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Evaluation of Putative Mutant Population of Papaya cv. Arka Prabhath for improved Biochemical Traits

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ABSTRACT: Papaya (*Carica papaya* L.) is one of the most important tropical fruit crop that has wide acceptance as a commercial crop for local and export purposes. It is being climacteric, perishable in nature and have low shelf life hence needs an improvement in terms of quality with improved shelf life. Mutation breeding has been an effective approach to produce improved varieties with desirable traits. In fruit crops, mutation breeding is preferable over conventional breeding because it prevents segregating progenies while refining the genetic composition through selection cycles. TILLING-based mutagenesis was undertaken to induce the variability by irradiating the seeds of papaya cv. Arka Prabhath with gamma rays ranging from 50 to 500 Gy. The outstanding mutant lines were evaluated for improved biochemical traits. Results revealed that there was significant increase in biochemical traits such as total soluble solids, Ascorbic acid, sugars, total carotenoids and lycopene content among various selected twenty-one mutant lines in comparison to control Arka Prabhath while there was reduction in acidity of the fruits. The highest ascorbic acid content of 65.63 mg/100g was recorded in line $R_{17}P_{19}$ with excellent taste among selected M_1 lines of papaya in contrast to control (33.13 mg/100g). Further, based on the estimation of fruit peel and pulp colour it was indicated that there was huge variation for pulp colour with delayed ripening. This indicates the efficacy of mutagen to induce variability in terms of fruit quality and shelf life.

Keywords: Papaya, Arka Prabhath, mutation, pulp colour, shelf life.

INTRODUCTION

Papaya (*Carica papaya* L.) belongs to the family Caricaceae and is native to tropical America, from southern Mexico to Costa Rica. It is one of the fruit crops which can be cultivated in both the tropical as well as subtropical regions of India with a significance of commercial as well as back yard crop. It is a rich source of vitamins having an approximate composition of 2020 IU of vitamin A, 40mg of vitamin B₁ and 46mg of vitamin C per 100g of fruit (Dinesh, 2010). The diversity coupled with varying agro-climatic conditions,

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especially with respect to duration allows the round the year availability of fruits. India stands first in production contributing 44.04 per cent of papaya in the world with production of 6.05 million tonnes from an area of 0.149 million hectares (FAOSTAT, 2018). Commercial cultivation in India can be seen in Andhra Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Assam, Odisha, Madhya Pradesh, Manipur, Tamil Nadu, Bihar and to a certain extent in Kerala (Anonymous, 2019). In India papaya production is revolutionized due to increasing demand of population and changes in technology but due to its climacteric behaviour, **14(4a): 723-728(2022)** 723

perishable in nature have low shelf life hence harvest and postharvest losses are reported together 7.36 percent wherein, the farm operation, storage, and transportation losses together account for 5.06, 2.28 and 1.13 percent, respectively (Kumar and Bhatnagar 2014). So far, increased efforts have been made to extend the shelf life through various coatings, modified storage and packaging conditions but developing new varieties through breeding strategy is the sole viable technique to extend the shelf life with improved quality. Mutation induction techniques such as irradiation are good tools for increasing variability in crop species because spontaneous mutations occur with an extremely low frequency. Mutation techniques have significantly contributed to plant improvement worldwide, and have made an outstanding impact on the productivity and economic value of some crops (Ahloowalia and Maluszynski 2001). In fruit crops, mutagenesis has already been used to introduce useful mutants related to dwarfing, blooming time and fruit ripening period, fruit colour, self-compatibility, self-thinning, improved quality and resistance to pathogens (Sanada and Amano 1998). TILLING (Targeting Induced Local Lesion IN Genome) a powerful reverse genetic strategy that allows the detection of induced point mutations in individuals of the mutagenized populations (McCallum et al., 2000). TILLING combines advantages of random mutagenesis and high throughput mutation discovery methods (Koornneef et al., 1982) and generates allelic series of the targeted genes which makes it possible to dissect the function of the protein as well as to investigate the role of lethal genes. Papaya is an ideal and attractive crop for TILLING as it is genomically the simplest fruit crop. Simple diploid genetics, small genome size (0.9 pg per haploid genome (Arumuganathan and Earle 1991), well studied genetics and a developing physical map, render papaya a better candidate for this technology. Keeping this as a baseline a study was conducted on evaluation of putative mutant population for improved biochemical traits.

