

EFFECT OF PHYSICAL AND CHEMICAL MUTAGENS ON MORPHOLOGICAL BEHAVIOUR OF PAPAYA CV. 'ARKA PRABHAT'

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ABSTRACT

The present investigation on “Effect of physical and chemical mutagens on the morphological behaviour of the papaya variety 'Arka Prabhat' was carried out at the CCS Haryana Agricultural University in Hisar during the 2020–21 period. The experiment was Completely Randomized Design (CRD) with twelve treatments and three replications. In papaya, plant height represents a significant problem at the time of harvest since tall plants are very susceptible to wind damage; nevertheless, no dwarf gynodioecious cultivar is currently available. In order to obtain variability for desirable morphological traits in the 'Arka Prabhat' variety, a mutation breeding programme was started using both physical (gamma rays) and chemical (ethyl methanesulfonate, EMS) mutagens. The treatments included T₁ at 100 Gy, T₂ at 200 Gy, T₃ at 300 Gy, T₄ at 400 Gy, T₅ at 500 Gy and T₆ at 600 Gy for gamma radiation, and T₇ at 0.2%, T₈ at 0.4%, T₉ at 0.6%, T₁₀ at 0.8% and T₁₁ at 1.0% for EMS, with T₁₂ serving as the untreated control. The seeds of 'Arka Prabhat' were obtained from freshly ripe fruit after controlled pollination and were used for the mutagenic treatments. The LD₅₀ was determined to be at T₄ (400 Gy) for gamma rays and at T₉ (0.60% EMS) for the chemical mutagen. The progeny from treatment T₁₂ (control) showed significantly better values for germination percentage, the number of days taken for germination, survival percentage, the number of leaves per seedling, shoot length, root length and seedling height when compared to the progeny obtained from the mutagen treated plants. The seedlings derived from treatment T₁ (100 Gy) had the highest total leaf chlorophyll content, whereas those from treatment T₄ (400 Gy) irradiated plants had the largest trunk circumference and canopy spread. The progeny from treatment T₈ (0.4% EMS) had the lowest plant height and dwarf plants were specifically noted at this treatment. No significant differences were found among the treatments with respect to the non-numerical morphological characteristics- the type of branching, the number of branches and leaf type and no morphological abnormalities were observed in any of the treated progenies.

KEYWORDS: Papaya; Gamma rays; Ethyl methanesulfonate; Morphological traits; LD₅₀

INTRODUCTION

Papaya (*Carica papaya* L.) is a member of the Caricaceae family, which includes 44 species, and is one of the most economically important fruit crops in the tropical and subtropical areas of the world. It originated in the area stretching from Mexico to Panama (Nakasone and Paull, 1998) and was brought to India in the early 16th century. The plant is diploid (2n = 18), has a small genome size of 372 Mbp/1C (Arumuganathan and Earle, 1991), and possesses nine pairs of chromosomes (Bennett and Leitch, 2005). India is now the largest producer of papaya in the world, with Brazil and Mexico coming next, and in the 2019–20 period the total area under cultivation and the total production amounted to 0.142 million hectares and 6.01 million metric tons, respectively (Anonymous, 2021). The tree is monopodial and has a single, orthotropic, non-branching stem, which enables it to be grown as a short-duration intercrop in the orchards of longer-duration fruit crops (Chattopadhyay, 2012); frost, waterlogging and strong winds are still the main obstacles to achieving a good crop yield (Dinesh and Reddy, 2002).

Because of the growing economic significance of papaya, there is still a need to develop high-yielding, dwarf varieties with good fruit quality that are suitable for a wide range of growing conditions, as the current genetic resources may not be enough to meet the food requirements of a human population expected to amount to nine billion by 2050 (Green et al., 2005), and since traditional breeding methods on their own have not completely succeeded in overcoming production bottlenecks through genetic improvement (Beyene et al., 2013). Induced mutation has been used as an additional breeding method, particularly appropriate for improving one or two traits that are easy to identify - for example, plant height in a cultivar that is already well accepted. The use of X-rays to induce genetic changes was first shown by Lewis John Stadler in the late 1920s and early 1930s, and this established induced mutation breeding as a separate plant breeding technique (Shu et al., 2012). In the case of papaya, this method has already resulted in a commercially available dwarf variety -Pusa Nanha, an dioecious, ultra-dwarf selection (with a fruit-bearing height of 60 cm and a total plant height of 106 cm) which was developed by ICAR-IARI, New Delhi, using gamma-ray mutation breeding (Mansharam, 1984) proving that it is possible to reduce plant height in this species.

