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AGGRESSIVENESS OF DIFFERENT ISOLATES MACROPHOMINA PHASEOLINA AND THEIR INTERACTION WITH SOYBEAN CULTIVARS

AGGRESSIVENESS OF MACROPHOMINA PHASEOLINA ISOLATES ON SOYBEAN

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ABSTRACT. The objective of this research was to evaluate the aggressiveness of ten *Macrophomina. phaseolina* isolates on two soybean cultivars. This fungus is the etiologic agent of charcoal rot and occurs on most of the crop areas in Brazil, causing losses in quality, productivity and rentability. The isolates, originating from different Brazilian states, were inoculated on soybean cultivars, Brasmax Bônus IPRO and Brasmax Desafio RR, through immersion of seeds on a mix of fungus spores, mineral oil and sugar solution. The research was conducted on a greenhouse with controlled temperature and irrigation system, where the evaluated variables were seedling emergence percentage, radicle and necrosis length (mm). An interaction between the factors of cultivar and isolates was observed for the seedling emergence percentage, but not for the radicle and necrosis length. All tested isolates were pathogenic to soybean, having differing levels of aggressiveness depending on the cultivar and isolate. The isolates MACRO – UFU 5 and MACRO – UFU 2 resulted in the death of 90 to 100% of Brasmax Bônus IPRO and Brasmax Desafio RR seeds, being considered the most aggressive. Although the resistance to *M. phaseoline* is quantitative, with a variable defense system depending on cultivar and isolate, the selection of a highly aggressive isolate can help with the analysis of resistant cultivars.

KEYWORDS: Germoplasm screening; *Glycine max*; Charcoal rot; Plant resistance

INTRODUCTION

The charcoal rot is one of the most destructive diseases on soybean [*Glycine max* (L.) Merr.], caused by the fungi *Macrophomina phaseolina* (Tassi) Goid (1947). Environmental conditions, such as low humidity, high temperatures and compacted soil can favor the pathogen development (Almeida et al., 2014; Marquez et al., 2021). Given the pathogens nature, being polyphagous, soil borne and capable of producing resistant structures (microsclerotia) that can stay in the soil for a long time, crop rotation is not an efficient way to manage this fungus. The chemical control can be viable economically but not environmentally, besides, in Brazil, *M. phaseolina* doesn't have a registered fungicide (Mengistu et al., 2007; Jordaan et al., 2019; Ministério da Agricultura e Pecuária, 2024). Recently, a biocontrol agent was registered, with a composition based on *Paenibacillus azotofixans*; *Bacillus subtilis*; *B. licheniformis* e *B. circulans* (Ministério da Agricultura e Pecuária, 2024).

Resistant cultivars could be the most viable strategy of control of this fungus, even though the resistance is quantitative (Da Silva et al., 2019; Basandrai et al., 2021). At this moment, only moderately resistant cultivars are available on the market, showing the importance of evaluating cultivars with higher resistance to *M. phaseolina* (Paris et al., 2006; Gupta et al., 2012; Almeida et al., 2014; Da Silva et al., 2019; Reznikov et al., 2019). According to Ribeiro do Vale et al. (2001), different cultivars show difference in the resistance to the fungus, being extremely susceptible to highly resistant, because of the quantitative resistance, that is regulated by multiple genes, that on their own have a small effect on the resistance, but when combined can have a larger effect, because they are additive. Therefore, the determination of quantitative resistance is complex and diverse in terms of number and nature of the involved genes (Pariaud et al., 2009).

The quantitative variation of phatogenicity in hosts can reflect the aggressiveness of the pathogen to a given genotype (Pariaud et al., 2009; Delmas et al., 2016). The aggressiveness is defined as the degree of the damage caused by the pathogen to the host, in other words, the more aggressive an isolate is, the more host tissue it can affect (Ribeiro Do Vale et al., 2001; Pariaud et al., 2009; Delmas et al., 2016). The aggressiveness of the pathogen is frequently evaluated based on multiple quantitative characteristics of its life cycle, like the efficiency of infection, latent period, sporulation rate, infectious period, toxin production and disease severity, through percentage of plant infection and size of injury (Pariaud et al., 2009). Thus, the knowledge of these quantitative characteristics, inherent to the pathogen in relation to the selective pressure of the host, are fundamental to identify resistant cultivars (Delmas et al., 2016).

