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ISOZYMIC VARIABILITY OF SELFPOLINATED SUNFLOWER (*Helianthus annuus* L.) LINES

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SUMMARY

Genetic variability in 22 selfpolinated sunflower lines resulting from NS selection was studied on the level of isozymes. Isozymes as genetic markers were used for estimation of breeding material, genetic identification and potential for its usage for hybrid development. On the basis of seven enzymic systems and seven loci from which certain isozymes were obtained, the homozygous lines and their variability were found. Seven polymorphous isozymic loci: Est1, Mdh2, Pgd1, Phi1, Pgm4, Gdh1 and Apc1, and 2-3 alleles of each locus were used for genetic identification. Genetic distance between the analyzed genotypes was determined on the level of a group of genetic markers and it presented a proof of their efficiency and reliability.

Key words: Genetic markers, *Helianthus*, isozymes, genetic variability, inbred lines.

INTRODUCTION

Usage of genetic markers such as isozymic loci and restriction fragment length polymorphism (RFLPs) presented a supplement to breeding and seed science and their conventional methods for development and better understanding of genetic base of divergency and variability of quantitative and qualitative traits.

Polymorphism of enzymes and isozymes as gene markers was an efficient way in studying breeding material and its genetic characteristic. Development of new genotypes depends very much on the starting material. Final results of selection depend first of all on variability level of source breeding material and parent components. The aim of this investigation was to make use of heterosis as a biological phenomenon, and it dependency on divergency of parent lines. Heterosis is a result of maximal organism heterozygosity, which was decreased by intensive selection. There has been a world-wide tendency for many years of using biological markers for studying genetic variability in plant breeding and in

seed science. Application of molecular markers in breeding programs presents a modern approach in genetics and plant breeding. Introduction of genetic markers is aimed at recognizing potential parental pairs (Hughes et al.,1992).

At the beginning, study of isozymes related to genetics of sunflower was smaller as compared with other field crops (Torres, 1983). However, as sunflower gained a greater significance as a main source of quality oil, application of isozymes as genetic markers became more intensive. Polymorphism of reserve and functional proteins of sunflower seed started gaining greater significance for identification and registration of inbred lines and their genetic purity, uniformity and variability (Anisimova et al., 1991). Lines of the same origin are very similar on the genetic level, so a large number of markers, from which some could be used for genotype differentiation, should be introduced.

Besides isozymes as genetic markers, several new molecular markers were introduced in sunflower restriction fragment length polymorphism (RFLPs) (Riesberg et al., 1990). They presented a proof that molecular and chromosomic evidences had a great advantage over morphological data for phylogenetic and systematic inference (Palmer et al., 1988).

The aim of this investigation was to determine genetic characteristics of certain selfpolinated genotypes and their variability using efficient and reliable polymorphic loci and their genetic markers-isozymes.

MATERIAL AND METHODS

The endosperm of individual seeds of selfpolinated sunflower lines (*Helianthus annuus* L.) was analyzed. The homozygosity of the lines was determined on the basis of 10 samples analyzed for seven isozyme loci.

Table 1: Electrode and gel buffers

System	Electrode buffer	Gel buffer
I	0.065 M L-histidine 0,02 M Citric acid pH 5.7	0.009 M L-histidine 0.003 M Citric acid pH 5.7
II	0.3 M Boric acid 0.1 M NaOH pH 8.6	0.015 M Triszma base 0.0035 M Citric acid pH 7.8

As genetic markers, isozymes as direct products of 7 loci: Acp1, Est1, Gdh1, Mdh2, Phi1, Pgd1 and Pgm4, each with 2-3 alleles, were analyzed. Electrophoretic analysis of the isozymes was done using starch gel (12%), on two systems (Table 1).

I L histidine; pH 5.7 (Cardy et al., 1981)

II Tris-citric - Na-borate; ph 7.8 and 8.6 (Torres and Diedenhofen, 1976).

These two systems were used for analysing sunflower lines on the level of polymorphism of the following enzymic systems: ACP (acid phosphatase); EST (esterase), GDH (glutamate dehydrogenase); MDH (malate dehydrogenase); PGD (6-phosphogluconate dehydrogenase); PHI (phosphohexosoisomerase) and PGM (phosphoglucomutase) (Table 2).

