J. Hortic. Sci.
Vol. 19(1), 2024

https://doi.org/10.24154/jhs.v19i1.2417

Original Research Paper

Morpho-physiological characterization of second generation colchiploids in sweet orange (*Citrus sinensis* (L.) Osbeck) cv. Mosambi

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ABSTRACT

Induction of tetraploidy in citrus is commonly meant for the development of triploid seedless cultivars as well as resistance against abiotic and biotic stresses. Three-year-old, 20 second-generation colchicine treated (0.05, 0.10, 0.15 and 0.20%) plants (colchiploids), established from the putative tetraploid branches of the first-generation colchiploids of sweet orange (*Citrus sinensis* (L.) Osbeck) cv. Mosambi vegetatively propagated on *Jatti khatti* rootstock, along with their wild (parent) type, were characterized based on morphological and physiological traits. Plant height and canopy volume were reduced, but stem girth, nodes per shoot and bark: wood increased in the majority of the second-generation colchiploids related to the wild type. Colchiploids also possessed improved flower characteristics in terms of length and width. The stomatal dimensions increased, but stomatal concentration reduced in all the colchiploids. Colchicine treatment also caused significant variations in leaf gas exchange parameters, including photosynthetic rate, intercellular CO_2 concentration, leaf net transpiration rates, stomatal conductance, and intrinsic water use efficiency in colchiploids affecting their photosynthetic activities. The solid tetraploids identified on the basis of morpho-physiological characterization can be used in future breeding programmes for the development of triploid seedless citrus cultivars or can be used for the mitigation of biotic and abiotic stresses.

Keywords:

INTRODUCTION

Citrus, the most delicious group of fruits, belongs to the family Rutaceae, and the sweet orange, mandarin, grapefruit, pummelo, lemon and acid lime are the commercially cultivated species. These fruits are widely cultivated in the sub-tropical and many tropical climates worldwide, but are mainly concentrated between 40° N and 40° S of the equator. Sweet orange has excellent potential source of vitamins ‘C’, ‘A’ and ‘B’, phytonutrients and sugars. Different cultivars of sweet orange are commercially cultivated across states in India, covering Andhra Pradesh, Assam, Gujarat, Himachal Pradesh, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttar Pradesh, Haryana, Punjab, and Karnataka. Among these cultivars, Mosambi is very prevalent in different Indian states particularly for the juice extraction. In 2022, sweet oranges were cultivated over an area of approximately 0.23 million hectares, yielding a production of 4.25 million tonnes (Anon., 2022). The commercial expansion of this cultivar is being hindered by its higher seed content and the seedlessness needs to be induced.

Ploidy manipulation is an important component in citrus breeding, directed for seedless fruit production and evolving biotic and abiotic stress tolerance in the newly bred varieties as the hyperploids have some inbuilt stress mitigating mechanisms. Therefore, the main objective for development of citrus varieties is to produce triploid seedless varieties and to develop varieties to mitigate abiotic and biotic stresses.

Several methods have been discovered to induce new tetraploids, including the selection of tetraploid nucellar seedlings and natural autotetraploid citrus genotypes from nursery seedbeds via flow cytometry. Autotetraploid citrus has also been produced by treating the budwood with some spindle fibre inhibiting chemicals like colchicine or oryzalin (Aleza et al., 2009). Most of the citrus colchiploids established from treating the propagative bud wood with colchicine were detected to be the cytochimeras, which are unstable and require dechimerization (Barrett & Hutchison, 1978). The chimeras can be detached following selection from vegetatively propagated axillary shoots (Aleza et al., 2009) and the citrus tetraploids were produced from the diploid-tetraploid cytochimeric citrus plants after dechimerization from the first-generation colchiploids.

Tetraploids have not yet been developed in the sweet orange cv. Mosambi in India and there is a necessity to induce them in this cultivar. The solid tetraploids can be identified in second generation colchicploids based on their easily identifiable morpho-physiological characteristics and by the possession of a greater number of distinct traits for hyperploidy, they can be considered as tetraploids. Therefore, the current investigation was carried out to identify solid tetraploids of Mosambi based on morpho-physiological characteristics.

