HELIA, **23**, Nr. 33, p.p. 65-76, (2000) UDC 633.854.78:631.523.1:577.152

GENETICS OF ISOZYMES AND ANALYSIS OF ISOZYMES LINKAGE AND MORPHOLOGICAL LOCI IN SUNFLOWER (*Helianthus annuus* L.)

Kirichenko, V.V. and Popov, V.N.*

Yurjev Plant Production Institute, Moskovskij prospect 142, Kharkov 310060, Ukraine

Received: January 27, 2000
Accepted: November 14, 2000

SUMMARY

The genetics of anodal esterase (Est), cathodal esterase (cEst), cathodal acid phosphatase (cAcp) and malate dehydrogenase (Mdh) has been studied in mature seeds and leaves (genetics of cAcp and Mdh has not been studied in leaves) of sunflower (*Helianthus annuus* L.). A total of ten loci (four loci of anodal esterase, two loci of cathodal esterase, three loci of malate dehydrogenase and one locus of cathodal acid phosphatase) have been identified and described. Five esterase loci (Est1, Est2, Est3, Est4, cEst5), three malate dehydrogenase loci and one locus of cathodal acid phosphatase are expressed in seeds. Three esterase loci (Est2, cEst5 and cEst6) are expressed in leaves. The analysis of linkage between these loci has been made. Two linkage groups have been found. The sequence of the loci in the first linkage group was Mdh2-Est1-Est2-Est3-cEst5. In the second linkage group it was Est4-cAcp1. Linkages have been analyzed between three isoenzymatic loci expressed in leaves and between two loci controlling morphological traits (branched stem and male fertility restoration). The linkage between morphological traits and isoenzymatic loci has not been revealed. It has been revealed in Br-Rf pair.

Key words: inheritance, isoenzymes, linkages, morphological traits, sunflower

INTRODUCTION

Among the agricultural crops the sunflower is one of the less studied ones as to its molecular and biochemical genetics, in contrast to other crops such as maize (Chandlee, 1982; Hoisington, 1985; Wendel *et al.*, 1985; Helentjaris, 1987), tomato (Tanksley, 1983; Paterson, 1988), and wheat (Jaaska, 1980; Hart, 1983, 1984; Chenicek, 1984; McIntosh, 1988; Liu *et al.*, 1991). There is no linkage map of classic genes in *Helianthus*, although the construction of physical maps of sunflower has been accelerated recently. Berry *et al.* (1995) reported the first RFLP map of

* Corresponding author, e-mail: vit@popov.kharkiv.com

the cultivated sunflower. Jan *et al.* (1998) constructed a map from the F_2 population of RHA271 and HA234. In addition, molecular markers are used in sunflower genetic analysis. Rieseberg *et al.* (1995) used up to 197 RAPDs in the investigation of introgression in the domesticated sunflower and its wild relatives. Gentzbittel *et al.* (1994) described the relationships among inbred lines of cultivated sunflower using RFLP markers. Phenetic and phylogenetic relationships in genus *Helianthus* were assessed with RAPD markers (Sossey-Alaoui *et al.*, 1999). Mouzeyar *et al.* (1995) reported on the linkage between a downy mildew resistance gene and RFLP and RAPD markers. Lawson *et al.* (1998) reported on the identification of RAPD markers linked to the R_{adv} gene for rust resistance. By now the genetics of several isozyme loci has been described in sunflower. Two loci controlling alcohol dehydrogenase synthesis (Torres, 1974a, 1974b) were identified and one locus of acid phosphatase was described (Torres *et al.*, 1976). Kahler and Lay (1985) studied the genetics of the following isozyme loci: peroxidase (Prx3), malate dehydrogenase (Mdh1), 6-phosphogluconate dehydrogenase (Pdh1), glucosephosphate isomerase (Gpi2), phosphoglucomutase (Pgm4) and isocitrate dehydrogenase (Idh2). Linkage was revealed only between Pgm4 and Prx3 loci with recombination frequency of 0.14. The genetic control of a single esterase locus was studied (Loskutov *et al.*, 1990) and so was the genetics of six loci governing helianthinin synthesis in sunflower seeds (Poperelya, 1994).

