Study Of Some Anatomical Features Of *Stevia Rebaudiana* (Bertoni) Bertoni Grown In Iraq

Ali T. AL-Taie Continuous Education Center, Mustansiriyah University, Baghdad, Iraq.



ARTICLE INFO

Received: 23 / 8 /2021 Accepted: 16 / 9 / 2021 Available online: 21 / 12 / 2021

DOI:10.37652/juaps.2022.172439

Keywords: Anatomy, *Stevia rebaudiana*, Asteraceae, Iraq .

(†)

Copyright©Authors, 2021, College of Sciences, University of Anbar. This is an open-access article under the CC BY 4.0 license (http://creativecommons.org/licens es/by/4.0/).

1. INTRODUCTION

The common name of *stevia rebaudiana* (Bertoni) Bertoni, from Asteraceae family, is candy leaf, sweetleaf, or sugar leaf. *Stevia* is a perennial native in Brazil and Paraguay having wet environments [1,2 and 3]. It was named after M.S Bertoni, who first identified this plant. [4]. It is cultivated primarily in South America and Asia. [5]. The plant grows to a height of 65 to 120 cm, with ellipse-shaped leaves and white blossoms. Stevia cultivation conditions vary greatly. Stevia thrives in both dark, wet, sandy soil and loam, permeable soils. Furthermore, it can be found on marsh banks, barren, acidic sands, or muck soils. [6 and 7]. The Indians have long known about it, referring to it as ka'ahee, which means "sweet herb."[8].

In the 1970s, stevioside was isolated and purified from *Stevia rebaudiana* leaves and was formally accepted in Japan as a sweetener. [9,10]. The European Commission issued a regulation on November 11, 2011, enabling steviol glycosides to be used in 31 different food categories, including beverages, desserts, sweets, and sweeteners. Previously, stevia products were offered in Poland as cosmetics for external use. Stevia or stevia-based products are currently permitted for use as a food ingredient and sweetener. [11]. The major advantage of the plant is the presence of steviol glycosides which provide a sensation of sweetness up to 450 times more intense than that of sucrose. [12].

ABSTRACT

The leaves of *Stevia rebaudiana*, from Asteraceae family, are a source of steviol glycosides, which are utilized as a sweeter, more favorite addition. Among the most important anatomical results that were reached through the research and which distinguish the plant from an anatomical point of view are uniseriate-multicellular of non-glandular trichomes that are covered the epidermis of stem and leaves. Also, the vertical walls of the ordinary epidermis cells in the upper and lower epidermis that are heavily sinuous and the stomata scattered in both of it, the type of the stomata anomocytic. In addition, the mesophyll which differentiated into 2-3 layers of palisade cells under the upper epidermis and the spongy cells after it and the cross-section of midrib region of the leaf as crescent shape and the vascular bundle central, crescent shape.

Stevia does not significantly alter blood glucose, and so it is attractive as a sweetener to diabetics and others on carbohydrates-controlled diets. [13]. Stevia is full of many important phytochemicals (Steviol, Steviosides, rebaudiosides, etc.) that have properties to reduce blood sugar levels. It possesses high anti-hyperglycemic activity and serves as a substituent for saccharose in diabetes patients. [14,15]. Previously, Stevia was introduced as a crop in many countries including Brazil, Korea, Mexico, United States, Indonesia, Tanzania, and Canada, since 1990. Its major production is currently centered in China and the major market is in Japan and become Stevia farming is widespread [16]. The *S. rebaudiana* is the only species that have an important sweetening feature from 150 species of *Stevia* [17].

There are limited anatomical studies available for the plant's parts; therefore, this study aims to clarify the tissue and cells of the epidermis of leaves and to studying the tissue of cross-section of stem and longitudinal section of leaf.

1. MATERIALS AND METHOD

The plant materials of *S. rebaudiana* stem and leaves were collected from the home garden in Baghdad, Iraq. For 24-28 hours, the fresh stems and leaves were saved in formalin acetic acid alcohol (FAA), which was made by mixed Ethyl alcohol (50 ml), Distilled water (35 ml), Glacial acetic acid (5 ml), Formaldehyde 37 - 40 % (10 ml) and, then changed the solution by 70% alcohol, according to [18].

^{*} Corresponding author at: Continuous Education Center, Mustansiriyah University, , Baghdad, Iraq; E-mail address: <u>alialtaie@uomustansiriyah.edu.iq</u>

The samples of stem and leaves were cut into small segments with a length by hand, using a razor blade and used distilled water to wash it, then put in 5% sodium hypochlorite for five minutes to remove the chlorophyll. Finally, the epidermal samples were put on the slides and mounted by cover slides with (D.P.X) Dextrin Plasticizer Xylene. According to [19], the samples were prepared with some modifications [20]. KRÜSS light microscope and AmScope microscope digital camera were used to examine and photograph all of the slides.

