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# Characterization of second generation colchiploids in sweet orange (*Citrus sinensis*) cv. Mosambi with respect to morpho-physio-biochemical traits

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ABSTRACT

Induction of tetraploidy in citrus is aimed for the development of triploid seedless cultivars, which are needed for meeting the requirements of the citrus industry. The present study was carried out during 2020 and 2021 at ICAR-Indian Agricultural Research Institute, New Delhi to identify suitable tetraploids in vegetatively propagated second generation of the colchiploids in sweet orange [*Citrus sinensis* (L.) Osbeck] cv. Mosambi. The 3-year-old, 20 2$^{nd}$ generation colchicine treated (0.05, 0.10, 0.15 and 0.20%) plants, developed from the putative tetraploid branches of the first generation colchiploids of sweet orange cv. Mosambi, budded on *Jatti khatti* rootstock along with their wild (parent) type, were characterized based on morphological and physiological traits. Leaf area, fresh weight and succulency increased, but density of foliar tissue and leaf dry weight reduced in majority of the colchiploids. Leaf length, width and thickness also increased in colchiploids in comparison to their wild type. Fruit weight, length, width and rind thickness also increased in the colchiploids in comparison to their wild type. Juice TSS reduced, but acidity increased in majority of these Mosambi colchiploids. Seed number was lowest in M-3 (7.33) colchiploid. Total carotenoids and chlorophyll fractions also increased in majority of the colchiploids. On the basis of morpho-physio-biochemical trait characterization, 8 solid tetraploids (M-1, M-3, M-2, M-5, M-4, M-7, M-13 and M-16) of Mosambi were identified, which can be used in future breeding programmes for the development of seedless triploids of Mosambi.

**Keywords**: Characterization, Morphological variation, Mosambi colchiploids, Mutagenesis, Physiological variation, Second generation, Sweet orange

Citrus is the third important commercially grown fruit crops in India, after only mango (*Mangifera indica* L.) and banana (*Musa* spp.). It belongs to family Rutaceae with a chromosome number of 2n = 2x = 18. The primarily cultivated *Citrus* spp. include sweet oranges, mandarins, lemons, acid limes, pummelos, grapefruits, sweet limes, and tangerines. Sweet oranges [*Citrus sinensis* (L.) Osbeck] are of particular significance, ranking second only to mandarins in terms of importance. In 2022, sweet oranges were cultivated in India over an area of approximately 0.23 million hectares, yielding a production volume of 4.25 million tonnes (Anonymous 2022).

The global citrus breeding community prioritizes cultivating seedless fruits due to their superior quality and suitability for post-harvest processing (Grosser *et al.* 2014). Achieving seedlessness in new citrus varieties is a challenging goal, especially through traditional breeding methods. Techniques like inter-ploidy hybridization between tetraploid and diploid parents can be employed to develop seedless varieties (Jaskani and Khan 1993), but there is a limited availability of suitable tetraploids for Mosambi. Moghbel *et al.* (2015) suggested that physiological and morphological markers can be used for preliminary screening of putative tetraploid populations to identify beneficial candidates before verifying tetraploid genotypes with advanced methods like flow cytometry, chromosome counting, and molecular characterization.

Identifying ploidy levels is a crucial step in polyploidization, and this can be achieved in a straight forward and quick manner by examining the physiological and morphological traits of the developed genotypes. Polyploids generally exhibit distinct morphological, biochemical, physiological and molecular traits that are typically absent in their predecessors. Therefore, a systematic characterization is necessary to select ideal colchiploids/colchi-mutants and identify solid tetraploids. This process requires the dechimerization of the colchiploids induced in the first generation by vegetatively propagating the

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identified putative tetraploids branches in the second generation, potentially leading to the production of solid tetraploids. Hence, present study was carried out to examine morphological and physiological traits to identify suitable solid tetraploids in the second generation of colchiploids.

