Investigation note

First report of pokkah boeng in sugarcane in the Huasteca Potosina

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Abstract

Sugarcane (*Saccharum officinarum*) is one of the main crops produced in the world, Mexico is the sixth producer worldwide, while San Luis Potosí ranks third in production nationwide. Sugarcane plants with wilt symptoms similar to those caused by pokkah boeng disease were collected in sugarcane fields of the Huasteca Potosina. This disease known as twisted top is caused by some species of the genus *Fusarium* and causes economic losses due to the decrease in the quality of the harvested crop. Two isolates of fungi with morphological characteristics typical of *Fusarium* sp. were obtained from the stems of these plants. The identification of the isolates was carried out through the morphological characteristics of the macroconidia, microconidia and the characteristics of the colony. The molecular identification of the species was carried out by sequencing the regions of the genes ITS, β-tubulin (BT) and elongation factor (TEF). Koch's postulates were fulfilled for the isolates obtained by inoculation in the sugarcane varieties My 55 and Mex 79-431. The morpho-molecular characterization of the isolates identified *Fusarium sacchari* as the causative agent of the disease. As far as is known, this is the first report of *Fusarium sacchari* as a causative agent of pokkah boeng disease in sugarcane in the Huasteca Potosina region.

Keywords: *Saccharum officinarum*, *Fusarium sacchari*, twisted top.

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Saccharum officinarum is one of the main cultivated plants in the world. Mexico is the sixth largest producer in the world (FAO, 2022). In 2020, 774 954 ha were harvested, of which 69 626 corresponded to the state of San Luis Potosí (SIAP, 2021). The sugarcane fields are distributed in 15 states, with San Luis Potosí being the third state with the highest production with a participation of 9% of the cultivated area of the national total, the region producing this crop is located in the Huasteca Potosina, in this region, Tamasopo has 12% of the cultivated state area (Arcudia *et al*., 2018).

The yield of this crop is affected by diseases such as pokkah boeng or twisted top disease, where the pathogen penetrates the plant tissue through natural orifices or lesions present, causing an infection characterized by the appearance of chlorotic spots towards the base of the young leaves. In acute cases, the infection continues down the leaf and penetrates through the growth point of the stem, causing its distortion and presenting external and internal lesions similar to cuts, in the late stage of infection the leaves become deformed, at their base the leaves wrinkle, twist and rot, reddish spots and stripes appear, in the last stage the growth point of the plant rots and it dies (Wishwakarma *et al*., 2013; Jeyakumar and Zhang, 2020).

The disease affects almost all varieties of sugarcane (Wishwakarma *et al*., 2013), causes the reduction of macro and micronutrient levels in the stem and diseased leaves, affects weight, the development of internodes and the accumulation of sugars in the juice (Singh *et al*., 2006), which causes the decline in the quality of the harvested crop (Dohare *et al*., 2003) and consequently, of the sucrose juice used for the production of sugar and other raw materials.

Pokkah boeng disease is caused by *Fusarium*, with controversy about the species involved (Jeyakumar and Zhang, 2020). *F. moniliforme* var. *subglutinans* was reported by Govender *et al*. (2010) in Malaysia and Patil *et al*. (2007) in India. In Asia, *F. sacchari* was reported by Bourne in 1953 (Jeyakumar and Zhang, 2020). According to O'Reilly (1998), *Fusarium* causes two different diseases, one in the stem and one in the leaves, *F. sacchari* and *F. verticillioides* are the causative species, respectively.

In the Huasteca Potosina, sugarcane plants show symptoms characteristic of pokkah boeng in leaves and stems, with severe damage of the vascular bundles characterized by the development of reddish coloration that extends along the stem, leading to the death of the plant by wilt; however, the etiology of this pathology is unknown, so the objective of this study was to determine the causative agent of this disease present in the sugarcane-growing region in the municipality of Tamasopo, San Luis Potosí, Mexico.

The work was carried out in the Laboratory of Biotechnology of the Faculty of Agronomy of the Autonomous University of Nuevo León and in the Laboratory of Phytopathology of the Faculty of Agrobiology of the Michoacan University of San Nicolás of Hidalgo. Plant material with symptoms of pokkah boeng was collected in two zigzag stratified samples in February and April 2019 in the Huasteca Potosina, in the area of El Aguacate in the ejido of Damián Carmona in Tamasopo, San Luis Potosí.

Samples were taken in nine sugarcane fields, collecting four plants one meter high per sampling point. From the stems of the plants, fragments of symptomatic tissue of 2 cm in length were sectioned to obtain the isolates; after disinfection with 2% sodium hypochlorite and rinsing with sterile distilled water, samples of 2-3 mm^2 of the fragments were obtained for sowing in Petri dishes with potato-dextrose-agar (PDA) medium acidified with 10% tartaric acid. The dishes were incubated at 25 °C for seven days in dark conditions.

