



## Effect of ionizing radiation on morphological characters and leaf nutrient content of sweet orange cv. Mosambi

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### ABSTRACT

In the present study, sweet orange cv. Mosambi mutants were developed by treating bud sticks with different doses of gamma irradiation (10, 15, 20, 25, 30 and 35 Gray) and budded in situ on Jatti Khatti rootstock. Two years after the establishment of plants, the impact of ionizing radiations on the mutated population was studied for the alterations in growth and plant nutrient status, showing differential stimulatory and inhibitory dose-dependent responses. Mutants developed with lower doses of gamma irradiation stimulated plant height in the mutants GS-3 (39.30 per cent) and GS-8 (40.80 per cent) developed from 15 and 20 Gy, respectively. Contrary to the stimulatory effects, higher irradiation doses inhibited plant height and other growth parameters in the mutants GS-16, GS-30, GS-32 and GS-34 developed from 35 Gy. The macro-nutrient concentrations were higher at lower doses of 10 and 15 Gy in the mutants GS-2, GS-3 and GS-8. At the same time, the micro-nutrient contents in the leaf tissue were higher in the mutants GS-21 and GS-14 developed from intermediate doses of 20 and 25 Gy. Although a lower accumulation of macro-nutrients was observed at higher dosimetry, the reverse trend was noticed for the micro-nutrients with the minimum at the lowest dosimetry, i.e., 10 Gy.

**Keywords:** *Citrus sinensis* (L.) Osbeck, Mutants, Growth, Leaf nutrients,  $\gamma$ -rays

### INTRODUCTION

*Citrus* species are the most widely cultivated fruit crops in the world under diverse agro-ecological conditions between latitude 35°N~35°S. In India, it is the third most important fruit industry after banana and mango, occupying an area of 0.97 million ha with an annual production of 12.25 million tonnes. Among citrus group, sweet orange [*Citrus sinensis* (L. Osbeck)] is the second largest amongst the *Citrus* species cultivated in India on 0.18 million hectare with an estimated production of 2.87 million tonnes (Anonymous, 1). This orange is valued for its antioxidant properties that builds the body immune system. Besides the antioxidant properties, the fruit is in great demand due to high juice recovery with low acid content. However, despite the several positive traits, some of the unwanted traits like low quality fruits, granulation of juice sacs and presence of large number of seeds (15-30 seeds/fruit) hinder its acceptability among processors and consumers. Attempt to develop cultivars/varieties for traits of interest have been the prime objectives of the breeders across the globe, and good strides have been made in this direction both through the conventional and non-conventional approaches (Bermejo *et al.*, 2, Mallick *et al.*, 11). Although, some of the world's contrasting varieties have been

developed through traditional breeding, the system is handicapped by its long juvenile phase, high degree of nucellar embryony, embryo abortion, lack of the knowledge of inheritance pattern etc. Contrary to the traditional breeding approaches, mutagenesis either physical or chemical have been used as a tool for creating variability, selecting traits of genetic interest and its use in the future breeding programme from the mutated population which results due to the alterations in morphological, physiological, biochemical or other plant characteristics (Mallick *et al.*, 11). The technique have made an outstanding impact on the productivity and economic value of some crops leading to the development of seedless mutants (Vardi *et al.*, 14; Cuenca *et al.*, 4), improved plant characteristics such as citrus canker tolerant clones (Latado *et al.*, 9), highly fruitful and compact canopy orange (Donini, 5) and spine-free Sunki mandarin (Kukimura *et al.*, 8).

The present investigation aimed to understand the morphological and nutrient alterations in the mutated population of sweet orange developed through different doses of gamma irradiations. The alteration observed to provide useful information for selecting putative mutants of sweet orange in the pre-bearing stage, and its use in the future breeding programme.

### MATERIALS AND METHODS

The present experiment was carried out on two year old sweet orange putative mutants developed

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with different dose of gamma rays viz., 10, 15, 20, 25, 30 and 35 Gray (Gy). The mutated buds were budded *in situ* on *Jatti Khatti* rootstock during the year 2015 at the spacing of 3m × 3m, and maintained at the main orchard of Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi. From the diverse population, six random putative mutants developed from 10 to 30 Gy, and four putative mutants created from 35 Gy based on differential growth characters were selected and compared with the non-irradiated, wild type (mother plant) of same age for different plant growth characters and leaf nutrient status. The putative mutants developed from the varying doses of irradiation were assigned codes between GS-0 to GS-34, where GS-0 represent control (wild type) whereas code GS-1 to GS-6 represent mutant developed from 10 Gy; GS-7 to GS-12 at 15 Gy; GS-13 to GS-18 at 20 Gy; GS-19 to GS-24 at 25 Gy; GS-25 to GS-30 at 30 Gy and GS-31 to GS-34 developed from 35 Gy irradiation dose.

