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Effect of gamma ray irradiation doses on pollen viability and *in-vitro* germination in *Citrus*

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ABSTRACT

Induction of haploid plants in fruit crops through gamma irradiated pollen technique is of paramount importance in modern fruit breeding to reduce the breeding cycle. But the major problem of this technique is the sensitivity of pollen grains to higher irradiation doses. Present work analyzed the viability, *in vitro* germinability and pollen tube growth of gamma ray irradiated pollens of 2 different *Citrus* species: *C. limetta*, and *C. sinensis*. Both viability and *in vitro* germination capacity of irradiated pollen decreased gradually with increasing concentrations of irradiation in both the pollen parent; however, at highest irradiation dose (400 Gy), reduction of pollen viability and *in vitro* germination capacity, as compared to respective controls was recorded minimum in *C. limetta* (11.07 and 29.78%, respectively). *In vitro* pollen tube length as measured at 24, 48 and 72 hr after incubation, was found maximum in *C. limetta* (267.83, 303.24 and 325.57 µm, respectively). Our experiment revealed that pollen of *C. limetta* has higher resistance to gamma ray irradiation as compared to *C. sinensis*, hence *C. limetta* can be employed as more reliable pollen parent in haploidy programme of *Citrus*.

Key words: *Citrus*, *In vitro* germination, Irradiation, Pollen, Pollen tube growth

In *Citrus* as well as in other woody species, a long reproductive cycle, heterozygosity, large canopy size and self-incompatibility pose major problems for breeding and genetic research (Germanà and Chiancone 2001). Although the problems of long reproductive cycle, large canopy size and self-incompatibility can be overcome by using different plant bio-regulators, training and pruning operation and by using different cross compatible pollen parents, respectively, but it is very difficult to overcome the problem of heterozygosity. This problem of heterozygosity ultimately hinders the varietal improvement programme because for the development of new varieties, we need homozygous parental population to cross. But in fruit crops like citrus development of homozygous inbred line by selfing each parental population is very difficult because it will produce different hybrid in every selfing due to their heterozygous nature. To overcome this problem, production of haploid progenies is of utmost importance as chromosome doubling of these haploid progenies will make them into complete homozygous diploid progenies. In *Citrus*, haploids have been produced by anther culture (Germanà and Chiancone 2003) and interploid hybridization (Germanà and Chiancone 2001). However, these methods have not been effective because these haploids are very weak and grow more slowly than diploid plants (Germanà 1997). This suggests an alternative method to develop to produce haploids in this recalcitrant genotype. Irradiated pollen technique (UV, gamma rays and X-rays) is currently used successfully to induce *in situ* haploid plants. Irradiated pollens are genetically inert, physiologically active and can be easily germinated on the stigma, but are not able to fertilize the egg cell and the polar nuclei. Hence, these pollens might be used to stimulate parthenogenesis including gynogenic haploid production. Due to its simple application, good penetrability, reproducibility, high mutation frequency and lower number of disposal problems, gamma rays are commonly used in haploidy programs (Chahal and Gosal 2002). But the major constraint of using gamma irradiation technique is the sensitivity of *Citrus* pollen to dehydration; hence, they may lose their viability when treated with gamma ray. Moreover, after successful germination of irradiated pollen on the stigmatic surface, pollen tube may get degenerated within the style before reaching to the ovarian wall, resulting poor fruit setting (Yahata *et al*. 2010 and Kundu *et al*. 2014). Hence, before starting the hoploidy programme in this crop, it is essential to study the response of *Citrus* pollen to different doses of gamma ray irradiation. Keeping this view in mind, present study aimed to examine the effect of gamma ray irradiation doses on pollen viability, *in vitro* germination and pollen tube growth in *Citrus*.

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MATERIALS AND METHODS

Two different *Citrus* species, viz. sweet orange (*Citrus sinensis* (L.) Osb.) cv. 'Mosambi' and sweet lemon (*C. limetta* Risso) were selected as pollen sources for the experiment based on our previous year's study on pollen viability. Twenty unopened flowers, approaching anthesis were collected separately from mature plants of each of these 2 pollen parents in the morning (09:00 to 09:30 A M). After removal of petals and stigma, the anthers with filament were put in glass petri dishes and kept under sunlight for proper dehiscence. Irradiation was performed on these anthers in a gamma chamber by Cobalt 60 gamma rays (Nuclear Research Laboratory, IARI, New Delhi, India) at 50, 100, 200, 300 and 400 Gray (Gy). Two petri-dishes with flower of each of these 2 pollen parents were kept separately in clean and dry place for the further use as non-irradiated pollen (control).

