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Parameters and genetic divergence to identify resistance to anthracnose and bacteriosis in cassava accessions

Abstract – The objective of this work was to estimate the genetic parameters of cassava (*Manihot esculenta*) genotypes cultivated in the field, as well as the genetic divergence between them, in order to identify which ones show a greater resistance to anthracnose and bacteriosis. Three independent experiments were carried out, evaluating 133 accessions and nine commercial cultivars of cassava distributed in five blocks, with five plants per plot in each experiment. In the first experiment, the plants were subjected to inoculation with *Xanthomonas phaseoli* pv. *manihotis* (bacteriosis). In the second, the plants were subjected to inoculation with *Colletotrichum gloeosporioides* f. sp. *manihotis* (anthracnose). In the third, carried out in an augmented block design and under naturally occurring diseases, visual evaluations of the plants were performed using a rating scale. Heritability was used as a genetic parameter, and the unweighted pair group method with arithmetic mean was applied to determine genetic divergence and clustering. In the third experiment, genetic variability was detected among accessions, which were evaluated for the area under the disease progress curve (AUDPC) for anthracnose and bacteriosis. In the first and third experiments of bacteriosis, the chances of success in the selection of resistant accessions are higher due to the high heritability values obtained. The BGM-1170 and BGM-1134 accessions show the lowest mean for AUDPC and are considered resistant to anthracnose and bacteriosis.

Index terms: *Manihot esculenta*, *Colletotrichum gloeosporioides* f. sp. *manihotis*, *Xanthomonas phaseoli* pv. *manihotis*, heritability, resistance.

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Parâmetros e divergência genética para identificação de resistência à antracnose e à bacteriose em acessos de mandioca

Resumo – O objetivo deste trabalho foi estimar os parâmetros genéticos de genótipos de mandioca (*Manihot esculenta*) cultivados em campo, bem como a divergência genética entre eles, para identificar quais apresentam maior resistência à antracnose e à bacteriose. Foram realizados três experimentos independentes, tendo-se avaliado 133 acessos e nove cultivares comerciais de mandioca distribuídos em cinco blocos, com cinco plantas por parcela, em cada experimento. No primeiro experimento, as plantas foram submetidas à inoculação de *Xanthomonas phaseolis* pv. *manihotis* (bacteriose). No segundo, as plantas foram submetidas à inoculação de *Colletotrichum gloeosporioides* f. sp. *manihotis* (antracnose). No terceiro, realizado em delineamento de blocos aumentados e sob ocorrência natural das doenças, foram feitas avaliações visuais das plantas por escala de notas. A herdabilidade foi utilizada como parâmetro genético, e o método

de grupos de pares não ponderados com média aritmética foi aplicado para determinação de divergência genética e agrupamento. No terceiro experimento, detectaram-se diferenças genéticas entre os acessos, que foram avaliados quanto à área abaixo da curva de progressão da doença (AUDPC) para antracnose e bacteriose. No primeiro e no terceiro experimento de bacteriose, as chances de sucesso na seleção de acessos resistentes são maiores, em razão dos altos valores de herdabilidade obtidos. Os acessos BGM-1170 e BGM-1134 mostram as menores médias de AUDPC e são considerados resistentes à antracnose e à bacteriose.

Termos para indexação: *Manihot esculenta*, *Colletotrichum gloeosporioides* f. sp. *manihotis*, *Xanthomonas phaseoli* pv. *manihotis*, herdabilidade, resistência.

Introduction

Cassava (*Manihot esculenta* Crantz) is originally from Brazil and is one of the main sources of energy for food, in addition to being the staple food for more than 500 million people around the world (Oliveira et al., 2015; Embrapa, 2022). Due to its rich carbohydrate content, the crop has several applications both in human food, in the form of derivatives or in natura, as well as in animal feed (Oliveira et al., 2014; Morgan & Choct, 2016).

