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# INTERGENERIC HYBRIDS BETWEEN CULTIVATED SUNFLOWER (*Helianthus annuus* L.) AND *Verbesina helianthoides* (GENUS *Verbesina*) - MORPHOLOGICAL AND BIOCHEMICAL ASPECTS

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SUMMARY

The direct organogenesis method in immature $F_1$ hybrid embryos from sunflower, used for the first time in this study, was successfully applied for production of new forms from the intergeneric cross *Helianthus annuus* (cv. Albena) × *Verbesina helianthoides* (genus *Verbesina*). A large number of new sunflower lines were produced after self-pollination and individual selection. Agronomic traits such as oil content in seed, 1000-seed weight, leaf width, leaf length, number of leaves, length of petiole, 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 above indices, the conclusion can be drawn that lines 131 R, 138 R, 140 R, 143 R and 144 R show 76.5% intermediate phenotype in comparison with the two parental forms. Some of the new restorer lines were successfully used in heterosis breeding of sunflower. The positive change was 35.3% and the negative change was 11.8%. Line 140 R is of positive breeding importance because it exhibited an oil value exceeding the cultural and wild parents by 2.9 and 24.6%, respectively.

**Key words:** **direct organogenesis, *Helianthus annuus*, *Verbesina helianthoides* (genus *Verbesina*), morphological and biochemical characteristics**

## INTRODUCTION

Difficult crossability, embryonic and post-embryionic interspecific and intergeneric incompatibility and sterilty 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

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overcoming the incompatibility between *H. annuus* and other alien wild species. It allows obtaining a large number of interspecific hybrids (Chander and Beard, 1983; Georgieva-Todorova, 1984a; Bohorova *et al.*, 1985; Kräuter *et al.*, 1991; Freidt, 1992; Dahlhoff *et al.*, 1996a). The methods mentioned above 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 to overcoming interspecific and intergeneric incompatibility in sunflower hybridization that has not been tried earlier (Encheva *et al.*, 1992). The aim of this study was to characterize morphologically and biochemically the $F_8$-$F_{10}$ generations of the hybrid progenies of the cross *H. annuus* × *Verbesina helianthoides* produced by the method of direct organogenesis.

## MATERIAL AND METHODS

### Plant material

Cultivated sunflower (the hybrid Albena, 2n=34) and the wild species *Verbesina helianthoides* accession VI (2n=34) (Figure 1) were grown under field conditions at DAI, General Toshevo. The 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-day old were cultivated on nutrition medium I (according to Volin *et al.*, 1989) for further embryo development. After one week the embryos were transferred to medium II (Freyssinet and Freyssinet, 1988) for production of somatic buds. After 2-3 weeks, the somatic buds that already appeared were excised and placed on medium III for rooting (Wilcox *et al.*, 1988). The fertile plants were self-pollinated by hand. The $F_3$ plants were subjected to biochemical study (Encheva *et al.*, 1992). As a result of long-term selfing and individual selection in the hybrid materials, fertility restorer lines were produced in the $R_{10}$ generation. All hybrid material possessed the *cms* source of *H. petiolaris* from Leclerq (1969).

The lines were investigated for the main breeding characteristics in sunflower. Biometric evaluation, fertility test and biochemical characterization of seeds were carried out in each generation. The breeding process was directed towards creation of the branched type of fertility restorer lines.

### Biometric evaluation and biochemical analysis of the parental forms and the new hybrid progenies R 131, R 138, R 140, R 143, R 144 and R 146

The biometric evaluation and biochemical analysis of the parental forms and the new lines was made each year on individual plants. The sample size was 17 main heads. Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) was

used to determine the oil content of dry seeds in the developed R lines and the parental forms.

## RESULTS AND DISCUSSION

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 intergeneric cross *H. annuus* × *Verbesina helianthoides* (Encheva *et al.*, 1992). After self pollination and long-terms individual selection, a large number of new sunflower fertility restorer lines were produced.

