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Evaluation and characterization of TILLING-based putative mutant populations of papaya cv. Arka Prabhath

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ABSTRACT

Enhancing virus resistance and extending shelf life, coupled with a dwarf stature, are critical factors for successful papaya cultivation in India. These attributes facilitate increased production and productivity and help reduce postharvest losses. Using mutagens in selected papaya cultivars is one of the methods to create variability in papaya. Hence, the TILLING-based mutant populations of papaya cv. Arka Prabhath was generated through irradiation of gamma rays ranging from 50 to 500 Gy dosage. This experiment evaluated and characterized the putative mutant populations based on morphological, fruit characters and physiological parameters. Desirable traits such as early flowering, reduced plant height and bearing height, papaya ring spot virus tolerant plants and fruits with extended shelf life were observed in the mutant population. Early flowering and reduced plant height with wide variation was found at 50 Gy, wherein the first flowering was on 57 days with 43 cm of plant height in contrast to 80 days in the control plant with 79 cm of plant height. Reduced bearing height (40 cm) was observed in 100 Gy with a mean bearing height of 70.65 cm compared to control plants (72.97 cm). Further, twenty-one lines were selected based on morphological traits for physiological and fruit character evaluation. Reduction in physiological loss in weight, ethylene evolution rate and respiration rate were observed in the mutant lines treated with 150 Gy dosage. The results advocate the efficacy of gamma-induced mutation for developing dwarf cultivars with improved shelf life of fruits.

Key words: *Carica papaya*, Arka Prabhath, Mutation, TILLING, Shelf life.

INTRODUCTION

Papaya is a tropical fruit crop of the Caricaceae family. It is believed to have originated in the lowlands of eastern Central America, from Mexico to Panama (Nakasone and Paul, 9). In the early 16th century, papaya was introduced to India from the Philippines through Malaysia. It is of great commercial importance because of its high productivity, short-duration nature, round-the-year availability, nutritive value and suitability for the preparation of various value-added products. One of the main drawbacks of papaya cultivation has been the occurrence of papaya ring spot virus (PRSV). Apart from this, papaya fruits are perishable with a short shelf life because of their climacteric behaviour. Despite a large production with the highest productivity among all fruits grown in India, a significant portion of the production goes into waste, which is reported to be around 40-60% of the total production. In mutagenesis, there is a chance of getting resistant or tolerant mutants to PRSV with improvement in other desirable traits, as has been achieved in peppers for broad-spectrum viruses (Ibiza *et al.*, 5). Recently, biotechnological tools have been applied to enhance fruit quality traits. However, aversion to transgenic technology made way for the development of non-transgenic potential reverse genetic strategies like TILLING (Targeting Induced Local Lesions in Genome), which allows the easy screening of induced point mutations in mutagenesis (McCallum *et al.*, 8). In this study, TILLING-based putative mutant populations were evaluated to determine the variability for various morphological, physiological, fruit characters and tolerance/resistance to PRSV of mutant populations to assimilate the sensitivity and effectiveness of mutagenesis for developing dwarf cultivars with improved shelf life and postharvest behaviour of fruits.

MATERIALS AND METHODS

The experiment was undertaken at the ICAR-Indian Institute of Horticultural Research (IIHR) from 2020 to 2022. The mutant seedlings of papaya cv. Arka Prabhath was generated by irradiating its seeds with five different doses of gamma radiation, viz.,

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T2-50 Gy, T3-100 Gy, T4-150 Gy, T5-250 Gy and T6-500 Gy, in a gamma chamber located at ICAR-IIHR, Bengaluru. The doses of gamma irradiation were fixed based on the LD50 value of gamma rays for papaya, which was worked out through linear regression considering the germination level < 50% (Bhat *et al.*, 2). A total of 600 plants from five treatments along with T1-control (untreated Arka Prabhath plants) were selected from the putative mutant population generated for taking observation in the mutant population (M1) and were advanced to the next generations where molecular screening of mutations is carried out through TILLING using ACC-synthase and ACC-oxidase gene-specific primers (data not published).

