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Effect of γ-irradiation on *in vitro* pollen germination of muskmelon (*Cucumis melo L.*)

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Abstract

Induction of haploid plants in vegetable crops through ã-irradiated pollen method is of utmost importance in contemporary vegetable breeding to accelerate the breeding cycle. But the major problem of this technique is the sensitivity of pollen grains to higher irradiation doses. Present work investigated the effect of different γ-irradiation dosage (250 to 400 Gy) on percent germination and tube length of pollen grains of muskmelon (*Cucumis melo* L.) genotype, MS-5 on Brewbakers medium. The observations were recorded after 1, 2 and 3 hours of incubation. Irradiated pollen didn't show any sign of germination at 1 and 2 hours, however germination initiation was observed in the non-irradiated pollen. A decrease in percent germination and pollen tube growth was observed with incremental irradiation dose. Very little germination or abnormal pollen tube growth was recorded for pollen grains irradiated at 400 Gy. This study advocates the need for deriving out the appropriate ã-irradiation dose specific for the muskmelon genotype besides obtaining the extent of the lethality for the irradiated pollen grains for its possible application in generation of haploids through parthenogenesis.

Key words: Irradiation dose, mutation, muskmelon (*Cucumis melo* L.), parthenogenesis, pollen germination, pollen tube abnormality

Introduction

Development of haploids may involve micro-propagation enabled generation of complete plantlets from the haploid genome bearing organs through androgenesis or gynogenesis (Reed 2005). However, both the techniques include a crucial step, differentiation of the calli to transform into plantlets, which may limit the success. Thus, parthenogenesis can lead to formation of haploid plants directly after rescuing the unfertilized embryos in the pre-mature fruit. Parthenogenesis can be induced either by pollination with inactivated pollen or by treating with a variety of chemicals (Ficcadenti and Sestili 1995). Irradiation of pollen grains using different ionizing radiations such as UV rays, gamma rays and X-rays is most preferred technique now a day for *in situ* induction of haploid plants (Ari et al. 2010). This involves irradiating pollens grains at higher irradiation doses such that they become biologically inactive and genetically inert (Hertwig 1920) as their germ nucleus is destroyed. On contact with stigma surface, such pollen grains remain physiologically active; get hydrated by absorption of water and nutrients and germinate to form a tube-like pro tuberance which delivers the biologically inactive sperm cells to the embryo sac in the ovary (Lord and Russell 2002). Thus, these pollen grains elicit cell division in the embryo sac without fusion/ recombination with germ nucleus of pollen i.e. without any active participation of the sperm nucleus to generate haploid embryo.

The EMR spectrum includes gamma rays which are most suitable for haploid production programs due to their ease of application, good penetration capability, reproducibility, higher mutation rate and lower disposal problems (Chahal and Gosal 2002). Cobalt (^{60}Co) is most commonly used γ-rays source. Depending on the plant species, the LD_{50} (dose to inhibit germination in 50% pollen) dosage may vary between 35 to 550 Krad (350 to 5500 Gy). Doses in-between this range lower pollen germination and greatly depresses pollen tube growth in the germinated pollens resulting in formation of short stout tubes with burst tips (Casarett 1968). Therefore, the extent of the radiobiological injury varies for the pollen parameters such as germination and ultimate survival of pollen.

The applied irradiation doses vary greatly among species or genotypes of same genera (Cunny et al. 1993).

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Haploids have been obtained by using lower irradiation doses of 5-20 Gy in barley (Powell et al., 1983), 25 and 50Gy in summer squash (Kurtar et al. 2002), 50 and 100 Gy in pumpkin (Kurtar et al. 2009) and 50 and 100 Gy in winter squash (Kurtar and Balkaya 2010). Whereas few crops require relatively higher irradiation doses for haploid embryo induction for instance, 200-1200 Gy in cabbage (Dore 1989), 500 Gy in cucumber (Claveria et al. 2005 and Dolcet-Sanjuan et al. 2006), 200-300 Gy in watermelon (Gursoz et al. 1991 and Sari et al. 1994) and typically about 300 Gy was shown to be effective in experiments carried out on melon (Ari et al. 2010, Gonzalo et al. 2011, Nasertorabi et al. 2012 and Baktemur et al. 2013). Thus, the radio-resistance of pollen besides the biological efficiency of irradiation exhibited a linear relationship with the pollen morphology (size, shape, sensitivity condition of dehydration) thickness of the pollen wall (Giles and Prakash 1987) and amount of DNA (Kurtar and Balkaya 2010).Therefore, vegetables like pumpkin and winter squash which have quite large pollens (average width 180 ìm) exhibit greater parthenogenetic potentials at lower irradiation dosage compared to muskmelon, watermelon, and cucumber that have smaller pollens (mean width 50, 60 and 65 ìm, respectively) (Sensoy et al. 2003). Occurrence of multiple apertures i.e. region of fused exine and intine layers of the pollen grain wall, can affect radio-resistance of pollen as these are the possible sites for emergence of the pollen tube. The greater the number of apertures, lower will be the radio-resistance, making pollen susceptible to dehydration, rapid loss of viability and hence, lower irradiation dosage will inactivate the pollen. Thus, pollen germination and tube growth studies are one among the most reliable parameters to determine the appropriate irradiation dose for a genotype. This manuscript evaluates the optimum gamma irradiation dose for biological inactivation of pollen grains of *Cucumis melo* genotype MS-5 using *in vitro* pollen germination study.

