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Evaluation of mutant progenies for improved morphological, fruit and yield traits

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Abstract
Mutation was induced to create variability for plant height, shelf life, PRSV resistance and other desirable traits. Different treatments were employed *viz*., T_1: Gamma-ray (Co^{60}) (0.0Gy) (control), T_2: Gamma-ray (Co^{60}) (50Gy), T_3: Gamma-ray (Co^{60}) (100Gy), T_4: Gamma-ray (Co^{60}) (250Gy), T_5: Gamma-ray (Co^{60}) (500Gy), T_6: Gamma-ray (Co^{60}) (750Gy), T_7: Gamma-ray (1000Gy), T_8: EMS (0.0%) (Distilled water), T_9: EMS (0.25 %), T_{10}: EMS (0.50 %), T_{11}: EMS (0.75 %) and T_{12}: EMS (1.0 %). Studies revealed that significant variation was observed for all characters *viz*., morphological, fruit and quality parameters studied among mutant progenies. Different desirable traits were observed among mutant population *viz*., ultra-dwarf (R_4P_2-100 Gy), perfect hermaphrodite (R_4P_8-100 Gy), fruits with yellow pulp ($R_{14}P_5$- 500 Gy) and extended shelf life (R_8P_{10}-0.25 %). Plants with desirable traits were selfed, sib-mated and were forwarded to next generation.

Keywords: Papaya, mutation, induction, variability

1. Introduction

Papaya with its botanical name as *Carica papaya* L. belongs to the family Caricaceae, is one amongst the economically vital fruit crops of both subtropical and tropical zones of the world. It is basically a tropical fruit crop and believed to have originated from Mexico to Panama (Nakasone and Paull., 1998) [13]. Papaya is a polygamous species with three basic sex forms as male (staminate), female (pistillate) and hermaphrodite forms of inflorescence (Storey, 1938; Singh, 1964) [19, 18]. It is a diploid species with a small genome of 372 Mbp/1C (Arumuganathan and Earle, 1991) [1] and nine pairs of chromosomes (Bennett and Leitch, 2005) [3]. The fruit of papaya has high nutritive and medicinal value (Azad *et al*., 2012) [2] especially vitamin A (2020 IU/100g). The proteolytic enzyme 'papain' obtained from raw fruits of papaya is used for meat tenderization, wool pre-shrinking, chewing gum preparation, removal of gum from natural silk and in preparation of many cosmetics. Papaya has been successfully cultivated in India, USA, Mexico, Peru, Brazil, Jamaica, Nigeria, China, Taiwan, Indonesia, Philippines and Thailand.

Mutation breeding is one of the approaches to create variability through novel recombination's using both chemical and physical mutagens. Pusa Nanha (earlier it was named as a mutant dwarf) an ultra-dwarf (60cmfruit bearing height, 106 cm total plant height) variety of dioecious nature developed by ICAR-IARI, New Delhi, through mutation breeding by using gamma irradiation (Mansharam, 1984) [11]. Hence, the same approach can be used for developing gynodioecious types, as there may be a chance of getting a dwarf mutant with tolerance or resistance for PRSV, good yield, quality and shelf life. Keeping this information as a baseline, an experiment was conducted with an objective to create variation using mutagens and evaluation of mutant progenies for improved morphological, fruit and yield traits. Keeping this information as a baseline, an objective was set to know the cross compatibility between two distant genera *viz*., *Carica* and *Vasconcellea* for PRSV tolerance or resistance.

2. Material and Methodology

2.1 Materials

Seeds extracted from fully ripe fruits of papaya Var. 'Arka Prabhath' obtained by controlled pollination were used for the experiment.

2.1.1 Gamma chamber

Induction of mutation in dry papaya seeds using gamma rays was facilitated by gamma chamber 5000 (Plate 1) installed at ICAR-IIHR, the details of which are furnished in Table 1.

Plate 1: View of gamma chamber used for induction of mutation in papaya

Table 1: Specifications of Gamma Chamber 5000

Parameters	Specifications
Maximum Co-60 source capacity	518 TBq (14000 Ci)
Dose rate at maximum capacity	~9 kGy/hr (0.9 Mega Rad/hr) at the center of sample chamber
Irradiation volume	5000cc approx.
Size of sample chamber	17.2cm (dia) X 20.5cm (ht)
Shielding material	Lead & stainless steel
Weight of the unit	5600 kg. approx.
Size of unit	125cm (l) X 106.5cm (w) X 150cm (ht)
Timer range	6 seconds onwards

2.2 Methodology
Seeds of *Carica papaya* var. Arka Prabhath were used for induction of mutation. Both physical (gamma ray) and chemical mutagens (EMS) were used for mutagenesis. For gamma-irradiation, dry seeds were exposed to different doses in gamma chamber at IIHR. For chemical mutagenesis, seeds were soaked in sterile water overnight and treated with different concentrations of Ethyl Methane Sulfonate (EMS). After germination, seedlings were hardened (Plate 2) and field planted for evaluation (Plate 3).

Plate 2: Hardened mutant seedlings

Plate 3: Field view of mutant progenies

Treatment details
T_1: Gamma-ray (Co^{60}) (0.0Gy) (control)
T_2: Gamma-ray (Co^{60}) (50Gy)
T_3: Gamma-ray (Co^{60}) (100Gy)
T_4: Gamma-ray (Co^{60}) (250Gy)
T_5: Gamma-ray (Co^{60}) (500Gy)
T_6: Gamma-ray (Co^{60}) (750Gy)
T_7: Gamma-ray (1000Gy)
T_8: EMS (0.0%) (Distilled water)
T_9: EMS (0.25 %)
T_{10}: EMS (0.50 %)
T_{11}: EMS (0.75 %)
T_{12}: EMS (1.0 %)

Various nursery, morphological and fruit characters were recorded.

2.3 Statistical analysis
The data on morphological and fruit characters were subjected to Fisher's method of analysis of variance (ANOVA) as given by Sundarraj *et al.*, (1972) [21]. A simple randomised complete block design was followed with 12 treatments and three replications. Wherever the F test was significant for comparison of treatment means, CD values were worked out at 0.05 probability level. The treatment means were separated by using Duncans Multiple Range Test (DMRT). Data obtained were subjected to arc sin transformation.

