Resistance to *Fusarium sacchari* associated with pokkah boeng in sugarcane genotypes in a semiarid environment in Brazil

Resistência à *Fusarium sacchari* associada à podridão do topo em genótipos de cana-de-açúcar em um ambiente semiárido do Brasil

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RESUMO

A Pokkah boeng é uma doença causada por *Fusarium sacchari* que, embora ainda emergente, tem despertado atenção devido a sintomatologia observada em diversas regiões canavieiras do Brasil. Este estudo teve como objetivo avaliar a resistência de genótipos de cana-de-açúcar à *F. sacchari* nas condições ambientais de temperatura e umidade relativa preponderantes na região canavieira do estado do Piauí, Brasil. Inicialmente foi conduzido um ensaio no período chuvoso da região usando 16 clones de cana-de-açúcar que foram inoculados com *F. sacchari* via suspensão de esporos. Os

níveis de resistência foram estabelecidos por scores (de 0-5) de uma escala diagramática e posterior classificação mediante o índice de severidade da doença (ISD). Posteriormente, quatro clones de cana-de-açúcar com os menores e quatro com os maiores ISD selecionados previamente no primeiro ensaio foram submetidos a um novo ensaio durante o período seco da região, utilizando-se a mesma metodologia do primeiro ensaio. Ficou demonstrado que a agressividade do fungo se torna mais expressiva em alguns genótipos, em geral, quando as médias de temperaturas estão em torno de 34 ^{0}C e umidade relativa do ar em torno de 75%. Assim, no período seco do ano, correspondente ao período de junho a novembro, a incidência desta doença é praticamente nula mesmo para os genótipos mais suscetíveis. Assim, nossos resultados demonstram que, apesar de haver inóculo do patógeno presente na região, o desenvolvimento de *F. Sacchari* não causa danos significativos à cana-de-açúcar em virtude das condições ambientais que limitam à doença.

Palavras-chave: fitopatologia, incidência do patógeno, *saccharum* spp, severidade da doença.

ABSTRACT
Pokkah boeng is a disease caused by *Fusarium sacchari* which, although emerging, has attracted attention due to the symptoms observed in several sugarcane regions of Brazil. This study aimed to evaluate the resistance of sugarcane genotypes to F. sacchari under the prevailing environmental conditions of temperature and relative humidity in the sugarcane region of the state of Piauí, Brazil. Initially, a trial was conducted in the rainy season of the region using 16 sugarcane clones that were inoculated with *F. sacchari* via spore suspension. Resistance levels were established by scores (from 0 to 5) on a diagrammatic scale and subsequent classification by the disease severity index (DSI). Subsequently, four sugarcane clones with the lowest and four with the highest DSI selected previously in the first trial were submitted to a new trial during the dry period of the region, using the same methodology of the first trial. It was demonstrated that the fungus aggressiveness becomes more expressive in some genotypes, in general, when the average temperatures are around 34 ^{0}C and relative humidity of the air around 75%. Thus, in the dry period of the year, corresponding to the period from June to November, the incidence of this disease is practically null, even for the most susceptible genotypes. Thus, our results demonstrate that, despite the inoculum of the pathogen present in the region, the development of *F. sacchari* does not cause significant damage to sugarcane due to the environmental conditions that limit the disease.

keywords: disease severity, pathogen incidence, phytopathology, *saccharum* spp.

1 INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is an important crop to the economic and social development of the country, contributing to the production of sugar, alcohol and other derivatives, in addition to generating employment and income in several producing regions (GILIO; MORAES, 2016).

Pokkah boeng, caused by *Fusarium* spp. (WANG et al., 2016) has shown occurrences in several sugarcane areas around world, especially in Brazil and India, drawing attention to possible damage to the crop in the medium and long term (WANG et al., 2016; ZHANG; JEYAKUMAR, 2018; COSTA et al., 2019). There are studies to define the best conditions for the development of this fungus, however the literature shows divergence mainly due to the studies being conducted in different environments. Some studies show that there is a temperature range between 20-30 °C and relative humidity between 75-85% as being optimal conditions for the development of fungi of these species (ANURADHA et al., 2018). What demonstrate a very large spectrum of possible variation and the need for more assays.

