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Physiological alteration in Kinnow developed through physical and chemical mutagen

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ABSTRACT

Alteration in plant growth, fruit yield and leaf gas exchange characteristics such as photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*) and intercellular CO_2 concentration (*Ci*) were investigated in five year old putative Kinnow developed from different doses of gamma irradiation and chemical mutagen (EMS) and compared with wild type (WT). The result showed dose-dependent decrease in plant height and yield in the developed from higher dosimetery. The plant height in the (G_{17}-G_{20}) developed from 30 Gy was inhibited by > 45.0%, while in the E_{15} and E_{16} developed from 0.02 and 0.05% EMS, it was reduced by >24%. Fruit yield was reduced by 66% in the G_{18} and G_{20} developed from 30Gy and >50% reduction in fruit yield were noted in E_6 and E_8 developed from intermediary dose of 0.1% EMS. Leaf gas exchange parameters recorded during different seasons of the year (April, August and December) exhibited differential response. Photosynthetic rate as compared to the wild type was reduced in the mutagenic population and maximum reduction without any seasonal variation were observed in the G_{12} (4.92, 5.20 and 4.92 µmol m^{-2} s^{-1}) and G_{13} (4.98, 5.04 and 4.96 µmol m^{-2} s^{-1}) developed from 25 Gy. Amongst the developed from chemical mutagen, photosynthetic rate was minimum in mutant E_{13} (5.15, 5.27 and 5.09 m^{-2} s^{-1}) developed at EMS 0.2%. Both stimulatory and inhibitory response were observed in the magnitude of transpiration rate, stomatal conductance and leaf intercellular CO_2 in the developed from higher doses of gamma rays and EMS. The result demonstrated that physiological alterations can also play a role in characterising genetic variability and pave way for selecting putative for future use in the breeding programme.

Key words: *Citrus nobilis* × *Citrus deliciosa*, gamma irradiation, EMS, plant growth, photosynthetic rate.

INTRODUCTION

Citrus is one of the third most important fruit crop of India after banana and mango. Amongst citrus group, Kinnow mandarin (*Citrus nobilis* Loureiro × *Citrus deliciosa* Tenora), mostly cultivated in the arid and semi-arid tracts of the Indian subcontinent has revolutionised the citrus industry of India (Kumar *et al*., 8; Sharma, *et al*., 15). The fruit is valued for its beautiful golden-orange fruits, higher juice recovery per cent, TSS:acid ratio, superior yield and better economic returns, Kumar *et al*. (9). Despite several positive traits, some inherent characters like alternate bearing and presence of large number of seeds hinders consumers adaptability and its processing into juice because of delayed bitterness resulting from high limonin content of the crushed seeds. Conventional breeding, although have been successfully used to improve citrus cultivars and develop newer and responsive varieties, it is constrained by its complicated genetic system and overall long juvenile phase (Grosser *et al*., 4; Sutarto *et al*., 17), thus restricting the development of varieties with desired traits in a shorter period of time.

Mutagenesis as a breeding tool on the other hand has contributed appreciably, made an excellent impact in enhancing genetic variability and have significantly assisted in development of improved varieties in array of crops (Ahloowalia and Maluszynski, 1). Morphological trait such as plant height although a low level but powerful taxonomic tool, has been utilised for grouping of germplasm, selecting promising genotypes and cultivars (Mallick *et al*., 10). Yield and fruit quality is yet another important parameter for selecting promising genotypes, particularly in fruit crops which have been developed through mutagenic tools (Goldenberg *et al*., 3; Rattanpal *et al*., 14). Several researchers have attempted mutagenesis in different citrus species using either physical or chemical mutagens for evolving new genotypes (Gulsen *et al*., 5) having desirable traits such as low seeded fruits which is the major focus of breeders around the world. Breeding efforts through mutagenesis have led to the development of several seedless in citrus (Vardi *et al*., 18; Rattanpal *et al*., 14). Besides low seeded varieties, mutagenesis as a tool have also been used to improve other characteristics such as 'Star Ruby' a deep-red-fleshed grapefruit (Hensz, 6),

