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Effect of colchicine on plant growth and leaf nutrient acquisition of sweet orange (*Citrus sinensis* (L.) Osbeck) cv. Mosambi

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Abstract
The present study was conducted on sweet orange cv. Mosambi mutants, developed by treating with different doses of colchicine (0.05%, 0.10%, 0.15% and 0.20%) and raised on *Jatti Khatti* rootstock. Two years after the field establishment of plants, the developed mutants were examined in terms of alterations in growth parameters and leaf nutrient acquisition. Colchicine had both inhibitory and stimulatory effects on the mutants developed at different doses. Reductions in stem girth and TCSA were recorded at 0.05% and 0.20% of colchicine treatment. Mutants developed at 0.10% and 0.15% colchicine concentration, had stimulatory effect on plant growth parameters. Higher nutrient acquisition was noticed in the leaves of mutants developed at 0.10%-0.15% doses, while lower macronutrient acquisition was recorded at 0.05% and 0.20% doses. Variation induced in the mutants by colchicine indicated that both lower and higher doses of mutagenic treatments may give rise to the mutants of economic importance and indicating its potential use as a mutagen.

Keywords: Mutants, growth, nutrient, colchicine, variation

Introduction
Citrus is considered as one of the most important group of fruits in the world and includes fruits such as sweet oranges, mandarins, grapefruits, pummelos, tangerines, tangors, citranges, lime, lemon, sour orange, etc. (FAOSTAT, 2017) [11]. The major citrus growing states in India are Uttar Pradesh, Anadhra Pradesh, Maharashtra, Punjab, Tamil Nadu, Rajasthan, West Bengal, Chhattisgarh and North-Eastern states. Sweet orange (*Citrus sinensis* (L.) Osbeck), the second most important group of citrus after mandarin, contributed more than 23% of total citrus production (Anonymous, 2018) [3] in India. Mosambi is the choicest variety because of its sweet taste and pleasant flavour and is widely grown in western and central India. Fresh fruit and juice of sweet orange (*Citrus sinensis*) are in great demand because of the low acidity, high juice recovery percentage and better antioxidant properties. Despite several positive traits, some unwanted traits like the excessive seededness (15-30 seeds/fruit) greatly hinders consumer's acceptability, as crushed seeds cause bitterness in the extracted juice.
Seedlessness is of paramount importance in citrus breeding and has been attempted by several workers using conventional and non-conventional breeding techniques (Bermejo *et al.*, 2015) [6]. Induced mutagenesis has scientific and commercial interests to improve the plant growth, yield and quality characteristics of commercially important plants. Mutation breeding provides raw materials for the genetic improvement of economic crops (Adamu *et al.*, 2004) [1]. It helps in designing crops having improved yield, increased stress tolerance, and longer life span with reduced agronomic inputs (Ahloowalia and Maluszynski, 2001) [2]. Seedlessness can be achieved by various breeding methods including the spontaneous selections or from the seedling populations, but the development of seedless triploids by crossing either tetraploids with diploid or vice versa is one of the common techniques used in breeding for seedlessness. But major problem in such breeding programmes is the non-availability of suitable tetraploids. Colchicine, an alkaloid extracted forms meadow saffron (*Colchicum autumnale* L.), is known to be a mitotic inhibitor (Blakeslee and Avery, 1937) [7] and is commonly used for doubling of chromosomes for increased ploidy level, inducing and enhancing genetic variability in different crops within a very short period of time.

Ploidy manipulation through colchicine has been used since long in citrus by treating the buds *in vivo* with this chemical (Barrett, 1974) [5]. Colchicine not only used for doubling of chromosomes, but it also induces mutations in plants. Plants mutated through colchicine are known as colchi-mutants (Ari *et al.* 2015) [4]. Mutagenic effects of colchicine on plant performance have been reported by various workers (Castro *et al.*, 2003) [9]. It was suggested that the citrus cultivars behaved differently to the colchicine treatment and this might be result of killing or growth retardation of treated buds (Barrett, 1974) [5].