Studies were conducted on the genetics of a number of genes controlling morphological traits (Fick, 1976; Heiser, 1954; Hockett, 1970; Barotti, 1995; Vrânceanu *et al.*, 1983; Luczkiwich, 1975). Several groups of linkage between morphological traits were determined (Stoenescu *et al.*, 1977; Iuoras *et al.*, 1982).

The isozyme use as genetical markers allows to determine linkage groups and compose genetic maps. However, little is said in literature about linkage groups for isozymes in sunflower. This paper reports the data on the inheritance of four anodal esterase loci, two cathodal esterase loci, three malate dehydrogenase loci and one acid phosphatase locus as well as the linkage between them and morphological traits. The results of the analysis of anodal esterase and cathodal esterase loci expression in seeds and leaves have been shown.

MATERIALS AND METHODS

The genetics of isozymes was studied in three F_2 seed populations derived from the crosses between inbred lines SKH 2552 x KH 982 (cross 1), SKH 2552 x KH 854 (cross 2) and SKH 2552 x KH 790 (cross 3).

To investigate esterase inheritance in sunflower leaves we used the combination from two inbred lines, SKH 2552 x KH 982.

Isozymes were extracted from individual seeds and leaves for an hour at 0 to +2°C with 0.02 M tris-HCl buffer (pH 7.5) containing 0.01 mM PVP, 0.006 mM EDTA, 0.1 mM DTT and 20% sucrose. The enzyme electrophoresis was carried out

on vertical slabs. The separation of anodal esterase and malate dehydrogenase enzymes was done with tris-EDTA-borate buffer system - 0.09 M tris, 0.09 M H_3BO_3, 0.0031 M EDTA (pH 8.3). For separation of anodal esterase the resolving gel containing 7% (w\v) acrylamide and 0.37% (w\v) bis-acrylamide was used. The running buffer for cathodal esterase and cathodal acid phosphatase contained 0.5% (w\v) tris and 1% (w\v) aluminum lactate. The stacking gel containing 5% (w\v) acrylamide and 0.34% (w\v) bis-acrylamide was used for isozyme separation of cathodal esterase and cathodal acid phosphatase.

Hystochemical staining of enzymes was done according to the procedures reported by Shaw and Prassad (1985).

Chi-square tests for goodness-of-fit between observed and expected single-locus numbers were used.

Linkage analysis was done using MAPMARKER versions (Lander *et al.*, 1987). Two-point analysis was conducted to determine the maximum likelihood recombination fraction and the LOD score for each of the possible pairs of loci. Linkage group were formed using "group" command with a minimum LOD score of 3.0 and $r_{max} = 0.316$.

RESULTS AND DISCUSSION

Anodal esterase

Figure 1 shows the electrophoretic patterns of anodal esterase enzymes in the F_2 population from the cross SKH 2552 x KH 854 as well as in the parent lines.

Four zones of isozyme activity were observed for esterase on the electrophoregrams of the parental lines.

Table 1: Segregation in loci encoding anodal esterase, cathodal esterase, cathodal acid phosphatase and malate dehydrogenase in seeds of F_2

Zone	SKH2552 x KH982	χ^2	P	SKH2552 x KH854	χ^2	P	SKH2552 x KH790	χ^2	P
Anodal esterase									
I	28:44:25	1.00	0.70-0.50	13:15:5	4.14	0.20-0.10	22:57:28	1.11	0.70-0.50
II	28:44:25	1.00	0.70-0.50	13:15:5	4.14	0.20-0.10	22:57:28	1.11	0.70-0.50
III	28:44:25	1.00	0.70-0.50	13:15:5	4.14	0.20-0.10	22:57:28	1.11	0.70-0.50
IV	32:49:16	5.27	0.10-0.05	8:15:10	0.50	0.70-0.50	23:54:31	1.18	0.70-0.50
Cathodal esterase									
I	26:41:30	2.63	0.30-0.20	12:16:5	2.99	0.30-0.20	29:45:24	1.15	0.70-0.50
Cathodal acid phosphatase									
I	30:44:23	1.83	0.50-0.30	8:16:9	0.07	0.98-0.95	17:48:18	2.04	0.50-0.30
Malate dehydrogenase									
I	-	-	-	-	-	-	62:46	0.05	0.90-0.80
III	-	-	-	-	-	-	75:33	1.77	0.20-0.10