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'Sunki' spine free mandarin (Kukimura *et al*., 7), 'Mal Secco' tolerance in lemon (Gulsen *et al*., 5) etc. It is well known that such modification are the results arising due to damage in cell structure which alter the physiological and biochemical composition of the plants, because the plant perception and response to a low dose may be different from the one caused by elevated doses of irradiation or chemical mutagen. The adaptive mechanism that may be functional and operating at the physiological level in citrus is poorly understood. To fill such knowledge gaps, this study although targeted for developing seedless Kinow mandarin mutant was undertaken to understand the alterations in plant growth, yield and leaf gas exchange patterns in putative Kinnow which could be also be used as a parameter for selecting desirable genotypes from a mutated population.

MATERIALS AND METHODS

In the present study, plant growth, yield and leaf gas exchange parameters were determined on five year old Kinnow. The were developed during September 2011 by exposing fresh non dormant mature bud sticks to different doses of gamma rays (15, 20, 25 and 30 Gray) using Co^{60} γ-irradiation chamber (Model GC-5000, BRIT, Mumbai) at Nuclear Research Laboratory (NRL), ICAR-Indian Agricultural Research Institute, New Delhi. Ethyl methanesulfonate (EMS) treatments to the fresh non dormant buds were given by soaking the buds in different concentration of 0.05%, 0.1%, 0.2% and 0.5% for a period of 12 h. The treated buds and the wild type were budded on *in situ* on *Jatti Khatti* rootstock on the same day and maintained at 3x3m spacing under uniform cultural practices at the main orchard of Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi. From the diverse population, putative were selected for recording the observations. Description of Kinnow are given in Table 1.

Morphological aberrations in terms of plant height (m) in the selected and WT were recorded two months after the emergence of spring flush during 2016 and

Table 1. Description of putative Kinnow developed through Gamma rays and ethylene methanesuplhonate (EMS).

Gamma Ray			Ethylene methanesuplhonate (EMS)		
Dose	Mutant population	Mutant code	Dose	Mutant population	Mutant code
Non treated	Wild type	WT	Non treated	Wild type	WT
15 Gray	15-1	G_1	0.05 %	0.05-1	E_1
15 Gray	15-2	G_2	0.05 %	0.05-2	E_2
15 Gray	15-3	G_3	0.05 %	0.05-3	E_3
15 Gray	15-4	G_4	0.05 %	0.05-4	E_4
15 Gray	15-5	G_5	0.05 %	0.05-5	E_5
20 Gray	20-1	G_6	0.1 %	0.1-1	E_6
20 Gray	20-2	G_7	0.1 %	0.1-2	E_7
20 Gray	20-3	G_8	0.1 %	0.1-3	E_8
20 Gray	20-4	G_9	0.1 %	0.1-4	E_9
20 Gray	20-5	G_{10}	0.1 %	0.1-5	E_{10}
25 Gray	25-1	G_{11}	0.2 %	0.2-1	E_{11}
25 Gray	25-2	G_{12}	0.2 %	0.2-2	E_{12}
25 Gray	25-3	G_{13}	0.2 %	0.2-3	E_{13}
25 Gray	25-4	G_{14}	0.2 %	0.2-4	E_{14}
25 Gray	25-5	G_{15}	0.2 %	0.2-5	E_{15}
30 Gray	30-1	G_{16}	0.5 %	0.5-1	E_{16}
30 Gray	30-2	G_{17}	0.5 %	0.5-2	E_{17}
30 Gray	30-3	G_{18}	0.5 %	0.5-3	E_{18}
30 Gray	30-4	G_{19}	0.5 %	0.5-4	E_{19}
30 Gray	30-5	G_{20}	0.5 %	0.5-5	E_{20}

