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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 04

Res. Jr. of Agril. Sci. (2022) 13: 1235–1243

CARAS

Res. Jr. of Agril. Sci. (Jul-Aug 2022)
13(4): 1235–1243

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Full Length Research Article

Effect of Gamma Radiation for Improving Morphological Parameters in Papaya (*Carica papaya* L.) cv. Arka Prabhath in M_1 Generation

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Received: 05 Jun 2022 | Revised accepted: 01 Aug 2022 | Published online: 11 Aug 2022

A B S T R A C T

The papaya (*Carica papaya* L.) cv. Arka Prabhath is an advanced generation gynodioecious cultivar developed at ICAR-IIHR-Bengaluru. The seeds of Arka Prabhath hybrid were treated with various doses (T_3-50Gy, T_4-100Gy, T_5-150Gy, T_6-250Gy and T_7-500Gy) of Gamma radiation to study its influence on morphological parameters of papaya along with T_1-control (non-mutagenized Arka Prabhath) and T_2-Red Lady among the M_1 population. Mutation was induced to create variability for morphological, and other desirable traits. Height to first flowering was recorded lowest in T_5 (26cm). Canopy spread (East –West direction) and canopy spread (North –South direction) were higher in treatments T_3 and T_6 (231cm). The maximum intermodal length was recorded in both T_5-150Gy and T_6-250Gy (8.9 cm). Significantly more number of leaves at first flowering was recorded in both T_4-100Gy and T_5-150Gy (28 leaves). Variations were observed in leaf type with coloration and leaf shape in mutant population. Extent of branching was observed among mutant populations which ranged from 2-5 branches per plant. The data on PRSV scoring showed that the most mutant progenies during M_1 generation have not shown any tolerance or resistance towards PRSV. Even though there were no symptoms after 6 months of planting. For most of the mutant progenies disease score was ranged from 3-5 indicating susceptibility for PRSV. There is a chance of getting resistant or tolerant mutants to PRSV. Studies revealed that significant variation was observed for morphological and yield parameters studied among the mutant progenies. Plants with desirable traits were selfed, sib-mated and were forwarded to next generation for breeding purpose.

***Key words*:** Gamma radiation, Mutation breeding, Arka Prabhath, Gynodioecious, PRSV incidence

Papaya (*Carica papaya* L.) is an economically important fruit crop belongs to family Caricaceae. It is having commercial importance because of its high nutritive and medicinal value [1]. Papaya fruit is a rich source of vitamin A and C. It is an excellent source of β-carotene which prevents cancer, diabetes and heart disease [2]. Papaya is the third most cultivated fruit crop in the world and India leads the world in papaya production. It has been successfully cultivated in India, USA, Brazil, Mexico, Nigeria, Jamaica, Indonesia, China, Taiwan and Philippines [3]. Area under papaya in India is around 142 thousand Ha and annual production stood at 6011 thousand MT [4]. In 2020, India produced 43% of the world supply of papayas. In India, it is commercially cultivated large scale in Andhra Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Assam, Odisha, Madhya Pradesh, Manipur, Tamil Nadu, Bihar and to a certain extent in Kerala [5].

Post-harvest losses are high (40-70%) in papaya mainly due to its high perishable nature which leads to rapid ripening (short shelf-life) and affected by papaya ring spot virus (PRSV). In recent years, the most serious disease of papaya worldwide is Papaya Ring Spot Virus (PRSV) type P, a definitive poty virus in potyviridae [6]. It is grouped into two major types, Type P (PRSV-P) infects both papaya and cucurbits and type-W (PRSV-W) infects only cucurbits and not papaya [7]. The incidence of PRSV has been reported to be more than 90 per cent in India [8] and almost all cultivated varieties of genus *Carica* are highly susceptible to PRSV. PRSV produces range of symptoms such as leaf mosaic, chlorosis and water-soaked oily streaks on the petiole and upper part of trunk, distortion of young leaves like shoe string that resembles mite damage, stunting and flower abortion. Results in decreased fruit production and reduces more than 50 per cent of sugar levels [9-10]. The incidence of PRSV has been reported to be more than 90 per cent in India [11]. Control measure includes rouging of diseased plants, cultural practices, cross protection and planting of tolerant cultivars [7]. But these methods are not

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successful in developing virus resistant/tolerant cultivars through conventional breeding and it is the only reliable tool for long term control of this disease.

