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VARIABILITY OF HELIANTHININ, THE MAJOR SEED GLOBULIN IN THE GENUS *Helianthus* L.

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SUMMARY

Polymorphism of the major storage protein of sunflower seed, 11S globulin (helianthinin), was studed using the method of one-dimensional sodium dodecylsulphate polyacrilamide gel electrophoresis. The examined materials included seventeen accessions of annual and perennial *Helianthus* species, one accession of *Tithonia sp.* and six simple and complex interspecific hybrids. The helianthinin polypeptide composition differed considerably among the species of the genus. The annual and perennial species were most different. A number of polypeptides were stable within the genus, the other ones were characterized by high variability. In the crosses of genetically close species, the polypeptide composition was inherited as a Mendelian character. However, by hybridization of distant species having non-homologous genomes (e.c., *H. rigidus x H. annuus*), poor polypeptide composition of hybrid protein was obtained. In hybrid seeds, only those polypeptides were expressed which were presents in both parents. This probably explains the seed non-viability in interspecific crosses of the genus *Helianthus L.*

Key words: *Helianthus* L. spp. - helianthinin - polypeptide composition - interspecific variability - inheritance.

INTRODUCTION

Wild species of the genus *Helianthus L.* have a number of useful agronomic characters therefore they are a valuable genefund for genetic improvement of the cultivated sunflower *H. annuus L.* Sunflower germplasm, especially North American species, are studied extensively for morphology, phulogeny, crossability, and other characters (Heiser et al., 1969; Georgieva-Todorova, 1976; Rogers et al., 1982; and others). However, the potentials of using genetic diversity of wild species in sunflower breeding programmes are limited because of their incompatibility with cultivated forms and difficulties in obtaining hybrids. In this connection, markers which allow to control the transmission of genetic material from one species to the other have great importance. Possibilities of using molecular markers in sunflower genetic and phylogenetic studies have been demonstrated in recent work on nuclear and cytoplasmic DNAs (Choumane and Heizmann, 1988; Perez and Berville, 1988; Rieseberg et al., 1988; 1990) and seed storage proteins (Anashchenko and Gavrilyuk, 1977; Anisimova, 1984; Anisimova and Gavrilyuk, 1985).

The most abundant protein of sunflower seed is 11S globulin (helianthinin). It is characterized by significant heterogeneity and polymorphism (Anisimova and Gavrilyuk, 1989). As it was shown earlier, helianthinin polypeptide composition revealed by the sodium dodecylsulphate polyacrilamide gel electrophoresis (SDS-PAGE) is genotype

specific and can be used for the identification of lines, varieties and hybrids (Anisimova et al., 1986; Anisimova et al., 1991).

Here, we analyze the helianthinin polypeptide composition of some *Helianthus* species and study the inheritance of this character in interspecific crosses.

MATERIAL AND METHODS

Seed accessions of annual and perennial sunflower species from the collections of Institute of Genetics (Sofia, Bulgaria), N.I.Vavilov Institute of Plant Industry (VIR) (St.Petersburg, Russia), Wheat and Sunflower Institute "Dobroudja" (General Toshevo, Bulgaria), and Institute of Oil Crops (Krasnodar, Russia) were used. Seeds of interspecific hybrids obtained in Bulgaria and Russia were also analyzed. The examined materials, their origins and years of harvesting are listed in Table 1.

Helianthinin was isolated from single seeds or seed samples using the method of cryoprecipitation (Schwenke et al., 1975). The isolated helianthinin was dissociated to polypeptides in the presence of SDS and ß-mercaptoethanol and then fractionated in 12,5% SDS-PAG according to the method of Laemmli (1970) as it was described earlier (Anisimova, 1991).

RESULTS AND DISCUSSION

Helianthinin SDS-PAGE patterns of all analyzed sunflower species included three distinct groups of polypeptides - the basic polypepetides with the molecular mass of about 20 kDa and two groups of acidic polypeptides (30 kDa and 40 kDa). We compared helianthinin patterns of the examined accessions with the standard pattern of the variety Peredovik (*H.annuus*) which has been characterized earlier (Anisimova et al., 1991). Positions of polypeptides in the standard pattern were enumerated according to the increase in their molecular mass. Furthermore, positions of all pattern bands observed in the analysis of helianthinin intraspecific variation were taken into account. The distributions of polypeptides in helianthinin patterns of wild annual and perennial species were similar to that in *H.annuus*. However, the number and arrangement of bands within individual molecular groups were very different.

