Short note [Nota corta]

AGENTE ASOCIADO CON EL TIZÓN TEMPRANO *Alternaria alternata* (FR.) KEISSLER EN EL CULTIVO DE TOMATE *Solanum lycopersicum* L. EN MÉXICO †

[AGENT ASSOCIATED WITH EARLY BLIGHT *Alternaria alternata* (FR.) KEISSLER IN THE *Solanum lycopersicum* L. TOMATO CROP IN MEXICO]

Abiel Sánchez-Arizpe, Ma. Elizabeth Galindo-Cepeda, José Luis Arispe-Vázquez* and Eduardo García-Tapia

Departamento de Parasitología, Universidad Autónoma Agraria Antonio Narro, Antonio Narro #1923, C.P. 25315, Buenavista, Saltillo, Coahuila, México. Tel. 844-4110226. Email: arispe_uaaan@hotmail.com
**Corresponding author*

SUMMARY

Background. Tomato is an important crop in Mexico, due mainly to its economic profitability. **Objective.** The objective of the research was to identify the agent associated with early blight in tomato at Morelos. **Methodology.** Samples of diseased plants were taken from a greenhouse at Jantetelco country of the Morelos state and taken to the laboratory. Later, 1 cm cuts of diseased and healthy tissue were made which disinfected with 3% sodium hypochlorite for 1 min and washed three times with sterile distilled water for 1 min. Later, four leaf cuts in were put in Petri dishes with PDA culture medium (Bioxon) kept at 25 °C ± 2 °C for 168 h, the pathogen purification was done by monoconidial cultures and pathogen identifying by morphocultural criteria and molecularly. **Results.** The agent associated with early blight was *Alternaria alternata*. **Implications.** Our result reveals the opportunity to improve the control of this pathogen. **Conclusion.** Although *A. alternata* to infect the fruit peduncle causing abortion, in this research the pathogen was found also in leaves.
Keywords: Agent; Identification; Incidence; Foliage; Software.

RESUMEN

Antecedente. El tomate es un cultivo importante en México, debido principalmente a su rentabilidad económica. **Objetivo.** El objetivo de la investigación fue identificar el agente asociado con el tizón temprano en tomate en Morelos. **Metodología.** Se tomaron muestras de plantas enfermas de un invernadero de la ciudad de Jantetelco, estado de Morelos, y se llevaron al laboratorio. Más tarde, se hicieron cortes de 1 cm de tejido sano y enfermo que se desinfectaron con hipoclorito de sodio al 3% durante 1 min y se lavó tres veces con agua destilada estéril durante 1 min. Después, se colocaron cuatro cortes de hojas en placas de Petri con medio de cultivo PDA (Bioxon) mantenidos a 25 °C ± 2 °C durante 168 h, la purificación del patógeno se realizó mediante cultivos monoconidiales y la identificación mediante criterios morfoculturales y molecularmente. **Resultados.** El agente asociado con el tizón temprano fue *Alternaria alternata*. **Implicaciones:** Nuestro resultado revela la oportunidad de mejorar el control de este patógeno. **Conclusión.** Aunque *A. alternata* infecta el pedúnculo de la fruta que causa el aborto, en esta investigación se encontró el patógeno en las hojas.
Palabras Clave: Agente; Identificación; Incidencia; Follaje; Software.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the main export vegetable of products of Mexico. In November 2019, the cummulative production in the spring-summer cycle was 1159936 t, that is, 2.2% higher than the obtained in the same period the previous year and the total harvested area per month October in this cycle was 16161 ha, which represents an increase of 9% over the previous year. Morelos with a production of 78914 t, occupates the seventh place of production, which represents 5.2% of the production national (SIAP, 2019).

One of the main fungal diseases of tomatos is the early blight since it is distributed in all frowing areas of the world and can become a major disease if favorable environmental conditions for its development occur (Momol and Pernezny, 2006; UC IPM, 2009). In addition to early blight, the late blight *Phytophthora*

† *Submitted August 1, 2020 – Accepted October 22, 2020.*
ISSN: 1870-0462.

infestans (Mont.) De Bary (Agrios, 2005) and the septoria leaf spot *Septoria lycopersici* Spegazzini (Ishjijima and Okawara, 2002) stand out among the relevant leaf diseases in tomato, and among the major vascular diseases are; *Fusarium oxysporum* f. sp. *lycopersici (*Ben *et al.,* 2016) and *Verticillium* spp. (Bubici and Cirulli, 2008).