Environmental variables are a key role on the manifestation of the disease and can have favorable, neutral or even negative results for the disease presence, considering that pathogens and plants have their own favorable environmental conditions for growth and development (HUA, 2013; VELÁSQUEZ; CASTROVERDE; SY, 2018). In particular, temperature affects the growth and development of the fungus and becomes the determining variable to favor the occurrence and development of diseases (COLHOUN, 1997). Therefore, it is necessary to study the process of manifestation of the disease in different environments.

Thus, the objective of this study was to previously screen for resistance of sugarcane genotypes to *Fusarium sacchari* and, later, to evaluate the incidence and development of the disease in a semi-arid region of northeastern Brazil.

2 MATERIALS AND METHODS

This study was carried out in two stages under semi-controlled conditions of an agricultural greenhouse from the Department of Plant Science of the Federal University of Piauí, in Teresina-PI, Brazil, whose local altitude is 72 m, with a latitude of 5º02’34” S and a longitude of 42º47’01” W. The first stage was carried out from June 6 to October 17, 2020, and the second was implemented in the autumn-winter period (May 1st to August 19th, 2021) in order to prove the response of the contrasting genotypes for resistance to *F. sacchari* based on the screening resulting from the first trial.

The temperature records (maximum, average and minimum) and relative humidity of the air were computed daily through a thermo-hygrometer installed inside the agricultural greenhouse.

2.1 SCREENING FOR *FUSARIUM SACCHARI* RESISTANCE IN SUGARCANE GENOTYPES

A screening was performed on 16 sugarcane genotypes (Table 1) in order to identify genotypes resistant and susceptible to pokkah boeng caused by *F. sacchari*. The experiment was carried out in a randomized block design with 32 treatments and four replications, with the treatments represented by the sugarcane genotypes submitted to foliar inoculation with suspension of *F. sacchari* conidia, and the control plants of the respective genotypes received, in the meristematic region, only sterilized water.

Table 1. List of sugarcane genotypes and respective encodings used in screening for resistance to pokkah boeng caused by *F. sacchari*

Code	Genotype	Code	Genotype
G01	RB04803	G09	RB05876
G02	RB015935	G10	RB863129
G03	RB867515	G11	RB021754
G04	RB0449	G12	RB041443
G05	RB975952	G13	RB0442
G06	RB05642	G14	RB036066
G07	SP933094	G15	RB977540
G08	RB027040	G16	RB92579

The trial was implanted in polyethylene pots with a capacity of 3.5 L of soil, through sugarcane grinding wheels containing a viable bud and treated with Trifloxystrobin® at 10% of the commercial formulation. The base fertilization was carried out based on a previous analysis of the soil, applying 150 mg dm-3 of nitrogen (N), phosphorus (P) and potassium (K), using ammonium sulfate, simple superphosphate and potassium chloride.

The spore suspension was prepared from the fungus grown in a Potato-Dextrose-Agar (B.D.A) type of culture medium and incubated in B.O.D with a photoperiod of 12 h and a temperature of 25 ± 2 °C for 7 days. At 90 days after planting, with the exception of the controls representing each of the genotypes inoculated only with sterile water, the plants were inoculated with a spore suspension of *F. sacchari* at a concentration of 1.0 x 10^6 at the height of the cartridge, with the aid of a sterile 1mL insulin syringe (100UI) with a type 8 fixed needle, adapted according to the protocol by Wang et al. (2017). Only the control plants received sterile water, and 100 µL of spore suspension was applied to the other plants. The isolate COUFPI 65, previously identified by Carvalho et al. (2017),

and used in this trial, is preserved in the Phytopathogenic Fungi Collection of the Phytopathology Laboratory of the Department of Plants Science/CCA-UFPI.

