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# Gamma Irradiation Effect on Leaf Gas Exchange and Hormones of Sweet Orange Cv. Mosambi

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**ABSTRACT: In the present study, the induced variation in respect to leaf gas exchange and phytohormone parameters were studied in the pre-bearing mutants of sweet orange cv. Mosambi. These mutants were developed through different doses (10, 15, 20, 25, 30 and 35 Gy) of gamma irradiation. A stimulatory and inhibitory effect in respect to the leaf gas exchange parameters was noticed in the mutants developed at lower and higher doses of gamma irradiation respectively. The mutants GS-32 and GS-31 developed from 35 Gy had shown reduction in the *A* by -41.35% and -31.37% respectively. *E*was witnessed lower in mutants GS-24 (-32.12%) and GS-33 (-27.97%) developed at 25 and 35 Gy respectively. Similar trend was observed to follow in case of *gs* and *Ci* values. The peak IAA and ABA content was assayed in the mutants developed at 25-35 Gy. IAA content in leaf tissue was varied between 91.3 ng $g^{-1}$ in GS-32 to 138.3 ng $g^{-1}$ in mutants developed with the higher dosimetries of 30 and 35 Gy. The ABA level was recorded maximum in the mutants GS-28 (714.3 ng $g^{-1}$) and GS-26 (694.8 ng $g^{-1}$) developed from 25 Gy. The study highlights that mutants developed at higher doses of gamma irradiation significant stimulatory and inhibitory effect with respect to leaf gas exchange and phytohormone (IAA and ABA). Thus, such induced alterations are of significance and would help in improvement of sweet orange.**

**Keywords**: γ-rays; mutants; leaf gas exchange IAA and ABA.

## INTRODUCTION

Citrus is one of the most important fruit crop grown between latitude 35°N~35°S. In India, citrus production is 13.20 million tonnes from 1.03 million ha. Citrus fruits including sweet orange *(Citrus sinensis* Osbeck), mandarin (*Citus reticulata* Blanco), limes (*Citrus aurantifolia* Swingle), lemon (*Citrus limon* (L) Burm. f), grapefruit (*Citrus paradise* Macf.) and pumello (*Citrus grandis* (L.) Osbeck) grown on commercial scale in India. Sweet orange occupies a second position followed by mandarin with a production of 3.40 million tonnes from 0.19 million hectare contributing 25.76% of total citrus production in the country (Anonymous, 2018). Since the sweet orange is a prime source of antioxidants and soluble sugar, its juice is highly recommended to a sick patient. However, the quality of juice is degraded as the seeds get crushed during juice extraction anddue to enzymatic conversion of a non-bitter compound Limonate A ring Lactone (LARL) into a bitter compound limonin which imparts bitterness to juice. Thus, the presence of large number of seeds/fruit is a major bottle neck to the citrus industry.

Several attempts have been made in past by the breeders through conventional breeding and developed of certain promising varieties in fruit crops (Spiegel-Roy *et al*., 2007; Bermejo *et al*., 2011). However, the varietal development through conventional breeding in citrus is slow because of the presence of apomixes, self and cross incompatibility, high heterozygosity, perennial nature and overall a long juvenile phase (Grosser *et al*., 2000). On the other hand, mutation breeding holds prime position in the varietal improvement of perennial fruit crops such as seedless mutants in orange, mandarin, grapefruit and lemon (Spiegel-Roy *et al*., 1990; Hearne, 1984; Wu *et al*., 1986). Besides the seedless varieties, mutagenesis has been utilized to developspine-free mandarin i.e."Sunki"

(Kukimura *et al*., 1976); a compact fruitful canopy in orange (Donini, 1982);dwarfness and biotic and abiotic stresses tolerance (Jain, 2000; Ahloowalia and Maluszymski, 2001). Apart from the varietal improvement, mutagenic agents rather physical or chemical havealso been reported to have a differentially effecton the plant physiology depending upon the mutagen dose (Mallick *et al*., 2016; Kumar *et al.*, 2020). Thus, Keeping in view of this, the present study was conducted to evaluate the alteration in leaf gas exchange and phytohormones parameters ofthe putative mutants developed from the different doses of gamma irradiation in comparison to wild-type and mutants.