Plant growth parameters of the mutants with respect to plant height (m), stem girth (cm) and canopy volume (m<sup>3</sup>) were recorded four months after the emergence of spring and rainy season flush i.e., June and November for two consecutive years (2017 and 2018). Plant height was measured with the help of measuring scale from the base of plant to the last fully opened leaf on the main stem. The stem diameter was measured with Digimatic vernier calipers 5 cm above the bud union marked with white paint. Canopy volume was calculated using tree height (H) and width in parallel (D<sub>1</sub>) and perpendicular (Dr) direction to the row with the following formulae  $V = (\pi/6) \times H \times D_1 \times Dr$  (Zekri, 15). Trunk cross-sectional area (TCSA) was measured using the formula  $TCSA = \pi (d/2)^2$ , Where d = average of east–west and north–south trunk diameter.

For determining the leaf nutrient status, as recommended by Srivastava and Singh (13), four months old leaf Samples were collected, brought to the laboratory and thoroughly washed in running water. The leaf samples were then dried in oven at 70±2 °C and then ground to obtain homogenous samples. Digestion block method as suggested by Bremner *et al.* (3) was used for measuring nitrogen content in leaves. Phosphorus content in leaves was assessed by vando-molybdo-phosphoric yellow colour method and Potassium by the flame photometry method (Jackson, 6). Calcium (Ca), magnesium (Mg) and micro-nutrients (Fe, Cu, Mn and Zn) content in leaf samples were ascertained as per the method of Jackson (6) by atomic absorption

spectrophotometer (Model- GBC, 904AA, GBC Scientific Equipment, Hampshire, Illinois, USA).

Statistical analysis ( $P \leq 0.05$ ) of the mean data (2017-2018) for growth parameters viz., plant height, stem girth and canopy volume, which comprised of thirty four mutants and one wild type plants (control) was done through augmented design. The statistical analysis of plant nutrient content, comprising of four replications in each treatment was carried out in completely randomized block design using SAS package (9.3 SAS Institute, INC., USA) followed by Tukey's Honest test.  $P$  values  $\leq 0.05$  were considered as significant. Relationship among plant growth and nutrient parameters was computed using Pearson's simple correlation using the SPSS software.

## RESULTS AND DISCUSSION

Growth parameters (plant height, stem girth and canopy volume) are important morpho-economic traits which contribute to biomass production. In the present study as compared to wild type, stimulated increase in plant height in the Mosambi mutants GS-3(39.30 per cent) and GS-8 (40.80 per cent) developed at 10 and 15 Gy of gamma irradiations, respectively. Contrary to the stimulated increase in plant height at the lower doses, a drastic reduction of 31.84-34.83 per cent was recorded in the mutants GS-16 and GS-30, GS-32 and GS-34 developed from 20, 30 and 35 Gy, respectively. It is also interesting to note that the 35 Gy dose stimulated plant height in the mutant GS-31 by 10.45 per cent which however, was significantly lower than the mutants GS-3 and GS-8 (Table 1). A trend similar to plant height was recorded with respect to the canopy volume and as compared to the wild type, it was 5.6 times more in mutant GS-11 developed at 15Gy, while it was 6.25-6.70 times less in the mutants GS-15, GS-16 at 20 Gy, 3.57times less in GS-20 (25 Gy), 3.16 times less in GS-30 (30 Gy) and 2.95 times less in GS-34 (35 Gy). Trunk cross sectional area (TCSA) exhibited significant variation amongst the mutant population. It was 3.16 time higher in the mutant GS-11(15 Gy), and 4.00 time lower in the mutant GS-30 (30Gy). Stem girth as compared to wild type was significantly higher by 79.26 per cent in the mutant GS-11 developed at 15 Gy and was statistically similar to the values recorded in the mutants GS-8 and GS-9 at the same irradiation dose. A reduction in stem girth of 50.35 per cent was recorded in GS-30 as compare to WT.

