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# TRAITS VARIABILITY OF NOVEL CITRUS HYBRIDS FOR ENHANCED FRUIT PRODUCTION

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## SUMMARY

The conducted study occurred on 24 interspecific citrus hybrids derived from pummelo (female parent) crosses with tangerine and sweet orange (male parents). The hybrids obtained assessments for 26 qualitative and 15 quantitative traits related to tree, leaf, fruit, and seed characteristics. Among the parental genotypes, Mosambi and Tangerine exhibited polyembryony, while pummelo was monoembryonic. Most of the hybrids (95.83%) were monoembryonic, except SCSH 9-14. Among different parents, PS-13 had the heaviest fruit (619.28 g) and Tangerine had the lightest (111.85 g). Fruit weight of hybrids ranged from 108.72 g (SCSH 2-3) to 1047.42 g (SCSH 3-10). Though a large variation emerged among the hybrids for different traits, including leaf, tree, seed, and fruit characters, SCSH 7-8 and SCSH 13-6 were appropriate for fruit weight, as well as fewer seeds than better parents. Principal component analysis identified five traits—leaf lamina length, width, their ratio, pulp thickness, and fruit axis diameter—explaining 56.10% of the total variation. High heritability and genetic advance achieved recordings for fruit weight, fruit length, pulp thickness, leaf lamina dimensions, and seed count. These traits are promising for selection in citrus breeding programs targeting improved fruit quality and yield potential.

**Keywords:** Interspecific citrus hybrids, heritability, qualitative traits, quantitative traits, PCA

**Key findings:** Peel color mainly transferred from pummelo, and traits like fruit weight, fruit length, pulp thickness, leaf lamina dimensions, and seed count demonstrated high heritability.

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## INTRODUCTION

*Citrus* is a genus within the Rutaceae family and Geraniales order. The primary centers of origin for citrus seemed to be in the tropical and subtropical regions of Southeast Asia (Zhong and Nicolosi, 2021; Pandey *et al*., 2022). Citrus cultivation happens in over 140 countries, and the global citrus production reaches 169.38 million metric tons, cultivated over an area of 10.55 million hectares, with Asia leading the way at 89.59 million metric tons (FAOSTAT, 2023). Today, widely popular citrus varieties consumed globally include sweet oranges (*Citrus sinensis*), mandarins (*Citrus reticulata*, *Citrus clementina*, and *Citrus unshiu*), lemons (*Citrus limon*), and grapefruits (*Citrus* × *paradisi*), either fresh or processed into products like juices and jams (Ma *et al*., 2020).

Sweet orange (*Citrus sinensis* [L.] Osbeck), a polyembryonic species, is a complex hybrid between pummelo and mandarin (Wu *et al*., 2014), originating in southern China (Albrigo *et al*., 2019). Pummelo (*C. maxima* Merr.), being a true *Citrus* species, is native to the Yunnan Province of Southeast Asia (Zhong and Nicolosi, 2020). It has a distinctive and complex phytochemical profile, including dietary fiber, vitamin C, β-complex, lycopene, and flavonoids, such as naringin, naringenin, hesperidin, neohesperidin, ellagic acid, caffeic acid, and epicatechin (Gupta *et al*., 2020).

Sweet oranges are valuable for their distinctive sensory qualities, as well as their rich nutritional and therapeutic benefits arising out of the richness in their bioactive compounds (Kiran *et al*., 2024). Tangerines are smaller and low in juice content but are particularly rich in flavonoids. Pummelo generally exhibits a massive fruit size and weight, whereas Mosambi and Tangerine possess relatively smaller fruits. Through interspecific hybridization, it is possible to develop progenies that exhibit moderately sized fruits with optimal fruit weight, balanced rind thickness (neither excessively thick nor thin), medium-sized leaves, and a reduced number of seeds, along with additional traits (desirable rind color). Monoembryony remains a major concern in citrus breeding; however, addressing this issue can proceed through interspecific hybridization.

The primary challenge in effectively utilizing germplasm lines lies in the lack of comprehensive passport data, as well as detailed characterization and evaluation (Xu *et al*., 2013; Devy *et al*., 2023). The Indian Council of Agricultural Research-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, has developed interspecific citrus scion hybrids with two different cross combinations—*Citrus maxima* Merr. × *Citrus sinensis* (L.) Osbeck and *Citrus maxima* Merr. × *Citrus reticulata* cv. Tangerine—to overcome the hurdles of the citrus industry. Therefore, the presented study aimed to assess qualitative and quantitative fruit characteristics in novel citrus hybrids for further implications in breeding and selection.

## MATERIALS AND METHODS

### Experimental site

The study transpired during 2023–2024 at the field of the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi (28°40′ N, 77°12′ E; 228.6 masl), which experiences a subtropical climate with hot summers (22.2 °C–38.3 °C) and cold winters (7.5 °C–22.8 °C). The experimental field had a level terrain, efficient drainage, and alluvial, slightly saline, clay loam soil with low organic matter having a pH value of 7.22 and 0.84% organic carbon content.

### Plant material

Twenty-four interspecific hybrids and four parent plants became samples under study, planted in a square system at 3 m × 3 m spacing. All the hybrids were between seven and 12 years old. The research followed uniform cultural practices throughout the study.

The assessment of all qualitative and quantitative pomological traits followed standard methodologies based on citrus descriptors provided by the UPOV (International Union for the Protection of New Varieties of Plants) and Biodiversity International (IPGRI, 1999). Fully developed leaves, flowers, fruits, and seeds reached systematic collections across seasons before cleaning with tissue paper and evaluation for quantitative traits according to standardized descriptors. The experiment followed a randomized complete block design with five replications, each consisting of a single tree per replication.

**Statistical analysis**

Performing statistical analysis used the analysis of variance (ANOVA) in SAS 9.3 (SAS, USA Inc.), with the mean comparison conducted using Duncan's multiple range test (DMRT) at $P \leq 0.05$. Principal component analysis (PCA) and Pearson's correlation analysis succeeded in R Studio (Version 2024.12.0-467, R Studio PBC). Descriptive statistics and broad-sense heritability estimates incurred computations using the variability package in R. Genetic similarity determination among genotypes utilized NTSYS-pc 2.1 software (Rohlf, 2000).