Gamma irradiation and EMS are generally considered to be effective methods for producing a high frequency of mutations in crop plants. The degree of radiosensitivity and the optimal mutagen dose depend on the species, genotype, plant part and developmental stage, as well as on the nuclear volume, the number of chromosomes and the ploidy level (Sparrow et al., 1967), and it is therefore necessary to establish standards for each new combination of crop and mutagen by determining the median lethal dose (LD₅₀), this being most frequently evaluated by examining the response of seed germination and seedling survival. In the case of papaya, LD₅₀ values for gamma rays have been reported to range from about 650 Gy in the cv. Eksotika/Sekaki (Chan, 2009) down to 1.5 krd in the cv. Coorg Honey Dew (Mahadevamma et al., 2015), showing a considerable variation in radiosensitivity at the cultivar level, while LD₅₀ values for EMS of about 0.3–0.5% have been given for both cv. Arka Surya and cv. Arka Prabhat (Santosh et al., 2008; Veena et al., 2014; Bharathi, 2011). Because of this sensitivity which is specific to each cultivar and in view of the limited amount of mutation-breeding work that has so far been done on papaya, the present study was carried out on the papaya cv. 'Arka Prabhat', a gynodioecious, semi-vigorous hybrid derived from (Arka Surya × Tainung-1) × Local Dwarf and released by ICAR-Indian Institute of Horticultural Research, Bengaluru, with the seeds being exposed to gamma rays and EMS in order to determine the LD₅₀ for this cultivar and to assess the morphological characteristics of the resulting mutant progenies, the particular aim being to investigate the effect of physical and chemical mutagens on the morphological traits of papaya seedlings.

MATERIALS AND METHODS

Experimental site

The field aspect of the experiment was conducted at the Precision Farming Development Centre, Department of Horticulture, and the laboratory work was carried out at the Department of Horticulture Laboratory, CCS Haryana Agricultural University, Hisar, in the period 2020–21. Hisar is located at an altitude of 215 metres above sea level and has a latitude of 29°09'N and a longitude of 75°42'E, in western Haryana on the alluvial Ghaggar–Yamuna plain. The area has a continental climate characterised by very hot summers (with daily maximum temperatures ranging from 40 to 46°C), relatively cool winters (between 1.5 and 4°C), scarce and irregular rainfall (the annual average being about 429 mm, most of which occurs in July–August), and large variations in both daily and seasonal temperatures.

Plant material

Seeds of papaya cv. 'Arka Prabhat' - a gynodioecious, advanced-generation hybrid derivative of the cross (Arka Surya × Tainung-1) × Local Dwarf, released by ICAR-Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru were used for the experiment. Seeds were extracted from freshly ripe fruit obtained through controlled pollination on plants maintained for this purpose.

Mutagenic treatments

Variability was induced using both a physical mutagen (gamma rays) and a chemical mutagen (EMS). The experiment was arranged according to a Completely Randomized Design (CRD) with twelve treatments and three replications; the gamma-ray doses were 100, 200, 300, 400, 500 and 600 Gy for treatments T₁ through T₆ respectively, EMS concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0% were used for treatments T₇ through T₁₁ respectively, and treatment T₁₂ was the untreated control.

The dry seeds used in the gamma-ray treatment were irradiated in a Gamma Chamber-5000 (which has a Co⁶⁰ source and emits gamma radiation of 1.33 and 1.17 MeV per disintegration; the maximum source capacity is 518 TBq; the dose rate is approximately 9 kGy/hr at the centre of the chamber) at the Punjab Agricultural University in Ludhiana, the exposure times varying from about 42 minutes (100 Gy) to 251 minutes (600 Gy). In the case of the EMS treatment, the seeds were first soaked in sterile water over night and then exposed to the appropriate EMS concentration (ranging from 0.2 to 1.0%, this being obtained by diluting pure EMS with distilled water in volumetric flasks) for 4 hours; 30 seeds were treated in each replication. The EMS was obtained from Sisco Research Laboratories Pvt. Ltd. in Mumbai. After treatment, the seeds were removed, thoroughly washed in distilled water and then sown.

Raising of seedlings and field transplanting

The treated seeds were sown in polybags (3" × 4") containing a mixture of soil, sand and perlite in equal amounts and were then kept in a polyhouse until germination started about 13 days after sowing. When the plants had grown for 60 days after sowing, they were transferred to the main field under protected conditions, with a spacing of 1.8 × 1.8 m, into soil that had been ploughed and had received farmyard manure 15 days before the transplanting. During the entire experiment the recommended package of practices was adhered to with regard to irrigation, nutrition and plant protection; superior plants, which had been chosen according to their morphological characteristics, were then sib-mated in order to proceed to the next generations.

Observations recorded

The growth parameters were as follows: the germination percentage was noted 45 days after sowing, the number of days taken for germination being calculated from the date of sowing to the date on which germination was first observed, and the survival percentage was recorded 15 days after the seedlings had been transplanted to the main field. At 15 and 30 days after germination (DAG), the shoot length, root length and seedling height (which is the total of shoot and root length) were measured using a centimetre scale, and the number of leaves per seedling was assessed by visual inspection at 30 DAG.

It is a physiological parameter and the total chlorophyll content in the leaves was measured 90 days after germination with a SPAD (Soil Plant Analysis Development)-502 chlorophyll meter, a non-destructive technique since the value obtained is proportional to the chlorophyll content in the leaves.

