

## IDENTIFICATION OF SOURCES OF RESISTANCE AGAINST CHARCOAL ROT IN COWPEA<sup>1</sup>

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**ABSTRACT** - Cowpea [*Vigna unguiculata* (L.) Walp.] is an important socioeconomic crop in Brazil, mainly in the Northeast and more recently in the Midwest of Brazil. Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid, is an important disease in semiarid regions, where edaphoclimatic conditions are favorable to the development of disease. The aim of this study was to evaluate the response of 100 cowpea lines to two isolates of *M. phaseolina*. The experiments were conducted in a completely randomized design, with five replications (two plants per pot). The main variables evaluated were lesion length and relative growth compared to control (RGCC). Among the evaluated accessions, 15% of the lines were resistant to isolate 59 and 11% of the lines were resistant to isolate CMM 2106 of *M. phaseolina*. Therefore, these accessions can be used as a source of resistance to *M. phaseolina* by farmers directly as new cultivars or in future hybridizations of cowpea genetic breeding programs.

**Keywords:** Cowpea breeding. Germoplasm. *Macrophomina phaseolina*. Genetic resistance. *Vigna unguiculata*.

## IDENTIFICAÇÃO DE FONTES DE RESISTÊNCIA À PODRIDÃO CINZENTA DO CAULE EM FEIJÃO-CAUPI

**RESUMO** – O feijão-caupi [*Vigna unguiculata* (L.) Walp.] é uma importante cultura socioeconomicamente, principalmente na região nordeste e, mais recentemente no centro-oeste do Brasil. Podridão cinzenta do caule causada pelo fungo *Macrophomina phaseolina* (Tassi) Goid, é importante em regiões semiáridas, onde as condições edafoclimáticas são favoráveis para o desenvolvimento da doença. O objetivo deste estudo foi avaliar a reação de 100 linhagens de feijão-caupi à dois isolados de *M. phaseolina*. Os experimentos foram conduzidos em delineamento inteiramente casualizado com cinco repetições (duas plantas por parcela). As principais variáveis analisadas foram comprimento da lesão e crescimento relativo a testemunha. Entre os acessos avaliados, 15% das linhagens foram resistentes ao isolado 59 e 11% foram resistentes ao isolado CMM 2106 de *M. phaseolina*. Portanto, os acessos podem ser utilizados como fontes de resistência a *M. phaseolina* pelos agricultores diretamente como cultivares ou em futuros cruzamentos de programas de melhoramento genético do feijão-caupi.

**Palavras-chave:** Melhoramento do feijão-caupi. Germoplasma. *Macrophomina phaseolina*. Resistência genética. *Vigna unguiculata*.

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## INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] has great socio-economic importance to Brazil, as it contributes to the generation of jobs and income for thousands of people, especially for family farms. The world production of cowpea reaches 7,233,408 tons in an area of 12,496,305 ha. Nigeria is the largest producer, with 2,577,393.32 tons (FAO, 2018; BOUKAR et al., 2018). Brazil has an area of 1,352,500 ha, a productivity of 494 kg ha<sup>-1</sup>, and a total production of 668,000 tons of cowpea (CONAB, 2021). The largest national production is in the state of Mato Grosso, followed by the states of Ceará, Bahia, and Piauí (CONAB, 2021). The low productivity of cowpea crop in Brazil is owed to the non-use of technologies and inputs for production because the non-use of certified seeds, inadequate cultural treatments, and occurrence of pests and diseases affect crops.

Charcoal rot is among the main diseases that affect cowpea crops, and it is caused by the fungus *Macrophomina phaseolina*. The fungus is a soil-borne pathogen that attacks the root system of several host plants and infects their vascular bundles, making translocation of nutrients and water difficult. The fungus has resistance structures known as micro-sclerotia, which remains after long periods of crop rotation, and once they are introduced into a cultivated area, they are hardly eradicated (CORREIA; MICHEREFF, 2018).

