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Multivariate analysis of resistance components of the saccharin and biomass sorghum to *Ramulispora sorghi*

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Abstract. The objective of this work was to evaluate the reaction of biomass and saccharin sorghum genotypes to a isolate of the *Ramulispora sorghi*. The experiment was conducted in a completely randomized design with three replications in a greenhouse. The variables analyzed were: area below the disease curve progress; lesion length (LL), lesions width (LW); normalized area below the curve of the lesion area expansion; incubation period and latency period. Data were submitted to analysis of variance, Pearson correlation and multivariate analysis based on Mahalanobis distance, using the Tocher and UPGMA clustering method. Significant differences were observed in the level of ($p < 0.01$) and ($p < 0.05$), for almost all variables analyzed. Of the 45 correlations, only 22 (48.89%) were significant by t-test, regardless of significance ($p < 0.01$ or 0.05), where it was observed that in all significant correlations were positive, indicating that the reaction symptoms correlate between the variables evaluated. The groupings generated by the Tocher and UPGMA clustering method were in agreement to group genotypes with the same behavior in relation to reaction to *Ramulispora sorghi*. From the observed results, it was verified that all evaluated genotypes presented ramulispora spot symptoms, thus demonstrating their susceptibility to the pathogen.

Keywords: *Ramulispora* spot; Genetic resistance; *Sorghum bicolor* (L.) Moench.

Introduction

In recent years, with the growing demand for renewable energy, the search for raw materials with low production costs and high energy efficiency has intensified. In this scenario, the sorghum culture fits in, with two types of sorghum that stand out among others: sweet and biomass sorghum. The main characteristic of sweet sorghum is that it has a sweet and succulent stem, suitable for the production of sugar and ethanol, while biomass sorghum has the particularity of being sensitive to photoperiod, has a high size and fibrous stem with the capability to provide energy in the biofuel market (Virmond, 2011; Durães, 2014).

Sorghum is an economically important crop in the world as in Brazil, but the occurrence of diseases is one of the limiting factors in its production. Depending on the environmental conditions, the susceptibility of the cultivar and the virulence of the pathogen, losses can reach approximately 46% in the final weight of the grains (Silva et al., 2013). Cúdom et al. (2016), evaluating

the development of the fungus *Ramulispora sorghi* in batches of sorghum during the 2012/2013 crop, observed an incidence of the disease in up to 80% of some batches susceptible to the pathogen, thus causing loss in production.

Among the diseases that affect sorghum in Brazil is the sooty stripe, which is a disease of great importance in China and the United States, but eventually detected in sorghum crops in Brazil (Ferreira et al., 2007). Sorghum species are the only host of the pathogen, which can affect the plant at all stages of its development, from seedling stage to maturity.

The initial symptoms of the disease are small circular-elliptical shape spots, with reddish-brown or brown color and yellow halo on the leaves and sheaths. These spots becomes elongated necrotic lesions measuring 5 to 14 cm long and 1 to 2 cm wide. In the center of the lesions, it is possible to observe numerous microsclerotia, which are resistance structures of the pathogen and plays an important role in the fungus survival in the leaves or

below the soil surface. (Williams et al., 1978; Frederiksen, 2000; Brady et al., 2011).

The crop rotation system and the destruction of infected leaves are effective alternatives to control the sooty stripe disease. These measures reduce the presence of the primary inoculum; however, the most effective way to prevent or reduce the disease is to cultivate resistant cultivars in regions where the pathogen is favored (Ramos et al., 2012; Silva et al., 2013; Cota et al., 2013).

The disease was observed in consecutive plantations of sweet and biomass sorghum in the region of Cáceres-Mato Grosso, at the experimental area of the Laboratory of Genetic Resources & Biotechnology in the year 2014, 2015 and 2016 with worrying severity. In this context, the objective of this work was to evaluate the reaction of biomass and sweet sorghum genotypes to an isolate of *Ramulispora sorghi*, through multivariate analysis of the resistance components to *Ramulispora sorghi*.

