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EFFECT OF FEMALE GENOTYPE ON THE EFFICIENCY OF γ-INDUCED PARTHENOGENESIS IN SUNFLOWER (*Helianthus annuus* L.)

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SUMMARY

A comparative study was carried out on the parthenogenetic reaction of *Helianthus annuus* L. lines and hybrids used as initial female material for parthenogenetic induction. It was established that the mean parthenogenetic responsiveness of the hybrids was considerably higher than that of the lines. The mean parthenogenetic responsiveness of the lines used as initial female material (2607 A, 2607 B, 1607 A, 1607 B, 1234 A and 1234 B) was lower than the mean responsiveness of the hybrids (Albena, Viki, Euroflor, Perla and San Luka). Within the investigated lines, no specific genotype reaction with regard to parthenogenetic induction was ascertained; such reaction was observed in the hybrids. The hybrids San Luka and Perla had the relatively highest parthenogenetic responsiveness.

The analysis of the results showed that the optimal expression of the parthenogenetic responsiveness of the female genotype depended mainly on the interaction with the pollen source. The pollen source Rf 673 induced on average 3.4 times better parthenogenetic reaction in the hybrids Perla, San Luka and Viki that the line Rf 147.

The γ-irradiation influenced the expression of parthenogenetic responsiveness of the female genotype through its effect on the parthenogenesis-inducing ability of the pollen source.

Key words: **γ-irradiation, induced parthenogenesis, genotype response, sunflower**

INTRODUCTION

In using γ-irradiation for induction of parthenogenetic doubled haploidy, some authors have reported effect of female genotype (Pandey and Phung, 1982; Sauton, 1989; Bouvier *et al.*, 1993; Faris *et al.*, 1999). Others (Çaglar and Abak, 1999; Lotfi *et al.*, 1999) claimed that the efficiency of the method was not influenced by

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pollen recipient. Female genotype effect can be expressed as lack of parthenogenetic response (Pandey and Phung, 1982; Bouvier *et al.*, 1993), as lower responsiveness (Sauton, 1989; Faris *et al.*, 1999), or as specific selectivity with regard to the type of parthenogenetic development (Pandey *et al.*, 1990).

This paper describes the parthenogenetic reaction of six sunflower lines and hybrids used as initial female material.

MATERIALS AND METHODS

Selfed lines with normal cytoplasm (2607 B, 1607 B and 1234 B), their sterile analogues, and hybrids Albena, Viki, Euroflor, Perla and San Luka were used in this study. The criterion for choice of lines was their good uniformity, but primarily their different origin. These lines had been developed at Dobroudja Agricultural Institute. The criteria for choosing the hybrids included also good economic characters, good productivity and resistance to the economically important diseases of sunflower.

The study included also the pollen sources Rf 673 and Rf 147.

The female plants were isolated immediately before flowering to avoid open pollination. To sterilize the male gametophyte, the pollen recipient plants of the hybrids were treated with a water solution of gibberellic acid (GA_3) in the concentration of 45 mg/l (0.0045%). The plants of the lines with normal cytoplasm were treated with 33 mg/l (0.0033%). The solution used was prepared one day prior to the treatment of plants. The plants were sprayed individually, applying the solution directly to the forming flower bud (1-1.5 cm in diameter) and the upper leaves. The treatment was carried out twice.

The pollen was collected from the pollen sources on alternate days and stored at 4^{o}C till irradiation. Pollen irradiation was performed with a gamma-irradiation apparatus GOU-3M, with ^{137}Cs as a source of ionizing radiation and the source position of 3.38 Gy/min. Pollen was irradiated with γ-rays at the doses of 600 and 900 Gy. Treatment was performed one day prior to pollination. After pollination, remaining pollen was again stored in a refrigerator. Plants with confirmed chemical castration without traces of own pollen were pollinated. Pollination was carried out manually. The irradiated pollen was applied on the pollen recipient plants twice. To check spontaneous apomixis and the efficiency of castration, 10 plants from each female (pollen recipient) genotype, preliminary castrated and isolated at the beginning of flowering, with the exception of the *cms* lines, were not pollinated with irradiated pollen.

The obtained parthenogenetic embryos were cultivated *in vitro* following the method of Azpiroz *et al.* (1988). Two plants from each investigated variant (female genotype × pollen source × gamma-irradiation dose) were left without embryo culture and were further grown in the field.

The young plants were transferred to soil and stabilized at 20°C/15°C day/night, 70% relative air humidity and photoperiod 16/8 h day/night for 30 days; after that, the daily temperature was increased to 25°C±2°C. The plants were grown under these conditions till maturity.

Three-factor dispersion analysis of the number of parthenogenetic embryos was performed to evaluate the effect of the studied female genotypes on parthenogenetic induction, and their interactions with the pollen sources and the γ-irradiation doses (Table 1).

