Scientific Note

Fusarium sacchari associated with stem rot in sweet corn in Brazil[1]

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ABSTRACT

Sweet corn is susceptible to the attack of various pathogens that affect its metabolism and compromise its quality and production. This study aimed to identify the causal agent of stem rot in sweet corn plants under greenhouse conditions. The identity of the pathogenic isolate was confirmed by sequencing of internal transcribed spacer (ITS1/ITS4), beta-tubulin (BT2A/BT2B), calmodulin (CL1/CL2) and translation elongation factor 1α (*TEF*1). The morphological and sequencing characteristics showed that *Fusarium sacchari*, which belongs to the *Fusarium fujikuroi* complex, is directly associated with the symptoms observed in the field. This fungal isolate has never been associated with diseases in *Zea mays* (saccharata group) in Brazil; therefore, this is the first report of the fungus infecting sweet corn in a cultivated area.

KEY-WORDS: *Fusarium fujikuroi*, *Zea mays*, fungal pathogen.

RESUMO

Fusarium sacchari associado à podridão do colmo em milho doce no Brasil

O milho doce está sujeito ao ataque de vários patógenos que afetam o seu metabolismo e comprometem a sua qualidade e produção. Objetivou-se identificar o agente causal da podridão do colmo em plantas de milho doce, em casa-de-vegetação. A identidade do isolado patogênico foi confirmada pelo sequenciamento do espaço interno transcrito (ITS1/ITS4), beta-tubulina (BT2A/BT2B), calmodulina (CL1/CL2) e fator de elongação da tradução 1α (*TEF*1). As características morfológicas e de sequenciamento revelaram que *Fusarium sacchari*, pertencente ao complexo *Fusarium fujikuroi*, está diretamente associado aos sintomas observados em campo. Esse isolado fúngico nunca foi associado a doenças em *Zea mays* (grupo saccharata) no Brasil; portanto, este é o primeiro relato do fungo infectando milho doce em área de cultivo.

PALAVRAS-CHAVE: *Fusarium fujikuroi*, *Zea mays*, patógeno fúngico.

INTRODUCTION

Cereals are among the most important agricultural products both for human and animal diets, leading to an increase in production areas worldwide. Among the cereals intended for human consumption, sweet corn (*Zea mays* L.; saccharata group) stands out. It is consumed as green corn at the stage in which the grains are milky and have a higher amount of sucrose, dextrin and vitamins than the common green corn. In addition, this product has a great commercial potential, because it is consumed both in the cob and in the processed form (Storck & Lovato 1991).

Sweet corn and common corn are susceptible to several diseases that may compromise their quality and production. Among the viral diseases, maize dwarf mosaic (MDM) is the major sweet corn disease in the United States (Williams & Pataky 2012). The *Maize dwarf mosaic virus* is often associated with the *Sugarcane mosaic virus*, the causal agent of the sugarcane mosaic disease (Meyer & Pataky 2010, Williams & Pataky 2012). Among the bacteria, *Pantoea stewartii* subsp. *stewartii* causes significant losses in both the common and sweet corn (Roper 2011).

Regarding the fungal etiological agents that cause rot in maize, the *Fusarium* species deserve to be highlighted, and most of these species are found in the *Gibberella fujikuroi* complex, such as *F. verticilioides*, *F. proliferatum* and *F. subglutinans*. Losses of approximately 10-30 % are caused by the stem rot complex (Agrios 2005).

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Despite the well-known viral and bacterial diseases in sweet corn crops, the main ones are those caused by fungi, which are the most prevalent pathogens in cereals. Among these, the *Fusarium* species stand out because of their incidence, ability to infect different host species, and severity of the disease (Moussa et al. 2017).

The *Fusarium* genus comprises different species complexes, such as the *Fusarium graminearum* and *Fusarium fujikuroi* (FFSC) complexes. The FFSC species, particularly *F. sacchari*, are associated with rot diseases in rice, sugarcane, sorghum and maize (Petrovic et al. 2013), and are morphologically indistinguishable from other FFSC species such as *F. subglutinans*, *F. circinatum* and *F. mangiferae*. Their major characteristics include the formation of conidia clavate in chains in the aerial mycelium and the absence of chlamydospores (Leslie & Summerell 2006).

