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Research note

Importancia de la capa cuticular durante la colonización del hongo causante de la negrilla en *Agave salmiana* Otto ex Salm-Dyck ssp. *salmiana*

Importance of the cuticular layer during the colonization of the fungus that causes *negrilla* in *Agave salmiana* Otto ex Salm-Dyck ssp. *salmiana*

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Resumen

Las plantas del género *Agave* representan un recurso forestal no maderable valioso para la recuperación del suelo. La epidermis de sus pencas contiene múltiples estomas y está cubierta por una capa cuticular. En la actualidad presentan una enfermedad fúngica que se caracteriza por la existencia de manchas grises circulares sobre las pencas, que con el tiempo se tornan necróticas; y en ocasiones, dichas lesiones terminan por secar las pencas. El objetivo de este trabajo fue describir la relevancia de la capa cuticular durante la colonización del hongo causante de la negrilla en *Agave salmiana* ssp. *salmiana*. La capa cuticular tiene un grosor de 121 ± 2.8 mm. Se observó una distribución homogénea de los estomas y se determinó la densidad (22.67-27.67 estomas $mm^{-2)}$ y el índice estomático (10.61-14.15). Los estomas observados son de tipo tetracítico, el tamaño de los ostiolos de 57.9 mm ± 5 de largo y 23.75 mm ± 1.25 de ancho y células epidérmicas poligonales isodiamétricas. Los cortes transversales y paradermales muestran que las hifas y los apresorios fúngicos quedan restringidos al lado anverso de la capa cuticular, por lo cual se corrobora la importancia de conservar la epidermis en las pencas del maguey pulquero.

Palabras clave: *Agave salmiana* Otto ex Salm-Dyck, *Asterina mexicana* Ellis & Everh., capa cuticular, estomas, negrilla, ostiolo.

Abstract

The *Agave* genus plants represent a non-timber forest resource, valuable for soil recovery. The epidermis of the agave leaves contains multiple stomata and is covered by a cuticular layer. Currently, agave plants have a fungal disease that is characterized by circular gray spots on the *maguey* leaves, which over time become necrotic and eventually, these lesions end up drying them. The aim of this work was to describe the cuticular layer during the fungus colonization that causes bold in *Agave salmiana* subsp. *salmiana*.

The cuticular layer is 121 mm ± 2.8 thick. A homogeneous distribution of the stomata was observed, and the density (22.67-27.67 stomata mm^{-2}) and the stomatal index (10.61-14.15) were determined. Tetracytic stomata and isodiametric polygonal epidermal cells were identified, the ostioles size were calculated as 57.9 mm ± 5 long and 23.75 mm ± 1.25 wide. The transverse and paradermal sections showed that the fungal hyphae and appressoria are restricted from the obverse side of the cuticular layer, which confirms the importance of preserving the epidermis in the *maguey pulquero* leaves.

Key words: *Agave salmiana* Otto ex Salm-Dyck, *Asterina mexicana* Ellis & Everh., cuticle layer, stomata, negrilla, ostiole.

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The plants of the *Agave* genus represent a valuable non-timber forest resource for soil recovery, particularly in arid and semi-arid zones. The leaves (*pencas*) of agaves are covered with a cuticular layer that performs relevant functions, such as: gas exchange, control of temperature changes, as a barrier against pathogens, among others (Tafolla-Arellano *et al*., 2013). The cuticular layer that covers the leaves of *Agave salmiana* Otto ex Salm-Dyck provides highly appreciated characteristics in order to cook some traditional-food dishes; it is made up of epidermal cells, cutin, polysaccharides, waxes and stomata (Reina-Pinto and Yephremov, 2009; Staiger *et al*., 2019; Pérez-España *et al*., 2019). Stomata are formed by two guard or occlusive cells, which give rise to a pore called an ostiole. Guard cells control the opening and closing of ostioles in response to hormonal and environmental signals to regulate gas exchange (Zeng and He, 2010; Biswapriya *et al.,* 2015). *Agaves* have stomata on the abaxial and adaxial face of the leaves (Hernández-Valencia *et al*., 2003) that open at night and remain closed during the day to prevent excessive loss of liquids in the plant (Lee, 2010).

Phytopathogenic fungi use different mechanisms to colonize plants (Gudesblat *et al.,* 2009) such as: adhesion of spores to the cuticular layer, formation of appressoria and adhesive, invasive and infective structures, development of a high

pressure of turgidity in the invaded cells (Mendgen *et al.,* 2016) and degradation of the cell wall and the cuticular layer through the secretion of enzymes. Other phytopathogens have the ability to modulate the opening or closing of stomata through the release of chemical compounds (Staples and Macko, 1980; Lee *et al*., 1999; Bury *et al.,* 2013).

