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RESEARCH ARTICLE

Performance of seedless and sparse-seeded mutants in sweet orange and mandarin genotypes induced through Gamma-irradiation mutagenesis

Ashutosh A. Murkute, A. Thirugnanavel*, Subhra Saikat Roy, J. Prasanth Tej Kumar, Prafulla Jalamkar, Manju Gurjar and Dilip Ghosh

Abstract

Citrus fruits, a globally significant crop, face consumer and processing challenges due to the presence of seed. Conventional breeding methods to develop seedless varieties are time-consuming and often unsuccessful due to the complex genetic nature of citrus. This study explores the potential of gamma-irradiation mutagenesis in producing seedless or sparsely-seeded mutants. Dormant budwoods of sweet orange cv. Mosambi (Tm-33), Kinnow, and Nagpur mandarin (N-4 and N-74) were irradiated with gamma doses (5–40 Gy) and grafted onto rough lemon rootstocks. Observations were recorded from mutant generations (MV2) and analyzed over two seasons. Significant variations in fruit weight, size, rind thickness, juice content, and total soluble solids (TSS) were observed. On average, the physical parameters of the fruit, such as weight, length, diameter, axis diameter, and rind thickness, were found to be inversely proportional to increasing doses of gamma-irradiation. Notably, a sweet orange mutant (Tm33-5-B2R2-P01) exhibited reduced seed count (3.67 seeds/fruit) with satisfactory TSS (9.70°Brix) compared to the parent (12.25 seeds/fruit). Kinnow mandarin mutant KM-40-B2R1-P19 showed the lowest seed count (1.50 seeds/fruit) and good TSS (9.03°Brix) compared to the parent (14.75 seeds/fruit). Nagpur mandarin mutant N4-5-B3R1-P05 was identified as seedless (0 seeds/fruit), maintaining favorable TSS (10.07°Brix) compared to parent (12.23 seeds/fruit). The results demonstrate the potential of gamma-irradiation in citrus breeding to meet consumer demands for quality seedless fruits.

Keywords: Sweet orange, fruit quality, mandarin, sweet orange, gamma-irradiation, mutants, seedlessness.

Introduction

Citrus is one of the most significant fruit crops globally, cultivated in 137 countries. According to the FAO (2023), global citrus production reached 166.30 million tonnes. The leading citrus-producing countries are China, with 48.77 million tonnes, Brazil, with 19.73 million tonnes, and India, with 14.76 million tonnes. Sweet orange represents the largest portion of citrus production worldwide, accounting for approximately 45.95% of the total. This is followed by mandarin, tangerine, and clementine (26.57%), lemon and lime (12.95%), pummelo and grapefruit (5.87%), and other citrus fruits (8.67%). In India, citrus is the third most important fruit crop, following banana and mango. It covers an area of 1.10 million hectares, producing 14.76 million tonnes of fresh fruits. Among the citrus varieties in India, mandarins (*Citrus reticulata* Blanco) are the most commercially significant, constituting 42.30% of the citrus crop, followed by sweet orange (*C. sinensis* Osbeck) with 26.89%, lime and lemon (*C. aurantifolia* Swingle and *C. limon* L.) with 25.93%, and other citrus fruits at 4.88%. Among mandarin oranges in India, Nagpur and Kinnow mandarin are the two most important varieties. Commercially, Kinnow mandarin is cultivated in Punjab, Haryana, Himachal Pradesh, the western part of Rajasthan, and Uttarakhand. In contrast, Nagpur mandarin is grown in the Vidarbha region of Maharashtra, the adjoining areas of Madhya Pradesh, and the Jhalawar areas of Rajasthan. Sweet orange is primarily grown in the Marathwada region of Maharashtra, including

ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, India.

***Corresponding Author:** A. Thirugnanavel, ICAR-Central Citrus Research Institute, Nagpur 440 033, Maharashtra, India, E-Mail: lotus.thiru@gmail.com

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the Aurangabad, Jalna, Parbhani, and Nanded districts, as well as in Andhra Pradesh, particularly in the Nalgonda, Prakasam, and Anantapur districts.

Both sweet orange and mandarin orange are widely consumed worldwide for their nutritional and health benefits. However, the presence of seeds in these fruits can be a major deterrent for consumers, especially in the fresh fruit market (Purba et al. 2021), as well as for processors. Most mandarin orange varieties in India have seeds (13–15 seeds per fruit). Among sweet oranges, the Mosambi cultivar is grown commercially, but it suffers from poor fruit quality, with low acidity (0.25%), colorless juice lacking flavor, and high seed content (20–25 seeds per fruit). Developing seedless or less-seeded citrus varieties with high sensory and quality attributes through traditional breeding methods can be a challenging and time-consuming process, often with limited success. The long juvenile period and the highly heterozygous, polygenic nature of citrus limit the success rate of conventional breeding methods.

In recent decades, mutation breeding has gained momentum due to its widespread success in producing improved crop varieties with desirable qualitative and quantitative traits. Gamma-irradiation technology has been successfully used in citrus to increase grapefruit yield (Pandey 2009), develop promising seedless lines of mandarin and pummelo (Sutarto et al. 2009), create resistant lines to citrus canker in mandarin and sweet orange (Junior et al. 2009) and low seeded Kinnow mandarin (Kumar et al. 2023). However, determining the optimal dose of gamma-irradiation is crucial to achieving the desired mutation, such as seedlessness, without causing significant damage to the plant (Purba et al. 2021). Despite the potential, there has been limited work on induced mutations through irradiation in citrus in India. Given this background, the present study aims to investigate the effect of gamma-irradiation dose that can effectively reduce the number of seeds in sweet orange, Kinnow mandarin and Nagpur mandarin while improving overall fruit quality.