Fusarium rot, caused by a complex of *Fusarium* species, is one of the major diseases of sweet corn, mainly due to the difficulty in its control and the inherent capacity of the pathogen to infect different host plants (Petrovic et al. 2013). Ridout et al. (2019) reported two *Fusarium* species that affect the sweet corn seed production in the United States: *F. verticillioides* and *F. proliferatum*. Costa et al. (2019) described the symptoms of pokkah poeng caused by *F. sacchari* in Brazil. However, their studies have been conducted on the etiology of diseases in sweet corn. Thus, the present study aimed to identify the causal agent of stem rot in sweet corn in Brazil.

Samples of sweet corn plants (*Zea mays* L.; saccharata group) showing symptoms of stem rot were collected from commercial fields in Morrinhos, Goiás state, Brazil, in 2016, and sent to laboratory analysis. Fragments of infected tissues were disinfected in 70 % alcohol for 30 s, 1 % sodium hypochlorite for 1 min and immersed thrice in sterile distilled water for 1 min, and transferred to Petri dishes containing potato-dextrose-agar (PDA) medium. The dishes were kept in a BOD-type growth chamber at 28 ºC, with a 12-h photoperiod. After 48 h, mycelium discs were removed from the ends of the colonies grown from the infected plant fragments and transferred to new Petri dishes containing PDA medium. The samples were kept in a BOD-type growth chamber for 7 days under similar conditions as previously described.

From the pure colonies of the isolates, a platinum loop was used on the aerial mycelium of the sporulating areas of the colonies and transferred to other Petri dishes containing PDA through zigzag movements, thereby promoting the dilution of the propagules. After 20 h, using light microscopy, the isolated germinated microconidia were located and transferred to another Petri dish containing PDA medium, to obtain monosporic cultures of the isolates, which were then morphologically characterized (Leslie & Summerell 2006), stored and maintained in a BOD chamber at 4 ºC (Castellani 1939).

To evaluate the pathogenicity of the isolates, sweet corn plants from seeds of the Tropical Plus variety were sown in 2.0-L pots containing sandy-clay soil previously autoclaved at 120 ºC, for 40 min. The plants were kept in a greenhouse with an average temperature of 25 ºC ± 5 and relative humidity of 60-80 %.

The isolates were inoculated at 45 days after sowing, using two methods for each isolate: method 1 - inoculation in the stem by depositing 10 mL of a spore suspension (1 x 10^6 conidia mL^{-1}) at 5 cm above the base of the plant stem through a hole made with a veterinary hypodermic needle 40 mm thick, at an inclination of approximately 45º to the stem, until reaching half the stem diameter (Figure 1A). After inoculation, the site was sealed with a plastic film to prevent desiccation and contamination by other

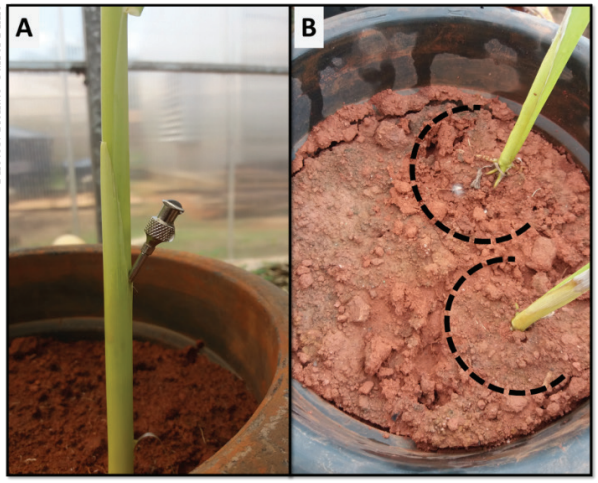


Figure 1. Methodology used to inoculate the conidial suspension from isolates obtained from sweet corn stem lesions. A) method 1 - stem inoculation using a veterinary hypodermic needle; B) method 2 - root inoculation by depositing the conidial suspension with the half-moon cutting method in the soil.

microorganisms; method 2 - inoculation at the root by the half-moon cutting method in the soil (Gurgel et al. 2005), in which each cut received 30 mL of the spore suspension (Figure 1B). As a negative control, plants were subjected to the two inoculation methods using sterile distilled water with no conidial presence.