Unlike helianthinin of the annual species, helianthinin of the perennial ones had multiple basic and acidic (40 kDa) polypeptides. In addition, polypeptides with similar mobilities were sometimes in adjacent positions and could be displayed as intensively stained regions. Such patterns were characteristic for the polyploid species *H.rigidus, H.resinosus, H.scaberimus* (Figure 1.). Significant helianthinin molecular heterogeneity in auto- and allopolypoid species was possibly a consequence of either genetic heterogeneity due to gene duplication or effects of gene doses but not the result of summing the polymorphic patterns of distinct genotypes. This suggestion was confirmed by the SDS-PAGE of helianthinin from individual seeds of *H.rigidus*. In these analyses, patterns of individual seeds were similar to the patterns of seed samples by the absence of discrete bands in 40 kDa region (Figure 2b). Another distinct feature of helianthinin of the perennial species was a significant reduction of 39 kDa acidic polypeptides when compared with helianthinin of the annual sunflowers. This can be explained by the more

Table 1. List of analyzed seed accessions

Name	2n	Origin	Year of seed harvesting
Annual			
H.annuus L	34	Institute of Genetics, Sofia, Bulgaria	1989
H.praecox E & G	34	Institute of Genetics, Sofia, Bulgaria, 1989	
H.lenticularis Ckll., ,	34	VIR Collection, Expeditionary accession, USA	1977
H.lenticularis Ckll.	34	Kuban Experimental Station VIR, Rusia	1978
H.annuus (cms rigidus)	34	Institute of Oil Grops, Krasnodar, Russia	1988
H.annuus (cms rigidus)	34	Wheat and Sunflower Institute "Dobroudja" General Toshevo, Bulgaria	1989
F_1 *(H.annuus x H.lenticularis)*	34	Kuban Experimental Station VIR, Russia	1978
F_2 *(H.lenticularis x H.annuus)*	34	Kuban Experimental Station VIR, Russia	1978
F_1 *(H.praecox x H.annuus)*	34	Institute of Genetics, Sofia, Bulgaria	1989
Perennial			
H.angustifolius L	34	Kuban Experimental Station VIR, Russia	1979
H.mollis Lam.	34	Kuban Experimental Station VIR, Russia	1979
H.mollis Lam	34	Institute of Genetics, Sofia, Bulgaria	1988
(H.nuttallii Tats.)	34	Kuban Experimental Station VIR, Russia	1979
H.nuttallii Tats	34	Institute of Genetics, Sofia, Bulgaria	1998
H.hirsutus Raf.	68	Institute of Genetics, Sofia, Bulgaria	1988
H.scaberimus Ell.	68	Institute of Genetics, Sofia, Bulgaria	1988
H.resinosus	102	Institute of Genetics, Sofia, Bulgaria	1979, 1987
H.rigidus (Cass.) Desf.	102	Institute of Genetics, Sofia, Bulgaria	1988
H.rigidus (Cass.) Desf.	102	Kuban Experimental Station VIR, Russia	1988
H.tuberosus L.	102	Kuban Experimental Station VIR, Russia Sofia, Bulgaria	1979
F_2 *(H.rigidus x H.annuus)*	102	Institute of Genetics, 2 *(H.rigidus x H.annuus)*	1988
F_1 *(H.tuberosus x H.annuus) x H.lenticularis*	Non-identified	Kuban Experimental Station VIR, Russia	1978
Tithonia Desf.	Non-identified	Kuban Experimental Station VIR, Russia	1978