Methods

The experiment was conducted in March 2016, in a greenhouse belonging to the Laboratory of Plant Genetic Improvement, at the University of the State of Mato Grosso (UNEMAT), Cáceres-MT Campus.

Seventeen sorghum genotypes from the Embrapa's Maize and Sorghum improvement program were evaluated, eight of which were saccharine type (Sugargraze (1); CV198 (2); BRS506 (3); BRS508 (4); BRS505 (5); BRS511 (6); BRS509 (7) and CV568 (8); and, eight biomass type (201429B015 (9); 201429B021 (10); 201429B022 (11); 201429B023 (12); 201429B029 (13); 201429B030 (14); 201429B024 (15); 201429B033 (16). The control was the genotype BRS505 (17) without inoculation.

The experiment was conducted as a completely randomized design (CRD), consisting of the sixteen sorghum genotypes inoculated with an isolate of *R. sorghi*, with three repetitions and the control genotype.

Five seeds of each genotype were sown in plastic pots with 40 cm high and 50 cm diameter, containing Topstrato HT Hortaliças® substrate. After the emergence, establishment and thinning of the plants, three plants were kept per pot. During the 20-day stages after the plants growth, the treatments received macronutrients fertilization, with 50 g of 20-05-20 N-P₂O₅-K₂O mineral formulated dissolved in 2 liters of water. Each pot received 50 mL of the solution, according to the culture's recommendation.

To inoculate the plants it was used a monosporic isolate from the mycoteca of the Laboratory of Genetic Resources and Biotechnology, UNEMAT, Cáceres-MT Campus. To produce the inoculum, the isolate of *R. sorghi* was replicate in Oatmeal-Agar and incubated for 10 days in a growth chamber at 27 °C and 12 hours' photoperiod. After sporulation, a suspension was prepared by performing a superficial scraping of the

fungal colony and releasing the conidia into a Petri dish with sterile water. The conidia were counted using a Neubauer chamber and the concentration of the conidia suspension was adjusted to 1x10⁶ spores/mL⁻¹.

Twenty-eight days after planting, the plants were inoculated by sprinkling the suspension of conidia on the leaves with a hand sprayer, totaling 10 mL per pot. After inoculation, the plants were covered with a plastic bag for a period of 18 hours in order to form a humid chamber to favor the process of infection of the plants, with a temperature of 26 ±2 °C and relative humidity of approximately 70 ±5%.

After the appearance of the first symptoms, the genotypes were analyzed based on ten variables to indicate the behavior of the genotypes regarding the reaction to the *R. sorghi* isolate. The plants of all three replications were evaluated daily taking into consideration:

a) incubation period (IP): number of days after inoculation until the first symptoms appears;

b) Latency period (LP): the time in days from inoculation to sporulation;

c) Length and Width of lesions: three lesions were randomly chosen from the plants in each pot, most of them in the third expanded leaf. The leaves were identified for assessment at 10, 14 and 18 days after inoculation (DAI), being: LL1- lesion length in the first measurement; LL2- lesion length in the second measurement; LL3- lesion length in the third measurement; LW1- lesion width in the first measurement; LW2- lesion width in the second measurement; LW3- lesion width in the third measurement;

d) Area under the disease progress curve (AUDPC): the evaluation was carried out considering each pot as plot, assigning grades of severity through the diagrammatic scale proposed by Agrocere (1996), ranging from 1 to 9, being: 1 - 0%; 2 - 1%; 3 - 10%; 4 - 20%; 5 - 30%; 6 - 40%; 7 - 60%; 8 - 80%; 9 - > 80% of severity. Once sooty stripe does not have a specific scale to evaluate its severity, it was used the scale used to evaluate helminthosporiosis in maize, for presenting symptoms with similar characteristics with sooty stripe lesions.

Four weekly evaluations were carried out. For the AUDPC calculation it was used the equation proposed by Shaner & Finney (1977).

e) Standardized area under lesion expansion curve (sAULEC): After measuring the length and width of the lesions, the estimated lesioned area (LA, mm²) was calculated considering the formula for calculating the area of an ellipse ($\pi \cdot L \cdot W / 4$), where L is the length of the lesion and W is the width of the lesion. After obtaining these data, Standardized area under lesion expansion curve (sAULEC) was calculated. The calculation of this variable was based on the equation proposed by Shaner & Finney (1977).