The morphological parameters included the type of branching (single, double, or triple), the number of branches and the type of leaf (narrow or papaya-type as opposed to broad or castor-type), all of which were noted by visual inspection. The leaf area was determined using a leaf area meter on two or three leaves selected at random from each plant, at 90 days after germination, and then averaged. The internodal distance was measured between the 3rd and 4th, 4th and 5th, and 5th and 6th nodes from the tip of the shoot and the values were averaged, the result being given in centimetres. The trunk circumference was recorded 30 cm above the base of the plant by measuring the trunk diameter (d) with a vernier calliper and then calculating the circumference as 3.14 times d. Canopy spread in the north-south and east-west directions was obtained by taking the distance between the opposite leaf tips with a measuring tape.

Statistical analysis

Data on the morphological and physiological characters recorded were subjected to Fisher's analysis of variance (ANOVA) for a Completely Randomized Design with twelve treatments and three replications, following Sundararaj *et al.* (1972). Percentage data were subjected to angular (arc-sine) transformation and count/length data to square-root transformation prior to analysis, as indicated in the corresponding tables. Where the F-test was significant, treatment means were compared using the critical difference (CD) at the 5% probability level, calculated as: $CD (5\%) = \sqrt{(2 \times \text{error mean square} / n)} \times t$ (at 5% error degrees of freedom), where n is the number of replications. All statistical analysis was carried out using OPSTAT statistical software.

RESULTS

Growth parameters

There were significant differences between the various treatments with regard to germination percentage, the number of days taken for germination, survival percentage, the number of leaves per seedling, shoot length, root length and seedling height (Tables 1 and 2).

The germination percentage varied significantly depending on the treatment, lying between 0 and 83.33 per cent. The highest rate of germination was found in the untreated control, T12, at 83.33 per cent, which was statistically similar to that of T1: 100 Gy (73.33 per cent), and then came T7: 0.2% EMS at 70 per cent. The lowest germination figures, 10 per cent, were observed in the cases of T5: 500 Gy and T10: 0.8% EMS, while no germination took place in T6: 600 Gy or in T11: 1.0% EMS. From this it was concluded that the LD₅₀ for gamma rays was T4 (400 Gy) and for the chemical mutagen was T9 (0.60% EMS), beyond which lethality in cv. 'Arka Prabhat' became severe.

The number of days required for germination varied greatly, going from 13.67 days with T12 (the control) to 43.33 days with T10 (0.8% EMS); the shortest germination period among the treated seeds was observed with T1 (100 Gy) at 24.33 days. The percentage of survival varied from 16.67% (T4: 400 Gy) to 66.67% (T12, control), with T1 (53.33%) and T7 (53.33%) falling in an intermediate position; T5, T6, T9, T10 and T11 all showed zero survival. The number of leaves per seedling ranged from 4.72 (T8: 0.4% EMS) to 6.75 (T12, control), the highest number being that of T2 (200 Gy) at 6.22.

The shoot length, root length and seedling height at both 15 and 30 days after germination showed a generally similar trend, the untreated control having the highest measurements at both times (3.47 and 6.77 cm for shoot length; 4.28 and 7.32 cm for root length; 7.75 and 14.09 cm for seedling height at 15 and 30 days after germination respectively) while the T8: 0.4% EMS treatment had the lowest values among the germinating treatments at both dates (2.28 and 3.85 cm for shoot length; 2.39 and 4.27 cm for root length; 4.67 and 8.12 cm for seedling height).

The germination and survival responses of papaya cv. 'Arka Prabhat' showed a clear dose-dependent decline following treatment with both gamma radiation and EMS (Figure 1). Germination percentage progressively decreased with increasing gamma-ray dose, declining from 73.33% at 100 Gy to complete inhibition at 600 Gy. Similarly, increasing EMS concentration resulted in a substantial reduction in germination, with complete inhibition observed at 1.0% EMS.

The untreated control recorded the highest germination percentage (83.33%) and survival percentage (66.67%). Among the mutagen-treated seeds, the highest germination was observed at 100 Gy (73.33%), followed by 0.2% EMS (70.00%). Higher doses of both mutagens resulted in progressively greater biological damage, as reflected by reduced germination and survival. Based on the dose-response pattern, 400 Gy gamma radiation and 0.60% EMS were identified as the approximate LD₅₀ levels for papaya cv. 'Arka Prabhat'.

The dose-response curves clearly demonstrate the differential sensitivity of papaya seeds to physical and chemical mutagens and indicate that higher doses caused severe inhibition of germination and seedling survival.

