Induced parthenogenesis in sunflower (*Helianthus annuus* L.):Effect of gamma-irradiation doses.

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Summary

Six gamma-irradiation doses were applied for pollen inactivation. As pollen donors were used three pollen donors as well as mixed pollen from them. Two hybrids and three cms lines were used as female genotypes. The total number of tested lines was 24. Each variant included a dose, a pollen donor and a mother genotype.

Concerning the number of embryos formed best results were obtained in pollen inactivation with doses 1100 Gy and 700 Gy. There are no statistically significant differences between these two doses according to this index.

When registering the number of fertile and sterile plants obtained we found out that at dose 1100 Gy considerably more fertile plants were produced while at dose 700 Gy the sterile plant predominated.

On the basis of these results it could be asserted that under the conditions of the experiment, best results were obtained in inactivation of the pollen with 1100 Gy.

Introduction

Pollen irradiation technique has been used in plant breeding for different purposes i.e., for mutational damage (Werner at al. 1984), overcoming incompatibility barriers (Pandey, 1974); "egg transformation" (Pandey 1978); gene transfer (Shizukuda et al. 1983); induction of haploids (Raquin, 1985), and nucleus substitution (Raquin et al., 1989).

In our preliminary investigations (Todorova et al., 1997) we used the pollen irradiation technique to induce parthenogenetic development on sunflower (*Helianthus annuus* l.). The results we have obtained reveal the method of irradiated pollen induced parthenogenesis followed by *in vitro* culture of immature embryos as an efficient method for production of sunflower doubled haploids. The method has been applied on several plants as Nicotiana (Pandey and Phung,1982), petunia (Raquin, 1985), musk melon (Sauton et al., 1987), onion (Dore et al., 1993), apple (Zhang et al., 1991), rose (Meynet et al., 1994) and kiwifruit (Pandey et al., 1990) more or less successfully. A common idea in these studies is that the efficiency of the method is genotype- and dose-dependent. To enhance the induction frequency of parthenogenetic embryos in sunflower an attempt has been made, by the present study, to analyze the effect of various gamma-irradiation doses on the pollen ability of different pollen donor genotypes to induce parthenogenesis.The effect of the irradiation doses is considered in its interaction with the pollen donor genotypes and mother genotypes used.

Material and methods

For pollen inactivation were applied the gamma-irradiation doses: 300 Gy, 500 Gy, 600 Gy, 700 Gy, 900 Gy and 1100 Gy. Three of the doses (300 Gy, 600 Gy and 900 Gy) have been applied in preceding experiments, too. The lines Rf 673, 147R, 939R, as well as mixed pollen from these lines were selected for pollen donors. The hybrids Albena, San Luka and the cms lines 738 A, 872 A, 2628 A were used as initial mother genotypes.

The collection, storage and irradiation of the pollen was made according the protocol, described from Todorova et al., 1998. Pollinations were carried out on field plants, bagged before anthesis. Four heads from each variant were pollinated. Embryo culture were applied 13 days after pollination. Two plants of each variant were left without embryo culture. Six to eight days later the young plantlets developed from the embryos were transferred to soil and were further grown under greenhouse conditions, by consequently increase of the night/ day temperature - 10/15°C, 15/20°C and 20/25°C. The ploidity of the obtained plants was determined flow-cytometrically at the stage second - third leaf. The parthenogenetic origin of the developed plants was studied according the technique, described by Todorova et al., 1997.

Results and discussion

The total number of embryos obtained from the hybrids Albena and San Luka was 46. Forty-one young plants developed out of them which were further grown under greenhouse

conditions. Forty plants reached maturity, 20 of which were fertile, and 20 - sterile. The results from the flow-cytometrical analysis revealed that the plants were diploid (dihaploid).

In assessing the effect of the radiation dose on the efficiency of the method, we based our study on the number of embryos formed and their development for each of the variants. The one-factor dispersion analysis applied to the results obtained showed that at doses 300, 600 and 900 Gy the differences in the frequency of induction of parthenogenetic development can not be statistically proved, i.e. they are random. The differences between each of these three doses and doses 500, 700 and 1100 Gy are statistically significant at p.0.001("c").

Concerning the number of embryos formed, when eliminating the specificity of the mother genotype and the pollen donor, we obtained best results in pollen inactivation with doses 1100 Gy and 700 Gy (Fig. 1). There are no statistically significant differences between these two doses according to this index.

