Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga
ISSN 1983-2125 (online)

http://dx.doi.org/10.1590/1983-21252025v3812519rc

Scientific Article

Resistance response of cowpea accessions to charcoal rot

Reação de acessos de feijão-caupi à podridão cinzenta do caule

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ABSTRACT - The objective of this study was to assess the resistance reaction of cowpea accessions grown in municipalities of Rio Grande do Norte, Brazil, to the fungus *Macrophomina phaseolina* (Tassi) Goid. A total of 53 cowpea accessions were evaluated in two greenhouse experiments (25 and 28 accessions, respectively), using a completely randomized design with five replications; each replication consisted of one plant per pot. The fungus was isolated from cowpea plants showing charcoal rot symptoms. Fungi were cultured and purified on a potato-dextrose-agar (PDA) medium with tetracycline (0.05 mg mL^{-1}) and then inoculated into cowpea plants using the toothpick method. Inoculation was carried out when the plants exhibited two true leaves. The disease response of accessions was evaluated 30 days after inoculation based on a disease severity scale. Transformed data were subjected to analysis of variance, and means were grouped using the Scott-Knott test ($p < 0.05$). Accessions 6, 14, 17, 30, 42, 43, 46, 48, and 50 were highly resistant to *M. phaseolina* and, therefore, can be included in breeding programs focused on resistance to pathogens. The results indicate a predominance of genes conferring resistance to *M. phaseolina* in cowpea accessions from the Agreste mesoregion of Rio Grande do Norte, Brazil.

Keywords: *Vigna unguiculata* (L.) Walp. *Macrophomina phaseolina*. Genetic resistance. Root diseases.

RESUMO - Objetivou-se com este trabalho avaliar a reação de resistência de acessos de feijão-caupi, coletados em municípios do Rio Grande do Norte, ao fungo *Macrophomina phaseolina* (Tassi) Goid. Foram realizados dois ensaios em casa de vegetação, avaliando-se 25 acessos no primeiro ensaio e 28 no segundo, totalizando 53 acessos de feijão-caupi. Ambos os ensaios foram realizados em delineamento inteiramente casualizado com cinco repetições, sendo cada repetição constituída por um vaso, com uma planta. O fungo foi proveniente de plantas de feijão-caupi que exibiam sintomas de podridão cinzenta do caule. Este foi cultivado e purificado em meio BDA (batata-dextrose-ágar) + tetraciclina (0,05mg/mL), e posteriormente utilizado nas inoculações utilizando o método do palito. A inoculação foi realizada quando as plantas estavam com duas folhas definitivas. A avaliação da reação dos acessos foi realizada 30 dias após a inoculação por meio de escala de notas de severidade, posteriormente foram determinadas as classes de reação à doença. Os dados transformados foram submetidos à análise de variância e as médias dos acessos foram agrupadas pelo método de Scott-Knott ($p<0,05$). Os acessos 6, 14, 17, 30, 42, 43, 46, 48 e 50 foram altamente resistentes à *M. phaseolina* e podem ser incluídos em programas de melhoramento visando resistência ao patógeno. Verificou-se predominância de genes de resistência à *M. phaseolina* na mesorregião Agreste do Rio Grande do Norte.

Palavras-chave: *Vigna unguiculata* (L.) Walp. *Macrophomina phaseolina*. Resistência genética. Doença radicular.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: February 24, 2024.
Accepted in: October 18, 2024.

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INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a widely cultivated legume crop species with significant economic importance in Brazil. It is an excellent source of proteins, amino acids, carbohydrates, vitamins, and minerals, and contains a high quantity of dietary fiber and a low-fat content (KOTUE et al., 2018). It is generally consumed as dry and green beans in human diets, and its stems and branches are used for animal feed (MULLINS; ARJMANDI, 2021).

India, Nigeria, Niger, and Brazil are the largest cowpea-producing countries (FAO, 2023). Cowpea production in Brazil is concentrated in the North and Northeast regions due to the species' favorable adaptation to local soil and climate conditions. Additionally, cowpea has an important socioeconomic function, as it is mainly cultivated in family farming systems, often with low technological level. In recent years, cowpea crops have been expanding to the Central-West region of the country and raising the interest of growers with high technology, who grow beans between soybean crop seasons (VALE; BERTINI; BORÉM, 2017).

