HELIA, **25**, Nr. 37, p.p. 85-92, (2002) UDC 633.854.78:631.523

CREATING GENETIC VARIABILITY IN SUNFLOWER THROUGH THE DIRECT ORGANOGENESIS METHOD, INDEPENDENTLY AND IN COMBINATION WITH GAMMA IRRADIATION

J. Encheva*, F. Tsvetkova and P. Ivanov

Dobroudja Agricultural Institute, General Toshevo 9520, Bulgaria

Received: May 30, 2002
Accepted: December 10, 2002

SUMMARY

Immature zygotic embryos from a self-pollinated sunflower line 147 R *(Helianthus annuus* L.) were used for donor material to induce direct organogenesis. A portion of the isolated immature embryos were treated with gamma radiation (^{137}Cs) at a dose of 5 Gy before plating. The range of the spontaneously induced somaclonal variation among the obtained regenerants was investigated and compared with that of the variants induced through irradiation. The genetic changes occurring spontaneously during the regeneration procedure included seventeen morphological and biochemical characters. The most significant changes were observed for the characters of plant height, stem diameter, head diameter, length of branches, oil content in seed (%) and 1000-seed weight.

The researches showed that the somaclonal (R9) and the radiation-induced (M9R9) variants revealed similar spectra of morphological and biochemical modifications, thought with different frequency.

Key words: ***Helianthus annuus*, organogenesis, somaclonal variation, gamma irradiation, mutagenesis, new breeding material**

INTRODUCTION

The development of variable initial breeding material is a primary task in the genetic and breeding programs of sunflower. The new approaches, tissue cultures in particular, allow to widen the genetic variability in this crop.

There are insufficient and contradictory data for sunflower with regard to the genetic variability produced independently through tissue culture or in combination with gamma irradiation. Absence of statistically significant changes after plant regeneration from immature zygotic embryos in sunflower has been reported by

* Corresponding author

Freyssinet and Freyssinet (1988) who used a system of direct organogenesis. Roseland *et al.* (1991) obtained statistically significant somaclonal variants in sunflower with an increased level of coumarin after stress induction folowing the technique of plant regeneration from a callus of zygotic embryo. Somaclonal variants in sunflower inherited in the next, R2 generation, were described by Pugliesi *et al.* (1993), but the authors did not present statistical data on the range and inheritability of the induced somaclonal variation.

Mutagenesis, both physical and chemical, proved favorable for mutation induction in tissue cultures. Positive results were obtained when induced mutagenesis and tissue cultivation were combined appropriately in maize (Gavazi *et al.*, 1987; Novak *et al.*, 1988) and wheat (Cheng *et al.*, 1990). Encheva *et al.* (1993) reported statistically significant changes of morphological and biochemical characters in plants regenerated from immature zygotic embryos from sunflower, independently and in combination with gamma irradiation.

The aims of this study were to determine the genetic variability that had occurred spontaneously among the regenerated plants of the genotype 147 R, produced through the direct organogenesis method, as well as to examine the possibility of enhancing this effect by combined use of direct organogenesis and gamma radiation ^{137}Cs. The study included economically important morphological and biochemical traits.

MATERIAL AND METHODS

The study included the fertility restorer line 147 R. The donor plants were grown under field conditions. Plants were self-pollinated under insulator and the immature embryos were isolated 12 days after pollination. A part of the embryos were treated with gamma-irradiation (^{137}Cs) before plating. A low dose of gamma irradiation (5 Gy) was used because we worked with functioning tissue. Cultivation and plant regeneration were realized on nutrition medium El (Encheva *at al.*, 1997), and the obtained somatic buds were developed on SIM1 medium (MS macro salts, MS micro salts, 1 mg l^{-1} K, 0.01 mg l^{-1} IAA, pH 5.7). The regenerated plants were grown under greenhouse conditions and self-pollinated. The seeds produced (R2 and R2M2) were sown in the field. Morphological characterization was carried out on 10 plants for each of the three years of study, and 1000-seed weight and oil in the kernel (%) were determined for the seeds produced by each plant. The oil content was quantified using NMR (Newport Instruments, Ltd. 1972). The control data were collected from plants of the original line 147 R which was grown in field together with the regenerants.

