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Evaluation of resistance levels to *Fusarium solani* in cassava genotypes

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ABSTRACT

The search for cassava varieties resistant to fungi of the genus *Fusarium* has proven to be a good option for producers. To achieve this, it is essential to identify sources of resistance that allow genetic parameters to be estimated, providing useful information for breeders. The genus *Fusarium* is recognized as one of the main responsible for causing significant damage and economic impacts on cassava plantations globally. The present study aimed to identify, in cassava genotypes, resistance levels to *F. solani* using the detached root inoculation methodology. The experiment was conducted in the laboratory, and evaluations were conducted every two days after inoculation. The experimental design adopted was completely randomized with three replications consisting of a cassava root disc with or without inoculation of pathogen spore suspension. We obtained genotypes grouped as moderately susceptible and extremely susceptible, using an easy-to-execute methodology in a controlled environment in this study.

Keywords: Fungi, Rot, Inoculation methodology, Resistance.

Avaliação dos níveis de resistência a *Fusarium solani* em genótipos de mandioca

RESUMO

A busca por variedades de mandioca com resistência aos fungos do gênero *Fusarium* tem se mostrado uma boa opção para os produtores. Para isto, é fundamental identificar fontes de resistência que permitam estimar parâmetros genéticos, fornecendo informações úteis para os melhoristas. O gênero *Fusarium* é reconhecido como um dos principais responsáveis por causar danos significativos e causar impactos econômicos consideráveis nas plantações de mandioca em escala global. Este trabalho teve como objetivo identificar os níveis de resistência a *F. solani*, utilizando a metodologia de raiz destacada em genótipos de mandioca. O experimento foi conduzido em laboratório e as avaliações foram realizadas de dois em dois dias após a inoculação. O delineamento experimental adotado foi inteiramente casualizado, com três repetições constituídas de um disco de raiz de mandioca com ou sem inoculação de suspensão de esporos do patógeno. Neste trabalho obtivemos genótipos agrupados como moderadamente susceptíveis e extremamente susceptíveis, através de uma metodologia de fácil execução e em ambiente controlado.

Palavras-chave: Fungos, Podridões, Metodologia de inoculação, Resistência.

1. Introduction

Cassava is a highly important food for the world diet and industry, especially in tropical regions. According to the Brazilian Institute of Geography and Statistics (IBGE, 2021), cassava production in Brazil in 2021 was 18.4 million tons, with this production coming from an area of 1.2 million hectares (ha). In countries with a tropical climate, it is one of the most cultivated and consumed foods due to its rusticity to edaphoclimatic factors (climate and soil), producing the greatest amount of starch in soils with low fertility and acid pH, making it viable to cultivation, unlike other crops, such as corn, soybeans, which are more demanding for their development (Stefanello et al., 2017).

Cassava belongs to the order Malpighiale, the family Euphorbiaceae, and the species *Manihot esculenta* Crantz, popularly known as cassava and yuca (Mattos; Farias e Filho, 2006). It is characterized by being table or industrial according to the amount of hydrocyanic acid (Alves et al., 2009). Table cassava is classified as having a low content (<100 mg kg^{-1}) of hydrocyanic acid, and industrial cassava as having a high content (> 100 mg kg^{-1}) of hydrocyanic acid; the high content of which is toxic and can lead to death, which is why fresh consumption is not recommended (Bolhuis 1954).

The detoxification of cassava in industrial processing occurs through dissolution in water or volatilization, which includes maceration, soaking in water, boiling, roasting, fermentation of the roots, or a combination of these processes (Chisté, Cohen, 2008). Endogenous detoxification takes place through the conversion of cyanide into thiocyanate, the main cyanide metabolite, in the presence of the enzyme rhodanase and cysteine. The resulting thiocyanate is non-toxic and is eliminated through the urine (Chisté et al., 2010).