3. Result and Discussion
The results pertaining to different parameters *viz.*, (nursery, morphological and fruit) and PRSV incidence recorded among the M_1 progenies of Arka Prabhath are presented (Table 2 to 7 and Plate 4 to 10) here under;

3.1 Nursery parameters
3.1.1 Germination (%)
The data on germination percentage among different dose of gamma radiation and concentrations of EMS, revealed significant difference among the treatments with a range of 1.76 to 81.50 per cent. Highest germination was recorded in wet control (water soaked seeds) (T_8, 81.50 %) which was

found to be significantly superior to other treatments. The next best treatments were T_1 (control, dry seeds @ 77.91 %) followed by T_4 (250 Gy @ 72.33 %). The lowest germination was recorded in the treatments EMS 0.75 per cent (T_{11}, 1.76 %), EMS 0.50 per cent (T_{10}, 3.46 %) and 500 Gy (T_5, 33.56 %), where the higher doses of gamma rays and EMS treatments resulted in complete lethality (Table 2). LD_{50} was found to be 500 Gy for gamma radiations and 0.25 per cent for EMS above which there was a maximum lethality in var. Arka Prabhath after mutation induction.

Germination (%) recorded significant difference among treatments in mutagenic population. As there will be some deleterious effect of mutagens on embryo development, lower per cent of germination was recorded in most of the mutagen treated seeds and with increased dose and concentration of gamma radiations and EMS has resulted in lower germination. This result was in confirmation with Shailendra *et al*., (2010) [17] in papaya var. Farm selection-1 and also in Arka Surya by Mahadevamma (1997) [10], Santosh (2002) [15] and in mango var. Dashehari stones highest seed germination was recorded with CO-60 @ 2.5 K-rad (Hafiz *et al*., 2005) [6]. Control untreated seeds recorded higher germination as compared to mutagen treated seeds among which seeds which were soaked in water overnight (control) recorded higher germination. The reason might be due to overnight soaking which helped in initiation of imbibition during which amylase enzyme would have got activated and helped in catalysis of starch which will be utilised by developing embryo leading to better germination.

3.1.2 Days taken for germination

Data on days taken for germination recorded among mutagenic treatments revealed significant difference, which was in the range of 14.66 to 44.66. Both control treatments took significantly lower number of days (T_8 @14.66 and T_1 @ 15.66) compared to other treatments. The next best treatment recorded lower number of days was 50 Gy irradiated seeds (T_2, 26.66) which was on par with 100 Gy treatment (T_3, 27.33). The treatment T_{11} (EMS @ 0.75%) has recorded higher number of days (44.66) which was on par with the treatment T_{10} (EMS @ 0.5 %, 41.66). None of the seeds germinated under high dose of EMS (T_{11} and T_{12}) and gamma radiation seeds (T_6 and T_7) which recorded 0.00 value (Table 2).

Different treatments recorded different days for germination which may be due to impact of mutagens on rate of embryo growth and development. Similar trend was observed for days to germination (%). Control seeds which were not treated with any mutagens has recorded earlier germination as there was no deleterious effects of mutagens on embryo development. In addition, soaking of seeds helps in initiation of germination much earlier. Increased concentration of EMS and gamma irradiation dose, resulted in longer time for germination which was in accordance with Kushwah *et al.* (2010) [9] and Shailendra *et al*. (2010) [17], they also recorded higher number of days for germination when high concentration of EMS was used.

3.1.3 Survival (%)

The data on survival (%) recorded among the M_1 progenies also recorded similar trend which was in the range of 1.72 to 81.03 per cent. Significantly highest survival percentage was recorded in wet control treatment (T_8, 81.03 %) followed by the treatment T_1: dry control (77.91 %) and T_4: 250 Gy. (72.13 %). Among the seeds germinated, the lowest survival percentage was recorded in T_{10}: EMS @ 0.50 (1.72 %) and T_5: 500 Gy (26.33 %). As there was no seed germination in T_6: 750 Gy, T_7: 1000 Gy, T_{11}: 0.75 % and T_{12}: 1.0 %, these treatments recorded 0.00 value (Table 2).

Significant difference was found for survival (%) of mutant population. Both the control treatments had recorded higher survival per cent than mutagen treated seeds. The reason behind this might be the accumulation of some deleterious genes or alleles in population by mutation that may lead to lethality of mutagen treated seedlings. As there will be no such deleterious effects in control seeds, higher survival per cent. This result is in accordance with Bharati (2011) [4], wherein low survival percentage after induction of mutation was noticed.

Table 2: Effect of gamma irradiation and EMS on germination (%), days taken for germination and survival (%) of Papaya var. Arka Prabhath

Treatments	Germination (%)	Days taken for germination	Survival (%)
T_1-Control (Dry seeds)	77.91^b (61.96)	15.66^d	77.91^b (61.95)
T_2- 50 Gy	67.43^d (55.20)	26.66^c	66.33^d (54.54)
T_3- 100 Gy	66.16^d (54.43)	27.33^c	63.93^e (53.09)
T_4- 250 Gy	72.33^c (58.13)	29.66^{bc}	72.13^c (58.26)
T_5- 500 Gy	33.56^f (35.40)	30.66^{bc}	26.33^g (30.86)
T_6- 750 Gy	0.00^i (0.65)	0.00^a	0.00^i (0.65)
T_7- 1000 Gy	0.00^i (0.65)	0.00^e	0.00^i (0.65)
T_8- Control (wet seeds)	81.50^a (64.52)	14.66^d	81.03^a (64.92)
T_9-0.25 %	46.11^e (42.76)	33.00^b	38.44^f (38.32)
T_{10}-0.50 %	3.46^g (10.72)	41.66^a	1.72^h (7.55)
T_{11}- 0.75 %	1.76^h (7.63)	44.66^a	0.00^i (0.65)
T_{12}- 1.0 %	0.00^i (0.65)	0.00^a	0.00^i (0.65)
CV (%)	1.51	9.90	1.38
CD (p=0.05)	0.84	4.78	0.72
S.Em±	0.29	1.63	0.25

Values in parenthesis indicate arc sin transformed ones
Values following same alphabets in the column are not significantly different at P= 0.05
As there was no germination recorded in treatments T_6, T_8 and T_{12}. The data pertaining to all observations after seedling emergence were recorded from other treatments excluding the treatments T_6, T_8 and T_{12}.

3.2 Morphological parameters

The results pertaining to morphological parameters such as days to first flowering, height to first flowering, plant height, trunk circumference, canopy spread (E-W and N-S), number of leaves, number of nodes to first flowering, sex type, type of leaf and branching pattern recorded during first flowering among the M_1 progenies of Arka Prabhath are presented in Table 3.

