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HERITABLE TISSUE CULTURE INDUCED GENETIC VARIATION IN SUNFLOWER (*Helianthus annuus* L.) AS A TOOL FOR CROP IMPROVEMENT

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SUMMARY

Immature zygotic embryos from the Bulgarian fertility restorer line R 147 (male component of the commercial hybrid Albena) were used as donor material for induction of direct organogenesis in sunflower (*Helianthus annuus* L.). Range of spontaneous somaclonal variation among the progenies of regenerants has been assessed. Genetic modifications observed in the regenerants included agronomic traits such as oil content in seed, 1000-seed weight, plant height, petiole length, internode length, number of branches, length of branches, number of ray florets, seed width and seed length. The somaclonal line R 12001 showed a modified architecture and higher oil content in seed than the standard R 147, and very good combining ability. The hybrid Julia, an offspring of the somaclonal line R 12001, showed seed and oil yields higher by 18.3% and 17.0%, respectively, than those in the standards Albena and Super Start. In the 2002 tests of the State Variety Testing Commission, the hybrid Julia had a higher seed yield than the standards` mean. Such combination of these favorable changes is desirable in breeding work on sunflower. Somaclonal variation through direct organogenesis facilitated the creation of genetically heritable variation in sunflower, which can be used with for effective production of highly productive and adaptable hybrids.

Key words: ***Helianthus annuus* L., direct organogenesis, somaclonal variation, new breeding material, combining ability**

INTRODUCTION

Genetic variability can be induced through polyploidy, hybridization, induced mutagenesis, tissue culture, etc. Plant cell and tissue culture may provide a promising approach for development of new breeding material exhibiting qualities valuable for agricultural production (Skirvin, 1978; Thomas and Pratt, 1982; Thomas *et al.*, 1982; Larkin and Scowcroft, 1981; Chaleff, 1983; Ahloowalia, 1983; Orton, 1985; Evans and Sharp, 1986; Gavazzi *et al.*, 1987; Karp, 1991). Studies on genetic variation in sunflower regenerants have begun comparatively recently and they are not very numerous. Roseland *et al.* (1991) used a technique aiding regeneration from embryo-obtained callus for production of somaclonal variants in sunflower with an increased level of coumarin following stress. Fambrini *et al.* (1993) characterized genetically and phenotypically an albino mutant in sunflower induced through direct organogenesis of cotyledons. The ratio between R3 heterozygous and homozygous plants confirmed that the albino phenotype was controlled by a single recessive gene. Pugliessi *et al.* (1991) observed morphological variants such as abnormal stem development, dwarf forms, plants with arrow leaves and albino plants among a self-pollinated progeny of regenerants. Significant changes in sunflower regenerants obtained through the direct organogenesis method in different genotypes were reported by Encheva *et al.* (1993) and Encheva *et al.* (2003). The genetic variation concerned a large number of indices and some of the somaclonal lines produced were used successfully in heterosis selection for development of highly productive hybrids.

The aims of this study were:

a) to carry out biochemical and biometric investigations on the fertility restorer line R 12001 (the R11 generation) produced through the direct organogenesis method from the genotype R 147 and
b) to study the production capacity and the morphological and biological specificities of the hybrid Julia produced from the somaclonal line R 12001.

MATERIALS AND METHODS

The study was performed during 1989-1999. A part of the experiments were carried out under laboratory conditions, another part in the fields of the Dobroudja Agricultural Institute near General Toshevo.

A main requirement to the initial plant material was genetically purity, *i.e.*, maximum homoyzygosity. We used the fertility restorer line R 147. It possesses very good morphological uniformity since it is the product of more than 20 generations of selfing.

In vitro cultivation of immature zygotic sunflower embryos

The control line R 147 was grown in the field and manually self pollinated. Immature zygotic embryos (9-13 days old) were aseptically isolated and plated on nutrient medium E1 (Encheva *et al.*, 1997) for induction of direct organogenesis and cultivated for 3-4 weeks in dark at 25-26^{o}C. The isolated somatic buds were transferred to SIM1 medium [modified SIM medium (Wilcox McCann *et al.*, 1988), without amino acids and with decreased concentration of sucrose] for further development under 16/8 h photoperiod at 25-26^{o}C. Two or three weeks later, regenerant plants - R0 (nomenclature according to Evans and Sharp, 1983) bigger than 2-5 cm were transferred to R medium for rooting (Wilcox McCann *et al.*, 1988). The plants which formed roots were transferred to pots containing a 3:1 mixture of soil and sand and grown under greenhouse conditions. The fertile plants were self-pollinated by hand. The seeds thus produced (R1) were planted and grown in the field.