The data obtained were submitted to analysis of variance to verify the existence of

variation among the sorghum genotypes; Tukey's test at 5% probability; Pearson's correlation; multivariate analysis based on Mahalanobis' generalized distance. Tocher grouping analysis (Rao, 1952) was also performed by the Genes software (Cruz, 2013) and UPGMA clustering by the R software.

Results and discussion

From the results obtained by the analysis of variance, significant differences ($p < 0.01$) were observed for treatments, genotypes and between genotypes x control for the lesion width in the third measurement (LW3) and standardized area under lesion expansion curve (sAULEC). It was also observed significant difference ($p < 0.01$) between genotype x control for the lesion length in the third measurement (LL3) and ($p < 0.05$) lesion width in the second measurement (LW2). These results indicate that the genotypes evaluated behaved differently from *R. sorghi*. The analysis of (AUDPC) and lesion length in the first measurement (LL1) showed significant difference ($p < 0.01$) only between genotype x control. Some studies have used these methods to evaluate genetic resistance in different cultures (Preisigke et al., 2015, Silva, 2009, Prates et al., 2001; Bellon et al., 2012).

For the variables latency period (LP) and lesion length in the second measurement (LL2), significant differences were observed ($p < 0.01$ and 0.05), for treatment and between genotype x control. These results are different from those observed by Guedes et al. (2013), which evaluated 23 genotypes of graniferous and forage sorghum and do not observed significant difference on the variable LP for the sooty stripe.

Only the variables incubation period (IP) and lesion width in the first measurement (LW1) showed no significant effect for treatment and genotypes x control, possibly due to the rapid spread of the pathogen in a short period for the development of lesions.

Table 1 shows the means of the seven variables that showed significant difference by the Tukey test at 5% probability, between the different genotypes grouped. It is possible to observe that all variables analyzed that showed significant difference, differed statistically from the control, for having highest means regarding the occurrence of the pathogen. The variables presented amplitude of: PL 1.66 to 7.66 days; CL1 0.34 to 7.35 cm; CL2 0.35 to 8.63 cm; CL3 0.15 to 10.19 cm; LL2 0.07 to 1.49 cm; LL3 0.16 to 2.05 cm and sAULEC 1.52 to 55.49 cm.

The LP variables for the 17 genotypes were allocated into three groups of means, thus revealing variability among them, with 14 genotypes presenting a lower rate of pathogen sporulation, thus differing significantly from the control that presented low incidence. According to Lobo et al (2005), longer latency periods may indicate a greater resistance of the plant to the colonization of the pathogen at the

end of the plant cycle. The genotype that presents a longer latent period is more likely to reduce the rate of progress of the disease, avoiding the epidemic or reducing the inoculum potential, when the conditions favorable to the occurrence of the disease persist for prolonged periods.

For LL1, the genotypes were grouped into three groups of means, and among the genotypes evaluated, the genotype 201429B030 had the longest lesion 7.33cm, thus expressing a higher incidence of the pathogen. The other genotypes presented a general mean of 4.61 cm, showing low incidence of the pathogen.

Regarding the variable LL2, genotypes were classified into three groups, with 201429B023, 201429B029, 201429B030 and 201429B033 averaging more than 8.63 cm. The other genotypes presented smaller size of lesions, because the shorter the length of the lesions the smaller the colonized area will be and, consequently, the lower the severity of the disease.

For LL3, the genotypes were distributed in three groups, 13 with mean over 10 cm, and this behavior was expected due to the length of the lesions showing gradual increase during the intervals of the evaluations. The other genotypes presented a general mean of 4.65 cm with less aggressiveness of the pathogen. Similar results were obtained by Brady et al. (2011), who observed a variation in the length of the *R. sorghi* lesions between 5 and 14 cm.

