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Original Research Paper

Determination of mutagenic sensitivity and its manifestations on papaya (*Carica papaya* L.) cv. Arka Prabhath

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ABSTRACT

Papaya is an important fruit crop of the family Caricaceae which needs the improvement in terms of virus resistance and shelf life with dwarf stature. Mutation breeding technique has been considered as an efficient tool adopted by plant breeders to create variability in papaya. The mutation frequency and population structure of the mutants directly depend upon the type of mutagen and the time of exposure. Irrespective of the used mutagens, the ultimate induced mutations are random and therefore determination of mutagenic sensitivity is important pre-requisite. Based on this, investigation on the induction of mutation in papaya cv. Arka Prabhath was carried out with the objective of creating genetic variability through physical mutagen. In this study, papaya seeds were irradiated with five different dose of gamma rays ranging from 50 Gy to 500 Gy. The results revealed that gradual reduction in germination, survival of seedlings and delayed germination with increase in dosage of gamma rays. Based on probit analysis, LD_{50} (Lethal dose) was fixed at 186.24 Gy. Total seven types of chlorophyll mutants were observed as a result of mutation. Mutagenic efficiency and effectiveness were higher in a lower dose of gamma treatment (50 Gy).

Keywords: Arka Prabhath, gamma rays, lethal dose, mutation, papaya

INTRODUCTION

Papaya (*Carica papaya* L.) is an economically important fruit crop of the family Caricaceae. It is having commercial importance because of its high nutritive value and productivity, varied medicinal and industrial uses, round the year availability, short duration nature and its suitability for the preparation of several value-added products. It is basically a tropical fruit crop and believed to have originated in the lowlands of eastern Central America, from Mexico to Panama (Nakasone and Paul, 1998). It has been successfully cultivated throughout the world and in India. Though, there is an increase in area under cultivation of papaya in the recent past, the corresponding increase in production has not been realized in India (Sharma and Tripathi, 2019). This might be due to the losses caused by diseases incited by fungi, bacteria, phytoplasma, viruses, various pests and abiotic factors such as variations in temperature, humidity, asymmetric rainfall and soil moisture stress (Rahman and Akanda, 2008). One of the main drawbacks for papaya cultivation has been the occurrence of papaya ring spot virus (PRSV). Development of virus resistant/ tolerant cultivars through conventional breeding is the only reliable tool for long-term control of this disease (Sharma *et al.*, 2017). Introgression of resistance genes from wild species into commercial cultivar background so far been unsuccessful. Among the breeding techniques, mutation is one of the important methods adopted by plant breeders to create variability in a number of species. In fruit crops, mutation breeding is advantageous over conventional breeding because it precludes segregation progenies while improving the genetic make-up during selection cycles.

The prime approach in mutation breeding has been to upgrade the well-adapted plant varieties by altering major agronomic traits, productivity and quality. The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half of the planting material survive) doses before massive irradiation of similar materials are accepted.

The selection of effective and efficient mutagen is of paramount importance in any mutagenic experiments to obtain promising stable mutants (Kumar *et al.*, 2021). Different type of mutagens can be employed to induce mutagenesis in fruit crops. Physical mutagens have been extensively used for the development of new cultivars with improved characteristics. The frequency and saturation of mutations can be regulated by varying the mutagen dose (Menda *et al.,* 2004) and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions (Kim *et al.,* 2006). Among various radiation sources, gamma rays are very important in creating genetic variability through mutagenesis (Hong *et al*., 2022). The usefulness of mutagen depends on the spectrum of chlorophyll mutations, lethality, sterility, mutagenic efficiency and effectiveness of the mutagen. Furthermore, reports on mutagenic efficiency in fruit crops are very few and no such studies have so far been done on papaya. Various studies suggest that more desirable mutations occur at the dose, which causes the death of 50% of organisms or 50% of growth reduction (Alvarez-Holguin *et al*., 2019). Therefore, optimization of LD_{50} (lethal dose 50) is very important for any mutation breeding experiment. LD_{50} is the dosage of mutagen which causes 50 % lethality in the organism and changes with the species, the nature of the plant material and the stage of the crop. Mutagenic effectiveness measures the mutations induced per unit dose of mutagen. Gustafsson (1951) defined that identifying chlorophyll mutants are the most convenient method for evaluating the genetic effect of a mutation in plants. Papaya is an ideal and attractive crop for mutation studies as it is genomically the simplest fruit crop. Simple diploid genetics, small genome size (0.9 pg per haploid genome [Arumuganathan and Earle, 1991]), well studied genetics and a developing physical map, render papaya a better candidate for this study. Based on this, the present study was framed to determine the mutagenic effectiveness, efficiency and optimum lethal dose (LD_{50}) for gamma radiation in papaya cv. Arka Prabhath.