Pollen viability was estimated by fluorescein diacetate (FDA) test as described by Heslop- Harrison and Heslop-Harrison (1970).For this, a stock solution (2 mg/ml) of fluorescein diacetate was prepared in acetone. In addition, 10% sucrose solution was used to prevent the bursting of pollen grains, while 300 mg/ml of calcium nitrate were added to improve their response. After putting 2-5 ml of this solution in a small glass vial, drops of FDA stock solution were added until the resulting mixture showed a persistent turbidity. After taking a drop of sucrose-FDA mixture on a micro slide, pollen grains were suspended on the drop and incubated in a humid chamber (> 90% RH) for 5-10 min. Thereafter, a cover glass was lowered and the preparation was observed under a fluorescent microscope. Pollen grains, fluoresce brightly were counted as viable pollens. For each irradiation dose, the pollen viability was evaluated on 200 pollen grains in each replication.

In vitro germination of those irradiated and non-irradiated pollen grains was performed in a liquid culture medium (150 g/l sucrose, 100 mg/l H_3BO_3, 1000 mg/l $Ca(NO_3)_2$, 300 mg/l $MgSO_4$ and 100 mg/l KNO_3 at pH 5.5) following the hanging drop technique according to Cavalcante *et al.* (2000). Thereafter, pollen grains, observed by a light microscope (40× magnifications), were considered germinated when the length of pollen tubes was measured at least equal to or greater than the grain diameter. For each irradiation dose, *in vitro* germination rate was evaluated on 200 pollen grains in each replication.

After germination of the pollen grains on growing medium, pollen tube length was measured at 24, 48 and 72 hr after incubation. It was measured directly by using an ocular micrometer fitted to the eyepiece of the microscope. Mean pollen tube length was calculated on 20 geminated pollen tubes for each replication. The experiment was laid out on complete randomized design with 5 replications; statistical analysis was performed using statistical analysis software (SAS 9.3; SAS Institute, Cary, NC, USA) and the means were compared using Tukey's Honest Significant Difference (THSD) Test at P≤0.05.

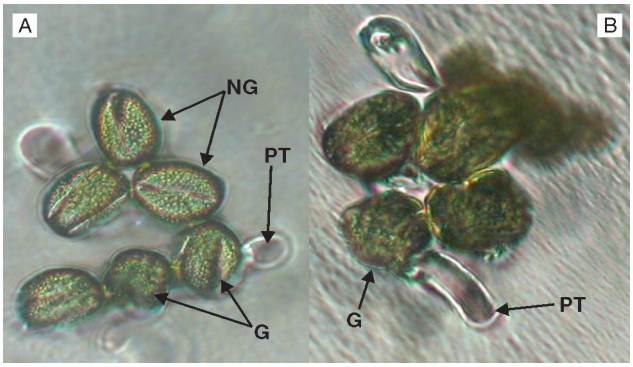

Fig 1 *In vitro* germination of gamma irradiated (400 Gy) treated pollen of *C. sinensis* (A) and *C. limetta* (B). G: germinated pollen; NG: non-germinated pollen; PT: pollen tube

RESULTS AND DISCUSSION

The results of viability and *in vitro* germinability, observed in irradiated as well as non-irradiated pollen by FDA and *in vitro* germination test (Fig 1) indicate a significant variation among the pollen parents. Regardless of irradiation doses, significantly higher viability and *in vitro* germination rate was recorded in *Citrus sinensis* (83.58% and 46.49%, respectively). These differences in pollen viability and *in vitro* germination capacity of two different pollen parent of *Citrus* were mainly due to the genotypic variation, which confirms the earlier findings of Chalak and Legave (1997) in kiwi fruit, Ali *et al*. (1998) in pomegranate and Kundu *et al*. (2014) in *Citrus*.

However, irrespective of the pollen parent, both viability and germination values decreased gradually with increasing concentrations of gamma ray irradiation and at highest irradiation dose (400 Gy), it was decreased by 12.13% and 35.59%, respectively as compared to respective controls. It is evident from the interactions of pollen parent and irradiation dose that non-irradiated *C. sinensis* pollen had maximum viability and *in vitro* germination capacity (89.88 and 63.39%, respectively). From the result, it was also evident that although the highest viability and *in vitro* germinability was recorded in non-irradiated *C. sinensis* pollen as compared to *C. limetta*, but with the increasing level of irradiation, the rate of reduction of pollen viability and *in vitro* germinability as compared to respective controls was much higher in *C. sinensis* and at 400 Gy, the reduction of pollen viability in *C. sinensis* as compared to respective controls was 13.16%; however, it was reduced only 11.07% in *C. limetta* for the same level of irradiation doses. Similarly, the rate of reduction of *in vitro* germination capacity at 400 Gy as compared to respective control was found maximum in *C. sinensis* (40.61%) than *C. limetta* pollen (29.78%).