Mutation breeding is one of the approaches to create variability through novel recombination's using both chemical and physical mutagens. Pusa Nanha (mutant dwarf) an ultra-dwarf variety of dioecious nature was developed by ICAR-IARI, New Delhi, through mutation breeding using gamma irradiation [12]. 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 Papaya Ring Spot Virus (PRSV) along with good yield, quality and prolonged shelf life. The present study evaluates the effect of gamma irradiation on the growth and morphological parameters of papaya cv. Arka Prabhath.

METHODS AND MATERIALS

The present study "Effect of Gamma Radiation on Improving Morphological Parameters of Papaya (*Carica papaya* L.) cv. Arka Prabhath in M_1 Generation" was carried out at ICAR-Indian Institute of Horticultural Research Station, Hesarghatta, Bengaluru during 2020-2022. The soil is red sandy loam with a pH 5.2-6.4. The climate of Hesaraghatta, Bengaluru is moderately warm with mild summer months.

Gamma chamber

Gamma chamber 5000 (Plate 1) installed at the ICAR-IIHR, was used for induction of mutation via gamma rays for dry papaya seeds. Specifications of Gamma Chamber 5000 are given below in the (Table 1).

Gamma irradiation methodology

The seeds of *Carica papaya* var. Arka Prabhath were treated with different doses of gamma rays. For seed treatment (100 seeds/ treatment) healthy seeds of uniform size were used. These papaya seeds were kept in 0.2 mm thick polyethylene bags and the sealed bags were exposed to different doses of gamma irradiation (50 Gy, 100 Gy, 150 Gy, 250 Gy and 500 Gy), temperature and exposure time of each sample were recorded. The seeds were sown in polyethylene bags with in 24 h to retain the vigour of the gamma treated seeds. The gamma treated and gamma untreated (control) seeds were grown under poly house condition.

Table 1 Specifications of gamma chamber 5000

Parameters	Specification
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

This experiment was carried out using the gynodioecious cultivar Arka Prabhath. It is an advanced generation of hybrid derivative from the cross of (Arka Surya × Tainung-1) × Local Dwarf released from ICAR-IIHR, Bengaluru. It is gynodioecious in nature, with large sized fruits of 900-1200g and smooth skin. The pulp is an attractive deep pink color with good keeping quality and high TSS (13-14°B) [13].

MATERIALS AND METHODS

The seeds of Arka Prabhath are gamma-irradiation with different doses of gamma rays (Table 2). Seeds of non-mutagenized and mutagenized Arka Prabhath along with non-mutagenized Red Lady were sown in polyethylene bags in green house.

Table 2 Treatment details of gamma irradiation

T_1: Arka Prabhath	(0.0Gy, control)
T_2: Red lady	(0.0Gy, control)
T_3: Gamma-ray (Co^{60})	50Gy
T_4: Gamma-ray (Co^{60})	100Gy
T_5: Gamma-ray (Co^{60})	150Gy
T_6: Gamma-ray (Co^{60})	250Gy
T_7: Gamma-ray (Co^{60})	500Gy

Transplanting

The seedlings non-mutagenized and mutagenized Arka Prabhath along with non-mutagenized Red Lady were transplanted 45 days after sowing and the following quantitative traits like height at first flowering, number of leaves, internodal length, canopy spread (E-W), canopy spread (N-S) and qualitative traits viz. sex type, type of leaf, type of branching, PRSV at flowering, intensity of PRSV were recorded (Plate 2).

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

The height of the plant was measured from ground level to first flowering with the help of measuring tape and expressed in centimeter (cm). The distance from one end of leaf tip to another end of leaf tip in both North-South and East-West direction was measured during flowering using a measuring

tape and expressed in centimeter (cm). The number of leaves in each tree was counted during first flowering and expressed in numbers. The sex type of tree was recorded during flowering, based on the type of flower produced as hermaphrodite or female. Type of leaf was recorded during flowering based on the nature of leaf produced as normal papaya type or broad (castor leaf type). Type of branching was recorded during flowering based on single trunk or branched. PRSV disease incidence was scored based on low, moderate, high and very high symptoms.

