Screening of chilli pepper genotypes against anthracnose (*Colletotrichum brevisporum*)

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A B S T R A C T

Anthracnose is the most important disease of chili pepper *pimenta-de-cheiro* (*Capsicum chinense* Jacq.). This species is widely cultivated in dryland areas in the Amazon, presenting high genetic diversity. Therefore, it presents a high potential for use in breeding. The objective of this study was to select pepper genotypes with potential resistance to anthracnose (*Colletotrichum brevisporum*). For this purpose, ripe fruits of pepper were acquired from the producing farms in the municipalities of Iranduba, Manacapuru, Rio Preto da Eva and Presidente Figueiredo. The experiments were conducted in seedlings (Phase I) and in mature and immature fruits (Phase II). The evaluation of the disease severity was performed using a scale of scores. The morphoagronomic characterization of the genotypes considered the fruits in the immature and mature stages and plant growth habit. The injured areas in the immature fruits ranged from 0.3 to 9.7 cm^2 and in the mature areas, it ranged from 0.2 to 9.9 cm^2. The genotypes RPE41 and MPU29 indicated resistance to anthracnose in both stages. The morphoagronomic characterization of the fruits revealed variability for mass (5.58 to 13.74 g), length [C] (4.08 to 8.16 cm), diameter [D] (1.74 to 2.54 cm), L/D ratio (1.88 to 4.70) and color of the fruit.

Keywords: *Capsicum chinense*; Morphoagronomic characterization; Severity

INTRODUCTION

Species of the genus *Capsicum*, family Solanaceae, are among the most cultivated annual herbaceous vegetables (Mishra et al., 2017). The Amazon is an important center of diversity of this genus, especially for the species of *Capsicum chinense* Jacq. (Fonseca et al., 2008). In Brazil, family farmers in the state of Amazonas obtain their income from the sale of chili pepper *pimenta-de-cheiro* fruits (*C. chinense* Jacq.), either as mono or polyculture. However, their productivity is limited by fruit losses due to anthracnose disease. Ali et al. (2016) state that high losses in *Capsicum* spp. are caused by several microorganisms, in which anthracnose is one of the main problems associated with the commercialization of high-quality fruits in many production areas.

It is known in the literature that chilies (*Capsicum* spp.) are susceptible to a *Colletotrichum* species complex. At least fifteen species have been cited for causing damage to pepper crops in the world (Diao et al., 2017). More recently, in the State of Amazonas, *C. brevisporum* was reported as aJB causal agent of anthracnose in pepper. Until then, this species had not been reported in this crop in the state of Amazonas (Almeida et al., 2017).

The first records of anthracnose caused by *C. brevisporum* were in bromeliads of the genus *Neoregalia* sp. and *Pandanus pygmaeus* Thouars, in Thailand (Noireung et al., 2012), in the endophytic form in *Lycium chinense* Miller, Korea (Paul et al., 2014), in papaya, *Carica papaya* L. (Vieira et al., 2013) and chayote, *Sechium edule* Jacq. (Bezerra et al., 2016), both in Brazil. Regarding chili, anthracnose had been described by Liu et al. (2016) and Silva et al. (2017) in bell peppers 'pimentões', *Capsicum annuum* L.

The use of synthetic chemicals is one of the most common practices for the control of anthracnose in peppers. However, it may be harmful to human health if used in post-harvest management, in addition to accumulating potential resistance in pathogens to fungicides (Ali et al., 2016).

Resistance was found in semiripe mature *Capsicum annuum* fruits against *Colletotrichum capsici* (Gupta et al., 2018); *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* (Mishra et al., 2019); *Colletotrichum acutatum* (ChunYing et al., 2015) and in fruits of other species of *Capsicum* (*Capsicum* spp.) against to anthracnose caused by *Colletotrichum* spp. (Parey et al., 2013; Begum et al., 2015; Arunakumara and Satyanarayana, 2016; Bento et al., 2017; Maracahipes et al., 2017).

The selection of plants with resistance genes is an important tool to solve phytosanitary problems caused by anthracnose in chili pepper 'pimenta-de-cheiro' plantations. For Lee et al. (2010), resistance introgression may be the best way to control anthracnose due to the possibility of selecting genotypes with characteristics of commercial importance.

In this context, the objective of this study was to screen genotypes of chili peppers *pimenta-de-cheiro* with potential resistance to anthracnose (*Colletotrichum brevisporum*) through the evaluation of severity in fruits and seedlings.

MATERIALS AND METHODS

Collection of chili pepper genotypes
In July and August 2016, samples of ripe fruits from presumably resistant and productive plants were collected from farmer's reference and visual evaluation (mass selection), in 37 producing farms of chilli pepper *pimenta-de-cheiro* in the municipalities of Iranduba, Manacapuru, Rio Preto da Eva and Presidente Figueiredo, being eight of them from the municipality of Iranduba (IRA); seven from Manacapuru (MPU); thirteen from Presidente Figueiredo (PF) and nine from Rio Preto da Eva (RPE).

Three to five fruits with peduncle were removed from each plant, stored in paper bags, labeled and transported to the Phytopathology Laboratory of the National Institute of Amazonian Research - INPA. Seeds were removed from fruits and submitted to asepsis according to Baraka et al. (2011). Then were stored in hermetically closed plastic bottles and identified according to their origin.