## MATERIALS AND METHODS

The present study was carried out during 2020 and 2021 at ICAR-Indian Agricultural Research Institute (77° 12'E, 28° 40'N, and at an altitude of 228.6 m amsl), New Delhi. A total of 20, 2nd generation colchiploids (M1-M22) and one wild type (mother type) of sweet orange cv. Mosambi were selected for the experiment. Second generation colchiploid population of Mosambi was developed from colchicine treated first generation colchiploids by vegetatively propagating the putative tetraploid branches on the rough lemon rootstock seedlings to get the solid tetraploids. The first generation colchiploids were developed by treating the budwood from a single elite plant of Mosambi through treating the budsticks with varying colchicine concentrations (0.00, 0.05, 0.10, 0.15 and 0.20%). The 2nd generation colchiploids were planted at a spacing of 4 m × 3 m in a randomized block design (RBD) with three replications. The leaf, plant and fruit analysis was conducted in the laboratories at ICAR-Indian Agricultural Research Institute, New Delhi.

Leaves were weighed directly after sampling to record their fresh weight followed by oven-drying at 70°C for 48 h, and their dry weight was taken. The physiological parameters, viz. specific leaf area [SLA ($cm^2/g$) = Leaf area/Dry weight], leaf succulency [S (mg $H_2O/cm$) = (Fresh weight-Dry weight)/Leaf area] specific leaf weight [(SLW ($g/cm^2$) = Dry weight/Leaf area)], and density of foliar tissue [DFT (g/kg) = Dry weight/Fresh weight × 1000] were determined using the formulae suggested by Ennajeh *et al.* (2010). The length, width of the leaf lamina and petiole length were measured using a measuring scale. The leaf lamina length to width ratio was obtained by dividing the leaf lamina length with the leaf lamina width.

Fruit and seed weights were measured using a digital electronic balance. The length and width of the fruit and rind thickness were measured with Vernier callipers. The fruit length to width ratio was detected by dividing the length by the width. The presence of oil glands and the appearance of the fruit rind were visually analysed and scored as prominent (1) or suppressed (0), and smooth (1) or rough (0), respectively, for statistical analysis. Juice percentage of the fruit was determined by dividing the total juice weight by the total fruit weight and multiplying by 100. The number of fruits per plant, number of segments per fruit, and number of seeds per fruit were counted manually. The total soluble solids (TSS) of the juice were measured using a manual hand-held refractometer. Titratable acidity was estimated through titration using phenolphthalein as an indicator. The TSS to acid ratio was determined by dividing the TSS values by the acidity values.

From the rainy season flush, fully matured and established leaf samples were collected during the month of August. The extraction of the chlorophyll fractions (chlorophyll '*a*', '*b*', and absolute chlorophyll) was carried out following the Hiscox and Isaraelstam (1979) method. The results were noted using spectrophotometer (UVD-3200, Labomed, USA) at wavelengths of 645, 663 for chlorophyll fractions and at 480 nm for total carotenoids using pure DMSO as a blank. The concentrations of chlorophyll '*a*', chlorophyll '*b*', total chlorophylls, as well as total carotenoids, were determined on the fresh weight basis, following the below-mentioned formulae:

$$\text{Chlorophyll '}a\text{' (mg/g FW)} = \frac{(12.7\times A663 - 2.69\times A645)\times \text{Volume}\times \text{Dilution}}{\text{Weight of Sampel}\times 1000}$$

$$\text{Chlorophyll '}b\text{' (mg/g FW)} = \frac{(22.9\times A645 - 4.68\times A663)\times \text{Volume}\times \text{Dilution}}{\text{Weight of Sampel}\times 1000}$$

$$\text{Total chlorophyll (mg/g FW)} = \frac{(20.2\times A645 + 8.02\times A663)\times \text{Volume}\times \text{Dilution}}{\text{Weight of Sampel}\times 1000}$$

$$\text{Total carotenoids } (\mu g/g \text{ FW}) = A_{480} + (0.114\times A_{663}) - (0.638\times A_{645})$$

## RESULTS AND DISCUSSION

Second-generation colchiploids of Mosambi, displayed substantial variations in leaf sclerophylly parameters (Table 1). Notably, genotype M-14 exhibited the maximum leaf area (399.21 $cm^2$) and succulency (0.0220 mg $H_2O/cm$). All induced colchiploids of Mosambi demonstrated higher leaf area values compared to the control (wild type). The fresh weight of M-14 was recorded the highest (12.84 g), followed by M-4 (12.20 g) and M-1 (12.16 g), significantly exceeding the wild type (5.49 g). Maximum leaf dry weight was registered in M-1 and M-14 (both 4.07 g). Wild type displayed the minimum leaf fresh and dry weight (5.49 g and 2.56 g, respectively). Colchiploid M-16 exhibited the highest specific leaf area (126.97 $cm^2/g$), while wild type had the minimum (90.01 $cm^2/g$). Wild type recorded the maximum specific leaf weight (SLW) (0.0111 $g/cm^2$) and density (467.01 g/kg), while M-16 showed the minimum SLW value (0.0080 $g/cm^2$). The variations in leaf characteristics, such as increased leaf area, might be attributed to increase in cell size because of higher accumulation of cytoplasm and large cell organelles due to polyploidation and because of which leaf fresh weight and succulency increased but the SLW and density decreased. Interestingly, the dry weight of the colchiploids was also higher but SLW and density was lower as expected. This finding aligns with the earlier reports of Lalit (2020) and Sabooni *et al*. (2022).