At present, *A. salmiana* plants show a disease known as smallpox or "*negrilla*"; caused by the *Asterina mexicana* Ellis & Everh. fungus (Ellis y Everhart, 1900; Cesaveg, 2008), of which there is little information. This disease causes dark spots on both sides of the leaves and can lead to necrosis and dryness. The objective of this work was to describe the importance of the cuticular layer during the colonization of the fungus that causes the *negrilla* in *Agave salmiana* Otto ex Salm-Dyck ssp. *salmiana.*

The cuticular layers were obtained from leaves of agave plantations in *Pachuca de Soto, Apan, Cardonal* (state of *Hidalgo*) and *Calpulalpan* (state of *Tlaxcala*) municipalities, from February to June 2019. Three leaves which carried symptoms of the disease were collected per ~8-year-old plant (according to the experience of the producers) by municipality. From the adaxial face of each *penca*, a sample was taken from the apical, middle and base sites (Figure 1a). Cuticular layer samples were removed from the collection site and kept in a 2 % glycerol solution until they were analyzed.

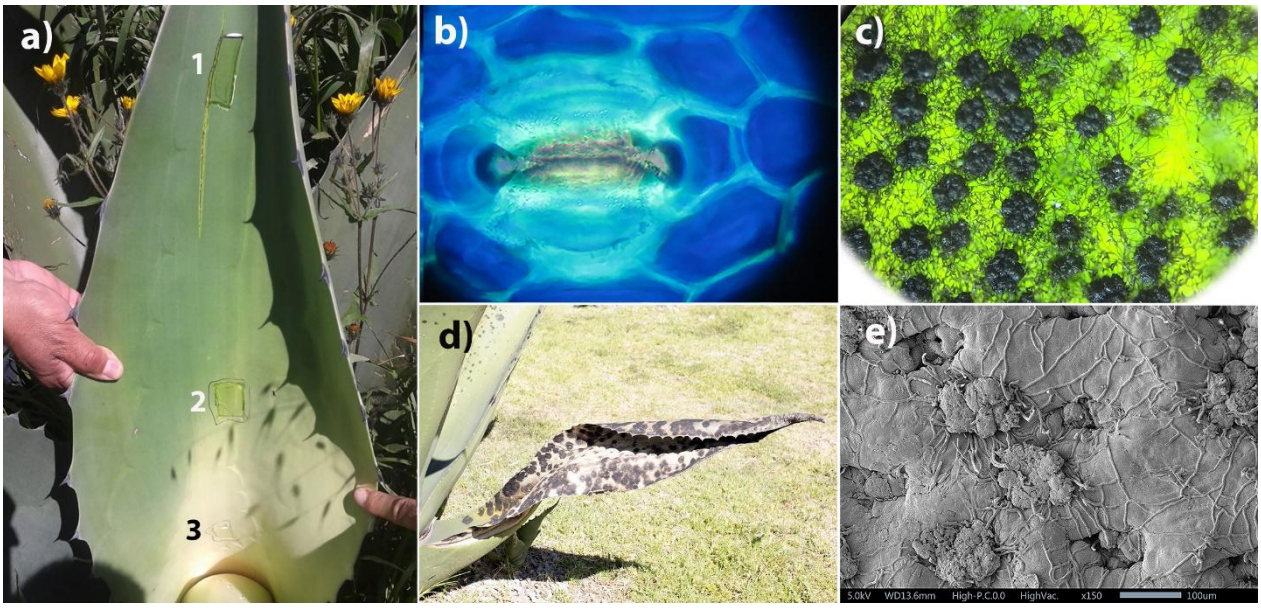


Figure 1. Cuticular layer of *Agave salmiana* Otto ex Salm-Dyck ssp. *salmiana*, (a) Extraction of the cuticular layer in three areas of the adaxial face: 1) distal area, 2) middle area and 3) proximal area; (b) Adverse of stomata, guard cells and epidermal cells; (c) appressoria observed in black spot (4X), (d) dehydrated *penca* due to invasion of the bold and (e) appressoria obstructing the stomata (SEM).

