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INDUCED PARTHENOGENESIS IN SUNFLOWER: EFFECT OF POLLEN DONOR

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SUMMARY

A rapid production of doubled haploid lines in the cultivated sunflower (*H. annuus* L.) is already possible by means of irradiated pollen - induced parthenogenesis (Todorova *et al.*, 1997). The genetic constitution of donor parents is one of the significant factors for the successful induction of parthenogenetic embryos. The effect of pollen donor was examined in eighty-eight combinations, involving 10 donor parents, a pollen mixture of them and two mother genotypes.

Pollen was treated with gamma-irradiation doses of 600 Gy and 900 Gy. In total, 705 embryos were cultivated, giving rise to 205 plants that reached maturity. After selfing, 104 plants produced seeds, while the others were sterile. Sixty-five agronomically useful doubled haploid lines were selected.

The ploidy level of all the regenerants was flow-cytometrically evaluated. Best results were achieved by the use of the pollen donor Z-8-A.

The mixed pollen, followed by two pollen donor lines yielded also very high numbers of parthenogenetic embryos, i.e., plants.

Key words: **sunflower, induced parthenogenesis, gamma-irradiation, embryo culture, mixed pollen**

INTRODUCTION

In situ parthenogenesis by irradiated pollen followed by *in vitro* culture of immature embryos has been first developed by Pandey and Phung (1982) on *Nicotiana*. The method has been applied on petunia (Raquin, 1985), musk melon (Sauton *et al.*, 1987), onion (Dore *et al.*, 1993), apple (Zhang *et al.*, 1991) and rose (Meynet *et al.*, 1994).

Todorova *et al.* (1997) successfully used the irradiated pollen technique for induction of haploids in sunflower (*Helianthus annuus* L.).

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In situ parthenogenesis seems to be the most advanced haploidization method in sunflower because the regeneration rates which the anther and microspore culture techniques propose are rather low. The former method induces *in situ* parthenogenesis by combining the use of heavily irradiated pollen and *in vitro* culture of pollinated ovaries.

In our preliminary studies we have found that the efficiency of parthenogenesis varied according to the pollen donor used. In the opinion of Pandey and Phung (1982) the genetic constitution of both maternal and paternal parents, particularly of the donor parents, is a significant factor in the successful induction of parthenogenetic embryos by using irradiated pollen.

The aim of the present study was to investigate the parthenogenesis-inducing ability of genotypically different lines preferred by the IWS breeders, and to select those which demonstrated best results for routine parthenogenetic production of sunflower dihaploid lines. The effect of the pollen donor is considered in its interaction with the irradiation doses and mother genotypes used.

MATERIAL AND METHODS

The study included 10 pollen donors and mixed pollen from them, 2 hybrids (Albena and San Luka) used as initial mother plants, and 2 gamma-irradiation doses (600 Gy and 900 Gy).

Pollen was collected from field plants bagged before anthesis. The collected pollen was stored in a refrigerator at 4°C till flowering of the mother plants. The pollen was irradiated one day prior to pollination of the mother plants. Mixed pollen was obtained by mixing equal amounts of pollen of the studied pollen donors.

Pollen was irradiated in petri dishes using a Cs 137 source of γ-irradiation (IWS "Dobroudja", General Toshevo) in doses of 600 and 900 Gy. The aim was to damage the pollen grains to the extent that they were still capable of germination but incapable of efficient fertilization. Pollinations were carried out on emasculated field plants. Four heads from each variant were pollinated (Table 1). Emasculated sunflower heads were bagged to eliminate contamination by foreign pollen. At the same time control emasculations were made on the genotypes used as maternal parents without subsequent pollination to see if any seed was produced by spontaneous parthenogenesis. None was, however, produced.

To ensure that as many embryos as possible survived, embryo cultures were applied 14 days after pollination. Two plants of each variant were left without embryo culture; however, no seeds were formed in any of these.

Six to eight days later the young plantlets developed from the embryos were transferred to soil and were further grown under greenhouse conditions (10-15°C). The ploidy of the obtained plants was determined flow-cytometrically at the stage of second - third leaf.