Few sources of resistance to *M. phaseolina* have been identified in cowpea genotypes. Muchero et al. (2011) evaluated 14 genotypes evaluated under moderate water stress conditions, and there were different genotypic responses to *M. phaseolina* infection. The genotypes IT98K-499-39, Suvita 2, IT93K-503-1, and Mouride were found to be the most resistant genotypes to the development of the disease, with mortality below 10%.

In Brazil, studies carried out by Noronha et al. (2010) and Lima et al. (2012), through severity analysis and comparison of lesion averages with the aid of a reference table, revealed some highly resistant (HR) and moderately resistant (MR) cowpea genotypes for *M. phaseolina*. Subsequently, Lima et al. (2017) used these genotypes previously classified as HR and MR: IT98K-1092-1, MNC 03-761F-1, MNC 02-675F-4-9, MNC 02-675F-4-10, and MNC 02-675-9-2 with other genotypes resistant to *Thanatephorus cucumeris*. Genotypes were crossed using the partial diallel method to obtain promising lines that are also resistant to both pathogens. Genotypes moderately resistant to the

pathogen were also found, and they include: MNCO4-769F-48, MNCO4-769F-30, MNCO4-769F-46, MNCO4-769F-45, MNCO4-774F-90, MNCO4-769F-62, and MNCO4-782F-104; these moderately resistant genotypes can be used as potential sources of resistance in cowpea breeding program (AMARAL; NORONHA, 2016).

The identification of sources of resistance to charcoal rot will enable the development of cultivars that are resistant to *M. phaseolina*, contributing to the sustainability of cowpea production chain and providing measures for producers to control this disease with reduced cost. The objective of this study was to identify lines that are resistant to charcoal rot, and the aim was to select parents for breeding programs for genetic resistance.

## MATERIALS AND METHODS

The experiments were carried out from July 2018 to August 2019 in the Plant Pathology Laboratory and the greenhouse of Universidade Federal do Vale do São Francisco (UNIVASF) in Petrolina, Pernambuco, Brazil. The experimental design was a completely randomized design (CRD). One hundred lines of cowpea developed by Embrapa Meio-Norte (Teresina, Piauí, Brazil) were used (Table 1). The experiment was divided into stages, each stage containing 20 lines per experiment. Each plot was a pot with two plants, with five replications (pot). For each line, there was a control (toothpick inoculated without the fungus). Isolates 59 of *M. phaseolina* were obtained from an infested area from the field of Agricultural Sciences Campus - Univasf and Isolate CMM 2106 was obtained from the Phytopathogenic Fungal Culture Collection of Professor Maria Menezes from Universidade Federal Rural de Pernambuco. These isolates were selected because they were more aggressive in previous studies (SOUZA et al., 2022).

### Seeding of cowpea lines

Two seeds of each cowpea line were sown in plastic pots (1 L) containing substrate and soil mixture (1:1) previously autoclaved at 120 °C for 1 h twice (2 days); the sowing was done in greenhouse underscreens (Sombrite®), which retained 50% brightness. After sowing, the pots were irrigated twice daily until inoculation. After inoculation, the pots were irrigated once a day with a fixed volume of 180 ml of water.

