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CHARACTERIZATION OF INTERSPECIFIC HYBRIDS BETWEEN CULTIVATED SUNFLOWER *H. annuus* L. (cv. ALBENA) AND WILD SPECIES *Helianthus tuberosus*

Encheva, J.*, M. Christov and P. Ivanov

Dobroudja Agricultural Institute, General Toshevo 9520, Bulgaria

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SUMMARY

By using the direct organogenesis method, applied for the first time at the Biotechnology Laboratory of DAI, General Toshevo, new sunflower forms were obtained through distant hybridization from the cross *H. annuus* L. (cv. Albena) × *Helianthus tuberosus* (Encheva *et al.*, 1992). Three to eight hybrid plants were produced from a single embryo through the direct organogenesis method. This is a valuable method because it allows to obtain more than one plant from a hybrid embryo, something which is impossible with the commonly used "*embryo rescue*" technique. A considerable number of new sunflower lines were produced after self-pollination and individual selection. After characterization of the hybrid progenies according to seventeen morphological and biochemical indices, the conclusion can be drawn that lines R101 and R104 show 58.8% and 70.6%, respectively, intermediate phenotype in comparison with the two parental forms. The positive transgressive forms were 29.4% and 23.5%, respectively, and the negative transgressive forms were 5.9% for both lines. Some of the R lines were directly included in heterosis breeding of sunflower.

***Key words*: direct organogenesis, *Helianthus annuus L.* (cv. Albena), *Helianthus tuberosus*, morphological and biochemical characteristics**

INTRODUCTION

The difficult crossability, embryonic and post-embryonic interspecific and intergeneric incompatibility and sterility in the F_1 hybrid progeny are the barriers to the use of the genetic potentials of the wild species for improving some characters of the cultivated 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

* Corresponding author

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.*, 1996a).

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 that had not been applied up to now (Encheva *et al.*, 1992).

Crosses with the species *H. tuberosus* have been obtained by Kräuter *et al.* (1991) and Dahlhoff *et al.* (1992) by the embryo culture method, but the authors have not presented data on the hybrid progenies at advanced generations.

The aim of this study was to characterize morphologically and biochemically the hybrid progenies in the F_8-F_{10} generations from the cross *H. annuus* L. (cv. Albena) × *H. tuberosus* produced by the method of direct organogenesis.

MATERIAL AND METHODS

The cultivated sunflower *Helianthus annuus* L. (cv. Albena) and the wild species *H. tuberosus* (2n=102) accession (M-004) were grown under field conditions at DAI, General Toshevo. Hybrid embryos were obtained under field conditions. Direct somatic buds and plants from the interspecific cross *H. annuus* L. (cv. Albena) × *H. tuberosus* were induced on nutrition media I, II, and III (Encheva *et al.*, 1992). As a result of long-term individual selection in the hybrid materials, fertility restorer lines were produced in the R10 generation; these were investigated with regard to all basic characteristics concerning sunflower breeding.

RESULTS AND DISCUSSION

The wild *Helianthus* species are potential sources of genes of resistance to diseases and pests, other important agronomic traits and seed properties (Thomson *et al.*, 1981) and can be included in the genome of the cultivated sunflower by means of interspecific and intergeneric crossings (Seiler *et al.*, 1992; Škorić and Rajčan, 1992; Škorić *et al.*, 1995; Köhler *et al.*, 1997; Thomson *et al.*, 1981).

Morphological and biochemical characterization of hybrid progenies from the cross *H. annuus* L. (cv. Albena) × *H. tuberosus*

The direct organogenesis method in immature F_1 hybrid embryos (Figure 2) from sunflower used for the first time in a study, was successfully applied for production of new forms from the interspecific cross *H. annuus* (cv. Albena) × *H. tuberosus* (Figure 1).

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

Figure 1: Species H. tuberosus (accession M-004)

Through the direct organogenesis method, 3 to 8 hybrid plants were produces 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, i.e., an entirely and well developed zygotic embryo with apical and root meristem is not necessary. After self-pollination and long-term individual selection, a large number of new sunflower fertility-restorer lines (R) were produced.

