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REACTIONS OF LIMA BEAN (*Phaseolus lunatus* L.) ACCESSIONS TO *Colletotrichum truncatum*[1]

ROMMEL DOS SANTOS SIQUEIRA GOMES[2], JOÃO VICTOR DA SILVA MARTINS[3], EDCARLOS CAMILO DA SILVA[2], HIAGO ANTÔNIO OLIVEIRA DA SILVA[4], LUCIANA CORDEIRO DO NASCIMENTO[2]*

ABSTRACT - Lima bean (*Phaseolus lunatus*) can be considered as an alternative income and food supply to farmers of the Brazilian Northeast. This crop has faced serious sanitary problems. Among the most important diseases that attack this crop, the anthracnose caused by *Colletotrichum truncatum* requires greater attention. This study aimed to determine the resistance of lima bean accessions to the isolates of *C. truncatum*. Detached leaves were used from ten lima bean accessions, originated from production fields in Paraíba State – Brazil. They were artificially inoculated with ten isolates of *C. truncatum*. The isolates were grown on bean-dextrose-agar medium under temperature 27 ± 2 °C and a photoperiod of 12 hours, for fourteen days. The evaluations of the accessions' resistance to the isolates were performed at 5, 7, 9, 11 and 13 days after inoculation (DAI), adopting a scale of notes, and the results were transformed in the area under the disease progress curve. The research was carried out in completely randomized design, in a 10x10 factorial arrangement (accessions x isolates) with 12 replications. The first symptoms of anthracnose on the detached leaves appeared from the third day after inoculation. The accessions were grouped from highly to moderately resistant or susceptive. The aggressiveness among *C. truncatum* isolates varied depending on the genetic variability of the lima bean accessions used. Accessions with significant resistance levels to anthracnose can be used as resistance sources in future breeding programs.

Keywords: *Phaseolus lunatus*. Anthracnose. Resistance. Severity.

REAÇÃO DE ACESSOS DE FEIJÃO-FAVA (*Phaseolus lunatus L.*) A *Colletotrichum truncatum*

RESUMO - A cultura do feijão fava (*Phaseolus lunatus*) pode ser considerada uma alternativa de renda e fonte de alimento aos agricultores do Nordeste brasileiro, no entanto depara-se com graves problemas sanitários. Dentre as principais doenças encontradas em campos de produção, destaca-se a antracnose causada por *Colletotrichum truncatum*. O objetivo do trabalho foi determinar a resistência de acessos de feijão fava a isolados de *C. truncatum.* Foram utilizadas folhas destacadas pertencentes a dez acessos de feijão-fava originados de campos de produção no Estado da Paraíba, inoculadas artificialmente por dez isolados de *C. truncatum.* Os isolados foram cultivados em meio de cultura feijão, dextrose e ágar, sob temperatura de 27 ± 2 ºC e fotoperíodo de 12 horas durante quatorze dias. As avaliações de resistência foram realizadas aos 5, 7, 9, 11 e 13 dias após a inoculação, adotando-se escala de notas e os resultados foram transformados em área abaixo da curva de progresso da doença. O delineamento experimental utilizado foi inteiramente casualizado, arranjados em esquema fatorial 10x10 com 12 repetições. Os primeiros sintomas da antracnose surgiram a partir do terceiro dias após a inoculação. A maior agressividade entre os isolados de *C. truncatum* aos acessos de feijão fava foi observada para o isolado CT35. Os acessos foram agrupados de altamente a moderadamente resistente ou suscetível. A agressividade entre os isolados de *C. truncatum* foi alterada em função da variabilidade de resistência dos acessos de feijão-fava. Os acessos com grau de resistência à antracnose podem servir de fontes de resistência em cruzamentos futuros.

Palavras-chave: *Phaseolus lunatus.* Antracnose. Resistência. Severidade.