Morphological observation on the number of days to first flowering (days), plant height (cm), height to first fruiting (cm), stem girth (cm), canopy spread (East-West and North-South) (cm), number of leaves, nodes to first flowering, average weight of the fruit (kg), number of fruits per plant, yield (kg/plant), flower type, branching type and PRSV tolerance/resistance for M1 population along with control were recorded based on papaya descriptor (IPGRI, 6). Based on the morphological characteristics, twenty-one lines along with control plants were selected, and fruit characters were further evaluated. The fruit characters such as fruit weight (kg), fruit size (length and breadth in cm), the thickness of pulp (cm), fruit volume (ml), fruit cavity per cent, shelf life and firmness were recorded. The instron-Universal testing machine of Model 4201, USA, was used to measure the fruit's firmness and expressed it as kgF.

The fruits at the breaker stage were harvested and stored at 20°C for postharvest studies. Storage life was determined by recording the number of days taken by harvested fruits to reach the full ripe stage. Further, physiological parameters such as physiological loss in weight (PLW %), respiration rate and ethylene evolution rate were determined. The respiration rate is determined using an auto gas analyzer (Model: Checkmate 9900 O_2/CO_2, Denmark) and expressed in mg kg^{-1} h^{-1}. The ethylene concentration was measured using an ethylene analyzer and expressed in µl kg^{-1} h^{-1} (Rao and Rao, 12).

The data on morphological parameters were subjected to descriptive statistics using standard procedure. Analysis of variance (ANOVA) was performed for data on fruit characters and physiological parameters such as PLW and shelf life at a 5% significant level using XL-stat (Addinsoft., 1).

RESULTS AND DISCUSSION

Gamma rays are the most commonly used physical mutagen since they have good efficiency, effectiveness, reproducibility, high mutation frequency and simple application. During the papaya genetic improvement programme through TILLING, a putative mutant population with considerable variation in different morphological traits was generated by gamma irradiation in papaya cv. Arka Prabhath.

Screening mutants with precocity in flowering can identify early-bearing mutant progenies. Further, this precocious flowering trait can also be used in breeding varieties with early fruiting and short duration. Variation in the number of days to first flowering compared to control plants in different gamma irradiation doses was observed, ranging from 115 days to 137 days. The highest mean number of days was observed in T4-150 Gy (109.40 days), whereas the lowest mean value for days to first flowering was observed in T5-250 Gy (105.88 days). Further, although the mean number of days to first flowering of population generated through T2-50 Gy was 108.54 days, the progenies it produced were early flowering in terminal position at 57 days (R3P3) in contrast to 80 days in control plants (Table 1. and Fig. 1b). This result was in congruence with Ramesh *et al.* (11) in papaya, who reported early flowering and maturing mutant in Arka Prabhath.

With increasing irradiation dosage, plant height, bearing height and trunk circumference were reduced compared to control plants. Plant height and bearing height seemed to be the most altered traits by gamma irradiation (Fig. 1c). The plant height range was 43 to 145 cm in 50 Gy treated plant progenies in dissimilarity to control where the plant height ranged from 79 to 200 cm (Table 1). Further, although the mean plant height of the population generated through 50 Gy treatment was 108.43 cm, the progenies it produced were as short as 43 cm in the R3P3 mutant plant, which was found to be dwarf compared to the control. These results are in congruence with past findings of reduced height of plants with rising irradiation doses in papaya, Husselman *et al.* (4) found dwarf plants at 80 Gy of gamma irradiation in papaya varieties, Perveen *et al.* (10) in mango. The bearing height range was 43 cm to 103 cm in 50 Gy treated plant progenies, although the mean bearing height was 71.70 cm in contrast to the control, where the bearing height range was 55 to 115 cm (Table 1). This result is in accordance with Hang and Chau (3), who made dwarf mutant selections, which were found to bear at a lower height from the ground.

The T2-50 Gy treatment produced the progeny with the maximum stem girth (38.00 cm), while the control had the highest mean trunk circumference (24.52 cm), which decreased to 18.75 cm in T5-250 Gy treatment (Table 1). The variation for canopy spread (N-S) was highest in control with the mean

Table 1. Effect of gamma irradiation on morphological traits in M_1 population of papaya.