Data on some morphological and biochemical indices for lines R101 and R104 are presented in Table 1 (a-c). The plants from these two lines showed an intermediate genotype with regard to the indices of leaf width (Figure 3), leaf length, petiole length, seed width, seed length (Figure 4), seed thickness, oil content in seed and 1000-seed weight. Concerning the above indices, the two studied lines demonstrated a high degree of significant differences with regard to the female parent hybrid Albena.

Table 1a: Characteristics of promising R lines from the interspecific cross *H. annuus* L. (cv. Albena) × *H. tuberosus* (1997-1999); average data

R form	Plant height (cm)	Number of leaves (no)	Leaf width (cm)	Leaf length (cm)	Petiole length (cm)
Cultivated sunflower (cv. Albena)					
	177.2	32.0	32.3	22.0	16.2
Wild species -*Helianthus tuberosus*					
	219.8	39.0	8.7	18.2	4.8
Hybrid progeny					
R101	122.8 -c	19.0 -c	20.0	20.6 -a	13.1 -c
R104	143.0 -c	32.0	19.8 -a	21.1	14.3 -c

Table 1b:

R form	Internodule length (cm)	Stem diameter (mm)	Head diameter (cm)	Number of branches (no)	Length of branches (cm)	Number of ray florets (no)
Cultivated sunflower (cv. Albena)						
	5.8	29.4	23.0	0	0	51.0
Wild species - *Helianthus tuberosus*						
	6.3	13.2	1.6	10.0	24.1	10.0
Hybrid progeny						
R101	7.6 +c	22.6 -c	11.0 -c	16.0 +c	31.2 +c	54.0 +a
R104	6.1 +a	25.1 -c	12.7 -c	22.0 +c	25.6	57.0 +c

Table 1c:

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 tuberosus*						
	1.7	3.0	5.9	1.7	27.4	10.0
Hybrid progeny						
R101	7.6 +c	5.2 -c	10.0 -c	3.1 -c	44.1 -c	33.6 -c
R104	8.5 +c	4.8 -c	9.7 -c	3.3 -c	43.0 -c	31.0 -c

a, b and c = significant of differences at the level of 0.05, 0.01 and 0.001, respectively

Positive transgressive forms were obtained for the indices of branch length (line R101), number of branches, branch head diameter and number of ray florets (Tables 1b and 1c), i.e., the above lines possess mean arithmetic values higher than those of the parent with higher values of the respective index. The differences in comparison with the female parent are mostly with highest significance.

Contrary to the results presented above, negative transgressive forms with high degree of significance (Table 1a) were produced for the indices of plant height and number of leaves (line R101).

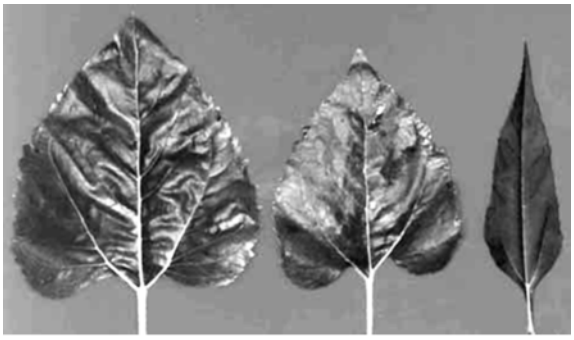

Figure 3: Shape and color of leaves of H. annuus L. (cv. Albena), line R104 and H. tuberosus (from left to right)

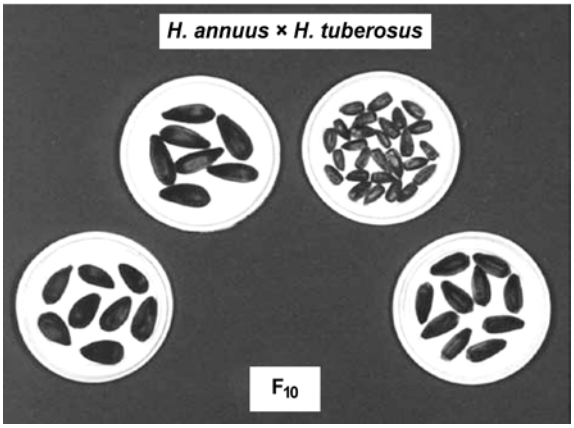

Figure 4: Seed shape of the parents H. annuus L. (cv. Albena) and H. tuberosus, and of lines R101 and R104 (from left to right)

CONCLUSION

The significance and role of interspecific and intergeneric hybrids in plant breeding is determined by the enormous variability observed in F_2 and the succeeding generations as a result of the remarkable heterozygosity of the hybrids. In the segregants of the interspecific and intergeneric hybrids plants can be observed, the characters of which are similar to those of either parent, intermediate forms, individuals with a completely new phenotype or those that resemble related species.