LW2, allowed the allocation of genotypes into 3 distinct groups, 16 of 17 genotypes evaluated presented lower incidence of the pathogen. This is a desired behavior, because the smaller the width of the lesions, the smaller the area colonized by the pathogen and, consequently, lower severity. However, the CV198 genotype showed greater aggressiveness, thus expressing greater susceptibility to the sooty stripe.

The LW3 variable allocated the genotypes into seven groups of means, with the genotypes 201429B015 and 201429B022 showing a lower incidence, similar to the mean of the control genotype, with a mean of more than 0.34 cm, indicating that these genotypes showed less colonization of the pathogen. On the other hand, CV198 had the highest mean in both evaluations of the width of the lesions, thus expressing greater colonization of the pathogen as to the expansion of the lesions.

Regarding the sAULEC variable, genotypes were allocated into five distinct groups. The genotypes 201429B015 and 201529B022 showed lower incidence of the disease, with an overall mean of 11.35 cm. The CV198 genotype showed greater colonization of the pathogen, which was observed in previous evaluations of LW2 and LW3, indicating that this genotype showed a higher incidence of the disease, standing out as the most divergent and susceptible to the pathogen.

In Table 2 are presented the Pearson's correlations coefficients, with a variation from -0.091 to 0.986. It is possible to observe that all significant correlations are positive, indicating that the reaction symptoms correlate among the evaluated variables. Of 45 Pearson's correlations,

only 22 (48.89%) were significant by the t test, independent of statistical significance (1% or 5%), with 20% being considered as very strong; 13.33% as strong and 15.56% as moderate.

Table 1. Epidemiological variables of seventeen genotypes of biomass and sweet sorghi, regarding the reaction to *Ramulispora sorghi*, Cáceres - Mato Grosso, 2016.

Genotypes	Variables ^{1/}						
	LP	LL1	LL2	LL3	LW2	LW3	sAULEC
Sugargraze	6.00 a	3.83 ab	4.80 ab	6.22 ab	0.59 ab	1.32 abcd	22.49 abc
CV198	6.33 a	5.00 ab	5.85 ab	7.30 a	1.49 a	2.05 a	55.49 a
BRS 506	6.66 a	3.55 ab	4.22 ab	5.97 ab	1.04 ab	1.57abc	33.95 abc
BRS508	6.33 a	5.22 ab	6.22 ab	7.84 a	0.93 ab	1.45abcd	39.43 abc
BRS505	7.33 a	4.50 ab	5.61 ab	6.72 a	0.41 ab	0.73 bcd	16.98 abc
BRS511	7.00 a	4.56 ab	6.24 ab	7.67 a	1.27 ab	1.85 ab	49.96 ab
BRS509	5.66 ab	3.83 ab	4.57 ab	6.26 ab	0.90 ab	1.34 abcd	27.91 abc
CV568	7.66 a	5.40 ab	6.32 ab	8.07 a	0.70 ab	1.16 abcd	32.23 abc
201429B015	7.33 a	4.66 ab	5.43 ab	7.47 a	0.21 ab	0.25 d	11.08 bc
201429B021	5.66 ab	4.51 ab	5.52 ab	7.33 a	0.92 ab	0.97 abcd	26.93 abc
201429B022	6.00 a	4.50 ab	5.12 ab	6.78 a	0.29 ab	0.34 d	11.61bc
201429B023	6.66 a	6.40 ab	7.55 a	8.81 a	0.65 ab	0.77abcd	33.53 abc
201429B029	6.00 a	6.33 ab	6.88 a	8.39 a	0.51 ab	0.62bcd	19.41 abc
201429B030	6.66 a	7.35 a	8.63 a	10.19 a	0.44 ab	0.73 bcd	25.99 abc
201429B024	6.33 a	4.96 ab	6.23 ab	7.82 a	0.91ab	1.46 abcd	40.55 abc
201429B033	6.00 a	6.27 ab	7.65 a	8.64 a	0.70 ab	1.29 abcd	35.84 abc
Control	1.66 b	0.34 b	0.35 c	0.15 b	0.07 b	0.16 d	1.52 c
Standard Deviation	1.73	2.26	2.41	2.24	0.52	0.63	17.35

* mean followed by the same letter in the same column are not significantly different by Tukey at 5%.