## MATERIALS AND METHODS

Plant materials consisted of thirty four mutants and non-treated Mosambi plant (wild type). The mutants were developed at the different doses of γ-irradiation from 10, 15, 20, 25, 30 and 35 Gy using $Co^{60}$ γ-irradiation chamber (Model GC-5000, BRIT, Mumbai) at Nuclear Research Lab-oratory (NRL), Indian Agricultural Research Institute, New Delhi. The developed mutants were assigned code GS-0 (wild type/control), GS-1 to GS-6 (10 Gy), GS-7 to GS-12 (15 Gy), GS-13 to GS-18 (20 Gy), GS-19 to GS-24 (25 Gy), GS-25 to GS-30 (30 Gy) and GS-31 to GS-34 (35 Gy) of gamma irradiation.The wild type and the mutant trees were grown under drip irrigation system and received the same cultural practices. The mutants were evaluated forleaf gas exchange and phytohormone parameters.

Leaf Net Photosynthesis (*A*), Stomatal Conductance (*gs*), Intercellular $CO_2$ concentration (*Ci*) and transpiration (*E*) was recorded on 60–70 days old leaf after emergence of spring season flush *i.e.,* April for year 2017 and 2018. The gas exchange traits were measured from 11.00AM to 12.00 PM (IST) using an LCi-SD Ultra Compact Photosynthesis System. Twelve mature leaves/plant from exterior canopy position was used for recoding the leaf gas exchange parameters.

Estimation of indole-3-acetic acid (IAA) from fresh leaf tissue was done as per the protocol of Fu *et al.* (2011) with modifications in process of extraction (repeated overnight extraction of IAA from samples with methanol) and final sample volume for IAA reconstitution after vacuum evaporation (300 µL methanol). The leaf extract in methanol was centrifuged at 15,000×g for 10 min at 4°C (model-HERMLE Z 323K). The obtained supernatant was concentrated until the volume decreased to less than $1/10^{th}$using a vacuum concentrator. Thereafter, HPLC grade water was added in the sample and then pH of the solution was adjusted between 9-10 with potassium hydroxide to keep IAA ionized and then partitioned with 100% ethyl acetate. The lower aqueous were separated by centrifugation (15,000× g for 2 min) and transferred to a new tube. Wherein, the pH was adjusted to < 3 with acetic acid to conserve IAA in protonated form. Now sample was partitioned again with ethyl acetate and cleared by centrifugation. The upper organic phase was recovered and dried completely and dissolved in 300 µL of methanol. The filtered samples in 20 µL volume were then injected and analysed by HPLC.For Chromatographic separation Agilent 1200 series HPLC system (Agilent Technologies Inc., USA) includingan auto-sampler, a degasser, a quaternary pump and FLD was used. A Zorbax Eclipse XDB-C18 reversed-phase column (5 µm, 4.6 × 250 mm, Agilent) used forIAA separation platform. The solventAmobile phase included methanol (90%) with acetic acid (0.3%) and solvent B includes methanol (10%) with acetic acid (0.3%). The Fluorescence Detection was set with excitation wave length and emission wave length at 280 nm 360 nm respectively.

Abscisic acid in the leaf tissue was estimated using HPLC, as the method suggested by Zeevaart (1980) with minor modifications. Frozen leaf samples were ground into a fine powder in liquid nitrogen and extracted in 10 ml of 80 % v/v acetone for three times. The extract was filtered through Whatman No. 1 filter and transferred to the boiling flask of rotary flash vacuum evaporator. The acetone was evaporated anda lipid soluble material remained at the bottom walls of the boiling flask. This remained was dissolved in 1ml of 1% acetic acid solution and transferred into small amber colored vials (1ml). This sample was used for injecting into HPLC after filtering with 0.45 µmPVDF membrane micro syringe filter. The preparative HPLC system was the same as that used for IAA extraction. However, the separation was carried out on ZORBAX Eclipse XDB-C18 column (250x4.6mm, 5 µm) at $30^oC$ with mobile phase composed of 1% acetic acid in 95% methanol in isocratic mode at a flow rate of 1ml $min^{-1}$. The detection was monitored at variable wavelength detector at 265 nm.

The statistical analysis of mean replicated data of leaf gas exchangefor years 2017 and 2018 was carried out in completely randomized block design with 4 replications to measure standarderror, subjected to analysis of variance (ANOVA) usingSAS package (9.3 SAS Institute, INC., USA) followed by Tukey's Honest test. P values ≤0.05 were considered as significant.