3.2.1 Day to first flowering

The data on days to first flowering recorded among the M_1 progenies revealed significant differences among the treatments, which ranged from 120.00 to 136.00. Among the seeds germinated under various treatments, significantly lower number of days (120.00) was recorded in the treatment T_5: 500 Gy, which was on par with control (T_8: wet seeds) recording 122.33 days. The EMS treated plants took highest number of days (T_{10}, 136.00 and T_9, 133.00) for first flowering after transplanting (Table 3).

Days to first flowering will help in identification of mutant progenies which can bear fruits earlier. Earlier flowering was recorded in 500 Gy treated seeds and control seeds. This may be due to the positive response of mutation which is comparable with the control treatments. Along with early bearing other important traits needs to be studied for 500 Gy treated seeds. This variation could be utilised for breeding varieties with precocity in flowering ultimately results in early fruiting. However, EMS treated seeds have recorded maximum days for first flowering, so if main aim is to develop varieties which are early bearer, EMS should not be taken into consideration. This result was in accordance with Stremmpl *et al*. (1988) [20], early flowering and early maturity with gamma irradiation in apple mutant (Golden Haidegg).

3.2.2 Height to first flowering (cm)

The data on days to first flowering revealed significant difference among the treatments and ranged from 68.66 to 87.33 cm (Table 3). Among the seeds germinated, the treatment T_{10}; EMS @ 0.50 % has recorded significantly lowest (68.66 cm) plant height. The next lowest height (75.33 cm) was recorded in the treatment T_9: EMS0.25 % followed by T_8: Control (wet seeds) (77.00). The highest plant height (87.33 cm) was recorded in the treatment T_3: 100 Gy which was on par with control (T_1: dry seeds @ 85.00 cm) (Plate 4).

Plate 4: Field view of Ultra-dwarf mutant observed in mutant population

In papaya, dwarf stature is important as this crop is prone to wind damage. So any mutations which can induce lower plant height to first flowering would be beneficial in breeding dwarf varieties. In this experiment, significant difference was found among treatments and EMS has resulted in lower plant height to first flowering than other treatments. Gamma irradiated plants have recorded higher fruit bearing height than other treatments. Nhat and Chau 2010 [14], reported first flowering height was 50-60 cm by gamma irradiation (10-60 Gy).

3.2.3 Plant height at first flowering (cm)

Data on plant height recorded at first flowering revealed significant difference among different mutagenic treatments (Table 3). It was in the range of 123.66 to 152.67 cm between the treatments. Significantly lowest plant height was recorded in control (T_1, dry seeds, 123.66 cm) treatment which was on par with the treatment T_9: EMS @ 0.25 % (125.00 cm) and T_4: 250 Gy (127.00 cm). However, highest plant height was recorded in EMS 0.50 % (T_{10}, 152.67 cm) treated plants and 500 Gy irradiated plants (T_5, 140.00 cm).

Plant height at first flowering also recorded similar trend as height to first flowering. Ems treated and control seedlings have recorded lower plant height. All gamma irradiated seedlings have recorded higher plant height except 250 Gy. The reason might be due to differential nature of induction of dwarfism in var. Arka Prabhath with different mutagens. This result is in confirmation with earlier report of Mansha ram (1984) [11], who has successfully developed dwarf plants by inducing gamma radiations and Kolesnikova and Zav Yalavo, 1989 recorded dwarf sweet cherry plants by 40 Gy dose. Lower dose of EMS has produced dwarf plants which was in accordance with research findings of Mahadevamma *et al*, (1997) [10] Shailendra *et al*., (2010) [17], Santosh *et al*, (2010) [16]. However high dose of EMS has resulted in production thin and lanky plants due to negative impact of EMS on plant height of var. Arka Prabhath which was in agreement with Bharati (2011) [4], they also recorded higher doses are deleterious for papaya.

3.2.4 Trunk circumference (cm)

Data on trunk circumference among different mutagenic treatments revealed significant differences, which varied from 25.33 to 33.00 cm (Table 3). Significantly superior trunk circumference (33.00 cm) was recorded in the treatment T_5 (500 Gy.) followed by T_4: 250 Gy (31.66 cm), which was on par with both control treatments (T_1, 31.00 and T_8, 31.33 cm). However, lowest trunk circumference was recorded in treatment T_{10}: EMS @ 0.50 % (T_{10}, 25.33 cm).

Significant difference was found for trunk circumference among treatments. Gamma irradiated plants had higher trunk circumference and lower plant height. This is well known trait as it combines the dwarfness and thicker girth to bear good size fruits as its bearing is cauliflorus nature. However, EMS has resulted in lower trunk circumference which could be due to the produced variation by EMS. This result is supported by

Veena (2012) [22], also recorded lower trunk circumference with EMS.

3.2.5 Canopy spread (E-W) (cm)

The data on canopy spread in east to west direction recorded among the progenies revealed significant differences which was in the range of 96.66 to 164.00 cm (Table 3). Significantly more (164.00 cm) canopy spread was recorded in plants irradiated with gamma radiations of 500 Gy (T_5) compared to other treatments. Next best treatments were EMS at 0.50 per cent ($T_{10,}$ 137.33 cm) and control (T_1- dry seeds, 137.00 cm). However, less canopy spread (96.66 cm) was recorded in control treatment (T_8, wet seeds).

Significant difference was found forcanopy spread directing towards E-W direction. Higher canopy spread was recorded in seeds germinated from 500 Gy irradiation dose and also in EMS treated seeds. As wider canopy helps in production of more photosynthates it helps in higher fruit production. This positive attribute of mutation effect is beneficial selecting the progenies with higher biomass. According to Veena (2012) [22], EMS treatment resulted in higher canopy development. Both gamma and EMS are strong mutagens that can act on chromosome resulting in chromosomal aberrations which might have exhibited higher canopy spread than normal canopy. However, still increased doses resulted in reduced canopy spread due to deleterious effect.

3.2.6 Canopy spread (N-S) (cm)

The data on canopy spread in north to south direction showed significant differences among the treatments and ranged from 94.66 to 153.66 cm. As recorded in east to west direction, similar trend was noticed here. Significantly more (153.66 cm) canopy spread was recorded in plants irradiated with gamma radiations of 500 Gy (T_5) compared to other treatments. Next best treatments were EMS at 0.50 per cent ($T_{10,}$ 150.66 cm) and control (T_1- dry seeds, 136.66 cm). However, less canopy spread (94.66 cm) was recorded in control treatment (T_8, wet seeds) (Table 3).