The present study was carried out in the pre-bearing colchicine induced mutants of sweet orange cv. Mosambi. The study aimed to measure the efficiency of chromosome doubling agents (colchicine) for creating variation and to determine the alterations in plant growth and leaf nutrient acquisition amongst the mutants in comparison to wild type. It was expected that if any desirable mutant was found, these parameters will be correlated with the selected mutant having economical superiority than wild type.

Materials and Methods

In the present experiment, growth and nutrient absorption study was carried out on two-year-old putative sweet orange cv. Mosambi mutants developed through different doses of colchicine (0.05%, 0.10%, 0.15% and 0.20%) along with the wild type. The plants were raised on *Jatti Khatti* (*Citrus jambhiri* Lush.) rootstock and maintained under uniform cultural practices at the main orchard of Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi. Detail of sweet orange cv. Mosambi mutants is given in Table 1.

Plant growth parameters viz., plant height (m), stem girth (cm) and canopy volume (m^3) of the mutants were recorded four months after the emergence of spring and rainy season flush i.e., June and November for two consecutive years *i.e.* 2017 and 2018. Plant height was measured by measuring scale from the base of plant to the top of fully opened leaf on the main stem. The diameter of the stem was measured 6 cm above budding union using Digimatic vernier calipers at a pre-marked point. Plant Spread (N-S) and (E-W) was recorded using meter scale and then canopy volume was calculated using tree height (H) and width in parallel (D_1) and perpendicular (Dr) with the following formulae V= (π/6) x H X D_1 X Dr (Zekri, 2000) [18]. Trunk cross-sectional area (TCSA) was measured using following formula.

$$TCSA = \pi (d/2)^2$$

Where d = average of east–west and north–south trunk diameter

Digestion block method as suggested by Bremner *et al.*, (1965) [8] was used for measuring the nitrogen content in leaves. For this, 500 mg of finely ground leaf sample was taken in digestion tube, sequentially digestion mixture and 6.0 ml of concentrated H_2SO_4 was added. Then digestion tubes were heated at 385^0C in digestion assembly. The digestion was continued till the black or brown colour disappeared. The digestion tubes were then taken to distillation unit and dilution, addition of alkali, steam generation and titration were performed. About 25.0 ml of 4% boric acid containing mixed indicator in a 250 ml conical flask taken and placed it under ammonia-receiving tube of the distillation assembly and distillation was run for 150 seconds. The distillate was titrated against N/10 H_2SO_4. Nitrogen in leaf samples was determined by using the formula;

$$N (\%) = [(T-B) \times N \times 1.4] /S$$

Where, T = Volume of standard H_2SO_4 taken for sample; B = Volume of standard H_2SO_4 taken for blank; N = Normality of acid; S = Weight of plant sample taken.

Phosphorus content in leaves was assessed by vando-molybdo-phosphoric yellow colour method as described by Jackson (1973) [14]. 0, 1, 2, 3, 4 and 5 ml of standard solution was taken into volumetric flask (50 ml) to get 0, 1, 2, 3, 4 and 5 ppm of P, respectively and 10 ml of vando-molybdate solution was added to each flask. The volume was made up with distilled water and the transmittance was recorded on spectrophotometer (UV-VIS double beam PC 8 scanning auto cell spectrophotometer (UVD-3200, Labomed, Inc., Culver city, USA) at 420 nm wavelength after 30 minutes. The absorbance thus recorded was plotted against concentration. The phosphorus concentration was calculated by using the standard curve.

$$P \text{ in sample } (\%) = A/100 \times V$$

Where, A = P concentration in µg as read against sample reading on the standard curve; V = Volume of aliquot taken (ml) for colour development.

