


DIFFERENTIAL RESPONSES OF VIGNA UNGUICULATA CULTIVARS TO ROOT KNOT NEMATODES (MELOIDOGYNE SPP.)

RESPOSTAS DIFERENCIAIS DE CULTIVARES DE VIGNA UNGUICULATA AOS NEMATÓIDES DAS GALHAS (MELOIDOGYNE SPP.)

Artigo Original

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RESUMO

Os nematóides das galhas são os fitonematódeos mais importantes do mundo, pois infectam as plantas cultivadas e se adaptam mais a diferentes tipos de ambientes, o que contribui para sua maior dispersão. No Brasil, existem mais de 20 espécies, com destaque para *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. enterolobii* e *M. konaensis*. Esses fitoparasitas afetam diversas culturas, incluindo o feijão-caupi [*Vigna unguiculata* (L.) Walp.], que é uma espécie bem adaptada do semiárido nordestino. O objetivo deste trabalho foi verificar a hospedabilidade das cultivares de feijão-caupi Pitiúba, Sempre Verde, João Paulo II e Setentão a *M. incognita*, *M. arenaria*, *M. enterolobii* e *M. konaensis*. Observou-se que a cultivar Pitiúba foi a única suscetível a *M. enterolobii*, enquanto João Paulo II apresentou resistência apenas a esta espécie. As cultivares João Paulo II e Setentão foram suscetíveis a *M. konaensis*. Sempre Verde apresentou resistência a *M. incognita*, *M. arenaria*, *M. enterolobii* e *M. konaensis*, sugerindo que esta cultivar, que está entre as cultivares recomendadas para a região Nordeste do Brasil, seria menos afetada em condições de campo infestadas por esta espécie de nematóides de galha.

Palavras-chave: Reação de cultivares. *Meloidogyne konaensis*. Relação parasita-hospedeiro.

Abstract

The root-knot nematodes are the most important phytonematodes in the world, as they infect cultivated plants and adapt more to different types of environments, what contributes to their greater dispersion. In Brazil, there are more than 20 species, notably *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. enterolobii*, and *M. konaensis*. Such phytoparasites affect crops differently, including cowpea [*Vigna unguiculata* (L.) Walp.], a well-adapted species of the Northeast semiarid. The objective of this work was to verify the hospedability of the cowpea cultivars Pitiúba, Sempre Verde, João Paulo II, and Setentão to *M. incognita*, *M. arenaria*, *M. enterolobii*, and *M. konaensis*. It was observed that the Pitiúba cultivar was the only one susceptible to *M. enterolobii*, whereas João Paulo II presented resistance only to this species. João Paulo II and Setentão cultivars were susceptible to *M. konaensis*. Sempre Verde showed resistance to *M. incognita*, *M. arenaria*, *M. enterolobii* and *M. konaensis*, suggesting that this cultivar, which is among the cultivars recommended for the Brazilian Northeast region, would be less affected in field conditions infested with this species of root-knot nematodes.

Keywords: Cultivars reaction. *Meloidogyne konaensis*. host-parasitic relationship.



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INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a dicot plant in the Fabaceae family and subfamily Faboideae of great importance for global food in tropical regions (ALVAREZ et al., 2021). Brazil is the third largest world producer of beans, which includes the production of common beans (*Phaseolus vulgaris* L.) and cowpea. In the country, mainly in the north and northeast regions, the cowpea crop is widely cultivated, occupying, in these regions, an area of 1,100,206 hectares with a production of 693,768 tons of grains in 2019 (CONAB, 2020). The northeastern region accounts for 67.21% of the national production of Fabaceae, with emphasis on the states of Bahia, Ceará and Piauí, which are responsible for more than 48% of the country's total production, due to the good adaptability of the culture to the conditions edaphoclimatic, to be a source of basic protein in the food of rural producers, in addition to being used as green manure and forage (CONAB, 2020). The state of Ceará is the largest producer of cowpea in the country, producing several cultivars including Pitiúba (CE-31), João Paulo II (CE-586), Semper Verde (CE-25) and, Setentão (CE-596) due to the good agronomic characteristics (PAIVA et al., 2014).

Of the phytosanitary problems in cowpea, fungal diseases, viruses, and nematosis are the most important, as they occur throughout the crop cycle. Among phytonematodes, associations with *Meloidogyne* spp., *Pratylenchus brachyurus* (Godfrey) Filipjev and Ste Khovem, and *Rotylenchus reniformis* (Linford and Oliveira) stand out. In Brazil, more than 20 species of *Meloidogyne* have been reported, with *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, and *M. enterolobii* Yang and Eisenback are the most frequent ones affecting different cultures in the country. Of these nematodes, the species *M. incognita* and *M. javanica*, have a wide distribution in areas of cowpea cultivation in northeastern Brazil, causing losses in production (NAMA; SHARMA, 2017). In Ceará, the species *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* were reported to be associated with fabaceae in the 1960s to 1980s (PONTE, 1987). However, information regarding the susceptibility to root-knot nematodes in cowpea currently exploited in the Northeast is scarce. Thus, the objective of this work was to study the behavior of cowpea cultivars Pitiúba, Semper Verde, João Paulo II and Setentão about the parasitism of *Meloidogyne* spp.