*Figure 1: The species Verbesina helianthoides (accession VI).*

*Figure 2: Multiple $F_1$ hybrid plants from the intergeneric cross.*

In the cross made, the female form had the sterile cytoplasm of *Helianthus petiolaris* and therefore only fertile forms were considered in this work *i.e.*, the ones possessing a restoration gene. It may originate both from the female (the hybrid Albena) and from the wild parent *Verbesina helianthoides.* According Christov and Vassilevska (1999), there is evidence that the wild form carried genes for restoration of this cytoplasm. No sterile plants were found in the $F_6$-$F_7$ generations, which showed that the selected plants were homozygous with regard to Rf genes.

No segregation was registered concerning the type of branching in the $F_4$, $F_5$ and $F_6$ generations. Large variability was observed among the individual lines both in the form and size in plant architecture. The plants of most progenies produced

large amounts of viable pollen. Vegetation period varied from 98 to 110 days and 1000-seed weight was within 37-55 g. This character shows that the plants had a normal genotype.

**Morphological and biochemical characterization of hybrid progenies**

Some morphological and biochemical indices for lines R 131 (Figure 3), R 138, R 140 (Figure 4), R 143 (Figure 5) and R 144 ($F_{10}$ generation) are presented in Tables 1a-1c.

*Figure 3: Line R 131.*

*Figure 4: Line R 140.*

*Figure 5: Line R 143.*

The plants from these lines showed an intermediate genotype with regard to the indices for plant height, number of leaves (R 143), leaf width, leaf length (Figure 6), petiole length, length of internodes (R 131, R 140 and R 144), stem diameter, number of ray flowers (R 131, R 138 and R 144), seed width (Figure 7), seed length (R 138), seed thickness, oil content in seed (R 131 and R 144) and 1000-seed weight. Concerning the above indices, the lines demonstrated a high degree of significant difference with regard to the female parent (the commercial hybrid Albena).

Positive transgressive forms (Table 1a-1b) were obtained for the indices length of internodes (R 138 and R 143), number of branches, length of branches, number of ray flowers (R 140 and R 143), seed length (R 131, R 140, R 143 and R 144), oil content in seed (R 138, R 140 and R 143) *i.e.*, the above lines possessed mean arithmetic values higher than those of the parent with higher values of the respective indices. The differences in comparison with the female parent were mostly with highest significance.

Contrary to the results presented above, negative transgressive forms with high degree of significance (Tables 1a-2b) were produced for the indices number of leaves (R 131, R 138, R 140 and R 144) and head diameter.

Table 1a: Morphological characteristics of advanced R lines ($F_{10}$) from intergeneric hybrids and their parents (1997-1999)

| R lines | Plant height (cm) | Number of leaves (n) | Leaf width (cm) | Leaf length (cm) | Length of petiole stems (cm) |
|---|---|---|---|---|---|
| Cultivated sunflower | | | | | |
| | 117.2 | 32.0 | 32.3 | 22.0 | 16.2 |
| Wild species - *Verbesina helianthoides* | | | | | |
| | 105.8 | 36.0 | 3.0 | 7.5 | 0 |
| Hybrid progenies | | | | | |
| R 131 | 136.1$^{c}$ | 25.0$^{c}$ | 18.0$^{c}$ | 17.5$^{c}$ | 12.0$^{c}$ |
| R 138 | 141.9$^{c}$ | 31.0$^{-b}$ | 20.1$^{c}$ | 22.0$^{c}$ | 13.8$^{c}$ |
| R 140 | 135.7$^{c}$ | 29.0$^{-c}$ | 18.3$^{c}$ | 19.4$^{c}$ | 13.1$^{c}$ |
| R 143 | 154.5$^{c}$ | 32.0 | 19.0$^{c}$ | 20.7$^{a}$ | 15.6$^{c}$ |
| R 144 | 121.5$^{c}$ | 27.0$^{-c}$ | 17.0$^{c}$ | 17.7$^{c}$ | 12.0$^{c}$ |

