

# eucarpia

XIII EUCARPIA Biometrics in Plant Breeding Section Meeting

30 August :: 1 September 2006

Zagreb :: Croatia

## Book of Abstracts



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XIII EUCARPIA Biometrics in Plant Breeding Section Meeting  
30 August - 1 September 2006 :: Zagreb :: Croatia

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Dear Colleagues,

Organizing Committee welcomes you to the XIII meeting of the EUCARPIA Biometrics in Plant Breeding Section, in Zagreb, Croatia.

It was a great pleasure to find out that joint effort of Scientific and Organizing Committee invested in preparation of the meeting yielded widespread interest. Therefore, we expect more than 100 participants from 30 countries. We have received abstracts of 66 contributions in addition to 7 invited lectures. All the abstracts have been thoroughly reviewed by the members of Scientific Committee and hereby we thank them for their time and efforts. They have selected 22 abstracts for oral presentations, while remainder will be presented as posters. Furthermore, 11 posters were selected for short (oral) presentations. As an outcome, we can expect that the Meeting will further contribute to the development of biometrical methods and models to be used in plant breeding and related plant sciences.

The meeting is hosted by Faculty of Agriculture of the University of Zagreb. Its Department of Plant Breeding, Genetics and Biometrics was founded in 1920. The first textbook for the course of Biometrics was published back in 1946, written by Alois Tavčar who was the Head of the Department for more than 50 years.

This Book of Abstracts has been published as a supplement to the journal *Agriculturae Conspectus Scientificus* (ACS), the oldest and the most prominent journal in the field of agriculture in Croatia. ACS publishes original scientific papers, scientific reviews and preliminary communications in the field of agricultural and related sciences. The journal is fully available at [www.agr.hr/smotra](http://www.agr.hr/smotra).

And last but not least, the Organizing Committee greatly appreciates support from the sponsors. Without their help the task of organizing this meeting would become extremely taunting.

We wish you all a very fruitful meeting and the pleasant stay in Zagreb.

Jerko Gunjača

On Behalf of the Organizing Committee

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# A Ruby in the Rubbish: Searching for Signatures of Selection Using Molecular Data

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Bruce WALSH

## Abstract

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Over the last dozen or so years, population geneticists have actively been developing tests for signatures of selection using molecular data. These tests fall into two broad categories. Tests of very recent (or ongoing) selection typically rely on within- and between-population data on the level of molecular polymorphisms around loci of interest. Tests for historical (i.e., over evolutionary time) selection involve wide between-species comparisons focusing on rates of substitution, in particular the synonymous vs. non-synonymous ratio. These tests have much to offer breeders, allowing for a retrospective analysis of the actual genomic regions that change as a result of artificial selection. They also offer a tool for screening natural populations of loci of interest, and potential introgression, into domesticated crops.

## Keywords

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selective sweeps, neutral theory, detecting selection

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# Linkage Analysis and QTL Mapping in Blackcurrant (*Ribes nigrum* L.) Using a Population of Full Sib and Selfed Offspring

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Linzi JORGENSEN<sup>2</sup>

Joanne RUSSELL<sup>2</sup>

## Abstract

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Blackcurrant (*Ribes nigrum* L.) is an important fruit crop in Europe, grown mainly for juice production. A cross was made between two SCRI breeding lines, and AFLP and SSR markers were scored on these and 125 progeny. Analysis of this marker data indicated that 43 of the progeny were almost certainly selfs, rather than from the intended cross.

A problem with linkage analysis in a full sib population from heterozygous parents is that the information about the recombination frequency varies with the segregation ratio of the markers, especially for dominant markers. Markers present in one parent but absent in the other segregate in a 1:1 ratio and are highly informative for constructing a linkage map for that parent. However, to bridge the maps for the two parents requires the calculation of a recombination frequency between a marker segregating in a 1:1 ratio and one segregating in a 3:1 ratio, and this type has very low information content. In the selfed population, all of these markers should segregate in a 3:1 ratio, which is highly informative when the markers are linked in coupling. The linkage map was therefore improved by combining weighted estimates of recombination frequencies from the selfed and full sib offspring.

Markers on two chromosomes showed unexpected segregation ratios in the selfs, and these can be explained by a hypothesis of two genes affecting viability, with different genetic models. An analysis of the quantitative trait hundred berry weight showed a significant difference between the selfed and full sib offspring, and that differences within these population appeared to be determined by QTLs at or very close to the locations of the two viability genes.

## Keywords

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blackcurrant, linkage, recombination frequency, information, QTL

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# Development of LD Mapping System for *Oryza sativa* L. Germplasm

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## Abstract

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Although there is a broad range of phenotypic variations in indigenous varieties of *Oryza sativa* L., only a part of the variations has been used in the development of commercial varieties. To use these variations more actively in breeding programs, it is necessary to establish efficient systems for searching and identifying key genes involved in the variations. Now, we are developing (1) *O. sativa* germplasm SNP/indel database covering whole genome and (2) linkage disequilibrium (LD) mapping system testing association between DNA polymorphisms and traits. SNP/indel polymorphisms are mapped to absolute locations on the rice whole-genome sequences and accumulated in the database. The LD mapping system developed as a Web service enables users to analyze latest SNP/indel data. In our system, to avoid false association due to cryptic population structure, the DNA polymorphisms-traits associations are investigated by a general linear model including each SNP/indel polymorphism and the mixed ancestries estimated by the Bayesian clustering as independent variables. The significance of the association is examined on *F*-test, generalized cross-validation and AIC, comparing a full model with a reduced model excluding the SNP/indel term. By using our system, breeders and researchers can perform whole-genome LD mapping easily, with just preparing a spreadsheet in which observed phenotypic values of the germplasm are recorded.

## Keywords

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*Oryza sativa* L., germplasm, SNP/indel database, LD mapping, web-service

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# Adjusting for Population Structure in Genetic Association Studies

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David BALDING

## Abstract

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The potential for false positives due to population structure has been a major topic of concern for those designing and implementing genetic association studies. In humans, it is plausible that any such effects are weak and may be negligible; this in a sense makes matters worse because possible effects can never be ruled out yet these are difficult to measure and hence adjust for. In plant populations, variation in the distributions of both genetic alleles and phenotypes with geography is often marked, and thus must be addressed if genetic association mapping strategies are to succeed in these populations. However, for both humans and plants this variation may not be well-characterised by a model of partially isolated subpopulations, which forms the basis for most discussions of the problem.

I will discuss the nature of the problem of spurious associations due to demography, and attempts to adjust for this that have been applied in human genetic association studies, including genomic control, structured association and other approaches. With the advent of a genome-wide association studies involving hundreds of thousands of markers, the possibility for an almost complete solution to the problem emerges, but this rests on a new understanding of the nature of the problem.

## Keywords

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genetic association studies, linkage disequilibrium, population stratification, spurious associations, statistics

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# Association Mapping of Key Traits in UK Barley Recommended List Trials

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David F. MARSHALL

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William (Bill) T.B. THOMAS (✉)

## Abstract

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Barley breeding in the UK is very competitive with over 35 spring and winter barley entries, derived from many European as well as UK breeding programmes, submitted to National List Trials yearly. The pedigrees of recommended cultivars clearly demonstrate that commercial breeders are achieving this progress by working within the elite gene pool. Marker Assisted Selection (MAS) has not been widely deployed by commercial breeders. We have used the Recommended List Trial (RLT) yield and Hot Water Extract (HWE) data collected over the period 1993 to 2003 to highlight the advances that are being made through conventional phenotypic selection as this sets the standard that MAS must be judged against.

Yield ( $\text{t ha}^{-1}$ ) data from a total of 228 spring and 237 winter trials grown since 1993 was available together with a more restricted set of malting quality data. From each site, the mean grain nitrogen (GN) content and hot water extract (HWE) were used to derive a hot water extract corrected to 1.5% grain nitrogen content (HWEc) using the formula  $\text{HWEc} = \text{HWE} + 11(\text{GN} - 1.5)$  as this gives a more accurate representation of varietal performance over contrasting seasons. These data were used to estimate overall mean plot yields (PY) and HWEc for each entry. For both spring and winter barley, significant improvements in yield potential were apparent over time. The same was true for HWEc in spring barley but no significant progress over the period was apparent for winter barley.

Genotypic data from 48 SSR markers was used to detect significant associations of 7 SSR loci with HWEc in winter barley. Pedigree analysis of the UK-bred winter cultivars reveals that they are all derived from Maris Otter which was introduced in 1965 and they exhibit considerable similarity in their alleles at the seven significant SSR loci. By contrast, two other ancestors in the pedigrees of the UK cultivars share few alleles at these loci and also possess at least one allele with a marked detrimental effect upon HWEc. These data suggest that several of the seven SSR loci associated with HWEc could be used in MAS for assembling an elite malting quality gene pool and then using phenotypic selection to pick out the best. Intriguingly, none of the cultivars possesses the best allele at all seven loci. Assuming that effects are estimated accurately and the loci act in an additive manner, assembling optimal alleles at all seven loci would increase HWEc by nearly 2 Lintner°  $\text{kg}^{-1}$ .

We thank the Home Grown Cereals Authority of Great Britain and Crop Evaluation Limited for use of data collected on the HGCA-funded UK spring and winter barley RLTs.

## Keywords

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Barley, Association Genetics, Yield, Quality, Marker assisted selection

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## Association Mapping in Potato (*Solanum tuberosum* L.)

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### Abstract

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Quality demands on current potato cultivars are stringent and therefore breeding companies have a keen interest in developing molecular markers for use in breeding programs on quality. Within the Centre for BioSystems Genomics, the Netherlands plant genomics network (CBSG, [www.cbsg.nl](http://www.cbsg.nl)), a linkage disequilibrium study was performed to identify markers associated with quality traits. A phenotypic data base was constructed for quality data for about 220 tetraploid cultivars that represent worldwide genetic variation for quality traits. The phenotypic data came from multi-environment trials performed by various breeding companies and an EU-project. The cultivars were genotyped with AFLPs, where bands were created by 10 primer combinations. Marker-trait associations were investigated by fitting various mixed models using AFLP information to the quality traits. The models we used have been described by Malosetti et al. (2006) and Yu et al. (2006). We will discuss the performance, usefulness and suitability of the different models. In addition, we will pay attention to how to extract information regarding allele dosages scoring from AFLP gels and how to incorporate this information in the statistical model.

### Keywords

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association mapping, AFLP®, linkage disequilibrium, quality, potato

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# Neutral Mutation and the Mechanism of Adaptation

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Krunoslav BRČIĆ-KOSTIĆ

## Abstract

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The mechanism underlying the maintenance of adaptive genetic variation is a long-standing question in evolutionary genetics. There are two concepts (mutation-selection balance and balancing selection) which are based on the phenotypic differences between alleles. Both, mutation-selection balance and balancing selection cannot properly explain the process of gene substitution, i. e. the molecular evolution of quantitative trait loci affecting fitness. I assume that such loci have nonessential functions (small effects on fitness), and that they have the potential to evolve into new functions and acquire new adaptations. Here I show that a high amount of neutral polymorphism at these loci can exist in real populations. Consistent with this, I propose a hypothesis for the maintenance of genetic variation in life history traits which can be efficient for the fixation of alleles with very small selective advantage. The hypothesis is based on neutral polymorphism at quantitative trait loci and both neutral and adaptive gene substitutions. The model of neutral-adaptive conversion assumes that neutral alleles are not neutral indefinitely, and that in specific and very rare situations phenotypic (relative fitness) differences between them can appear. In this paper I focus on neutral-adaptive conversion due to phenotypic plasticity of neutral alleles. The important evolutionary consequence of neutral-adaptive conversion (NAC) could be the increased adaptive potential of a population. Loci responsible for adaptation should be fast evolving genes with minimally discernible phenotypic effects and the recent discovery of genes with such characteristics implicates them as suitable candidates for loci involved in adaptation.