Among the limiting factors in cassava production, diseases are one of the main causes of economic damages, among which anthracnose and bacteriosis are the prevalent ones (Oliveira et al., 2016, 2020; Silva et al., 2018). Anthracnose is caused by the fungus *Colletotrichum gloeosporioides* f. sp. *manihotis* (Sangpueak et al., 2018), and bacteriosis is caused by *Xanthomonas phaseoli* pv. *manihotis* (Bernal-Galeano et al., 2018; Mora et al., 2019). The most efficient control measure for these diseases is through the use of resistant cultivars, since the associated costs are lower than those of agrochemicals (Oliveira et al., 2013, 2016; Tremacoldi, 2016).

In genetic breeding programs, resistant genotypes are selected through the genetic variability available to perform a selective process aimed to advance segregating populations and to future acquiring of new cassava cultivars (Oliveira et al., 2014; Santiago et al., 2018). Variability is achieved by obtaining estimates of genetic parameters such as heritability, genetic variance, phenotypic variance, and expected gains from selection (Andrade et al., 2020). In addition, the knowledge of genetic diversity helps with the identification of parents and can be applied in cluster analysis of the unweighted pair group method with arithmetic mean (UPGMA) (Babu Rao et al., 2018).

The objective of this work was to estimate the genetic parameters of cassava genotypes cultivated in the field, as well as the genetic divergence between them, in order to identify which ones show a greater resistance to anthracnose and bacteriosis.

Materials and Methods

The research was conducted at the experimental farm of the Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados (UFGD), in the municipality of Dourados, in the state of Mato Grosso do Sul (MS), Brazil (22°48'53"S, 54°44'31"W, at 452 m altitude). According to the Köppen-Geiger's classification, the climate is Cwa (wet mesothermal climate), with hot summers and dry winters. The meteorological data of the experimental period (September 2016 to August 2017) came from the meteorological station of Embrapa Agropecuária Oeste, Dourados, MS (Figure 1). The soil in the region is classified as a Latossolo Vermelho distrófico, with a clayey texture, according to the Brazilian Soil Classification System (Santos et al., 2018), i.e., Oxisol.

The experimental trials that make up this work are part of the first cassava accession selection cycle of the UFGD Breeding Program in partnership with Embrapa Mandioca e Fruticultura (located in Cruz das Almas, in the state of Bahia, Brazil). On September 5, 2016, accessions from the active germplasm bank of Embrapa Mandioca e Fruticultura were evaluated in three independent field experiments in the same area, with the same design, drawing, and genetic material. Plants of the first experiment were subjected to inoculation with *Xanthomonas phaseoli* pv. *manihotis* (bacteriosis); plants in the second experiment underwent inoculation with *Colletotrichum gloeosporioides* f. sp. *manihotis* (anthracnose), and the third experiment was performed with plants without inoculation which were subjected to naturally occurring diseases.

The experimental design was carried out in augmented blocks, with 142 cassava genotypes (treatments), 133 accessions (noncommon treatments), and nine commercial cultivars (common treatments), distributed in five blocks; only the commercial cultivars of cassava were repeated within each block,

totaling 178 plots in each experiment, and 534 plots. The commercial cultivars BRS Caipira, BRS Formosa, BRS Kiriris, Cigana, Dourada, Gema de Ovo, IAC-90, IAC-576, and Mulatinha. The plot was composed of five plants spaced at 0.80 m, totaling 3.6 m^2 per plot. The spacing between rows was 0.90 m. Hand weeding was performed to control weeds during the experiment. No fungicide or bactericide was applied, as the objective was to obtain data on the disease for selection of resistant accessions.

On February 5 and 6, 2017, the accessions of the first experiment were inoculated with *X. phaseoli* pv. *manihotis*, and those of the second experiment, with *C. gloeosporioides* f. sp. *manihotis*, to increase the amount of inocula and ensure the presence of symptoms.