Dose-dependent response of papaya cv. 'Arka Prabhat' to physical and chemical mutagens

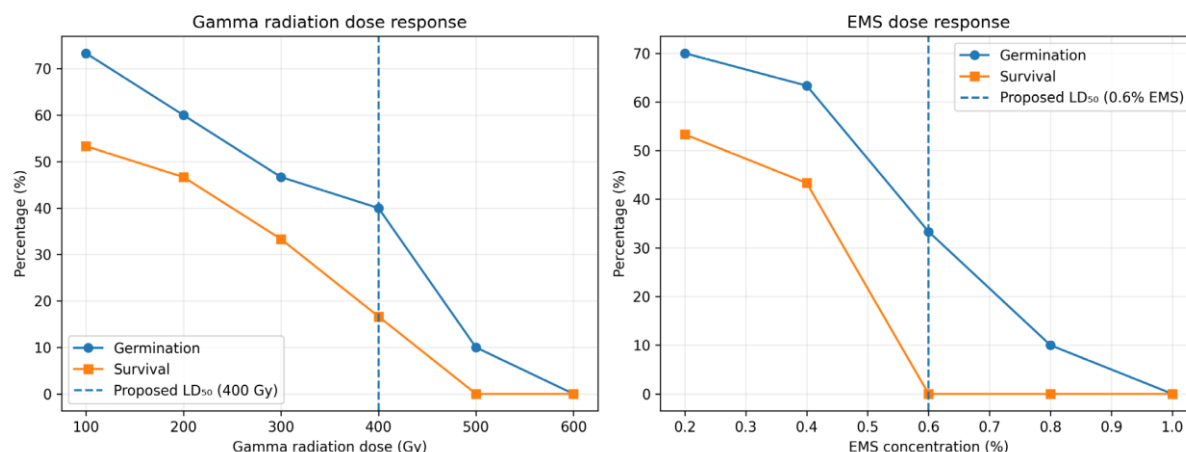


Figure 1. Dose-dependent effects of gamma radiation and EMS on germination and survival of papaya (*Carica papaya* L.) cv. 'Arka Prabhat' {Dashed vertical lines indicate the approximate LD₅₀ levels identified for gamma radiation (400 Gy) and EMS (0.60%)}.

Table 1. Effect of gamma radiation and EMS on germination (%), days taken for germination, survival (%) and leaves per seedling of papaya cv. 'Arka Prabhat'

Treatments	Germination (%)	Days taken for germination	Survival (%)	Leaves seedling per
T1: 100 Gy	73.33 (58.98)	24.33 (5.03)	53.33 (46.90)	6.22 (2.69)
T2: 200 Gy	60.00 (50.83)	25.67 (5.16)	46.67 (43.06)	5.66 (2.58)
T3: 300 Gy	46.67 (43.06)	26.33 (5.22)	33.33 (35.20)	5.26 (2.50)
T4: 400 Gy	40.00 (39.13)	27.67 (5.35)	16.67 (23.84)	5.07 (2.46)
T5: 500 Gy	10.00 (18.43)	30.67 (5.63)	0.00 (0.00)	0.00 (1.00)
T6: 600 Gy	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)
T7: 0.2% EMS	70.00 (56.97)	35.33 (6.03)	53.33 (46.90)	6.19 (2.68)
T8: 0.4% EMS	63.33 (52.75)	38.00 (6.24)	43.33 (41.05)	4.72 (2.39)
T9: 0.6% EMS	33.33 (35.20)	39.67 (6.38)	0.00 (0.00)	0.00 (1.00)
T10: 0.8% EMS	10.00 (18.43)	43.33 (6.65)	0.00 (0.00)	0.00 (1.00)
T11: 1.0% EMS	0.00 (0.00)	0.00 (1.00)	0.00 (0.00)	0.00 (1.00)
T12: Control	83.33 (66.12)	13.67 (3.83)	66.67 (54.76)	6.75 (2.78)
CD (p=0.05)	11.65 (7.17)	0.85 (0.08)	10.19 (6.17)	0.17 (0.03)

Table 2. Effect of gamma radiation and EMS on shoot length, root length and seedling height (cm) of papaya cv. 'Arka Prabhat' at 15 and 30 days after germination (DAG)

Treatments	Shoot length 15 DAG	Root length 15 DAG	Seedling height 15 DAG	Shoot length 30 DAG	Root length 30 DAG	Seedling height 30 DAG
T1: 100 Gy	3.23 (2.05)	4.19 (2.28)	7.42 (2.90)	5.88 (2.62)	6.47 (2.73)	12.35 (3.65)
T2: 200 Gy	3.02 (2.00)	3.65 (2.16)	6.67 (2.77)	5.20 (2.50)	5.73 (2.59)	10.94 (3.45)
T3: 300 Gy	2.75 (1.94)	3.23 (2.06)	5.98 (2.64)	4.80 (2.40)	5.13 (2.48)	9.93 (3.30)
T4: 400 Gy	2.45 (1.85)	3.06 (2.01)	5.51 (2.55)	4.15 (2.27)	4.73 (2.39)	8.88 (3.14)