Reactivation and obtaining inoculum
The INPA 2787 strain, previously identified as *Colletotrichum brevisporum* and with proven pathogenicity in chili pepper *pimenta-de-cheiro* (Almeida, 2015) was reactivated. This strain was preserved by the Castellani method (Castellani, 1967) and deposited in the fungi collection at the Laboratory of Plant Pathology of INPA.

The reactivation occurred by transferring the fungus to Petri dishes containing potato-dextrose-agar (PDA)

culture medium maintained at 27 ºC for 10 days for conidia production. Preparation of the inoculum was performed according to Coutinho et al. (2011) with adaptations, being the concentration adjusted for 10^6 conidia/mL.

Anthracnose severity in seedlings
The experiment was carried out in a greenhouse at the National Institute of Amazonian Research - INPA, Campus III. A completely randomized design was used, with 102 treatments (genotypes) and 4 replicates for each treatment, one plant per experimental plot. Two of these genotypes were used as controls. The positive control, a genotype collected with symptoms of anthracnose, therefore considered as susceptible, was inoculated with the pathogen. The negative control, a genotype collected without anthracnose symptoms, was inoculated only with sterile distilled water.

Sowing was performed in 128-cell trays with Plantmax® organomineral substrate and then transplanted into plastic cups with capacity for 300 ml containing sifted latosol. After reaching 15 cm in height and with six to eight leaves, the seedlings were inoculated with the suspension of the inoculum, with the aid of a spray near the point of flowing through the leaves. The seedlings were kept for 24 h in a humid chamber in a greenhouse with controlled irrigation.

Anthracnose symptoms on leaf were evaluated at 7, 14 and 21 days after inoculation (DAI) using the scale of scores as described by Mahasuk et al. (2009a), with some changes, where grade 1 = absence of symptoms; grade 2 = localized cell death, lesions (> 1 mm) with defined margin, hypersensitivity reaction; grade 3 = Small isolated necrotic lesions, covering about 1% of the leaf area; grade 4 = Discrete increase in necrotic lesions, covering about 5% of the leaf area; grade 5 = Lesions covering approximately 10% of the leaf area and with the presence of the acervulum; grade 6 = Lesions covering approximately 25% of the leaf area and with abundant presence of acervuli.

Anthracnose severity in fruits
From the seedling inoculation results, 28 genotypes were selected, which were transplanted to the field at the Experimental Station of Hortaliças Dr. Alejo Von der Pahlen located at Km 14, BR174, Rodovia AM 010, at the National Institute of Amazonian Research - INPA, Manaus, AM.

The transplanting was performed in soil fertilized with chicken manure (2 kg per pit) following the spacing and fertilization recommendations according to Filgueira (2013). The topdressing fertilization was performed with 30 g of NPK (4-14-8). Foliar fertilization was performed every 15 days using 6 g per liter of Plantafol (Valagro®)

(NPK-20-20-20). Flowering began at 65 days after sowing and fruit were harvested at approximately 85 days.

The experiments were conducted in a completely randomized design with 28 treatments (genotypes), 3 replicates per treatment (one plant as an experimental unit), in which the fruits were the sub-replicates. Five fruits from each plant were collected, making 15 fruits per treatment, for each maturity stage [mature and immature]. The fruits were taken to Phytopathology Laboratory of INPA, where the fruits severity to anthracnose was evaluated in two periods (7 and 14 days).

The fruits were soaked in sodium hypochlorite solution 1% NaClO (v/v) for one minute and washed in distilled water. The dry fruits were stored under previously sterilized towel paper in gerbox boxes containing cotton soaked with sterile water to maintain 100% relative humidity (RH). The inoculation in the fruit was performed according to Lin et al. (2002).

Resistance of the chili pepper fruits to the anthracnose was evaluated according to the following considerations:

(i)   Area of the lesion measured by means of images of the fruits photographed at 7 DAI and 14 DAI, in immature and mature stages, with the help of Autocad 2016 software tools; so, it became possible to classify the genotypes into the following groups: resistant with <10% of the injured area (R), moderately resistant with 11-20% (MR), susceptible with 21-40% (S) and highly susceptible > 41% (HS) (Park et al., 2009).

(ii) Number of days for the emergence of the first symptoms of the pathogen, incubation period.

(iii) Counting of conidia on 14 DAI with the help of the Neubauer chamber. The fruit was washed with 1 mL of sterilized distilled water on a Petri dish with the aid of a number zero brush to help detach the conidia from the conidiophores. Afterwards, a sample was removed from the suspension for the quantification of conidia (Alfenas and Mafia, 2007). The results were calculated to obtain the number of conidia per cm² of the lesion.

Morphoagronomic characterization of chili pepper genotypes

The characterization of the genotypes and evaluations from a sample of 150 fruits per genotype considered the fruits in the immature stage, green coloration, mature stage, the appearance of ripe fruit color (senescence phase) and plant growth habit according to IPGRI (1995).

Statistical analyzes

Seedling severity scores were tabulated, and the data were compared by analysis of variance (ANOVA) using the Scott and Knott (1974) test (P≤0.05) using the Sisvar software, version 5.6 (Ferreira, 2014).

Fruit severity and morphoagronomic characterization data were submitted to analysis of variance (ANOVA) and to the Scott and Knott (1974) test (P≤0.05) using Genes software (Cruz, 2001).

