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Morphological characterization of citrus cultivars in the subtropical region of Himachal Pradesh

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Abstract
The present investigation was conducted during 2019-2021 at the Experimental Farm and Laboratory of the Department of Fruit Science, College of Horticulture and Forestry, Neri, Hamirpur (Himachal Pradesh), to assess the morphological diversity among seven citrus cultivars, comprising mandarins and sweet oranges. A total of 32 qualitative traits related to foliage, fruits and seeds were characterized using standard descriptors developed by the International Plant Genetic Resources Institute (IPGRI). While certain traits like leaf division and seed embryony remained uniform across cultivars, considerable variability was observed in leaf shape, fruit apex, surface texture, albedo colour and seed shape. Distinctive features such as clavate seeds in Kinnow and W. Murcott and rounded fruit apices with yellow pulp in Mosambi and Valencia Late, highlighted cultivar-specific characteristics. These morphological differences facilitate field-level identification and provide a valuable resource for germplasm conservation and breeding programmes aimed at citrus improvement under subtropical conditions.

Keywords: Citrus, morphological diversity, qualitative characters, genetic variability, sweet orange, mandarin

Introduction
Citrus is one of the most important fruit crops globally, ranking third in total fruit production after banana and grapes (FAO, 2021) [8]. India, being part of its centre of origin, holds considerable genetic diversity in citrus, particularly in the North-Eastern Himalayan and Western Himalayan regions (Swingle and Reece, 1967; Singh and Chadha, 1993) [25, 21]. Among citrus species, mandarin (*Citrus reticulata* Blanco) and sweet orange (*Citrus sinensis* L. Osbeck) dominate the Indian citrus industry, contributing significantly to fruit production, processing industries and farmer livelihoods.

Citrus fruits are also valued for their high nutritional content, being excellent sources of vitamin C, carotenoids and bioactive antioxidants that help to reduce the risk of chronic diseases such as cardiovascular disorders, diabetes and certain cancers (Gil-Izquierdo *et al.*, 2001; Abd-Ghafar *et al.*, 2010) [9, 1]. Botanically classified as hesperidia, citrus fruits show remarkable variability in fruit morphology, rind colour, pulp texture and seed traits, making phenotypic characterization an essential tool for cultivar identification, breeding, conservation and germplasm management (IPGRI, 1999) [10].

With the increasing demand for quality planting material and high-density orchard systems, precise varietal identification and characterization have become critical. However, the highly heterozygous nature of citrus, presence of polyembryony, frequent bud mutations and intergeneric hybridization often complicate species delimitation and cultivar identification (Talon and Gmitter, 2008) [26]. In such cases, standardized morphological descriptors provide a practical and reliable means of distinguishing closely related cultivars, especially under diverse agro-climatic conditions.

Morphological characterization plays a crucial role in the assessment of genetic diversity, cultivar identification and improvement programmes. It offers a cost-effective and practical approach for evaluating phenotypic variability among citrus genotypes. Important traits such as tree habit, leaf morphology, fruit shape, rind texture, pulp characteristics and seed traits serve as reliable markers for distinguishing cultivars (Jaskani *et al.*, 2006; Khan *et al.*, 2008)

[11, 13]. Understanding morphological variation under specific environmental conditions helps growers to select suitable cultivars and enables breeders to identify promising parents for hybridization.

The present investigation was conducted to characterize the variability among seven citrus cultivars, including three cultivars of mandarin (Kinnow, Daisy, W. Murcott) and four cultivars of sweet orange (Blood Red Malta, Jaffa, Mosambi, Valencia Late), under the subtropical conditions of Himachal Pradesh. The study aimed to evaluate tree, foliage, fruit and seed traits using standardized IPGRI descriptors to identify cultivar-specific characteristics and assess phenotypic diversity. The findings will aid in effective germplasm management; guide cultivar selection and support breeding programmes adapted to specific agro-climatic conditions.

Materials and Methods

Planting material and site

The investigation was carried out during 2019-2021 at the Experimental Farm and Laboratory of the Department of Fruit Science, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh. It is situated in subtropical climate on geographical coordinates 31º41'47.6" N, 76º28'6.3" E and at an altitude of 650 m above mean sea level. The research work was conducted on three cultivars of mandarin (*Citrus reticulata* Blanco) namely Kinnow, Daisy and W. Murcott and four cultivars of sweet orange (*Citrus sinensis*), namely Blood Red Malta, Jaffa, Mosambi and Valencia Late. The trees were planted during the year 2016 under an Ultra High-Density Plantation (UHDP) system with a spacing of 2.5 × 2.0 m. Four plants of each cultivar that were uniform in growth characteristics, free from insect pests and diseases and growing under healthy conditions were selected for recording observations on various characteristics.

