# Morphological characterization of newly developed orangelo (*Citrus* spp.) hybrids

RAUSHAN KUMAR<sup>1</sup>, NIMISHA SHARMA<sup>1</sup>\*, A K DUBEY<sup>1</sup>, R M SHARMA<sup>1</sup>, HATKARI VITTAL<sup>1</sup>, SANDEEP<sup>1</sup>, GYAN P MISHRA<sup>1</sup>, SHRUTI SETHI<sup>1</sup>, MUKESH SHIVRAN<sup>1</sup> and DEEPAK<sup>1</sup>

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 21 September 2023; Accepted: 28 November 2023

#### **ABSTRACT**

Present study was carried out during 2021–23 (*Ambe* and *Mrig bahar* season) at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi to analyse the morphological diversity of 24 orangelo (*Citrus* spp.) hybrids and their 4 parents based on 17 quantitative traits such as leaves, flowers, fruits and seed characters. Cluster analysis based on 17 morphological traits divided 28 genotypes of citrus into two main groups. A higher range of variation with average Euclidean distance coefficients ranging from 0.02–0.26 was observed between groups as well as clusters, thereby indicating that genotypes within and among clusters were highly diverse. The PCA for morphological quantitative traits showed that five components leaf lamina length (LLL); leaf lamina width (LLW); LLL: LLW; leaf lamina thickness and pedicel length, of the 17 principal component axis had Eigen-values>1 and all together accounted for over 77.76% of the total variability. Analysing the genetic relationships among the hybrids yielded important insights for potential use in future breeding programmes as valuable sources of desirable traits.

**Keywords**: Diversity, Morphological, Orangelo, Quantitative variation

Citrus (Citrus spp.) is one of the world's most important fruit crops. It is widely grown in the tropical, subtropical and borderline subtropical areas of the world. The genus Citrus belongs to the order of the Geraniales, family Rutaceae and subfamily Aurantioideae (Swingle et al. 1967). It has a haploid genome of about 367 Mb and 2n = 18 chromosomes (Inglese et al. 2019). Citrus fruits are very rich in vitamin C. In terms of global citrus fruit output in 2023, China is the leading country with a production of 46.7 million tons, accounting for around 28.77% of global citrus fruit production (USDA, FAS 2022–23). Furthermore, the other five nations, which account for 59.13% of world output, are Brazil, India, Mexico, and the United States of America. Annually, India produces 14,245 thousand metric tons (MT) of Citrus (Mandarin, acid lime/lemon, sweet orange, and other citrus) from an area of 1097 thousand ha with a productivity level of 12.98 MT/ha (Anonymous 2020-21). Morphological characteristics have served as primary attributes for identifying and defining plant categories. Vegetative characteristics, leaves, flowers and fruits have been characterized to differentiate between citrus varieties and their hybrids. The first and most significant

<sup>1</sup>ICAR-Indian Agricultural Research Institute, New Delhi. \*Corresponding author email: nims17sharma@gmail.com

step in the description and characterization of hybrids is the morphological characterization of trees and fruits (Devi *et al.* 2021). Assessment of genetic variability and identification of superior parents among a genetically diverse population is the preliminary objective of any crop improvement programme. The present investigation was carried out with the objective of characterization using different parameters of orangelo hybrids based on DUS guidelines.

### MATERIALS AND METHODS

The experiments were conducted during 2021-23 (Ambe and Mrig bahar season) at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi. A total of 24 interspecific orangelo hybrids and 4 parents were used in the present study (Table 1). A total of 17 quantitative morphological traits were recorded by using standard methods as per citrus descriptors set out by UPOV and IPGRI (1999). Samples were collected each season from the field, the fully developed leaves, flowers, fruits, and seeds were cleaned from tissue papers and quantitative traits were recorded as per descriptors. The experiment was laid out in a randomized block design (RBD). There were 5 replications with one tree in each replication. The data were subjected to statistical analysis of variance (ANOVA) using SAS 9.3 version software (SAS, USA INC) and significant differences were compared followed by DMRT at  $P \le 0.05$ . Principal component analysis and Pearson's correlation

Table 1 The details of orangelo hybrids including parents used in the present study

Hybrid	Parent	Year of
CCCII 2 10	n in i	planting
SCSH 3-10	Red Pummelo × sweet orange cv.	2016
SCSH 3-14	Mosambi	2015
SCSH 3-15		2016
SCSH 5-5		2016
SCSH 9-2		2012
SCSH 9-20		2016
SCSH 13-13		2016
CRH 20-11		2015
SCSH 13-14		2016
SCSH 11-15	White Pummelo ×	2012
SCSH 11-19	sweet orange cv.	2012
SCSH 13-4	Mosambi	2013
SCSH 7-12		2013
SCSH 9-10		2012
SCSH 13-17		2012
SCSH 15-2		2012
SCSH 15-3		2014
SCSH 15-18		2014
SCSH 15-19		2012
SCSH 17-9		2014
SCSH 19-2		2012
SCSH 19-6		2014
SCSH 19-8		2014
SCSH 21-10		2012
Parentage		
Sweet orange cv. Mosambi	3	2003
PS-2 (Pummelo red)	2	2003
PS-5 (Pummelo white)	2	2003
PS-10 (Pummelo white)	2	2003

were performed using R Studio (R Studio, PBC, Version 2020.07.1–554). The similarity between individual pairs of genotypes was analyzed by using the NTSYS pc 2.1 Software (Rohlf *et al.* 2000).

