27 132	2 461
Onion	*Allium*	25 288	1 545
Beet	*Beta*	24 085	2 509
Goat grass	*Aegilops*	21 360	1 537

and, therefore, have lost their germinability it is still possible to extract DNA for comparative studies.

A second aspect of this article is the characterization of large germplasm collections. Infact, the efficiency of exploitation of the germplasm depends on the quality of characterisation. However, phenotypic screening is time consuming and done only sporadic. To make screening feasible for large collections, we wanted to develop molecular marker(s) in order to conduct marker assisted screening. Data are presented using the abiotic stress salt tolerance in barley as a case study.

Table 2. Details on geographical origins, multiplications and regeneration frequencies of the wheat and rye accessions analysed

Species	Accession number	Geographical origin	Years of first and last multiplication	Regeneration frequency
T. aestivum L.	TRI 11742	Pakistan	1978, 1997	5
	TRI 12922	China	1979, 1992	6
	TRI 249	Germany	1946, 1995	11
	TRI 2292	Greece	1952, 1995	11
	TRI 4599	Albania	1952, 1996	15
	TRI 3342	China	1951, 1995	16
	TRI 1634	Albania	1948, 1996	17
	TRI 2519	Tibet	1950, 1996	24
S. cereale L.	R 793	Germany	1988, 1995	2
	R 784	Spain	1986, 1996	3
	R 52	Austria	1963, 1998	8
	R 200	Germany	1954, 1993	12
	R 78	Germany	1954, 1993	12
	R 197	Italy	1954, 1993	14

Table 3. Wheat (GWM) and rye (RMS) microsatellite markers used and their chromosomal location

Species	Microsatellite marker	Chromosome location
T. aestivum L.	GWM 3	3D
	GWM 186	5A
	GWM 261	2D
	GWM 357	1A
	GWM 437	7D
	GWM 445	2A
	GWM 619	2B
	GWM 631	7A
	GWM 680	6B
S. cereale L.	RMS 7	4RL
	RMS 10	1R
	RMS 12	7RS
	RMS 18	7RL
	RMS 20	7RL
	RMS 28	3RL
	RMS 104	6R
	RMS 107	1RL
	RMS 115	5RL
	RMS 121	6RL

Studies on the genetic integrity

Self pollinating species. From the Gatersleben wheat (*Triticum aestivum* L.) collection randomly selected accessions differing in their frequency of multiplication were investigated. Details on geographical origins, years of first and latest multiplication and regeneration frequencies of the material investigated are given in Table 2. Five grains of each accession derived from the first and last regeneration cycle were pooled for DNA extraction according to the procedure described by Plaschke *et al*. (1995). Nine Gatersleben wheat microsatellites (GWM) with different chromosomal location were chosen for analysis (Table 3). Details of the GWM used (fragment sizes, annealing temperatures for PCR) are described by Börner *et al*. (2000). PCR reactions and fragments detection were performed as described by Röder *et al*. (1995, 1998).

Comparing the genetic fingerprints of the stocks multiplied up to 24 times, a high degree of identity was detected (Börner *et al*. 2000). No contamination due to cross pollination or erroneous handling during harvesting, threshing or labelling was detected. For one accession (TRI 4599), however, genetic drift occurred. For three GWM markers, two alleles

