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Short Communication

Studies on mutagenic sensitivity of seeds of pummelo (*Citrus maxima* Merr.)

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ABSTRACT

Mutation breeding is a key method of generating large number of heritable variations. Effective dose (LD^{50}) needs to be standardized for inducing sufficient variation in a crop. In the present study, seeds were irradiated with different doses of Gamma rays and found that 66.94 Gy could suppress germination close to 50 per cent (LD^{50}) in pummelo. This 60 Gy gamma dose can effectively be used for raising the mutant populations to identify a desirable mutation in pummelo.

Keywords : Gamma Irradiation, Germination, Gray (Gy), LD^{50}, Mutation and Pummelo

Induced mutation plays a significant role in the crop improvement of horticultural crops. It is an important tool for induction of variation in quantitative and qualitative characters. It can be a supplement to conventional breeding methods when it is desired to improve one or two characters in a well-adapted variety. Induced mutation improving crops cultivars, enhancing biodiversity and Gamma irradiations are safe for human and environment and can be used widely to develop new varieties in fruit crops. In the recent past, mutation using gamma rays is regularly attempted in the banana breeding program (Smith *et al.,* 2006 and Mishra *et al.,* 2007). Among the different strategies to enhance crop improvement programs, induced mutagenesis has contributed immensely by creating mutant varieties with improved and desirable genetic changes in agronomically important traits in crops. Much progress has been made in generating superior genotype with favourable attributes through induced mutations in fruit crops. Pummelo is the largest citrus fruit and known in the western world mainly as the principal ancestor of the grapefruit. Pummelo fruit, like all fruits of the citrus family has several health benefits because of its super – rich Vitamin C and Vitamin B content. It also contains Vitamin A, Vitamin B1, B2 and C, bioflavonoid, healthy fats, protein, fibre, antioxidants and enzymes. It bears medium sized fruits (1-1.5kg) with good TSS (8-10 ^{0}B) value. The pulp texture of pummelo fruit is fleshy and pulp colour is pink. Excessive bitter taste in citrus juice is a major problem in citrus industry worldwide because it reduces the quality and commercial value of the product (Mongkolkul *et al.,* 2006). The bitterness in citrus fruit is affected by limonin and naringin, which are generally recognized as the major two bitter compounds. Limonin is the bitter limonoid found in major citrus cultivars such as grapefruit, the Navel orange, and the Shamouti orange (Guadagni *et al.,* 1973). Naringin is not transported after being synthesized in the fruit or leaves. However, till date, there is no information about availability of sweet pummelo varieties or bitter free pummelo varieties across the globe. Hence, the present investigation is aimed to determine the optimal dosage of gamma irradiation (LD50) for mutation induction in seeds of pummelo genotype "Kallar Selection" (Deep pink pulp with high bitterness) for developing a mutant with desirable horticultural traits.

The present investigation was carried out at ICAR-Indian Institute Horticultural Research, Bengaluru-560089. The seeds of Kallar Selection (Accession-6) pummelo were irradiated with different doses of gamma rays. The fresh and physically pure quality seeds of pummelo fruit were used for the irradiation purpose. In first step of experiment, a preliminary study was conducted to know the sensitivity of pummelo dry seeds to gamma radiation in which, pummelo seeds irradiated with gamma rays at the doses of 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy. In second step of the experiment, based on preliminary results the pummelo fresh seeds were irradiated with gamma doses of 25Gy, 50Gy, 60Gy, 75Gy and 100Gy to determine the exact LD50. The irradiated seeds along with non-irradiated seeds (control) were sown in protrays filled with cocopeat and each protray was marked with given gamma dose. Thereafter, water was sprinkled over the protray to provide enough moisture for seed germination and it was kept in controlled condition. Different parameters (seed germination per cent, number of seed germinated and number of seed deformities during germination) related to determining LD50 was recorded in 60 days after sowing. The data were subjected to Chi-Square analysis and Chi-Square table was used for the calculation error degrees of freedom.

For any induced mutagenesis programme, it is necessary to fix the LD50 value based on which larger population can be raised to isolate the desirable mutant progeny/progenies. The LD50 value varies according to crop species, varieties, seeds or other planting materials, nature of treatment, method of raising, climate, cultural practices and other parameters (Singh, 1994). In Citrus, several attempts to induce variability with some traits of seedless, thorn less, color changed fruits and juices (Maluszynski *et al.*, 2000). The radio-sensitivity (LD50) of acute citrus exposure ranges from 40 to 100 Gy (Sanada and Amano, 1998; Sparrow *et al.*, 1968), depending on the species and variety. The bud wood of pummelo Nambangan was irradiated with the dosage of 20, 40, and 60 Gy. The buds then grafted to Japanish Citron rootstocks. After three years of selection based on performance and fruit evaluation on MV2 generation, a mutant plant derived from 20 Gy irradiation treatments were obtained with improved character on the number of seeds. Pummelo Nambangan has more than 40 seeds/fruit and the mutant has less than 10 seeds/fruit on average and it shows higher volume of juice compared to that in pummelo Nambangan. However, the fruit appearance between mutant plant and parent in term of the fruit shape had no difference, showing a combination of spheroid and pyriform shape. The difference between them was shown prominently when the fruit was cut, indicating the less seed contained and more intense red color of flesh of the mutant fruit compared to that of parent (Mariana *et al.*, 2018.) In the initial study, pummelo seeds were treated with 100, 200, 300, 400 and 500 Gy). The effect of different doses of gamma rays ranging from 0 to 100 Gy on seed germination are shown in Table 1 and shown in Fig.1. The percentage of seed germination ranged from 18.31 per cent to 95.07percent with different doses of gamma rays in pummelo (Acc-06) as compared to 97.96 per cent in control.