## RESULTS AND DISCUSSION

Results pertaining to quantitative parameters suggested in DUS guidelines for the conduct of test for distinctiveness, uniformity, and stability on citrus descriptors, UPOV/IPGRI. Orangelo hybrids along with their parentage indicated the existence of wide morphological variability between the genotypes. Leaf length (cm), leaf width (cm), length:width ratio, leaf thickness (mm), and pedicel length (mm) presented higher variation (P<0.05) in the studied genotypes. Our findings suggested 17 hybrids possessed medium (8–10 cm) and very few hybrids such as SCSH 13-14, SCSH 15-18, SCSH 19-2 and CRH 20-11 expressed

short lamina length (<8 cm). Among parents, only PS-10 (Pummelo white) showed long leaf length and the rest parents expressed medium length. Leaf lamina width was also measured broad (>6 cm) in SCSH 3-10, SCSH 3-15, SCSH 5-5 hybrids and medium (5-6 cm) in 10 hybrids and narrow (<5 cm) in 11 hybrids. Only PS-10 (Pummelo white) parent had broad lamina width and PS-2 (Pummelo red) found narrow leaf lamina width. Our key findings suggested that the ratio of leaf lamina length to width data clearly showed a considerable difference between hybrids and their parents. All hybrids have a leaf lamina length to width ratio between 1.48-2.05, being lowest in SCSH 3-14 and highest in SCSH 11-15. Among parents, PS-2 (Pummelo red) had a ratio that was considerably higher than the other three parents. Similar to leaf lamina length, hybrids relative to the parents showed both lower and greater values of the leaf lamina length and width ratio. Leaf lamina thickness varied from 0.22 mm to 0.35 mm in hybrid SCSH 21-10 exhibited thickest and SCSH 3-14 measured thinnest. White pummelo parents such as PS-5 (Pummelo white) and PS-10 (Pummelo white) had much higher leaf lamina thickness when compared to the other two parents (Table 2 and Fig. 1). It is noteworthy to mention that hybrids exhibited higher and lower values of leaf lamina length than parents. Leaf shapes can differ due to both genetic and environmental factors. The combination of genetic interactions, changes in gene expression patterns, involvement of micro-RNAs, and hormonal regulation contribute to the development and various forms of leaves. Similarly, environmental conditions during a plant's growth, including factors like nutrient availability, water, and light, influence variations in leaf color (Chan et al. 2022). Similar variations in leaf traits have been documented in other studies (Gaikwad et al. 2018, Dubey et al. 2021, Kandowangko et al. 2023). These findings collectively underscore the diverse nature of leaf characteristics in citrus trees. Significant variation was observed in the flower parameters among hybrids and parents. Hybrids like SCSH 3-10, SCSH 3-15, SCSH 13-4, and all female parent recorded long (>15 mm) pedicel length, medium (10–15 mm) in SCSH 5-5, SCSH 7-12, SCSH 9-10, SCSH 15-3, SCSH 15-19, SCSH17-9, SCSH 19-6, SCSH 19-8, CRH 20-11 hybrids and male parent Mosambi and shorter (<10 mm) in 12 hybrids. Most of the hybrids and all female parents recorded long (>20 mm) petal length, and medium (15-20 mm) in SCSH 3-14, SCSH 11-19, SCSH 13-14, SCSH 15-3, SCSH 15-18, SCSH 19-6 hybrids and male parent Mosambi and shorter (<15 mm) in SCSH 9-2, SCSH 15-2. Data on petal width showed a considerable variation between the hybrids and their parents. Additionally, the petal width of the PS-10 (Pummelo white) had a much higher petal width than the other three parents.

The data (Table 3), indicated great variation with regard to fruit characters among the hybrids and parents. The larger fruit weight (>500 g) was recorded in hybrids SCSH 3-10, SCSH 5-5, SCSH 9-10, SCSH 15-3, PS-5 (Pummelo white). The larger (>100 mm) fruit length was measured in SCHS 3-10, SCHS 3-14, SCHS 5-5, SCHS7-12, SCHS 9-2,

Table 2 Leaf and flower parameters of orangelo hybrids (Pummelo × Sweet orange) and their parents