When registering the number of fertile and sterile plants obtained (Fig. 1) we found out that at dose 1100 Gy considerably more fertile plants were produced while at dose 700 Gy the sterile plant predominated. On the basis of these results it could be asserted that under the conditions of the experiment, best results were obtained in inactivation of the pollen with 1100 Gy.

The Fig. (1/1; 1/2; 1/3; 1/4; 1/5 and 1/6) present the effect if the interaction between each of the studied gamma-irradiation doses and the pollen donors. The indices which give the effect of the dose on the parthenogenesis efficiency are the following: number of embryos obtained; number of sterile plants and number of fertile plants. The results presented in Figs. 1/1 - 1/6 confirm the hypothesis about the relativity of the irradiation effect power and the genotype specificity of the pollen donor; in this experiment the genotype specificity consisted in the following:

■ pollen donor Rf 673 induced parthenogenesis in treating the pollen with the relatively higher radiation doses - 700 Gy, 900 Gy and 1100 Gy. Best response was obtained in irradiation with 700 Gy;

■ pollen donor 147 R induced parthenogenesis in treating the pollen with the relatively highest radiation dose - 1100 Gy;

■ the mixed pollen induced parthenogenesis in treatment with the lowest radiation doses - 300 Gy, 500 Gy and 600 Gy. Best response was obtained at 500 Gy.

■ Donor 939 R was active when treated with doses 500 Gy, 600 Gy, 700 Gy and 1100 Gy. The highest induction frequency of parthenogenetic development, the product of which are

DH-R lines of breeding importance was registered in the interaction of this donor with dose 1100 Gy.

The idea forms that under the effect of doses 500 Gy, 700 Gy and 1100 Gy, having in mind the specificity of the pollen donors, more intensive parthenogenetic induction and development are observed. The relatively highest parthenogenesis frequency was established under the effect of dose 1100 Gy (Fig. 1). Assessment was made on the basis of the obtained DH-R lines of breeding importance.

In summarising the behaviour of pollen donors (Fig. 2) under the conditions of this experiment, it becomes clear that 993 R induced parthenogenesis with the highest frequency.

Fig. 3 shows the specific parthenogenetic reaction of the mother hybrids under the conditions of the experiment. It could be seen that hybrid San Luka demonstrated considerably higher responsiveness than hybrid Albena. In the latter, parthenogenesis was induced in treating the pollen with radiation doses 500 Gy, 600 Gy and 1100 Gy. In San Luka induction was observed under the effect of all 6 radiation doses, but the highest number of DH-R lines of breeding importance were obtained at 1100 Gy (Fig. 3/1).

In the cms-lines our previous observations were confirmed, i.e. the frequency of induced parthenogenesis was low under the conditions we have created for the purposes of the experiment. The total number of embryos obtained was 11, nine of which developed into sterile plants, resembling mother genotypes.

In all variants in which embryo culture was not applied, the seeds obtained were empty. This is most probably due to the fact that the parthenogenetic embryos formed were not capable to develop further without artificial cultivation.

References

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Meynet, J., R. Barrade, Duclos A. & R. Siadous, 1994. Dihaploid plants of roses obtained by parthenogenesis induced using irradiated pollen and *in vivo* culture of immature seeds. Agronomie 2: 162-175.

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Fig. 1: Iinfluence of gamma - radiation doses on the induced parhenogenesis in sunflower.

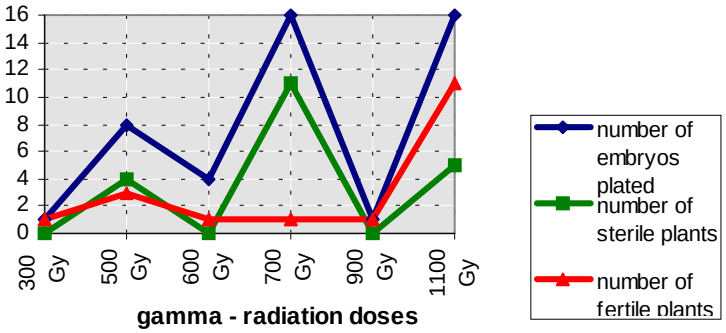


Fig. 1/3: Influence of gamma -irradiation dose 600 Gy on pollen donors (Rf 673, 147 R and 939 R) activity and ability to induce parthenogenic development in sunflower hybrids Albena and San Luka.