**Table 1.** One hundred lines of cowpea developed by Embrapa Meio-Norte and their codes used in the study.

Line	Code	Line	Code
L1	MNC 11-1005E-20	L51	MNC 11-2023E-28
L2	MNC 11- 1005E-28	L52	MNC 11-1023E-60
L3	MNC 11-1005E-37	L53	MNC 11-1023E-48
L4	MNC 11- 1006E-10	L54	MNC 11-1023E-26
L5	MNC 11- 1008E-9	L55	MNC 11-1024E-18
L6	MNC 11- 1012E-7	L56	MNC 11-1024E-1
L7	MNC 11- 1013E-18	L57	MNC 11-1024E-16
L8	MNC 11- 1013E-33	L58	MNC 11-1026E-15
L9	MNC 11- 1013E-27	L59	MNC 11-1026E-5
L10	MNC 11- 1013E-8	L60	MNC 11-1026E-19
L11	MNC 11-1013E-16	L61	MNC 11-1028E-16
L12	MNC 11-1013E-25	L62	MNC 11-1028E-34
L13	MNC 11-1015E-2	L63	MNC 11-1028E-95
L14	MNC 11-1015E-5	L64	MNC 11-1029E-9
L15	MNC 11-1015E-7	L65	MNC 11-1029E-13
L16	MNC 11-1015E-15	L66	MNC 11-1029E-15
L17	MNC 11-1015E-28	L67	MNC 11-1031E-5
L18	MNC 11-1015E-29	L68	MNC 11-1031E-8
L19	MNC 11-1015E-35	L69	MNC 11-1031E-9
L20	MNC 11-1016E-12	L70	MNC 11-1031E-11
L21	MNC 11-1016E-16	L71	MNC 11-1031E-13
L22	MNC 11-1017E-3	L72	MNC 11-1031E-15
L23	MNC 11-1017E-8	L73	MNC 11-1033E-14
L24	MNC 11-1017E-10	L74	MNC 11-1033E-30
L25	MNC 11-1017E-26	L75	MNC 11-1034E-1
L26	MNC 11-1017E-30	L76	MNC 11-1034E-2
L27	MNC 11-1017E-31	L77	MNC 11-1036E-3
L28	MNC 11-1017E-33	L78	MNC 11-1036E-4
L29	MNC 11-1017E-37	L79	MNC 11-1037E-1
L30	MNC 11-1018E-2	L80	MNC 11-1036E-5
L31	MNC 11-1018E-4	L81	MNC 11-1037E-4
L32	MNC 11-1018E-17	L82	MNC 11-1037E-5
L33	MNC 11-1018E-20	L83	MNC 11-1039E-4
L34	MNC 11-1019E-8	L84	MNC 11-1042E-1
L35	MNC 11-1019E-12	L85	MNC 11-1042E-4
L36	MNC 11-1019E-15	L86	MNC 11-1043E-4
L37	MNC 11-1019E-16	L87	MNC 11-1044E-8
L38	MNC 11-1019E-40	L88	MNC 11-1046E-3
L39	MNC 11-1019E-46	L89	MNC 11-1046E-8
L40	MNC 11-1020E-29	L90	MNC 11-1046E-9
L41	MNC 11-1020E-18	L91	MNC 11-1047E-4
L42	MNC 11-1020E-16	L92	MNC 11-1047E-6
L43	MNC 11-1020E-5	L93	MNC 11-1048E-2
L44	MNC 11-1020E-16	L94	MNC 11-1052E-3
L45	MNC 11-1020E-6	L95	MNC 11-1052E-4
L46	MNC 11-1021E-27	L96	MNC 11-1053E-3
L47	MNC 11-1021E-17	L97	BRS Tumucumaque
L48	MNC 11-1022E-1	L98	BRS Pajéu
L49	MNC 11-1022E-9	L99	Inmuna
L50	MNC 11-1022E-58	L100	Pingo-de-Ouro 1-2

### Inoculum production

The infested toothpick method was used to inoculate *M. phaseolina* isolates (COHEN; ELKABETZ; EDELSTEIN, 2016). Isolates 59 and CMM 2106 were plated in Petri dishes containing potato dextrose agar (PDA) medium and maintained in a biological oxygen demand (BOD) at 25 °C for seven days for mycelium growth. Afterward, isolates were plated in new Petri dishes containing PDA medium and toothpick previously autoclaved. Petri dishes with a toothpick and the isolates were maintained in a BOD for 15 days until the fungus colonized all the surfaces of the plate and toothpick.