Parameter		T_1	T_2	T_3	T_4	T_5	T_6	Parameter		T_1	T_2	T_3	T_4	T_5	T_6
Days to first flowering (days)	Mean	106.91	108.54	107.53	109.40	105.88	0.00	Plant height (cm)	Mean	110.08	108.43	107.35	107.20	104.38	0.00
	St. Err.	1.65	0.96	0.58	0.93	1.88	0.00		St. Err.	79.00	43.00	68.00	76.00	97.00	0.00
	Min	80.00	57.00	91.00	89.00	100.00	0.00		Min	79.00	43.00	68.00	76.00	97.00	0.00
	Max	131.00	137.00	136.00	137.00	115.00	0.00		Max	200.00	145.00	184.00	183.00	116.00	0.00
	CV	12.42	9.39	8.27	9.45	5.01	0.00		CV	15.26	11.09	16.47	17.01	6.10	0.00
Height to first flowering (cm)	Mean	72.97	71.70	70.65	67.57	64.50	0.00	Trunk circumference (cm)	Mean	24.52	21.13	20.66	19.16	18.75	0.00
	St. Err.	1.34	0.91	00.92	1.27	2.67	0.00		St. Err.	0.43	0.32	0.25	0.34	1.24	0.00
	Min	55.00	43.00	40.00	42.00	53.00	0.00		Min	20.00	11.00	13.00	13.00	15.00	0.00
	Max	115.00	103.00	117.00	109.00	73.00	0.00		Max	36.00	38.00	34.00	28.00	25.00	0.00
	CV	14.80	13.50	20.15	20.76	11.69	00.0		CV	13.36	16.27	19.04	19.96	18.84	0.00
Canopy spread (N-S) (cm)	Mean	173.08	169.10	167.34	166.71	165.50	0.00	Canopy spread (E-W) (cm)	Mean	177.29	168.33	167.70	166.98	164.75	0.00
	St. Err.	2.36	1.99	1.20	1.70	6.11	0.00		St. Err.	2.31	2.10	1.27	1.88	3.45	0.00
	Min	134.00	110.00	70.00	109.00	144.00	0.00		Min	142.00	75.00	85.00	121.00	152.00	0.00
	Max	232.00	220.00	220.00	216.00	194.00	0.00		Max	230.00	220.00	220.00	216.00	179.00	0.00
	CV	11.00	12.54	11.10	11.32	10.44	0.00		CV	10.49	13.25	11.70	12.48	5.93	0.00
No. of leaves at first flowering	Mean	25.05	24.11	22.94	22.03	20.75	0.00	Nodes to first flowering	Mean	16.31	15.91	15.17	13.70	12.50	0.00
	St. Err.	0.53	0.51	0.50	0.64	1.03	0.00		St. Err.	0.32	0.26	0.20	0.30	0.27	0.00
	Min	18.00	13.00	14.00	13.00	18.00	0.00		Min	12.00	8.00	8.00	7.00	12.00	0.00
	Max	48.00	49.00	82.00	68.00	25.00	0.00		Max	24.00	22.00	24.00	22.00	14.00	0.00
	CV	17.21	22.47	33.39	32.11	14.05	0.00		CV	15.67	17.57	20.21	24.46	6.05	0.00
Average fruit weight (kg)	Mean	1.31	1.47	1.27	1.33	1.16	0.00	No. of fruits per plant	Mean	25.75	20.93	24.58	26.15	22.50	0.00
	St. Err.	0.02	0.02	0.02	0.03	0.05	0.00		St. Err.	0.98	0.69	0.45	0.49	2.11	0.00
	Min	0.925	0.954	0.210	0.618	0.943	0.00		Min	10.00	8.00	4.00	12.00	16.00	0.00
	Max	1.577	2.076	2.323	2.404	1.318	0.00		Max	41.00	42.00	44.00	46.00	32.00	0.00
	CV	10.23	14.37	25.75	25.74	11.41	0.00		CV	30.80	35.15	28.16	20.85	26.56	0.00
Yield (kg/plant)	Mean	33.69	30.64	30.93	34.02	25.84	0.00								
	St. Err.	1.33	1.04	0.65	0.69	2.35	0.00								
	Min	11.10	10.50	0.84	11.24	19.86	0.00								
	Max	54.21	54.89	57.11	54.80	38.42	0.00								
	CV	31.74	36.22	32.70	22.48	25.71	0.00								

canopy spread of 173.08 cm, while the lowest mean canopy spread (N-S) was observed in T5-250 Gy (165.50 cm) (Table 1). The outcomes are consistent with the findings of Kumar *et al.* (7) in kinnow mandarin. Further, a reduction in the number of leaves compared to control plants with increased doses of gamma irradiation was observed with the highest mean number of leaves in control (Table 1). The mean number of nodes to bearing was highest in control (16.31), while the lowest was recorded in T5-250 Gy (12.50). Albeit the mean nodes to bearing observed in the population generated through T4-150 Gy was 13.70, the progenies it produced had the minimum number of nodes to bearing (7.00) (Table 1). Variations in growth traits are very promising for breeding purposes. Increasing gamma-ray doses has been found to decrease growth parameters. The reduction was observed even from the lower doses of irradiations, which might be due to certain ill effects of mutagens on plant biochemical activities. These results are consistent with the outcomes of Ramesh *et al.* (11) in papaya and Perveen *et al.* (10) in mango. Differential killing of meristematic cells due to genetic injury damages cells, producing fewer cell progenies and reducing plant growth.