Plate 2 An overview of experimental field – M_1 population

Statistical analysis

The data on germination of seeds, days taken for germination and survival percentage were analyzed using Fisher's method of analysis of variance (ANOVA) as given by Sundarraj *et al.* [14]. The field observations of M_1 populations were analyzed using descriptive analysis. The observations on mean, standard error of mean, standard deviation, minimum value, maximum value and co-efficient of variance (0.05%) were recorded.

RESULTS AND DISCUSSION

Morphological parameters

The results pertaining to morphological parameters such as height to first flowering, canopy spread (E-W and N-S), intermodal length, number of leaves along with non-numerical morphological parameters like sex type, type of leaf shape, Incidence of PRSV disease and number of branches were recorded during first flowering among the M_1 progenies of papaya cv. Arka Prabhath.

Height to first flowering (cm)

The data on height to first flowering revealed significant difference among the treatments ranged from 51.78 to 60.23 cm (Table 3). The highest height to first flowering (124 cm) was recorded in the treatment T_1 (control population) and lowest height to first flowering was observed in T_5: 150 Gy (26 cm). The highest error of variance was observed in treatment T_5-150Gy induced M_1 population (Fig 1).

Gamma irradiated plants has recorded significantly shorter height to first flowering compared control population. Plant height to first flowering significantly varied among the treatments this may be due to differential nature of induction of dwarfism in cultivar Arka Prabhath with different doses of gamma radiations. These results were in confirmation with the earlier report of Mansha Ram *et al.* [12], who successfully developed dwarf plants by inducing gamma radiations in papaya crop. Similarly, Deepa *et al.* [15], reported 250Gy gamma radiated papaya population resulted lowest height to first flowering.

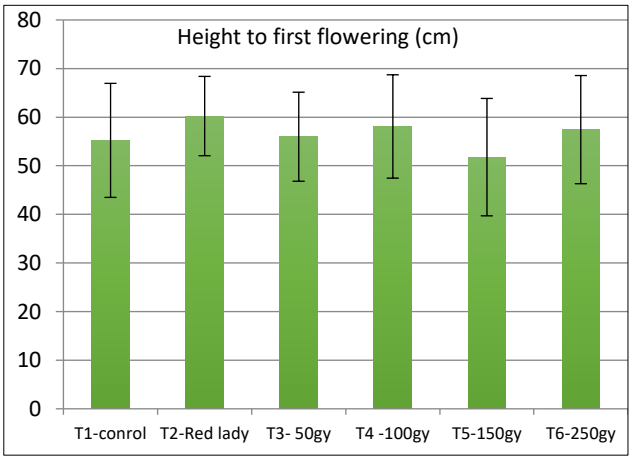

Fig 1 Graph depicting days to first flowering and height to first flowering among M_1 population

Table 3 Effect of gamma radiation on height to first flowering of papaya in M_1 generation

Treatments	Height to 1st flowering (cm)					
	Mean	Std. Error	Std. deviation	Min.	Max.	CV (%)
T_1: Control	55.23	1.54	11.73	18.00	124.00	24.86
T_2: Red Lady (Control)	60.23	1.60	8.16	43.00	80.00	13.55
T_3: 50 Gy	55.98	0.89	9.16	30.00	78.00	16.36
T_4: 100 Gy	58.09	1.15	10.63	30.00	81.00	18.29
T_5: 150 Gy	51.78	1.42	12.08	26.00	77.00	23.32
T_6: 250 Gy	57.44	1.26	11.13	36.00	98.00	19.38
T_7: 500 Gy	00	00	00	00	00	00

Canopy spread (East-West) (cm)

The data on canopy spread in east-west direction recorded among the mutant progenies resulted significant differences, mean values ranging from 170.94 to 194.56 cm (Table 4). Higher canopy spread of 231 cm was recorded in T_3-50Gywhereas, lesser canopy spread of 108.60cm was recorded in control T_1. The highest error of variance was observed in T_5-150Gy but lowest error of variance was observed in T_6-250Gy (Fig 2).

Canopy spread (North-South) (cm)

The data on canopy spread in east-west direction recorded among the mutant progenies revealed significant

differences, mean values ranging from 168.51 to 192.90 cm (Table 5). Significantly higher canopy spread of 231 cm was recorded in treatment T_6- 250 Gy whereas, lesser canopy spread of 102.50cm was recorded in control T_1. The highest error of variance was observed in T_5- 150Gy but lowest error of variance was observed in T_6-250Gy (Fig 2). Both canopy spreads east to west and north to south directions revealed similar results. The high canopy spread enhances good yield and quality fruits (Plate 3). Main cause behind this may be due to mutagen gamma radiation which may act on chromosome resulting in chromosomal aberrations that resulted in exhibiting higher canopy spread than the control population. But higher doses of gamma radiation resulted in reducing canopy length may be due to increased dose range that resulted in some deleterious effect combined with higher dose treatments [16-17].