Pollen viability in terms of stainability and *in vitro* germination not only depends upon the genotype, pollen maturity, plant physiological status, and growing media composition (Ferri *et al*. 2008), but also upon the internal conditions of pollen grains in relation to environmental factors like air temperature, humidity, grain water content, reserve substances, gametic maturity and their interactions

(Pacini *et al*. 2006). On the basis of all these factors, the pollen grains of different crop species have been classified into 2 categories: partially hydrated pollen, having water content >30% (like recalcitrant seeds) and partially dehydrated pollen, with a water content <30% (like orthodox seeds) (Kermade and Finch-Savage 2002). Unfortunately, due to scanty research work, there is scarce evidence regarding the interaction between the water content of pollen grains and irradiation doses, especially in *Citrus*. According to the morphological similarities of *Citrus* pollen grains with pollen grains of other Angiosperm species, classified by Franchi *et al*. (2002), it is possible to consider *Citrus* pollen grains as partially dehydrated pollen. Now, the exposure of pollen grains to different doses of gamma ray irradiation might reduce the water content of those pollen grains which in turn reduce the capability of those pollens to inter convert carbohydrate reserves; this is strictly linked to a change in the state of the cytoplasmic water of pollen grains, resulting in abnormal meiosis, irregular gametes formation that may cause significant changes in pollen properties (Nepi and Pacini 1993), and a consequent reduction in their viability. This result confirmed the earlier findings reported by Zhang and Lespinasse (1991) in apple and Kurtar (2009) in pumpkin and winter squash and kundu *et al*. (2014) in *Citrus*.

In vitro pollen tube length also differed significantly in these two different pollen parents during the entire period of observation, viz. 24, 48 and 72 hr after incubation of the pollen grains on the germinating medium. Pollen tubes remained intact without any rupturing up to 72 hr after incubation. Irrespective of gamma irradiation doses, after 24 hr of incubation, maximum pollen tube length was recorded in *C. sinensis* (263.67 µm), while after 48 and 72 hr after incubation, it was measured maximum in *C. limetta* (299.69 and 322.25 µm, respectively). However, pollen tube length decreased gradually with the increased dose of irradiation during the entire period of observation in both the pollen parents. After 24 hr of incubation, minimum pollen tube length was recorded in 400-Gy-treated *C. limetta* pollen (90.70% as compared to control), while after 48 and 72 hr of incubation the maximum decrease of pollen tube length were measured in 400-Gy-treated *C. sinensis* pollen (8.30 and 6.54%, respectively).

Variation in pollen tube growth in two different pollen parents could be due to the variation in their pollen carbohydrate concentration as carbohydrates are responsible for pollen development and especially, pollen cytoplasmic carbohydrates and sucrose are involved in protecting pollen viability during exposure and dispersal (Pacini 1996). Regardless of genotypic variation, pollen tube growth was also decreased with increased dose of gamma ray irradiation, which may be due to increasing sensitivity of the pollen grains to higher exposure doses and their inability to inter convert carbohydrate reserves. According to Cresti *et al*. (1977) at higher irradiation doses, although the pollen grains started to germinate but at the initial stage of tube growth, the inner wall of the tube disappeared; several bipartite particles accumulated at the tip of the tube; alteration of endoplasmic reticulum into a whorl of concentric circles occurred which ultimately leads to the inhibition of protein synthesis, resulting slow growth of pollen tube. In our study, minimum pollen tube length, measured at highest irradiation doses (400 Gy) for both the pollen parent might be due to the above mentioned facts which confirm the earlier findings of Swaminathan and Murty (1959), Cresti *et al*. (1977). On the other hand, although after 24 hr of incubation, maximum pollen tube length was recorded in *C. sinensis* but later on it was found higher in *C. limetta* which might be due to higher resistance capacity and higher ability to inter convert carbohydrate reserves of *C. limetta* pollen to higher exposure doses than *C. sinensis* during the later phase of pollen tube growth.

From the experiment, it can be concluded that at the higher irradiation dose of 400 Gy, the pollen of *Citrus limetta* showed higher resistance to gamma-ray irradiation than *C. sinensis*, as evidenced by viability, *in vitro* germinability and pollen tube growth data. Hence, this species can be employed as more reliable pollen parent in haploidy programme of *Citrus*.