As recorded in east west direction similar trend was observed for north south direction also. Gamma radiation of about 500 Gy resulted in higher canopy spread than other treatments. Consistently 500 Gy irradiated plants showing wider canopy, so this trait can be fixed in particular variety for crop breeding programme. However less canopy was recorded in control treatment as normal. This result is contradictory with Veena (2012) [22], recorded higher canopy spread with EMS treatment.

3.2.7 Number of leaves at first flowering

The data on number of leaves at first flowering presented revealed significant difference among the treatments. It ranged from 20.33 to 27.33 between treatments. Significantly highest number of leaves were produced in 500 Gy irradiated plants (T_5, 27.33) which was on par with 100 Gy irradiated plants (T_3, 27.00) and 50 Gy irradiated plants (T_2, 25.66). However, lowest number of leaves were produced in EMS 0.50 per cent (T_{10}, 20.33) (Table 3).

Significant difference was found for number leaves at first flowering (20.33 to 27.33) among all treatments. Number of leaves is directly related to yield potential of crop as it can accumulate more photosynthates ultimately leading to fruit growth and development. Any variation with respect to increased number of leaves would be beneficial for crop improvement programme. Lower dose of gamma radiations has resulted in lower number of leaves that might be due to low dose of gamma radiations.

Table 3: Effect of gamma irradiation and EMS on morphological characteristics of papaya var. Arka Prabhath in M_1 generation

Treatments	Days to first flowering	Height to first flowering (cm)	Plant height at first flowering (cm)	Trunk circumference (cm)	Canopy spread (E-W) (cm)	Canopy spread (N-S) (cm)	Number of leaves at first flowering	Number of nodes to first flowering
T_1-Control (Dry seeds)	123.00^{cd}	85.00^{bc}	123.66^{g}	31.00^{bc}	137.00^{b}	136.66^{c}	24.66^{bc}	11.00^{ab}
T_2- 50 Gy	132.33^{b}	82.66^{d}	134.33^{d}	29.66^{cd}	109.66^{d}	109.33^{e}	25.66^{ab}	11.00^{ab}
T_3- 100 Gy	131.00^{b}	87.33^{a}	136.66^{c}	31.00^{bc}	98.00^{f}	106.33^{f}	27.00^{a}	11.66^{ab}
T_4- 250 Gy	125.33^{c}	85.66^{b}	127.00^{f}	31.66^{ab}	105.00^{e}	101.66^{g}	23.33^{c}	10.66^{ab}
T_5- 500 Gy	120.33^{e}	83.66^{cd}	140.00^{b}	33.00^{a}	164.00^{a}	153.66^{a}	27.33^{a}	10.00^{b}
T_6- 750 Gy	0.00^{f}	0.00^{h}	0.00^{h}	0.00^{f}	0.00^{g}	0.00^{i}	0.00^{e}	0.00^{c}
T_7- 1000 Gy	0.00^{f}	0.00^{h}	0.00^{h}	0.00^{f}	0.00^{g}	0.00^{i}	0.00^{e}	0.00^{c}
T_8- Control (wet seeds)	122.33^{de}	77.00^{e}	131.00^{e}	31.33^{b}	96.66^{f}	94.66^{h}	25.00^{bc}	11.33^{ab}
T_9-0.25 %	133.00^{b}	75.33^{f}	125.00^{fg}	28.66^{d}	118.33^{c}	114.66^{d}	23.33^{c}	12.33^{a}
T_{10}-0.50 %	136.00^{a}	68.66^{g}	152.67^{a}	25.33^{e}	137.33^{b}	150.66^{b}	20.33^{d}	10.66^{ab}
T_{11}- 0.75 %	0.00^{f}	0.00^{h}	0.00^{h}	0.00^{f}	0.00^{g}	0.00^{i}	0.00^{e}	0.00^{c}
T_{12}- 1.0 %	0.00^{f}	0.00^{h}	0.00^{h}	0.00^{f}	0.00^{g}	0.00^{i}	0.00^{e}	0.00^{c}
CV (%)	1.70	1.47	1.45	4.60	1.38	1.09	6.93	13.61
CD (p=0.05)	2.46	1.34	2.19	1.57	1.89	1.50	1.92	1.70
S.Em±	0.84	0.45	0.75	0.53	0.64	0.51	0.65	0.57

The values in parenthesis indicates arc sin transformed ones

Values following same alphabets in the column are not significantly different at P= 0.05

might not have been optimum for inducing variation. This is in contradictory with Veena (2012) [22], recorded higher number of leaves with EMS treatment.

3.2.8 Number of nodes to first flowering

The data on number of nodes to first flowering, revealed significant difference among the treatments. It was in the range of 10.00 to 12.33 between treatments. Significantly highest number of nodes to first flowering (12.33) was recorded in EMS at concentration of 0.25 per cent (T_9). Next best treatment was 100 Gy (T_3, 11.66) irradiated plants which was on par with control treatment (T_8, 11.33). However, lowest number of nodes to first flowering (10.00) was recorded in 500 Gy irradiated plants (T_5) (Table 3).

Low concentration of EMS has recorded high number of nodes to first flowering. However, lower number of nodes were recorded in gamma irradiated plants. This result is in contradictory with results of Bharati (2011) [4], recorded higher number of nodes were recorded in gamma irradiated plants and lower nodes in EMS treated plants. As mutation induced variation are random this type of variation was expected in the progenies.

3.3 Fruit characteristics

The results pertaining to different fruit and quality parameters *viz.*, fruit weight, fruit length, fruit width, fruit volume, fruit cavity index, pulp thickness, TSS, shelf life, number of fruits per tree, yield per tree and PRSV incidence. Results pertaining to all fruit, quality parameters and PRSV incidence among the M_1 progenies of Arka Prabhath are presented (Table 4) here under. Variation with respect to fruit characteristics were observed among mutant populations (Plate 5)