Table 4 Effect of gamma radiation on canopy spread (East-west direction) of papaya in M_1 generation

Treatments	Canopy spread East-west (cm)					
	Mean	Std. Error	Std. deviation	Min.	Max.	CV (%)
T_1: Control	182.57	2.56	22.77	108.60	220.00	12.47
T_2: Red Lady (Control)	183.77	4.06	20.71	120.00	220.00	11.27
T_3: 50 Gy	184.34	2.59	26.59	190.00	231.00	14.42
T_4: 100 Gy	185.00	2.49	23.13	110.00	225.00	12.51
T_5: 150 Gy	170.94	3.65	31.00	180.00	224.00	18.14
T_6: 250 Gy	194.56	2.02	17.81	135.00	226.00	9.16
T_7: 500 Gy	00	00	00	00	00	00

Table 5 Effect of gamma radiation on canopy spread (North-South direction) in M_1 generation

Treatments	Canopy spread North-South (cm)					
	Mean	Std. Error	Std. deviation	Min.	Max.	CV (%)
T_1: Control	179.79	3.37	22.77	102.50	220.00	16.67
T_2: Red Lady (Control)	175.66	7.71	21.71	122.50	205.00	22.38
T_3: 50 Gy	185.09	1.92	25.59	117.00	222.00	10.62
T_4: 100 Gy	186.48	2.45	23.13	110.00	222.00	12.18
T_5: 150 Gy	168.51	3.89	30.50	80.00	226.00	19.58
T_6: 250 Gy	192.90	2.15	18.81	126.00	227.00	9.84
T_7: 500 Gy	00	00	00	00	00	00

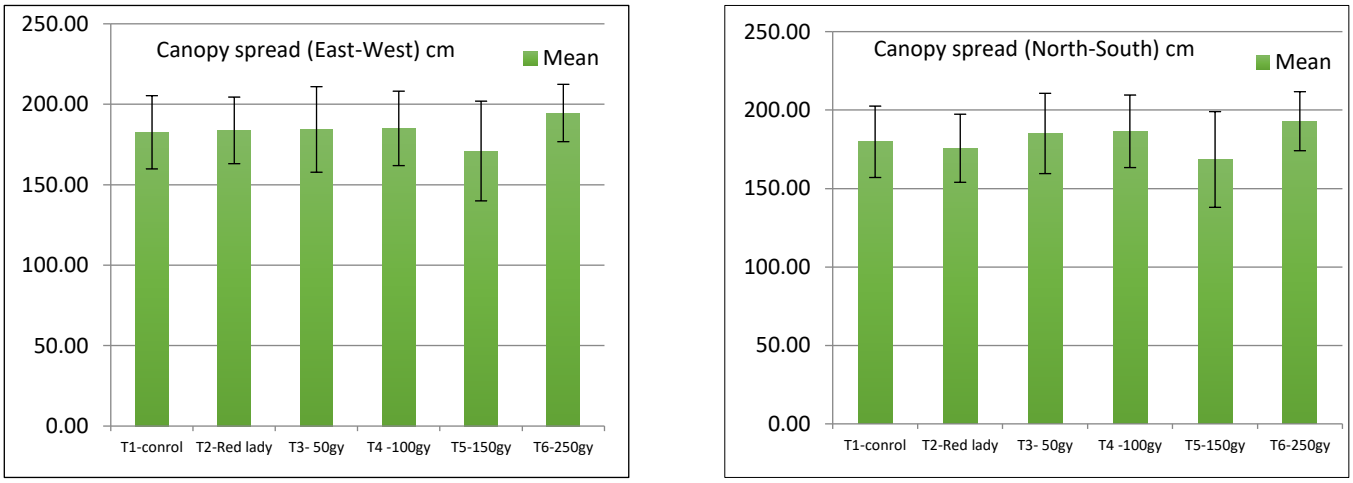

Fig 2 Graph depicting days to canopy spread east-west and north-south directions among m_1 population

Internodal length (cm)

The data on intermodal length results revealed significant difference among the treatments and mean values ranging from 4.20 to 5.81 cm (Table 6). The maximum intermodal length was recorded in both T_5-150Gy and T_6-250Gy (8.9 cm) whereas minimum intermodal length was recorded in control T_1, T_2 (Red Lady) and treatment T_3-50Gy (2cm) (Fig 3). Diversified variations are observed and noted in intermodal length parameter among control and treated population.