RESULTS AND DISCUSSION

The general evaluation of the obtained data by the criterion F showed significant differences in the parthenogenetic reaction of the pollen recipient (female) genotypes. This reveals that the differences in the parthenogenetic responsiveness of the initial material were due to the specific genotype reaction of the female, and not due to some casual factors. The interactions, initial female genotype × pollen source and initial female genotype × pollen source × gamma-irradiation dose, were also statistically significant.

Table 1: Dispersion analysis of parthenogenetic reaction of *Helianthus annuus* L. lines and hybrids using two γ-irradiation doses and two pollen sources

Factors	SS	MS	df	$F_{exp.}$	F	
					P=5%	P=1%
General	2297.9					
Replication	6.01					
Initial female material (IM)	840.52	84.05	10	17.94	2.06	2.77
Pollen source (PS)	17.28	17.28	1	3.98	4.06	7.26
Irradiation dose (ID)	10.92	10.92	1	2.33	4.06	7.26
IM × PS	994.7	99.47	10	21.23	2.06	2.76
IM × ID	114.34	11.43	1	2.44	4.06	7.26
PS × ID	6.01	6.01	1	1.28	4.06	7.26
IM × PS × ID	106.61	10.66	10	2.28	2.06	2.76
Error (E)	201.49	4.69	43			

SS - sum of squares df - degree of freedom
MS - mean squares F - criterion of Fisher

Among the investigated female genotypes, the hybrids Perla and San Luka demonstrated comparatively good parthenogenetic responsiveness (Table 2).

The weakest response was that of the hybrid Euroflor. The hybrids can be conditionally divided into three groups according to their parthenogenetic reaction: hybrids with low responsiveness, including Euroflor; hybrids with moderate responsiveness, including Viki and Albena; and hybrids with good responsiveness, including Perla and San Luka.

The general parthenogenetic reaction of the lines was very low. Parthenogenetic embryos were obtained from all lines, but with very low frequency. No significant differences were established between the mean values of parthenogenetic induction. Therefore, no specific genotype reaction was detected in the investigated lines.

Table 2: Initiation of parthenogenetic development using two γ-irradiation doses and two pollen sources

	Pollen source			
	Rf 673		Rf 147	
Gamma-irradiation dose	600 Gy	900 Gy	600 Gy	900 Gy
Initial female material	Number of parthenogenetic embryos (X - mean)			
Albena	2	3.5	6.5	4
Viki	12	6	2.5	3.0
Euroflor	2	2	3.5	1.5
Perla	19.5	13.5	4.5	7
San Luka	20.5	11.5	1	7
2607 A	2	0	0	2
2607 B	0	1	1	0
1607 A	1	0	0	0
1607 B	0	0	1	0
1234 A	1	1	1	0
1234 B	0	3	2	2

$GD_{5\%}$=4.37 $GD_{1\%}$=5.84 $GD_{0.1\%}$=7.65

When comparing the averaged mean values of the parthenogenetic reaction of the lines against those of the hybrids, it was established that the efficiency of the method was 8.9 times lower when using the lines as initial female material (Figure 1).

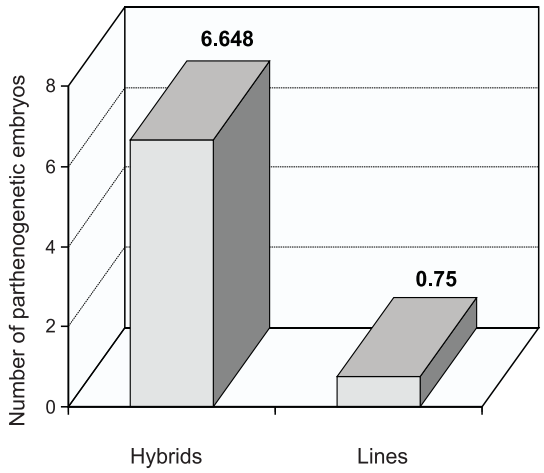

Figure 1: Differences in response of Helianthus annuus L. lines and hybrids to γ-induced parthenogenetic development

We assumed that the better pathenogenetic responsiveness of the hybrids in comparison with the lines was due to the heterosis effect. Our assumption was supported also by the fact that one of the investigated *cms* lines, 2607 A, was the female line of the hybrid San Luka. The hybrid demonstrated the highest responsiveness in this study, while the parthenogenetic response of its female line (2607 A) was low, which was a part of the general tendency determined in the rest of the lines included in this investigation. We supposed that the

better response of the hybrids resulted from the combined genomes of their parental lines, which generated the heterosis effect.

Similarly to the results obtained by Bouvier *et al.* (1993), Sauton (1989) and Faris *et al.* (1999), we observed effects of the female genotype which were expressed as different responses to induction of parthenogenesis between the lines and hybrids and between the individual hybrids.

