

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

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### 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 (Anonymous, 1999). 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.

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**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) which is grown commercially in both tropical and subtropical regions of the world (Wu *et al.*, 2018) and is known worldwide for its characteristic flavour, attractive

evergreen foliage and flower as well as extraordinary fragrance, which are added aesthetic values (Rabha *et al* 2013). 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).

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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 ~~is~~are an essential dietary supplements 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*) ~~are~~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

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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).



### 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<sup>2</sup>) was observed in pummelo which was statistically at par with grapefruit (34.3 cm<sup>2</sup>), 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 par with pummelo (19.3) Similar results were 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 no. 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 <sup>2</sup> )	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

<b>5.</b>	<b>Lime</b>	81.3	49.2	1.65	24.8	Absent
<b>6.</b>	<b>Grapefruit</b>	101.7	61.7	1.65	34.3	19.6
<b>C.D.</b>		0.202	0.248	0.155	0.125	0.227
<b>SE(m)</b>		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.

## 5. References:

- Adamu U, Yusha'u M, Bahauddeen S and Hussain A M (2020). Phytochemical screening, antibacterial potentials and gas chromatography-mass spectrometry analysis (GC-MS) of *Citrus sinensis* leaves extracts. *Microbes Infectious Diseases*: Article In Press, DOI: 10.21608/MID.2020.32010.1019.
- Ahmed S, Rattanpal H S, Gul K, Dar A R and Sharma A (2019). Chemical composition, antioxidant activity and GC-MS analysis of juice and peel oil of grapefruit varieties cultivated in India. *Journal of Integrative Agriculture* **18**(7): 1634-1642.
- Anonymous (1999) Descriptors of Citrus. International Plant Genetic Resource Institute. Rome, Italy. [Available from <http://www.cgiar.org/ipgri/>].
- Anonymous (2020). Area and Production of fruit in Punjab. *Package and Practices for Cultivation of Vegetable Crops*. Punjab Agricultural University, Ludhiana pp 1-1
- Arora M and Kaur P (2013). Antimicrobial & antioxidant activity of orange pulp and peel. *International Journal of Science and Research* **2**(1): 412-415.
- Benavente-Garcia O and Castillo J (2008). Update on uses and properties of Citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. *Journal of Agricultural and Food Chemistry* **56**(15): 6185–6205.
- Buachan P, Chularojmontri L and Wattanapitayakul S (2014). Selected activities of *Citrus maxima* merr. Fruits on human endothelial cells: enhancing cell migration and delaying cellular aging. *Nutrients* **6**(4):1618-1634.
- Dorji K and Yapwattanaphun C (2011). Morphological identification of mandarin (*Citrus reticulata* Blanco) in Bhutan. *Kasetsart Journal (Natural Science)* **45**(5): 793-802.
- Estellena N T and Odtojan R C (1992). Pomological characterization of eight pummelo cultivars, *Citrus maxima* (Burm.) Merr. *Philippine Journal of Crop Science* **17**(3): 137-42.
- Germana M A, Aleza P, Grosser J W, Dutt M, Wang N, Cuenca J and Kaur P (2020). Citrus biotechnology. *In The Genus Citrus*. Woodhead Publishing: 171-192.
- Jaskani M J, Haider A, Khan M M, Shahzad U and Hussain Z (2006). Morphological description of three potential Citrus rootstocks. *Pakistan Journal of Botany* **38**(2): 311-317.



- Juan M J F, Omar G, Monica A R, Patricia C E and Maria D C (2016). Chemistry and pharmacology of *Citrus sinensis*. *Molecules* **21**:247.
- Khan M M, Mumtaz S, Ahmad S, Abbas M and Khan I A (2008). Some studies on the morphology of Kinnow mandarin and Feutrell's Early. *Pakistan Journal of Agricultural Sciences* **45**(4): 424-431.
- Kumar M, Parthiban S, Sarala Devi D and Ponnuswami V (2013). Genetic diversity analysis of acid lime (*Citrus aurantifolia* Swingle) cultivars. *The Bioscan* **8**(2): 481-484.
- Kumar R, Vijay S and Khan N (2011). Comparative nutritional analysis and antioxidant activity of fruit juices of some Citrus spp. *Octa Journal of Biosciences* **1**(1): 44-53.
- Namani Al J, Baqir E, Abri Al A, Hubaishi T Al, Husain A and KHAN S A (2018). Phytochemical screening, phenolic content and antioxidant activity of *Citrus aurantifolia* L. Leaves grown in two regions of Oman. *Iranian Journal of Pharmaceutical Sciences* **14**(1): 27-34.
- NHB. (2018-2019). Fruit Production Database *National Horticulture Board*, New Delhi: India (Available online: [www.nhb.in](http://www.nhb.in)).
- Nicolosi E, Deng ZN, Gentile A, La Malfa S, Continella G and Tribulato E (2000). Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theoretical and Applied Genetics* **100**(8): 1155-66.
- Rabha A, Wangchu L and Singh B (2013). Studies on genetic diversity of citrus in East Siang district of Arunachal Pradesh. *International Journal of Agriculture Environment and Biotechnology* **6** (1): 131-137.
- Rahman M M, Rabbani M G, Khan A S M M R, Ara N and Rahman M O (2003). Study on physico-morphological characteristics of different local pummelo accessions. *Pakistan Journal of Biological Sciences* **6**(16): 1430-1434.
- Saunt J (1990). Citrus varieties of the world. *Sinclair International Ltd*: 49-61.
- Singh H, Rattanpal H S, Sidhu G S and Chahal T S (2010). Study on physio-morphological characteristics among six Rangpur Lime (*Citrus limonia* Osbeck.) strains. *Journal of Tree Sciences* **29**(1&2): 48-56.
- Singh S and Rajam M V. (2009). Citrus biotechnology: achievements, limitations and future directions. *Physiology and Molecular Biology of Plants* **15**(1): 3-22.

Swingle W T and Reece P C (1967). The botany of Citrus and its wild relatives. In: W Reuther, H J Webber and L D Batchelor (Eds.), *The Citrus industry*, Vol 1 (rev). University of California Press, Berkley, California: 190-430.

Tripoli E, La Guardia M, Giammanco S, Di Majo D and Giammanco M (2007). Citrus flavonoids Molecular structure, biological activity and nutritional properties: A review *Food Chemistry* **104**(2): 466–479.

Wu G A, Terol J, Ibanez V, López-García A, Pérez-Román E, Borredá C, Domingo C, Tadeo F R, Carbonell-Caballero J, Alonso R and Curk F (2018). Genomics of the origin and evolution of Citrus. *Nature* **554**(7692): 311-6.

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