A critical analysis shows that plant growth stimulation in the tested mutants in general got stimulated at the lower doses of gamma irradiation, while at higher dose inhibitory effects were apparent. It is logical to imply that induced stimulation or

inhibition of plant growth as observed in the present study might be a consequence of affected due to gamma rays induced changes in physiological and biochemical alterations. Damage done to the target tissue by ionizing radiation which directly or indirectly

regulates cell division and cell elongation might have led to the stimulatory/ inhibitory effects on plant growth in a dose dependent manner. Alteration in the hormonal signaling network in the plant cells which stimulate certain enzymes and hormones responsible

**Table 1.** Variation in plant growth parameters of gamma rays induced cv. Mosambi mutants.

Treatment	Plant height (m)	Canopy volume (m <sup>3</sup> )	TCSA	Stem girth (mm)
GS-0	2.01 <sup>ij</sup>	2.75 <sup>hijk</sup>	73.74 <sup>jl</sup>	54.63 <sup>jk</sup>
GS-1	2.58 <sup>abcdef</sup>	5.62 <sup>cdef</sup>	164.82 <sup>bc</sup>	81.57 <sup>bcd</sup>
GS-2	2.70 <sup>abcde</sup>	7.18 <sup>bc</sup>	119.14 <sup>efg</sup>	69.38 <sup>defgh</sup>
GS-3	2.80 <sup>ac</sup>	6.86 <sup>c</sup>	120.73 <sup>efg</sup>	69.84 <sup>cdefgh</sup>
GS-4	2.28 <sup>bdefghij</sup>	4.74 <sup>defg</sup>	127.41 <sup>def</sup>	71.74 <sup>cdefg</sup>
GS-5	2.08 <sup>ghijklm</sup>	2.98 <sup>ghijklm</sup>	106.09 <sup>efghi</sup>	65.48 <sup>fghij</sup>
GS-6	2.50 <sup>abcdefg</sup>	4.45 <sup>defgh</sup>	109.60 <sup>efgh</sup>	66.55 <sup>efghi</sup>
GS-7	2.30 <sup>cdefghi</sup>	3.54 <sup>fghijk</sup>	103.29 <sup>fghi</sup>	65.01 <sup>fghij</sup>
GS-8	2.83 <sup>ab</sup>	9.72 <sup>b</sup>	187.41 <sup>b</sup>	87.78 <sup>ab</sup>
GS-9	2.45 <sup>abcdefgh</sup>	7.77 <sup>bc</sup>	176.89 <sup>b</sup>	85.27 <sup>abc</sup>
GS-10	2.20 <sup>efghij</sup>	6.14 <sup>cde</sup>	161.86 <sup>bcd</sup>	81.55 <sup>bcde</sup>
GS-11	2.70 <sup>abcd</sup>	15.40 <sup>a</sup>	233.11 <sup>a</sup>	97.93 <sup>a</sup>
GS-12	2.45 <sup>abcdefgh</sup>	6.50 <sup>cd</sup>	137.62 <sup>cde</sup>	75.16 <sup>bcdef</sup>
GS-13	1.60 <sup>klmnopqrs</sup>	1.62 <sup>ijklm</sup>	56.21 <sup>ijklmnopqr</sup>	47.87 <sup>klmnopr</sup>
GS-14	1.87 <sup>hijklmnoprs</sup>	1.65 <sup>ijklm</sup>	57.14 <sup>ijklmnopqr</sup>	48.25 <sup>klmnop</sup>
GS-15	1.52 <sup>lmnopqrs</sup>	0.44 <sup>lm</sup>	43.54 <sup>klmnopqrs</sup>	42.34 <sup>lmnopqrst</sup>
GS-16	1.35 <sup>qt</sup>	0.41 <sup>lm</sup>	24.92 <sup>pqr</sup>	32.61 <sup>qrstv</sup>
GS-17	1.75 <sup>ijklmnopqrs</sup>	2.17 <sup>ghijklm</sup>	67.85 <sup>ijklmno</sup>	52.45 <sup>ijklmno</sup>
GS-18	1.72 <sup>ijklmnopqrs</sup>	1.86 <sup>ijklm</sup>	41.54 <sup>klmnopqrs</sup>	41.40 <sup>mnopqrst</sup>
GS-19	2.10 <sup>fghijkl</sup>	2.32 <sup>ghijklm</sup>	67.51 <sup>ijklmno</sup>	52.36 <sup>ijklmno</sup>
GS-20	1.35 <sup>st</sup>	0.77 <sup>m</sup>	28.21 <sup>opqr</sup>	34.97 <sup>pqrstuv</sup>
GS-21	1.93 <sup>ghijklmnopq</sup>	1.99 <sup>hijklm</sup>	73.18 <sup>hijklm</sup>	54.43 <sup>hijklm</sup>
GS-22	1.95 <sup>ghijklmno</sup>	2.88 <sup>ghijkl</sup>	38.38 <sup>nopqr</sup>	40.16 <sup>nopqrst</sup>
GS-23	1.90 <sup>hijklmnopq</sup>	1.98 <sup>hijklm</sup>	41.03 <sup>klmnopqrs</sup>	41.41 <sup>nopqrst</sup>
GS-24	1.55 <sup>mnopqrs</sup>	1.34 <sup>ijklm</sup>	23.35 <sup>qt</sup>	32.22 <sup>rstv</sup>
GS-25	1.36 <sup>pqrst</sup>	0.78 <sup>lm</sup>	24.18 <sup>pqr</sup>	31.16 <sup>st</sup>
GS-26	1.94 <sup>ghijklmno</sup>	2.44 <sup>ghijklm</sup>	80.75 <sup>ghijk</sup>	57.21 <sup>ghijkl</sup>
GS-27	2.16 <sup>defghijk</sup>	3.85 <sup>efghi</sup>	61.63 <sup>klmnopq</sup>	49.95 <sup>klmnp</sup>
GS-28	1.71 <sup>ijklmnopqrs</sup>	1.67 <sup>ijklm</sup>	46.29 <sup>lmnopqrs</sup>	43.26 <sup>mnopqrs</sup>
GS-29	1.49 <sup>nopqrs</sup>	1.14 <sup>klm</sup>	33.98 <sup>mnopqr</sup>	37.02 <sup>opqrstu</sup>
GS-30	1.31 <sup>st</sup>	0.87 <sup>lm</sup>	18.39 <sup>rt</sup>	27.12 <sup>t</sup>
GS-31	2.22 <sup>defghij</sup>	3.91 <sup>efghij</sup>	107.97 <sup>efghi</sup>	66.67 <sup>defghi</sup>
GS-32	1.32 <sup>st</sup>	1.23 <sup>klm</sup>	34.88 <sup>mnopqr</sup>	36.46 <sup>pqrstuv</sup>
GS-33	1.89 <sup>hijklmnopqr</sup>	2.54 <sup>ghijklm</sup>	57.73 <sup>ijklmnopr</sup>	48.11 <sup>klmnopq</sup>
GS-34	1.37 <sup>ost</sup>	0.93 <sup>lm</sup>	23.81 <sup>qt</sup>	29.05 <sup>st</sup>
CD (P=05)	0.47	2.10	33.56	12.80
SED	0.17	0.74	11.86	4.53