Cassava adapts well to various growing locations, but due to the interaction between genotype and environment, it adapts differently in specific regions (Lessa et al., 2017). According to Notaro et al. (2013), several factors reduce production performance, whether it is the acquisition of propagative material for the production of seedlings (stem cuttings) of lower quality, low management technology, physiological aging, and others, and these factors favor the spread of pests and diseases.

Among the phytosanitary problems that reduce cassava root yield are diseases caused by fungi, such as anthracnose, overgrowth, and root rot, identified as one of the main limiting factors. These diseases reduce the efficiency of water and nutrient absorption, showing symptoms of wilting and toppling, affecting the roots, the main organ of interest, due to its use for human consumption, implying disposal due to changes in its morphology since it reduces quality and makes it difficult to market (Ceballos et al., 2004; Boas et al., 2016). Root rots are classified as dry rot (dry-looking symptoms and a yellowish color) and soft rot (watery tissue and a strong odor, and black rot, with black damage and exudation of liquid albumen). Both can infect cassava plants at any stage of development (Bandyopadhyay et al., 2006).

The species that cause the most damage in Brazil are *Phytophthora drechsleri*, known for soft root rot, and *Fusarium* spp., which causes dry root rot, both of which can cause 100% losses in favorable conditions for the diseases (Oliveira et al., 2013). The conditions that favor the development of dry rot are times of lower rainfall, in rainier months, the symptoms intensify in the shoot with wilting and yellowing, similar to soft rot, however, to differentiate one disease from the other, the tuberous roots of the plant must be sampled (Tremacoldi, 2016).

Fungi of the *Fusarium solani* species complex cause dry rot, showing symptoms of root striae that block the vascular tissues for sap circulation, causing indirect and dry rot of the roots with coloration varying from yellow to brown (Ghini et al., 2011; Hohenfeld et al., 2018). This fungus can stay in the soil for a long because it can survive through resistance structures called chlamydospores (Nogueira et al., 2019). As it is a soil borne fungus, it is difficult to control, and there are no records of chemical products recommended for controlling the disease in cassava (Agrofit, 2024).

Some methods identify resistance through plant tissue, such as the soil infestation method, soaking stem cuttings, soaking roots in a suspension containing conidia of the fungus, and wounding the stem (Hohenfeld et al., 2018; Paiva et al., 2022). However, for this study, we chose the detached root inoculation method proposed by Oliveira et al. (2013), which is widely used to identify resistant genotypes, requiring complete root formation to inoculate the pathogen. This method is conducted in the laboratory. This study aims to identify the levels of resistance of cassava ethnovarieties to the fungus *Fusarium solani* by inoculating detached roots under laboratory conditions.

2. Material and Methods

The experiment was conducted in the Phytopathology laboratory of the Federal Institute of Mato Grosso do Sul, Nova Andradina Campus (IFMS-NA). Nineteen cassava ethnovarieties collected from the IFMS-NA germplasm bank from the locations shown in Figure 1 were used.

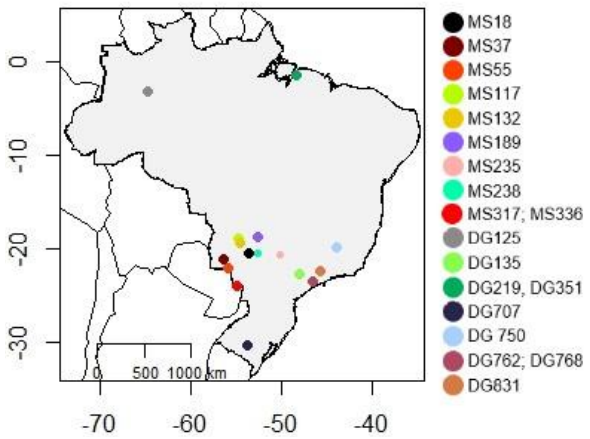

Figure 1. Location of origin of cassava genotypes: MS 18 Bodoquena; MS 37 Bonito; MS 55 Bela Vista; MS 117 Rio Verde de MS; MS 132 S. Gabriel D´Oeste; MS 189 Chapadão do Sul; MS 235 Paranaíba; MS 238 Ribas do R. Pardo; MS 317 Sete Quedas; MS 336 Tacuru; DG 125 R.Solimões/lag.Mamiá (AM); DG 135 Anhembi; DG 219 Belém/Embrapa/cpatu; DG 351 Belém/EMBRAPA/CPATU; DG 707 Vila Nova (SP); DG 750 MG; DG 762 São Paulo do Coraci (AM); DG 768 São Paulo do Coraci (AM); DG 831 Conceição dos Ouros / Ouros Velho (MG).

