

Macro and micro morphological features of *Citrus reticulata* Blanco cultivated in eastern Libya

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Abstract:

Citrus reticulata Blanco is economically and medically important and cultivated in Al-Marj city (Libya). In this study, the morphological, epidermal and anatomical characteristics of *Citrus reticulata* were considered in a laboratory experiment through staining of cut sections and microscope visualization with the aim of providing detailed description of characteristics of this plant species. Morphological characteristics of leaves, fruits, and seed are very important in comparing between *Citrus* species. Epidermal attributes such as a variety of cell shapes, hypostomatic, anamocytic stomata, and the presence of secretory cavities on the upper surface of the epidermis are valuable characteristics in distinguishing *Citrus* species. Also, the mesophyll structure, the shape of vascular bundle, the presence of druses and the secretory cavities features have taxonomic value in the *Citrus* genus. Additionally, the petiole features, the presence of trichomes and druses and characteristics of the vascular bundles are important for determining phylogenetic relationships between *Citrus* species.

Key words: *Citrus reticulata*, morphology, anatomy, secretory cavities and druses, Al- Marj (Libya).

الصفات الظاهرية والدقيقة لـ *Citrus reticulata* Blanco المزروع

في شرق ليبيا

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الملخص

نبات اليوسفي *Citrusreticulata* نبات ذو أهمية اقتصادية و طبية و يزرع في مدينة المرج (ليبيا). تم في هذه الدراسة، فحص الصفات الظاهرية و التشريحية و صفات البشرة لنبات اليوسفي بواسطة تجربة مختبرية من خلال صبغ المقاطع و التصوير المجهرى بهدف تقديم وصف تفصيلي لصفات هذا النوع النباتي. تعتبر الصفات الظاهرية للأوراق، و الفواكه و البذور مهمة جدا في المقارنة بين أنواع الحمضيات. تعد صفات البشرة مثل التنوع في أشكال الخلايا، و وجود الثغور في البشرة السفلية، و الثغور عديمة الخلايا، و وجود تجاويف إفرازية على السطح العلوي للبشرة من الصفات القيمة في التمييز بين أنواع الحمضيات. كذلك النسيج الوسطي للورقة، و شكل الحزم الوعائية، و وجود الدروز و التجاويف الإفرازية، هذه الصفات لها قيمة تصنيفية في جنس الحمضيات. تعتبر صفات عنق الورقة من وجود الشعيرات و الدرز و شكل الحزم الوعائية صفات مهمة لتحديد العلاقات التطورية بين أنواع الحمضيات.

الكلمات الدالة: *Citrusreticulata*، علم الشكل الظاهري، علم التشريح، التجاويف الإفرازية والدروز، المرج (ليبيا).

Introduction

The Rutaceae family, which is well known due to its economic importance, is a large family, widely distributed in the tropics regions, including 154 genera and approximately 2100 species characterized by oil glands in its leaves (Kubitzki et al. 2010). *Citrus* L. is one of the very important genera in the Rutacea family. In Libya, *Citrus* is represented by 9 Species, all of which are cultivated (Jafari and EL gadi, 1985). Most *Citrus* members are of importance because of their fruits, which are eaten alone as fresh fruit, or they may be processed as fruit juice and in some cuisines, lemon are added to dishes and drinks. Most of the members in the genus have traditional medicinal importance (Okeke and Mbagwu, 2001). Because of the great economic value of *Citrus* species, many agricultural cultivars have been developed which has led to series of identity conflict between the parent plants and cultivars (Morton, 1987; Goldschmidt, 1996). Therefore, the phylogeny and classification of *Citrus* remains a matter of controversy (Herrero et al., 1996). The leaves possess many morphological characters with potential taxonomic value that are frequently diagnostic at the genus and species level (Ashfaq et al, 2019). Many studies use anatomical characters to distinguish between species they worked on (Okoli, 1987, Edeoga and Osawe, 1996; Heo, 1996). Taxonomic studies are incomplete without the micro-morphological characters of epidermal anatomy (Khalid et al., 2009; Metcalfe and Chalk, 1950). Epidermal and micro-morphological characters have provided useful systematic data for the species separation and classification of the *Citrus* L. species (Mbagwu et al., 2007).

Although the *Citrus* genus is common, not much study have been done on the morphology and anatomy of the genus *Citrus*. Therefore, the current study described the morphology and the anatomy of leaf and petiole of *Citrus reticulata*.

Materials and Methods

Plant samples of *C. reticulata* Blanco were collected from farm in the Bata area of AL-Marj city. The plant was identified and named following (Jafri and EL-Godi ,1985) and (IPNI)International plant names index.

