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Characterization of EMS Induced Putative Kinnow Mandarin Mutants for Growth, Yield and Quality Traits

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A B S T R A C T

In the present study, twenty putative bearing mutants of Kinnow mandarin developed from different concentration (0.05%, 0.1%, 0.2% and 0.5%) of ethyl methanesulfonate (EMS) were evaluated for phenological, yield and quality traits and compared with the wild type (WT). Plant height as compared to WT was stimulated by almost 10% in the mutants E-2 and E-5 developed from the lower doses of 0.05% EMS, while it was curtailed by 25% in the mutants E-16 and E-17 developed from higher dosimetry of 0.5% EMS. The inhibited plant however, did not affect the fruiting intensity. A dose-dependent decrease in fruit weight varying from 22.04 to 41.50% was noticed in the mutants between E-1 (0.05%) to E-15 (0.2%). Mutants E-1 and E-16 developed from the two contrasting doses of EMS had fruits with thicker peel similar to WT. The number of seeds/fruit was significantly reduced in the putative mutants E-16 and E-19 (<15.0 seeds/fruit) as compared to WT which had almost 32.0 seeds/fruit. The juice recovery per cent was however, slightly lower in the EMS induced mutants. This mutagenic approach has led to the identification of mutant with positive traits such as diminutive plants coupled with higher fruiting intensity and lower number of seeds per fruit.

Introduction

Kinnow mandarin, a first-generation hybrid between King orange (*Citrus nobilis* Loureiro) and Willow leaf mandarin (*Citrus deliciosa* Tenora) has become the most favourite choice cultivar of citrus growers of India because of its precocious and prolific bearing habit, better yield, higher economic return and wider adaptability to arid and semi-arid climate (Kumar *et al.*, 2019). It is a preferred fruit crop by the consumers because of higher juice recovery and appropriate sugar: acid blend. Despite the several positive traits, vigorous growth, alternate bearing and more number of seeds/fruit are some of the negative traits of Kinnow (Khalil *et al.*, 2011). To improve the productivity and full fill the consumer's demand of low seeded mandarin and processing industry, there is an urgent need to develop a Kinnow plant having dwarf stature and seedless or low seeded fruits. For decades, great progress on citrus breeding was made by traditional approaches

such as sexual hybridization, seedling and bud sport selection. However, due to the peculiarities of citrus reproductive biology such as long juvenile period and nucellar polyembryony, traditional breeding is inefficient and costly (Zheng *et al.,* 2011).

Mutation induction techniques such as irradiation or chemical mutagens are good tools for increasing variability in crop species because spontaneous mutations occur with an extremely low frequency. Mutation techniques have significantly contributed to plant improvement worldwide, and have made an outstanding impact on the productivity and economic value of some crops (Ahloowalia & Maluszynski 2001). In fruit crops, mutagenesis has already been used to introduce useful mutants related to dwarfing, blooming time and fruit ripening period, fruit colour, self-compatibility, self-thinning, and resistance to pathogens (Sanada & Amano 1998). Chemical mutagens lead to more specific and predictable mutation, and the procedures are easier to manage without specialized, expensive equipment. Ethyl methanesulfonate (EMS), as a chemical mutagen, can be used as a supplementary approach to improve desired identifiable characters such as yield-related characters (Botticella *et al.,* 2011). It produces random point mutations in genetic material. Creation of genetic variability through induced mutagenesis is certainly beneficial to develop a new phenotype and to understand and exploit the variability present in the germplasm by adopting different methods of characterization.

Hence, the present study was undertaken to characterize genetic variability in the putative mutant populations of Kinnow based on morphological and fruit quality parameters and to identify some desirable dwarf mutant with fewer seeds for future use in the breeding programme.