MATERIALS AND METHODS

The experiment was undertaken at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru-560089, located at 13°58' North latitude and 78° East longitudes and at an altitude of 890 m above mean sea level. The soil is red sandy loam with a pH 6.0- 6.7. The mutant seedlings of papaya cv. Arka Prabhath were generated by treating its seeds with five different doses gamma radiation viz., 50 Gy, 100 Gy, 150 Gy, 250 Gy and 500 Gy. From the putative mutant population thus generated, a total of twenty-one outstanding plants based on the morphological characters were selected along with control (untreated Arka Prabhath plants) for further quality analysis.

Five fruit per replication were selected at random from each treatment and analysed, to study biochemical changes after storage and ripening. Small pieces of pulp

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were cut and immediately stored at -21 °C until chemical analysis. The samples were thawed and homogenised using vir Tishear (Virtis Company Inc., Gardiner, NY, USA), before estimating total soluble solids (TSS) as "Brix, sugars (total and reducing), ascorbic acid (mg/100 g), titrable acidity (TA %) and total carotenoids (mg/100 g) using standard methods of analysis (AOAC, 1995). TSS was measured using a hand refractometer (Erma Inc., Tokyo, Japan) and values were corrected to 20°C. Total carotenoids and lycopene were extracted with acetone until the pulp became colourless and pigments were then transferred into the petroleum ether phase. The absorbance was measured at 452 nm and 502 nm using a Spectronic 1201 spectrophotometer (Milton Rov Co., NY, USA).

The fruit peel and pulp colour was measured by using a colorimeter, Color Reader, CR-10 (Minolta Co., Ltd, Osaka, Japan; measuring area of 8 mm with 8/d viewing geometry using CIE Standard illuminant and expressed in terms of L* (lightness), a*, b*, C (chroma) and H (hue) values. L represents lightness of the color and ranges from 0 (black) to 100 (white). which refers to the ability of the object to reflect or transmit the light incident on it; the closer the value is to zero, the darker the sample. In fruits, luminosity is related to freshness, and the higher the luminosity value is, the fresher the fruits tend to be. The variable a* is the coordinate indicative of the color variation from green to red. Negative values indicate greener colors, and positive values indicate redder colors. The variable b* is the coordinate indicative of the color variation from yellow to blue. Negative values indicate bluer colors, and positive values indicate yellower colors. Hue is the actual colour of an object such as red, green, orange, vellow, blue, etc. On a colour wheel subtending 360°, red-purple are placed at the far right (or an angel of 0°); vellow, bluish-green and blue follow at 90° 180° and respectively. Chroma 270°. represents the purity/saturation of a colour, how pure or intense the hue is. The fruit surface colour was measured by taking readings at three different positions on the fruit surface. Similarly, pulp colour was examined by taking the homogenized papaya pulp into a transparent glass cup. Analysis of variance (ANOVA) was performed for biochemical data at 5% significant level using XL-stat (Addinsoft, 2021) and least significant difference was used to compare means at the 5% significance level.

RESULTS AND DISCUSSION

Papaya is a climacteric fleshy fruit characterized by fast ripening after harvest. During the relatively short postharvest period, papaya fruit undergoes several changes in metabolism that results in pulp softening and sweetening, as well as the development of characteristic aroma. The wall disassembling of the papaya cell wall appears to help in fruit sweetness, while glucose and fructose are also produced by acidic invertases. The increase in ethylene production also results in carotenoid 14(4a): 723-728(2022)

accumulation due to the induction of cyclases and hydroxylases, leading to yellow and red/orange-colored pulp phenotypes. Moreover, the production of volatile compounds is an important biological marker for papaya's sensorial quality, which is also induced by ethylene (Fabi *et al.*, 2019). In this study the data pertaining to biochemical traits in selected mutant lines of M_1 indicated that there was significant difference in comparison to control (Table 1).

Significantly lower acidity was recorded in mutant line $R_{16}P_5$ (0.064%) while control fruits highest in acidity (0.256%). The other quality attributes. Total soluble solids and total sugars was significantly highest in $R_{17}P_5$ (15.43 °B & 13.90%) whereas, lowest was recorded in $R_{19}P_{13}$ (10.10°B) and $R_{15}P_{22}$ (6.69%) respectively. The most affected trait was found to be ascorbic acid with significant increase in amount of 65.63 mg/100g in $R_{17}P_{19}$ when compared to control (33.13 mg/100g). The results are in close proximity with the findings of Kumar *et al.* (2021) in Kinnow mandarin with improved TSS