Despite its hardy nature, several pathogens can damage cowpea crops, including the soilborne fungus *Macrophomina phaseolina* (Tassi) Goid. This mitosporic (asexual spore-producing) fungus from the family Botryosphaeriaceae forms two types of asexual structures: pycnidia and microsclerotia (LINHARES et al., 2020). *M. phaseolina* has high pathogenic variability, a strong capacity for survival under adverse conditions, and a wide geographical distribution. This makes it a cosmopolitan pathogen with higher incidence in regions with high air temperatures and water deficit (MARQUEZ et al., 2021), common conditions in the Semiarid region of Brazil.

Charcoal rot or stem canker is one of the diseases caused by *M. phaseolina*. Symptoms initially appear as irregular, slightly sunken, dark necrotic lesions on the plant stem, which turn grayish and can lead to chlorosis, wilting, and eventual death of branches or the entire plant (MARQUEZ et al., 2021). The progression of lesions can weaken the stem, resulting in plant breakage. Severely infected plants die due to toxins produced by the fungus and the blockage of xylem vessels (BASANDRAI et al., 2021). Consequently, the ideal plant population is significantly reduced, directly impacting the crop yield (LINHARES et al., 2020; SOUZA et al., 2022).

The most important and cost-effective strategy for managing *M. phaseolina* in cowpea crops is the use of resistant cultivars, which does not significantly impact production costs and can be integrated with other control methods like crop rotation or biological control (LODHA; MAWAR, 2019; ARAÚJO et al., 2022). Several studies have reported the existence of cultivars of cultivated species with varying levels of resistance to *M. phaseolina*, including soybean (REZNIKOV et al., 2019), sorghum (CHATTANNAVAR; VINAYAKA, 2020), fava bean (GARCÍA et al., 2019), and mung bean (PANDEY et al., 2021). However, research on cowpea cultivars remains scarce.

In this context, the objective of this study was to assess the resistance response of cowpea accessions from municipalities of Rio Grande do Norte, Brazil, to *Macrophomina phaseolina*, aiming to identify those with potential for use in breeding programs.

MATERIAL AND METHODS

Two experiments were conducted in a greenhouse at the Departamento de Ciências Agronômicas e Florestais (DCAF) of the Universidade Federal Rural do Semi-Árido (UFERSA), in Mossoró, Rio Grande do Norte (RN), Brazil (5°12'48"S, 37°18'44"W, altitude of 37 meters). During the experimental period, maximum, minimum, and mean temperatures were 42.5, 30.2, and 36.3 ºC, respectively, whereas maximum, minimum, and mean relative air humidities were 59, 22, and 41%, respectively.

A total of 53 cowpea genotypes were evaluated: 25 accessions in Experiment 1 and 28 accessions in Experiment 2. These accessions were from the UFERSA germplasm bank and were originally collected from different municipalities in the state of Rio Grande do Norte. Both experiments were conducted in a completely randomized design with five replications, each replication consisting of a pot with one plant. Each treatment (accession) included one plant serving as a control, which remained uninoculated to compare against the inoculated plants. The subsequent procedures described were the same for both experiments. Thus, sequential numbers were assigned to the genotypes to facilitate their identification by municipality and mesoregion of origin (Table 1).

The fungal inoculum was isolated in the same year as the experiments to prevent loss of pathogenicity due to long storage periods combined with certain preservation methods. Therefore, cowpea plants showing disease symptoms caused by *Macrophomina phaseolina* were collected at the Rafael Fernandes experimental station of UFERSA, in the Alagoinha community, Mossoró. These plants were taken to the Laboratório de Microbiologia e Fitopatologia at UFERSA where the presence of the pathogen was confirmed and subsequently isolated.

The inoculation was performed following the infested toothpick method, which is more efficient in distinguishing resistant and susceptible genotypes (MEDEIROS et al., 2015; SOUZA et al., 2022). Petri dishes, along with approximately 1.0 cm-long pieces of toothpicks, were autoclaved on two consecutive days. Subsequently, a potato-dextrose-agar (PDA) culture medium with tetracycline (0.05 mg mL^{-1}) was poured. Once the medium solidified, mycelium discs from the cultured fungus were transferred onto Petri dishes with PDA culture medium, and toothpicks inserted vertically into the medium. The plates were incubated in a BOD chamber at 25 °C with a 12-hour photoperiod until the fungus had colonized all toothpicks, which occurred 10 days after subculturing.

