ARTIGOS

Reaction of lima bean genotypes to *Macrophomina phaseolina*

Mynor Efraín Munguía García[1], Enayra Silva Sousa[1], Jayara Dayany da Costa Silva[1], Maruzanete Pereira de Melo[1], José Monteiro da Mota[1], Antônio Dias de Almeida Neto[1], Regina Lúcia Ferreira Gomes[1], José Evando Aguiar Beserra Jr[1]

[1]Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Fitotecnia, Campus Universitário Ministro Petrônio Portella, s/n Bairro - Ininga, Teresina - PI - Brasil, CEP64049-550

Autor para correspondência: José Evando Aguiar Beserra Jr (evando@ufpi.edu.br).

Data de chegada: 14/09/2017. Aceito para publicação em: 27/03/2018.

10.1590/0100-5405/185340

ABSTRACT

García, M.F.M.; Souza, E.S.; Silva, J.D.D.; Melo, M.P.; Mota, J.M.; Almeida Neto, A.D.; Gomes, R.L.F.; Beserra Jr., J.E.A. Reaction of lima bean genotypes to *Macrophomina phaseolina.* ***Summa Phytopathologica,*** v.45, n.1, p.11-17, 2019.

Charcoal rot, caused by *Macrophomina phaseolina,* is an important disease of lima bean (*Phaseolus lunatus* L.) in the Northeast of Brazil. Considering that there are no reports of resistance to this disease in Brazil, 37 lima bean accessions were evaluated for their resistance reaction and resistance stability against isolates of *M. phaseolina* in two periods of the year (February-March and July-August 2016), with the aim of selecting genotypes with the potential for use in the management of this disease. Seeds were inoculated at sowing, using rice grains with husk that were colonized by the pathogen. The evaluations of genotypes were carried out at every five days, from the tenth day after sowing, using a score scale and dividing them into six reaction classes. From a population of 37 accessions of *P. lunatus*, no immunity reaction to *M. phaseolina* was seen; however, thirteen accessions presented the lowest severity levels and greatest stability in the two seasons: UFPI 892, UFPI 908 and UFPI 905, which were considered resistant to the isolate COUFPI 06. The resistance reaction of accessions considered resistant varied depending on the isolate of *M. phaseolina.* Accession UFPI 908 expressed the most promising results for resistance and stability of resistance against the isolates COUFPI 06, COUFPI 08 and COUFPI 10. Therefore, accession UFPI 908 constitutes a promising source of resistance to *M. phaseolina*, making possible its use in breeding programs and in integrated management of charcoal rot.

Keywords: *Phaseolus lunatus* L., charcoal rot, genetic resistance

RESUMO

García, M.F.M.; Souza, E.S.; Silva, J.D.D.; Melo, M.P.; Mota, J.M.; Almeida Neto, A.D.; Gomes, R.L.F.; Beserra Jr., J.E.A. Reação de genótipos de feijão-fava a *Macrophomina phaseolina*. ***Summa Phytopathologica,*** v.45, n.1, p.11-17, 2019.

A podridão cinzenta do caule, causada por *Macrophomina phaseolina,* é uma importante doença do feijão-fava (*Phaseolus lunatus* L.) no Nordeste brasileiro. Considerando que no Brasil não existem relatos de resistência a esta doença, 37 acessos de feijão-fava foram avaliados pela reação de resistência e estabilidade da resistência contra isolados de *M. phaseolina* em duas épocas do ano (fevereiro-março e julho-agosto de 2016), visando selecionar genótipos com potencial de utilização no manejo da doença. As sementes foram inoculadas no momento da semeadura, com grãos de arroz com casca colonizados pelo patógeno. As avaliações dos genótipos foram realizadas a cada cinco dias, a partir do décimo dia após a semeadura, com auxílio de escala de notas, discriminando-os em seis classes de reação. De uma população de 37 acessos de *P. lunatus* não foi observada reação de imunidade a *M. phaseolina*, no entanto, treze acessos apresentaram os menores níveis de severidade e maior estabilidade nas duas épocas, dentre eles UFPI 892, UFPI 908 e UFPI 905, tendo sido considerados resistentes ao isolado COUFPI 06. A reação de resistência dos acessos considerados resistentes variou conforme o isolado de *M. phaseolina.* O acesso UFPI 908 expressou os resultados mais promissores de resistência e estabilidade da resistência contra os isolados COUFPI 06, COUFPI 08 e COUFPI 10. Portanto, o acesso UFPI 908 constitui uma fonte promissora de resistência a *M. phaseolina,* sendo possível a sua utilização em programas de melhoramento e no manejo integrado da podridão cinzenta do caule.