Total potassium content in the leaves was estimated, by method suggested by Jackson, (1980) [15], from diacid digested leaf samples using a microprocessor-based flame photometer (Flame Photometer-128, Systronics, Ahmedabad). Suitably diluted sample was fed to an atomizer capillary tube and concentration of potassium was directly read on the display monitor. Potassium present in the leaf samples was calculated by multiplying the reading with the dilution factor and expressed in percentage on dry weight basis.

Calcium, Magnesium and micronutrient (Cu, Fe, Zn and Mn) contents in leaf samples were ascertained by atomic absorption spectrophotometer (Model- GBC, 904AA, GBC Scientific Equipment, Hampshire, Illinois, USA) according to Jackson (1980) [15].

Statistical analysis ($P \leq 0.05$) of the data for growth parameters viz., plant height, stem girth and canopy volume, comprising of twenty mutants and one wild type (control), was done following augmented design. The statistical analysis of plant nutrient content, comprising of four replications in each treatment was carried out in completely randomized block design using SAS package (9.3 SAS Institute, INC., USA) followed by Tukey's Honest test. P values ≤ 0.05 were considered as significant.

Table 1: Description of sweet orange cv. Mosambi mutants developed through chemical mutagen (colchicine)

Code of Mutant	Colchicine concentration	Population
CS 0	Untreated	Wild type
CS 1	0.05%	0.05-1
CS 2	0.05%	0.05-2
CS 3	0.05%	0.05-3
CS 4	0.05%	0.05-4
CS 5	0.05%	0.05-5
CS 6	0.05%	0.05-6
CS 7	0.05%	0.05-7
CS 8	0.05%	0.05-8
CS 9	0.05%	0.05-9
CS 10	0.10%	0.10-1
CS 11	0.10%	0.10-2
CS 12	0.10%	0.10-3
CS 13	0.10%	0.10-4
CS 14	0.10%	0.10-5
CS 15	0.15%	0.15-1
CS 16	0.15%	0.15-2
CS 17	0.15%	0.15-3
CS 18	0.20%	0.20-1
CS 19	0.20%	0.20-2
CS 20	0.20%	0.20-3

Results and Discussion

Growth parameters (plant height, stem girth and canopy volume) are important morpho-physiological traits, which contribute to increased biomass. Statistical tests on plant growth characteristics of mutants, developed through different doses of colchicine, revealed non-significant differences in plant height and plant canopy (Table 2), whereas significant differences in stem girth and TSCA were recorded. A general decrease in plant height without any significant difference was recorded among the mutants. A significant decrease in stem girth was recorded among the mutants as compared to control, which was recorded minimum at 0.20% concentration of colchicine in CS 20 mutant by -49.82% compared to control. A general decrease in TCSA was recorded with significant difference in the mutants as compared to control. A minimum TCSA was recorded by -77.49% in CS 20, which differed significantly to control. Chemical mutagenesis induced by the varying colchicine concentrations have shown non-significant variation in the plant height and canopy volume, but reduction in stem girth and TCSA might prove beneficial in the improvement of some of its selected growth traits. Uno *et al.* (2001) [17] suggested that mutations induced by colchicine lead to an alteration of the plant genome through an increased cellular division rate and growth of the meristematic regions, and this may happen probably due to modification of the signaling pathway. While conducting research on jute (*Corchorus olitorius* L.), Nura *et al.* (2011) [16] found stimulatory and inhibitory effects of lower and higher doses colchicine on plant growth parameters. El-Nashar and Ammar (2016) [10] also reported the similar results in *Calendula officinalis* L.

Table 2: Variation in plant growth characteristics in the mutants developed through colchicine.