Seeds of cowpea accessions were disinfected in a 1% NaClO (sodium hypochlorite) for 2 minutes, rinsed in running water, and dried under aseptic conditions for 45 minutes. They were sown in 0.8 kg plastic pots (11 cm in diameter), each containing 0.7 kg of autoclaved commercial substrate (Tropstrato®), using four seeds per pot. The commercial substrate had been autoclaved at 120 °C for 1 hour and then autoclaved again for 1 hour after 24 hours. The plants were thinned at 9 days after planting, leaving only one plant per pot.

The pathogen was inoculated 10 days after planting, when the plants had two true leaves. The fungus-colonized toothpicks were inserted into the plants at a height of 1.0 cm from the soil at a 45º angle. Autoclaved, non-infested toothpicks were inserted into the control plants.

Considering that irrigation is one of the main sources of variation in plant disease experiments (CAFÉ-FILHO; LOPES; ROSSATO, 2019; LODHA; MAWAR, 2019), plants were manually irrigated to ensure uniform water distribution across all plots, minimizing variation in disease progression. Water stress was imposed 3 days after inoculation (DAI) by gradually extending the watering intervals up to four days, based on visual assessment of plant moisture needs, until the evaluations (30 DAI).

Levels of resistance and susceptibility were determined using rating scale adapted from Abawi and Pastor-Corrales (1990): Score 1, plants with no visible symptoms; Score 3, plants with up to 25% of the area lesioned; Score 5, plants with up to 50% of the area lesioned; Score 7, plants with up to 75% of the area lesioned; Score 9, plants that died due to pathogen-induced damage.

The disease response was assessed based on the mean of the five replications for each treatment, classifying the cowpea accessions as follows: immune (means equal to 1); highly resistant (means between 1.1 and 3.9); moderately resistant (means between 4 and 6.9); susceptible (means between 7 and 8.9), and highly susceptible (means equal to 9).

Table 1. Evaluated cowpea accessions and their respective municipalities/mesoregions of collection. Rio Grande do Norte, Brazil.

Experiment 1			Experiment 2		
Accession	Municipality	Mesoregion	Accession	Municipality	Mesoregion
1	Macaíba	East	26	Alexandria	West
2	Martins	West	27	Angicos	Central
3	Monte Alegre	Agreste	28	Angicos	Central
4	Mossoró	West	29	Apodi	West
5	Nova Cruz	Agreste	30	Baraúna	West
6	Passa e Fica	Agreste	31	Boa saúde	Agreste
7	Pedra Preta	Central	32	Bodó	Central
8	Pedro Velho	East	33	Campo Grande	West
9	Santa Cruz	Agreste	34	Campo Redondo	Agreste
10	Santana do Mato	Central	35	Carnaúba dos Dantas	Central
11	São Bento do Trairi	Agreste	36	Carnaúba dos Dantas	Central
12	São José do Campestre	Agreste	37	Carnaubais	West
13	São José do Mipibu	East	38	Ceará Mirim	East
14	São Miguel	West	39	Currais Novos	Central
15	São Paulo do Potengi	Agreste	40	Currais Novos	Central
16	São Tomé	Agreste	41	Currais Novos	Central
17	Senador Eloi de Souza	Agreste	42	Currais Novos	Central
18	Serra do Mel	West	43	Felipe Guerra	West
19	Serrinha	Agreste	44	Itaú	West
20	Tangará	Agreste	45	Jaçanã	Agreste
21	Tenente Ananias	West	46	Japi	Agreste
22	Tenente Laurentino Cruz	Central	47	José da Penha	West
23	Vera Cruz	Agreste	48	Lagoa D'anta	Agreste
24	Umarizal	West	49	Lagoa de Pedra	Agreste
25	Upanema	West	50	Lagoa Salgada	Agreste
			51	Lajes	Central
			52	Luís Gomes	West
			53	Macaíba	East

Symptomatic plants during evaluation were taken to the Laboratório de Microbiologia e Fitopatologia at UFERSA to isolate and confirm the presence of *M. phaseolina* in the lesions. Fragments were collected from the area between diseased and healthy tissue, subjected to surface disinfestation in 70% alcohol for 30 seconds, and 2% sodium hypochlorite for one minute, and then washed in sterilized distilled water. Five fragments were then transferred to Petri dishes containing PDA medium supplemented with tetracycline (0.05 g L^{-1}). After five days, the plates were examined under an optical microscope (Olen 1600x), and presence of the fungus was confirmed by morphological characterization.