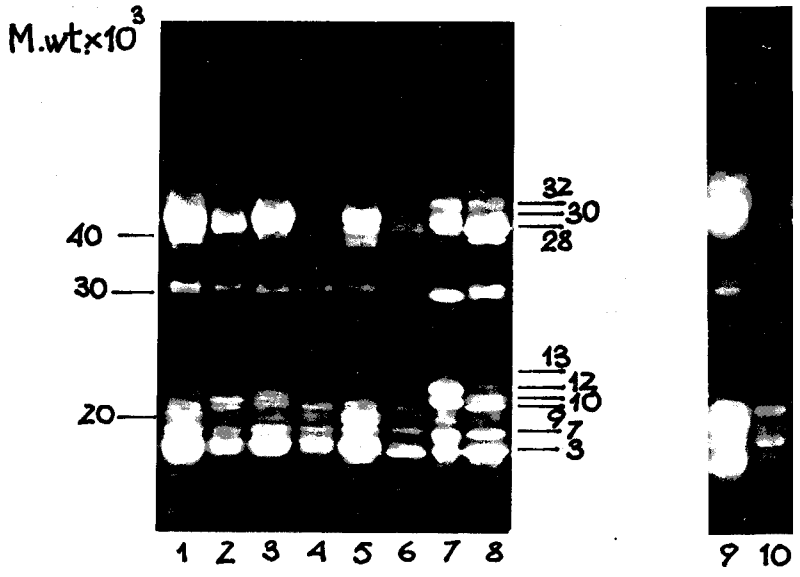

Figure 1.SDS-PAGE patterns of helianthinin of H.rigidus(1), H.resinosus(2), H.scaberimus(3), H.hirsutus(4), H.nuttallii(5), H.mollis(6), H.annuus variety Peredovik (7), H.praecox(8), H.tuberosus(9) and SDS-PAGE pattern of 11S globulin of Tithonia sp.

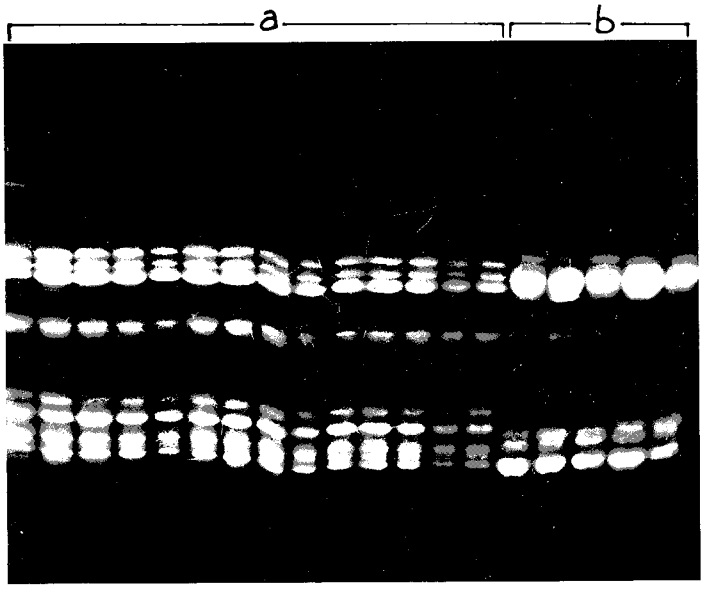

Figure 2.SDS-PAGE patterns of helianthinin from single seeds of H.annuus (cms rigidus) (a) and H.rigidus (b).

ancient "evolutionary age" of encoding genes in the perennial species. It should be notd that the 30 kDa polypeptides were lacking in the homologous protein of *Tithonia sp.* (Figure 1) which is a proposed progenitor of the genus *Helianthus* (Anashchenko, 1979). Polypeptides 3,7, 10 and apparently several 40 kDa polypeptides were common for all the analyzed sunflower species. Furthermore, bands 7 and 10 were also observed in the protein of *Tithonia sp.* The other polypeptide bands were very variable among different *Helianthus* species. Helianthinin patterns were identical between the accessions of the same species from different collections. Thus, for instance, helianthinin of *H.mollis* from the collection of Institute of Genetics was very similar to helianthinin of the same species from VIR collection.. This was also true for the two analyzed accessions of *H.rigidus*. However, the two accessions defined as *H.nuttallii* had different helianthinin polypeptide compositions. It can probably be explained by a mistaken field identification of one of them.

Certain parallelism was observed in helianthinin variability among the annual sunflowers. Thus, for example, polypeptide variants 9, 11, 13, 29 which were the most usual for the wild annual forms, were also frequent in the cultivated sunflower genefund. It should be pointed out that the results of our previous genetic analysis indicate the allelism of polypeptides 11 and 29 to the variant forms 12 and 30, respectively, which are characteristic of the standard type helianthinin. The low molecular weight polypeptides 11 - 13 were strongly reduced in the perennial polyploid species and absent in the perennial diploids (Figure 1). Therefore, a rise of the above polypeptides may be considered as a late evolutionary event which accompanied the origin of annual sunflowers and probably the proces of polyploidization in perennial species.

