HELIA, **26**, Nr. 38, p.p. 91-98, (2003) UDC 633.854.78:631.523

A COMPARISON BETWEEN SOMACLONAL VARIATION AND INDUCED MUTAGENESIS IN TISSUE CULTURE OF SUNFLOWER LINE Z-8-A (*Helianthus annuus* L.)

Julia Encheva*, Fota Tsvetkova and Peter Ivanov

Dobroudja Agricultural Institute, General Toshevo 9520, Bulgaria

Received: February 10, 2003
Accepted: June 21, 2003

SUMMARY

Immature zygotic embryos from the Spanish self-pollinated sunflower line Z-8-A (*Helianthus annus* L.) were used as 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 during the regeneration procedure included sixteen morphological and biochemical characters. The most significant changes were observed for the following characters: plant height, number of leaves, stem diameter, head diameter, length of branches, oil content in seed (%) and 1000-seed weight.

The study showed that the somaclonal- (R_9) and the radiation-induced (M_9R_9) variants revealed similar spectra of morphological and biochemical changes, thought with different frequency.

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

INTRODUCTION

The main sources of variability for sunflower breeders have been mutation and crosses with unrelated or wild species followed by selection for the desired characters. More recently a new approach such as tissue culture, allowed to widen the genetic variability in this crop. There are insufficient and contradictory data for sunflower with regard to the genetic variability produced independently trough tissue culture or in combination with gamma irradiation. While Freyssinet and Freyssinet (1988) did not observe significant changes after plant regeneration from sunflower cotyledons, Roseland *et al.* (1991) obtained statistically significant soma-

* Corresponding author

clonal variants in sunflower with an increased level of coumarin after applying stress. Somaclonal variants in sunflower inherited in the next R_2 generation were described by Pugliesi *et al.* (1993).

Mutagenesis, both physical and chemical, proved favorable for mutation induction in tissue cultures. Positive results have been obtained when induced mutagenesis and tissue cultivation were appropriately combined in maize (Gavazi *et al.*, 1987; Novak *et al.*,1988) and wheat (Cheng *et al.*, 1990). Besides the "potato leaf" mutation in tomato, Gavazi *et al.* (1987) reported statistically significant changes in indices of large economic importance such as early maturation, reduced stem and male sterility. Encheva *et al.* (1993, 2002) reported statistically significant changes in morphological and biochemical characters of plants regenerated from immature zygotic embryos of sunflower, independently and in combination with gamma irradiation.

The aim of this study was to compare the genetic variability that occurred spontaneously among the regenerated plants of genotype Z-8-A produced through direct organogenesis with that induced by a combined use of tissue culture and gamma radiation ^{137}Cs at a dose of 5 Gy. The study included economically important morphological and biochemical traits, as well as data on the frequency and spectrum of mutation cases.

MATERIAL AND METHODS

The study included the Spanish fertility restorer line Z-8-A. The donor plants were grown under field conditions, self-pollinated under insulator and 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 functional tissue. Cultivation and plant regeneration were realized on nutrition medium E1 (Encheva *et al.*, 1997) and the obtained somatic buds were developed on SIM1 medium (MS macro salts, MS micro salts, 1 mg/l K, 0.01 mg/l IAA, pH 5.7). The regenerated plants R_0 (nomenclature according Evans and Sharp, 1983) and R_0M_0 (nomenclature according Novak *et al.*, 1988) were grown under greenhouse conditions and self-pollinated. The seed produced (the R_1 and R_1M_1 generations) were sown in the field. Morphological characterization of the new lines (the R_9 and R_9M_9 generations) was carried out on 10 plants for each of the three years of study. The weight of 1000 seeds was determined in three samples with 50 seeds from each head. The oil content was quantified using a NMR analyzer (Newport Instruments, Ltd.1972), from seeds produced by each plant. The characterization of the indices of seed width, thickness and length was done on 25 seeds from each plant. The control data were collected from plants of the original line Z-8-A which was grown in field together with the regenerants. The morphological and biochemical traits of the new lines R 1275 and R 1279 produced through direct organogenesis and lines R 1404 and R 1410 produced through direct organogenesis in combination with gamma irradiation were studied during 1998-2000. The statistical analysis of results was carried out according to Snedecor and Cochran (1957).