Early blight damages stem, leaves, and fruits of the tomato, beging with small circular spots brown or black on the older leaves; lesions may be surrounded by a chlorotic halo, and as the disease develops, spots become larger 8-10 mm in diameter or length. As the disease develops, can damage stems and fruits, the stains in fruits are similar to those on leaves with brown color and dark concentric rings; in these rings, dusty and dark spores are produced (Cepeda, 2009). This it is a disease that affects the aerial part of tomato plants, mainly leaves in all growing stages (Sepulveda, 2018).

Species within *Alternaria* that have been reported causing early blight of tomato are: *A. alternata* (Fr.) Keissler, *A. tomatophila* Simmons, *A. solani* Sorauer and *A. tomato* (Cooke) LR Jones (Blancard, 2012; Snowdon 1990). *A. solani* is a plant pathogen and causal agent of early blight in solanum species (Russell *et al*., 2010); however, there are reports of *A. alternata* causing early blig**h**t of tomato at Pakistan and China (Akhtar *et al.*, 2004; Ren *et al.*, 2017). Due the above mentioned the objective of the research was to identify the agent associated with early blight in tomato at Morelos.

MATERIALS AND METHODS

Location of the area of study

Diseased tomato plants were collected (in the first stage of fruit development) in a greenhouse at Jantetelco, Morelos (Figure 1), whose coordinates are (18°42'44.7" N 98°46'31.0" W). On the first sampling September 2017 24 plants were collected; on the second in October 2018, 16 plants were collected for a total of 40 disease plants for this research, placed in paper bags and taken to the phytopathology laboratory of Universidad Autonoma Agraria Antonio Narro and stored at 4 °C for further analysis.

Disinfection

1 cm cuts of healthy and diseased tissue (foliage with concentric circular spots) were made and disinfected with 3% sodium hypochlorite for 1 min and washed three times with sterile distilled water for 1 min.

Isolation

Four leaf cuts in were put in Petri dishes with PDA culture medium (Bioxon) + Antibiotic (Oxytetracycline, 1 mL L^{-1}), to prevent bacterial growth, with a total of three replicates by plant, kept at 25 °C ± 2 °C for 168 h.

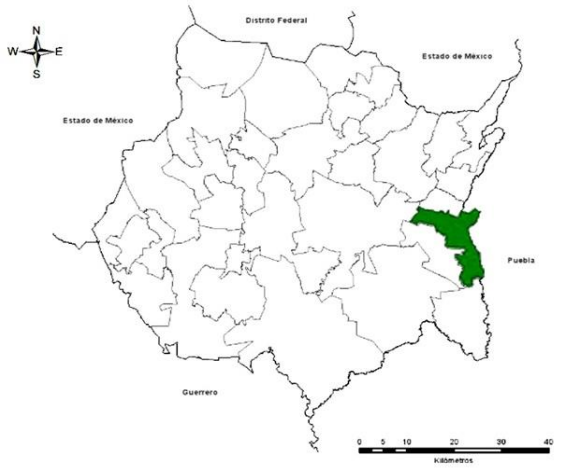

Figure 1. Location of the study area "Jantetelco, Morelos, Mexico".

Purification

Six mm diameter discs were taken from the pathogen colonies, and put in test tubes with 9 mL of sterile distilled water and 2 µL of tween 20, stirring in the bortex for 20 s and then 50 µL put in a Petri dish with PDA culture medium. Twenty four h later, one taken germinated conidia were placed in Petri dishes with PDA, kept at 25 C ± 2 °C for 120 h.