The developed colchiploids exhibited a significant variety in leaf morphology (Table 2). The length of the leaf blade in these colchiploids varied from 11.38 (M-5 and M-22) to 8.75 cm (wild type), while the width ranged from 7.22 (M-3) to 4.82 cm (M-18). The ratio of length to width was between 2.12 (M-18) and 1.44 (M-6), and the leaf thickness varied from 0.44 (M-5) to 0.22 mm (wild type) (Table 2). Variations were also observed in the length of

Table 1 Variability in leaf sclerophylly characteristics of the 2nd generation colchiploids of sweet orange cv. Mosambi

| Colchiploid no. | Leaf area ($cm^2$) | Leaf fresh weight (g) | Leaf dry weight (g) | Specific leaf area ($cm^2$/g) | Specific leaf wt. (g/m) | Density of foliage tissue (g/kg) | Succulency (mg $H_2O$/ $cm^2$) |
|---|---|---|---|---|---|---|---|
| Wild type | 230.37 | 5.49 | 2.56 | 90.01 | 0.0111 | 467.01 | 0.0127 |
| M1 | 386.26 | 12.16 | 4.07 | 94.82 | 0.0105 | 337.33 | 0.0209 |
| M2 | 367.24 | 10.79 | 3.43 | 107.02 | 0.0093 | 318.76 | 0.0200 |
| M3 | 367.95 | 11.41 | 3.69 | 99.91 | 0.0100 | 324.11 | 0.0210 |
| M4 | 383.02 | 12.20 | 3.90 | 98.32 | 0.0102 | 320.82 | 0.0217 |
| M5 | 344.04 | 8.74 | 3.21 | 106.83 | 0.0094 | 373.43 | 0.0160 |
| M6 | 286.74 | 6.95 | 2.87 | 99.77 | 0.0100 | 413.42 | 0.0142 |
| M7 | 306.61 | 8.94 | 3.03 | 101.22 | 0.0099 | 343.60 | 0.0192 |
| M8 | 310.14 | 7.77 | 2.92 | 106.03 | 0.0095 | 377.89 | 0.0156 |
| M10 | 326.17 | 7.40 | 2.96 | 110.32 | 0.0091 | 399.79 | 0.0137 |
| M12 | 289.63 | 8.10 | 2.98 | 97.06 | 0.0103 | 368.40 | 0.0177 |
| M13 | 337.05 | 10.07 | 3.34 | 100.89 | 0.0099 | 331.41 | 0.0200 |
| M14 | 399.21 | 12.84 | 4.07 | 97.89 | 0.0102 | 317.41 | 0.0220 |
| M15 | 314.40 | 8.42 | 3.27 | 95.86 | 0.0105 | 388.69 | 0.0165 |
| M16 | 335.22 | 7.66 | 2.73 | 126.97 | 0.0080 | 351.72 | 0.0148 |
| M17 | 323.65 | 7.84 | 3.02 | 108.31 | 0.0093 | 383.97 | 0.0149 |
| M18 | 302.25 | 7.60 | 2.77 | 110.93 | 0.0092 | 364.99 | 0.0159 |
| M19 | 318.85 | 7.38 | 2.91 | 109.64 | 0.0092 | 395.40 | 0.0140 |
| M20 | 316.84 | 6.90 | 2.60 | 121.71 | 0.0082 | 377.33 | 0.0136 |
| M21 | 317.93 | 7.79 | 3.03 | 104.79 | 0.0095 | 389.96 | 0.0150 |
| M22 | 311.79 | 7.19 | 2.72 | 114.90 | 0.0087 | 378.32 | 0.0143 |
| LSD (*P*= 0.05) | 57.66 | 1.81 | 0.58 | 13.20 | 0.0010 | 44.39 | 0.0030 |