RESULTS AND DISCUSSION

In addition to the traditional breeding approaches, genetic variability in sunflower can be induced also by mutagenesis and tissue cultures.

The biometric characters presented in this study are commonly studied in the process of traditional breeding. The investigation on the progenies of the regenerants obtained by the direct organogenesis method independently and in combination with gamma-ray treatment revealed considerable deviations.

Table 1: Effect of tissue cultivation and gamma-ray treatment on some morphological characteristics (plant height, number of leaves, leaf width, leaf length and petiole length) of R lines (R_9 and R_9M_9 generation) produced through the direct organogenesis method from genotype Z-8-A. Harvest years 1998-2000, average data.

Genotype	Plant height (cm)	Number of leaves (no)	Leaf width (cm)	Leaf length (cm)	Petiole length (cm)
Control Z-8-A	119.40	21.00	21.95	21.62	12.23
Line R 1275	121.71	28.00+c	17.42-c	18.26-c	14.63+c
Line R 1279	128.25	23.00+c	21.91	20.63+a	12.36-a
Line RM 1404	135.77+c	21.00	22.36	22.63+a	14.05+a
Line RM 1410	135.15+c	21.00	21.93	21.78	14.00+a

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

Table 1 presents data showing the considerable and with highest degree of significance increase of the character plant height in the progenies of the zygotic embryos treated with gamma rays. The increase in plant height was from 15.8 cm to 16.4 cm, while in the somaclonal variants this characteristic was changed to a lesser degree. In contrast to Novak *et al.* (1988) who reported plant height reduction after treatment of immature zygotic embryos of maize with 5 Gy, a considerable increase of this parameter was observed in our experiment. Cheng *et al.* (1990) found that the dose of 5 Gy caused most significant increases in the number of leaves in the somaclonal lines in relation to the control. A more significant difference was observed in line R 1275 (Figure 1); its value was 28.0, that of the control being 21.0. Conversely, the variants with gamma irradiation demonstrated stability with regard to this index (Table 1).

Significant decreases of the indices for leaf width and leaf length were observed in the two investigated somaclonal lines (Table 1). In line R 1275, the reductions were 4.5 and 3.4 cm, respectively, in relation to the control Z-8-A. A positive and statistically significant difference in the index for leaf length was observed in the variant with gamma irradiation of line RM 1404. Its mean value was 22.63 cm while that in the control was 21.62 cm. All lines under study showed significant differ-

Figure 1: Control line Z-8-A and somaclonal line R 1275

Figure 2: Differences between control line Z-8-A and somaclonal line R 1275 in petiole and leaf base lengths

Figure 3: γ-radiation induced line R 1410

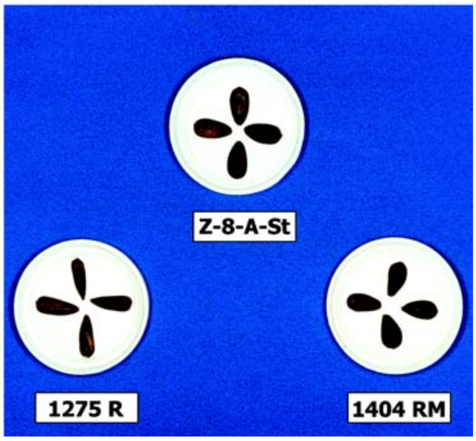

Figure 4: Forms of seed in control line Z-8-A and somaclonal lines R 1275 and RM 1404

ences concerning leaf petiole length as compared with the control. While only positive changes were observed in the irradiated variants, both positive and negative changes were observed in the somaclonal lines (Table 1). The most significant positive difference of 2.4 cm in relation to the control Z-8-A was demonstrated by somaclonal line R 1275 (Figure 2). The results obtained for internode length were similar to those for plant height. The tendency of significant increase in the irradiated variants remained, varying from 1.9 to 2.2 cm in this case; no changes were registered in the somaclonal lines (Table 2).

Table 2: Effect of tissue cultivation and gamma-ray treatment on some morphological characteristics (internode length, stem diameter, head diameter, number of branches, length of branches and number of ray florets) of R lines (R_9 and R_9M_9 generation) produced through the direct organogenesis method from genotype Z-8-A. Harvest years 1998-2000, average data.