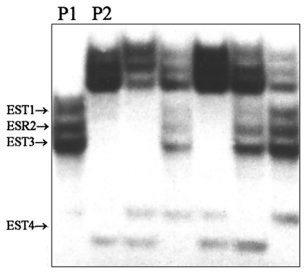

Figure 1: Zymogram of anodal seed esterase observed in hybrid population SKH2552 x KH854 (P1-SKH2552; P2-KH854)

Three enzyme phenotypes with a 1:2:1 ratio were observed in zones I, II, III and IV (crosses 1, 2 and 3) that was in good conformity with the expected segregation 1:2:1 (Table 1).

Anodal esterase isoenzymes in the leaves of the investigated sunflower initial inbred lines were distinct for electrophoretic mobility (Figure 2). Three phenotypic classes were observed in that zone. Chi-value shows that isoenzymes are governed by a single locus (Table 2). The isozymes observed in zone I seem to be monomers. In additional zone for isoenzymatic activity were not revealed.

Table 2: Segregation in loci encoding anodal esterase and cathodal esterase in leaves of F_2 population SKH2552 x KH982

Zone	Genotype			χ^2	P
	AA	AB	BB		
Anodal esterase					
I	49	74	37	2.69	0.30-0.20
Cathodal esterase					
I	35	77	42	0.62	0.70-0.50
II	35	59	40	2.50	0.30-0.20

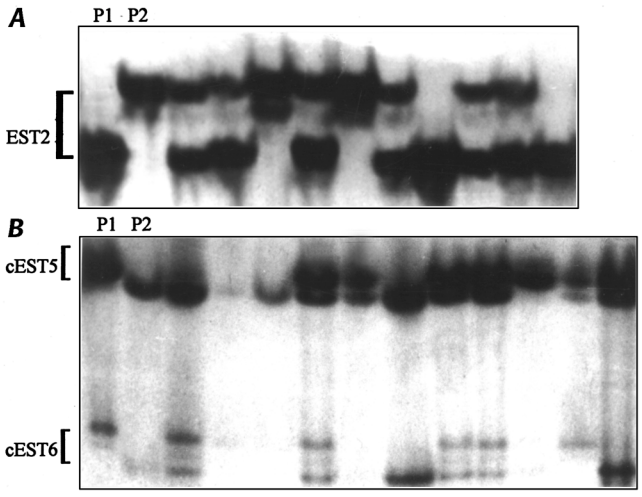

Figure 2: Zymograms of anodal esterase (A) and cathodal esterase (B) observed in hybrid population SKH2552 x KH982 (P1-SKH2552; P2-KH982)

The obtained results permit to suggest that at least four loci govern the anodal esterase synthesis in dry seeds and one locus in leaves of sunflower.

We did not investigate the correspondence of the studied esterase loci with an earlier published paper (Loskutov *et al.*, 1990).

Cathodal esterase and cathodal acid phosphatase

One zone of isozyme activity was observed for cathodal esterase (Figure 3). Chi-square values for goodness-of-fit corresponds to the 1:2:1 ratio in this zone, indicating monohybrid codominant inheritance (Table 1).

Two zones of cathodal esterase activity were found in the leaves of initial parent lines. Three isozyme phenotypes were observed in these zones (Figure 2). Chi-square tests of goodness-of-fit to the 1:2:1 ratio indicate that enzyme activity in each zone is determined by a single locus (Table 2). We did not notice the formation of additional activity zones in heterozygotes in zone I, which evidently proves the monomeric structure of the enzyme. The presence of an additional active zone in heterozygotes in zone II indicates the probable dimeric structure of the enzyme.