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REFERENCES

Ali M A, Bacha M A and Farahat F A. 1998. Pollen viability, germination and rates of pollen tube growth in some pomegranate cultivars (*Punica granatum* L.*). Journal of King Saud University. Agricultural Science* **10**: 73–81.

Cavalcante H C, Schifro-Wittmann M T and Dornelles A L C. 2000. Meiotic behaviour and pollen fertility in an open-pollination of 'Lee' mandarin [*Citrus Clementine* X (*C. paradise* X *C. tangerine*)]. *Scientia Horticulturae* **86**: 10–4.

Chahal G S and Gosal S S. 2002. *Principles and Procedures of Plant Breeding: Biotechnological and Conventional Approaches.* Alpha Science International Ltd, United Kingdom.

Chalak L and Legave J M. 1997. Effects of pollination by irradiated pollen in Hayward kiwifruit and spontaneous doubling of induced parthenogenetic trihaploids. *Scientia Horticulturae* **68**: 83–93.

Cresti M, Ciampolini F and Pacini E. 1977. Ultrastructural aspects of pollen tube growth inhibition after gamma irradiation in *Lycopersicum peruvianum*. *Theoretical and Applied Genetics* **49**: 297–303.

Ferri A, Giordani E, Padula G and Bellini E. 2008. Viability and *in vitro* germinability of pollen grains of olive cultivars and advanced selections obtained in Italy. *Advances in Horticultural Science* **22**: 116–22.

Franchi G G, Nepi M and Pacini E. 2002. Partially hydrated pollen: taxonomic distribution, ecological and evolutive significance. *Plant Systematics and Evolution* **234**: 211–27.

Germanà MA. 1997. Haploidy in *Citrus*. (*In*) *In vitro* Haploid Production in Higher Plants, 5th Edn, pp 195–217. Jain S M, Sopory S K and Veilleux R E (Eds). Kluwer Academic Publisher, Dordrecht, Netherland.

Germanà M A and Chiancone B. 2001. Gynogenetic haploids of *Citrus* after *in vitro* pollination with triploid pollen grains. *Plant Cell Tissue and Organ Culture* **66**: 59–66.

Germanà M A and Chiancone B. 2003. Improvement of *Citrus clementina* Hort. ex Tan. microspore-derived embryoid induction and regeneration. *Plant Cell Reports* **22**: 181–7.

Heslop-Harrison J and Heslop-Harrison Y. 1970. Evaluation of pollen viability by enzymetically induced fluroscence; Intracellular hydrolysis of fluorescein diacetate. *Biotechnic and Histochemistry* **45**: 115–20.

Kermade A R and Finch-Savage B E. 2002. Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development. (*In*) *Desiccation and Survival in Plants: Drying without Dying*, pp 49–184. Black M and Pritchard H W (Eds). Wallingford: CABI Publishing, Reading.

Kundu M, Dubey A, Srivastav M, Mallik S and Singh B. 2014. Effect of gamma ray irradiation and cryopreservation on pollen stainability, *in vitro* germination, and fruit set in *Citrus*. *Turkish Journal of Biology* **38**: 1–9.

Kurtar E S. 2009. Influence of gamma irradiation on pollen viability, germination ability, and fruit and seed-set of pumpkin and winter squash. *African Journal of Biotechnology* **8**: 6 918–26.

Nepi M and Pacini E. 1993. Pollination, pollen viability and pistil receptivity in *Cucurbita pepo* L. *Annals of Botany* **72**: 527–36.

Pacini E. 1996. Types and meaning of pollen carbohydrate reserves. *Sexual Plant Reproduction* **22**: 362–6.

Pacini E, Guarnieri M and Nepi M. 2006. Pollen carbohydrates and water content during development, presentation and dispersal: a short review. *Protoplasma* **228**: 73–7.

Swaminathan M S and Murty B R. 1959. Effect of X-irradiation of pollen tube growth and seed setting in crosses between *Nicotiana tabacum* and *N. rustica*. *Molecular and General Genetics* **90**: 393–9.

Yahata M, Yasuda K, Nagasawa K, Harusaki S, Komatsu H and Kunitake H. 2010. Production of haploid plant of 'Banpeiyu' pummelo [*Citrus maxima* (Burm.) Merr.] by pollination with soft X-ray-irradiated pollen. *Journal of Japanese Society of Horticultural Science* **79**: 239–45.

Zhang Y X and Lespinasse Y. 1991. Pollination with gamma irradiated pollen and development of fruits, seeds and partheno-genetic plants in apple. *Turkish Journal of Botany* **54**: 101–9.