for growth as a cause have also been reported by Kim *et al.* (7). Ling *et al.* (10) and Mallick *et al.* (11) also reported both stimulatory and inhibitory plant growth response in *Citrus sinensis* and Kinnow mandarin due to gamma irradiations.

Leaf macro nutrient viz., N, P, K, Ca, Mg and micronutrients Fe, Cu, Mn, and Zn showed differential response in relation to leaf nutrient content in the mutated population, despite being grown on the same rootstock. The macro nutrients (N, P, and Mg) content

**Table 2.** Variation in leaf macro and micro nutrient of gamma rays induced cv. Mosambi putative mutants.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
GS-0	2.31 <sup>j</sup>	0.14 <sup>efg</sup>	1.18 <sup>ik</sup>	3.53 <sup>onm</sup>	0.39 <sup>hg</sup>	80 <sup>gih</sup>	6 <sup>no</sup>	43 <sup>o</sup>	38 <sup>ih</sup>
GS-1	2.60 <sup>f</sup>	0.16 <sup>d</sup>	1.40 <sup>ef</sup>	4.37 <sup>d</sup>	0.49 <sup>cb</sup>	77 <sup>ijk</sup>	5 <sup>o</sup>	34 <sup>r</sup>	26 <sup>o</sup>
GS-2	3.00 <sup>a</sup>	0.22 <sup>a</sup>	1.65 <sup>cb</sup>	4.60 <sup>b</sup>	0.53 <sup>a</sup>	70 <sup>no</sup>	5 <sup>o</sup>	45 <sup>mn</sup>	23 <sup>p</sup>
GS-3	2.91 <sup>b</sup>	0.19 <sup>b</sup>	1.65 <sup>cb</sup>	4.40 <sup>cd</sup>	0.50 <sup>b</sup>	81 <sup>gfh</sup>	5 <sup>o</sup>	38 <sup>q</sup>	35 <sup>ml</sup>
GS-4	2.42 <sup>i</sup>	0.15 <sup>ed</sup>	1.35 <sup>gf</sup>	3.65 <sup>k</sup>	0.46 <sup>ed</sup>	85 <sup>dfe</sup>	5 <sup>o</sup>	48 <sup>l</sup>	34 <sup>m</sup>
GS-5	2.40 <sup>i</sup>	0.15 <sup>ef</sup>	1.33 <sup>gf</sup>	3.95 <sup>hg</sup>	0.44 <sup>f</sup>	79 <sup>jih</sup>	7 <sup>m</sup>	53 <sup>kj</sup>	43 <sup>ef</sup>
GS-6	2.71 <sup>d</sup>	0.18 <sup>c</sup>	1.55 <sup>cd</sup>	4.22 <sup>ef</sup>	0.50 <sup>cd</sup>	92 <sup>b</sup>	7 <sup>m</sup>	55 <sup>hi</sup>	46 <sup>d</sup>
GS-7	2.65 <sup>e</sup>	0.18 <sup>cb</sup>	1.60 <sup>cd</sup>	4.00 <sup>g</sup>	0.48 <sup>cd</sup>	75 <sup>lk</sup>	7 <sup>nm</sup>	34 <sup>r</sup>	42 <sup>fg</sup>
GS-8	2.93 <sup>b</sup>	0.19 <sup>b</sup>	1.75 <sup>b</sup>	4.50 <sup>cb</sup>	0.52 <sup>a</sup>	78 <sup>jkih</sup>	11 <sup>ih</sup>	64 <sup>ef</sup>	38 <sup>ihj</sup>