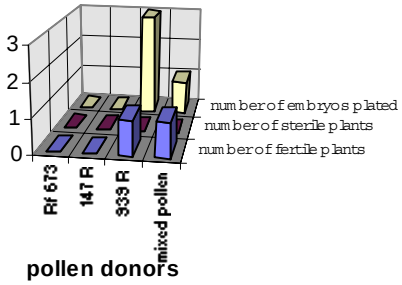


Fig. 1/1: Influence of gamma - irradiation dose 300 Gy on pollen donors (Rf 673, 147 R and 939 R) activity and ability to induce parthenogenetic development in sunflower hybrids Albena and San Luka.

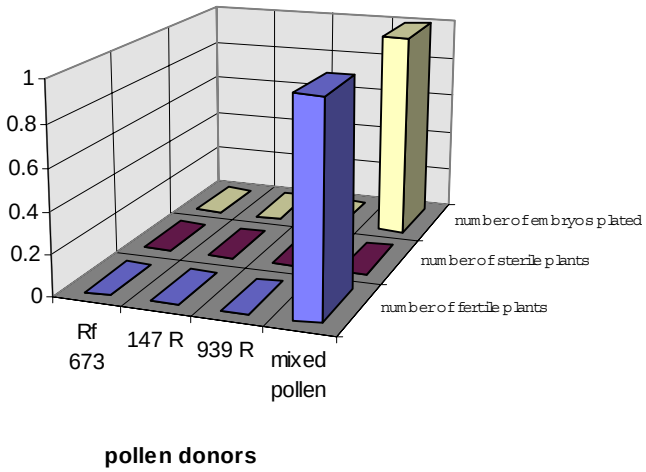


Fig. 1/4: Influence of gamma -irradiation dose 700 Gy on pollen donors (Rf 673, 147 R and 939 R) activity and ability to induce parthenogenetic development in sunflower hybrids Albena and San Luka.

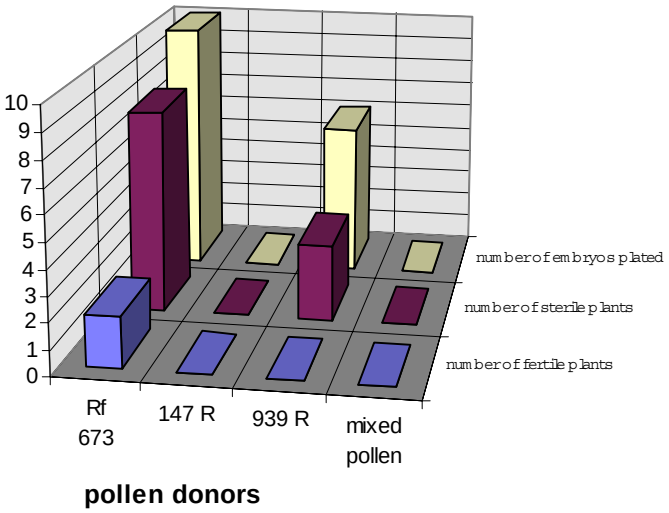


Fig. 1/2: Influence of gamma - irradiation dose 500 Gy on pollen donors (Rf 673, 147 R and 939 R) activity and ability to induce parthenogenic development in sunflower hybrids Albena and San Luka.

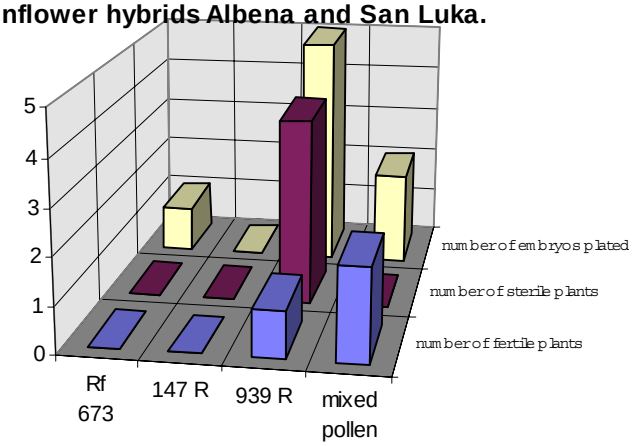


Fig. 1/5: Influence of gamma - irradiation dose 900 Gy on pollen donors (Rf 673, 147 R and 939 R) activity and ability to induce parthenogenetic development in sunflower hybrids Albena and San Luka.

