HELIA, **29**, Nr. 45, p.p. 107-116, (2006) UDC 633.854.78:631.527.5

INTERSPECIFIC HYBRIDS BETWEEN CULTURAL SUNFLOWER *Helianthus annuus* L. AND *Helianthus salicifolius* L. - MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

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Received: January 31, 2006
Accepted: December 05, 2006

SUMMARY

The direct organogenesis method in immature F_1 hybrid embryos from sunflower used for the first time in a study, was successfully applied for production of new forms from the intergeneric cross *Helianthus annuus* L. (cv. Albena) × *H. salicifolius*. A considerable number of new sunflower lines were produced after self-pollination and individual selection. Agronomic traits such as oil content in seed, 1000 seed weight, plant height, leaf width, leaf length, number of leaves, length of petiole stems, internode length, head diameter, stem diameter, diameter of branch head, number of branches, length of branches, number of ray florets, seed width, seed length, and seed thickness were investigated. After characterization of the hybrid progenies according to the indices, the conclusion can be drawn that lines 107 R, 114 R and 120 R show 76.5% intermediate phenotype in comparison with the two parental forms. The positive change was 15.5% and the negative change was 21.5%. Some of the new restorer lines were successfully used in heterosis breeding of sunflower.

Key words: **sunflower, direct organogenesis, *Helianthus annuus*, *Helianthus salicifolius*, morphological and biochemical characteristics**

INTRODUCTION

Wild sunflower species are a source of genes for resistance to diseases and pests in cultural species, of genes for early maturation and resistance to unfavorable environmental factors and of sources of cytoplasmic male sterility, *Rf* genes and other useful characters (Putt and Sackston, 1963; Leclercq, 1969; Pustovoit, 1975; Morized *et al*., 1984; Škorić, 1985; etc.).

Hybridization, however, is often restricted by genetic incompatibility in crossing, genetic distance and different ploidy levels. *Helianthus annuus* (2n=2×=34)

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can be easily crossed to annual diploid species, but its crossing to diploid and polyploid perennial species is more difficult (Georgieva-Todorova, 1993). The difficult crossability, the embryonic and post-embryonic interspecific and intergeneric incompatibility and sterility in the F_1 hybrid progeny are barriers to the use of the genetic potential of the wild species for improving some characters in the cultural sunflower. The embryo rescue technique is most commonly used for overcoming the incompatibility between *H. annuus* and other alien wild species. It allows to obtain a large number of interspecific hybrids (Chandler and Beard, 1983; Georgieva-Todorova, 1984a; Bohorova *et al.*, 1985; Kräuter *et al.*, 1991; Freidt, 1992; Dahlhoff *et al.*, 1992; Dozet *et al.*, 1996; Sukno *et al.*, 1999). The methods of embryo culture, ovular culture, somatic hybridization and callus culture applied up to now in interspecific and intergeneric hybridization do not always contribute to the production of hybrid plants. This gave us grounds to investigate the possibilities of the direct organogenesis method as an approach for overcoming interspecific and intergeneric incompatibility in sunflower hybridization, which has not been tried before (Encheva *et al.*, 1992).

The cross *H. salicifolius* × *H. annuus* L. has been obtained by Georgieva-Todorova (1990) by the embryo culture method, but the author has not presented data on the hybrid progenies at advanced generation. In comparison to the author mention above, our study presents data on the hybrid progenies of *H. annuus* L. (cv. Albena) × *H. salicifolius* up to the F_{10} generation.

The aim of this study was to present the morphological and biochemical characters of the F_{10} hybrid progenies from the cross *H. annuus* L. (cv. Albena) × *H. salicifolius* produced by the method of direct organogenesis.

MATERIAL AND METHODS

Plant material

Cultural sunflower (the hybrid Albena, 2n=34) and the wild species *H. salicifolius*, accession M-078 (2n=34) (Figure 1), were grown under field conditions at DAI-General Toshevo. Hybrid embryos were obtained after sterilization of female plants with GA_3 and hand pollination with pollen of the male parent.