RESULTS

Anthracnose severity in seedlings

The evaluations indicated a progressive increase in the levels of the disease severity, with averages of 2.70 to 4.41. After 21 days, there was colonization of the pathogen in 100% of the genotypes, but with different levels of severity. The negative control genotype, inoculated with water, did not show any symptoms due to anthracnose, whereas the positive control genotype showed symptoms due to anthracnose after 7 DAI (Table 1).

The distribution of genotypes in evaluation were the following: 38.61% lower and 61.39% greater than the average of 2.70 on the first week, 32.67% lower and 54.46% greater than the average of 3.50 on the second week and 40.59% lower and 59.41% greater than the average of 4.41 on the third week. MPU10, IRA03, and RPE33 genotypes stood out as they presented an injured area of less than 1 mm up to 14 DAI (Table 1).

Anthracnose severity in fruits

In the fruits with more advanced anthracnose symptoms, at 14 DAI, circular lesions and tissue deepening were observed. The lesions formed concentric rings that evolved until the involvement of the entire fruit, characteristic of anthracnose. Another characteristic observed was the production of conidial masses of orange color, sometimes white or grayish, that made the surface of the fruit wet (Fig. 1a-e).

The amplitude of injured areas in immature fruits at 7 DAI ranged from 0.3 and 3.2 cm². According to the anthracnose severity assessment, in these fruits, at 7 DAI, the genotypes that presented averages of injured areas with amplitude ranging from 0.3 to 0.8 cm² were considered resistant, those that had averages of injured areas between 0.9 and 1.8 cm² were considered moderately resistant and those with an average between 1.9 and 3.2 cm² were susceptible (table 2).

Following the reactions classification in the fruits at 7 DAI, the results showed that resistant (R), moderately resistant (MR) and susceptible (S) genotypes at the stage of immature fruit comprised 17.86%, 32.14% and 50%, respectively (Table 2). The resistant genotypes were: RPE02, MPU29, IRA03, RPE33, and RPE41. In contrast, susceptible

Table 1: Anthracnose severity on *capsicum chinense* seedlings inoculated with *colletotrichum brevisporum* (inpa 2787 strain)

Genotype	Anthracnose severity[1]		
	7 DAI	14 DAI	21 DAI[2]
NC[3]	1.00a	1.00a	1.00a
MPU10	1.25a	1.25a	3.25b
IRA03	1.25a	1.75a	2.75b
RPE33	1.50a	1.50a	3.50b
MPU05	1.25a	2.50b	3.50b
MPU04	1.75a	2.25b	3.50b
MPU16	1.75a	2.25b	3.50b
MPU03	1.75a	2.25b	3.75b
RPE16	1.75a	2.50b	2.75b
MPU06	1.75a	2.50b	3.25b
IRA24	1.75a	2.50b	3.50b
MPU08	2.00a	2.50b	3.00b
MPU29	2.00a	3.00b	3.75b
MPU31	2.00a	3.00b	4.00b
RPE02	2.50a	2.50b	3.50b
MPU09	1.50a	2.50b	4.25c
MPU12	1.50a	3.00b	4.50c
IRA02	1.75a	2.75b	4.50c
MPU01	2.00a	2.50b	4.25c
IRA20	2.25a	2.75b	4.75c
IRA9	2.25a	3.00b	4.25c
IRA25	2.75b	2.75b	4.00b
IRA01	3.00b	3.00b	4.00b
IRA28	2.75b	2.75b	4.75c
MPU13	2.00a	3.25c	4.00b
IRA04	2.00a	3.50c	4.00b
RPE07	2.00a	3.75c	4.00b
RPE37	2.50a	3.25c	3.50b
PF18	1.75a	3.25c	4.25c
IRA1b	1.75a	3.75c	4.50c
IRA21	2.00a	3.50c	4.25c
MPU31b	2.00a	3.50c	4.25c
MPU23	2.00a	3.75c	5.00c
PF09	2.25a	4.00c	5.00c
PC[3]	2.25a	4.25c	4.50c
MPU33	2.50a	3.25c	4.50c
IRA05	2.50a	3.50c	4.25c
MPU34	2.50a	3.75c	4.50c
PF4a	2.50a	3.75c	4.75c
RPE20	2.50a	3.75c	4.75c
IRA19	2.50a	4.00c	4.25c
RPE15	2.50a	4.00c	4.50c
MPU24	2.50a	4.50c	5.00c
IRA06	2.75b	3.25c	4.00b
PF29	2.75b	4.00c	4.00b
IRA23	3.00b	3.25c	3.50b
PF25	3.50b	3.50c	4.00b
RPE4a	3.50b	3.50c	4.00b
RPE18	2.75b	3.25c	4.25c
RPE30	2.75b	3.25c	5.00c
MPU25	2.75b	3.75c	4.25c
RPE17	2.75b	3.75c	4.25c
MPU02	2.75b	3.75c	4.75c
MPU26	2.75b	4.00c	4.75c

(Contd...)