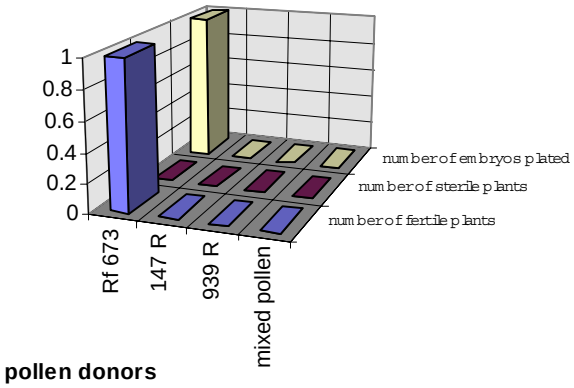


Fig. 1/6: Influence of gamma - irradiation dose 1100 Gy on pollen donors (Rf 673, 147 R and 939 R) activity and ability to induce parthenogenetic development in sunflower hybrids Albena and San Luka.

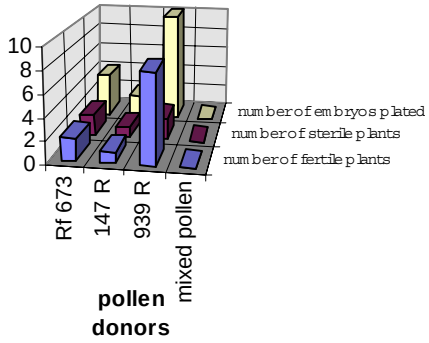


Fig. 3: Responsiveness of mother genotypes to parthenogenetic development induced by gamma irradiatiated pollen.

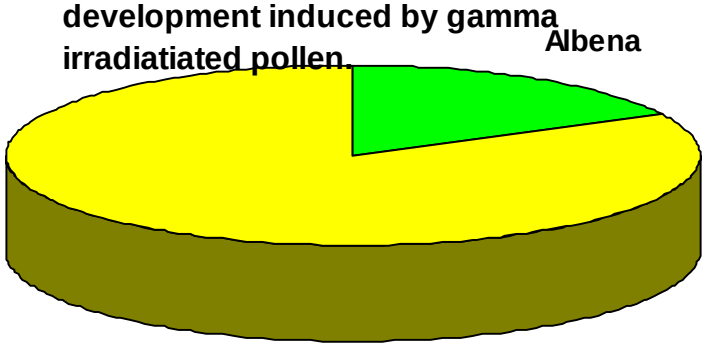


Fig. 2: Effect of pollen donor on the induced parthenogenesis in sunflower.

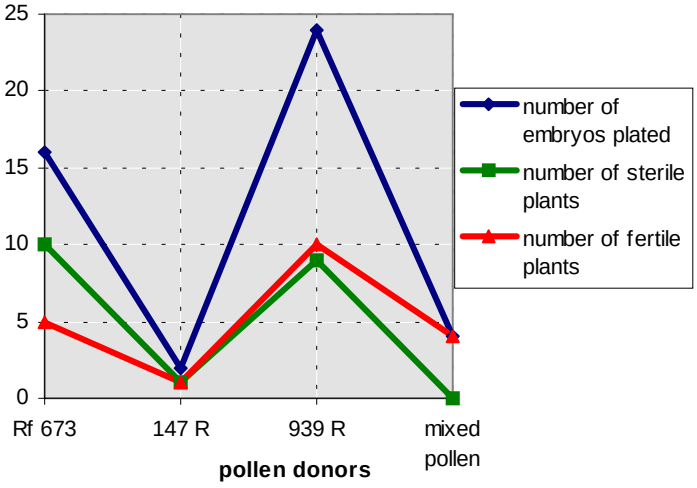
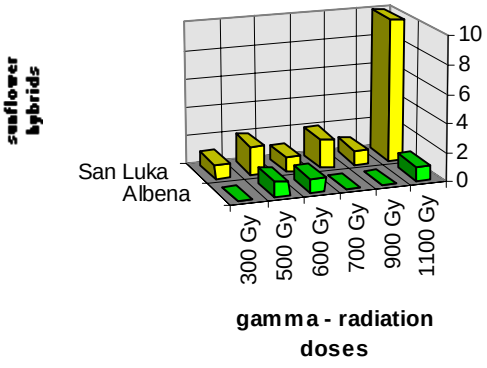


Fig. 3/1: Parthenogenetic response, expressed as a number of useful DH lines produced, of two sunflower hybrids as mother plants and three pollen donors (Rf 673, 147 R and 939 R).



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