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

Table 1b: Morphological characteristics of advanced R lines ($F_{10}$) from intergeneric hybrids and their parents (1997-1999)

| R lines | Length of internodes (cm) | Stem diameter (mm) | Head diameter (cm) | Number of branches (n) | Length of branches (cm) | Number of ray flowers (n) |
|---|---|---|---|---|---|---|
| Cultivated sunflower | | | | | | |
| | 5.8 | 29.4 | 23.0 | 0 | 0 | 51.0 |
| Wild species - *Verbesina helianthoides* | | | | | | |
| | 3.5 | 5.6 | 14.4 | 8.0 | 6.4 | 10.0 |
| Hybrid progenies | | | | | | |
| R 131 | 5.5 | 22.0$^{c}$ | 12.2$^{-c}$ | 23.0$^{+c}$ | 29.2$^{+c}$ | 48.0$^{a}$ |
| R 138 | 6.7$^{+c}$ | 26.0$^{c}$ | 10.0$^{-c}$ | 24.0$^{+c}$ | 50.2$^{+c}$ | 47.0$^{c}$ |
| R 140 | 5.2$^{a}$ | 21.0$^{c}$ | 11.6$^{-c}$ | 19.0$^{+c}$ | 28.6$^{+c}$ | 54.0$^{a}$ |
| R 143 | 6.5$^{+b}$ | 28.0 | 10.6$^{-c}$ | 19.0$^{+c}$ | 26.8$^{+c}$ | 58.0$^{+c}$ |
| R 144 | 5.0$^{c}$ | 21.0$^{c}$ | 12.0$^{-c}$ | 19.0$^{+c}$ | 28.5$^{+c}$ | 48.0$^{b}$ |

Table 1c: Morphological and biochemical characteristics of advanced R lines ($F_{10}$) from intergeneric hybrids and their parents (1997-1999)

| R lines | Diameter of branch head (cm) | Width of seed (mm) | Length of seed (cm) | Thickness of seed | Oil in seed (%) | 1000-seed weight (g) |
|---|---|---|---|---|---|---|
| Cultivated sunflower (the hybrid Albena) | | | | | | |
| | 0 | 5.9 | 11.2 | 3.9 | 47.7 | 78.3 |
| Wild species - *Verbesina helianthoides* | | | | | | |
| | 1.64 | 4.3 | 5.9 | 1.6 | 26.0 | 5.4 |
| Hybrid progenies | | | | | | |
| R 131 | 8.67$^{+c}$ | 5.0 | 12.2$^{+c}$ | 3.5$^{b}$ | 47.4 | 37.4$^{c}$ |
| R 138 | 7.67$^{+c}$ | 5.6$^{c}$ | 10.0$^{c}$ | 3.9 | 48.8 | 46.2$^{c}$ |
| R 140 | 8.13$^{+c}$ | 5.0$^{c}$ | 12.3$^{+c}$ | 3.6$^{a}$ | 50.6$^{+b}$ | 46.0$^{c}$ |
| R 143 | 7.50 | 5.4$^{b}$ | 12.6$^{+c}$ | 4.0 | 48.2 | 51.2$^{c}$ |
| R 144 | 8.60$^{+c}$ | 5.1$^{c}$ | 12.6$^{+c}$ | 3.8 | 47.4 | 47.3$^{c}$ |

