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ISOZYMES, TOCOPHEROLS AND FATTY ACIDS AS SEED BIOCHEMICAL MARKERS OF GENETIC PURITY IN SUNFLOWER

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SUMMARY

Isozyme (eight loci), tocopherol (two genes) and fatty acid (one gene) phenotypes are shown to be a suitable seed biochemical marker of genetic purity in sunflower.

Isozyme loci Est 1, Gdh 1, Gpi 1, Mdh 2, Mdh 5, Pgd 1 and Pgm 4 have an independent inheritance with Tph 1 and Tph 2 genes, controling tocopherol composition, and Est 1 and Pgd 1 loci - with Ol 1 gene, controlling the oleic/linoleic ratio in seed oil.

Fourteen inbred lines of sunflower were identifed with these biochemical markers.

***Key words*: Sunflower seeds, biochemical markers, genitic purity.**

INTRODUCTION

Sunflower (*Helianthus annuus L.*) is one of the most important oil crops in the world. However, its genetics has not been truly studied in comparison with other crops such as wheat, corn or tomato.

Cultivated sunflower has 2n=34 chromosomes, but only few linkage pairs have been identified for approximately eighty genes known.

The genes for morphological traits have been considered as directly visible markers in the development of sunflower hybrids (Bochkarev et al., 1991).

Molecular-genetic markers are introduced into modern plant breeding as reliable parameters of genetic purity of inbred lines and hybrids. Isozymes from sunflower seeds are widely used for these purposes (Geric et al., 1989). There are data on isozyme genetics in sunflower (Torres, 1983; Kahler and Lay, 1985; Borovkova et al., 1991). DNA and storage proteins are considered to be prospective.

On the other hand, sunflower seeds contain some breeding-valuable substances which influence oil quality directly and which belong to biochemical markers. Tocopherols and fatty acids of storage oil could be recognized from that point of view.

MATERIALS AND METHODS

Fourteen inbred lines of sunflower were used in this study. Six of them (M 41, VK 464, VK 541, VK 571, VK 639 and VK 678) are parents of commercial hybrids.

Horizontal gel electrophoresis was performed on 12% starch gel. The enzyme systems included esterase (EST), malate dehydrogenase (MDH), glucosephosphate isomerase