## Keywords

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neutral mutation, DNA polymorphism, life history traits, adaptive evolution, molecular evolution

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# QTL by Genetic Background Interaction: Application to Predicting Progeny Value

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Jean-Luc JANNINK

## Abstract

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Association studies are designed to identify main effects of alleles across a potentially wide range of genetic backgrounds. To control for spurious associations, effects of the genetic background itself are often incorporated into the linear model, either in the form of sub-population effects in the case of structure, or in the form of genetic relationship matrices in the case of complex pedigrees. In this context epistatic interactions between loci can be captured as an interaction effect between the associated locus and the genetic background. In this talk I describe genetic and statistical models to tie the locus by genetic background interaction idea back to a standard concept of additive by additive epistasis when genetic background is modeled using an additive relationship matrix. I also present results from simulation studies using this model to evaluate the power of the model to detect epistasis and its ability to predict genotypic values of unphenotyped progeny. The model detects epistasis with the same power whether the epistatic effect is the result of a single pairwise interaction or the sum of many pairwise interactions, each generating a fraction of the total epistatic variance. Otherwise the power and predictive ability of the model depends on the pedigree size (the bigger the better), the level of linkage disequilibrium between the causal polymorphism and the typed polymorphism (the higher the better), the allele frequency of the causal polymorphism (the closer to 0.5 the better) and the degree of inbreeding of the phenotyped individuals (the more inbred the better). Under favorable conditions, the epistatic model can predict the genotypic value of progeny for which marker data but no phenotypic data is available better than a standard additive model.

## Keywords

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complex pedigree, epistasis, genetic background, linkage disequilibrium, QTL interaction

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# QTL Detection and Marker-assisted Selection in a Multiparental Maize Design

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## Abstract

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There is a growing consensus towards the need to address QTL detection and Marker Assisted Selection (MAS) in diverse populations obtained from the cross between several parental inbred lines. Connected multiparental populations (i.e. coming from crosses sharing common parent(s)) allow one, for a given population size, to increase the power and accuracy of QTL detection, to compare for each QTL different alleles and to test for epistatic interactions with the genetic background (Rebai and Goffinet, 1993, Charcosset et al., 1994; Jannink and Jansen, 2001). MAS should be particularly in this context to select individuals carrying favorable alleles and to conduct crosses to assemble favorable alleles issued from different parents.

The objective of this study was to validate experimentally the interest of a connected multiparental design for QTL detection and MAS. Six F<sub>2</sub> populations, with 150 individuals each, were obtained from a diallel cross between four unrelated maize inbreds. QTL detection was performed with MCQTL software (Jourjon et al., 2005). We observed a gain of power and an increase in the accuracy of QTL positions when QTL detection was performed considering connections, when compared to detection without taking into account connections or on individual populations (Blanc et al., 2004). Significant epistatic interactions were detected and found especially important for grain yield (epistasis explained 10% of the phenotypic variance). Using these results we performed three MAS cycles for two schemes following different objectives: (i) improving earliness and (ii) improving grain yield while keeping grain moisture at harvest constant. To follow the transmission of parental alleles at the QTL during selection generations, we developed a program to calculate identity by descent probabilities adapted to our design. Experimental evaluation of genetic progress revealed a significant improvement for both designs (3 days for earliness and 3.2 q/ha for grain yield). This result differs from the lack of efficiency of MAS observed in a previous experiment carried out in a biparental population (Moreau et al., 2004). To compare the efficiency of the selection in the multiparental design to a selection performed in each biparental populations independently, stochastic simulations were carried out using parameters (QTL positions and effects,  $h^2$ ...) close to our experimental results. Selection in the multiparental design overtakes selection in biparental populations considered independently, especially when heritability is low and selection performed using markers. So both experimental and theoretical results highlighted the interest of multiparental connected design over biparental populations for QTL detection and MAS.

## Keywords

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maize, *Zea mays*, marker assisted selection (MAS), quantitative trait locus (QTL), epistasis, cross optimization

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# Changes of Heterotic Pattern and Allele Frequencies in the M3S Maize Population after Two Cycles of Selfed Progeny Recurrent Selection Revealed by SSR Markers

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Marijana BARIĆ

Vinko KOZUMPLIK

## Abstract

Maksimir 3 Synthetic (M3S) maize population was developed at the Faculty of Agriculture University of Zagreb by intercrossing inbred lines, which origin traces back to several open-pollinated varieties and local populations from different regions of the former Yugoslavia. The population was subjected to two cycles of selfed progeny recurrent selection, which resulted in a significant increase in grain yield of 12% per cycle with no significant changes in lodging resistance. The objectives of this study were: i) to determine genetic relationship among the parental inbred lines of the M3S population (M3S progenitors), the M3S population, and elite inbred lines representing the BSSS and Lancaster heterotic groups and ii) to examine the effect of two cycles of recurrent selection on allele frequency changes in the population. Nine M3S progenitors, three BSSS lines, and three Lancaster lines were genotyped at 24 SSR loci, out of which nine randomly chosen loci were used for genotyping 96 individuals from M3SC0 (the population before selection) and from M3SC2 (the population after two cycles of selection). A total number of 101 alleles were detected across 24 loci in the 15 lines, whereas 83 alleles were found in the nine M3S progenitors. Among the latter 83 alleles 31 were unique, i.e. found only in one of the progenitors. Mean genetic distance among nine M3S progenitors was 0.61 indicating a broad genetic base of the M3S population. High mean genetic distance was found between M3S progenitors and BSSS lines (0.69) and M3S progenitors and Lancaster lines (0.71). This indicates that the M3S population represents germplasm source unrelated to both the BSSS and Lancaster germplasm. Mean genetic distance between the M3S population and BSSS as well as Lancaster lines slightly decreased after two cycles of recurrent selection suggesting the need of introducing testers from both groups in future selection in the M3S population in order to maintain heterotic complementarity of the M3S population to these groups. A test of selective neutrality identified several non-neutral loci in the population whose allele frequency changes from the C0 to the C2 cannot be explained by genetic drift. The majority of non-neutral alleles, whose frequency increased after two cycles of selection, were present at least in one line from the BSSS or Lancaster heterotic group.

## Keywords

maize, exotic germplasm, recurrent selection, allele frequency, heterotic pattern

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# Analysis of Quantitative Traits in an F4 Population Derived from Crossing Four Accessions of *Arabidopsis thaliana*

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## Abstract

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Following a similar initiative in mice, a complex intercrossed population was created from a set of 8 accessions of *Arabidopsis thaliana* (Col, Kyo-1, Cvi, Sha, Eri-1, Ler and C24). The created population consists of 12 intercrossed subpopulations of about 100 recombinant inbred advanced intercross lines (RIAILs) each. The subpopulations exhibit very different degrees of kinship. The population as a whole will offer increased power and resolution in QTL mapping, with a focus on epistatic interactions.

We will discuss advantages and properties of this type of population for breeding and physiological research. In addition, we will present preliminary results of analyses on the first subpopulation, obtained from the cross CviXSha and LerXC24, that is presently genotyped and measured for traits like flowering time, number of leaves and leaf size and shape parameters.

## Keywords

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*Arabidopsis thaliana*, RIAILs, intercrossed population

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# Use of Haplotype Relationships in Genome-wide LD-mapping Methods

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## Abstract

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Within the context of the Bioseeds Breeding by Design program, it is aimed to identify all QTL regions for large numbers of traits by marker LD mapping in a wide germplasm. In genomewide LD studies, which may yield large numbers of significant results, it is important to apply methods that minimize type I error rates by optimal use of the available information. We have implemented and tested two QTL mapping methodologies based on marker haplotypes, rather than single markers, derived from Kruskal-Wallis test and Haplotype Sharing Statistic. Both methods have clearly different characteristics in terms of detection power, false discovery rate, and type of associations found. An additional source of information that has not been used so far is the reconstructed relationship between haplotypes. The inclusion of this type of information in association tests may substantially increase the signal-to-noise ratio in trait association studies, which is demonstrated using both simulated and real data.

## Keywords

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LD mapping, detection power, false discovery rate

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# Bayesian Analysis of Complex Traits in Pedigreed Plant Populations

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## Abstract

Breeders and geneticists have developed statistical methods to identify quantitative trait loci (QTL) by utilising molecular markers. These methods have sought to answer basic questions concerning QTL (e.g. number, mode of action and size of action) and to map QTL on the genome to facilitate their manipulation for breeding purposes. In plants, populations derived from single crosses of inbred lines have predominantly been used in QTL mapping experiments (Jansen 2001). Major incentives do exist to study more complex populations those derived from multiple founders or collected from ongoing breeding programs, for example: (1) Highly improved exploration of QTL variation since multiple alleles are present at a high probability when a population arises from many founders. (2) Applied context of identified QTL alleles since experimental line crosses often do not represent the (commercial) breeding populations. (3) Improved cost effectiveness of QTL mapping by using available phenotypes from selection experiments, since the cost of obtaining marker data likely continues to decline and evaluating phenotypes becomes relatively more expensive. Breeding programs routinely evaluate the phenotypes of many progeny with replication at several locations.

These incentives should convince plant geneticists and breeders to better exploit the data from pedigreed populations as available from ongoing breeding programs. However, the analysis of this type of data has been hampered by the absence of flexible and robust statistical tools and methods. Important criteria for QTL mapping in complex data may be summarised as: (A) Robustness and flexibility to possible structures in the data, especially in pedigree; i.e. individuals may cover multiple generations, the population may cover multiple families, with large differences in size and relationships in between. (B) The number of QTL, across a single chromosome and across all chromosomes, is in fact unknown and should be treated as such in the analysis. Also, the mode of action of QTL is unknown and/or may interact with the genetic or environmental background in which it is expressed. Also, many small QTL may not be detectable but may be grouped into a polygenic background component. (C) Incomplete marker information; this holds on multiple levels, i.e. DNA on an individual may be absent, markers are partially scored on an individual, markers may be partially informative (e.g. dominant scoring) or markers may not be informative on an individual (e.g. its parents sharing similar homozygous genotypes). (D) Environmental factors may contribute to the observed phenotypic variation in the quantitative trait. Pre-correction for these factors may eliminate uncertainty in these factors and can introduce bias in parameter estimates. Simultaneous analysis seems to be more appropriate.

Here we attempt to accommodate all these criteria by the application of a Bayesian approach. That is, a Bayesian framework with Markov chain Monte Carlo (MCMC) algorithms provides a powerful tool for estimating the chromosomal location and contribution of genes affecting complex traits and, potentially, gene-by-gene and gene-by-environment interactions as well. After describing the probability model and its variables with their prior distributions, we will present empirical results from the analyses of simulated and real data to dissect complex traits into their underlying genetic components.

## Keywords

quantitative traits, Bayesian analysis, multiple QTL models, polygenes, pleiotropy, pedigree

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# Searching for Interacting QTL in Pedigree-related Populations of an Outbreeding Species

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## Abstract

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Many important crop species are outbreeding. The search for genes affecting traits is complicated by the fact that in each cross up to four alleles may be present at each locus. This paper is concerned with the search for interacting loci, perhaps in the presence of non-interacting loci. The material that is used consists of populations which have been obtained by crossing a number of parent individuals (*e.g.* using an incomplete diallel cross). In practice, parent individuals may be related by pedigree. However, in this paper it will be assumed that the parents are unrelated. The consequences of genetic relationships between parents will be shown. The approach has two goals: (1) finding loci that are possibly interacting with other loci, and also finding loci that behave additively; (2) finding parent individuals which segregate at the interacting loci, and which can be used to study interactions in greater detail. In this paper QTL analysis is carried out by means of regression on predictions of QTL genotypes. The research for this paper was carried out within the EU funded project Hidras, a collaborative effort of 11 European groups to breed high-quality, disease resistant apples.

## Keywords

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interacting QTL, related populations, regression analysis, segregating parents

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# Identification of QTLs Underlying Resistance to Soybean Cyst Nematode Races 3 and 5 in Soybean PI 494182

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## Abstract

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Soybean Cyst Nematode (SCN) is the most important soybean pest throughout the world. Soybean plant introduction (PI) 494182 is a new source of resistance to SCN HG Types 0 and 2 from Japan. The objectives of this study were to find QTLs for resistance to HG Types 0 and 2 in PI 494182 and evaluate efficiency of marker assisted selection for SCN resistance. The mapping population consisted of 162 F3:4 lines from a cross between Skylla X PI 494182. One hundred and thirty seven polymorphic SSR markers covering 18 Linkage Groups (LG) were used in this study. In Single Marker Analysis (SMA), 12 markers from LGs A2, F, G, and N showed significant association with resistance to HG Type 0 and 7 markers from LGs A2, G, and N showed significant association with resistance to HG Type 2. In Composite Interval Mapping (CIM), one significant QTL found in LG G near *rhg1* gene and another significant QTL was found in LG A2 near I locus and *Rhg4*. Indication of QTL also found in LG F even though its LOD did not exceed the threshold. CIM of HG Type 2 did not detect any significant QTL. Marker assisted selection using two markers from LGs A2 and G showed to be efficient for SCN HG Type 0.