MATERIALS AND METHODS

Plant material and treatments

In the current investigation, 20 second-generation colchiploids and one control (wild type/mother type) of sweet orange cv. Mosambi were selected and the second-generation population of colchiploids was developed from colchicine treated first generation colchiploids by vegetatively propagating the identified putative tetraploid branches on the rough lemon (*Citrus jambhiri* Lush.) rootstock seedlings. The colchiploids in the first generation were produced by treating the scion sticks from a single elite plant of Mosambi with varying colchicine concentrations (0.00, 0.05, 0.10, 0.15 and 0.20%). The second-generation colchiploids were planted at a spacing of 4 m x 3 m in a randomized block design with three replications and maintained at the experimental plot of Division of Fruits and Horticultural Technology's Main Orchard, located at 77° 12'E Longitudes, 28° 40'N latitude, and at an altitude of 228.6 m above mean sea level. The leaf, plant and fruit analysis were conducted in the Fruits and Horticultural Technology Genetics and Agronomy divisions, ICAR-IARI, New Delhi.

The plant growth was measured in terms of plant height (m), stem girth (mm) and canopy volume (m^3). Bark to wood ratio was determined by dividing bark weight by wood weight. The shoot inter-nodal length (cm) was recorded by counting the number of nodes on a 30 cm long stick. The length and width of the flower and stigma length were measured with Vernier callipers. The number of petals and stamens per flower was counted manually.

The stomata distribution was examined using epidermal imprints of the abaxial surface of mature, fully- grown leaves, following the method proposed by Sampson (1961). The stomata's density and size were measured using a digital fluorescence microscope equipped with an inbuilt camera [Leadz camera-GSS (UK)]. Stomatal density was recorded by counting the stomata number per unit area (mm^2) at 10x magnification, whereas, stomatal length and width were measured to the nearest micrometer division at 40x magnification. Gas exchange parameters, including internal CO_2 concentration (Ci), photosynthetic rate (A), stomatal conductance of water (gs), and transpiration (E), were analyzed *in vivo* using an infrared gas analyzer (IRGA) (LI-6200, LICOR Biosciences, Lincoln, NE, USA) and these observations were recorded between 9:00 a.m. and 11:00 a.m.

Statistical analysis of the data for plant growth parameters, *viz*., plant height, canopy volume, stem girth, and other morphological and physiological traits was analysed in completely randomized block design with three replications. Data were analysed using statistical analysis system software (SAS version 2).

RESULTS & DISCUSSION

The statistical analysis exhibited significant variations in vegetative growth characteristics *viz*., canopy volume (m^3), plant height (m), and stem girth (cm) in the population of colchiploids in contrast to their wild type (Table 1). Maximum plant height was significantly higher in the wild type (2.80 m) than in other induced colchiploids and the lowest plant height (1.45 m) was recorded in MO-12, which was 48% lower than the check. Canopy volume (m^3) was maximum in the colchiploid MO-1 (5.03 m^3) compared to the wild type (2.83 m^3). Similarly, stem diameter of the colchiploids was highest in MO-15 (23.57 cm). These growth parameters are significant morphological characters, as they directly contribute to the plant's photo-assimilate production and are the major factors responsible for the amplified plant biomass. The developed colchiploids revealed decreased plant height, while, the effects on canopy volume displayed a varied response following colchicine treatment. Similar observations were made in *Pinellia ternate*, *Paulonia tomentosa* and *Vitis* spp. (He et al., 2012; Sinski et al., 2014; Tang et al., 2010), where induced tetraploids exhibited changes in stem

Table 1 : Plant growth characteristics in the 2nd generation colchiploids of Mosambi

