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Original Article

Characterization of stomata in the species *Juglans jamaicensis* ssp. *insularis* (Griseb.) H. Schaarschm. (walnut tree)

Caracterización de los estomas en la especie *Juglans jamaicensis* ssp. *Insularis*(Griseb.) H. Schaarschm. (nogal del país.)

Caracterização dos estômatos da espécie *Juglans jamaicensis* ssp. *insularis* (Griseb.) H. Schaarschm. (Nogueira)

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ABSTRACT

Juglans jamaicensis subsp insularis, is an endemism of Pinar del Río, protected by Resolution 330/1999 of the Ministry of Agriculture included in the Red List of Cuban Vascular Flora. It is a species that has been little studied in its physiology and anatomy. The present study aims to characterize the stomas of *Juglans jamaicensis subsp. insularis*. Epidermal impressions were made with nail polish on both sides of the leaflets. These were observed with a novel optical microscope (NLCD-307 B). The length and width of the stomas were measured with a 40x lens, the density per unit area (cm²) and the stomatic index. The average size of the stomas is 16.5 µm wide and 19.9 µm long. The stomas were considered small, with a high density and a stomatic index of 15.82

Keywords: Stomatic index; stomatic density; stomata size.

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RESUMEN

Juglans jamaicensis subsp *insularis*, es un endemismo de Pinar del Río, protegido por la Resolución 330/1999 del Ministerio de la Agricultura e incluido en la Lista Roja de la Flora Vascular Cubana. Es una especie que ha sido poco estudiada en su fisiología y anatomía. El presente estudio tiene como objetivo la caracterización de los estomas de *Juglans jamaicensis* subsp. *insularis.* Se realizaron impresiones epidérmicas con esmalte de uñas, en ambas caras de los folíolos. Estas fueron observadas con un microscopio óptico Novel (NLCD-307 B). A los estomas se les midió el largo y ancho con lente de 40x, la densidad por unidad de superficie (cm²) y el índice estomático. El tamaño promedio de los mismos es de 16,5 ìm de ancho por 19,9 ìm de longitud, considerándose estomas pequeños, con una alta densidad y un índice estomático de 15,82.

Palabras clave: Índice estomático; densidad estomática; tamaño de estomas.

RESUMO

Juglans jamaicensis subsp *insularis*, é um endemismo de Pinar del Río, protegido pela Resolução 330/1999 do Ministério da Agricultura e incluído na Lista Vermelha da Flora Vascular Cubana. É uma espécie que tem sido pouco estudada em sua fisiologia e anatomia. O presente estudo visa caracterizar os estômatos de *Juglans jamaicensis* subsp. *insularis*. As impressões epidérmicas foram feitas com esmaltes em ambos os lados das cúspides. Estes foram observados com um novo microscópio óptico (NLCD-307 B). O comprimento e largura dos estômatos foram medidos com uma lente de 40x, a densidade por unidade de área (cm²) e o índice estomático. O tamanho médio dos estômatos é de 16,5 ìm de largura por 19,9 ìm de comprimento. Os estômatos foram considerados pequenos, com uma alta densidade e um índice estomático de 15,82.

Palavras-chave: Índice estomático; densidade estomática; tamanho do estoma.

INTRODUCTION

The gender *Juglans*, groups the plants of the family *Juglandaceae* known as walnut trees. About 50 species have been reported from China, Japan, India, Persia, and much of the Americas (Acevedo et al., 2012). There are 12 species in that area, two in the West Indies and several in South America (León and Alain, 1951). There are also isolated individuals with an even wider distribution in semi-deciduous tropical forests (López, 2000).

Juglans jamaicensis subsp. insularis, is an endemism of Pinar del Río. Its distribution is restricted to the regions of Sierra del Rosario and the base of the mogotes of Sierra de los Órganos, grows in an isolated way, never reaching the top, preferring clayey soils between the plains and near the rivers. In forests at the base of mogotes it grows on a substrate of humic yields from shady places and serpentine. It is reported in Minas de Matahambre (Sumidero), Viñales (San Vicente, Hoyo del Ruiseñor), La Palma: (San Andrés de Caiguanabo), Los Palacios (San Diego de los Baños) and Bahía Honda (Urquiola *et al.,* 2010).