Morphological characterization

Morphological characterization was carried out based on 32 qualitative traits, following the Descriptors of Citrus by International Plant Genetic Resources Institute (IPGRI, 1999) [10], now renamed as Bioversity International. Morphological data pertaining to tree, foliage, fruit and seed traits were collected during two successive growing seasons.

Tree vegetative characters

Spine density was assessed by counting the number of spines per 50 cm of shoot length on five randomly selected shoots per tree and classified as absent, low, medium or high. Spine shape was recorded as curved or straight.

Foliage characters

Vegetative life cycle was classified as evergreen, deciduous or semi-persistent by visually observing the leaf retention pattern throughout the season. Ten leaves per tree were evaluated for leaf division (simple to pentafoliate), intensity of green colour (light to dark), lamina attachment (sessile, brevipetiolate or longipetiolate) and shape (elliptic to obcordate). Leaf margins were categorized as crenate, dentate, entire or sinuate, while the shape of leaf apex ranged from attenuate to emarginate. Presence or absence of petiole wings was also recorded.

Fruit characters

Five fully mature fruits from each plant were evaluated for fruit shape (spheroid to ovoid), base (necked to concave-collared) and apex (mammiform to depressed). Fruit skin colour was recorded using a 12-point colour scale (green to red-orange). Fruit surface texture was scored as smooth, rough, papillate, pitted, bumpy or grooved. Density of oil glands was calculated by counting the number of oil glands per square centimetre on five fruit surfaces per replication and categorized as low, intermediate or high. Albedo colour and presence of areola was recorded based on visual inspection. Adherence of segment walls was determined based on the ease of segment separation and classified as weak, medium or strong. Segment wall thickness, fruit axis (solid to hollow), pulp colour (white to purple) and pulp firmness (soft to firm) were also evaluated visually.

Seed characters

Ten seeds per replication were analysed for shape (fusiform to semi-spheroid), surface texture (smooth to hairy) and colour (white to brown). Seed embryony was assessed by dissecting seeds and classifying them as monoembryonic, polyembryonic or mixed.

Results and Discussion

The morphological characterization of seven citrus cultivars was carried out to evaluate the phenotypic variability in tree, foliage, fruit and seed traits. The results presented here are based on observations recorded over two consecutive years and are discussed in relation to existing descriptors and relevant literature.

Tree vegetative characters

Among the seven citrus cultivars evaluated, low spine density was observed in Kinnow, Blood Red Malta, Jaffa, Mosambi and Valencia Late, while spines were completely absent in Daisy and W. Murcott (Table 1). No variation was recorded in spine shape among the mandarin and sweet orange cultivars. These results are in line with the earlier observations of Singh (2006) [23] and Singh *et al.* (2010) [22].

Foliage characters

The citrus cultivars under study exhibited both uniformity and subtle variation in their foliage traits (Table 2). All cultivars showed an evergreen growth habit, with simple leaf division and brevipetiolate leaf lamina attachment. Petiole wings were present in all cultivars. These results are consistent with earlier findings reported by Khan *et al.* (2008) [13], Talukder *et al.* (2015) [27] and Kaur *et al.* (2016) [12].

In terms of leaf blade colour, slight variability was observed. Kinnow, Blood Red Malta, Jaffa, Mosambi and Valencia Late exhibited a dark green colour, whereas Daisy and W. Murcott had medium green foliage. Jaskani *et al.* (2006) [11] reported comparable results, recording dark green leaf blades in Bitter sweet orange and Sour orange and medium green in Yuma citrange.

Leaf lamina shape exhibited moderate diversity among cultivars. W. Murcott, Blood Red Malta, Jaffa and Valencia Late were characterized by an elliptic lamina, while Daisy and Mosambi had ovate-shaped leaves. Kinnow was distinct in having a lanceolate leaf shape. Similar variation in leaf form was documented by Marboh *et al.* (2015) [15] in different Indian citrus genotypes and by Egbadzor *et al.*

(2022) [7] in a morphological assessment of citrus accessions in Ghana.

Variations were also recorded in the leaf margin and apex shape. Dentate leaf margins were observed in Kinnow, Daisy, W. Murcott and Jaffa; crenate margins were found in Blood Red Malta and sinuate leaf margins in Mosambi and Valencia Late. These patterns align with Dubey *et al.* (2019) [6], who described similar margin types in *Citrus maxima* accessions. The leaf apex was predominantly acute across all cultivars, except for Mosambi, which exhibited an obtuse apex. Similar trends were documented by Nurzaman *et al.* (2024) [17] in a phenotypic analysis of citrus genotypes.