Morphocultural and molecular identification

Identification was made in a compound microscope using DinoCapture 2.0 software (Dino-lite, 2020), based on the mycelium characteristics, color and shape of the colony, color, long and wide of 100 conidia, following Rotem (1994), Barnett and Hunter (2006), and the update key on the Classification Systems and Identification of Hypomycete Fungi (Castaneda, 2004). Molecular analyses were performed in the Instituto Potosino de Investigación Científica y Tecnológica, primers ITS1 (5´TCCGTAGGTGAACCTGCGG-3´) and ITS4 (5´TCCTCCGCTTATTGATATGC-3´) for molecular identification of isolates, for the DNA extraction by method Dellaporta (Dellaporta *et al*., 1983) based on the use of SDS (Sodium Dodecyl Sulfate), visualization of the DNA obtained was carried out by electrophoresis in an agarose gel to 2% (p/v), in

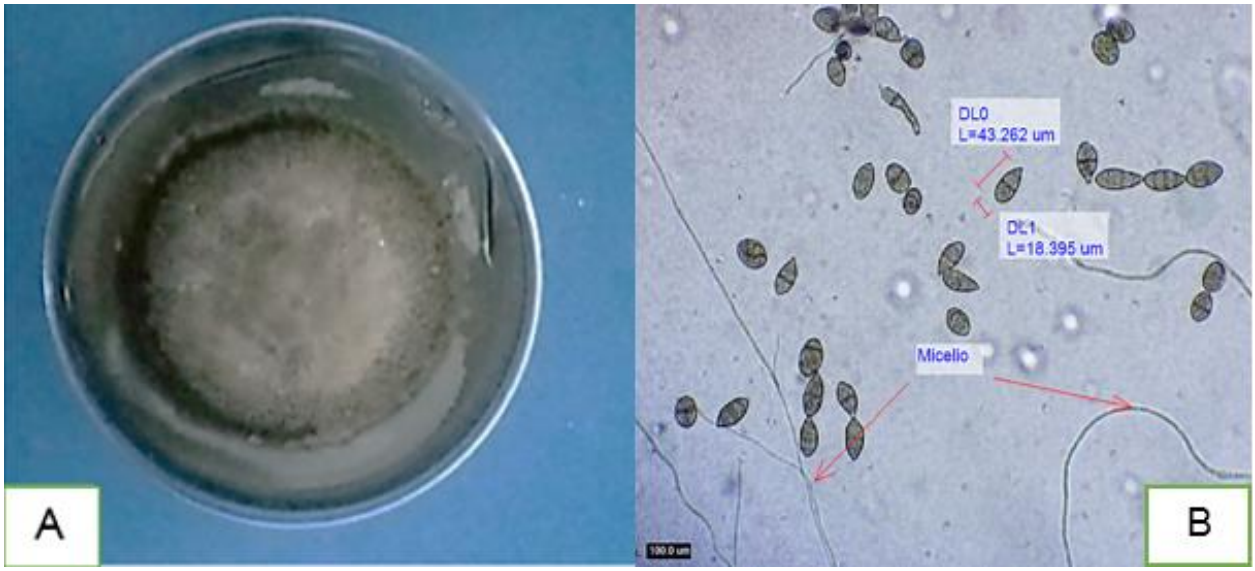

Figure 2. *A. alternata;* A) Colony, B) Conidia.

addition DNA was quantified on a NanoDrop 1000 Spectrophotometer (Thermo Scientific) and the Thermal cycler Veriti for end point PCR. The samples were sequenced with the dideoxynucleotide method labeled in the 3130 Genetic Analyzer sequencer, and the sequence obtained was assembled and compared with those available in the database of the National Center for Biotechnology Information (NCBI).

RESULTS AND DISCUSSION

The amplified regions were approximately 600 pb in size and of all the tomato foliage samples analyzed, the presence of *A. alternata* (Figure 2) was found on 20%, of then determined morphoculturally and molecularly, presenting particular mycelium macroscopic characteristics as; velvety, flattened, slow-growing and gray to dark-gray colony color. Microscopically, simple, linear, light brown conidiophores were observed and brown-colored conidia with transverse and vertical septa, 20.103 to 54.776 µm in length and 4.010 to 16.592 µm width (Figure 2), results that agree with to Ramjegathesh and Ebenezar, (2012) and Agrios, (2005) reports.