Materials and methods

Plant materials and irradiation treatment

The study was conducted at the research farm of ICAR-Central Citrus Research Institute in Nagpur, Maharashtra. It included sweet orange var. Mosambi (Tm-33), Kinnow mandarin, and Nagpur mandarin (N-4 and N-74). In 2015, gamma-irradiation of dormant budwoods was performed using a cobalt (^{60}Co) source at doses of 5, 10, 15, 20, 30 and 40 Gy. The irradiated buds were grafted onto a one-year-old rough lemon (*C. jambhiri* Lush) rootstock. These budlings were then planted in containers and maintained under protected conditions. Buds from the first mutant vegetative generation (MV_1) were further used to raise budlings of the MV_2 generation on rough lemon rootstock. Since citrus trees require substantial space and time to produce results, mutation breeding necessitates not only determining the optimal irradiation dosage but also minimizing the number of resulting plants. This approach ensures a reasonable probability of finding seedless mutants that produce high-quality fruits. In the present experiment, a total of 221 budlings were planted in the experimental field (21°15′N, 79°02′E, and 335 m above mean sea level) in a randomized block design with three replications. Separate blocks for the mutant populations of sweet orange, Kinnow mandarin, and Nagpur mandarin were maintained alongside non-irradiated mother parents as control. Recommended crop management practices were followed to raise the plants having identical soil, environmental and cultural conditions, ensuring growth was not influenced by varying climatic factors or cultivation techniques. After four years, good fruiting was observed in the mutant populations.

Recording of observations

Observations were recorded from the representative fruit samples (15 fruits per mutant) harvested over two seasons (2021 and 2022) and the pooled data were used for analysis. Fruits were harvested in November at optimal maturity, determined by fruit size and peel color. Fruit weight was measured with a semi-analytical weighing balance, and fruit height, diameter, and rind thickness were measured with millimetric Vernier calipers. The number of segments and seeds per fruit was recorded after cutting the fruits in two halves and extracting the juice with a simple hand extractor. Total soluble solids (TSS) in the juice were measured using a thermostat-based refractometer DR6000 Series (A KrussOptronic, Germany) having a scale range of 0 to 95 °Brix. The titratable acidity of samples was determined according to the method outlined by Ranganna (1987).

Data analysis

All data were expressed as means. One-way ANOVA analyses were performed using the SPSS statistical package, and the Tukey HSD test method ($p < 0.05$) was applied to estimate significant differences among observations. Figures were constructed using Microsoft Office 365 software.

Results and discussion

The present study aimed to study the effect of gamma-irradiation dose for effectively reducing the seed count in these fruits while enhancing the fruit quality with the ultimate goal of developing superior varieties. The perusal data indicates that gamma-irradiation significantly impacted the physico-chemical properties of sweet orange var. Mosambi (Tm-33), Kinnow mandarin, and Nagpur mandarin (N-4 and N-74).

Sweet orange

Gamma-irradiation at doses of 5, 10, 15, 20, and 30 Gy resulted in significant variations in various physico-chemical

Table 1a. Effect of gamma-irradiation on weight, length, diameter and axis diameter of sweet orange mutants

Mutant code	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit axis diameter (mm)
Tm33-5-B2R2-P15	113.00^{u}	59.13^{klm}	59.75^{pq}	11.82^{abcd}
Tm33-5-B2R2-P03	155.00^{lm}	67.60^{cdef}	64.41^{klmn}	$10.56^{cdefghij}$
Tm33-5-B2R2-P01	151.67^{mno}	66.66^{cdefgh}	66.96^{ghijkl}	$10.64^{cdefghi}$
Tm33-5-B2R2-P06	276.50^{a}	82.89^{a}	81.83^{a}	13.04^{a}
Tm33-10-B2R2-P12	152.75^{mn}	64.51^{fghij}	67.51^{fghijk}	$9.65^{fghijklmn}$
Tm33-10-B2R2-P08	247.50^{c}	74.82^{b}	79.35^{ab}	12.45^{abc}
Tm33-10-B2R2-P07	111.75^{u}	58.37^{lm}	58.12^{q}	$8.99^{hijklmnop}$
Tm33-10-B2R2-P11	254.00^{b}	82.94^{a}	79.98^{ab}	$9.24^{ghijklmno}$
Tm33-10-B2R2-P15	151.50^{mno}	66.49^{defgh}	65.53^{ijklmn}	$9.31^{ghijklmno}$
Tm33-10-B2R2-P14	150.25^{nop}	64.89^{fghij}	64.87^{jklmn}	8.22^{lmnop}
Tm33-10-B2R2-P10	147.50^{p}	63.53^{hij}	63.58^{mno}	7.94^{nop}
Tm33-10-B2R2-P09	114.50^{u}	57.60^{lm}	61.01^{opq}	$10.41^{defghij}$
Tm33-10-B2R2-P16	214.00^{e}	73.59^{b}	75.04^{c}	11.02^{bcdefg}
Tm33-15-B2R2-P25	157.75^{kl}	69.90^{c}	$66.65^{ghijklm}$	$8.73^{jklmnop}$
Tm33-15-B2R2-P24	174.50^{hi}	66.87^{cdefg}	70.71^{de}	$10.00^{defghijkl}$
Tm33-15-B2R2-P17	136.00^{r}	63.63^{ghij}	64.38^{klmn}	$10.25^{defghijk}$
Tm33-15-B2R2-P18	183.00^{g}	69.28^{cd}	71.86^{d}	11.26^{abcdef}
Tm33-15-B2R2-P23	148.00^{op}	65.25^{efghi}	$65.75^{hijklmn}$	8.05^{mnop}
Tm33-15-B2R2-P21	142.25^{q}	63.62^{ghij}	65.03^{jklmn}	8.45^{klmnop}
Tm33-15-B2R2-P26	172.50^{i}	67.56^{cdef}	69.06^{defg}	11.07^{bcdefg}
Tm33-15-B2R2-P19	141.75^{q}	62.17^{ijk}	64.27^{lmn}	$9.68^{fghijklmn}$
Tm33-15-B2R2-P27	227.25^{d}	76.49^{b}	76.83^{bc}	12.92^{ab}
Tm33-15-B2R2-P22	89.50^{x}	51.85^{n}	58.54^{q}	10.79^{cdefgh}
Tm33-20-B2R3-P10	163.00^{j}	65.98^{efgh}	68.72^{defgh}	$9.52^{fghijklmno}$
Tm33-20-B2R4-P03	135.75^{r}	60.11^{kl}	62.95^{no}	$9.89^{efghijklm}$
Tm33-20-B2R3-P01	182.25^{g}	68.37^{cde}	70.19^{def}	11.61^{abcde}
Tm33-20-B2R3-P07	106.00^{v}	57.34^{lm}	57.89^{q}	7.66^{op}
Tm33-20-B2R3-P08	160.00^{jk}	65.58^{efgh}	67.87^{efghij}	$10.34^{defghijk}$
Tm33-20-B2R3-P06	188.67^{f}	69.39^{cd}	69.61^{defg}	$8.76^{ijklmnop}$
Tm33-20-B2R3-P09	176.75^{h}	68.31^{cde}	68.38^{efghi}	10.82^{cdefgh}
Tm33-30-B2R3-P16	131.75^{g}	61.74^{jk}	63.22^{no}	$9.57^{fghijklmn}$
Tm33-30-B2R3-P14	125.75^{t}	60.12^{kl}	62.78^{nop}	7.16^{p}
Tm33-30-B2R3-P13	95.50^{w}	56.18m	58.55^{q}	$9.65^{fghijklmn}$
Tm33-Control (Mother)	161.00^{jk}	65.98^{efgh}	67.72^{efghij}	$9.62^{fghijklmn}$
Range	89.50-276.50	51.85-82.94	57.89-8183	7.16-13.04
CD ($P \leq 0.05$)	3.952	2.844	2.664	0.966
SEM (±)	1.396	1.005	0.941	0.341
CV (%)	1.512	2.644	2.432	5.927