Palavras-chave:*Phaseolus lunatus* L., podridão cinzenta do caule, resistência genética

Lima bean (*Phaseolus lunatus* L.) is the second most important species belonging to the genus *Phaseolus* (*P. vulgaris, P. coccineus, P. accutifolius* and *P. plianthus*) and presents a strong potential for supplying plant-origin protein of high nutritional value (7, 15). However, in Brazil, lima bean is grown especially by smallholders in association with other crops, and little technology has been used to increase its yield (25, 30). Considering this situation, charcoal rot is a problem for this crop (28).

The fungus *Macrophomina phaseolina* (Tassi) Goid. is the etiological agent of the disease known as grey stem rot or charcoal rot (22). This pathogen, frequently associated with leguminous seeds (16), is considered one of the most destructive pathogens in tropical and subtropical regions, in arid and semi-arid climates (8). It causes diseases in 500 botanical species and is responsible for low yields in crops of great economic importance such as beans, soybean, sugarcane, cotton and maize (6).

For lima bean, the symptoms of this disease are diverse, including seed rot, necrotic lesions on the hypocotyl and epicotyl, as well as drying

and death of seedlings. In the stem, it produces abundant microsclerotia, which reduce the root system. Due to colonization of the root system, yellowing is noted, followed by wilting and drying of the plant, and subsequent formation of numerous pycnidia and microsclerotia (28). Microsclerotia are produced in the stem base and roots of the plants, constituting the first inoculum source (6). As these tissues decompose, the structures are incorporated in the soil and may be in contact with new plant tissues. However, infected seeds are the main vehicle for spreading the fungus to new cultivation areas, and these are also the first organs exposed to the fungus (23).

Crop rotation is not considered an effective management strategy for charcoal rot (5) because there is a large range of hosts for *M. phaseolina*, and other methods are technically and economically unfeasible, including chemical control. This fact limits management to alternatives such as suppression, cultural control by means of modifying conditions both before and after planting, and use of resistant materials (6). The use of resistant varieties is a viable alternative in crops such as soybeans (3).

In the search for disease management mechanisms, it is common to consider the use of resistant varieties since this is one of the most efficient methods for managing pathogens and does not inflict high environmental impacts as it reduces applications of agricultural pesticides (21). For this reason, a number of studies have evaluated host resistance to *M. phaseolina* (12, 14, 18, 29). However, in Brazil there are no lima bean varieties that are resistant to *M. phaseolina.* Therefore, the aim of this study was to evaluate the resistance reaction of 37 lima bean accessions to *M. phaseolina*, focusing on the selection of accessions that have potential for use in the management of charcoal rot.

MATERIAL AND METHODS

Plant material, pathogen isolates and installation of experiments

The resistance of lima bean accessions to *M. phaseolina* was evaluated in three experiments. Initially, the most promising fungal isolate was selected to discriminate the resistance reaction among accessions; then, the resistance reaction of the accessions to the selected fungal isolate was evaluated; finally, the resistance stability of these genotypes was analyzed in relation to different isolates of the pathogen.

For the inoculations, seven isolates of *M. phaseolina*, previously characterized as pathogenic (data no shown), and 37 lima bean accessions were used. These isolates are part of the phytopathogenic fungus collection at the Phytopathology Laboratory, Universidade Federal do Piauí (UFPI), Teresina, Piauí State, Brazil, and were obtained from lima bean plants from smallholders in the states of Piauí and Ceará, with symptoms of charcoal rot. The identity of isolates was previously confirmed by amplification and sequencing of the translation elongation factor gene 1α (TEF-1α) (data not shown). The used lima bean accessions belong to the Germplasm Active Bank at the Genetic Resources and Plant Breeding Laboratory in the Plant Technology Department, UFPI.

The experiments were installed under greenhouse conditions, in the city of Teresina, Piauí. The seeds were planted in 500-mL cups containing substrate (70% soil, 15% burned rice straw and 15% cattle manure) autoclaved twice at 121 °C for one hour.

The adopted inoculation method was that of rice grain with husk inoculated with the pathogen, adapted from Songa et al. (27), which consisted of sterilizing rice grains with husk, moistened with distilled water (1:1 ratio). Then, five disks of 5-mm diameter of potato dextrose agar medium (PDA) colonized with *M. phaseolina* were added. The Erlenmeyers were maintained in an incubator for 15 days. From the third day after colonized disks had been deposited, the Erlenmeyers were manually shaken every day to guarantee a homogeneous colonization of the grains. In the inoculations, five grains colonized with the pathogen were deposited in the planting trenches (adapted from Songa et al. (27)). Controls of each treatment consisted of autoclaved rice grains that were not colonized by the fungus, deposited in the same way.