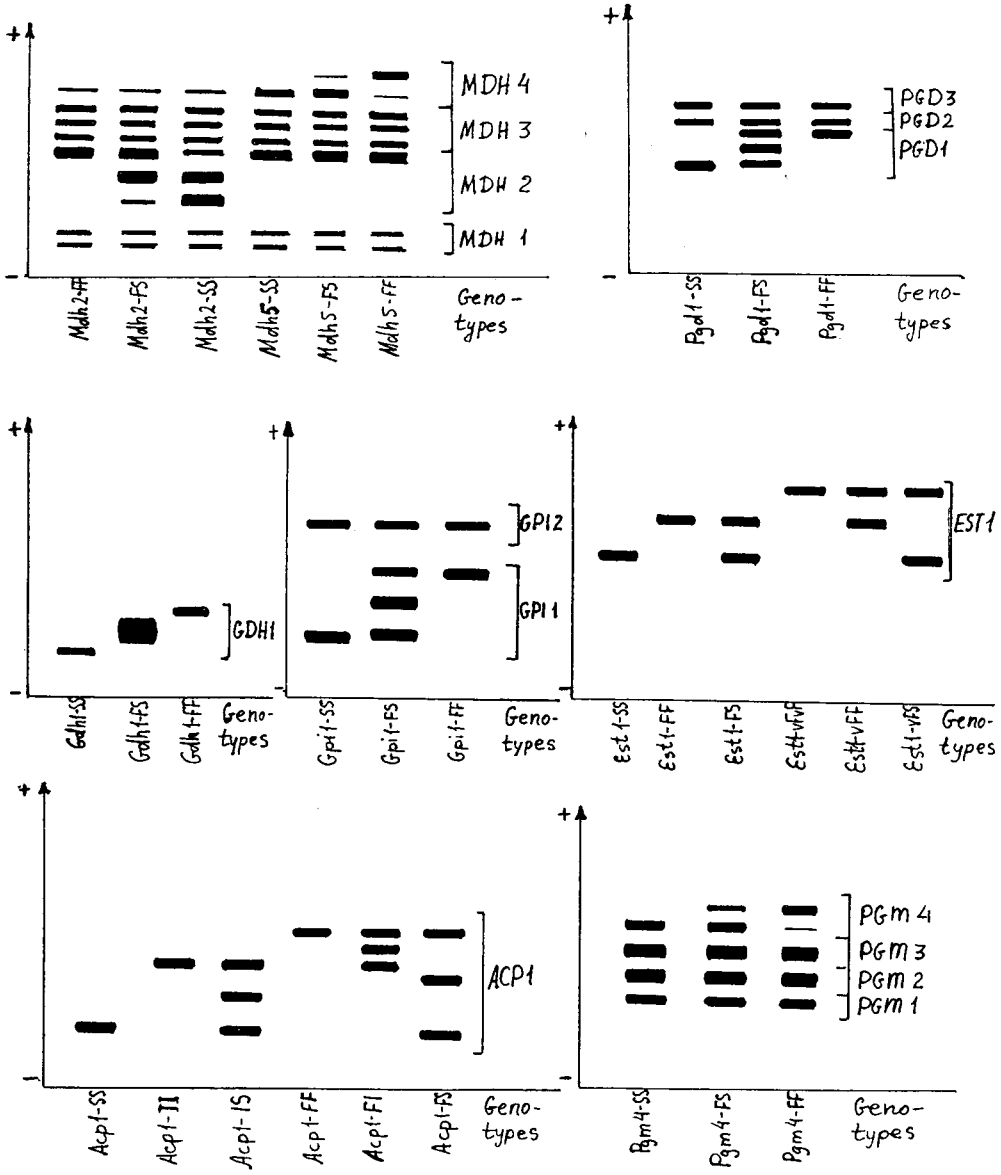

Figure 1. The scheme of isozyme variations for eight loci in sunflower

(GPI), 6-phosphogluconate dehydrogenase (PGD), glutamate dehydrogenase (GDH), acid phosphatase (ACP) and phosphoglucomutase (PGM).

A part of dehulled achene was crushed in 40 μl extraction buffer (0.009 M tris, 0.003 M citric acid, pH 7.0; 0.1% polyvinyl pyrrolidone). The extract was absorbed onto rectangles of 3 MM Whatman paper wick (3x10mm), the wick was placed in a cut (4 cm from the cathodal end) of a gel slab (15x13x1 cm).

Two different electrode and gel buffer systems were used. System I - electrode buffer pH 5.7, 0.065 M L-histidine, 0.02 M citric acid; gel buffer pH 5.7, 0.009 M L-histidine, 0.003 M citric acid, was used for EST, MDH, GPI, PGD, PGM, and system II-electrode buffer pH 8.6, 0.3 M boric acid, 0.1 M NaOH, gel buffer pH 8.6, 0.045 M tris, 0.001 M EDTA, 0.025 M boric acid, was used for GDH and ACP. Gels were allowed to run from 15 to 16 h at constant amperage (10 mA for system I and 13 mA for system II). After electrophoresis has been done, the gels were cut into slices from 1 to 2 mm thick which were immersed in specific staining solution according to the procedure described by Vallejos (1983).

The nomenclature used to describe the isozyme systems and encoding loci was in accordance with Kahler and Ley (1985) and Levites (1986).

Tocopherols were determined by TLC followed by Emmerie-Engel reaction. GLC of fatty acid methyl esters was applied.

Composition of isozymes, tocopherols and fatty acids was analyzed in single seed of inbred lines, F_1 hybrids or F_2 progeny.

RESULTS AND DISCUSSION

Isozyme patterns and inheritance. Seven enzyme systems have been used. An illustration of isozyme variations for eight loci is shown in Figure 1. Data on genetic analysis are given in Table 1.

A detailed description of these enzyme systems and corresponding loci have been presented by Torres (1983), Kahler and Lay (1985), Loskutov et al., (1990) and Loskutov (1993).

Tocopherols and fatty acids. Tocopherols (vitamin E) are natural fat-soluble antioxidants. They exist in four forms - alpha, beta, gamma and delta, which differ in activity. Genetic alteration of tocopherol composition in sunflower seed is included in breeding programs to increase oil oxistability.

Two non-allelic unlinked genes, Tph 1 and Tph 2, controlling tocopherol composition, were identified (Demurin, 1993).

Tocopherol complex of common sunflower contains above 90% of alpha form, while the content of beta form amounts to 50% for recessive gomozygote tph 1 tph 1 and the content of gamma form to 90% for recessive gomozygote tph 2 tph 2 due to a decrease in the alpha tocopherol portion. So, the mutant phenotypes are well-distinguished.