Stomatal index (*SI*) and stomatal density (*SD*) were determined with an optical microscope (Zeigen, ZB-7300). Cuticles were stained with methylene blue for better definition. The SI was calculated according to what was recommended by Sosa *et al*. (2014) from the following formula:

$$IE = (NE)/(CE + NE) \times 100$$

Where:

NE = Number of stomata per observation field

CE = Number of epidermic cells in the same observation field

SD was determined by calculating the number of stomata found in 19.635 mm^{-2}, equivalent to the field observed (40X) in a 2.5 mm radius. The SI and SD results were analyzed with ANOVA and the significant differences ($P < 0.05$) between the groups with the Bonferroni test (GraphPad Prism Software version 5.00 for Windows, GraphPad Software Inc.).

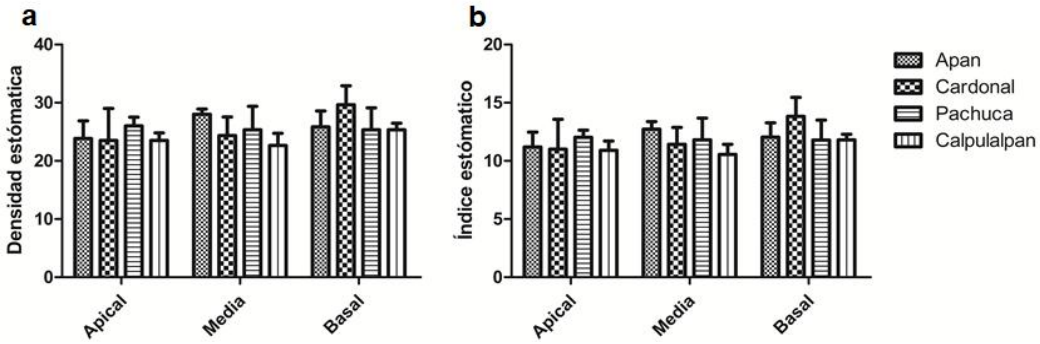
For the analysis of the microstructure, the cuticular layers were dehydrated in a vacuum oven (Shel-Lab, Mod. 1407) at 40 °C and 45 kPa. Subsequently, they were fixed with carbon tape to an aluminum slide, covered with gold-palladium and observed with a scanning electron microscope (SEM; Model JEOL JMS-5600LV).

The distribution of the stomata in the cuticular layer of the abaxial face showed homogeneity in the analyzed areas. In the micrographs of the cuticular layer of *A. salmiana* ssp. *salmiana* with a 121 ± 2.8 mmascomata thickness, the presence of waxes, epidermal cells and stomata was recognized. The average thickness recorded for the cuticle of *A. tequilana* F. A. C. Weber is 8.67 µm for the abaxial face (Hernández-Valencia *et al*., 2003).

SD showed homogeneity in the analyzed areas. The tetracytic-type stomata, the size of the ostioles of 57.9 mm ± 5 long and 23.75 mm ± 1.25 wide, and the isodiametric polygonal epidermal cells (Figure 1b) are similar to those reported by Vargas-Rodríguez *et al.* (2017) and by Chávez-Güitrón *et al.* (2019). The difference in the size of the ostioles is attributed to variations in the thickness and distribution of the cuticular wax, to the species and to the environmental conditions in which the plants grow (García, 2007; Bernardino-Nicanor *et al*., 2012).

Macroscopically, bold disease is seen as gray spots that turn black over time (Figure 1d). Inside the stain, the existence of ascomata with hyphae specialized in the invasion of plant tissue is identified (Figure 1c) that accumulate in the *Agave* leaf and obstruct the stomata; in addition, other hyphae adhered to the cuticular layer are observed (Figure 1e).

SD (22.67 to 27.67 stomata mm^{-2}) did not show significant differences between the areas of the adaxial face of the analyzed leaves or between municipalities (Figure 2a); the values (mm^{-2}) were similar to those recognized for other *Agave* species, such as: *A. mapisaga* Trel in L. H. Bailey (20-50 stomata; Nobel, 1994), *A. deserti* Engelm. (34 stomata; Gentry and Sauck, 1978) and *A. atrovirens* Karw. Ex Salm-Dyck (29-30 stomata; Bernardino-Nicanor *et al.,* 2012); but they differ from *A. promontorii* Trel. (18 stomata) and *A. sisalana* Perrine (11 stomata; Neto and Martins, 2012).



Densidad estomática = Stomatal density; *Índice estomático* = Stomatal index; *Apical* = Apical; *Media* = Middle; *Basal* =Base.

Figure 2. Index and stomatal density in the cuticular layer of *Agave salmiana* Otto ex Salm-Dyck ssp. *salmiana* in different municipalities.

SI for *A. salmiana* ssp. *salmiana* is 10.61-14.15 (Figure 2b) while for *A. tequilana* it is between 5.72 and 6.68 stomata/epidermal cells (Hernández-Valencia *et al.,* 2003). Both parameters (*IE* and *SD*) are influenced by some environmental and nutritional conditions (Wilkinson, 1979; Roth *et al*., 1986).

