DOI : 10.5958/0974-0112.2016.00063.3

Indian J. Hort. 73(2), June 2016: 291-293

Short communication

Effect of physical and chemical mutagens on leaf sclerophylly and stomatal characteristics of Kinnow mandarin mutants

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ABSTRACT

In the present study, Kinnow buds were treated with different doses of gamma irradiation (5, 10, 15, 20 Gy) and ethyl methane sulphonate (EMS) (0.05, 0.1, 0.2, 0.5%). The mutated buds were chip budded *in situ* on *Jattik khatti* rootstock. Two years after the establishment of plants, the developed mutants were examined in terms of alterations in leave sclerophylly and stomatal characteristics. Irradiation doses at 10 and 15 Gy resulted in more succulent leaves as compared to EMS treated plants. The maximum reduction in density of foliar tissue (DFT) was observed at 10 and 15 Gy (12.81%) and 0.2% EMS (7.27%). Stomata length and number was significantly reduced in plants at higher doses of irradiation i.e., 15 and 20 Gy and 0.2% EMS treatment. A general diminution of 11% in stomata nmber was observed under the EMS treatment while at 20 and 15 Gy stomata number was reduced by 40.16 and 32.73% respectively. Varaition in leaf sclerophylly and stomatal characteristics of the mutants indicate that both lower and higher doses of mutagenic treatments may give rise to the mutants of economic imporatance indicating their potential use as mutagens in future breeding programmes.

Key words: Leaf area, fresh mass, succulency, stomata number, mutagenesis.

Mutagenesis has contributed significantly to plant improvement worldwide and has made an outstanding impact on the productivity and economic value of some crops (Ahloowalia, 1). Several workers have attempted for induction of mutation in citrus species using either physical or chemical mutagens for evolving new genotypes (Gulsen *et al.*, 4). Besides, the development of improved varieties, mutagenic agents have also been reported to differentially affect the morphology, anatomy, biochemistry and physiology of the plants depending upon the mutagen dose. The symptoms frequently observed in the low or high dose mutated plants are enhancement or inhibition of plant growth, alteration in leaf sclerophylly, shoot length, leave density, stomata number and size, and other biological and physiological responses (Kovacs and Keresztes, 5; Mallik *et al*., 6). Till date there is no major information about the degree and direction of variation caused by physical and chemical mutagen on leaf sclerophylly and stomatal characteristics of Kinnow mandarin (*C. nobilis* Lour x *C. deliciosa* Tenora). In this backdrop, the present study was undertaken to study the alterations in leaf sclerophylly and stomatal characteristics in the newly developed mutated plants of Kinnow mandarin.

Plant material for the experiment included eight mutants and untreated Kinnow plants (control). For inducing mutations, fresh non dormant mature budsticks of Kinnow were collected during the last week of September, 2011 and were exposed to different doses of irradiation at 5, 10, 15 and 20 Gy using ^{60}Co Gamma irradiation Chamber (Model GC-5000, BRIT, Mumbai) at Nuclear Research Laboratory (NRL), ICAR-IARI, New Delhi. The ethyl methane sulphonate (EMS) treatments to the fresh non dormant buds were given by soaking the buds in different concentrations of 0.05, 0.1, 0.2 and 0.5% EMS for a period of 12 h. The treated buds were chip budded *in situ* on *Jatti khatti* rootstock planted at the spacing of 3x3 m on the same day. Two years after the establishment of the plants, one plant in each treatment was selected and observations on leaf sclerophylly and stomatal characteristics were recorded. Ten mature, two and half-month-old leaf of the rainy season flushes were collected from each treatment in three replicates. Several indices of leaf physiological parameters were calculated by the formulae suggested by Ennajeh *et al*. (3). These included specific leaf area (SLA= LA/ DM: in $cm^2\ g^{-1}$ DW), specific leaf weight (SLW = DW/LA: in g cm^2 LA), density of foliar tissue (D = DW/FW X 1000: in g kg^{-1}) and succulency [S = (FW-DW)/LA: in mg $H_2O\ cm^{-2}$].

Stomatal distribution was studied from epidermal impressions of the abaxial surfaces of mature, fully expanded leaves (Sampson, 8). Stomata length and width were measured to the nearest micrometer viewed at 40x magnification. Stomatal density was assessed by counting the number of stomata per field

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of view at 40x magnification. Nine measurements per treatment were made for recording the observations.

The statistical analysis of the data which comprised of nine treatments including non-irradiated Kinnow (control) with single plant as one unit and three replications were analysed in completely randomized block design using statistical analysis system software (SAS version 2.) followed by Tuckey's Honest Test. P values ≤ 0.05 were considered as significant.

Statistical test on SLW did not show any significant variation among the EMS treatments over control. Among the gamma irradiated plants, SLW was found to be significantly higher by 25% at 20 Gy followed by rest of the other treatment without any significant variation. Density of foliar tissue exhibited reducing trend with increasing dose of EMS and irradiation treatment. The maximum reduction in DFT (12.81%) was observed in the plants irradiated with 10 and 15 Gy gamma rays followed by reduction of 7.27% at 0.2% EMS, which was statistically at par with the irradiation dose of 20 Gy. As compared to control, although a decreasing trend was noted in SLA values; they did not differ significantly among themselves except with 0.5% EMS where a reduction of 6.94% was noticed. Significant reductions in SLA were however, observed in the leaves of gamma ray irradiated plants and the maximum reduction of 22% was observed at 20 Gy (Table 1). Literature data on changes in leaf sclerophylly induced by the mutagens are limited. The performed analysis, however, indicates the existence of differences in leaf sclerophylly between the mutants. Swaminathan (9) reported that the radiation was found to cause malfunctioning of various phyto-hormones and cause changes in chemical patterns leading to morphological variations.