M1-M22, 20 2nd generation colchiploids of sweet orange cv. Mosambi.

the petiole, which ranged from 2.14 (M-5) to 1.42 cm (wild type). These results were similar with previous studies on different citrus fruits (Tan *et al*. 2019, Lalit 2020, Usman *et al*. 2021 and Sabooni *et al*. 2022) who reported that colchicine treatment slowed plant growth and the leaves in tetraploids were wider with a smaller leaf index (length/breadth ratio). Additionally, the leaves were observed to be thicker in tetraploids because of the augmentation in size of leaf cells.

Fruit characteristics are one of the most important traits as they ultimately determine the preferences of consumers. In this study, significant changes were observed in fruits of polyploids compared to the wild type (Fig. 1 and Table 3 and Supplementary Table 1). The fruit weight, length, and width ranged from 91.17–225.50 g, 52.56–74.97 mm, and 54.97–76.07 mm, respectively. The heaviest fruits were produced by the M-1 plant, with an average fruit weight of 225.50 g, a length of 74.97 mm, and a width of 76.07 mm. In contrast, the wild type had a fruit weight of 158.07 g, length of 63.74 mm, and a width of 68.81 mm. The highest fruit length: width ratio was recorded in M-17 (1.05). Rind thickness increased significantly in most of the induced colchiploids compared to the wild type, with the highest increase observed in M-5 (5.19 mm) and M-17 (5.14 mm), respectively, and lowest rind thickness was observed in M-16 (2.24 mm), which was not significantly different from wild type (2.96 mm). Juice weight and juice percentage

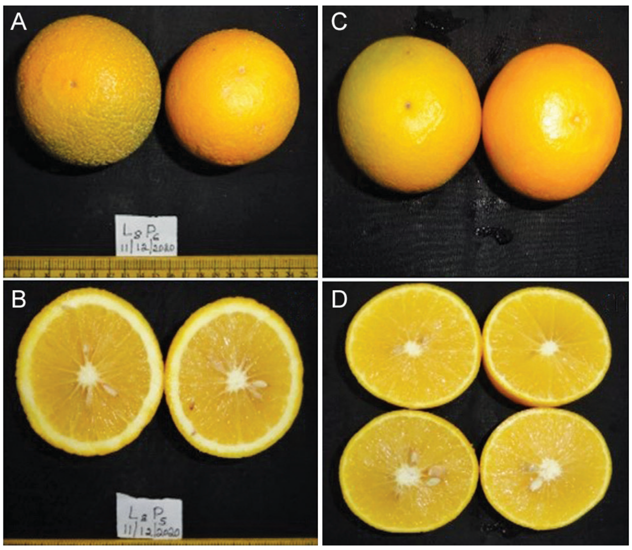


Fig. 1 Variability in fruit characteristics of the 2nd generation colchiploids of sweet orange cv. Mosambi (A and B) and its wild type (C and D).

Table 2 Variability in leaf characteristics of the 2nd generation colchiploids of sweet orange cv. Mosambi

| Colchiploid no. | Leaf blade length (cm) (L) | Leaf blade width (cm) (W) | Leaf L:W ratio | Leaf thickness (mm) | Petiole length (cm) |
|---|---|---|---|---|---|
| Wild type | 8.75 | 4.89 | 1.81 | 0.22 | 1.42 |
| M1 | 10.61 | 6.92 | 1.53 | 0.38 | 1.87 |
| M2 | 10.18 | 6.92 | 1.47 | 0.38 | 1.85 |
| M3 | 10.59 | 7.22 | 1.47 | 0.33 | 2.06 |
| M4 | 10.55 | 6.69 | 1.58 | 0.35 | 1.64 |
| M5 | 11.38 | 6.24 | 1.86 | 0.44 | 2.14 |
| M6 | 9.08 | 6.32 | 1.44 | 0.34 | 1.75 |
| M7 | 9.52 | 6.03 | 1.58 | 0.34 | 1.89 |
| M8 | 9.20 | 6.05 | 1.53 | 0.31 | 1.82 |
| M10 | 10.92 | 5.59 | 1.97 | 0.40 | 1.64 |
| M12 | 8.94 | 6.01 | 1.49 | 0.32 | 1.62 |
| M13 | 10.04 | 6.66 | 1.50 | 0.39 | 1.66 |
| M14 | 8.81 | 5.80 | 1.52 | 0.32 | 1.50 |
| M15 | 9.62 | 5.33 | 1.81 | 0.38 | 1.54 |
| M16 | 9.72 | 6.14 | 1.58 | 0.39 | 2.02 |
| M17 | 10.58 | 6.07 | 1.75 | 0.42 | 1.62 |
| M18 | 10.19 | 4.82 | 2.12 | 0.41 | 1.53 |
| M19 | 11.16 | 6.46 | 1.73 | 0.38 | 1.92 |
| M20 | 10.19 | 5.42 | 1.89 | 0.33 | 1.57 |
| M21 | 10.56 | 5.20 | 2.04 | 0.40 | 1.49 |
| M22 | 11.38 | 5.83 | 1.95 | 0.41 | 1.83 |
| LSD ($P$= 0.05) | 1.49 | 0.69 | 0.28 | 0.10 | 0.32 |