Materials and Methods

The pollen grains of the male parent in this study, muskmelon genotype MS-5 were evaluated for the radio-resistance to gamma irradiation. The plants of the genotype MS-5 were raised in the field at Vegetable research farm, Punjab Agricultural University, Ludhiana, India. The pollens were collected from dehiscent anther lobes of the male flowers of 35 to 40 days old plants during night hours.

The male buds were collected one day before anthesis i.e. in the evening. The petals were removed to expose the anthers. The exposed anthers were irradiated in a γ-chamber next morning with gamma rays (Cobalt-60 source, manufactured by BRIT, Department of Atomic Energy of India, Mumbai, India) at four different doses i.e. 250, 300, 350 and 400Gy. The control pollen grains were also collected in a similar manner, placed in petri dish and held for 1 day before pollination. To assess the pollen germination potential, both irradiated and control pollen were placed on the pollen germination medium having following composition (per L); 10% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)$ $4H_2O$, 200 ppm $MgSO_4 \cdot 7H_2O$, 100 ppm KNO_3 and phytagel (4%) (Brewbaker and Kwack 1963). This assembly containing pollen grains on media in petri plates was kept in incubator at 28°C±2° C temperature to initiate proper germination. The observations for the germination of pollens were recorded after 1, 2 and 3 hrs of incubation. The optical research microscopy study of the pollen grains stained with 1% acetocarmine-glacial acetic acid-ferric chloride solution (Sunderland and Wicks 1971). The stained pollens were then placed on the slide along with the cover slip to observe them under the optical research microscope (Leica DM 5000B) at 400X magnification. Data on percent germination and pollen tube length at control and different irradiation doses was recorded. Data on percent germination and pollen tube length was subjected to descriptive analysis using generalized linear model procedure of SAS software (Version 9.3, Cary NC, USA).

Results and Discussion

No pollen germination was observed after 1 and 2 hrs at any irradiation dose while some germination was observed in non-radiated pollen. Similarly, Cuny et al. (1993) reported some pollen germination after 20 mins in non-radiated pollen only while, irradiated pollen took more time for germination. However, after 3 hrs, germination was recorded in both non-radiated pollen

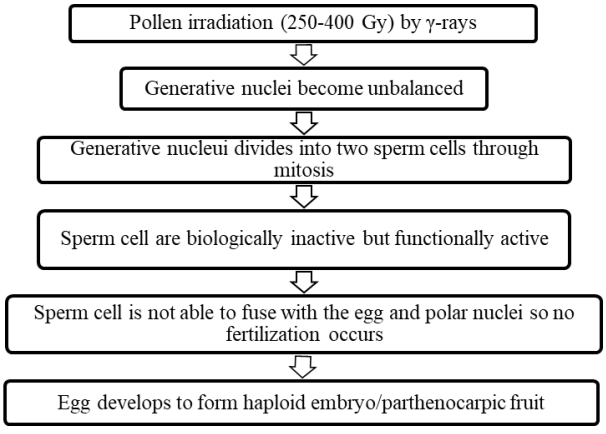

Fig. 1: Flow diagram indicating the crucial steps for the generation of parthenogenic fruit through pollen irradiation technique

and radiated pollen (250 Gy to 400 Gy). So, the data was recorded for two parameters, pollen germination and pollen tube length after 3 hrs.

Effect on pollen germination: A decreasing trend in pollen germination (%) was observed, as the γ-irradiation dose was increased from 0 to 400 Gy. The highest pollen germination was recorded in control treatment (90.06%), while the lowest pollen germination (6.65%) was exhibited at 400 Gy dose. Further, at 250, 300 and 350 Gy γ-irradiation dose, the pollen germination was observed to be 65.68%, 53.72% and 41.81%, respectively (Fig. 2). Thus, these results indicate that pollen germination was decreased with incremental dose of ã-irradiation. This decrease in pollen germination can be attributed to biological inactivation of the pollen (Kurtar 2009). Similarly, Cuny et al. (1993) reported that at high γ-irradiation doses, the pollen germination in Vedrantais cultivar, of the Cantaloupe Charentais group was significantly decreased. Further, at 300 Gy and 350 Gy doses moderate pollen germination was recorded, thus these two doses were selected assuming generation of a desirable level of mutated pollens which could induce haploid embryos. Similarly, Sauton and Dumas De vaulx (1988), Ari et al. (2010), Godbole and Murthy (2012) and Baktemur et al. (2013) used 300 Gy γ-irradiation dose to induce haploids in muskmelon. Contrary to above reports, Cuny et al. (1993) reported no effect on pollen germination in muskmelon cv. Vedarantais even at 500 Gy. These variations in the extent of radio-resistance may be attributed to the difference in the genotype belonging to diverse melon groups. The pollen grains of *charantias* melon have higher radio-resistance than the *reticulates* melon used in the present study. Thus, the effect of irradiation dose on pollen germination can vary with the crop/ species and genotype (Cunny et al. 1993).

