# Isozymes as genetic markers in maize breeding

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## Abstract

The major objective of the research is to identify and locate quantitative trait loci ($QTL_s$) in the Yugoslav maize population. The plants (F2) were selected for the analysis at seedling stage and were selfed to obtain F3 generation. The analysis covered about 15 enzymes controlled by about 30 loci. The seeds of F3 family planted in the greenhouse for measuring some quantitative traits, recorded tasselling and silking during vegetation. At the end of vegetation grain yield, and some other quantitative traits of grain in F3 family were assessed. The relationship between marker loci and the loci for quantitative traits ($QTL_s$) were estimated by computerized statistical method.

*Additional key words*: allozymes, marker loci, quantitative traits, *Zea mays* L.

## Introduction

Breeding programs for agricultural crops are geared toward improvements in quantitatively inherited traits. These traits are polygenic so that the conventional biometric methods are only capable of assessing the total effect of genes that control certain quantitative traits. Enzymic polymorphism and restriction fragment length polymorphism (RFLP), allow the identification of individual genes which control certain quantitative traits, the assessment of the effect of individual genes, as well as the establishment of their locations on chromosomes.

Association between allozyme loci and the loci for several quantitative traits showed that the most allozyme loci are closely associated with quantitative traits, that a single allozymic marker locus may explain for more than 17 % of phenotypic variability, and that the cumulative effect of marker loci stands for 8 % to 40 % of total phenotypic variability (Stuber and Edwards 1986, Edwards *et al.* 1987, Stuber *et al.* 1987, Stuber 1989).

Some of the physiologically different quantitative traits concerning maize development and its yield are mapped by using molecular markers. So the putative associations of developmental genes generally coincide with the location of homeotic genes (Khavkin and Coe 1997). Drought-stressed plants as quantitatively inherited trait, controlling leaf abscisic acid (ABA) concentration in maize, are tested under drought conditions using genetic map with different RFRP loci (Tuberosa *et al.* 1998).

These investigations established the connection between marker loci and loci for quantitative traits of maize both in general and in Yugoslav germplasm. Allozymic genotypes were determined in some of the F1 hybrids, which had been developed at the Institute of Field and Vegetable Crops in Novi Sad. The associations between genotypes at enzyme marker loci and quantitative traits in F3 families of a single-cross hybrids of maize were investigated.

## Materials and methods

A group of F1 hybrids was analyzed for polymorphism of 21 isozymic loci. Two hybrids were chosen for further work, those with largest numbers of heterozygous loci. The two F1 populations were selfed in field to produce F2 populations for further study.

The assessment of quantitative traits was made on the basis of allozymic loci for seedlings of F2 populations, and after that the same plants were transplanted in a greenhouse for 10 d and then to a field until the end of the vegetation.

*Received* 23 July 1999, *accepted* 6 December 1999.
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Table 1. Enzymes used to characterise maize hybrids.

| Enzymes | Locus | Chromosomal location |
|---|---|---|
| Aconitase | Aco 1 | 4 |
| Acid phosphatase | Acp 1 | 9L |
| Alcohol dehydrogenase | Adh 1 | 1L |
| Arginine aminopeptidase | Amp 1 | 1 |
| Diaphorase-glucosidase | Dia 1 | 2 |
| Diaphorase-glucosidase | Dia 2 | 4 |
| Glutamate oxaloacetate transaminase | Got 1 | 3L |
| Glutamate oxaloacetate transaminase | Got 2 | 5L |
| Hexokinase | Hex 2 | 6L |
| Isocitrate dehydrogenase | Idh 1 | 8L |
| Isocitrate dehydrogenase | Idh 2 | 6L |
| Malate dehydrogenase | Mdh 1 | 8L |
| Malate dehydrogenase | Mdh 2 | 6L |
| Malate dehydrogenase | Mdh 3 | 3L |
| Malatedehydrogenase | Mdh 5 | 5S |
| Glucosidase | Glu 1 | 10L |
| Phosphoglucomutase | Pgm 1 | 1L |
| Phosphoglucomutase | Pgm 2 | 5S |
| 6-phosphogluconate dehydrogenase | Pgd 1 | 6L |
| 6-phosphogluconate dehydrogenase | Pgd 2 | 3L |
| Phosphohexose isomerase | Phi 1 | 1L |

Each family contained up to seven plants in one row. During vegetation period some morphological observation were made. The families were selfed and cultivated until the full maturity of seeds.