The raw data were transformed using $\sqrt{(x + 0.5)}$ to meet the assumption of homogeneity of residual variances for analysis of variance (ANOVA). The transformed data were subjected to ANOVA, and the statistical means of the different accessions were grouped using the Scott-Knott test at a 5% confidence level. Accuracy was estimated as $A = (1 - 1 / F)^{1/2}$, where F corresponds to the F-test value for genotypes (RESENDE; DUARTE, 2007). All statistical analyses were carried out using the program R 4.3.2 (R CORE TEAM, 2023).

RESULTS AND DISCUSSION

According to the ANOVA results, significant differences at a 5% significance level were found for both experiments, indicating that at least two cowpea accessions in each experiment differed significantly in their resistance to *Macrophomina phaseolina* (Table 2). This result denotes the presence of genetic variability among the evaluated cowpea accessions. All controls showed no disease symptoms.

Table 2. Analysis of variance for the effect of inoculating different cowpea accessions with *Macrophomina phaseolina* in two experiments.

SV	Experiment 1		Experiment 2	
	DF	MS	DF	MS
Treatment	25	0.2538*	28	1.2423*
Residual	104	0.0959	116	0.2295
CV (%)		14.14		16.34
SA		0.78		0.90

SV: Source of variation; DF = degrees of freedom; MS = mean squares; CV = coefficient of variation; SA = selective accuracy.

Table 2 shows two measures of experimental accuracy for both experiments: the coefficient of variation and selective accuracy. According to Pimentel-Gomes (1985), the coefficient of variation (CV) can be classified as low (< 10%), medium (10% to 20%), high (20% to 30%), or very high (> 30%). The CV classification is inversely related to the experimental accuracy; thus, the higher the CV, the lower the experimental accuracy. Therefore, based on the CV, both experiments showed medium experimental accuracy.

Selective accuracy (SA) ranges from 0 to 1, reflecting the correlation between the true genotypic value of the genetic treatment and the predicted value based on experimental data (RESENDE; DUARTE, 2007). SA can be classified as very high (> 0.90), high ($0.70 \leq SA < 0.90$), moderate ($0.50 \leq SA < 0.70$), or low (< 0.50). The estimated SA values in the present study were high, 0.78 (Experiment 1) and 0.90 (Experiment 2). This indicates a high experimental accuracy and a strong correlation between the estimated and actual resistance levels expressed by the genotypes (Table 2). Table 3 shows the grouping of genotypes by the Scott-Knott test ($p<0.05$) for both experiments.

Three groups were formed in Experiment 1 regarding disease response (Table 3): Group 1 consisted of highly resistant accessions (6, 14, and 17); Group II consisted of moderately resistant accessions (5, 12, 1, 11, 4, and 21); and Group III consisted of genotypes classified as moderately resistant (16, 24, 18, 13, and 28), susceptible (15, 8, 9, 25, 2, 19, 20, and 23), and highly susceptible (7, 10, 3, and 22).

In Experiment 2, two significantly different groups were identified: Group I comprised highly resistant (46, 42, 50, 48, 43, and 30) and moderately resistant accessions (44, 34, 45, and 51); Group II consisted of the remaining genotypes, classified as moderately resistant (38, 29, 52, 36, 49, 37, 33, 41, 39, 53, 32, 47, 31, and 28), susceptible (40, 26, and 35), and highly susceptible (27). The formation of distinct groups in both experiments confirms the presence of genetic variability among the evaluated cowpea accessions in terms of response to *M. phaseolina*.

Araújo et al. (2022) evaluated the response of 100 cowpea lines to two *M. phaseolina* isolates (59 and CMM 2106) under greenhouse conditions and identified two distinct groups. However, three genotype groups were identified in Experiment 2 of the in the present study. Despite using the same inoculation method, differences between these studies may be due to variations in evaluation methods and genotypes.

Another factor that may have affected the results of the present study was the 30-day interval between inoculation and evaluation, which was used to ensure the disease expression. Ishikawa et al. (2018) investigated the resistance of soybean cultivars to *M. phaseolina* and reported that the first symptoms in the root system appeared 30 days after inoculation. More severe symptoms, such as leaf necrosis and plant death, were observed during the evaluations in the present study, supporting the reliability of the results.

Overall, 53% of the evaluated cowpea accessions were classified as moderately resistant, while 21% and 9% were classified as susceptible and highly susceptible to the pathogen, respectively. Only 17% of the accessions (6, 14, 17, 30, 42, 43, 46, 48, and 50) exhibited high resistance, with means below 3.9 (Table 3; Figure 1). These results highlight the challenge of obtaining genetic materials highly resistant to *M. phaseolina*, possibly due to the complexity of the pathogenesis process.