Isolates of *X. phaseoli* pv. *manihotis* were obtained from the experimental farm of Agricultural Sciences of the UFGD. Inoculation was performed only in the first experiment, using wooden sticks previously immersed for 10 min in a bacterial suspension at 10^8 mL^{-1}, which was introduced in the petiole insertion region of the oldest leaf (Nery-Silva et al., 2007). The severity in cassava accessions was performed by visual inspection of five plants per plot, using a scale from 0 to 5, for which: 0 represents plants and stems without symptoms of bacteriosis; 1, plants with symptoms only on the leaves (angular spot); 2, plants with symptoms on leaves and/or necrotic lesions on stems or petioles; 3, plants with necrotic lesions with gum exudation on petioles and stems; 4, plants with wilted leaves and descending death and/or the presence of necrotic lesions with gum exudates; 5, plants with total leaf loss, apical death and/or plant death (Oliveira et al., 2016).

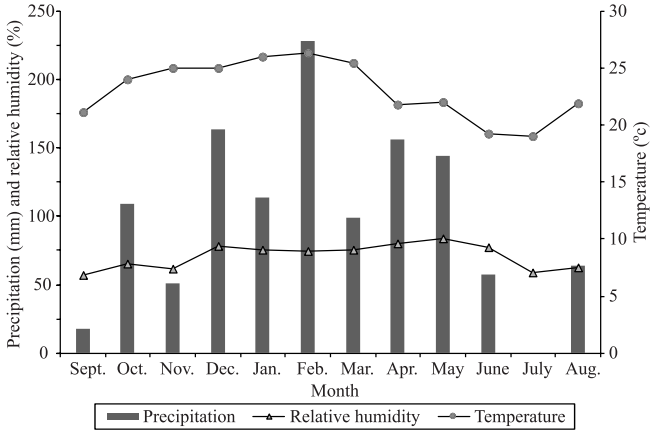

Figure 1. Monthly weather data collected from September 2016 to August 2017, at Embrapa Agropecuária Oeste weather station, in the municipality of Dourados, in the state of Mato Grosso do Sul, Brazil. Source: Guia Clima (2021)

For anthracnose, isolates of *C. gloeosporioides* f. sp. *manihotis* were obtained from Embrapa Mandioca e Fruticultura and inoculated in the second experiment. The plants were subjected to an increase in inocula pressure by spraying the aerial part with a costal atomizer, and with a spore suspension calibrated at 10^6 conidia mL^{-1}. The severity was performed by the visual analysis of five plants per plot, using a scale from 1 to 5, in which: 1, no symptoms of anthracnose; 2, angular leaf spots on the underside of the plant and/or small or old cankers on the lower half of the plant; 3, extensive leaf spot in the upper part of the plant and/or deep cankers in the upper half of the plant; 4, deep cankers with sporulation, distortion and/or wilting of new leaves, and apex drying; 5, severe defoliation, apical death or total plant death (Oliveira et al., 2016).

The third experiment (under naturally occurring diseases) was used to verify the symptoms of anthracnose and bacteriosis in uninoculated cassava accessions, since in the Dourados region the presence of these diseases has been verified in commercial areas. For this, five plants per plot were evaluated, and the anthracnose and bacteriosis severity scales described above were used.

In the three experiments, disease severity assessments started on February 17, 2017 and ended on May 11, 2017, totaling 12 assessments per experiment. For each disease, the area under the disease progress curve (AUDPC) (Campbell & Madden, 1990) was calculated, according to the following equation:

$$AUDPC = \sum_{i=1}^{n-1}\left[\frac{y_i + y_{i+1}}{2}\right]\times(t_{i+1} - t_i)$$

where: y_i e y_{i+1} correspond to the mean disease severity in the assessments i and (i + 1); t_i and t_{i+1} correspond to the time in days of the assessments i and (i + 1); n is the total number of reviews.

Subsequently, the AUDPC data were subjected to the analysis of variance, and the genetic parameters were estimated. In the analysis of variance, the significance by the F-test was 5 and 1% probability. The

genetic parameters were of phenotypic and genotypic variances were determined by the following equations:

$$\sigma^2_p = \sigma^2_g + \sigma^2_e,$$

$$\sigma^2_g = \sigma^2_f + \sigma^2_e$$

where: σ^2_g is the genotypic variance; σ^2_p is the phenotypic variance; and σ^2_e is the environmental variance. The heritability in the broad sense (h^2_b) was estimated according to the following equation:

$$h^2_b(\%) = \frac{\sigma^2_g}{\sigma^2_f} \times 100.$$

The selection gain (SG) was obtained by

$$SG = h^2_b \times K \times \sigma_p,$$

where: (h^2_b) is the heritability in the broad sense; K is the selection differential between 2.06 e 5% of selection intensity; and σ_p is the phenotypic standard deviation. The selection gain in percentage of the mean (SGM) was obtained considering

$$SGM = \frac{SG}{\bar{X}} \times 100,$$

where: X is the general average of the characteristic.