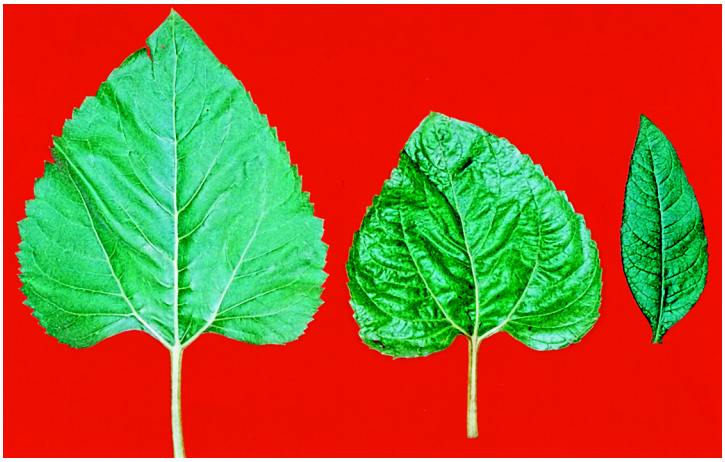

*Figure 6: Shape of leaves of the female parent Albena, line R 131 and the species Verbesina helianthoides.*

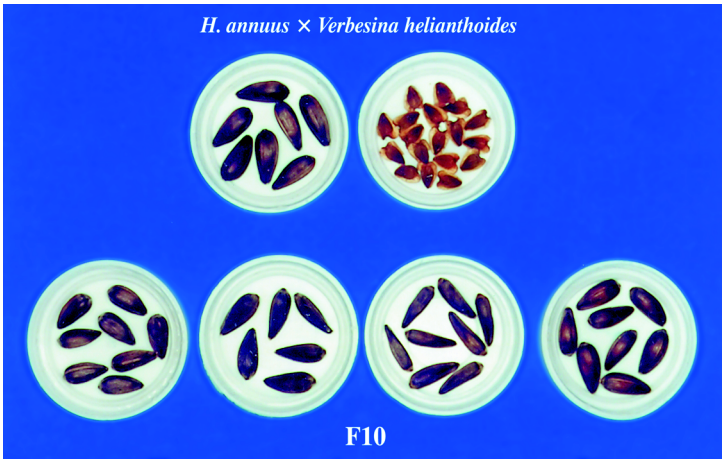


*Figure 7: Shape of seeds of the female parent Albena, the species Verbesina helianthoides and hybrid progenies R 144, R 140, R 138 and R 131.*

The plants of three lines, R 138, R 140 and R 143 (Table 1b), possessed the mean arithmetic value of the index of oil content in seed of 49.2%, the values of the female and male parents being 47.7% and 26.0%, respectively. Line R 140 differed from all other lines because it was positively changed and demonstrated a high degree of significant difference with regard to the female parent, the hybrid Albena. This line is valuable for breeding because it exhibited an oil value exceeding the cultivated and wild parents by 2.9% and 24.6%, respectively, *i.e.*, a positive transgressive form was evidently obtained.

## CONCLUSION

The role of interspecific and intergeneric hybrids in increasing the genetic variability of cultivated sunflower is rather important. The methods of embryo culture, ovule culture, somatic hybridization and callus culture applied up to now in interspecific and intergeneric hybridization have not always contributed to the production of hybrid plants. This gave us grounds to investigate the possibilities of applying the direct organogenesis method to immature zygotic embryos, as an approach for overcoming interspecific and intergeneric incompability in sunflower hybridization, that had not been applied earlier (Encheva *et al.*, 1992). Application of the direct organogenesis method can help overcome these problems. In our study, 3 to 8 hybrid plants were produces from a single embryo by the direct organogenesis method. 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 better efficiency of the direct organogenesis method is that adventitious buds can be produced, without going through callus tissue techniques.

Lines R 131, R 138, R 140, R 143 and R 144 developed with the help of the direct organogenesis method from the intergeneric cross showed an intermediate phenotype between the parents with regard to the seventeen characters investigated. Positive forms were observed in 35.3% of the studied indices and negative ones in 18%. Having in mind the great economic importance of oil content in seed, the line R 140 is important because it exceeded the oil contents of the cultivated and wild parents by 2.9% and 24.6%, respectively.