The highest efficiency of the method was achieved when pollinating the hybrids San Luka and Perla with pollen from the line Rf 673 irradiated with 600 Gy (Table 2). When the same pollen source was irradiated with 900 Gy, the induced parthenogenetic responsiveness was lower in the two hybrids, the differences being significant at P=0.001 (San Luka) and P=0.01 (Perla). The parthenogenetic reaction of the hybrid Viki initiated by the pollen of Rf 673 treated with 600 Gy was also considerably better than after treatment with the dose of 900 Gy. In the combination of San Luka with the pollen source Rf 147 and the dose of 900 Gy, the average number of parthenogenetic embryos produced was higher (P=0.01) than in the combination San Luka × Rf 147 × 600 Gy. The above data show that the same pollen source induced different responsiveness of the female genotype in dependence of γ-irradiation treatment.

Table 3: Plants obtained from the pollen recipients trough initiation of parthenogenetic development by γ-irradiated pollen

Index		Plantlet		Mature plant ("*"-haploid plant)	
Female genotype	Gamma irradiation dose	No. of diploid plants	No. of haploid plants	No. of fertile plants	No. of sterile plants
Albena	Rf 673	9	1	4	3
	Rf 147	14	-	6	5
Viki	Rf 673	22	2	11+2*	8
	Rf 147	7	-	4	2
Euroflor	Rf 673	8	-	3	4
	Rf 147	9	1	5	2
Perla	Rf 673	47	4	23+1*	17
	Rf 147	18	1	10	7
San Luka	Rf 673	45	2	23	16+1*
	Rf 147	6	-	2	3
2607 A	Rf 673	2	-	0	2
	Rf 147	2	-	0	0
2607 B	Rf 673	1	-	0	1
	Rf 147	0	-	0	0
1607 A	Rf 673	1	-	0	1
	Rf 147	0	-	0	0
1607 B	Rf 673	0	-	0	0
	Rf 147	1	-	0	0
1234 A	Rf 673	3	-	0	2
	Rf 147	2	-	0	0
1234 B	Rf 673	5	-	1	2
	Rf 147	6	-	2	1
Σ		208	11	97	77

In contrast to the results obtained by Pandey and Phung (1982) and Bouvier *et al.* (1993), parthenogenetic embryos were developed from all investigated genotypes. In this study, the effect of the female was expressed in the different parthenogenetic responsiveness of the investigated genotypes. Similar results were reported by Sauton (1989) and Faris *et al.* (1999) in cucumber. As far as the type of parthenogenetic development is concerned, it is difficult to say that there is genotypically dependent selectivity of the female such as that observed in different kiwi varieties (Pandey *et al.*, 1990). In this investigation, the relative share of haploid plants was considerably smaller than that of the parthenogenetic doubled haploid plants. Simultaneously, haploids were produced from all hybrids (Table 3).

No haploid plants were obtained from the lines. This can be explained by the comparatively low parthenogenetic responsiveness they demonstrated. It is interesting to mention the fact that in the hybrids Albena, Viki and San Luka haploid development was induced only by the pollen source Rf 673, while in the hybrid Euroflor only the combination with the line Rf 147 led to the initiation of haploid parthenogenesis. Haploid plants were obtained from the hybrid Perla using both pollen sources, the interaction with Rf 673 being more productive.

Some authors have pointed out that the genotype of the female line affects the type of parthenogenetic development, registering in parallel with this the effect of the pollen source (Pandey and Phung, 1982; Pandey *et al.*, 1990). Our data confirmed the hypothesis that both the female genotype and the pollen source genotype were important, the type of parthenogenetic development being determined by their interaction.

On the basis of the obtained results and their analysis, it can be summarized that the parthenogenetic reaction of the initial female material is determined by the genotype.

The investigated hybrids demonstrated better parthenogenetic response than the studied lines. The hybrids Perla and San Luka showed the highest responsiveness.

The optimal expression of parthenogenetic responsiveness of the female genotype depended mainly on the interaction with the pollen source. The pollen source Rf 673 induced on average 3.4 times better parthenogenetic reaction in the hybrids Perla, San Luka and Viki as compared with the line Rf 147.

The γ-irradiation influenced the expression of parthenogenetic responsiveness of the female genotype through its effect on the parthenogenesis-inducing ability of the pollen source.

The combinations San Luka $\times$ Rf 673 $\times$ 600 Gy, Perla $\times$ Rf 673 $\times$ 600 Gy and Viki $\times$ Rf 673 $\times$ 600 Gy were much more productive than the same combinations of female genotypes and pollen sources and the irradiation dose of 900 Gy.