The roots were inoculated according to the methodology described by Oliveira et al. (2013). To do this, fully formed roots with a diameter of approximately 7.5 cm were collected and washed under running water, dried with paper towels, and cut into 1.5 cm thick disks. The disks were washed, immersed in 0.5% sodium hypochlorite for three minutes, and placed on filter paper to dry.

A morphologically characterized isolate of *F. solani* was used, grown in Petri dishes containing BDA culture medium at a temperature of 25±2ºC for seven days to form mycelium and sporulation. The conidia suspension was obtained by adding 10 mL of sterilized distilled water to the Petri dishes containing the fungal growth, and the conidia were released by scraping them off with a glass rod. The conidia suspension was filtered through a double layer of gauze, and the spore concentration was determined by counting using an optical microscope and a hemocytometer (Neubauer chamber), adjusted to $2x10^5$ conidia mL^{-1}.

Inoculation was conducted in a wound in the center of the disc by depositing 20 µL of the conidia suspension. A disk with 20 µL of distilled water and tween (1%) was used as a control. The inoculated discs were placed in transparent plastic trays with lids and kept in a humid chamber with absorbent cotton at 26°C ± 2 for ten days, under a 12-hour photoperiod, keeping the absorbent cotton moist throughout the experiment with autoclaved distilled water. The experiment was set up in a completely randomized design (CRD) with three replications of a cassava root disc with or without inoculating the pathogen spore suspension.

Evaluations occurred every two days after inoculation to determine the disease progress curve and the Area Under the Disease Progress Curve (AUDPC) up to ten days after inoculation, totaling 5 evaluations. To determine the colonized area, a 750 x 1334 pixels resolution camera was used to obtain the images, and ImageJ software was used to measure the lesion.

The data were subjected to analysis of variance and cluster analysis using the Euclidean distance and average method with the aid of the R software (R Core team, 2023). A scale adapted from the McKinney index (1923) was used to measure the colonization of the cassava discs:

0= cassava disks without symptoms;

1= cassava disks with less than 10% to 25% symptoms;

2= cassava discs with 25% to 50% symptoms;

3= cassava disks with 50% to 75% symptoms;

4= cassava disks with 75% to 100% symptoms.

Where 0=Resistant; 1=Moderately resistant; 2=Susceptible; 3=Moderately susceptible and 4=Extremely susceptible.

Using the symptom scores on the inoculated disks, the area under the disease progress curve (AUDPC) was calculated using the equation described by Shaner and Finney (1977):

$$\sum_{i}^{n-1}[(\frac{y_i + y_{i+1}}{2})(t_{i+1} - t_i)$$

Where: n is the number of evaluations, y_i is the severity or incidence of the disease in the i-th evaluation, and t_i is the time in the i-th evaluation.

3. Results and Discussion

The lesions caused by *F. solani* were analyzed in this study. The lesions had a dry appearance and a brownish halo (Figure 1- A, B, C). Hohenfeld et al., (2018) studied the varieties BRS Kiriris, BRS Poti Branca, and Salangó, which also showed the same symptoms of a dry appearance on the cassava disks. The disease progressed as the size of the lesion in the tissue increased over the days, and the severity of the pathogen increased. Symptoms evolved over the evaluation period, and it was possible to verify the infection of the pathogen in the cassava root discs through re-isolation.