*Corresponding author
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[2]Department of Fhytotechnics and Environmental Sciences, Universidade Federal da Paraíba, Areia, PB, Brazil; pratacca@gmail.com – ORCID: 0000-0001-7596-3221, edcarloscamilo@bol.com.br – ORCID: 0000-0001-9990-8630, luciana.fitopatologia@gmail.com – ORCID: 0000-0002-5706-7041.
[3]Department of Agronomy, Universidade Federal de Viçosa, Viçosa, MG, Brazil; eng.agro.martins@gmail.com – ORCID: 0000-0001-5388-2543.
[4]Departament of Plant Pathology, Universidade Federal de Viçosa, Viçosa, MG, Brazil; hiagooliveirasilva@gmail.com – ORCID: 0000-0002-9159-1744.

INTRODUCTION

Phaseolus lunatus L., popularly known as lima bean, is a leguminous vegetable widely cultivated in tropical regions and constitutes an excellent source of food supply due to its vegetable protein potential, and also for its use as a complementary income to small producers (SOARES, 2010; LUCENA et al., 2018)

In the United States, the world's largest lima bean producer, the grains are still commercialized as preserved green, such as in canned, packaged or frozen beans, which adds more value to the product. In Brazil, the consumption of lima bean is preferably in the form of cooked dried beans (BITENCOURT; SILVA, 2010), where it is cultivated in several states in the northeast region, responsible for 96% of the national production (COSTA et al., 2021). Paraíba state is the second producer after the Ceará state, with an average yield of 306 kg ha^{-1} (IBGE, 2020).

Although adapted to edaphoclimatic conditions of the Brazilian Northeast, lima bean has a low yield due to the occurrence of diseases and lack of modern technologies for its production (SANTOS et al., 2002; FEIJÓ et al., 2017; SOUSA et al., 2018). Among the diseases, anthracnose caused by *Colletotrichum truncatum* is frequently found in Brazilian bean crops (MOTA et al., 2019).

The symptoms of anthracnose in lima bean are characterized by reddish spots, initially observed along the veins on the abaxial side of the leaves, on young stems and petioles. In the pods, the lesions are depressed, large and reddish, in which the whitish acervuli are formed with numerous setae of the pathogen (CAVALCANTE et al., 2019).

The use of resistant cultivars is one of the methods with the most promising results for pathogen control and has great importance in integrated management of diseases, since it has lower costs and environmental impact (GARCÍA et al., 2019). In addition, few studies indicate resistance among lima bean genotypes to this disease (CAVALVANTE et al., 2012, CARMO et al., 2015, SANTOS et al., 2015). The first step in management programs with a focus on reducing anthracnose damage through genetic resistance is the identification of resistance sources in crop varieties to be incorporated into genetic breeding programs (SPONHOLZ et al., 2016; SOUSA et al., 2018).

Thus, the aim of this study was to determine the reactions of lima bean accessions to *C. truncatum* isolates in the state of Paraíba, Brazil.

MATERIAL AND METHODS

The experiment was carried out at the Laboratory of Phytopathology, Universidade Federal da Paraíba, Department of Plant Science and Environmental Sciences, Areia city, Paraiba state, Brazil. Ten accessions of lima bean (Table 1) inoculated with ten isolates of *C. truncatum* were evaluated.

Treatments and selection of isolates

Fragments of leaf, stem and fruit tissues with typical anthracnose symptoms were used to obtain the pathogen isolates, collected in commercial production fields. For that, they were disinfested in 70% alcohol for 30 seconds and 1% sodium hypochlorite for one minute. The fragments were plated in potato-dextrose-agar medium (PDA) and maintained under 27 ± 2 ºC and 12-h photoperiod for 14 days. To promote the abundant conidia production, seven days after incubation, scraping was performed on the colonies of the isolates, and the surface mycelium was removed (SOUZA et al., 2013).

The monosporic cultures of the isolates were obtained according to Souza et al. (2013). The monosporic isolates were transferred to lima bean-dextrose-agar medium (LBDA - 200 g of common bean [*Phaseolus vulgaris* (L.)]) according to Cavalcante et al. (2012) and remained under the same conditions as described previously.