The morphological and biochemical traits of the new lines №№ 46, №№ 60 and №№ 66 (R9 generation) produced through the direct organogenesis method, and lines №№ 81 and №№ 85 (R9M9 generation) produced through direct organogenesis in combination with gamma irradiation were studied during 1998-2000.

RESULTS

The genetic variability in sunflower can be realized also through mutagenesis and tissue cultures, besides the traditional breeding approaches.

The biometric characters presented in this study are among the most commonly studied in the process of traditional breeding. The statistically significant changes concerning plant height included both positive and negative deviations (Table 1).

Table 1: Effect of cultivaton and gamma-ray treatment on some morphological characteristics of R lines (R9 and R9M9 generation) produced through the direct organogenesis method from genotype 147 R (Harvest years 1998-2000)

Genotype	Plant height	Number of leaves	Leaf width	Leaf length	Petiole length
	Mean (cm)	Mean (no)	Mean (cm)	Mean (cm)	Mean (cm)
Control -147 R	140.4	28.0	19.2	21.1	14.8
Line 46 R9	139.1	28.0	19.0	19.8 -b	15.2
Line 60 R9	147.7 +c	28.0	19.2	21.5	15.5 +a
Line 66 R9	134.1 -c	27.0	21.0 +b	22.6 +b	16.1 +c
Line 83 R9M9	126.2 -c	28.0	20.9 +c	21.4	12.8 -c
Line 85 R9M9	126.5 -c	27.0	20.7 +c	21.6	13.4 -c

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

Increase of plant height from 4.1 to 7.7 cm was observed only in lines produced through the direct organogenesis method. Conversely, in the variants with gamma irradiation, significant decreases this index, from 13.9 to 14.2 cm, were registered. The maximum value of the variation coefficient in the initial genotype 147 R (Figure 1) was 4.8%, which points to a high homogeneity of the control line grown in the field.

The change went in opposite directions for both indices: plant height and internodule length (Table 2). However, significantly lower values (5.3 cm, the control being 6.4 cm) were observed only in lines Nº 83 and Nº 85 (Figure 4) produced through combination of direct organogenesis and gamma irradiation.

Table 1 presents data on the changes of the indices for leaf width and leaf length. Highest and statistically significant exceeding of the standard concerning these two indices was demonstrated by somaclonal line Nº 66 (Figure 3). The variants with gamma irradiation also showed increased mean values of the two indices, but only the data on leaf width were statistically significant.

On the basis of the good homozygosity of the initial line 147 R (VC=11.0%), the range of the formative process in the somaclonal and mutation induced lines was followed with regard to the leaf petiole length index. The changes observed in the three lines, Nº 46, Nº 60 (Figure 2) and Nº 66, were invariably towards increase. The was statistically significant highest value of 16.1 cm in line Nº 66, the control

Figure 1: Control line 147 R

Figure 2: Somaclonal line Nº 46

Figure 3: Somaclonal line Nº 66

Figure 4: γ-Radiation induced line Nº 85

being 14.8 cm. Opposite to the above data, the variants with the highest degree of significance, Nº 83 and Nº 85, deviated towards decrease of the mean value.

Table 2: Effect of cultivation and gamma-ray treatment on some morphological characteristics of R lines (R9 and R9M9 generation) produced through the direct organogenesis method from genotype 147 R (Harvest years 1998-2000)

Genotype	Internode length	Stem diameter	Head diameter	Number of branches	Length of branches	Number of ray florets
	Mean (cm)	Mean (mm)	Mean (cm)	Mean (number)	Mean (cm)	Mean (number)
Control -147 R	6.4	25	12.2	20	27.5	53
Line 46 R9	6.1	19.3 -c	15.3 +c	23.0 +c	30.9 +b	53
Line 60 R9	7	24.7	12.1	24.0 +c	33.8 +c	59.0 +c
Line 66 R9	6.5	26	11.7	23.0 +c	42.4 +c	54
line 83 R9M9	5.3 -c	18.0 -c	11.5 -b	24.0 +c	34.5 +c	51.0-b
Line 85 R9M9	5.3 -c	16.8 -c	11.2 -c	24.0 +c	34.8 +c	57

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

The changes of the index for head diameter are given in Table 2. Somaclonal line Nº 46 showed a head diameter which was 3.0 cm higher and statistically different from the control genotype with a mean value of the index of 12.2 cm. Both variants produced through gamma irradiation showed values significantly lower than the standard.