## RESULTS AND DISCUSSION

Mutants were witnessed with decrease in leaf gas exchange parameters like photosynthetic rate (*A*), Transpiration rate (E), internal carbon dioxide concentration (Ci) and stomatal conductance (gs) at higher dosimetry however*E* and *gs* were recorded higher the mutants developed at lower dosimetery in comparison to wild type (Table 1). Photosynthesis rate documented a significant decrease in the photosynthetic rate as compared to the WT was observed in the mutants except GS-17. Maximum decrease in the

photosynthetic rate was registered in the mutant GS-32 (-41.35%) and GS-31 (-31.37%) developed from 35 Gy. *E* and *gs* were higher by 33.67 and 36 per cent in the mutant GS-1 developed from the lower dosimetry of 10 Gy. However, a different trend was recorded in the mutants developed from higher irradiation doses. Mutants GS-24 and GS-33 developed at higher irradiation dose of 25 and 35 Gy witnessed lower transpiration of -32.12 and -27.97 per cent respectively. Similarly lower *gs* values without any statistical difference were indexed in mutants GS-21, GS-24, and GS-32 created from 25Gyand GS-34 from 35 Gy.*Ci* concentration was higher by 8.54 and 4.62 per cent in the mutants GS-20 and G-19 generated from 25 Gy, while a decrease of 14.23 per cent was registered in GS-31 and GS-32 with statistical equivalence.