M1-M22, 20 2nd generation colchiploids of sweet orange cv. Mosambi.

were highest in M-13 (115.01 g) and M-15 (53.54%), respectively, and lowest in M-16 (57.83) and M-22 (39.07), respectively. Although juice weight was higher in most of the colchiploids, juice percentage decreased with increasing rind thickness, indicating an inverse relationship between juice content and rind thickness. These results were similar to the earlier findings of Tan *et al*. (2019), who reported that fruit weight, height and diameter of tetraploid fruits increased in Ponkan mandarin, whereas the seed number per fruit decreased by more than 50%.

The number of segments per fruit decreased in colchiploids, with the lowest value observed in M-16 (10.33), indicating an increase in segment size and a decrease in their number compared to the wild type. The minimum number of seeds was recorded in M-19 (7.33 seeds/fruit), while the maximum was found in the wild type (22.11 seeds/fruit). The lowest seed weight and test weight were recorded in M-19 (1.00 and 13.43 g, respectively). In all the colchiploids, the seed number per fruit displayed a decreasing pattern. While, seed weight varied significantly in most of the induced colchiploids compared to their wild types and was maximum in colchiploids, indicating the presence of bold seeds. Oil glands were also prominent in most of the polyploids, with M-1 (0.59) displaying the maximum mean value. More rough-rinded fruits were observed in colchiploids and the fruits of M-4 were all rough-rinded. These findings align with the findings of Ollitrault *et al.* (2008), who recorded tetraploid fruit with thicker rind, fewer seeds, less juice, and prominent oil glands. They also observed that they were less fertile than corresponding diploids, which might be responsible for the reduced seed number in most of the induced colchiploids noted in this study. Similar findings were noted in groundnut and cowpea by Chimdi *et al.* (2022) and Nawalkar and Verma (2023), respectively. The reduced seed number and increased seed size might be due to the mutagenic action of colchicine, which acts as a mutagen in addition to its chromosome doubling work, leading to the development of colchiploids (Ari *et al*. 2015).

Chlorophyll content is a vital component for photosynthesis mechanism and essential component of the leaves. The photosynthetic efficiency of the plant is directly affected by chlorophyll content present in its leaves. The concentration of photosynthetic pigments significantly changed in the second-generation Mosambi colchiploids (Supplementary Fig. 1). The highest chlorophyll '*a*' fraction (1.50 mg/g FW) was recorded in M-12, which was higher than all colchiploids and the wild type (0.90 mg/g FW). The lowest chlorophyll '*a*' was recorded in M-19 (0.88 mg/g FW). Chlorophyll '*b*' was maximum (0.59 mg/g FW) in the M-16 colchiploid, which was higher than the wild type