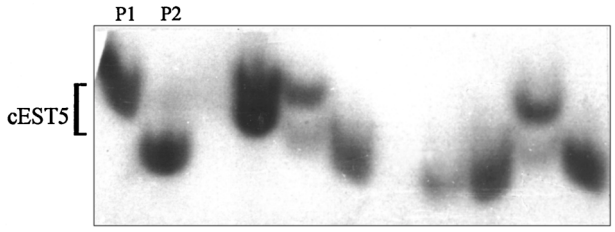

Figure 3: Zymogram of cathodal seed esterase observed in hybrid population SKH2552 x KH854 (P1-SKH2552; P2-KH854)

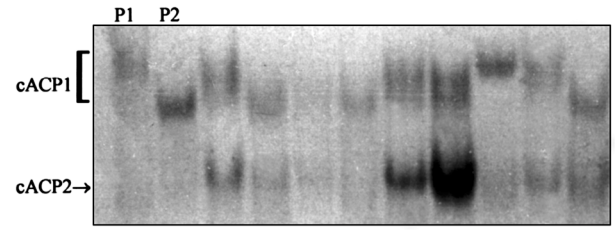

Figure 4: Zymogram of cathodal seed acid phosphatase observed in hybrid population SKH2552 x KH854 (P1-SKH2552; P2-KH854)

Two zones of isozyme activity were observed for cathodal acid phosphatase (Figure 4). Three phenotype classes with the 1:2:1 ratio were detected in zone I. Chi-square value shows that the isozymes of this zone are governed by one locus with two codominant alleles (Table 1). The presence of the additional zone of cathodal acid phosphatase activity in heterozygotes can indicate a probable dimeric structure of the enzyme. We were not able to establish the genetics of cathodal acid

phosphatase in zone II as the components did not differ in mobility. Genetics of cathodal acid phosphatase was not studied in leaves.

Malate dehydrogenase

Isozymes were observed in three zones on MDH zymograms. Initial parents of inbred lines differed in malate dehydrogenase activity (Figure 5). Two ("active band":"null") isozyme phenotypes were observed in zone III. Chi-square values for goodness-of-fit to a 3:1 ratio indicate that zone III was controlled by a single locus.

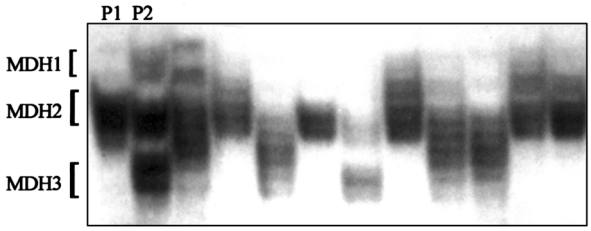

Figure 5: Phenotypes of malate dehydrogenase observed in hybrid population SKH2552 x KH790 (P1-SKH2552; P2-KH790)

Furthermore, two-locus segregation was observed in malate dehydrogenase zone I. Two (9 "active band" : 7 "null") phenotypes were observed in this zone. In this case isozymes in zone I seem to be encoded by two independent loci. The recombination has not been revealed between isozymes in each zone. The absence of recombination is likely to indicate that those products of a single locus (zone III) and 2 loci (zone I) are influenced by posttranslation modifications. The observed isozymes (zone II) are probably interlocus hybrid bands due to dimerization between Mdh1 (zone I) and Mdh2 (zone III).

Linkage

The linkage test showed the absence of recombinations between Est1, Est2 and Est3 loci. These loci form a close linkage group. Cathodal esterase (cEst5) was linked to Est1, Est2 and Est3 with recombination percentages of 0.5%, 11.2%, 6.7% in crosses 1, 2 and 3, respectively. Mdh2 was also linked to Est1, Est2, Est3 and cEst5 with recombination percentages from 25.6% to 28.0% (cross 3).