Hybrid	Leaf lamina length (cm)	Leaf lamina width (cm)	Leaf lamina length/ width	Leaf lamina thickness (mm)	Pedicel length (mm)	Petal length (mm)	Petal width (mm)	Diameter of open flower (mm)
SCSH 3-10	10.58 <sup>b</sup>	6.44 <sup>a</sup>	1.64 <sup>ghi</sup>	0.32 <sup>cd</sup>	17.50 <sup>d</sup>	26.90 <sup>a</sup>	9.20°	57.40 <sup>a</sup>
SCSH 3-14	8.22ghi	5.54 <sup>e</sup>	1.48 <sup>j</sup>	$0.22^{1}$	6.90 <sup>no</sup>	$18.40^{j}$	8.50 <sup>cde</sup>	$41.40^{j}$
SCSH 3-15	9.46 <sup>d</sup>	6.18 <sup>abc</sup>	1.53 <sup>ij</sup>	0.26hij	17.00 <sup>d</sup>	21.20 <sup>hi</sup>	10.50 <sup>b</sup>	$40.20^{j}$
SCSH 5-5	9.28 <sup>def</sup>	6.06 <sup>bcd</sup>	1.53 <sup>ij</sup>	0.30 <sup>de</sup>	14.70 <sup>ef</sup>	21.60ghi	8.50 <sup>cde</sup>	$41.60^{ij}$
SCSH 7-12	9.10 <sup>def</sup>	$4.90^{ij}$	1.86 <sup>cde</sup>	$0.28^{efgh}$	14.60 <sup>ef</sup>	25.20 <sup>b</sup>	8.50 <sup>cde</sup>	51.90 <sup>cd</sup>
SCSH 9-2	8.32ghi	$4.82^{ijk}$	1.74 <sup>efg</sup>	$0.23^{kl}$	7.20 <sup>no</sup>	13.20 <sup>n</sup>	$7.30^{ghij}$	23.00°
SCSH 9-10	8.40gh	5.48 <sup>e</sup>	1.53 <sup>ij</sup>	$0.27^{fghi} \\$	$10.10^{ijk}$	22.70 <sup>efg</sup>	9.20°	47.80 <sup>ef</sup>
SCSH 9-20	9.58 <sup>d</sup>	5.86 <sup>d</sup>	1.63 <sup>ghi</sup>	$0.26^{hijk}$	6.80°	25.20 <sup>b</sup>	13.20 <sup>a</sup>	52.60 <sup>bc</sup>
SCSH 11-15	$8.74^{\rm efg}$	4.26 <sup>m</sup>	2.05a	$0.24^{jkl}$	8.76 <sup>klm</sup>	22.70 <sup>efg</sup>	12.90 <sup>a</sup>	43.90 <sup>hi</sup>
SCSH 11-19	$8.72^{efg}$	$4.54^{1}$	1.92abcd	$0.24^{jkl}$	9.80 <sup>ijk</sup>	15.90 <sup>lm</sup>	$6.90^{ij}$	31.20 <sup>n</sup>
SCSH 13-4	10.16 <sup>c</sup>	5.08ghi	2.00abc	$0.28^{efg}$	15.40 <sup>e</sup>	$22.20^{efgh} \\$	10.50 <sup>b</sup>	45.60 <sup>fgh</sup>
SCSH 13-13	9.16 <sup>ed</sup>	5.38ef	$1.70^{fgh}$	0.29ef	$9.40^{ijkl}$	21.40ghi	$6.50^{j}$	$40.20^{j}$
SCSH 13-14	6.26 <sup>k</sup>	3.46 <sup>n</sup>	1.81 <sup>def</sup>	$0.24^{jkl}$	9.90 <sup>ijk</sup>	16.40 <sup>lm</sup>	7.70 <sup>efghi</sup>	$36.90^{l}$
SCSH 13-17	8.12hi	$4.70^{jkl}$	1.73 <sup>efg</sup>	$0.27^{fghi} \\$	8.20 <sup>lmn</sup>	21.20 <sup>hi</sup>	11.30 <sup>b</sup>	44.40gh
SCSH 15-2	$8.72^{efg}$	5.02 <sup>ghi</sup>	1.74 <sup>efg</sup>	0.32 <sup>cd</sup>	7.66 <sup>mno</sup>	13.52 <sup>n</sup>	8.44 <sup>cdef</sup>	34.10 <sup>m</sup>
SCSH 15-3	9.56 <sup>d</sup>	$4.68^{jkl}$	2.04 <sup>ab</sup>	$0.25^{hijk}$	13.10 <sup>g</sup>	$17.00^{kl}$	$7.10^{hij}$	$40.60^{j}$
SCSH 15-18	7.24 <sup>j</sup>	4.20 <sup>m</sup>	1.73 <sup>efg</sup>	$0.24^{jkl}$	$9.30^{jkl}$	$18.00^{jk}$	$8.13^{defg}$	37.42 <sup>kl</sup>
SCSH 15-19	9.46 <sup>d</sup>	5.06 <sup>ghi</sup>	1.89 <sup>cd</sup>	$0.23^{kl}$	11.50 <sup>h</sup>	24.20 <sup>bcd</sup>	$8.00^{efgh}$	50.20 <sup>cde</sup>
SCSH 17-9	$8.62^{fgh}$	5.02 <sup>ghi</sup>	$1.72^{fgh}$	$0.22^{1}$	11.50 <sup>h</sup>	$23.00^{def}$	10.40 <sup>b</sup>	50.60 <sup>cd</sup>
SCSH 19-2	6.52 <sup>k</sup>	3.64 <sup>n</sup>	1.80 <sup>def</sup>	$0.25^{hijk}$	9.60 <sup>ijk</sup>	$20.60^{i}$	9.00 <sup>cd</sup>	44.40gh
SCSH 19-6	9.46 <sup>d</sup>	$4.64^{jkl}$	2.04 <sup>ab</sup>	$0.25^{jkl}$	10.70 <sup>hi</sup>	$17.90^{jk}$	$6.90^{ij}$	$39.80^{jk}$
SCSH 19-8	8.40 <sup>gh</sup>	5.28 <sup>efg</sup>	1.59 <sup>ghij</sup>	$0.27^{ghi}$	13.10 <sup>g</sup>	$22.30^{efgh}$	$8.20^{defg}$	$46.40^{fg}$
SCSH 21-10	11.32 <sup>a</sup>	5.94 <sup>cd</sup>	1.91 <sup>bcd</sup>	$0.35^{b}$	$9.50^{ijkl}$	23.40 <sup>cde</sup>	9.20°	50.00 <sup>de</sup>
CRH 20-11	$7.84^{i}$	4.98hi	1.58 <sup>hij</sup>	$0.24^{jkl}$	10.50 <sup>hij</sup>	25.00 <sup>b</sup>	10.90 <sup>b</sup>	54.80 <sup>b</sup>
Parentage								
Sweet orange cv. Mosamb	i 8.46 <sup>gh</sup>	$5.20^{fgh}$	1.63 <sup>ghi</sup>	$0.32^{c}$	14.01 <sup>fg</sup>	15.57 <sup>m</sup>	$7.50^{fghi}$	33.10 <sup>mn</sup>
PS-2 (Pummelo red)	$8.62^{fgh}$	4.62 <sup>kl</sup>	1.87 <sup>cde</sup>	0.34bc	24.30 <sup>b</sup>	21.20 <sup>hi</sup>	11.10 <sup>b</sup>	44.38gh
PS-5 (Pummelo white)	8.40gh	5.46 <sup>ef</sup>	1.54 <sup>ij</sup>	$0.38^{a}$	22.80°	$21.80^{fghi}$	11.20 <sup>b</sup>	45.20gh
PS-10 (Pummelo white)	10.12 <sup>c</sup>	6.24 <sup>ab</sup>	1.63ghij	$0.38^{a}$	28.50 <sup>a</sup>	24.60 <sup>b</sup>	12.40 <sup>a</sup>	50.50 <sup>cd</sup>
LSD ( <i>P</i> ≤0.05)	0.52	0.27	0.14	0.02	1.36	1.30	0.94	2.42

