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Effect of Gamma Radiation on Pollen Viability and Pollen Germination of Marigold Cultivar 'Pusa Narangi Gainda'

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Abstract

Irradiated pollen can be used to arouse gynogenetic haploid production. The dose of radiation is one of important factor for controlling haploid production. In order to know whether gamma irradiated pollen is viable or non-viable or they have germination capacity or not, experiment on effect of gamma radiation on marigold pollen viability and germination percentage was conducted. Pollen of marigold cultivar 'Pusa Narangi Gainda' was irradiated with 0, 50,100, 150, 200, 250, 300 Gy gamma radiation in Gamma Radiation Chamber 5000, ICAR-IIHR, Bengaluru. Pollen viability percentage of these pollens was recorded by staining with Alexander stain and aceto carmine separately. Pollen germination test was done using hanging drop method. Maximum pollen viability of 98.67% and 99.33% was reported when control pollen (which was not gamma irradiated) was stained with Alexander stain and aceto carmine stain respectively. When pollen grain was subjected to 200Gy gamma radiation, the resultant pollen was 28.67% and 32.00% (Alexander stained and aceto carmine stained respectively) viable. Only 9.33% and 10.00% (Alexander stained and aceto carmine stained respectively) pollen was found to be viable when these were irradiated with 300Gy. Maximum pollen germination (87.67%.) was reported in control. Gamma irradiation of 200Gy dose, reported 18.33% germination. Only 1% pollen was found to be germinated when pollen was irradiated with 300Gy gamma ray radiation.

Introduction

Marigold is botanically known as *Tagetes* spp. It is an economically important flower crop that is grown for loose flower, garden flower, medicinal and therapeutic values. In India, at present the area under marigold is 55.89 thousand ha with a production of 511.31 thousand metric tons. Karnataka alone occupies 8,500 ha under its cultivation with the production of 74,000 metric tons. Its productivity varies from 2 tons per acre in Uttar Pradesh to 8.2 tons per acre in Karnataka (NHB, 2015). Recently, many companies released F_1 hybrids and they are popular among farmers, their productivity is very high. This variation in marigold productivity is due to its heterozygous nature. Marigold breeding programs require large numbers of homozygous plants. Producing homozygous lines by conventional methods of breeding is time consuming in this crop (Vogel, 2015). Therefore, haploid lines in marigold can be used for obtaining homozygous doubled haploid true breeding lines which, in conventional breeding, would take multiple generations.

Pollination with irradiated pollen is one of the possibilities for inducing the formation of maternal haploids. Irradiated pollens are genetically inactive but physiologically active. They are unable to fertilize the egg cell but can germinate on the stigma, therefore can induce parthenogenesis. The dose of radiation is the main factor controlling *in situ* haploid production (Kundu *et al*., 2016). In order to know whether gamma irradiated pollen is viable or non-viable or they have germination capacity or not, experiment on effect of gamma radiation on pollen viability and germination is very important. Haploid has been obtained in apple, cacao, melon, barley, onion (Sestili and Ficcadenti, 1996), petunia (Raquin, 1985; Raquin and co-worker, 1989), rose (Meynet and co-worker, 1994), sunflower (Todorova and Ivanov, 2000) and carnation (Sato *et al*., 2000) by this method. To the best of our knowledge, there appears to be no report on gamma radiation effects on pollen viability and pollen germination of marigold or *Tagetes* spp.

Material and Methods

Flower of 'Pusa Narangi Gainda' were bagged before anthesis in order to avoid contamination from the unwanted pollen. Alexander stain (Alexander, 1969) was prepared. Aceto carmine stain was prepared. 100 ml of 45% acetic acid solution was heated for boiling, 0.5 g of carmine powder was added in to this solution and continued heating for 15-20 min while stirring the solution. This resulting solution was cooled and then filtered to remove any precipitate, and stored in brown bottle.

Preparation of pollen germinating media

In vitro pollen germination of marigold (*Tagetes* sp.) cultivar 'Pusa Narangi Gainda', was carried out in media containing sucrose 10%, polyethylene glycol (PEG) 15%, calcium nitrate 300 ppm, magnesium sulphate 200 ppm, potassium nitrate 100 ppm and boric acid 100 ppm. pH of the media was adjested to 6.5.

Collection of pollen and gamma irradiation of pollen

Pollen from the bagged flower (when fully open) was collected in morning hr. Pollen was irradiated with 50,100, 150, 200, 250, 300 Gy gamma radiation in Gamma Radiation Chamber 5000, ICAR-IIHR, Bengaluru. Pollen without gamma irradiation was used as control treatment. Three replications of each treatment were used.

