Morphological Characterization of citrus leaves (*Citrus species*) under sub-tropical conditions of Punjab, India

ABSTRACT

A field experiment was conducted during 2019-2021 at Sri Guru Granth Sahib World University, Fatehgarh sahib, Punjab to study the morphological characterization of citrus leaves (*Citrus* species) under sub-tropical conditions of Punjab. For morphological studies, leaves samples collected from the Citrus orchard. Samples were randomly collected from 10 leaves samples which were randomly collected from 5 plants in order to obtain a representative sample. All leaves sample were observed for integrity and absence of dust and insect contamination. The leaves of six different citrus species were used for the morphological characterization. Morphological characters of citrus accessions were observed on basis of UPOV (International Union for the Protection of New Varieties of Plants) descriptors. The experimental results revealed that there were significant variations for morphological characterization in different citrus species. There are no variations in vegetative life cycle, leaf division, leaf colour, leaf lamina attachment in lime, lemon, pummelo, mandarin, grapefruit and sweet orange leaves. Vegetative life cycle was evergreen and leaf division was found to be simple in all the six citrus species. However, maximum mean leaf lamina length, leaf lamina width and leaf lamina length to width ratio was recorded in pummelo followed by grapefruit and mandarin. The maximum leaf area was observed in pummelo which was statistically at par with grapefruit, mandarin, sweet orange, lemon. Thus, there was no significant variation morphological characterization among citrus leaves.

Keywords: Citrus, Morphological character, Lemon, Mandarin, Grapefruit, Leaf division, Leaf colour.

1. Introduction:

Citrus is the third largest fruit crop in the world (Singh and Rajam, 2009) and is commercially grown in tropical and subtropical regions (Wu et al., 2018). It is known for its flavor, attractive evergreen foliage and flower, and extraordinary fragrance, which add aesthetic value. It is the third largest fruit crop in the world after apples and

bananas (Singh and Rajam, 2009). It is a long- lived perennial crop and is cultivated in more than 100 countries around the world (Saunt, 1990). This genus may be further divided into two subgenera (Citrus and Papeda), based on leaf, flower and fruit properties. Swingle and Reece (1967) opined that the genus *Citrus* has only three 'basic' true species *viz.* citron (*Citrus medica* L.), mandarin (*Citrus reticulata* Blanco) and pummelo (*Citrus grandis* Osbeck), while the rest of the species are hybrid derivatives of any one of the true species and species belonging to sub genus papeda (Nicolosi *et al* 2000).

In India, citrus fruit is cultivated in an area of 1,034 thousand hectares with an annual production of 13,200 thousand tons and in Punjab, it is cultivated in an area of 59,980 hectares with an annual harvest of 13, 49,523 tonnes (NHB, 2018-2019). Citrus fruits are an essential dietary supplement used in several countries around the world and it is taken in the form of processed juice, beverages and fresh fruit (Kumar *et al* 2013). Citrus have a potent source of significant bioactive secondary metabolites having antioxidant, lipid anti-peroxidation activities and anti-inflammatory activities. Citrus is not only the source of phenolic compounds and flavonoids but citrus leaves are a significant source of bioactive compounds including antioxidants such as ascorbic acid, phenolic and flavonoid compounds, minerals, vitamins including macronutrients and micronutrients (Buachan *et al*., 2014). Citrus flavonoid compounds have anticancer and anti-inflammatory properties (Benavente-Garcia and Castillo, 2008; Germana *et al* 2020).

Sweet orange (*C. sinensis*) is grown worldwide and its leaves are generally used in treatment of infections one which is the typhoid fever. It has antibacterial, anti-inflammatory, antiparasitic, antiproliferative, antifungal activity and antioxidant properties (Juan *et al* 2016; Arora *et al* 2013; Adamu *et al* 2020) and also the leaves of *C. aurantifolia* have been used conventionally for skin care, relief constipation, weight loss and treatment of scurvy, etc. (Namani *et al* 2018). Citrus flavonoids possess biological and healthy effects as antioxidants as reported (Tripoli *et al* 2007). Citrus flavonoids can prevent cancer through selective apoptosis, cytotoxicity and antiproliferative actions. Acetone and hexane extracts of *C. sinensis* leaf exhibited inhibition zones of 27mm towards Helicobacter pylori (Juan *et al* 2016). Thus, keeping the above fact in view, an experiment was conducted to study the morphological characterization of leaves in different citrus species.

