



صفات بشرة الورقة والصفات التشريحية لنبات *Citrus limon* المزروع في المرج (ليبيا)

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Foliar epidermal and anatomical characters of *Citrus limon* (Rutaceae) cultivated in Al-Marj (Libya)

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الملخص:

تم دراسة صفات البشرة و الصفات التشريحية لنبات الليمون الحامض *Citrus limon* (L.) Osbeck المزروع في مدينة المرج ، والهدف من هذه الدراسة الحالية هو تزويد صفات جديدة لتمييز ووصف هذا النوع. وتعتبر صفات البشرة كتنوع أشكال الخلايا، ووجود الثغور على البشرة السفلى فقط، نوع الثغور عديمة الخلايا، الثغور المتجاورة و الثغور الضخمة من الصفات المهمة لتمييز هذا النوع. ترتيب النسيج الوسطي، و شكل الحزم الوعائية، ووجود الدرروز و التجاويف الإفرازية، هذه الصفات لها قيمة تصنيفية عالية على مستوى تحت الجنس. ونلاحظ وجود شعيرات غدية و لا غدية في عنق الورقة. لشعيرات قيم تصنيفية مهمه جدا.

الكلمات الدالة: *Citrus limon*، البشرة، التشريح، الدرروز و الشعيرات، المرج (ليبيا).

Abstract

Epidermal and anatomical features of *Citrus limon* (L.) Osbeck, cultivated in Al-Marj city were studied. The goal of the current study is provide new characteristics for the identification and description of this species. Epeidermal characteristics such as a variety of cell shapes, hypostomatic, anamocytic stomata, contiguous stomata and mega stomata are important in distinguish *C. limon*. Mesophyll arrangement, the shape of vascular bundle, the presence of druses and secertory cavities these features have high taxonomic value at infrageneic level. We abserve glandular and non glandular trichomes in petiole. Trichomes have very important taxonomic values.

Keywords: *Citrus limon*, epidermis, anatomy, druses and trichomes, Al-Marj (Libya).

Introduction:

The genus citrus belongs to Rutaceae which is considered to be the largest family in order spindles with about 2100 species and 154 genera (kubitzki *et al*, 2010). Many species of this genus are cultivated almost all over the world for their fruits or aromatic oils; 9 Species are known from cultivation in Libya (Jafari and EL gadi, 1985). Citrus plants are shrubs or small to medium_sized tree and cultivated throughout the tropic and subtropics they are native in some parts of India, north Australia (Harley *et al.*, 2006). The species of genus have medicinal importance, for example, leaves of *C. limetta* Risso used to treat swollen limbs, *C. aurantifolia* (Cristm.), Swingle juice is used to treat dermatitis, and *C. limon* (L.) Burm.f. juice is used to treat hookworm (Göthesson, 1997; Ogundare and Saheed, 2012).

The leaves possess a variety of morphological characters with potential taxonomic relevance that are frequently diagnostic at the genus and species level (Ashfaq et al, 2019) . Many studies have emphasized the taxonomic significance of anatomical features (Hameed et al., 2020; Naik and Nirgude, 1981; Raza et al., 2020; Shaheen et al., 2022; Taia, 2005).

Foliar anatomical and micro- morphological characters have provided useful systematic data for the species demarcation and classification of the *Citrus* L. species(Mbagwu et al.,2007).

Taxonomic investigations are incomplete without the microscopic characters of epidermal anatomy (Khalid et al., 2009; Metcalfe and Chalk, 1950).

In this study , *Citrus limon* was chosen to be the subject of the current study because its economic and medicinal importance and the epidermal and anatomical studies of citrus genus is very limited. Thus, this investigation aimed to study the anatomical structures of vegetative organs of *C. limon*.

Materials and Methods

Fresh leaves of Citrus *C. Limon* (L.) Osbeck were collected from farm in AL-Marj city (Bata). The plant was identified using the Libyan flora (Jafri and EL-Godi ,1985). Nomenclature of plant followed International Plant Names Index .

I. Epidermal study

Upper and lower pidermal peels were obtained manually using forceps and dissecting needles. 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 mount in dilute (10%) glycerol solution on a glass slide for microscopic examination (Ogundare and Saheed, 2012).

II. Anatomical study

The transverse sections of leaf blade and petiole median regions were obtained by free hand using a common razor blade. The sections were dehydrate in alcohol series, staine dwith safranin and then mount in dilute (10%) glycerol solution on slides. Theslides were then studied under microscope at different magnification and photographs (Chalise el at. 2022) .