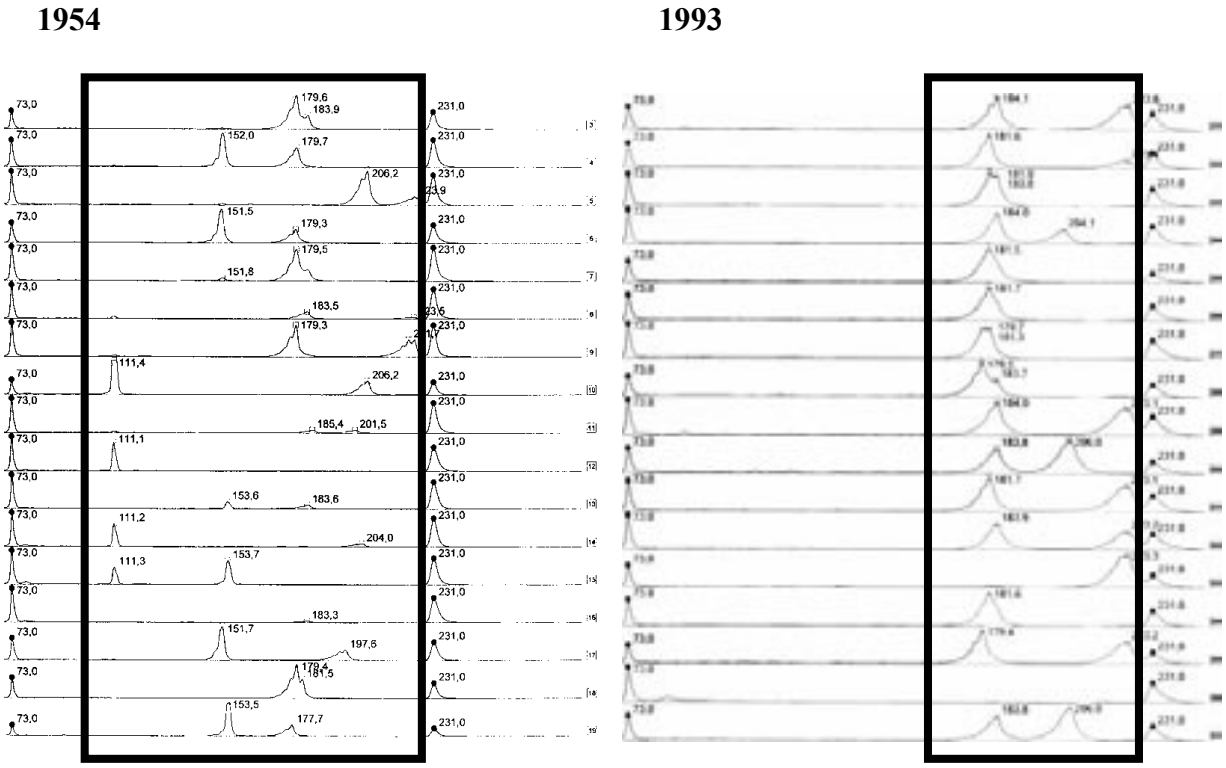

Fig. 1. PCR-amplified fragments from the primer RMS12 for accession R78, originated from regenerations of 1954 (left) and 1993 (right)

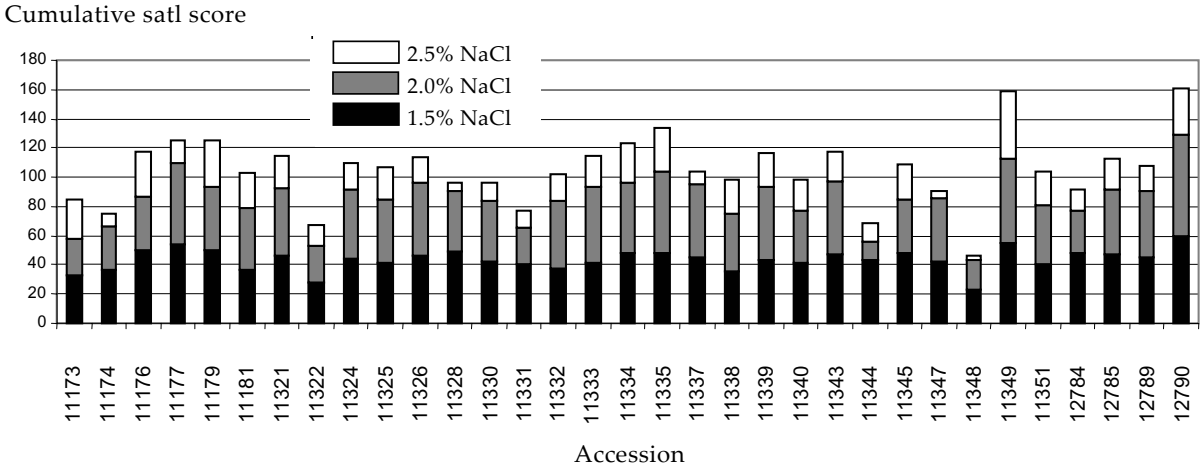

Fig. 2. Results of a screening of 33 barley accessions of the Gatersleben genebank for salt tolerance. The salt score shown here is cumulative for different salt concentrations (1.5%, 2.0%, 2.5%) tested. Higher cumulative values for a genotype suggest higher tolerance for salt

were detected in the bulk of the original seeds, whereas in the most recent sample only one allele remained. In another accession (TRI 249) a heterogenous situation for two markers was maintained over the years. Summarising the results a high quality of maintaining self-pollinating genebank accessions in Gatersleben for more than 50 years is demonstrated.

Open pollinating species. From the Gatersleben rye (*Secale cereale* L.) collection six accessions were analysed, regenerated 2, 3, 8, 12 (twice) or 14 times. Details on geographical origins, the years of the first and most recent regeneration and the regeneration frequencies are given in Table 2. Since the rye accessions represent populations, 36 and 60 single grains from the first and most recent regeneration cycle, respectively, of each accession were used for extracting DNA (Plaschke *et al*. 1995). Seven rye microsatellites (RMS) listed and described in Table 3 were chosen for analysis (for details see Röder *et al*. 1995, 1998; Chebotar *et al*. 2003). Allele frequencies of microsatellite markers (loci) were calculated for each sub-population separately.

The results clearly indicated major changes in the genetic integrity of the open pollinating species rye maintained for up to 45 years in an *ex situ* genebank (Chebotar *et al*. 2003). Four out of the six accessions investigated showed significantly different allele frequencies after having been multiplied between 7 and 13 times. One example of such cases is shown in Figure 1. From the 242 alleles discovered in total in the original samples of that four accessions 118 (nearly 50%) were not found in the material stored today. On the other hand, 26 alleles were detected in the sub-populations regenerated recently, which were not observed in the investigated plants of the first harvest.