The genetic divergence between cassava genotypes (accessions and commercial cultivars) was evaluated on the basis of the AUDPC, obtained in the three experiments, by grouping the UPGMA obtained with the dissimilarity matrix by Euclidean distance. Cassava genotypes which were genetically similar to each other for AUDPC in the three experiments tended to be in the same group, and the most divergent ones, in different groups. The formation of groups is based on the cut obtained by the significance through the methodology of Mojena (1977). The groups of cassava genotypes were represented by dendrogram graphs and their means in each experiment, by boxplot graphs. Statistical analyses were performed using the R software (R Core Team, 2020).

Results and Discussion

There was a significant difference for the source of variation of accesses in the experiment under naturally occurring diseases, when they were evaluated for AUDPC of anthracnose ($p \leq 0.05$) e bacteriosis ($p \leq 0.01$), which reveals the genetic variability among cassava accessions for the trait under study (Table 1). Significant differences were also observed for the sources of variation of the commercial cultivars in the experiment under naturally occurring diseases, when these cultivars were evaluated for anthracnose ($p<0.01$); and for the sources of variation of the commercial cultivars plus block in the experiment under naturally occurring, for bacteriosis ($p<0.01$).

The highest mean of anthracnose (127.77) was observed in the experiment subjected to the inoculation of *C. gloeosporioides* f. sp. *manihotis* (anthracnose); and highest mean for bacteriosis (257.39) was found in the experiment with inoculation of *Xanthomonas phaseoli* pv. *manihotis* (bacteriosis). Although in the experiment without inoculation (under naturally occurring diseases) the means of AUDPC for the two diseases were lower, even so they were found in values close to those of the experiments inoculated with their respective causal agents. This factor is explained by the environmental

Table 1. Mean squares and genetic parameters of the mean of the area under the disease progress curve (AUDPC) of 133 accessions and 9 commercial cultivars (CC) of cassava (*Manihot esculenta*) subjected to anthracnose (*Colletotrichum gloeosporioides* f. sp. *manihotis*), bacteriosis (*Xanthomonas phaseolis* pv. *manihotis*), and under naturally occurring diseases.

Source of variation	Mean squares of the AUDPCs			
	Anthrac-nose	Bacteriosis	Naturally occurring diseases	
			Anthracnose	Bacteriosis
Blocks	1270.47	12617.10	4578.90	79.44
Accessions	159.66^{ns}	241.70^{ns}	697.30*	79.49**
CC	176.63^{ns}	164.00^{ns}	1723.00**	6.74^{ns}
CC + Blocks	158.64^{ns}	246.30^{ns}	635.60^{ns}	83.86**
Error	115.17	241.40	460.00	6.04
Mean	127.77	257.39	92.00	230.65
CV (%)	8.40	6.00	23.30	1.10
Genetic parameter				
σ^2_p	186.97	508.38	764.89	85.63
σ^2_g	71.80	266.94	304.89	79.58
σ^2_e	115.17	241.44	460.00	6.04
h^2_b (%)	38.40	52.51	39.86	92.94
SG	10.83	24.42	22.74	17.74
SGM (%)	8.44	9.48	24.58	7.70

*, ** Significant at 5% and 1% probability, respectively, by the F-test. ^{ns}Nonsignificant. CV, coefficient of variation. Genetic parameters: σ^2_p, phenotypic variance; σ^2_g, genotypic variance; σ^2_e, environmental variance; h^2_b, broad-sense heritability. SG, selection gain, SGM: selection gain in percent of the mean.

conditions in the region, as they are favorable to the development and propagation of those pathogens.