The numerical values with same alphabetical notations are not statistically significant at P ≤ 0.05as per Tukey's HSD test

Table 1b. Effect of gamma-irradiation on rind thickness, number of segments, juice content and titratable acidity of sweet orange and mutants

Mutant code	Rind thickness (mm)	No. of segments	Juice content (%)	Titratable acidity (%)
Tm33-5-B2R2-P15	5.39^{cde}	11.00^{abcd}	32.76^{i}	0.38^{bc}
Tm33-5-B2R2-P03	5.84^{bcd}	12.00^{abc}	30.32^{m}	0.64^{bc}
Tm33-5-B2R2-P01	4.86^{defg}	10.67^{bcd}	33.68^{fg}	0.38^{bc}
Tm33-5-B2R2-P06	6.93^{b}	10.75^{bcd}	26.69^{q}	0.52^{bc}
Tm33-10-B2R2-P12	4.02^{ghijklm}	11.00^{abcd}	32.65^{i}	0.49^{bc}
Tm33-10-B2R2-P08	6.30^{bc}	12.50^{ab}	28.98^{n}	0.41^{bc}
Tm33-10-B2R2-P07	3.68^{hijklm}	10.75^{bcd}	31.59^{l}	0.41^{bc}
Tm33-10-B2R2-P11	4.53^{efghij}	10.75^{bcd}	15.75^{u}	0.44^{bc}
Tm33-10-B2R2-P15	3.43^{klm}	10.5^{bcd}	38.26^{a}	0.35^{c}
Tm33-10-B2R2-P14	8.22^{a}	3.20^{e}	16.25^{t}	0.37^{c}
Tm33-10-B2R2-P10	5.32^{cde}	11.00^{abcd}	35.03^{e}	0.48^{bc}
Tm33-10-B2R2-P09	3.64^{hijklm}	10.75^{bcd}	36.46^{c}	0.56^{bc}
Tm33-10-B2R2-P16	3.51^{ijklm}	11.50^{abcd}	26.65^{q}	0.48^{bc}
Tm33-15-B2R2-P25	3.99^{ghijklm}	10.75^{bcd}	33.28^{h}	0.65^{bc}
Tm33-15-B2R2-P24	3.81^{ghijklm}	10.75^{bcd}	35.22^{e}	0.49^{bc}
Tm33-15-B2R2-P17	4.19^{fghijkl}	10.50^{bcd}	27.63^{op}	0.61^{bc}
Tm33-15-B2R2-P18	3.81^{ghijklm}	10.75^{bcd}	27.40^{p}	0.46^{bc}
Tm33-15-B2R2-P23	3.38^{klm}	10.25^{cd}	29.21^{n}	0.48^{bc}
Tm33-15-B2R2-P21	3.20^{lm}	10.25^{cd}	36.87^{b}	0.57^{bc}
Tm33-15-B2R2-P26	4.33^{efghijk}	11.75^{abcd}	33.25^{h}	0.48^{bc}
Tm33-15-B2R2-P19	3.05^{m}	10.75^{bcd}	30.31^{m}	0.72^{b}
Tm33-15-B2R2-P27	5.39^{cde}	13.00^{a}	23.15^{r}	0.64^{bc}
Tm33-15-B2R2-P22	4.82^{defg}	10.50^{bcd}	23.41^{r}	1.12^{a}
Tm33-20-B2R3-P10	3.44^{jklm}	10.75^{bcd}	30.07^{m}	0.35^{c}
Tm33-20-B2R4-P03	4.36^{efghijk}	11.50^{abcd}	32.19^{jk}	0.54^{bc}
Tm33-20-B2R3-P01	5.15^{def}	11.00^{abcd}	32.51^{ij}	0.37^{c}
Tm33-20-B2R3-P07	3.47^{ijklm}	11.25^{abcd}	21.41^{s}	1.07^{a}
Tm33-20-B2R3-P08	4.54^{efghi}	9.75^{d}	33.39^{gh}	0.44^{bc}
Tm33-20-B2R3-P06	4.02^{ghijklm}	10.67^{bcd}	34.00^{f}	0.51^{bc}
Tm33-20-B2R3-P09	4.67^{efgh}	11.75^{abcd}	33.20^{h}	0.40^{bc}
Tm33-30-B2R3-P16	3.57^{ijklm}	11.00^{abcd}	32.10^{k}	0.48^{bc}
Tm33-30-B2R3-P14	3.85^{ghijklm}	10.75^{bcd}	35.75^{d}	0.41^{bc}
Tm33-30-B2R3-P13	4.13^{fghijklm}	11.5^{abcd}	27.95^{o}	0.62^{bc}
Tm33-Control (Mother)	3.44^{jklm}	10.75^{bcd}	32.07^{k}	0.35^{c}
Range	3.05-8.22	3.20-13.0	15.75-38.26	0.35-1.12
CD ($P \leq 0.05$)	0.318	1.107	0.039	0.030
SEM (±)	0.113	0.391	0.014	0.011
CV (%)	4.409	6.29	0.079	3.516