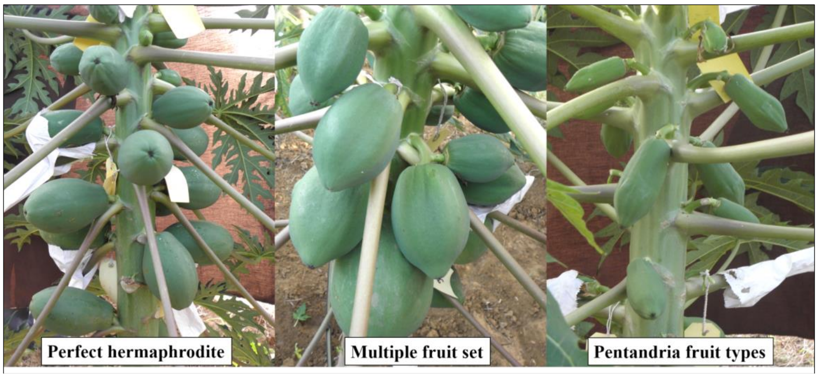

Plate 5: Variation observed among mutant populations for fruit characters

3.3.1 Fruit weight (kg)

The data on fruit weight revealed significant differences among the treatments, which ranged from 0.78 to 1.28 kg. The fruit weight was significantly high (1.28 kg) in control treatment (T_8, wet seeds) as compared to other treatments. The next best treatment was 500 Gy irradiated plants (T_5, 1.15 kg) which was on par with control treatment (T_1, dry seeds) with a fruit weight of 1.12 kg. However, low (0.78 kg) fruit weight was recorded in 50 Gy irradiated plants (T_2) which was on par with EMS treated plants at concentration of 0.25 per cent (0.84 kg) (Table 4).

Table 4: Effect of gamma irradiation and EMS on fruit characteristics of papaya var. Arka Prabhath in M_1 generation

Treatments	Fruit weight (kg)	Fruit length (cm)	Fruit width (cm)	Fruit volume (ml)	Fruit cavity index (%)	Pulp thickness (cm)	TSS (˚B)	Shelf life (days)	Number of fruits per tree	Yield per tree (kg)
T_1-Control (Dry seeds)	1.12^{b}	13.00^{c}	12.27^{b}	636.33^{de}	15.6^{bc} (23.26)	2.13^{de}	12.73^{a}	5.66^{b}	37.33^{a}	42.30^{a}
T_2- 50 Gy	0.78^{d}	11.62^{d}	11.70^{c}	769.33^{b}	18.43^{a} (25.42)	2.43^{ab}	12.66^{a}	4.73^{c}	24.66^{c}	22.23^{d}
T_3- 100 Gy	1.03^{c}	13.30^{bc}	12.66^{ab}	627.00^{e}	15.7^{bc} (23.34)	2.33^{bc}	12.80^{a}	5.50^{b}	37.66^{a}	37.16^{b}
T_4- 250 Gy	1.03^{c}	13.70^{b}	12.83^{a}	630.33^{e}	15.9^{b} (23.49)	2.01^{ef}	12.66^{a}	5.50^{b}	37.66^{a}	35.83^{bc}
T_5- 500 Gy	1.15^{b}	11.93^{d}	11.56^{c}	732.00^{c}	14.7^{c} (22.54)	1.86^{f}	11.53^{e}	5.83^{b}	36.00^{a}	36.00^{bc}
T_6- 750 Gy	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{d} (0.10)	0.00^{g}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{e}
T_7- 1000 Gy	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{d} (0.10)	0.00^{g}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{e}
T_8- Control (wet seeds)	1.28^{a}	14.40^{a}	12.60^{ab}	659.00^{d}	15.00^{bc} (22.77)	2.50^{a}	11.73^{d}	5.33^{bc}	37.00^{a}	40.86^{a}
T_9-0.25 %	0.84^{d}	11.73^{d}	9.76^{d}	721.66^{c}	17.33^{a} (24.59)	2.20^{cd}	12.20^{c}	5.63^{b}	18.66^{d}	22.70^{d}
T_{10}-0.50 %	0.99^{c}	14.26^{a}	12.46^{ab}	950.66^{a}	15.06^{bc} (22.82)	2.23^{cd}	12.46^{b}	6.66^{a}	30.33^{b}	34.66^{c}
T_{11}- 0.75 %	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{d} (0.10)	0.00^{g}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{e}
T_{12}- 1.0 %	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{d} (0.10)	0.00^{g}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{e}
CV (%)	6.52	3.31	3.02	3.50	3.45	6.60	1.40	9.56	7.01	4.70
CD (p=0.05)	0.07	0.48	0.40	28.31	0.91	0.16	0.19	0.60	2.56	1.80
S.Em±	0.02	0.16	0.13	9.65	0.31	0.05	0.06	0.20	0.87	0.61

Values in parenthesis indicates arc sin transformed ones, the values following same alphabets in the column are not significantly different at P= 0.05

3.3.2 Fruit length (cm)

The data on fruit length showed significant difference among the treatments which was in the range of 11.62 to 14.40 cm. Control treatment (T_8, wet seeds) recorded significantly high fruit length (14.40 cm) which was on par with EMS (0.50 %) treated plants (14.26 cm). Next best treatment found was 250 Gy irradiated plants (13.70 cm). However, low (11.62 cm) fruit length was recorded in 50 Gy irradiated plants (T_2) which was on par with EMS treated plants at concentration of 0.25 per cent (11.73 cm) (Table 4).

3.3.3 Fruit width (cm)

The data on fruit width revealed significant difference among the treatments. It ranged from 9.76 to 12.83 cm between treatments. Significantly high (12.83 cm) results were recorded in 250 Gy irradiated plants (T_4) which was on par with 100 Gy irradiated plants (T_3, 12.66 cm), EMS at 0.50 per cent (T_{10}, 12.46 cm) and control (T_8, wet seeds) (12.60 cm). However, lowest fruit width was recorded in 0.25 per cent EMS treated plants (T_9, 9.76 cm) (Table 4).

3.3.4 Fruit cavity index (%)

The data on fruit cavity index revealed significant difference among the treatments. It ranged from 14.7 to 18.43 per cent between treatments (Table 4). Plants irradiated with 50 Gy (T_2) gamma radiations recorded high fruit cavity index (18.43 %) which was on par with 0.25 per cent EMS (T_9, 17.33 %). Next best treatment was control ($T_{1,}$ dry seeds) treatment which recorded 15.6 per cent of fruit cavity index and control

(T_8, wet seeds, 15.00 %). However, lowest (14.7 %) fruit cavity index was recorded in 500 Gy (T_5) irradiated plants.

3.3.5 Fruit volume (ml)

The data on fruit volume revealed significant difference among the treatments which was in the range of 627.00 to 950.66 ml. Plants treated with 0.50 per cent EMS has recorded significantly higher (T_{10}, 950.66 ml) fruit volume. Next best treatment found was 50 Gy irradiated plants (T_2, 769.33 ml). However, lowest fruit volume was recorded in 100 Gy irradiated plants (T_3, 627.00 ml) which was on par with 250 Gy irradiated plants (T_4, 630.33 ml) and control (dry seeds) treatment (T_1, 636.33 ml) (Table 4).