The statistical analysis was made according to Snedecor and Cochran (1957).

Table 1: Table 1. Design of the trial

Maternal genotypes	Albena				San Luka			
γ - irradiation doses	600 Gy		900 Gy		600 Gy		900 Gy	
Embryo culture	+	-	+	-	+	-	+	-
Pollen donors	Number of pollinated heads							
Rf 673	2	2	2	2	2	2	2	2
147 R	2	2	2	2	2	2	2	2
2607 R	2	2	2	2	2	2	2	2
937 R	2	2	2	2	2	2	2	2
1457 R	2	2	2	2	2	2	2	2
Z-8-A	2	2	2	2	2	2	2	2
1395 R	2	2	2	2	2	2	2	2
939 R	2	2	2	2	2	2	2	2
1398 R	2	2	2	2	2	2	2	2
19 R	2	2	2	2	2	2	2	2
Mixed pollen	2	2	2	2	2	2	2	2

+ embryo culture applied

- embryo culture not applied

Table 2: Results of pollinations with irradiated pollen involving two maternal parents and eleven pollen donors

Maternal hybrids	Albena								San Luka							
γ-radiation doses	600 Gy				900 Gy				600 Gy				900 Gy			
Pollen donors	1*	2*	3*	4*	1*	2*	3*	4*	1*	2*	3*	4*	1*	2*	3*	4*
Rf 673	2	2	-	2	6	3	2	1	44	20	5	8	24	22	7	9
147 R	11	1	-	1	7	3	-	3	-	-	-	-	11	10	4	2
2607 R	-	-	-	-	39	22	1	20	-	-	-	-	-	-	-	-
937 R	10	-	-	-	-	-	-	-	24	11	3	2	28	5	2	2
1457 R	7	4	-	4	5	-	-	-	8	3	2	1	38	5	3	2
Z-8-A	7	2	1	1	18	10	5	6	68	11	6	3	21	9	4	3
1395 R	38	12	5	7	40	6	1	5	12	9	6	3	2	1	-	1
939 R	19	6	3	3	2	1	-	1	10	7	4	3	1	1	1	-
1398 R	27	6	1	3	21	6	1	4	1	-	-	-	7	1	1	-
19 R	37	14	3	6	2	-	-	-	5	2	1	1	3	2	1	1
Mixed pollen	6	4	-	1	4	1	-	1	74	62	28	22	16	7	3	4

1* - no. of embryos formed

2* - no. of plantlets obtained

3* - no. of fertile plants

4* - no. of sterile plants

RESULTS

The total number of embryos obtained from all pollen donors and maternal genotypes was 705 (Table 2). Of that number, 205 reached maturity, 104 yielded seeds and the rest, 101, were sterile. The flow-cytometrical study revealed that the obtained plants were dihaploid. On the basis of the 104 plants tested for resistance to mildew under greenhouse conditions, 65 agronomically useful doubled haploid lines were selected. The aim of this selection was practical - to select lines of potential economic importance, i.e., which are fertile and are 100% resistant to mildew. On the other hand, this is an additional test for the gynogenetic origin of the obtained plants (Todorova *et al.*, 1997).