Ten isolates of *C. truncatum*, CT01, CT02, CT11, CT12, CT12, CT20, CT33, CT35, CT36 and CT62, were previously selected from a collection of 66 monosporic isolates obtained from leaves with anthracnose symptoms in the cities of Alagoa Grande, Areia, Campina Grande, Juarez Távora, Alagoa Nova and Queimadas, from Paraíba state - Brazil. The aggressiveness of the isolates to lima bean accessions, the mycelial growth of the colonies and the production of conidia were used as criteria for selection of isolates, and the ones that showed greater aggressiveness to the accessions, faster mycelial growth, regular and greater sporulation were selected. For this, the isolates were cultivated in the LBDA at 27 ± 2 ºC for 14 days (CAVALCANTE et al., 2012), and the aggressiveness in the leaves (CARMO et al., 2015) was evaluated in ten lima bean genotypes (Table 1).

Reaction of lima bean accessions

The reaction of lima bean to *C. truncatum* was evaluated using detached trifoliate leaves of the accessions (Table 1). The genotypes were selected based on the agricultural importance and potential cultivation in the Brazilian northeast.

Plants of each accession were grown under greenhouse conditions at temperatures that ranged from 20.4 to 39.4 °C and relative humidity from 34 to 83%. Five seeds were sown in polypropylene pots containing 3 dm^3 of soil from lima bean fields. Twenty Days After Sowing (DAS), thinning was performed, two plants per pot were maintained and first irrigation was checked and adjusted to 60%. The irrigation occurred at every 72 hours (MAPA, 2009). At 45 DAS, when the genotypes were in the phenological stage V3 (primary trifoliate leaf), fully expanded leaves were selected, with similar diameter (OLIVEIRA et al., 2010), and detached on the day of the inoculation. The leaves were incubated in a Gerbox® containing a double layer of filter paper and a cotton swab sterilized and moistened with 10 mL of Sterile Distilled Water (SDW). The leaves remained on sterile wooden rods (toothpicks) inside the boxes, avoiding direct contact of the leaves with the moistened paper. This procedure was carried out on the day of inoculation of *C. truncatum* isolates.

Table 1. Detached leaves belonging to lima bean (*Phaseolus lunatus* L.) plants.

[1]Accessions	Popular name	Tegument color	Weight of 100 seeds (g)	Growth habit	Origin of the accessions
UFPB02	Amarela cearense	Yellow, white hilum	56.04	Indeterminate	Queimadas
UFPB04	Branca pequena	White to yellowish	37.05	Determinate	Remígio
UFPB05	Branca grande	White	64.89	Indeterminate	Campina Grande
UFPB06	Cara larga	Yellowish, red hilum	41.91	Indeterminate	Areia
UFPB11	Eucalipto	Grey speckled with black dots	47.05	Indeterminate	Massaranduba
UFPB13	Orelha de vó	White with black stripes	68.43	Indeterminate	Campina Grande
UFPB14	Moita	Yellowish, red hilum	39.07	Determinate	Areia
UFPB18	Rainha	White with red stripes	100.56	Determinate	Alagoa Grande
UFPB19	Rosinha	Pink with black spots	31.98	Indeterminate	Queimadas
UFPB20	Roxinha	Purple, yellowish hilum	36.46	Indeterminate	Queimadas

[1]Accessions available in the Germplasm Bank of the Universidade Federal da Paraíba, Brazil.

The inoculum suspension was prepared by adding 10 mL of SDW to the fungal colonies of each isolate, grown in FDA at 27 ± 2 ºC and under photoperiod of 12 hours. After 14 days, the pathogen mycelia were released using soft brush and the suspension was filtered on a sterile gauze. After that, the inoculum concentration was adjusted to $1x10^6$ conidia.mL^{-1} (CAVALCANTE et al., 2012). The inoculum suspension (1.5 mL) was distributed on the abaxial and adaxial sides of the leaves, until the point of drainage. After the inoculation, the boxes with the inoculated leaves were sealed with PVC plastic film to provide a humid chamber environment, maintained for 48 h at 27 ± 2 °C and under photoperiod of 12 hours. After this process, the plastic film was removed and the leaves remained under these ambient conditions for 13 days.