3.3.6 Pulp thickness (cm)

The data on pulp thickness revealed significant difference among the treatments which ranged from 1.86 to 2.50 cm. Significantly thicker pulp (2.50 cm) was recorded in control treatment (T_8, wet seeds) which was on par with 50 Gy irradiated plants (T_2, 2.43 cm). EMS at 0.50 per cent was found to be the best treatment (T_{10}, 2.23 cm) which was on par with EMS at 0.25 per cent by recording almost same pulp thickness (2.20 cm). However, lowest (1.86 cm) pulp thickness was found in 500 Gy irradiated plants (T_5) which was on par with 250 Gy irradiated plants (2.01 cm) (Table 4 and Plate 6).

Plate 6: Variation in pulp colour and pulp thickness observed among mutant population

3.3.7 TSS (˚B)

The data on TSS exhibited significant difference among the treatments, which was in the range of 11.53 to 12.73 ˚B. Gamma irradiated plants with 100 Gy (T_3) dose recorded significantly superior result (12.80 ˚B) which was on par with control (T_1, dry seeds), 50 (T_2) and 250 Gy (T_4) irradiated plants (12.73, 12.66 and 12.66 ˚B). Low (11.53 ˚B) TSS was recorded in 500 Gy irradiated plants (T_5) (Table 4).

Variations were observed for different fruit traits such as fruit weight, fruit length, fruit width, pulp thickness, fruit volume, fruit cavity index, pulp colour, TSS, among mutant populations. Most of them were recorded high values when optimum (500 Gy gamma radiations and 0.50 % of EMS) doses of gamma and EMS were treated. Both low and too high concentrations did not produce any variation or no germination at all. This indicated optimum concentration is good for induction of variation for different fruit traits without affecting growth and health of plant. This result was in close proximity with earlier research findings of Hoang and Tunyh, 1989 [7], recorded larger fruit size upon induction of mutation with N-methyl N-nitroso urea mutagen (0.02-0.04 %). TSS recorded was high in gamma irradiates plants. This result was supported by Nhat and Chau (2010) [14] in irradiated papaya with 10-60 Gy gamma radiations.

3.4 Shelf life (days)

Significant difference was found among the treatments for shelf life trait which ranged from 4.73 to 5.83 days (Plate 7). Plants treated with EMS (T_{10}, 0.50 %) showed highest shelf life (6.66 days) which was significantly superior to other treatments. Next best treatment was 500 Gy gamma irradiated plants (T_5) which had shelf life of 5.83 days and was found to be on par with control (T_1, dry seeds), EMS (T_9, 0.25 %), 100 (T_3) and 250 Gy (T_4) irradiated plants (5.66, 5.63, 5.50 and 5.50 days respectively). However, shelf life 4.73 days was recorded in 50 Gy (T_2) irradiated plants (Table 4 and Plate 8). Optimum dose of gamma (500 Gy) and EMS (0.50 %) had recorded more number of days to ripen from maturity. Lower concentration did not affect variation for shelf life. The reason may be due to too low concentration results in no variation and too higher concentration results in lethality of seedlings before emergence. This result was in close proximity with Shailendra *et al*., (2010) [17], increased concentration resulted in abnormalities with respect morphological traits, sterility and fruit shape. Mutation can be effective breeding method for improvement of shelf life as this is a major problem in highly perishable fruit crops. Successful mutants with increased shelf life was produced in apple var. Golden Delicious (Stremmpl *et al*., 1988) [20] tomato, melon (Mccallum *et al*., 2008) [12] upon gamma irradiation.

Plate 7: Shelf life studies; a) Fruit at harvest taken for shelf life studies b) Fruit at fully ripen firm stage recorded for shelf life studies

Plate 8: Shelf life studies in mutant progenies

3.5 Number of fruits per tree

The data on number of fruits per tree recorded exhibited significant difference among treatments, which ranged from 18.66 to 37.66 (Table 4). More (37.66) fruits were recorded in gamma irradiated plants (T_3) which was on par with 250 Gy irradiated plants (T_4), control (T_1, dry seeds) control (T_8, wet seeds) and 500 Gy (T_5) irradiated plants (37.66, 37.33, 37.00 and 36.00 fruits/tree). However, less (18.66) fruits per tree was recorded in EMS (T_9, 0.25 %).

3.6 Yield (kg/tree)
The data on fruit yield showed significant difference among treatments, which ranged from 22.23 to 42.30 kg/tree (Table 4). The T_1 plants-control (dry seeds) recorded high yield which was on par with the treatment T_8 (control- wet seeds, 40.86 kg/tree). Next best treatment found was 100 Gy (T_3) irradiated plants by recording 37.16 kg/tree. However, low (22.33 kg/tree) yield was recorded in 50 Gy irradiated plants (T_2). Varied yield was recorded among mutant populations of both gamma and EMS treated plants. Even though number of fruits per tree were recorded high in 250 Gy irradiated plants but final yield/tree was recorded high in control treatments. This is due to most irradiated plants had produced fruits with lower weight and size. However, control plants had recorded higher yield even though number of fruits were low. The main reason being deleterious effects of mutagens on yield and yield attributes which was supported by results of Shailendra *et al.* (2010) [17], recorded some abnormalities and reduced size of fruits upon mutagen treatment.

3.7 PRSV incidence
Disease (PRSV) incidence was recorded by scoring technique. The data on PRSV scoring, showed that most mutant progenies during M_1 generation has not shown any tolerance or resistance towards PRSV. Even though there was no symptom expression in some of the M_1 progenies but all the progenies after field planting expressed symptoms after field planting. For most of mutant progenies disease score ranged from 3-5 indicating susceptibility for PRSV (Table 5).

3.8 Morphological (Non-numerical) parameters
3.8.1 Type of branching
Branching was observed among mutant populations. Extent of branching ranged from 2-5 to the main stem (Plate 9) (Table 5). Erect growth habit was also observed.
Branching is phenomenon which occurs upon induction of stress. In this experiment induction of mutation might have acted like stress inducer in papaya var. Arka Prabhath resulting in branched plants. Again, extent of branching was observed among mutant populations which ranged from 2-5 branches per plant. This result was in accordance with research findings of Froneman (1999) [5], observed higher frequency of branches by gamma irradiation (30-75 Gy).