Colchiploid No.	Plant height (m)	Canopy volume (m^3)	Stem girth (cm)	No. of nodes/ shoot	Shoot internodal length (cm)	Bark/ wood ratio
Wild type	2.80±0.05	2.83±0.15	18.17±0.83	15.33±0.27	1.96±0.04	0.48±0.03
MO1	2.14±0.04	5.03±0.17	22.33±0.72	17.00±0.47	1.74±0.03	0.69±0.03
MO2	1.92±0.05	3.81±0.60	20.90±0.68	18.00±0.94	1.67±0.07	0.67±0.07
MO3	1.92±0.11	3.84±0.68	21.13±1.43	19.67±0.27	1.54±0.02	0.69±0.06
MO4	2.15±0.06	4.76±0.15	23.27±0.50	20.00±1.79	1.56±0.18	0.67±0.04
MO5	1.84±0.09	3.48±0.78	21.20±0.71	15.33±1.09	1.94±0.11	0.92±0.09
MO6	1.64±0.11	2.04±0.37	18.50±0.56	19.00±0.82	1.59±0.06	0.71±0.04
MO7	1.85±0.08	3.09±0.48	22.47±1.49	18.67±0.98	1.62±0.08	0.71±0.05
MO8	1.75±0.13	2.38±0.45	19.07±0.95	15.33±0.88	1.95±0.19	0.74±0.07
MO10	1.64±0.15	2.68±0.36	18.13±0.58	15.67±0.95	1.90±0.11	0.66±0.09
MO12	1.45±0.12	1.17±0.21	16.67±1.37	17.33±0.82	1.75±0.08	0.74±0.06
MO13	1.83±0.07	2.57±0.48	20.20±0.17	15.00±0.94	1.98±0.13	0.75±0.07
MO14	1.66±0.02	1.89±0.08	19.87±0.75	16.33±0.72	1.84±0.08	0.59±0.04
MO15	1.88±0.08	2.16±0.15	23.57±0.97	18.00±0.82	1.70±0.07	0.71±0.06
MO16	1.77±0.16	2.03±0.26	18.85±0.74	17.33±0.76	1.72±0.08	0.74±0.03
MO17	1.88±0.09	2.66±0.06	22.63±0.76	14.00±0.82	2.14±0.11	0.66±0.05
MO18	1.86±0.07	2.38±0.34	19.53±0.90	14.68±0.52	2.04±0.03	0.67±0.03
MO19	2.16±0.03	4.76±0.11	22.57±0.33	19.00±1.05	1.60±0.12	0.66±0.02
MO20	1.92±0.08	2.77±0.14	20.83±0.33	15.33±0.98	1.94±0.07	0.72±0.04
MO21	1.71±0.03	1.86±0.07	18.70±0.57	16.33±0.27	1.85±0.10	0.60±0.05
MO22	2.10±0.04	3.00±0.14	18.87±0.53	17.33±0.42	1.71±0.04	0.69±0.03
SEm±	0.10	0.43	1.26	1.28	0.12	0.075
LSD at 5%	0.28	1.25	3.60	3.66	0.34	0.21
CV (%)	10.71	25.99	10.71	13.14	11.59	18.75

characteristics and reduced plant height. Reduced tree height and canopy spread in tetraploids were also conveyed by Barrett & Hutchison (1978) in citrus. Colchicine, a toxic chemical, interferes with microtubule formation during cell division, averting the normal separation of chromosomes. The reduced plant height observed in colchicine-treated plants might be due to this interference on cell division. The disruption of normal cell division processes could lead to a reduction in the elongation of plant cells, resulting in shorter plants (Ren et al., 2018). An increase in the volume of individual cells might cause an increased canopy volume and stem girth. Polyploid cells, having more genetic material, tend to be larger than their diploid counterparts. This augmenting cell size could lead to thicker stems and broader leaves, contributing to a larger canopy volume.

The data presented in Table 1 highlight variations in the number of nodes per shoot, shoot inter-nodal length, and bark-to-wood ratio among all the colchiploids. The maximum number of nodes per shoot (30 cm long) was recorded in MO-4 (20.00), which also revealed the shortest shoot internodal length (1.50 cm). Although, MO-17 recorded longest shoot internodal length (2.14 cm), this was not statistically dissimilar from the wild type. The bark-to-wood ratio was highest in MO-5 (0.92), significantly differing from the wild type (0.48). This ratio improved in all colchiploids, indicating a consistent effect of the colchicine treatment. These results are in harmony with the findings of Blasco et al. (2015), who recorded that the colchicine induced tetraploids exhibited a higher number of internodes and shorter internodal distances than their wild type. Ari et al. (2015)