Materials and Methods

Experimental site

The experiment was conducted at the experimental orchard of Division of Fruits and Horticultural Technology, ICAR- Indian Agricultural Research Institute (IARI), New Delhi (India), located at an altitude of 228.61 m above mean sea level with 77° 12' E and 28° 40' N. The climate is characterised as semiarid and subtropical, with hot and dry summers and cold winter. The mean annual rainfall is 710 mm of which more than 75 per cent is received during monsoon season (July to September). The soil type was sandy loam with bulk density 1.58 g cm^{-3}, pH of 7.4 and electrical conductivity [EC; 1:2 (W/V) in water] of 0.34 dSm^{-1} and organic carbon content of 0.39% (w/w) and a soil N, P and K concentration of 159.23, 536.1 and 314.78 kg ha^{-1}. The mean available Fe, Mn, Cu and Zn concentration in the soil was 9.35, 22.67, 7.52 and 4.83 mg kg^{-1} soil respectively.

Plant material

Five-year-old mutagenic populations of Kinnow mandarin which was earlier subjected to different treatments of 0.05% (E-1 to E-5), 0.1% (E-6 to E-10), 0.2% (E-11 to E-15) and 0.5% (E-16 to E-20) ethyl methanesulfonate (EMS) (SRL Chem., Mumbai) were used for the study. Five putative mutants developed from each treatment were compared with non-treated Kinnow plants (wild type). The wild type and the mutant trees were grown under drip irrigation system and received the same cultural practices. Observations on growth, yield and fruit quality were recorded for two consecutive years, *i.e.* 2016-17 and 2017-18. To prevent chimerism, four bearing-fruit branches in each direction were labelled with a unique number for documentation and observed for further evaluation.

Plant growth and yield

Plant growth in terms of plant height (m) and plant spread [N-S (D1)] and [E-W (Dr)] of the mutants and wild type were recorded two months after the emergence of the spring flush. Canopy volume (V) was determined by the formulae V= (π/6) x H (height) x D1 (width in parallel) x Dr (width in perpendicular) (Zekri 2000). Stem diameter was measured with millimetric calipers. Fruits were harvested at maturity from each mutant and wild type. The fruit yield was calculated by multiplying the fruit weight (average of 20 fruits) to the number of fruits tree^{-1} and then the resultant was converted into Kg tree^{-1}. The fruiting intensity was calculated by dividing the number of fruits with canopy volume.

Fruit quality parameters

Fruits were harvested at optimal maturity in the first week of January as the breeding team's previous experience. A random sample of 20 fruits from the tagged branches of each mutant was selected and evaluated for different fruit quality parameters. Fruit weight was measured with a semi-analytical balance (Citizen, Mumbai, India), and fruit height, diameter and thicknesses of total peel (flavedo and albedo) were measured with a vernier caliper. Peel weight was measured with a semi-analytical balance. The number of segments and seeds per fruit were evaluated after cutting the fruits in half and extracting the juice with a hand extractor. Seed weight/fruit was measured with a semi-analytical balance. Juice retrieved was measured with a measuring cylinder to calculate the juice recovery percentage from the relationship between the weight of the extracted juice and the fruit sample weight.

Total soluble solids (°Brix) were measured using an ATC-1E ATAGO handheld refractometer. Titratable acidity was estimated according to Rangana (1986). Vitamin C content (mg/100 ml of juice) was determined using a dye (2, 6-dichlorophenol indophenol) according to Rangana (1986).

Statistical analyses

The statistical analysis of the data for plant growth and yield was done through one-way ANOVA using SPSS software. The statistical analysis of fruit quality parameters was carried out in a completely randomized block design using statistical analysis system software (9.3 SAS Institute, INC., USA) followed by Tuckey's Honest Test. P values ≤ 0.05 were considered as significant. The dissimilarity distance matrix of the growth, yield and fruit quality parameters was estimated using the programme DARwin with considering bootstraps analysis with 1,000 replications (Perrier & Jacquemoud-Collet 2006). The dendrogram was constructed using the programme DARwin by adopting the unweighted Neighbour-joining method (Gascuel 1997).