In the paradermal and transverse sections, it was noted that the cuticular layer acts as a mechanical barrier and prevents the passage of the reproductive structures of the pathogenic fungus into the interior of the *Agave* leave. With the SEM micrographs, it was detected that the hyphae and appressoria of the phytopathogen only appear on the obverse side of the cuticular layer since they could not be

identified on the reverse side. In addition, it was observed that the appressoria are located mainly in the stomata, which suggests that the mechanism of invasion of this fungus consists of drying the leaves through the modulation of the stomata, by preventing them from completing the cycle (day-night) in a normal way for affected plants.

SEM micrographs show the importance of the cuticular layer as a mechanical barrier by preventing hyphae from entering the innermost layers of the maguey leaf. Therefore, the infection is restricted to the obverse side of the cuticular layer.

On the other hand, *SD* coincides with the records for other species of the *Agave* genus. However, this is the first report on the importance of the cuticular layer of *Agave salmiana* ssp. *salmiana* during its interaction with the phytopathogenic fungus responsible for the bold disease. Complementary studies are necessary to propose alternatives to prevent or control fungal invasion.

Conflict of interests

The authors declare no conflict of interest.

Contribution by author

Víctor Hugo Pérez Spain: research development, data analysis and correction of the manuscript; Jaime Alioscha Cuervo Parra: data analysis, structure and design of the manuscript; José Esteban Aparicio Burgos and Martín Peralta Gil: data interpretation and correction of the manuscript; Mario Alberto Morales Ovando: structure and design of the manuscript; Teresa Romero Cortes: coordination of the work, development of the research, analysis and interpretation of results.

References

Bernardino-Ninacor, A., R. Mora-Escobedo, J. L. Montañez-Soto, S. Filardo-Kerstupp and L. González-Cruz. 2012. Microstructural differences in *Agave atrovirens* Karw leaves and pine by age effect. African Journal of Agricultural Research 7(24):3550-3559. Doi:10.5897/ AJAR11.1185.

Biswapriya, B. M., R. A. Biswa, D. Granot, S. M. Assmann and S. Chen. 2015. The guard cell metabolome: functions in stomatal movement and global food security. Frontiers in Plant Science 6:1-13. Doi:10.3389/fpls.2015.00334.

Bury, M., A. Andolfi, B. Rogister, A. Cimmino, V. Mégalizzi, V. Mathieu, O. Feron, A. Evidente and R. Kiss. 2013. Fusicoccin A, a phytotoxic carbotricyclic diterpene glucoside of fungal origin, reduces proliferation and invasion of glioblastoma cells by targeting multiple tyrosine kinases. Translational Oncology 6(2):112-23. Doi: 10.1593/tlo.12409.

Comité Estatal de Sanidad Vegetal de Guanajuato A.C. (CESAVEG). 2008. Manual de plagas y enfermedades del *Agave*. Casa editorial. Irapuato, Gto., México. 28p.

Chávez-Güitrón, L. E., F. C. Salinas-Pérez, E. A. Pérez-Salinas, J. Caballero, A. Vallejo-Zamora y E. Sandoval-Zapotitla. 2019. Variación de caracteres epidérmico-foliares de *Agave salmiana* subsp.*salmiana* (Asparagaceae) en el centro de México. Botanical Sciences 97 (4): 711-724. Doi: 10.17129/botsci.2159.

Ellis J. B. and B. M. Everhart. 1900. New species of fungi from various localities with notes on some published species. Bulletin of the Torrey Botanical Club. 27(2):49-64. https://www.jstor.org/stable/2478343.

García M., A. 2007. Los agaves de México. Ciencias 87:14-23. https://www.redalyc.org/pdf/644/64408704.pdf (20 de enero de 2021).

Gentry, H. S. and J. R. Sauck. 1978. The stomatal complex in *Agave*: groups Deserticolae, Campaniflorae, Umbelliflorae. Proceedings of the California Academy of Sciences series 41:371–387. https://ia800206.us.archive.org/0/items/biostor-78394/biostor-78394.pdf (25 de octubre de 2020).

Gudesblat, E. G., S. P. Torres and A. A. Vojnov. 2009. Stomata and pathogens: warfare at the gates. Plant Signaling & Behavior 4(12):1114-1116. Doi: 10.4161/psb.4.12.10062.