Table 1: (*Continued*)

Genotype	Anthracnose severity[1]		
	7 DAI	14 DAI	21 DAI[2]
RPE06	2.75b	4.00c	4.75c
PF4c	2.75b	4.00c	5.00c
MPU22	2.75b	4.25c	4.75c
PF11	2.75b	4.25c	5.25c
PF15	2.75b	4.25c	5.25c
RPE19	2.75b	4.25c	5.50c
PF33	3.00b	3.25c	4.50c
RPE10	3.00b	3.50c	4.25c
MPU30	3.00b	3.50c	4.50c
RPE03	3.00b	3.50c	4.75c
PF2	3.00b	3.50c	5.50c
RPE41	3.00b	3.75c	4.50c
RPE01	3.00b	3.75c	5.25c
IRA18	3.00b	4.00c	4.75c
PF3	3.00b	4.00c	4.75c
IRA4b	3.00b	4.00c	5.00c
PF5c	3.00b	4.25c	4.75c
PF9b	3.00b	4.25c	5.00c
RPE05	3.25b	3.25c	4.25c
PF19	3.25b	3.50c	4.50c
PF31	3.25b	3.50c	4.75c
IRA22	3.25b	3.75c	4.50c
RPE31	3.25b	3.75c	5.00c
IRA17	3.25b	4.00c	4.25c
RPE24	3.25b	4.00c	4.25c
IRA11	3.25b	4.00c	4.50c
PF22	3.25b	4.00c	4.75c
RPE18c	3.25b	4.00c	4.75c
IRA14	3.25b	4.25c	5.00c
PF34	3.25b	4.50c	5.50c
RPE38	3.25b	4.75c	5.00c
PF08	3.25b	4.75c	5.25c
MPU07	3.50b	3.50c	4.75c
PF35	3.50b	3.75c	4.50c
RPE04	3.50b	3.75c	4.50c
IRA10	3.50b	3.75c	4.75c
IRA12	3.50b	3.75c	5.00c
RPE42	3.50b	4.00c	4.50c
PF28	3.50b	4.00c	4.75c
PF07	3.50b	4.25c	5.25c
PF14	3.50b	4.25c	5.50c
IRA7	3.75b	3.75c	4.50c
PF20	3.75b	4.00c	4.25c
IRA15	3.75b	4.00c	4.75c
RPE34	3.75b	4.00c	5.00c
PF5a	3.75b	4.00c	5.50c
PF26	3.75b	4.25c	5.00c
PF17	3.75b	4.50c	5.25c
Pr>F	2.40**	2.64**	2.77**
CV	32.5	25.6	18.8

**Significant at the 5% probability level
[1]Score scale: 1–no symptoms; 2–lesion>1 mm; 3 lesion 1 % of leaf area; 4–lesion 5 % of leaf area; 5–lesion 10 % of foliar area; 6–lesion 25 % of foliar area
[2]Averages of four replicates. Columns with the same letter do not differ statistically from each other by the Scott and Knott (1974) test at the 5% probability level
[3]NC-negative control (inoculated with water) and PC-positive control (inoculated with the INPA 2787 strain)

Table 2: Anthracnose severity in immature fruits of *capsicum chinense* inoculated with *colletotrichum brevisporum* (inpa 2787 strain)

Genotype	7 DAI[1,2]	Reation[3]	14 DAI	Reaction	Incubation (days)
Anthracnose severity in immature fruits					
Injured area (cm²)					
MPU02	3.2[a]	S	7.0[b]	AS	6
IRA18	3.0[a]	S	9.0[a]	AS	2
IRA21	2.9[a]	S	8.3[a]	AS	2
IRA01	2.9[a]	S	8.0[a]	AS	3
MPU06	2.9[a]	S	8.0[a]	AS	3
IRA24	2.8[a]	S	9.7[a]	AS	3
MPU08	2.6[a]	S	7.6[b]	AS	4
IRA05	2.6[a]	S	6.6[b]	AS	5
IRA04	2.1[b]	S	8.4[a]	AS	2
IRA25	2.1[b]	S	5.3[c]	AS	4
IRA06	2.0[b]	S	7.3[b]	AS	3
RPE07	2.0[b]	S	7.0[b]	AS	3
IRA02	1.9[b]	S	7.2[b]	AS	2
MPU03	1.9[b]	S	4.9[c]	AS	3
IRA23	1.8[b]	MR	8.5[a]	AS	3
MPU13	1.8[b]	MR	7.2[b]	AS	3
IRA15	1.7[c]	MR	7.2[b]	AS	5
RPE03	1.6[c]	MR	3.6[d]	S	3
MPU31	1.5[c]	MR	6.4[b]	AS	6
MPU25	1.5[c]	MR	4.9[c]	AS	6
RPE04	1.3[c]	MR	6.2[b]	AS	4
PF09	1.2[c]	MR	5.8[c]	AS	3
PF25	1.1[c]	MR	2.6[d]	S	2
RPE41	0.8[d]	R	5.8[c]	AS	5
RPE33	0.7[d]	R	5.2[c]	AS	4
IRA03	0.7[d]	R	5.4[c]	AS	3
MPU29	0.6[d]	R	4.6[c]	AS	2
RPE02	0.3[d]	R	2.1[d]	S	3
Pr>F	7.8**		14.0**		
CV	27.2		13.5		

**Significant at the 5% probability level
[1]Means followed by distinct letters in the same column do not differ by the Scoktt and Knott (1974) test at the 5% probability significant level
[2]Media of injured area of 15 fruits per genotype
[3]Reaction: R-resistant; MR-moderately resistant; S-susceptible; AS-highly susceptible

genotypes were the following: MPU02, IRA18, IRA21, IRA01, MPU06, IRA24, MPU08 and IRA05 (Table 2).