Experiment I: Evaluation of the aggressiveness of *M. phaseolina* isolates in lima bean

The experiment was conducted in December 2015 and January 2016, using seven isolates of *M. phaseolina* (COUFPI 05, COUFPI 06, COUFPI 07, COUFPI 08, COUFPI 09, COUFPI 10 and COUFPI 11) and the creole lima bean variety, "Branquinha" (accession UFPI 890), which is susceptible to the pathogen. The accession was seeded in 2.8-L plastic pots containing the previously described substrate. In each pot four seeds were planted, and one seedling was removed seven days after planting. Experimental design was completely randomized, with seven treatments, seven replicates and one control. The experimental plot consisted of one pot with three seedlings.

Experiment II: Evaluation of the resistance reaction of lima bean accessions to an isolate of *M. phaseolina*

Thirty-seven lima bean accessions were evaluated and classified for their resistance to an aggressive isolate selected in Experiment I, in two periods: February and July 2016. The used experimental design was completely randomized, with 37 treatments, four replicates and one control. Each replicate consisted of plots of six plants seeded individually in 500-mL cups, and each control consisted of four cups with one plant each.

Experiment III: Evaluation of the resistance stability of lima bean accessions in relation to seven isolates of *M. phaseolina*

Based on the results obtained in Experiment II, six lima bean accessions were selected for analysis of their resistance stability to *M. phaseolina*: three resistant and three susceptible accessions. The accessions were evaluated in relation to their resistance to infection by seven isolates of *M. phaseolina*, used in Experiment I. This experiment was carried out in October and November 2016. Experimental design was completely randomized, with 42 treatments and four replicates, and each replicate was constituted by three plants sown individually in 500-mL cups and one control, which was one cup with one plant.

Data analysis

The disease severity was evaluated on the 25th and 35th days after inoculation, Experiment I and Experiments II and III, respectively. The resistance reaction of plants to infection by *M. phaseolina* was estimated with a severity scale adapted from Pastor-Corrales & Abawi (19), to evaluate charcoal rot in common beans, in which: 0 = absence of symptoms; 1 = lesions limited to the cotyledon tissues; 2 = lesions on roots, cotyledons and/or reaching the hypocotyl tissues at approximately 2.0 cm; 3 = lesions above 2.0 cm in length in the region of the plant stem base; 4 = stem with entire diameter colonized by the fungus and/or with the presence of pycnidia; 5 = ungerminated seeds and collapse of seedlings.

The disease severity (SEV) was calculated in accordance with McKinney index (11), by using the expression: , in which SEV: proportion of the weighted mean of grades; F_k: represents the number

of individuals in the sample with infection level x_k, k=1,..,K, in the plot; x_k: numerical value of the used scale; n: total number of individuals in the sample; x_K: maximum numerical value of the used scale.

In Experiment I, the severity data were subjected to ANOVA and means were compared according to Tukey's test at 5% probability. In Experiment II, the severity data were subjected to ANOVA at 5% probability. Then, the homogeneity of variances was tested according to Hartley F maximum at 5% probability for the two periods of the experiment and a joint analysis of the same. For the joint analysis of Experiment II, the severity data were subjected to ANOVA, considering the factors Period, Accession and Period-Accession interaction, and the means of the accessions were compared according to Scott-Knott test at 5% probability. In Experiment III, the severity data were subjected to Kruskal-Wallis non-parametric analyses, considering factors of each analysis the accessions and the isolates, partitioned for each accession and each isolate, respectively, and means were compared to the pairs at 5% probability.

Severity data were used to calculate the resistance reaction for each accession, expressed as arithmetical mean of the severity of the evaluated plants. This characteristic was used to discriminate the accessions in six classes of reaction to *M. phaseolina* (adapted from Pastor-Corrales; Abawi (19)): 0 = immune (I); 0.1-20 = resistant (R); 20.01-40 = medium-resistant (MR); 40.01-60 = medium- susceptible (MS); 60.01-80 = susceptible (S); 80.01-100 = highly susceptible (HS).

For statistical analysis and interpretation, in accordance with the evaluated variables, frequency tables were constructed with the program Microsoft Excel® 2016 and the statistical program InfoStat® 2011 (4).

RESULTS AND DISCUSSION

Experiment I: Evaluation of the aggressiveness of *M. phaseolina* isolates on lima bean

Accession UFPI 890 was susceptible (S) and highly susceptible (HS) for six isolates (Table 1). The aggressiveness expressed by isolate COUFPI 05 was significantly lower ($p<0.05$) when compared to that of the other isolates.