Results

I. Leaf epidermal surface

The anticlinal walls of the epidermis are straight and thick-walled at both upper and lower epidermis. Epidermal cells are variedly shaped. It is rectangular to cuboidal to triangular to pentagonal to hexagonal to trapezial in the upper epidermis. and rectangular to triangular to pentagonal to hexagonal to trapezial in the lower epidermis.

Hypostomatic with anomocytic type of stomata, in addition to two types of special stomata: contiguous stomata and mega stomata. The epidermis shows no trichome or subsidiary cells. Sheath cells are found covering the vein areas. The secratory cavities are present in the upper epidermis and absent in the lower epidermis .

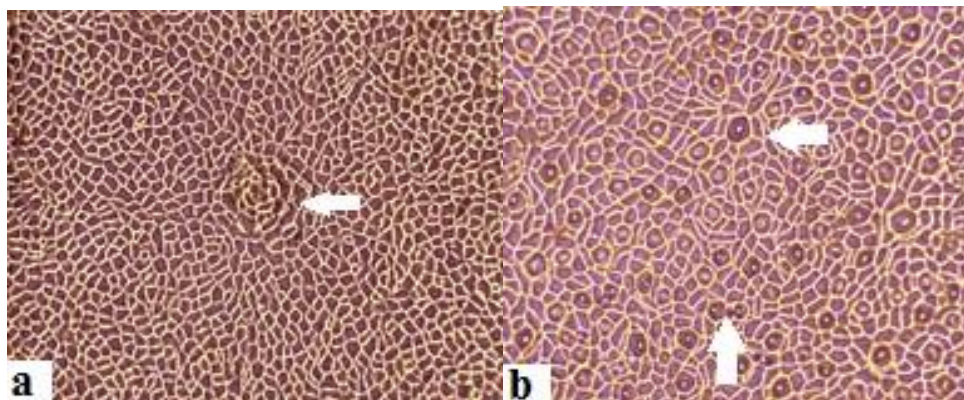


Figure1: Epidermal characteristics: a. Upper epidermis. (Arrow):Secretory cavity. b. Lower epidermis.(Arrows): Contiguous stomata and mega stomata.

II. Leaf anatomy

In transverse section of leaf ,the mesophyll is bifacial with two layers of cylindrical palisade cells and four to eight layers of round spongy cells. Midrib region is round shaped, occupied by angular collenchyma (3-4 layers) followed by (6-8 layers)of parenchyma and (2-3 layers) of sclerenchyma. The vascular bundle is collateral and arranged in a close arc .Druses and 3 layers of secretory cells are present.

III. Petiole anatomy

Median region outline is circle. Uniseriate epidermis. Glandular and non glandular trichomes are present. The cortex contains 5_7 layered angular collenchyma cells , followed by 11_14 layered parenchyma cells. A layer of sclerenchyma cells surrounds the vascular bundles.. Druses and 2_3 layers of Secertory cells are present. The collateral vascular bundles are arranged in a ring form.