Results and Discussion

Growth and yield

Significant variations in growth and yield parameters were observed in the mutants (Table 1). Compared to WT (2.88m), plant height was simulated by 9.38 per cent in the mutants E-2 and E-5, while it was reduced by 25.34 per cent in E-16 with statistical parity in the mutants E-15 and E-17. Contrary to the inhibitory response in plant height at higher doses of EMS concentration, stem diameter was significantly higher with maximum values in the mutants E-20 followed by E-16 and maintained statistical similarity with the values recorded in E-18 and E-19. With reverence to WT (11.81 m^3), reduction in canopy volume was also witnessed in the mutants to the tune of 54.45% in E-15 (0.5%

EMS) which was not significantly different from E-11 (0.2% EMS) and E-10 (0.1% EMS) Reduction in the number of fruits per plant and yield (Kg/plant) was observed in all mutants as compared to WT (289.00 fruits, 70.81 Kg). Maximum yield amongst the mutated population was obtained in E-19 (49.01 Kg). The fruiting intensity was however, higher in the mutants created from 0.2% EMS and was maximum in E-15.

Fruit quality parameters

Reduction in fruit weight was observed in all the mutants and as compared to WT (245.30 g) (Table 2), minimum fruit weight (143.50 g) was observed in E-15 and was statistically similar with the values recorded in E-20 (156.48 g) and E-18 (160.48 g) developed through higher concentrations of 0.2 and 0.5% EMS. The fruit shape index (0.98) as compared to WT (0.90) was maximum in E-6 (0.98) and minimum in E-13 (0.83). Fruits with thinner peel were noticed in the mutants E-17 (3.42 mm) to E-20 (2.95 mm) generated from 0.5% EMS and the difference between them was not significant. Observation on seed number, an important observation in the present study showed that the mutants E-16 and E-19 created from the highest dosimetry doses of 0.5% EMS had almost 50 per cent fewer seeds as compared to the WT (31.75 seeds/fruit). Reduced number of seed/fruit was also witnessed in the mutants E-12 (14.75) and E-8 (15.50). The corresponding decrease in seed weight was also documented in these mutants. Fruit obtained from the mutagenic population had lower juice recovery per cent, except E-20 (57.40 %) which was parallel to the juice recovery per cent obtained from the WT (57.21 %).

The total soluble solids (TSS) as equated against the WT (11.49 ^{o}B) was significantly higher in the mutants E-6 (13.73^{o}B), E-15 (13.63 ^{o}B) and E-8 (13.41 ^{o}B) (Table 3). Vitamin C content was lower in the mutagenic population, while the acidity was towards the higher side in the majority of the mutants except for E-6 (0.84 %) and E-15 (0.84 %). TSS: acid ratio in most of the mutants increased as compared to WT (13.42: 1). Maximum TSS: acid ratio (16.45: 1) was found in E-6 followed by E-15 (16.26: 1) and E-8 (15.79:1).

Cluster analysis

The dendrogram of putative Kinnow mutants including wild type was constructed based on twenty-eight growth, yield and fruit quality parameters. As evident, the different putative Kinnow mutants were grouped into three major clusters (Fig. 1). The cluster A was divided into two sub-clusters; E-13, E-7 and E-9 formed sub-cluster A-I and E-18, E-20, E-6 and E-8 were grouped in sub-cluster A-II. The cluster B further divided into two sub-clusters; E-1 and E-12 were formed sub-cluster B-I and E-3, E-4, WT, E-2 and E-5 were grouped in sub-cluster B-II. Similarly, cluster C was further grouped into two sub-clusters; *i.e.* E-17 and E-19 were retained in sub-cluster C-I, while E-11, E-15, E-16, E-10 and E-14 in sub-cluster C-II.