### Inoculation using infested toothpick method

The inoculated toothpicks were used for plant inoculation 15 days after sowing by stabbing the crown approximately three cm above the ground. Plant height (cm) was evaluated after inoculation. Three evaluations were made every three days by measuring the lesion length (cm) caused by the fungus and plant height (cm) with a ruler. The variables obtained were lesion length, plant height, area under the disease progress curve (AUDPC), severity, and relative growth compared to the control (RGCC). The length of the lesion was converted to a note as described by You, Colmer and Barbetti (2011) where the plants were assessed for incidence/severity of disease using 0 to 5 scale for lesions and discoloration on the hypocotyls; where: 0 = no disease; 1 = 1 cm lesion/discoloration; 2 = >1 to 1.5 cm; 3 = >1.5 –3 cm; 4 = >3 to 5 cm lesion/discoloration or plant collapsed.

### Description of the variables

1) Height Difference (HD):  $HD = H_f - H_i$ , where  $H_f$  = Average final plant height (last evaluation),  $H_i$  = average initial plant height (first evaluation).

2) Area Under the Disease Progress Curve (AUDPC) =  $\sum Y_f + Y_i \times (X)$ , where  $Y_f$  is the average final lesion length,  $Y_i$  is the average initial lesion length,  $X$  is the number of days between first and last evaluations.

3) Severity (SEV):  $SEV = Y(H) \times 100$ , where:  $Y$  = Average final lesion length,  $H$  = Average final height

4) Relative Growth Compared to Control (RGCC):  $RGCC = \frac{\sum H_f - H_i}{\sum H_{cf} - H_{ci}} \times 100$ , where  $H_f$  is the average final height,  $H_i$  is the average initial height,  $H_{cf}$  is the average final length of the control,  $H_{ci}$  is the average initial length of the control. Control is the treatment (accession)

inoculated without the isolates of *M. phaseolina*.

### Statistical analysis

Data from each experiment using 20 lines were subjected to analysis of variance, and the means were grouped using the Scott-Knott test ( $\alpha=0.05$ ) of the SISVAR software (FERREIRA, 2011). Assumptions underlying the analysis of variance has been checked.

## RESULTS AND DISCUSSION

There was a significant difference ( $P < 0.05$ ) between lines 1 to 20 for the variable HD (Table 2) when inoculated with isolate 59 and CMM 2106, with averages ranging from 1.97 cm to 7.80 cm and 3.14 cm to 10.65 cm, respectively. Lines 61 to 80 also had a significant effect ( $P < 0.05$ ) on HD when inoculated with isolate 59, with averages ranging from 6.55 cm to 8.75 cm. However, HD was not different for lines 61 to 80 when inoculated with isolate CMM2106, with averages ranging from 4.65 cm to 10.50 cm. Among the lines that had a significant effect on HD when inoculated with isolate 59, the ones with the highest means were L1, L6, L7, L8, L9, L10, L11, L12, L20, L61, L64, L65, L71, L72, L73, L74, L75, L76, L77, L78, L79, and L80. For isolate CMM 2106, only lines L3, L10, L18, L25, L27, L29, L30, L31, L32, and L40 had the highest means. It has been reported in literature that plants, which are infected during their early stages of development, are smaller than normal in soybean crop (ISHIKAWA et al., 2018). Plant growth can be affected by the pathogen because *M. phaseolina* enters the host tissue by dissolving the cell wall through the secretion of toxins or enzymes, making plant development difficult, which consequently results in small plant height (MEDEIROS et al., 2015).

No difference was observed between lines for AUDPC ( $P > 0.05$ ). Severity for both isolates was only different in the experiment of lines 1 to 20. For isolate 59, severity ranged from 3.04% to 12.60% with line 11 having the highest mean severity (12.60%), whereas for isolate CMM 2106, the mean severity ranged from 1.79% to 14.31% with lines L1, L5, L7, L10, L11, L12, L14, L15, L16, L17, L19, and L20 having the highest averages ranging from 6.44% to 14.31%. Thus, line 11 had one of the highest averages of severity, and it can be characterized as a susceptible line for both isolates 59 and CMM 2106. The absence of immunity to this pathogen is common among legumes, as it is a generalist species (GARCÍA et al., 2019).