This species has been classified as Critically Endangered (González-Torres *et al.,* 2016), due to the loss and degradation of habitat by agricultural activity, livestock and the effect of exotic plants and animals, and is protected by Resolution 330/1999 of the Ministry of Agriculture.

The effects of climate change on flora in general, and on threatened flora in particular, force us to study the morphoanatomy of species in order to understand how they evolve and adapt. Currently, the water deficit, increased by the possible consequences of climate change, is considered the main environmental factor limiting the growth and productivity of plantations (Silva *et al.,* 2017).

The morphological and physiological mechanisms involved in the processes of adaptation-acclimatisation to stress operate at different levels of the plant, with the aim of ensuring water functionality in the new environmental conditions, the stomas being vital for the homeostasis of the plant (Torala et al., 2010). Because they are responsible for the exchange of gases between the atmosphere and the leaf and thus prevent excessive water loss, hence the importance of knowing the number, shape and factors that control their opening or closing (Vázquez y Torres, 2007).

Therefore, it is necessary to know the anatomy and characteristics of stomas as entities in charge of gas exchange and transpiration and the response to adjustments in them, as a result of changes in environmental conditions. Therefore, the aim of the study was to characterize the stomas of the species *Juglans jamaicensis subsp. insularis.*

MATERIALS AND METHODS

The plant material used was leaves from individuals of *Juglans jamaicensis ssp insularis* collected in San Andrés de Caiguanabo, La Palma Municipality, Pinar del Río Province. To characterize the stomas, epidermal impressions were made on both sides of the leaflets, using nail polish, to a total of 20 samples, taken at random. They were observed with a Novel brand optical microscope, model NLCD-307 B and measured in length and width, with an ocular micrometer calibrated for the 40x lens.

The stoma count was performed to determine the density per unit area (mm²) and to calculate the stoma index according to (Reyes *et al.,* 2015) (Equation 1)

$$IE = (NEx100) /(CE+NE) \quad (1)$$

Where:

IE = Stomatic Index;
NE = Number of stomas per field of view:
EC = Number of epidermal cells in the field of view.

The length and width of the stomas were measured and classified by size according to Bory *et al.,* (2008).

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RESULTS AND DISCUSSION

Stoma size

The number of stomas on the adaxial (beam) surface compared to the abaxial (back) surface is a distinguishing feature of different species. Plants with bundle stomas are called epistomata, typical of aquatic plants (Reyes *et al.,* 2015), if they are present only on the underside, hypestomata, from mesophytic habitats and those with bundle and underside stomas, ampistomata, common from arid environments (Bieto and Talón, 2008; Reyes *et al.,* 2015). The hypostomatic *Juglansjamaicensises* leaf (Figure 1) (Rivera *et al.,* 2018), this typical character in many dicotyledons is considered a feature of primitivism Camargo, (2009).

The average stomach width was 16.5µm and the average length was 19.9µm. According to Bory *et al.,* (2008) these stomas are small. Stoma size is a key factor in the process of acclimatization to water stress, as there is an inverse relationship between this character and drought resistance (Toral *et al.*, 2010).

The species *J. jamaisensis ssp.insularis* lives mainly in the base and holes of hummocks, where water easily infiltrates through the yields developing then the edaphic drought, therefore, the reduction of the stomach size is considered an adaptation to these conditions.

The increase in the number of small stomas, together with the presence of glandular hairs and trichomes with no apparent secretory function, are catalogued as features of xeromorphism (Ely *et al.,* 2005). This characteristic of the presence of glandular hairs present in the species is associated with the function of accumulating water that is deposited on the epidermis (Rivera *et al.*, 2018).