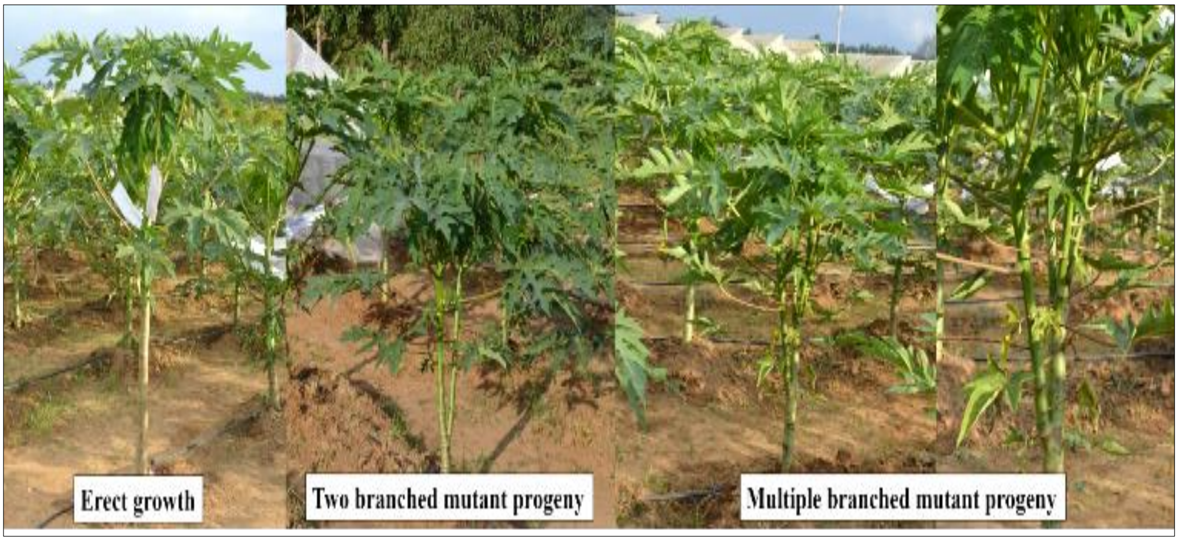

Plate 9: Variations in growth habit observed among mutant populations

3.8.2 Sex type
Sex type among mutagenic population segregated for female and hermaphrodite as the parent Arka Prabhath used is gynodioecious line (Table 6).
Since Arka Prabhath is a gynodioecious variety it had segregated for female and hermaphrodite plants. In addition, sterile hermaphrodite types, more pentandria types and perfect hermaphrodite types were noticed due to the mutagenic effect which have targeted some sex related genes leading to variation in sex forms among mutant populations. This resultwas in close proximity with Chan (2009), they also observed sterility in hermaphrodite plants of Eksotika var of papaya which were exposed to gamma irradiation.

3.8.3 Type of leaf
The leaf type recorded among the mutagenic population revealed narrow leaved (papaya type) and broad leaf (castor type) progenies (Plate 10 and Table 6).
Meagre variations with respect to type of leaf was recorded in mutant populations. As not much variations occur in M_1 progenies. Most of the mutagen treated plants had recorded papaya type leaf, however, some plants exhibited broad castor type leaf similar to wild species of Caricaceae family. This might be due to the upgradation of ancestor genes in production of broad castor type leaves. This is in contradictory with Veena (2012) [22], recorded papaya type leaf among mutant populations.

Plate 10: Field view of broad leaved mutant progeny observed in mutant population

Table 5: Effect of gamma irradiation and EMS on branching pattern and PRSV incidence of papaya var. Arka Prabhath in M_1 generation

Treatments /Plants	T_1		T_2		T_3		T_4		T_5		T_8		T_9		T_{10}	
	BT	PI	BT	PI	BT	PI	BT	PI	BT	PI	BT	PI	BT	PI	BT	PI
P_1	S	3	S	3	S	3	S	3	B	3	S	3	B	3	S	3
P_2	S	4	S	4	S	4	S	3	S	4	S	4	S	4		
P_3	S	4	S	4	S	4	S	3	S	4	S	3	S	4		
P_4	S	3	S	3	S	3	S	3	B	3	S	3	S	3		
P_5	S	5	S	4	S	5	S	3	S	4	S	3	B	3		
P_6	S	3	S	3	S	3	S	3	S	3	S	3	S	3		
P_7	S	3	S	3	S	3	S	3	S	3	S	3	S	3		
P_8	S	4	S	4	S	5	S	3	S	5	S	4	S	3		
P_9	S	3	S	4	S	4	S	3	S	5	S	4	S	4		
P_{10}	S	3	S	3	S	3	S	3	S	3	S	3	S	3		
P_{11}	S	2	S	4	S	5	S	3	S	4	S	3	S	4		
P_{12}	S	4	S	4	S	4	S	4	S	4	S	4	B	4		
P_{13}	S	4	S	4	S	4	S	4			S	4	B	4		
P_{14}	S	3	S	3	S	3	S	3			S	3	S	3		
P_{15}	S	4	S	4	S	4	S	5			S	4	S	5		
P_{16}	S	5	B	4	S	4	S	5			S	4	S	4		
P_{17}	S	4	S	3	S	3	S	5			S	3	S	4		
P_{18}	S	3	S	3	S	3	S	3			S	3	S	3		
P_{19}	S	4	S	2	S	4	S	4			S	5	S	5		
P_{20}	S	3	S	3	S	3	S	3			S	3	S	3		
P_{21}	S	5	S	3	S	4	S	4			S	5	B	4		
P_{22}	S	5	S	5	S	4	S	4			S	4	S	4		
P_{23}	S	4	S	4	S	4	S	5			S	3				
P_{24}	S	3	S	4	S	4	S	5			S	4				
P_{25}	S	3	S	4	S	4	S	3			S	4				
P_{26}	S	4	S	4	S	4	S	3			S	4				
P_{27}	S	4	S	4	B	4	S	3			S	5				
P_{28}	S	3	S	3	S	3	S	3			S	3				
P_{29}	S	3	S	3	S	3	S	3			S	3				
P_{30}	S	4	S	4	S	4	S	4			S	4				
P_{31}	B	4	S	4	S	4	B	4			B	4				
P_{32}	S	4	S	5	S	4	S	4			S	4				
P_{33}	S	3	S	3			S	3			S	3				
P_{34}			S	3												
P_{35}			B	4												
P_{36}			S	3												
P_{37}			S	3												
P_{38}			S	4												

BT- Branching type: S- Single, B- Branched, PI- PRSV incidence- 0-5 score

Table 6: Effect of gamma radiation and EMS on sex type and type of leaf characteristics of papaya var. Arka Prabhath in M_1 generation