Table 3 Variability in fruit characteristics of the 2nd generation colchiploids of sweet orange cv. Mosambi

| Colchiploid no. | Fruit weight (g) | Fruit length (mm) | Fruit width (mm) | L:W ratio | Rind thickness (mm) | Segments/ fruit | Seeds/ fruit | Seed weight/ fruit (g) | Test weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| Wild type | 158.07 | 63.7 | 68.81 | 0.93 | 2.96 | 11.67 | 22.11 | 3.49 | 15.77 |
| M1 | 225.50 | 74.97 | 76.07 | 0.99 | 4.58 | 11.41 | 9.56 | 1.97 | 20.44 |
| M2 | 164.60 | 64.89 | 67.26 | 0.96 | 3.59 | 11.56 | 11.11 | 1.84 | 16.18 |
| M3 | 205.07 | 71.44 | 71.05 | 1.01 | 3.71 | 11.19 | 8.26 | 1.34 | 16.15 |
| M4 | 181.74 | 68.36 | 69.21 | 0.99 | 4.09 | 11.37 | 7.81 | 1.33 | 16.20 |
| M5 | 194.69 | 70.68 | 71.78 | 0.99 | 5.19 | 11.30 | 12.81 | 2.37 | 18.56 |
| M6 | 188.28 | 70.39 | 70.64 | 1.00 | 5.13 | 11.15 | 18.22 | 3.76 | 20.82 |
| M7 | 188.98 | 69.46 | 71.18 | 0.98 | 4.73 | 11.26 | 16.15 | 3.45 | 21.21 |
| M8 | 177.52 | 68.04 | 69.28 | 0.98 | 4.10 | 11.74 | 18.78 | 3.38 | 18.27 |
| M10 | 186.91 | 69.24 | 70.69 | 0.98 | 5.08 | 11.41 | 15.78 | 2.97 | 18.12 |
| M12 | 178.97 | 68.55 | 70.00 | 0.98 | 3.90 | 11.26 | 14.63 | 2.38 | 16.54 |
| M13 | 224.95 | 73.31 | 74.42 | 0.98 | 4.05 | 11.70 | 8.63 | 1.70 | 19.19 |
| M14 | 195.53 | 70.78 | 71.93 | 0.98 | 4.04 | 11.33 | 14.96 | 2.81 | 18.95 |
| M15 | 182.83 | 61.50 | 68.99 | 0.89 | 2.94 | 12.00 | 9.67 | 1.50 | 15.56 |
| M16 | 127.33 | 57.30 | 62.69 | 0.91 | 2.24 | 10.33 | 9.00 | 1.50 | 16.62 |
| M17 | 177.29 | 69.34 | 66.29 | 1.05 | 5.14 | 11.44 | 10.72 | 1.96 | 18.49 |
| M18 | 216.63 | 73.97 | 72.01 | 1.03 | 3.61 | 11.00 | 14.00 | 2.17 | 15.46 |
| M19 | 124.50 | 57.58 | 61.87 | 0.93 | 2.85 | 12.00 | 7.33 | 1.00 | 13.43 |
| M20 | 91.17 | 52.56 | 54.97 | 0.96 | 3.63 | 11.67 | 16.33 | 2.33 | 14.28 |
| M21 | 214.67 | 71.63 | 74.03 | 0.97 | 4.16 | 11.33 | 18.33 | 3.67 | 20.03 |
| M22 | 180.33 | 65.93 | 72.73 | 0.91 | 4.32 | 11.00 | 17.67 | 2.83 | 16.63 |
| LSD ($P$= 0.05) | 27.96 | 3.93 | 4.84 | 0.05 | 0.85 | 0.80 | 5.81 | 1.13 | 3.09 |

M1-M22, 20 2nd generation colchiploids of sweet orange cv. Mosambi.

(0.40 mg/g FW) and many other colchiploids. The total chlorophyll (‘*a*’+‘*b*’) fraction was maximum (2.16 mg/g FW) in M-12, which was higher than all other colchiploids and the wild type (1.21 mg/g FW). The minimum total chlorophyll (1.10 mg/g FW) was recorded in M-19. The maximum value (4.19 mg/g FW) for the chlorophyll ‘a’:‘b’ ratio was found in M-19, which was statistically higher than the wild type. Its lowest value (2.04 mg/g FW) was recorded in M-7. The highest total carotenoids were observed in M-12 (2.01 µg/g FW), which was higher than the wild type (1.20 µg/g FW). The lowest value (1.03 µg/g FW) was found in M-19. This increase in chlorophyll content might be due to an increased leaf area and thickness in colchiploids compared to the wild type. Most of the induced colchiploids exhibited an increase in the chlorophyll ‘a’:‘b’ ratio. The total carotenoids content also increased in the majority of the second-generation Mosambi colchiploids. These results were similar to the earlier findings of Wang *et al*. (2018), who reported that the overall leaf chlorophyll content in Chinese jujube was reduced in diploids compared to their counterpart tetraploids. Lalit (2020), while studying the first generation of Mosambi colchiploids, recorded the similar observations. Similarly, Abdolinejad *et al*. (2021) observed that the tetraploids of fig had higher chlorophyll content, a greater number of chloroplasts in guard cells, and a higher photosynthesis rate compared to their diploids. Microscopic imprints of the abaxial leaf surface of second-generation Mosambi colchiploids, revealed the significant variation in stomatal characteristics (Fig. 2). Stomatal density was reduced in Mosambi colchiploids in comparison to wild type whereas, size of the stomata was increased compared to the wild type. Similar type of observations on stomatal characteristics were also recorded by Lalit (2020) while studying the first generation colchiploids of Mosambi.