In epidermal study, the method of (Ogundare and Saheed, 2012) was used. The upper and lower epidermal peels were obtained manually using forceps. The peels were stained with 1% Safranin for about 5-10 minutes, rinsed carefully in several changes of water to remove excess stains and then placed on a glass slide, and examined under the microscope.

For anatomical investigations, the method of (Chalise et al. 2022) was followed. Leaf blade cross-section parallel to the midrib was prepared. The leaf and petiole sections of 2-3 Mm were obtained by free hand using a common razor blade. The sections were dehydrate in alcohol series (70-100%), stained with safranin and then mount in dilute (10%) glycerol solution on slides. The slides were examined using the microscope at different magnification power.

Results

I. Morphological features

Spinous tree is up to 280cm tall. The leaves are 7.1x2cm, with alternate position on the stem. The petiole is 0.6cm. The lamina is simple, and elliptic in shape, with dark green on upper surface and green on lower surface, having crenulate margin, with acuminate apex and obtuse base. The fruit is orange, sub-spherical in shape, had a thin, easily removable shell, and a sarcocarp of 10 segments. The seeds are ovoid, acuminate apex and base rounded (Figure1).



Figure1: Morphological features of *C. reticulata*. a. Leaf morphology. b. Fruit morphology. c. Seed morphology.

II. Epidermal features

Hypostomatic, with anomocytic type of stomata. The antichinal walls of epidermis are straight-sinuuous and thick-walled at both upper and lower epidermis. The shape of epidermis cells are variedly. It is rectangular to cuboidal to pentagonal to trapezial to pyramidal in both the upper lower epidermis. The secretory cavities are present in the upper epidermis and absent in the lower epidermis (Figure 2).

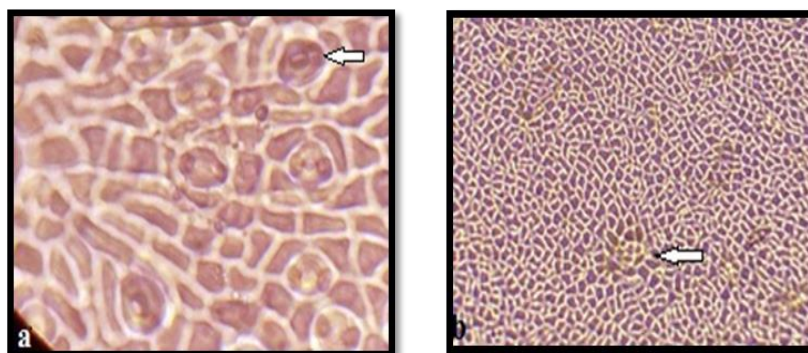


Figure2: Epidermal features of *C. reticulata*. a. Adaxial epidermis. (Arrow): Stomata. B. abaxial epidermis. (Arrow): Secretory cavity.

III. Anatomical features

In transverse section of leaf, the mesophyll structure is bifacial of two rows of palisade parenchyma and four to eight rows of spongy parenchyma. Druses are present in the palisade parenchyma. Midrib region is round shaped, surrounded by 2-3 rows of angular collenchyma followed by 5-7 rows of parenchyma. The vascular bundle is collateral, arranged in open arc and surrounded by 2-3 rows of sclerenchyma. From one to two layers of secretory cells are present (Figure 3a &b).

In transverse section of petiole, Median outline is convex with short ribs. One layer of epidermis with thick cuticle, Trichomes are absent. The cortex contains 4 - 6 rows angular collenchyma cells, followed by 6-8 rows parenchyma cells. The vascular bundle is collateral, surrounded by a layer of sclerenchyma cells. Druses and two layers of secretory cells are present (Figure 3c).

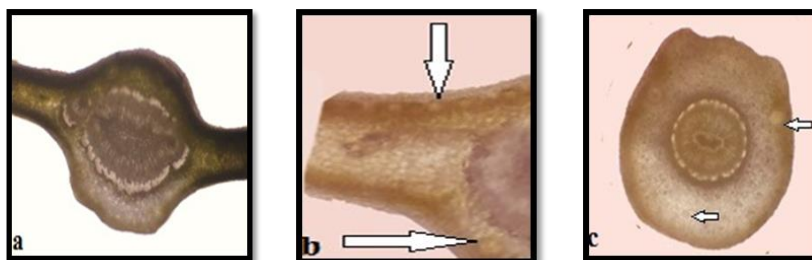


Figure3: Anatomical features of *C. reticulata*. a. Leaf anatomy.
b. Arrows: Druses and secretory cavity. c. Petiole anatomy. (Arrows):
Arrows: Druses and secretory