## Keywords

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soybean, QTL mapping, SCN, SSR markers

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# The Importance of GCA and SCA in a Context of Gene Expression

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## Abstract

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Gene surveys for regulatory variation are a first step in identifying the specific nucleotide changes that govern gene expression differences, although they leave the underlying mechanisms unexplored. Here, we propose a quantitative genetics approach to detect and ascribe imbalance in allelic expression to cis regulatory variation. We took advantage of a diallel design that is often used in plant breeding programs in which the goal is to estimate general combining abilities (GCAs) of specific inbred lines and to identify high yielding hybrid combinations of parents based on their specific combining abilities (SCAs). In a context of gene expression, the values of GCA and SCA parameters allow cis and trans regulatory changes to be distinguished on a large scale and to ascribe imbalances in gene expression to cis regulatory variation. The method is demonstrated for *Arabidopsis thaliana*, but it can also be used to explore naturally occurring cis regulatory variation in other species where inbred lines and genomic tools are available, such as corn.

## Keywords

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general combining abilities, *Arabidopsis thaliana*, specific combining abilities, cis regulatory variation

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# Support Vector Machine Regression for Hybrid Prediction

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Bernard DE BAETS<sup>2</sup>

## Abstract

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The phenomenon of heterosis is widely used in breeding programmes of various crops, despite the fact that no satisfactory molecular explanation is available. By studying the genetic relationships between individuals of the breeding pool, plant breeders have developed strategies based on heterotic groups and early testing of inbred lines. These often heuristic methods allow to considerably increase the frequency of finding a combination of inbreds whose offspring exemplify an elevated level of hybrid vigour. This observation has become the underlying idea of the presented research. We do not focus on the exploration of heterosis from the molecular perspective but merely try to model the occurrence of the phenomenon using linear mixed models and non-linear kernel-based methods like Support Vector Machine Regression (SVR).

Linear mixed model analysis is a widely accepted method for the genetic evaluation of livestock and crops. Best Linear Unbiased Predictors (BLUPs) of numerous Specific Combining Abilities (SCAs) were obtained using a subset of the database of a private company (RAGT R2n) containing all field evaluation results from their maize breeding program since 1984. These predicted SCA values were consequently used as training examples for building a predictive model using SVR. The basic idea of this technique is to map the data into a high-dimensional feature space via a nonlinear mapping (kernel function) and to do an insensitive linear regression in this newly constructed space. Molecular marker scores of both parents (inbreds) are used as features since genetic distances form the basis of heterotic groups. Each parental line is therefore characterized by 101 SSR markers and 15 AFLP primer combinations. Experiments with various standard kernels and kernel parameters as well as preliminary results are presented. Future improvements using specifically designed kernels are proposed.

## Keywords

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heterosis, maize, linear mixed models, support vector machine regression, SSR, AFLP

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# Population Genetical Simulation and Data Analysis with Plabsoft

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Matthias FRISCH

## Abstract

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Random mating, infinite population size, and absence of selection are often assumed in the derivation of closed analytical solutions for population genetical problems. However, in plant breeding finite populations are derived from planned crosses, and selection is carried out to achieve breeding progress. Therefore, regularly population genetical problems do occur in plant breeding programs, for which no suitable analytical solutions are available. Computer simulations can be employed to solve such problems.

We developed software Plabsoft, which combines simulation and data analysis routines in an integrated program. Plabsoft allows the simulation of arbitrary crossing schemes with finite populations and various selection methods. All breeding techniques typically employed in plant breeding programs are implemented. Data analysis routines comprise measures of linkage disequilibrium, tests for Hardy-Weinberg and linkage equilibrium, and a wide range of genetic distance measures and similarity coefficients. Standard errors of estimated parameters can be determined with bootstrap and jackknife methods. The analysis routines are designed to analyze simulated as well as experimental datasets.

The functionality of Plabsoft is illustrated at the example of the following studies: Heckenberger et al. (2005) simulated populations of single-seed descent lines and determined the probability distribution of genetic distances to obtain threshold values for plant variety protection. Stich et al. (2005) analyzed linkage disequilibrium in European maize to assess the prospects of association mapping in plant breeding. Stich et al. (2006) simulated the breeding history of European maize to evaluate a new association test. We conclude that our software can be employed in a broad range of applications in genetical research and applied breeding.

## Keywords

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breeding informatics, computer simulations, population genetical analyses

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# Optimization of DH-line Based Recurrent Selection Procedures in Maize

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## Abstract

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The use of doubled haploid (DH) lines is increasingly replacing the traditional development of inbred lines in commercial hybrid maize (*Zea mays* L.) breeding. The success of employing DH lines depends on the efficiency of the breeding strategy implemented and the optimum allocation of monetary and technical resources. In addition, when optimizing recurrent selection (RS) schemes, the breeder has to limit the decline of genetic variance in order to ensure long-term selection gain. A software package has been developed allowing to optimize alternative DH breeding schemes including various RS procedures (Gordillo and Geiger, this conference). Using model calculations, the software maximizes the expected genetic gain per year as a function of the pertinent variance and covariance components and various operational variables under the restriction of a given annual budget and an upper limit for the decay of genetic variance. Optimization results are presented for two RS schemes differing in cycle length. In the longer scheme, the DH lines are pre-evaluated for line *per se* performance before testcross selection is started, whereas in the shorter scheme, the lines *per se* are evaluated in parallel to the first stage of testcross selection. Taking advantage of winter nurseries, the two procedures require two resp. three years for completing an RS cycle. Employing a second stage of testcross selection extends the cycle length by one year. Results show that cycle length has a striking effect on the relative merits of the compared RS procedures. Given a standard set of parameter estimates and input variables, much faster breeding progress is achieved with the shorter scheme, and in both schemes one-stage selection is more efficient than two-stage selection. Moreover, we studied the impact of deviations from the optimum number of testers and test locations. Generally, near-optimum allocations lead to only minor reductions in the expected genetic gain.

## Keywords

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doubled haploid lines, optimization of breeding procedures, gain from one and two-stage selection, effective population size

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# MBP: A Software Package to Optimize Hybrid Maize Breeding Procedures

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## Abstract

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A software package called MBP is presented, which allows the maize breeder to optimize alternative breeding schemes for recurrent selection (RS) as well as for developing parent lines of hybrid varieties. Though primarily developed for procedures based on doubled haploid (DH) lines, it also includes traditional schemes based on selfed lines. Here we limit ourselves to DH lines. MBP maximizes the expected annual genetic gain in general combining ability by means of quantitative genetic model calculations under the restriction of a given breeding budget. Exact formulae are applied to predict the gain from one-, two-, and three-stage testcross selection. Pre-selection for line-*per-se* performance is considered as a matter of expense but is not considered in calculating the response to testcross selection. The effective population size ( $N_e$ ) is predicted by applying exact formulae for the joint effect of drift and selection according to Santiago and Caballero (1995) extended to account for the specifics of the present breeding schemes. The coefficient  $1/(2N_e)$  is used to set upper limits for the decay of genetic variance. The number of testcross evaluation stages as well as the number of selected lines may be specified independently by the user for both RS and hybrid parent line development procedures. MBP uses standard values for the required genetic parameters, labour costs and haploid induction parameters as estimated from numerous experiments and information provided by breeders. These parameters as well as the budget and the limit value of  $1/(2N_e)$  may be varied by the user in accordance with the available genetic resources and technical and financial restrictions. Optimum values are determined for the number of crosses, S0 plants per cross, testers, test units and locations, and replicates at each selection stage. Optimization examples are given in an accompanying paper by Geiger and Gordillo (this conference). MBP needs comparatively little computing time and is therefore a valuable tool for designing breeding schemes and determining their relative merits.

## Keywords

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software package, doubled haploid lines, recurrent selection, hybrid breeding, optimum allocation, selection gain, effective population size

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# Comparison of Tree Architecture Using a Tree Edit Distance: Application to 2-year-old Apple Hybrids

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Pascal FERRARO<sup>2</sup>

Evelyne COSTES<sup>1</sup>

## Abstract

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In fruit tree species, understanding genetic determinisms of architectural traits is considered as a promising manner to control vegetative development and yield regularity via the integration of these traits in selection schemes. Within this context, our study aimed to (i) analysing genetic parameters of architectural traits on a segregating progeny and (ii) classifying hybrids on the basis of their architectural traits. The present paper focuses on this second objective.

Using the methodology defined by Godin et al. (1997), a topological description was performed on apple hybrids which derived from a cross between two varieties with contrasted architectures. Each tree topology was represented as a multi-scale tree graph with attributed geometrical variables. In order to evaluate tree similarity, a comparison method based on edit operations (substitution, insertion and deletion) was applied. This method is based on an algorithm which computes a distance between two tree graphs by minimising the sum of the costs of edit operations applied to transform the first tree into the second. Several distance matrices were calculated taking into account different combinations of topological and geometrical attributes. Then, from each distance matrix, trees were clustered using Ward's method. An interpretation of the results is proposed through the analysis of 3D representations of the trees. On the one hand, within-tree local similarities were analysed by identifying the entities which were matched between pairwise trees. On the other hand, similarities between trees were analysed within and between clusters. Furthermore, the variability of clustering was analysed regarding the different combinations of geometrical or topological attributes which were taken into account in the comparison. Discussion will focus on the prospects given by this approach in a tree breeding context.

## Keywords

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*Malus x domestica*, topology, geometry, tree graph comparison

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# Design a Marker-based Pedigree Selection Strategy for Parent Building in Barley Using Computer Simulation

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Maarten VAN GINKEL

## Abstract

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Pyramiding multiple desirable genes is an important method for the development of improved breeding materials and/or new cultivars. When the number of genes to be pyramided is many, or the genes are tightly linked in repulsion, it is impossible to recover the desirable recombinants in a single generation using realistic population size, and continual selection at several generations is required. The availability of markers tightly linked to the desirable genes makes it possible to conduct effective individual selection at early generations. This reduces the number of lines tested in the later generations and increases the desirable genotype frequency in the selected progeny. Computer simulation was used to develop such a marker-based pedigree selection strategy for the development of a barley line that contains six genes from three breeding lines (H: 221222; P: 212222; S: 122111 - 1 and 2 represents desirable and undesirable allele, respectively), using top cross H/P//S. Under the assumption that perfect markers were available for all the six genes, a TC<sub>1</sub> population of 300 plants was required to obtain three or more lines of the best genotype '211222/122111'. When SSD was used from TC<sub>2</sub> generations until to homozygosity, the probability to obtain lines of the desirable genotype (fixed for the desirable alleles at all six loci) was low due to the tight repulsion linkage between some of the genes. About 4000 individuals were required to ensure with 99% probability to recover at least one line of the desirable genotype. The total number of lines genotyped was at least 5000. When pedigree method was used in all test cross generations, many schemes resulted in more lines of the desirable genotype by genotyping less than 3000 lines. The best scheme consisted of the following steps: 1) 500 TC<sub>2</sub> plants were genotyped and individuals fixed for the desirable alleles at three loci were selected. 2) 100 TC<sub>3</sub> plants per TC<sub>2</sub> plants were genotyped and individuals fixed for the desirable alleles at five loci were selected. 3) 25 TC<sub>4</sub> plants per TC<sub>3</sub> plants were genotyped and individuals of the desirable genotype were selected. Using this scheme, in average, 320 desired TC<sub>5</sub> lines were obtained by genotyping less than 2000 lines. When markers were tightly linked to the target genes but not diagnostic (perfect), not only more genotyping was required but also phenotyping at the end of marker selection was necessary to confirm the presence of the target genes. Under the assumption that the recombination between marker and target gene was 5%, the best selection scheme identified, in average, produced 30 desirable lines by genotyping 8000 lines and phenotyping 700 TC<sub>5</sub> lines. If double haploid lines were produced using F<sub>1</sub> between H and P, and marker and phenotypic screening were conducted, the total genotyping and phenotyping could be halved.

## Keywords

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*Hordeum vulgare*, marker-assisted selection, pedigree selection, simulation

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# Parental Selection in Self-pollinating Crops Using Best Linear Unbiased Prediction (BLUP)

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Jens LÉON

## Abstract

The selection of favourable parental lines is an essential requirement for the breeding success. As the breeders get only the phenotypic information of the parental lines and the data structure is highly unbalanced, the selection decision will be erroneous. Furthermore, neither pedigree information nor molecular marker data can be considered in the selection. Best linear unbiased prediction (BLUP), commonly applied in animal breeding, is able to account for these problems.

In our study we generated a virtual parental population by computer simulation using the software SAS/IML 9.1 (SAS Institute 2004). In the simulation, the population was composed of 500 inbred lines. The genotypic value of a line arose from the action of 150 loci with 2 until 7 alleles per locus. The parental lines were simulated to be tested in 5 different environments each with 5 replications. Then the phenotypic value of a line was obtained by adding an overall mean and normally distributed environmental, genotype by environment interaction and residual effects to the genotypic value which was influenced by additive effects and additive x additive epistatic effects. Furthermore we simulated three different traits with the heritabilities  $h^2 = 0.9$ ; 0.5 and 0.1 and balanced as well as unbalanced data sets.