Table 2 : Flower characteristics in 2nd generation colchiploids of Mosambi

Colchiploid No.	Flower length (mm)	Flower width (mm)	Stigma length (mm)	No. of petals	No. of stamens
Wild type	15.15±0.06	6.89±0.07	6.46±0.05	5.00	19.73
MO1	17.65±0.15	7.93±0.08	8.33±0.02	4.98	20.31
MO2	16.57±0.13	7.39±0.16	7.85±0.18	4.95	20.78
MO3	17.07±0.30	7.48±0.11	7.87±0.39	5.02	20.91
MO4	16.88±0.06	7.10±0.08	6.99±0.34	4.93	20.51
MO5	17.49±0.27	7.81±0.09	7.60±0.33	4.87	21.11
MO6	15.21±0.52	7.34±0.11	6.43±0.35	4.94	21.22
MO7	16.66±0.16	7.21±0.01	7.24±0.22	5.00	20.84
MO8	17.10±0.16	7.36±0.09	6.84±0.16	4.98	20.93
MO10	16.53±0.19	7.05±0.12	7.14±0.22	4.98	20.94
MO12	16.45±0.35	7.25±0.12	7.62±0.39	4.98	20.80
MO13	15.89±0.34	7.05±0.11	6.42±0.77	4.99	21.01
MO14	15.76±0.45	6.91±0.04	6.73±0.67	5.00	21.08
MO15	16.20±0.33	7.04±0.12	6.72±0.12	5.00	21.20
MO16	16.02±0.39	7.03±0.19	7.35±0.21	4.67	21.11
MO17	16.36±0.28	8.02±0.16	7.11±0.23	5.00	21.88
MO18	15.46±0.14	7.14±0.06	7.43±0.09	4.95	20.73
MO19	17.06±0.24	7.15±0.09	7.20±0.05	5.00	21.57
MO20	16.32±0.22	7.09±0.14	6.78±0.14	4.67	20.30
MO21	17.45±0.04	7.67±0.08	7.49±0.25	5.00	20.56
MO22	16.54±0.09	7.20±0.12	7.22±0.12	4.67	21.00
SEm±	0.33	0.13	0.38	-	-
LSD at 5%	0.96	0.37	1.10	NS	NS
CV (%)	3.52	3.09	9.34	-	-

suggested that colchicine might have operated as a mutagen and might have induced wide variations noticed in the studied parameters. However, the consistent results across all colchiploids may not solely be attributable to mutations. Instead, polyploidy can be accountable for influencing hormonal imbalance in plants. Altered levels of phytohormones, with augmented cytokinin and decreased auxins may be a key factor, which necessitates further studies (Tossi et al., 2022; Fakhrzad et al., 2023).

Some of the floral characters, *viz*., flower length, width, and stigma length (Table 2) also displayed significant differences in the colchiploids. The longest flower and stigma were observed in MO-1, measuring 17.65 and 8.33 mm, respectively. The widest flower was found in MO-17, (8.02 mm). These findings align with previous studies conducted on gladiolus, chrysanthemum, and Ponkan mandarin (Kushwah et al., 2018; Manzoor et al., 2018; Tan et al., 2019). However, no significant changes were observed in the number of petals and stamens.

Microscopic imprints of the abaxial leaf surface of colchiploids, disclosed significant variation in stomatal characteristics, including density, length, width, and length-to-width ratio (Table 3 and Fig. 1). The average number of stomata per mm^2 leaf area was significantly reduced in all the colchiploids. The lowest value for stomatal density was recorded in MO-18 (373.33). The longest stomata were recorded in MO-1 (19.43 µm), while, the shortest length was observed in the wild type (14.62 µm). The widest stomata were recorded in MO-22 (15.10 µm), whereas, the narrowest width was noticed in the wild type (9.81 µm). The highest stomata length-to-width ratio