MATERIALS AND METHODS

This study was undertaken at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru during 2020-2021. An attempt was made to induce genetic variability in papaya cv. Arka Prabhath, an advanced generation hybrid derived from the cross of (Arka Surya x Tainung-1) x Local Dwarf released from ICAR- Indian Institute of Horticultural Research. It is gynodioecious in nature, with large sized fruits of 900-1200 g and smooth skin. The pulp is an attractive deep pink colour with good keeping quality and high TSS (13-14^{0}B). The seeds extracted from fully ripe fruits which were obtained by controlled self-pollination were used for the experiment. The seeds (80 seeds/ treatment) were irradiated with gamma rays in gamma chamber located at Indian Institute of Horticultural Research (IIHR), Bengaluru. This gamma chamber installed with the support of BRIT (Board of Radiation and Isotope Technology) and AERB with Co-60 source capacity of 518 Terabecquerel at the dosage rate of 9 KGY/hour or 0.9 Mega Rad/hour. The exposure time in the gamma chamber was 28, 57, 86, 143, 286 seconds for five different doses of gamma rays *viz*., 50 Gy (T_2), 100 Gy (T_3), 150 Gy (T_4), 250 Gy (T_5) and 500 Gy (T_6) respectively. The gamma treated seeds were soaked in GA_3 @ 100 ppm solution for 12 hours and sown in polyethylene bags containing Red soil: FYM: Sand in the ratio of 1:1:1 within 24 hours to retain the vigour of the gamma treated seeds. The gamma treated and gamma untreated (control-T_1) seeds were maintained separately in mist chamber for germination. The germination percentage, survival percentage and days taken for germination were recorded. The LD_{50} value was calculated based on probit analysis.

Probit analysis: LD_{50} values of gamma radiation was determined based on Finney's method (Finney, 1978). Probit analysis was carried out in MS excel by following procedure with some modification in log-doses (mentioned in the procedure). The dose concentration of mutagen was transformed into log10^3 value. The mortality percentage of seeds due to treatment doses were worked out and rounded to the nearest whole number. The corrected mortality percentage was calculated using Abbott's formula given below.

Corrected mortality (%) =
[M observed - M control x 100] /
100 – M control

All the corrected values are rounded to the nearest whole number. The corrected values were converted to the probit transformation. Probit values (Y-axis) were graphed against Log concentration (X-axis) and a straight line passing through most of the plotted points is drawn; then this line was used to estimate the Log10 concentration associated with a probit of 5. Antilog to the $Log10^3$ value corresponding to the probit 5 was taken and the arrived value was divided by 10^{-3} (1000), thus LD_{50} for the particular mutagen under study was determined.

Analysis of variance: data obtained for nursery parameters were subjected to analysis of variance (ANOVA) at the significant level of 5% using Addinsoft., 2021. When statistical differences were found, the least significant difference (LSD) was used to compare means at the 5% significance level.

Further, M_1 progenies were screened for a spectrum of chlorophyll mutations. The chlorophyll mutants were classified as the scheme of Gustafson (1940) and Blixt (1972). Types of mutants found in this study explained and categorized as follows: Albina mutants were completely devoid of chlorophyll. Xantha consists of pale-yellow colored leaves due to disruption in chlorophyll. The viridis were represented by light green color in the nursery stage. This color gradually changed to the normal green color during the subsequent period of growth and found viable in nature. The chlorina mutants were yellowish green in color Xantha-viridis mutants were characterized by both viridine green color and bright yellow color occurring in the same leaf.