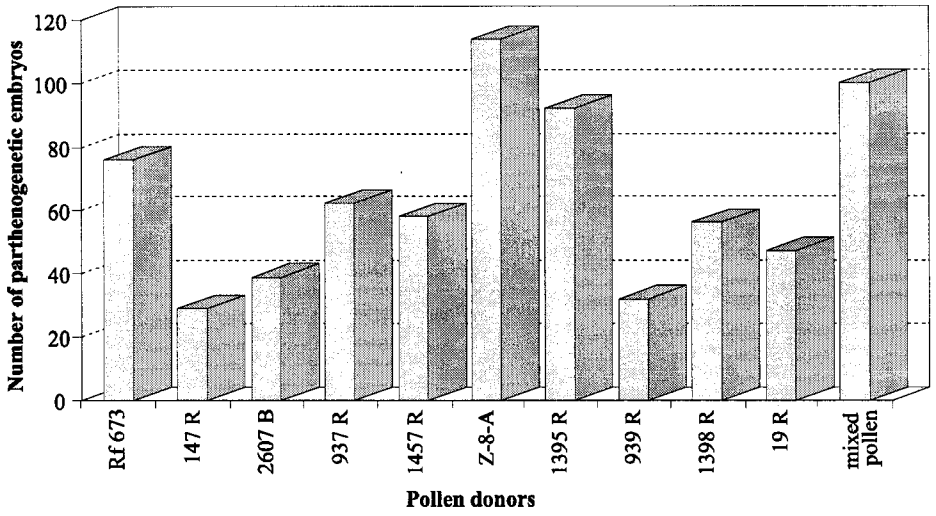

Figure 1: The effect of pollen donor genotype on parthenogenetic embryo production in sunflower after γ-irradiation of the pollen

Figure 1 presents the results from the study on the parthenogenetic induction abilities of the pollen donors according to the two parental hybrids, Albena and San Luka, and the two gamma-irradiation doses. The maximal number of gynogenetic embryos was obtained with the pollen donor Z-8-A, followed by the mixed pollen and lines 1395 R and *Rf* 673. The observed differences in the parthenogenetic and induction abilities of the pollen donors were with the degree of significance "c".

When considering in detail the results with regard to the used doses of pollen irradiation, it is evident that the parthenogenetic induction abilities of the various pollen donors are not uniformly affected by the doses of gamma-irradiation (Figure 2).

Lines 1395 R, 939 R, 19 R and the mixed pollen gave better results when treating pollen with 600 Gy, while lines 147 R and 2607 R did not show parthenogenetic response to the mentioned dose. They induced parthenogenesis at 900 Gy.

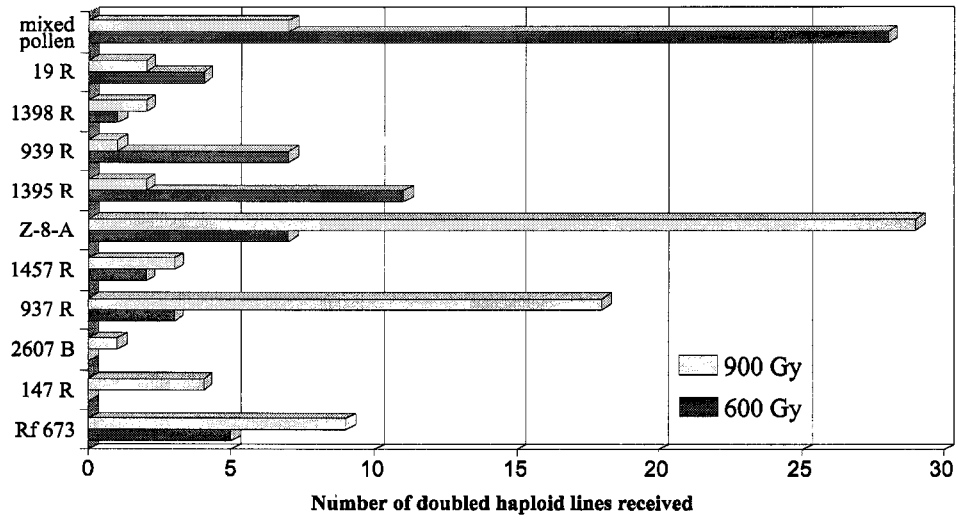

Figure 2: The effect of γ- irradiation doses on parthenogenesis inducing ability of some pollen donors

The data for the most prominent pollen donors are presented in Figures 3 and 4. From the DH-R-lines obtained with the participation of Z-8-A, 67% resulted from the interaction of this donor with the dose of 900 Gy (Figure 3).

The mixed pollen demonstrated higher parthenogenesis-inducing activity with the dose 600 Gy. 80% of the mixed pollen DH-R-lines were obtained by treating pollen with this dose (Figure 4).