Hernández-Valencia, R. E. M., R. López-Franco and A. Benavides-Mendoza. 2003. Micromorfología de la epidermis foliar de *Agave tequilana* Weber. Agrofaz 3(2):387-396. https://www.researchgate.net/publication/283995003 (15 de octubre de 2020).

Lee S., H. Choi, S. Suh, I.S. Doo, K. Y. Oh, E. J. Choi, A. T. S. Taylor, P. S. Low and Y. Lee. 1999. Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and Commelina communis. Plant Physiology 121:147-52. Doi:10.1104/pp.121.1.147.

Lee, J. S. 2010. Stomatal opening mechanism of CAM plants. Journal of Plant Biology 53:19–23. Doi:10.1007/s12374-010-9097-8.

Mendgen, K., M. Hahn and H. Deising. 1996. Morphogenesis and mechanisms of penetration by plant pathogenic fungi. Annual Review of Phytopathology 34:367–86. Doi: 10.1146/annurev.phyto.34.1.367.

Neto, I. L. C. and F. M. Martins. 2012. Anatomia dos órgãos vegetativos de Agave sisalana Perrine ex Engelm (Agavaceae). Revista Caatinga 25(2):72-78. https://www.redalyc.org/pdf/2371/237123825011.pdf (12 de febrero de 2021).

Nobel, P.S. 1994. Remarkable Agaves and Cacti. Cambridge University Press. New York, NY, USA. 166 p.

Pérez-España, V. H., J. A. Cuervo-Parra, C. Paz-Camacho, M. A. Morales-Ovando, C. A. Gómez-Aldapa, G. C. Rodríguez-Jimenes, V. J. Robles-Olvera, P. A. López-Pérez and T. Romero-Cortés. 2019. General characterization of cuticular membrane

isolated from *Agave salmiana*. International Journal of Bio-resource and Stress Management 10(1):046-052. Doi:10.23910/IJBSM/2019.10.1.1950.

Reina-Pinto, J. J. and A. Yephremov. 2009. Surface lipids and plant defenses. Plant Physiology and Biochemistry 47(6):540-549. Doi:10.1016/j.plaphy.2009.01.004.

Roth I., T. Merida y H. Lindorf. 1986. Morfología y anatomía foliar de plantas de la Selva Nublada de Rancho Grande. Parque Nacional "Henry Pittier". El ambiente físico, ecología general y anatomía vegetal. Fondo Editorial Acta Científica Venezolana. Caracas, Venezuela. pp. 205-241.

Sosa, C. M., G. S. Alemán, H. Y. Pérez, C. E. Abreu, C. D. Sosa and O. G. González. 2014. Caracterización de la lámina foliar de plantas de *Agave fourcroydes* Lem. obtenidas por propagación asexual. Biotecnología Vegetal 14(1):37-44. https://revista.ibp.co.cu/index.php/BV/article/view/40/433 (12 de febrero de 2021).

Staiger, S., P. Seufert, K. Arand, M. Burghardt, C. Popp and M. Riederer. 2019. The permeation barrier of plant cuticles: uptake of active ingredients is limited by very long-chain aliphatic rather than cyclic wax compounds. Pest Management Science 75(12):3405-3412. Doi: 10.1002/ps.5589.

Staples, R. C. and V. Macko. 1980. Formation of infection structures as a recognition response in fungi. Experimental Mycology 4(1):1-15. Doi:10.1016/0147-5975(80)90045-6.

Tafolla-Arellano, J. C., A. González-León, M. E. Tiznado-Hernández, L. Zacarías G. and R. Báez-Sañudo. 2013. Composición, fisiología y biosíntesis de la cutícula en plantas. Revista Fitotecnia Mexicana 36(1):3-12. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802013000100001&lng=es&tlng=es (20 de noviembre de 2020).

Vargas-Rodríguez, L., M. I. García-Vieyra, B. I. León-Bata and P. Lozano-Sotomayor. 2017. Physical properties and microscopic structure of the *Agave salmiana* cuticle (mixiote). Revista Chapingo Serie Zonas Áridas 17(2):1-9. Doi: 10.5154/r.rchsza.2017.12.017.

Wilkinson, H. 1979. The plant surface (mainly leaf). *In:* C. R. Metcalfe and L. Chalk (eds.). Anatomy of Dicotiledons. Oxford Claredous Press. London, UK. pp. 97-16.

Zeng, W. and S. Y. He. 2010. A prominent role of the flagellin receptor FLAGELLIN-SENSING2 in mediating stomatal response to *Pseudomonas syringae* pv tomato DC3000 in *Arabidopsis*. Plant Physiology 153:1188–1198. Doi: 10.1104/pp.110.157016.