At 24 hours after inoculation, a rate of progress of the disease was observed in the cassava genotypes DG 135, DG 219, DG 351, DG 707, DG 768, DG 831, MS 132, MS 18, MS 235, MS 317, MS 336, and MS 55 (Figure 2). The growth stabilized on the second day of evaluation due to the fungus having already evolved over the area of the cassava disc. In the genotypes DG 125, DG 750, DG 762, DG 117, MS 189, MS 238, and MS 37, the fungus grew more slowly than the genotypes already mentioned, reaching stability from the third and fourth evaluation days.

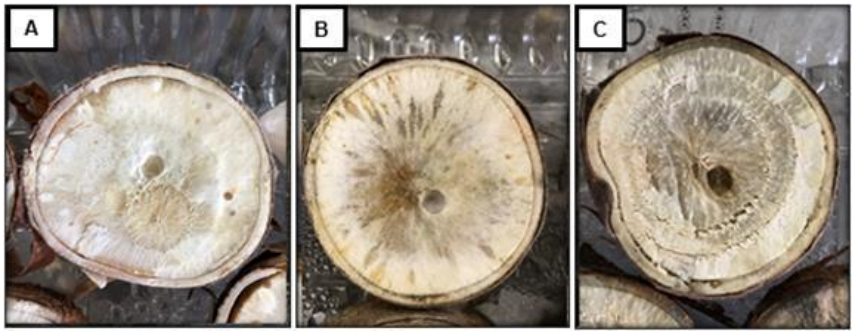

Figure 1. Cassava roots inoculated with dry rot pathogen (*Fusarium solani*). "A" MS 238, "B" DG 750, and "C" MS 336.