At 14 DAI, the amplitude of injured areas in immature fruits was between 2.1 and 9.7 cm². In this case, highly susceptible genotypes were observed, with areas between 4.2 and 9.7 cm² according to Table 2.

The amplitude of injured areas in mature fruits, at 7 DAI, was between 0.2 and 3.8 cm². The genotypes that presented injured areas between 0.2 and 1.0 cm² were considered resistant; between 1.1 and 1.8 cm² considered moderately resistant and between 2.2 and 3.8 cm², susceptible genotypes (Table 3). The results showed that resistant (R), moderately resistant (MR), susceptible (S) genotypes in the mature fruit stage constituted 17.86%, 50%, and 32.14% respectively (Table 3).

In mature fruits, the resistant genotypes were: MPU29, PF09, IRA02, IRA01, and RPE41. Only the RPE02 genotype was considered susceptible at this stage and the MPU29 genotype showed the lowest mean of injured area (0.2 cm²) (Table 3).

The amplitude of injured areas at 14 DAI was between 4.2 and 9.9 cm². Highly susceptible genotypes were found with damaged areas of 4.3 and 9.9 cm² (Table 3). The incubation period ranged from 2 to 6 DAI for both mature and immature fruits (Table 2 and 3).

In laboratory conditions, maximum production of 19.57 x 10^6 conidia/cm² and a minimum production of 3.27 x 10^6 conidia/cm² was detected after 14 DAI (Table S1).

By comparing the results of sporulation of *C. brevisporum* with the lesion reaction in mature fruits (Table 3), it is observed that there is some correspondence of the data, however, this parameter can be a reference, but not determinant. The genotypes RPE02, MPU13 and RPE07

Fig 1. [a-e] Anthracnose symptoms of (*Colletotrichum brevisporum*) in two chili peppers genotypes, at 14 DAI.

Table 3: Anthracnose severity in mature fruits of *Capsicum chinense* inoculated with *Colletotrichum brevisporum* (INPA 2787 strain)

Genotype	7 DAI[1,2]	Reaction[3]	14 DAI	Reaction	Incubation (days)
RPE02	3.8[a]	S	9.2[a]	AS	3
RPE07	3.1[b]	S	8.9[a]	AS	3
MPU13	3.0[b]	S	5.8[c]	AS	2
MPU02	2.7[c]	S	5.8[c]	AS	5
IRA04	2.6[c]	S	9.7[a]	AS	2
IRA18	2.3[c]	S	6.8[b]	AS	2
RPE03	2.2[c]	S	9.0[a]	AS	2
MPU06	2.2[c]	S	7.4[b]	AS	5
IRA23	2.2[c]	S	9.9[a]	AS	3
PF25	1.8[d]	MR	8.0[a]	AS	4
MPU25	1.7[d]	MR	7.5[a]	AS	5
IRA21	1.6[d]	MR	9.2[a]	AS	2
IRA05	1.6[d]	MR	6.5[b]	AS	3
IRA24	1.6[d]	MR	9.4[a]	AS	3
IRA03	1.5[d]	MR	8.5[a]	AS	4
RPE04	1.5[d]	MR	5.8[c]	AS	3
MPU03	1.5[d]	MR	5.8[c]	AS	2
MPU08	1.4[d]	MR	7.2[b]	AS	4
IRA06	1.4[d]	MR	4.3[c]	AS	2
RPE33	1.4[d]	MR	4.2[c]	AS	4
MPU31	1.3[d]	MR	4.8[c]	AS	6
IRA15	1.1[e]	MR	6.7[b]	AS	5
IRA25	1.1[e]	MR	5.7[c]	AS	3
RPE41	1.0[e]	R	6.4[b]	AS	3
IRA01	1.0[e]	R	5.9[c]	AS	3
IRA02	0.6[e]	R	6.2[b]	AS	2
PF09	0.5[e]	R	5.5[c]	AS	4
MPU29	0.2[e]	R	5.9[c]	AS	2
Pr>F	10.7**		9.3**		
CV	25.2		13.7		

The header note: Anthracnose severity in mature fruit / Injured area (cm²)

**Significant at the 5% probability level
[1]Means followed by distinct letters in the same column do not differ by the Scoktt and Knott (1974) test at the 5% probability significant level
[2]Media of the injured area of 15 fruits per genotype
[3]Reaction: R-resistant; MR-moderately resistant; S-susceptible; AS- highly susceptible

Table 4: Morphoagronomic characterization of 28 chili pepper (*Capsicum chinense*) genotypes