Mutagenic effectiveness, mutagenic efficiency and mutation rate were calculated based on the formulae proposed by Konzak *et al.* (1965) by incorporating the mutation frequency values recorded for each mutagenic treatment.

Mutagenic effectiveness = Mutagenic frequency / Dose/Concentration of the mutagen

Mutagenic efficiency =
Mutagenic frequency / Biological damage

Mutation rate = Sum of values of efficiency or effectiveness of particular mutagen / Number of treatments of a particular mutagen

Biological damage refers to the lethality or reduction percentage over control (survival) and germination percentage reduction over control in this study. Biological damage is contributed by germination and survival of seedlings which has primary role in the establishment of crop.

RESULTS AND DISCUSSION

Gamma rays are the most widely used physical mutagen employed in mutation breeding of crop plants and are well known for bringing about morphogenetic and endomorphic changes in plants (Yasmeen *et al.*, 2020). The results indicated that there was a gradual reduction in germination percent with increase in dosage of gamma irradiation with highest germination percentage being in control (92.50%). Among the treatments, highest germination percent was recorded in 50 Gy (82.19%) and higher doses above 250 Gy were lethal in papaya. Further, although lower irradiation doses did not have a marked effect, higher doses (250 Gy) delayed seed germination considerably with maximum number of days taken for germination being 23 days (T_5) and minimum being 9 days in control. Reduction in survival percentage with increased dosage with higher survival percentage was recorded in control (90 %), among the treatments, higher survival per cent was recorded in 50 Gy (80 %) (Fig. 1 and Table 1 & 2). Similar results were elicited in papaya by Aiswarya Ravi *et al.* (2022), Pujar *et al.* (2019) and Ramesh *et al.* (2019), Sahu *et al.* (2019), in mango by Parveen *et al.* (2023), pummelo (Sankaran *et al.*, 2021), Rough lemon (Kaur and Rattanpal, 2010), Saini and Gill (2009) and Sharma *et al.* (2013) in Rough lemon. This might be

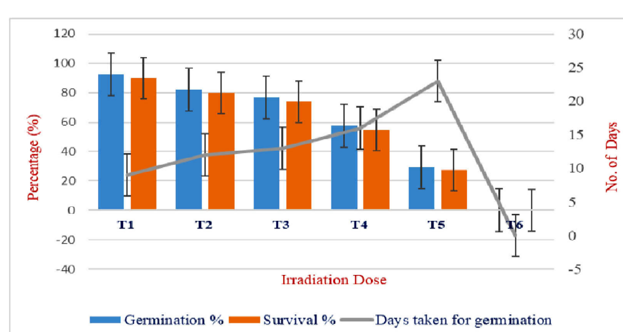


Fig. 1 : Effect of gamma irradiation on germination, survival and days taken for germination in M_1 population of papaya

Table 1 : Effect of gamma irradiation on germination percentage in papaya

Dose (Gy)	Germination count (out of 80)	Germination %	% over control	% of reduction over control
T_1 (Control)	74.00	92.50	100.00	0.00
T_2 (50Gy)	65.75	82.19	88.85	11.15
T_3 (100 Gy)	61.25	76.56	82.77	17.23
T_4 (150 Gy)	46.00	57.50	62.16	37.84
T_5 (250 Gy)	23.50	29.38	31.76	68.24
T_6 (500 Gy)	0.00	0.00	0.00	100.00

Table 2 : Effect of gamma irradiation on survival of seedlings percentage in papaya

Dose (Gy)	Survival Nos. (Out of 80)	Survival %	% over control	% reduction over control
T_1 (Control)	72.00	90.00	100.00	0.00
T_2 (50Gy)	64.00	80.00	88.89	11.11
T_3 (100 Gy)	59.00	73.75	81.94	18.06
T_4 (150 Gy)	44.00	55.00	61.11	38.89
T_5 (250 Gy)	22.00	27.50	30.56	69.44
T_6 (500 Gy)	0.00	0.00	0.00	100.00

due to the altered enzyme activity (Zou *et al.*, 1999), metabolic disturbances (Ananthaswamy *et al.*, 1971), inactivity of plant hormones (Sideris *et al.*, 1971) and chromosomal aberrations (Nurmansyah *et al.*, 2018).