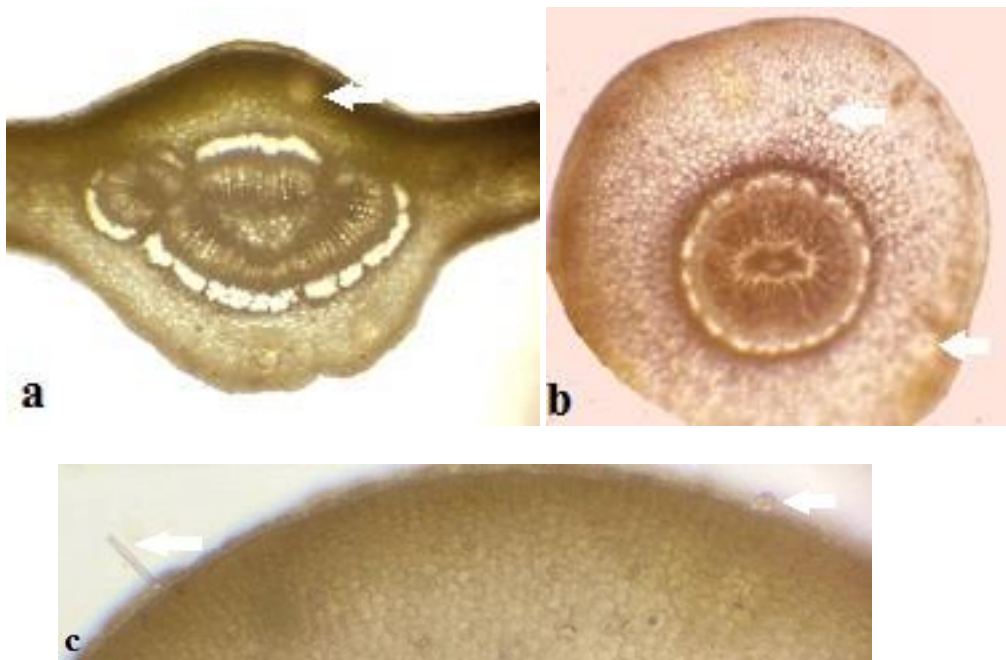


Figure2: Anatomical characteristics: a. Leaf anatomy. (Arrow):Secretory cavity. b. Petiole anatomy.(Arrows): Secretory cavity and druses. c. Trichomes.

Discussion

Taxonomically most important foliar epidermal characteristics are epidermal cell shape, stomatal type, stomatal pore, stomatal index and trichomes (Ullah et al., 2018). On the upper epidermis, the shape of cells was diverse. It is rectangular to cuboidal to triangular to pentagonal to hexagonal to trapezoidal. (Inyama ,2015) observed the presence of rectangular, triangular, hexagonal and trapezoidal epidermal cells. On the lower epidermis ,the anomocytic stomata observed in our study agreed with Mbagwu,et al,(2008) who reported same types of stomata in *C. limon*,*C. sinensis* ,*C. aurantifolia* and *C. maxima*. On the contrary, the result disagreed with obiremi et al. (2001)who reported paracytic stomata in *C. limon*. (Inyama ,2015) noticed the presence contiguous stomata and mega stomata of *C. limon*. This result is arrangement with our results. Patel and shah(1971) who used shape of epidermal cell as taxonomic tool in differentiating different species they studied.

According to the mesophyll structure of *C.limon* is bifacial (Gupta and Singh, 2019). Also they observed collateral vascular bundle in midrib. These results are consistent with our study. (Riani et al , 2023) noted in their study on the genus citrus that the anatomy and morphology of the leaf have high taxonomic value at infrageneic level. Among these characteristics were the type of stomata , mesophyll arrangement , number of palisade layers , shape of the midrib and sheath around vascular tissue .

The epidermis of the petiole of two species studies is one layer thick. Below the epidermis ,is a multi layered hypodermis which consists of collenchym, parenchyma and caps of sclerenchyma cells around vascular bundle .These results are in harmony with (Osuoha et al 2015) who showed that shape of the vascular bundles is important for determining phylogenetic relationships between citrus species. In our study, Two types of trichoms are present. Trihomes have often been used in plant taxonomy (Atalay et al., 2016; Gul et al., 2019). (Ogundare and saheed ,2012) noticed , when they studied the epidermal features and the petiole anatomy of Citrus species , that the presence and absence of secretory cavities on the upper surface of the epidermis separated some species of citrus from others , and the presence of trichomes and crystals distinguished one species from other species .

Conclusion

Citrus limon belongs to Rutaceae. It has been used in medicine and food. In our study, epidermal characteristics, stomata types, shape of epidermal cell and the presence of secretory cavities are very important characteristics to comparing and distinguishing between Citrus species. The anatomical features, the mesophyll type, shape of vascular bundle of leaf and the presence of trichomes in petiole have potential taxonomic values.

Our study proposes in the future a taxonomic study of the genus Citrus using morphological, anatomical and chemical characters.

References:

1. Atalay, Z., Celep, F., Bara, F., & Doğan, M. (2016). Systematic significance of anatomy and trichome morphology in Lamium (Lamioideae; Lamiaceae). *Flora–Morphology, Distribution, Functional Ecology of Plants*, 225, 60–75
2. Ashfaq, S., Ahmad, M., Zafar, M., Sultana, S., Bahadur, S., Ullah, F., Zaman, W., Ahmed, S., Nazish, M., 2019. Foliar micromorphology of Convolvulaceous species with special emphasis on trichome diversity from the arid zone of Pakistan. *Flora* 255, 110–124.
3. Chalise P;paneru Y.R.and josh L. (2022). Anatomical study of Shorou rabusia gairtn. *J, pLant Reso* .20(1),113–120
4. Gul, S., Ahmad, M., Zafar, M., Bahadur, S., Celep, F., Sultana, S., Begum, N., Hanif, U., Zaman, W., & Shuaib, M. (2019). Taxonomic significance of foliar epidermal morphology in Lamiaceae from Pakistan. *Microscopy Research and Technique*, 82(9), 1507–1528.
5. 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.
6. Göthesson, L.–Å., 1997. Plants of the Pitcairn Islands including local names and uses. University of New South Wales, pp.397.
7. Hameed, A., Zafar, M., Ullah, R., Shahat, A. A., Ahmad, M., Cheema, S. I., Haq, I. U.,Sultana, S., Usma, A., Majeed, S., 2020. Systematic significance of pollen