Table 1. Mean severity (SEV) and resistance reaction of accession UFPI 890 of lima bean (*Phaseolus lunatus* L.) inoculated with seven isolates of *Macrophomina phaseolina*

Isolate	SEV (%)	Resistence reaction
COUFPI 05	42.86 a	MS
COUFPI 08	76.19 b	S
COUFPI 11	88.57 b	HS
COUFPI 06	89.52 b	HS
COUFPI 10	90.48 b	HS
COUFPI 07	95.24 b	HS
COUFPI 09	100.00 b	HS

Means with the same lowercase letter are not significantly different according to Tukey's test ($p>0.05$). Resistance Reaction: immune (I), resistant (R), medium-resistant (MR), medium-susceptible (MS), susceptible (S) and highly susceptible (HS).

The different aggressiveness found among isolates obtained from the same country of origin (Mexico, Italy, Australia, the USA, Colombia and Brazil), and collected from the same hosts (24), revealed high pathogenic variability among the isolates of *M. phaseolina*, similar to that observed in this study. There is a report of variability in the resistance reaction of mung bean (*Vigna radiata* L.) genotypes against infection by a single isolate of *M. phaseolina*, as well as various levels of aggressiveness among isolates (8).

This result indicates that there is a variation in aggressiveness among isolates of *M. phaseolina,* which may oscillate, in this case, depending on the level of host resistance.

Experiment II: Evaluation of the resistance reaction of lima bean accessions to *M. phaseolina*

Considering that there was no significant difference ($p>0.05$) in aggressiveness among six isolates of *M. phaseolina,* evaluated in Experiment I (Table 1), none of these isolates was suitable for use in Experiment II. Isolate COUFPI 06 was chosen as inoculum to evaluate the resistance reaction of 37 lima bean accessions. Although no accession presented an immune reaction, the found resistance levels to *M. phaseolina* were promising (Table 2). Absence of immunity to this pathogen seems to be common among legumes such as common bean (12, 13), cowpea (10) and soybean (29), since *M. phaseolina* is known as a generalist species because no specific resistance genes are reported. There is no immunity reported for this pathogen among the host species probably because resistance is quantitatively controlled.

Based on the comparison of means, lima bean accessions were separated into four groups in interaction with the period. The groups were associated with five of the six resistance reaction categories, based on the transformation of severity means recorded for each accession for resistance reactions (Table 2). Studies reporting levels of resistance to *M. phaseolina* in other bean species generally use only two resistance categories (resistant and susceptible) (1, 14, 27). This approach may result in discarding materials with partial resistance, which could have been used in breeding programs.

Among the 37 evaluated accessions, only UFPI 892 was classified as resistant (R) in the two evaluation periods. Accessions UFPI 905, UFPI 908, UFPI 902b, UFPI 902a, UFPI 904, UFPI 897 and UFPI 916 also expressed resistance reactions, continuing to be medium-resistant (MR) or resistant (R) in the two periods (Table 2). Only 21.62% studied accessions presented some stable resistance over time against isolate COUFPI 06. These results demonstrated the difficulty in obtaining sources that have high levels of stable resistance to *M. phaseolina,* which is a necrotrophic pathogen, and that are capable of producing the numerous enzymes responsible for destroying it in the host tissue (9).

For the other accessions, resistance reactions varied between the two evaluation periods, as in the case for accessions UFPI 915, UFPI 882a and UFPI 917, which were demonstrated to be susceptible (S) or medium-susceptible (MS) in the first period and resistant (R) in the second period (Table 2). The pathogenicity loss by the isolate due to maintenance is unlikely; otherwise, this behavior should have occurred with the other varieties that were previously susceptible. In general, there was some reduction in the disease severity from the first to the second period, possibly due to the higher temperatures of the period, which may have influenced the infectious process of the pathogen. About 67% accessions changed their resistance category between the first and the second period. This variation in the resistance expression among accessions reveals the difficulty in identifying sources with high

Table 2. Mean severity (SEV) and resistance reaction of 37 accessions of lima bean (*Phaseolus lunatus* L.) in two periods (February – March and July - August) after inoculation with isolate COUFPI 06 of *Macrophomina phaseolina*