One hundred families of NS-B originated population were planted in a greenhouse to produce F4 generation ($S_2$) during winter period.

At the stage of full development, the ear leaf was sampled from all plants of F2 populations and stored in ultra-deep freezer for subsequent analyses. Fresh mass of these samples will be used for DNA isolation and RFLP analyses which will, together with alozymic marker, serve to establish their eventual association with quantitative characters primarily the yield.

The hybrid labelled NS-B was chosen according to the maximum number of segregating allozyme loci.

Coleoptile tissue from 5 d old F2 seedlings was sampled and used for electrophoretic analyses according to Stuber *et al.* (1988).

Genotypes at 21 enzyme loci (Table 1) were determined by starch gel electrophoresis for maize hybrids. For NS-B hybrids only six loci were segregated: Aco 1 - 1/4; Acp 1 - 2/4; Idh 2 - 4/6; Mdh 2 - 3/6; Pgm 1 - 9/16; Phi 1 - 4/5.

Quantitative traits were measured for each plant including mass, plant height, total number of leaves on plant, ear leaf area, plant grain mass, top ear grain mass, top ear length, mass of 100 kernels, moisture of kernels at harvest, grain index (grain mass/ear mass).

Table 2. Mean values of homozygous and heterozygous marker classes and variation values of $R^2$ calculated for each marker locus and for plant height, total number of leaves, and ear leaf area. Significance level of $R^2$ determined by F-test: ** - $P = 0.001$, PD - partial dominance; D - dominance, OD - over dominance.

| Trait | Marker locus | Marker classes $P_1$ homozygote | $R1^2$ | heterozygote | $P_2$ homozygote | $R2^2$ | F-test | gene action |
|---|---|---|---|---|---|---|---|---|
| Plant height [cm] | Aco1 | 189.8 | 1.26 | 181.1 | 193.6 | 0.67 | ** | - |
| | Acp1 | 194.0 | 0.54 | 193.1 | 164.4 | 1.26 | ** | D |
| | Idh2 | 180.8 | 1.31 | 188.3 | 191.6 | 0.56 | ** | D |
| | Mdh2 | 184.4 | 0.95 | 189.4 | 189.1 | 0.98 | ** | D |
| | Pgm1 | 185.3 | 1.09 | 185.7 | 192.9 | 0.79 | ** | -PD |
| | Phi1 | 181.4 | 1.38 | 188.3 | 189.4 | 0.53 | ** | D |
| Number of leaves [plant$^{-1}$] | Aco1 | 10.1 | 1.09 | 10.6 | 11.2 | 0.40 | | |
| | Acp1 | 11.0 | 1.00 | 10.0 | 10.5 | 0.87 | ** | - |
| | Idh2 | 10.7 | 0.92 | 10.8 | 10.6 | 0.92 | ** | OD |
| | Mdh2 | 10.6 | 1.07 | 10.7 | 10.7 | 0.86 | ** | D |
| | Pgm1 | 10.5 | 1.07 | 10.8 | 10.9 | 0.82 | ** | D |
| | Phi1 | 10.9 | 0.01 | 10.7 | 10.8 | 0.06 | ** | - |
| Ear leaf area [cm$^2$] | Aco1 | 393.1 | 1.12 | 386.7 | 424.4 | 0.79 | ** | - |
| | Acp1 | 380.7 | 0.86 | 422.5 | 374.2 | 1.09 | ** | OD |
| | Idh2 | 380.4 | 0.65 | 423.7 | 410.6 | 1.27 | ** | OD |
| | Mdh2 | 385.0 | 0.96 | 436.5 | 392.1 | 0.98 | ** | OD |
| | Pgm1 | 377.8 | 1.40 | 437.2 | 359.6 | 0.53 | ** | OD |
| | Phi1 | 381.5 | 11.32 | 407.1 | 503.0 | 8.85 | | |