In the present study, LD_{50} values were determined with the help of probit analysis based on their survival rate of the seed after treatment with different doses of gamma rays compared with untreated control. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. The probit curve analysis shown that the LD_{50} value for gamma rays was 186.24 Gy (Table 3 & Fig. 2). The minor difference was observed in LD_{50} doses which ranged from 300 to 350 Gy in three different varieties of papaya by Aiswarya Ravi *et al.* (2022). Lethal dose differs with biological materials, nature of treatment and subsequent environmental conditions. Several studies of mutation by gamma ray exposure in different papaya cultivars were reported. Hang and Chau (2008) exposed the seeds of the papaya variety Dai Loan Tim to gamma rays ranging from

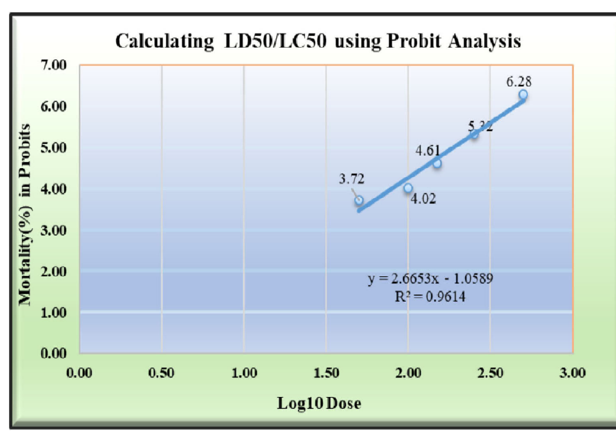


Fig. 2 : Plot of log-doses versus Probits for calculations of LD_{50} for gamma rays for papaya

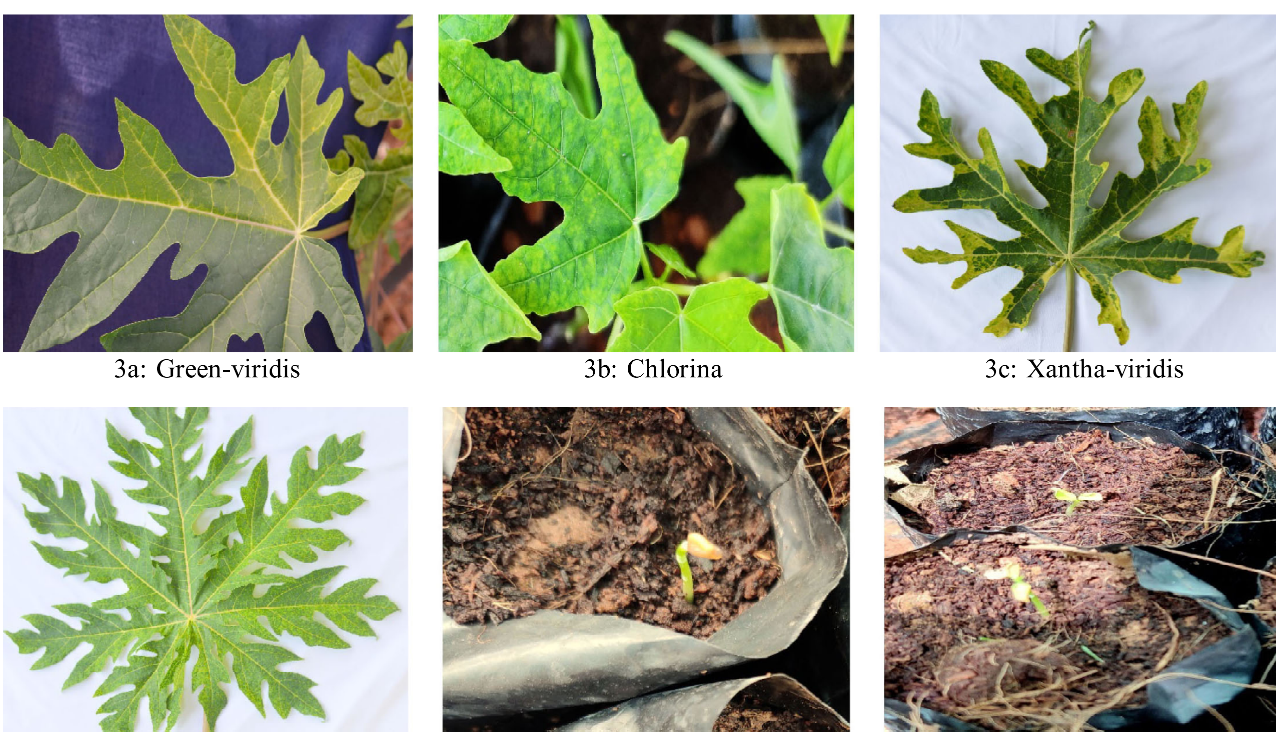
Table 3 : Probit analysis for cv. Arka Prabhath irradiated with gamma radiation