## RESULTS

**Qualitative traits based on morphology**
***Tree morphology***

Most hybrids (70.83%) exhibited a spheroid tree shape, while others displayed obloid (20.83%) or ellipsoid (8.34%) forms (Table 1). Growth habits varied, with 70.83% showing a spreading habit, 16.67% drooping, and 12.50% erect. Most of the hybrids (62.50%) and all parents had medium branch density, with a few hybrids displaying sparse (8.33%) or dense (29.17%) branching. Spine density ranged from spineless (8.33%) to medium (79.17%), with PS-13 being the only parental genotype exhibiting low spine density.

***Leaf shape***

All hybrids and parental lines were evergreen with simple leaf division. Leaf lamina shape was predominantly ovate (75%), while some hybrids displayed elliptic or obovate forms (Table 1). Margins were either crenate (62.5%) or dentate (37.5%). Petiole wing shapes varied across hybrids and parents, with 50.34% showing an obdeltate shape, while others exhibited obcordate or obovate wings.

***Fruit and seed characteristics***

Fruit shape varied, with 45.84% spheroid, 33.33% pyriform, and the rest exhibiting obloid or ellipsoid forms, as shown in Figure 1. Most hybrids (83.33%) had green-yellow peels, while Tangerine was the only parent with light-orange skin. Fruit pulp color was predominantly yellow (87.5%), except for three hybrids and PS-8, which exhibited red pulp. The fruit axis was primarily hollow (50%), while some hybrids showed semi-solid or solid axes. Seed shape was mainly clavate (41.67%) or semi-deltoid (37.5%), with a few cuneiform seeds. The hybrids (95.83%) were mostly monoembryonic, except for SCSH 9-14, which was polyembryonic. Among parents, Mosambi and Tangerine were polyembryonic, while pummelo parents were monoembryonic (Table 2).

**Quantitative trait variability in citrus hybrids**

***Leaf traits***

Significant variation was evident in leaf traits among hybrids and parents. The longest leaf lamina recorded was in SCSH 3-10 (154.72 mm), while the shortest was in SCSH 3-6 (68.11 mm). Among parents, PS-13 had the longest (110.69 mm) and Tangerine the shortest (69.52 mm). Leaf lamina width also varied, with SCSH 3-10 exhibiting the widest (86.16 mm) and SCSH 7-3 the narrowest (45.66 mm). PS-13 had the highest leaf width among parents (57.93 mm). The highest leaf lamina length-to-width ratio was visible in

**Table 1.** The morphological traits related to the tree and leaf of citrus hybrids and parents.

| Hybrids | Growth habit | Tree shape | Density of branches | Branch angle | Leaf division | Leaf attachment | Leaf apex | Leaf shape | Leaf margin | Petiole wing shape | Petiole wing width |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SCSH 2-2 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obcordate | Medium |
| SCSH 2-3 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Medium |
| SCSH 2-6 | Drooping | Obloid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Narrow |
| SCSH 3-5 | Erect | Spheroid | Medium | Narrow | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obdeltate | Narrow |
| SCSH 3-6 | Spreading | Obloid | Medium | Narrow | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obcordate | Medium |
| SCSH 3-9 | Erect | Ellipsoid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obcordate | Broad |
| SCSH 3-10 | Drooping | Obloid | Dense | Wide | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obcordate | Broad |
| SCSH 4-13 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Narrow |
| SCSH 6-4 | Spreading | Spheroid | Dense | Narrow | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obdeltate | Narrow |
| SCSH 6-6 | Drooping | Obloid | Dense | Narrow | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obdeltate | Narrow |
| SCSH 7-2 | Spreading | Spheroid | Sparse | Wide | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Narrow |
| SCSH 7-3 | Spreading | Spheroid | Dense | Medium | Simple | Brevipetiolate | Obtuse | Elliptic | Dentate | Obovate | Narrow |
| SCSH 7-4 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Medium |
| SCSH 7-7 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Medium |
| SCSH 7-8 | Drooping | Obloid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Medium |
| SCSH 8-4 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Obtuse | Obovate | Crenate | Obdeltate | Narrow |
| SCSH 8-11 | Spreading | Spheroid | Dense | Medium | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obdeltate | Narrow |
| SCSH 8-15 | Erect | Ellipsoid | Dense | Narrow | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obdeltate | Narrow |
| SCSH 8-18 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Obtuse | Elliptic | Crenate | Obovate | Narrow |
| SCSH 8-19 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obovate | Narrow |
| SCSH 9-14 | Spreading | Spheroid | Sparse | Wide | Simple | Brevipetiolate | Obtuse | Elliptic | Crenate | Obovate | Narrow |
| SCSH 11-16 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Obtuse | Elliptic | Crenate | Obcordate | Medium |
| SCSH 13-4 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Obtuse | Elliptic | Crenate | Obovate | Narrow |
| SCSH 13-6 | Spreading | Spheroid | Dense | Narrow | Simple | Brevipetiolate | Acute | Ovate | Dentate | Obdeltate | Medium |
| **Parents** | | | | | | | | | | | |
| MOSAMBI | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Acute | Ovate | Crenate | Obdeltate | Narrow |
| PS-8 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Obtuse | Obovate | Crenate | Obcordate | Medium |
| PS-13 | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Obtuse | Obovate | Crenate | Obcordate | Medium |
| TANGERINE | Spreading | Spheroid | Medium | Medium | Simple | Brevipetiolate | Obtuse | Elliptic | Crenate | Obovate | Narrow |