Fruit characters

The analysis of fruit-related traits across the citrus cultivars revealed noticeable variability in several morphological characteristics, while some traits remained consistent among the cultivars.

The overall fruit shape was found to be spheroid in all the cultivars except W. Murcott, where fruits were obolid (Table 3). Fruit base was reported as truncate in all the cultivars except for Daisy where it was recorded as convex in shape. The fruit apex was reported as truncate in Kinnow, Daisy, W. Murcott, Blood Red Malta and Jaffa, while rounded apices were found in Mosambi and Valencia Late. Similar findings were reported by Altaf *et al.* (2014) [3] and Sunaiana *et al.* (2018) [24].

Fruit skin colour (epicarp) varied among the cultivars. Kinnow exhibited a typical orange skin colour, Daisy and W. Murcott had dark orange colour, while light orange colour was observed in Blood Red Malta, Jaffa, Mosambi and Valencia Late. These observations correspond with the findings by Patel *et al.* (2016) [18], who reported diverse skin colours in both sweet orange and mandarin groups. Surface texture exhibited moderate variation. Fruits of Kinnow, Daisy and W. Murcott exhibited smooth rind texture, whereas Blood Red Malta was characterized by a distinctly rough and uneven rind. Pitted textures were observed in Jaffa, Mosambi and Valencia Late. Dorji and Yapwattanaphun (2011) [5] similarly reported smooth-skinned fruits in mandarins, while Usman *et al.* (2020) [28] observed smooth texture across all studied grapefruit cultivars.

No significant variation was observed in oil gland density or presence of areola. All cultivars had a low density of oil glands (<40/cm^2) and an areola was consistently present. These findings are in agreement with those of Baswal *et al.* (2015) [4] and Sunaiana *et al.* (2018) [24], who reported similar traits in sweet orange cultivars. Albedo (mesocarp) colour showed slight variability. White coloured albedo was observed in all the cultivars except for W. Murcott where it was recorded as orange in colour. These results support earlier findings by Jaskani *et al.* (2006) [11].

Segment-related traits also varied among cultivars. Kinnow, Daisy and W. Murcott had medium adherence between segment walls, while strong adherence was seen in Blood Red Malta, Jaffa, Mosambi and Valencia Late (Table 4). Thin segment walls were present in the former group, whereas the latter cultivars had medium-thick segment walls. Fruit axis was solid in Kinnow, Daisy and Valencia Late and semi-hollow in the remaining cultivars. These findings align with previous studies by Talukder *et al.* (2015) [27], Singh *et al.* (2016) [20] and Raheb *et al.* (2018) [19], who documented similar segment traits in citrus varieties.

Pulp characteristics also showed variation. Pulp (flesh) colour was orange in Kinnow, Daisy and W. Murcott; orange-red in Blood Red Malta and yellow in Jaffa, Mosambi and Valencia Late. Pulp firmness was soft in the mandarin cultivars and intermediate in the remaining cultivars. These results align with those of Usman *et al.* (2020) [28] and Zacarías-García *et al.* (2021) [31], who reported comparable differences in pulp colour and firmness in different citrus cultivars.

Among all the traits evaluated, fruit morphology exhibited the greatest degree of variability, underscoring its significance in differentiating citrus cultivars.

Seed characters

The citrus cultivars studied exhibited considerable diversity in seed morphological traits. Seed shape varied across the cultivars: Kinnow and W. Murcott had clavate shaped seeds, Daisy and Valencia Late were characterized by semi-deltoid seeds, while Blood Red Malta, Jaffa and Mosambi had ovoid seeds (Table 5). These differences highlight the morphological diversity present among the evaluated citrus cultivars. Seed surface texture also varied among cultivars. A smooth surface was observed in Kinnow, Daisy and W. Murcott, whereas the remaining cultivars had a wrinkled seed surface. These results are in agreement with those reported by Malik *et al.* (2012) [14] and Ahmed *et al.* (2015) [2].

In contrast to shape and surface texture, no variation was observed in seed colour and embryony among the studied cultivars. All cultivars consistently had cream-coloured seeds that were polyembryonic in nature. This aligns with genomic evidence from Wang *et al.* (2022) [29], which highlights widespread nucellar embryony in cultivated citrus. Woo *et al.* (2019) [30] and Martínez-Ochoa *et al.* (2022) [16] also documented consistent polyembryony in sweet orange accessions.