Methods

Immature hybrid zygotic embryos 13-15-days old were cultivated on nutrition media I (according to Volin *et al.*, 1989) for further embryo development. After one week the embryos were transferred to medium II (according to Freyssinet and Freyssinet, 1988) for production of somatic buds. After 2-3 weeks, the somatic buds which had already appeared were excised and placed on medium III for rooting (according to Wilcox *et al.*, 1988). The fertile F_1 plants were self-pollinated manually (Figure 2). The F_3 plants were subjected to biochemical study (Encheva *et al.*,

Figure 1: Species H. salicifolius (accession M-078)

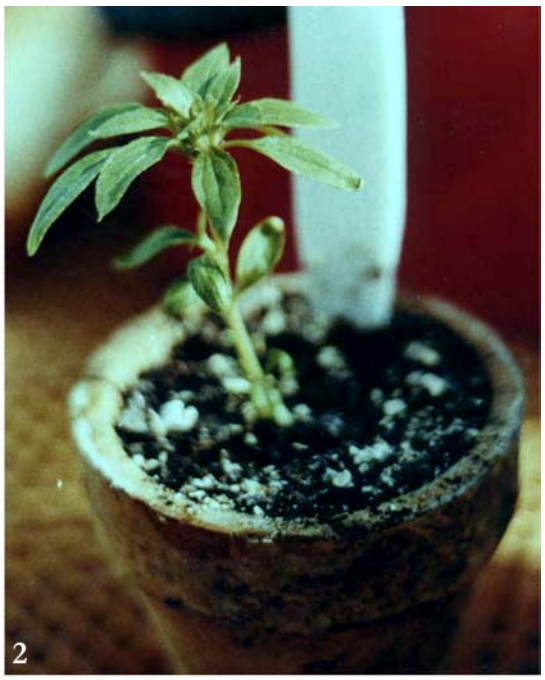

Figure 2: An F_1 hybrid plant from the cross H. annuus L. (cv. Albena) × H. salicifolius

Figure 3: Shape and color of leaves of H. annuus L. (cv. Albena), line R114 and H. salicifolius

Figure 4: Seed shape of the parents H. annuus (cv. Albena) and H. salisifolius, and of lines R120, R114 and R107

1992). As a result from long-term selfing and individual selection in the hybrid materials, fertility restorer lines were produced in the R_{10} generation. All hybrid materials possessed a *cms* source of *H. petiolaris* from Leclerq (1969).

The lines were investigated for some important characteristics concerning breeding in sunflower. Biometric studies, fertility test and biochemical characterization of seeds were carried out in each generation. The breeding process was directed towards creation of branched fertility restorer lines.

Biometrical evaluation and biochemical analysis of the parental forms and the new hybrid progenies R107, R114 and R120

The biometrical evaluation and biochemical analyses of the parental forms and the new developed lines was made on plants from individual year, and they included 17 main agronomic characters. Thousand-seed weight (g) was determined in three samples of 50 seeds per head. Nuclear-magnetic resonance (Newport Instruments *Ltd.*, 1972) was used to determine oil content of dry seeds in the developed R lines, as well as in the parental forms.

RESULTS AND DISCUSSION

The significance and role of interspecific and intergeneric hybrids in plant breeding is due to the large variability observed in the F_2 and subsequent generations as a result of extreme heterozygosity in the hybrids. Among segregants of interspecific and intergeneric hybrids, plants can be observed with characters approximating the characters of either parent, individuals with a completely new phenotype or those similar to related species. This large genetic variability provides the basis necessary for successful plant breeding.

The direct organogenesis method allows breeders to produce 3 to 8 hybrid plants from a single embryo. This is a valuable method because it allows to obtain more than one plant from a hybrid embryo, which is not possible with the commonly used embryo rescue technique (a method for production of one plant only from a hybrid embryo). The reason for the more efficient use of the direct organogenesis method is that adventive buds can be produced, and subsequently plants from each cell of the epidermic layer of explants such as hypocotyl or cotyledons of the hybrid tissue may be developed, *i.e.*, it is not necessary to have an entire and well developed zygotic embryo with apical and root meristems.

In this study, a large number of new sunflower fertility-restorer lines (R) were produced after self-pollination and long-term individual selection. It is evident from Table 1, which presents biological characterization of the studied genotypes, that the hybrid progenies of the cross *H. annuus* (hybrid Albena) × *H. salicifolius* possessed a value of the index “beginning of flowering” two to three days shorter than that of the female form.

The obtained change in the index "beginning of flowering" will be of economic value since earliness is desirable in traditional breeding.