As the coefficient of coancestry seems to be a biased estimator of the relationship information among parental inbred lines, in this study we compared the consideration of genetic similarities instead of coefficient of coancestry in BLUP of self-pollinating crops.

Regarding traits with a medium or low heritability, BLUP where pedigree information or genetic similarities were accounted for in the prediction is superior to the conventional approach in plant breeding (Bauer et al. 2006a, b). Assuming unbiased conditions, BLUP using genetic similarities leads to a marginal higher breeding success than BLUP based on coefficient of coancestry. Hence, coefficient of coancestry can be replaced by genetic similarities in BLUP of self-pollinating crops if the pedigree of the parental lines is unknown.

## Keywords

BLUP, coefficient of coancestry, genetic similarity, inbred lines, selection methods

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## Discriminating Maize Inbred Lines Using Molecular and DUS Data

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### Abstract

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To be eligible for the protection in form of the Plant Breeders' Rights (PBR), a potential new variety must meet the criteria of Distinctness, Uniformity, and Stability (DUS). In most of the European countries, testing for DUS is, or will become, the consistent part of variety trials. It is based on a set of traits suggested by UPOV, which are traditionally, and predominantly, morphological characters that should have some agronomic relevance. Being affected by environmental factors, morphological markers require repeated testing over years. Multiplied by the growing numbers of candidate varieties, it would inevitably cause either dramatic increase of trial costs, or equal decrease of trial precision. Necessity of the cost reduction, due to general limitation of resources, raises the need for the alternative system of DUS testing. Proven to have high discriminative power, unaffected by environmental factors, molecular markers become the obvious candidates. However, they are still not widely accepted for use in DUS testing, due to non-uniformity within varieties, often observed at the molecular level.

In this study total of 41 maize inbred lines were scored for 32 DUS characters prescribed by UPOV. The same set of inbred lines was genotyped at 28 SSR loci. The aim of the study is to compare the results obtained by using DUS data and SSR markers to discriminate maize inbred lines. Further comparisons will be done with available pedigree information, in order to assess power of both data types to detect the degree of relatedness between lines. We will also try to establish parallels between morphological and molecular markers, and point out morphological traits that could be associated with the variability at the molecular level. Suitability of the SSR markers for DUS testing will be reviewed from the aspects of their discriminative power, cost reduction, and others.

### Keywords

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maize, DUS, SSR

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# A Comprehensive Database Management System for Integrating Phenotypic and Genomic Data in Scientific and Applied Plant Breeding Programs

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Matthias FRISCH

## Abstract

Data management and analysis systems, currently available in plant breeding were developed mostly for dealing with just one or a few specific problems. Some software packages offer tools for administration and biometric analyses of molecular marker data, but these do not implement links to phenotypic and pedigree data. Other software packages cover special tasks in plant breeding, such as administration of pedigree records and nursery books, but lack links to genomic data. However, no database system or structure is currently available integrating all the functions needed for efficient data management in modern plant breeding programs. Due to this lack of an integrated database system, breeders generally exploit only part of their data for selection decisions (*e.g.*, only data from the current season is used) or retrieve only part of the information present in the data (*e.g.*, information on related genotypes is ignored). Most approaches in genomics (*e.g.*, pedigree-based or haplotype-based QTL mapping), however, develop their full power only when they are based on analyses of large numbers of genotypes from multiple crosses and current as well as past generations.

These types of integrated analyses require highly sophisticated data structures ensuring best possible data integrity and quality. We have developed a flexible data management and -analyses system for storage, quality control, and integrated analyses of phenotypic and genomic data from breeding programs. It is implemented using the PostgreSQL database management system, linked to the R software environment for statistical analyses.

The database structure is capable of managing the following types of data observed in contemporary breeding programs of all major crops:

- Otu data of any species including pedigree data
- Phenotypic data of any trait and trait complexity
- Trial management data for any field and trial design (*e.g.*, randomization, field plans, fertilization, pesticide application, *etc.*)
- Molecular marker data for all common types of markers (*e.g.*, SSRs, AFLPs, RFLPs, SNPs, *etc.*)
- Some project-, and study management data

Data entry and -retrieval from defined input formats is implemented either as specific functions in R or as semi-automatic database functions. In addition, the interface to the R software environment offers the possibility to perform integrated analyses of phenotypic, molecular, and other data, directly retrieved from the database (*e.g.*, association mapping, advanced phenotypic analyses using BLUP, genetic diversity analyses, plant breeding simulations, *etc.*). The presented software is based on (a) a large body of phenotypic and genomic data specially generated for the purpose of this project from applied breeding programs of seven crops and (b) information about the structure and dimensions of these breeding programs. This guarantees practical relevance of the methods and software for all crops. Furthermore, we established a tight networking with other genome research projects for which our methods and software are indispensable tools.

## Keywords

breeding informatics, database, statistical analyses

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## Quantifying Tomato Flavour and its Metabolic Basis

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Pim LINDHOUT<sup>1</sup>, Fred A. VAN EEUWIJK<sup>1</sup>

### Abstract

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Improving tomato quality has been a challenge for quite some time. Modern high throughput techniques will further clarify the biological background and might boost developments in flavour breeding. We investigated metabolite levels and flavour sensation in a collection of tomato fruits. Our main questions were: how do metabolite levels and sensory trait scores vary between tomatoes, are there clear patterns in this variation, can we link patterns from the metabolic to the sensory level, and is there a genetic basis for the variation and patterns? From 94 tomato hybrids/cultivars with repetition over three experiments fruits were collected and processed for metabolic profiling and flavour assessment by a taste panel. We analyzed these data using mixed models to determine variance components and to calculate genotypic means for all traits/metabolites. Heritabilities were estimated for all traits/metabolites and these showed to be relatively low for many traits/metabolites in our study. We tried to find patterns in the metabolite space by various multivariate techniques and investigated a possible basis in biochemical pathway structure and regulation. For the sensory traits we tried to identify patterns in the flavour space. Furthermore we tried to clarify which metabolites or metabolic pathways might play a role in which sensory traits or sensory axes in the sensory space. For this we used redundancy analysis and partial least squares regression. Based on variation in metabolite content as well as sensory trait scores, we found a clear separation between cherry and non-cherry tomatoes. Especially sucrose and the phenylalanine derived volatiles, have higher levels in cherry tomatoes. These tomatoes also have a sweeter and more “spicy” sensation. Other combinations of metabolites, e.g. the (iso)leucine derived volatiles and the phenylpropanoids, that explained considerable parts of the metabolite space, did not separate types of tomato. But they could be linked to “corners” in flavour space. So indeed we do find high correlated metabolites that share a known biochemical pathway and they sometimes can be linked to specific areas in the flavour space.

### Keywords

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tomato, flavour, metabolic, mixed model, PCA, RDA

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# Evaluating Plant Breeding Strategies by Gene Based Crop Growth Simulation

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Scott CHAPMAN

## Abstract

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In recent years, there have been impressive achievements in engineering and/or selection for single genes associated with simply controlled traits associated with processes like disease, insect and herbicide tolerance and changes in biochemical product composition. Now, genomics is rapidly uncovering the biochemical function of many of the genes involved in plant growth and development in response to environmental conditions. Selection or engineering these genes to sustain or increase agricultural production is challenging, given the complex interaction of gene action with the environment, i.e. it is difficult to understand which of these genes is most important, so an alternative approach is to dissect the processes of growth and development down to a point at which gene action can be interpreted. Simulation models of plant processes enable us to capture the interactions between the sensing of signals at an organ level (e.g. drought affecting roots or leaves), the response of the plant at a biochemical level (change in development rate) and the result of the response at the organ (or crop) level (reduced growth). If we can explain these major processes in terms of gene action, then it is possible to analyse the process of plant breeding and selection by simulating the phenotypes (e.g. crop yield) of millions of genetic combinations grown in different environments. These simulation datasets are the basis for running selection programs using this phenotypic data or knowledge of the underlying genetic inputs. This system then becomes a 'testbed' for statistical techniques to determine which methods are able to reveal the underlying genetic and environment structure that was used as input to the simulations.

## Keywords

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genomics, simulation models, selection

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# Modelling Nitrogen Stress with Probe Genotypes to Assess Genetics Parameters in Winter Wheat

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Maryse BRANCOURT-HULMEL (✉)

Anne LAPERCHE

Jacques LE GOUIS

## Abstract

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Wheat varieties adapted to low input production systems are favoured in Europe due to low market prices and increasing environmental concerns. To efficiently breed for such varieties, our objective was to model environmental nitrogen stress to estimate genetics parameters of winter wheat tested under diverse nitrogen supplies. 222 doubled-haploid lines, from the cross between Arche (nitrogen stress tolerant) and Récital (susceptible), were tested in France at three locations, during two years, and under high (N+) and low (N-) nitrogen supplies. The environments were characterized using probe genotypes, composed of the two parents and two controls, to detect environmental yield limiting factors. Yield and nitrogen traits were studied. A factorial regression aimed to assess the tolerance (slope) of the lines to nitrogen stress and their performance under low nitrogen supply (ordinate).

Environment nitrogen status was best modelled by the nitrogen nutrition index at flowering of the probe genotype Récital. Heritabilities of yield and nitrogen traits for each nitrogen level were always above 0.6. Heritabilities decreased and the genotype x interaction variances increased when the nitrogen stress increased. Lower heritabilities in N- were mainly explained by lower genetic variances. Lines showed variation for tolerance to nitrogen stress and for performance under low nitrogen conditions, indicating the capacity to breed lines adapted to low input systems. These characteristics will be used to analyze QTL x environment interaction.

## Keywords

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winter wheat, nitrogen stress, genotype x environment interaction, QTL x environment interaction

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# Multiplicative Cultivar Effects in Trial Series in Different Environments

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## Abstract

98 wheat cultivars, tested by the Czech Plant Variety office in 554 regional trials from 1976 to 2005, were analysed for breeding progress, yielding ability and adaptation to particular environments. The environments could be distinguished by years, yielding level, altitude, precipitation and other parameters. The trials were sorted by value of the particular parameter and separated into groups of similar size. Within each group cultivar effects and variance components were estimated using a Least Squares procedure. Between trials grouped by yield the genetic variance increased with increasing yield level, while the residual variance changed only slightly. There was no evidence of additive main cultivar effects reproducible across environments, as expected from standard statistical models. The cultivar means of all kinds of trial groups were highly correlated. The genetic performance of cultivars could be described by their relative ranking, which was well reproducible across environments, if the actual data from individual trials or trial groups were properly standardised. Since primarily cultivar behaviour had to be estimated, we used a procedure which minimised the residual variance relative to the genetic variance. The standardisation consisted in multiplying the actual cultivar means of a trial or trial group by a factor, which adjusted the variance of the cultivar means to the variance expected from the respective general cultivar means. The adjusted data could then be visualised by converting them after subtraction of column (i.e. environment) effects to percents of the general mean. These “standardised relative yields” were surprisingly stable over environments for the majority of cultivars. A few cultivars showed adaptations to particular environments, expressed as significant trends in relative yield across the ranked environments. A few cultivars showed also instability of relative yield. Cultivar adaptation or instability was judged from the F-value, calculated as the relation of the variance of standardised relative yield across environments (within row) to the residual variance.

The standardised relative yields of cultivars were well suited, to visualise the pure breeding progress, free of environmental effects.

## Keywords

trial series, cultivars, variance components, adaptation, multiplicative model

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# A mixed model strategy for the detection of Quantitative Trait Loci in Multiple Environmental Trials

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Marcos MALOSETTI<sup>2</sup>

## Abstract

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In recent literature, mixed models are used for the analyses of quantitative trait loci (QTLs) in multi-environment trials (MET) (see e.g. Malosetti et al. 2004). In this talk we describe a general mixed model framework for the analyses of this type of data. Basically, the approach consists of the following three steps:

1. Selection of a model for the genetic correlations between the environments. As we will show in this presentation, selection of an appropriate model is important in the next steps of the analyses of the MET data.
2. Genome scan for QTLs and QTL by environment interactions (QxE), and the calculation of environment specific QTL effect. We will discuss relevant aspects such as the use of False Discovery Rates, and the modeling of the QTLs as fixed or random effects.
3. For the QTLs with a significant QxE effect, the calculation of QTL responses to environmental covariables such as temperature, rainfall.