A conclusion was drawn on the basis of the restoration ability test that all lines restored at 100%. Lines R 131, R 138 and R 140 were successfully included in heterosis breeding of sunflower. The good agronomic indices of the new lines, their resistance to some economically important diseases including parasitic broomrape reveal the large importance of intergeneric hybridization for improvement of cultivated sunflower.

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## HÍBRIDOS INTERGENÉRICOS ENTRE EL GIRASOL CULTIVADO (*Helianthus annuus* L.) y *Verbesina helianthoides* (GÉNERO *Verbesina*) - CARACTERÍSTICAS MORFOLÓGICAS Y BIOQUÍMICAS

RESUMEN

El método de organogénesis directa de embriones inmaduros de los híbridos de girasol $F_1$, fue aplicado por la primera vez con éxito en esta investigación para la creación de nuevas formas del cruzamiento intergenérico de *Helianthus annuus* (cv. Albena) × *Verbesina helianthoides* (género *Verbesina*). Tras la autopolinización y la selección individual, fue creado un gran número de nuevas líneas de girasol. Fueron investigadas las siguientes características agronómicas: contenido de aceite en la semilla, peso de 1000 granos, ancho de la hoja, longitud de hoja, número de hojas, longitud del pecíolo, longitud de internodio, diámetro de la cabeza, diámetro del tallo, diámetro del cuello de la cabeza, número de ramas, longitud de ramas, número de flores liguladas, ancho de la semilla, longitud de semilla y grosor de la semilla. Tras la caracterización de la descendencia híbrida de acuerdo con los índices arriba mencionados, podía deducirse que las líneas 131 R, 138 R, 140 R, 143 R y 144 demostraron el fenotipo intermediario 76,5% entre dos progenitores. Algunos de los nuevos restauradores fueron utilizados con éxito para la hibridización heterótica de girasol. Los cambios positivos ascendían a 35,3%, y los cambios negativos, ascendían a 11,8%. La línea 140 R es importante para la selección, debido a que su contenido de aceite superaba los contenidos de los progenitores cultivado y salvaje por 2,9 y 24,6% respectivamente.

## HYBRIDES INTER GÉNÉRIQUES ENTRE LE TOURNESOL DE CULTURE (*Helianthus annuus* L.) ET *Verbesina helianthoides (*GENRE *Verbesina*) - CARACTÉRISTIQUES MORPHOLOGIQUES ET BIOCHIMIQUES

RÉSUMÉ

La méthode d'organogenèse directe des embryons immatures F1 de l'hybride de tournesol a été appliquée avec succès pour la première fois dans cette recherche sur la création de nouvelles formes de croisement inter

générique *Helianthus annuus* (cv. Albena) × *Verbesina Helianthoides* (genre *Verbesina)*. Un grand nombre de nouvelles lignées de tournesol ont été produites après autofécondation et sélection individuelle. Les caractéristiques agronomiques comme le contenu d'huile dans la graine, le poids de 1000 graines, la largeur de la feuille, la longueur de la feuille, le nombre de feuilles, la longueur des pétioles, le diamètre de la tête, le diamètre de la tige, le diamètre du réceptacle floral, le nombre de branches, le nombre de fleurons ligulés, la largeur de la graine, le longueur de la graine et l'épaisseur de la graine ont été examinés. Après caractérisation de la progéniture hybride d'après ces indices, on peut conclure que les lignées 131 R, 138 R, 140 R, 143 R et 144 R montrent 76,5% de phénotype intermédiaire en comparaison avec deux formes parentales. Quelques-uns des nouveaux restaurateurs ont été utilisés avec succès dans l'hybridation hétérosis du tournesol. Les changements positifs étaient de 35,3% et négatifs, de 11,8%. La lignée 140 R est importante pour la sélection car son contenu d'huile surpasse celui du parent de culture et du parent sauvage de 2,9 et de 24,6% respectivement.