The inheritance of helianthinin polypeptide composition in the crosses of closely related cultivated (*H.annuus*) and wild (*H.praecox, H.lenticularis*) annual sunflowers was similar to that in intraspecific crosses. For example, in the F_1 generation of the crosses between a CMS form of *H.annuus* and different accessions of wild sunflower *H.lenticularis*, the polypeptides of both parental species were inherited codominantly. Polypeptides 11 and 12 were used as markers for definition of hybrid genotypes in these crosses. The female *H.annuus* plants had polypeptide 12 whereas the male *H.lenticularis* parents had a proposed allelic variant 11. Both variants were displayed in the SDS-PAGE patterns of all F_1 hybrid seeds analyzed. In F_2 samples of the hybrid *H.annuus x H.lenticularis*, homo- an heterozygotes were observed in variants 11 and 12. This suggests a possible Mendelian inheritance of helianthinin characters in crosses of phylogenetically close forms. In another combination. *H.praecox x H.annuus*, helianthinin of the maternal parent, *H.praecox*, had the lacking polypeptides 11 and 12. Eight out of the eighteens seeds analyzed had variant 12 originated from the paternal form and apparently were F_1 hybrids (Figure 3). The other ten seeds were free from variants 11 and 12 and probably resulted from self-pollination. A distinct type of helianthinin inheritance was observed in crosses of genetically incompatible species, for example, in F_2 hybrids between the perennial hexaploid *H.rigidus* and the cultivated *H.annuus*. The F_1 plant was a perennial but also a diploid with seventeen chromosome pairs and very poor seed setting. Its main head was of an intermediate phenotype between the parental species, and the side branches were of wild type. In one of the three F_2 seeds analyzed, helianthinin was not found. In the two other seeds the protein patterns were imperfect. The identified

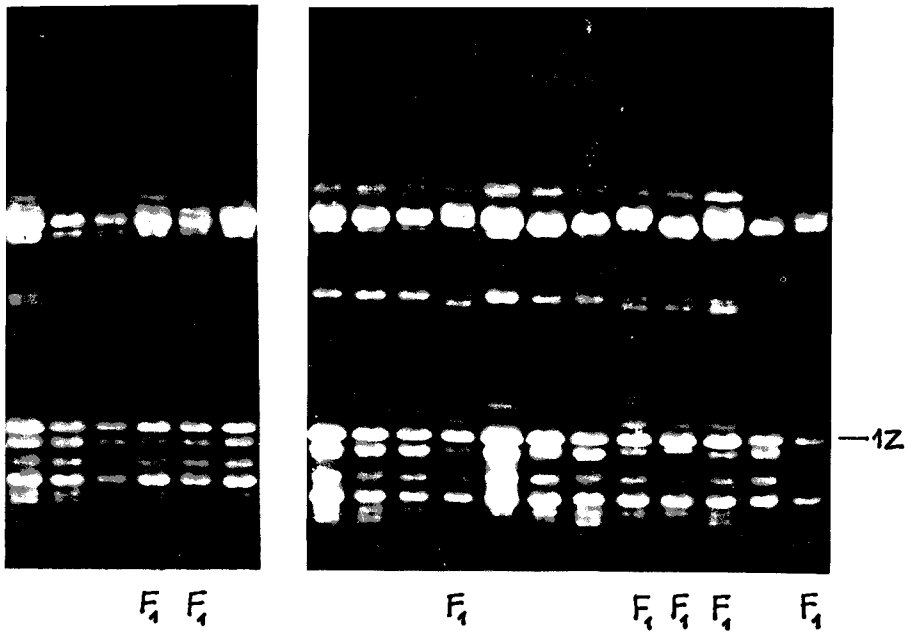

Figure 3.SDS-PAGE patterns of helianthinin from single seeds of the cross H.praecox x H.annuus.