The reaction of the lima bean accessions was evaluated by the quantification of the anthracnose severity at 5, 7, 9, 11 and 13 Days After Inoculation (DAI), adopting the scale of notes, where: 0 = Absence of symptoms; 1 = traces at 10% of infected leaf area; 2 = 11 to 25% of infected leaf area; 3 = 26 to 50% of infected leaf area without leaflet fall; 4 = 51 to 75% of infected leaf area, without or with one of the leaflets falling; 5 = 76 to 100% of infected leaf area, with or without two or three leaflets (CAVALCANTE et al., 2012). From the severity quantifications, the Area Under the Disease Progress Curve (AUDPC) was calculated according to Campbell and Madden (1990). The accessions were grouped according to the severity evaluation in five classes of reaction to CT35 isolate, namely: Immune (IM) - 0; Highly resistant (HR) - 0.1 to 1.4; Moderately resistant (MR) - 1.5 to 2.4; Moderately susceptible (MS) - 2.5 to 3.0; and Highly susceptible (HS) - above 3.0 (CARMO et al., 2015).

Statistical analysis

The experimental design was completely randomized in a simple 10x10 factorial arrangement (isolates x accessions) with 12 replications. The analysis of variance was performed with the data transformed to $\sqrt{x+0.5}$ and the means were grouped by the Scott-Knott test at 5% probability level, using the statistic program SISVAR® (FERREIRA, 2019).

RESULTS AND DISCUSSION

A significant effect (p<0.01) was observed for the interaction between isolates and lima bean accessions (Figure 1), where the severity of the lima bean anthracnose revealed that the 10 accessions produced different levels of susceptibility to the pathogen isolates, five DAI (Figure 1). This information corroborates the findings of Carmo et al. (2015), who identified similar behavior in the reaction of eleven bean accessions to *C. truncatum*, under detached leaf conditions, at the 5th day.