Genotype	Mass/Fruit (g)[1]	Fruit length (cm)	Fruit diameter (cm)	Length/Diameter
MPU03	5.6[a]	4.10[a]	2.14[b]	1.90[A]
IRA01	6.1[a]	5.49[a]	1.74[a]	3.25[B]
MPU02	7.3[a]	4.08[a]	2.18[b]	1.88[A]
IRA04	7.3[a]	7.11[b]	1.61[a]	4.44[B]
MPU08	7.5[a]	4.88[a]	1.95[a]	2.44[A]
IRA06	7.9[a]	6.66[b]	1.85[a]	3.64[B]
RPE41	7.9[a]	6.41[b]	2.09[a]	3.08[A]
RPE04	8.1[a]	5.50[a]	2.10[a]	2.61[A]
MPU13	8.4[a]	5.60[a]	2.19[b]	2.57[A]
IRA05	8.7[a]	6.93[b]	1.81[a]	3.88[B]
MPU06	9.2[a]	5.27[a]	2.38[b]	2.23[A]
RPE07	9.4[a]	6.81[b]	1.92[a]	3.55[B]
IRA15	9.4[a]	7.27[b]	1.80[a]	4.08[B]
MPU29	9.9[b]	6.33[b]	2.38[b]	2.73[A]
MPU31	9.9[b]	5.78[a]	2.24[b]	2.58[A]
RPE02	10.2[b]	5.59[a]	2.27[b]	2.46[A]
IRA02	10.4[b]	7.03[b]	2.07[a]	3.44[B]
PF09	10.4[b]	7.51[b]	1.99[a]	3.93[B]
RPE03	10.7[b]	5.90[a]	2.35[b]	2.51[A]
IRA21	10.7[b]	8.16[b]	1.76[a]	4.70[B]
IRA23	11.0[b]	7.20[b]	2.19[b]	3.30[B]
MPU25	11.1[b]	6.67[b]	2.24[b]	2.98[A]
RPE33	11.2[b]	4.68[a]	2.48[b]	1.93[A]
IRA24	11.3[b]	7.66[b]	2.03[a]	3.81[B]
PF25	11.7[b]	5.39[a]	2.54[b]	2.16[A]
IRA25	12.6[b]	7.79[b]	2.04[a]	3.79[B]
IRA03	13.6[b]	6.47[b]	2.54[b]	2.55[A]
IRA18	13.7[b]	7.33[b]	2.42[b]	3.08[A]
Pr>F	2.09**	4.98**	2.66**	6.91**
CV	24.5	13.67	12.75	16.91

**Significant at the 5% probability level
[1]Average mass of 150 fruits per genotype
[2]Means followed by distinct letters in the same column do not differ by the Scoktt and Knott (1974) test at the 5% probability significant level

were classified as highly susceptible (HS) and presented conidia concentrations of 19.57 x 10⁶, 16.57 x 10⁶ and 12.36 x 10⁶ per cm² of the lesion, respectively (Table S1). The genotype MPU29, classified as resistant (R), presented concentration of conidia/cm² of the lesion of 4.61 x 10⁶ (Table S1). However, this behavior cannot be considered the standard, because the RPE41 genotype, classified as resistant (R), despite low sporulation (0.51 x 10⁶), showed large lesion area and conidia production of 12.55 x 10⁶ conidia/cm² (Table S1).

Morphoagronomic characterization of chili pepper genotypes

The fruit mass values varied between 5.58 g (MPU03) and 13.74 g (IRA18), the fruit length values ranged from 4.08 cm (MPU02) to 8.16 cm (IRA21), the diameter values varied between 1.61 (IRA04) and 2.55 (IRA03 and PF25), the length/diameter ratio ranged from 1.88 (MPU02) to 4.70 (IRA21) (Table 4).

The genotypes classified as resistant presented average lengths of 4.68 (RPE33), 5.49 (IRA01), 5.59 (RPE02), 6.33 (MPU29), 6.41 (RPE41), 6.47 (IRA03), 7.03 (IRA) and 7.51 (PF09). The genotypes IRA25, MPU25, IRA24, PF25, IRA21 indicated tolerance to anthracnose, since they had normal development and good productivity, with averages of mass/fruit ranging from 10.43 (PF09) to 12.59 (IRA25) (Table 4).

Regarding the shape of the fruits (Table 5 and Fig. S1), genotypes were distinguished in four types: elongated, triangular, campanulate and block. Regarding fruit color, three red color variations were observed, light-red, red and dark-red, as well as lemon-yellow. Regarding the habit of

Table 5 : Characteristics of fruit shape and color, plant growth habit of chilli pepper (*Capsicum chinense*) genotypes

Genotype	fruit shape	mature fruit color	growth habit
RPE07	campanulate	dark-red	prostrate
MPU13	campanulate	dark-red	prostrate
IRA25	elongated	dark-red	compact
RPE04	triangular	dark-red	erect
RPE41	campanulate	dark-red	erect
IRA02	elongated	clear-red	compact
IRA18	triangular	dark-red	erect
MPU06	triangular	dark-red	prostrate
MPU02	block	lemon-yellow	compact
MPU31	triangular	dark-red	erect
MPU25	triangular	dark-red	erect
RPE03	triangular	dark-red	erect
IRA23	triangular	red	compact
IRA06	elongated	red	erect
MPU03	block	lemon-yellow	compact
IRA04	elongated	clear-red	erect
IRA01	triangular	dark-red	compact
MPU08	block	dark-red	prostrate
IRA24	elongated	red	prostrate
RPE02	block	dark-red	compact
RPE33	block	dark-red	erect
PF25	block	red	compact
IRA21	elongated	dark-red	erect
IRA05	elongated	clear-red	compact
PF09	elongated	dark-red	compact
IRA03	triangular	clear-red	erect
IRA15	elongated	dark-red	compact
MPU29	elongated	dark-red	erect

plant growth, genotypes differed in the following: erect, compact and prostate.