The numerical values with same alphabetical notations are not statistically significant at $P \leq 0.05$as per Tukey's HSD test characteristics of sweet orange fruits. However, irradiation at 40 Gy did not produce any viable results (no flowering). On average, fruit weight was found to be inversely proportional to the increasing doses of gamma-irradiation. Similar trends were observed for fruit length, diameter, axis diameter, and rind thickness. In the mutant population, fruit weight ranged from 89.50 g (Tm33-15-B2R2-P22) to 276.50 g (Tm33-5-B2R2-P06), whereas the fruit weight in control plants was 161.00 g. Likewise, the fruit length, diameter, and axis diameter ranged from 51.85 mm (Tm33-15-B2R2-P22) to 82.94 mm (Tm33-10-B2R2-P11), 57.89 mm (Tm33-20-B2R3-P07) to 81.83 mm (Tm33-5-B2R2-P06), and 7.16 mm (Tm33-30-B2R3-P14) to 13.04 mm (Tm33-5-B2R2-P06), respectively. For the control plants, these values were 65.98, 67.72, and 9.62 mm, respectively (Table 1a).

Data in Table 1b describes the effect of gamma-irradiation on the rind thickness, number of segments, juice content, and titratable acidity of the sweet orange fruits. There was no substantial variation in rind thickness and number of segments per fruit among the different irradiation doses; however, significant differences were recorded among the mutant selections.

The thinnest rind (3.05 mm) was observed in Tm33-15-B2R2-P19, while the thickest (8.22 mm) was found in Tm33-10-B2R2-P14, compared to the control (3.44 mm). Among the mutant population, the minimum number of segments (3.20) was recorded in Tm33-10-B2R2-P14, whereas the maximum segment count (13.00) was found in Tm33-15-B2R2-P27. In the control group, fruits had an average of 10.75 segments per fruit. The irradiation treatment of budwood also resulted in significant differences in juice content, ranging from 15.75% in Tm33-10-B2R2-P11 to 38.26% in Tm33-10-B2R2-P15, compared to an average juice content of 32.07% in the control plants. Regarding titratable acidity of the juice, the mutant population showed significant variation, with levels ranging from 0.35% (Tm33-10-B2R2-P15, Tm33-20-B2R3-P10, and the control) to 1.12% (Tm33-15-B2R2-P22).

One of the major objectives of the experiment was to induce seedlessness in sweet orange through gamma-irradiation. Although no completely seedless mutants were

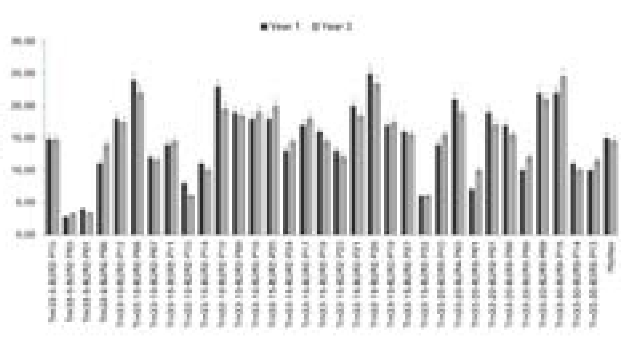

Fig. 1. Effect of gamma-irradiation on number of seeds in sweet orange mother plant and mutants

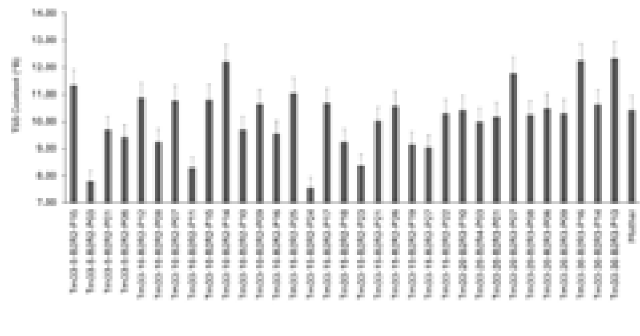

Fig. 2. Effect of gamma-irradiation on TSS content in sweet orange mother plants and mutants

observed, gamma-irradiation at 5 Gy proved to be the most effective in reducing the average number of seeds per fruit (Fig. 1). A promising low-seeded mutant, Tm33-5-B2R2-P01, was identified, with an average of only 3.67 seeds (4.0 seeds in 2021 and 3.34 seeds in 2022) compared to 14.75 seeds per fruit in the control mother plants. This mutant also exhibited a satisfactory juice total soluble solids (TSS) content of 9.70°Brix. Additionally, irradiation with a higher dose of 30 Gy resulted in an increase in TSS content, with the highest value (12.33°Brix) recorded in Tm33-30-B2R3-P13 (Figure 2).