GS-9	2.60 <sup>f</sup>	0.15 <sup>ed</sup>	1.60 <sup>cd</sup>	4.30 <sup>ed</sup>	0.47 <sup>ed</sup>	86 <sup>dc</sup>	13 <sup>bedc</sup>	55 <sup>hij</sup>	44 <sup>ed</sup>
GS-10	2.48 <sup>h</sup>	0.13 <sup>hg</sup>	1.38 <sup>gf</sup>	3.80 <sup>ji</sup>	0.43 <sup>f</sup>	93 <sup>b</sup>	13 <sup>bac</sup>	47 <sup>ml</sup>	29 <sup>n</sup>
GS-11	2.85 <sup>c</sup>	0.19 <sup>b</sup>	1.90 <sup>a</sup>	4.78 <sup>a</sup>	0.46 <sup>e</sup>	72 <sup>nm</sup>	13 <sup>bdc</sup>	51 <sup>k</sup>	37 <sup>ikhj</sup>
GS-12	2.54 <sup>g</sup>	0.13 <sup>hg</sup>	1.53 <sup>d</sup>	4.30 <sup>ed</sup>	0.49 <sup>cb</sup>	76 <sup>lk</sup>	7 <sup>nm</sup>	76 <sup>ba</sup>	46 <sup>d</sup>
GS-13	2.02 <sup>sr</sup>	0.11 <sup>lkj</sup>	1.00 <sup>nm</sup>	3.35 <sup>rq</sup>	0.30 <sup>qp</sup>	76 <sup>ljk</sup>	12 <sup>fgedh</sup>	54 <sup>ij</sup>	43 <sup>efg</sup>
GS-14	2.25 <sup>lk</sup>	0.12 <sup>ihj</sup>	1.18 <sup>jik</sup>	3.80 <sup>ji</sup>	0.37 <sup>kij</sup>	95 <sup>ba</sup>	10 <sup>ji</sup>	64 <sup>ef</sup>	35 <sup>ml</sup>
GS-15	2.05 <sup>qr</sup>	0.10 <sup>l</sup>	1.02 <sup>nml</sup>	3.68 <sup>k</sup>	0.35 <sup>ml</sup>	83 <sup>gfe</sup>	9 <sup>jk</sup>	46 <sup>mn</sup>	48 <sup>c</sup>
GS-16	2.09 <sup>qp</sup>	0.11 <sup>lk</sup>	1.13 <sup>ilk</sup>	3.85 <sup>hi</sup>	0.32 <sup>o</sup>	84 <sup>dfe</sup>	14 <sup>a</sup>	65 <sup>def</sup>	43 <sup>efg</sup>
GS-17	2.21 <sup>lm</sup>	0.12 <sup>ikl</sup>	1.20 <sup>ijh</sup>	3.60 <sup>lkm</sup>	0.36 <sup>kij</sup>	86 <sup>dce</sup>	8 <sup>lm</sup>	40 <sup>p</sup>	35 <sup>ml</sup>
GS-18	2.26 <sup>k</sup>	0.12 <sup>ihj</sup>	1.33 <sup>gf</sup>	3.43 <sup>oqp</sup>	0.34 <sup>mn</sup>	94 <sup>ba</sup>	14 <sup>a</sup>	44 <sup>on</sup>	52 <sup>b</sup>
GS-19	2.35 <sup>i</sup>	0.14 <sup>fg</sup>	1.20 <sup>ijh</sup>	4.15 <sup>f</sup>	0.42 <sup>f</sup>	88 <sup>c</sup>	12 <sup>fgedc</sup>	68 <sup>c</sup>	36 <sup>kij</sup>
GS-20	2.05 <sup>qr</sup>	0.11 <sup>lk</sup>	1.10 <sup>jmik</sup>	2.90 <sup>t</sup>	0.31 <sup>op</sup>	77 <sup>lki</sup>	14 <sup>ba</sup>	77 <sup>a</sup>	43 <sup>ef</sup>
GS-21	2.25 <sup>lk</sup>	0.13 <sup>ih</sup>	1.20 <sup>ijh</sup>	3.23 <sup>s</sup>	0.37 <sup>hkij</sup>	97 <sup>a</sup>	12 <sup>fgeh</sup>	57 <sup>hg</sup>	38 <sup>ih</sup>