Table 1: Phenological observation of promising R lines from the interspecific cross *H. annuus* L. (cv. Albena) × *H. salicifolius* (1997-1999)

Material	Beginning of flowering of central head (days from germination)	Mass flowering (days from germination)	Duration of central head flowering (days)	Vegetation period (days from germination)
Cultivated sunflower (cv. Albena)				
	54	54	5	108
Wild species - *H. salicifolius*				
	108	115	6	142
Hybrid progeny				
R107	51	53	4	95
R114	51	52	6	93
R120	52	53	6	104

Concerning the index "full flowering", lines with intermediate inheritance of the character were obtained, but with regard to the cultivated parent line R114, the beginning of full flowering was two days earlier. Lines R107 and R114 were equal with the wild parent regarding the value of the index "duration of central head flowering".

The three-year results for the character "vegetation period" varied in the hybrid progenies (the F_{10} generation) from 93 to 104 days (Table 1). There was an evident decrease of the values of the investigated character in comparison with the two parents, in which the duration of the vegetation period was 108 and 208 days, respectively.

Morphological characterization of hybrid progenies from the cross *H. annuus* (hybrid Albena) × *H. salicifolius*

Data on some morphological indices of the three R lines developed through interspecific hybridization are given in Tables 2a-2c.

Tables 2a, 2b and 2c present the mean values of the most frequently analyzed sunflower breeding characters. The data for the hybrids of the selected plants showed intermediacy with regard to the indices for leaf width (Figure 3), petiole length, stem diameter, head diameter, number of ray florets (R107 and R120), seed width (Figure 4), seed length (R107 and R120), seed thickness, oil content in seed and 1000-seed weight. Concerning the above indices, most of the lines showed highly significant differences in respect to the female parent Albena.

A negative transgression was established for the characters plant height, number of leaves (R107 and R120), leaf length and internodule length (Tables 2a and 2b). The three investigated hybrid progenies possessed mean arithmetic values lower than those of the two parents, *i.e.*, the character was less expressed. After analyzing the data, it becomes clear that in most of the cases the differences in respect to the female form had the highest degree of statistical significance.

A positive transgression was determined for the indices number of branches, length of branches and diameter of head on branches [R107 and R114 (Tables 2b and 2c)]. The above hybrid progenies had mean arithmetic values higher than those of both parental forms, *i.e.*, the index was more strongly expressed. The data from the two tables showed that the differences in comparison with the cultural form had the highest significance.

Table 2a: Characteristics of promising R lines from the interspecific cross *H. annuus* L. (cv. Albena) × *H. salicifolius* (1997-1999); averaged data

R form	Plant height (cm)	Number of leaves (nb)	Leaf width (cm)	Leaf length (cm)	Petiole length (cm)
Cultivated sunflower (cv. Albena)					
	177.2	32.0	21.1	22.0	16.2
Wild species - *Helianthus salicifolius*					
	236.5	261.0	0.6	20.7	0
Hybrid progeny					
R107	121.6***	28.0***	16.7***	17.5***	14.4***
R114	129.4***	32.0	15.1***	16.0***	14.2***
R120	90.3***	20.0***	16.7***	17.2***	10.2***

Table 2b: Continued

R form	Internodule length (cm)	Stem diameter (mm)	Head diameter (cm)	Number of branches (nb)	Length of branches (cm)	Number of ray florets (nb)
Cultivated sunflower (cv. Albena)						
	5.8	29.4	23.0	0	0	51.0
Wild species - *Helianthus salicifolius*						
	15.6	12.1	1.2	14.0	12.8	16.0
Hybrid progeny						
R107	5.8	22.0***	13.2***	25.0***	28.4***	50.0
R114	5.5	25.0***	12.6***	24.0***	21.2***	53.0
R120	4.7***	21.0***	15.0***	0	0	46.0***

Table 2c: Continued

R form	Diameter of branch head (cm)	Seed width (mm)	Seed length (mm)	Seed diameter (mm)	Oil percent (%)	1000 seed weight (g)
Cultivated sunflower (cv. Albena)						
	0	5.9	11.2	3.9	48.0	78.3
Wild species - *Helianthus salicifolius*						
	1.1	2.2	5.0	1.3	28.4	5.8
Hybrid progeny						
R107	7.1***	5.0***	11.0	3.5*	45.4**	35.4***
R114	8.6***	5.3**	11.5	3.6*	45.0**	37.3***
R120	0	5.0***	8.8***	3.5*	44.1***	40.3***

a, b and c = significance of differences at the levels 0.05, 0.01 and 0.001, respectively

The highest percent of intermediate phenotype with regard to all investigated characters was observed in line R120 (76.5%) followed by R107 (58.8%) and R114 (47.0%).