The temperatures observed during the period of the experiment – average of 23.5ºC between the first inoculation and the last evaluation – were favorable to the development of anthracnose and bacteriosis, (Figure 1). According to Massola Jr. & Bedendo (1997), bacteriosis develops at temperatures between 22 and 26ºC. For anthracnose temperature vary from 18 to 28ºC in long periods of rain. As to relative humidity, during the evaluation period the average was 78%, which is below the range considered optimal (90 to 100% for bacteriosis), but even so it was still favorable for the development of diseases.

The influence of environmental variation resulted in low magnitudes of the heritability coefficient in the experiments subjected to anthracnose (38.40%) and in the experiment under naturally occurring diseases for anthracnose (39.86%). The experiments subjected to bacteriosis (52.51%) showed a moderate heritability estimate (Table 1). However, the high heritability based on the lowest AUDPC means of the cassava accessions favors the selection of more resistant clones to anthracnose and bacteriosis. These clones can be used as parents in the future. Therefore, in the experiment under naturally occurring bacteriosis, heritability showed high magnitude (92%), which makes it possible the success of the selection of resistant bacterial accessions. For anthracnose, both in the experiments subjected to inoculation and that under naturally occurring diseases, the values of environmental variance (σ^2_e) were high in relation to the genetic variance (σ^2_g) (Table 1), making the cassava genotypes susceptible to environmental conditions for their phenotypic expression.

Heritability is an indication of success in choosing resistant genotypes. As the selection gain (SG) is expressed as a function of heritability with the standard deviation of the phenotypic variance, the values for SG is influenced by the values of the phenotypic variance. Thus, for the highest heritability estimate, the highest values for SG were not obtained. However, comparing the two situations – inoculation and natural occurring diseases –, the expected gain value in percentage of the mean (SGM %) was greater for the experiment under naturally occurring diseases evaluated for anthracnose (24.58%) than the SGM in the experiment subjected to inoculation (8.44%) with the respective pathogen, since this experiment showed the largest phenotypic standard deviation with lower mean than the experiment with inoculation. The behavior was the opposite for the bacteriosis experiment, which showed higher SGM with inoculation of the bacteriosis pathogen (9.48%) than the SGM under naturally occurring diseases (7.70%), since the inoculation experiment showed a higher phenotypic standard deviation that resulted in higher SG (24.42), thus explaining the higher value for SGM.

For the genetic diversity between accessions and commercial cassava cultivars, in the UPGMA dendrogram, a cut was performed on the basis of the significance obtained by the methodology of Mojena (1977), forming five groups (Figure 2). Group 4 grouped 60 accessions and six commercial cultivars (BRS Caipira, Cigana, BRS Kiriris, BRS Formosa, Mulatinha, and IAC-90), representing 42.25% of the total. Groups 1, 2, 3, and 5 were composed of 26, 36, 2, and 18 cassava genotypes, respectively, representing