Dose (Gy)	Log value of dose	Observed mortality	Corrected mortality %	Empirical value of probit unit	LD_{50} Value
T_1 (Control)	0.00	10.00	0.00	0.00	
T_2 (50Gy)	1.70	20.00	11.11	3.72	
T_3 (100 Gy)	2.00	26.25	18.06	4.02	186.24 Gy
T_4 (150 Gy)	2.18	45.00	38.89	4.61	
T_5 (250 Gy)	2.40	72.50	69.44	5.32	
T_6 (500 Gy)	2.70	100.00	100.00	6.28	

10 to 60 Gy. They indicated that LD_{50} of gamma rays in germinated papaya seeds was 30 Gy. Husselman *et al.* (2014) found that increased doses of gamma rays of 100 and 120 Gy were lethal to papaya when treated with dosages ranging from 0 to 120 Gy. Sahu *et al.* (2019) reported that LD_{50} value was 28.35 Gy and 33.13 Gy for pre-soaked seeds & 24.05 Gy and 23.78 Gy for seeds immersed in water for Ranchi local and Arka Surya varieties of papaya respectively. The lethal dose of gamma rays for different fruit crops was reported by Surakshitha *et al*. (2017) in grapes and Murti *et al.* (2013) in strawberries.

Chlorophyll mutants are not desirable in crop improvement programs but serve as an important parameter to determine the mutagenic efficiency (Eswaramoorthy *et al*., 2021). In the present study, a wide spectrum of chlorophyll mutants such as Xantha, Viridis, Chlorina, Xantha-viridis, Maculata and Green-viridis (Fig. 3). Most of the chlorophyll deficient mutants died after a few days of germination whereas some showed retarded growth because of the deficiency of chlorophyll. The highest mutation frequency of chlorophyll phenodeviants was found in 250 Gy (30.42) and least in 50 Gy (9.22) (Table 4). The results are in accordance with Aiswarya Ravi *et al.* (2022), who observed retarded growth and mortality in chlorophyll deficient plants with wide spectrum of chlorophyll mutants such as xantha, chlorina, striata, virescent viridis and albino. The similar results were also observed by Naveena *et al.* (2020) and Seemanthini *et al.* (2022), in hibiscus. The occurrence of chlorophyll mutants might be attributed to a variety of factors, including defective chlorophyll biosynthesis, chlorophyll degradation, and carotenoid deficiency (Goyal *et al*., 2019). Chlorophyll mutations are crucial for determining gene function as well as understanding chlorophyll metabolism and regulation in plants (Dwivedi *et al*., 2021). Hence chlorophyll mutations can be used as the most reliable marker for assessing the genetic impact of mutagenic treatments in different crops.