A positive transgression was established for lines R114 (29%) and R107 (17.6%), and negative for lines R107 and R120 (23.5%) and R114 (17.6%).

Based on the above data, a conclusion can be drawn that the greater part of the indices had values close to those of the female form. This was an expected result due to the long selfing of the investigated lines (F_{10}) and regression to the cultural parent.

CONCLUSIONS

Lines R107, R114 and R120 developed by the direct organogenesis method from the cross *H. annuus* (hybrid Albena) × *H. salicifolius* showed an intermediate phenotype (60.8%) between the two parental forms for the investigated seventeen characters. A positive transgression was observed in 15.5% of the investigated indices, and a negative one in 21.5%.

The developed lines R107, R114 and R120 were earlier with regard to the index "beginning of flowering" and they had a shorter vegetation period than their parental components. The three lines possessed 100% restoration ability. Lines R107 and R114 were included in heterosis breeding of sunflower.

REFERENCES

Todorova, J.G., 1990. Genetic and cytogenetic study of genus *Helianthus*. Sofia, BAS (Bg).

Bohorova, N., Atanassov, A. and Georgieva-Todorova, J., 1985. *In vitro* organogenesis, androgenesis and embryo culture in genus *Helianthus*. Z. Pflanzenzuchtg. 95: 35-44.

Chandler, J.M. and Beard, B.H., 1983. Embryo culture of *Helianthus* hybrids. Crop Sci. 23: 1004-1007.

Dahlhoff, M., Kohler, H. and Friedt, W., 1992. New interspecific hybrids of sunflower. Proceedings of the 13th International Sunflower Conference, Piza, Italy, 7-11 Sept., Vol. II: pp. 1438-1443.

Dozet, B., Atlagić, J. and Vasić, D., 1996. Transferring stem canker resistance from *Helianthus tuberosus* L. into inbred line of sunflower by embryo rescue technique. Helia 19(25): 87-94.

Encheva, J., Christov, M. and Ivanov, P., 1992. Use of direct organogenesis *in vitro* from immature embryos of interspecific and intergeneric hybrids of *Helianthus annuus* L. Proceedings of the 13th International Sunflower Conference, Piza (Italy), 7-11 Sept., Vol. II: 1455-1460.

Freyssinet, M. and Freyssinet, G., 1988. Fertile plant regeneration from sunflower (*H. annuus* L.) immature embryos. Plant Science 56: 177-181.

Friedt, W., 1992. Present state and future prospects of biotechnology in sunflower breeding. Field Crops Research 30: 425-442.

Georgieva-Todorova, J., 1984a. Interspecific hybridization in the genus *Helianthus*. Z. Pflanzenzuchtg. 93: 265-279.

Georgieva-Todorova, J., 1993. Interspecific hybridization and its application in sunflower breeding. Biotechnology and Biotechnology Equipment 7(4): 153-157.

Kräuter, R., Steinmetz, A. and Friedt, W., 1991. Efficient interspecific hybridization in the genus *Helianthus* via "embryo-rescue" and characterization of the hybrids. Theor. Appl. Genet. 82: 521-525.