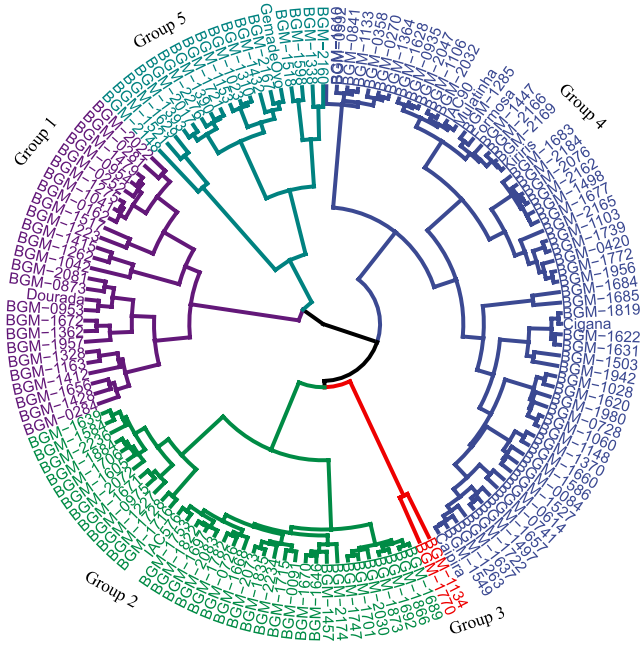

Figure 2. Dendrogram of dissimilarity of 142 cassava genotypes (accessions and commercial cultivars) determined by the UPGMA method, established by the Euclidean distance through the evaluations of experiments with plants subjected to the inoculation of *Colletotrichum gloeosporioides* f. sp. *manihotis* (anthracnose) and *Xanthomonas axonopodis* pv. *manihotis* (bacteriosis), and under naturally occurring diseases.

25.35%, 18.31%, 12.68%, and 1.41% of the total cassava genotypes per formed group.

The cassava genotypes in groups 1 and 5 (the most distant) are the most divergent ones, considering the mean values of AUDPC. Group 1 is formed by cassava genotypes that make up closer AUDPC lowest values, while group 5 is made up of accessions with AUDPC highest values.

The results of the boxplot analysis (Figure 3) allow to show the distribution of the groups originated from the dendrogram (Figure 2), in relation to the means of AUDPC, for inoculation experiments of the bacteriosis and anthracnose causal agents, and the experiment under naturally occurring diseases.

In the experiment subjected to inoculation of the anthracnose pathogen, groups 2 and 3 had AUDPC the