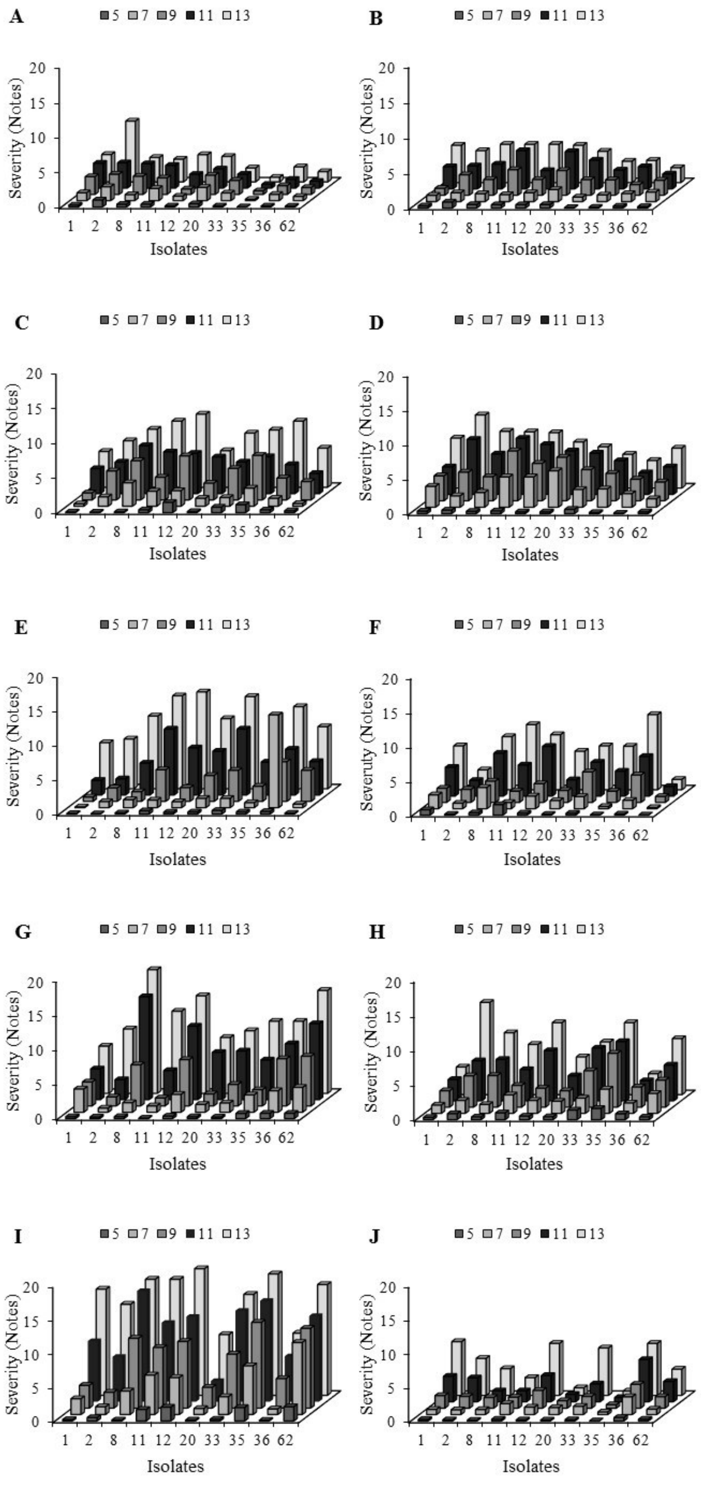

Figure 1. Aggressiveness of *Colletotrichum truncatum* isolates against lima bean (*Phaseolus lunatus* L.) accessions UFPB02 (A), UFPB04 (B), UFPB05 (C), UFPB06 (D), UFPB11 (E), UFPB13 (F), UFPB14 (G), UFPB18 (H), UFPB19 (I) and UFPB20 (J).

The CT35 isolate was selected because it was the most aggressive to the evaluated accessions. The lowest area under the anthracnose progress curve was obtained by the UFPB13 accession, reaching 3% of severity. The highest susceptibility was observed in the accession UFPB04, with 100% of severity (Figure 1B). This result is highly important, since the UFPB13 accession is one of the most cultivated, due to its seed size and acceptance by the consumers, as well as the indeterminate growth habit, which facilitates the cultivation in a intercropped system with maize (*Zea mays* L.), using it as a natural support. However, it is not the most productive in a intercropped production system, as already observed by Santos et al. (2002).

The isolates CT08, CT12 and CT35 caused the highest damaged leaf area in the evaluated lima bean accessions, where the typical lesions ranged from 51 to 100% of the leaf area infected by the pathogen (Figure 1). Similar behavior in three isolates, PGA-PR1, PGA-PR2 and REG-SP2, was observed by Mafacioli et al. (2008), who verified that the largest damaged leaf area among 17 isolates of *C. gloeosporioides* associated with anthracnose on leaves of peach palm (*Bactris gasipaes* Kunth.) was attributed to these isolates.

Significant interactions between isolates of *Sclerotium rolfsii* and bean genotypes were reported by Silva et al. (2014), who reinforce the need for the use of different isolates in the resistance evaluation for this pathogen. When evaluating the partial resistance of sorghum (*Sorghum bicolor* L.) hybrids and their parental lineages regarding the aggressiveness of the isolates of *Colletotrichum sublineolum*, Pereira et al. (2011) concluded that none of the isolates were virulent to all genotypes. Similar results were observed in this research, where several levels of anthracnose in lima bean occurred by inoculation of the ten *C. truncatum* isolates, analyzing the infected leaf area.

Due to the wide variability of the disease, observed in the different accessions of lima bean evaluated, which has already been verified in previous studies of genetic resistance by Guimarães et al. (2007), further studies should be performed, involving a greater number of isolates from different sources and other types of bean, such as common bean and cowpea (*Vigna unguiculata* L.). These studies are necessary for the identification of the virulence of different *C. truncatum* populations associated with this crop and the obtaining of new sources of resistance to anthracnose in lima bean.

Reaction of the lima bean accessions

There was significant interaction ($p<0.01$) of the disease severity and the reaction of the accessions evaluated (Figure 2). The first symptoms of anthracnose in the leaves of the lima bean genotypes started from the 3rd DAI.