^{1/}LP- Latency period, LL1- lesion length in the first measurement; LL2- lesion length in the second measurement; LL3-lesion length in the third measurement; LW1- lesion width in the first measurement; LW2- lesion width in the second measurement; LW3- lesion width in the third measurement and sAULEC - standardized area under the lesion expansion curve.

Table 2. Pearson's linear correlation coefficient estimates from ten reaction variables to *Ramulispora sorghi* fungus, cultivated in Cáceres, 2016.

Variables ¹	IP	LP	LL1	LL2	LL3	LW1	LW2	LW3	sAULEC
AACPD	0.684**	0.852**	0.760**	0.762**	0.823**	0.103	0.404	0.424	0.506*
IP	1	0.669**	0.435	0.475	0.527*	-0.091	0.397	0.530*	0.455
PL		1	0.690**	0.724**	0.805**	0.154	0.319	0.335	0.437
CL1			1	0.986**	0.968**	0.101	0.176	0.146	0.401
CL2				1	0.979**	0.164	0.247	0.232	0.479*
CL3					1	0.143	0.293	0.266	0.485*
LL1						1	0.533*	0.454	0.645**
LL2							1	0.941**	0.931**
LL3								1	0.914**

^{1/}AACPD - area below the disease progress curve, IP - incubation period, LP - latency period, LL1- lesion length in the first measurement; LL2- lesion length in the second measurement; LL3-lesion length in the third measurement; LW1- lesion width in the first measurement; LW2- lesion width in the second measurement; LW3- lesion width in the third measurement and sAULEC - standardized area under the lesion expansion curve.

**,* significant respectively at 1% and 5% probability levels by the F test.

Strong correlations were observed between AUDPC with PI (0.684), LL1 (0.760) and LL2 (0.762) and very strong for LP (0.852) and LL3 (0.823), except for the variable sAULEC (0.506), which obtained a correlation considered moderate, indicating that the greater the severity of the disease, the greater the damage caused by the pathogen.

The IP variable showed a strong correlation for LP (0.669) and moderate for LL3 (0.527) and LW3 (0.530), this can be explained by the fact that the longer the incubation period, the longer the time for sporulation. The other variables that showed moderate correlation indicate that the increase in the incubation period will not necessarily imply increases in LL3 and LW3.

For the variable LP, it was observed correlations classified as strong between LL1 (0.690) and LL2 (0.724) and only for the variable LL3 (0.805) was observed a correlation considered very strong, indicating that the longer the latency period the greater the gradual lengths of the size of the lesions. As proposed by Amorim (1995), the differences in the time variation of these variables may be associated with the variety of host species, pathogen variability and environment.

According to Wesp et al. (2008), longer incubation and latency periods are related to a lower final severity of the disease in the field. This fact was not observed in this study and, as the experiment was conducted in a greenhouse, only a single generation cycle of the pathogen was analyzed.

The variable LL1 presented a very strong correlation between LL2 (0.986) and LL3 (0.968), showing how much these variables are interconnected. LL2 and LL3 showed a strong correlation (0.979) and a moderate correlation between LL2 and sAULEC (0.479). The variable LL3, on the other hand, showed a moderate correlation only with the variable sAULEC (0.485). This behavior was expected, due the gradual growth of the pathogen colonization during the intervals of measurements.

Regarding the LW1 variable, it was observed a moderate correlation with LW2 (0.533) and a strong correlation with sAULEC (0.645). The LW2 variable presented a very strong correlation between LW3 (0.941) and sAULEC (0.931), while LW3 presented a very strong correlation only between sAULEC (0.914), showing that the increase of this variable will imply a gradual increase of the others.

Correlations between width and length are an expected result, as the fungus expands from the point of infection in various directions (longitudinal and transverse). These variables are fundamental for assessing the indicator of resistance and the progress of the disease in sorghum genotypes

regarding to the pathogen's reaction to the sooty stripe.