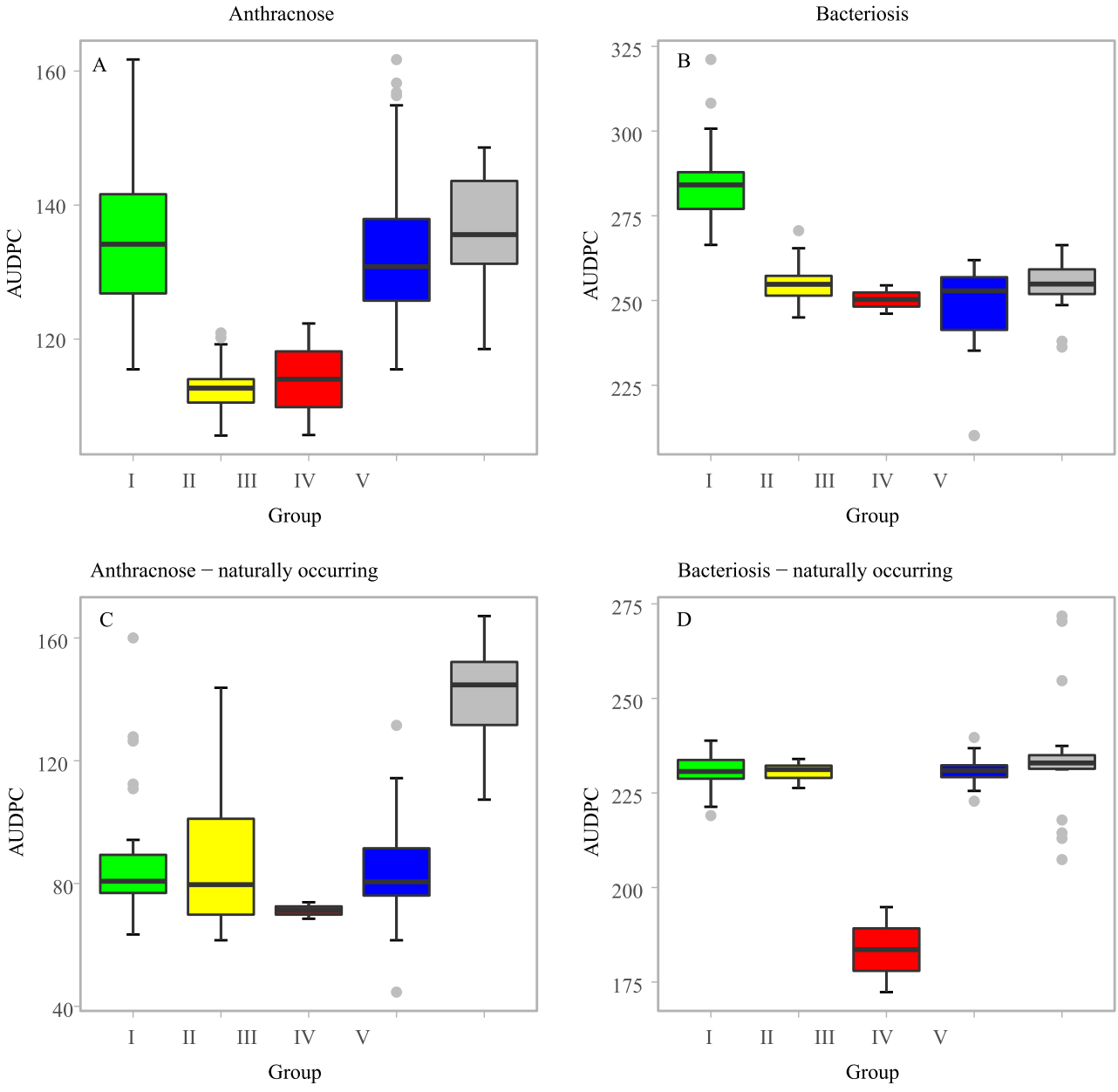

Figure 3. Boxplots of the severity of two leaf diseases – anthracnose and bacteriosis –, as a function of AUDPC evaluated in experiments with plants subjected to inoculation with the following pathogens: A, *Colletotrichum gloeosporioides* f. sp. *manihotis* (anthracnose); B, *Xanthomonas axonopodis* pv. *manihotis* (bacteriosis); C, naturally occurring anthracnose infestation; D, naturally occurring bacteriosis infestation.

lowest values, indicating that they are the most resistant to anthracnose (Figure 3 A). When anthracnose was evaluated in the experiment under naturally occurring diseases, an overall mean of AUDPC was observed as relatively close to those of the constituent accessions of groups 1, 2, 3, and 4; and group 5 had an upper mean approximately twice as high as those of the others groups (Figure 3 C)

In the experiment subjected to inoculation with the bacteriosis causal agent, groups 2, 3, 4 and 5 showed very close general means for AUDPC; and group 1 showed the highest mean (Figure 3 B). An inverse situation is observed for the means behavior of the groups in the experiment under naturally occurring disease for bacteriosis, since group 3, formed by only two accessions, had the lowest mean compared to the others (Figure 3 D).

Except for the experiment subjected to inoculation with the anthracnose causal agent, group 3 (which had the second lowest mean), represented by the accessions BGM1170 and BGM1134, showed the lowest means of AUDPC in the experiments and, therefore, it is considered resistant. It is noteworthy that for all experiments, group 3 had a lower mean than group 4, formed by the largest number of accessions and where most commercial cultivars are also found, indicating possible selection success for these two genotypes.

The highest means of AUDPC were identified in group 5, except for the experiment subjected to inoculation with *Xanthomonas phaseoli* pv. *manihotis*. The variation of the amplitude of the means was greater for the inoculation with *Colletotrichum gloeosporioides* f. sp. *manihotis* and for the experiment under naturally occurring diseases evaluated for anthracnose, with more discrepant values observed for the individual means of each accession in relation to the general mean of each group.

Conclusions

1. The cassava (*Manihot esculenta*) accessions in the experiment under naturally occurring diseases, evaluated for the area under the disease progress curve (AUDPC) of anthracnose and bacteriosis show genetic variability among the accessions.

2. In the experiments subjected to inoculation with *Xanthomonas phaseoli* pv. *manihotis* and the experiment under naturally occurring diseases evaluated for bacteriosis, the chances of success in the selection for resistant accessions are higher due to the high heritability values.

3. Five groups are formed through the unweighted pair group method with arithmetic mean for genetic divergence and clustering, in which group 3 – composed by the accessions BGM-1170 and BGM-1134 – shows the lowest mean for AUDPC, therefore, it is considered resistant to anthracnose and bacteriosis.

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