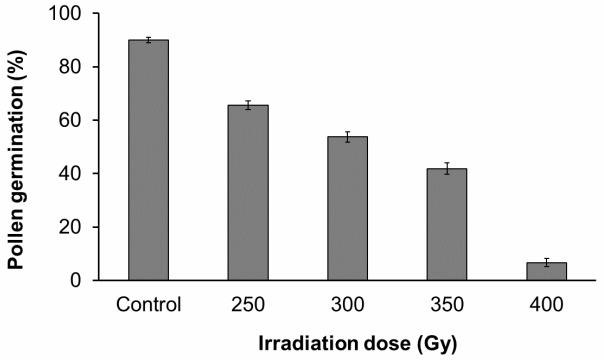

Fig. 2: Effect of irradiation dose on pollen germination

Effect on pollen tube length: A similar decreasing trend for pollen tube length was observed with increase in irradiation dose from 0 to 400 Gy (Fig. 3). The highest pollen tube length was observed in control treatment

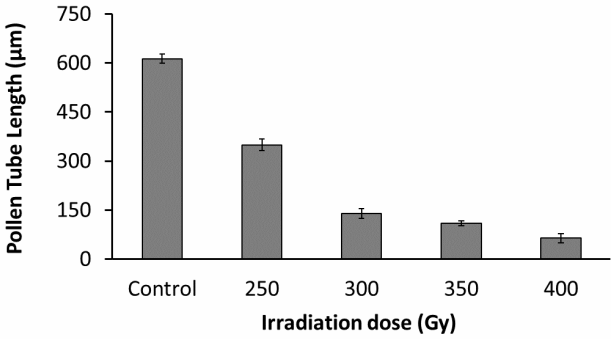

Fig. 3: Effect of different irradiation dose on pollen tube length

(613 µm) while the lowest pollen tube length was recorded at 400 Gy (63.8 µm). Similar observations on decreased germination, depression in pollen tube length and occurrence of short stout tips on irradiating pollens with 350 to 5500 Gy have been reported by Casarett (1968). Further, Cuny et al. (1993) reported decrease in the pollen tube growth with incremental γ-irradiation dose. At 400 Gy the pollen tube formed was distorted and stout. At 300 and 350 Gy ã-irradiation doses abnormalities like burst pollen tube tip and pollen tube growth from two sides were observed.

Further, the efficiency of γ-irradiation dose and radio resistance of pollen is dependent on the size and shape of the pollen grain (Giles and Prakash 1987). As the mean width of the pollen grains of genotype MS-5 was 46.1±0.9 µm, it has optimum irradiation dosage in range of 300 to 350 Gy and thus exhibited a little higher radio resistance. Moreover, presence of lower number of pollen apertures (generally three in number), further improve radio-resistance as the pollen are less prone to dehydration and rapid loss of viability compared to squash (Kerhoas et al. 1986). This *in vitro* pollen germination study concludes that both pollen germination and pollen tube length decreased with increase in the ã-irradiation dose in muskmelon genotype MS-5. The radio resistance of the pollen grains was recorded to be proportional to the morphology (size, shape) of the pollen grain and ultra-structural characteristics of the pollen wall (thickness and number of apertures per pollen grain). Therefore, to ensure the induction of gynogenic haploid embryo in muskmelon

Table 1 Pollen germination after 1 and 2 hrs at different irradiation doses

S. No.	Irradiation dose	Pollen germination after 1 hr	Pollen germination after 2 hrs
1	Control	25.02 %	44.44%
2	250 Gy	Not detected	Aperture visible
3	300 Gy	Not detected	Aperture visible
4	350 Gy	Not detected	Aperture visible
5	400 Gy	Not detected	Aperture visible