Rodríguez-Roa *et al.*, (2013) reported *A. alternata*, with measures 11.8 to 52.6 µm length and 7.1 to 23.4 µm width on tomato leaves in Bogotá (Suba), Colombia. Our results are similar to those reported by Flores *et al*., 2013, who identified this phytopathogen from arugula leaves (*Eruca vesicaria* ((Linnaeus) Cavanilles)) with similar symptoms on the leaves at Morelos, Mexico, with conidia measures 19.2 to 55.9 µm in length and 6.1 to 11.8 µm width, obtaining a of 99.0% similarity percentage. Cova and Rodriguez (2003) identified *A. alternata* with measures of conidia of 25.42 ± 3.78 µm x 9.83 x 1.62 µm with a length-width ratio of 1: 2.6, in onion leaves at Lara, Venezuela.

The sequence obtained whet at compared to BLAST resulted in *A. alternata* (Table 1). Espinoza *et al*., 2009 isolated this pathogen, likewise from concentric circular leaf spots, collected in this case of watermelon and tomato. Sankar *et al*., (2012) using the ITS, region identified *A. alternata* in *Rumex vesicarius* L. plants, with a 100% similarity percentage, as in this research (Table 1).

Table 1. Compared Sequence in BLAST.

Pathogen	Access Key	Top Score	Identity (%)
A. alternata	MH992147.1	983	100%

It should be noted that *A. solani* is more frequently found in Morelos; however, its damage and importance in rapidly increasing incidence of *A. alternata* should not be ruled out since. Although *A. alternata* is often associated with *A. solani*, in this research, this pathogen was not found, *A. alternata* the main disease of ripe tomato fruits for the industry in North Sinaloa, Mexico (Felix and Galvez, 2002). Whose pathogen is responsible for serious economic losses, especially in the product for the industry (Mejia and Hernández, 2001), Both species of *Alternaria* are responsible for early blight in tomato, whose pathology can decrease yields between 20 to 30% (Martinez *et al*., 2002) and from 50 to 80% (Piña, 1979; Fernandez *et al.*, 1996).

The importance of this pathogen lies in its ability to survive up to a year in the crop and soil residues (Seminis, 2020; Sela, 2020), as well as in remains of diseased plants, in seed covers and various weed species, which can host of the fungus without showing symptoms (asymptomatic), being an important source of infection (Reyes, 2016). In addition to mycotoxins production such as alternariol, alternariol monomethyl ether, (tenuazonic acid), and altertoxin I and II that cause health problems, affect the safety of processed products (Hasan, 1995; Motta *et al.*, 2000; Sempere and Santamarina, 2007), it is very necessary to implement quality control measures which guarantee harmless tomato production.

CONCLUSIONS

A. alternata was identified in tomato leaves. *A. alternata* is associated with *A. solani* since there is confusion that *A. alternata* is only found in the peduncle of the fruit causing abortion, therefore, it should be noted that this pathogen can also be found in leaves.

Acknowledgements
The authors want to thank the farmers of the greenhouse at Jantetelco, Morelos.

Funding. This research was funded by the Universidad Autonoma Agraria Antonio Narro.

Conflict of interests. The authors declare no conflict of interests

Compliance with ethical standards. This research complies with ethical standard required for the research according to the handling of biological material.

Data availability. Data are available with the corresponding author (arispe_uaaan@hotmail.com) upon reasonable request.

REFERENCES

Agrios, G.N. 2005. *Plant Pathology*. 5ta Ed. Estados Unidos: Elsevier Academic Press**.**

Akhtar, K., Saleem, M.Y., Asghar, M., Haq, M.A. 2004. New report of *Alternaria alternata* causing leaf blight of tomato in Pakistan. Plant Pathology. 53816-816. https://doi.org/10.1111/j.1365-3059.2004.01099.x

Barnett, L.H., Hunter, B.B. 2006. Illustrate genera of imperfect fungi. 4th Ed. Minnesota. The American Phytopathology Society Press. 978-0-89054-192-0. 200-220.