The changes in the somaclonal lines with regard to the index stem for diameter (Table 2) were positive and negative, with a significant decrease by 5.7 cm in line Nº 46. Significant differences were observed in lines Nº 83 and Nº 85, their changes being towards a considerable decrease of 7.0 and 8.2 cm, respectively.

Table 3: Effect of cultivation and gamma-ray treatment on some morphological and biochemical characteristics of R lines (R9 and R9M9 generation) produced through the direct organogenesis method from genotype 147 R (Harvest years 1998-2000)

Genotype	Diameter of branch head	Seed width	Seed length	Seed diameter	Oil in the kernel	1000 seed weight
	Mean (cm)	Mean (mm)	Mean (mm)	Mean (mm)	Mean (%)	Mean (g)
Control -147 R	8.2	4.4	10.5	2.8	44.6	36.3
Line 46 R9	7.2 -a	4.1	10.0 -b	2.8	45.1	26.6 -c
Line 60 R9	8.3	4.0 -a	9.7 -c	2.8	47.0 +a	31.4 -c
Line 66 R9	8.4	4.1	10.1 -a	2.9	45.7	29.6 -c
Line 83 R9M9	9.0 +b	4.0	9.6 -c	2.8	41.7 -c	41.7
Line 85 R9M9	9.6 +c	3.8 -a	9.6 -c	2.6 -b	42.2 -c	42.3

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

In the index for the number of branches, as well as for the length of branches (Table 2) the changes in all variants were only positive, with a highest degree of significance. The differences from the control concerning the length of branches varied from 3.4 to 14.9 cm, the highest deviation being that in somaclonal line Nº 66.

Negative changes of the indices for seed width, seed length and seed thickness were observed in all variants included in this study (Table 3). The data characterizing the three indices show that direct organogenesis used both independently and in combination with gamma irradiation leads to production of smaller seeds.

When analyzing the lines from the two variants, it was established that the arithmetical mean values of the character 1000-seed weight (Table 3) deviated in both positive and negative directions in comparison to the control. Statistically significant negative differences were demonstrated only by the somaclonal lines.

Oil content in the kernel (%) is another important index; data concerning the changes in the investigated lines are given in Table 3. The highest variation coefficient value of the initial control genotype 147 R was VC=5.8, which was a proof for the high homogeneity of the initial line with regard to the studied character under field conditions. Increases in oil content in the kernel (%) were observed in the three somaclonal lines, but a significant positive difference of 2.4 % was registered only in line № 60. Both variants with gamma irradiation showed significant decrease in comparison to the control.

The absence of significant changes for the number of leaves was observed in all studied variants, which proved its stability.

DISCUSSION

The combined use of gamma radiation and *in vitro* cultivation, with intention was to widen the genetic variability, applied by Novak *et al.* (1988) on maize, by Sheng *et al.* (1990) on wheat, and by Encheva *et al.* (1993) on sunflower with regard. A considerable positive effect from the use of this approach has been observed. The optimal dose causing changes of many characters in R2M2 wheat plants proved to be 5 Gy. Sheng *et al.*, 1990, found that the dose of 5 Gy was optimal for the occurrence of modified forms in wheat. The dose of 7 Gy proved more efficient than 10 Gy for causing changes in the greater part of the studied characteristics of sunflower (Encheva *et al.*, 1993).

The present research showed that the significant positive changes in plant height, leaf length, petiole length, head diameter, number of ray florets, oil in the kernel (%) occured only in the somaclonal variants. Significant positive changes for all variants of the somaclonal and induced lines were observed for the number of branches and the length of branches, while the values were decreased for the seed length.

Gamma irradiation with a dose of 5 Gy caused significant decreases of some characters such as petiole length, internodule length, head diameter, number of ray florets, seed diameter and oil in the kernel (%).

Our result confirmed the results obtained by Novak *et al.* (1988) that quality, radiation induced plants (M9R9) and somaclonal plants (R9) manifested similar spectra of chlorophyll and morphological variants.

Somaclonal variation as well as its combination with induced mutagenesis leads to genetically heritable variations of sunflower that are suitable for use in a programs for the development of initial breeding material.

REFERENCES

Barov, B. and J. Shanin, 1965. Metodika na polskia opit, Sofia.