2017. For determining the fruit yield, marketable fruits upon maturity were harvested from WT and and data were added to get final yield. The net Photosynthetic rate (*A*), stomatal conductance (g_s), transpiration rate (*E*) and intercellular CO_2 concentration (*Ci*) of fully expanded leaves were recorded sixty days after the emergence of the spring flush followed by rainy and autumn season flush *i.e.,* April, August and December for two consecutive years. 2016 and 2017. Four mature leaves/plant (4th leaf from tip of shoot) from exterior canopy position (one leaf each in North, South, East and West direction) per treatment were selected for measuring the leaf gas exchange traits between 09.30 am to 11.30 am (IST) on clear day using an LCi-SD Ultra Compact Photosynthesis System (ADC Bio Scientific Ltd., Global House, Hoddesdon, U.K.).

Data of two years 2016 and 2017 were expressed as means. One way ANOVA analyses using SPSS software was applied to experimental data on plant height and fruit yield which comprised of 20 mutants for each treatment and one wild type and means were compared at ($P \leq 0.01$). The data on leaf gas exchange parameters were analysed in completely block design (CBD) with 4 replications using statistical analysis system software (SAS version 2) followed by Tukey's Honest test and mean values compared at $P \leq 0.05$ level of significance are presented.

RESULTS AND DISCUSSION

The plant height in developed from different doses of gamma ray and EMS concentration was significantly different by exhibiting lesser plant height in response to the higher doses. As compared to WT, comparable reduction in plant height ranging from 46.18-49.65% were recorded in the developed from 30 Gy, while 24.30-25.34% reduction with statistical similarity were recorded in the E_{15} and E_{16} developed from 0.02 and 0.05% EMS (Table 2). The reduction in plant height may be attributed to cellular injury which

might have taken place at the time of treating the bud sticks and in later phase due to hormonal disturbances leading to plant disruption, such as short internodal distances and subsequently disturbed physiological attributes as observed in the dwarf in our study. Inhibitory effect in plant height at higher doses have also been reported in different *Citrus spp* (Ling *et al*., 2008, Mallick *et al*., 10) and in mango Rime *et al*. (15).

Fruit yield as compared to the WT followed a dose-dependent decrease in the mutagenic population raised from different doses of irradiation and EMS treatments. The magnitude of decrease was significantly more apparent in the G_{18} and G_{20} with almost 66% reduction in fruit yield as compared to the WT. Similar decrease in fruit yield were also recorded in the developed from different concentration of EMS, but the reduction was more perceptible in the E_6 and E_8 developed from intermediary dose of 0.1% EMS thus exhibiting a reduction of 57.39% and 50.99% in these respectively over the WT (Table 2). A dose-dependent decrease in fruit yield with increasing dose of gamma rays and EMS concentration in the putative may be associated with the concurrent decrease in plant height, lower canopy volume and fruiting intensity. In addition, decrease in fruit yield at higher doses may be a consequence of lower phothosynthetic activity and other gas exchange related parameters as detected in our study which regulate carbon metabolism and water relation and guide growth activity. Decrease in fruit yield similar to those observed in the present study has been reported in a dose-dependent manner by Bermejo *et al*. (2) and Goldenberg *et al*. (3) in citrus.