In this research the agressiveness of ten isolates of *M. phaseolina* were evaluated through the seedling emergence percentage, radicle and necrosis length of soybean seedlings.

MATERIAL AND METHODS

Evaluated isolates and soybean cultivars

The ten *M. phaseolina* isolates were obtained from the collections of the Universidade Federal de Uberlândia (UFU) and the Instituto Federal Goiano (Table 1). The cultivation of *M. phaseolina* isolates and inoculum preparation were carried out at the Mycology and Plant Protection Laboratory (LAMIP) – UFU, Campus Umuarama, Minas Gerais.

The experiment was conducted in a greenhouse at the same location, using the Brasmax Bônus IPRO and Brasmax Desafio RR cultivars, to verify the pathogenicity and aggressiveness level of the isolates.

Table 1. Macrophomina phaseolina isolates obtained from different Brazilian states.

Code	**Origin**
MACRO - UFU 1	Cambé, PR
MACRO - UFU 2	Vianópolis, GO
MACRO - UFU 3	Uberlândia, MG
MACRO - UFU 4	Passo Fundo, RS
MACRO - UFU 5	Vianópolis, GO
MACRO - UFU 6	Alto Uruguai Gaúcho, RS
MACRO - UFU 7	Uberlândia, MG
MACRO - UFU 8	Londrina, PR
MACRO - UFU 9	Uberlândia, MG
MACRO - UFU 10	São Paulo, SP

Inoculum Preparation

Each *M. phaseolina* isolate was cultivated on seven 9 cm Petri dishes containing potato dextrose agar (PDA), for 14 days, at a temperature of 28°C, without light. The pure grown fungal cultures were dried in a hermetically sealed container with silica gel, for 15 days. After the drying period, the cultures were scraped using a Drigalski spatula. Approximately 1 g of dried culture was mixed with 14 g of cornmeal and blended in an industrial blender (Figure S1 A-D). To the blended material, 15 mL of sugar solution (50 g of sugar to 50 mL of distilled water) and 15 mL of mineral oil were added (Depieri, 2009). This mixture was then used for the inoculation of soybean seeds.

Inoculation

Seeds of both soybean cultivars were immersed in the prepared mixture for 30 minutes. Subsequently, they were sown in 200 mL cups, containing organic substrate, and placed in a greenhouse (Figure S1 E-F) with a controlled temperature of 32°C ±2 and manual irrigation via hose, occurring on alternate days (adapted from Depieri, 2009). In the control treatments, seeds of both cultivars were immersed only in mineral oil and sugar solution. The experimental design was completely randomized in a 2 x 10 + 2 factorial scheme (cultivars x isolates + control treatments), with three replicates, and five seeds per replicate.

Verification of isolate aggressiveness

The evaluation of disease severity was carried out 14 days after inoculation by measuring the emergence percentage, root length (mm) and necrosis length (mm) using a ruler. Isolates that induced a reduction in emergence and more severe disease symptoms were considered more aggressive.

Statistical analysis

The collected data were subjected to the assumption tests before the analysis of variance, verifying the homogeneity of variances and the normality of residuals (Shapiro121 Wilk). After checking the assumptions, the data were subjected to the analysis of variance 122 using the F-test (p=0.05). Means were compared in two distinct ways: grouped by Tukey's 123 test (p=0.05) and Dunnett's test (p=0.05). In cases where assumptions were not met at the 0.05 significance level, the data were transformed using the square root of (x + 1). All data obtained were analyzed using R software (R Core Team, 2020).

RESULTS AND DISCUSSION

In Table 2, a significant difference was observed between *M. phaseolina* isolates, as well as a significant interaction between cultivars and isolates, indicating that the isolates behaved differently depending on the cultivar, when emergence percentage was evaluated. No significant difference was found for root and necrosis length.