SCHS 15-3, SCHS 15-18 and in PS-10 (Pummelo white). The higher fruit width (>100 mm) was measured in SCHS 3-10, SCHS 5-5, SCHS 15-3, PS-10 (Pummelo white). The variation in fruit peel thickness with maximum (>10 mm) in SCHS 3-10, SCHS 3-14, SCHS 7-12, SCHS 9-20, SCHS 15. The higher (>25 mm) fruit core diameter was observed in SCHS 3-10, SCHS 9-2, SCHS 9-20, PS-5 (Pummelo white), PS-2 (Pummelo red), medium (15–25 mm) in 17 hybrids and sweet orange cv. Mosambi and lower (<15 mm) in SCHS 3-15, SCHS 11-15, SCHS 13-14, and PS-10 (Pummelo white). The hybrid SCSH 3-10, PS-2 (Pummelo red), and

PS-5 (Pummelo white) had a higher (15–18) number of segments per fruit and, 20 hybrids and parents such as sweet orange cv. Mosambi, PS-10 (Pummelo white) was found to be the medium (10–14), however lower (<10) in SCSH 3-14, SCSH 11-15, and SCSH 19-8. It is important to mention that the hybrids expressed higher and lower values than either of the parents. The female red pummelo parents showed more characters than male parents. These findings corroborate earlier studies that have documented variations in fruit parameters across different citrus cultivars (Gaikwad *et al.* 2015). Notably, in the case of orangelo hybrids, the observed

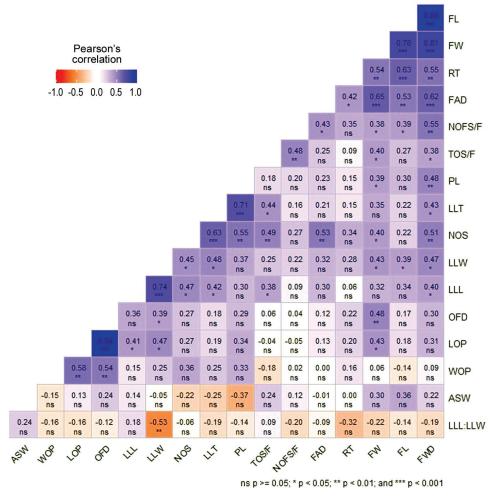


Fig. 1 Pearson correlation between studied morphological quantitative traits.