Induced colchiploids of sweet orange cv. Mosambi were found exhibiting greater phenotypic variations in comparison to their wild type. From the compilation of 24 different traits, putative tetraploids were identified in the population of induced second generation colchiploids. Tetraploids showed many changes in morphological characteristics such as increased leaf area, wider blade, irregular edges and strong stems. Tetraploids were also recorded to exhibit wider and thicker leaves in contrast to their diploids. Chlorophyll has been observed to be a vital trait and proposed as a physiological index for identifying the growth and development of the plants and its amount was recorded higher in tetraploids as compared to diploids. Eight putative tetraploids (M-1, M-3, M-2, M-5, M-4, M-7,

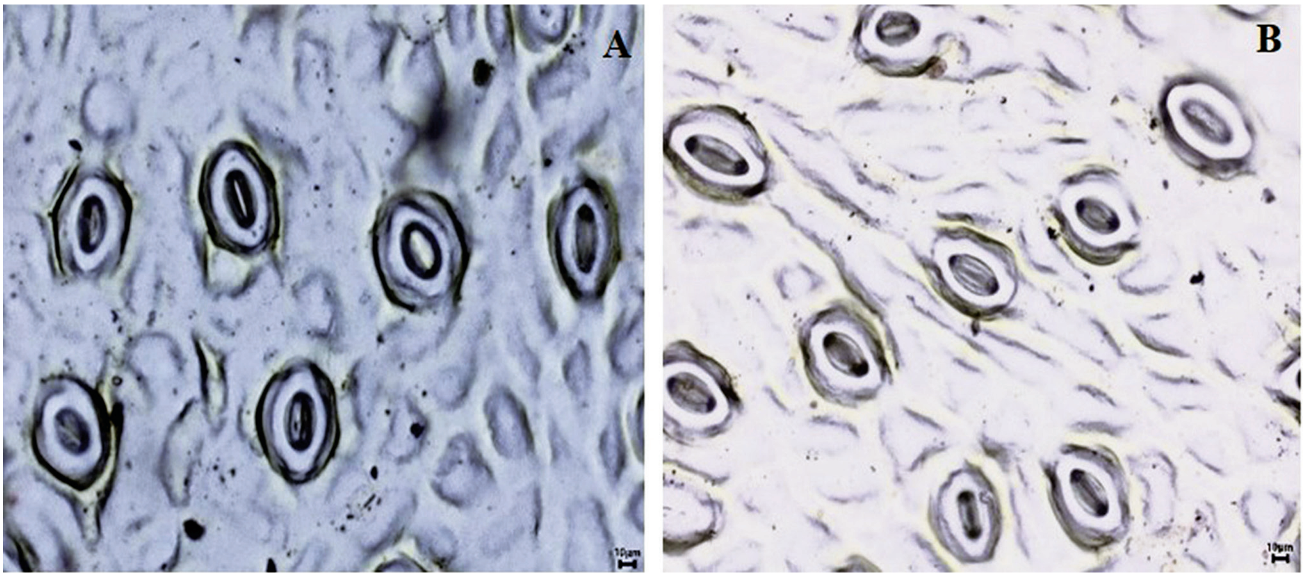


Fig. 2 Leaf stomata density of the 2nd generation colchiploids of sweet orange cv. Mosambi (A) and its wild type (B) at 40X magnification.

M-13 and M-16) in Mosambi were identified based on overall morphological and physiological (Supplementary Table 2 and 3) trait characterization. The identified colchi-mutants and solid tetraploids displaying genetic diversity represents their potentiality to be utilized as parents in future breeding programmes aimed to develop seedless/ triploid citrus cultivars.

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