- morphology and foliar epidermal anatomy of medicinal plants using SEM and LM techniques. *Microscopy Research and Technique* 83, 1007–1022.
8. Harley, M. I., Richard, B. S., Virginia, E. S., Ward, D. and Elevitch, C. R. 2006 citrus (citrus) and fortunella (kumquat) Rutaceae (Rue family) in: Elevitch, C. R. (ed). species profile for Pacific Island agroforestry. permanent Agriculture resources, (PAR), Hōlualoa Hawai'i, pp 1–27
 9. Inyama CN, Osuoha VON, Mbagwu FN and Duru CM. 2015. Comparative morphology of the leaf epidermis in six Citrus species and its biosystematic importance. *Med. aromal plants*. 4 (3):1–5
 10. Jafri, S., & El-gadi, A. Flora of Libya. (ed) Al Faateh University. Faculty of Science. Department of Botany, Tripoli, Libya, 1985;
 11. Khalid, A., Mir, A. K., Mushtaq, A., Muhammad, Z., Muhammad, A., Farooq, A., 2009. 326 Taxonomic diversity of stomata in dicot flora of a district tank (NWFP) in Pakistan. 327 *African journal of Biotechnology* 8, 1052–1055.
 12. Kubitzki, K., Kallunki, J., Duretto, M., Wilson, P. G., 2010. Rutaceae. in *flowering plants eudicots*.
 13. Mbagwu FN, Okafor VU, Okeke AE (2008) Comparative palynological characters in four species of the family Poaceae (Gramineae). *Int Sc Res Journal* 1: 127–129.
 14. Mbagwu, F., Nwachukwu, C., Ubochi, B., 2007. Leaf epidermal characteristics of four species of the genus Citrus (Rutaceae). *Life Sci. J.* 4, 68–70.
 15. Metcalfe, C. R., Chalk, L., 1950. *Anatomy of the Dicotyledons*. At The Clarendon Press; Oxford.
 16. Naik, V., Nirgude, S., 1981. Anatomy in relation to taxonomy of Chlorophytum (Liliaceae). *Indian Journal of Botany* 4, 48–60.
 17. Obiremi EO, Oladele FA (2001) Water conserving stomatal system in selected Citrus species. *South African Journ of Bot* 67: 258–260.
 18. Ogundare, C., Saheed, S., 2012. Foliar epidermal characters and petiole anatomy of four species of Citrus L. (Rutaceae) in South-western Nigeria. *Bangladesh Journal of Plant Taxonomy* 19, 25–31

19. Osuoha VUN, Mbagwu FN, Inyama CN and Ukpai KU. 2015. Systematic Characterisation of six Citrus Using petiole anatomy. *Med. Aromat. plants* S1. doi:10.4172/2167-0412.S1-005.
20. Patel JD, Shah JJ (1971) Studies in stomata of Chilli and Bringal. *Ann Bot* 35: 197-201.
21. Raza, J., Ahmad, M., Zafar, M., Athar, M., Sultana, S., Majeed, S., Yaseen, G., Imran, M., Nazish, M., Hussain, A., 2020. Comparative foliar anatomical and pollen morphological studies of Acanthaceae using light microscope and scanning electron microscope for effective microteaching in community. *Microscopy Research and Technique* 83, 1103-1117.
22. Shaheen, S., Sharifi-Rad, J., Ali, M., Shamim, Z., Rasool, B., Ashfaq, M., Mukhtar, H., Harun, N., Hanif, U., Siddique, R., 2022. Light and scanning electron microscopy-based foliar morpho-anatomical comparison of selected family Rosaceae members distributed in district Lahore, Punjab, Pakistan. *Microscopy Research and Technique* 85, 1597-1610.
23. Taia, W. K., 2005. Modern trends in plant taxonomy. *Asian Journal of Plant Sciences* 4, 2005.
24. 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 J. Plant Taxon.* 19(1): 25-31. Ullah, F., Zafar, M., Ahmad, M., Shah, S. N., Razaq, 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.