Number of leaves at first flowering

The data on number of leaves at first flowering results revealed significant difference among the treatments and mean values ranging from 9 to 17 (Table 7). Significantly more number of leaves at first flowering was recorded in both T_4-100Gy and T_5- 150Gy (28 leaves) whereas, less number leaves at first flowering was recorded in T_2- Red Lady (Control). More number of leaves at first flowering was observed in all treated plants when compared to control plants (Fig 3). Since number of leaves is directly proportional to yield potential of the crop which return helps in higher rate of photosynthesis, ultimately resulting in fruit set, growth and development. Any variation with increased number of leaves would be beneficial for crop breeding and improvement programme. Gamma radiation induced plants resulting in increased number of leaves at first flowering (Plate 3) also been reported by Deepa *et al.* [15], Ramesh *et al.* [17], as they obtained more number of leaves at first flowering in mutation induced population.

Table 6 Effect of gamma radiation on intermodal length of papaya in M_1 generation

Treatments	Internodal length (cm)					
	Mean	Std. Error	Std. deviation	Min.	Max.	CV (%)
T_1: Control	4.20	0.15	1.30	2.00	8.00	31.04
T_2: Red Lady (Control)	4.90	0.15	0.77	2.00	5.90	15.70
T_3: 50 Gy	5.31	0.57	0.79	2.00	6.00	19.03
T_4: 100 Gy	5.81	0.54	1.98	3.40	8.00	25.72
T_5: 150 Gy	5.78	0.15	1.26	3.60	8.90	21.87
T_6: 250 Gy	5.50	0.11	0.96	3.70	8.90	17.45
T_7: 500 Gy	00	00	00	00	00	00

Table 7 Effect of gamma radiation on number of leaves of papaya in M_1 generation

Treatments	Number of leaves					
	Mean	Std. Error	Std. deviation	Min.	Max.	CV (%)
T_1: Control	15.90	0.54	4.76	5.00	26.00	29.94
T_2: Red Lady (Control)	9.31	1.11	5.66	3.00	26.00	60.83
T_3: 50 Gy	16.66	0.36	3.67	7.00	26.00	22.02
T_4: 100 Gy	17.67	0.34	3.18	12.00	28.00	17.96
T_5: 150 Gy	17.22	0.48	4.07	8.00	28.00	23.64
T_6: 250 Gy	17.32	0.36	3.22	8.00	26.00	18.57
T_7: 500 Gy	00	00	00	00	00	00