2. RESULTS AND DISCUSSION:

The stem of *S. rebaudiana* is cylindrical in the crosssection. The epidermis consists of one layer of ovoid cells and a uniseriate-multicellular of non-glandular trichomes. The cortex consists of 2-3 layers of collenchyma tissue under the epidermis and parenchyma tissue. The Pericycle has many vascular bundles connecting with as by the interfascicular cambium, each bundle consists of the xylem and phloem and the bundle covered by the bundle cap fiber from the upper of the phloem tip, the result agrees with [21], that shear to the Pericycle of stem were simple and the cortical parenchyma is thick. The pith located in the center of the stem consists of ordinary parenchyma cells (Fig. 1).



Fig. 1: Cross section of stem of S. rebaudiana

The epidermis of leaves consists of the upper and lower surface, the vertical walls of the ordinary epidermis cells in the upper and lower epidermis are heavily sinuous and the stomata scattered in the upper and lower epidermis, the type of the stomata anomocytic (Fig. 2).



Fig. 2: Surface view of leaf epidermis of *S. rebaudiana*, appear the stomata when the A: Upper epidermis, B: Lower epidermis and C: The parts of stomata complex

Also, the non-glandular trichomes are uniseriatemulticellular, and they are distributed on both surfaces of the epiderm(Fig. 3).



Fig. 3: Surface view of leaf epidermis of *S. rebaudiana*, appear the trichomes when the A: Upper epidermis and B: Lower epidermis

The cross section of the leaf consists from the upper and lower uniseriate epidermis. And the upper epidermis is covered by the cuticle and the mesophyll, which is differentiated into 2-3 layers of palisade cells under the upper epidermis and the spongy cells after it, it is related with [21].The cross section of midrib region of the leaf as crescent shape showed upper and lower epidermis after it located the cortex, which mainly composed of ordinary parenchyma cells, the vascular bundle central, collateral, open, and crescent shape include xylem with variable size of vessels and phloem



(Fig. 4).

Fig 4: Longitudinal section of leaf show the midrib and blade part in the leaf of *S. rebaudiana*.

3. CONCLUSION

The *S. rebaudiana* is the only species that has an important sweetening feature from 150 species of Stevia as a whole. Integrative, this study provides a novel perspective on the microscopic structure of plants. The study contributions to appear the plant anatomy of the stem, leaves, and epidermis of leaves, respectively and because there are limited anatomical studies available for the plant's parts; therefore, this study aims to clarify the tissue and cells of the epidermis of leaves also studying the tissue of cross-section of stem and longitudinal section of leaf. The stem of *S. rebaudiana* is cylindrical, in the cross-section. The epidermis is consisted of uniseriate-multicellular of non-glandular trichomes, and the

epidermis of leaves consist of the upper and lower surface, the vertical walls of the ordinary epidermis cells in the upper and lower epidermis are heavily sinuous and the stomata scattered in the upper and lower epidermis, the type of the stomata anomocytic, also the non-glandular trichomes are uniseriate-multicellular and they are distributed on both surfaces of the epidermis. The cross section of leaf consists from the upper and lower uniseriate epidermis, and the mesophyll which differentiated into 2-3 layers of palisade cells under the upper epidermis and the spongy cells after it. The cross section of midrib region of leaf as crescent shape.

4. References

- Soejarto, D.D. (2002). Ethnobotany of Stevia and Stevia rebaudiana. In: inghorn, A.D.(Ed.), Stevia: The Genus Stevia. Taylor and Francis, London and New York, pp.40–67.
- [2] Brandle, J.E. and Telmer, P.G. (2007). Steviol glycoside biosynthesis. Phytochemistry 68,1855–1863.
- [3] Madan, S.; Ahmad, S.; Singh, G.N.; Kohli, K.; Kumar, Y.; Singh, R. and Garg, M. (2010). *Stevia rebaudiana* (Bert.) Bertoni – a review. Indian J. Nat. Prod. 1, 267– 286, IPC code; int.cl A61K 36/00, A23L 1/09.
- [4] Abeer Kazmi, Mubarak Ali Khan*, Sher Mohammad, Amir Ali and Huma Ali. (2019) "Biotechnological Production of Natural Calorie Free Steviol Glycosides in Stevia rebaudiana: An Update on Current Scenario", Current Biotechnology; 8(2). https://doi.org/10.2174/2211550108666191210100751
- [5] Ranjan, R.; Jaiswal, J. and Jena, J. (2011). Stevia as a natural sweetener. IJRPC 1(4), 1199-1202.
- [6] Mishra, P., Singh, R., Kumar, U., Prakash, V. (2010). Stevia rebaudiana – A magical sweetener. Global J. Biotechn. Biochem., 5, 62–74.
- [7] Lemus-Mondaca, R., Vega-Galvez, A., Zura-Bravo, L., Ah-Hen, K. (2012). Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chem., 132, 1121–1132.
- [8] Tavarini, S. and Angelini, L.G. (2013). Stevia rebaudiana Bertoni as a source of bioactivecompounds: the effect of harvest time, experimental site and crop age on steviolglycoside content and antioxidant properties. J. Sci. Food Agric. 93, 2121–2129
- [9] Dacome, A.S.; Silva, C.C.; Costa, S.C.; Fontana, J.D.; Costa, C.E. and Adelmann, J. (2005). Sweet diterpenic glycosides balance of a new cultivar of *Stevia rebaudiana* (Bert.) Bertoni. Isolation and quantitative distribution by chromatographic, spectro-scopic, and electrophoretic methods. Process Biochem. 40, 3587– 3594.