Access	SEV (%)		Resistence reaction	
	Period I	Period II	Period I	Period II
UFPI 892	12.50 a A	5.00 a A	R	R
UFPI 908	25.00 b B	5.00 a A	MR	R
UFPI 905	27.50 b B	5.00 a A	MR	R
UFPI 902a	38.33 b B	14.00 a A	MR	R
UFPI 902b	34.17 b B	19.00 a A	MR	R
UFPI 904	39.17 b B	16.00 a A	MR	R
UFPI 917	55.83 c B	14.00 a A	MS	R
UFPI 915	57.50 c B	10.00 a A	MS	R
UFPI 882a	65.00 d B	14.00 a A	S	R
UFPI 897	22.50 a A	35.00 b B	MR	MR
UFPI 916	24.16 a A	29.00 b B	MR	MR
UFPI 893	21.66 a A	51.00 c B	MR	MS
UFPI 889	37.50 b A	49.00 c B	MR	MS
UFPI 898	38.34 b A	50.00 c B	MR	MS
UFPI 907b	25.84 b A	71.00 d B	MR	S
UFPI 913	41.66 b A	31.00 b A	MS	MR
UFPI 896	41.67 b A	31.00 b A	MS	MR
UFPI 900	48.34 c B	35.00 b A	MS	MR
UFPI 909	51.66 c B	38.00 b A	MS	MR
UFPI 910	64.17 d B	40.00 b A	S	MR
UFPI 885	82.50 d B	29.00 b A	HS	MR
UFPI 911	47.50 c A	50.00 c A	MS	MS
UFPI 912	50.00 c A	50.00 c A	MS	MS
UFPI 899	54.16 c A	45.00 c A	MS	MS
UFPI 891	55.00 c A	58.00 c A	MS	MS
UFPI 881	57.50 c B	41.00 b A	MS	MS
UFPI 888	57.50 c A	56.00 c A	MS	MS
UFPI 914	45.00 c B	78.00 d A	MS	S
UFPI 906	48.33 c A	68.00 d B	MS	S
UFPI 890	53.34 c A	74.00 d B	MS	S
UFPI 918	60.00 c A	66.00 d B	MS	S
UFPI 882b	75.83 d B	40.00 b A	S	MS
UFPI 886a	58.33 c A	52.00 c A	S	S
UFPI 907a	65.00 d A	79.00 d A	S	S
UFPI 886b	70.00 d A	79.00 d A	S	S
UFPI 887	61.67 c A	90.00 d B	S	HS
UFPI 880	90.00 d A	75.00 d A	HS	S
MEAN	48.76 A	43.11 B	--	--

Means with the same letter are not significantly different according to Scott-Knott test ($p>0.05$). Uppercase letters indicate differences between columns and lowercase letters, between lines. Resistance Reaction: immune (I), resistant (R), medium-resistant (MR), medium-susceptible (MS), susceptible (S) and highly susceptible (HS).

resistance to *M. phaseolina* in lima bean, partly due to the pathogenesis process of this pathogen (18).

The different resistance levels expressed in the experiments and the variation in resistance between periods also suggest that resistance to the pathogen is of the polygenic type. Furthermore, the variation in the resistance expressed in the two periods is comparable with the broad variation in the results reported for resistance among genotypes of common bean to *M. phaseolina* in Colombia (19), Kenya (27) and Mexico (14) and of mung bean in Pakistan (8). In these cases, the alteration in the expression of resistance in the genotypes was mainly related to the infective capacity of the *M. phaseolina* isolate under the different environmental conditions. In this study, although the average temperature in the two periods was similar (28.29 and 28.07 ºC), the thermal amplitude was lower in the first period: 8 °C (T min 24.03 °C and T max 32.55 °C), in contrast to 16 °C (T min 20.01 °C and T max 36.16 °C) in the second period. In the first period, the mean air humidity (RH) recorded was higher (72.26%) compared to 52.51% recorded for the second.

The high genetic diversity of lima bean was believed to be a factor that influenced the different resistance expression between periods because the accessions mostly came from the Northeast of Brazil, where mixed crossing systems are prevalent and genetic diversity is high among accessions and within the populations of each accession; in addition, cross-pollination levels are up to 38.10% and total diversity values are high (0.596) (20). These values suggest that the resistance characteristics of the accessions may be masked among replicates of the experiment, resulting in variation in the disease severity for the same populations.

EXPERIMENT III: Evaluation of the resistance stability of lima bean accessions in relation to seven isolates of *M. phaseolina*

The seven isolates of *M. phaseolina* were pathogenic to the lima bean accessions that had been selected based on their resistance reactions (UFPI 892, UFPI 905 and UFPI 908) and susceptibility reactions (UFPI 886b, UFPI 907a and UFPI 890) expressed in Experiment II. Results reveal statistically significant differences among accessions for some isolates and differences among isolates, suggesting the existence of various resistance levels against infection by *M. phaseolina*, which resulted in different severity levels in lima bean accessions (Table 3).

The variation in the resistance reaction among accessions was lower when compared to the result of Experiment II, indicating that the resistance expressed by the lima bean accessions is not stable for the isolates of *M. phaseolina* (Tables 2 and 3). Absence of resistance stability to necrotrophic pathogens in lima bean seems to be common (2, 26). In the case of common bean, various results have been reported in relation to the lack of stability in the resistance expression (17). The saprophytic characteristic of the fungus also influences parasitic specialization among populations as there is no need for the fungus to establish specialized interactions with the host (6).

Su et al. (29) suggest that the variation in the aggressiveness of isolates may be confined to populations that have their limits defined by the host species, considering that DNA polymorphism has been reported among isolates of *M. phaseolina* obtained from four host species, associating the genetic isolation of the isolates with the original host. Thus, the variation in the aggressiveness of *M. phaseolina* observed in our study may be associated with the variety that was originally collected.