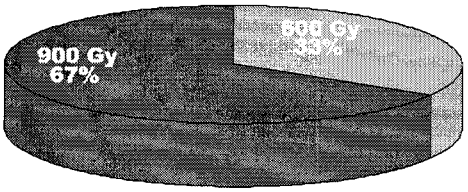

Figure 3: The interaction between the pollen donor Z-8-A and the γ-irradiation doses - 600 Gy and 900 Gy

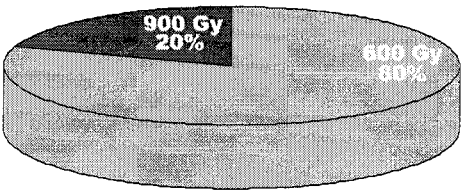

Figure 4: The interaction between mixed pollen and the γ-irradiation doses

Figure 5 shows the specific parthenogenetic reaction of the mother hybrids under the conditions of the trial. It is evident that the hybrid San Luka demonstrated considerably higher responsiveness than the hybrid Albena. The only exception was observed when using the pollen donors 1398 R and 19 R, in which the relative share of the DH lines originating from hybrid Albena is higher than that of San Luka.

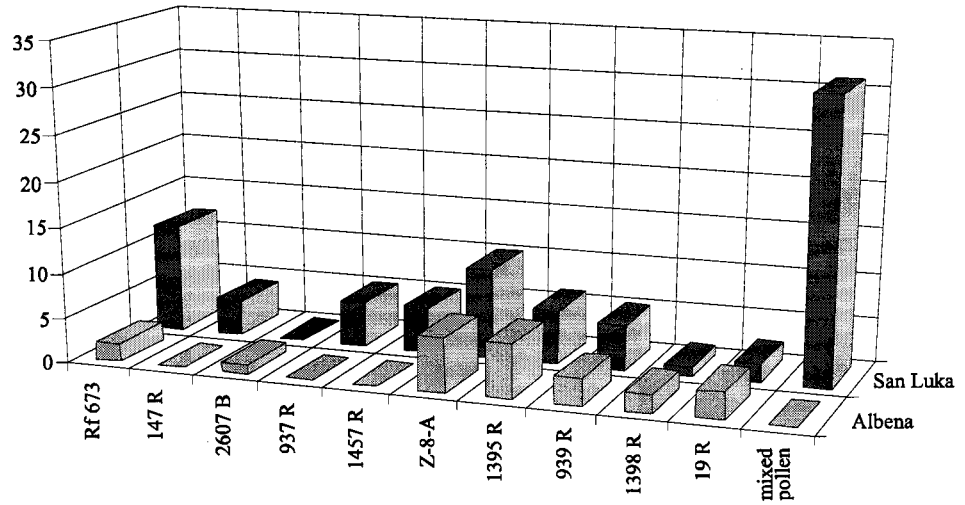

Figure 5: The pollen donor effect on parthenogenetic development of economically important DH-lines of the sunflower hybrids Albena and San Luka

Four of the studied pollen donors did not induce parthenogenesis in the hybrid Albena, mixed pollen included.

In the hybrid San Luka, the parthenogenetic induction was successful under the effect of all pollen donors except 2607 B. The highest number of DH lines was obtained when using mixed pollen. 78% of the total number of DH lines obtained originated from San Luka, and the rest originated from Albena.

DISCUSSION

On the basis of the results obtained in the experiment, we can draw the conclusion that the pollen donor Z-8-A in interaction with the irradiation dose 900 Gy stands out for the best parthenogenesis-inducing ability among the studied lines. Mixed pollen in combination with the irradiation dose of 600 Gy also realized a high induction frequency of parthenogenesis.

The effect of the pollen donor genotype on the parthenogenesis efficiency is a fact established by researchers who have applied *in situ* parthenogenesis in combination with *in vitro* cultivation of the parthenogenetic embryos. Pandey and Phung have as early as 1982 reported that when using *N. alata* as pollen donor, parthenogenetic plants were obtained from *N. langsdorfii*, but no parthenogenetic plants were produced when using *N. langsdorfii* as pollen donor. Similar observations have been made in kiwi fruit (Pandey *et al.*, 1990) and onion (Dore and Marie, 1993), too. The results we have obtained in this as well as in previous studies (Todorova *et al.*, 1997) confirm the presence of genotype specificity concerning parthenogenesis-inducing ability of pollen donors in sunflower. The parthenogenetic