Biometric evaluation of donor genotype R 147 and somaclonal line R 12001 produced through direct organogenesis

The biometric evaluation and biochemical analysis of the donor line and the lines obtained through direct organogenesis were carried out on 10 plants each year and they involved 16 basic agronomic indices. The weight of 1000 seeds (g) was determined in three samples, with 50 seeds from each head. The biometric evaluations were carried out for a three-year period (1998-2000), with the exception of the oil content in seed, head diameter and vegetation period, which were measured for eight years (1993-2000).

Biochemical analysis

Oil content in air-dry seeds from the materials included in the study was determined by nuclear magnetic resonance (Newport Instruments Ltd.).

Hybridization

To assess the combining ability of the somaclonal sunflower line R 12001, the sterile analogue of the Bulgarian self-pollinated line 2607 was used. Two Bulgarian commercial hybrids, Albena and Super Start, were used as standards for comparison against the new hybrid Julia.

RESULTS AND DISCUSSION

Evaluation according to quantitative traits in somaclonal line R 12001

The aim of this experiment was to investigate some agronomically important traits of the sunflower somaclonal line R 12001 (Figure 2) produced by the direct organogenesis method.

Figure 1: Control line R 147

Figure 2: Somaclonal line R 12001

Somaclonal lines originating from the fertility restorer R 147 (Figure 1) were selected on the basis of good combining ability or statistically significant morphological and biochemical changes (Figures 3, 4, 5 and 6 and Tables 1, 2 and 3).

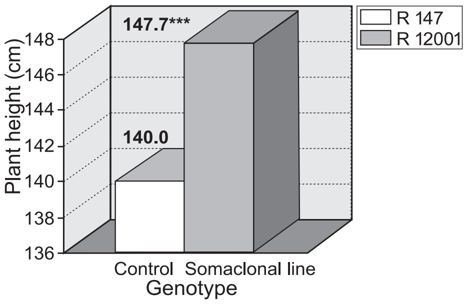

*Figure 3: Changes in plant height index in R11 somaclonal line R 12001 in relation to the source genotype R 147 (1993-2000), (***P=0.1%)*

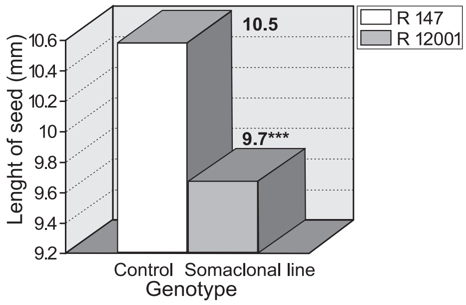

*Figure 4: Changes of seed length index in R11 somaclonal line R 12001 in relation to the source genotype R 147 (1998-2000), (***P=0.1%)*

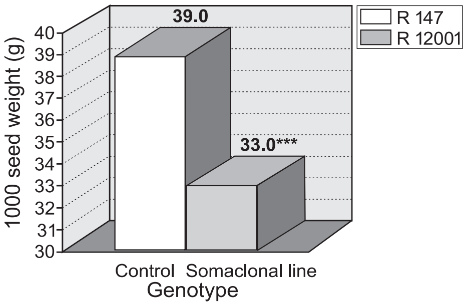

*Figure 5: Changes in 1000-seed weight index in R11 somaclonal line R 12001 in relation to the source genotype R 147, (1998-2000) (**P=1%)*

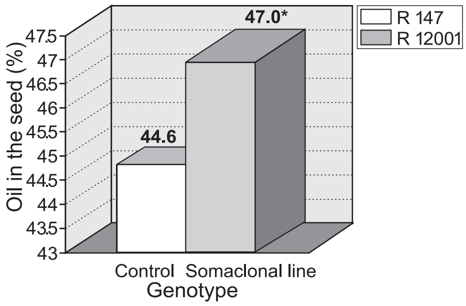

*Figure 6: Changes in oil content in seed index in R11 somaclonal line R 12001 in relation to the source genotype R 147, (1998-2000) (*P=5%)*

Plant height is one of the morphological indices most often investigated in traditional breeding (Figure 3) in cultural sunflower. It is considered as a quantitatively inherited character controlled by 57% dominant (Marinković, 1982; Putt and Sackston, 1966) and 30% additive genes (Lay and Khan, 1985), or by dominant to superdominant genes (Kovačik and Škaloud, 1990).