Table 3. Mean values of homozygous and heterozygous marker classes and variation values of $R^2$ calculated for each marker locus and for plant grain mass, top ear grain mass, and top ear length. Significance level of $R^2$ determined by F-test: ** - $P = 0.001$, PD - partial dominance; D - dominance, OD - over dominance.

| Trait | Marker locus | Marker classes $P_1$ homozygote | $R1^2$ | heterozygote | $P_2$ homozygote | $R2^2$ | F-test | gene action |
|---|---|---|---|---|---|---|---|---|
| Grain mass [g plant$^{-1}$] | Aco1 | 56.8 | 1.18 | 45.2 | 51.4 | 0.75 | ** | - |
| | Acp1 | 73.8 | 0.66 | 52.2 | 36.0 | 0.62 | | |
| | Idh2 | 60.1 | 0.74 | 43.4 | 49.0 | 1.14 | ** | - |
| | Mdh2 | 51.0 | 1.21 | 41.3 | 56.9 | 0.72 | ** | - |
| | Pgm1 | 41.5 | 1.13 | 50.2 | 65.9 | 0.36 | | |
| | Phi1 | 46.8 | 1.37 | 46.3 | 56.8 | 0.49 | ** | -D |
| Top ear grain mass [g ear$^{-1}$] | Aco1 | 84.5 | 1.12 | 73.7 | 72.7 | 0.77 | ** | -D |
| | Acp1 | 97.3 | 0.80 | 78.7 | 59.0 | 0.55 | | |
| | Idh2 | 80.6 | 0.89 | 70.5 | 77.9 | 1.05 | ** | - |
| | Mdh2 | 78.9 | 1.06 | 68.5 | 85.5 | 0.87 | ** | - |
| | Pgm1 | 68.8 | 1.65 | 75.9 | 78.4 | 0.24 | ** | PD |
| | Phi1 | 70.7 | 1.51 | 74.3 | 87.3 | 0.28 | ** | -D |
| Top ear length [cm] | Aco1 | 12.3 | 0.86 | 12.3 | 13.5 | 1.02 | ** | -D |
| | Acp1 | 13.2 | 1.22 | 13.3 | 11.9 | 0.65 | ** | D |
| | Idh2 | 12.0 | 0.93 | 13.0 | 13.0 | 0.96 | ** | D |
| | Mdh2 | 12.6 | 0.76 | 12.9 | 12.9 | 1.18 | ** | D |
| | Pgm1 | 12.8 | 0.88 | 12.5 | 15.5 | 0.67 | ** | -D |
| | Phi1 | 12.4 | 1.19 | 13.0 | 12.8 | 0.73 | ** | D |

Table 4. Mean values of homozygous and heterozygous marker classes and variation values of $R^2$ calculated for each marker locus and for kernel mass, moisture at harvest, and grain index. Significance level of $R^2$ determined by F-test: ** - $P = 0.001$, PD - partial dominance; D - dominance, OD - over dominance.