The varying response of EMS treatment on plant growth exhibited an inhibitory effect on plant height at a higher dose of EMS concentration in contrast to the mutants developed from lower doses where a stimulated response was observed. The reduction in plant height may be attributed to reduced internodal distances as also reported earlier in mango (Rime *et al.*, 2019) and in barley dwarf mutant (Sethi 1974). Reduction in plant height might have also taken place due to the damage incurred by EMS in the GA biosynthesis pathway (Arisha *et al.*, 2015) leading to a reduced level of gibberellic acid (GA) which was also observed in the present study (data not given).

Table.1 Variation in growth and yield characteristics of putative Kinnow mutants

Code of mutant	Plant height (m)	Plant spread (m)		Diameter (mm)	Canopy volume (m^3)	Number of fruits	Yield (Kg)	Fruiting intensity (m^3)
		East-west	North-south					
WT	2.88	2.72	2.85	106.56	11.81	231.20	56.71	19.58
E-1	2.68	2.50	2.29	69.14	8.09	186.40	35.65	23.03
E-2	3.15	2.55	3.04	62.68	13.45	184.40	32.03	13.71
E-3	2.45	2.58	2.72	74.52	9.10	172.50	31.04	18.95
E-4	2.75	2.48	2.53	60.53	9.16	170.20	32.04	18.57
E-5	3.15	2.89	3.23	70.87	16.18	196.00	35.81	12.11
E-6	2.60	2.49	2.64	73.35	9.34	152.40	24.14	16.32
E-7	2.85	3.20	3.10	79.50	15.48	202.40	35.88	13.08
E-8	2.70	3.35	3.02	90.52	15.01	178.00	27.76	11.86
E-9	2.70	3.26	2.85	84.88	13.76	196.80	31.06	14.30
E-10	2.23	2.21	2.64	79.52	6.85	213.60	32.70	31.18
E-11	2.35	2.00	2.37	82.14	6.08	193.20	30.90	31.79
E-12	2.56	2.80	2.67	81.45	10.22	152.40	27.60	14.91
E-13	2.63	2.89	2.64	79.58	10.59	210.80	35.88	19.90
E-14	2.65	2.44	2.22	91.64	7.72	216.40	37.44	28.03
E-15	2.18	2.28	2.06	96.90	5.38	211.20	30.31	39.27
E-16	2.15	2.50	2.65	102.94	7.71	196.00	33.52	25.43
E-17	2.23	2.29	2.68	97.28	7.34	186.40	32.94	25.39
E-18	2.43	2.93	3.10	101.50	11.74	209.80	33.67	17.87
E-19	2.33	2.55	2.73	100.30	8.56	209.60	39.21	24.47
E-20	2.53	2.72	3.13	105.32	11.58	187.90	29.40	16.23
CD @ 5%	0.149	0.241	0.221	2.575	2.504	6.417	1.573	5.137