Leaf gas exchange parameters measured across WT and mutant revealed significant alterations (Fig 1 and 2). Irrespective of seasons, the photosynthetic rate (*A*) in WT had significantly the highest value (6.33, 6.68 and 6.29 µmol m^{-2} s^{-1}) compared to . Maximum reduction in photosynthetic rate without any seasonal variation were observed in the G_{12} (4.92, 5.20 and 4.92 µmol m^{-2} s^{-1}) and G_{13} (4.98, 5.04 and 4.96 µmol m^{-2} s^{-1}) (Fig. 1a) developed at 25 Gray and the values maintained statistical parity with other . Among the chemical mutagen, photosynthetic rate was minimum in mutant E_{13} (5.15, 5.27 and 5.09 m^{-2} s^{-1}) developed at 0.2% EMS (Fig. 2a) and showed insignificant change in other irrespective of season. Reduction in photosynthetic rate in the as compared to wild type and its ability to acclimate to seasonal variability may be attributed to stomatal/non-stomatal limitations, thus conferring protective mechanism to the plants. The results are in conformity with the findings of Moghaddam *et al*. (14) who reported decline in photosynthetic activity with increasing dose or irradiation treatment in *Centella asiatica*. The sensitivity of to transpiration rate (*E*) showed varied response as compared to WT (2.65, 2.20 and 0.79 mmol m^{-2} s^{-1}) (Fig. 1b and 2b). The transpiration rate was invariably higher during April followed by August and December. In general, the transpiration rate reduced with increasing doses of gamma irradiation and EMS except in the G_4 (3.64 mmol m^{-2} s^{-1}), G_6 (3.52 mmol m^{-2} s^{-1}) and G_9 (2.94 mmol m^{-2} s^{-1}) developed at 15 and 20 Gray during April (Fig. 1b). Similar higher transpiration pattern during April was recorded in the E_4 (2.66 mmol m^{-2} s^{-1}), E_6 (3.24 mmol m^{-2} s^{-1}) and E_{20} (2.70 mmol m^{-2} s^{-1}) (Fig. 2b). Fluctuating trends

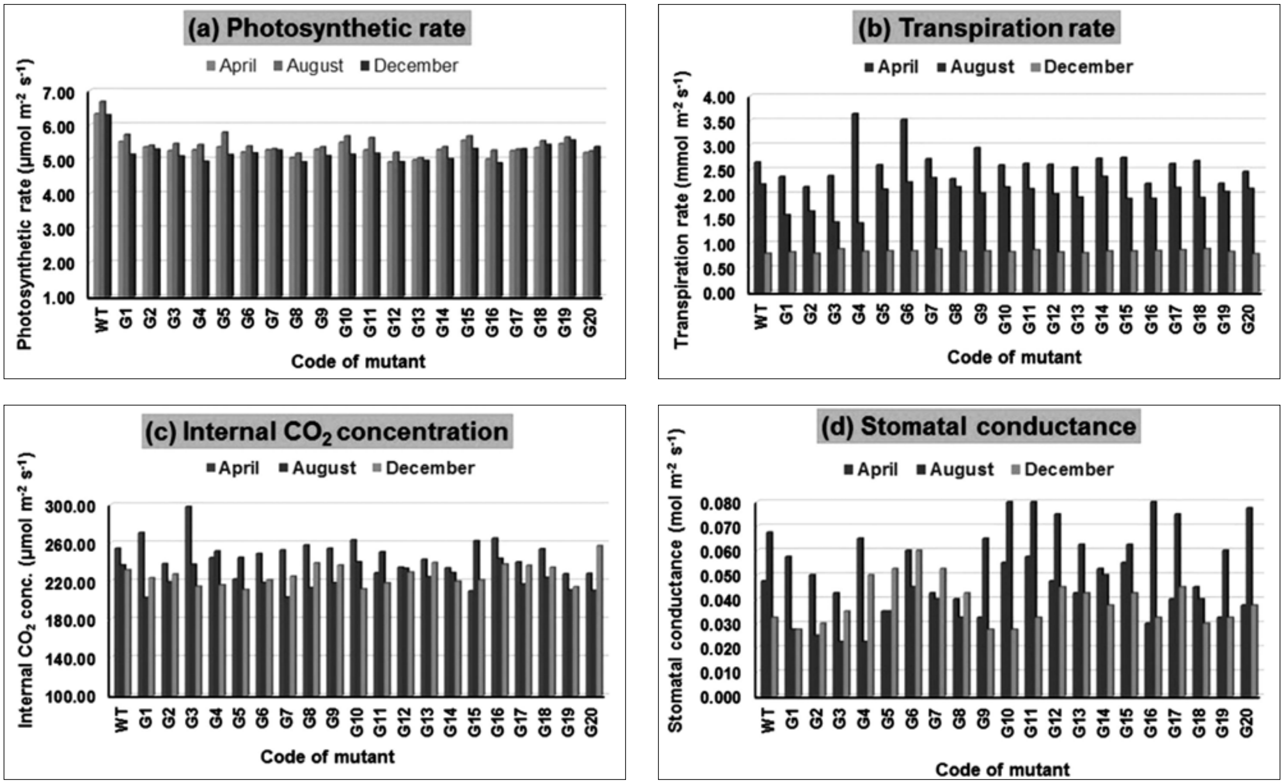

Fig. 1. Alteration in leaf gas exchange characteristics of γ-rays induced putative Kinnow.