The highest average fruit weight was observed in T2-50 Gy (1.47 kg). The highest mean number of fruits per plant was observed in T4-150 Gy (26.15), which

1a. Multiple branching of main stem

1b. Terminal early flowering mutant 1c. Ultra-dwarf mutant 1d. Single branching of plants

Fig. 1. Morphological variations in M_1 population of papaya

also produced the progeny with the highest number of fruits per plant (46.00 cm) (Table 1). The highest mean yield was observed in T4-150 Gy (34.02 kg/plant). The mean yield in the population generated through T3-100 Gy treatment was 30.93 kg/pant. However, yield was 57.11 kg/plant in the progenies. In control, yield ranged from 11.10 to 54.21 kg/plant (Table 1). The highest fruit weight and size are observed in most irradiated plants due to the mutagenesis effects such as Indels, transition, transversion and chromosomal re-arrangements, which affect yield and yield-attributing traits. The results are supported by the findings of Ramesh *et al.* (11) in papaya and Kumar *et al.* (7) in Kinnow mandarin.

The sex type of flowers did not show any significant variation among mutant populations as they segregated for females and hermaphrodites since Arka Prabhath is gynodioecious. The branching range was 2-11 to the main stem (Fig. 1a and 1d). The results are juxtaposed with the findings of Ramesh *et al.* (11), who also reported branching in mutant progenies in Arka Prabhath, which might be due to the induction of stress by the effect of mutagen.

A major bottleneck in the commercial cultivation of papaya is PRSV. In this experiment, most of the M1 progenies had developed PRSV symptoms. However, only some plants had shown initial resistance, but this resistance was broken with time. Among the selected twenty-one progenies, one progeny viz., R25P10 developed only a few chlorotic spots, which was found to be field tolerant (Fig. 2). Further, the progenies (M2) of R25P10 were tested for its

Fig. 2. Virus tolerant plant in the field and its fruiting coloumn

tolerance by challenge inoculation using PRSV through artificial inoculation method and progenies which showed resistance were transplanted to the field for further evaluation of quality traits (data not shown).

Mutant lines with superior morphological traits selected from M1 were further evaluated for fruit characters. Among 21 selected lines of M1 population along with control, the highest weight of fruit (kg), fruit size (length and breadth in cm), thickness of pulp (cm), volume of fruit (ml), fruit cavity per cent, firmness was recorded in most of the selected lines in comparison to control (Table 2 and Fig. 3). This result is in contiguity with the earlier findings of Hang and Chau (3), Ramesh *et al.* (11) in papaya.

Table 2. Fruit quality parameters of selected lines of M_1 population of papaya.