Pollen viability test

Pollen irradiated with different gamma irradiation doses (0, 50, 100, 150, 200, 250, 300 Gy) were placed on glass slide, stained with Alexander stain and aceto carmine separately and finally covered with cover slip. These slides were then observed under microscope. The treatment was replicated three times and ten microscopic fields were taken for each replication. The pollen viability percentage for each treatment was calculated. In case of aceto carmine stain, pollen viability was reported according to red colour staining. Pollen with red colour are viable whereas colourless are non-viable. In case of Alexander stain, pollen viability was reported according to differential stain. Pollen with blue colour are viable and pollen with green colour are non-viable.

Pollen germination

Pollen was germinated using hanging drop method. The germinating media was placed on a cover slip to which the pollen was placed. The cover slip was then placed on a cavity slide sealed with petroleum jelly. After the pollen was left to germinate in the media, the slides were kept for incubation in moist condition for germination. The slides were left for incubation for about two hrs. The treatment was replicated three times and ten microscopic fields were taken for each replication. The pollen grain was considered germinated when its pollen tube length was greater than its diameter. The pollen germination percentage for each treatment was calculated.

Result and Discussion

As the doses of gamma irradiation increases, pollen viability (PV) continuously decreased as compared to non-irradiated pollens (Table 1and Fig 1). Maximum pollen viability of 98.67% and 99.33% was reported when control pollen (which was not gamma irradiated) was stained with Alexander stain and aceto carmine stain respectively. There were about 50% reductions in pollen viability when pollen grain was subjected to150Gy gamma radiation. As the dose increase from 150Gy to 200Gy there was sudden decline in pollen viability. When pollen grain was subjected to 200Gy gamma radiation, the resultant pollen was 28.67% and 32.00% (Alexander stained and aceto carmine stained respectively) viable. Only 9.33% and 10.00% (Alexander stained and aceto carmine stained respectively) pollen was found to be viable when these were irradiated with 300Gy.

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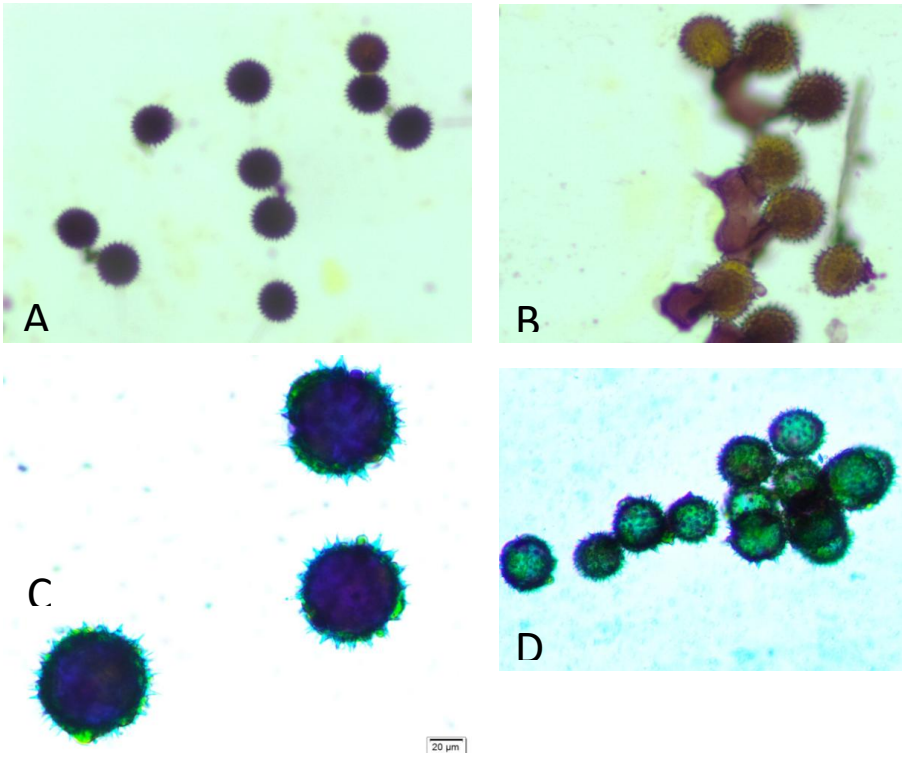

Fig 1. Viable and non-viable pollen grain of marigold cv. 'Pusa Narangi Gainda'. A, B) Viable and non viable pollen respectively with aceto carmine stain; C, D) Viable and non viable pollen respectively with alexander staining. 100 Gy; C) Pollen grain irradiated with 200 Gy; D) Pollen grain irradiated with 300 Gy.