Table 2: Active isozymes used for characterization of sunflower genotypes

Enzyme	Locus	Alleles	Buffer system
Acid phosphatase (ACP)	Acp1	S,F,I	II
Esterase (EST)	Est1	S,F,vF	I
Glutamate dehydrogenase (GDH)	Gdh1	S,F	II
Malate dehydrogenase (MDH)	Mdh2	S,F	I
Phosphohexose isomerase (PHI)	Phi1	S,F	I
6-Phosphogluconate dehydrog. (6-PGD)	Pgd1	S,F	I
Phosphoglucomutase (PGM)	Pgm4	S,F	I

Buffer system I (Cardy et al., 1981)
Buffer system II (Torres, Diedenhofen, 1976)

Part of endosperm of individual seeds was extracted in Tris-citric buffer with 0.1% PVP (pH 7.0), 40 µl/sample and this crude extract was deposited onto gel in order to achieve separation of proteins at the constant power strength (15mA - I and 10 mA - II).

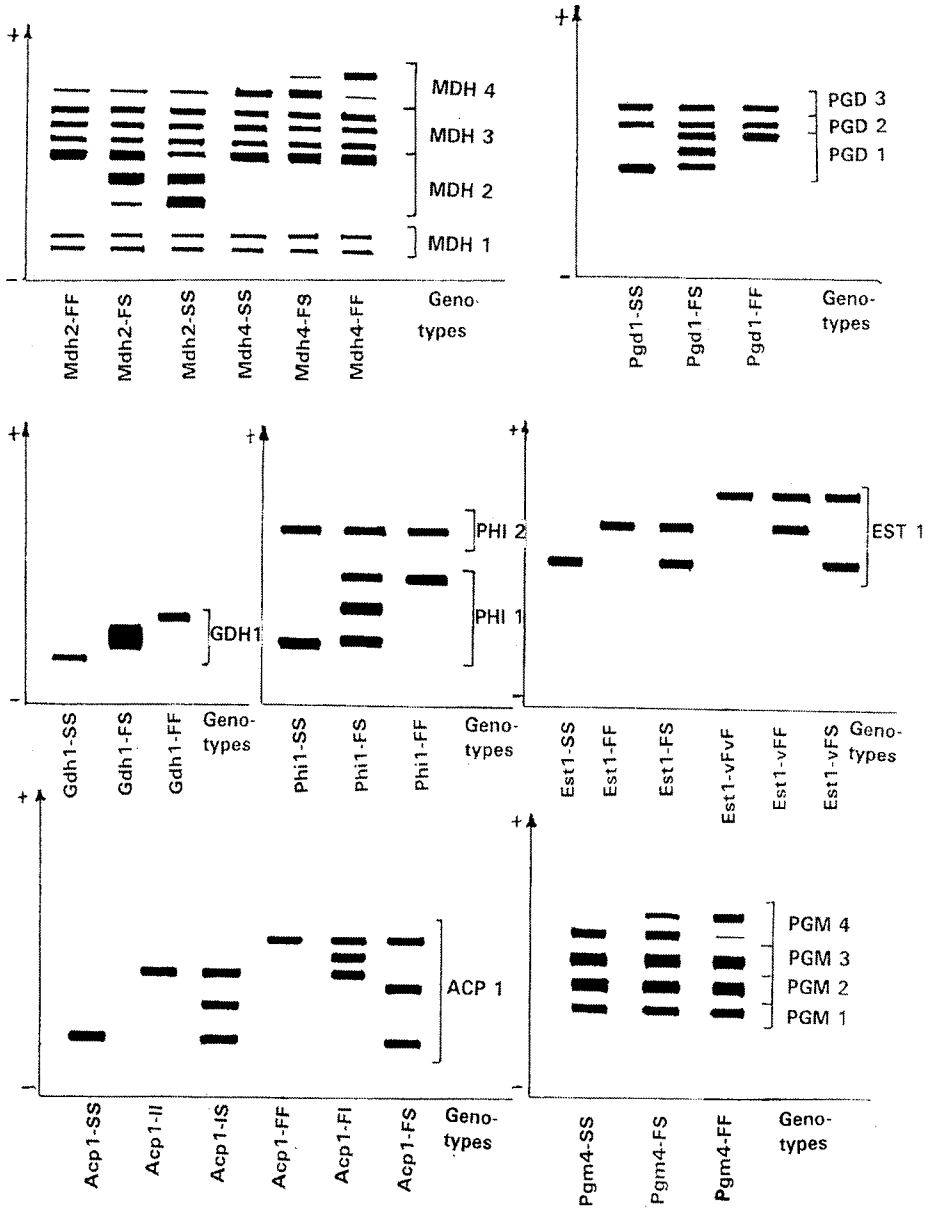
After electrophoretic separation was done, gels were divided into several slices for incubation of the analyzed enzymic systems.