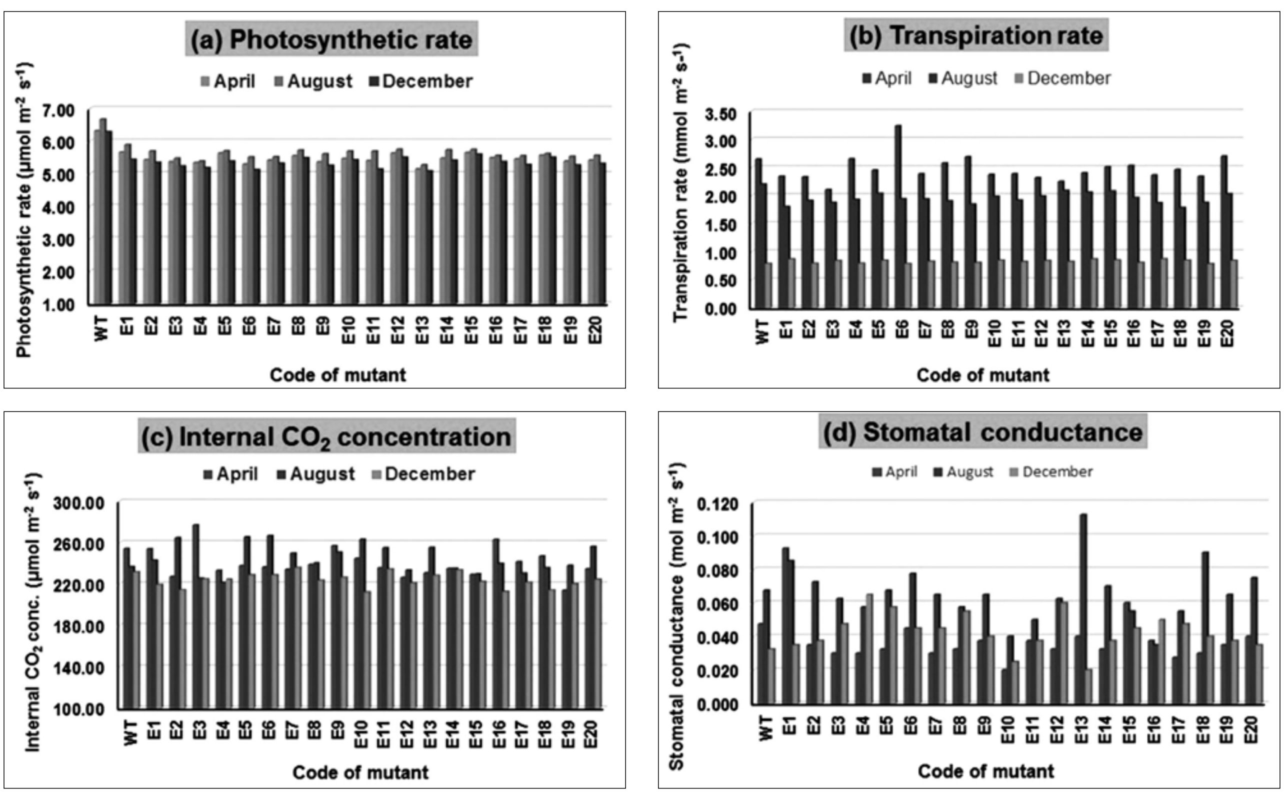

Fig. 2. Alteration in leaf gas exchange characteristics of EMS induced putative Kinnow.

