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Impact of ionising irradiation on physio-biochemical traits of Kinnow mandarin

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ABSTRACT

In our study, Kinnow mutants developed by exposing bud-wood to gamma rays at a dose range from 15-30Gy showed both stimulatory and inhibitory responses and even within the mutants developed with same dosimetery variations were observed. Irradiation exceeding 15Gy augmented the magnitude of radiation advantage for recording the maximum alteration in the physiological and biochemical traits in the putative mutants. Compared to wild type (WT), leaf area was recorded maximum and minimum in the mutants G_6 and G_{14} developed from 20 and 25Gy. Photosynthetic pigments, chlorophyll *a* and *b* were degraded maximum in mutant G_{20} developed from 30Gy, while total phenol was stimulated in the mutants G_{18}-G_{20} derived from 30Gy. Proline accumulation was higher in G_{14} and G_{15} and total soluble protein (TSP) in G_{15} developed from 25Gy. Antioxidant enzymes (SOD, CAT, POX and GR) were up-regulated maximum in the mutants generated from a higher dosimetery of 30Gy. Collectively these results confirm the dosimetry effects of irradiation on alteration of physiological and biochemical traits which can be used as a pre-selection criterion for selecting desirable mutants.

Key words: *Citrus nobilis* Loureiro × *Citrus deliciosa* Tenora, gamma irradiation, phenol, antioxidant enzymes.

INTRODUCTION

Kinnow (*Citrus nobilis* Loureiro × *Citrus deliciosa* Tenora) has emerged as a pre-dominant mandarin, and is widely grown in the semi-arid and lower foothills of the Indian subcontinent. It is the most favoured mandarin from the consumers and grower's point of view because of its attractive appearance, higher juice recovery, extended market availability, better yield and high economic returns. All these attributes have enabled this mandarin to occupy a place of eminence having a major share in the area and production of citrus grown in India. Despite the various qualities, the plant needs to be improved for various traits such as low seeded fruits, small tree size, compact canopy, regular bearing, tolerant to diverse biotic and abiotic stresses, which however, are not easy to achieve through conventional breeding because of their high heterozygosity, quantitative inheritance and overall a long juvenile phase.

Mutagenesis on the other hand has the potential for creating variability by changing one or a few trait-specific genes that may result in the development of superior varieties. Gamma irradiation is considered as one of the potential tools for generating plant variability. The variability normally observed in the irradiated plants is in the form of physiological and biochemical alterations, which results due to disturbances in hormonal imbalance, protein synthesis, enzyme action etc., depending on the engrossed irradiation dose (Kiong *et al.,* 12). To analyse the variance of new Kinnow mandarin phenotypes and to understand the adaptation mechanism to irradiation, physiological and biochemical parameters were analysed in the putative mutants developed through different doses of gamma rays.

MATERIALS AND METHODS

The investigation included twenty putative mutants and a non-irradiated wild type (WT). For developing mutants, fresh non-dormant mature bud sticks were selected from healthy Kinnow plant (wild type) during last week of September 2011, and exposed to different doses of γ-irradiation at 15, 20, 25 and 30Gy using Co^{60} γ-irradiation chamber (Model GC-5000, BRIT, Mumbai) at Nuclear Research Laboratory (NRL), Indian Agricultural Research Institute, New Delhi. The treated buds and wild type were then shield budded *in situ* on *Jatti Khatti* rootstock and planted at the spacing of 3m × 3m on the same day. The mutants and wild type were maintained at the experimental orchard of Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi. Five years after the establishment of mutated plants, based on the morphological observations, five putative mutants were selected from the established population developed from different doses of gamma rays *i.e.* 15Gy (G_1 to G_5), 20Gy (G_6 to G_{10}), 25Gy (G_{11} to G_{15}) and 30Gy (G_{16} to G_{20}). The selected mutants were compared with the WT of the same age for various physio-biochemical characteristics for two successive years (2017 and 2018). To avoid the risk of chimera, four branches in each direction were

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labelled with a unique number for documentation, and observed for further evaluation.