**Table 2.** The qualitative fruit and seed parameters of citrus hybrids and parents

| Hybrids | Fruit shape | Shape of fruit base (Stalk end) | Shape of fruit apex (Stylar end) | Fruit skin color | Fruit pulp color | Fruit surface texture | Fruit axis | Seed surface | Seed color | Seed shape | Seed embryony |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SCSH 2-2 | Spheroid | Truncate | Truncate | Green Yellow | Red | Rough | Hollow | Wrinkled | White | Clavate | Monoembryonic |
| SCSH 2-3 | Spheroid | Convex | Truncate | Green Yellow | Red | Rough | Hollow | Smooth | White | Clavate | Monoembryonic |
| SCSH 2-6 | Spheroid | Truncate | Truncate | Green Yellow | Yellow | Smooth | Hollow | Wrinkled | Cream | Semi-deltoid | Monoembryonic |
| SCSH 3-5 | Spheroid | Convex | Truncate | Green Yellow | Red | Rough | Hollow | Wrinkled | Yellowish | Clavate | Monoembryonic |
| SCSH 3-6 | Pyriform | Collard | Truncate | Green Yellow | Yellow | Rough | Semi-solid | Wrinkled | Cream | Clavate | Monoembryonic |
| SCSH 3-9 | Obloid | Truncate | Truncate | Green Yellow | Yellow | Smooth | Hollow | Wrinkled | Cream | Semi-deltoid | Monoembryonic |
| SCSH 3-10 | Spheroid | Convex | Truncate | Green Yellow | Yellow | Smooth | Solid | Wrinkled | Cream | Cuneiform | Monoembryonic |
| SCSH 4-13 | Pyriform | Concave | Truncate | Green Yellow | Yellow | Rough | Semi-solid | Wrinkled | Yellowish | Clavate | Monoembryonic |
| SCSH 6-4 | Pyriform | Collard | Truncate | Green Yellow | Yellow | Smooth | Solid | Wrinkled | Yellowish | Clavate | Monoembryonic |
| SCSH 6-6 | Obloid | Convex | Depressed | Green Yellow | Yellow | Smooth | Hollow | Wrinkled | Yellowish | Semi-deltoid | Monoembryonic |
| SCSH 7-2 | Obloid | Truncate | Truncate | Green Yellow | Yellow | Bumpy | Hollow | Wrinkled | Yellowish | Semi-deltoid | Monoembryonic |
| SCSH 7-3 | Spheroid | Convex | Truncate | Green Yellow | Yellow | Smooth | Solid | Wrinkled | Cream | Cuneiform | Monoembryonic |
| SCSH 7-4 | Spheroid | Convex | Depressed | Green Yellow | Yellow | Rough | solid | Smooth | Yellowish | Clavate | Monoembryonic |
| SCSH 7-7 | Pyriform | Collard | Truncate | Yellow | Yellow | Bumpy | Hollow | Wrinkled | Yellowish | Clavate | Monoembryonic |
| SCSH 7-8 | Spheroid | Truncate | Truncate | Yellow | Yellow | Smooth | Hollow | Wrinkled | Cream | Cuneiform | Monoembryonic |
| SCSH 8-4 | Pyriform | Collard | Truncate | Green Yellow | Yellow | Rough | Hollow | Wrinkled | Cream | Semi-deltoid | Monoembryonic |
| SCSH 8-11 | Spheroid | Truncate | Truncate | Green Yellow | Yellow | Smooth | Semi-solid | Wrinkled | Yellowish | Semi-deltoid | Monoembryonic |
| SCSH 8-15 | Ellipsoid | Collard | Truncate | Green Yellow | Yellow | Rough | Semi-solid | Wrinkled | Cream | Semi-deltoid | Monoembryonic |
| SCSH 8-18 | Ellipsoid | Collard | Rounded | Green Yellow | Yellow | Bumpy | Semi-solid | Wrinkled | Cream | Cuneiform | Monoembryonic |
| SCSH 8-19 | Pyriform | Necked | Truncate | Green | Yellow | Rough | Semi-solid | Smooth | Yellowish | Clavate | Monoembryonic |
| SCSH 9-14 | Pyriform | Necked | Rounded | Green | Yellow | Rough | Solid | Wrinkled | Cream | Semi-deltoid | Polyembryonic |
| SCSH 11-16 | Spheroid | Concave | Truncate | Green Yellow | Yellow | Pitted | Semi-solid | Wrinkled | Cream | Clavate | Monoembryonic |
| SCSH 13-4 | Spheroid | Truncate | Depressed | Green Yellow | Yellow | Smooth | Hollow | Wrinkled | Yellowish | Cuneiform | Monoembryonic |
| SCSH 13-6 | Pyriform | Necked | Truncate | Green Yellow | Yellow | Smooth | Hollow | Wrinkled | Cream | Semi-deltoid | Monoembryonic |
| Parents | | | | | | | | | | | |
| MOSAMBI | Spheroid | Convex | Truncate | Green Yellow | Yellow | Smooth | Semi-solid | Smooth | Cream | Clavate | Polyembryonic |
| PS-8 | Pyriform | Collard | Depressed | Green Yellow | Red | Bumpy | Solid | Wrinkled | Cream | Semi-deltoid | Monoembryonic |
| PS-13 | Spheroid | Convex | Truncate | Green Yellow | Yellow | Rough | Semi-solid | Wrinkled | Cream | Semi-deltoid | Monoembryonic |
| TANGERINE | Spheroid | Convex | Truncate | Light Orange | Yellow | Pitted | Semi-solid | Wrinkled | Yellowish | Clavate | Polyembryonic |