Table 2. Plant growth and yield variation in putative Kinnow developed through gamma irradiation and chemical mutagen (EMS).

Wild Type/ Gamma ray	Plant growth (m)	Yield (Kg)	Wild Type/ EMS Mutant	Plant growth (m)	Yield (Kg)
WT	2.88	70.81	WT	2.88	70.81
G_1	2.41	42.59	E_1	2.68	44.56
G_2	2.33	49.06	E_2	3.15	40.04
G_3	2.10	45.85	E_3	2.45	38.80
G_4	2.35	54.75	E_4	2.75	40.06
G_5	2.58	43.38	E_5	3.15	44.36
G_6	1.83	31.24	E_6	2.60	30.17
G_7	1.75	34.85	E_7	2.85	44.85
G_8	1.78	41.40	E_8	2.70	34.70
G_9	1.95	30.74	E_9	2.70	38.82
G_{10}	1.88	33.12	E_{10}	2.23	40.87
G_{11}	1.58	32.14	E_{11}	2.35	38.62
G_{12}	1.68	25.60	E_{12}	2.56	34.81
G_{13}	1.83	27.86	E_{13}	2.63	44.85
G_{14}	1.58	30.95	E_{14}	2.65	46.80
G_{15}	1.53	36.34	E_{15}	2.18	37.88
G_{16}	1.60	33.62	E_{16}	2.15	41.90
G_{17}	1.50	27.52	E_{17}	2.23	41.17
G_{18}	1.48	23.41	E_{18}	2.43	42.09
G_{19}	1.55	26.69	E_{19}	2.33	49.01
G_{20}	1.45	24.11	E_{20}	2.53	36.75
SEm	0.05	0.52	--	0.050	0.52
CD @ 5%	0.15	1.57	--	0.149	1.57

in the transpiration rate of developed with different doses of irradiation and EMS may be due to the imbalances in stomatal conductance as observed in our study. The findings are in consonance with Nobel (13) who reported that transpiration rate is greatly affected by stomatal conductance. In terms of leaf intercellular carbon dioxide concentration (*Ci*), there were also significant differences between and WT (254.25, 236.75 and 231.75 μmol m^{-2} s^{-1}). In general, it was observed that leaf intercellular carbon dioxide declined significantly in (Fig. 1c and 2c) as compared to wild type. Among , the highest *Ci* was found in G_3 (298.00, 237.50 and 214.50 μmol m^{-2} s^{-1}) and E_3 (277.00, 254.00 and 225.50 μmol m^{-2} s^{-1}), while the lowest was found in G_{19} (228.00, 210.50 and 214.10 μmol m^{-2} s^{-1}) and E_{19} (214.00, 230.00 and 214.00 μmol m^{-2} s^{-1}) irrespective of the seasonal variation (Fig. 1c and 2c). As shown in Fig. 1d and 2d, stomatal conductance (g_s) in exhibited both increasing and decreasing trends. For WT, its value was found to be 0.048, 0.068 and 0.033 mol m^{-2} s^{-1}. The lowest *gs* were detected in G_{16} (0.030, 0.040 and 0.033 mol m^{-2} s^{-1}) and E_{17} (0.028, 0.035 and 0.048 mol m^{-2} s^{-1}) of April and December flush, whereas it was detected highest in G_4 (0.065, 0.080 and 0.050 mol m^{-2} s^{-1}) and E_{13} (0.040, 0.113 and 0.060 mol m^{-2} s^{-1}). The findings of the study are in consonance with Marcu *et al*. (11) and Moghaddam *et al*. (12) who reported alteration in leaf gas exchange characteristics in different plant species when exposed with physical and chemical mutagens.

Considering the above facts in view, present study elucidates the response of putative at the physiological level and delves into the dose effect relationship on leaf gas exchange parameters which govern the growth and yield behaviour of plant. Further, wide range of variation induced both by the physical and chemical mutagen has enhanced the scope for in-depth study of other biochemical and quality traits of the desirable obtained in the bearing stage to obtain superior .

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