We will discuss the mixed model framework using both simulated and real data sets.

## Keywords

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GxE, QTLxE, multiple environment trials, QTL responses to environmental covariables, mixed models, genetic correlations between environments

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# Assessing the Importance of Genotype x Environment Interaction for Root Traits in Rice Using QTL Analysis by Mixed Models

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Adam PRICE<sup>2</sup>

Hans-Peter PIEPHO<sup>1</sup>

## Abstract

The phenotypic analysis of agronomical field experiments includes information about the experimental design, the randomization structure and a number of putative dependencies of treatment and design factors on the trait investigated. Especially the genetic correlation across environments, which arises when the same set of lines is tested in multiple environments, plays an important role.

The analysis of QTLs for root traits indicates that different results are obtained if varying genetic populations are used and also if different environmental conditions are included. An experiment with 168 RILs and parents as checks was conducted under 4 environmental conditions (low light, low nitrogen, drought and a control environment).

We propose a model that incorporates all relevant experimental information into a composite interval mapping approach based on a mixed model, which especially considers the correlation of genotype by environment effects. An extensive sequential model selection procedure was applied based on the phenotypic model, using the AIC to determine an appropriate random structure for the (REML) analysis and type 3 Wald F-tests for selection of fixed effects. In a first step a number of random (nested) design effects were extracted. Secondly, an appropriate covariance structure is chosen for genotype x environment interaction. In a third step Box-Cox transformations were applied based on residual analysis.

We used the efficient S-Plus Module SAMM for fitting the mixed models. This language interface for S-Plus applies the fitting routines of ASReml and the estimation process is much faster than in SAS or S-Plus which used up to 36 hours for a single fit (on one QTL position).

We compare FDR-based profiles of composite interval mapping scans with and without the inclusion of genotype x environment interaction and the phenotypic information. Some distinct differences in profiles indicate that insufficient modeling of the non-QTL part can lead to an overly optimistic interpretation of QTL effects in interval mapping.

## Keywords

QTL, interval mapping, genotype x environment interaction, mixed model, FDR

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# Multi-trait QTL Mixed Modelling with an Application to Drought Stress in Maize

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## Abstract

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The combined use of several traits in a multi-trait QTL mapping approach has shown to be more powerful than the single trait QTL mapping strategy (Jiang and Zeng, 1995; Knott and Haley, 2000). In addition, multi-trait QTL modelling can be useful to investigate whether closely linked QTLs or pleiotropic QTLs are causing genetic correlations between traits. Several methods have been proposed for multi-trait QTL mapping, either based on multivariate regressions (Knott and Haley, 2000), mixture models (Jiang and Zeng, 1995), or by reducing the dimensionality of the problem by, for example, principal components (Mangin et al., 1998). In this paper we discuss a multi-trait QTL mapping based on a multivariate mixed model approach. Mixed model QTL models are particularly useful for multi-trait approaches as the variance-covariance between traits can be straightforwardly modelled. In addition, extra complications as multi-environment evaluations can easily be accommodated. The approach is illustrated with an application to drought stress trials in maize produced by CIMMYT. With the objective of mapping QTLs for drought tolerance in maize, several traits were evaluated in a range of environments including both water-stressed and non-stressed conditions. Based on single-trait QTL analysis, significant QTLs and QTL-by-environment were reported in this population (Ribaut et al. 1996; Ribaut et al. 1997; Vargas et al. 2006). Here, we extend previous single-trait analysis to a multi-trait analysis in which we address more specifically the issue of pleiotropy or genetic linkage as causing genetic correlations between traits. This later information can be exploited in indirect selection strategies.

## Keywords

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mixed models, multi-trait, QTL, maize, drought stress

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# BLUP in Plant Breeding

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## Abstract

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Best linear unbiased prediction (BLUP) is a standard method for estimating random effects of a mixed model. This method was originally developed in animal breeding for estimation of breeding values and is now used widely in many areas of research. It does not, however, seem to have gained the same popularity in plant breeding as in animal breeding. In plant breeding and variety testing, application of mixed models with random genetic effects has up until recently been mainly restricted to the estimation of genetic and non-genetic variance components, whereas estimation of genotype effects is mostly based on a model with fixed genetic effects. This paper reviews recent developments in the application of BLUP in plant breeding and variety testing. It demonstrates the advantages of BLUP compared to other procedures in terms of predictive accuracy. Particular emphasis is given to methods that exploit pedigree information and to the analysis of series of experiments.

## Keywords

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mixed model, breeding value, pedigree, genetic effect

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# Understanding the Relationship between Flowering Overlapping and Outcrossing: Lessons Learned from *Vicia faba* L.

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Fouad MAALOUF<sup>1</sup>

## Abstract

In several breeding procedures, such as recurrent selection methods, it is compulsory to facilitate inter-mating among selected genotypes. Outcrossing depends on many plant traits and their inter-relationships. Various traits of phenology may have a critical effect on outcrossing because they determine the reproductive synchrony with potential mates and synchrony with or attractiveness towards pollinators. Flowering overlapping has been considered as an indicator of the “opportunity for crossing”. Increased flowering overlapping can potentially increase the level of outcrossing, but this idea has received little empirical testing and it is based largely on suppositions.

An approach that combines isozyme codominant loci, the mixed mating model and multivariate regression models were used to predict the relative importance of flowering overlapping and phenology traits in outcrossing level in two synthetic populations of *Vicia faba* L. Both synthetic populations showed great intra-population variability in flowering overlapping and outcrossing. Significant trait predictors of outcrossing varied with the population. In one of the synthetic populations, floral traits significant predictors of outcrossing were the number of opened inflorescences and the flowering overlapping at the second week of flowering and the flowering duration. Combined, these three traits accounted for 20% of outcrossing variation. However, in the other population the number of opened inflorescences at the first week of flowering and flowering overlapping at the first and third week of flowering contributed significantly to outcrossing prediction and accounted for 40% of the variation but only in nectar-producing plants. There is a lack of consistent association between outcrossing and flowering overlapping. In addition, the relative low coefficient of determination values of the models indicates that this trait should not be regarded as having a critical effect on the level of outcrossing instead other floral traits such as floral display should be taken into account.

## Keywords

flowering overlapping, mixed mating, multiple regression, outcrossing

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# Evaluation of environment as a background for plant breeding

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Alexander V. KILCHEVSKY

## Abstract

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An important aspect of the genotype x environment interaction is evaluation of the environment as a background for breeding. The major parameters defining usefulness of the environment as a background are as follows: typicality nature of the environment, the environmental ability to reveal variability in bred population (differentiating ability), environmental productivity, recurrence of environmental parameters through the years and by change in group of genotypes and predicting ability (Kilchevsky, Khotyleva, 1985, 1989).

The analysis of the environment as a background for selection was made at various breeding stages of vegetable crops and potato on the basis of quantitative evaluation of environmental parameters after method of A. Kilchevsky, L. Khotyleva.

The following regularities of genotype x environment interaction were established:

- The environment channels variability in terms of productivity and stability. Genotype variability in the response rate and maximum selection efficiency to the general adaptive ability remain in the medium-productive environments. Selection in rich and poor environments can result in loss of ecological stability and isolation of narrow adapted genotypes.
- The sequence of genotype passage through test environments affects greatly the breeding efficiency.
- The «in vitro» environment can channel variability in an undesirable direction.
- There is no a universal environment for testing genotypes of diverse species for a complex of traits.
- Application of 2-3 environments is desirable for analyzing ecological stability in early generations.

It is advisable to use tester varieties, tested earlier in the state varietal tests for controlling major environmental parameters (typical nature, differentiating and predicting ability), as well as for realizing the principle of ecological purposefulness for the final set of environments.

## Keywords

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genotype x environment interaction, parameters of environment, typicality, differentiating and predicting ability, ecological optimization, plant breeding

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# Genotype by Environment Correlation and Genotype by Environment Interaction: Statistical Aspects of Collaborative Breeding

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Johann SÖLKNER

## Abstract

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Participatory plant breeding (PPB) has been proposed to complement or, in some cases, replace formal plant breeding (FPB). In PPB, farmers practice selection on their own fields while in FPB selection decisions are centrally taken by a breeder based on information gathered in a controlled testing environment. The logistic advantages of FPB are obvious, the major disadvantages are the potential misconception of selection criteria important to farmers and the loss of genetic gain when the target environments (i.e., the fields of farmers) are considerably different from the testing environment. From an animal breeders' point of view, pig and poultry breeding operations are similar to FPB and discussions about the utility of such operations are along similar lines as in the plant breeding community. The breeding of dairy cattle is different. The testing environments are the stables of farmers but the most important breeding decisions (the selection of bulls and bull sires) are taken centrally, within countries. The flow of information about the relative performance of daughters of bulls is international and extremely sophisticated, INTERBULL accumulates data for several breeds from all participating countries and provides rankings of bulls for each member county, taking the genotype x environment interaction across countries into account.

I will compare approaches of acquiring and analysing information on breeding objectives of farmers in terms of number and relative importance of traits, with a focus on low input systems (organic farmers and farmers in developing countries), using examples from animal and plant breeding. For the analysis of genotype x environment interactions, I will present a relatively new approach used in animal breeding, the reaction norm model. Finally I will consider the problem of genotype-environment-correlation and its effect on the estimates of heritabilities.

## Keywords

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animal breeding, selection criteria, breeding decision, INTERBULL

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# Participatory Variety Evaluation and Selection is an Effective Approach to Accelerate Breeding Process and Seed Dissemination: a Case Study of Common Beans in Uganda

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Fina OPIO<sup>1</sup>, Rose TAKUSEWANYA<sup>1</sup>, Richard SSEKABEMBE<sup>1</sup>, Frank BAGAZONZYA<sup>4</sup>

## Abstract

In this paper, we present experiences in participatory variety evaluation and selection in common bean in order to identify common bean genotypes adapted to low soil fertility combined with tolerance/resistance to the root rot disease and acceptable seed quality attributes for home consumption and the market. 89 bush bean genotypes were first grown in an on-station trial during the second rains of 2002 and in a community on-farm plot during the first rains of 2003. In addition to the researchers, 53 farmers and 4 grain traders participated in the evaluation and selection of the genotypes at critical stages of crop growth. At the end of these seasons, bean seed corresponding to genotypes each farmer and trader had selected was provided to them for further evaluation on their individual fields during the subsequent seasons. Results indicated that four to five seasons later, the evaluators had dropped most genotypes and retained others. Out of those retained by at least each farmer and grain trader were either genotype RWR 1946 or RWR 2075 or both. These two genotypes are selections from the Bean Improvement for Low Soil Fertility in Africa nursery. Through the participatory trials involving both farmers and grain traders they were tested and verified to be adapted to the prevailing low fertile soils characteristic of farmers' fields and also tolerant to the bean root rot disease. They also possessed the desirable and acceptable large seed size and red colours and a high yield potential. The evaluators also shared some common selection criteria. Large seeded beans with either red or red mottled or cranberry colours were the most preferred. Use of participatory variety evaluation and selection approach was also found to result into fast diffusion of the selected bean genotypes.

## Keywords

BILFA, common bean, community on-farm, grain traders, participatory variety evaluation and selection

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## Statistical Aspects of Imposed Collaborative Research

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Snježana BOLARIĆ  
Vinko KOZUMPLIK

### Abstract

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Purpose of this study is to describe specific aspects of research done within the framework of the projects sponsored through the Fund for Applied and Development Research, governed by the Agricultural Research Council (ARC), in terms of the requirements related to design and statistical analysis of experiments. The ARC and the Fund itself are components of the Farmer Support Services Project (FSSP), developed by the Ministry of Agriculture and Forestry of Republic of Croatia, and funded through the loan agreement with the World Bank signed in 1996. One of the specific objectives of ARC is “to introduce rational planning of research projects to resolve readily identifiable constraints at farm level, with due consideration of the total socio-economic farm system”. In order to meet this objective, ARC sponsored projects imposed trials that have to be carried out in on-farm, productional, or generally far less controlled conditions than requested by standards for scientific research.

The ARC project survey shows that five different types of plant breeding related projects covering different species, goals, methodologies and statistical tools used, were realized within Dept. of Plant Breeding, Genetics and Biometrics, Faculty of Agriculture in Zagreb from 1998. Here we emphasize some of them: in order to explore the possibilities of improving the efficiency of official Croatian variety trials through the use of alternative trial design, RCBD was substituted by alpha design and comparison of statistical models on six species in 50 trials was investigated; the effects of genotype and environment on quality traits of bread winter wheat (*T. aestivum* L.) cultivars, where stability was evaluated in 12 environments on family farms in different parts of Croatia. Four stability parameters, covering a wide range of statistical approaches, were used to estimate cultivar stability; study on genetic potential of yield, stability and quality of clover-grass mixtures in order to select and recommend the best mixtures to producers on family farms and to use them as breeding germplasm. In this type of collaborative research limited funds and the need for adaptation to production technology of the local producers was found as the executive difficulty in comparison to the scientific research.