GS-22	2.18 <sup>nm</sup>	0.11 <sup>lkj</sup>	1.28 <sup>gih</sup>	3.30 <sup>rs</sup>	0.39 <sup>hg</sup>	83 <sup>gfe</sup>	12 <sup>fg</sup>	58 <sup>g</sup>	35 <sup>ml</sup>
GS-23	2.24 <sup>lk</sup>	0.15 <sup>ed</sup>	1.30 <sup>gfh</sup>	3.53 <sup>onm</sup>	0.40 <sup>g</sup>	75 <sup>lm</sup>	14 <sup>ba</sup>	47 <sup>ml</sup>	43 <sup>fg</sup>
GS-24	2.11 <sup>op</sup>	0.10 <sup>l</sup>	1.18 <sup>jik</sup>	3.20 <sup>s</sup>	0.36 <sup>mkl</sup>	86 <sup>dc</sup>	9 <sup>lk</sup>	64 <sup>ef</sup>	54 <sup>a</sup>
GS-25	2.04 <sup>qr</sup>	0.10 <sup>l</sup>	1.08 <sup>g</sup>	3.66 <sup>lk</sup>	0.29 <sup>q</sup>	95 <sup>ba</sup>	12 <sup>fgedh</sup>	36 <sup>r</sup>	49 <sup>c</sup>
GS-26	2.22 <sup>lk</sup>	0.14 <sup>ef</sup>	1.38 <sup>gf</sup>	3.80 <sup>ji</sup>	0.38 <sup>hi</sup>	87 <sup>dc</sup>	13 <sup>bac</sup>	55 <sup>hi</sup>	39 <sup>h</sup>
GS-27	2.31 <sup>j</sup>	0.13 <sup>hg</sup>	1.20 <sup>ijh</sup>	3.70 <sup>lk</sup>	0.37 <sup>hij</sup>	93 <sup>b</sup>	10 <sup>j</sup>	65 <sup>def</sup>	42 <sup>fg</sup>
GS-28	2.22 <sup>lkm</sup>	0.12 <sup>ikj</sup>	1.40 <sup>ef</sup>	3.45 <sup>onp</sup>	0.37 <sup>hkij</sup>	84 <sup>dfe</sup>	9 <sup>jk</sup>	75 <sup>b</sup>	48 <sup>c</sup>
GS-29	2.04 <sup>qr</sup>	0.10 <sup>l</sup>	1.20 <sup>ijh</sup>	3.40 <sup>rap</sup>	0.32 <sup>o</sup>	76 <sup>lk</sup>	12 <sup>fg</sup>	66 <sup>dc</sup>	52 <sup>b</sup>
GS-30	1.98 <sup>st</sup>	0.10 <sup>l</sup>	1.00 <sup>ijh</sup>	3.55 <sup>lnm</sup>	0.34 <sup>ml</sup>	67 <sup>po</sup>	11 <sup>gh</sup>	44 <sup>on</sup>	34 <sup>m</sup>
GS-31	2.41 <sup>i</sup>	0.15 <sup>ef</sup>	1.50 <sup>ed</sup>	3.85 <sup>hi</sup>	0.44 <sup>f</sup>	87 <sup>dc</sup>	12 <sup>fg</sup>	55 <sup>hij</sup>	42 <sup>g</sup>
GS-32	1.89 <sup>u</sup>	0.12 <sup>ikj</sup>	1.00 <sup>nm</sup>	3.40 <sup>rap</sup>	0.33 <sup>on</sup>	76 <sup>ljk</sup>	9 <sup>jk</sup>	65 <sup>de</sup>	37 <sup>ikj</sup>
GS-33	2.14 <sup>on</sup>	0.13 <sup>hg</sup>	1.05 <sup>nml</sup>	3.60 <sup>lkm</sup>	0.35 <sup>ml</sup>	66 <sup>p</sup>	13 <sup>fbcd</sup>	48 <sup>l</sup>	46 <sup>d</sup>
GS-34	1.94 <sup>t</sup>	0.10 <sup>l</sup>	0.98 <sup>n</sup>	3.23 <sup>s</sup>	0.29 <sup>q</sup>	78 <sup>ljkih</sup>	7 <sup>m</sup>	63 <sup>f</sup>	36 <sup>kl</sup>
LSD (P ≤ 0.05)	0.05	0.01	0.11	0.12	0.02	3	1	2	2