Wu *et al.,* (2018) found that two subtropical forest tree species, *Schima superbay Syzygium rehderianumse*, show adjustments in anatomy and stoma size at different temperatures and water stress. For Schima superbaen increased temperature led to a reduction in stoma density, while in *Syzygium rehderianumse* reduced stoma size.

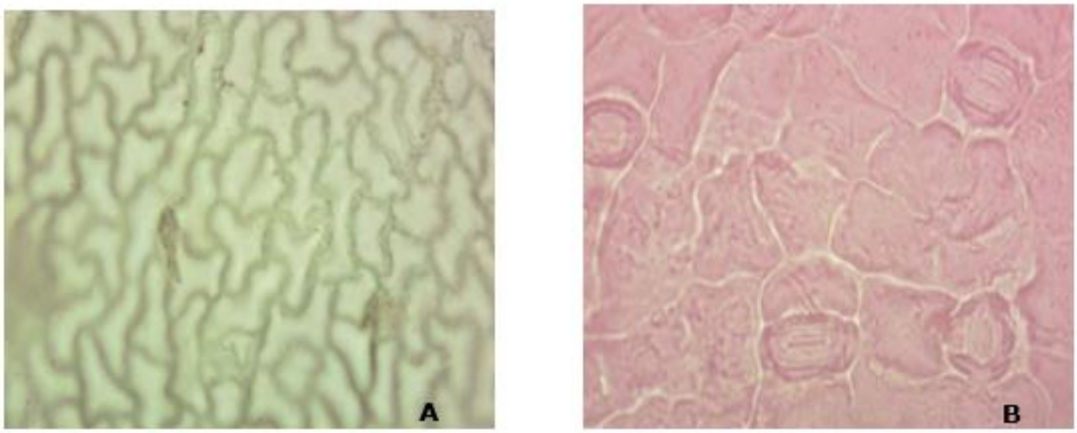

Figure 1. - Adaxial epidermal impression (A) and abaxial epidermal impression of the sheet (B) (640x)
Source: Photos of the author.

Stomatic index and density

The average stoma count was 22 per field and 117 epidermal cells. By determining stoma density in the leaves, 440 stomas/mm² were obtained. For other species it has been reported that the number of stomas per leaf area (density or stomatal frequency) is recorded between 10 and 2000 per mm² (Naizaque *et al.,* 2014) and indicates for *Accasellowiana*, a shrub species of *Myrtaceae,* stomatal density values of 91/mm^2 to 78/mm2 depending on the plant strata. Although studies on stomatic density are limited in tree species, it could be considered that, in the species, the stomatic density is high (Figure 2).

The average stomach index calculated was 15.82, which coincides with those calculated in *Annona muricata* L. and *Annona montana* MADFAC (Parés *et al*., 2004).

Kivimäenpää *et al.,* (2017) relate high stomatic density to the mesophilic formed by several layers of chlorophyll parenchyma in deciduous species with sunny habitats, which guarantees photosynthetic efficiency.

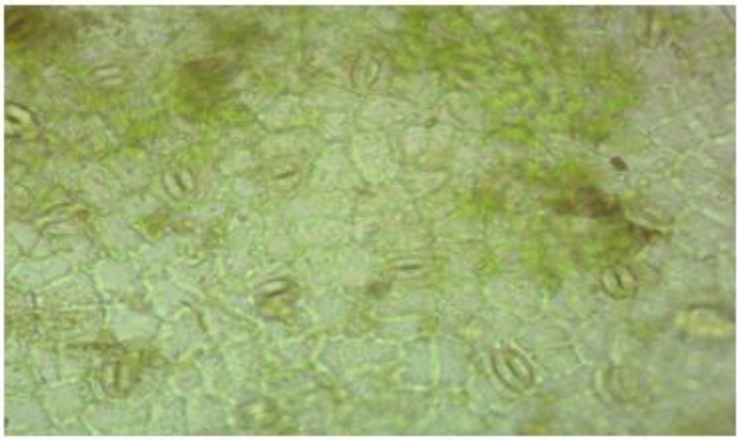

Figure 2. - Distribution of stomas on the abaxial side of the leaf (160x)
Source: Author's photos.