Treatments /Plants	T_1		T_2		T_3		T_4		T_5		T_8		T_9		T_{10}	
	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT	ST	LT
P_1	F	N	F	N	F	N	F	N	B	N	F	N	B	N	B	N
P_2	B	N	B	N	B	N	B	N	F	N	B	N	F	N		
P_3	F	N	B	N	B	N	F	N	F	N	F	N	F	N		
P_4	F	N	B	N	B	N	F	N	B	N	F	N	B	N		
P_5	F	N	B	N	B	N	F	N	B	N	F	N	B	N		
P_6	B	N	F	N	B	N	B	N	B	N	B	N	B	N		
P_7	B	N	F	N	F	N	B	N	B	N	B	N	F	N		
P_8	B	N	B	N	B	N	B	N	B	N	B	N	B	N		
P_9	B	N	B	N	B	N	B	N	F	N	B	N	B	N		
P_{10}	B	N	B	N	B	N	B	N	B	N	B	N	B	N		
P_{11}	F	N	B	N	B	N	F	N	B	N	F	N	B	N		
P_{12}	B	N	F	N	B	N	B	N	B	N	B	N	M	N		
P_{13}	F	N	B	N	B	N	F	N			F	N	B	N		
P_{14}	B	N	B	N	B	N	B	N			B	N	F	N		
P_{15}	B	N	B	N	B	N	B	N			B	N	F	N		
P_{16}	B	N	F	N	B	N	B	N			B	N	B	N		
P_{17}	F	N	B	N	B	N	F	N			F	N	F	N		
P_{18}	B	N	B	N	B	N	B	N			B	N	B	N		
P_{19}	B	N	B	N	B	N	B	N			B	N	B	N		
P_{20}	B	N	F	N	B	N	B	N			B	N	B	N		
P_{21}	B	N	B	N	B	N	B	N			B	N	F	N		
P_{22}	B	N	F	N	B	N	B	N			B	N	B	N		

P_{23}	B	N	B	N	B	N	B	N			B	N				
P_{24}	B	N	B	N	B	N	B	N			B	N				
P_{25}	B	N	B	N	B	N	B	N			B	N				
P_{26}	B	N	B	N	B	N	B	N			B	N				
P_{27}	B	N	F	N	F	N	B	N			B	N				
P_{28}	F	N	B	N	F	N	F	N			F	N				
P_{29}	B	N	B	N	F	N	B	N			B	N				
P_{30}	B	N	F	N	F	N	B	N			B	N				
P_{31}	B	N	B	N	B	N	B	N			B	N				
P_{32}	B	N	F	N	B	N	B	N			B	N				
P_{33}	F	N	F	N			F	N			F	N				
P_{34}			B	N												
P_{35}			B	N												
P_{36}			B	N												
P_{37}			F	N												
P_{38}			F	N												

ST- Sex type: F- Female, B- Bisexual, LT- Leaf type: N- Normal leaf, B- Broad leaf

3.8.4 Pulp colour

In all the treatments, pulp colour was found to be pale yellow to orange red except one progeny in which pulp colour recorded was yellow (EMS-0.25 %) (Table 8 and Plate 6).

Table 8: Effect of gamma irradiation and EMS on pulp colour of papaya var. Arka Prabhath in M_1 generation

Treatment/Plants	T_1	T_2	T_3	T_4	T_5	T_8	T_9	T_{10}
P_1	OG	OG	OG	OG	OG	ORG	OG	ORG
P_2	ORG	ORG	ORG	ORG	ORG	ORG	OG	
P_3	OG	OG	OG	OG	ORG	OG	ORG	
P_4	ORG	ORG	ORG	ORG	ORG	ORG	ORG	
P_5	OG	OG	OG	OG	OG	OG	ORG	
P_6	OG	OG	OG	OG	ORG	ORG	OG	
P_7	ORG	ORG	ORG	ORG	ORG	OG	Y	
P_8	ORG	ORG	ORG	ORG	ORG	OG	OG	
P_9	ORG	OG	ORG	ORG	OG	ORG	ORG	
P_{10}	OG	OG	OG	OG	ORG	ORG	ORG	
P_{11}	ORG	ORG	ORG	ORG	OG	OG	OG	
P_{12}	OG	OG	OG	OG	OG	OG	ORG	
P_{13}	OG	ORG	OG	OG		OG	OG	
P_{14}	ORG	OG	ORG	ORG		ORG	ORG	
P_{15}	OG	OG	OG	OG		OG	OG	
P_{16}	ORG	ORG	ORG	ORG		ORG	ORG	
P_{17}	OG	OG	OG	OG		OG	OG	
P_{18}	OG	OG	OG	OG		OG	OG	
P_{19}	OG	OG	OG	OG		OG	ORG	
P_{20}	ORG	ORG	ORG	ORG		OG	ORG	
P_{21}	ORG	ORG	ORG	ORG		ORG	OG	
P_{22}	OG	OG	OG	OG		OG	OG	
P_{23}	ORG	ORG	ORG	ORG		OG		
P_{24}	OG	OG	OG	OG		OG		
P_{25}	ORG	ORG	ORG	ORG		ORG		
P_{26}	OG	OG	OG	OG		OG		
P_{27}	OG	OG	OG	OG		OG		
P_{28}	ORG	ORG	ORG	ORG		OG		
P_{29}	OG	OG	OG	OG		ORG		
P_{30}	OG	OG	OG	OG		OG		
P_{31}	OG	OG	OG	OG		OG		
P_{32}	OG	OG	OG	OG		OG		
P_{33}	ORG	ORG	ORG	ORG		OG		
P_{34}		ORG						
P_{35}		ORG						
P_{36}		ORG						
P_{37}		OG						
P_{38}		ORG						

Pulp colour- Given based on Royal horticultural society (RHS) colour chart: OG- Orange Group (28B), ORG Orange Red Group (30C) and Y-Yellow

4. Conclusion

Mutation was induced to create variability for plant height, shelf life, PRSV resistance and other desirable traits. Studies revealed that significant variation was observed for all characters *viz*., morphological, fruit and quality parameters studied among mutant progenies. Different desirable traits were observed among mutant population *viz*., ultra-dwarf (R_4P_2-100 Gy), perfect hermaphrodite (R_4P_8-100 Gy), fruits with yellow pulp ($R_{14}P_5$- 500 Gy) and extended shelf life (R_8P_{10}-0.25 %). Plants with desirable traits were selfed, sib-mated and were forwarded to next generation.

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