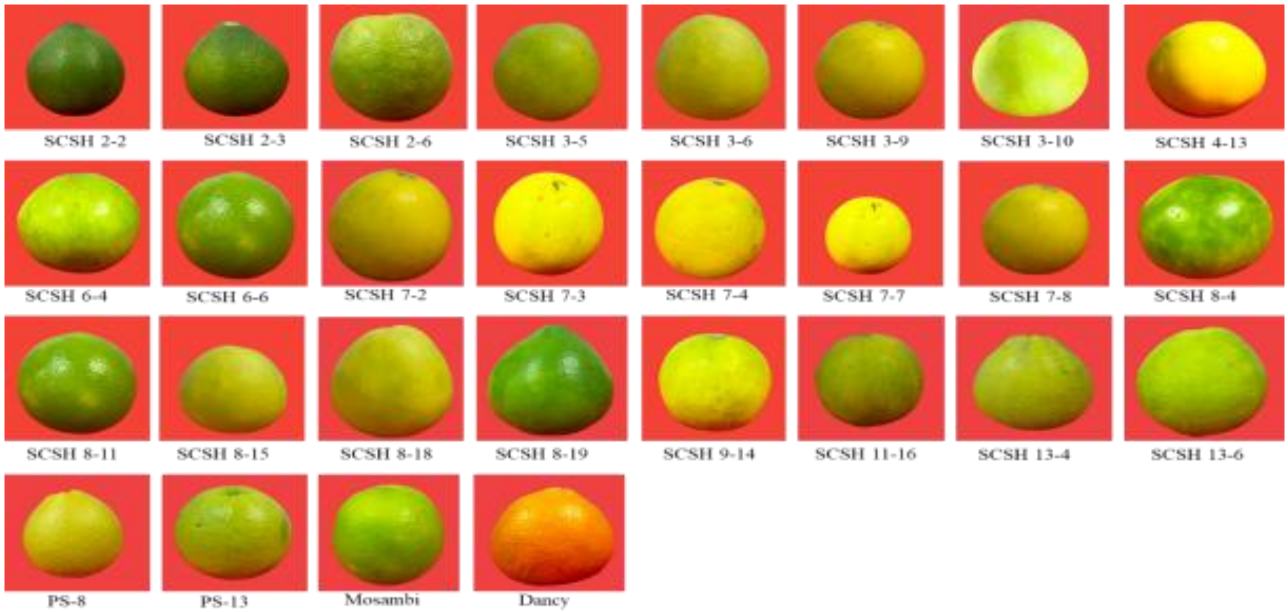


**Figure 1.** Variation in fruit shape and color of interspecific citrus hybrids and their parents.

SCSH 9-14 (1.95), while the lowest was in SCSH 3-6 (1.34).

***Fruit characteristics***

Fruit weight ranged from 1047.42 g in SCSH 3-10 to 108.72 g in SCSH 2-3. Among parents, PS-13 produced the heaviest fruit (619.28 g), while Tangerine had the lightest (111.85 g). The longest fruit resulted in SCSH 3-10 (136.87 mm), and the shortest in SCSH 2-3 (64.13 mm). PS-8 had the longest fruit (116.61 mm) among parents. The widest fruit appeared in SCSH 3-10 (142.20 mm), while the narrowest was in SCSH 2-3 (55.50 mm).

***Peel and pulp traits***

Rind thickness varied significantly, with SCSH 3-10 having the thickest peel (14.63 mm) and SCSH 2-3 the thinnest (2.88 mm). Among parents, PS-8 had the thickest rind (12.10 mm), while Tangerine had the thinnest (2.37 mm). Pulp thickness was highest in SCSH 6-4 (110.43 mm) and lowest in SCSH 2-3 (48.93 mm). Among parents, PS-13 had the thickest pulp (87.38 mm).

***Seed characteristics***

The highest number of seeds per fruit occurred in SCSH 3-6 (70.20), while the lowest was in SCSH 3-5 (6.6). Among parents, PS-8 had the highest seed count (49.00), and Mosambi had the lowest (10.80) (Table 3). False seed numbers ranged from 11.00 (SCSH 2-6 and SCSH 4-13) to none in SCSH 3-5. Among parents, PS-13 had the topmost false seed count (7.20). The heaviest seeds were notable in SCSH 8-18 (2.92 g), while the lightest were in SCSH 3-5 (1.15 g). Among parents, PS-13 had the heaviest seeds (2.57 g). Seed length was supreme in SCSH 3-6 (17.33 mm) and lowest in SCSH 2-2 (12.14 mm). Among parents, PS-13 had the longest seeds (17.93 mm), while Mosambi had the shortest (12.10 mm).

**Correlation analysis**

Pearson's correlation analysis quantified the relationships among key traits, with significant correlations denoted at $P < 0.05$, $P < 0.01$, and $P < 0.001$. Strong positive correlations ($r > 0.8$) were apparent between fruit width and