Table 1: Effect of cultivation on some morphological characteristics (number of leaves, leaf width, leaf length and length of petiole) of R12 somaclonal line R 12001 originated from the genotype R 147 (harvest years 1998-2000)

R line	Number of leaves	Leaf width	Leaf length	Length of petiole
	Mean, (no)	Mean, (cm)	Mean, (cm)	Mean, (cm)
		Control		
R 147	28.00	19.80	21.20	14.00
		Somaclonal line		
R 12001	28.00	19.20	21.50	15.50*

*, ** and *** = significant differences at the levels of 0.05, 0.01 and 0.001, respectively

In our study, the mean value of plant height in the line R 12001 was 7.7 cm, significantly higher than that in the control R 147.

The two-factor dispersion analysis for the seed length index showed a decrease by 0.8 mm in relation to the control. This difference was highly significant (Figure 4). A decrease of 0.4 mm in the seed width index value was also observed (Table 3). Consequently, the somaclonal line R 12001 produced smaller seeds than the initial genotype R 147.

Table 2: Effect of cultivation on some morphological characteristics (length of internodes, stem diameter, head diameter and number of branches) of R12 somaclonal line R 12001 originated from the genotype R 147 (harvest years 1998-2000)

R line	Length of internodes	Stem diameter	Head diameter	Number of branches
	Mean, (cm)	Mean, (mm)	Mean, (cm)	Mean, (no)
		Control		
R 147	6.40	25.00	12.60	20.00
		Somaclonal line		
R 12001	6.90**	24.70	13.40	24.00***

Table 3: Effect of cultivation on some morphological characteristics (length of branches, number of ray florets, seed width and seed thickness) of R12 somaclonal line R 12001 originated from the genotype R 147 (harvest years 1998-2000)

R line	Length of branches	Number of ray florets	Seed width	Seed thickness
	Mean, (cm)	Mean, (no)	Mean, (mm)	Mean, (mm)
		Control		
R 147	27.50	53.00	4.40	2.80
		Somaclonal line		
R 12001	33.80***	60.00***	4.00*	2.80

Data on the 1000-seed weight index are presented in Figure 5. The observed decrease of 6 g in relation to the control was statistically significant. Numerous authors have reported variation in seed weight per plant among regenerants from different crops. Unlike Ahloowalia et al. (1982a, 1985) who found no wheat somaclones with increased weight, Ryan and Scowcroft (1987) did find such somaclonal lines. Zheng *et al.* (1989) established both positive and negative change in the grain weight of rice regenerants.

Oil content in seed (%) is an important biochemical index in sunflower (Figure 6). Our analysis showed that the oil content in the somaclonal line R 12001 was 2.4% higher than that in the control. This difference was statistically significant. The maximum variation coefficient value of the control line R 147 was VC=5.8%, which confirmed the high homogeneity with regard to the studied index under field conditions.

In our study, negative values in relation to the control were registered for the indices leaf width, stem diameter, seed width, seed length and 1000-seed weight, which make 31.3% of the studied characters.

The opposite, *i.e.*, positive values in relation to the control, were registered for the indices plant height, leaf petiole length, internode length, head diameter, number of branches, length of branches, number of ray florets, and oil content in seed, which make 56.3% of all characters studied. Three of the negative and seven of the positive changes were statistically significant, covering 62.5% of all indices included in the investigation. Stability after *in vitro* cultivation was demonstrated by the characters number of leaves and seed thickness. As a result of the positive changes in the indices for plant height, length and number of branches, the produced line R 12001 was superior to the control R 147.