Table.2 Variation in fruit physical characteristics of putative Kinnow mutants

Code of mutant	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit shape Index (length/width)	Peel thickness (mm)	Peel weight (g)	No. of Segments	No. of seeds	Seed weight (g)	Juice content (ml)	Juice recovery (%)
WT	245.30^{a}	79.79^{a}	88.62^{a}	0.90^{fcebd}	4.54^{ba}	83.83^{a}	10.88^{bac}	31.75^{a}	6.52^{a}	140.13^{a}	57.21^{ba}
E-1	191.23^{b}	65.77^{cb}	77.76^{b}	0.84^{ih}	4.75^{a}	67.06^{b}	10.63^{bedc}	18.50^{dce}	3.05^{ji}	82.25^{ef}	43.14^{g}
E-2	173.72^{gfh}	63.66^{ced}	68.42^{ij}	0.93^{b}	3.53^{ghi}	66.50^{cb}	10.75^{bdc}	20.00^{dc}	4.61^{cb}	94.63^{cb}	54.24^{bac}
E-3	179.92^{gfed}	62.80^{gcfed}	69.28^{hi}	0.91^{cebd}	3.94^{de}	58.88^{ef}	11.13^{ba}	19.38^{dc}	4.73^{cb}	97.13^{b}	53.31^{c}
E-4	188.28^{cb}	61.43^{ghfed}	69.25^{hi}	0.89^{fcebdg}	3.65^{ghf}	56.45^{gf}	10.88^{bac}	20.25^{dc}	4.42^{cd}	85.88^{ed}	45.34^{gf}
E-5	182.73^{ced}	64.57^{cbd}	69.75^{hi}	0.93^{cb}	3.90^{def}	66.95^{b}	11.38^{a}	21.25^{c}	5.00^{b}	98.00^{b}	53.11^{c}
E-6	158.38^{i}	67.25^{b}	68.71^{hij}	0.98^{a}	3.81^{gef}	49.57^{ij}	10.00^{f}	17.50^{dfe}	3.45^{hig}	70.00^{jhi}	45.41^{gf}
E-7	177.27^{gfeh}	63.13^{cfed}	75.67^{c}	0.83^{ih}	4.36^{bc}	63.60^{cd}	10.88^{bac}	19.50^{dc}	3.89^{ef}	77.25^{gf}	43.65^{g}
E-8	155.94^{i}	60.17^{ghghf}	69.83^{hi}	0.86^{feihg}	3.19^{jkl}	47.40^{j}	10.75^{bdc}	15.50^{gfe}	2.45^{k}	75.50^{gh}	48.50^{edf}
E-9	157.82^{i}	60.95^{ghfe}	71.60^{gf}	0.85^{fihg}	4.16^{dc}	54.84^{gh}	10.63^{bedc}	18.25^{dce}	3.28^{hi}	73.50^{ghi}	46.61^{gf}
E-10	153.08^{i}	62.18^{ghfed}	70.05^{hg}	0.89^{fcebdg}	4.06^{de}	49.55^{ij}	10.38^{fedc}	21.25^{c}	4.13^{ed}	75.00^{ghi}	49.01^{edf}
E-11	159.92^{i}	62.95^{gcfed}	71.73^{ef}	0.88^{fcebhdg}	4.55^{ba}	65.52^{cb}	10.13^{fe}	19.50^{dc}	3.54^{hfg}	82.50^{ef}	51.80^{dc}
E-12	181.07^{cfed}	61.28^{ghfe}	73.13^{edf}	0.84^{ih}	4.14^{dc}	62.04^{ed}	10.88^{bac}	14.75^{gf}	3.03^{ji}	64.75^{j}	35.80^{h}
E-13	170.20^{h}	59.91^{gh}	72.23^{ef}	0.83^{i}	3.25^{jki}	52.25^{ih}	10.25^{fed}	25.25^{b}	4.04^{ed}	86.00^{ed}	50.79^{edc}
E-14	173.01^{g}	62.98^{gcfed}	73.28^{ed}	0.86^{feihg}	3.52^{hi}	50.55^{ih}	10.00^{f}	19.00^{dc}	3.28^{hi}	83.00^{ef}	48.42^{edf}
E-15	143.50^{j}	59.35^{h}	69.83^{hi}	0.85^{fihg}	3.95^{de}	40.85^{k}	10.63^{bedc}	15.50^{gfe}	2.53^{k}	68.50^{ji}	47.84^{ef}
E-16	171.02^{h}	62.32^{ghfed}	74.02^{d}	0.84^{ih}	4.73^{a}	57.14^{gf}	10.25^{fed}	13.00^{g}	2.46^{k}	75.00^{ghi}	43.70^{g}
E-17	176.70^{gfeh}	63.23^{cfed}	72.27^{ef}	0.88^{fceihdg}	3.42^{jhi}	57.37^{gf}	10.38^{fedc}	19.50^{dc}	3.03^{ji}	82.50^{ef}	46.76^{gf}
E-18	160.48^{i}	62.44^{ghfed}	68.28^{ij}	0.92^{cbd}	3.16^{jkl}	48.54^{j}	10.50^{fedc}	19.50^{dc}	3.74^{efg}	86.25^{ed}	53.45^{bc}
E-19	187.05^{cbd}	63.63^{ced}	72.87^{edf}	0.87^{feihdg}	2.96^{kl}	58.67^{f}	11.13^{ba}	14.00^{g}	2.53^{k}	96.00^{cb}	51.08^{edc}
E-20	156.48^{i}	61.39^{ghfe}	67.15^{j}	0.92^{cbd}	2.95^{l}	59.39^{ef}	10.88^{bac}	18.25^{dce}	2.68^{jk}	90.38^{cd}	57.40^{a}
LSD (P ≤ 0.05)	7.93	3.18	1.60	0.04	0.28	3.31	0.59	3.22	0.44	6.66	3.86