LLL, Leaf lamina length; LLW, Leaf lamina width; LLL:LLW, Leaf lamina length: width ratio; LLT, Leaf lamina thickness; PL, Pedicel length; LOP, Length of petal; WOP, Width of petal; OFD, Diameter of opened flower; FL, FWD, Fruit length; fruit width; FW, Fruit weight; ASW, 10 average seed weight; TOS/F, Total number of seeds/fruit; NOFS/F, Number of false seeds/fruit; NOS, Number of segments; RT, Rind or peel thickness; and FAD, Fruit axis diameter.

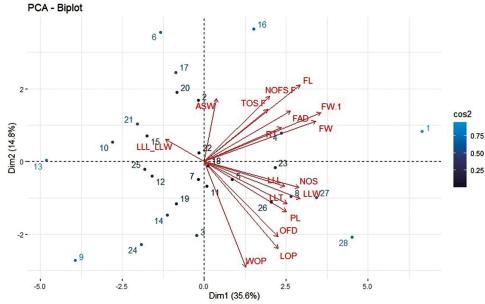


Fig. 2 Principal component analysis of studied morphological traits.

traits indicated reduced rind thickness, elevated juice content, and enhanced fruit quality attributes compared to the parent trees. Based on the results, it becomes apparent that many of the observed traits in hybrids are linked to the characteristics of the female parent trees. Moreover, alterations in fruit size often coincide with changes in fruit shape, implying a potential shared genetic regulation between these two traits. Recognizing the significance of fruit traits, they are a crucial focus in breeding efforts (Zhang et al. 2022). The total number of seeds was recorded higher (<50) in SCSH 15-3, SCSH 21-10 and medium (30-50) in SCSH 3-10, SCSH 15-2, SCSH 19-2, PS-5 (Pummelo white), PS-10 (Pummelo white). However, lesser (<30) was noted in most of the hybrids and parents like sweet orange cv. Mosambi, PS-2 (Pummelo red). Data indicated significantly the highest number of false seeds in hybrid SCSH 3-10 (10.00) followed by SCSH 3-14, while it was found to be lowest in hybrid SCSH 17-9 (0.67). The heavy seed weight (>1.5 g) was measured in SCSH 5-5, SCSH 13-4, SCSH 15-3, SCSH 15-18, SCSH 17-9, SCSH 19-2, SCSH 19-6, SCSH 21-10. Pearson's correlation coefficient (r) value among the different morphological quantitative parameters is shown in Fig. 1. The fruit length (FL) expressed strongly positive correlation with fruit width (FWD) (r = 0.88, P < 0.001), fruit weight (FW) correlated positively to FL and FWD (r = 0.78 and r = 0.81, P < 0.001); total number of seeds/fruit (TOS/F) showed positive correlation with number of

Table 3 The quantitative fruit and seed parameters of orangelo hybrids (Pummelo × Sweet orange) and their parents