The results presented allow to draw the conclusion that tissue cultivation, and direct organogenesis in particular, may contribute to the occurrence of mutant sunflower plants with modified architecture and increased oil content in seed.

Figure 7: Hybrid Julia, composed from the line ms2607 and the somaclonal line R 12001

Due to its high combining ability, the somaclonal line R 12001 was included in the sunflower breeding program which produced the hybrid Julia.

Contrary to our results, Freyssinet and Freyssinet (1988) observed no significant changes after plant regeneration from immature zygotic embryos of sunflower. Modified sunflower forms (dwarfs) were obtained by Pugliesi *et al.* (1991) through direct organogenesis from cotyledons. The authors followed the heritability of the character in the progeny of R1 self-pollinated regenerants and determined its recessive character.

Use of somaclonal variation for induction of single mutation controlled by one or several genes while preserving all other positive characters of a given line is one of the most useful applications of this technique.

Morphological and biological specificities and the production potential of the hybrid Julia derived from the somaclonal line r 12001 R developed by the direct organogenesis method from the genotype R 147

A five-year test of the somaclonal line R 12001 showed 100% restoration and very good combining ability. The sterile analogue of the Bulgarian self-pollinated line 2607 had been selected as a tester. The results of the dispersion analysis are given in Figures 8, 9 and 10.

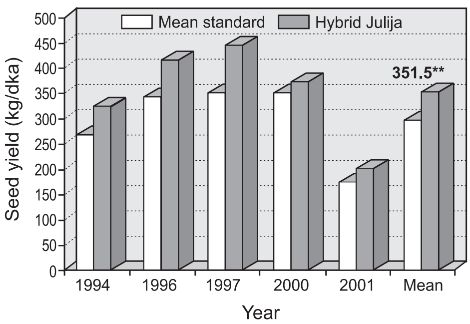

*Figure 8: Comparison of seed yields of the hybrid Julia against the mean value of the standards (hybrids Albena and Super Start) during 1994-2001 (**P=0.1%)*

The seed yield of the hybrid Julia (Figure 7), on average for the five-year period of testing of the somaclonal line R 12001 was 54.4 kg higher (18.3%), than the mean yield of the standards (the commercial hybrids Albena and Super Start); the difference was highly significant (Figure 8).

The results of the dispersion analysis of oil yield showed that the increase of hybrid Julia in relation to the standards' mean value was 23.2 kg or 16.9% (Figure 9). This difference was significant. In addition to the increased higher seed and oil yield, the hybrid Julia was characterized also by the increased oil content in seed (%) and that difference too was statistically significant (Figure 10). The hybrid exceeded the standards' mean values in all years of the study. An exception was observed in 1996, when an insignificant decrease was registered.

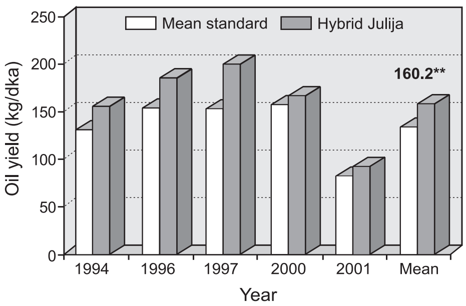

*Figure 9:. Comparison of oil yields of the hybrid Julia against the mean value of the standards (hybrids Albena and Super Start) during 1994-2001 (**P=0.1%)*

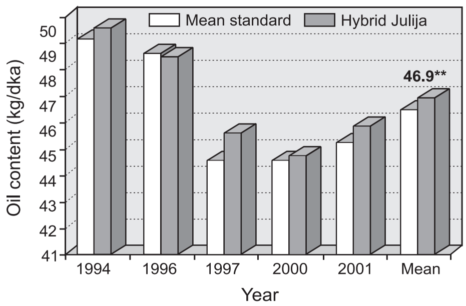

*Figure 10: Comparison of oil content (%) of the hybrid Julia against the mean value of the standards (hybrids Albena and Super Start) during 1994-2001 (**P=0.1%))*

It is worth mentioning that 1997 was the year with the highest average precipitation - 708.9 $cm^3\ m^{-2}$, and 2001 was the driest - 316.8 $cm^3\ m^{-2}$ as compared with the long-term average for the period - 519.2 $cm^3\ m^{-2}$ (1953-2001).