### Keywords

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collaborative research, ARC projects in Croatia, family farms, statistical analysis

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# Genetic Research in a Public-private Research Consortium: Prospects for Indirect Use of Elite Breeding Germplasm for Academic Research

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## Abstract

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The creation of a public-private research partnership between plant breeding industry and university can be beneficial for all parties involved. University benefits from the contributions by industry while industry benefits from increased insights and methodology that is developed tailored to a relevant set of data. However, plant breeding industry is highly competitive and there are obvious limits to the data and material companies are willing to share. These usually include current and historic released cultivated materials, but will very often not include the elite germplasm used in-house to create the released cultivated materials. Especially for crops where hybrid cultivars dominate the market, the parental lines of hybrid cultivars are considered core 'treasures' and are never provided to outside parties. Still, this limitation often does not apply for DNA or genetic fingerprints of these parental lines. We developed a stepwise procedure to take advantage of elite breeding materials for the creation of new promising research populations, through indirect selection. The procedure starts with association studies being applied to an available set of hybrid cultivars, resulting in the identification of relevant traits and genomic regions. Next, regression analysis is used within the set of hybrid cultivars to identify markers linked to the traits. Fingerprint data of the parental lines is then used to predict parental phenotypes from the regression results. Finally parental lines are selected and new promising combinations identified. Breeding industry can then be asked to create and provide progeny of promising line combinations for further research. This approach will be illustrated with a case study in tomato.

## Keywords

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public-private partnership, quantitative genetics, research populations

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# Genetic Structure and Differentiation in Hop (*Humulus lupulus* L.)

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## Abstract

Hop is cultivated for its female inflorescence (hop cones) with lupulin glands that provide aroma, flavour and bitterness to beer. Breeding for improved cultivars in terms of yield, high resin content and disease resistance has been a principle aim of hop breeding programs. Germplasm collections have been established in main hop breeding centres, providing valuable starting material in hop breeding. These collections are fundamental resources for studies in genetics and breeding and their exploitation requires a detailed knowledge of the levels and distribution of genetic diversity present and of genetic relationships among available genotypes. We analyzed genetic structure and diversity among hop genotypes using microsatellite markers and a set of wild and cultivated hop accessions. Model-based clustering (Structure) allocated the accessions into five gene pools that correspond to major breeding groups and to accessions showing mixed origin. A Fitch-Margoliash least-square tree shows good agreement with pedigree information and five gene pools, and further assigned eight accessions into two additional gene pools. The cultivated hops differ significantly from the wild ones, although most of the variability was found within groups. Molecular variances within groups of cultivated and wild hops were homogeneous, suggesting that a similar level of molecular variability is found in both groups of accessions. Core sets of cultivated hop accessions that capture maximum allelic richness were defined, generally including hop cultivars of 'mixed origin' that combine alleles originating from different gene pools and thus have the greatest allelic richness. The above results are consistent with two biogeographically separated germplasms and with the known history of accessions and have so far given a most detailed insight into the structure and extent of genetic diversity present in hop germplasm collections.

## Keywords

model-based clustering, genetic diversity, hop, *Humulus lupulus* L.

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## Effects of Different Cytoplasms on Quantitative Characters in Maize

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### Abstract

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The study of cytoplasmic effects on the expression of quantitative characters is important in learning about cytoplasmic-nuclear interactions and their influence on breeding and genetic programs. The genotypes of six early-time maize inbreds T 248, TC 243, TC 209, TB 367, A654, TC 102, T 291 were backcrossed eight times into ten cytoplasms other than their own. Thus, each cytoplasmic source of seed was assumed to have the same nuclear genotype. Five characters were studied over two years and two locations. Significant differences among the cytoplasms occurred for plant vigor, date of tasseling and silking, stalk lodging resistance and kernel dry matter.

Differences in grain yield among cytoplasms were not significant, or their expression was of low magnitude and probably was modified by interaction with environments. Differences that did exist were in the direction expected on the basis of inbred performance. The results indicated that differences in performance of the inbreds were not necessarily an indication of cytoplasmic effects in hybrid combination. Results from these experiments provide further support for cytoplasmic effects on some agronomic characters in maize. They also emphasize that the environment can influence the performance of cytoplasm from one year to another.

### Keywords

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maize, cytoplasmic effects, environmental-cytoplasmic interaction

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# Line x Tester Analysis for Yield Components in Sunflower (*Helianthus annuus* L.)

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## Abstract

The development of new hybrids with a high genetic potential for seed and oil yields based on interspecific hybridization requires information on the mode of inheritance and combining abilities of the newly developed inbred lines for total seed number per head (TSN) and 100 seed mass (100SM). The interdependence between yield components and seed yield (SY) in order to select promising lines to be used subsequently as components of future sunflower hybrids. In the present study, we used seven new divergent (A) cytoplasmically sterile inbred lines obtained by interspecific hybridization, three Rf-restorer lines used as testers, and 21 F1 hybrids developed at the Institute of Field and Vegetable Crops in Novi Sad. The female inbred lines had been developed by interspecific hybridization, while the three male restorer inbreds with good combining abilities were used as testers in the form of fertility restorers. A trial was set up at the Rimski Šančevi Experiment Field of the Institute of Field and Vegetable Crops in Novi Sad using a randomized block design with three replications. The mean values and coefficient of correlation ( $r$ ) as indicators of interdependence between two variables were determined according to Hadživuković (1991). Analysis of combining abilities was done by the line x tester method (Singh and Choudhary, 1976). Significant differences in TSN and 100 SM were found between the A-lines and the Rf-testers and their F1 hybrids. Analysis of combining abilities showed that there were significant differences between the A-lines and Rf-testers in the GCA for both traits under study. Highly significant positive GCA values were found in the A-lines NS-GS-4 and NS-GS-5, while the NS-GS-6 inbred had a highly significant negative value of the GCA for both traits. Among the Rf-testers, highly significant positive GCA values were found in RHA-N-49 for TSN and RHA-R-PL-2/1 for 100 SM. The highest and highly significant positive SCA value for both traits was recorded in the hybrid NS-GS-5 x RHA-R-PL-2/1. The nonadditive component of genetic variance had the main role in the inheritance of TSN and 100 SM, further supporting this was the GCA to SCA ratio in the F1 generation of less than one (0.11, 0.24). The largest average contribution in the expression of TSN was found in the Rf-testers (55.8%), whereas with 100 SM, the contribution of the A-lines was more significant (70.6%). Significant positive interdependence was established between SY and TSN (0.376\*). Between SY and 100 SM, there was highly significant positive interdependence (0.823\*\*).

## Keywords

sunflower, yield components, combining abilities, gene effects, correlations

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# Generalized Linear Model in Analysis of Grasspea Mutants (*Lathyrus sativus* L.)

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## Abstract

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Grasspea (*Lathyrus sativus* L.) is characterized by low habitat requirements, high content of protein and favorable composition of fatty acid in seed. Simultaneously, grasspea possesses a lot of unfavorable traits in agronomy point of view. Essential is to modify this plant on the genetic level for example through mutation induction. The object of investigation constituted three grasspea mutants and their initial cultivar Derek, analyzed for eight characters in Experiment Station in Cerekwica. The field trial was conducted according to method of randomized block design with three replication. All mutants were obtained after grain irradiation of cultivar Derek with laser light and two chemomutagen treatment: N-nitroso-N-methylurea (MNU) and sodium azide (NaN<sub>3</sub>). The aim of the work was to compare the variety Derek with its mutants with respect to two selected traits: the number of pods per plant and the number of branches per plant. Both considered traits are discrete, so in the analysis the logistic model which belongs to the class of generalized linear models was applied.

## Keywords

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generalized linear model, grasspea mutants

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# Comparison of Two Methods of Estimation of Nonallelic Interaction of QTL Effects on the Basis of Doubled Haploid Lines in Barley

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## Abstract

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The paper presents numerical comparison of two methods of estimation of nonallelic interaction of QTL effects. In the first method we assume that we observe only the plant phenotype, while in the second method we have additional information from the molecular markers observations. In this paper we analysed phenotypic data on 120 barley doubled haploid lines, derived from cross Clipper × Sahara and data concerning 183 molecular markers. The analysed traits were beta-amylase activity, alpha-amylase activity, beta-glucanase activity and cyst nematode resistance. Results obtained for three from four traits show that by using molecular marker observations we obtain estimators which have smaller absolute values than estimators obtained by the phenotypic method.

## Keywords

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nonallelic interaction, quantitative trait loci, doubled haploid lines

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# Genetic Diversity Evolution through Participatory Maize Breeding

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## Abstract

Maize arrived in Portugal from America during the XVI century and spread rapidly throughout the country. Numerous open pollinated traditional varieties (landraces) have been developed during the centuries of cultivation, adapted to specific regional growing conditions as well as farmer's needs, and represent a valuable genetic resource for breeding and genetic studies. Concern has been expressed that genetic diversity might be reduced by natural and artificial (human) selection. However, traditional Portuguese farmers were trained to preserve variability as a way to guarantee production under any circumstance. In 1984, Pêgo started, with CIMMYT support, an on-farm participatory maize breeding project at the Portuguese Sousa Valley region (VASO). It concerned mainly flint-type open pollinated landraces with technological ability for production of the traditional maize bread called "broa". "Broa" production still plays an important economic and social role in Central and Northern Portuguese rural communities. This bread making ability depends on a range of particular traits not found on the available commercial hybrid varieties and this is probably why traditional maize landraces have not, in these regions, been totally replaced by hybrid varieties. In the VASO project, a particular landrace 'Pigarro' was selected from the traditional populations cultivated in this region. Participatory plant breeding using mass selection was carried out each year and seed was stored from each selection cycle. Production and ear size increased and root lodging decreased along this selection process. Our present objective is to evaluate the impact of participatory plant breeding upon 'Pigarro' genetic diversity over an interval of 20 years of selection. For this purpose, 30 randomly selected individuals from each of three different selection cycles (1984, 1993 and 2004) were fingerprinted with 10 SSR markers uniformly distributed across the maize genome. Allelic richness and gene diversity will be assessed for each selection cycle and genic and genotypic differentiation among cycles will be tested and discussed.

## Keywords

participatory plant breeding, maize, open-pollinated varieties, genetic diversity

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## Prediction of Winter Wheat Crossing Performance (F4) on the Base of Parents

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### Abstract

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Winter wheat bulk populations in F3 and F4 generations and parental varieties, including control varieties were evaluated in two replications on three localities – Piestany, Viglas, Maly Saris in the years 2003/04, 2004/05. 18 hybrid populations and 10 parental varieties were evaluated in F3 generation, only 10 hybrid populations were selected but 17 parental varieties were included into F4 generation. High correlations were found among average parental values and their hybrid populations, for grain yield ( $r=0.646$ ), protein content ( $r=0.612$ ), grain hardness ( $r=0.914$ ), SPAD index ( $r=0.645$ ), but low correlations for wet gluten ( $r=0.283$ ). On the base of population average of parental varieties and varietal effects estimated from the set of parental varieties it is possible to forecast productivity of hybrid populations. Interaction genotype x environment decreased reliability of estimation of hybrid population productivity.

### Keywords

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winter wheat, parental varieties, bulk populations, prediction

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# An Integrated Data Standard for Genetic Data Types

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## Abstract

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In contrast to sequence annotation and microarray analysis, in the field of genetics, data standardization is still in its infancy. This is surprising, as characteristic genetic studies, such as QTL or association mapping, require the integrative co-analysis of different data types. Within a typical genetic data type, several, mutually incompatible, data formats exist, that are usually defined by the applied analysis software. Moreover, genetic datasets are usually composed sets from different data origins, which, by lack of standardization, have to be integrated manually with the risk of introducing errors and of losing background context.

The POS $\Sigma$  (Possum) initiative was set up to develop and promote an integrated data standard in the field of genetics. Within the POS $\Sigma$  community, consisting of developers and researchers of institutes and companies worldwide, an open data standard is under development that is capable of holding different data types. Central in POS $\Sigma$  is the development of a universal data file or POF (Portable Omics Format). The various data contents of the POF can be viewed using free reader software resembling the Acrobat reader functionality with the PDF standard. Methods to read and convert currently used file standards into POF will be provided. Third parties will be able to employ the data standard either by writing plug-ins for this reader, or by using the open reading/writing interface within their own software context. Use of this interface will be made as easy as possible in order to make it attractive for use in both commercial and academic software. In this way, the format can act as a reliable intermediate between various genetical analysis packages.