Treatment	Plant height (m)	Stem girth (mm)	Canopy volume (m3)	TCSA
CS0	1.98	54.47^{a}	2.70	73.28^{a}
CS1	1.93	39.89^{bcdef}	1.60	39.24^{bcde}
CS2	1.93	35.56^{ef}	1.73	31.18^{def}
CS3	1.81	47.99^{abcde}	2.00	56.80^{abcde}
CS4	1.63	47.37^{abcde}	1.21	55.35^{abcde}
CS5	1.46	49.90^{abcd}	1.57	61.42^{abcd}
CS6	1.59	35.93^{def}	1.80	33.80^{bcde}
CS7	1.49	38.08^{def}	1.68	37.48^{bcde}
CS8	1.66	45.49^{abcde}	2.73	51.89^{abcde}
CS9	1.44	35.09^{def}	1.63	32.42^{cef}
CS10	1.99	51.63^{abc}	3.02	65.88^{abd}
CS11	1.94	36.63^{cdef}	1.50	32.56^{def}
CS12	2.19	36.70^{cdef}	1.86	32.70^{def}
CS13	1.89	54.02^{ab}	2.77	72.06^{abc}
CS14	1.94	38.22^{cdef}	1.60	35.56^{def}
CS15	1.84	33.82^{ef}	1.31	27.58^{def}
CS16	1.97	35.93^{def}	2.07	30.55^{def}
CS17	1.65	34.38^{def}	1.21	27.74^{def}
CS18	1.65	34.96^{def}	1.51	28.79^{def}
CS19	1.92	46.07^{abcde}	2.69	51.81^{abcd}
CS20	1.42	27.33^{f}	0.94	16.49^{e}
CD (P=05)	NS	12.81	NS	33.30
SED	0.16	3.88	0.66	10.10

Table 3: Variation in leaf macronutrients in the mutants developed through colchicine.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
CS0	2.31^{b}	0.13^{hi}	1.10^{ih}	3.1^{i}	0.33^{h}
CS1	2.16^{e}	0.16^{bc}	1.45^{b}	3.7^{f}	0.43^{c}
CS2	2.10^{hg}	0.15^{dce}	1.30^{fed}	3.9^{e}	0.40^{edf}
CS3	1.94^{j}	0.14^{hgfe}	1.20^{fhg}	3.4^{h}	0.36^{g}
CS4	1.90^{kl}	0.13^{hgi}	1.18^{hg}	3.1^{i}	0.37^{g}
CS5	1.86^{m}	0.11^{k}	1.02^{i}	2.9^{jk}	0.31^{ji}
CS6	1.90^{kl}	0.14^{dgfe}	1.23^{feg}	3.0^{ji}	0.23^{h}
CS7	1.86^{m}	0.13^{i}	1.00^{i}	2.8^{l}	0.29^{jk}
CS8	1.92^{kj}	0.15^{dfe}	1.15^{hg}	3.5^{g}	0.36^{g}
CS9	1.88^{ml}	0.12^{kj}	1.00^{i}	3.0^{ji}	0.28^{k}
CS10	2.15^{fe}	0.15^{dfe}	1.40^{cbd}	4.1^{c}	0.44^{cb}
CS11	2.28^{c}	0.17^{ba}	1.60^{a}	4.2^{cb}	0.47^{a}
CS12	2.52^{a}	0.18^{a}	1.65^{a}	4.3^{b}	0.45^{b}
CS13	2.10^{hg}	0.15^{dfe}	1.33^{ced}	4.2^{cb}	0.40^{ed}
CS14	2.23^{d}	0.17^{a}	1.43^{cb}	4.0^{d}	0.43^{c}
CS15	2.08^{h}	0.14^{hgfi}	1.18^{hg}	4.2^{cb}	0.41^{d}
CS16	2.12^{fg}	0.14^{hgfe}	1.10^{ih}	4.0^{d}	0.39^{ef}
CS17	1.90^{kl}	0.13^{ji}	1.00^{i}	4.7^{a}	0.39^{f}
CS18	1.95^{j}	0.14^{hgfe}	1.10^{ih}	3.4^{hg}	0.34^{h}
CS19	2.05^{i}	0.15^{dc}	1.20^{fhg}	3.8^{e}	0.41^{d}
CS20	1.86^{m}	0.11^{k}	1.00^{i}	2.9^{jk}	0.31^{i}
LSD (P≤0.05)	0.03	0.01	0.12	0.1	0.02