Kinnow mandarin

Similar to the results observed in sweet orange, gamma-irradiation led to significant variations in various physico-chemical characteristics of Kinnow mandarin fruits, except at a dose of 5 Gy where no flowering was observed (Table 2a). Following the same trend as in sweet orange, fruit weight and fruit length in Kinnow mandarin were inversely proportional to the increasing doses of gamma-irradiation. However, no definite trend was observed in the fruit diameter and axis. Among the mutant population, the maximum fruit weight and diameter were recorded in KM-15-B2R1-P28 (162.00 g and 72.55 mm, respectively). The minimum fruit weight (77.00 g) was found in KM-30-B2R1-P11, compared to the control (155.26 g). This mutant selection also registered the minimum fruit length and diameter (45.11 mm and 57.12 mm, respectively). The maximum fruit length of 60.35 mm was recorded in KM-15-B2R1-P28, while control plants showed an average fruit length of 58.26 mm. Regarding fruit axis diameter, the thickest axis (12.30 mm) was found in KM-40-B2R1-P19, and the thinnest axis (9.23 mm) was observed in the control plants.

Gamma-irradiation did not induce significant variations in rind thickness, number of segments per fruit, and titratable acidity in juice among the mutant population. However, there was a significant difference observed in juice content per fruit, ranging from 26.65% in KM-20-B2R1-P10 to 41.85% in KM-20-B2R1-P26, whereas fruits from control plants exhibited 35.36% juice content (Table 2b).

On average, higher doses of gamma-irradiation have

Table 2a. Effect of gamma-irradiation on physicochemical attributes of Kinnow mandarin mutants

Mutant code	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit axis diameter (mm)
KM-10-B2R1-P03	104.00^{d}	51.89^{bc}	61.41^{de}	11.36^{abc}
KM-15-B2R1-P28	162.00^{a}	60.35^{a}	72.55^{a}	11.55^{ab}
KM-20-B2R1-P24	134.25^{c}	54.19^{b}	67.95^{bc}	11.06^{abc}
KM-20-B2R1-P10	84.50^{f}	48.58^{cd}	58.46^{ef}	9.74^{bc}
KM-20-B2R1-P26	157.75^{b}	58.19^{a}	71.39^{ab}	10.32^{abc}
KM-20-B2R1-P08	105.00^{d}	49.93^{cd}	61.73^{de}	10.75^{abc}
KM-30-B2R1-P11	77.00^{g}	45.11^{e}	57.12^{f}	9.34^{bc}
KM-40-B2R1-P19	97.00^{e}	48.05^{de}	64.56^{cd}	12.30^{a}
KM-Control	155.26^{b}	58.26^{a}	62.15^{de}	9.23^{c}
Range	77.0-162.0	45.11-60.35	57.12-72.55	9.23-12.30
CD ($P \leq 0.05$)	6.012	3.984	5.984	1.828
SEM (±)	1.988	1.317	1.979	0.604
CV (%)	2.878	4.328	5.343	9.85

The numerical values with same alphabetical notations are not statistically significant at $P \leq 0.05$as per Tukey's HSD test

been observed to reduce the number of seeds per fruit. One promising mutant, KM-40-B2R1-P19 (derived from budwood irradiated with 40 Gy), exhibited the lowest number of 1.50 seeds per fruit (1.0 seeds in 2021 and 2.0 seeds in 2022). Additionally, two other mutant selections, KM-20-B2R1-P10 and KM-30-B2R1-P11, showed fewer seeds per fruit (3.50), compared to 12.25 seeds per fruit in control mother plants (Figure 3). KM-20-B2R1-P10 and KM-30-B2R1-P11 resulted from irradiation at 20 Gy and 30 Gy, respectively.

The mutant KM-20-B2R1-P10 also displayed the highest TSS content (11.00°Brix), whereas KM-40-B2R1-P19 and KM-30-B2R1-P11 showed TSS contents of 9.03 and 10.65°Brix, respectively. The lowest TSS content (8.25°Brix) was recorded in KM-15-B2R1-P28, compared to 9.56 °Brix in control plants (Figure 4).