DISCUSSION

Anthracnose symptoms in peppers occur mainly in fruits. In leaves, the symptoms are not severe, besides being restricted, which may be advantageous for the selection of resistance at the early stage of development (Mahasuk et al., 2009b). In similar experiments, anthracnose resistance sources have been found in *C. chinense* in the seedling phase (Mahasuk et al., 2009a; Silva et al., 2014). This indicates that breeding of this species can be performed by selection or by genetic crosses.

On the other hand, it is verified that the selection based only on the reaction of seedlings to anthracnose is not enough. Mahasuk et al. (2009b) verified the independence of the *Co3* gene in relation to the *Co1* and *Co2* genes, suggesting that there was no correlation between seedling resistance and fruits resistance of *Capsicum* spp., thus, the selection process for anthracnose resistance still needs to be carried out in fruits.

In the present study, genotypes with early resistance to anthracnose were considered those that showed a lack of symptoms of the disease or with isolated necrotic lesions covering leaf area up to 1% or hypersensitivity reaction. According to Mukhtar et al. (2016), plants detect molecular components of the invading pathogens and frequently respond with successful immune responses. Dickman and Fluhr (2013) mention that practically all cells have an intrinsic program for genetically encoded cell suicide. Cell deaths are genetically programmed, sometimes requiring the induction of specific genes to stimulate cell death machinery.

Only the MPU29 and RPE41 genotypes were classified as resistant at both maturation stages. The differential reactions in fruits may be the result of the activity of enzymes related to the pathogenesis. Prasath and Ponnuswami (2008) demonstrated that the total content orthodihydroxy phenols were significantly higher in resistant and moderately resistant genotypes to anthracnose caused by *C. capsici*. The activity of the enzymes peroxidase, polyphenol oxidase, and phenyl ammonia lyase was also higher in the resistant genotype followed by moderately resistant hybrids. In addition, the lowest enzymatic activity was recorded in the susceptible genotype.

The cuticular layer of the fruits is another factor that may play an important role in the prevention of infection and colonization of the fungus. Ranathunge et al. (2012) described the mechanisms of colonization and infection of *C. truncatum* and observed dissolution of the cell walls on the 6th DAI, causing the appearance of sunken on the surface of the fruit and only on the 7th-9th DAI, the epidermis then breaks and reveals the presence of setae and conidia.

In contrast, Ridzuan et al. (2018) state that the manifestation of resistance or susceptibility of the host to a pathogen is restricted to the pathogen genotype and to its degree of virulence in the host genotype. The corresponding gene pairs regulate the result of some particular genotype-genotype interaction. The authors also state that the host genotype, the pathogen genotype, time and environment may be responsible for the anthracnose development. For Mongkolporn et al. (2010) different genes with distinct expression mechanisms differentially react to *Colletotrichum* species.

In this study, no difference was found in severity between immature and mature fruits at 7 DAI, but the difference was found on 14 DAI. Mahasuk et al. (2009b) found similar behavior regarding susceptibility to anthracnose in plants F1 and F2, regardless of the stage of the fruits (green and red).

Nevertheless, Silva et al. (2014) suggest the existence of distinct genes responsible for resistance in different stages of the fruit in development. The authors also verified the influence of environmental conditions on the severity of the disease in some of the evaluated accesses.

Oh et al. (1998) observed a successful invasion and colonization of *C. gloeosporioides* occurring in green fruits, but not in red fruits. Although the severity did not present a significant difference on the 7th DAI, numerically speaking, the green fruits presented a larger average of injured area in relation to the mature fruits.

According to Adikaram, Brown and Swinburne (1983), the infection may start by spore germination and formation of spores and appressoria on green fruits, which may remain quiescent and continue the infection process and colonization of the host only after maturation of the fruits.

Nevertheless, in observations made on the anthracnose severity in *Capsicum annuum* L. at 130 and 145 days after transplantation, Begum et al. (2015) noted that all genotypes/varieties reacted differentially and significantly to this disease.

The incubation period (time elapsed between inoculation to the emergence of symptoms) and the latency period (time elapsed between inoculation to the emergence of the first fungal structures) are indices that can infer if the pathogen is more or less aggressive to a certain genotype. In nature, a polycyclic system such as anthracnose can generate a high variability (Amorim, 1995). In this experiment, it was not possible to relate the incubation period to the resistance classification since the genotypes classified as resistant did not indicate a pattern, the symptoms manifested early, with two days or late, with 6 days.

The fungus sporulation potential is an index that allows to determine the capacity of fungal species proliferation in nature with greater efficiency and thus, to guarantee a greater number of generations of the pathogen per cycle of the host. After deposition on the host surface, the conidia emit the germ tube and penetration begins. However, higher conidial production does not necessarily indicate a susceptibility reaction.

In Amazonas, farmers show preferences for green fruits, which are most sought after for fresh consumption in local cuisine, and mature fruits are reserved for seed withdrawal (self-observation).

Genotypes classified as resistant with good agronomic performance were observed in this study. Oliveira et al. (2011) characterized genotypes from the States of Amazonas, Pará, and Rondônia and obtained fruits that showed variation in fruit length from 3.2 cm to 7.5 cm and fruit diameter from 1.9 cm to 3.2 cm. Domenico et al. (2012) characterized accessions of *C. chinense* from the pepper germplasm bank of Campinas, SP, and obtained *C. chinense* fruits with mean values ranging from 2.3 to 7.7 cm and for width, the mean values varied from 1.1 to 2.5 cm. Fonseca et al. (2008) characterized 38 accessions from the Alto Rio Negro region, state of Amazonas and categorized the different lengths into five classes, whose measures ranged from 1 to 12 cm, while for the width, there were three classes, ranging from 1.0 to 2.5 cm. These variations of length and width were also observed in this study.