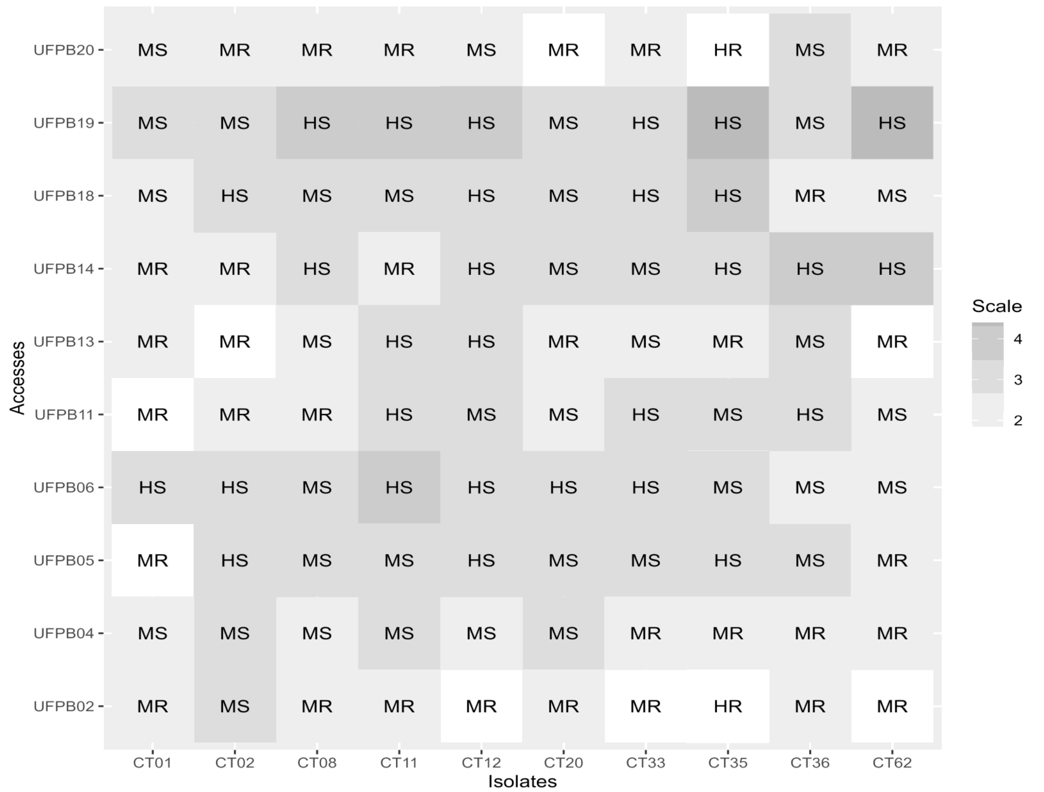

Figure 2. Reaction scale of lima beans (*Phaseolus lunatus*) artificially inoculated with *Colletotrichum truncatum* isolates. HR = highly resistant; MR = moderately resistant; MS = moderately susceptible; HS = highly susceptible.

This is an indication of homogeneity of inoculation of the isolates in the lima bean accessions and that all accessions inoculated were infected by *C. truncatum*. Similar results were observed by Carmo et al. (2015) and Santos et al. (2015), who observed anthracnose symptoms on detached leaves of lima bean, started from the third to fourth DAI and severity evaluations were performed at five and seven DAI. However, extensive disease variations has been reported, such as *C. truncatum*-induced symptoms in susceptible lentil (*Lens esculenta* Moench) cultivars varying from three to six days and up to 14 DAI in resistant cultivars (CHONGO; GOSSEN; BERNIER, 2002).

Sanitary levels in soybean (*Glycine max* L.) seeds and reactions to *C. truncatum* were evaluated by Noetzold et al. (2014), who concluded that the fungus can stay in latency for almost the entire crop cycle and that this depends on the soil and climatic conditions, so identification and quantification of the symptoms can be difficult. Thus, the adoption of preventive methods of control is essential. Similarly, the conditions of the present research may have influenced the reaction of the lima bean accessions evaluated. Thus, more studies should be carried out with a higher number of *C. truncatum* isolates from different regions, and under different environmental conditions.

Considering the total of possible reactions, in the influence of the ten isolates of *C. truncatum* tested, approximately 33% were moderately resistant (MR), 41% were moderately susceptible (MS), 24% corresponded to highly susceptible (HS) and only 2% were classified as highly resistant (HR) to *C. truncatum*. There was no identification of material with no symptoms, but it was possible to distinguish differentiated levels of resistance (Figure 2). Similar results were obtained by Cavalcante et al. (2012), among the reactions caused by 30 subsamples of *C. truncatum* and by Santos et al. (2015), with the reaction of 57 subsamples of lima bean to the severity of the anthracnose in detached leaves.