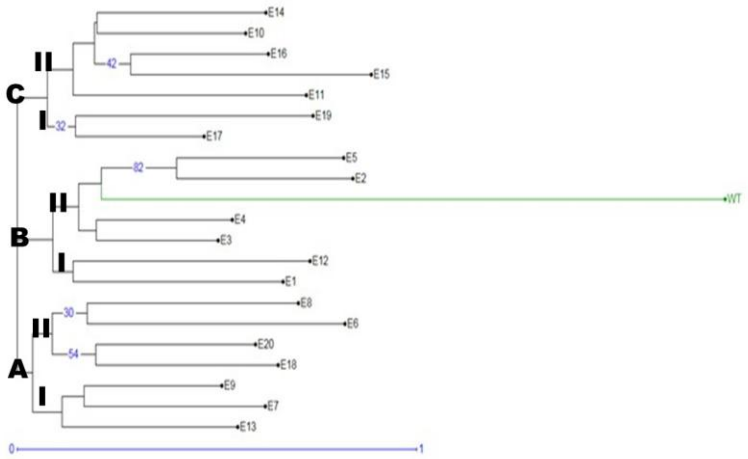
Notes: Superscript in small letters indicate significant difference at P < 0.05

Table.3 Variation in fruit chemical characteristics of putative Kinnow mutants

Code of mutant	TSS (°B)	Vitamin C (mg/100g)	Acidity (%)	TSS: Acid ratio
WT	11.49^{i}	41.43^{a}	0.86^{feg}	13.42^{gfdec}
E-1	11.08^{i}	35.38^{b}	1.01^{b}	11.11^{ih}
E-2	11.41^{ih}	33.00^{cebd}	1.12^{a}	10.38^{i}
E-3	12.19^{gfdeh}	34.75^{cb}	0.97^{cbd}	12.64^{gfeh}
E-4	11.83^{gfih}	33.75^{cebd}	0.99^{cb}	11.96^{gih}
E-5	12.51^{gfde}	31.00^{e}	1.01^{b}	12.48^{gfeh}
E-6	13.73^{a}	36.13^{b}	0.84^{g}	16.45^{a}
E-7	12.95^{bdac}	33.75^{cebd}	0.87^{feg}	15.01^{bac}
E-8	13.41^{bac}	30.88^{e}	0.86^{feg}	15.79^{ba}
E-9	12.58^{gfdec}	31.13^{e}	0.92^{fcegd}	13.75^{fedc}
E-10	12.55^{gfde}	31.13^{e}	0.93^{cebd}	13.54^{gfdec}
E-11	12.13^{gfdeh}	33.63^{cebd}	0.91^{fcegd}	13.41^{gfedc}
E-12	11.78^{g}	30.63^{e}	0.97^{cbd}	12.20^{gfh}
E-13	12.65^{fdec}	33.13^{cebd}	0.90^{fegd}	14.20^{bedc}
E-14	12.74^{dec}	34.50^{cbd}	0.91^{fcegd}	14.13^{bedc}
E-15	13.63^{ba}	31.63^{ced}	0.84^{fg}	16.26^{a}
E-16	12.75^{dec}	31.00^{e}	0.89^{fegd}	14.43^{bdc}
E-17	11.98^{gfeh}	31.50^{ed}	0.93^{fcebd}	13.04^{gfed}
E-18	12.88^{bdc}	31.00^{e}	0.93^{fcebd}	14.14^{bedc}
E-19	11.84^{gfih}	31.38^{ed}	0.97^{cbd}	12.65^{gfeh}
E-20	12.65^{fdec}	30.75^{e}	0.91^{fcegd}	14.06^{bedc}
LSD (P ≤ 0.05)	0.84	3.16	0.08	1.75