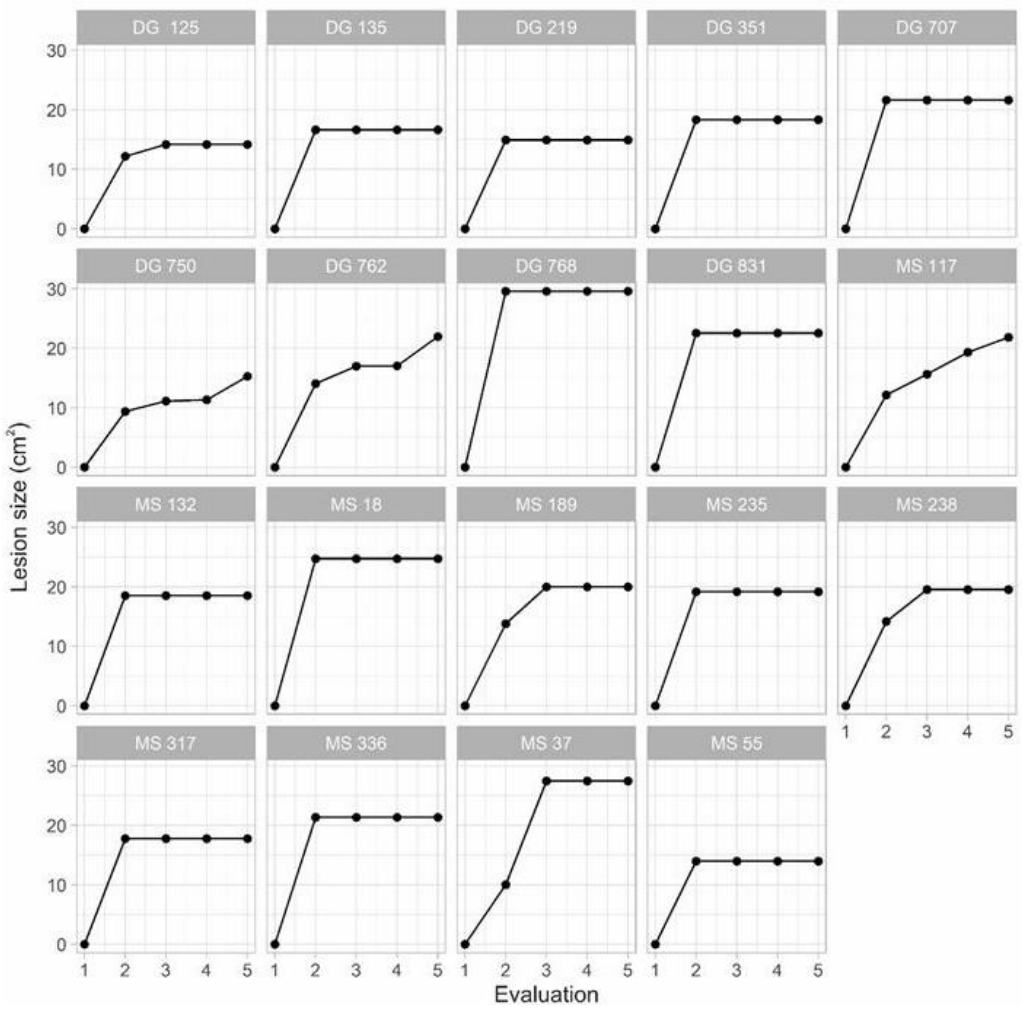

Figure 2. Evolution of fusariosis caused by *Fusarium solani* inoculated on cassava root disks and kept for ten days under laboratory conditions at 26°C ± 2 and 12-hour photoperiod.

All the cassava genotypes showed symptoms caused by the growth of *Fusarium solani* on the first day of evaluation. The DG 768 and MS 18 genotypes showed the highest infection rate, with fungal growth all over the root disk as it is shown in Figure 3. The other genotypes, on the other hand, behaved similarly to each other, with fast initial growth and soon stabilized from the third and fourth day; the decline in the growth of the fungus was due to the smaller healthy area available to be infected and continue growing.

Juliatti et al. (2019) states that many researchers use AUDPC to classify resistant and susceptible genotypes, which reaffirms the importance of this type of analysis. All the genotypes studied regarding AUDPC behaved as susceptible to the disease. In Lima et al. (2019), larger areas of AUDPC were also found, showing susceptibility to pineapple fusariosis and fusarium wilt in castor beans. Even with all the pathogenicity of the disease during the experiment, among the 19 cassava genotypes, DG 768 showed the highest AUDPC and DG 750 had the lowest AUDPC (Figure 4).