in the leaf tissue as compared to the wild type was significantly higher in the mutants GS-2 and GS-3 and GS-8 developed with lower doses of gamma rays i.e. 10 and 15 Gy, whereas the K and Ca content was recorded more in the mutants GS-11 developed at 15 Gy. A concomitant decrease in the macronutrient content was recorded in the mutant GS-34 developed at 35 Gy (Table 2). The micronutrients in the leaf tissue exhibited different trend showing increase in the Fe contents in the mutants GS-14 and GS-21 at 20 and 25 Gy, respectively. Similar trend was recorded with respect to Cu, Mn and Zn at the same irradiation doses, except that Cu was higher in the mutants GS-16, GS-18 GS-20 and GS-23; Mn (GS-20) and Zn (GS-24). In comparison to wild type, significant decrease in Fe, Zn and Mn was recorded in the mutant GS-2, GS-2 and GS-1, respectively, developed at 10 Gy in (Table 2).

Analysing the gamma radiation induced variation in macro-nutrients level of mosambi tissue, an increase in N, P, K, Ca and Mg was evident in some of the mutants developed at the lower doses of 10 and 15 Gy. However, the macro-nutrients studied showed reverse trend in mutant GS-34 at higher radiation dose of 35 Gy. The findings of the study with respect to micro-nutrient showed that the intermediate doses 20 and 25 Gy in certain mutants enhanced the accumulation of Fe, Zn, Mn and Cu contents in their leaf tissue. Interestingly, the micro-nutrients unlike the macro-nutrients showed reduced accumulation of these nutrients at the lowest dosimetry of 10 Gy. Better accumulation of macro- and micro-nutrients at lower doses might be attributed to the better photosynthetic efficiency. Restricted plant growth with lower foliage in the mutants developed at 35 Gy might have hampered the photosynthetic efficiency thus resulting in low macro nutrient status in leaf tissue. Singh and Datta (12) also observed the alterations in stomatal conductance, transpiration and photosynthetic rate and plant nutrition. Gamma irradiation improved plant nutrition but did not improve the nutritional quality of grains particularly relating to micronutrients. There are no reports regarding the impact of ionizing radiation on leaf nutrient status in fruit crops. The reason for the reduction of micronutrients in the leaf tissue at the lower dosimetry is not understood, except that lower Fe concentration might have decreased in the content other micro-nutrients.