**Table 2.** Average Height Difference (HD) to cowpea lines 1-20 inoculated with isolates 59 and CMM 2106.

Line	Isolate 59	Line	Isolate CMM 2106
L15	1.97 a*	L16	3.14 a
L16	2.24 a	L8	3.30 a
L18	2.43 a	L1	3.83 a
L13	2.87 a	L4	3.84 a
L4	3.00 a	L17	4.06 a
L2	3.02 a	L14	4.10 a
L19	3.29 a	L15	4.33 a
L3	3.41 a	L6	4.35 a
L17	3.83 a	L9	4.58 a
L5	4.29 a	L11	4.71 a
L12	5.66b	L7	4.84 a
L1	5.73b	L5	5.02 a
L9	5.75b	L13	5.10 a
L7	5.84b	L20	5.44 a
L20	6.40b	L19	5.62 a
L11	7.38b	L12	5.65 a
L6	7.38b	L2	6.43 a
L8	7.40 b	L18	7.80 b
L10	7.80 b	L10	8.10 b
L14	-	L3	10.65 b

\*Means followed by the same letter, belonging to the same group and do not differ by the Scott-Knott test at 5% probability.

For length of lesion, no significant difference was observed between lines for most experiments ( $P > 0.05$ ). The average length of lesion for isolate 59 ranged from 0.86 to 1.88 cm for lines 1 to 20, 0.70 to 1.66 cm for lines 21 to 40, 0.52 to 0.91 cm for lines 41 to 60, and 0.71 to 1.46 for lines 61 to 80, and 0.48 to 1.02 cm for lines 81 to 100. For isolate CMM 2106, average lesion ranged from 1.20 to 4.0 cm for lines 1 to 20, 1.0 to 1.80 cm for lines 21 to 40, 0.40 to 2.80 cm for lines 41 to 60, and 1.20 to 2.80 cm for lines 81 to 100; for lines 61 to 80, only the score of the lesion was made. The hot and dry weather conditions (temperatures between 28 °C and 35 °C) are favourable to the development of *M. phaseolina* (CRUCIOL; COSTA, 2018). Recently, a study carried out with melon accessions showed that increase in temperature increased the severity of symptoms caused by *M. phaseolina* in most genotypes evaluated, thus the effect of the temperature on disease severity should be considered during the genetic studies (LINHARES et al., 2020). According to the UNIVASF Meteorology laboratory, the temperatures during the evaluation periods of the experiments were within ideal conditions for the development of the pathogen, with a maximum average temperature of 32.2 °C and a minimum of 21.1 °C.

Analysis of lesions was performed by converting the lesion length into notes according to the scale of You, Colmer and Barbetti (2011). There were significant differences between lesion scores in

lines 1 to 20 and 81 to 100. For lines 1 to 20, there was variability for both isolates (Table 3).

The highest average lesion scores were observed in CMM 2106 isolate, and it was considered the most aggressive isolate. Isolate CMM 2106 was obtained from the cowpea host plant, while isolate 59 was obtained from the common bean host plant (SOUZA et al., 2022). For isolates 59 and CMM 2106, 9 and 8 lines were obtained, respectively, with the lowest average scores of the injury caused by *M. phaseolina* (Table 3). Line 8 was the only one to be in the group with the lowest average scores for both isolates; therefore, it can be a promising source of resistance. The average scores for lines 81 to 100, inoculated with isolate 59, ranged from 1.00 to 1.75. Lines L81, L83, L84, L86, L87, L88, L89, L90, L91, L92, L93, L94, L95, L96, L97, L98, and L99 had the lowest averages. There was no variation for CMM 2106 in this step. For the lines evaluated during the other stages, there was no significant difference for both isolates ( $P > 0.05$ ).