The evaluation of plants was performed 30 days after inoculation (DAI), using a diagrammatic scale described by WANG et al. (2016) following a scale of disease severity indices (DSI) as follows: grade 0 - Highly resistant (HR), defined as those with an DSI ≤ 1.0; grade 1 - Resistant (R), with an DSI from 1.1 to 5.0; grade 2 - moderately resistant (MR), with an DSI from 5.1 to 10.0; grade 3 - moderately susceptible (MS), with an DSI between 10.1 to 15.0; grade 4 - susceptible (S) with DSI between 15.1 and 20.0; and grade 5 - highly susceptible (HS), considering DSI ≥ 20.1. To obtain the DSI, the formula DSI = ∑ (ni x vi) / N x 100% is applied, where n refers to the number of culms evaluated with a grade, v is the grade, and N is the total number of plants observed.

2.2 CONTRASTING GENOTYPES SUBJECTED TO CONDITIONS OF HIGH TEMPERATURE AND LOW RELATIVE HUMIDITY

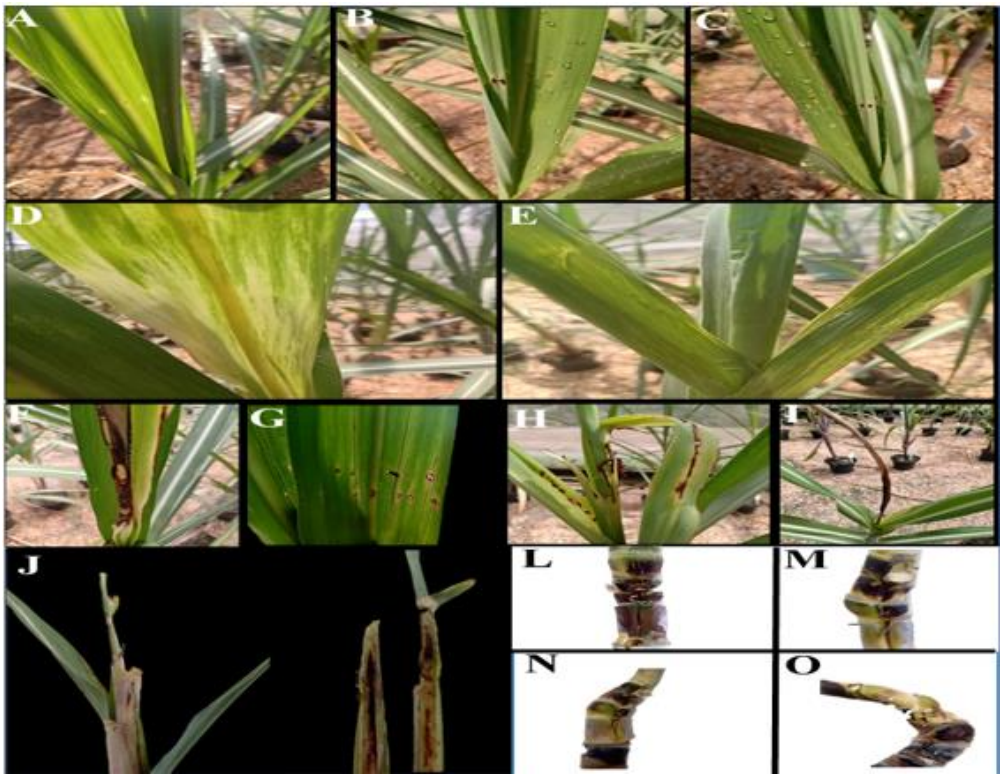
In the second trial, the four genotypes with the highest incidence and the four with the lowest incidence of *F. sacchari* were submitted to a new inoculation, this time in the dry season to verify if they would maintain the same level of resistance. The experimental design established for this trial was similar to the first one, changing only the number of treatments that was reduced by half (16 treatments).