Monosporic cultures of the isolates (H1, first sampling and H2, second sampling) were obtained using the streak plate technique from dilutions of spore solutions. The morphological characterization was based on the macroscopic (colony color and pigmentation) and microscopic (conidia, phialides and hyphae) characteristics of colonies grown for seven days in PDA medium at 25 °C in dark conditions. Fungal structures were mounted with lactophenol on slides to be examined with a compound light microscope with magnifications of 40 x and 100 x (Marques *et al*., 2013).

For the molecular identification of the isolates, the following regions were amplified: internal transcribed spacer (ITS), translation elongation factor (TEF) and β-Tubilin (BT), after DNA extraction (Cenis, 1992) from monosporic colonies. The primers used for amplifications were: ITS4/ITS5 (White *et al*., 1990), TEF1α/TEF2 (Carbone and Kohn, 1999) and BT3/BT5 (Chala *et al*., 2019). PCR reactions were carried out in a volume of 12.5 µl containing 1X Taq buffer, $MgCl_2$ 2.5 mM, dNTPs 0.2 mM, primers 0.2 pM each, Taq polymerase 0.25 U and DNA 10 ng. The thermal program used was the one reported by Chala *et al*. (2019) with alignment temperatures of 52 °C for ITS and TEF and 60 °C for BT.

The amplified fragments were sent to bidirectional sequencing to Macrogen (Seoul, South Korea). In the editing of sequences and obtaining consensus sequences, the programs Chromas Lite v2.6.1 and Reverse Complement (Stothard, 2000), were used, respectively, the Basic Local Alignment Search Tool (BLAST) program was used for comparison with the nucleotide sequences of the National Center for Biotechnology Information (NCBI).

To perform the pathogenicity tests, 25 cm long sugarcane stems of the Mex 79-431 and M55-14 varieties were inoculated with 10 µl of spore suspension 1×10^6 ml^{-1} of both isolates, 2 cm above and below the internode. Six treatments with eight repetitions were evaluated: T1) Mex 79-43_H1; T2) Mex 79-431_H2; T3) Mex 79-431_distilled water; T4) My 55-14_H1; T5) My 55-14_H2; T6) My 55-14_distilled water, a stem was used per repetition. The inoculated stems were incubated in a wet chamber for 24 h and after removal from the chamber cover, they were incubated for seven more days, in both cases at room temperature (≈25 °C). The re-isolation of the pathogen from the inoculated stems with symptoms of disease was performed in plates with PDA medium added with streptomycin sulfate 0.05 mg ml^{-1}.

The colonies of the monosporic cultures H1 and H2 obtained from the collected material, as well as from the re-isolates of the inoculated stems, showed purple-violet coloration (Figure 1A) and pinkish coloration (Figure 1B), respectively. The mycelial growth of the colonies was abundant, of fluffy consistency and pale initial coloration that turned violet over time.

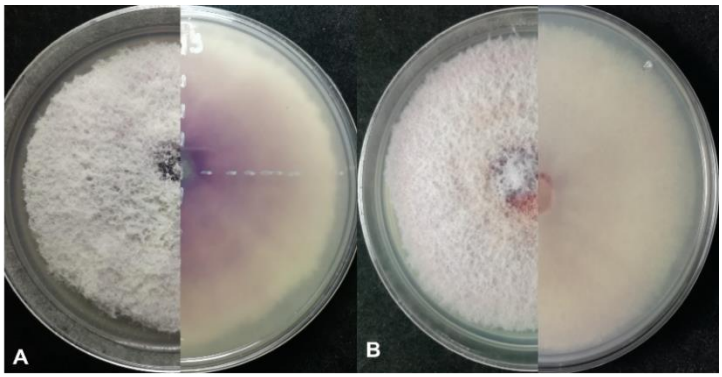

Figure 1. Monosporic cultures of *Fusarium sacchari* obtained from sugarcane stems with symptoms of pokkah boeng after seven days of incubation. A) H1 and B) H2.

The microconidia showed oval shape and without septa, the macroconidia showed elongated shape with one or two septa (Figure 2A-C), the formation of microconidia was observed, which were present abundantly in the false heads of the monophialides and also of the polyphialides (Figure 2F). These characteristics exhibited by H1 and H2 correspond to those described by Leslie and Summerell (2006); Nordahliawate *et al*. (2008) for *Fusarium sacchari* isolates.

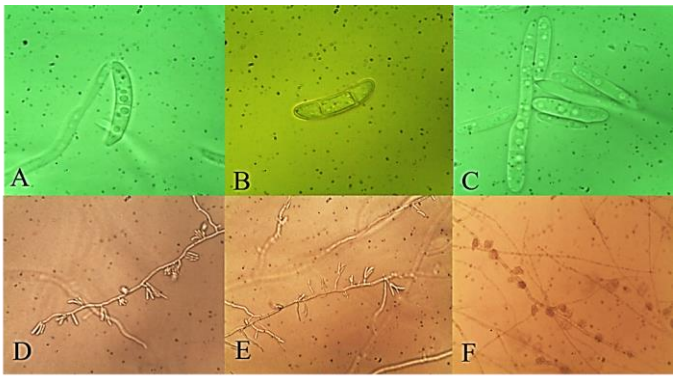

Figure 2. *Fusarium*. A-C) microconidia and macroconidia; D, E) hyphae; and F) false heads.