Table 3 : Stomatal characteristics in 2nd generation colchiploids of Mosambi

Colchiploid No.	No. of stomata/ (mm^2)	Stomatal length (µm)	Stomatal width (µm)	Stomata length: width ratio
Wild type	588.00±11.78	14.62±0.40	9.81±0.12	1.49±0.04
MO1	462.00±4.32	19.43±0.23	14.81±0.63	1.32±0.05
MO2	482.67±4.06	17.16±0.69	13.87±0.72	1.25±0.05
MO3	503.00±3.86	16.66±0.40	11.82±0.07	1.41±0.04
MO4	539.67±6.67	16.28±0.53	12.24±0.39	1.33±0.06
MO5	483.67±3.54	17.69±0.21	12.71±0.29	1.39±0.05
MO6	510.00±5.25	17.03±0.25	11.47±0.22	1.49±0.01
MO7	391.67±1.78	17.79±0.30	12.31±0.13	1.45±0.03
MO8	451.00±2.36	18.58±0.33	12.18±0.72	1.54±0.09
MO10	467.00±2.16	16.57±0.35	10.92±0.20	1.52±0.04
MO12	504.00±6.18	16.81±0.40	11.89±0.38	1.42±0.01
MO13	405.33±2.88	18.18±0.24	12.71±0.37	1.44±0.06
MO14	417.33±2.18	18.10±0.26	13.00±0.38	1.40±0.05
MO15	471.00±4.99	18.24±0.52	12.77±0.16	1.43±0.03
MO16	490.67±2.88	19.02±0.44	12.73±0.70	1.50±0.06
MO17	489.33±2.78	18.09±0.11	13.23±0.41	1.37±0.05
MO18	373.33±3.18	18.23±0.64	11.95±0.20	1.52±0.03
MO19	441.33±2.88	18.36±0.32	12.70±0.54	1.45±0.05
MO20	424.00±1.89	17.18±0.26	11.64±0.23	1.48±0.02
MO21	452.00±1.65	17.93±0.32	12.56±0.38	1.43±0.02
MO22	470.67±4.75	19.38±0.68	15.10±0.34	1.29±0.07
SEm±	5.22	0.50	0.56	0.058
LSD at 5%	15.00	1.45	1.62	0.16
CV (%)	1.93	4.96	7.81	7.1

was recorded in MO-8 (1.54) and the lowest length-to-width ratio was recorded in MO-2 (1.25), which was statistically lower than the wild type (1.49). Based on these parameters, it was inferred that the colchicine significantly affected stomatal density, length, width, and length-to-width ratio. All other stomatal features were generally higher in the colchiploids except for stomatal density. The decreased stomatal density and increased dimensions might be due to induced mutation occurrences or ploidy level changes. These findings align with earlier studies (Blasco et al., 2015; Bhuvaneswari et al., 2020; Suliman et al., 2020; Usman et al., 2021; Kashtwari et al. 2022; Bora et al., 2023) conducted on loquat, lemon, citrange, black locust, grapefruit, saffron and acid lime.

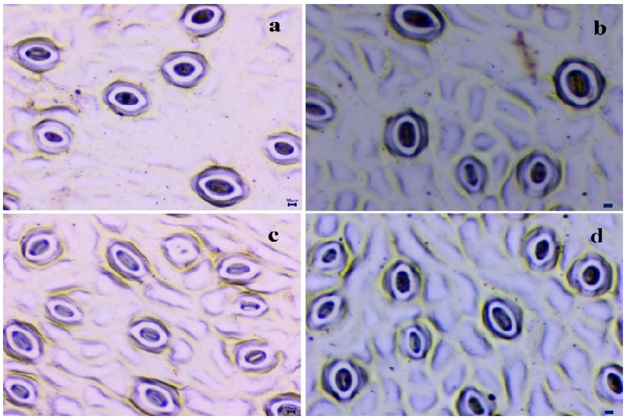

Fig. 1 : Leaf stomata density of second generation Mosambi colchiploids (a and b) and their wild types (c and d)