There was a significant difference in relative growth compared to control (RGCC) for both isolates. The lines considered most resistant were those which growth was not affected by inoculation. For lines 1 to 20, seven lines were considered resistant because they had an RGCC greater than 100% for isolate 59 (Figure 1 A). For isolate CMM 2106, 2 lines were considered resistant, which RGCC was higher by 206.19% compared to control (Figure 1B).

**Table 3.** Average lesion scores for charcoal rot by inoculation of isolates 59 and CMM 2106 in cowpea lines.

Line	Isolate 59	Line	Isolate CMM 2106
L8	1.20 a*	L8	1.20 a
L10	1.20 a	L17	1.60 a
L18	1.20 a	L6	1.80 a
L4	1.20 a	L2	2.00 a
L12	1.40 a	L15	2.00 a
L19	1.40 a	L3	2.20 a
L20	1.60 a	L14	2.20 a
L1	1.80 a	L13	2.40 a
L5	1.80 a	L7	2.60 b
L7	2.00 b	L5	2.80 b
L3	2.00 b	L4	2.80 b
L2	2.00 b	L18	2.80 b
L17	2.40 b	L16	3.20 b
L15	2.40 b	L1	3.20 b
L9	2.40 b	L19	3.20 b
L6	2.40 b	L12	3.20 b
L13	2.60 b	L9	3.40 b
L16	2.60 b	L10	3.40 b
L11	3.00 b	L11	3.40 b
L14	-	L20	4.00 b

\*Means followed by the same letter, belonging to the same group and do not differ by the Scott-Knott test at 5% probability.

Of the lines 21 to 40 evaluated for isolate 59, 7 resistant lines were considered to have RGCC greater than 101% (Figure 1C). For CMM 2106 isolate, only one line was resistant (Figure 1D). In lines 41-60, 3 resistant lines were observed for isolate 59 (Figure 1E) and had RGCC greater than 184%. Regarding the CMM 2106 isolate, 4 resistant lines were considered (Figure 1F).