Accession UFPI 892 presented resistance stability for the periods

Table 3. Mean (%) severity (SEV) and resistance reaction of six accessions of lima bean (*Phaseolus lunatus* L.) after inoculation with seven isolates of *Macrophomina phaseolina*

	Isolate													
	COUFPI 05		**COUFPI 06**		**COUFPI 07**		**COUFPI 08**		**COUFPI 09**		**COUFPI 10**		**COUFPI 11**	
Access														
UFPI 892	83.33	a AB	36.67	a A	100.00	a B	100.00	b B	100.00	a B	58.30	ab A	78.33	a AB
UFPI 905	100.00	a B	68.33	a A	100.00	a B	88.33	ab AB	100.00	a B	91.70	bc AB	91.67	a AB
UFPI 908	85.00	a C	25.00	a A	100.00	a C	58.33	a ABC	75.00	a BC	33.30	a AB	66.67	a ABC
UFPI 886b	100.00	a A	66.67	a A	100.00	a A	100.00	b A	100.00	a A	100.00	c A	91.67	a A
UFPI 907a	100.00	a A	66.67	a A	100.00	a A	100.00	b A	100.00	a A	100.00	c A	100.00	a A
UFPI 890	100.00	a A	78.33	a A	100.00	a A	83.33	ab A	100.00	a A	86.70	bc A	100.00	a A
UFPI 892	HS	a AB	MR	a A	HS	a B	HS	b B	HS	a B	MS	ab A	S	a AB
UFPI 905	HS	a B	S	a A	HS	a B	HS	ab AB	HS	a B	HS	bc AB	HS	a AB
UFPI 908	HS	a C	MR	a A	HS	a C	MS	a ABC	S	a BC	MR	a AB	S	a ABC
UFPI 886b	HS	a A	S	a A	HS	a A	HS	b A	HS	a A	HS	c A	HS	a A
UFPI 907a	HS	a A	S	a A	HS	a A	HS	b A	HS	a A	HS	c A	HS	a A
UFPI 890	HS	a A	S	a A	HS	a A	HS	a b A	HS	a A	HS	bc A	HS	a A

Means with the same letter are not significantly different at 5% probability according to Kruskal-Wallis test. Uppercase letters indicate differences between columns and lowercase letters, between lines. Resistance Reaction: immune (I), resistant (R), medium-resistant (MR), medium-susceptible (MS), susceptible (S) and highly susceptible (HS).

in Experiment II (Table 2), but its resistance varied against the same isolate (COUFPI 06) in Experiment III (Table 3) possibly due to the higher temperatures of the last quarter of the year, characterized by higher temperatures and lower humidity in the region, which may have influenced the infectious process of the pathogen. Moreover, it was the accession with the second lowest severity levels for four isolates (COUFPI 05, COUFPI 06, COUFPI 10 and COUFPI 11). Accession UFPI 908 presented less stable resistance reactions than accession UFPI 892 in Experiment II and its severity and resistance levels in Experiment III were equal to those from the first period in Experiment II for the same isolate (Tables 2 and 3), being classified as medium-resistant (MR).

Accessions UFPI 892 and UFPI 908 presented the lowest infection severity levels for the majority of isolates (Table 3). Significant differences were found ($p=0.0029$) in the severity levels expressed for isolates COUFPI 08 and COUFPI 10. Accession UFPI 908 presented the lowest disease severity levels for most isolates, differing significantly ($p<0.05$) for isolates COUFPI 06, COUFPI 08 and COUFPI 10. The highest resistance reactions were thus recorded for accession UFPI 908. However, accession UFPI 905, considered resistant or medium-resistant in Experiment II, was classified as susceptible and highly susceptible without significant differences in relation to those accessions considered susceptible.

This is the first study in Brazil that has evaluated the resistance reaction of lima bean accessions to *M. phaseolina*, as well as the resistance stability over time and against different isolates of the pathogen. Despite being susceptible to some isolates, accession UFPI 908 presented results that were most promising and stable over time for the different isolates; thus, it constitutes an important source of resistance to *M. phaseolina*. However, it will be important to devote more attention to the evaluation and selection of more promising accessions against the different isolates under various environmental conditions.

The indicated materials may function as sources of resistance in crosses for the transfer of this character to genotypes of lima bean that present desirable agronomic characteristics. They are thus a source of resistance to *M. phaseolina* with the potential to be used in breeding programs and in integrated management of the disease.

ACKNOWLEDGEMENTS

The authors thank the Coimbra Group of Brazilian Universities and the Department of Human Development and Education of the Organization of American States (PAEC-OEA-GCUB), the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), and the Program for Genetic Improvement of Sugarcane (PMGCA-UFPI), for making their greenhouse available for experiments.