2. Materials and Method

A field experiment was conducted during 2019-2021 at Sri Guru Granth Sahib World University, Fatehgarh sahib, Punjab. Geographically, the experimental site is located at an elevation of 246 meters above the mean sea level. (AMSL) which is having a value of 30.60° N latitude and 76.39° E longitude. For morphological studies, leaves samples were collected from the Citrus orchard which is well maintained and followed the package and practices as recommended by Punjab Agricultural University for growth and development. Samples were randomly collected from 10 leaves samples which were randomly collected from 5 plants in order to obtain a representative sample. All leaves sample were observed for integrity and absence of dust and insect contamination. The leaves of six different citrus species were used for the morphological characterization. Morphological characters of citrus accessions were observed on basis of UPOV (International Union for the Protection of New Varieties of Plants) descriptors (Anonymous, 1999).

For statistical analysis, Pearson correlation coefficients were calculated with the SPSS version 15.0 software (SPSS Inc., Chicago, USA). Data from the correlation matrix was represented as a heat map by means of the Acuity 4.0 program.

3. Results and discussion

Morphological characterization of different citrus species

3.1 Leaf characterization on the basis of qualitative characters

Data pertaining to qualitative leaf characters viz., leaf lamina shape, leaf lamina margin, absence/presence of petiole wing and petiole wing shape are presented in Table 2 revealed that there was no variation for vegetative life cycle, leaf division, leaf colour, leaf lamina attachment in lime, lemon, pummelo, mandarin, grapefruit and sweet orange leaves. Vegetative life cycle was evergreen and leaf division was found to be simple in all the six citrus species. Similar results were reported for vegetative life cycle to be evergreen by Singh, (2010) in Red Fleshed Pummelo, Mosambi and grapefruit Saharanpur Special. Similarly, Khan *et al* (2008) reported simple leaf division in Kinnow mandarin and Feutrell's Early. Kumar *et al* (2011) also reported simple unifoliate leaves in box orange, Cleopatra mandarin, Pectinifera, Rangpur Lime, Karun Jamir, Rough Lemon and Billi Kitchli and trifoliate in case of Carrizo and Troyer citrange. Leaf division was simple among six different strains of Rangpur Lime as

reported by Singh *et al* (2010).

Data presented in Table 1 also revealed that there was no variation for intensity of green colour of leaf blade recorded in the five citrus species *viz.* lemon, sweet orange, mandarin, grapefruit and pummelo. While only, in lime the intensity of green colour of leaf blade was medium in colour. Dark green intensity was observed of the leaf blade in all the other five citrus species. Singh, (2006) reported medium to dark leaf colour intensity in Trifoliate orange and Red Fleshed pummelo, respectively.

The data presented in Table 1 revealed that there is variation in all citrus species for leaf lamina shape. Ovate shape of leaf lamina was observed in lemon, sweet orange and grapefruit leaf while obovate leaf lamina shape was recorded in lime and pummelo leaf. In mandarin leaf, lanceolate shape of leaf lamina was observed. Similar results were reported by Estellana and Odtojan, 1992. Further, Little variation was observed in all six citrus species for leaf lamina margin. Crenate shape of leaf lamina margin was observed in lime, sweet orange and lemon leaf. While dentate leaf lamina margin was there in all other three citrus species leaf. Likewise, among grapefruit, mandarin and pummelo, no variation was observed for leaf lamina margin among these three citrus species. Acute leaf apex was detected in mandarin, lime and lemon leaf whereas, rounded leaf apex was recorded in pummelo leaf. Similar results were reported by Khan *et al* (2008). Petiole wing was absent in the lime, sweet orange, mandarin and lemon leaf. Likewise, petiole wing was present in all other citrus species (grapefruit and pummelo). Similar results were reported by reported by Jaskani *et al* (2006) and Khan *et al* (2010).