**Table 3.** The quantitative leaf, fruit, and seed parameters of citrus hybrids and parents.

| Hybrids | Leaf lamina length (mm) | Leaf lamina width (mm) | Leaf lamina length/ width | Leaf lamina thickness (mm) | Fruit weight (g) | Fruit length (mm) | Fruit width (mm) | Rind thickness (mm) | Pulp thickness (mm) | No. of segments/ fruit | Fruit axis diameter (mm) | No. of seeds per fruit | No. of false seeds per fruit | Seed weight (10 seeds) (g) | Seed length (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SCSH 2-2 | 79.63 | 49.33 | 1.60 | 3.54 | 261.90 | 87.49 | 82.37 | 5.83 | 73.15 | 10.00 | 11.56 | 56.80 | 3.00 | 1.53 | 12.14 |
| SCSH 2-3 | 81.48 | 59.23 | 1.37 | 3.40 | 108.72 | 64.13 | 55.50 | 2.88 | 48.93 | 8.00 | 6.26 | 18.40 | 2.60 | 1.72 | 14.22 |
| SCSH 2-6 | 78.22 | 56.10 | 1.38 | 3.40 | 639.97 | 124.66 | 103.46 | 11.56 | 80.42 | 11.80 | 8.17 | 45.80 | 11.00 | 2.29 | 15.52 |
| SCSH 3-5 | 114.74 | 66.98 | 1.70 | 2.52 | 469.11 | 103.64 | 108.80 | 11.45 | 82.62 | 14.20 | 20.21 | 6.60 | 0.00 | 1.15 | 17.22 |
| SCSH 3-6 | 68.11 | 50.51 | 1.34 | 2.76 | 952.32 | 135.63 | 131.45 | 11.42 | 105.56 | 13.60 | 25.18 | 70.20 | 1.40 | 2.58 | 17.33 |
| SCSH 3-9 | 95.05 | 65.46 | 1.44 | 3.04 | 346.33 | 83.31 | 80.95 | 7.39 | 68.92 | 13.00 | 11.17 | 44.80 | 8.40 | 2.08 | 17.06 |
| SCSH 3-10 | 154.72 | 86.16 | 1.79 | 3.52 | 1047.42 | 136.87 | 142.20 | 14.63 | 105.80 | 14.60 | 24.79 | 69.00 | 3.20 | 2.47 | 16.17 |
| SCSH 4-13 | 81.05 | 47.69 | 1.69 | 3.40 | 532.60 | 105.30 | 97.97 | 6.65 | 86.49 | 13.20 | 26.42 | 46.20 | 11.00 | 1.60 | 15.37 |
| SCSH 6-4 | 70.88 | 49.31 | 1.43 | 3.28 | 749.40 | 107.90 | 124.02 | 6.12 | 110.43 | 11.40 | 34.75 | 36.40 | 1.20 | 2.16 | 16.63 |
| SCSH 6-6 | 74.79 | 49.58 | 1.50 | 3.52 | 348.99 | 79.09 | 91.99 | 4.24 | 86.79 | 13.20 | 31.94 | 27.00 | 0.00 | 1.65 | 14.32 |
| SCSH 7-2 | 76.05 | 51.75 | 1.46 | 2.66 | 332.90 | 87.38 | 90.67 | 12.06 | 74.33 | 12.60 | 20.10 | 34.40 | 3.60 | 1.47 | 15.34 |
| SCSH 7-3 | 77.52 | 45.66 | 1.69 | 2.66 | 523.43 | 102.26 | 93.07 | 7.68 | 84.26 | 13.60 | 20.17 | 42.60 | 2.40 | 2.45 | 16.18 |
| SCSH 7-4 | 105.41 | 55.59 | 1.89 | 2.74 | 229.11 | 79.28 | 77.24 | 10.14 | 59.57 | 12.80 | 15.08 | 33.20 | 2.60 | 1.43 | 14.26 |
| SCSH 7-7 | 105.50 | 55.20 | 1.90 | 3.24 | 280.88 | 78.96 | 82.68 | 6.16 | 67.42 | 11.20 | 9.98 | 47.40 | 5.40 | 2.24 | 17.27 |
| SCSH 7-8 | 92.53 | 55.53 | 1.66 | 3.26 | 259.84 | 77.35 | 81.25 | 5.58 | 67.61 | 11.60 | 10.26 | 26.40 | 0.00 | 1.35 | 16.67 |
| SCSH 8-4 | 87.28 | 59.54 | 1.46 | 3.38 | 551.46 | 97.74 | 111.57 | 8.77 | 103.64 | 13.40 | 33.16 | 34.40 | 1.80 | 2.28 | 16.31 |
| SCSH 8-11 | 101.78 | 56.13 | 1.81 | 3.54 | 439.68 | 98.01 | 101.57 | 7.92 | 88.49 | 9.60 | 33.04 | 32.60 | 4.60 | 2.62 | 15.61 |
| SCSH 8-15 | 102.22 | 55.86 | 1.82 | 3.56 | 732.19 | 113.08 | 119.53 | 6.10 | 107.03 | 11.80 | 31.98 | 24.40 | 1.60 | 2.31 | 16.22 |
| SCSH 8-18 | 75.24 | 47.59 | 1.57 | 2.76 | 636.97 | 117.64 | 105.49 | 3.59 | 98.65 | 10.40 | 26.36 | 34.80 | 3.80 | 2.92 | 16.20 |
| SCSH 8-19 | 101.40 | 64.82 | 1.55 | 2.54 | 650.58 | 114.50 | 112.96 | 5.40 | 100.62 | 11.40 | 31.36 | 43.60 | 7.80 | 2.27 | 15.92 |
| SCSH 9-14 | 116.19 | 59.29 | 1.95 | 2.42 | 450.81 | 88.78 | 96.87 | 5.92 | 83.77 | 12.60 | 23.99 | 62.80 | 4.00 | 2.24 | 15.71 |
| SCSH 11-16 | 96.30 | 55.42 | 1.73 | 3.44 | 462.63 | 94.95 | 93.57 | 4.16 | 81.78 | 11.80 | 9.40 | 66.40 | 2.80 | 2.42 | 12.69 |
| SCSH 13-4 | 79.98 | 46.50 | 1.71 | 3.46 | 426.67 | 100.30 | 87.05 | 7.01 | 71.67 | 11.20 | 12.59 | 9.20 | 0.60 | 2.30 | 16.26 |
| SCSH 13-6 | 79.11 | 48.18 | 1.64 | 3.54 | 241.27 | 87.88 | 75.90 | 7.55 | 62.03 | 10.60 | 9.81 | 25.20 | 4.80 | 2.25 | 12.65 |
| Mosambi | 85.54 | 45.95 | 1.85 | 3.22 | 150.75 | 66.14 | 60.42 | 2.71 | 55.37 | 11.40 | 9.10 | 10.80 | 2.80 | 1.29 | 12.10 |
| PS-8 | 93.36 | 49.11 | 1.89 | 3.44 | 582.76 | 116.61 | 97.09 | 12.10 | 71.20 | 13.80 | 13.34 | 49.00 | 6.20 | 2.39 | 15.96 |
| PS-13 | 110.69 | 57.93 | 1.90 | 3.74 | 619.28 | 111.41 | 122.05 | 12.00 | 87.38 | 14.00 | 11.45 | 42.40 | 7.20 | 2.57 | 17.93 |
| Tangerine | 69.52 | 45.81 | 1.51 | 3.10 | 111.85 | 47.37 | 55.64 | 2.37 | 50.31 | 10.20 | 13.59 | 20.80 | 2.60 | 1.10 | 12.59 |
| *LSD(P ≤ 0.05)* | 1.61 | 1.11 | 0.04 | 0.10 | 16.64 | 5.58 | 6.05 | 1.53 | 4.79 | 1.21 | 2.37 | 4.03 | 1.03 | 0.21 | 0.74 |