| Trait | Marker locus | Marker classes $P_1$ homozygote | $R1^2$ | heterozygote | $P_2$ homozygote | $R2^2$ | F-test | gene action |
|---|---|---|---|---|---|---|---|---|
| Mass [g kernel$^{-1}$] | Aco1 | 35.0 | 1.37 | 34.4 | 33.0 | 0.54 | ** | A |
| | Acp1 | 35.6 | 1.19 | 34.0 | 32.1 | 0.63 | ** | A |
| | Idh2 | 35.7 | 1.18 | 34.0 | 33.2 | 0.72 | ** | A |
| | Mdh2 | 33.7 | 0.76 | 33.1 | 37.3 | 1.05 | ** | -D |
| | Pgm1 | 33.8 | 1.10 | 33.1 | 31.7 | 0.80 | ** | D |
| | Phi1 | 33.4 | 1.20 | 34.0 | 34.4 | 0.72 | ** | D |
| Kernel moisture [%] | Aco1 | 49.2 | 1.16 | 55.2 | 54.6 | 0.61 | * | OD |
| | Acp1 | 50.2 | 0.43 | 55.0 | 54.0 | 1.42 | ** | OD |
| | Idh2 | 55.1 | 0.62 | 51.6 | 56.2 | 1.32 | ** | - |
| | Mdh2 | 57.1 | 1.13 | 52.1 | 53.6 | 0.74 | ** | - |
| | Pgm1 | 54.7 | 1.19 | 50.9 | 51.9 | 0.69 | ** | - |
| | Phi1 | 54.6 | 1.51 | 53.3 | 54.6 | 0.43 | ** | - |
| Grain index | Aco1 | 0.65 | 0.37 | 0.53 | 0.58 | 1.53 | ** | - |
| | Acp1 | 0.67 | 0.31 | 0.60 | 0.55 | 1.38 | * | A |
| | Idh2 | 0.70 | 0.46 | 0.57 | 0.61 | 1.26 | * | - |
| | Mdh2 | 0.62 | 1.36 | 0.55 | 0.67 | 0.56 | ** | - |
| | Pgm1 | 0.57 | 1.25 | 0.58 | 0.69 | 0.16 | | |
| | Phi1 | 0.62 | 1.45 | 0.55 | 0.65 | 0.44 | ** | - |

The association coefficients between each of segregating marker loci and quantitative traits were calculated. For each genotypic class at each marker locus, a mean value for each of analyzed traits was computed. Then, for each marker locus and each trait, a single factor analysis of variance was computed for the estimation of the significance level of the variations among marker-locus class means. F-test was used as a measure of significance to indicate segregation of genotypes at loci for quantitative traits and linkage to the marker locus. The variation attributed to each marker-locus was considered as a proportion of the total variation for each trait and this proportion was the association value of $R^2$.

## Results and discussion

Relation between marker loci and phenotypic expression of quantitative traits is given as total variance values ($R^2$):

$$R^2 = \frac{S^2T}{S^2n}$$

Where: $S^2T$ - square standard deviation for marker locus, $S^2n$ - total square standard deviation, $R^2$ were calculated for each marker locus and parental marker class for several quantitative traits and yield-related traits in $F_3$ family plants (Tables 2, 3, 4).

Segregating marker loci are: Aco1 (chromosome 4), Acp1 (chromosome 9L), Idh2 (chromosome 6L), Mdh2 (chromosome 6L), Pgm1 (chromosome 1L) and Phi1 (chromosome 1L).

$R^2$ values were used to indicate the association of each segregation marker loci and quantitative traits. The values are in the range from 0.01 to 1.53 except for ear leaf area and Phi1 marker locus where $R^2$=11.32, but F-test was not significant. Distribution is not high but F-test indicates significant probability level.

According to Edwards *et al.* (1987) small $R^2$ values may reflect either quantitative trait loci ($QTL_s$) having only a small effect, or $QTL_s$ having a larger effect but being more loosely linked to the marker locus.

Gene action represents depression of inbreeding of $F_3$ selfed plants with the stress laid more to yield-related traits than to the traits like plant height, number of leaves and ear leaf area.

Mišević *et al.* (1990) made an attempt to find the association between allozyme marker loci and new favourable alleles for grain yield in maize but they failed in most of the cases. They suspected that some or all of the analysed enzyme loci were not linked to loci for grain yield estimated in the studied germ plasm.