T5: 500 Gy	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T6: 600 Gy	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T7: 0.2% EMS	3.11 (2.02)	3.57 (2.13)	6.68 (2.77)	5.10 (2.47)	5.67 (2.58)	10.77 (3.43)
T8: 0.4% EMS	2.28 (1.81)	2.39 (1.84)	4.67 (2.38)	3.85 (2.20)	4.27 (2.29)	8.12 (3.01)
T9: 0.6% EMS	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T10: 0.8% EMS	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T11: 1.0% EMS	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T12: Control	3.47 (2.11)	4.28 (2.30)	7.75 (2.96)	6.77 (2.79)	7.32 (2.88)	14.09 (3.88)
CD (p=0.05)	0.18 (0.05)	0.20 (0.04)	0.26 (0.05)	0.34 (0.07)	0.45 (0.08)	0.47 (0.07)

The effect of mutagen treatments on seedling height at 15 and 30 days after germination is presented in Figure 2. A progressive increase in seedling height was observed between 15 and 30 DAG in all surviving treatments. However, the magnitude of growth differed considerably among treatments.

The untreated control maintained the highest seedling height at both observation stages, followed by the lower doses of gamma radiation and EMS. Among the surviving mutagen-treated progenies, 0.4% EMS produced the lowest seedling height at both 15 and 30 DAG. The graphical representation clearly demonstrates the inhibitory effect of increasing mutagen intensity on early seedling growth.

The reduced seedling height observed under the 0.4% EMS treatment is particularly noteworthy because dwarf progenies were also observed under this treatment, indicating its potential importance for developing dwarf breeding material in papaya.

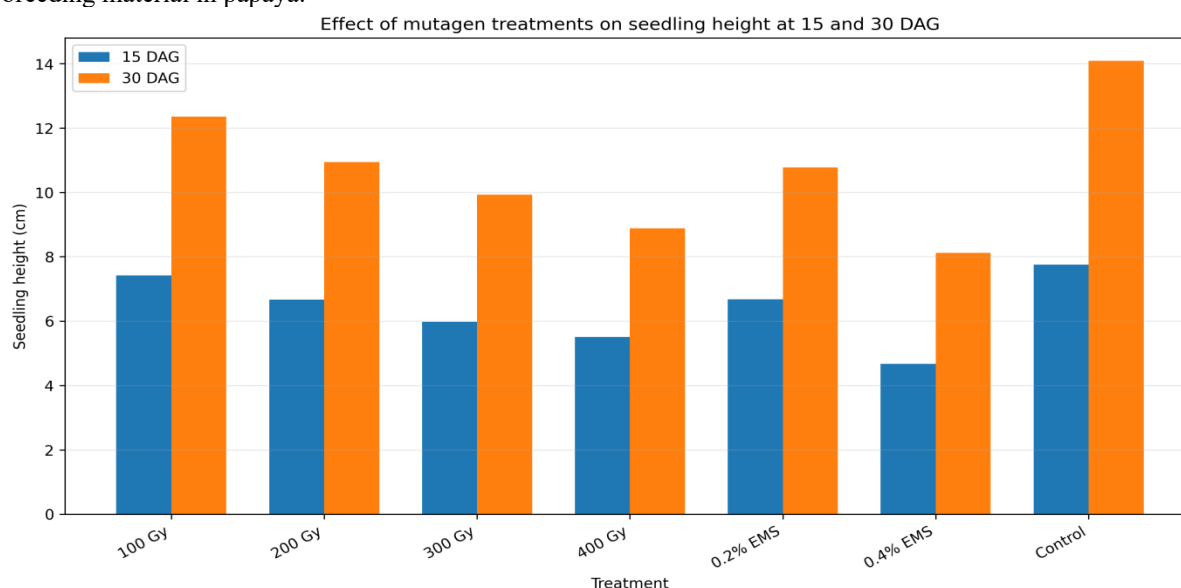


Figure 2. Effect of gamma radiation and EMS treatments on seedling height of papaya cv. 'Arka Prabhat' at 15 and 30 days after germination (DAG).

Physiological parameter

There were significant differences in the total chlorophyll content of the leaves among the various treatments (Table 3). The lowest value was obtained at T8: 0.4% EMS (41.63%), while the highest was found at T1: 100 Gy (51.13%), this being statistically similar to that at T2: 200 Gy (50.2%); the control (T12) had an intermediate value of 46.2%.