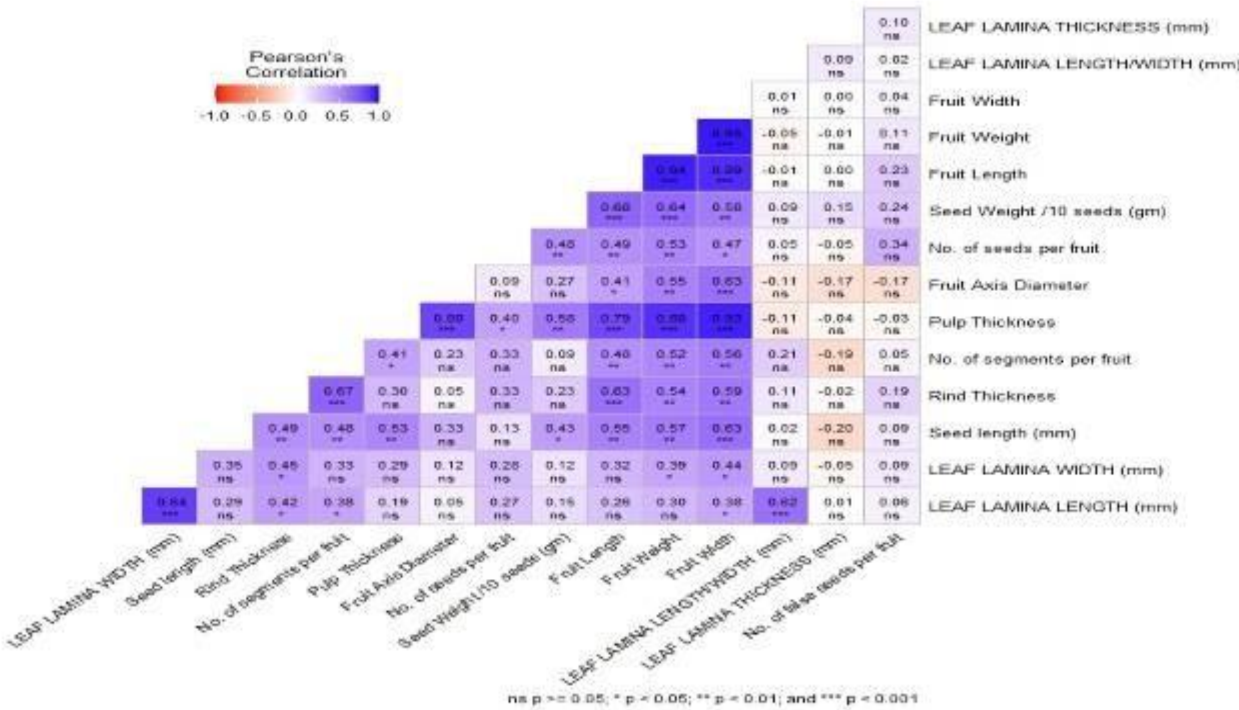


**Figure 2**. Pearson correlation between studied morphological quantitative traits of citrus hybrids and parents.

fruit weight ($r$ = 0.95, $P$ < 0.001), indicating that wider fruits tend to be heavier (Figure 2). Similarly, fruit length and fruit weight ($r$ = 0.89, $P$ < 0.001) exhibited a strong positive correlation, demonstrating longer fruits contribute significantly to higher weight and yield. A similar trend emerged between fruit length and fruit width ($r$ = 0.80, $P$ < 0.001), suggesting proportional growth in both dimensions.

Moderate positive correlations (0.5 < r < 0.8) were noticeable between seed weight (10 seeds) and fruit weight ($r$ = 0.68, $P$ < 0.001), indicating heavier seeds have an association with larger fruits. Pulp thickness and fruit weight ($r$ = 0.63, $P$ < 0.001) showed a strong association, suggesting thicker pulp contributes to higher fruit weight, a desirable trait for breeding programs. Additionally, a moderate relationship was evident between the number of segments per fruit and rind thickness ($r$ = 0.55, $P$ < 0.01), indicating that rind thickness may play a role in fruit segmentation (Figure 2).

**Cluster analysis**

Cluster analysis revealed two primary groups: Cluster A (13 hybrids) and Cluster B (14 hybrids), with hybrids in both clusters exhibiting superior leaf lamina length and width, fruit weight, fruit dimensions, and seed weight. However, the outstanding hybrid SCSH 3-9 attained a classification as an outgroup, suggesting distinct morphological and genetic characteristics.

**Principal component analysis**

The PCA biplot (Figure 3) explained 56.1% of total variance, with Dim1 (41.9%) primarily associated with fruit size and weight traits (e.g., fruit length, width, and weight, and seed weight). However, Dim2 (14.2%) represented variation in seed and rind traits (e.g., rind thickness, number of seeds, and false seeds). Genotypes positioned at the positive extreme of Dim1 (e.g., SCSH 3-10) exhibited larger fruit dimensions and higher weights, making