Mutant Lines	Fruit weight (kg)	Fruit Length (cm)	Fruit Breadth (cm)	Pulp thickness (cm)	Fruit Volume (ml)	Fruit cavity index (%)	Firmness (kg/cm^2)
R_5P_4	1.23	18.07	12.53	2.77	958.85	27.63	4.11
R_7P_8	1.27	16.77	13.57	3.10	952.96	28.13	5.74
R_9P_{17}	1.50	18.37	13.77	2.67	1290.25	20.93	4.39
$R_{14}P_{22}$	1.00	16.07	12.60	2.67	949.14	27.39	3.99
$R_{15}P_{22}$	1.82	28.23	18.89	2.97	1560.00	15.51	2.38
$R_{16}P_4$	1.65	24.87	18.83	3.93	1625.85	15.38	3.51
$R_{16}P_5$	1.46	18.30	14.33	2.73	1269.13	20.67	3.69
$R_{17}P_5$	1.08	18.73	15.62	2.63	944.36	28.81	3.85
$R_{17}P_{18}$	1.43	23.73	18.85	2.73	1257.00	19.81	4.49
$R_{17}P_{19}$	1.20	22.77	20.53	2.63	962.38	26.19	4.33
$R_{18}P_5$	1.23	17.33	13.87	3.20	924.86	29.20	4.60
$R_{18}P_7$	1.66	19.20	13.93	3.43	1611.98	16.50	3.28
$R_{18}P_8$	1.39	19.33	15.77	3.07	1013.88	25.28	2.20
$R_{18}P_{16}$	1.22	26.37	12.47	3.60	918.78	29.31	1.82
$R_{19}P_{13}$	1.04	13.33	11.57	2.40	971.06	28.32	4.72
$R_{20}P_4$	1.50	18.23	14.73	3.73	1332.14	20.20	4.26
$R_{20}P_6$	1.49	22.83	12.37	3.17	1255.30	18.72	3.45
$R_{23}P_5$	1.34	19.83	13.73	3.43	967.01	26.37	4.89
$R_{23}P_{13}$	1.96	20.13	12.67	3.00	1575.09	15.05	5.75
$R_{25}P_{10}$	1.14	23.63	10.83	3.03	835.92	25.91	2.25
$R_{26}P_{21}$	1.83	27.47	11.83	3.47	1760.30	14.15	4.09
Control	1.38	16.50	12.67	2.90	978.91	27.28	2.77
Mean	1.40	20.46	14.36	3.06	1168.63	23.03	3.84
Sem	0.02	0.82	0.73	0.15	4.38	0.37	0.38
CD @ 5%	0.05	2.33	2.09	0.43	12.49	1.04	1.09

Fig. 3. Variation for fruit characters in M_1 population of papaya.

In the present investigation, among 21 selected lines of M_1 population along with the control, the highest PLW (%) was noticed in line $R_{19}P_{13}$ (14.84 %) whereas the lowest PLW (%) was noticed in $R_{25}P_{10}$ (5.96 %) on 10th day of storage at 20⁰C. The highest shelf life (days) was observed in line $R_{25}P_{10}$ (16.00 days) whereas, lowest shelf life (days) was recorded in control (10.00 days) (Table 3). This might be due to effect of mutagen on metabolic processes which lead to varied metabolic activities in mutant lines. These results are in congruence with outcomes of Ramesh *et al*. (11) in papaya.

In the present experiment shelf life studies were laid out by selecting some of the lines based on qualitative and quantitative traits. The harvested fruits were weighed and kept in cold storage at 20⁰C for ethylene and CO_2 observations. The fruits were observed for 10-15 days, weight, ethylene production and the CO_2 rate, which varied each day. As the days advanced, the fruit weight was measured initially more than the weight measured during the ripening stages of the fruits, which explains that fruits are supposed to get lighter as the physiological changes involved in the fruit, especially the respiration. Significant differences were observed in the shelf life, respiration rate & ethylene evolution rate of selected mutant lines fruits stored at 20⁰C. The low CO_2 and ethylene production, as evident from Fig. 4 & 5 in mutant lines. ACC-synthase and ACC-oxidase are the two key enzymes in the biosynthesis of ethylene, by knocking down their activities might be resulted in declined ethylene levels due to the effect of mutagens. This resulted in an extended shelf life up to 16 days in the mutant population. These results are in congruence with the outcome of Ramesh *et al.* (11).