For each inoculation method, four plants were used for each isolate and periodically inspected for systemic symptoms after inoculation. From the observed lesions in roots and stems, new isolations from the infected plant parts were performed using the aforementioned methodology.

After the pathogenicity test, the isolate that was found to be pathogenic was grown in an agar-clove medium for 10-14 days (Nelson et al. 1983). Monosporic cultures of the obtained strains were morphologically characterized. Traits such as colony color and texture, conidia size and shape, type of hyphae and formation of reproductive and resistance structures were analyzed (Leslie & Summerell 2006).

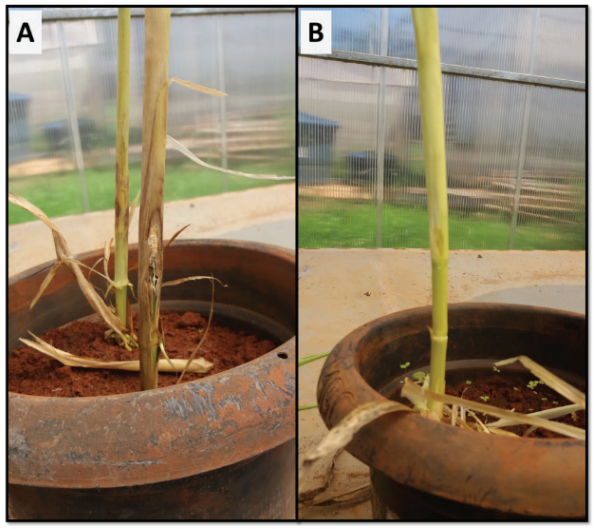
To confirm the identity of the pathogenic isolate, genomic DNA was extracted from the mycelia obtained from monosporic cultures grown on PDA and from the respective isolate obtained after re-isolation from the pathogenicity test. DNA was extracted using the Dellaporta method (Dellaporta et al. 1983), quantified using a spectrophotometer (NanoDrop Lite Invitrogen), and standardized to a concentration of 30 ηg μL^{-1}.

The extracted DNA was identified by polymerase chain reaction (PCR) using ITS1/ITS4 primers for the internal transcribed spacer (ITS) region of the rDNA (White et al. 1990), BT2A/BT2B for β-tubulin (Glass & Donaldson 1995), CL1/CL2 for calmodulin (O'Donnell et al. 2000) and the transcription elongation factor 1α (*TEF*1) (O'Donnell et al. 1998), with separate reactions for each pair of primers. Each final reaction volume of 20 µL was composed of 10 µL of Mastermix EmeraldAmp (Takara®), 0.4 µL of each primer (2.5 µM) and 7.2 µL of dH_2O. The PCR products were sequenced by Macrogen (Seoul, Korea).

The ITS region sequences were analyzed and compared with the sequences deposited in the GenBank database from the National Center for Biotechnology Information (NCBI) using the BLAST tool (Altschul et al. 1990). The species identification was performed by phylogenetic analysis, and the sequences of the β-tubulin, calmodulin and *TEF*1 regions checked and edited manually using the SeqAssem (Hepperle 2004). Multiple alignments for each region were performed using the ClustalW tool implemented in the MEGA 7 (Kumar et al. 2016). A phylogenetic tree was generated by combining the alignments of the three regions with the available reference isolates. The method used for phylogenetic analysis was neighbor-joining (Saitou & Nei 1987) with 10,000 bootstraps, using the MEGA 7 (Kumar et al. 2016).

Fusarium sp. strains were detected in fragments of sweet corn stem samples collected from commercial fields in the Goiás state, Brazil. From this isolation process, 10 monosporic isolates (UFG1-UFG10) were obtained. Knowledge regarding the species diversity of the *F. fujikuroi* complex and its significance as an etiological agent of various diseases is well known; however, for some pathosystems, it is still limited (Petrovic et al. 2013). There is a lack of basic literature on *Fusarium* species that are pathogenic to sweet corn in Brazil.

Among the isolates subjected to the pathogenicity test, only the isolate identified as UFG10 proved to be pathogenic, which had the ability to damage sweet corn plants. The first symptoms were observed in the stem, with reddish-brown spots and necrotic lesions at 45 days after inoculation (Figure 2). After careful removal and washing under running water, light to dark brown spots were observed on the root surfaces.