Hybrid	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Peel or rind thickness (mm)	Fruit core diameter (mm)	No. of segments	Total seed/ fruit	False seed/ fruit	10 seed weight (g)
SCSH 3-10	814.00 <sup>a</sup>	120.20 <sup>a</sup>	117.91 <sup>a</sup>	11.45 <sup>a</sup>	31.02a	15.00 <sup>a</sup>	37.33 <sup>bc</sup>	10.00 <sup>a</sup>	1.35 <sup>defg</sup>
SCSH 3-14	$390.00^{i}$	104.04 <sup>cd</sup>	92.35 <sup>fg</sup>	11.02 <sup>ab</sup>	21.34 <sup>c</sup>	9.67 <sup>kl</sup>	11.00 <sup>lmn</sup>	8.33abc	1.35 <sup>defg</sup>
SCSH 3-15	$251.00^{l}$	74.94 <sup>kl</sup>	$78.08^{kl}$	$6.47^{fghi}$	14.66 <sup>ij</sup>	13.33 <sup>bcd</sup>	24.00 <sup>efg</sup>	5.67 <sup>efgh</sup>	$0.86^{hijk}$
SCSH 5-5	550.00 <sup>c</sup>	108.81 <sup>bc</sup>	104.58 <sup>b</sup>	8.32°	22.86 <sup>c</sup>	13.33 <sup>bcd</sup>	$22.33^{fgh} \\$	$3.67^{ijk}l$	1.80 <sup>abc</sup>
SCSH 7-12	$340.00^{j}$	103.96 <sup>cd</sup>	92.06 <sup>fg</sup>	11.57 <sup>a</sup>	16.73 <sup>fg</sup>	12.67 <sup>cdef</sup>	24.33efg	$2.00^{lmn}$	1.43 <sup>def</sup>
SCSH 9-2	240.00 <sup>lm</sup>	101.96 <sup>de</sup>	99.80°	$5.33^{ijkl}$	25.59 <sup>b</sup>	11.67 <sup>efghi</sup>	28.33 <sup>def</sup>	9.33 <sup>ab</sup>	1.35 <sup>defg</sup>
SCSH 9-10	532.00 <sup>d</sup>	91.29gh	$81.31^{jk}$	7.16 <sup>cdefg</sup>	17.10 <sup>efg</sup>	$10.33^{ijkl}$	23.67 <sup>efg</sup>	$3.00^{klm}$	1.43 <sup>def</sup>
SCSH 9-20	451.00g	90.40gh	98.57 <sup>cd</sup>	11.42 <sup>a</sup>	25.45 <sup>b</sup>	14.33 <sup>ab</sup>	25.00 <sup>efg</sup>	7.33 <sup>cde</sup>	1.32 <sup>defg</sup>
SCSH 11-15	138.80°	64.27 <sup>mn</sup>	65.80 <sup>m</sup>	4.19 <sup>lmno</sup>	11.85 <sup>k</sup>	9.67 <sup>kl</sup>	8.67 <sup>n</sup>	1.33 <sup>mn</sup>	1.30 <sup>feg</sup>
SCSH 11-19	201.00 <sup>n</sup>	69.68 <sup>lm</sup>	73.98 <sup>1</sup>	7.15 <sup>cdefg</sup>	19.52 <sup>d</sup>	12.67 <sup>cdef</sup>	29.67 <sup>de</sup>	$4.00^{hijk}$	$0.62^{k}$
SCSH 13-4	416.50 <sup>h</sup>	97.66 <sup>ef</sup>	93.22 <sup>efg</sup>	$5.18^{jklm}$	18.94 <sup>d</sup>	11.00ghijk	10.00 <sup>mn</sup>	$3.33^{jkl}$	1.61 <sup>abcde</sup>
SCSH 13-13	$289.70^{k}$	88.46 <sup>gh</sup>	$83.04^{ij}$	$4.64^{klmn}$	17.26 <sup>efg</sup>	10.67 <sup>hijk</sup>	17.33 <sup>hijk</sup>	1.00 <sup>n</sup>	$1.17^{fgh}$
SCSH 13-14	193.40 <sup>n</sup>	65.35 <sup>mn</sup>	$74.96^{l}$	4.00mno	9.45 <sup>1</sup>	10.67 <sup>hijk</sup>	15.67 <sup>ijklm</sup>	$4.33^{hijk}$	$1.07^{ghij}$
SCSH 13-17	$332.90^{j}$	81.48ij	89.08gh	7.78 <sup>cde</sup>	15.82 <sup>ghi</sup>	13.33 <sup>bcd</sup>	12.00 <sup>klmn</sup>	1.00 <sup>n</sup>	$1.13^{fghi}$
SCSH 15-2	230.80 <sup>m</sup>	75.15 <sup>kl</sup>	77.51 <sup>kl</sup>	6.66efgh	15.98 <sup>gh</sup>	13.00 <sup>bcde</sup>	33.00 <sup>cd</sup>	5.67 <sup>efgh</sup>	1.41 <sup>def</sup>
SCSH 15-3	581.90 <sup>b</sup>	111.83 <sup>b</sup>	100.84 <sup>bc</sup>	$7.02^{defg}$	22.85 <sup>c</sup>	13.00 <sup>bcde</sup>	59.33 <sup>a</sup>	8.33abc	1.93 <sup>a</sup>
SCSH 15-18	486.30e	112.28 <sup>b</sup>	97.77 <sup>cde</sup>	10.17 <sup>b</sup>	17.04 <sup>efg</sup>	$10.00^{jkl}$	16.67 <sup>hijkl</sup>	$5.33^{fghi}$	1.61 <sup>abcde</sup>
SCSH 15-19	468.60 <sup>f</sup>	89.88g <sup>h</sup>	93.84 <sup>ef</sup>	3.74 <sup>no</sup>	22.85 <sup>c</sup>	12.33 <sup>defg</sup>	27.33 <sup>def</sup>	$3.33^{jkl}$	1.35 <sup>defg</sup>
SCSH 17-9	$398.90^{i}$	86.66ghi	$86.24^{ij}$	$5.02^{jklm}$	19.10 <sup>d</sup>	12.00 <sup>defgh</sup>	14.33 <sup>ijklmn</sup>	0.67 <sup>n</sup>	1.65 <sup>abcd</sup>
SCSH 19-2	475.30 <sup>ef</sup>	88.04gh	92.15 <sup>fg</sup>	8.24 <sup>cd</sup>	18.31 <sup>def</sup>	11.33 <sup>fghij</sup>	36.67 <sup>bc</sup>	8.00 <sup>bcd</sup>	1.84 <sup>ab</sup>
SCSH 19-6	$286.50^{k}$	85.54hi	$83.08^{ij}$	5.50 <sup>hijk</sup>	16.89 <sup>efg</sup>	10.67 <sup>hijk</sup>	19.33ghij	5.00ghij	1.57 <sup>bcde</sup>
SCSH 19-8	447.00g	91.56 <sup>fgh</sup>	94.40 <sup>def</sup>	6.09ghij	18.03 <sup>def</sup>	$9.00^{1}$	19.00ghij	7.00 <sup>cdef</sup>	1.33 <sup>defg</sup>
SCSH 21-10	419.30 <sup>h</sup>	85.80 <sup>hi</sup>	94.45 <sup>def</sup>	4.58 <sup>klmno</sup>	18.33 <sup>de</sup>	14.00 <sup>abc</sup>	56.67 <sup>a</sup>	6.33 <sup>defg</sup>	1.93 <sup>a</sup>
CRH 20-11	196.60 <sup>n</sup>	61.56 <sup>n</sup>	$73.85^{1}$	3.390	14.26 <sup>ij</sup>	11.00ghijk	20.33ghi	7.00 <sup>cdef</sup>	1.47 <sup>cdef</sup>
Parentage									
Sweet orange cv. Mosambi	286.83 <sup>k</sup>	78.89 <sup>jk</sup>	$81.43^{jk}$	$5.02^{jklm}$	16.39 <sup>g</sup>	11.33 <sup>fghij</sup>	28.33 <sup>def</sup>	$2.00^{lmn}$	$0.85^{hijk}$
PS-2 (Pummelo red)	444.41 <sup>g</sup>	87.89 <sup>gh</sup>	97.73 <sup>cde</sup>	7.55 <sup>cdef</sup>	30.52 <sup>a</sup>	15.33 <sup>a</sup>	13.33 <sup>jklmn</sup>	6.33 <sup>defg</sup>	$0.78^{jk}$
PS-5 (Pummelo white)	576.02 <sup>b</sup>	92.57 <sup>fg</sup>	97.55 <sup>cde</sup>	8.37 <sup>c</sup>	26.55 <sup>b</sup>	15.00 <sup>a</sup>	41.67 <sup>b</sup>	6.33 <sup>defg</sup>	$0.81^{ijk}$
PS-10 (Pummelo white)	446.30 <sup>g</sup>	108.94 <sup>bc</sup>	114.55a	8.10 <sup>cd</sup>	$12.72^{jk}$	14.33 <sup>ab</sup>	36.00 <sup>cd</sup>	8.33abc	$1.03^{\mathrm{ghij}}$
LSD ( <i>P</i> ≤0.05)	16.00	6.24	4.57	1.24	1.58	1.57	6.28	1.88	0.34