The UFPB02 and UFPB20 accessions were classified with HR reaction level, with values of 1.1 and 1.2 of severity compared to the CT35 isolate, besides showing differentiated levels of resistance to the other isolates. Reaction corresponding to HS was expressed by the accessions UFPB06 and UFPB19 in relation to the isolates CT11, CT12, CT33 and CT35, and the severity of anthracnose was estimated between 3.2 and 4.4 for these accessions (Figure 2). These results showed that there is a high variability of the virulence of *C. truncatum* to the lima bean accessions evaluated and, with this, it is possible to obtain anthracnose resistance sources in future studies. Thus, more isolates of *C. truncatum* from different locations in Paraíba state should be added to the studies.

Among the ten accessions evaluated, seven (UFPB02, UFPB04, UFPB05, UFPB11, UFPB13, UFPB14 and UFPB20) showed different levels of resistance to at least five of the ten inoculated isolates, where the severity ranged from 1.1 to 2.4 and the reaction level was considered from HR to MR (Figure 2). The accessions UFPB06, UFPB18 and UFPB19 behaved as MS to HS for all *C. truncatum* isolates evaluated (Figure 2). Thus, it was possible to conclude that many of the accessions used have different levels of susceptibility to anthracnose, but there are also sources of resistance to this disease among the accessions evaluated. Similarly, Carmo et al. (2015) observed the reaction of 12 lima bean accessions to anthracnose using detached leaves and the results revealed two accessions, UFPI641 and UFPI644, which behaved as HR at five DAI. However, there was a change in the reaction level for MR at seven DAI. Likewise, seventeen genotypes resistant to *C. truncatum* were observed by Costa et al. (2009), when evaluating forty-eight commercial soybean cultivars under greenhouse conditions.

Due to the high variability of disease levels detected in this study, it is possible to infer that there are virulence differences (SOUZA et al., 2013) among the isolates of *C. truncatum* inoculated. In addition, all reaction classes were observed in the accessions as a function of the variability of resistance detected and the conditions of the environment used in the evaluations. Nonetheless, the accessions showed different levels of resistance to anthracnose and could be explored in future studies as sources of resistance together with studies of structuring of *C. truncatum* virulence.

This study is notable for describing potential sources of anthracnose resistance of lima bean and for the evidence of structuring virulence in populations of *C. truncatum*. Thus, the evaluated accessions can be explored in the obtaining of anthracnose resistant cultivars in future breeding programs, which is very important for the Brazilian Northeast region. In addition, the isolates used in this research and other populations of *C. truncatum* should be evaluated for virulence to the different lima bean accessions available in germplasm banks, in order to verify the prevalence of pathogens in plantations and to efficiently manage the lima bean production.

Area Under the Disease Progress Curve (AUDPC)

The evaluation of anthracnose severity in detached leaves revealed that the accessions of lima beans were susceptible to isolates of *C. truncatum*, at five days after inoculation (DAI), showing different levels of aggressiveness (Figure 3). Carmo et al. (2015) also observed similar behavior for tested isolates when they evaluated the reaction of 11 accessions of lima beans to *C. truncatum*, in detached leaves, at five DAI.

According to the AUDPC, a significant effect (p<0.01) was observed in the isolates versus accessions interaction (Figure 3). For the isolates CT 01, CT 02 and CT 20 there was no statistical difference in relation to the accessions used. The isolate CT35 was responsible for causing higher disease severity to the UFPB19 accession with AUDPC of 91.49 when compared to the other accessions. The lowest AUDPC was obtained for UFPB 02, UFPB 04, UFPB 13 and UFPB 20 accessions.

Significant interactions between *Sclerotium rolfsii* isolates and lima bean genotypes have also been reported by Silva et al. (2014) and reinforce the use of isolates from different sources in assessments aimed at resistance to this pathogen. Pereira et al. (2011), when evaluating the partial resistance of sorghum hybrids and their parent strains subjected to the aggressiveness of *C. sublineolum* isolates, concluded that none of the isolates were virulent to sorghum genotypes.