Reasons for loosing alleles may be, that the population sizes used for regeneration of the material were too small or decreased by damage during periods of environmental stress like e.g. frost. New alleles may be due to contamination with foreign pollen. It, therefore, may be suggested that genetic changes occure in other rye accessions as well and, most probably, also in accessions of other cross pollinating species maintained in *ex situ* genebanks.

Marker assisted evaluation of genebank collections

One of the target traits considered for evaluation in the Gatersleben genebank is the salt tolerance of cereal crops. Using a germination test described by Mano *et al*. (1996) screenings of the wheat and barley collections were initiated. Three different concentrations of sodium chloride (NaCl) solution (1%, 1.5%, 2% for wheat and 1.5%, 2%, 2.5% for barley, respectively) and distilled water as control were used to screen for salt tolerance (Weidner *et al*. 2005). From each accession ten seeds per treatment were germinated on filter paper in plastic boxes. Germination tests were carried out in climatic chambers with a constant temperature of 20°C and a 12 h light/12 h dark photoperiod. Salt tolerance was scored after ten days, using a

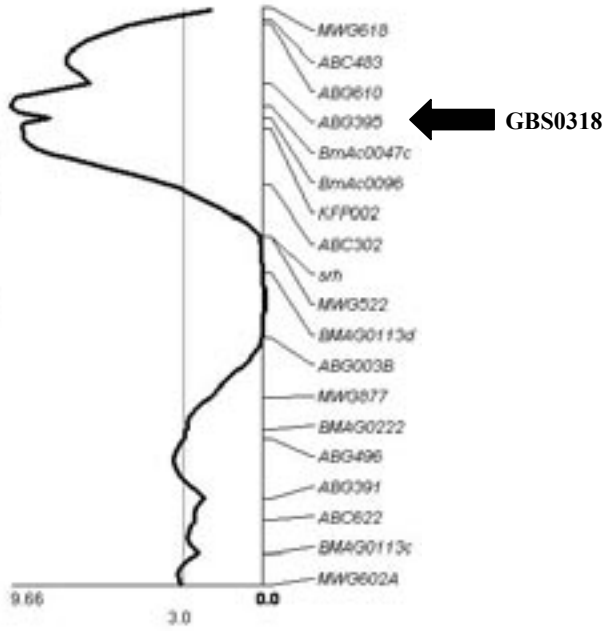

Fig. 3. Molecular linkage map of chromosome 5H showing the position of a major QTL for salt tolerance, linked to the functional molecular marker GBS0318

modified scale of Mano *et al*. (1996) from 1 to 9. Results obtained from a screening of 33 barley accessions are given in Figure 2. With the capacity available at the moment in our genebank we are able to screen 84 accessions within 10 days. Considering the 21,244 barley accessions maintained in Gatersleben we would need more than 7 years to handle the whole collection.

In order to make the screening more efficient we were interested to find molecular markers for performing a marker assisted screening. A 'model population', the 'Oregon Wolfe Barley' (OWB) mapping population (Costa *et al*. 2001) was phenotyped applying the seedlings test described above. A quantitative trait locus (QTL) analysis of the OWB mapping population revealed a major QTL on linkage group 5H, contributing up to 42% of the phenotypic variation for salt tolerance in different replications of different salt concentrations and two minor QTLs on 2H and 7H, respectively. For the OWB population a functional molecular marker map comprising of > 500 EST-based markers was developed as a part of the preparation of the transcript map at IPK Gatersleben (A. Graner, person. commun.; Varshney *et al*. 2005). Using this data the marker GBS0318 (function = hypersensitive induced reaction pro-tein 3) was found to be associated with a major QTL on chromosome 5H (Figure 3).

Subsequently, GBS0318 was used for screening about 100 accessions collected from Pakistan, Tunisia, Libya and Afghanistan to conduct the marker validation. Although about 60% of the salt tolerant accessions could be identified by using the GBS0318 marker, some (~30%) susceptible accessions also showed the tolerance allele of that candidate marker (black color). Screening of the genotypes with other candidate molecular markers (linked to the minor QTLs) as well as the utilization of other mapping populations (Igri × Franka; Steptoe × Morex) for which we have the functional molecular maps too are underway in order to identify perfect molecular marker(s) for characterising genebank collections.

The present study, therefore, underlines the utility of molecular markers in both management as well as characterization of germplasm available in genebanks. However, it is important to note that allelic stability for cross pollinating species like rye is in dynamic phase, while the genetic integrity in self pollinating species like wheat is maintained during more than 50 years of their storage in genebank. Molecular marker resources available, at present, in large number especially for cereal species (Varshney *et al*. 2004) provides the opportunites for facilitating the systematic characterization of genebank material especially cereal species, as demonstrated here for salt tolerance in barley.

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