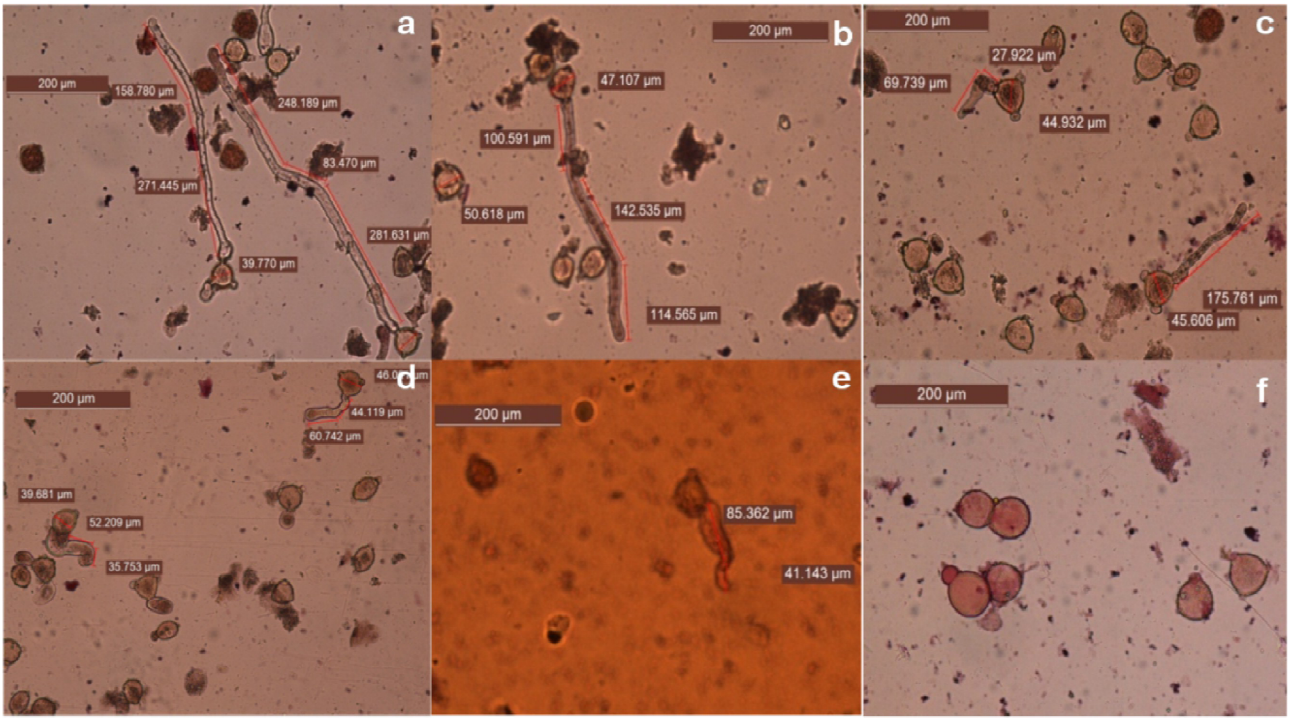

Fig. 4: Effect of ã-irradiation on pollen tube growth of muskmelon genotype MS-5 at different irradiation dosages (a) Control (b) 250 Gy (c) 300 Gy (d) 350 Gy (e) 400 Gy (f) Abnormality at 400 Gy

(*Cucumis melo* var *reticulatus*), pollen grains should be irradiated with higher doses (300 and 350 Gy) of gamma rays.

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समकालीन सब्जी प्रजनन में विकिरणित पराग विधि के माध्यम से सब्जी फसलों में अगुणित पौधों की प्रेरणा प्रजनन चक्र को तेज करने के लिए अत्यधिक महत्व है। लेकिन इस तकनीक की प्रमुख समस्या पराग कणों की उच्च विकिरण दर की संवेदनशीलता है। वर्तमान अध्ययन में विभिन्न विकिरण की दर (250–400 जीवाई) का प्रभाव परागकणों के प्रतिशत अंकुरण और पराग कण नलिका विकास खरबूजा के प्रभेद एमएसम–5 पर ब्रिउबेकर्स माध्यम से किया गया। आंकड़ें 1, 2 व 3 घण्टे उपचार उपरान्त एकत्रित किये गये। विकिरणित परागकण 1 व 2 घण्टे में अंकुरण का कोई संकेत प्रदर्शित नहीं किये जबकि गैर विकिरणित परागकण में अंकुरण स्पष्ट देखा गया। इस प्रकार वृद्धिशील विकिरण दर के साथ प्रतिशत अंकुरण एवं परागकण नलिका विकास में कमी देखी गयी। विकिरणित 400 जीवाई की दर परागकणों के अंकुरण हेतु कम पाया गया या असामान्य पराग नलिका की वृद्धि पायी गयी। इस अध्ययन से स्पष्ट होता है कि उपयुक्त γ–विकिरण की दर खरबूजा की विशिष्ट प्रभेदों हेतु परागकणों की घातकता की सीमा के अलावा पाथोजेनेसिस के माध्यम से हैप्लायड्स विकास हेतु किया जा सकता है।

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