Photos: Renato Carrer Filho

Figure 2. Symptoms of stem rot in a sweet corn plant, at 45 days after inoculation, with the UFG10 isolate, using the direct stem inoculation method (A), and plant used as negative control (B), inoculated with sterile water.

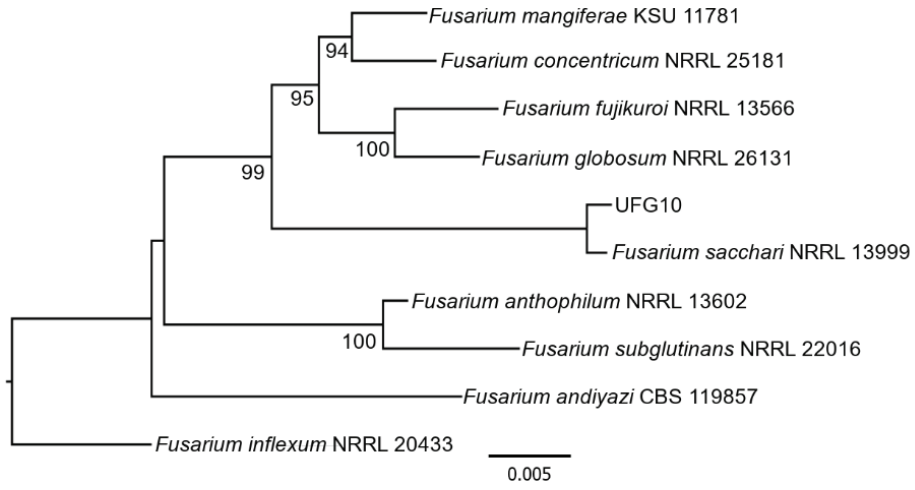


Figure 3. Evolutionary relationships among the sequences of β-tubulin, calmodulin and TEF1 regions of the UFG10 isolate, in comparison with *Fusarium* species, using the neighbor-joining method. Bootstrap value with 10,000 repetitions (expressed in %) are shown next to the nodes.

All the 10 isolates obtained by indirect isolation presented morphological characteristics belonging to the *Fusarium* genus, according to the identification key by Nelson et al. (1983) and Leslie & Summerell (2006). For the UFG10 isolate, which proved to be pathogenic after cultivation in clove-agar leaf medium (Nelson et al. 1983), the presence of abundant aerial mycelium, false-headed microconidia, polyphialids and slightly falconate macronidia was observed. Septation bands consisted of 3-5 septa.

To confirm the isolate UFG10 species within the *F. fujikuroi* complex, sequences of the β-tubulin, calmodulin and TEF1 regions were used to construct a combined tree of the three genes. Based on phylogenetic analysis, the isolate in this study was grouped with the reference isolate of *Fusarium sacchari* (Figure 3) (GenBank accession numbers: TEF1-α OK490376, TUB OK490378 and CAL OK490377).

Within the FFSC complex, *F. fujikuroi* is responsible for causing the well-known "bakanae disease" in rice, and *F. proliferatum* and *F. verticillioides* for causing stem and root rot in corn (Choi et al. 2018). In addition, FFSC is a large group of species of the *Fusarium* genus in various environments, and molecular data suggest that over 50 species within the Fujikuroi complex are phylogenetically isolated from different environments (Moussa et al. 2017).

F. sacchari commonly occurs in tropical regions and is an economically important pathogen for some plant species in the Poaceae family. For example, it is a causal agent of pokkah poeng in sugarcane in Brazil (Costa et al. 2019) and in several countries, but it has also been reported to cause diseases in rice, sorghum and common corn (Petrovic et al. 2013).

In this study, through pathogenicity and molecular analyses, it was possible to confirm that the *F. sacchari* belonged to the FFSC and was the causal agent behind stem rot in sweet corn (*Zea mays* L.; saccharata group) in Brazil.

The fungal isolate identified as *Fusarium sacchari*, which belongs to the *Fusarium fujikuroi* complex, caused stem rot in the sweet corn. To our knowledge, this is the first report on *F. sacchari* in sweet corn in Brazil.

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