Observation on leaf sclerophylly was taken using ten mature leaves (two months old). To have a uniform representative sample, leaf sampling was done from the different directions (N-S and E-W), and observations on leaf area (LA) was recorded using a Li-Cor, LI 3100 area meter (Li-Cor, USA). Several indices of leaf sclerophylly were calculated by the formulae suggested by Ennajeh *et al.* (9). Chlorophyll and carotenoids were analysed as per the method suggested by Barnes *et al.* (3). The total phenolic content in leaf was determined following the protocol of Malik and Singh (14). The leaf tissue was estimated for proline by acid ninhydrin method of Bates *et al.* (4). Sample for the estimation of protein was prepared by the process described by Bradford (5). The activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR) were estimated by following the standard protocols suggested by Dhindsa *et al.* (7), Aebi (1), Castillo *et al.* (6) and Smith *et al.* (15), respectively.

The experiment was conducted in a complete block design (CBD) with 4 replications. The pooled data of 2 consecutive years were subjected to analysis of variance using the SAS package (9.3 SAS Institute, INC., USA). For the comparison of statistics, Tukey's Honest test was performed. Means were considered significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

Physiological and biochemical alterations have been reported to serve as a quantifiable response to varying irradiation doses (Kiong *et al*., 12). Variation in leaf sclerophylly recorded in the mutants was compared with wild type (WT). Leaf area was recorded maximum in the mutants G_6 developed from 20Gy and was 22.31 per cent more than the leaf area recorded in the WT, while reduction of 29.98 per cent was recorded in mutants G_{14} (25Gy) (Table 1). The fresh (6.47 g), and dry (2.30 g) weight of leaf was more in

Table 1. Effect of gamma-rays induced variation on leaf sclerophylly characteristics of putative Kinnow mutants (Pooled mean for two years).

Mutant code	Leaf area (cm^2)	Leaf fresh weight (g)	Leaf dry weight (g)	Specific leaf area (cm^2/g)	Specific leaf weight (g/cm^2)	Density of foliage tissue (g/kg)	Succulency (mg H_2O/ cm^2)
WT	168.65^{e}	4.49^{l}	1.77^{cd}	95.56^{g}	0.0105^{f}	392.88^{a}	0.0162^{o}
G_1	169.65^{e}	4.72^{k}	1.35^{lk}	125.91^{a}	0.0080^{l}	285.79^{gf}	0.0199^{m}
G_2	160.54^{f}	5.59^{ef}	1.48^{hi}	108.30^{e}	0.0092^{h}	265.70^{ij}	0.0256^{ef}
G_3	151.93^{hg}	4.50^{l}	1.29^{lm}	117.84^{b}	0.0085^{k}	286.70^{f}	0.0211^{kjl}
G_4	146.51^{i}	4.35^{l}	1.19^{n}	123.69^{a}	0.0081^{l}	272.40^{ih}	0.0216^{j}
G_5	141.27^{j}	5.06^{j}	1.26^{m}	112.17^{dc}	0.0089^{ih}	249.23^{k}	0.0269^{d}
G_6	206.29^{a}	6.09^{b}	1.82^{cb}	113.37^{c}	0.0088^{ij}	298.95^{ed}	0.0207^{kml}
G_7	164.07^{f}	5.16^{ij}	1.65^{f}	99.62^{f}	0.0101^{g}	319.56^{c}	0.0214^{kj}
G_8	155.04^{g}	5.94^{cb}	1.87^{b}	82.84^{i}	0.0121^{c}	315.19c	0.0262^{ed}
G_9	132.17^{k}	4.69^{k}	1.40^{jk}	94.27^{g}	0.0106^{f}	299.42^{ed}	0.0248^{gf}
G_{10}	177.04^{d}	5.21^{ihj}	1.58^{g}	112.07^{dc}	0.0089^{ih}	303.25^{d}	0.0205^{ml}
G_{11}	129.36^{k}	5.89^{c}	1.68^{ef}	76.92^{kj}	0.0130^{b}	285.52^{gf}	0.0326^{b}
G_{12}	128.50^{lk}	5.97^{cb}	1.73^{ed}	74.17^{k}	0.0135^{a}	290.06^{ef}	0.0330^{ba}
G_{13}	191.18^{b}	6.07^{b}	1.74^{ed}	109.73^{de}	0.0091^{ih}	286.99^{f}	0.0226^{i}
G_{14}	118.08^{m}	3.47^{m}	1.36^{k}	87.20^{h}	0.0115^{d}	392.28^{a}	0.0179^{n}
G_{15}	177.06^{d}	6.47^{a}	2.30^{a}	77.16^{j}	0.0130^{b}	355.03^{b}	0.0236^{h}
G_{16}	125.09^{l}	5.71^{ed}	1.53^{hg}	81.85^{i}	0.0122^{c}	267.68^{ihj}	0.0335^{a}
G_{17}	183.34^{c}	5.27^{ih}	1.46^{ji}	125.80^{a}	0.0080^{l}	276.68^{gh}	0.0208^{kjl}
G_{18}	164.28^{f}	5.38^{gh}	1.40^{jk}	117.46^{b}	0.0085^{kj}	260.03^{j}	0.0243^{gh}
G_{19}	184.54^{c}	5.49^{gf}	1.66^{f}	111.52^{dc}	0.0090^{ih}	301.78^{d}	0.0208^{kl}
G_{20}	150.52^{h}	5.87^{cd}	1.69^{ef}	89.33^{h}	0.0112^{e}	287.17^{f}	0.0278^{c}
LSD (0.05)	3.82	0.17	0.07	2.78	0.0003	9.75	0.0009