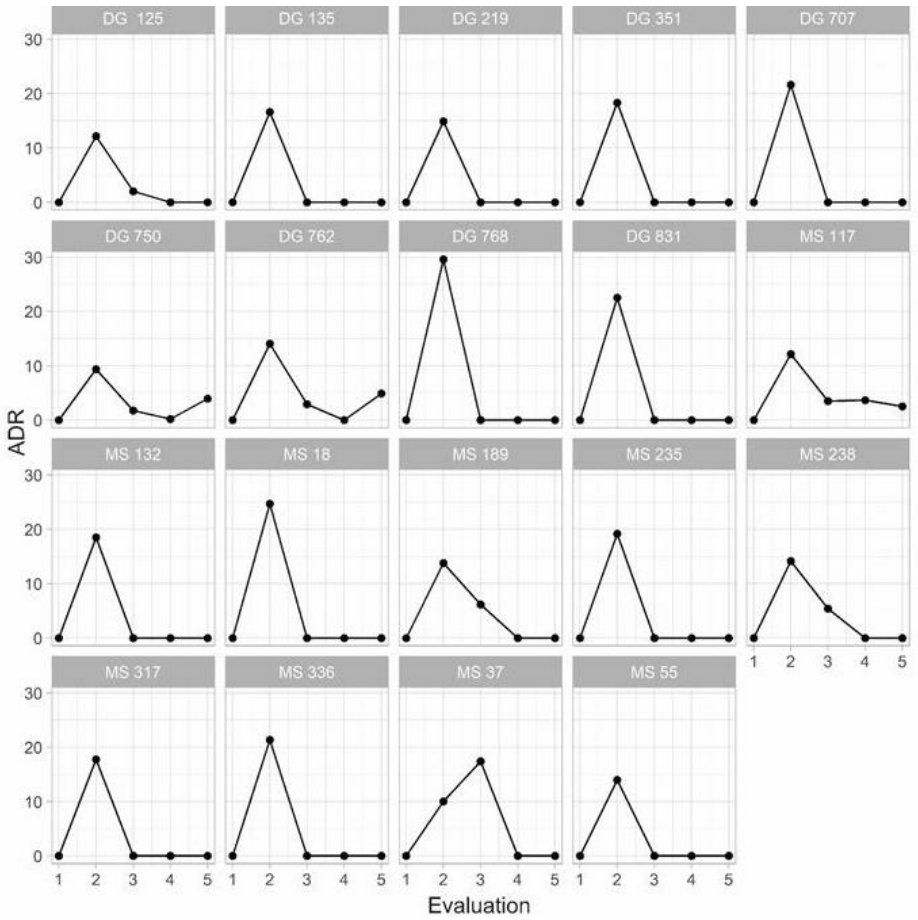

Figure 3. Absolute disease rate (ADR) caused by *F. solani* inoculated in cassava root discs and kept for ten days under laboratory conditions at 26°C ± 2 and 12-hour photoperiod.

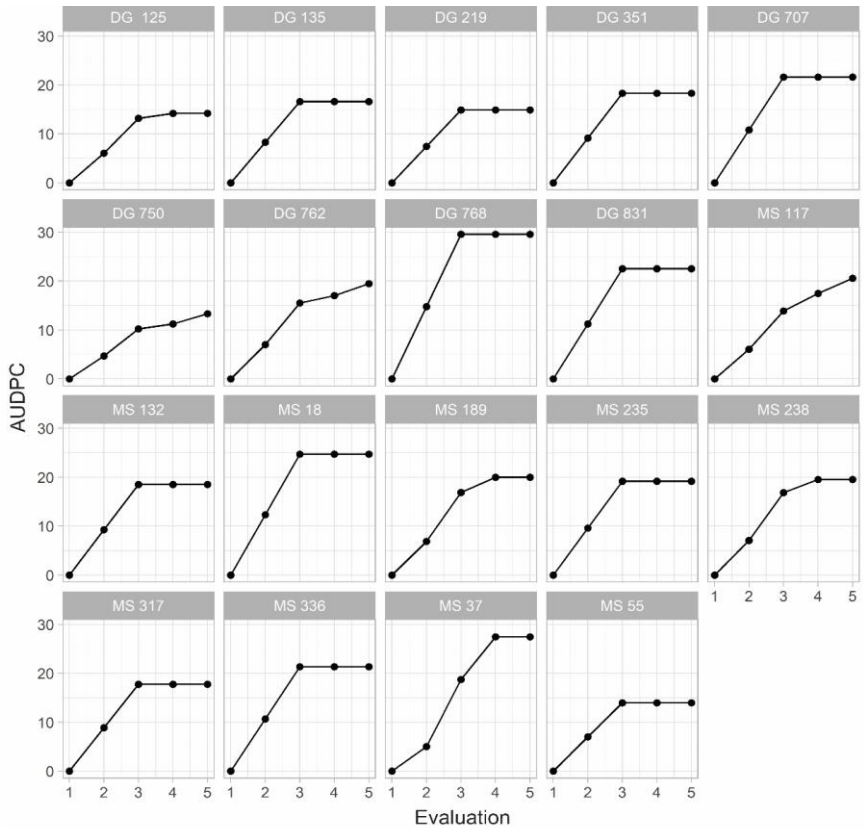

Figure 4. Area under the disease progress curve (AUDPC) caused by *F. solani* inoculated in cassava root discs and kept for ten days under laboratory conditions at 26°C ± 2 and 12-hour photoperiod.

According to Santos and Café Filho (2005), the AUDPC can be used in the laboratory to evaluate plants for breeding programs, but they report the importance of taking the genotypes to the field so that they can be compared with the results obtained in this study and present reliable genotypes for cultivation, in line with the producer reality. It is easier to experiment in the laboratory because it is a controlled environment, whereas in the field, we cannot control temperature, humidity, climate, soil, etc.