Table 3. Effect of gamma radiation and EMS on total chlorophyll content of papaya cv. 'Arka Prabhat'

Treatments	Total chlorophyll content
Control	46.2
T ₁ 100 Gy	51.133

T₂ 200 Gy	50.2
T₃ 300 Gy	44.133
T₄ 400 Gy	42.667
T₅ 500 Gy	0
T₆ 600 Gy	0
T₇ 0.20 %	44.633
T₈ 0.40 %	41.633
T₉ 0.60 %	0
T₁₀ 0.80 %	0
T₁₁ 1.0 %	0
C.D	1.285

Morphological parameters

There were significant differences among the treatments with respect to leaf area, internodal distance, trunk circumference and canopy spread (N-S and E-W) (Table 4). Leaf area varied from 582.43 cm² (in the case of T₈: 0.4% EMS, the lowest value) to 985.62 cm² (for T₁₂, the control, the highest value). Internodal distance went from 3.37 cm (T₇: 0.2% EMS, the lowest) to 6.27 cm (T₄: 400 Gy, the highest); T₈: 0.4% EMS was intermediate at 5.07 cm. Trunk circumference ranged from 24.17 cm (T₈: 0.4% EMS, the lowest) to 33.00 cm (T₄: 400 Gy, the highest), with T₃: 300 Gy being the next highest at 31.83 cm. Canopy spread in the N-S direction was between 101.67 cm (T₃: 300 Gy, the lowest) and 153.17 cm (T₄: 400 Gy, the highest), with T₈: 0.4% EMS being intermediate at 148.67 cm; canopy spread in the E-W direction ranged from 103.33 cm (T₂: 200 Gy, the lowest) to 164.17 cm (T₄: 400 Gy, the highest), and the control was the next highest at 137.33 cm.

Table 4. Effect of gamma radiation and EMS on leaf area (cm²), internodal distance (cm), trunk circumference (cm), and canopy spread N-S and E-W (cm) of papaya cv. 'Arka Prabhat'

Treatments	Internodal distance(cm)	Trunk circumference(cm)	Canopy spread(N-S) (cm)	Canopy spread(E-W) (cm)	Leaf area(cm²)
Control	6.27	30.33	136.17	137.33	985.62
T₁ 100 Gy	5.83	29.17	108.83	109.17	844.63
T₂ 200 Gy	5.63	31.167	106.33	103.33	781.69
T₃ 300 Gy	5.33	31.83	101.67	104.83	657.05
T₄ 400 Gy	5.07	33	153.17	164.17	596.06
T₅ 500 Gy	0	0	0	0	0
T₆ 600 Gy	0	0	0	0	0
T₇ 0.20 %	3.37	28.17	111.67	116.67	674.14
T₈ 0.40 %	5.27	24.17	148.67	137	582.43
T₉ 0.60 %	0	0	0	0	0
T₁₀ 0.80 %	0	0	0	0	0
T₁₁ 1.0 %	0	0	0	0	0
C.D	0.25	0.81	1.93	1.781	34.33

The comparative response of the major morphological traits among the surviving papaya progenies is illustrated in Figure 3. Considerable variation was observed among the treatments with respect to trunk circumference, canopy development and leaf area.

The 400 Gy gamma radiation treatment exhibited the highest trunk circumference and canopy spread, indicating a relatively vigorous vegetative response among the surviving progenies. In contrast, the 0.4% EMS treatment was associated with reduced trunk circumference and the lowest leaf area among the surviving treatments.

The progressive reduction in leaf area with increasing mutagen intensity indicates the adverse effect of mutagenic treatments on vegetative development. The contrasting responses of the gamma-ray and EMS treatments demonstrate that physical and chemical mutagens produced distinct morphological effects in papaya cv. 'Arka Prabhat'.

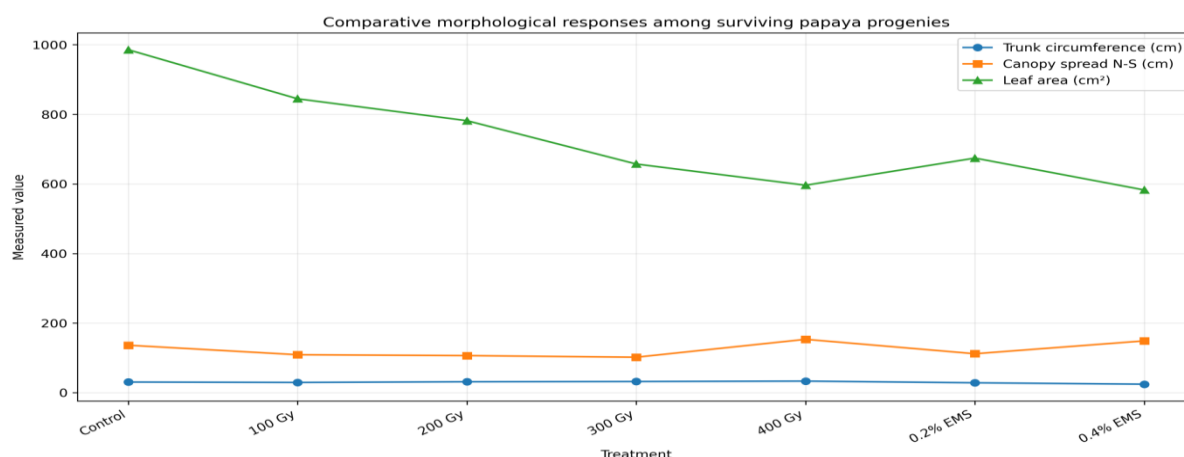


Figure 3. Comparative response of major morphological traits among surviving progenies of papaya cv. ‘Arka Prabhat’ following gamma radiation and EMS treatments.