CONCLUSIONS

Screening of resistant chilli pepper genotypes to anthracnose caused by *Colletotrichum brevisporum* should be performed based on seedlings and fruits. The use of the anthracnose severity scale for seedlings and fruits contributes to the screening of resistant genotypes. Genotypes from the municipalities of Iranduba, Manacapuru, Rio Preto da Eva and Presidente Figueiredo are still in the process of genetic segregation for anthracnose resistance. Sporulation can be used as a reference for the study of fruit severity, but not as a response pattern for resistance or susceptibility. Resistant genotypes can be used in breeding programs because they have good agronomic performance.

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Authors' contributions
Leonor C. S. Souza conducted the whole experiment and wrote the first draft of the manuscript; Luiz A. G. Assis collaborated in conducting the experiment; Aricléia de M. Catarino contributed for the preparation and organization of the manuscript and Rogério E. Hanada idealized the work, guided the first author and contributed greatly for the preparation of the manuscript.

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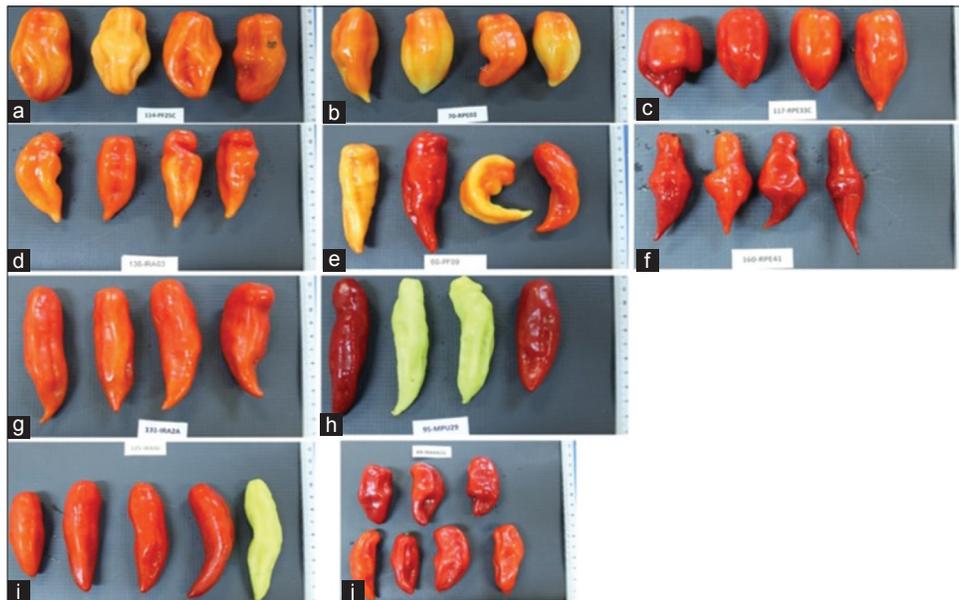
SUPPLEMENTARY

Fig S1. [a-j] Morphological characterization of chili peppers genotypes

Table S1: *Colletotrichum brevisporum* (INPA 2787 strain) sporulation 14 DAI under chili pepper fruits (*Capsicum chinense*)

Genotype	Final area[1]	Sporulation[2]	Conidia.10⁶ per cm² of lesion[3]
MPU03	5.8	0.35[a]	16.57
MPU13	5.8	0.35[a]	16.57
RPE02	9.2	0.47[a]	19.57
MPU25	7.5	0.50[a]	15.00
IRA03	8.5	0.50[a]	17.00
RPE41	6.4	0.51[a]	12.55
IRA05	6.5	0.52[a]	12.50
IRA02	6.2	0.61[a]	10.16
RPE33	4.2	0.64[a]	6.56
RPE07	8.9	0.72[a]	12.36
MPU31	4.8	0.73[a]	6.58
PF25	8.0	0.78[a]	10.26
IRA01	5.9	0.95[a]	6.21
IRA25	5.7	0.95[a]	6.00
IRA21	9.2	1.02[a]	9.02
PF09	5.5	1.05[a]	5.24
MPU02	5.8	1.09[a]	5.32
RPE03	9.0	1.16[b]	7.76
IRA06	4.3	1.27[b]	3.39
MPU29	5.9	1.28[b]	4.61
IRA18	6.8	1.31[b]	5.19
IRA04	9.7	1.42[b]	6.83
IRA24	9.4	1.49[b]	6.31
IRA15	6.7	1.51[b]	4.44
IRA23	9.9	1.53[b]	6.47
RPE04	5.8	1.72[b]	3.37
MPU08	7.2	1.86[b]	3.87
MPU06	7.4	2.26[b]	3.27
Pr>F		3,11**	

**significant at the 5% probability level. Final injured area average of the mature fruit after 14 days; ²concentration of conidia in 1 mL; ³ratio of injured area/amount of conidia. Means followed by distinct letters in the same column differ by the Scoktt & Knott test at the 5% probability level