Superscript in small letters indicate significant difference at $P < 0.05$.

mutant $G_{15.}$ Higher specific leaf area (125.91 cm^2/g) without any statistical variation were witnessed in mutants G_1 (125.91 cm^2/g), G_4 (123.69cm^2/g) and G_{17} (125.80cm^2/g) developed from the lower and higher doses of 15 and 30Gy, while the mutants G_{12} (74.17 cm^2/g) and G_{11} (76.92 cm^2/g) created from 25Gy had lower specific area. Specific leaf weight followed a reciprocal trend with SLA with the maximum and minimum values in the above mutants. In our study, leaf sclerophylly characteristics varied dramatically in the mutants and highlight both stimulatory and inhibitory effects of irradiation (Table 1). Even in the mutants developed with same irradiation dose, variations were observed which may be attributed to metabolic disturbances, such as changes in chemical patterns or improper functioning of different phytohormones leading to morphological variation. Variation in leaf characteristics of *Citrus* species such as grapefruit, sweet orange, mandarin and lemon treated with gamma rays have been reported previously (Hearne, 11).

The range of measured photosynthetic pigments varied among the mutants investigated in the study (Table 2). Chlorophyll *a* estimated in the mutated population was significantly lower than the WT and it was lower in mutants G_{20} and G_{11} (0.84 -0.91 mg g^{-1} FW) generated from the higher dosimetery doses of 30 and 25Gy. Chlorophyll *b* showed invariable trends and the maximum chlorophyll *b* content was registered significantly higher in mutants G_{14} (0.52 mg g^{-1} FW) and G_{13} (0.46 mg g^{-1} FW) created from 25Gy than others, while it was minimum in G_{20} (0.21 mg g^{-1} FW). The total chlorophyll recorded maximum in the WT (1.97 mg g^{-1} FW) maintained statistical parity with the mutants G_3 and $G_{6,}$ while it was lowest in G_{20} (1.05 mg g^{-1} FW). The mutant G_{18} (30Gy) had the highest total carotenoids content (1.51 mg g^{-1} FW), while it was lowest without any statistical disparity in the mutants G_8 and G_{10} developed from 20Gy. Chlorophyll pigments are the key component for photosynthesis and are considered as a reliable indicator for evaluating genetic alterations in a mutated population. In our study, although the pigments followed an inhibitory or stimulatory response within the mutated population, the level of chlorophyll '*a'* recorded in the mutants was invariably higher than chlorophyll '*b'* (Table 2). The similar elevated trend in chlorophyll '*a*' was observed by Ling *et al.* (13) in sweet orange. The comparative study of the pigments within the mutated population and on comparison with the WT showed a reduction in chlorophyll '*a*' as compared to the stimulatory or inhibitory response in chlorophyll 'b'. Such alterations might have taken place due to the disturbances in hormone balance, leaf gas exchange, water exchange and enzymatic activity.

Table 2. Effect of gamma-ray induced variation in the photosynthetic pigments of putative Kinnow mutants (Pooled mean for two years).