Genotype	Internode length (cm)	Stem diameter (mm)	Head diameter (cm)	Number of branches (no)	Length of branches (cm)	Number of ray florets (no)
Control Z-8-A	5.85	22.60	12.58	17.00	30.67	55.00
Line R 1275	5.92	25.60+c	10.89-c	18.00	33.65	53.00
Line R 1279	5.89	22.45	13.53+a	13.00-c	40.44+b	53.00
Line RM 1404	7.75+c	21.00-a	10.04-c	19.00+c	33.13	55.00
Line RM 1410	8.08+c	22.10	9.64-c	19.00+c	25.46	53.00

Both positive and negative changes in stem diameter were observed among the investigated lines. A significant positive difference of 3.0 cm was registered in somaclonal line R 1275 (Table 2). Stem breakage due to unfavorable growing conditions such as rainfall and stormy weather may cause up to 100% losses in sunflower. Therefore, the increased stem diameter strengthens the plants. A stable negative tendency concerning head diameter was observed in the two irradiated variants, a marked decrease of 2.94 cm occurring in line R 1410 (Figure 3; Table 2). The changes in the somaclonal lines were both positive and negative, but the differences in relation to the control were non-significant. The considerable and statistically significant increase in the length of branches, by 10.3 cm, as well as the reduced number of branches in line R 1279 (13 in contrast to 17 of the control) lead to changes in stem architecture (Table 2). Positive and negative changes were observed for the indices of seed length and diameter in some of the investigated variants. The statistically significant negative change in seed diameter and the positive change in seed length allowed us to conclude that somaclonal line R 1275 had longer and narrower seeds as compared with the control Z-8-A (Figure 4; Table 3). Regarding 1000-seed weight, both positive and negative changes occurred but only the decrease of 3.93 g in somaclonal line R 1275 (Table 3) was significant.

Data on oil content in seed, one of the most important agronomic indices, are given in Table 3. A steady tendency towards increase of the mean value of this index was observed in all variants included in the study. The total mean increase of this character for both somaclonal lines was 6.3% in relation to the control. The corresponding increase in the irradiated variants was 5.3%. The most significant change was registered in somaclonal line R 1279 which exceeded the control Z-8-A by 8.2%. This considerable increase of oil content in the somaclonal line in combination with the higher 1000-seed weight (although statistically insignificant) is very useful for sunflower improvement program.

Table 3: Effect of tissue cultivation and gamma-ray treatment on some morphological and biochemical characteristics (seed width, seed length, seed diameter, oil in the kernel and 1000-seed weight) of R and RM lines (R_9 and R_9M_9 generation) produced through the direct organogenesis method from genotype Z-8-A. Harvest years 1998-2000, average data.

Genotype	Seed width (mm)	Seed length (mm)	Seed diameter (mm)	Oil in the kernel (%)	1000-seed weight (g)
Control Z-8-A	2.74	10.25	4.63	42.15	31.10
Line R 1275	2.65	10.64+c	4.01-c	46.56+c	27.17-a
Line R 1279	2.72	10.13	4.39-a	50.38+c	32.95
Line RM 1404	2.86	10.13	4.53	47.58+c	31.62
Line RM 1410	2.59	10.35	4.87+a	47.36+c	34.38

The lack of changes for the number of ray florets and seed thickness was observed in all studied variants (Tables 2 and 3). The presents study also showed that positive significant changes occurred only in the somaclonal variants for the number of leaves, stem diameter, head diameter, length of branches and length of seed, which make 31.3% of all investigated characters. Positive changes only in the irradiated variants were registered for plant height, leaf length, internode length, number of branches and seed diameter, i.e., in 31.3% of all characters. Negative changes only in the somaclonal lines were registered for the leaf width, leaf length, number of branches, seed diameter and 1000-seed weight, i.e., in 31.3% of all characters. In the variants with gamma-irradiation,a negative change was registered for stem diameter. A positive and significant change was registered for oil content in seed in all investigated variants, i.e., in 6.3% of the total number of indices, while a negative change was not observed. The highest number of significant differences (62.5%) in relation to the control were observed in somaclonal line R 1275. Considerable and significant changes were observed for plant height, number of leaves, stem diameter, head diameter, length of branches, oil content in seed and 1000-seed weight. With the exception of the number of leaves, a similar conclusion was drawn genotype R 147 in a previous study of ours (Encheva *et al.*, 2002), which allows us to presume that these indices of sunflower are considerably mutable.