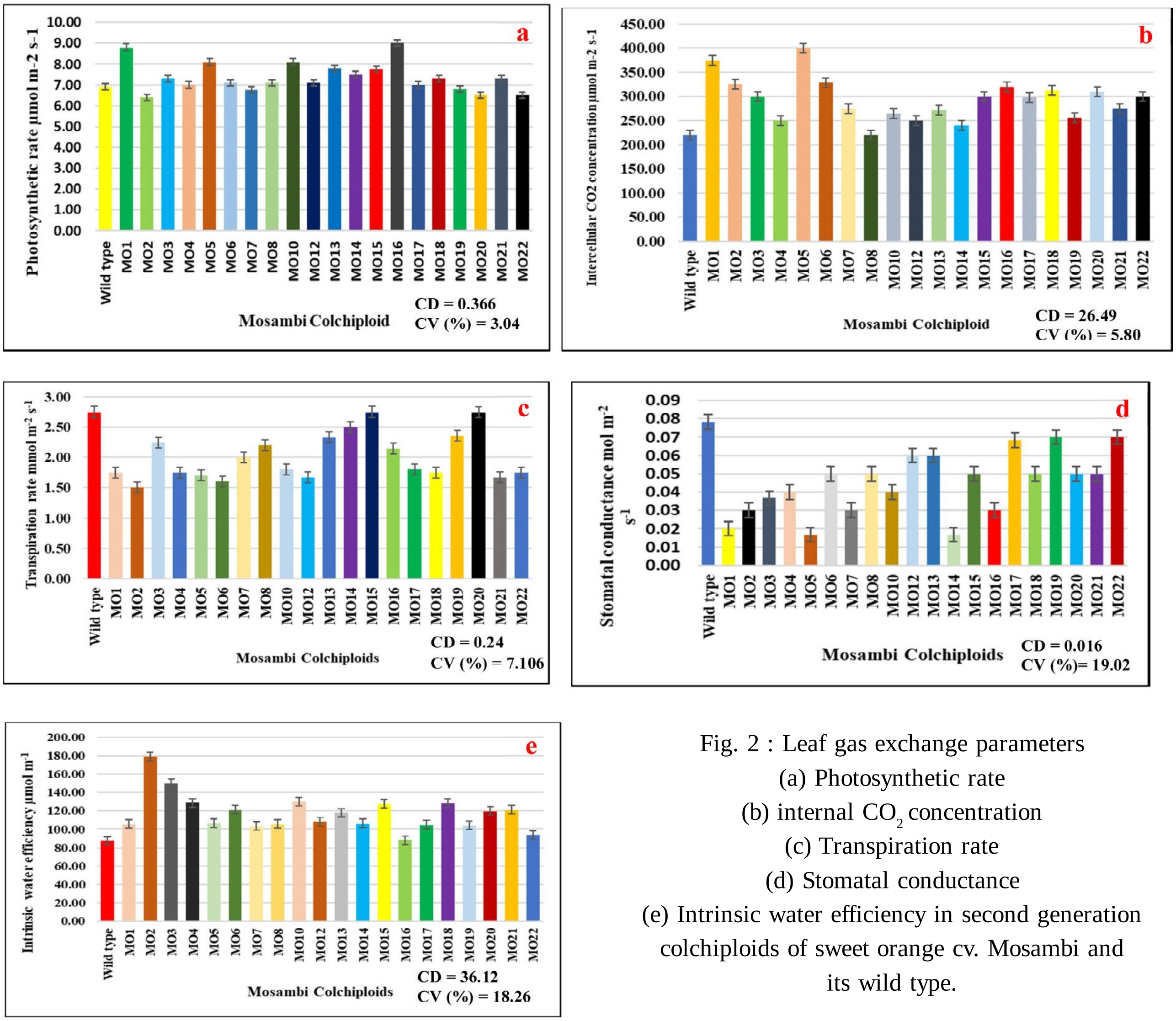

Fig. 2 : Leaf gas exchange parameters
(a) Photosynthetic rate
(b) internal CO_2 concentration
(c) Transpiration rate
(d) Stomatal conductance
(e) Intrinsic water efficiency in second generation colchiploids of sweet orange cv. Mosambi and its wild type.

The data presented in Fig. 2 showed variations in leaf gas exchange parameters in the leaves of Mosambi colchiploids compared to their control. The photosynthetic rate (A) was highest in MO-1 (8.77 µmol m^{-2} s^{-1}), higher than the wild type (7.00 µmol m^{-2} s^{-1}), while, the lowest value was noticed in MO-2 (6.20 µmol m^{-2} s^{-1}).

Differences were also noticed for the intercellular CO_2 concentration, with the highest value recorded in MO-5 (400 µmol m^{-2} s^{-1}) and the lowest value was recorded in the wild type and MO-8 (220 µmol m^{-2} s^{-1}). The average leaf net transpiration rates were also significantly affected under different colchicine treatments. The highest transpiration (2.75 mmol m^{-2} s^{-1}) was found in the wild type, which was higher than all colchiploids but MO-20 and MO-15. The lowest transpiration (1.50 mmol m^{-2} s^{-1}) was registered in MO-2. The maximum value for stomatal conductance was recorded in MO-16 (0.097 mol m^{-2} s^{-1}), which was *at par* with MO-1, but significantly higher than the wild type (0.080 mol m^{-2} s^{-1}). The minimum value was recorded in MO-2 (0.037 mol m^{-2} s^{-1}). For intrinsic water use efficiency, the maximum value was recorded in MO-2 (179.11 µmol m^{-1}), higher than the wild type (87.50 µmol m^{-1}). The colchicine treatment led to significant alterations in leaf gas exchange parameters. There was less variation observed in the photosynthetic rate among the Mosambi colchiploids compared to the wild type. However, an increase in photosynthetic rate was noticed in a few colchiploids. The internal CO_2 concentration augmented in all the Mosambi colchiploids. The increase in stomatal size and cell size, which accumulated more CO_2 because of stomatal closure and non-stomatal restriction compared to their respective wild types might be the reason for the alteration in the transpiration rate and the variation in stomatal conductance in colchiploids. These observations align with Nobel (1999), who noticed that stomatal conductance affected the transpiration rate. Ainsworth et al. (2004) also reported that an increased leaf area provides an increased surface area for gaseous exchange. Abdolinejad et al. (2021) reported that the tetraploid figs had a 57.89 per cent elevation for the photosynthetic rate compared to the wild type.