The grouping analysis using the Tocher Optimization method, based on the dissimilarity matrix, allowed the distribution of the 17 genotypes into two distinct groups (Table 3). Group I was composed by all genotypes that received the inoculation (93.75%), demonstrating that all genotypes in this group presented susceptibility to the pathogen. The greatest dissimilarity in this group was between the genotypes Sugargraze and BRS 511 (982.18) and the least dissimilar in this group was between BRS 506 and CV 509 (2.06). In Group II only the control genotype was allocated, differing from the genotypes that presented greater pathogen aggressiveness, for not having received the inoculum.

Table 3 - Representation of the grouping generated by the Tocher Optimization method based on epidemiological components among the evaluated sorghum genotypes cultivated in Cáceres - Mato Grosso, 2016.

Group	Genotypes	(%)Genotypes
I	BRS505, BRS506, BRS508, 201429B015, CV198, Sugargraze, 201429B022, BRS511, 201429B033, BRS509, 201429B024, 201429B029, 201429B023, 201429B030, 201429B021.	93,75
II	Control - BRS505	6,25
Total	16	100,00

The analysis performed by the UPGMA Hierarchical method presented a cophenetic correlation coefficient (CCC), applied to the UPGMA grouping methods, by the t test, of $r= 0.90$ and significant to 1% probability, demonstrating reliability in the relationship between the dissimilarity matrix and the dendrogram generated by UPGMA (Figure 1).

This method allowed the formation of two distinct groups, representing well the most divergent and the most similar combinations, identified by the generalized distance of *Mahalanobis*. The Tocher and UPGMA groupings were concordant, not only in the number of groups established, but also in the genotypes constituting the groups.

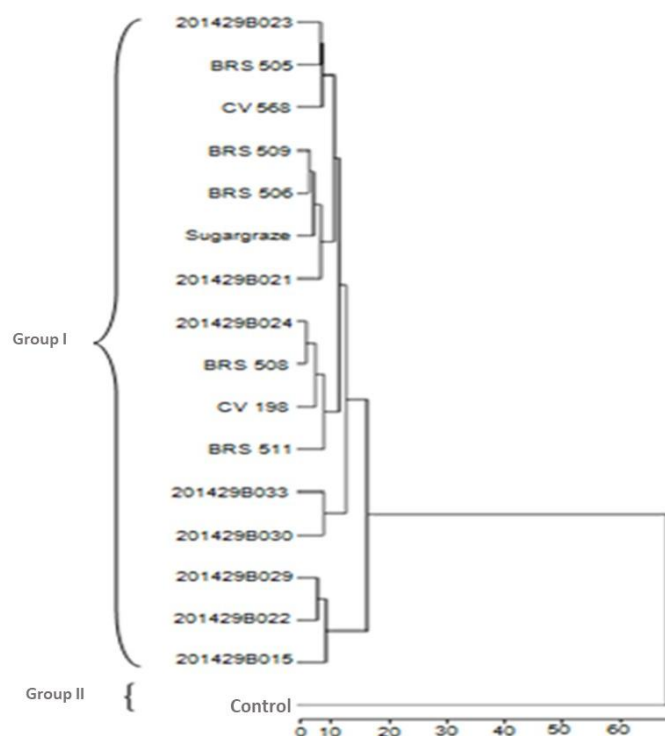


Figure 1. Representative dendrogram of the grouping of 17 *sorghum* genotypes, obtained by the UPGMA Method, based on epidemiological components estimated from ten variables regarding the reaction to *Ramulispora sorghi*.

The Group I observed in the dendrogram, submitted to a 63% cut, was composed of the 16 genotypes that received the inoculation, thus demonstrating that all presented symptoms of the disease. The genotypes 201429B015 and 201429B022 showed an indicator of tolerance to the sooty stripe, because they presented a smaller total area affected by the pathogen. The CV198 genotype was the most divergent and susceptible to the pathogen. The group II was composed by only the control genotype, which did not received the inoculum.

All sorghum genotypes evaluated presented susceptibility to the pathogen, some of it showed an indicator of resistance, once it was observed less aggressiveness when the pathogen was colonized.

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