**Table 1: Alteration in leaf gas exchange parameters in the mutants in comparison to wild type.**

| Treatment | Photosynthetic rate (A) (µmol $m^{-2}$ $s^{-1}$) | Transpiration rate (E) | Stomatal conductance (Gs) | Internal $CO_2$ concentration (Ci) |
|---|---|---|---|---|
| GS-0 | $9.02^{a}$ | $1.93^{lejbidhkgcnfm}$ | $0.075^{edf}$ | $281^{ebdagcf}$ |
| GS-1 | $8.21^{bdec}$ | $2.58^{a}$ | $0.102^{a}$ | $266^{ejdihgf}$ |
| GS-2 | $7.64^{fidehg}$ | $2.17^{ebdhagcf}$ | $0.085^{dc}$ | $263^{ejihgf}$ |
| GS-3 | $8.25^{bdec}$ | $2.34^{ba}$ | $0.077^{ed}$ | $271^{ebdihgcf}$ |
| GS-4 | $8.29^{bdac}$ | $2.21^{ebdacf}$ | $0.090^{bc}$ | $268^{edihgcf}$ |
| GS-5 | $8.02^{fbdfc}$ | $2.31^{bac}$ | $0.100^{ba}$ | $282^{ebdagcf}$ |
| GS-6 | $8.31^{bdac}$ | $2.20^{ebdagcf}$ | $0.085^{dc}$ | $277^{ebdhgcf}$ |
| GS-7 | $8.02^{fbdec}$ | $1.70^{ljoikpnm}$ | $0.062^{ijhgk}$ | $274^{ebdhgcf}$ |
| GS-8 | $8.33^{bdac}$ | $2.23^{ebdacf}$ | $0.075^{edf}$ | $267^{edihgcf}$ |
| GS-9 | $8.17^{bdec}$ | $1.63^{lopnm}$ | $0.055^{jlk}$ | $284^{ebdacf}$ |
| GS-10 | $7.61^{fideh}$ | $2.15^{ebidhagcf}$ | $0.075^{edf}$ | $278^{ebdhgcf}$ |
| GS-11 | $7.24^{ijhkg}$ | $2.26^{bdac}$ | $0.077^{ed}$ | $269^{ebdihgcf}$ |
| GS-12 | $7.61^{fideh}$ | $1.81^{lejoidhkgnfm}$ | $0.065^{ijhgf}$ | $282^{ebdagcf}$ |
| GS-13 | $8.01^{fbdec}$ | $1.88^{lejidhkgcnfm}$ | $0.060^{ijhgk}$ | $268^{eddihgcf}$ |
| GS-14 | $7.85^{fbdehcg}$ | $2.16^{ebdhagcf}$ | $0.072^{egf}$ | $284^{ebdacf}$ |
| GS-15 | $8.43^{bac}$ | $2.09^{lejbidhkgcf}$ | $0.085^{dc}$ | $260^{jihgf}$ |
| GS-16 | $7.73^{fdehcg}$ | $2.13^{ejbidhagcf}$ | $0.097^{ba}$ | $277^{ebdhgcf}$ |
| GS-17 | $8.59^{ba}$ | $2.24^{ebdac}$ | $0.092^{bac}$ | $266^{edihgf}$ |
| GS-18 | $7.89^{fbdecg}$ | $1.90^{lejidhkgcnfm}$ | $0.075^{edf}$ | $260^{jihgf}$ |
| GS-19 | $7.17^{ijhkg}$ | $1.64^{opnm}$ | $0.055^{jlk}$ | $294^{ba}$ |
| GS-20 | $5.95^{mn}$ | $2.14^{ebidhagcf}$ | $0.070^{ehgf}$ | $305^{a}$ |
| GS-21 | $7.13^{ijhk}$ | $1.57^{opnm}$ | $0.050^{l}$ | $274^{ebdhgcf}$ |
| GS-22 | $6.55^{mlk}$ | $1.72^{ljoihkpnm}$ | $0.067^{iehgf}$ | $258^{jihg}$ |
| GS-23 | $6.26^{ml}$ | $1.85^{lejidhkgnfm}$ | $0.052^{lk}$ | $255^{jih}$ |
| GS-24 | $6.59^{mlk}$ | $1.31^{p}$ | $0.050^{l}$ | $286^{ebdac}$ |
| GS-25 | $6.60^{mlk}$ | $1.78^{ljoihkgnfm}$ | $0.055^{jlk}$ | $270^{ebdihgcf}$ |
| GS-26 | $7.37^{fijhg}$ | $1.69^{ljokpnm}$ | $0.065^{ijhgf}$ | $289^{bdac}$ |
| GS-27 | $7.71^{fidehcg}$ | $1.97^{lejbidhkgcfm}$ | $0.065^{ijhgf}$ | $292^{bac}$ |
| GS-28 | $7.51^{fieng}$ | $2.10^{ejbidhkgcf}$ | $0.065^{ijhgf}$ | $287^{ebdac}$ |
| GS-29 | $7.80^{fdehcg}$ | $1.84^{lejoidhkgnfm}$ | $0.075^{edf}$ | $269^{ebdihgcf}$ |
| GS-30 | $6.96^{iljk}$ | $1.66^{lopnm}$ | $0.065^{ijhgf}$ | $270^{ebdihgcf}$ |
| GS-31 | $6.19^{m}$ | $1.80^{lejoihkgnfm}$ | $0.057^{ijlk}$ | $241^{j}$ |
| GS-32 | $5.29^{n}$ | $1.75^{ljoihkgpnm}$ | $0.050^{l}$ | $247^{ji}$ |
| GS-33 | $6.62^{mljk}$ | $1.39^{op}$ | $0.060^{ijhgk}$ | $281^{ebbdagcf}$ |
| GS-34 | $8.06^{fbdec}$ | $1.49^{opn}$ | $0.050^{l}$ | $275^{ebbdhgcf}$ |
| LSD ($P \leq 0.05$) | 0.75 | 0.45 | 0.012 | 25.02 |

Notes:Superscript in small letters on the value of each leaf gas exchange parameter indicates significant difference at $P < 0.05$.

Critical examination of the data shows that both the photosynthetic rate and stomatal conductance were inhibited at 30-35 Gy. It is logical to imply that the lower photosynthetic rate and stomatal conductance in the mutants at higher radiation dose may be a consequence of disturbances in chloroplast function thus inhibiting the enzymatic activities of chlorophyll biosynthesis and $CO_2$ fixation. Similar decline in photosynthetic rate of mutants developed from higher irradiation doses have been reported in Kinnow mandarin (Kumar *et al.,* 2020); in sweet orange (Singh *et al.,* 2021); in *Centella asiatica* (Moghaddam*et al*., 2011) and in buck wheat (Jia and Li, 2008). Higher transpiration in the mutant GS-1 may be attributed to stomatal conductance and free radical scavenging in the investigated doses which however, could not be met out in the mutants developed at higher irradiation. The findings are in consonance with Nobel (1999) who

reported that transpiration rate is greatly affected by stomatal conductance.