In the present study, plant height showed significant positive correlation with the stem girth ( $r= 0.91$ ), canopy volume ( $r= 0.84$ ), TCSA ( $r= 0.88$ ), nitrogen ( $r= 0.96$ ), phosphorus ( $r= 0.89$ ), potassium

( $r= 0.88$ ), calcium ( $r= 0.83$ ) and magnesium ( $r= 0.94$ ), while it was significantly negatively correlated with copper ( $r= -0.36$ ) and zinc ( $r= -0.41$ ). Stem girth was significantly and positively correlated with canopy volume ( $r= 0.89$ ), TCSA ( $r= 0.99$ ), nitrogen ( $r= 0.86$ ), phosphorus ( $r= 0.78$ ), potassium ( $r= 0.83$ ), calcium ( $r= 0.80$ ) and magnesium ( $r= 0.85$ ), while it was significantly negatively correlated with zinc ( $r= -0.39$ ). Canopy volume showed positive correlation with TCSA ( $r= 0.93$ ), nitrogen ( $r= 0.84$ ), phosphorus ( $r= 0.75$ ), potassium ( $r= 0.86$ ), calcium ( $r= 0.80$ ), magnesium ( $r= 0.75$ ), while it showed complete negative correlation with zinc ( $r= -0.35$ ). Nitrogen was significantly and positively correlated with phosphorus ( $r= 0.93$ ), potassium ( $r= 0.92$ ), calcium ( $r= 0.86$ ), magnesium ( $r= 0.94$ ); while the complete negative correlation with copper ( $r= -0.36$ ) zinc ( $r= -0.38$ ). Phosphorus showed positive correlation with potassium ( $r= 0.85$ ), calcium ( $r= 0.80$ ) and magnesium ( $r= 0.88$ ); while it was negatively correlated with copper ( $r= -0.37$ ) and zinc ( $r= -0.44$ ). Potassium showed positive correlation with calcium ( $r= 0.79$ ), magnesium ( $r= 0.86$ ). Calcium was positively correlated with magnesium ( $r= 0.82$ ); while it was negative correlation with zinc ( $r= -0.36$ ). Magnesium was negatively correlated with copper ( $r= -0.46$ ) and zinc ( $r= -0.40$ ). Copper was positively correlated with zinc ( $r= -0.35$ ). The other parameters were not significantly correlated for the present set of sweet orange cv. Mosambi mutants (Table 3).

The findings of the study suggest that mutants developed from different doses of gamma rays exhibited significant variability in the sweet orange cv. Mosambi with respect to the parameters studied. Putative mutant generated from 15 Gy where positive alterations have taken place with respect to plant growth and leaf tissue nutrient will be further selected for understanding its impact on yield, quality and other traits. Furthermore, from the variability developed at 35 Gy, promising dwarf putative mutant will be selected for exploring its suitability in high density orcharding (HDO) and other quality traits.

## **AUTHORS' CONTRIBUTION**

Recording and analyses of data on morphological and tissue nutrient (KS), Interpretation of the results and editing of the manuscript (OPA and AKD), Providing lab facilities for tissue nutrient analysis (VK), Providing assistance in laboratory work (SK and TM).

## **DECLARATION**

No potential conflict of interest was reported by the author.

**Table 3.** The Pearson correlation of coefficient among the plant growth and nutrient parameters of gamma rays induced cv. Mosambi mutants.

	PH	SG	CV	TCSA	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
PH	1												
SG	0.91**	1											
CV	0.84**	0.89**	1										
TCSA	0.88**	0.99**	0.93**	1									
N	0.96**	0.86**	0.84**	0.85**	1								
P	0.88**	0.78**	0.75**	0.75**	0.93**	1							
K	0.88**	0.84**	0.86**	0.84**	0.92**	0.85**	1						
Ca	0.83**	0.80**	0.80**	0.81**	0.86**	0.80**	0.79**	1					
Mg	0.94**	0.85**	0.75**	0.82**	0.94**	0.88**	0.86**	0.82**	1				
Fe	-0.05	-0.04	-0.19	-0.08	-0.07	-0.21	-0.06	-0.15	-0.11	1			
Cu	-0.36*	-0.24	-0.13	-0.21	-0.36*	-0.37*	-0.19	-0.29	-0.46**	0.16	1		
Mn	-0.23	-0.22	-0.14	-0.20	-0.26	-0.31	-0.14	-0.25	-0.22	0.08	0.26	1	
Zn	-0.41*	-0.39*	-0.35*	-0.39*	-0.38*	-0.44**	-0.20	-0.36*	-0.40*	0.17	0.35*	0.29	1

\*Correlation is significant at the 0.05 level (2-tailed).

\*\*Correlation is significant at the 0.01 level (2-tailed).

Abbreviation; as PH= plant height, SG= stem girth, CV= canopy volume, TCSA= Trunk cross section area, N= nitrogen, P= phosphorus, K= potassium, Ca= calcium, Mg= magnesium, Fe= iron, Cu= copper, Mn= manganese, Zn= zinc.

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