Table 1: Effect of gamma irradiation in pummelo seed germination

Treatment	No of seeds sown	No of seeds germinated	No of under developed seedlings	Days to germination	Germination percentage
Control	98	96	0	10	97.96
Dosage 25 Gy	98	93	0	11	94.90
Dosage 50 Gy	98	88	0	11	89.90
Dosage 60 Gy	98	46	21	14	46.94
Dosage 75 Gy	98	24	16	20	24.49
Dosage 100 Gy	98	18	14	20	18.37

Gradual reduction/decrease in pummelo seed germination was observed with increase in gamma irradiation dose. The inhibitory effect on seed germination was directly proportional to the dose of gamma radiation. Similar results were reported by Dhatt *et al*. (2000) and Latado *et al*. (2001) with gamma radiation in citrus. Decrease in percent seed germination by gamma irradiation might be due to its effect on genetical and cytological processes coupled with the changes induced in metabolic processes. The decrease in seed germination was mainly due to the interference of mutagens with metabolic activities of the seeds (Sjodin, 1962). Sinha and Godward (1972) opined that the reduction in percentage of seed germination was due to the disturbances caused at the physiological level coupled with chromosomal damage. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatments (Kulkarni,2011). Gamma radiation is well known for their action causing point mutations, enzyme inhibitions and chromosomal aberrations. The observed reduction in seed germination in pummelo as a result of gamma radiation might be due to point mutations or the injuries caused to the genetic material. This may eventually lead to decrease the rate of respiration and energy production, which finally caused decrease in seed germination. Days taken to germination and number of seeds with deformity were increased with increasing dose of gamma radiation. Based on probit analysis, 66.94Gy dose was found to be effective as LD50 for irradiation of pummelo seeds (Table 2) to induce sufficient variation. This finding may assist as reference dose for large scale gamma irradiation of pummelo genotypes to induce genetic variation. However, considering the practical difficulty, its better fix 60 Gy as LD50 to induce sufficient mutation to select the desirable one. This result was in line with other studies on the effect of gamma rays in citrus. On lemon, Gulsen *et al*. (2007) obtained most seedless fruit from 50 Gy treatment while Spiegel-Roy *et al*. (2007) successfully obtained seedless fruit with 13.3 Gy. On mandarin, Kafa *et al*. (2015) obtained most mutant plants with seedless fruit with 30 Gy while Montanola *et al*. (2015) obtained seedless fruit with 40-50 Gy.

Table 2: Lethal dose calculation

Pummelo	n^a	LD50 LCL-UCL (95% confidence limit)	LD90 LCL-UCL (95% confidence limit)	χ^2	df
Gamma irradiation	98	66.94 (60.06-74.61)	164.27 (127.03-212.42)	9.4	4

LD50= lethal dose that kill 50% of the population; n^a= population number; LCL=lower confidence limit; UCL= upper confidence limit

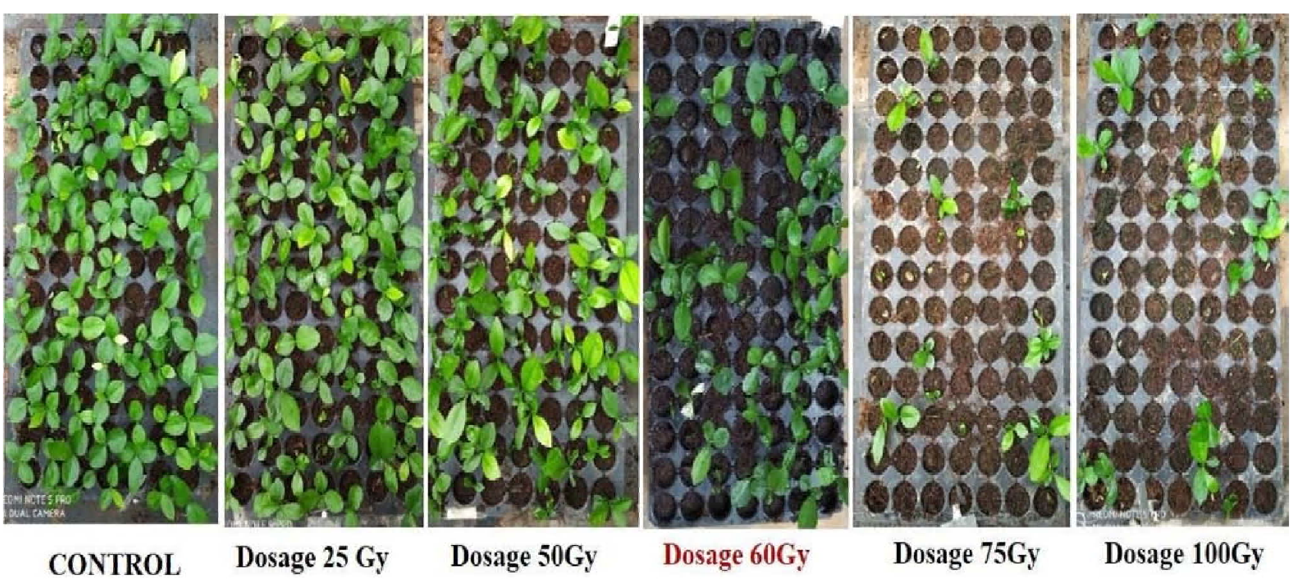

Fig.1: Mutagenic sensitivity of pummelo seedlings to different doses of gamma radiation

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