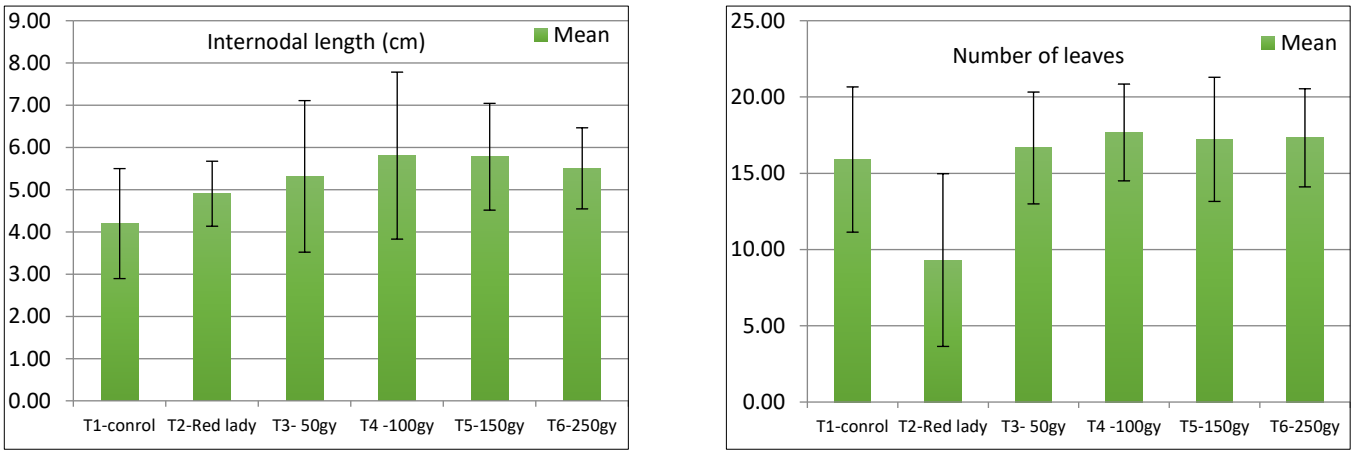

Fig 3 Graph depicting intermodal length and number of leaves among M_1 population

Plate 3 Continuous fruit column observed in female and perfect hermaphrodite in mutant population

Morphological (non-numerical) parameters

Sex type

Sex type among mutagenic population was not shown any significant difference; they were segregated for female and hermaphrodite (Table 8). Since Arka Prabhath is a gynodioecious line which had segregated for female and hermaphrodite plants (Plate 4). Apart from this there were some abnormalities recorded like sterile hermaphrodite flowers and some pentandria type hermaphrodites (Plate 5). This might be due to mutagens might have targeted some sex related genes leading to variation in sex forms among mutant populations. This result was in close proximity with Chan [18], observed sterility in hermaphrodite plants of Eksotika variety of papaya which were exposed to gamma irradiation.

Plate 4 Profuse flowering at early stage, fruit set and breeding programme (selfing and sib-mating)

Plate 5 Variations in fruit size observed in mutant population

Type of leaf shape

The leaf type recorded among the mutagenic population revealed narrow leaved progenies (papaya type) except some which have shown broad leaf (castor type) and some variation with petiole color and erect growth habit also observed (Table 8). This might be due to mutations some ancestor genes might have up regulated resulting in production of broad castor type leaves (Plate 6). Veena [16], recorded that papaya leaf type among mutant populations showed variations.

Plate 6 Variations in leaf shape and color observed in mutant population

Table 8 Effect of gamma radiation on sex type and leaf characteristics of papaya cv. Arka Prabhath in M_1 generation

Plants	T_1		T_2		T_3		T_4		T_5		T_6	
	ST	LT	ST	LT	ST	ST	LT	LT	ST	LT	ST	LT
P_1	H	N	F	B	H	F	N	N	H	N	H	N
P_2	F	N	H	B	H	H	N	N	H	N	H	N
P_3	H	N	H	N	H	H	N	N	H	N	F	N
P_4	H	N	F	B	F	F	N	N	F	B	H	N
P_5	H	N	H	B	H	H	B	N	F	N	H	N
P_6	F	N	H	N	F	H	N	N	H	N	F	N
P_7	H	N	H	N	F	H	N	N	H	N	F	N
P_8	H	N	H	N	H	H	N	B	F	N	F	N
P_9	F	N	H	B	F	H	N	N	H	N	H	B
P_{10}	H	N	H	B	H	H	N	N	F	N	H	N
P_{11}	F	B	F	N	H	F	N	N	F	N	H	N
P_{12}	H	N	F	B	F	F	N	N	F	N	H	N
P_{13}	H	N	H	B	F	H	N	N	H	N	H	N
P_{14}	H	N	H	N	F	H	N	N	H	N	H	N
P_{15}	H	N	H	B	H	H	N	N	H	N	F	N
P_{16}	F	N	H	N	F	H	N	N	H	N	F	N
P_{17}	F	N	H	B	H	H	N	N	H	N	F	N
P_{18}	F	N	F	B	F	F	N	N	F	N	H	B
P_{19}	F	N	H	B	F	H	N	N	H	N	H	N
P_{20}	F	N	H	N	F	H	N	B	F	N	H	N
P_{21}	F	N	H	N	H	H	N	N	H	N	H	N

ST- Sex type; F- Female and H- Hermophrodite; LT- Leaf type; N- Normal and B- broad leaf

Plate 7 Multiple branching observed in mutant population

Plate 8 PRSV disease symptoms and scoring of papaya cv. Arka Prabhath in M_1 generation

Type of branching

Branching was observed among mutant populations. Extent of branching was ranged from 2-5 to the main stem (Table 9). Erect growth habit was also observed. Branching is phenomenon which occurs upon induction of stress. In this experiment mutation inducing might have acted like stress inducer in papaya cv. Arka Prabhath resulting in branched plants. Extent of branching was observed among mutant populations which ranged from 2-5 branches per plant (Plate 7). These results are in contradictory with the research findings of Deepa *et al*. [15], Ramesh *et al*. [17], both observed higher frequency of branches by gamma irradiation with a dose range of 30-75Gy.