There were nine highly resistant accessions, one (accession 42) was collected in the Central mesoregion, three (14, 30, and 43) in the West mesoregion, and five (6, 17, 46, 48, and 50) in the Agreste mesoregion (Table 3). This denotes a predominance of resistant alleles in the Agreste mesoregion of Rio Grande do Norte and provide valuable information for future searches for *M. phaseolina*-resistant materials within the cowpea germplasm. Contrastingly, one of the five highly susceptible accessions was from Monte Alegre, in the Agreste mesoregion (accession 3), while the others (7, 10, 22, and 27) were from the Central mesoregion. This indicates a predominance of genes conferring susceptibility to *M. phaseolina* in cowpea genotypes grown in the Central mesoregion of Rio Grande do Norte (Figure 2).

Different responses of bean genotypes to *M. phaseolina* have been reported in other studies (GARCÍA et al., 2019; ARAÚJO et al., 2022). Araújo et al. (2022) evaluated the response of 100 cowpea genotypes to two *M. phaseolina* isolates under greenhouse conditions and found resistance in 26 lines: 15 were resistant to isolate 59, and 11 to isolate CMM 2106. García et al. (2019) assessed the resistance response of 37 *Phaseolus lunatus* accessions to *M. phaseolina* in protected-environment experiments at the Universidade Federal do Piauí (UFPI), PI, Brazil, and identified only three accessions resistant to the pathogen isolate COUFPI 06.

Table 3. Mean resistance response of cowpea accessions to *Macrophomina phaseolina* in two experiments.

Experiment 1				Experiment 2			
Accession	Mean	SE	Response	Accession	Mean	SE	Response
6	3.0 a	0.0	HR	42	3.0 a	0.0	HR
14	3.0 a	1.4	HR	46	3.0 a	0.0	HR
17	3.0 a	0.0	HR	50	3.4 a	0.4	HR
5	4.2 b	1.2	MR	30	3.8 a	0.5	HR
12	4.2 b	1.2	MR	43	3.8 a	0.8	HR
1	4.6 b	1.2	MR	48	3.8 a	0.8	HR
11	4.6 b	0.7	MR	34	4.2 a	0.5	MR
4	5.4 b	1.5	MR	44	4.2 a	0.5	MR
21	5.4 b	1.5	MR	45	4.6 a	1.2	MR
16	6.2 c	1.4	MR	51	4.6 a	1.0	MR
18	6.2 c	1.4	MR	38	4.6 a	0.4	MR
24	6.2 c	1.4	MR	29	5.0 b	0.9	MR
13	6.6 c	1.5	MR	36	5.4 b	1.2	MR
8	7.0 c	1.3	S	52	5.4 b	1.5	MR
9	7.0 c	1.9	S	33	5.8 b	1.4	MR
15	7.0 c	1.3	S	37	5.8 b	1.4	MR
2	7.8 c	1.2	S	39	5.8 b	1.4	MR
25	7.8 c	1.2	S	41	5.8 b	1.4	MR
19	8.2 c	1.2	S	49	5.8 b	1.4	MR
20	8.2 c	0.8	S	32	6.2 b	1.2	MR
23	8.6 c	0.4	S	47	6.2 b	1.2	MR
3	9.0 c	0.0	HS	53	6.2 b	1.4	MR
7	9.0 c	0.0	HS	31	6.6 b	1.0	MR
10	9.0 c	0.0	HS	28	6.6 b	1.2	MR
22	9.0 c	0.0	HS	40	7.0 b	1.3	S
				26	7.8 b	1.2	S
				35	8.2 b	0.8	S
				27	9.0 b	0.0	HS

Means followed by the same letter indicate a statistically homogeneous group according to the Scott-Knott test ($p < 0.05$); HR = highly resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible; SE = standard error of the mean.