More info on the data standard and the Possum community is available at <http://www.possum-datastandard.org/>.

## Keywords

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data standardization, data exchange, data types, file formats

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# Molecular Marker Diversity of SCN Resistant Sources in Soybean

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## Abstract

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Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is one of the most destructive pests of soybean (*Glycine max* (L) Merr) in the United States. Over 100 SCN resistant accessions within the USDA Soybean Germplasm Collection have been identified, but little is known about the genetic diversity of this SCN resistant germplasm. The objective of this research was to evaluate the genetic variation and determine the genetic relationships among the SCN resistant accessions. One hundred and twenty-two genotypes were evaluated by 85 simple sequence repeat (SSR) markers from 20 linkage groups. Nonhierarchical (VARCLUS) and hierarchical (Ward's) clustering were combined with multidimensional scaling (MDS) to determine relationships among tested lines. The 85 SSR markers produced 566 allelic fragments with a mean PIC value of 0.35. The 122 lines were grouped into seven clusters by two different clustering methods and the MDS results are consistently corresponded to the assigned clusters. Assigned clusters were dominated by genotypes that possess one or more unique SCN resistance genes and were associated with geographical origins. The results of AMOVA showed that the variation differences among clusters and individual lines were significant, but the differences among individuals within clusters were not significant.

## Keywords

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soybean cyst nematode resistance, genetic diversity, SSR markers, cluster analysis, soybean

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# Use of Computer Image Analysis for Evaluation of Brown Rice Quality

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## Abstract

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Chalkiness is a major concern in the rice trade as it is one of the key factors in determining rice quality and price. A major cause of chalkiness is exposure to high temperature immediately after flowering and global warming is expected to exacerbate the problem in the future. While breeders are carrying out various studies to obtain a better understanding of the genetic and physiological mechanisms that govern chalkiness, evaluation of chalkiness is traditionally judged through human visual inspection and there is no standard objective method to effectively classify kernels into the different categories, such as white-core, white-back and white-based rice. Recent improvements in computer performance and significant reductions in the cost of digital imaging hardware and software are triggering the widespread use of digital image analysis in biological and agricultural research. This study evaluates the effectiveness of digital image analysis in measuring and categorizing chalkiness in brown rice and assesses its viability as an alternative procedure to human visual assessments. Transmitted light images of kernel were collected using image scanner so that the contrast between chalky and non-chalky area became clear. In order to evaluate localization of chalky area, the 256 mean grayscale values of pixels on the straight lines originating from the centre of gravity to the arbitrary points on the contour taken from 0 to 360 degree in a counter clockwise manner. Then, Fourier coefficients were computed by fast Fourier transformation to evaluate the sequential pattern of the mean grayscale values from 0 to 360 degree. Support Vector Machine (SVM) with a linear kernel on the Fourier coefficients generated an accuracy rate of approximately 75-90% in discriminating the level of chalkiness. The results show considerable promise of this technique for application in scientific research and breeding programs.

## Keywords

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chalkiness, Fourier transformation, image information, *Oryza sativa* L., Support Vector Machine

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# Identification of Chestnut (*Castanea sativa* Mill.) Genotypes by Neural Networks

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## Abstract

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The aim of our research was to find out the dependence of pollen germination of selected genotypes of chestnut (*Castanea sativa* Mill.) on a genotype and a date of pollen gathering. For evaluation were selected 10 genotypes from locality Radosina, the Slovak Republic. This orchard is historically important. There are one hundred years old trees cultivated from seeds. The selection of genotypes for our research was realized according to the variability in the phase beginning of flowering. The set of our genotypes were evaluated in a one quantitative trait (pollen germination %) in three repetitions and in a one qualitative trait (date of pollen gathering). The pollen was gathered according to a date of flowering of the individual genotypes. The experimental set of genotypes was then divided on two groups.

Methodologically was used the mathematical-statistical method Neural Networks for the testing of the dependence.

The most suitable neural network for determination of the pollen germination dependence on the genotype is the MLP 10-4-1, i.e. the multilayer perceptron neural network with 4 neurons in hidden layers.

Figured out from the results, the pollen germination is determinate by the genotype and the information about the date of pollen gathering is not necessary. It can be notified, that the genotype identification is possible by the pollen germination.

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-026704 Biological and reproduction characterization of neglected and by genetic erosion endangered plant species with economical utilization of their pollen.

## Keywords

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chestnut, *Castanea sativa* Mill., pollen germination, neural networks, genotype

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# Determination of Oil and Fiber Types of Flax (*Linum usitatissimum* L.) by Discriminate Analysis

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## Abstract

Our task was oriented on separation of flax genotypes (*Linum usitatissimum* L.) according to their economic use on oil and fiber flax. The task was realized on the set of 252 genotypes. A biological material was obtained from the Slovak National Gene Bank in Piestany (139 genotypes) and from the Agritec Ltd. Sumperk (113 genotypes) from Czech Republic. From the Agritec Ltd. at our disposal were 86 fiber genotypes and 27 oil genotypes. The experimental set of flax genotypes was evaluated and characterized at 23 quantitative traits. For separation of oil and fiber flax genotypes was used a discriminate analysis. In the case of the set of 113 genotypes was known economic use. The individual genotype was exactly classified to oil or fiber types. In the first step were selected two traits – Z2 technical length of stem (mm) and Z61 weight of thousand seeds (g). According to literature, in these traits genotypes of oil and fiber flax are distinctly distinguished. The discriminate analysis was performed at the base of these traits. The result of the discriminate analysis was to verified suitability of discrimination on two economic types of flax by these two traits and to obtained discriminate functions. The discriminate analysis correctly classified 104 genotypes i.e. 92% in the consistency with known economic use of 113 genotypes with use of traits (Z2, Z61). Obtained discriminate functions were used for separation of 139 genotypes, which economic use was not known. In the set of 139 genotypes were according to values of discriminate functions identified the 69 oil genotypes and the 70 fiber genotypes of flax. In the next step were used 23 evaluated traits for discriminate analysis. In the set of 113 genotypes was a difference of separation on oil and fiber flax minimal. Comparing with results for two traits (Z2, Z61) dissimilarly were classified only 9 genotypes. These new discriminate functions about 23 evaluated traits were used also for separation of the set of 139 genotypes. In this case were dissimilarly classified 44 genotypes in comparison with separation by two traits (Z2, Z61). The application of discriminate functions, calculated from two selected characters (Z2, Z61), is adequate for separation of set of flax genotypes according to their economic use. Figured out from the results, by application of discriminate functions calculated from two selected traits (Z2, Z61) is separation of genotype set (113 genotypes) according to economic use only 3% worse than in the case of all 23 evaluated traits. For a mathematical and statistical processing of data were used the program SAS. The results are obtained by support of the project no. aAV/1121/2004 Department of education of the Slovak Republic.

## Keywords

flax, *Linum usitatissimum* L., discriminate function, quantitative characters, genotype

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# Participatory Selection of Plantain-Like Hybrids in Ivory Coast

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## Abstract

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Plantain consumption growth rate reaches 3% in West Africa. Nevertheless, cultivated varieties are unproductive and susceptible to pests and diseases. In order to respond to the growing need and contributing to food security, 14 plantain-like hybrids resistant to the Black Sigatoka disease have been identified to be introduced into the traditional farming system. Plantain growers are cultivating the hybrids as part of their usual farming system based on the three main crops Cocoa, Rice and Yam. Prior to hybrids delivery, a preliminary survey was conducted in 5 regions in Ivory Coast to access to small scale farmers' preferences and labor conditions. The survey emphasized traditional knowledge of *Musa* diversity as well as traditional uses of the over organs of plantain. Ethnobotanic uses and diversity synonymy have been accessed as well. The National Extension Service (ANADER) plays a key role in the selection process. ANADER contributed in plantain growing zone selection and in managing assays at farmer level. Additional investigations are issued at research station level by a team composed of a breeder, a pathologist, a Socio-economist and a Post-harvest researcher. Peasant Field Days are organized at flowering and harvest. This participatory approach is facilitating plantain-like hybrids adoption by small scale growers.

## Keywords

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participatory selection, plantain, farming system, Ivory Coast

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# Handling Gene Bank Data Using the Rareness Parameter

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## Abstract

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Landrace collections should be evaluated from the breeding point of view and a population is valuable for plant breeders if they can find within it useful attributes for their program. The value of a population starts to increase if these levels of traits are of “low frequency” within the specific collection. Aiming to the measurement of this value, the parameter  $R$  (‘rareness’) was proposed. For each trait, a ‘partial rareness’ ( $R_i$ ) parameter was calculated using only those trait levels that can be considered as having ‘low frequency’. The parameter  $R$  is the sum of the ‘partial rarenesses’. Populations with high frequencies of the ‘lowest frequency’ levels of traits in the collection are characterized by high values of  $R$  and can, therefore, be more easily identified.

The “rareness” parameter was applied in order to evaluate and classify a collection of Greek faba bean landraces. Observation of ten morphological and ten agronomical traits was performed. For classification purposes UPGMA and Neighbor-joining cluster methods were applied with the use of the Manhattan dissimilarity coefficient. The populations with high frequencies of the ‘lowest frequency’ levels of traits in the collection were characterized by high values of  $R$  and could be identified. One group with populations that had the “low frequent” characteristics was the result of the classification.

## Keywords

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landrace, classification, evaluation, germplasm collection, heterogeneity, rareness

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## Use of Additive-Dominant Model in Genetic Analysis of some Quantitative Characteristics in Sunflower

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### Abstract

A simple link between the expected means of quantitative traits of different generations (progenies) can be established if the mean values are dependant exclusively on additive and dominant gene effects. This study aims to prove the correctness of the hypothesis that progeny means depend solely on additive and dominant genes, which can be tested in several ways, one of which is the applied tests. Seven  $F_1$  hybrids obtained by crossing five sunflower inbred lines were used to analyze the impact of genes with additive and dominant effects and their interactions on the inheritance of plant height, leaf number per plant and head diameter. The linkage among the expected progeny means was tested using the scaling tests method, while the estimates of gene effects and mode of inheritance were made by Generation Mean Analysis. The additive-dominant model proved adequate only for leaf number per plant in crosses  $C_5$  and  $C_7$ . Besides the main gene effects (additive and dominant), epistatic gene effects were also of great importance in the inheritance of all three traits. In the inheritance of plant height, duplicate epistasis between dominant increasers was found in  $C_2$ , while  $C_1$ ,  $C_4$  and  $C_7$  had duplicate epistasis between dominant decreaseers. In the case of leaf number per plant, we only found duplicate epistasis between dominant decreaseers in  $C_1$ ,  $C_3$  and  $C_6$ , while in the inheritance of head diameter both types of duplicate epistasis were found, namely duplicate epistasis between dominant decreaseers in  $C_1$  and duplicate epistasis between dominant increasers in  $C_2$ . It can be concluded that the additive-dominance model was not adequate in explaining the differences between various generation means. In addition to of additive and dominance gene effect, digenic epistatic effects were also found to be important in the inheritance of the characters studied. Therefore, geneticists and breeders working on sunflower should make provisions for the occurrence of epistatic gene effects in their research programs.

### Keywords

sunflower, quantitative traits, genetic analysis, additive-dominant model

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# Genetic Diversity and Relationships among Wild Olive Populations

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## Abstract

Wild olives (*Olea europaea* subsp. *europaea* var. *sylvestris*) include true oleasters (corresponding to the wild forms present in natural areas) and feral forms, which may be seedlings of the cultivated clones or result from hybridisation between the true oleasters and olive cultivars. The knowledge of the genetic variability of wild olive populations and the study of their relationships may be of great interest for their possible use as a new source of variability in breeding programmes. For that purpose, eight microsatellite markers have been used to study the genetic variation within and between 11 wild olive populations from Southern and North-Eastern Spain and mainland and insular Italy. Our study uncovered abundant allelic variation over eight loci with an average of 18.5 per locus alleles and high values of observed and expected heterozygosity (mean values of 0.717 and 0.843 respectively). Although most of the genetic diversity was attributable to differences among individuals within populations (91.8%), significant  $\phi$ -values among populations suggested the existence of genotypic differentiation. The analysis of structure allowed the detection of four different gene pools:  $O_S$ ,  $O_I$ ,  $F_1$  and  $F_2$  despite the overall low interpopulation differentiation of this tree species. The gene pools  $O_S$  and  $O_I$  predominantly found in southern Spain and Italian islands, respectively may correspond to genuine wild olive germplasm. The gene pools  $F_1$  and  $F_2$  detected in North-Eastern regions of Spain and in continental Italy likely represent feral gene pools. The existence of four gene pools and the evidence of differences between the genuinely wild olive germplasm and the feral forms were in overall concordance with the Fitch-Margoliash tree based on Nei's genetic distance.