CONCLUSIO\N

Induced colchiploids of sweet orange cv. Mosambi were found to reveal more significant phenotypic variations in comparison to their wild type. The tetraploids exhibited changes in morphological characteristics such as canopy volume, plant height, stem girth, nodes per shoot, shoot inter-nodal length, bark-to-wood ratio, and variations in floral characteristics such as flower length and width and stigma length. Tetraploids exhibited significant physiological disparities in stomatal density and stomatal size. The photosynthetic rate was increased, leading to good growth of the tetraploids. It can be concluded that the morphological and physiological characteristics are useful in screening induced second-generation colchiploids, dissociating chimaeras and preliminary selection of putative tetraploids in Mosambi, mainly when dealing with large populations. The identified colchi-mutants and solid tetraploids displaying genetic diversity represented their potentiality to be utilized as parents in future breeding programmes aimed to develop seedless/ triploid citrus cultivars or to combat biotic and abiotic stress that can be utilized directly as new cultivars.

REFERENCES

Abdolinejad, R., Shekafandeh, A., & Jowkar, A. (2021). *In vitro* tetraploidy induction creates enhancements in morphological, physiological and phytochemical characteristics in the fig tree (*Ficus carica*). *Plant Physiology and Biochemistry*, *166*, 192-202. doi: 10.1016/j.plaphy.2021.05.047

Ainsworth, E. A., Rogers, A., Nelson, R., & Long, S. P. (2004). Testing the “source—sink” hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in *Glycine max*. *Agricultural and Forest Meteorology*, *122* (1-2), 85-94.

Aleza, P., Juarez, J., Cuenca, J., Ollitrault, P., & Navarro, L. (2009). Extensive citrus triploid hybrid production by 2x times 4x sexual hybridizations and parent-effect on the length of the juvenile phase. *Plant Cell Reports*, *31*(9), 1723-1735. doi: 10.1007/s00299-012-1286-0

Anonymous (2022). Indian Horticulture Databases. Published by National Horticulture Board.

Ari, E., Djapo, H., Mutlu, N., Gurbuz, E., & Karaguzel, O. (2015). Creation of variation through gamma irradiation and polyploidization in *Vitex agnuscastus* L. *Scientia Horticulturae*, *195*, 7-81. https://doi.org/10.1016/j.scienta.2015.08.039

Barrett, H.C., & Hutchison, D.J. (1978). Spontaneous tetraploidy in apomictic seedlings of Citrus. *Economic Botany*, *32*(1), 27-45.

Bhuvaneswari, G., Thirugnanasampandan, R., & Gogulramnath, M., (2020). Effect of colchicine induced tetraploidy on morphology, cytology, essential oil composition, gene expression and antioxidant activity of *Citrus limon* (L.) Osbeck. *Physiology and Molecular Biology of Plants*, *26*(2), 271-279. doi: 10.1007/s12298-019-00718-9.