All the genotypes studied showed some degree of susceptibility to *F. solani*. However, DG 831 showed the highest susceptibility, and the DG 135 genotype showed the highest resistance (Figure 5). This variation may be related to producing the fungus's extracellular enzymes to degrade the cell wall, allowing it to penetrate and colonize the plant tissue (Sunitha et al., 2013). Cassava has starch in its composition, so the enzymes can convert starch into smaller polymers, allowing the fungus to obtain food and thus infect the root easily (Lacerda et al., 2008).

Temperature may be another factor that contributed to the growth of *F. solani*. The optimum growth temperature for the fungus ranges from 24 to 28°C and for sporulation, from 25 to 32°C (Hohenfeld et al., 2018). This study used an incubation temperature of 26°C ± 2 for ten days under a 12-hour photoperiod, i.e., between the most suitable growth and sporulation temperatures for the fungus development. It can be seen that two distinct groups were formed (Figure 6). The advances in this research can be seen in several fields, driven by a combination of emerging technologies and innovative approaches. Methods for inoculating pathogens and evaluating resistance have broadened horizons and facilitated the work of plant breeders and phytopathologists. As we continue to explore the limits of knowledge, prospects are full of possibilities, shaped by the constant flow of discoveries.

Group 2 (right side) comprised ten genotypes classified as extremely susceptible, including the DG 831 genotype with the highest susceptibility and the DG 768 genotype with the highest AUDPC. Characterizing cassava genotypes helps breeders to sample cultivars to be evaluated for particularities of agronomic interest since obtaining improved populations involves the selection and recombination of individuals or families (Bruckner et al., 2002).

The advances in this research can be seen in several fields, driven by a combination of emerging technologies and innovative approaches. Methods for inoculating pathogens and evaluating resistance have broadened horizons and facilitated the work of plant breeders and phytopathologists. As we continue to explore the limits of knowledge, prospects are full of possibilities, shaped by the constant flow of discoveries.