Extensive investigations conducted by Stuber *et al.* (1987), Stuber (1989) on associations between allozymic loci and the loci for some quantitative traits showed that some of the loci were closely associated with quantitative traits, that a single allozymic marker locus may explain for more than 17 % of phenotypic variability and that the cumulative effect of marker loci stands for 8 - 40 % of total phenotypic variability.

The studies in maize are being conducted for many years now. The isoenzymes and their allelic variants were studied and on the basis of their loci the relation with quantitative traits such as yield was found (Stuber 1997). This author focused on studying the phenomena of genetic basis, such as heterosis and interaction between genotypes and environment.

Determination of genetic diversity on the basis of molecular markers such as allozyme and RFLP could serve for expression of heterosis in selection programs. Searching for extremely diverse parents on the basis of genetic markers is used for creation of new population, and gene location affecting the morphological traits is studying for individual plants of F2 populations and their F3 progenies (Lee 1992).

Allozymes and RFLP in particular are reliable tools for understanding of plant genome organisation and for marking of genes for agronomic significant traits (Young *et al.* 1992).

Covering of genome with these marker loci is small, and quantitative trait loci, especially yield-related traits loci are polygenic and distributed over all genome. Limitation of distribution of variation values that is significant indicates a kind of association between some of marker loci and quantitative trait loci.

## References

Edwards, M.D., Stuber, C.W., Wendel, J.F.: Molecular facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. - Genetics **116**: 113-125, 1987.

Khavkin E., Coe, E.: Mapped genomic locations for developmental functions and $QTL_S$ reflect concerted groups in maize (*Zea mays* L.). - Theor. appl. Genet. **95**: 343-352, 1997.

Lee, M.: [Development and application of DNA - based marker mutations for *Sorghum* improvement.] - In: Seminar on the Use of Mutation and Related Biotechnology for Crop Improvement in the Middle East and the Mediterranean Regions. Pp. 46-47. Zaragoza 1992. [In Span. ]

Mišević, D., Gerić, I., Tadić, B.: Allozyme marker loci associated with favourable alleles for grain yield in maize - Theor. appl. Genet. **80**: 518-522, 1990.

Stuber, C.W.: Marker-based selection for quantitative traits. - In: Science for Plant Breeding. Proc. of the XII Congress of Eucarpia. Pp. 31-50. 1989.

Stuber, C.W.: 25 years of searching and manipulating $QTL_S$ in maize. - In: Tsaftaris, A.S. (ed.): Genetics, Biotechnology and Breeding of Maize and Sorghum. Pp. 43-51. 1997.

Stuber, C.W., Edwards, M.D.: Genotypic selection for improvement of quantitative traits in corn using molecular marker loci. - In: Proc. of 41st Annual Corn and Sorghum Research Conference. Pp. 70-83. 1986.

Stuber, C.W., Edwards, M. D., Wendel J. F.: Molecular marker-facilitated investigations of quantitative trait loci in maize. II Factors influencing yield and its component traits. - Crop Sci. **27**: 639-648, 1987.

Stuber, C.W., Wendel, J.F., Goodman, M.M., Smith, S.C: Techniques and scoring procedures for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). - N.C. State Univ. Tech. Bull. **286**: 1-95, 1988.

Tuberosa, R., Sanguineti, M.C., Landi, P., Salvi, S., Casarini, E., Conti, S.: RFLP mapping of quantitative trait loci controlling abscisic acid concentration in leaves of drought-stressed maize (*Zea mays* L.). - Theor. appl. Genet. **97**: 744-755, 1998.

Young, N.D., Fatokun, C., Menancio-Hautea, D.: Genome mapping in cowpea and mungbean with $RFLP_s$. - In: Seminar on the Use of Mutation and Related Biotechnology for Crop Improvement in the Middle East and Mediterranean Regions. Pp. 48-49. Zaragoza 1992.