Blasco, M., Badenes, M. L., & Naval, M. (2015). Colchicine-induced polyploidy in loquat (*Eriobotrya japonica*). *Plant Cell, Tissue and Organ Culture*, *120*(2), 453-461. https://doi.org/10.1007/s11240-014-0612-3

Bora, L., Vijayakumar, R. M., Ganga, M., Meenakshi, N., Sarkar, M., & Kundu, M. (2023). Induction of polyploids in acid lime (*Citrus aurantifolia*) through colchicine treatment of *in vitro* derived shoot tip explants. *Plant Breeding*, *142*(1), 118-127. https://doi.org/10.1111/pbr.13068

Fakhrzad, F., & Jowkar, A. (2023). Water stress and increased ploidy level enhance antioxidant enzymes, phytohormones, phytochemicals and polyphenol accumulation of tetraploid induced wallflower. *Industrial Crops and Products*, *206*, 117612. https://doi.org/10.1016/j.indcrop.2023.117612

He, L., Ding, Z., Jiang, F., Jin, B., Li, W., Ding, X., Sun, J., & Lv, G. (2012). Induction and identification of hexadecaploid of *Pinellia* *ternate*. *Euphytica*, *186*(2), 479-488. doi: 10.1007/s10681-012-0642-z

Kashtwari, M., Jan, S., Wani, A. A., & Dhar, M. K. (2022). Induction of polyploidy in saffron (*Crocus sativus* L.) using colchicine. *Journal of Crop Improvement*, *36*(4), 555-581. doi: 10. 1080/15427528.2021.1994502

Kushwah, K. S., Verma, R. C., Patel, S., & Jain, N. K. (2018). Colchicine induced polyploidy in *Chrysanthemum carinatum* L. *Journal of Phylogenetics and Evolutionary Biology*, *6*(193), 2. doi: 10.4172/2329-9002.1000193

Manzoor, A., Ahmad, T., Bashir, M. A., Baig, M. Q., Quresh, A. A., Shah, M. K. N., & Hafiz, I. A. (2018). Induction and identification of colchicine induced polyploidy in *Gladiolus grandiflorus* 'White Prosperity'. *Folia Horticulturae*, *30*(2), 307-319. doi: 10.2478/ fhort-2018-0026

Nobel, P. S. (1999). *Physicochemical and Environmental Plant Physiology* 2nd ed, Academic Press, San Diego, CA. https:// doi.org/10.1046/j.1365-2664.1999.00459-5.x

Ren, J. I. A. N., Wu, X., Song, C., Liang, Y., Gao, W., and Wang, Y. (2018). Induction of polyploid tillered onion using colchicine and pendimethalin. *Sains Malaysiana*, *47*(11), 2617-2624. http://dx.doi.org/10.17576/jsm-2018-4711-04

Sampson, J. (1961). A method of replicating dry or moist surfaces for examination by light microscopy. *Nature*, *191*(4791), 932-933. doi: 10.1038/191932a0.

Sinski, I., Dal Bosco, D., Pierozzi, N. I., Maia, J. D. G., Ritschel, P. S., & Quecini, V. (2014). Improving *in vitro* induction of autopolyploidy in grapevine seedless cultivars. *Euphytica*, *196*(2), 299-311. doi: 10.1007/s10681-013-1034-8

Suliman, H. H., & Asander, H. S. (2020). Polyploidy induction by colchicine in *Robinia pseudoacacia* L. and its effect on morphological, physiological and anatomical seedling traits. *The Iraqi Journal of Agricultural Science*, *51*(3), 829-847.

Tan, F. Q., Zhang, M., Xie, K. D., Fan, Y. J., Song, X., Wang, R., Wu, X. M., Zhang, H. Y., & Guo, W. W. (2019). Polyploidy remodels fruit metabolism by modifying carbon source utilization and metabolic flux in Ponkan mandarin (*Citrus reticulata*). *Plant Science*, *289*, 110276.

Tang, Z. Q., Chen, D. L., Song, Z. J., He, Y. C., & Cai, D. T. (2010). *In vitro* induction and identification of tetraploid plants of *Paulownia tomentosa*. *Plant Cell, Tissue and Organ Culture*, *102*(2), 213-220. doi: 10.1007/s11240-010-9724-6

Tossi, V. E., Martinez, T. L. J., Laino, L. E., Iannicelli, J., Regalado, J. J., Escandon, A. S., & Pitta, A. S. I. (2022). Impact of polyploidy on plant tolerance to abiotic and biotic stresses. *Frontiers in Plant Science*, *13*, 869423. https:/ /doi.org/10.3389/fpls.2022. 869423

Usman, M., Fatima, B., & Rana, I. A. (2021). Morphological and stomatal diversity in colchiploid germplasm of grapefruit. *Pakistan Journal of Agriculture Science*, *58*(2), 555-560. doi: 10.21162/pakjas/21.1499

(Received :06.01.2024; Revised :28.02.2024; Accepted :02.03.2024)