Cheng, X.Y., M.W. Gao, Z.Q. Ling and K.Z. Lin, 1990. Effect of mutagenic treatments on somaclonal variation in wheat (*Triticum aestivum* L.). Plant Breeding 105: 47-52.

Encheva, J., P. Ivanov, F. Tsvetkova and V. Nikolova, 1993. Development of a new initial breeding material in sunflower (*Helianthus annuus* L.) using direct organogenesis and somatic embryogenesis. Euphytica 68: 181-185.

Encheva, J. and P. Ivanov, 1997. Sunflower genotype reaction to direct and indirect organogenesis and somatic embryogenesis using three media and gamma ray treatment. Helia 20: 135-142.

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

Gavazzi, G., C. Tonelli, G. Todesco, E. Arreghini, F. Raffaldi, F. Vecchio, G. Barbuzzi, M. Biasini and F. Sala, 1987. Somaclonal variation versus chemically induced mutagenesis in tomato (*Licopersicum esculentum* L.). Theor. Appl. Genet. 74: 733-738.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassaus with tobacco tissues cultures. Plant Physiol. 15: 473-497.

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.

Novak, F.J., S. Daskalov, H. Brunner, M. Nestincky, R. Afza, M. Dolezelova, S. Lucretti, Herichova and T. Hermelin, 1988. Somatic embryogenesis in maize and comparison of genetic variability induced by gamma radiation and tissue culture techniques. Plant Breeding 101: 66-79.

Pugliesi, C., F. Cecconi, A. Mandolfo and S. Baroncelli, 1991. Plant regeneration and genetic variability from tissue cultures of sunflower (*Helianthus annuus* L.). Plant Breeding 106: 114-121.

Roseland, C.R., A. Espinasse and T.J. Grosz, 1991. Somaclonal variants of sunflower with modified coumarin expression under stress. Euphytica 54: 183-190.

CREACIÓN DE VARIABILIDAD GENÉTICA EN GIRASOL POR EL MÉTODO DE ÓRGANO- GÉNESIS DIRECTO CON Y SIN RADIACIÓN DE RAYOS GAMA

RESUMEN

Los embriones cigóticos inmaduros, de la línea auto fertilizante de girasol 147 R (*Helianthus annuus*) se han utilizado como el material de donación para la inducción del órgano-génesis directa. Una parte de los embriones inmaduros aislados, antes de plantación, ha sido expuesta a la radiación gama (^{137}Cs) de 5 Gy. El rango de las variaciones somaclónicas inducidas espontáneamente entre los regenerantes obtenidos ha sido investigado y comparado con las variantes inducidas mediante radiación. En 17 características morfológicas y bioquímicas, se han presentado durante el proceso de regeneración, los cambios genéticos espontáneos. Los cambios más significativos se han observado en la altura de la planta, diámetro del tallo, diámetro de la cabeza, longitud de ramas, contenido de aceite en la semilla (%) y en el peso de 1000 semillas.

Las variantes somaclónicas (R9) y radiadas (M9R9) han tenido el espectro similar de las modificaciones morfológicas y bioquímicas, aunque con diferentes frecuencias.

CRÉATION DE VARIABILITÉ GÉNÉTIQUE DANS LE TOURNESOL PAR LA MÉTHODE D'ORGANOGENÈSE DIRECTE AVEC ET SANS IRRADIATION GAMMA

RÉSUMÉ

Des embryons zygotes immatures de la ligne de tournesol 147 R (*Helianthus annuus* L.) autofertilisée ont été utilisés comme donneurs pour l'induction d'organogenèse directe. Une partie des embryons immatures isolés ont été traités par irradiation gamma (^{137}CS) à une dose de 5 Gy avant d'être plantés. La portée des variations somaclones spontanément induites parmi les régénérants obtenus a été examinée et comparée aux variantes induites à l'aide d'irradiation. Les changements génétiques apparaissant spontanément pendant le processus de régénération incluent dix-sept caractéristiques morphologiques et biochimiques. Les changements les plus significatifs ont été observés pour la hauteur de la plante, le diamètre de la tige, le diamètre de la tête, la longueur des rameaux, le contenu d'huile dans la graine (%) et le poids de 1000 graines.

Les recherches ont montré que les somaclones (R9) et les variantes induites par irradiation avaient un spectre de modifications morphologiques et biochimiques semblable bien qu'avec une fréquence différente.