Mutant code	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Total chl (mg g^{-1} FW)	Chl *a*:*b*	Total carotenoids (mg g^{-1} FW)
WT	1.66^{a}	0.31^{def}	1.97^{a}	5.39^{ba}	1.31^{cd}
G_1	1.45^{b}	0.36^{dc}	1.81^{bdc}	4.08^{fecd}	1.15^{hg}
G_2	1.33^{c}	0.27^{gef}	1.60^{gf}	5.07^{bcd}	1.00^{ji}
G_3	1.48^{b}	0.41^{bc}	1.90^{ba}	3.61^{fegh}	1.27^{cfde}
G_4	1.39^{cb}	0.36^{dc}	1.74^{edc}	3.98^{fecd}	1.02^{i}
G_5	1.41^{cb}	0.45^{ba}	1.86^{bac}	3.13^{figh}	1.14^{hg}
G_6	1.43^{cb}	0.46^{ba}	1.89^{ba}	3.14^{figh}	1.20^{fg}
G_7	1.43^{cb}	0.41^{bc}	1.83^{bc}	3.57^{fegh}	1.15^{hg}
G_8	1.13^{efg}	0.23^{g}	1.37^{ji}	5.07^{bcd}	0.82^{l}
G_9	1.03^{g}	0.21^{g}	1.24^{jk}	5.04^{bcd}	1.34^{cb}
G_{10}	1.17^{ef}	0.34^{de}	1.51^{hg}	3.56^{fegh}	0.88^{lk}
G_{11}	0.91^{h}	0.32^{def}	1.23^{k}	2.93^{igh}	1.08^{hi}
G_{12}	1.32^{cd}	0.22^{g}	1.54^{hg}	6.49^{a}	1.23^{fde}
G_{13}	1.22^{ed}	0.46^{ba}	1.69^{edf}	2.70^{ih}	1.22^{fge}
G_{14}	1.10^{fg}	0.52^{a}	1.62^{egf}	2.14^{i}	1.28^{cfde}
G_{15}	1.19^{ef}	0.23^{g}	1.42^{hi}	5.21^{b}	1.41^{b}
G_{16}	1.21^{e}	0.31^{def}	1.53^{hg}	3.90^{feg}	0.94^{jk}
G_{17}	1.13^{efg}	0.25^{gf}	1.38^{i}	4.64^{becd}	1.33^{cb}
G_{18}	1.20^{ef}	0.24^{g}	1.44^{hi}	5.18^{bc}	1.51^{a}
G_{19}	1.05^{g}	0.32^{de}	1.37^{ji}	3.32^{fgh}	1.32^{c}
G_{20}	0.84^{h}	0.21^{g}	1.05^{l}	4.09^{fecd}	1.30^{cde}
LSD (0.05)	0.10	0.06	0.12	1.12	0.08

Superscript in small letters indicate significant difference at $P < 0.05$.

The results of the total phenol distribution in the foliar tissue depicted a dose-dependent ascending increase in the mutated population (Table 3). In contrast to the wild type, 43-47% increase in the total phenol content (TPC) were recorded in the mutants developed between 15-25 Gy, whereas more than two-fold increase in contrast to the WT were recorded in the mutants developed with 30Gy. Phenolic compounds have antioxidant defence properties and can alleviate intermediary radicals. The results obtained indicated that the mutants accumulated more phenol than the wild type especially in the mutants developed at 30Gy. Higher phenol accumulation in the mutants developed at 30Gy may be attributed to the increased phenylalanine ammonia-lyase activity, a key enzyme for phenolic

Table 3. Effect of gamma-ray induced variation in the biochemical parameters of putative Kinnow mutants (Pooled mean for two years).