High oleic sunflower is widely used in breeding programs. Faty acid composition of common sunflower seed oil includes approximately 30% of oleic acid. A dominant Ol 1 gene increases the content of oleic acid up to 85% (Urie, 1985). This mutant phenotype can be easily separated from "wild type".

Table 1. F_2 and BC segregation for isozyme loci in sunflower

Locus	Allele	Segregation	Expected ratio	P
Mdh 2	F,S	162:344:189	1:2:1	0.34
Mdh 5	F,S	22:49:16	1:2:1	0.38
6Pgd 1	F,S	65:112:65	1:2:1	0.51
Gdh 1	F,S	178:330:177	1:2:1	0.63
Gpi 1	F,S	33:32	1:1	0.89
	F,S	133:247:128	1:2:1	0.79
Est 1	F,S	64:78	1:1	0.24
	F,S	270:491:283	1:2:1	0.14
	vF,S	97:168:90	1:2:1	0.52
	vF,F	28:73:34	1:2:1	0.49
Acp 1	F,S	21:60:37	1:2:1	0.15
Pgm 4	F,S	74:147:90	1:2:1	0.28

Table 2. Joint segregation analysis of the F_2 population (3:6:3:1:2:1 ratio tested) for isozyme loci with tocopherol and fatty acid genes in sunflower seeds.

Pair of genes	Phenotype						P
	D/FF	D/FS	D/SS	r/FF	r/FS	r/SS	
Tph1-Gdh1	46	95	40	11	24	17	0.65
Tph1-Est1	40	68	27	5	19	13	0.24
Tph1-Mdh2	21	42	17	3	10	6	0.67
Tph1-Pgd1	27	68	44	9	20	9	0.34
Tph1-Gpi1	16	31	15	4	8	3	0.92
Tph1-Pgm4	13	26	24	5	7	4	0.20
Tph1-Mdh5	18	38	13	2	9	3	0.33
Tph2-Gdh1	32	57	33	7	24	10	0.82
Tph2-Est1	41	83	30	6	24	13	0.29
Tph2-Mdh2	45	75	33	14	24	8	0.56
Tph2-Gpi1	25	43	17	2	9	7	0.24
Tph2-Pgd1	18	37	20	2	11	9	0.49
Tph2-Pgm4	28	57	38	11	14	10	0.50
Tph2-Mdh5	17	35	13	4	11	3	0.81
Ol1-Est1	12	28	11	7	9	7	0.66
Ol1-Pgd1	11	23	18	4	17	6	0.12
D-dominant, r-recessive							

Linkage test. Joint segregation was screened for seven isozyme loci with Tph 1, Tph 2 and Ol 1 genes. Linkage relationships were evaluated on the base of the F_2 populations.

Due to the codominance of isozyme loci, dihybrids are expected to segregate in a ratio of 3:6:3:1:2:1.

Sixteen two-locus combinations (out of twenty one possible) were examined. No linkage was found (Table 2).

Line biochemical identification. Isozyme and fatty acid "fingerprint" of the fourteen inbred lines is given in Table 3. Est 1 and Acp 1 isozyme loci have three alleles, the others two alleles.

The simultaneous use of ten biochemical markers makes a good discrimination between the inbred lines. Seven isozyme loci cannot distinguish VK 639 and VK 678 lines which have the same genetic background but different tocopherol composition.

On the base of results of the identification, genetic purity of hybrids and its parent lines has been estimated. Codominance of isozyme loci, recessive character of tocopherol mutations and dominance of high oleic trait provide a suitable approach to find the undesirable outcross genotypes in the inbred lines and outcross or parent genotypes in the F_1 hybrids. Any unexpected seeds are easily obesrved.

Obviously there are two ways for further research - increasing the number of traits in the "fingerprint" pattern and using new unique alleles of the genes.

Table 3. Biochemical identification of sunflower lines from the VNIIMK collection

Line	Gene									
	Est1	Mdh2	Gpi1	Pgd1	Gdh1	Acp1	Pgm4	Tph1	Tph2	Ol1
M41	F	S	F	S	F	S	S	D	D	r
VK464	S	S	F	S	F	S	S	D	D	D
VK639	S	S	F	S	F	S	F	D	D	r
VK678	S	S	F	S	F	S	F	r	D	r
VK541	F	S	F	F	S	S	S	D	D	D
VK571	F	F	S	F	F	F	S	r	D	r
LG15	F	F	F	S	F	I	F	r	D	r
LG17	F	F	F	S	S	S	S	D	r	r
LG18	vF	S	F	F	S	F	F	D	D	D
LG21	F	F	F	S	S	F	F	r	D	D
LG24	S	S	F	S	F	S	F	r	r	r
LG25	F	F	F	S	S	S	S	D	r	D
K824B	S	S	S	S	S	S	F	D	D	r
L2121-2	F	F	S	F	S	S	S	D	D	r
D-dominant, r-recessive										