Table 2. Mean squares from the analysis of variance for the parameters of seedling emergence (E), root length (CR), necrosis length (CN), of the 10 *Macrophomina phaseolina* isolates in relation to two cultivars, Brasmax Bônus IPRO and Brasmax Desafio RR.

Source of Variation	**df**	**GL – MS**	**GL – Pr > Fc**	**E (%) – MS**	**E (%) – Pr > Fc**	**CR (mm) / CN (mm) – MS**	**CR (mm) / CN (mm) – Pr > Fc**
Cultivar	1	0.03580	0.9133	35.90845	35.908	1.19474	1.1947
Isolate	9	9.66307	0.0389*	81.91370	35.908	0.87054	0.2746
Cultivar × Isolate	9	6.34744	0.0052*	113.49178	0.0979	0.44104	0.7495
Control × Factors	1	135.78800	0.0000	11.78183	0.6687	0.02582	0.8464
Control$_1$ × Control$_2$	1	1.96025	0.4224	1.96025	0.8613	0.16663	0.6231
Residue	44	2.98853	—	63.48070	—	0.68006	—
C.V. (%)	—	56.78	—	—	—	—	—
Total	65	6.35961	—	70.79155	—	0.66330	—

* Significant at $p \leq 0.05$.

Mean Squares (MS) for the factors of Cultivar, Isolate, and their interactions. Coefficient of Variation (C.V. (%)); Asterisks (*) denote significance at 5% by the F-test.

In the Dunnett test, comparisons were made between the control (non-inoculated seeds) and the treatments (seeds inoculated with *M. phaseolina* isolates) within each cultivar (Table 3). There was variation in isolate aggressiveness on both cultivars. In the cultivar-pathogen interaction, when the cultivar's defense system recognized the pathogen, the infection efficiency was reduced, resulting in greater seedling emergence, increased root length, and shorter necrosis length; the following isolates were considered less aggressive: MACRO - UFU 1, MACRO - UFU 3, MACRO - UFU 4, MACRO - UFU 6, MACRO - UFU 8, MACRO - UFU 10. However, when the pathogen was not recognized, seed mortality reached 100%. The isolates MACRO - UFU 5 and MACRO - UFU 2 were capable of killing 90-100% of the seeds of the Brasmax Bônus IPRO and Brasmax Desafio RR cultivars, being considered the most aggressive for both cultivars. The isolate MACRO - UFU 7 was less aggressive when inoculated in the Brasmax Bônus IPRO cultivar, as there was a higher percentage of seedling emergence compared to the other isolates. However, it caused 100% of seed mortality in Brasmax Desafio RR. Although seedling emergence occurred in the Brasmax Bônus IPRO cultivar when inoculated with isolate MACRO - UFU 9, root development of the seedling did not occur.

Table 3. Means of seedling emergence (E), root length (CR), necrosis length (CN), from cultivars Brasmax Bônus IPRO and Brasmax Desafio RR inoculated with ten *M. phaseolina* isolates.

Treatment	(%)	(%)	(%)	(%)	(%)	(%)
MACRO - UFU 1	20bc	13.00a	0.00a	46a	10.77a	0.27a
MACRO - UFU 2	6.66cd*	6.66a	0.16a	0.00c*	0.00a	0.00a
MACRO - UFU 3	13.33bc*	11.33a	0.00a	20.00ab*	9.83a	0.00a
MACRO - UFU 4	13.33bc*	8.66a	0.16a	20.00ab*	8.75a	0.00a
MACRO - UFU 5	0.00d*	0.00a	0.00a	6.66bc*	4.33a	0.00a
MACRO - UFU 6	6.66cd*	10.50a	0.00a	6.66bc*	0.33a	0.16a