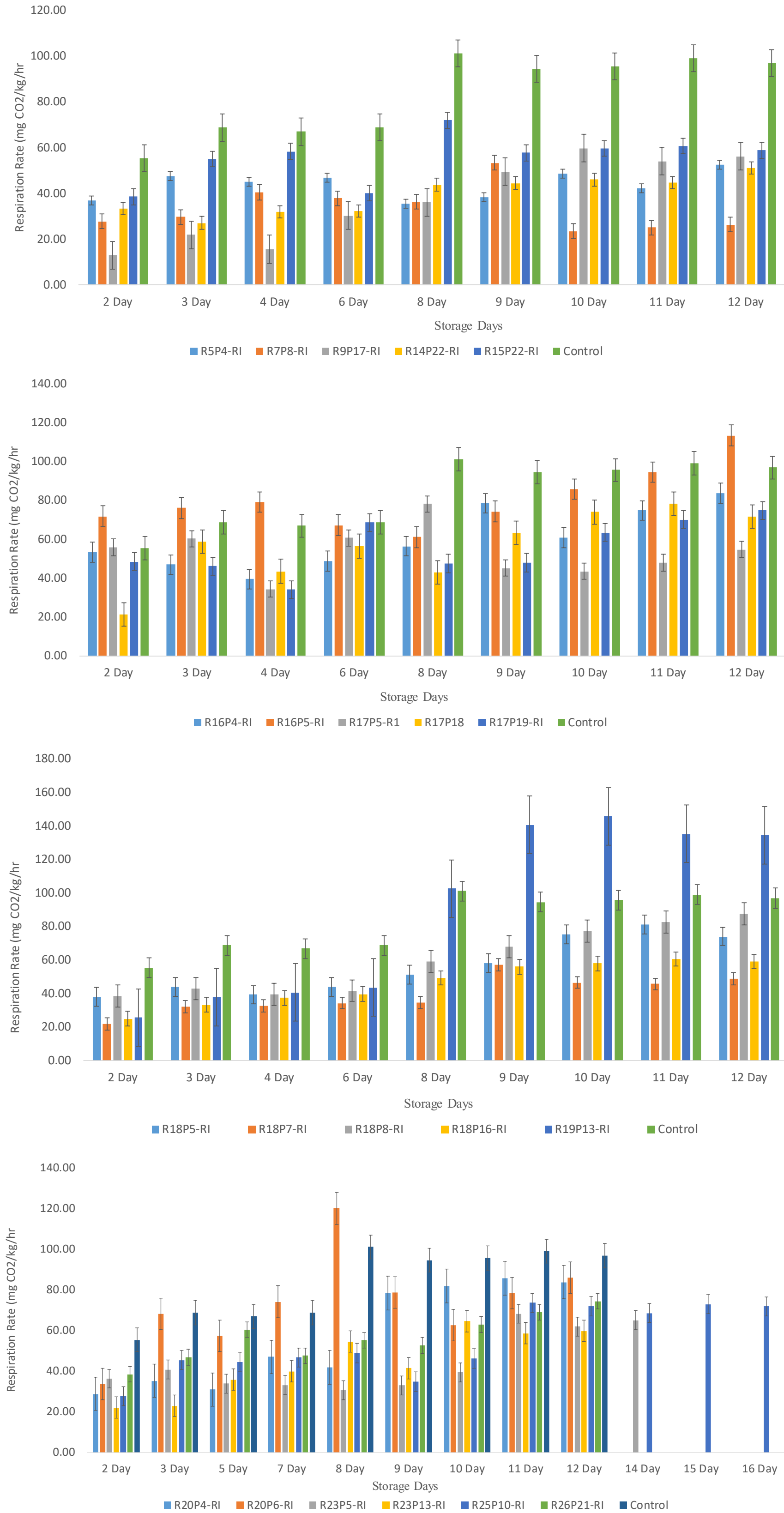

Fig. 4. Respiration rate of M_1 selected lines of papaya at 20°C storage.