and ascorbic acid content. Papaya is a rich source of carotene and lycopene which is readily available source of pro vitamin A. The highest total carotenoid and lycopene content was recorded in $R_{14}P_{22}$ (3.15 mg/100g) and R₁₈P₈ (1.90 mg/100g) respectively. Out of 21 selected lines among M₁ population, 9 lines were observed to be excellent in taste other mutant lines with good and moderate taste. This might be due to mutagens, which might have targeted some key enzymes in the metabolic processes leading to up-regulation and downregulation of metabolism and reduced enzyme activity among mutant populations responsible for higher and lower retention of biochemical constituents. The changes in chemical attributes are due to the stimulatory and inhibitory effect of enzymes depending on the biological damage caused by the mutagen dose. The finding of the present study aligns with Kumar et al. (2017); Pujar et al. (2019); Ramesh et al. (2019); Hussain et al. (2019); Panou et al. (2020) in papaya and Bhat et al. (2017) in strawberry.

Mutant Lines	Acidity %	TSS (°Brix)	Ascorbic acid (mg/ 100g)	Carotene (mg/100g)	Lycopene (mg/100g)	Reducing Sugar %	Total Sugar %	Non- Reducing Sugar (%)	Taste
R5P4	0.128	12.83	51.25	3.06	1.70	8.07	9.04	0.97	Good
R7P8	0.128	12.00	53.13	2.28	1.31	7.41	8.29	0.88	Intermediate
R ₉ P ₁₇	0.128	13.53	33.13	1.89	1.15	7.06	8.90	1.84	Excellent
R14P22	0.128	13.97	42.50	3.15	1.76	8.95	9.37	0.42	Excellent
R15P22	0.128	11.33	33.13	1.85	1.02	6.03	6.69	0.67	Intermediate
R ₁₆ P ₄	0.128	14.47	60.63	2.11	0.94	8.77	12.05	3.28	Excellent
R ₁₆ P ₅	0.064	10.60	58.13	2.33	1.29	7.01	8.61	1.60	Good
R 17 P 5	0.128	15.43	49.38	1.81	1.00	9.04	13.90	4.87	Excellent
R 17 P 18	0.128	10.80	53.75	1.97	1.04	6.46	6.69	0.24	Intermediate
R 17 P 19	0.128	12.13	65.63	2.35	1.43	7.53	8.82	1.29	Excellent
R18P5	0.128	14.10	52.50	3.00	1.73	9.51	9.77	0.26	Good
R 18 P 7	0.128	13.27	38.75	1.86	1.21	8.53	10.04	1.52	Good
R 18 P 8	0.128	15.17	48.13	3.07	1.90	8.45	8.82	0.37	Excellent
R ₁₈ P ₁₆	0.256	12.80	45.00	1.60	0.66	10.89	12.05	1.16	Good
R 19 P 13	0.128	10.10	38.75	1.99	1.25	6.90	7.12	0.22	Intermediate
R20P4	0.192	11.53	41.25	2.06	1.26	7.66	10.33	2.67	Intermediate
R20P6	0.192	12.60	61.25	4.46	1.90	7.53	8.61	1.08	Good
R ₂₃ P ₅	0.064	12.77	50.63	3.07	1.79	7.29	9.51	2.23	Excellent
R ₂₃ P ₁₃	0.128	12.10	45.00	1.84	1.22	6.32	6.46	0.14	Good
R25P10	0.128	13.00	41.25	2.26	1.14	6.23	7.29	1.06	Excellent
R26P21	0.128	12.57	46.25	1.76	1.05	7.41	9.67	2.26	Good
Control A.P	0.256	11.70	33.13	2.31	0.94	6.46	8.22	1.76	Excellent
Mean	0.140	12.67	47.39	2.37	1.30	7.70	9.10	1.40	
SEM	0.000	0.35	2.20	0.03	0.02	0.41	0.39	0.04	
CD	0.013	1.00	6.28	0.10	0.07	1.17	1.11	0.12	
Treatment	*	*	*	*	*	*	*	*	

Table 1: Biochemical parameters of selected M₁lines of papaya.

In the selected lines of M_1 population varied fruit peel & pulp colour was observed for values L*, a*, b*, C* (NBS units) and hue angle (h⁰). In M_1 population for fruit peel colour, the highest L* value of 58.90 (NBS units) was observed in line $R_{20}P_6$ whereas, lowest L* value of 50.10 (NBS units) was observed in line $R_{23}P_{13}$. Mean colour

space value a* ranged from 23.37 in $R_{18}P_{16}$ to 9.40 in $R_{18}P_7$. Mean colour space value b* ranged from 61.63 in $R_{20}P_6$ to 41.80 in $R_{15}P_{22}$. C*, Chroma/chromaticity ranged from 64.22 in $R_{20}P_6$ to 44.84 in $R_{15}P_{22}$. Hue angle ranged from 78.43° in $R_{18}P_7$ to 65.04° in $R_{18}P_{16}$ (Table 2).