Comparison of the amplified sequences ITS (485 bp), TEF (641 bp) and BT (339 bp) showed 99% identity with NCBI sequences of *F. sacchari* (ITS: MF063030.1; TEF: MT010988.1; BT: MT011039.1), species identified as a causative agent of pokkah boeng (Leslie and Summerell, 2006; Nordahliawate *et al*., 2008; Lin *et al*., 2014; Viswanathan *et al*., 2017; Zhang and Jeyakumar, 2018).

Both isolates induced the symptoms characteristic of pokkah boeng disease in sugarcane stems inoculated with treatments T1, T2, T4 and T5 after seven days (Figure 3A), while stems inoculated with control treatments (T3 and T6) remained asymptomatic. From the inoculation of the stems, the re-isolation of the fungus and its morphological identification was carried out, complying with Koch's postulates.

The Mex 79-431 variety showed symptoms and signs characteristic of the disease that were visually less severe than those showed by the My 55-14 variety (Figure 3B-C); Mex 79-431 is one of the sugarcane varieties grown in the Huasteca Potosina (Arcudia *et al*., 2018), with adaptation to different conditions of soil, climate and management of the sugarcane region (López, 2005), which allows assuming that because the isolate of *F. sacchari* is native to this area, Mex 79-431 may show resistance to the pathogen.

The My 55-14 variety exhibited reddish brown coloration both in the internode and in the rest of the stem, with the center of the lesion turning black, characteristic color of the necrotic tissue, the coloration of the edges was brown (Figure 3D-E), malformations of the stem and sprouting of lateral buds also occurred.

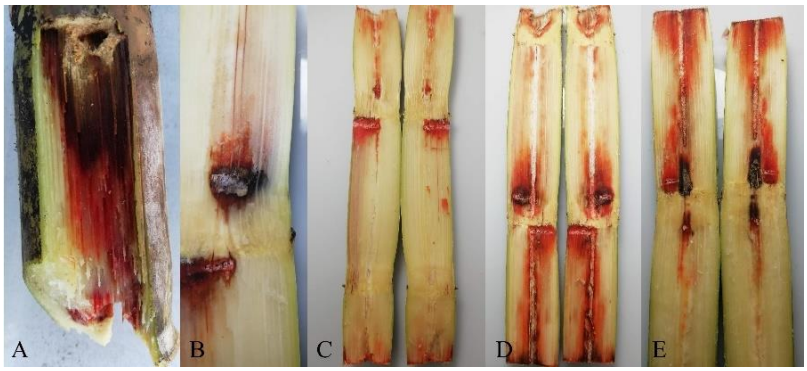

Figure 3. Symptoms shown in sugarcane stems eight days after inoculation with *Fusarium sacchari*. A) stem collected in the field; B, C) stems of the Mex 79-431 variety; D, E) stems of the My 55-14 variety.

The symptoms showed by the plants in the field, as well as those shown by the stems inoculated in the laboratory, coincide with what was reported by Nordahliawate *et al*. (2008); Wishwakarma *et al*. (2013) for pokkah boeng disease caused by *F. sacchari*. According to Nordahliawate *et al*. (2008), only *F. sacchari* causes pokkah boeng disease, other species such as *F. proliferation* and *F. subglutinans* are not pathogenic for sugarcane crop; however, *F. proliferatum* and *F. verticillioides* species were reported as causative agents of pokkah boeng in sugarcane in Veracruz (Rosas-Guevara *et al*., 2014). *F. sacchari*, *F. proliferatum* and *F. verticillioides* have been isolated from sugarcane roots with symptoms of wilt in Morelos (Martínez-Fernández *et al*., 2015).

Pokkah boeng disease becomes a problem after plant stress (Zhang and Jeyakumar, 2018). *F. sacchari* grows in decaying plant material, producing a large number of conidia that are propagated by wind and rain, spores colonize the leaves, flowers and stems of plants, the curved shape of the macroconidia of *Fusarium* species facilitates their dispersion by rain (Jeyakumar and Zhang, 2020), therefore, there is the risk that the presence of the fungus in the soil or in the seed could contaminate the new plantations. The varieties of sugarcane grown in Mexico do not show tolerance to the disease (Rosas *et al*., 2014), the phase in which the crop is as well as the climate of the areas where it develops are decisive for it to occur with severity (Viswanathan *et al*., 2017). Although no significant economic damage has been reported in the cultivation in Mexico due to pokkah boeng,

preventive measures must be taken, the search and use of resistant varieties, as well as certified or pathogen-free seed and comprehensive measures must be adopted as necessary for the production of sugarcane free of the disease.

Conclusions

The causative agent of pokkah boeng disease in sugarcane in the Huasteca Potosina region is *Fusarium sacchari*. The pathogen isolated from the stems with symptoms of the disease reproduced the symptoms in the inoculated healthy plants, the molecular identification of the isolates allowed the definition of the species. This is the first report of the presence of the disease in sugarcane fields in this region and of the identification of its causative agent.

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