Leclerq, P., 1969. Use sterile male cytoplasmique chez le turnesol. Ann. Amelior. Plant. 19: 99-106.
Morizet, J., Gruiziat, P., Chatenoud, J., Picot, P., and Leclercq, P., 1984. Improvement of drought resistance in sunflower by interspecific crossing with a wild species *Helianthus argophyllus*. Agronomie 4(6): 577-585.
Newport Instruments Ltd., 1972. Use of the Newport quantity analyzer as a replacement for solvent extraction for measuring the oil and fat content of oil seeds, chocolate, meat and other materials. Newport Pagnell, England.
Pustovoit, G.V. and Krasnokutskaya, O.N., 1975. Protein content of sunflower meal. *In*: Immunitet S.-Kh. Rast. K. Bolesnyam. Vreditelyam, Moskova (Plant Breed. Abstr. No.152).
Putt, E., Sackston, W., 1963. Studies on sunflower rust IV. Two genes R_1 and R_2 for resistance in the host. Can. J. Plant Sci. 43: 490-496.
Škorić, D., 1985. Sunflower breeding for resisting to Diaporthe (*Phomopsis helianthi*), Helia 8: 21-23
Sukno, S., Ruso, J., Jan, C.C., Melero-Vara, J.M. and Fernandez-Martinez, J.M., 1999. Interspecific hybridization between sunflower and wild perennial *Helianthus* species via embryo rescue. Euphytica 106: 69-78.
Volin, J., Espinasse, A. and Lay, C., 1989. *In*: Proceedings of the Sunflower Research Workshop. January, 9-10, 1989, Fargo, ND, USA.
Wilcox, A. McCann., Cooley, G. and Van Dreser, J., 1988. A system for routine plantlet regeneration of sunflower (*Helianthus annuus* L.) from immature embryo derived callus. Plant Cell Tissue Organ Culture 14: 103-110.

HÍBRIDOS INTERESPECIES ENTRE EL GIRASOL CULTIVADO *Helianthus annuus* L. Y *Helianthus salicifolius* – CARACTERÍSTICAS MORFOLÓGICAS Y BIOQUÍMICAS

RESUMEN

El método de organogénesis directa, aplicado por la primera vez en este investigación de los embriones F_1 de híbrido de girasol no madurados, fue aplicado con éxito para engendrar nuevas formas de cruzamiento intergenérico de *Helianthus annuus* L. (cv. Albena) × *H. salicifolius*. Tras la autofecundación y selección individual, fue obtenido el significante número de nuevas líneas de girasol. Estas líneas fueron analizadas en cuanto a las características agronóicas, como son el contenido de aceite en la semilla, el peso de 1000 granos, altura de la planta, ancho de la hoja, longitud de la hoja, número de hojas, longitud del pecíolo, longitud del internodio, diámetro de cabeza, diámetro del tallo, diámetro de cabezas secundarias, número de ramos, longitud de ramos, número de flores lingüiformes, ancho de la semilla, longitud de la semilla y grosor de la semilla. Tras la caracterización de la descendencia híbrida, de acuerdo con estos índices, fue concluido que las líneas 107 R, 114 R y 120 R demuestran 76,5% de intermediaridad del fenotipo en relación con las formas parentales. El cambio positivo fue 15,5%, negativo 21,5%. Algunas de estas líneas restauradoras fueron utilizadas con éxito en la selección de girasol heterótica.

HYBRIDES INTERSPÉCIFIQUES ENTRE LES TOURNESOLS DE CULTURE *Helianthus annuus* L. ET *Helianthus salicifolius* L. – CARACTÉRISTIQUES MORPHOLOGIQUES ET BIOCHIMIQUES

RÉSUMÉ

La méthode d'organogénèse directe utilisée pour la première fois dans cette tude des embryons de tournesol immatures F_1 a été appliquée avec succès dans la production de nouvelles formes à partir du croisement intergénérique *Helianthus annuus* L. (cv. Albena) × *H. salicifolius*. Après autofécondation et sélection individuelle, un grand nombre de nouvelles lignées de tournesol a été obtenu. Les caractéristiques agronomiques suivantes ont été analysées: contenu d'huile dans la graine, poids de 1000 graines, hauteur de la plante, largeur de la feuille, longueur de la feuille, nombre de feuilles, longueur des tiges de pétiole, longueur internodale, diamètre de la tête, diamètre de la tige, diamètre des têtes secondaires, nombre de ramifications, longueur des ramifications, nombre de fleurons ligurés, largeur de la graine, longueur de la graine et épaisseur de la graine. Après caractérisation de la progéniture hybride relativement à ces indices, on peut conclure que les lignées 107 R, 114 R et 120 R montrent 76,5% de phénotype intermédiaire en comparaison aux deux formes parentales. Le changement positif a été de 15,5% et le changement négatif de 21,5%. Certaines des nouvelles lignées restauratrices ont été utilisées avec succès dans la sélection hétérosis du tournesol.