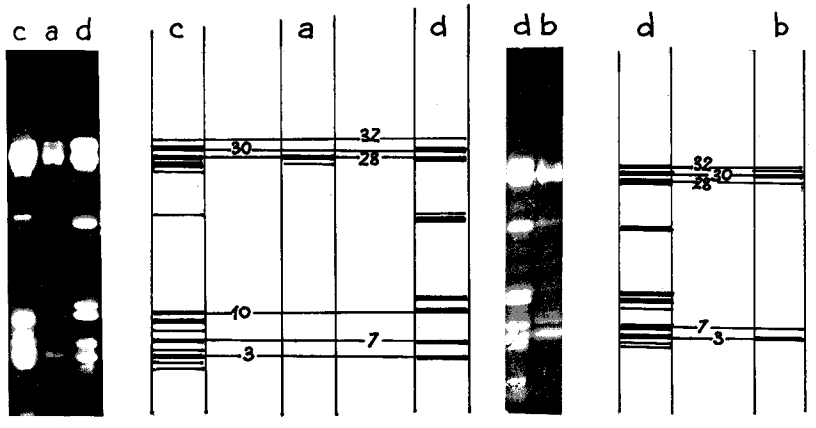

Figure 4.SDS-PAGE patterns of helianthinin from seeds of the F_2 hybrid H.rigidus x H.annuus (seed 1 - a, seed 2 - b) and its parental species (H.rigidus - c, H.annuus - d).

polypeptides coincided by their electrophoretic mobilities with the stable variants 3, 7, 10 (weak), 28, 30 and 32 in one case. In the other seeds, variants 3, 7, 28 and very weak 30 and 32 were observed (Figure 4). The patterns were also characterized by the absence of acidic 30 kDa polypeptides and all the species specific variants. The above data suggest that in hybridization of unrelated species differing in genome constitution the helianthinin polypeptide composition is inherited not codominantly but in accordance with the principle of homology. It may be that the synthesis of major protein polypeptides needs homologous DNA encoding sequences derived from both parents. Abscence of species specific polypeptides in hybrid protein is probably explained by the following reasons: i)elimination of a large part of cromosome material from hybrid nucleus (as in our case); ii) disturbances in the forming of olygomeres from the products of divergent or non-allelic genes (Gavrilyuk, 1986). Such low polypeptide heterogeneity observed in the above instance can lead to alteration of conformation and therefore to disturbances in protein biological functions, the main of which is the nutrition of the germinating embryo. Seed shrivelling or inviability of seedlings in distant phylogenetic crosses are probably in great part caused by underaccumulation of the major storage globulin usually averaging up to seventy per cent of sunflower seed protein fraction. The above assumptions are well illustrated by analyzing helianthinin from single seeds of crosses between the F_1 hybrid (*H.tuberosus x H.annuus*) and wild sunflower (*H.lenticularis*). The female F_1 plant of the cross *H.tuberosus x H.annuus* was a perennial of an intermediate phenotype. By hybridization with wild annual sunflower, seed setting was 23,8% (compared with 24,8% in the cross *H.tuberosus x H.lenticularis* and 1,2% in the cross *H.mollis x H.lenticularis*). The patterns of single seeds of the triple hybrid included all three polypeptide groups which

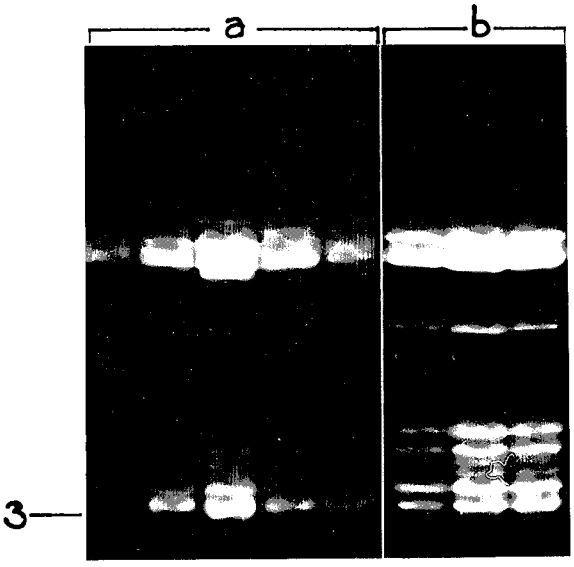

Figure 5.SDS-PAGE patterns of helianthinin from single seeds of the cross (H.tuberosus x H.annuus) x H.lenticularis (a) and H.annuus (b).

were considerably reduced compared with that of the parental species. All six examined seeds were similar to the annual sunflower in their basic polypeptides, however all bands in this region were very weakened except the third one. In hybrid helianthinin, the 30 kD acidic polypeptides were significantly reduced but the 40 kD ones were highly heterogenous due to the heterozigosity of encoding genes (Figure 5). It should be noted that *H.tuberosus* is a natural allopolyploid at least one of the genomes of which was originated from annual sunflower (Kostoff, 1939; Anashchenko, 1979; Anisimova, 1984).