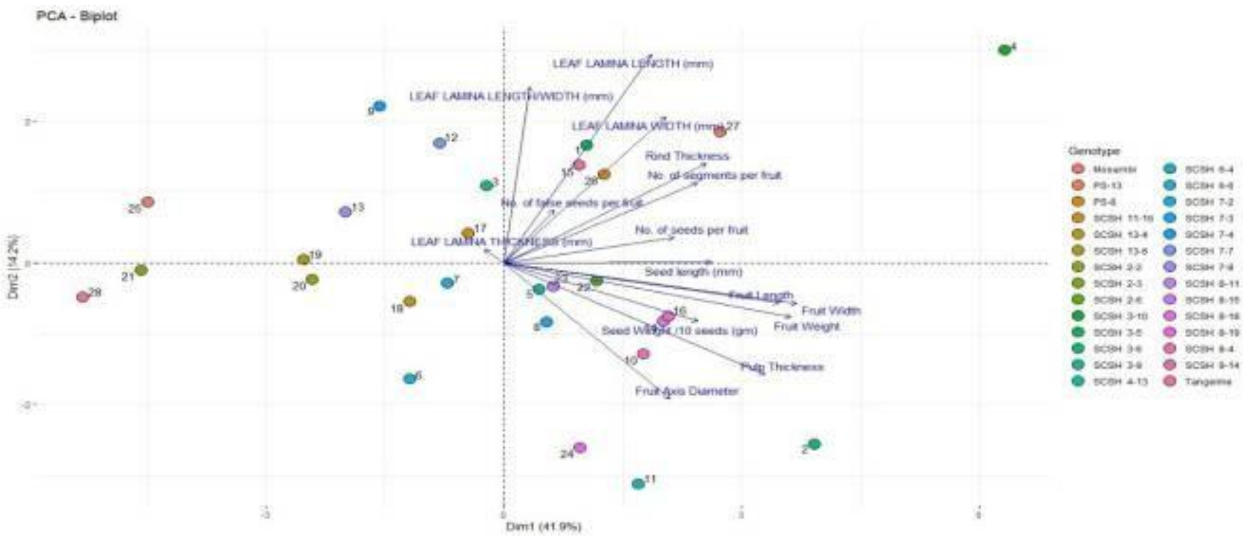


**Figure 3.** Principal component analysis of studied morphological traits of citrus hybrids and parents.

them ideal candidates for breeding high-yield citrus varieties. Meanwhile, Dim2 captured variability in seed and rind traits, with SCSH 6-4 characterized by thicker rinds and more seeds. Trait vectors closely aligned in the biplot (e.g., fruit length and fruit weight) indicated strong correlations, while orthogonality between leaf lamina dimensions and fruit traits suggested independent regulation of these characteristics.

**Heritability studies**

In this study, broad-sense heritability ($H^2$) was highest for fruit weight (82.4%), fruit length (79.6%), pulp thickness (75.3%), and seed weight (73.2%), indicating strong genetic influence and high potential for selection-driven improvement (Table 4). Traits, such as fruit width (68.9%), rind thickness (62.1%), and the number of segments per fruit (58.7%), exhibited moderate heritability, suggesting both genetic and environmental factors contribute to their expression. Genetic advance as a percentage of the mean (GAM) further supported the selection potential of high-heritability traits, with fruit weight (21.5%), fruit length (19.7%), and pulp thickness (16.8%) displaying the highest genetic gain. The moderate genetic advance observed in rind thickness (12.3%) signifies that while selection can lead to improvements, considering environmental variability is a must. The highest PCV observed came for the number of false seeds per fruit (81.89%), followed by fruit weight (50.23%), fruit axis diameter (49.59%), rind thickness (46.71%), and the number of seeds per fruit (46.47%). GCV was high (>40%) for traits such as the number of false seeds per fruit, fruit weight, fruit axis diameter, rind thickness, and the number of seeds per fruit (Table 4).

**DISCUSSION**

This study revealed significant variability in tree characteristics, including tree shape, growth habit, branch density, and branch angle, consistent with findings in other crops (Dubey *et al*., 2019; Siddique *et al*., 2022; Kumar *et al*., 2024). Moreover, a spreading branching pattern is more adaptable for cultivation and also for better growth of the tree. Among the hybrids, 70% showed a spreading growth habit. Variation in spine traits was remarkable, with parents Mosambi, PS-8, and Tangerine being spineless, while PS-13 exhibited low spine density. Among hybrids, 91.67% had straight spines, while SCSH 7-3, SCSH 7-4, and spineless parental genotypes lacked spine morphology, aligning with

**Table 4.** Estimation of descriptive statistics, heritability, and genetic advance of morphological character of citrus hybrids.

| Morphological parameters | Range | Mean | SEm | CV (5%) | GCV (%) | PCV (%) | $H^2$ (%) | GA | GA as % mean |
|---|---|---|---|---|---|---|---|---|---|
| Fruit Weight (g) | 108.72-1047.42 | 469.27 | 5.76 | 16.15 | 50.15 | 50.23 | 0.99 | 48.17 | 103.17 |
| Fruit Length (mm) | 47.37-136.87 | 96.70 | 1.99 | 5.58 | 21.76 | 22.24 | 0.95 | 42.41 | 43.86 |
| Fruit Width (mm) | 55.50-142.20 | 95.83 | 2.15 | 6.05 | 22.35 | 22.91 | 0.95 | 43.06 | 44.93 |
| Rind Thickness (mm) | 2.37-14.63 | 7.47 | 0.54 | 1.53 | 43.72 | 46.71 | 0.87 | 6.30 | 84.32 |
| Pulp Thickness (mm) | 48.93-110.43 | 80.86 | 1.71 | 4.79 | 21.70 | 22.71 | 0.95 | 35.33 | 43.69 |
| No. of Segments per Fruit | 8.00-14.60 | 12.03 | 0.43 | 1.21 | 12.61 | 14.96 | 0.70 | 2.63 | 21.88 |
| Fruit Axis Diameter (mm) | 6.26-34.75 | 19.11 | 0.84 | 2.37 | 48.59 | 49.59 | 0.96 | 18.74 | 98.07 |
| Leaf Lamina Length (mm) | 68.11-154.72 | 91.22 | 0.57 | 1.61 | 20.61 | 20.66 | 0.99 | 38.65 | 42.37 |
| Leaf Lamina Width (mm) | 45.66-86.16 | 54.86 | 0.39 | 1.11 | 15.78 | 15.86 | 0.98 | 17.74 | 32.34 |
| Leaf Lamina Length/Width | 1.34-1.95 | 1.65 | 0.01 | 0.04 | 11.20 | 11.38 | 0.96 | 0.37 | 22.71 |
| Leaf Lamina Thickness (mm) | 2.42-3.74 | 3.18 | 0.03 | 0.10 | 12.09 | 12.38 | 0.95 | 0.77 | 24.31 |
| No. of Seeds per Fruit | 6.60-70.20 | 37.91 | 1.44 | 4.03 | 45.69 | 46.47 | 0.96 | 35.08 | 92.53 |
| No. of False Seeds per Fruit | 0-11.00 | 3.80 | 0.37 | 1.03 | 78.93 | 81.89 | 0.92 | 5.95 | 156.73 |
| Seed Weight /10 seeds (g) | 1.10-2.92 | 2.04 | 0.07 | 0.21 | 24.32 | 25.68 | 0.89 | 0.97 | 47.45 |
| Seed Length (mm) | 12.10-17.93 | 15.42 | 0.26 | 0.74 | 10.72 | 11.39 | 0.88 | 3.20 | 20.77 |

SEm (standard error of mean), CV (coefficient of variation), GCV (genotypic coefficient of variation), PCV (phenotypic coefficient of variation), H2 (broad-sense heritability), GA (genetic advance), and GAM% (genetic advance as percentage of mean).

previous reports (Longkumer and Kabir, 2014; Kumar *et al*., 2024). Leaf traits displayed broad variation, with all hybrids and parents exhibiting evergreen characteristics and a 'brevipetiolate' attachment. The majority (75%) had ovate leaf lamina shapes, while Tangerine displayed an elliptic shape, and PS-8 and PS-13 exhibited ovate forms, mirroring previous findings (Kumar *et al*., 2024).