Table 2: Fruit peel colour indexes L*, a*, b*, C* and hue angle (h°) of selected lines of M₁population of papaya.

Mutant Lines	L*	a*	b*	Chroma	Hue angle
R5P4	51.57 ± 1.90	17.03 ± 3.29	45.63 ± 2.08	48.75 ± 3.02	69.64 ± 2.93
R 7 P 8	51.40 ± 1.04	13.63 ± 2.46	47.10 ± 0.36	49.07 ± 0.37	73.87 ± 2.86
R 9 P 17	53.43 ± 1.25	18.83 ± 2.27	52.00 ± 0.44	55.33 ± 0.81	70.11 ± 2.25
R14P22	54.97 ± 2.56	17.50 ± 2.86	56.00 ± 2.86	58.71 ± 3.19	72.68 ± 2.47
R15P22	50.33 ± 1.57	16.17 ± 0.85	41.80 ± 2.65	44.84 ± 2.18	68.78 ± 2.19
R16P4	54.40 ± 0.89	21.53 ± 3.95	47.80 ± 1.61	52.49 ± 2.87	65.85 ± 3.40
R16P5	54.03 ± 2.38	15.80 ± 2.56	49.70 ± 1.11	52.18 ± 1.84	72.42 ± 2.30
R17P5	53.83 ± 3.01	19.97 ± 0.71	50.17 ± 1.99	54.01 ± 1.59	68.27 ± 1.46
R17P18	48.80 ± 0.44	15.60 ± 2.87	43.60 ± 2.52	46.33 ± 3.33	70.42 ± 2.27
R ₁₇ P ₁₉	50.83 ± 2.83	16.17 ± 2.38	46.27 ± 3.84	49.02 ± 4.39	70.81 ± 1.23
R ₁₈ P ₅	50.87 ± 0.55	19.50 ± 2.78	47.50 ± 1.15	51.40 ± 0.97	67.70 ± 3.19
R ₁₈ P ₇	52.10 ± 3.01	9.40 ± 2.75	45.40 ± 3.24	46.40 ± 3.68	78.43 ± 2.63
R ₁₈ P ₈	53.63 ± 0.67	22.50 ± 1.21	49.63 ± 1.33	54.50 ± 1.63	65.62 ± 0.80
R 18 P 16	52.27 ± 1.72	23.37 ± 1.03	50.23 ± 1.48	55.42 ± 0.91	65.04 ± 1.62
R 19 P 13	54.07 ± 2.50	13.27 ± 2.73	53.07 ± 3.95	54.75 ± 3.86	75.93 ± 2.94
R ₂₀ P ₄	57.53 ± 1.70	18.43 ± 2.22	57.97 ± 0.91	60.85 ± 1.47	72.38 ± 1.78
R20P6	58.90 ± 0.69	17.97 ± 2.50	61.63 ± 1.31	64.22 ± 1.94	73.78 ± 1.83
R23P5	53.83 ± 2.02	14.80 ± 0.96	56.37 ± 1.72	58.29 ± 1.46	75.27 ± 1.30
R23P13	50.10 ± 2.98	17.43 ± 2.65	47.57 ± 2.71	50.68 ± 3.43	69.95 ± 1.86
R25P10	56.40 ± 2.86	19.83 ± 1.23	58.40 ± 3.26	61.69 ± 3.20	71.22 ± 1.27
R26P21	53.40 ± 1.80	15.37 ± 8.21	43.57 ± 3.53	46.54 ± 5.73	71.31 ± 8.84
Control A.P	50.43 ± 0.76	15.63 ± 1.76	48.53 ± 0.73	51.01 ± 0.26	72.14 ± 2.13



Fig. 2. Pulp colour variation in M1 putative mutant population.Bhat et al.,Biological Forum – An International Journal14(4a): 723-728(2022)

For fruit pulp colour, the highest L* value of 38.37 (NBS units) was observed in line R_5P_4 whereas, lowest L* value of 32.83 (NBS units) was observed in $R_{23}P_{13}$. Mean colour space value a* ranged from 15.23 in $R_{18}P_8$ to 5.43 in $R_{23}P_{13}$. Mean colour space value b* ranged from 30.07 in $R_{18}P_5$ to 16.80 in $R_{23}P_{13}$. C*, Chroma/chromaticity ranged from 33.26 in $R_{18}P_5$ to 17.66 in $R_{23}P_{13}$. Hue angle

ranged from 72.11° in $R_{23}P_{13}$ to 59.60° in $R_{18}P_8$ (Table 3 & Fig. 1). This might be due to variation in carotenoid accumulation due to the induction of cyclases and hydroxylases, leading to yellow and red/orange-colored pulp phenotypes The results are in accordance with Ramesh *et al.* (2019), Pujar *et al.* (2019).