Nagpur mandarin

The experiment was conducted using Nagpur mandarin (N-4 and N-74). The mutant population derived from N-4 budwood, irradiated with doses ranging from 10 to 40 Gy,

Table 2b. Effect of gamma-irradiation on physicochemical attributes of Kinnow mandarin mutants

Mutant Code	Rind thickness (mm)	Nos. of segments	Juice content (%)	Titratable acidity (%)
KM-10-B2R1-P03	4.72^{ab}	11.33^{ab}	36.29^{bc}	0.78^{a}
KM-15-B2R1-P28	5.55^{a}	10.00^{b}	33.37^{c}	0.99^{a}
KM-20-B2R1-P24	4.48^{ab}	11.25^{ab}	35.72^{bc}	1.21^{a}
KM-20-B2R1-P10	5.71^{a}	10.5^{ab}	26.65^{d}	1.31^{a}
KM-20-B2R1-P26	4.8^{ab}	12.25^{a}	41.85^{a}	1.21^{a}
KM-20-B2R1-P08	4.91^{ab}	11.33^{ab}	39.65^{ab}	1.28^{a}
KM-30-B2R1-P11	5.13^{ab}	12.00^{a}	27.93^{d}	1.22^{a}
KM-40-B2R1-P19	5.68^{a}	12.25^{a}	39.32^{ab}	0.99^{a}
KM-Control	3.56^{b}	11.56^{ab}	35.36^{bc}	0.89^{a}
Range	3.56-5.71	10.0-12.25	26.55-41.85	0.78-1.31
CD ($P \leq 0.05$)	NS	NS	6.736	NS
SEM (±)	0.407	0.425	2.228	0.048
CV (%)	14.241	6.465	10.984	7.566

The numerical values with same alphabetical notations are not statistically significant at $P \leq 0.05$as per Tukey HSD test; NS: Non-significant at $P \leq 0.05$

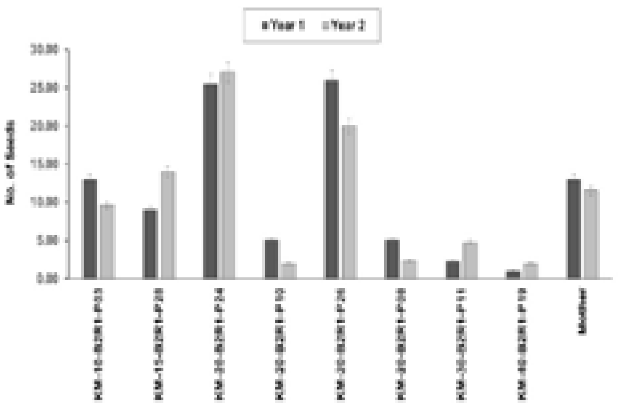

Fig. 3. Effect of gamma-irradiation on number of seeds in Kinnow mandarin mutants

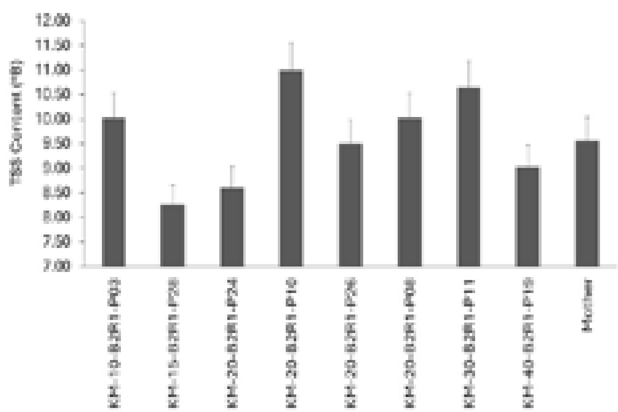

Fig. 4. Effect of gamma-irradiation on TSS content in Kinnow mandarin mutants

and N-74 budwood, irradiated with doses ranging from 20 to 40 Gy, did not produce flowers and fruits. Similar to sweet orange and Kinnow mandarin, the weight, length, diameter, and rind thickness of Nagpur mandarin fruits decreased with increasing doses of gamma irradiation. Among the mutant selections, the highest fruit weight and diameter were recorded in N4-5-B3R1-P07 (178.25 g and 78.52 mm, respectively). Conversely, the fruits with the minimum weight (97.50 g), length (57.66 mm), and diameter (65.13 mm) were found in N74-15-B3R4-P07. The maximum and minimum axis diameter was recorded in N4-5-B3R1-P06 (22.48 mm) and N74-5-B3R3-P02 (14.79 mm), respectively (Table 3a).

There was no statistically significant variation observed among the different irradiation doses regarding rind thickness; however, the rind thickness in mutant selections (ranging from 7.35–8.62 mm) was higher compared to that of control plants (5.36 mm). The irradiation treatment also did not yield significant differences in terms of the number of segments and titratable acidity. Nonetheless, plants derived from budwood irradiated with 5 Gy exhibited relatively higher juice content, with the maximum juice content (48.05%) recorded in N4-5-B3R1-P09, in contrast to the control plants (44.25%).

Figures 5 and 6 illustrate the significant impact of gamma irradiation on seed count and TSS content in Nagpur mandarin fruits. Notably, gamma irradiation markedly reduced the number of seeds in Nagpur mandarin. In addition to one seedless mutant, N4-5-B3R1-P05, five near-seedless mutants, *viz*. N4-5-B3R1-P07 and N74-5-B3R3-P02 (2.00 seeds per fruit), N4-5-B3R1-P09 (2.25 seeds per fruit), N74-10-B3R1-P11 (2.75 seeds per fruit), and N74-5-B3R3-P12

Table 3a. Effect of gamma-irradiation on physicochemical attributes of Nagpur mandarin mutants

Mutant code	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit axis diameter (mm)
N4-5-B3R1-P06	157.25^{b}	68.47^{a}	74.59^{b}	22.48^{a}
N4-5-B3R1-P07	178.25^{a}	67.38^{ab}	78.52^{a}	20.77^{ab}
N4-5-B3R1-P09	112.50^{f}	58.40^{c}	67.84^{c}	17.67^{bcde}
N4-5-B3R1-P05	116.00^{e}	58.71^{c}	67.84^{c}	17.10^{cde}
N74-5-B3R3-P12	150.75^{c}	66.69^{ab}	74.98^{b}	20.50^{ab}
N74-5-B3R3-P02	102.00^{g}	64.80^{b}	66.03^{c}	14.79^{e}
N74-10-B3R1-P11	112.25^{f}	60.10^{c}	67.00^{c}	18.29^{bcd}
N74-15-B3R4-P07	97.50^{h}	57.66^{c}	65.13^{c}	20.13^{abc}
N4-Control (Mother)	140.25^{d}	69.26^{a}	72.26^{b}	15.26^{de}
Range	97.50-178.25	57.66-69.26	65.13-78.52	14.79-22.48
CD ($P \leq 0.05$)	3.529	4.023	4.352	3.701
SEM (±)	1.167	1.331	1.439	1.224
CV (%)	1.559	3.630	3.538	11.425