Table 1. Leaf characterization on the basis of Qualitative leaf characters of different citrus Species

S. No.	Leaf Characters	Lemon	Grapefruit	Mandarin	Pummelo	Lime	Sweet orange
1	**Vegetative life cycle**	Evergreen	Evergreen	Evergreen	Evergreen	Evergreen	Evergreen
2	**Leaf division**	Simple	Simple	Simple	Simple	Simple	Simple
3	**Intensity of green colour of leaf blade**	Dark	Dark	Dark	Dark	Medium	Dark
4	**Leaf colour variegation**	Absent	Absent	Absent	Absent	Absent	Absent
5	**Leaf lamina attachment**	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate
6	**Leaf lamina shape**	Ovate	Ovate	Lanceolate	Obovate	Obovate	Ovate
7	**Leaf lamina margin**	Crenate	Dentate	Dentate	Dentate	Crenate	Crenate
8	**Leaf apex**	Acute	Attenuate	Acute	Rounded	Acute	Attenuate
9	**Absence/presence of petiole wings**	Absent	Present	Absent	Present	Absent	Absent
10	**Petiole wing shape**	Absent	Obdeltate	Absent	Obcordate	Absent	Absent
11	**Junction between petiole and lamina**	Articulate	Articulate	Articulate	Articulate	Articulate	Articulate

3.2 Leaf characterization on the basis of qualitative characters

The data presented in Table 2 revealed that significant variation in leaf lamina length, width, leaf lamina length to width ratio was observed among all selected citrus species (sweet orange, mandarin, grapefruit, lime, pummelo and lemon. The maximum mean leaf lamina length (111.8 mm), leaf lamina width (64.7mm) and leaf lamina length to width ratio (1.73mm) was recorded in pummelo followed by grapefruit and mandarin. Similar results were reported by Singh *et al* (2010) in six Rangpur Lime. These variations were exhibited due to environment and genotype interaction. Similar results were reported by Dorji and Yapwattanaphun (2011), Singh *et al* (2010) in six Nagpur lime.

The data pertaining to the leaf area of citrus varieties revealed that maximum leaf area (43.2 cm^2) was observed in pummelo which was statistically at par with grapefruit (34.3 cm^2), mandarin, sweet orange, lemon. Similar variation in leaf area was reported by Ahmed *et al* (2019). Significant variation was observed in petiole wing width among six citrus species. The maximum mean petiole wing width was observed by grapefruit (19.6) which was statistically at per with pummelo (19.3) Similar results was reported by Rahman *et al* (2003) in thirty local pummelo accessions and found that the accession number five had the highest petiole wing width, while accession number seventeen had the minimum petiole wing width. Moreover, Ahmed *et al* (2019) reported that the Petiole wing width was maximum in Devanpalli variety of grapefruit species with 27.4 mm and minimum in CHS Pink variety of Pummelo with 8.9 mm.

Table 2: Leaf characterization on the basis of Quantitative characters of different citrus species

S. No.	Citrus species	Leaf lamina length (mm)	Leaf lamina width (mm)	Leaf lamina length: width ratio (mm)	Leaf area (cm^2)	Petiole wing width (mm)
1.	**Sweet orange**	89.4	59.41	1.5	26.55	Absent
2.	**Mandarin**	90.66	60.3	1.5	33.45	Absent
3.	**Lemon**	82.4	55.15	1.49	25.5	Absent
4.	**Pummelo**	111.8	64.7	1.73	43.2	19.3

5.	**Lime**	81.3	49.2	1.65	24.8	Absent
6.	**Grapefruit**	101.7	61.7	1.65	34.3	19.6
C.D.		0.202	0.248	0.155	0.125	0.227
SE(m)		0.063	0.078	0.049	0.039	0.035

Conclusion

On the basis of experiment studies, it was concluded that there were significant variations for morphological characterization in different citrus species. There is no variation for vegetative life cycle, leaf division, leaf colour, leaf lamina attachment in lime, lemon, pummelo, mandarin, grapefruit and sweet orange leaves. Vegetative life cycle was evergreen and leaf division was found to be simple in all the six citrus species. However, maximum mean leaf lamina length, leaf lamina width and leaf lamina length to width ratio was recorded in pummelo followed by grapefruit and mandarin. The maximum leaf area was observed in pummelo which was statistically at par with grapefruit, mandarin, sweet orange, lemon. Thus, there was no significant variation morphological characterization among citrus leaves.

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5. References:

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