Preliminary results of a comparison of progenies of gamma treated immature zygotic sunflower embryos and the somaclonal variation that occurred were published by Encheva *et al.* (1993). The present study also confirms our previous conclusion drawn for two other genotypes that somaclonal variation occurs with higher frequency than the radiation-induced variation, but the rate of change is similar.

The combined use of gamma radiation and *in vitro* cultivation was also tried by Novak *et al.* (1988) on maize and by Cheng *et al.* (1990) on wheat. Sandford *et al.*

(1984) pointed out the similarity between many regenerants produced from protoplast culture of the potato variety R. Burbank and those obtained through mutagenesis. Some authors have directed their investigations towards direct comparison of somaclonal variants and mutants produced through irradiation or chemical mutagenesis using EMS (Gavazi *et al.*, 1987). Regeneration from *in vitro* culture (R_2 regenerants) leads to a higher number of mutations than when a chemical mutagen (EMS) is used. The difference between somaclonal variation and chemical mutagenesis is not only in the frequency of variation, but also with regard to qualitative changes. It became clear that the "potato leaf" mutation obtained by the author after *in vitro* cultivation of tomatoes was induced by neither of the investigated mutagenes, EMS including.

Our result confirmed the results obtained by Gavazi *et al.* (1987) and Novak *et al.* (1988) that somaclonal variation occurred with higher frequency but quality, radiation induced and somaclonal variants manifested similar spectra of chlorophyll, morphological and biochemical variants.

The advantage of somaclonal variation in respect to induced mutagenesis is that the organogenetic way of formation of plant regenerants allows to presume that their monocell origin rejects the possibility for occurrence of chimeric plants and thus the mutant plants may be identified among the progenies of self-pollinated regenerants at an earlier stage of breeding. Although somaclonal variation and tissue culture in combination with induced mutagenesis are unpredictable and unstable, they result in genetically heritable variation in sunflower that is suitable for use in breeding programs.

REFERENCES

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

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

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

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

Echeva, J., Tsvetkova, F. and Ivanov, P., 2002. Comparison between somaclonal variation and tissue culture induced mutagenesis in sunflower (*Helianthus annuus* L). Helia 25, Nr. 37.

Evans, D.A. and Sharp, W.R., 1983. Single gene mutations in tomato plants regenerated from tissue culture. Science 221: 949-951.

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

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

Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays 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., Daskalov, S., Brunner, H., Nestincky, M., Afza, R., Dolezelova, M., Lucretti, S., Herickova, A. and Hermelin, T., 1988. Somatic embryogenesis in maize and comparison of genetic variability induced by gamma radiation and tissues culture techniques. Plant Breeding 101: 66-79.

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

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

Sandford, J.C., Weeden, N.F. and Chyi, Y.S., 1984. Regarding the novelty and breeding value of protoplast-derived variants of Russet Burbank (*Solanum tuberosum* L.). Euphytica 33: 709-715.

Snedecor, G. and Cochran, W., 1957. Statistical Methods. The Iowa State College Press, Ames, Iowa.

COMPARAISON ENTRE LA VARIATION SOMACLONÉE ET LA MUTAGÉNJÈSE DANS LA CULTURE DE TISSU DE LA LIGNE Z-8-A DE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Des embryons zygotes immatures de la ligne Z-8-A de tournesol autofécondés (*Helianthus annuus* L.) ont été utilisés comme donneurs pour induire une organogénèse directe. Une partie des embryons immatures isolés a d'abord été traitée par radiation gamma (^{137}Cs) à une dose de 5 Gy. La portée de la variation somaclonale spontanée induite parmi les régénérateurs obtenus a été analysée et comparée à celle des variants induits par irradiation. Les modifications génétiques apparaissant pendant la procédure de régénération incluaient seize caractères morphologiques et biochimiques. Les modifications les plus importantes ont été observées pour la hauteur de la plante, le nombre de feuilles, le diamètre de la tige, le diamètre de la tête, la longueur des branches, le contenu d'huile dans la graine (%) et le poids de 1000 graines.

Les recherches ont montré que les variants somaclonés (R_9) et induits par radiation (M_9R_9) révélaient un spectre similaire de modifications morphologiques et biochimiques, mais ayant une fréquence différente.