The higher seed and oil yields and the higher oil content in seed of the hybrid Julia in comparison to the standards' means for 1997 and 2001, which were years with extreme precipitation levels, indicated that this hybrid was considerably plastic with regard to water stress.

In addition to the indices mentioned above, the hybrid Julia had a vegetation period 2 days shorter than the standards' mean. There was no significant difference between the hybrid and the standards' means for the indices of plant height and head diameter.

The hybrid Julia was registered with the State Variety Testing Commission in 2002 and it had a seed yield higher than the standards' mean.

It can be summed up in conclusion that, with the help of the direct organogenesis method, the sunflower fertility restorer line R 12001, derived from the genotype R 147, has confirmed positive modifications and high production potential on hybridization.

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LA VARIACIÓN GENÉTICA HEREDABLE CREADA POR EL CULTIVO DE TEJIDO COMO MEDIO PARA LA MEJORA GENÉTICA DE GIRASOL (*Helianthus annuus* L.)

RESUMEN

Los embriones cigóticos inmaduros de la línea restauradora búlgara R 147 (la forma paterna del híbrido comercial Albena) fueron utilizados como el material para la inducción de organogénesis directa de girasol (*Helianthus annuus* L.). Se estudiaba el rango de la variación somaclonal espontánea en la descendencia de los regenerantes. Las modificaciones genéticas, observadas en los regenerantes, incluían las características agronómicas, como el contenido de aceite en la semilla, el peso de 1000 granos, la altura de la planta, la longitud del pecíolo, la longitud del internodio, el número de ramas, la longitud de ramas, el número de flores lingüiformes, el ancho de semilla y la longitud de semilla. La línea somaclonal R 12001 ha mostrado una arquitectura modificada, el contenido de aceite en la semilla aumentado en relación con el estándar R 147, y muy buena habilidad de combinación. El híbrido Julia, creado con la participación de la línea somaclonal R 12001, tenía los rendimientos de semilla incrementados para 18.3% y de aceite para 17.0% en relación con los estándares Albena y Super Start. En el ano 2002, el híbrido Julia fue entregada para la investigación a la Comisión Estatal de Variedades, donde mostró el mayor rendimiento de semilla que el valor medio del estándar. La combinación de estos cambios favorables es deseable en la selección de girasol. La variación somaclonal mediante la organogénesis directo, facilitó la creación de la variación genéticamente heredable en girasol, que puede utilizarse con éxito en creación de los híbridos altamente productivos y adaptables.

VARIATION GÉNÉTIQUE TRANSMISSIBLE CRÉÉE PAR LA CULTURE DE TISSU EN TANT QUE MOYEN D'AMÉLIORER LE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Des embryons zygotes immatures de la ligne restauratrice bulgare R 147 (forme paternelle commerciale de l'hybride Albena) ont été utilisés comme matériel d'induction d'organogenèse directe du tournesol (*Helianthus annuus* L.). La gamme des variations somaclonales spontanées de la descendance des régénérants a été étudiée. Les modifications génétiques observées chez les régénérants comprenaient des caractéristiques agronomiques comme le contenu d'huile dans la graine, le poids de 1 000 graines, la hauteur de la plante, la longueur du pétiole, la longueur internodum, le nombre de ramifications, le nombre de fleurs ligulées, la largeur et la longueur de la graine. La ligne somaclonale R 12001 a montré une architecture modifiée, un contenu d'huile augmenté dans la graine comparativement à la ligne standard R 147 et une très bonne aptitude combinatoire. L'hybride Julia créé avec la participation de la ligne somaclonale R 12001 avait des rendements de graines augmentés de 18, 3% et d'huile de 17,0% par rapport aux lignes standard Albena et Super Start. En 2002, l'hybride Julia a été soumis aux tests de la commission de sélection de l'État où il a montré un plus grand rendement de graines que le standard moyen. La combinaison de ces changements favorables est désirable dans la sélection du tournesol. La variation somaclonale par organogenèse directe facilite la création de variation génétique transmissible dans le tournesol et peut être utilisée avec succès dans la création d'hybrides à grande productivité et à grande capacité d'adaptation.