The numerical values with same alphabetical notations are not statistically significant at $P \leq 0.05$ as per Tukey's HSD test

Table 3b. Effect of gamma-irradiation on physicochemical attributes of Nagpur mandarin mutants

Mutant code	Rind thickness (mm)	No. of segments	Juice content (%)	Titratable acidity (%)
N4-5-B3R1-P06	7.80[a]	10.00[a]	42.53[b]	0.65[a]
N4-5-B3R1-P07	7.78[a]	10.00[a]	45.47[ab]	0.94[a]
N4-5-B3R1-P09	7.53[a]	9.75[a]	48.05[a]	0.73[a]
N4-5-B3R1-P05	7.61[a]	9.67[a]	45.34[ab]	0.72[a]
N74-5-B3R3-P12	8.62[a]	10.25[a]	31.06[d]	0.70[a]
N74-5-B3R3-P02	7.35[a]	10.00[a]	44.24[b]	0.94[a]
N74-10-B3R1-P11	7.54[a]	10.50[a]	37.81[c]	0.70[a]
N74-15-B3R4-P07	7.50[a]	10.25[a]	36.89[c]	1.12[a]
N4-Control	5.36[b]	11.23[a]	44.25[b]	0.89[a]
Range	5.36-8.62	9.67-11.23	31.06-48.05	0.65-1.12
CD ($P \leq 0.05$)	0.763	NS	3.705	NS
SEM (±)	0.252	0.409	1.225	0.041
CV (%)	5.866	6.957	5.085	8.749

The numerical values with same alphabetical notations are not statistically significant at $P \leq 0.05$ as per Tukey's HSD test; NS: Non-significant at $P \leq 0.05$

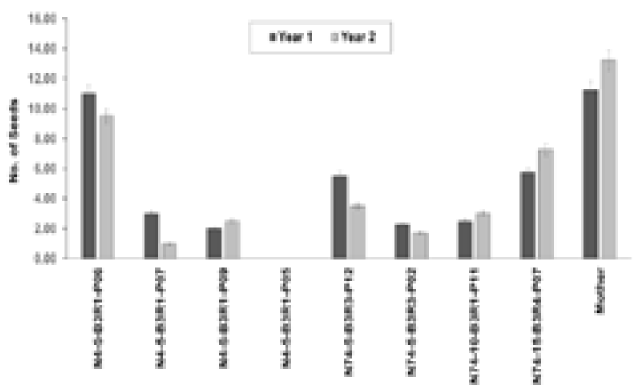

Fig. 5. Effect of gamma-irradiation on number of seeds in Nagpur mandarin mutants and mother plant

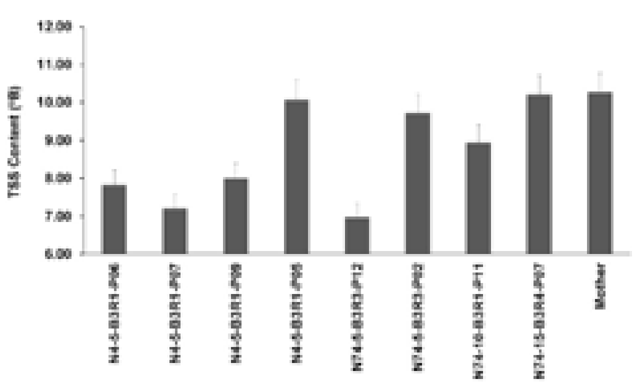

Fig. 6. Effect of gamma-irradiation on fruit TSS content in Nagpur mandarin mutants and mother plant

(4.50 seeds per fruit) were identified. The seed count in control mother plants was 12.23 per fruit. The seedless mutant, N4-5-B3R1-P05, also exhibited favorable TSS content (10.07°Brix), which was statistically comparable to that of control plants (10.26°Brix).

The observed inverse relationship between irradiation dose and fruit physical parameters, such as reduced fruit weight, length, diameter, axis diameter, and rind thickness, likely results from a complex interplay of multiple factors. One possible explanation is the disruption of nutrient and carbohydrate partitioning within the plant. Gamma-ray irradiation is known to induce genetic variability in various plant species, including citrus, by causing spontaneous DNA damage (Musa et al. 2021; Purba et al. 2021; Çelik and Atak 2017). This damage can lead to physiological and biochemical changes in plants (Khaerani et al. 2021), such as altered enzyme activities and metabolic pathways (Alexandre et al. 2012), which can disrupt cell division, growth, and development, ultimately affecting the fruit size and morphology.

The changes in metabolic pathways related to nutrient and carbohydrate distribution within the plant may result in altered translocation and utilization of essential nutrients and carbohydrates, impacting fruit growth and development (Kim et al. 2004). Additionally, irradiation affects the plant's hormonal balance, influencing fruit development and growth (Singh et al. 2022). For instance, gamma-irradiation can alter the levels of hormones such as auxins and gibberellins, which might reduce fruit size by inhibiting cell elongation and expansion. Irradiation can also affect the activity of cell wall-modifying enzymes, impacting fruit firmness and texture (Song et al. 2004). Goldenberg et al. (2014) found that gamma-irradiation reduced the fruit weight of seven out of eight tested low-seeded mandarin genotypes by 6-41 g per fruit.