REFERENCES

1. Abawi, G.S.; Pastor-Corrales, M.A. **Root rots of bean in Latin America and Africa:** diganosis, research methodologies and management strategies. Cali: Centro Internacional de Agricultura Tropical, 1990. 114p.
2. Assunção, I.P.; Nascimento, L.D.; Ferreira, M.F.; Oliveira, F.J.; Michereff, S.J. Gaus, G.S.A. Reaction of faba bean genotypes to *Rhizoctonia solani* and resistance stability. **Horticultura Brasileira**, Vitória da Conquista, v.29, n.4, p.492-497, 2011.
3. Coser, S.M.; Reddy, R.V.C.; Zhang, J.; Mueller, D.S.; Mengistu, A.; Wise, K.A.; Allen, T.W.; Singh, A.; Singh, A.K. Genetic architecture of chacoal rot (*Macrophomina phaseolina*) resistence in soybean revealed using a diverse painel. **Frontiers in Plant Science**, Lausanne, v.8, p.1-12, 2017.
4. Di Rienzo, J.A.; Casanoves, F.; Balzarini, M.G.; Gonzalez, L.; Tablada, M.; Robledo, C.W. InfoStat. Version 2011. Córdoba: Grupo InfoStat, 2011.
5. Francl, L.J.; Wyllie, T.D.; Rosenbrock, S.M. Influence of crop rotation on population density of *Macrophomina phaseolina* in soil infested with *Heterodera glycines*. **Plant Disease**, Saint Paul, v.72, n.9, p.760-764, 1988.
6. Gupta, G.K.; Sharma, S.K.; Ramteke, R. Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill). **Journal of Phytopathology**, Maldem, v.160, n.4, p.167-180, 2012.
7. IBGE. Banco de dados agregados: pesquisa: produção agrícola municipal: culturas temporárias. Rio de Janeiro, 2016. Disponível em: <http://www.ibge.gov.br/estadosat/index.php>. Acesso em nov 2016.
8. Iqbal, U.; Mukhtar, T. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiata* L.) Wilczek from Pakistan. **The Scientific World Journal**, Cairo, v.2014, p.1–9, 2014.
9. Islam, M.; Haque, M.S.; Islam, M.M.; Emdad, E.M.; Halim, A.; Hossen, Q.M.M.; Hossain, M.Z.; Ahmed, B.; Rahim, S.; Rahman, M.S.; Alam, M.M.; Hou, S.; Wan, X.; Saito, J.A.; Alam, M. Tools to kill: Genome of one of the most destructive plant pathogenic fungi *Macrophomina phaseolina*. **BMC Genomics**, London, v.13, p.493, 2012.
10. Lima, L.R.L.; Damasceno-Silva, K.J.; Noronha, M.A.; Schurt, D.A.; Rocha, M.M. Diallel crosses for resistance to *Macrophomina phaseolina* and *Thanatephorus cucumeris* on cowpea. **Genetics and Molecular Research**, Ribeirão Preto, v.16, n.3, p.1-11, 2017.
11. McKinney, H.H. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. **Journal of Agricultural Research,** Washington, v.26, n.5, p.195-218, 1923.
12. Mayek, N.P.; López, E.; Cupián, J.; Acosta, J.A. Resistencia del frijol a *Macrophomina phaseolina* en condiciones de riego-secano. **Agronomia Mesoamericana**, Alajuela, v.15, n.1, p.45-51, 2004.
13. Mayek-Pérez, N.; López-Castañeda, L.; Gonzáles-Chavira, M.; García-Espinosa, R.; Acosta-Gallegos, J.; Martínez, O.M.V.; Simpson, J. Variability of Mexican isolates of *Macrophomina phaseolina* based on pathogenesis and AFLP genotype. **Physiological and Molecular Plant Pathology**, New College, v.59, n.5, p.257-264, 2001.
14. Mayek-Pérez, N.; López-Castañeda, C.; López-Salinas, E.; Cupián-Gutiérrez, J.; Joaquín-Torres, I.C.; Padilla-Ramírez, J.S.; Acosta-Gallegos, J.A. Effect of *Macrophomina phaseolina* (Tassi) Goid. on grain yield of common beans (*Phaseolus vulgaris* L.) and its relationship with yield stability parameters. **Revista Mexicana de Fitopatologia**, Texcoco, v.21, n.2, p.168–175, 2003.
15. Moses, O.; Olawuni, I.; Jo, I. The proximate composition and functional properties of full-fat flour and protein isolate of Lima bean (*Phaseolus lunatus* L.). **Open Access Scientific Reports**, Henderson, v.1, n.7, p.1–5, 2012.
16. Mota, J.M.; Melo, M.P.; Silva, F.F.S.; Sousa, E.M.J.; Sousa, E.S.; Barguil, B.M.; Beserra Jr, J.E.A. Fungal diversity in lima bean seeds. **Braziliam Journal of Biosystems Engineering,** Campinas, v.11, n.1, p.79-87, 2017.
17. Muñoz-Cabañas, R.M.; Hernández-Delgado, S.; Mayek-Pérez, N. Análisis patogénico y genético de *Macrophomina phaseolina* (Tassi) Goid. en diferentes hospedantes. **Revista Mexicana de Fitopatología**, Texcoco, v.23, n.1, p.11–18, 2005.
18. Noronha, M.A.; Silva, K.J.D.; Gonçalves, S.R.; Lima, R.R.L. Avaliação da resistência de genótipos de feijão-caupi a *Macrophomina phaseolina*. **Comunicado Técnico Embrapa Meio-Norte**, Teresina, n.126, p.1-4, 2012.
19. Pastor-Corrales, M.A.; Abawi, G.S. Reactions of selected bean accessions to infection by *Macrophomina phaseolina*. **Plant Disease**, Saint Paul, v.72, n.1, p.39-41, 1988.
20. Penha, J.S.; Lopes, A.C.A.; Gomes, R.L.F.; Pinheiro, J.B.; Assunção Filho, J.R.; Silvestre, E.A.; Viana, J.P.G.; Martínez-Castillo, J. Estimation of natural outcrossing rate and genetic diversity in Lima bean (*Phaseolus lunatus* L. var. *lunatus*) from Brazil using SSR markers: implications for conservation and breeding. **Genetic Resources and Crop Evolution**, Berlin, v.64, n.6, p.1355-1364, 2017.
21. Piquerez, S.J.M.; Harvey, S.E.; Beynon, J.L.; Ntoukakis, V. Improving