Ben, A.R.A., Mokni-Tlili, S., Nefzi, A., Jabnoun-Khiareddine, H., Daami-Remadi, M. 2016. Biocontrol of *Fusarium* wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Nicotiana glauca* organs. Biological Control. 2016; 97:80–88. https://doi.org/10.1016/j.biocontrol.2016.03.005

Bubici, G., Cirulli, M. 2008. Integrated Management Of *Verticillium* Wilt Of Tomato. https://doi.org/10.1007/978-1-4020-8571-0_12

Blancard, D. 2012. Tomato diseases: identification, biology, and control: A Color Handbook. London, UK: CRC Press. 9781138034259.

Castañeda, R. 2004. Update in the classification and identification systems of hyphomycete fungi. National Institute of Agricultural Research (INIA). Maracaibo. 3-61.

Cepeda, M.S., 2009. Red tomato crop and parasitological control. First edition. Trillas. Mexico DF.

Cova, J., Rodriguez, D., 2003. Fungi Associated with the burned foliar of the onion (*Allium* cepa L.) in the state of Lara, Venezuela. Bioagro. Vol. 15, n.3. 1316-3361.

Dino-lite, 2020. DinoCapture 2.0: software de imágenes de microscopio. [online] Available at: <https://www.dinolite.us/en/dinocapture/> [Accessed 15 Ene 2019].

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA minipreparation version II. Plant Molecular Biology reporter. 18: 61-64 pp.

Espinoza, L., Jiménez, M., Peralta, E. 2009. Conservation of Pathogens and Epiphytes Present in Tomato, Watermelon, and Banana Crops and their Research Benefits. ESPOL - RTE Technology Magazine. 22, N.1, 51-56.

Felix, G.R., Galvez, F.C.A. 2002. Black Mold Control, *Alternaria alternata* (Fr.:Fr.) In the Tomato Fruit (*Lycopersicon esculentum* Mill.) Considering Heat Units and Environmental Variables for Application. Mexican Journal of Phytopathology. 20 (1), 72-76.

Flores, C.M.A., Martínez, D.M.T., Nieto, Á.D, Rodríguez, P.J.E., Colinas, L.M.T., Martínez, S.J. 2013. Reducción en la Germinación in vitro de Conidios de *Alternaria alternata* aislada de *Eruca sativa* con Jugo de Brócoli. 31(2), 180-190.

Fernández, A., Solorzano, E., Peteira, B., Fernández, E. 1996. Peroxidase induction in tomato

leaves with different degrees of susceptibility to *A. solani*. Protection Vegetal. 11 (2): 79-83, Cuba.

Hasan, H.A.H., 1995. *Alternaria* mycotoxins in black rot lesion of tomato fruit: conditions and regulation of their production. Mycopathologia.130 (3), 171-177. https://doi.org/10.1007/BF01103101

Ishjijima, T., Okawara, Y. 2002. Manual de Técnicas de cultivo de hortalizas de fruta (Tomate, Melón, Frutilla). Caacupé, Paraguay. MAG-JICA. 240 p.

Martínez, B., Bernal, A., Pérez, S., Muñiz, Y. 2002. Pathogenic variability of isolates of *Alternaria solani* Sor. Plant Protection Journal. 17 (1): 45-53.

Mejía, A.J., Hernández, M.M. 2001. Evaluación de azoxystrobin en el control de la candelilla temprana (*Alternaria solani*) en el cultivo de tomate Rev. Fac Agron. (LUZ). 18: 106-116. [online] Available at: <https://produccioncientificaluz.org/index.php/agronomia/article/view/26388> [Accessed 29 Mar 2020].

Momol, T., Pernezny, K. 2006. Florida Plant Disease Management Guide: Tomato. Publication PDMG-V3-53. Florida Cooperative. [pdf] Available at: <https://plantpath.ifas.ufl.edu/rsol/RalstoniaPublications_PDF/IFASExt2006_FloridaPDMG-V3-53.pdf> [Accessed 26 Dic 2019].

Motta, S., Valente, S.L.M. 2000. A method for the determination of two *Alternaria* toxins, alternariol and alternariol monomethyl ether, in tomato products. Brazilian Journal of Microbiology. 31, 315-320.