3 RESULTS AND DISCUSSION

Our results showed that even in humid weather conditions for the study region, conditions are not stable for long enough for the fungus to have full development, there is a large oscillation in temperature and relative humidity. Regarding the symptoms of the test in the wet period, the maximum temperatures reached averages that ranged from 37 to 40 ^{0}C. These are very high temperatures. when related to lowest relative humidity (MOHSEN; JANABI; JEBOR, 2016). Studies in this direction have already shown that no disease development was observed for temperatures above 40 °C (VISHWAKARMA et al., 2013). Visual symptoms can be seen as small necrotic halos at the inoculum point (Figure 1A-E) and initial symptoms of chlorotic central in leaf base, as well as leaf curling and wrinkling. Costa et al. (2019) report similar symptoms, with the presence of reddish black stripes, chlorosis and necrosis, as well as curling and reduction of leaf area, top and stem rot. It is possible to observe (Figure 1G-H) that the symptoms progressed to lesions in the form of lens and reddish strip in the central vein of the leaf. In more advanced

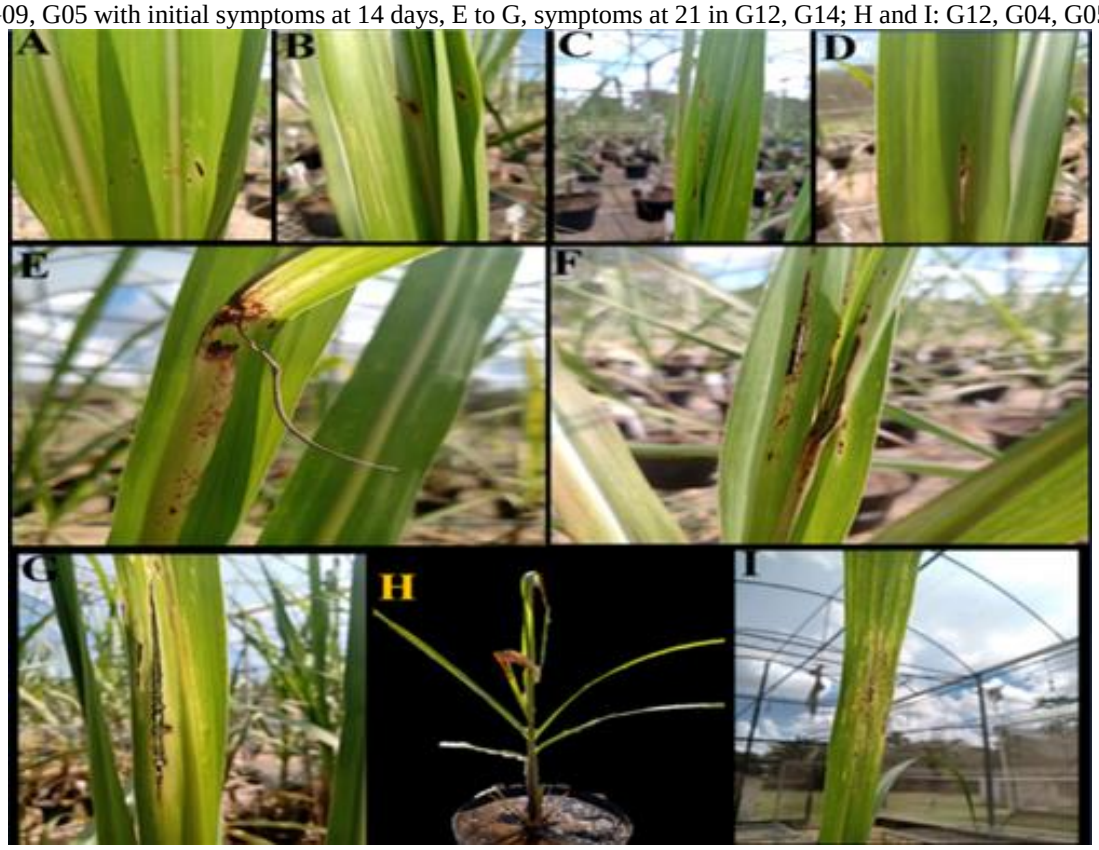
symptoms, at 14 days after inoculation, the central leaves showed damage throughout the leaf blade, in addition to cracks and lesions in the leaf tissue. In Figures 1H and I, it is possible to observe the progress of the disease that culminates in the death of the leaf.