Notes: Superscript in small letters indicate significant difference at P < 0.05

Fig.1 Dendrogram for the studied putative Kinnow mutants including wild type. The bootstrap values ≥30 were depicted on the dendrogram



Stimulated plant height in the mutants at the lower doses suggest lower biological damage to the tissues. The upregulation of the antioxidant enzymes such as superoxidase dismutase (SOD), catalase (CAT) and peroxidase (POX) must have also played an important role in triggering the plant growth. Alteration in plant morphology (plant height, stem diameter and canopy volume) in the EMS induced mutants at varying concentration have also been reported by other researchers in different fruit crops such as mango, citrus and papaya (Rime *et al.*, 2019; Kaur & Rattanpal 2010; Kumar *et al.*, 2013).

Reduction in the number of fruit per plant was observed in all the mutants irrespective of the mutants developed from the varying concentration of EMS. The fruiting intensity was however, higher in the mutant E-15 which resulted due to reduced canopy area. There are no reports on the effect of EMS induced mutants on the yield of fruit crops, except few reports indicating a stimulated increase in yield and related parameters in chilli and fenugreek (Jabeen & Mirza 2002; Basu *et al.*, 2008) and inhibitory effect in *Jatropha curcas* (Dhakshanamoorthy *et al.*, 2010).

EMS treatment significantly affected the fruit physico-chemical parameters in mutants. Fruit weight was considerably lower in the mutants which could be due to the negative relationship between fruit size and EMS, Bhat *et al.*, (2017). Varied effect of EMS on fruit quality parameters as observed in the present study clearly explains that EMS has both the stimulatory and inhibitory effect which may be due to the pleiotropic effects.

A similar alteration in fruit quality parameters has been reported in papaya (Kumar *et al.*, 2017) and strawberry (Bhat *et al.*, 2017). The lower number of seeds in the mutants E-16 and E-19 created from 0.5%, might be due to arrest in the ovary growth before pollination and curtailed the supply of auxin after the fertilization of the ovules because the auxin content assayed in the present study (data not given) was significantly higher in the EMS induced mutants and its supply to the ovary should not have been a constraint. The role played by auxin in harmonizing the shift from the flower to the fruit and its role on fertilization and development have been confirmed through molecular markers (Dorcey *et al.*, 2009).

The obtained result on chemical attributes of fruits showed that the total soluble solids (TSS), vitamin C and TSS: acid ratio was maximum in the mutant E-6 generated from 0.1% EMS. The Vitamin C content was however lower than the WT. Juice acidity per cent was determined maximum and minimum in the mutants E-3 and E-6 developed from dissimilar doses of 0.05 and 0.1%. Such variation in chemical attributes of mutants induced by EMS has been related to the stimulatory and inhibitory effect of enzymes depending on the biological damage caused by the mutagen dose. The finding of the present study aligns with Kumar *et al.*, (2017) and Bhat *et al.*, (2017) in papaya and strawberry respectively.

From the above findings, it can be concluded that the chemical mutagen, EMS was able to generate enormous variability in the Kinnow population. Long term evaluation and characterization of mutants led to the identification of mutant E-16 having dual trait i.e., dwarfness and fruits with the reduced number of seed and E-19 (reduced number of seeds/fruit) which can be used in the future breeding programme. Further, EMS can also be tried in other citrus spp for generating variability and developing mutants with traits of interest.

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