crop disease resistance: lessons from research on *Arabidopsis* and tomato. **Frontiers in Plant Science**, Melbourne, v.5, n.671, p.1-13, 2014.

22. Phillips, A.J.L.; Alves, A.; Abdollhzadeh, J.; Slippers, B.; Wingfield, M.J.; Groenewald, J.Z.; Crous, P.W. The Botryosphaeriacea: genera and species known from culture. **Studies in Mycology**, Ultrecht, v.76, n.1, p.51-167, 2013.
23. Rao, T.V.; Rajeswari, B.; Prasad, A.L.; Keshavulu, K. Seed transmission studies on seedborne fungi of soybean. **International Journal of Scientific and Research Publications**, Paris, v.5, n.10, p.1-6, 2015.
24. Reyes-Franco, M.C.; Hernández-Delgado, S.; Beas-Fernández, R.; Medina-Fernández, M.; Simpson, J.; Mayek-Pérez, N. Pathogenic and genetic variability within *Macrophomina phaseolina* from Mexico and other countries. **Journal of Phytopathology**, Maldem, v.154, n.7/8, p.447–453, 2006.
25. Santos, D.; Collett, F.M.F.; Mendes, J.E.M.F.; Wanderley-Junior, J.S.A. Produtividade e morfologia de vagens e sementes de variedades de fava no Estado da Paraíba. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.37, n.10, p.1407–1412, 2002.
26. Silva, J.A.; Oliveira, M.G.; Sousa, L.T.; Assunção, I.P.; Lima, G.A.; Michereff, S.J. Reação de genótipos de feijão-fava a *Sclerotium rolfsii.* **Horticultura Brasileira**, Vitória da Conquista, v.32, n.1, p.98-101, 2014.
27. Songa, W.; Hillocks, R.J.; Mwango'mbes, A.; Buruchara, R.; Ronno, W.K. Screening common bean accessions for resistance to charcoal rot (*Macrophomina phaseolina*) in eastern Kenya. **Experimental Agriculture**, Cambridge, v.33, n.4, p.459-468, 1997.
28. Sousa, E.S.; Melo, M.P.; Pires, L.L.; Silva, B.A.; García, M.E.M.; Sousa, E.M.J.; Mota, J.M.; Beserra Jr, J.E.A. First report of *Macrophomina phaseolina* **causing charcoal rot in Lima bean (***Phaseolus lunatus*) in Brazil. **Plant Disease**, Saint Paul, v.101, n.8, p.1551, 2017.
29. Su, G.; Suh, O.S.; Schneider, R.W.; Russin, J.S. Host specialization in the charcoal rot fungus, *Macrophomina phaseolina.* **Phytopathology**, Saint Paul, v.91, n. 2, p.120–126, 2001.
30. Vieira, R.F. A cultura de feijão-fava. **Informe Agropecuário**, Belo Horizonte, v.16, n.174, p.30-37, 1992.