A significant difference was observed between treated and untreated plants for different stomatal characteristics. Signinificant reduction in stomatal length was observed under treatment both 15 and 20 Gy treatments. As compared to non-irradiated plants, 3.66 and 5.29% higher reduction was recorded respectively in these treatments. Reduction in stomata length were also observed under different EMS treatments with the maximum (2.66%) reduction noted at 0.2%. In rest of the treatments, a general reduction of 1.52% was recorded with insignificant differences. Nominal increase of 1.46% in stomata width was observed at 15 Gy while in the other treatments the difference was not significant. Stomata number was recorded to be significantly higher in untreated buds. A general diminution of 11% in stomata number, irrespective of the treatment, was observed under the EMS treatment while in the gamma irradiated leaves, the maximum dimunution in stomata number was recorded at 20 and 15 Gy. The percentage reduction with respect to the number of stoamta in these treatment was to the tune of 40.16 and 32.37% , respectively followed by 19.39% reduction at 5 and 10 Gy (Table 2).

Reduction and enhancement in stomata length and width at higher doses of irradiation was possibly due to the induced genetic damage and mutation frequencies. Literature abounds in reports of increased stomata width in plants (Qosim *et al*., 7). There was no inverse relationship between the stomatal width and number of stomata/mm^2. Significant reduction in stomata number with increasing dose of irradiation

Table 1. Leaf Sclerophylly characteristics of Kinnow (control) and mutants.

Treatment	Succulency (mg H_2O/cm^{-2})	SLW (g/cm^{-2})	DFT (g/kg^{-1})	SLA (cm^2 g^{-1} DW)
Control	0.019 ± 0.000^{e}	0.012 ± 0.00^{d}	384.95 ± 1.00^{a}	84.14 ± 0.54^{a}
Chemical mutagen				
0.05% EMS	0.019 ± 0.000^{e}	0.012 ± 0.000^{cd}	388.07 ± 0.85^{a}	81.30 ± 1.44^{ba}
0.1% EMS	0.022 ± 0.000^{d}	0.012 ± 0.000^{cd}	365.32 ± 0.91^{c}	80.79 ± 0.80^{bac}
0.2% EMS	0.022 ± 0.001^{d}	0.012 ± 0.000^{d}	356.93 ± 1.24^{d}	82.96 ± 0.95^{a}
0.5% EMS	0.021 ± 0.000^{d}	0.013 ± 0.000^{cb}	376.76 ± 2.81^{b}	78.30 ± 0.66^{bc}
Gamma irradiation				
5 Gray	0.023 ± 0.000^{c}	0.014 ± 0.000^{b}	371.46 ± 3.16^{b}	73.77 ± 2.25^{d}
10 Gray	0.027 ± 0.000^{a}	0.013 ± 0.000^{b}	335.62 ± 0.66^{e}	74.25 ± 0.50^{d}
15 Gray	0.026 ± 0.000^{b}	0.013 ± 0.000^{cb}	334.56 ± 1.92^{e}	77.81 ± 0.95^{c}
20 Gray	0.027 ± 0.000^{a}	0.015 ± 0.000^{a}	356.75 ± 1.36^{d}	65.52 ± 0.93^{e}
LSD (P ≤ 0.05)	0.001	0.001	5.28	3.38

Table 2. Stomatal characteristics of Kinnow (control) and mutants.

Treatment	Stomata length (µm)	Stomata width (µm)	No. of stomata (mm^{-2})
Control	37.94 ± 0.23^{a}	32.13 ± 0.02^{dc}	7.32 ± 0.02^{a}
Chemical mutagen			
0.05% EMS	37.26 ± 0.12^{cb}	32.23 ± 0.14^{bdc}	6.78 ± 0.31^{b}
0.1% EMS	37.13 ± 0.06^{cbd}	32.36 ± 0.06^{bac}	6.48 ± 0.04^{b}
0.2% EMS	36.93 ± 0.06^{ced}	32.50 ± 0.11^{ba}	6.45 ± 0.02^{b}
0.5% EMS	37.36 ± 0.08^{b}	32.43 ± 0.12^{ba}	6.45 ± 0.05^{b}
Gamma irradiation			
5 Gray	37.20 ± 0.20^{cbd}	32.00 ± 0.11^{d}	5.90 ± 0.11^{c}
10 Gray	36.83 ± 0.08^{ed}	32.50 ± 0.00^{ba}	5.58 ± 0.08^{c}
15 Gray	36.60 ± 0.11^{e}	32.60 ± 0.05^{a}	4.95 ± 0.05^{d}
20 Gray	35.93 ± 0.06^{f}	32.30 ± 0.10^{bc}	4.38 ± 0.07^{e}
LSD (P ≤ 0.05)	0.39	0.28	0.36

and chemical mutagen are again consistent with the altered variations recorded in leaf sclerophylly. Reduced number of stomata with increasing dose of mutagenic treatment has also been reported in grape cultivars Cabernet Sauvignon and Merlot (Demir Kok, 2) and mangosteen (Qosim *et al*., 7). It is concluded that both irradiation treatment and alkylating agent cause alterations in leaf sclerophylly and stomata and may be a way to create superior mutants for future improvement programmes.

ACKNOWLEDGEMENTS

The authors are grateful to ICAR-IARI, New Delhi, for financial assistance and Dr Parimal Sinha, Pr. Scientist, Division of Plant Pathology, IARI for providing the necessary facilities.

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Received : April, 2015; Revised : March, 2016;
Accepted : May, 2016