Gamma-irradiation reduced the number of seeds in the fruits, a desirable trait in citrus for consumer appeal and convenience. The identification of seedless or less-seeded mutants in sweet orange, Kinnow mandarin, and Nagpur mandarin following gamma-irradiation of dormant budwood is a significant finding (Figure 7). The reduction in seed number can be attributed to disrupted cellular processes and DNA damage inhibiting seed development. Gamma-irradiation can induce mutations and chromosomal aberrations that disrupt normal seed formation, leading to seedlessness in some crops (Baktemur et al. 2014). Disruption of hormonal balance in mutant plants can also result in failed seed development. Furthermore, epigenetic modifications triggered by irradiation, such as histone methylation, acetylation, and ubiquitination, may silence genes crucial for seed formation (Nonogaki 2014). Saadati et al. (2022) reported that irradiation doses of 20 to 60 Gy effectively induced seedlessness in various citrus cultivars. Khalil et al. (2011) identified a sparsely seeded Kinnow mandarin (2 to 8 seeds per fruit) from 20 Gy plants, compared to its parent (20–30 seeds per fruit). Bermejo et al. (2012) also reported a reduction in seed count in Murcott mandarin obtained by bud irradiation. Promising mutant lines showing seedlessness were identified in SoE mandarin and Nambangan pummelo, and nearly seedless cultivars in SoE mandarin, Garut mandarin, and Nambangan pummelo when bud woods were irradiated at 20 and 40 Gy (Sutarto et al. 2009). Lower pollen fertility, distorted and shrunken pollen, and self-incompatibility resulted in seedlessness in the mutant 'Hongjiangcheng' sweet orange (Yin et al., 2023) and the 'Juxiangyuan' seedless orange (Zhang et al., 2024). Wen et al. (2024) identified the genes Cs2g16620 (PIN1), Cs7g06410 (NPH3), and Cs3g23070 (MYB) as being associated with female sterility in a seedless mutant of 'Meiguicheng' sweet orange. These genes are involved in the regulation of auxin metabolism in ovules, thereby leading to sterility.

The irradiation process also led to an improvement in fruit total soluble solids (TSS) content, an important indicator of fruit quality. Gamma-irradiation is known to induce various physiological and biochemical changes in plants, including alterations in enzyme activity, hormone regulation, and metabolite production (Mustapha et al. 2020). These changes can lead to the breakdown of complex carbohydrates and the release of simple sugars, as observed in other irradiated fruits and vegetables (Baktemur et al. 2014). The significant variation in TSS observed in mutant plants can be attributed to radiation-induced modifications in the activity of enzymes involved in carbohydrate synthesis and degradation pathways. Gamma-irradiation has been reported to alter the activity of enzymes like sucrose synthase, invertase, and amylase, which play crucial roles in carbohydrate metabolism (Fernandes and Rodrigues 2021). These changes can lead to variations in the accumulation of sugars, affecting the TSS content.

Similarly, changes in juice content may result from alterations in the water-holding capacity and osmotic balance within fruit tissues (Nakabayashi and Asai 2001) or changes in the expression and activity of aquaporins, responsible for water transport and regulation in plants (Fernandes and Rodrigues 2021). Such changes in water-related processes can directly influence the juice content of citrus fruits. These findings align with previous research on mandarin oranges (Goldenberg et al. 2014; Rattanpal et al. 2019; Bastianel et al. 2021; Eun et al. 2024). Another explanation for these variations is that gamma-irradiation can cause mutations in the plant's genetic material, altering the fruit's composition and quality. This is supported by research on sweet oranges, where gamma-irradiation-induced mutations result in changes to the fruit's sugar content and juice quality (Singh et al. 2022). Gamma-irradiation can also alter the levels of hormones such as ethylene and auxins, which are crucial for fruit growth and ripening (Çimen et al. 2020). The changes in these hormone levels could have contributed to the observed variations in TSS and juice content. The results of this study are consistent with previous research on Kinnow mandarin (Abdullah et al. 2018), which could be related to the observed variations in TSS and juice content.

Fig. 7. Fruits of promising mutants and their mother

Overall, this study demonstrated that gamma-irradiation mutagenesis effectively induces seedlessness or sparse seediness in citrus fruits. However, careful consideration must be given to its potential impact on reduced fruit weight and size, as well as its varied effects on fruit quality.

Under the present study, three promising mutant selections, *viz*. Tm33-5-B2R2-P01 (Sweet orange), KM-40-B2R1-P19 (Kinnow mandarin) and N4-5-B3R1-P05 (Nagpur mandarin) derived from budwood irradiation have been identified. These achievements hold potential for the development of new varieties of sweet orange, Kinnow mandarin, and Nagpur mandarin that are either seedless or have reduced seed count, meeting the increasing consumer demand for high-quality, convenient, and nutritious citrus fruits. Moreover, the successful application of gamma-irradiation technology in citrus breeding opens avenues for enhancing other economically important traits, such as longer shelf life, enhanced nutritional value, and resistance to biotic and abiotic stresses. Future research efforts should continue in this direction to breed high-yielding varieties with desirable multifaceted traits.

Author's contribution

Conceptualization of research (AAM); Designing of the experiments (AAM and AT); Contribution of experimental materials (AT and JPT); Execution of field/lab experiments and data collection (AAM, AT, and PJ); Analysis of data and interpretation (SSR, MG, and DG); Preparation of the manuscript (AAM, SSR, AT, and MG).

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