Fruit traits demonstrated extensive diversity, particularly in surface texture, color, fruit axis structure, and pulp composition. As 41.67% of hybrids had smooth surfaces, another 41.67% displayed rough textures, with the remainder showing bumpy or pitted surfaces. Mosambi had a smooth texture, PS-8 was bumpy, PS-13 was rough, and Tangerine had a pitted surface, contrasting with previous studies that reported a higher proportion of hybrids with smooth textures (Kumar *et al*., 2024). The predominant peel color among hybrids was green-yellow (RHS N-144A) in 83.33% of cases, consistent with prior findings (Kumar *et al*., 2024).

Significant differences in fruit weight succeeded in recording, with 10 hybrids and two parents producing fruits >500 g, while six hybrids and two parents produced fruits <300 g. The heaviest fruit resulted in SCSH 3-10 (1047 g). Notably, none of the parents fell into the medium fruit weight (300–500 g) category, whereas previous studies grouped pummelo parents in this range (Kumar *et al*., 2024). Rind and pulp thickness also showed substantial variation, with seven hybrids and the pummelo parents exhibiting rind thickness >10 mm. Reports of a similar pattern came from Kumar *et al*. (2024), emphasizing the genetic variability of this trait.

Seed traits demonstrated high heterozygosity, contributing to genotypic and phenotypic diversity (García-Lor *et al*., 2012; Wu *et al*., 2018). Most hybrids (95.83%) were monoembryonic, with SCSH 9-14 being the only polyembryonic hybrid. Among parents, Mosambi and Tangerine were polyembryonic, while pummelo parents were monoembryonic. These results contrast with Kumar *et al*. (2024), where 66.67% of hybrids were monoembryonic and 33.33% were polyembryonic, reflecting potential differences in parental combinations and hybridization outcomes. Morphological clustering only interprets the diversity; the hybrid SCSH 3-9 showed a distinct group. Moreover, morphological traits received influences from the environment, and it can affect expression.

Correlation studies revealed a strong positive association between fruit length, width, and weight, indicating larger fruits contribute to higher individual fruit weight and overall yield, a key concern for growers. The study is in agreement with previous studies of Kumar *et al.* (2018), where fruit length showed a significant correlation ($r$ = 0.54) with yield and other morphological parameters of *Ziziphus mauritiana.* Similarly, Abdel-Sattar *et al.* (2024) reported that larger fruits exhibited higher yields in different sweet orange (*Citrus sinensis*) cultivars. Neupane *et al.* (2023) found fruit length and fruit width have a significant positive correlation, and they are principal parameters to determine fruit weight, potentially finding an appropriate size-to-weight relationship in sweet orange cultivars. Likewise, several germplasm-characterization and path-analysis studies reported rind thickness and segments-per-fruit together and identified them as correlated or co-contributing traits to fruit weight and quality (Marboh *et al.* 2015; Nandi *et al.* 2019; Sekhar *et al.* 2022).

This study also identified key fruit and leaf traits with high heritability and genetic advance, making them ideal targets for breeding elite citrus cultivars. Morphological traits, such as fruit weight, fruit length, pulp thickness, fruit axis diameter, fruit width, leaf lamina length, and the number of seeds per fruit, exhibited high heritability and genetic advance, suggesting strong additive genetic effects with minimal environmental influence (Basawal *et al*., 2016; Rajae *et al*., 2019; Angami *et al*., 2022; Singh *et al*., 2022). Similarly, Khan *et al.* (2024) reported high heritability for fruit traits in sweet orange with broad-sense heritability, which allows for meaningful expected selections among the genotypes. The presented study aligns with findings of Hanyuan *et al.* (2024), where broad-sense heritability reported in citrus fruits revealed fruit diameter, fruit shape, peel thickness, and seed number exhibited high heritability. This indicates these traits possess a strong genetic influence on progeny and are amenable to selection-driven improvement. Variation occurred for most traits in all hybrids, which can be beneficial for the selection of superior genotypes in the future for breeding programs. Hybrids, such as SCSH 7-8 and SCSH 13-6, have proven superior for the fruit characters; also, other hybrids can be applicable for imparting other characters in progeny. These traits, being governed by additive genetic variance, respond well to selection, making them highly suitable for hybrid improvement programs.

## CONCLUSIONS

This study highlights the extensive genetic diversity in citrus hybrids, especially in fruit morphology, seed traits, and embryo development. The high heritability of important fruit traits indicates strong genetic control, making them valuable for breeding high-yielding, quality cultivars. Though a large variation occurred for almost all the traits, it helps for selection of superior genotypes. SCSH 7-8 and SCSH 13-6 emerged to be significant for ideal fruit weight and lesser seed content than one of the better parents. Tangerine and Mosambi both proved ideal as parents, but the addition of pummelo as a female parent has proven very significant to study. The use of such fruit is very rare due to its heavy fruit weight, larger trees with uneven tree shape, spiny character, higher seeds per fruit, and larger leaves. Although it has various health properties compared with Mosambi and Tangerine, being one of the primitive *Citrus* spp. Variation in polyembryony and seed characteristics suggests opportunities to develop seedless hybrids through targeted selection. Integrating molecular marker-assisted and genomic-selection approaches could further improve breeding efficiency and speed up elite citrus varieties' development.

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