## Keywords

wild olives, oleasters, feral forms, microsatellites, genetic structure

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## Evaluation of Gene Expression Data for Drought Stress in Common Bean

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### Abstract

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Drought is one of the major factors that affect the growth and development of plants. It is an important contributor to reducing the yield of food legumes; common bean is, compared to other grain legumes, moderately sensitive to water deficit. The plant cell responds to drought stress by the expression of genes encoding specific stress proteins with possible protective function or regulatory proteins involved in further regulation of signal transduction. While the list of genes which are induced or suppressed under drought stress has mainly been fulfilled for the model plant *Arabidopsis thaliana*, in common bean the first step was to identify the up- and down- regulated genes under drought stress. For the identification of differentially expressed transcripts we used a differential display RT-PCR method. cDNAs fragments were amplified using an anchored oligo-dT primer and short 10- to 13-mers arbitrary primers. Bands of interest, i.e. those differing between the patterns from control and drought stressed plants were recovered from the agarose gel, purified and sequenced. Identified differentially expressed transcripts were confirmed with the quantitative PCR. We designed specific oligonucleotide primers for the SYBR<sup>®</sup> Green I assay and validated 18S rRNA,  $\alpha$ -tubulin, actin 1 and ubiquitin for the reference gene (endogenous control). Quantitative PCRs were carried out on the Applied Biosystems 7500 Real Time PCR System. Gene expression data (Ct – threshold cycle) were evaluated using several statistical methods including the comparative Ct method and the standard curve method. Whether there is a significant difference between the drought-stressed and the control samples it was determined using the REST 2005 software tool (Hermann (Corbett Research) and Pfaffl, 2005). Different statistical models were also developed and tested by SAS GLM procedure.

### Keywords

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*Phaseolus vulgaris*, differential display, quantitative PCR, gene expression analysis

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# QTL Analysis of Resistance to *Mycosphaerella pinodes* in Pea

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## Abstract

*Mycosphaerella pinodes* causes necrotic spots on all aerial parts of the pea plant and is responsible for important yield and seed quality losses. The objective of this study was to gain a better knowledge of the genetic factors controlling pea resistance to *M. pinodes*. A population of 111 recombinant inbred lines (RILs), derived from the cross between *P. sativum* L. ssp. *syriacum* Berg (= *P. humile* Boiss and Nöe) line P-665 partially resistant to *M. pinodes* and the susceptible cultivar *P. sativum* L. cv. Messire was genotyped with morphological, isozyme, RAPD and STS markers.

Reaction to *M. pinodes* was evaluated in both growth chamber and field conditions after inoculation with a single monospore isolate from Córdoba, Spain. In growth chamber conditions disease rating was scored only on leaves according to a 0-5 scale (Ch) while in field conditions this disease rating scale was scored separately on leaves (L) and stems (St; on a 0-5 scale). In addition disease severity was assessed in the field by a visual estimation of the percentage of plant area covered by symptoms (Sa). Flowering dates (DF) were also scored, as the number of days to 50% bloom.

Of the 251 marker loci segregating in the RIL population, 246 could be mapped into nine linkage groups covering 1214 cM of the pea genome. Using composite interval mapping approach, five QTLs significantly associated with *M. pinodes* resistance were localized on linkage groups II, III, and V. Three QTLs (*mp2*, *mp4*, *mp5*) were detected on linkage group III. Some QTLs (*mp1* and *mp3*) were detected only when disease was checked in growth chamber conditions (Ch), some in both chamber and field conditions (*mp2* for Ch and Sa; *mp4* for Ch and St) while the QTL *mp5* was identified only in field conditions (for L, St, and Sa). Four QTLs for flowering date were also detected. The major QTL detected in field conditions (*mp5*) explaining 31, 42, and 46% of the phenotypic variation of L, St, and Sa, respectively, co-localized with the major QTL for flowering date (*df1*).

## Keywords

*Pisum sativum* L., *Mycosphaerella pinodes*, disease resistance, genetic mapping, quantitative trait loci

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# Adaptability of Introduced Grass-Clover Mixtures to Croatian Environments

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Vinko KOZUMPLIK

## Abstract

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Main goals in grass and clover breeding, aimed at their utilisation in mixtures are persistency, frost tolerance and competitiveness. The value of a particular cultivar in monoculture is not the indicator of the capacity of its performance in mixtures. Therefore, the insight into features of grass and clover cultivars in mixtures in specific growing conditions is important in selection of germplasm which will be used in further breeding work. The objectives of this investigation were to estimate *mixture x environment* interactions for important agronomic traits of fifteen clover grass mixtures, to assess germplasm valuable for breeding development of new cultivars, and to determine the most suitable mixtures for growing under specific environmental condition. A field trial was established in 1998 at three locations in Croatia (Vrbovec, Otočac, Medvednica). Fifteen grass-clover mixtures were evaluated. Five mixtures originated from Austria, three from Germany, six from Slovenia, and one from Croatia. Green matter yield, dry matter yield and crude protein content were studied in 1999, 2000 and 2001. Crude fibre and fat content were studied in 2000 and 2001. Various stability parameters were used for assessment of stability of investigated traits. Investigated mixtures varied significantly in the investigated traits, more in the green and dry matter yield, and less in the content of crude protein, crude fibre, and fat. Statistically significant differences were found between locations and years. Interactions *mixture x location*, *mixture x year*, *location x year* and *mixture x location x year* were also significant. Three mixtures appeared to be interesting for breeding purposes. Within these mixtures the most adaptable and the most persistent were *Dactylis glomerata* L. and *Festuca pratensis* among the grass species, and *Trifolium pratense* L. among the clover species. Low correlation between yield and protein content showed the possibility of developing new cultivars based on yield breeding without affecting protein content. Different mixtures were selected and recommended for commercial production for each of experimental location.

## Keywords

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grass-clover mixtures, agronomic traits, *mixture x environment* interactions

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# Using Stability Indices for the Assessment of Adaptation

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## Abstract

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Models used in the analysis of the genotype by environment interaction differ primarily in the level of specific adaptation which they can account for. Simple models, used as a basis for the estimation of various stability parameters, have their drawback in the assumption of no adaptation, or only general adaptation. Although they can be extended to allow for the assessment of specific adaptation of each genotype, it can lead to the models of extreme complexity. Avoiding (un)necessary complexity, number of studies in the past preferred probably simplest model for the assessment of adaptation, i.e. Finlay-Wilkinson (FW) model. Even at the present moment, despite of many specialized computer programs designed to support the complex models, and waste literature to help with the interpretation, plant breeders still tend to favor simple FW model.

This need for simplification inspired some authors to create stability indices. They require even less computation than stability parameters, however, they did not gain much popularity, and were only obscurely used in rare studies. Comparing the values of a wide range of measures of stability and adaptation, estimated using yields from the cereal variety trials, we have previously detected very high correlation between the two stability indices (yield ranges – Langer et al. 1979) and coefficient of regression from the FW model. Conclusions from that study had only limited value, because they were based on a low number of environments. Therefore, in this study we will try to obtain more environments by combining years to form clusters of three consecutive years. Besides real data, we will also use simulations in order to establish the limits for the use of stability indices for the assessment of the genotypic adaptation.

## Keywords

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stability indices, adaptation, simulation

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# Statistical Approaches to Analyse Genebank Data Using a Lentil Germplasm Collection as a Case Study

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## Abstract

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Normally in a plant gene bank a large number of accessions per each crop and/or taxon is stored. During their characterization and preliminary evaluation, several quantitative and qualitative data are recorded and, usually, a wide intra accession variation is observed. The management of all this information becomes very difficult without effective statistical methods combining these different types of data. At the Institute of Plant Genetics, CNR, in Bari (Italy) this problem has been tackled testing many statistical approaches. The present contribution describes one of these approaches, which to date has proven to be highly adequate; a case study describing a lentil germplasm collection has been used for demonstration. A valuable application of this method is the determination of core subsets important to increase the utilization and accessibility of plant genetic resources.

The application of a statistical method able to cluster similar accessions in a reliable way can be the starting point for further analyses on the germplasm available in genebanks. Characterization and preliminary evaluation data generally relate to agronomic performance, but give little information on the genetic constitution of the examined material. Conversely, molecular markers precisely define the genetic constitution of a sample, but give no information on productive attitude. In the presented case study, therefore, a subset of the lentil germplasm collection was chosen to perform molecular analysis based on ISSR markers. The samples were selected on the basis of both morpho-agronomic evaluation and geographical origin. These markers proved to be useful for distinguishing among closely related genotypes and for possibly substantiating the genetic peculiarity of some interesting material.

## Keywords

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statistical method, agrobiodiversity, ISSR markers, germplasm collection, lentil

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# Yield Stability Analysis of Maize Single Crosses by Different Nonparametric Methods

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## Abstract

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According to existence of different climate conditions in corn fields of Iran, determination of varieties with more stability and identification of adaptation patterns including specific or general adaptation is very importance for maize agronomists and plant breeders. One of the simplest and efficient approaches to determine stability is nonparametric methods so, twelve maize (*Zea mays* L.) Hybrids were assessed at 24 environments including twelve locations and two years in IRAN by the Institute of Plant Breeding and Improvement Research. The experiments layout was a randomized complete block design with four replications. Statistical analysis was done on biological maize yield. Simple and combined analysis of variances showed that there were significant differences between genotypes. Seven different nonparametric methods were used to analyze hybrid stability and then were compared. Between all genotypes, SC 725, SC 726 and SC 73 had a mean yield greater than 11, 7 kg/ha. Almost all nonparametric measures showed Sc 76 as the most stable hybrid. Finally different measures were compared and correlations between them were accounted.

## Keywords

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stability analysis, nonparametric methods, maize

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# Management of Research Departmental Data

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## Abstract

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DORIANE SAS has developed an expertise in database management for vegetal agronomy since its creation in 1984. Thanks to the contribution of several generations of developers and analysts and the strong relationship with industrials involved in vegetal breeding in a large range of crops and in laboratory activities, DORIANE concepts are now recognised as leaders in the domain. The resulting tool, called LABKEY™, is an integrated data management standard software organised in modules. These modules are organized in three groups 1) management of organisms (plants, animals, micro-organisms) with LABKEY-SELEC (breeding), LABKEY-EXPE (seed-testing) and LABKEY-PLANNING (promotion and dispatching); 2) management and analysis of samples with LABKEY-EXPE (analytical), LABKEY-FLOW (sample process) and LABKEY-MOLECULAR-MARKERS; 3) common tools with LABKEY-AUTOMATE (tasks, chaining, connection to apparatus, robot piloting), LABKEY-STAT (agronomy, molecular markers, ACP, graphics), LABKEY-TOOLS (report, labels, interfaces, presentation), LABKEY-MOBILE (hand held data collect), LABKEY-FINANCE. LABKEY follows the concept of Research Resource Planning (RRP), derived from Enterprise Resource Planning (ERP), where each resource must have only one occurrence in the database and all resources can be compared. Its client-server architecture facilitates the transmission of information between researchers and laboratories.

Two innovative concepts\* were found to solve 1) the complexity of breeding and laboratory activities with the management of huge amount of data, 2) the flexibility required to handle crops with very different biology or to handle very different experiment protocols in the laboratory, 3) the traceability management necessary for industrials and 4) the security and performance of the SQL standard database able to run on low cost PC server platform.

The first concept is described as “experiment cycle” in relationship with the research department activity. The materials (seeds, plants, DNA, molecular markers, reagents...) belong first to a “stock system”. Then these materials are grouped to be set up in one or several field or laboratory experiments. These experiments follow one protocol or process depending on the action we want to realise on this group of materials (crosses, self polination, PCR, specific measurements, simple observations...). New materials can be automatically generated and reintroduced in the stock and close the experiment cycle. Then a new experiment cycle can start.

The second concept is called “multi-level architecture” in relationship with the hierarchical organisation of experiments and stocks. This concept permits to link and store data with a level of observation which can be more or less accurate (level genotype, level plant, level flower, level molecular marker, level allele...).

These concepts make it possible to constitute a well organised library adapted for the whole research department and a powerful tool for assisted decision making. Currently, LABKEY is used by about 250 users in fifteen industrials companies.

## Keywords

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biotechnology, breeding, database, experiments management, molecular markers

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