Table 9 Effect of gamma radiation on branching type and PRSV incidence of papaya in M_1 generation

Plants	T_1		T_2		T_3		T_4		T_5		T_6	
	BT	PI	BT	PI	BT	BT	PI	PI	BT	PI	BT	PI
P_1	S	L	S	L	S	S	L	L	S	M	S	L
P_2	S	L	S	L	S	S	L	M	S	L	S	L
P_3	S	L	S	L	S	S	L	L	S	L	S	L
P_4	S	M	S	VL	S	S	M	L	S	M	S	M
P_5	S	M	S	VL	S	B-3	M	L	S	M	B-3	M
P_6	S	L	S	L	S	S	L	L	S	L	S	L
P_7	S	L	B-5	L	S	S	L	L	S	L	S	L
P_8	S	L	B-4	L	S	S	M	L	S	L	S	M
P_9	S	L	S	L	B-5	S	M	L	B-2	L	S	M
P_{10}	S	VL	S	L	S	S	L	M	S	VL	S	L
P_{11}	S	VL	S	M	S	S	L	M	S	VL	S	L
P_{12}	S	L	S	L	S	S	L	L	S	L	S	L
P_{13}	S	L	S	L	S	S	L	L	S	L	S	L
P_{14}	B-4	L	B-3	L	S	S	L	L	S	L	S	L
P_{15}	S	L	S	L	S	S	H	L	S	L	S	H
P_{16}	S	L	S	L	B-3	S	L	L	B-3	L	S	L
P_{17}	S	M	S	L	S	S	H	M	S	M	S	H
P_{18}	S	M	S	H	S	S	VL	VL	S	M	S	VL
P_{19}	B-2	M	S	M	S	S	H	VL	S	M	S	H
P_{20}	S	L	S	L	S	B-5	L	L	S	L	B-5	L
P_{21}	S	L	S	L	S	S	L	L	S	L	S	L

BT- branching type; S-single and B- branched, PI- PRSV intensity; low (L) and marginal (M), very low (VL), High (H)

Incidence of PRSV

PRSV Disease incidence was recorded by scoring technique. The data on PRSV scoring showed that the most mutant progenies during M_1 generation have not shown any tolerance or resistance towards PRSV. Even though there were no symptoms after 6 months of planting. For most of the mutant progenies disease score was ranged from 3-5 indicating susceptibility for PRSV (Table 9). There is a chance of getting resistant or tolerant mutants to PRSV**,** which was achieved in peppers and tomato for broad spectrum viruses.

CONCLUSION

Mutation was induced to create variability for plant height, days taken for first flowering, branches, yield and other desirable traits. Studies revealed that significant variation was observed for all characters viz., morphological, number of branches, type of leaf, fruit and quality parameters studied among the mutant progenies. Extent of branching was observed among mutant populations which ranged from 2-5 branches per plant. The data on PRSV scoring showed that the most mutant progenies during M_1 generation have not shown any tolerance or resistance towards PRSV. Even though there were no symptoms after 6 months of planting. For most of the mutant progenies disease score was ranged from 3-5 indicating susceptibility for PRSV. There is a chance of getting resistant or tolerant mutants to PRSV. Different desirable traits were observed among mutant population viz., $R_{12}P_{14}$ (100Gy) - perfect hermaphrodite with elongated and continuous fruit column, $R_{13}P_{19}$ and $R_{14}P_{15}$ (100Gy) - high yielding and dwarf plant, $R_{15}P_{19}$ (150Gy) - multiple branching with 5 branches. $R_{16}P_{14}$ (150Gy) – ultra dwarf mutant line, $R_{17}P_{18}$ and $R_{19}P_{14}$ (150Gy) – high yielding with uniformed fruit size and continuous fruit column. Plants with desirable traits were selfed, sib-mated and were forwarded to next generation for breeding purpose.

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