CONCLUSION

Biochemical markers of seeds such as isozymes, tocopherols and fatty acids can be effectively used to determine the genetic purity of sunflower inbred lines and hybrids. The possibility of determinantion is based on the xenia phenomenon and also on the opportunity of "half-seed technique" to be applied.

REFERENCES

Bochkarev N.I., Tolmachev V.V., Tsukhlo L.G. 1991. Plant and seed marker characters. Sunflower Biology, Plant Breeding and Growing Technology, Moscow, Agropromizdat: 39-44 (in Russ.)

Borovkova I.G., Loskutov A.V., Tolmachev V.V. 1991. Analysis of inheritance and linkage of morphological and isozyme loci in sunflower Helianthus annuus L. Genetics, v.27, n.10: 1773-1780 (in Russ.)

Demurin Ya. 1993. Genetic variability of tocopherol composition in sunflower seeds. Helia, v.16, n.18: 59-62

Geric I., Zlokolica M., Geric C. 1989. Seed isozymes in the crop genetic purity control. Poljoprivredne aktualnosti, v.33, n.3-4: 555-564 (in Serb.)

Kahler A. L., Lay C.L. 1985. Genetics of electrophoretic variants in the annual sunflower. J.Heredity, v.76: 335-340

Levites E.B.1986. Plant Isozyme Genetics, Novosibirsk (in Russ.)
Loskutov A.V. 1993. Isozyme systems in hybridological analysis of sunflower. Ph.D. thesis. VIR, S.Peterburg (in Russ.)
Loskutov A.V., Borovkova I.G., Borovkov A.Yu. 1990. Isozyme genetics in sunflower *Helianthus annuus* L. Inheritance and linkage of three isozyme loci. Genetics, v.26, n.11: 2079-2083 (in Russ.)
Torres A.M. 1983. Sunflower (*Helianthus annuus* L.). Isozymes in Plant Genetics and Breeding, Part B., Amsterdam, Elsevier: 329-338
Urie A. 1985. Inheritance of high oleic acid in sunflower. Crop Science, 25: 986-989
Vallejos C.E. 1983. Enzyme activity staining. Isozymes in Plant Genetics and Breeding, Part A., Amsterdam, Elseveir: 469-516

ISOENZIMAS, TOCOFEROLES Y ACIDOS GRASOS COMO MARCADORES BIOQUIMICOS DE SEMILLAS ARA LA PUREZA GENETICA DE GIRASOL

RESUMEN

Fenotipos de isoenzimas (ocho loci), tocoferoles, dos genes, y àcidos grasos, un gen, mostraran ser marcadores bioquimicos adecuados para la pureza genética en girasol.

Los loci Est 1, Gdh 1, Gpi 1, Mdh 2, Mdh 5, Pgd 1 y Pgm 4 mostraron una herencia independiente de los genes Tph 1 y Tph 2 que controlan composción de tocoferoles y los loci Est 1 y Pgd 1, con el gen OI, que controla la proporción oleico/linoleico en el aceite de la semilla. Catorce lineas puras de girasol fueron identificadas con estos marcadores.

UTILISATION DES ISOZYMES, TOCOPHEROLS ET ACIDES GRAS DE LA GRAINE COMME MARQUEURS BIOCHIMIQUES DE LA PURETE GENETIQUE CHEZ LE TOURNESOL

RÉSUMÉ

Les phénotypes liés aux isoenzymes (8 loci), aux tocophérols (2 gènes) et aux acides gras (1 gène) de la graine sont des marqueurs biochimiques appropirés de la pureté génétique du tournesol.

Les loci enzymatiques Est 1, Gdh 1, Gpi 1, Mdh 2, Mdh 5, Pgd 1 et Pgm 4 montrent une hérédité indépendante des gènes Tph 1 et Tph 2, contrôlant la composition en tocophérols, ainsi que les loci Est 1 et Pgd 1 avec le gène OI 1, contrôlant le raport oléique /linoléique de l'huile.

14 lignées inbred de tournesol ont été identifiées avec ces marqueurs biochimiques.