With the increasing doses of gamma ray, pollen germination percentage continuously decreased as compared to non-irradiated pollens (Table 1 and Fig 2). Maximum pollen germination was reported in control with the germination of 87.67%. As the dose increase from 200Gy to 300Gy there was sudden decline in pollen germination. When pollen grain was subjected to gamma irradiation of 200Gy dose, the resultant pollen reported 18.33% germination. Only 1% pollen was found to be germinated when pollen was irradiated with 300Gy gamma ray radiation. This result confirmed the previous finding recounted by Zhang and Lespinasse (1991) in apple, Meynet and co-worker (1994) in rose, Kurtar (2009) in winter squash and pumpkin, Kundu and co-worker (2014, 2016) in citrus and Grouh *et al.*, (2015) in iris.

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Table 1: Effect of different doses of gamma radiation on pollen viability and pollen germination of marigold cv. 'Pusa Narangi Gainda'

Gamma radiation (Gy)	Per cent pollen viability with Alexander stain	Per cent pollen viability with aceto carmine stain	Per cent pollen germination
0	98.67[a]	99.33[a]	87.67[a]
50	79.67[b]	81.67[b]	68.67[b]
100	70.33[c]	73.00[c]	59.00[c]
150	48.67[d]	50.00[d]	40.67[d]
200	28.67[e]	32.00[e]	18.33[e]
250	17.67[f]	20.00[f]	9.00[f]
300	9.33[g]	10.00[g]	1.00[g]

Data followed by different letters are significantly different at 1 % level of significance.

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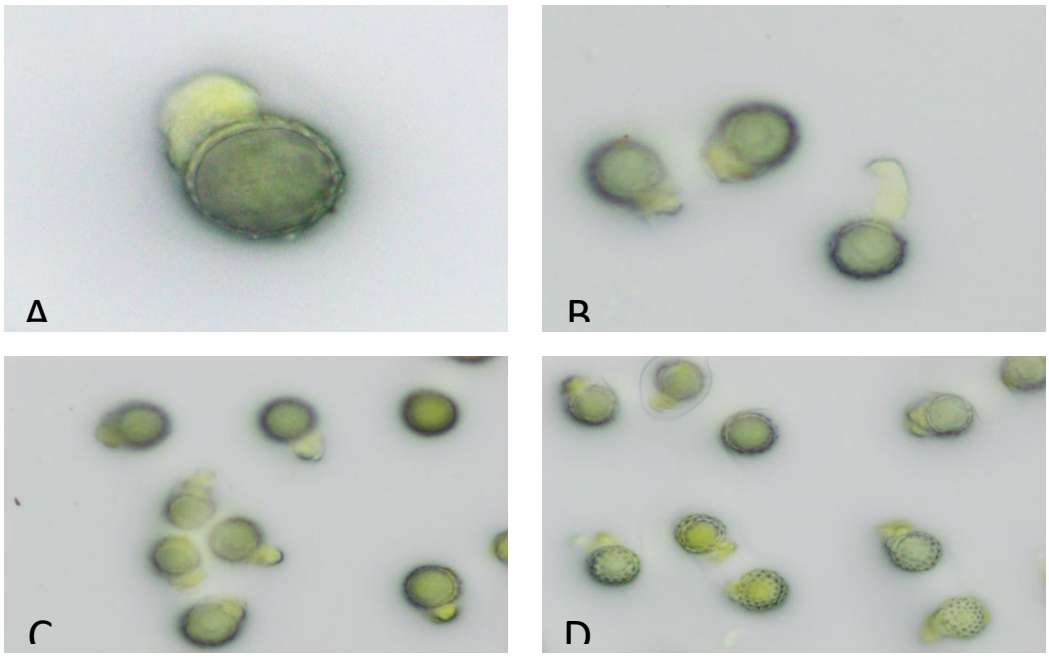

Fig 2. Effect of gamma radiation on pollen germination of marigold cv. 'PusaNarangiGainda'. A) Untreated pollen grain; B) Pollen grain irradiated with 100 Gy; C) Pollen grain irradiated with 200 Gy; D) Pollen grain irradiated with 300 Gy.

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