The stomas of the species Juglans *jamaicensis ssp.insularis* are small and occur in a high density per field with a stomach index of 15.82.

REFERENCES

ACEVEDO, RODRIGUEZ, P., STRONG, M.T. 2012 Catalogue of seed plants of the West Indies**.** Smithsonian Institution Scholarly Press. Smithsonian contributions to Botany number 98. ISSN: 0081-024X (print); 1938-2812. Disponible en: http://repository.si.edu/xmlui/handle/10088/17551

BIETO, J.A., CUBILLO, M.T., MANGAS, I.B. y ORMAECHEA, A.G., 2008. *Fundamentos de fisiología vegetal* [en línea]. McGraw-Hill Interamericana de España. ISBN 978-84-481-5168-3. Disponible en: https://dialnet.unirioja.es/servlet/libro?codigo=556962.

BORY, S., L. LUBINSKY, A.M. RISTERUCCI, J.L. NOYER, M. GRISONI, M.F. DUVAL, Y P. BESSE. 2008. Patrones de introducción y diversificación de *Vanillaplanifolia* (Orchidaceae) en Islas Reunión. Am. *J. Bot*. 95:805-815.

CAMARGO, M.A.B., 2009. *Stomatal characteristics in tree species of Central Amazonia*. Disseertacao de mestrado. Manaus, Amazonas, Brasil: Instituto Nacional de Pesquisas da Amazonia (INPA)/Universidad Federal do Amazonas.

ELY, F., TORRES, F. y GAVIRIA, J., 2005. Relación entre la morfoanatomía foliar de tres especies de *Miconia* (melastomataceae) con su hábitat y distribución altitudinal en el parque nacional sierra nevada de mérida, venezuela. *Acta Botánica Venezuélica* [en línea], vol. 28, no. 2, pp. 275-299. [Consulta: 27 enero 2020]. ISSN 0084-5906. Disponible en: https://www.jstor.org/stable/41740685.

GACETA OFICIAL DE LA REPÚBLICA DE CUBA. No 57. Resolución 330/1999. MINISTERIO DE LA AGRICULTURA. [Consulta: 14 de febrero de 2020]. ISSN 1682-7511. Disponible en: https://www.gacetaoficial.gob.cu/codbuscar.php

GONZÁLEZ- TORRES, L.R., BÉCQUER, E.R., PALMAROLA, A. 2016. Lista Roja de la Flora de Cuba. *Bissea*, 10, (Número especial 1). P.141. ISSN 1998-4189.

KIVIMAENPAA, M., SUTINEN, S., VALOLAHTI, H., HAIKIO, E., RIIKONEN, J., KASURINEN, A., GHIMIRE, R P., HOLOPAINEN, J K., HOLOPAINEN T.2017. Warming and elevated ozone differently modify needle anatomy of Norway spruce (*Piceaabies*) and Scots pine (*Pinus sylvestris*). Can. J. For. Res. 47: 488499. Disponible en: https://erepo.uef.fi/handle/123456789/3704

LÓPEZ GARCÍA, P.I., 2000. El estado de conservación de *Juglans jamaicensis* C.D.C en Cuba: evaluación de las subespecies *jamaicensis e insularis*. Jard. Bot. Nac, vol. 21.