Morphological (non-numerical) parameters

There were no significant differences among the treatments with regard to the type of branching, all the plants that survived in both the control and the treated progenies exhibiting a single, erect branching pattern and no lateral branches being noted in either the control or the treated seedlings. Leaf type was also unchanged by the treatment, all the progenies having narrow, papaya-type leaves. None of the mutagen-treated progenies showed any morphological abnormalities.

Integrated phenotypic response to mutagen treatments

The overall phenotypic response of papaya cv. ‘Arka Prabhat’ to gamma radiation and EMS treatments is presented as a standardized heatmap in Figure 4. The heatmap provides an integrated visualization of the variation observed across germination, survival, seedling growth, physiological and morphological traits.

The untreated control showed consistently superior performance for several growth-related traits, particularly germination, survival, seedling height and leaf area. Lower doses of gamma radiation and EMS generally produced intermediate responses, whereas higher mutagen doses resulted in severe inhibition of growth and survival.

The heatmap also highlights distinct treatment-specific responses. The 400 Gy gamma radiation treatment was characterized by relatively greater trunk circumference and canopy development, whereas the 0.4% EMS treatment showed a distinct phenotype associated with reduced seedling growth, reduced chlorophyll content and lower leaf area. These contrasting responses indicate that gamma radiation and EMS generated different patterns of phenotypic variation in papaya.

Overall, the integrated phenotypic analysis supports the potential usefulness of lower and intermediate mutagen doses for generating desirable variability, while higher doses caused substantial biological damage.

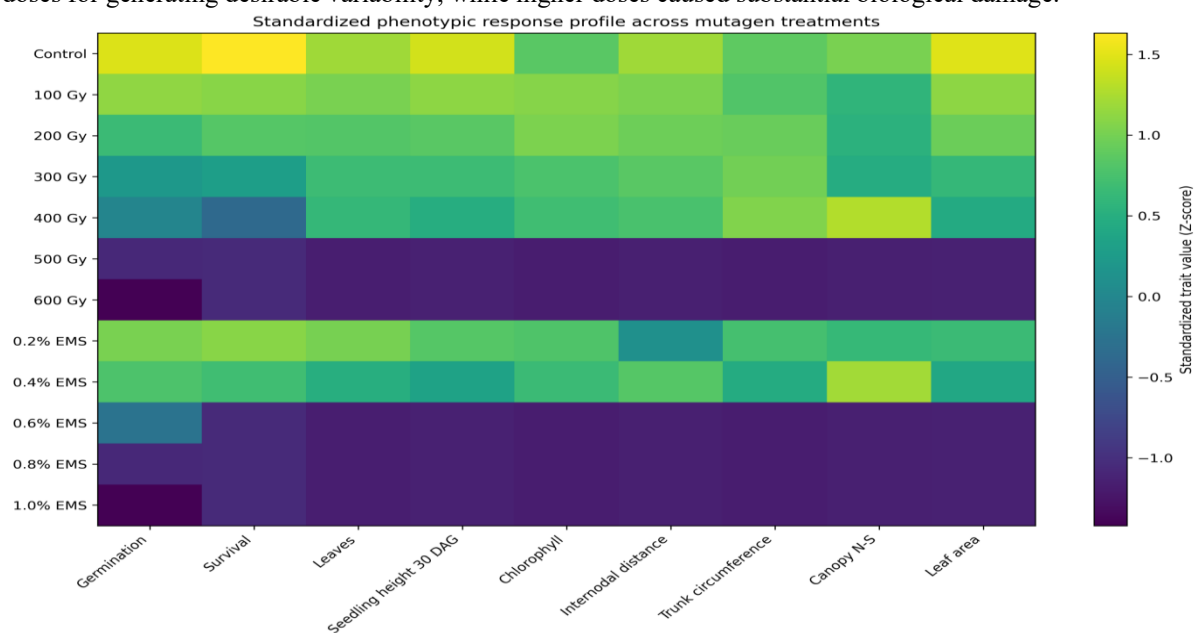


Figure 4. Heatmap showing the standardized phenotypic responses of papaya cv. ‘Arka Prabhat’ to gamma radiation and EMS treatments across growth, physiological and morphological traits.