Dyed isozymic tapes presented gene expression and genotypes were read off on the basis of the seven polymorphic loci, according to Kahler and Lay (1985) and Levites (1986).

RESULTS AND DISCUSSION

On the basis of seven isoenzymic loci and their alleles, 22 inbred sunflower lines were chosen (Table 3). According to certain number of samples for each genotype, genetically homozygous lines were separated by analyzed loci from larger groups. Polymorphism of all seven enzymic systems was presented by 1-4 isozymes, as gene expression (Scheme 1). Polymorphism of EST, ACP, GDH, PGD, PHI and PGM was genetically defined by one locus, and MDH by two loci. The highest degree of polymorphism was found in MDH and PGM systems and, accordingly, they were most suitable for determination of genetic characteristics of breeding materials and their final products.

The analyzed genotypes, selfpollinated lines, homozygous on the level of genetic markers, were determined on the basis of alleles for each locus. This pointed out the genetic identification and genotype variability, which was one of



Scheme 1.Polymorphism of enzymic systems: MDH, PGD, GDH, PHI, EST, ACP and PGM

Table 3: Genetic identification of sunflower lines from NS collection

	GENE						
Line	Est1	Mdh2	Pgd1	Phi1	Pgm4	Gdh1	Acp1
2A	FF	SS	FF	FF	SS	SS	SS
5	FF	FF	SS	FF	SS	SS	SS
5A	FF	SS	SS	FF	FF	SS	SS
7	SS	SS	SS	FF	SS	SS	FF
7A	FF	SS	SS	SS	SS	SS	SS
8	SS	SS	SS	FF	SS	FF	SS
8A	FF	SS	SS	FF	SS	SS	SS
9	SS	SS	SS	FF	SS	FF	SS
11A	FF	SS	SS	SS	SS	SS	SS
14A	SS	SS	FF	FF	SS	FF	SS
16A	SS	FF	FF	FF	SS	SS	SS
17	SS	SS	SS	FF	FF	FF	SS
20A	FF	SS	FF	SS	SS	SS	SS
21	FF	FF	FF	FF	SS	SS	FF
41	SS	SS	SS	SS	SS	FF	SS
49	vFvF	SS	SS	FF	SS	SS	SS
57	SS	SS	FF	FF	SS	SS	FF
59	FF	SS	FF	FF	SS	SS	FF
61	FF	SS	FF	FF	SS	SS	FF
67	FF	FF	SS	FF	SS	FF	FF
73	FF	SS	SS	FF	FF	SS	FF
4	FF	SS	SS	FF	SS	SS	FF
Gen. freq.	FF-13 SS-8 vFvF-1	FF-4 SS-18	FF-8 SS-14	FF-18 SS-4	FF-3 SS-19	FF-6 SS-16	FF-8 SS-14

the ways for their introduction into the improvement process. For each loci two allelic variants were found, and for Est1, three, but frequency of alleles was rather different (Table 4). For each locus, one allele was very frequent and another was of lesser or insignificant frequency (Est1-vF:0.045). Isoenzymic variants, as an expression of rare alleles, were very significant means for identification of divergent parent pairs, which are essential for the occurrence of heteroic effects. Rare allele variants such as the expression of Est1-vF, Mdh2-F, Pgm 4-F,

Phi1-S, Gdh1-F presented genetic variability and diversity of the analyzed genotypes, and their advantage when used for combining ability. So the line 49 had one allelic variant, Est1-vF, and only the genotypes 5A, 17 and 73 had the variant Pgm 4-F, which pointed out the specificity of the given genotypes and their diversity in relation to the others.