Table 3: Fruit pulp colour indexes L*, a*, b*, C* and hue angle (h°) of selected lines of M1population of papaya.

Mutant Lines	L*	a*	b*	Chroma	Hue angle
R 5 P 4	38.37 ± 0.67	12.77 ± 0.58	26.93 ± 1.44	29.81 ± 1.54	64.63 ± 0.26
R ₇ P ₈	35.20 ± 0.87	10.60 ± 0.60	24.17 ± 0.86	26.39 ± 0.90	66.32 ± 1.16
R 9 P 17	36.17 ± 0.06	10.87 ± 0.42	25.33 ± 0.40	27.57 ± 0.50	66.79 ± 0.62
$R_{14}P_{22}$	34.00 ± 0.66	10.57 ± 1.53	19.97 ± 0.59	22.61 ± 1.21	62.21 ± 2.84
R 15 P 22	36.50 ± 0.46	10.17 ± 0.29	26.47 ± 0.35	28.35 ± 0.42	68.99 ± 0.36
$R_{16}P_4$	36.47 ± 0.21	11.10 ± 0.26	28.37 ± 0.15	30.46 ± 0.24	68.63 ± 0.36
R ₁₆ P ₅	34.23 ± 0.31	9.53 ± 0.42	23.30 ± 0.26	25.18 ± 0.39	67.75 ± 0.70
R ₁₇ P ₅	34.53 ± 0.42	8.70 ± 1.73	23.03 ± 0.71	24.65 ± 1.27	69.41 ± 3.16
R17P18	37.80 ± 0.10	10.33 ± 0.06	28.30 ± 0.17	30.13 ± 0.15	69.94 ± 0.19
R ₁₇ P ₁₉	35.73 ± 0.21	13.53 ± 0.32	25.40 ± 0.26	28.78 ± 0.38	61.95 ± 0.32
R ₁₈ P ₅	36.60 ± 0.36	14.13 ± 1.59	30.07 ± 5.42	33.26 ± 5.34	64.61 ± 3.09
R ₁₈ P ₇	35.57 ± 1.42	10.37 ± 0.25	23.90 ± 1.40	26.05 ± 1.36	66.52 ± 0.93
R18P8	35.67 ± 0.21	15.23 ± 0.25	25.97 ± 0.38	30.11 ± 0.35	59.60 ± 0.54
R18P16	33.77 ± 0.68	8.90 ± 0.87	20.33 ± 1.17	22.20 ± 1.42	66.40 ± 0.91
R 19 P 13	34.33 ± 0.38	7.63 ± 0.15	17.80 ± 0.40	19.37 ± 0.35	66.78 ± 0.71
R20P4	33.07 ± 1.27	8.93 ± 1.68	19.07 ± 1.70	21.07 ± 2.24	65.06 ± 2.32
R20P6	37.80 ± 0.30	12.57 ± 0.06	26.37 ± 0.47	29.21 ± 0.44	64.51 ± 0.34
R23P5	35.27 ± 0.68	12.30 ± 1.14	24.07 ± 3.52	27.03 ± 3.64	62.80 ± 1.44
R ₂₃ P ₁₃	32.83 ± 0.38	5.43 ± 0.55	16.80 ± 0.78	17.66 ± 0.91	72.11 ± 0.95
R25P10	35.73 ± 0.12	12.13 ± 0.38	26.47 ± 7.75	29.21 ± 7.21	64.49 ± 5.37
R ₂₆ P ₂₁	33.20 ± 1.13	11.33 ± 0.25	22.27 ± 1.01	24.99 ± 0.80	62.99 ± 1.56
Control A.P	33.05 ± 0.93	12.20 ± 0.66	25.47 ± 1.08	28.24 ± 1.02	64.39 ± 1.51

CONCLUSION

The results from the evaluation of putative mutant population indicates that gamma rays an effective tool to inducing mutation. It provides an opportunity to develop varieties with improved quality and taste with good biochemical composition. Considerable changes in the fruit peel and pulp colour was observed in this study which makes a way for developing attractive cultivars which are rich in antioxidants and it also meets the different market preferences with increased shelf life and delayed ripening.

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Conflict of Interest. None.

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