P-ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access 2021,15 (2) : 11–14

- [10] Barriocanal, L., Palacios, M., Benitez, G., Benitez, S., Jimenez, J., Jimenez, N. (2008). Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans, a pilot study of repeated exposures in some normotensive and hypotensive individuals and in type 1and type 2 diabetics. Regul. Toxicol. Pharm., 51, 37–41.
- [11] Singh, S. D., Rao, G. P. (2005). Stevia: The herbal sugar of the 21st century. Sugar Technol., 7, 17–24.
- [12] Kobus-Moryson, M., Gramza-Michałowska, A. (2015). Directions on the use of stevia leaves (Stevia rebauidana) as an additive in food products. Acta Sci. Pol. Technol. Aliment., 14(1), 5–13.
- [13] Panja P, Mukhopadhyay M (2019) Extraction of Natural Sweetener from Stevia Leaves Using Pressurized Hot Water. J Nutraceuticals Food Sci Vol.4 No.1:3.
- [14] Ahmad, U., Ahmad, R.S. (2018). Anti-diabetic property of aqueous extract of Stevia rebaudiana Bertoni leaves in Streptozotocin-induced diabetes in albino rats. BMC Complement Altern Med 18, 179 https://doi.org/10.1186/s12906-018-2245-2
- [15] Jan, S.A., Habib, N., Shinwari, Z.K. et al. (2021). The anti-diabetic activities of natural sweetener plant Stevia: an updated review. SN Appl. Sci. 3, 517.

https://doi.org/10.1007/s42452-021-04519-2

- [16] Hossain, MF; Islam, MT; Islam, MA and Akhtar, S
 (2017). CULTIVATION AND USES OF STEVIA
 (Stevia rebaudiana Bertoni): A REVIEW. Afr. J. Food Agric. Nutr. Dev.; 17(4):12745-12757
- [17] Soejarto, D.D; Kinghorn, A.D. and Farnsworth, N.R. (1982). Potencial sweetening agentsof plant origin. III. Organoleptic evaluation of Stevia leaf herbarium samples for sweetness. J. Nat. Prod. 45, 590–599.
- [18] Johanson, A. D. (1940). Plant Microtechnique .1st ed. Mc. Graw-Hill Book Company, New York and London, 523 PP.
- [19] Al-Khazraji, T. O., & Aziz, F. M. (1989). Practical in plant anatomy and microscopic preparations. Press of the Ministry of Higher Education and Scientific Research. University of Salahuddin, 321pp. (In Arabic).
- [20] AL-Hadeethi, M. A.; AL-Taie, A. T. and Ali, J. K. (2020). Anatomical study of Combretum indicum (L.) DeFilipps cultivated in Iraq. Sys Rev Pharm 11(8):736-741.
- [21] Salama, A.M.A (2012). Morphological, anatomical and chemical studies on Steviaplant and its response to the growth regulators GA3 and IBA. Thesis Ph.D. in Agricultural Botany, p.215-229

دراسة بعض السمات التشريحية لنبات Bertoni (Bertoni) المزروع في العراق العراق على العراق المرابع في العراق

مركز التعليم المستمر, الجامعة المستنصرية / بغداد – العراق alialtaie@uomustansiriyah.edu.ig

الخلاصة:

تعتبر أوراق Stevia rebaudiana ، من عائلة Asteraceae ، مصدرًا لكلوكوسايد الستيفول ، والتي تستخدم كمحليات ومنكهات مضافة . من أهم النتائج التشريحية التي تم التوصل إليها من خلال البحث والتي تميز النبات من وجهة نظر تشريحية هي وجود الشعيرات والتي تكون غير غدية من نوع احادية الصف متعددة الخلايا تغطي بشرة الساق والأوراق ، وكذلك الجدران الخارجية لخلايا البشرة الاعتيادية ، المعقد الثغري من النوع الشاذ ، بالإضافة إلى الطبقة المتوسطة (الميزوفيلية) التي تميزت إلى 2-3 طبقات ثم طبقة الخلايا الإسفنجية الى الاسفل منها ، تميز المقطع العرضي للمنطقة الوسطى للورقة (منطقة العرق الوسطي) بكونه هلالي الشكل اما بالنسبة للحزمة الوعائية المركزبة فكانت هلالية الشكل ايضا .

الكلمات المفتاحية: علم التشريح , Asteraceae, Stevia rebaudiana , العراق