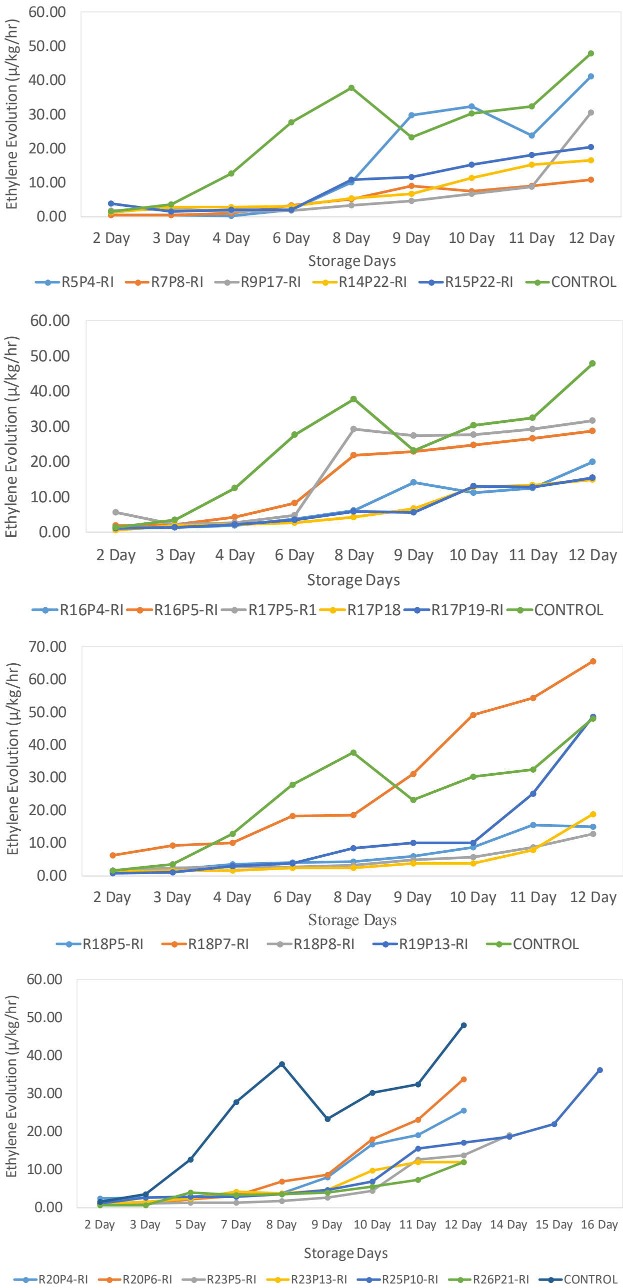

Fig. 5. Ethylene evolution rate of of M_1 selected lines of papaya at 20°C storage.

In the present investigation, among 21 selected lines of the M1 population along with the control, the highest PLW (%) was noticed in line R19P13 (14.84 %), whereas the lowest PLW (%) was noticed in R25P10 (5.96 %) on the 10th day of storage at 200C. The highest shelf life (days) was observed in line R25P10 (16.00 days), whereas the lowest shelf life (days) was recorded in control (10.00 days) (Table 3). This might be due to the effect of mutagen on metabolic processes, which leads to varied metabolic activities in mutant lines. These results are in congruence with the outcomes of Ramesh *et al.* (11) in papaya.

Table 3. PLW (%) and shelf life of selected lines of M_1 population of papaya stored at 20°C.

Mutant Lines	PLW % (4th Day storage)	PLW % (6th Day storage)	PLW % (8th Day storage)	PLW % (10th Day storage)	Shelf life (days)
R_5P_4	1.23	6.88	10.19	11.99	10.67
R_7P_8	2.32	4.15	7.64	9.06	12.33
R_9P_{17}	2.20	4.38	6.00	7.51	13.06
$R_{14}P_{22}$	1.82	3.75	6.16	8.30	12.67
$R_{15}P_{22}$	1.91	2.98	4.71	6.60	14.33
$R_{16}P_4$	2.90	5.08	6.99	8.85	13.00
$R_{16}P_5$	4.54	7.21	9.65	11.32	11.33
$R_{17}P_5$	2.51	4.18	6.60	8.90	12.00
$R_{17}P_{18}$	2.44	3.77	5.99	7.38	12.67
$R_{17}P_{19}$	2.26	3.74	5.35	7.50	13.33
$R_{18}P_5$	2.29	6.05	7.77	9.98	12.33
$R_{18}P_7$	1.67	2.56	3.65	8.86	13.33
$R_{18}P_8$	3.67	8.90	12.19	14.83	10.67
$R_{18}P_{16}$	1.36	2.95	4.27	9.74	12.33
$R_{19}P_{13}$	4.04	8.53	11.78	14.84	11.00
$R_{20}P_4$	1.79	3.63	5.36	8.52	13.00
$R_{20}P_6$	1.28	2.61	3.69	7.59	14.33
$R_{23}P_5$	5.79	8.17	11.63	12.21	12.00
$R_{23}P_{13}$	3.03	6.61	9.02	11.63	10.33
$R_{25}P_{10}$	1.76	3.60	4.75	5.96	16.00
$R_{26}P_{21}$	1.62	5.48	7.27	8.65	14.00
Control	2.56	4.27	6.68	10.72	10.00
Mean	2.50	4.98	7.15	9.59	12.49
Sem	0.11	0.23	0.32	0.14	0.56
CD @ 5%	0.32	0.65	0.92	0.39	1.59

AUTHORS' CONTRIBUTION

Research conceptualization (MRD and HSV); experiment designing (NVB, MRD and HSV); contribution of experimental materials (MRD, HSV and DVS); field/lab experiments execution and collection of data (NVB); Analysis of data and interpretation (GCA and NVB); manuscript preparation (NVB).

DECLARATION

The authors declare that there is no conflict of interest

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