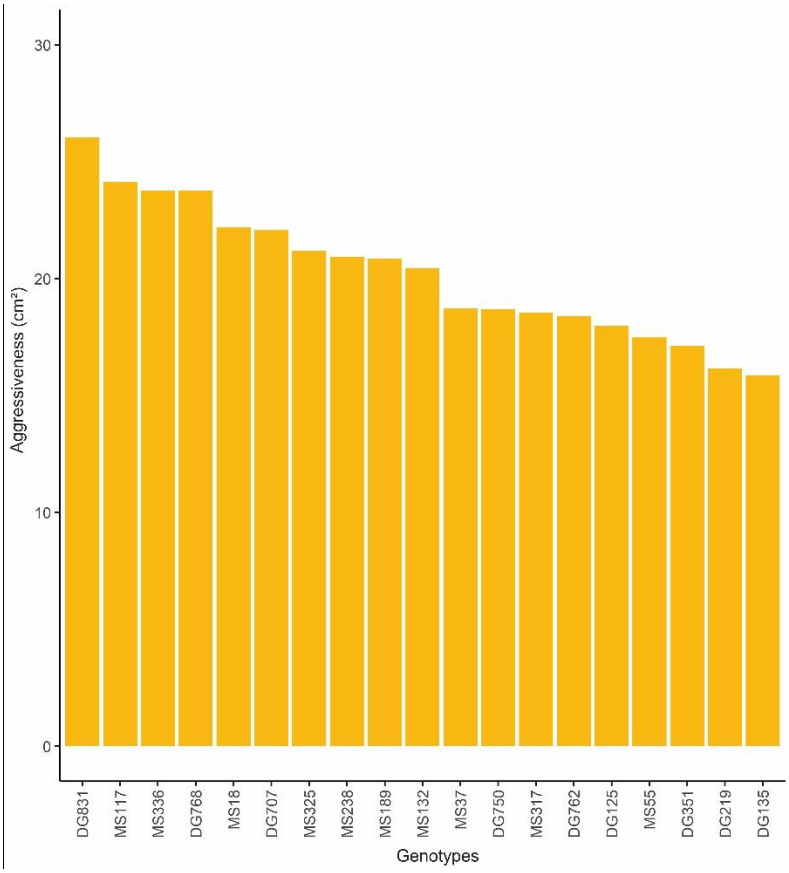

Figure 5. Aggressiveness (lesion area cm²) caused by *F. solani* inoculated in cassava root discs and kept for ten days under laboratory conditions at 26°C ± 2 and 12-hour photoperiod.

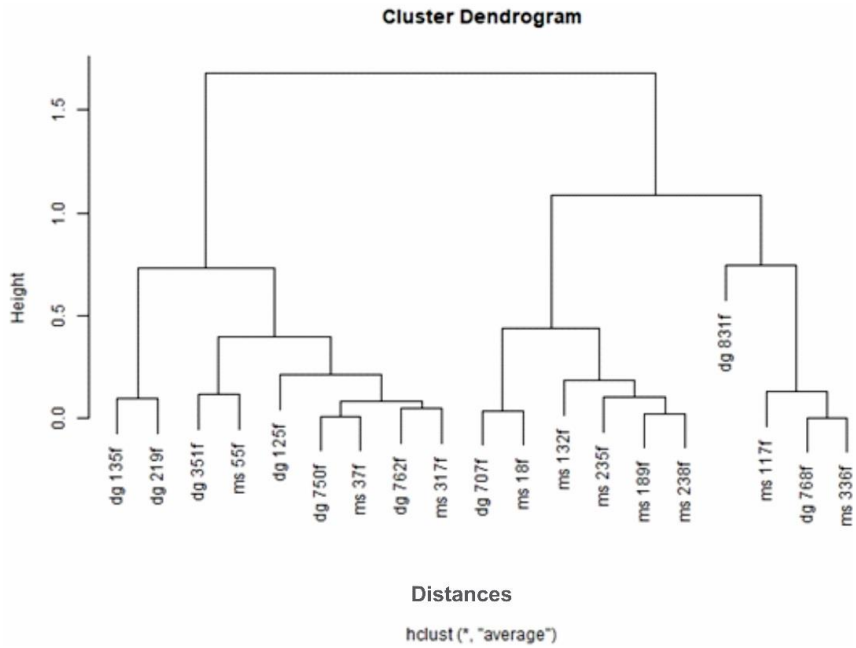

Figure 6. Dendrogram of genetic resistance among the 19 cassava genotypes associated with *Fusarium solani* inoculated in cassava root discs kept for ten days under laboratory conditions at 26ºC ± 2 and 12-hour photoperiod.

4. Conclusions

We can conclude from this work the importance of knowing cassava genotypes and their performance when subjected to inoculation with Fusarium solani. The inoculation method used on the roots is an efficient way of knowing the behavior of the genotype, contributing to the work of plant breeders and phytopathologists and generating more accessible and faster methodologies for evaluating cassava genetic materials.

Authors' Contribution

Nancy Farfan Carrasco; Francisco José Teixeira Gonçalves; Gabriel Ferreira Paiva; Francisco José Teixeira Gonçalves: Idea conception. Brenda Virgínia Sanches Silva; Gabriel Ferreira Paiva; Gustavo Henrique Silveira de Souza; Angelica Rodrigues Alves: Experiment execution and data collection and tabulation. Nancy Farfan Carrasco and Gabriel Ferreira Paiva: Experimental design, data analysis, interpretation, and drawing graphs. Brenda Virgínia Sanches Silva; Gustavo Henrique Silveira de Souza; Gabriel Ferreira Paiva; Nancy Farfan Carrasco; Angelica Rodrigues Alves; Luiz Henrique Costa Mota; Francisco José Teixeira Gonçalves: Writing and revising the manuscript.

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