false seeds per fruit (NOFS/F), FW, FWD (r = 0.48, P < 0.01; r = 0.40, P < 0.05; r = 0.38, P < 0.05); pedicel length (PL) showed positively correlated with FWD and FW (r = 0.48, P < 0.01; r = 0.39, P < 0.05); leaf lamina thickness (LLT) expressed positively correlated with pedicel length (PL), TOS/F and FWD (r = 0.71, P < 0.001; r = 0.44, P < 0.05; r = 0.43, P < 0.05). The ratio of leaf lamina length to leaf lamina width (LLL:LLW) negatively correlated with LLW (r = 0.53, P < 0.01). The genetic coefficient of variance (GCV) exhibited the lowest value (9.381) for the ratio of leaf lamina length to

leaf lamina width (LLL:LLW) and the highest value (42.571) for pedicel length. Similarly, the phenotypic coefficient of variance (PCV) was minimal (11.439) for the LLL:LLW and maximal (43.446) for pedicel length (Data not shown). Principal Component Analysis (PCA) serves a crucial role as a data reduction tool, aiding in the identification of key traits that contribute to the differentiation between genotypes. In the present study, the result of the PCA for morphological quantitative traits showed that five principal components of the 17 principal components had Eigen-values>1 and all

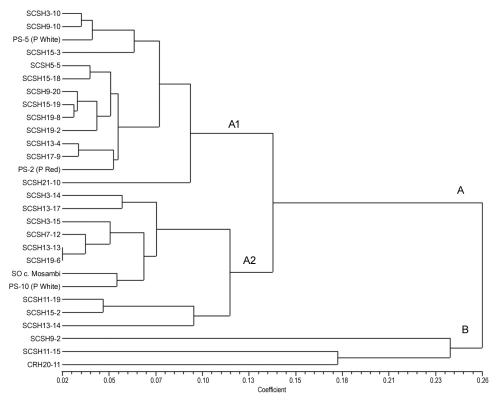


Fig. 3 Dendrogram depicting morphological relationship among 24 orangelo hybrids and their 4 parents.

together accounted for over 77.76% of the total variability (Fig. 2). The first component (35.62%) is followed by 2<sup>nd</sup> component (14.80%) and 3<sup>rd</sup> component (11.43%). Cluster analysis clearly showed that cluster A1 consists mainly the hybrids which are good in leaf lamina length and width, fruit weight, length, width, and seed weight however, outstanding performing hybrids SCSH 21-10 and SCSH 9-2 were presented as an out-group (Fig. 3). As per studies, the pummelo is a mono-embryonic as well as self-incompatible species, hence producing wide variability within pummelo and the sweet orange cv. Mosambi originated as a natural hybrid and white pummelo is one of the parents hence it may be possible that Mosambi is closer to white pummelo. Many researchers studied the diversity in different citrus species and the study exhibits substantial heterozygosity (Marboh et al. 2015, Wu et al. 2018, Munankarmi et al. 2023). On the other hand, a study by Dubey et al. (2021) demonstrated that parameters related to fruit, such as weight, length, diameter, peel thickness, core diameter, and segments, were major principal components, contributing to 80.62% cumulative variance in an Indian pummelo collection.