Table 4: Alleleic frequencies in analyzed lines

Locus	Allele	Frequency
Acp1	F	0.364
	S	0.636
Est1	F	0.591
	S	0.409
	vF	0.045
Gdh1	F	0.273
	S	0.727
Mdh2	F	0.182
	S	0.818
Phi1	F	0.818
	S	0.182
Pgd1	F	0.364
	S	0.636
Pgm4	F	0.136
	S	0.864

Identification and differentiation between homozygous or heterozygous genotypes, as well as between different cultivars on the basis of isozymes were very reliable. Identification of genotypes for two species, sorghum and sunflower, and recognition of potential line pairs on the basis of genetic markers were done by Hughes et al. (1992).

Isozymes, besides other parameters, were suitable chemical markers for sunflower seed identification and for genetic purity determination of parent components and hybrids (Loskutov et al., 1990; Gerić et al., 1989). On the basis of this possibility we also separated only uniform, homozygous lines of the observed loci, but it was not a proof of their absolute genetic purity. If this information is to be statistically supported, a certain number of individuals should be analyzed in order to determine the percentage of impurities in the tested seed.

For genetic improvement and development of new genotypes with desirable quantitative and quantitative traits, wild species should be introduced as donors

On the basis of such genetic markers and some new ones, on the molecular level, their introduction in the process of gene mapping for quantitative and qualitative traits becomes wider and more complete.

CONCLUSION

On the basis of polymorphism of seven enzymatic systems, and seven loci, twenty-two homozygous genotypes were chosen from a larger group of sunflower lines.

According to the frequency of individual alleles, which for most of the loci were present with greater and smaller frequency, the genetic variability and degree of divergency of analyzed genotypes could be discussed.

Combining abilities could be predicted on the basis of genetic characteristics of the analyzed lines.

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VARIABILIDAD ISOENZIMÁTICA DE GIRASOL AUTOFECUNDADO

RESUMEN

La variabilidad genética en 22 líneas puras de girasol procedentes de una selección NS fue estudiada en el nivel de isoenzimas. Las enzimas como marcadores moleculares fueron utilizadas para la estimación del material de mejora, identificación genética y potencial para la creación de híbridos. En base a siete sistemas enzimáticos y siete loci a partir de los cuales se obtuvieron ciertos isoenzimas, se encontró variabilidad en las líneas homocigotas. Siete loci isoenzimáticos polimórficos Ist 1, Mdh 2, Pgd 1, Phil, Pgm 4, Gdh1 y Apc 1 y 2 o tres alelos de cada locus fueron utilizados para identificación genética. La distancia genética en los genotipos analizados fue determinada en nivel de un grupo de marcadores genéticos y presentaron una prueba de su eficiencia y exactitud.

VARIABILITÉ ISOENZYMATIQUE CHEZ LES LIGNÉES AUTOFÉCONDÉES DE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

La variabilité génétique isoenzymatique a été étudiée chez 22 lignées autofécondées de tournesol sélectionnées à NS. Les marqueurs génétiques isoenzymatiques sont utilisés pour la caractérisation du matériel en sélection, pour l'identification génétique et comme prédicteurs pour la création d'hybrides. Sur la base de sept systémes enzymatiques et de sept loci a partir desquels certaines formes isoenzymatiques ont été obtenues, on a évalué la variabilité des lignées homozygotes. Sept loci isoenzymatiques polymorphes: Est1, Mdh2, Pgd1, Phi1, Pmg4, Gdh1, Acp1 ainsi que 2 à 3 alléles à chaque locus ont été utilisés pour l'identification génétique. La distance génétique entre génotypes évalués a été déterminée sur la base d'un groupe de marqueurs génétiques, elle constitue une preuve de leur efficacité et fiabilité.