Hybridization of genetically distant sunflower species particularly perennial and annual ones has been shown to be accompanied by a loss of genetical material of one of the parents. The resulting fertile forms, if obtainable, are morphologically similar to the other parent. The inheritance of helianthinin in such cases is like the inheritance of morphological characters. Thus, for instance, we have analyzed helianthinin of three accessions from hybridization *H.rigidus x H.annuus*, all having cytoplasm of the perennial species. One of the hybrids, obtained at the Institute of Genetics, Bulgarian Academy of Sciences, was morphologically close to the maternal species. Helianthinin isolated from seeds of this plant was identical with helianthinin of *H.rigidus*. Two other accessions, which were typical *H.annuus*, represented a new cms *rigidus* source and were reproduced in 1988 in Russia and in Bulgaria (see Table 1). Moreover, the material reproduced in Bulgaria was originated from Romania and Bulgaria. As it was expected, helianthinin from individual seeds of that sample was like the standard type helianthinin of *H.annuus* (Figure 2). In all three accessions, polypeptide 9 was found which is characteristic of wild species including *H.rigidus*. The frequencies of this polypeptide were about 6% in the Russian accession and 24% and 34% in the samples from Bulgaria.

CONCLUSION

Storage proteins accumulating in abundance in cotyledones of developing sunflower seed are necessary for providing sustenance during germination. Studies of seed proteins and especially of the major globulin are therefore important in relation with the problem of seed viability in interspecific crosses in the genus *Helianthus*. Comparative analyses of helianthinin from different species suggest a great polymorphism of the protein within the genus. The degree of differences in the polypeptide composition corresponds to the phylogenetic proximity of the species. In crosses of genetically related species certain helianthinin polypeptides are inherited codominantly and can be used as a marker character for controlling transmission of genetic material from one parental species to the other. The picture is different in the hybridization of genetically distant species. A combination of non-homologous genomes in hybrid nucleus leads to irregularities in protein synthesis and to disturbances in the association of polypeptides to the protein molecule. In this case, SDS-PAGE patterns of hybrid helianthinin are defective in their polypeptide composition. This allows identifying a proposed hybrid and predicting possible inviability of hybrid seed. Thus, certain helianthinin polypeptides can serve as molecular markers in interspecific hybridization within the genus *Helianthus*.

REFERENCES

Anashchenko A.V.,1979. Phylogenetic relations in the genus *Helianthus L.* Bull.Appl.Bot.Genet.Plant Breed. (in Russian), 64:146 - 156.

Anashchenko A.V. and I.P.Gavrilyuk, 1979. Immunochemical analysis of species of genus *Helianthus L.* Dokladi VASKHNIL (in Russian), 10: 17 - 19.

Anisimova I.N.,1984. Genome analysis in genus *Helianthus L.* Genetics (in Russian), 20: 1925 - 1933.

Anisimova I.N. and I.P.Gavrilyuk,1985. Helianthinin and proteins homologous to it in the family *Asteracea Dumort.* Bot.Journ. (in Russian), 70: 1029 - 1096.

Anisimova I.N., I.P.Gavrilyuk and V.G.Konarev,1986. Subunit and polypeptide composition of sunflower seed 11S globulin. Dokladi VASKHNIL (in Russian), 8: 4 - 8.

Anisimova I.N. and I.P.Gavrilyuk,1989. Heterogeneity and polymorphism of the 11S globulin of sunflower seeds. Genetics (in Russian), 25: 1248 - 1255.

Anisimova I.N., I.P.Gavrilyuk and V.G.Konarev, 1991. Identification of sunflower lines and varieties by helianthinin electrophoresis. Plant Varieties and Seeds, 4: 133 - 141.

Choumane W. and P.Heizmann, 1988. Structure and variability of nuclear ribosomal genes in genus *Helianthus.* Theor.Appl.Genet. 76: 481 - 489.

Gavrilyuk I.P.,1986. Immunochemistry of seed storage globulins of dicotyledonous plants. Autoref.doct.dis., Moscow: 56 p.