REFERENCES

Azpiroz, H.S., Vincourt, P., Serieys, H. and Gallais, A., 1988. La culture *in vitro* des embryons immatures dans l'acceleration du cycle de selection des lignees de tournesol et ses effets morphovegetatifs. Helia 10: 35-38.

Bouvier, L., Zhang, Y.X. and Lespinasse, Y., 1993. Two methods of haploidization in pear, *Pyrus communis* L.: greenhouse seedling selection and *in situ* parthenogenesis induced by irradiated pollen. Theoretical and Applied Genetics, Berlin 87: 229-232.

Çaglar, G. and Abak, K., 1999. Progress in the production of haploid embryos, plants and doubled haploids in cucumber (*C.sativus* L.) by gamma irradiated pollen in Turkey. Acta Hort. 492: 317-322.

Faris, N.M., Nikolova, V. and Niemirowicz-Szczytt, K., 1999. The effect of gamma irradiation dose on cucumber (*Cucumis sativus* L.) haploid embryo production. Acta Physiologiae Plantarum 21(4): 301-396.

Lotfi, M. and Kashi, A., 1999. Induction of parthenogenetic embryos by irradiated pollen in cucumber. Proc. of the 1st Int. Symp. on Cucurbits, Acta Hort. 492: 323-326.

Pandey, K.K. and Phung, M., 1982. Hertwig effect in plants: induced parthenogenesis through the use of irradiated pollen. Theor. Appl. Genet. 62: 295-300.

Pandey, K.K., Przywara, L. and Sanders, P.M., 1990. Induced parthenogenesis in kiwifruit (*Actinidia deliciosa*) through the use of lethally irradiated pollen. Euphytica, 51: 1-9.

Sauton, A., 1989. Haploid gynogenesis in *Cucumis sativus* induced by irradiated pollen. Cucurbit Genetics Cooperative, 12: 22-23.

INFLUENCIA DEL GENOTIPO FEMENINO EN LA EFICIENCIA DE PARTENOGÉNESIS γ-INDUCIDA EN GIRASOL (*Helianthus annuus* L.)

RESUMEN

Se ha realizado un estudio comparativo sobre la reacción partenogenética de las líneas e híbridos *Helianthus annuus* L. que se utilizan como el material materno inicial para la inducción de la partenogénesis. Se determinó que la reacción partenogenética media de los híbridos era significativamente más alta que la reacción de las líneas. La reacción partenogenética media de las líneas utilizadas como el material materno inicial (2607 A, 2607 B, 1607 A, 1607 B, 1234 A y 1234 B) era más baja que la reacción de los híbridos (Albena, Viki, Euroflor, Perla y San Luka). En las líneas investigadas no había reacción genotípica específica en relación con la inducción de la partenogénesis; pero, tal reacción se observó en los híbridos. Los híbridos San Luka y Perla tenían relativamente más alta reacción partenogenética.

El análisis de los resultados ha demostrado que la óptima expresión de la reacción partenogenética del genotipo femenino en la mayor parte dependía de la interacción con la fuente de polen. La fuente de polen Rf 673 inducía en promedio 3,4 veces mejor reacción partenogenética en los híbridos Perla, San Luka y Viki que la línea Rf 147.

La radiación γ influye en la expresión de la reacción partenogenética del genotipo femenino a través de su influencia en la aptitud de inducción de partenogénesis de la fuente de polen.

EFFET DU GÉNOTYPE FEMELLE SUR L'EFFICACITÉ DE LA PARTHÉNOGENÈSE PAR INDUCTION-á DANS LE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Une étude comparative sur la réaction parthénogénétique des lignes et hybrides (*Helianthus annuus* L.) utilisées comme matériel initial maternel pour l'induction de la parthénogenèse a été effectuée. Il a été établi que la réaction parthénogénétique moyenne des hybrides était significativement plus élevée que la réaction des lignes. La réaction parthénogénétique moyenne des lignes utilisées comme matériel maternel initial (2607 A, 2607 B, 1607 B, 1234 A et 1234 B) était inférieure à celle des hybrides (Albena, Viki, Euroflor, Perla et San Luka). Dans les lignes examinées aucune réaction génotypique particulière n'a été observée par rapport à l'induction parthénogénétique; cependant, une telle réaction a été constatée dans les hybrides. Les hybrides San Luka et Perla ont eu la réaction parthénogénétique relativement la plus élevée.

L'analyse des résultats a démontré que l'expression optimale de la réaction parthénogénétique du génotype femelle dépend surtout de l'interaction avec la source de pollen. La source de pollen Rf 673 a induit une réaction parthénogénétique en moyenne 3,4 fois meilleure dans les hybrides Perla, San Luka et Viki que la ligne Rf 147.

L'irradiation-γ a un effet sur l'expression de la réaction parthénogénétique du génotype femelle par son effet sur l'aptitude d'induction parthénogénétique de la source de pollen.