Mutant code	Total phenol (mg g^{-1} FW)	Proline (µM g^{-1} FW)	Total soluble protein (mg g^{-1} FW)	Superoxide dismutase (mg^{-1} protein min^{-1})	Catalase (µmol of H_2O_2 hydrolysed mg^{-1}protein min^{-1})	Peroxidase (µmol tetra-guaiacol formed mg^{-1} protein min^{-1})	Glutathione reductase (mg^{-1} protein min^{-1})
WT	48.79^{g}	0.36^{i}	9.11^{l}	0.92^{e}	24.39^{h}	1.30^{f}	1.15^{ihg}
G_1	85.59^{f}	0.42^{g}	13.24^{gh}	1.14^{d}	26.82^{g}	1.51^{e}	1.15^{ihg}
G_2	85.59^{f}	0.38^{hi}	14.65^{cfed}	1.15^{d}	26.87^{g}	1.50^{e}	1.05^{i}
G_3	85.22^{f}	0.39^{hgi}	12.59i^{h}	1.15^{d}	26.71^{g}	1.51^{e}	1.09^{i}
G_4	85.41^{f}	0.40^{hg}	12.15^{ij}	1.10^{d}	26.63^{g}	1.49^{e}	1.13^{ih}
G_5	85.55^{f}	0.39^{hgi}	10.93^{k}	1.10^{d}	25.76^{hg}	1.52^{e}	1.02^{i}
G_6	87.10^{e}	0.59^{e}	14.78^{ced}	1.40^{c}	31.34^{fe}	1.54^{ed}	1.31^{fhg}
G_7	88.97^{d}	0.52^{f}	13.78^{gf}	1.45^{c}	30.41^{f}	1.54^{ed}	1.34^{feg}
G_8	88.68^{d}	0.52^{f}	14.37^{fed}	1.46^{c}	30.93^{fe}	1.53^{ed}	1.35^{feg}
G_9	87.87^{ed}	0.53^{f}	12.52^{ih}	1.47^{c}	31.92^{e}	1.57^{ced}	1.54^{de}
G_{10}	88.24^{ed}	0.52^{f}	11.77^{ikj}	1.50^{c}	31.27^{fe}	1.51^{e}	1.50^{fe}
G_{11}	91.21^{c}	0.71^{bc}	12.44^{ihj}	1.99^{b}	34.68^{d}	1.73^{b}	1.75^{c}
G_{12}	90.81^{c}	0.72^{ba}	13.83^{gfe}	2.03^{b}	35.80^{d}	1.70^{cb}	1.80^{c}
G_{13}	91.77^{c}	0.72^{ba}	14.89^{cbd}	2.06^{b}	35.34^{d}	1.68^{cbd}	1.73^{dc}
G_{14}	91.65^{c}	0.75^{a}	12.67^{ih}	2.05^{b}	35.06^{d}	1.80^{b}	1.78^{c}
G_{15}	91.36^{c}	0.76^{a}	17.03^{a}	2.05^{b}	34.66^{d}	1.67^{cbd}	1.75^{dc}
G_{16}	101.18^{b}	0.67^{d}	15.85^{b}	2.55^{a}	38.82^{c}	2.00^{a}	2.03^{b}
G_{17}	101.73^{b}	0.67^{cd}	15.40^{cb}	2.63^{a}	39.57^{bc}	2.07^{a}	2.09^{ba}
G_{18}	103.20^{a}	0.67^{cd}	12.27^{ij}	2.63^{a}	39.82^{bc}	2.11^{a}	2.23^{ba}
G_{19}	103.42^{a}	0.70^{bcd}	11.47^{kj}	2.64^{a}	40.38^{ba}	2.09^{a}	2.24^{a}
G_{20}	103.57^{a}	0.62^{e}	14.68^{cfed}	2.65^{a}	41.51^{a}	2.12^{a}	2.03^{b}
LSD (0.05)	1.32	0.03	0.97	0.11	1.42	0.15	0.21

Superscript in small letters indicate significant difference at $P < 0.05$.

compound synthesis in plant tissues, which however, was not a part of this study. A similar increase in the TPC in plants in response to gamma irradiation has been reported by Dixit *et al.* (8). The leaf proline content followed a trend similar to the phenol content and as compared to the WT (0.36 µM g^{-1} FW) which registered a minimum proline content in the leaf tissue, was almost two-fold higher in the mutants G_{12}-G_{15} developed from 25Gy irradiation dose and the difference was not significant. In the mutants generated from 30Gy almost 1.72-1.94 fold increase in the TPC content was recorded (Table 3). Proline accumulation in plants is being used as a protective criterion for tolerance to a variety of stresses such as salinity, drought and heat. Biochemical analysis of proline content demonstrated that the mutants G_{12}-G_{15} developed at 25Gy gamma rays accumulated almost two-fold more proline in their foliar tissues followed by mutants developed with 30Gy. The increase in proline content in the mutants developed with higher irradiation dose suggests protective mechanism and ability to protect the cells during osmoticum by scavenging OH^- radicals and thus may be an important source from the drought tolerance point of view. A similar relationship between proline and gamma-ray dosimetery effect has been reported in *Trigonella foenum-graecum* by Hanafy and Akladious (10). Results presented in Table 3 revealed that the TSP in foliar tissue were in the range of 9.11mg g^{-1} FW in WT to 17.03mg g^{-1} FW in mutants G_{15} (25Gy). In comparison to WT, almost 1.86 and 1.73 fold increase in the TSP content were recorded in the mutants G_{15} and G_{16} (30Gy). The minimum TSP content although recorded in mutants G_5 (10.93mg