Treatment	(%)	(%)	(%)	(%)	(%)	(%)
MACRO - UFU 7	33.33b	17.66a	0.76a	0.00c*	0.00a	0.00a
MACRO - UFU 8	6.66cd*	0.33a	0.50a	6.66bc*	0.66a	0.50a
MACRO - UFU 9	6.66cd*	0.00a	0.00a	6.66bd*	14.33a	0.00a
MACRO - UFU 10	13.33bc*	4.00a	0.66a	6.66bd*	7.66a	0.00a
CONTROL	73.33a	14.26a	0.00a	53.33a	13.00a	0.00a

Means followed by different letters in the columns are significantly different according to Tukey test at 5% significance. Asterisks (*) in the mean shows significant differences between Isolates and Control, according to Dunnet test at 5% significance.

DISCUSSION

The difference in aggressiveness between isolates may be related to their ability to respond to specific environmental factors, as well as to genetic, physiological, and biological characteristics of the host, which allows the activation of mechanisms that produces multiple secondary metabolites, promoting the infection process (Reyes-Franco et al., 2006; Sarr et al., 2014; Chen et al., 2017). Considering that the *M. phaseolina* isolates in this study were collected from collections from different states and soybean growing areas, the observed difference in aggressiveness could be attributed to environmental conditions, as the most aggressive isolates, MACRO - UFU 5 and MACRO - UFU 2, were obtained from Vianópolis, GO. A study by Reyes-Franco et al. (2006) involving *M. phaseolina* isolates from eight countries and different hosts also found significant variability in isolate aggressiveness when inoculated in 12 common bean genotypes. The most aggressive isolates were from Mexico, Colombia, and Brazil, primarily obtained from common bean. Thus, the environment can affect the development of plant diseases.

An important aspect of studies aimed at assisting breeding programs is the evaluation of a pathogen's genetic diversity, crucial in helping understand the mechanisms that generate genetic variation, host-pathogen coevolution, and resistance management (Aradhya et al., 2001). This is even more important when the breeding goal is to provide resistance against multiple pathogen genotypes, as variability serves as a survival source for the pathogen (Kumar and Verma, 2019). According to Almeida et al. (2003), the high genetic variability among Brazilian *M. phaseolina* isolates complicates the attainment of root infection resistance in commercial soybean cultivars in Brazil, as specific interactions between soybean genotypes and *M. phaseolina* isolates may occur (Reznikov et al., 2019). The Brasmax Bônus IPRO and Brasmax Desafio RR cultivars were found to be susceptible to the MACRO - UFU 5 and MACRO - UFU 2 isolates. However, Silva (2019) showed that the Brasmax Desafio RR cultivar was moderately tolerant to the isolate he used. Given this, there may be differences in symptom severity in different genotypes depending on the isolate used in resistance screening.

When the cultivars were inoculated with isolate MACRO - UFU 9, seedling emergence with root development occurred in Brasmax Desafio RR, but root development of the seedling did not occur in Brasmax Bônus IPRO. This is due to differences between moderately tolerant and susceptible cultivars in terms of root colonization by *M. phaseolina*. In more resistant cultivars, root tissue disintegration occurs, but adventitious root development also takes place in response to the pathogen (Hemmati et al., 2018). Thus, pathogen population aggressiveness alone should not be considered independently of the host's resistance nature and level (Suffert et al., 2017).

Based on these results, the variability in aggressiveness displayed by the *M. phaseolina* isolates was due to specific interactions between each soybean cultivar and the genetic differences of each isolate, as all treatments were exposed to the same environmental conditions. Reznikov et al. (2019) also demonstrated the existence of specific interactions between soybean cultivars and *M. phaseolina* isolates. Therefore, during cultivar resistance screening, caution must be taken when evaluating disease severity, as pathogenic interactions can result in different symptoms in different genotypes depending on the isolate. Although recognizing quantitative resistance is challenging due to its nonspecific defense system, using highly aggressive isolates contributes to the selection of resistant cultivars.

CONCLUSION

The *M. phaseolina* isolates varied in aggressiveness according to the seedling emergence percentage of the Brasmax Bônus IPRO and Brasmax Desafio RR cultivars.

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