Linkage test showed that the loci of Est4 and cathodal acid phosphatase were linked with the recombination percentages of 9.8% (cross 1), 1.5% (cross 2) and 10.9% (cross 3). The linkage estimate demonstrated that Est4 and cAcp1 were inherited independent of loci Est1, Est2, Est3, cEst5 (crosses 1, 2), Mdh1 and Mdh2 (cross 3). Linkage tests showed independently inheritance of Est1, Est2, Est3, cEst5 and Mdh2 in relation to Mdh1. The linkage between the loci governing enzymatic activity in leaves was studied as well. The linkage test indicated an independent inheritance of cEst6 in relation to Est2 and cEst5. Two esterase loci, Est2 and cEst5, were placed in the same linkage group. The recombination percentage between them was 5.6%.

According to the obtained data the most probable distribution of loci is given in Figure 6.

The results indicates that these ten enzyme loci mark different linkage groups in the sunflower genome.

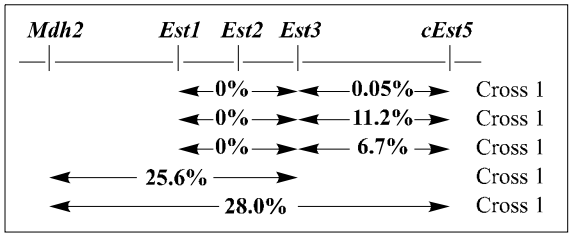

Figure 6: Schematic representation of isozyme loci linkage observed in three crosses

The genetic control of branched stem was investigated previously (Hockett, 1970). Leclercg (1969) was first to describe CMS (cytoplasmic male sterility) in sunflower, in interspecific crosses between *H.petiolaris* and *H.annuus*. Many authors reported on the genetic control of fertility restoration in different CMS sources. In most cases male fertility restoration is governed by single dominant or two dominant complementary independent Rf genes (Serieys, 1987, 1991, 1994; Jan, 1997; Leclercg, 1984; Miller, 1996; Fick *et al.*, 1974). Sometimes modifier genes can also participate in genetic control (Serieys, 1999). When crossing unbranched CMS maintainer (SKH 2552) and branched CMS restorer (KH 982), inbred lines in F_2 we observed segregation at the ratio 3:1 for these traits ($\chi^2 = 0.13$; 0.63, respectively). The test on the linkage between enzymatic loci (Est2, cEst5 and cEst6) and morphological traits demonstrated independent inheritance. Linkage was found for a pair of genes controlling branched stem and male fertility restoration. The recombination percentage was 24.9%.

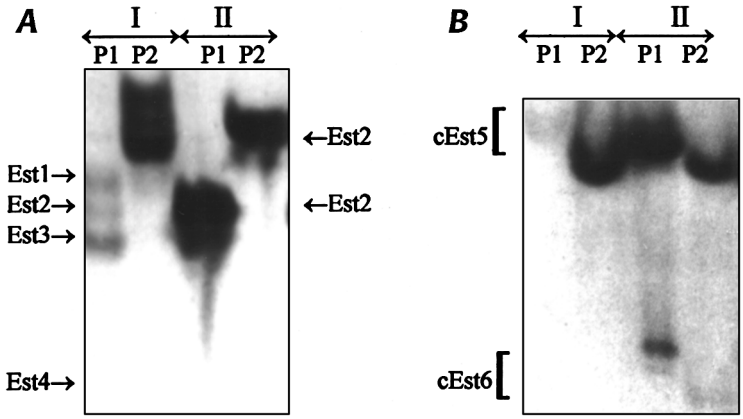

Figure 7: Zymograms of anodal esterase (A) and cathodal esterase (B) observed in seeds (I) and leaves (II) (P1-SKH2552; P2-KH982)

Figure 7 shows the isoenzymic pattern of anodal and cathodal esterase for two inbred lines (SKH 2552 and KH 982) in mature seed and leaves. Loci Est1, Est2, Est3, Est4 were expressed in seeds. Only Est2 was expressed in leaves. cEst5 locus was active in seed and leaf tissues. cEst6 was expressed in leaves. The expression of this locus has not been shown in seeds.