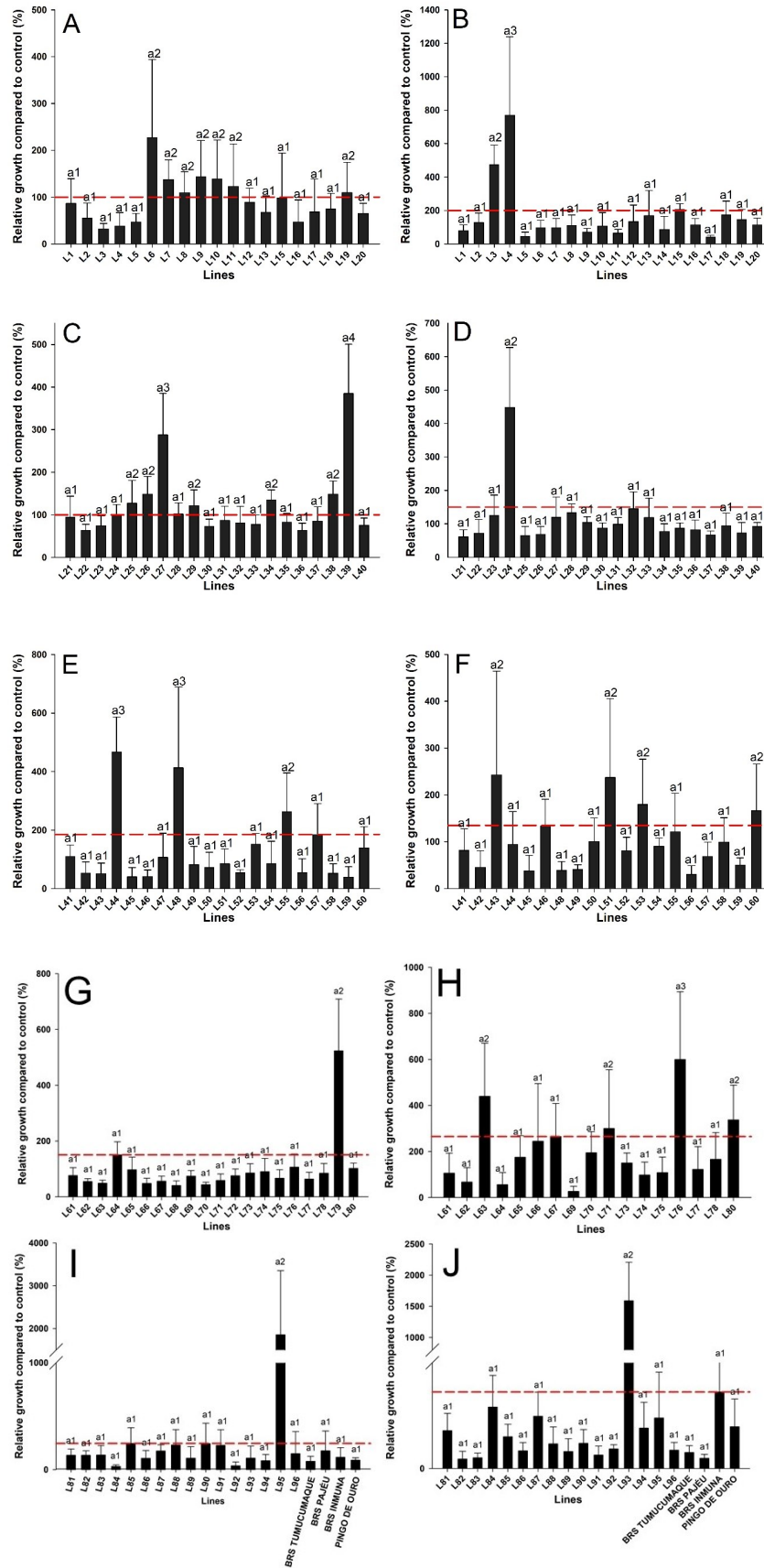
Among lines 61 to 80, one resistant line was identified, with an RGCC greater than 150% for isolate 59 (Figure 1G). For isolate CMM 2106, 4 resistant lines presented an RGCC of 265% greater than the control (Figure 1H). For lines 81 to 100, only one resistant line was identified for both isolates 59 (Figure 1I) and CMM 2106, with RGCC greater than 245% and 286.85%, respectively (Figure 1J). Among these lines, two are commercial cultivars: BRS Tumucumaque and BRS Pajéu, and were susceptible to charcoal rot for both isolates.

The cultivar BRS Tumucumaque showed no resistance to *M. phaseolina* (LIMA et al., 2017). However, the recombination of BRS Tumucumaque with other accessions generated progenies with resistance alleles to *M. phaseolina*. It has been reported that the use of hybridization in breeding programs allows recombination, which generates greater genetic variability in plants, resulting in highly resistant or moderately resistant strains. Lima et al. (2017) used combinations of commercial cultivars BR 14-Mulato, BRS Tumucumaque, and BRS Guariba with the lines IT98K-1092-1, MNC 03

-761F-1, MNC 02-675F-4-9, MNC 02- 675F-4-10, and MNC 02-675-9-2 to produce populations with great potential and obtained cultivars resistant to *M. phaseolina*.

There are still few reports of cowpea lines that are resistant to *M. phaseolina*. Some studies reported genotypes that are moderately resistant or highly resistant to this pathogen. Muchero et al. (2011) evaluated 14 cowpea genotypes under moderate water stress conditions and identified them in 4 genotypes: IT98K-499-39, Suvita 2, IT93K-503-1, and Mouride as a source of resistance to *M. phaseolina*, which exhibited the highest levels of resistance to charcoal rot. In another study, of the 40 cowpea genotypes evaluated, none showed high resistance; however, a group of 7 genotypes (MNCO4-769F-48, MNCO4-769F-30, MNCO4-769F-46, MNCO4-769F -45, MNCO4-774F-90, MNCO4-769F-62, and MNCO4-782F-104) were moderately resistant to charcoal rot (AMARAL; NORONHA 2016).