DISCUSSION

A mutagen has an immediate effect on treated seed by inhibiting germination, the degree of this effect varying with the dose or concentration used. As the dose of both gamma rays and EMS increased, the germination percentage decreased gradually, something most plausibly due to mutagen-induced chromosomal aberrations, damage to the organelles, obstruction of cell division, or direct adverse effects on embryo development (Mahadevamma et al., 2012); the total failure to germinate at T6 (600 Gy) and T11 (1.0% EMS) is in agreement with cv. 'Arka Prabhat' being relatively sensitive to the higher doses in the range tested. This trend is generally in line with earlier findings in papaya — Santosh et al. (2008) noted that the control had the highest germination rate while higher concentrations of EMS resulted in no germination in cv. Arka Surya, Singh et al. (2008) also found the highest germination in the control for cv. Farm Selection-1, and Pujar et al. (2019) observed the control outperforming both gamma- and EMS-treated seed in cv. 'Arka Prabhat' the same pattern being seen in mango, where Hafiz et al. (2005) reported maximum germination at a low gamma dose (2.5 krd). Along with this, the number of days required for germination increased as the mutagen dose rose, a finding that agrees with that of Singh et al. (2008) and Mahadevamma et al. (2012), while the survival percentage decreased as the mutagen concentration increased, following a pattern similar to that reported by Kumar et al. (2016) in cv. Pusa Dwarf and by Yadav et al. (2016) in cv. Kesar King.

The number of leaves per seedling, as well as shoot length, root length and seedling height, all displayed a general decreasing trend as the mutagen concentration increased, irradiation being consistently associated with the highest values in the case of the untreated control. This agrees with the idea that higher doses of mutagen cause a disruption of the endogenous levels of auxin and abscisic acid, lead to enzymatic changes, or interfere with meristematic cell division (Mahadevamma et al., 2012), and is in line with the pattern found by Sahu et al. (2019) in the papaya varieties Arka Surya and Ranchi Local, where the lowest gamma doses tested resulted in the greatest shoot and root length. The present findings regarding the number of leaves per seedling correspond very closely to the decreasing trend observed by Mahadevamma et al. (2012) in the varieties Coorg Honey Dew and Sunrise Solo, although Abd El-Latif et al. (2018) found the opposite trend at higher EMS concentration in the Solo variety, showing that the direction of this response can depend on the cultivar.

The occurrence of chlorophyll mutations is a frequently noted result of mutagenic treatment. In the cases examined, the total amount of chlorophyll decreased at the higher doses of both mutagens, which is in agreement with the idea that chlorophyll synthesis is inhibited via mutagenic effects on DNA, RNA or protein synthesis, or due to the mutagen having a preferential effect on the genes associated with chlorophyll that are situated near the centromere (Mahadevamma et al., 2012). A similar downward trend has also been observed in the papaya variety Coorg Honey Dew (Mahadevamma et al., 2015) and in the mango variety Arka Puneet after treatment with EMS (Rime et al., 2019), although Ahmed et al. (2018) found the opposite trend when the gamma dose was increased in acid lime, once more indicating that the response varies according to the species or cultivar.

Since leaf area is directly connected with photosynthetic capacity and therefore with the total productivity of the plant, it decreased at the higher mutagen concentrations, the smallest leaves being observed at the highest EMS concentration tested (T8: 0.4%). Internodal distance, trunk circumference and canopy spread were all at their maximum in T4 (400 Gy), which means that although this dose reduced survival and germination it also encouraged more vigorous secondary growth among the seedlings that did establish - a pattern that can plausibly be linked to mutation-induced changes in the polygenic control of vegetative growth (variations of this type are generally regarded as the result of phenotypically constructive, multidirectional mutation of polygenes). Notably, T8 (0.4% EMS) had both the smallest leaf area and the shortest seedling height together with dwarf progeny among the surviving treatments, which is why this concentration is of particular interest for breeding purposes with regard to reducing plant height. The fact that there were no significant treatment effects on branching type, branch number and leaf type shows that these specific qualitative traits were relatively resistant to the mutagen doses used in this study.

CONCLUSION

Mutation was achieved in the papaya variety 'Arka Prabhat' by means of both gamma rays and EMS, resulting in a great deal of variability with respect to growth, physiological and morphological traits. The different reactions of the progeny to the two mutagens are in agreement with the fact that certain genes throughout the genotype show different sensitivities depending on the character in question; generally, higher doses of mutagen caused an inhibitory effect due to biological damage, organelle failure, a blockage of cell division and cell death, while lower doses seemed to have a relatively stimulatory effect, this being in line with a positive response in terms of growth. The LD₅₀ was found to be at T4 (400 Gy) for gamma rays and at T9 (0.60% EMS) for the chemical mutagen. The untreated control (T12) showed the best performance in terms of germination percentage (83.33%), days required for germination (13.67), survival percentage (66.67%), number of leaves per seedling (6.75), total chlorophyll content and leaf area; shoot length, root length and seedling height were also highest in the control and lowest in T8 (0.4% EMS). Trunk circumference and canopy spread were both highest at T4: 400 Gy, whereas internodal distance was smallest at T7: 0.2% EMS. There was no significant effect of the treatments on the non-numerical morphological characters — namely, the type of branching, the number of branches and leaf type. In general, the efficiency and effectiveness of both mutagens were higher at the lower end of the dose range than at the higher end, and T8 (0.4% EMS), which produced dwarf progenies along with

reduced plant height, should be further evaluated in the advanced generations as a possible source of material for dwarf breeding.

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Conflict of Interest

No potential conflict of interest was reported by the author.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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