CONCLUSIONS

We studied the genetics of esterase, malate dehydrogenase and cathodal acid phosphatase. The obtained data show presence of six esterase loci (four anodal esterase loci and two cathodal esterase loci), four of which are distributed in the same linkage group. The locus of cathodal acid phosphatase is in one linkage group with Est4. One locus malate dehydrogenase (Mdh2) is placed in the same linkage group with esterase loci (Est1, Est2, Est3, cEst5). There was revealed a group of linkages between genes governing branched stem and male fertility restoration. The isoenzymatic pattern of esterase in seeds and leaves of inbred lines was analyzed.

These studied enzyme loci may serve as markers in sunflower breeding, research and genetics.

ACKNOWLEDGMENTS

The authors express their sincere acknowledgments to N.R. Sharopova, and V.A. Portyanko, for assistance in research and discussion of the results.

REFERENCES

Barotti, S., Fambrini, M., Pugliesi, M., Lenzi, A., 1995. Genetic variability in plants regenerated from *in vitro* culture of sunflower. Plant Breeding, 104: 275.

Berry, S.T., Leon, A.J., Hanfrey, C.C., Challis, P., Burkholz, A., Barnes, S.R., Rufener, G.K., Lee, M., Cqaligari, P.D.S., 1995. Molecular marker analysis of *Helianthus annuus* L. 2. Construction of an RFLP linkage map for cultivar sunflower. Theor. Appl. Genet., 91: 195-199.

Chandlee, J.M., Scandalios, J.M., 1982. Some properties of genetically defined proteins in *Zea mays*. Isozyme Bulletin, 15: 74-84.

Chenicek, K.J., Hart, G.E., 1984. Genetic control of hexaploid wheat aconitase isozymes. Isozyme Bulletin, 17: 40.

Fick, G.N., 1976. Genetics of floral color and morphology in sunflower. Journal of Heredity, 67: 4.

Gentzbittel, L., Zhang, Y.X., Vear, F., Griveau, Y., Nicolas, P., 1994. RFLP studies of genetic relationship among inbred lines of cultivated sunflower (*Helianthus annuus* L.): evidence for distinct restorer and maintainer germplasm pools. Theor. Appl. Genet., 89: 419-425.

Hart, G.E., 1983. Hexaploid wheat (*Triticum aestivum* L. Em Thell.). In: Isozymes in plant genetics and breeding. Part B, Amsterdam, Elsevier, pp. 35-56.

Hart, G.E., 1984. Biochemical loci of hexaploid wheat (*Triticum aestivum*, 2n=42, genomes AABBDD). In: St. J. O'Brien (editor). Genetic Maps, 3: 485-490.

Heiser, C., 1954. Variation and subspeciation in the common sunflower *H.annuus*. The American Midl. Nat., pp. 287-305.

Heun, M., Kennedy, A.E., Andreson, J.A., Lapitan, N.L.V., Sorrels, M.E., Tanskley, S.D., 1991. Construction of a restriction fragment length polymorphism map for barley (*Horeum vulgare*). Genome, 34: 437-447.

Hockett, E.A., Knowles, P.F., 1970. Inheritance of branching in sunflower (*H.annuus*). 10: 432-436.

Hoisington, D.A., 1985. Maize working maps. Maize Genet. Coop. News Letter, 59: 110.

Iuoras, M., Stoenescu, F.M., 1982. Linkage studies in sunflower (Romanian). Probl. de Genet. Theor. Sci. Aplic., 14: 145.

Jaaska, V.E., 1980. Electrophoresis survey of seedling esterases in wheats in relation to their phylogeny. Theor. Appl. Genet., 56: 273-284.

Jan, C.C., 1997. FAO report of the working group "Identification, study and utilization in breeding programs of new CMS sources", for the period 1995-1996.

Jan, C.C., Vick, B.A., Miller, J.F., Kahler, A.L., Butler, E.T., 1998. Construction of an RFLP linkage map for cultivated sunflower. Theor. Appl. Genet., 96: 15-22.