Piña, A. 1779. Methodology of early blight signaling (*Alternaria solani* (Ell and Martin) Jones and Grout) in potato and tomato crops. Methodologies for Signaling and Forecasting of Pests and Diseases, Inisav, Habana.

Ramjegathesh, R., Ebenezar, E. 2012. Morphological and Physiological Characters of *Alternaria alternata* causing Leaf blight of onion. International Journal Pathology. 3(2), 34-44. [online] Available at: <https://scialert.net/abstract/?doi=ijpp.2012.34.44> [Accessed 10 Feb 2020].

Ren, Z.J., Cao, A.C., Li, J., Guo, M.X. , Wang, Q.X., Yan, D.D., Li, Ouyang, C.B., Li, .Y. 2017. First Report of *Alternaria alternata* Causing Black Spot on Tomato (*Solanum lycopersicum*) in Tongzhou, China. Plant Disease. 101:9, 1680. https://doi.org/10.1094/PDIS-03-17-0346-PDN

Reyes, M.M. 2016. Agricultural Research Institute. Regional Raihuén Research Center. "Control of blight in industrial tomato through an early warning system". INIA BULLETIN - N ° 338. 0717-4829. [pdf] Available at: <http://biblioteca.inia.cl/medios/biblioteca/boletines/NR40563.pdf> [Accessed 02 Aug 2019].

Rodríguez-Roa, J.H., Cardenas, M., Jiménez, P. 2013. Especies de *Alternaria* de la Sabana de Bogotá, Colombia. Revista Facultad de Ciencias Básicas. 9. 228. https://doi.org/10.18359/rfcb.361

Rotem, Y. 1994. The Genus *Alternaria*: Biology, Epidemiology, and Pathogenicity. APS Press, American Phytopathological Society. 326.

Russell, J.C., Thomas, J., Thomas J.S., 2010. Comprehensive Natural Products II. Natural Products Structural Diversity-I Secondary Metabolites: Organization and Biosynthesis. [online] Available at: <https://www.sciencedirect.com/referencework/9780080453828/comprehensive-natural-products-ii#book-info> [Accessed 05 Dic 2019].

Sankar, N.R., Devamma, M.N. and Giridhar, D. 2012. First report of *Alternaria alternata* causing leaf spot on *Rumex vesicarius* in India. Australasian Plant Disease. Notes. 7, 17-18. https://doi.org/10.1007/s13314-011-0036-4

Sela, G., 2020. Tizón Temprano del tomate. [online] Available at: <https://cropaia.com/es/blog/tizon-temprano-del-tomate/> [Accessed 20 Feb 2020]

Seminis, 2020. Cancro de Tallo por *Alternaria*. 2020. [online] Available at: <https://www.seminis-andina.com/recursos/guias-de-enfermedades/tomates/alternaria-stem-canker/> [Accessed 10 Feb 2020]

Sempere, F., Santamarina, M.P. 2007. *In vitro* biocontrol analysis of *Alternaria alternata* (Fr.) Keissler under diferent environmental conditions. Mycopathologia. 163, 183-190. https://doi.org/10.1007/s11046-007-0101-x

Sepulveda, R.P. 2018. Agricultural Research Institute. Regional Raihuén Research Center. Integrated Management of Pest and *Alternaria* Diseases in tomato. Technical Sheet 12.

SIAP, 2019. Agrifood and Fisheries Information Service. Closure of Agricultural Production

by Crop "Irrigation + temporary modality". SAGARPA, D.F., Mexico. [online] Available at: <http://infosiap.siap.gob.mx:8080/agricola_siap_gobmx/ResumenDelegacion.do> [Accessed 19 Aug 2019].

Snowdon, A,L. 1990. A color atlas of post-harvest diseases and disorders of fruits and vegetables. Vol. 2: Vegetables. London, UK: Manson Publishing Ltd. 1

UC Statewide IPM Program., 2019. Pest management guidelines: tomato. University of California, Davis. 2009 [online] Available at: <http://ipm.ucanr.edu/index.html> [Accessed 12 Sep 2019].