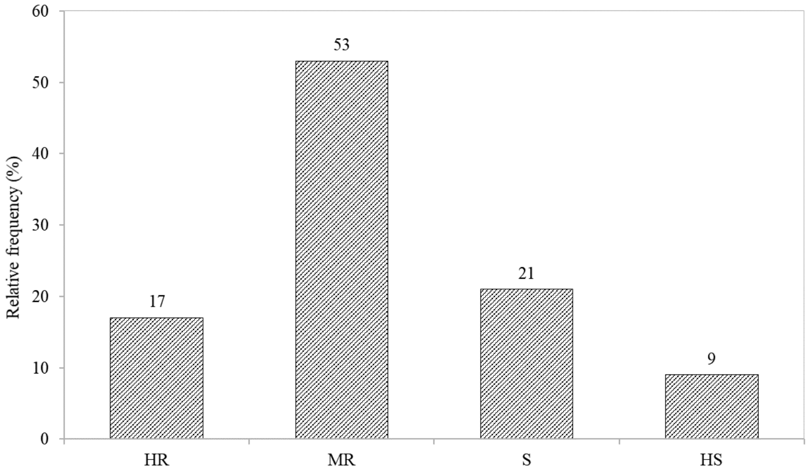

Figure 1. Distribution of cowpea accessions by disease response class after inoculation with *Macrophomina phaseolina*: HR = highly resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible.

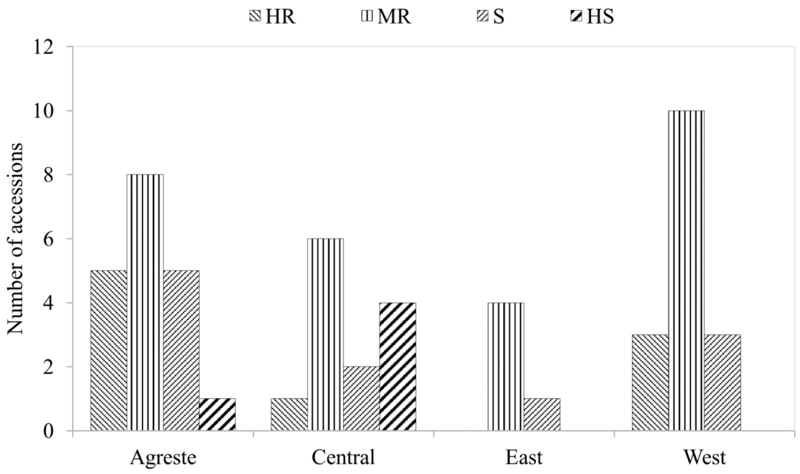

Figure 2. Distribution of cowpea accessions by disease response class after inoculation with *Macrophomina phaseolina,* based on the collection mesoregion in Rio Grande do Norte, Brazil: HR = highly resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible.

Silva Filho et al. (2013) studied the severity of *M. phaseolina* in 30 cowpea accessions collected from different municipalities of Rio Grande do Norte and found 90% of the accessions susceptible to the fungus, while three accessions (A6, A17, and A22) were moderately resistant. Although the geographic collection region was the same as in the present study, the results differed markedly between the studies. For example, genotypes collected from the municipalities of Currais Novos and São Miguel showed different responses to *M. phaseolina* in the present study; they were classified as susceptible (accession 40), moderately resistant (accessions 39 and 41), and highly resistant (42 and 14). However, Silva Filho et al. (2013) reported high susceptibility to the pathogen for accessions from these municipalities. These divergent results can be attributed to the use of different *M. phaseolina* isolates for inoculation.

Another possible explanation for these results is the exploration of accessions from family farming areas, which are expected to exhibit high genetic variability due to individual selection practices of each farmer and the small rate of cross-pollination, even among self-pollinating crop species such as beans (SANTOS et al., 2022).

Several studies have reported a scarcity of resistant genotypes to *M. phaseolina* that could be used in bean breeding programs (GARCÍA et al., 2019; LODHA; MAWAR, 2019; ARAÚJO et al., 2022). Additionally, Lima et al. (2017) state that cowpea resistance to *M. phaseolina* is controlled by two additive dominant genes, which allows for a relatively straightforward breeding program.

Therefore, the cowpea accessions identified as resistant and highly resistant to *M. phaseolina* in the present study should undergo further testing to confirm these results. They could then be included in breeding programs focused on thoroughly exploring this observed resistance. New collections of genotypes are recommended, particularly in regions where resistant genotypes have been identified. The seed-saving practices of family farmers may have generated many genotypes with potential for inclusion in breeding programs focused on resistance to *M. phaseolina*.

CONCLUSIONS

Nine cowpea accessions (6, 14, 17, 30, 42, 43, 46, 48, and 50) were identified as highly resistant to *Macrophomina phaseolina*, indicating their potential for inclusion in breeding programs targeting pathogen resistance. A predominance of genes conferring resistance to *M. phaseolina* was observed in cowpea accessions from the Agreste mesoregion of Rio Grande do Norte, Brazil.

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