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

Lander, E.S., Green, P., Abrahamson, J., Barlow, H., Daly, M., Lincoln, S., Newsbury, L., 1987. MAPMARKER: an interactive computer program for constructing genetic maps of experimental and natural populations. Genomics, 1: 174-181.

Lawson, W.R., Goulter, K.C., Henry, R.J., Kong, G.A., Kochman, J.K., 1998. Marker-assisted selection for two rust resistance genes in sunflower. Mol. Breeding, 4: 227-234.

Leclercq, P., 1969. Une stérilité male cytoplasmique chez le tournesol. Ann. Amél. Plant, 19: 99-106.

Liu, Y.G., Tsunewaki, K., 1991. Restriction fragment length polymorphism (RFLP) analysis in wheat. II Linkage maps of the RFLP sited in common wheat. Jpn. J. Genet., 66: 617-633.

Loskutov, A.V., Borovkova, I.G., Borovkov, A.U., 1990. Genetics of isoenzymes in sunflower (*Helianthus annuus* L.). Genetics (Russia), 26: 2079-2082.

Luczkiwich, T., 1975. Inheritance of some characters and properties in sunflower (*Helianthus annuus* L.). Genetica Polonica, 16: 167-184.

McIntosh, R.A., 1988. Catalogue of gene symbols for wheat. Proc. 7th Int. Wheat Genet. Symp., pp. 1225-1323.

Miller, J., 1996. Inheritance of restoration of *Helianthus petiolaris* sp. *fallax* (PEF1) cytoplasmic male sterility. Crop. Sci., 36: 83-86.

Mouzeyar, S., Roeckel-Drevet, P., Gentzbittel, L., Philippon, J., Tourvieille de Labrouhe, D., Vear, F., Nicolas, P., 1995. RFLP and RAPD mapping of the sunflower *Pl1* locus for resistance to *Plasmopara halstedii* race 1. Theor. Appl. Genet., 91: 733-737.

Poperelya, F.A., Netsvetaev, V.P., 1994. Genetic control of helianthinin in sunflower seeds (*Helianthus annuus* L.). Cytology and Genetics (Ukraine), 28: 59-63.

Reiseberg, L.H., Linder, C.R., Seiler, G.J., 1995. Chromosomal and genic barriers to introgression in *Helianthus*. Genetics, 141: 1163-1171.

Serieys, H., Vincourt, P., 1987. Characterization of some new CMS sources from *Helianthus* genus. Helia, 10: 9-13.

Serieys, H., 1991. In FAO progress report of the working group "Identification, study and utilization in breeding programs of new CMS sources". VII Consultation of the European Cooperative Research Network on Sunflower. Pisa (Italy), May 11-13.

Serieys, H., 1994. Report on the past activities of the FAO working group: "Identification, study and utilization in breeding programs of new CMS sources", for the period 1991-1993. Helia, 17: 159-163.

Serieys, H., 1999. FAO progress report of the working group:"Identification, study and utilization in breeding programs of new CMS sources". IX Consultation of the European Cooperative Research Network on Sunflower. Dobrich (Bulgaria), July 27-30.

Shaw, C.R., Prassad, R., 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. 4: 297-320.

Sossey-Alaoui, K., Serieys, H., Tersac, M., Lambert, P., Schilling, E., Griveau, Y., Kaan, F., Bervillé, A., 1999. Molecular relationships of *Helianthus* based on RAPD markers. Helia, 22: 1-18.

Stoenescu, F.M., Vrânceanu, A.V., 1977. Linkage studied between ms and marker genes in sunflower. Proc. 7th Inter. Sunfl. Conf., Krasnodar, pp. 315-321.

Tanskley, S.D., 1983. Gene mapping. In: Isozymes in Plant Genetics and Breeding. Part A, Amsterdam, Elsevier, pp. 111-140.