efficiency when using the line Z-8-A is four times higher than the efficiency of the method when using 147 R as pollen donor (Figure 1). The inclusion of mixed pollen in the trial was an attempt to overcome the pollen donor specificity. It is evident from Figure 1 that mixed pollen is second in induction ability as compared with the two parental hybrids as a whole, and the two irradiation doses. However, when considering the specific effect of the maternal genotype (Figure 5), it becomes clear that the mixed pollen does not induce parthenogenetic development in the hybrid Albena, while the seven pollen donors incorporated in the mixed pollen realised induction in the hybrid San Luka. The same figure shows that the relative share of mixed pollen for induction of parthenogenetic DH lines from the hybrid San Luka exceeds several times the shares of the individual pollen donors. A possible explanation of these data is the suggestion that mixed pollen should not be considered only as a mechanical mixture of equal amounts, but as a sum total of parts that interact with one another.

As far as we know, the mechanism for induction of parthenogenetic development under the effect of irradiated pollen has not been clarified in detail; therefore it is difficult to talk about a "mechanism" of pollen donor genotype specificity. According to Pandey and Phung (1982), the parthenogenetic plants obtained at higher doses had received specific genetic segments from the irradiated pollen that were conductive to parthenogenetic development. These segments may have been incorporated into the genome of the plants which lacked additional fragments.

Further experiments on the use of biochemical and molecular techniques must be carried out to check if certain parental genes can be inserted and fixed under such conditions.

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PARTENOGENESIS INDUCIDA EN EL GIRASOL: EFECTO DEL DONADOR DE POLEN

RESUMEN

La producción rapida de dobles líneas haploidas en el girasol cultivado (*H. annuus* L.) es ya posible con la utilización de la partenogenesis causada por el polen irradiado (Todorova y otr., 1997). La constitución genética del padre donador es un factor importante para la obtención eficaz de embriones partenogenéticos. La influencia del donador de polen era investigada en 88 combinaciónes creadas a base de 10 donadores de polen, de la miezcla de su polen y dos genótipos madre.

Polen era tratado por los rayos gamma en doses de 600 Gy y 90 Gy.

705 embriones en todo eran cultivados, de los cuales 205 plántas llegaron a la madurez. Después de la autofecundación 104 plántas producierón semillas, mientras otras eran esteril. Fueron obtenidas 65 dobles plántas haploidas utilizables agronomicamente.

El nivel de ploidez de todos regenerantes fué evaluado fluidamente por la citometria. Los mejores resultados fueron alcanzados por la utilización del donador de polen Z-8-A.

La miezcla de polen y dos líneas de donadores de polen han dado también un gran número de embriones partogeneticos, es decir de plántas.

PARTHENOGENESE PROVOQUEE CHEZ LE TOURNESOL: INFLUENCE DU DONNEUR DE POLLEN

RÉSUMÉ

La production rapide de lignes haploïdes doubles du tournesol de culture (*H. annuus* L.) est déjà possible au moyen de parthogénèse provoquée par pollen irradié (Todorova *et al.*, 1997). La constitution génétique du parent donneur est un facteur important pour l'obtention réussie d'embryons parthogéniques. L'influence du donneur de pollen a été examinée dans le cas de 88 combinaisons créées sur la base de 10 doinneurs de pollen, un mélange de leur pollen et deux génotypes mère.

Le pollen a été traité aux rayons gamma à des doses de 600 Gy et de 900 Gy.

Au total, 705 embryons ont été cultivés et ont donné 205 plantes ayant atteint la maturité. Après autofécondation, 104 plantes ont produit des graines et le reste est resté stérile. Soixante-cinq lignes haploïdes doubles utilisables en agronomie ont été obtenues.

Le niveau de fertilité des regénérants a été évalué par flux cytométrique. Les meilleurs résultats ont été atteints par l'utilisation du donneur de pollen Z-8-A.

Le mélange de pollen et deux lignes de donneurs de pollen ont eux aussi donné un grand nombre d'embryons parthénogéniques, i.e., de plantes.