Discussion

The morphological and anatomical attributes are important in the classification of flowering plants (Hutchinson et al.,1954; Metcalfe and Chalk,1979;Wahua and Sam, 2013;Olorode et al.,2015). In current study, most of the morphological characteristics (Figure 1a,b &c) were mentioned by (Jafri and El- gadi,1985), but some of them

not mentioned, such as leaf apex shape, base shape, leaf and petiole measurements and seed morphology. Budiarto et al., (2021) reported that leaf characteristics such as wing, spine, color, hair and fragrance, can be used as morphological markers within the citrus genus. Leaf shape is successful to solve some misunderstanding in the classification of some species of angiosperms (Huang and Liu 2014). Susandarini et al.,(2013) explained that fruit characteristics were very important in comparing between *Citrus* species.

In our results, the stomata are on the abaxial surface of leaves, and are the anomocytic type. According to Obiremi et al. (2001) *C. limon* has paracytic stomata while *C. reticulata* has paracytic to anomocytic stomata. Epidermal cell shape, stomatal type, stomatal pore, stomatal index and trichomes are taxonomically important characteristics (Ullah et al., 2018).

In *C. reticulata*, the shape of epidermis cells was variedly (Figure 2b). Inyama,(2015) observed that the shape of epidermal cells is important in distinguishing the species of *Citrus* genus. The presence of secretory cavities on the upper surface of the epidermis separated species of *Citrus* from others (Ogundare and Saheed ,2012).This result was observed in our study (Figure 2b).

Riahi et al.,(2023) observed bifacial mesophyll arrangement in most of the *Citrus* species they studied and isobilateral mesophyll arrangement in the rest.He also showed the presence of druses in the mesophyll of some species of *Citrus*, and its absence in the rest. These characteristics were observed in our study (Figure 3b). Gupta and Singh, (2019)noticed collateral vascular bundle in midrib of *C. limon*. This result is consistent with our study (Figure 3a). The number of palisade layers and the presence of glandular cavities in the midrib have taxonomic value in the *Citrus* genus (Riani et al., 2023).

Petiole characteristics of *C. reticulata*, uniseriate epidermis, hypodermis consists of collenchym, parenchyma, a layer of

sclerenchyma cells around vascular bundle and the presence of druses in cortex (Figure 3c). These results are in agreement with (Ogundare and saheed ,2012). They also explained that the presence of trichomes and druses distinguished the species of *Citrus*. Characteristics of the vascular bundles is important for determining phylogenetic relationships between *Citrus* species (Osuoha et al 2015).

Conclusion

Citrus reticulata is cultivated in Libya. It has many uses as juice, fruit, and in medicine. In current study, morphological characteristics of leaf, fruit and seed are attributes of valuable diagnostic value for describing *C. reticulata*. Also epidermis analysis, diversity in cell shape, hypostomatic, stomata and the presence of secretory cavities are taxonomically important features. In addition to the anatomical characteristics of leaf, such as the type of mesophyll structure and its containment of druses, and the type of vascular bundle in the midrib, these are characteristics that have taxonomic value in the *Citrus* genus. Through our study, we noticed that petiole attributes are of remarkable importance in distinguishing between *Citrus* species.

The study suggests that, the *Citrus* genus in Libya must be studied morphologically, anatomically, chemically, and molecularly to find phylogenetics between *Citrus* species.

References:

- Ashfaq, S., Ahmad, M., Zafar, M., Sultana, S., Bahadur, S., Ullah, F., Zaman, W., Ahmed, S. N., Nazish, M. 2019. Foliar micromorphology of Convolvulaceae species with special emphasis on trichome diversity from the arid zone of Pakistan. *Flora*, 255, 110-124.
- Budiarto, R., Poerwanto, R., Santosa, E. and Efendi, D. 2021. Morphological evaluation and determination keys of 21 *Citrus*