We have to conclude that 24 hybrid varieties of orangelo, along with their four parent plants, underwent assessment using DUS (Distinctness, Uniformity, and Stability) guidelines as a means to gauge diversity through various parameters related to genetic variability. Utilizing a bi-plot generated through principal component analysis proved to be a valuable tool for pinpointing superior hybrid varieties in terms of fruit weight. Notable examples of such hybrids

include SCSH 3-10, SCSH 5-5, SCSH 9-10, SCSH 15-3, and PS-5 (Pummelo white). The exploration of genetic relationships among the hybrid varieties yielded crucial insights that could potentially be harnessed for future breeding programs, offering promising sources of desirable traits.

#### REFERENCES

Anonymous. 2020–21. Indian Horticulture Database (Area and Production of Horticulture crops). Ministry of Agriculture, Government of India.

Chan S R O S, Achmad B S and Ferdinant. 2022. Morphological characterization of Gunung Omeh Citrus (Citrus nobilis L.) in Guguak District, Lima Puluh Kota Regency. IOP Conference Series: Earth and Environmental Science 1097: 012032. https://doi.org/10.1088/1755-

## 1315/1097/1/012032

Devi E J. 2021. Rediscovery and characterization of *Citrus Indica* Tanaka, a wild endangered and progenitor species in Dailong forest, Manipur and recommendations for its conservation. https://doi.org/10.21203/rs.3.rs-191171/v1

Dubey A K, Kholia A, Sharma N and Sharma R M. 2021. Assessing genetic diversity in Indian pummelo collections utilizing quantitative traits and simple sequence repeat markers (SSRs). *Indian Journal of Horticulture* **78**: 3–8. https://doi.org/10.5958/0974-0112.2021.00001.3

Gaikwad K A, Haldavanekar P C, Parulekar Y R and Haldankar P M. 2015. Survey and characterization of pummelo genotypes (Citrus grandis L. Osbeck) grown in coastal region of Maharashtra. Ecoscan 8: 371–80.

Gaikwad K A, Patil S R, Nagre P K and Potdukhe N R. 2018. Morphological characterization of citrus rootstock genotypes. *International Journal of Chemical Studies* **6**: 516–29.

IPGRI. 1999. *Descriptors for Citrus*, pp. 66. International Plant Genetic Resources Institute.

Inglese P and Sortino G. 2019. Citrus history, taxonomy, breeding, and fruit quality. Oxford Research Encyclopedia of Environmental Science. https://doi.org/10.1093/ acrefore/9780199389414.013.221

Kandowangko N Y and Febriyanti F. 2023. Genetic diversity of the genus citrus in Tomini Bay coastal areas, Indonesia based on morphological characters. *Biodiversitas Journal of Biological Diversity* 24(5). https://doi.org/10.13057/biodiv/d240549

Marboh E S, Singh A K, Dubey A K and Prakash J. 2015. Analysis of genetic variability among citrus (*Citrus* spp.) genotypes using morphological traits. *The Indian Journal of Agricultural Science* **85**: 203–11.

- Munankarmi N N, Rana N, Joshi B K, Bhattarai T, Chaudhary S, Baral B and Shrestha S. 2023. Characterization of the genetic diversity of *Citrus* species of Nepal using simple sequence repeat (SSR) markers. *South African Journal of Botany* **156**: 192–201. https://doi.org/10.1016/j.sajb.2023.03.014
- Rohlf F J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, New York. https://doi.org/10.2307/2684761
- Swingle W T. 1967. The Botany of Citrus and its wild relatives as a Guide for their use in Breeding. Florida State Horticultural Society.
- USDA-FAS Citrus statistics. 2022–23. https://www.fas.usda.gov/data/citrus-world-markets-and-trade
- Wu G A, Terol J, Ibanez V, Lopez-Garcia A, Pérez-Román E, Borreda C and Talon M. 2018. Genomics of the origin and evolution of *Citrus*. *Nature* 554: 311–16. https://doi. org/10.1038/nature25447
- Zhang T, Hong Y, Zhang X, Yuan X and Chen S. 2022. Relationship between key environmental factors and the architecture of fruit shape and size in near-isogenic lines of cucumber (*Cucumis sativus* L.). *International Journal of Molecular Science* 23: 14033. https://doi.org/10.3390/ijms232214033