Alteration in the IAA and ABA concentration among the mutants was recorded at different doses of gamma irradiation (Fig. 1 IAA). In distinction with the WT (39.7g ng $g^{-1}$), IAA content in leaf tissue was assayed maximum in mutants developed with the higher dosimetries of 30 and 35 Gy and varied between 91.3 ng $g^{-1}$ in GS-32 to 138.3 ng $g^{-1}$ in GS-27, except GS-26 (68.7 ng $g^{-1}$) which did not group in this range. A momentous increase between 2.29-3.5 fold was registered in the mutants developed by the irradiation doses between 30 and 35 Gy.ABA concentration was recorded significantly higher as compare to control at different doses of gamma irradiation (Fig. 1 ABA). In contrast to the WT (287.0 ng $g^{-1}$), the ABA level in the mutants GS-28 (714.3 ng $g^{-1}$) and GS-26 (694.8 ng $g^{-1}$) developed from 25 Gy was stimulated significantly by almost 2.5±0.06 fold, followed by 1.80 fold increase in the mutants GS-32 (514.3 ng $g^{-1}$) and GS-27 (513.7 ng $g^{-1}$) developed from 35 and 30 Gy respectively.

In citrus species, several economically important processes are controlled by phytohormones (Quecini *et al.*2007). In the present study, a fluctuation was observed in the phytohormone studied. The peak value for IAA was assayed at 30Gy and ABA was assayed at 30Gy, shows the plant response to the irradiation stress. Bhatt *et al.* (2008) stated that radiation can increase the level of endogenous level of hormones either by *de novo* synthesis of free hormone to reduce the effect of stress caused by radiation or by converting the conjugated form to free form. The results of the present study are supported by the finding of Latif *et al.* (2011); Qi *et al.* (2015), reported increase in the level of phytohormone when treated with different doses of gamma irradiation.

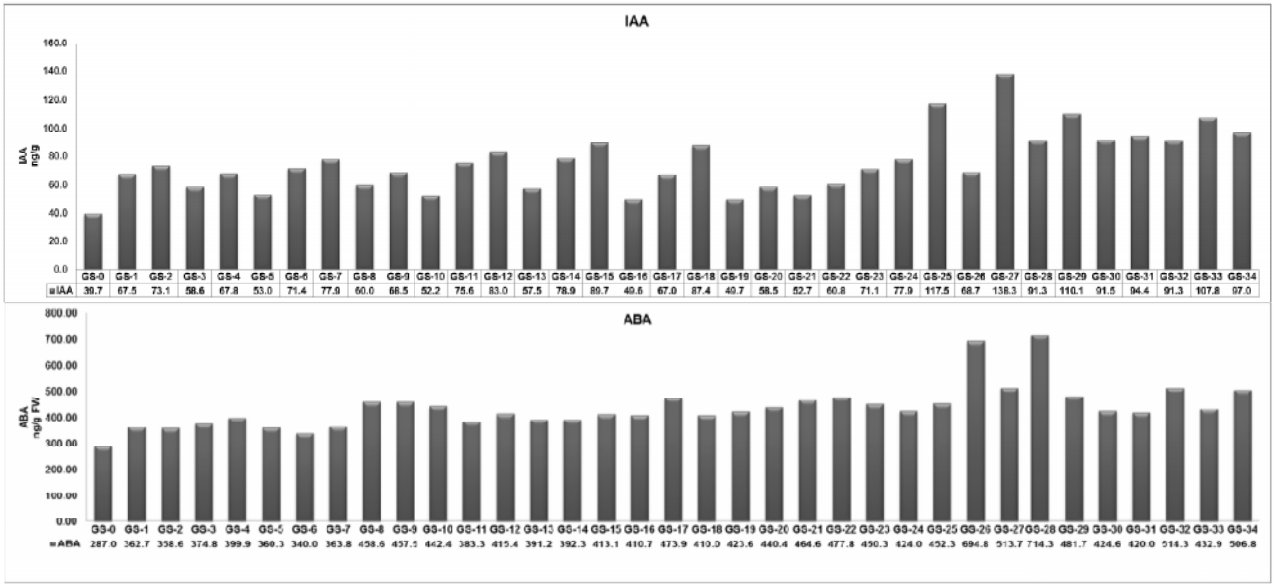


**Fig. 1.** Alteration in IAA (A) and ABA (B) content in the mutant in comparison to wild type.

## CONCLUSION

Gamma irradiation had shown inhibitory and simulative effect on the mutants. It would thus help in evolving desired mutants for economic traits and subsequent use in future breeding. Besides that, responsible gene for these traits can be identified, and transformed in new genetic backgrounds.

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**Conflict of Interest.** None.

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