The plant nutrients have important role to play in plant growth and development. Fredeen *et al.* (1989) [12] demonstrated that P deficiency led to reduction in leaf size and hence, decreased photosynthetic capacity leading to growth inhibition. Potassium is an important nutrient because it has an important role in carbohydrate translocation and regulation of plant water relations. Micronutrients are important for plant growth and are involved in various activities including energy metabolism, primary and secondary metabolism, cell protection, gene regulation, hormone perception and reproduction (Hancsh and Mendel, 2009) [13]. Increment in the major and micronutrients in the mutants developed through colchicine was recorded at 0.10%-0.15%, while decrease was recorded in mutants obtained from 0.05% and 0.20% colchicine concentration (Table 3 and Table 4). Mutants developed at 0.10%-0.15% colchicine treatment have higher growth along with high nutrient uptake, hinting at better efficiency of these mutants for the production of vegetative and reproductive mass. The increased mineral nutrient concentration in leaf tissues indicated a higher nutrient requirement of scion of colchicine treated plants for which the root part explores larger soil volume for extraction of nutrients. These mutants might be having higher photosynthetic leaf area and photosynthetic rate that lead to high nutrient uptake for their growth and development. This is true also because the colchiploids in general have bigger leaves and high chlorophyll content and thus exhibit higher photosynthetic efficiency.

Table 4: Variation in leaf micronutrient in the mutants developed through colchicine.

Treatment	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
CS0	57^{j}	7^{jki}	38^{h}	22^{n}
CS1	79^{ed}	7^{lk}	52^{d}	34^{h}
CS2	85^{c}	9^{g}	44^{fg}	41^{e}
CS3	76^{egf}	8^{hjgi}	66^{b}	38^{f}
CS4	78^{edf}	13^{b}	46^{fe}	29^{j}
CS5	85^{c}	6^{l}	66^{b}	29^{j}
CS6	74^{hg}	8^{hgi}	57^{c}	43^{d}
CS7	72^{h}	9^{g}	66^{b}	43^{d}
CS8	78^{edf}	8^{hji}	43^{g}	27^{lk}
CS9	78^{edf}	7^{jki}	38^{h}	35^{hg}
CS10	75^{gf}	11^{cd}	53^{d}	45^{c}
CS11	91^{b}	13^{b}	47^{e}	35^{g}
CS12	87^{c}	12^{b}	43^{g}	27^{k}
CS13	85^{c}	10^{f}	44^{fg}	31^{i}
CS14	96^{a}	11^{ed}	53^{d}	47^{b}
CS15	76^{egf}	9^{g}	65^{b}	49^{a}
CS16	88^{c}	8^{hg}	71^{a}	26^{lm}
CS17	87^{c}	14^{a}	44^{fg}	34^{h}
CS18	77^{egf}	12^{cb}	66^{b}	43^{d}
CS19	80^{d}	10^{ef}	58^{c}	38^{f}
CS20	68^{i}	7^{jk}	35^{i}	25^{m}
LSD (P≤0.05)	3	1	2	1

In conclusion, colchicine has significantly created variability in the sweet orange cv. Mosambi for the studied parameters. Increase in stem girth and TCSA observed in the mutants having good amount of nutrient absorption hints at better efficiency for the production of vegetative and reproductive biomass. Growth restriction and less nutrient availability were also observed in the mutants developed at different doses of colchicine. Mutagenic treatments may result in the development of mutants of significant economic importance for obtaining compact type of mutants having high nutrient use efficiency and ultimately improved yield and quality production of fruits. The obtained mutants may not be of direct use as a variety of sweet orange, but they can be used as parents in future breeding programmes for improving the nutrient use efficiency and sturdy plants.

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