Mutagenic effectiveness indicates the frequency of mutations induced by a unit dose of mutagen and mutagenic efficiency is a measure of the proportion of mutation in relation to lethality and sprouting percentage reduction. Biological damage is purely dose-dependent which increased with increased concentration/dose of gamma rays. In the present study, the mutagenic effectiveness found to be highest (18.44) at a lower dose (50 Gy) and lowest (12.17)



3a: Green-viridis 3b: Chlorina 3c: Xantha-viridis

3d: Maculata 3e: Xantha 3f: Viridis

Fig. 3 : Chlorophyll mutants obtained from gamma irradiated papaya seeds

Table 4 : Spectrum of chlorophyll mutants, Mutagenic effectiveness and efficiency in papaya

Treatment	Chlorophyll mutants						M_f	Biological damage		Mutation effective-ness	Mutation efficiency	
	Xantha	Chlorina	Viridis	Xantha-viridis	Maculata	Green-viridis		Survival reduction over control (%)	Germi-nation reduction over control (%)		Based on survival reduction over control	Based on germination reduction over control
T_2 (50Gy)	2	1	2	0	1	0	9.22	11.11	11.15	18.44	82.99	82.69
T_3 (100 Gy)	2	1	2	2	0	1	13.12	18.06	17.23	13.12	72.64	76.15
T_4 (150 Gy)	2	3	4	1	1	1	26.07	38.89	37.84	17.38	67.04	68.89
T_5 (250 Gy)	1	2	2	2	0	0	30.42	69.44	68.24	12.17	43.81	44.58
T_6 (500 Gy)	-	-	-	-	-	-	0.00	100.00	100.00	0.00	0.00	0.00

Table 5 : Mutation rate of gamma radiations in papaya

Treatments	Mutation rate in terms of effectiveness	Mutation rate in terms of efficiency	
		Lethality	Germination reduction
Nos. 5	12.22	53.30	54.46

at higher dose (250 Gy) of gamma rays. Mutagenic efficiency determined based on the lethality of embryos recorded highest value (82.69) at 50 Gy. Likewise, mutagenic efficiency calculated based on survival reduction (Biological damage) also found in the same pattern in which mutagenic efficiency was maximum (82.99) in the lower dose of gamma radiation (Table 4). Similar results of mutation efficiency with the lower dose of gamma rays were also reported by Aiswarya Ravi *et al*. (2022) in papaya, Naveena *et al*. (2020) in hibiscus, Seemanthini (2022) in hibiscus and Padmadevi (2009) in chrysanthemum.

The overall mutation rate in terms of effectiveness and efficiency was outstanding in gamma radiation treatment in papaya cv. Arka Prabhath. The maximum mutation rate in terms of effectiveness and efficiency reflects the usefulness of mutagen (Table 5). For obtaining high efficiency, the mutagenic effect should overcome other effects in the cells such as chromosomal aberrations and toxic effects. High mutation rate accompanied by minimal deleterious effects is desirable for a successful mutation programme. But generally, the mutagen that gives the higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects (Blixt *et al*., 1964).

CONCLUSION

The results indicate that induced mutation through gamma irradiation was found to be effective and efficient, which can be employed in enhancing the variability in papaya. Chlorophyll deficient mutants were observed among the irradiated population. All biological parameters showed a steady increase with increasing gamma irradiation dosages. The mutagenic efficiency and effectiveness of gamma irradiation were found highest at lower dose (T_2-50 Gy) and was decreasing with an increase in doses (T_3-100 Gy to T_6-500 Gy). Based on the current findings, the LD_{50} value (186.24 Gy) of gamma rays was optimized for papaya cv. Arka Prabhath. This information will be useful for further mutagenesis experiments for developing mutants with desirable characteristics in papaya since determination of mutagenic sensitivity is the pre-requisite for any mutation breeding programme.

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