Figure 1 - Stages of pokkah boeng throughout the experiment. A to E: initial symptoms in genotypes G15, G7 and G16; chlorotic base and wrinkling with slight torsion in G7 and G5, respectively; F to I: death of the central leaf of G10; G and H: progression of leaf limb laceration; J: external view and cross-section of G10, L and M: knife-cut in G03 and G05; N and O: knife-cut with twisted culms for G05.



The results of the second experiment (Figure 2), during the dry period of the year, some plants, as in the first experiment, showed some levels of disease expression. However, due to conditions of high temperature and low relative humidity, the progression of symptoms was suppressed. As seen, some plants, as in the first experiment, showed some level of disease expression.

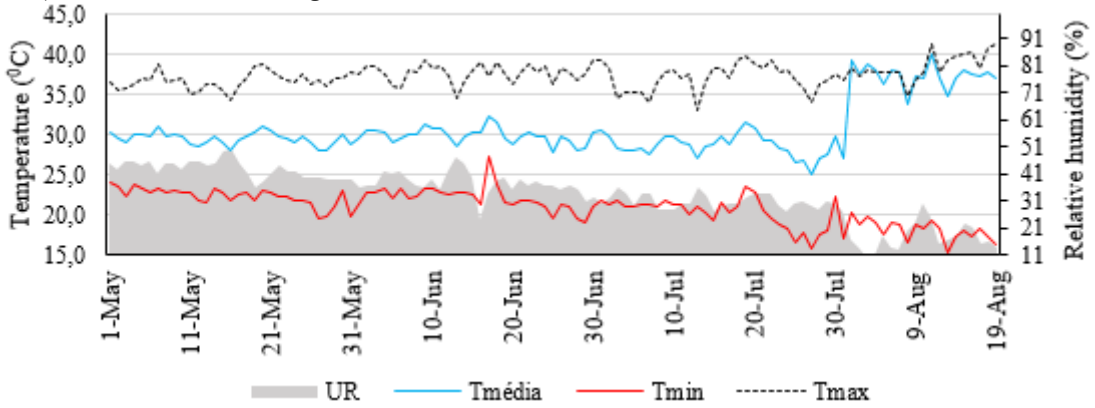
Figure 2 – Initial and final stages of the genotypes selected in the first phase and submitted to a new experiment under the same conditions in the following year. A to D correspond respectively to G12, G11, G09, G05 with initial symptoms at 14 days, E to G, symptoms at 21 in G12, G14; H and I: G12, G04, G05.



However, due to conditions of high temperature and low relative humidity (Figure 3), the progression of symptoms was suppressed. When considering the climatic conditions of this period, the averages reached a high peak in the last months of evaluation. This allied to the record of lower relative humidity either.

Such suppression can be explained by the high temperatures measured at this time of year, especially in the second half of the year, where it is observed that from July onwards, the average temperature had a drastic increase and the relative humidity decreased significantly. These two conditions were already expected for the second semester, where high temperatures and low relative humidity are common in the region from the month of July onwards (Figure 3).

Figure 3 - Data on average, minimum, maximum temperatures and minimum relative humidity (secondary axis) for the months of testing in 2021.



This factor is an ally so that this disease does not cause significant damage to the sugarcane crop in this study region. In general, our results allow us to infer that BP expression does not evolve when the etiological agent is inoculated into sugarcane under the environmental conditions described in this study.

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