Georgieva-Todorova J., 1976. Interspecific relationships in genus *Helianthus L.* Sofia, Bulgarian Academy of Sciences: 169 p.

Heiser C.B., D.M.Smith, S.B.Clevenger and W.C.Martin, 1969. The North American Sunflowers (*Helianthus*). Mem.Torrey Bot.Club, 22: 218 p.

Konarev V.G.,1983. Plant proteins as genetic markers. Moscow: 320 p.

Kostoff D.,1939. Autosynthesis and structure hybridity F_1 - hybrid *H.tuberosus x H.annuus L.* and their sequences. Genetica, 11: 285 - 300.

Perez C. and A.Berville, 1988. The mitochondrial plasmid of sunflower: its use to establish relationships for the *Helianthus petiolaris* species. Proc. 12th International Sunflower Conference. Novi Sad, Yugoslavia: 324 - 327.

Rieseberg L.H., D.E.Solits and J.D.Palmer,1988. A molecular reexaminanation of introgression between *H.annuus* and *H.bolanderi* (Compositae). Evolution, 42 227 - 238.

Rieseberg L.H., R.Carter and S.Zona,1990. Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* species (Asteraceae). Evolution, 44: 1498 - 1511.

Rogers C.E., T.E.Thompson and G.J.Seilor, 1982. Sunflower species of the United States. National Sunflower Association, Bismark, ND: 75 p.

Schwenke K.D., M.Schultz and K.J.Linow,1975. Isolierung und characterisierung des 11S globulins aus Sonnenblumensamen (*Helianthus annuus L.*). Nahrung, 19: 817 - 822.

VARIABILIDAD EN HELIANTININA, LA MAYOR GLOBULINA DE LA SEMILLA EN EL GÉNERO HELIANTHUS

RESUMEN

Polimorfismos de la mayor proteina de reserva de la semilla de girasol, HS globulina (heliantinina), fueron estudiados utilizando el método de electroforesis unidimensional sodio dodecilsulfato policrilamida. Los materiales examinados incluyeron diez y siete entradas de especies anuales y perennes del género *Helianthus* una entrada de especies de *Tithonia* y sus hibridos simples y complejos. La composición de polipéptidos de la heliantinina difirió considerablemente entre las especies del género. Las especies anuales y perennes fueron las que mas se diferenciaron. Un número de polipéptidos fueron estables dentro del género y los otros se caracterizaron por una alta variabilidad. En cruces de especies genéticamente cercanos la composición de polipéptidos se heredó como un carácter mendeliano. Sin embargo por hibridación de especies distantes sin genomios comunes (*H. rigidus x H. annuus*) la composición de polipéptidos de la proteina del hibrido fue pobre. En las semillas de hibridos solo se expresaron los polipéptidos que estuvieron presentes en ambos padres. Esto explica probablemente la inviabilidad en cruces interspecificos del género *Helianthus*.

VARIABILITÉ DE L'HELIANTHININ, PRINCIPALE GLOBULINE DES GRAINES DU GENRE *Helianthus* L.

RÉSUMÉ

Le polymorphisme de la principale protéine de réserve de la graine de tournesol – la 11S globuline (Helianthinin) – a été étudié par électrophorèse à une dimension sur gel polyacrilamide SDS. Le matériel étudié comprenait 17 *Helianthus* (espèces annuelles et pérennes), un représentant de l'espèce *Tithonia* ainsi que six hybrides interspécifiques simples et complexes. La composition polypeptidique de l'helianthinin varie considérablement selon les espèces, les espèces annuelles et pluriannuelles présentant entre elles les plus importantes différences. Un certain nombre de polypeptides sont spécifiques du genre, les autres se caractérisent par une forte variabilité. Les croisements entre espèces génétiquement proches montrent que la composition en polypeptides est un caractère dont l'héritabilité est de type mendélien. Cependant, l'hybridation d'espèces génétiquement éloignées, ne possédant pas de génome homologué (par exemple *H. rigidus* x *H. annuus*), produit des individus caractérisés par une composition polypeptidique pauvre. Dans les graines hybrides, seuls les polypeptides présents chez les deux parents à la fois sont exprimés. Cela explique probablement la non viabilité des graines issues de croisements interspécifiques au sein du genre *Helianthus*.