Toress, A.M., 1974a. Sunflower alcohol dehydrogenase: Adh-1 genetics and dissociation-recombination. Biochem. Genet., 11: 17-24.
Toress, A.M., 1974b. Genetics of sunflower alcohol dehydrogenase: Adh-2, non-linkage to Adh-1 and Adh-2 early alleles. Biochem. Genet., 12: 385-392.
Toress, A.M., Diedenhofen, U., 1976. The genetics control of sunflower seed acid phosphatase. Can. J. Genet. Cytol., 18: 709-716.
Vrânceanu, A.V., Iuoras, M., Stoenescu, F.M., 1983. Genetic study of short petiole trait and its use in sunflower breeding. Proc. 12th Inter. Sunfl. Conf., 11: 429.
Wendel, J.F., Stuber, C.W., Goodman, M.M., 1985. Twelve new isozyme loci in maize: progress report on chromosomal location, the subunit composition and subcellular localization of their products. Maize Genet. Coop. News Letter, 59: 87-88.

GENETICA DE ISOFERMENTOS Y EL ACOMPLAMIENTO DE LÓCUSES MORFOLOGICOS E ISOFERMENTICOS EN GIRASOL

RESUMEN

Ha sido estudiato el control genetico de las esterasas anódica (Est) y catódica (cEst), la fosfatasa acida catódica (cAcp) y malatdehidrogenasa (Mdh) en las semillas maduras y las hojas de girasol (*Helianthus annuus* L.). No hemos estudiado la genetica de Acp y Mdh en las hojas. En total 10 lócuses han sido identificado y descrito (cuatro lócuses de la esterasa anódica, dos locus de la esterasa catódica, tres lócuses de la malatdehidrogenasa y uno locus de la fosfatasa acida anodica). Se expresan en semillas cinco lócuses de esterasa (Est1, Est2, Est3, Est4, cEst5), tres lócuses de malatdehidrogenase y uno locus de fosfatasa acida catódica. En las hojas se expresan tres lócuses de esterasa (Est2, cEst5 y cEst6). Hemos analizado el acomplamiento entre esos lócuses. Han sido revelado dos grupos de acomplamiento. La disposicion de lócuses en primer grupo de acomplamiento fue siguiente: Mdh2-Est1-Est2-Est3-cEst5 y en segundo grupo: Est4-cAcp1. El analisis de acomplamiento se realisó entre tres grupos de isofermentos que se expresan en hojas, y dos grupos que controlan a los signos morfologicos (ramificacion, restablecimiento de la fertilidad masculina). No hemos encontrado el acomplamiento entre los signos morfologicos y los lócuses isofermenticos. Se ha revelado en la pareja Br-Rf.

GENETIQUE DES ISOFERMENTS ET LIAISON DES LOCUS MORPHOLOGIQUES ET CEUX-CI D'ISOFERMENTS AU TOURNESOL

RÉSUMÉ

On a étudié le contrôle génétique de l'estérase anodique (Est), de l'estérase cathodique (cEst), de la phosphatase acide cathodique (cAcp) et de la malatedéshydrogénase dans les graines mûres et dans les feuilles (la génétique cAcp et Mdh dans les feuilles n'est pas étudié) du tournesol (*Helianthus annuus* L.). En total on a identifié et on a décrit 10 locus (quatre locus de l'estérase anodique, deux locus de l'estérase cathodique, trois locus de la malatedéshydrogénase et un locus de la phosphatase acide cathodique). Cing locus de l'estérase (Est1, Est2, Est3, Est4, cEst5), trois locus de la malatedéshydrogénase et un locus de la phosphatase ont l'expression dans les

graines. Trois locus de l'estérase (Est2, cEst5, cEst6) ont l'expression dans les feuilles. On a fait l'analyse sur la liaision entre ces locus. On a révélé deux groupes de liaision. La disposition des locus dans le premier groupe de liaison était Mdh2-Est1-Est2-Est3-cEst5. Le deuxiéme groupe de liaison était Est4-cAcp1. On a fait l'analyse de liaison entre les trois locus d'isoferments, que ont l'expression dans les feuilles et les deux locus qui, à leur tour, contrôle les signes (la ramification, le rétablissement de la fertilité masculine). La liaison entre les signes morphologiques et les locus d'isoferments n'est pas découverte. Mais elle est découverte pour la paire Br-Rf.