NAIZAQUE J., GARCÍA G., FISCHER G., MELGAREJO L. M. 2014. Relación entre la Densidad Estomática, la Transpiración y las Condiciones Ambientales en feijoa (*Accasellowiana* [O. BERG] BURRET) Revista U.D.C.A Actualidad & Divulgación Científica 17 (1): 115 121. Disponible en: https://revistas.udca.edu.co/index.php/ruadc/article/view/946

PARÉS, J., ARIZALETA, M., SANABRIA, M.E. y BRITO, L., 2004. Características de los estomas, densidad e índice estomático y su variación en función a la enjertación en *Annonamuricata* L. y *A. montana* MADFAC. Bioagro.V [en línea], vol. 16, no. 3. Disponible en: https://www.redalyc.org/service/r2020/downloadPdf/857/85716309/1

REYES-LÓPEZ, D., QUIROZ-VALENTÍN, J., KELSO-BUCIO, H.A., HUERTA-LARA, M., AVENDAÑO-ARRAZATE, C.H., LOBATO-ORTIZ, R., REYES-LÓPEZ, D., QUIROZ-VALENTÍN, J., KELSO-BUCIO, H.A., HUERTA-LARA, M., AVENDAÑO-ARRAZATE, C.H. y LOBATO-ORTIZ, R., 2015. Caracterización Estomática de Cinco Especies del Género *Vanilla*. Agronomía Mesoamericana [en línea], vol. 26, no. 2, pp. 237-246. [Consulta: 28 enero 2020]. ISSN 1659-1321. DOI 10.15517/am.v26i2.19279. Disponible en: http://www.scielo.sa.cr/scielo.php?script=sci_abstract&pid=S1659-13212015000200237&lng=en&nrm=iso&tlng=es

RIVERA, C., PÉREZ, C., PÉREZ, A., ARMAS, I., VALDÉS, N. y SUAREZ, A.G., 2018. Estudio anatómico foliar de la especie *Juglans jamaicensis*subsp*insularis* (Griseb.) H. Schaarschm. San Andrés. La Palma, Cuba. *Revista Forestal Baracoa*, vol. 37.

SILVA, M., RUBILAR, R., ESPINOZA, J., YÁNEZ, M., EMHART, V. y QUIROGA, J.J., 2017. Respuesta en parámetros de intercambio gaseoso y supervivencia en plantas jóvenes de genotipos comerciales de *Eucalyptus*spp sometidas a déficit hídrico. *Bosque (Valdivia)* [en línea], vol. 38, no. 1, pp. 79-87. [Consulta: 28 enero 2020]. ISSN 0717-9200. DOI 10.4067/S0717-92002017000100009. Disponible en: https://scielo.conicyt.cl/scielo.php?script=sci_abstract&pid=S0717-92002017000100009&lng=es&nrm=iso&tlng=es.

TORAL, M., MANRÍQUEZ, A., NAVARRO-CERRILLO, R., TERSI, D. y NAULIN, P., 2010. Características de los estomas, densidad e índice estomático en secuoya (*Sequoia sempervirens)* y su variación en diferentes plantaciones de Chile. *Bosque (Valdivia)* [en línea], vol. 31, no. 2, pp. 157-164. [Consulta: 28 enero 2020]. ISSN 0717-9200. DOI 10.4067/S0717-92002010000200009. Disponible en: https://scielo.conicyt.cl/scielo.php?script=sci_abstract&pid=S0717-92002010000200009&lng=es&nrm=iso&tlng=es.

URQUIOLA, J.A., GONZÁLEZ, L., NOVO, R., ACOSTA, Z. 2010. *Libro rojo de la flora vascular de la provincia de Pinar del Río.* Edición. Universidad de Alicante. ISBN 978-84-97-17-061-1.

VÁZQUEZ, E. y TORRES, S., 2007. *Fisiología Vegetal*. La Habana, Cuba: Editorial Félix Varela.

WU, G., LIU, H., HUA, L., LUO, Q., LIN, Y., HE, P., FENG, S., LIU, J. y YE, Q., 2018. Differential Responses of Stomata and Photosynthesis to Elevated Temperature in Two Co-occurring Subtropical Forest Tree Species. *Frontiers in Plant Science*, vol. 9, pp. 467. ISSN 1664-462X. DOI 10.3389/fpls.2018.00467. Disponible en: https://frontiers.altmetric.com/details/41410160

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The authors declare not to have any interest conflicts.

Authors' contribution:
The authors have participated in the writing of the work and analysis of the documents.