- genotypes of seeding stage . *Biological Diveristy*. 22(3):1570 – 1579.
- Chalise P.,Paneru Y.R., Joshi L. 2022. Anatomical study of *Shorea robusta* Gaertn. *Journal of plant Resources*, 20(1): 113-120.
- Edeoga, H.O and Osawe, P.J. 1996. Cuticular studies of some Nigerian species of *Senna* Tourn. Ex Mill. (Syn *Casia* Tourn. Ex. L): Leguminosae Caesalpinoideae. *Acta Phytotaxonomica et Geobotanica*, 47:41-46.
- Goldschmidt, E.E. 1996. Biology of *Citrus*. Cambridge University Press, Cambridge, UK.
- Gupta,v. and Singh,v. 2019. Pharmacognostic studies of the leaves of *Citrus limon* linn.*Journal of pharmacognosy and phytochemistry*.8(4).1758-1763 12.
- Herrero, R., Asíns, M.J., Carbonell, E.A. and Navarro, L. 1996. Genetic diversity in the orange subfamily Aurantioideae. I. Intraspecies and intragenus genetic variability. *Theoretical and Applied Genetics*. 92(5): 599-609.
- Heo k. 1996.Reproductive structures and phylogeny in Lauraceae. Kyoto University. Ph.D. dissertation.
- Huang LJ, Liu YC. 2014. Understanding diversity in leaf shape of Chinese *Sagittaria* (Alismataceae) by geometric tools. *Pakistan Journal of Botany*, 46 (6): 1927-1934.
- Hutchinson J., Dalziel J.M. 1954. Flora of west Tropical Africa. Crown Agents for overseas Governments Administration , London, UK.
- Jafri, S.& El-gadi, A. 1985. Flora of Libya. (ed) Al FaatehUniveristy. Faculty of Science Department of Botany, Tripoli, Libya.
- Khalid, A., Mir, A. K., Mushtaq, A., Muhammad, Z., Muhammad, A., Farooq, A., 2009. Taxonomic diversity of stomata in dicot flora of a district tank (NWFP) in Pakistan. *African journal of Biotechnology*, 8, 1052-1055.
- Kubitzki k., kallunki j., duretto M., wilson p.G. 2010. Rutaceae. in flowering plants eudicots.

- Mbagwu, F., Nwachukwu, C., Ubochi, B., 2007. Leaf epidermal characteristics of four species of the genus *Citrus* (Rutaceae). *Agricultural Journal*. .2(6): 713-716.
- Metcalfe, C. R., Chalk, L., 1950. Anatomy of the Dicotyledons. At The Clarendon Press; Oxford.
- Metcalfe C R, Chalk L. 1979. Anatomy of the Dicotyledon: Systematic anatomy of the Leaf and stem. vol. I oxford University press ,New york.
- Morton, J. 1987. In: Morton, J.F. (ed.), Fruits of Warm Climates. Miami, Florida.
- Naik, V., Nirgude, S., 1981. Anatomy in relation to taxonomy of *Chlorophytum* (Liliaceae). *Indian Journal of Botany*, 4, 48-60.
- Obiremi EO, Oladele FA (2001) Water conserving stomatal system in selected *Citrus* species. *South African Journal of Botany*, 67: 258-26.
- Ogundare, C.S. and Saheed S.A. 2012. Foliar epidermal characters and petiole anatomy of four species of *Citrus* L. (Rutaceae) from south-western Nigeria. *Bangladesh Journal of Plant Taxonomy*, 19(1): 25-31.
- Okeke, S.E. and Mbagwu, F.N. 2001. Herbalism in Njaba local government area of Imo State of Nigeria. *Journal of Engineering Science and Technology*, 2: 168-174.
- Okoli B.E. 1987. Morphological and cytological studies in *Telfairia* Hooker (Cucurbitaceae). *Feddes Repertorium*, 98:505-508.
- Olorode O., Olayanju S., Garba A. 2013. *Physalis* (Solanaceae) in Nigeria. *Ife Journal of Science*, 15(1):101-109.
- Osuoha VUN, Mbagwu FN, Inyama CN, Ukpai KU. 2015. Systematic Characterisation of six *Citrus* Using petiole anatomy. *Medical and Aromatic plants*, S1. doi:10.4172/2167-0412.S1-005.
- Riahi M., Elham Kahdoui E., Tavakkoli Z. and Ghahremaninejad F. 2023. Comparative anatomical and morphological studies on leaves of some *Citrus* species and their systematic implications. *Plant Biosystems-an*

international Journal Dealing with all asPects of Plant Biology.1-8.

- Susandarini R, Subandiyah S, Rugayah, Daryono BS, Nugroho LH. 2013. Assessment of taxonomic affinity of Indonesian pummelo (*Citrus maxima* (Burm. Merr.) based on morphological characters. *American Journal Agricultural and Biological Sciences*, 8 (3): 182-190.
- Ullah, F., Zafar, M., Ahmad, M., Shah, S. N., Razzaq, A., Sohail, A., Zaman, W., Çelik, A., Ayaz, A., Sultana, S., 2018. A systematic approach to the investigation of foliar epidermal anatomy of subfamily Caryophylloideae (Caryophyllaceae). *Flora*, 246, 61-70.
- Wahua C., Sam S. M. 2013. Comparative chemotaxonomic investigations on *Physalis angulata* Linn.and *Physalis micrantha* Linn.(Solanaceae). *Asian Journal of Applied Science*, 1(5):220-228.