The lesion score and RGCC were considered the best forms of assessment to allow the identification of resistant accessions. Thus, lines classified as resistant according to their RGCC for isolate 59 of *M. phaseolina* were L6, L7, L8, L9, L10, L11, L19, L25, L26, L27, L29, L34, L38, L39, L44, L48, L55, L79, and L95. For strain CMM 2106, the lines L3, L4, L24, L43, L51, L53, L60, L63, L71, L76, L80, and L93 were considered resistant.



**Figure 1.** Relative growth compared to control (RGCC) for 100 lines inoculated with isolated 59 (A, C, E, G, I) and CMM2106 (B, D, F, H, J). Red line is the limit between the groups, below red line was considered susceptible. Means followed by same letter belonging to the same group and do not differ by the Scott-Knott test at 5% probability. Bar: Standard error.

Lines 8, 10, and 19 had the lowest average scores (Table 2) with the highest RGCC greater than 100% for isolate 59 (Figure 1A). The other lines had the lowest average scores and had their growth affected by the pathogen, that is, their RGCC was less than 100%. However, lines 6, 7, 9, and 11 were ranked to have the highest average of lesions, and their growths were not affected. For the isolate CMM 2106, only line 3 (Table 2) has the lowest averages of lesion score and was in the group that did not have its growth affected by the pathogen, with an RGCC greater than 206% (Figure 1). Lines 2, 13, and 15 had lower average scores and their RGCC ranged from 128.6% to 206%. Even though line 4 belonged to the highest average score, its height was not affected by the pathogen.

For both isolates, line 8 obtained the lowest average lesion scores; the line also had an RGCC of 109.63% for isolate 59 and 110% for isolate CMM 2106. Line 11 obtained one of the highest averages of the lesion scores and had greater severity for both isolates. Line 10 had a greater difference in height for the two isolates; however, this line belongs to the group with the highest severity for isolate CMM 2106. Lines 1 to 20, which had the highest scores of injuries, also had the highest severity. Lines 17 and 15, which had greater severity, had the lowest lesion scores for isolate CMM 2106. For isolate 59, line 11, which had greater severity, also had higher average lesion scores. The growth of lines L25, L26, L27, L29, L34, L38, L39, L44, L48, L55, L79, and 95 were not affected by the isolate 59; their average scores ranged from 1.00 to 1.60 and were also considered resistant. For isolate CMM 2106, the average scores of lines L24, L51, L63, L71, L76, L80, and L93 ranged from 1.00 to 2.33.

In soybean, a study for the identification of candidate genes involved in charcoal rot resistance and unravels the complexity of this resistance (COSER et al., 2017). Additional research is needed to determine resistance mechanisms in cowpea. The genetic entries identified in this paper will be useful for improving charcoal rot resistance in cowpea crop.

## CONCLUSIONS

Promising cowpea lines identified as resistant sources of resistance to *M. phaseolina* can be used by farmers directly as new cultivars or in future hybridizations of cowpea genetic breeding programs.

The lines L8, L10, L19, L25, L26, L27, L29, L34, L38, L39, L44, L48, L55, L79, and L95 (15% of the evaluated lines) are resistant to *M. phaseolina* isolate 59;

The lines L2, L3, L13, L15, L24, L51, L63, L71, L76, L80, and 93 (11% of the evaluated lines) are resistant to *M. phaseolina* isolate CMM 2106.

The variables lesion scores and relative growth compared to control were more promising in

assessing resistance of the cowpea lines to isolates 59, and CMM 2106 of *M. phaseolina* using the toothpick inoculation method.

The *M. phaseolina* isolate CMM 2106 is more aggressive than isolate 59.

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