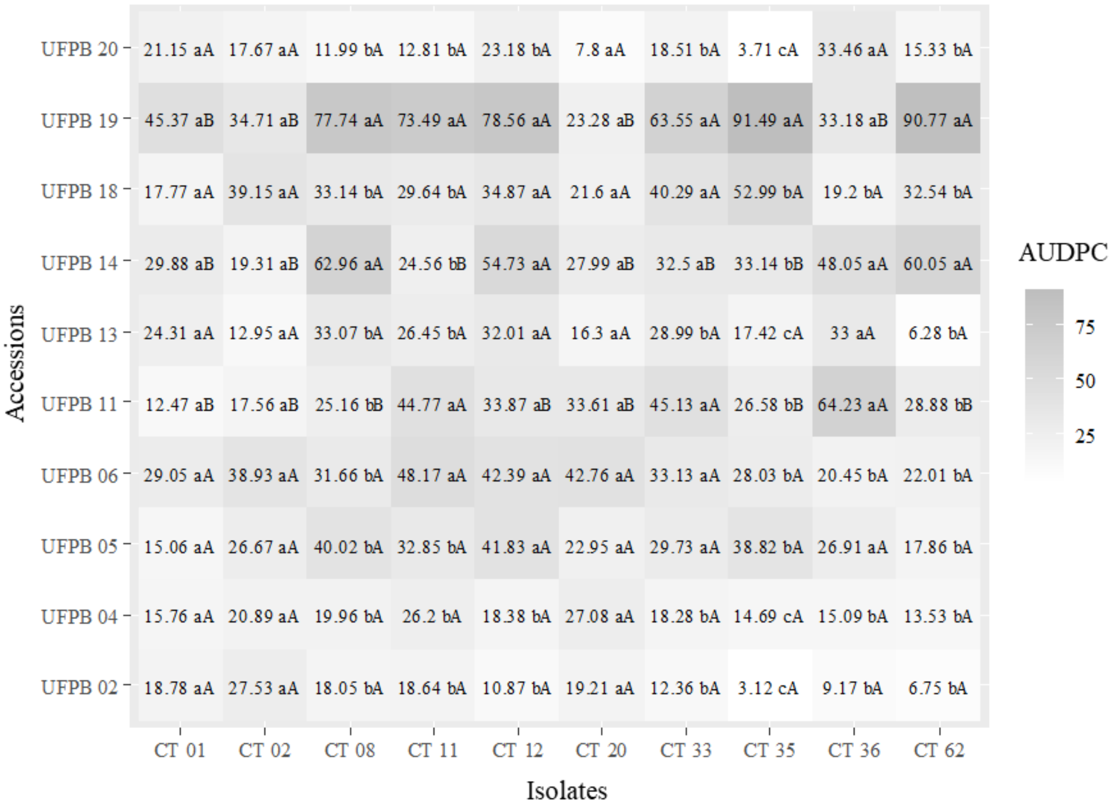

Figure 3. Area under the disease progress curve (AUDPC) of anthracnose of lima bean accessions (*Phaseolus lunatus* L.) inoculated with *Colletotrichum truncatum* isolates. Means followed by the same lowercase letter in the columns and uppercase letter in the rows are not statistically different by the Scott-Knott test at 1% probability.

From the ten isolates tested, three (CT 08, CT 12 and CT 35) showed higher aggressiveness to lima bean accessions, where typical anthracnose lesions ranged from 51 to 100% of infected leaf area, based on the scale of notes.

Mafacioli et al. (2008) also found a similar behavior in three isolates (PGA-PR1, PGA-PR2 and REG-SP2), which showed higher aggressiveness among 17 isolates of *C. gloeosporioides* associated with anthracnose in leaves of peach palm from states in the North, Southeast and South of Brazil.

Due to the wide genetic diversity found in different genotypes of lima beans (GUIMARÃES et al., 2007), new studies involving a higher number of isolates with peculiar characteristics of location and frequency of occurrence in crops of the same genus must be carried out. This study is important to identify virulence and aggressiveness of isolates and possible sources of resistance among accessions to anthracnose.

CONCLUSIONS

The aggressiveness among *C. truncatum* isolates changed depending on the genetic variability of the lima bean accessions evaluated.

Accessions with significant levels of resistance to anthracnose can be used as resistance sources for future breeding programs.

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