Table 1: Spine Characteristics of Citrus Cultivars

Cultivars	Spine density on adult tree	Spine shape
Kinnow	Low	Straight
Daisy	Absent	-
W. Murcott	Absent	-
Blood Red Malta	Low	Straight
Jaffa	Low	Straight
Mosambi	Low	Straight
Valencia Late	Low	Straight

Table 2: Leaf Morphological Traits of Citrus Cultivars

Cultivars	Vegetative life cycle	Leaf division	Intensity of green colour of leaf blade	Leaf lamina attachment	Leaf lamina shape	Leaf lamina margin	Leaf apex	Absence/presence of petiole wings
Kinnow	Evergreen	Simple	Dark	Brevipetiolate	Lanceolate	Dentate	Acute	Present
Daisy	Evergreen	Simple	Medium	Brevipetiolate	Ovate	Dentate	Acute	Present
W. Murcott	Evergreen	Simple	Medium	Brevipetiolate	Elliptic	Dentate	Acute	Present
Blood Red Malta	Evergreen	Simple	Dark	Brevipetiolate	Elliptic	Crenate	Acute	Present
Jaffa	Evergreen	Simple	Dark	Brevipetiolate	Elliptic	Dentate	Acute	Present
Mosambi	Evergreen	Simple	Dark	Brevipetiolate	Ovate	Sinuate	Obtuse	Present
Valencia Late	Evergreen	Simple	Dark	Brevipetiolate	Elliptic	Sinuate	Acute	Present

Table 3: Fruit Morphological Traits of Citrus Cultivars

Cultivars	Fruit shape	Shape of fruit base	Shape of fruit apex	Fruit skin (epicarp) colour	Fruit surface texture	Density of oil gland on fruit surface	Albedo colour	Absence/presence of areola
Kinnow	Spheroid	Truncate	Truncate	Orange	Smooth	Low (<40/cm^2)	White	Present
Daisy	Spheroid	Convex	Truncate	Dark Orange	Smooth	Low (<40/cm^2)	White	Present
W. Murcott	Obloid	Truncate	Truncate	Dark Orange	Smooth	Low (<40/cm^2)	Orange	Present
Blood Red Malta	Spheroid	Truncate	Truncate	Light Orange	Bumpy	Low (<40/cm^2)	White	Present
Jaffa	Spheroid	Truncate	Truncate	Light Orange	Pitted	Low (<40/cm^2)	White	Present
Mosambi	Spheroid	Truncate	Rounded	Light Orange	Pitted	Low (<40/cm^2)	White	Present
Valencia Late	Spheroid	Truncate	Rounded	Light Orange	Pitted	Low (<40/cm^2)	White	Present

Table 4: Segment and Pulp Characteristics of Citrus Cultivars

Cultivars	Adherence of segment walls to each other	Thickness of segment walls	Fruit axis	Pulp (flesh) colour	Pulp firmness
Kinnow	Medium	Thin	Solid	Orange	Soft
Daisy	Medium	Thin	Solid	Orange	Soft
W. Murcott	Medium	Thin	Semi-hollow	Orange	Soft
Blood Red Malta	Strong	Medium	Semi-hollow	Orange Red	Intermediate
Jaffa	Strong	Medium	Semi-hollow	Yellow	Intermediate
Mosambi	Strong	Medium	Semi-hollow	Yellow	Intermediate
Valencia Late	Strong	Medium	Solid	Yellow	Intermediate

Table 5: Seed Morphology and Embryony of Citrus Cultivars

Cultivars	Seed shape	Seed surface	Seed colour	Seed embryony
Kinnow	Clavate	Smooth	Cream	Polyembryonic
Daisy	Semi-deltoid	Smooth	Cream	Polyembryonic
W. Murcott	Clavate	Smooth	Cream	Polyembryonic
Blood Red Malta	Ovoid	Wrinkled	Cream	Polyembryonic
Jaffa	Ovoid	Wrinkled	Cream	Polyembryonic
Mosambi	Ovoid	Wrinkled	Cream	Polyembryonic
Valencia Late	Semi-deltoid	Wrinkled	Cream	Polyembryonic

Conclusion

The study revealed considerable morphological variability among the evaluated citrus cultivars, particularly in traits related to leaves, fruits and seeds, whereas certain vegetative characteristics remained largely uniform. These phenotypic variations hold practical significance for cultivar identification, trait-based selection and crop improvement programmes. Morphological traits, being simple and field-adaptable, provide an effective tool for preliminary screening and genetic resource management. The diversity observed, especially in commercially relevant traits such as fruit skin and pulp colour, can be utilised to select potential donors for future citrus breeding initiatives.

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