g^{-1} FW) G_{19} (11.47mg g^{-1} FW) and G_{10} (11.77 mg g^{-1} FW) was higher than the WT (9.11mg g^{-1} FW). The protein content in the foliar tissue of mutants as compared to wild type in the present study was found to be significantly stimulated in the mutants G_{15} and G_{16} developed from higher irradiation doses of 25 and 30Gy (Table 3). The stimulative cause for an increase in the protein content at higher irradiation dose may be attributed to the increased amino acid concentration. Increase in soluble protein as a protective mechanism against gamma irradiation in plants has been reported by Al-Rumaih and Al-Rumaih (2).

To evaluate the contribution of the inductive response of the antioxidant enzyme, we measured the activities of the antioxidant enzymes in the mutated population and WT Kinnow. The response of antioxidant enzymes in the foliar tissue of putative mutants was comparatively higher in the mutated populations in contrast to the WT which registered minimum activities of SOD, CAT and POX (Table 3). In general, a dose-dependent increase in the SOD activity was observed in the mutated population and it was recorded maximum in the mutants G_{16}-G_{20} (30Gy) with almost 2.77-2.88 fold increase and had parallel values. A similar dose-dependent increase in the CAT activity was also observed in the mutated population and it was up-regulated maximum in the mutants G_{17}-G_{20} developed from 30Gy with almost 1.62-1.70 fold increase over the WT. Peroxidase activity (POX) followed a pattern similar to SOD exhibiting an increase of 1.14-1.63 fold in the mutants G_{16}- G_{20} in contrast to the WT without any statistical variation. As compared to WT (1.15 mg^{-1} protein min^{-1}), the basal GR activity was almost doubled in the mutants G_{17}-G_{19} (30Gy) with statistical parity. GR activity although decreased in the mutants G_3 and G_4 it did not differ significantly with the wild type. Antioxidant enzymes SOD, CAT, POX and GR prevent the plant cells from oxidative damage under different stresses by scavenging the reactive oxygen species (ROS). SOD catalyses the partitioning of $O_2^-H_2O_2$ and O_2, while CAT and POX can decompose H_2O_2 into H_2O and O_2. The present study confirmed that the activities of SOD, CAT, POX and GR were invariably higher in the putative mutants G_{16}-G_{20} developed at higher irradiation doses of 30 Gy followed by a corresponding decrease in the values of mutants developed at lower irradiation. Enhanced activity of scavenging enzymes in the mutants imply its lower vulnerability to irradiation effect and its ability to scavenge the excess ROS and guard the cellular components.

The study delves that the gamma radiation mutagenesis altered the leaf physiological and biochemical traits in the putative mutants of Kinnow mandarin. The physiological traits demonstrated both stimulatory and inhibitory alterations and were maximum in the mutants developed between 25-30 Gy. The biochemical parameters erstwhile were significantly elevated in the mutants developed from 30 Gy signifying that the mutants developed a stress mechanism for mitigating the cellular mechanism disorder occurred during irradiation of bud wood. The range of variability observed in the putative mutants has enhanced the scope for isolating mutants with multiple traits for tolerance to various stresses. From the breeding point of view, it would be useful for improving the qualitative and quantitative traits via altering various pathways and the genes responsible for these changes can be tagged, isolated and transformed into new genetic backgrounds. The study suggests that irradiation dose beyond 25 Gy enhances the bio-chemical constituent in Kinnow mutant with concomitant increase in antioxidant compounds. The mutants (G_{15}-G_{20}) developed from 30 Gy was superior in terms of these compounds and can be evaluated for its tolerance to the various abiotic stresses. A similar dosimetry of 25-30 Gy can also be employed in other citrus species for understanding the physio-biochemical relation and identifying trait of interest which could be used in the future breeding programmes.

AUTHORS' CONTRIBUTION

Conceptualization of research (O. P. Awasthi); Design of experiments (O. P. Awasthi and Sunil Kumar); Contribution of experimental material (O. P. Awasthi); Execution of field/lab experiments and data collection (Sunil Kumar); Analysis of data and interpretation (Sunil Kumar, O. P. Awasthi, A. K. Dubey and Awtar Singh); Preparation of manuscript (Sunil Kumar, O. P. Awasthi and Renu Pandey)

DECLARATION

The authors declare no conflict of interest.

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