Lines R101 and R104 created with the help of the direct organogenesis method from the interspecific cross *H. annuus* L. (cv. Albena) × *H. tuberosus*, showed to a highest degree an intermediate phenotype between the parents according to the seventeen characters investigated. A positive transgressive forms were observed in 29% of the studied indices, and negative in 5.9%. Among the plants from the two lines a difference in the size and shape of seeds was registered. The vegetation period varied from 94 to 101 days. On the basis of the test carried out on the restorer ability, a conclusion was drawn that both lines restored at 100%. Lines R101 and R104 were successfully included in heterosis breeding of sunflower.

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CARACTERIZACIÓN DE HÍBRIDOS INTERESPECIES ENTRE EL GIRASOL *H. annuus* L. (cv. ALBENA) CULTIVADO Y LA ESPECIE *Helianthus tuberosus*

RESUMEN

Utilizando el método de organogénesis directa, que en esta ocasión fue aplicada por la primera vez en el Laboratorio de biotecnología en DAI, en General Tosevo, se obtuvieron nuevas formas de girasol, mediante hibridización alejada del cruzamiento *H. annuus* L. (cv. Albena) × *Helianthus tuberosus*

(Encheva *et al.*, 1992). El método fue utilizado para la obtención de tres a ocho plantas híbridas de cada embrión. El valor de este método está en el hecho de que el mismo permite la obtención de varias plantas de un embrión híbrido, lo que no es posible lograr aplicando la técnica acostumbrada de "salvación" de embriones. Con autofecundación y selección individual, se obtuvo el número significante de las nuevas líneas de girasol. A base de la caracterización de la descendencia híbrida según 17 índices morfológicos y bioquímicos, puede deducirse que las líneas R101 y R104 demostraron la intermediaridad de fenotipos en el nivel de 58,8% (R101) y 70,6% (R104) en comparación con dos formas parentales. Las formas transgresivas positivas eran 29,4% en la primera línea y 23,5% en la segunda línea, y negativas 5,9% en ambas líneas. Algunas de las líneas R están incluidas directamente en la mejora genética en heterosis en el girasol.

CARACTÉRISATION DES HYBRIDES INTERSPECIES ENTRE LE TOURNESOL CULTIVÉ *H. annuus* L. (cv. ALBENA) ET L'ESPÈCE *Helianthus tuberosus*

RÉSUMÉ

En utilisant la méthode d'organogenèse directe, appliquée pour la première fois au Laboratoire de biotechnologie de DAI, General Toshevo, de nouvelles formes de tournesol ont été obtenues par hybridation à distance du croisement *H. annuus* L. (cv.Albena) × *Helianthus tuberosus* (Encheva *et al.*, 1992). La méthode a été utilisée pour l'obtention de trois à huit plantes hybrides de chaque embryon. La valeur de cette méthode réside en ce qu'elle permet d'obtenir plus de plantes d'un seul embryon hybride, ce qui n'est pas possible par l'application des techniques habituelles de "sauvetage" d'embryon. Un grand nombre de nouvelles lignes de tournesol ont été produites par autofécondation et sélection individuelle. D'après la caractérisation de la descendance hybride selon 17 index morphologiques et biochimiques, on peut conclure que les lignes R101 et R104 ont montré un phénotype intermédiaire de 58,8% et 70,6% respectivement en comparaison des deux formes parentales. Les formes de transgression positive étaient 29,4% et de 23,5% dans les autres lignes et les formes de transgression négative étaient de 5,9% dans les deux lignes. Certaines lignes R étaient directement incluses dans la culture sur l'hétérosis du tournesol.