MATERIALS AND METHODS

For inoculation, inoculants of the species *M. incognita*, *M. arenaria*, *M. enterolobii* and, *M. konaensis* were used. The latter species was included in the study because it was first observed in the country, occurring in four microregions in Ceará state. All four species were previously identified by molecular techniques (MONTEIRO et al., 2016). The species were cultivated in coleus (*Plectranthus scutellarioides* (L.) R.Br.) aiming at the multiplication of populations. The extraction of eggs was carried

out according to the methodology of Bonetti and Ferraz (1981).

The cultivars of cowpea Pitiúba, Semper Verde, João Paulo II and, Setentão were obtained from the Germplasm Bank of Cowpea of the Federal University of Ceará. One seed of each cultivar was sown in a 1.5 L pot, containing sterile substrate (70% sand and 30% organic matter). Seedlings of cowpea cultivars aged 8 days after sowing were inoculated with 5,000 eggs of each species of *Meloidogyne*, distributing the suspension in three holes around the plant at a depth of 3 cm. The experiment was conducted in a greenhouse, using a completely randomized design, with 16 treatments and 8 repetitions, totaling 128 plants evaluated. The temperature of the greenhouse was $30 \pm 4^{\circ}\text{C}$, with an average of 32°C . Tomato seedlings (*Solanum lycopersicum* L.) were inoculated to confirm the infectivity of the inoculum.

After 35 days of inoculation, the plants were removed from each pot and the roots were washed in running water to remove the substrate. Then, the roots were evaluated for the following variables: fresh weight (FW), length (L), number of galls (NG) and, number of egg masses (NEM). To obtain the gall index (IG) and egg mass index (EMI), the Taylor and Sasser scale (1978) was used, which ranges from 0 to 5, where 0 = 0 galls or egg masses; 1 = 1 to 2 galls or egg masses; 2 = 3 to 10 galls or masses of eggs, 3 = 11 to 30 galls or masses of eggs; 4 = 31 to 100 galls or egg masses; and 5 = ≥ 100 galls or egg masses per root system. The eggs were extracted by the method of Bonetti and Ferraz (1981) to calculate the reproduction factor (RF). This parameter was obtained by dividing the final population by the initial inoculated population (F_p / I_p , where $I_p = 5,000$ eggs). The susceptibility of the plants was evaluated according to the criteria of Oostenbrink (1966): immune ($RF=0$), susceptible ($RF > 1$) or resistant ($RF < 1$). The cultivars were classified according to the methodology of Sasser et al. (1984) where: $RF \geq 1$ = good host; $0.1 < RF < 1.0$ = poor host; $RF \leq 0.1$ = non-host. The quantitative data were transformed into " $(x + k)^{1/2}$ " with $k = 0.01$ and subjected to analysis of variance (ANOVA) and the means to the Tukey test at the level of 5% probability.

RESULTS AND DISCUSSION

All cowpea cultivars allowed the reproduction of *Meloidogyne* ssp. at different levels, according to the statistical analysis presented in Table 1.

Table 1 - Means of cowpea cultivars (*Vigna unguiculata*) related to agronomic characteristics and parasitism by *Meloidogyne* spp.

Cultivar	Species	FW(g)	L(cm)	IG	EMI	NE	EGR	RF	Reaction
Pitiúba	<i>M. incognita</i>	5.54 ab	17.04c	4.6	2.3	3,200.0b	577.62	0.64	R
	<i>M. arenaria</i>	4.70 ab	16.76c	2.7	1.1	1,866.7b	397.17	0.37	R
	<i>M. enterolobii</i>	6.35 a	27.49a	5.2	4.2	37,000.0a	5826.77	7.40	S
	<i>M. konaensis</i>	3.95 b	24.56ab	4.3	2.0	5,600.0b	1417.72	1.65	S
CV		25,45%	15,56%			26,93%			
Sempre Verde	<i>M. incognita</i>	3.68 ab	22.29ab	3.6	3.1	1,733.3ab	471.01	0.35	R
	<i>M. arenaria</i>	4.24 a	22.95ab	3.2	2.0	666.7b	157.24	0.13	R
	<i>M. enterolobii</i>	1.57 b	21.87ab	4.2	3.1	4,133.3a	2632.68	0.83	R
	<i>M. konaensis</i>	2.67 ab	19.4ab	4.1	2.6	2,66.7ab	998.76	0.53	R
CV		52,30%	20,47%			47,75%			
João Paulo II	<i>M. incognita</i>	3.87 ab	17.79ab	5.7	5.0	40,666.7a	10,508.19	8.13	S
	<i>M. arenaria</i>	4.48 a	19.29a	4.9	4.2	14,933.3b	3,333.33	2.99	S
	<i>M. enterolobii</i>	5.81 a	24.29a	4.4	2.9	1,066.7c	183.60	0.21	R
	<i>M. konaensis</i>	1.69 b	14.41 b	5.1	4.1	16,800.0b	9,940.83	3.36	S
CV		42,41%	13,39%			38,75%			
Setentão	<i>M. incognita</i>	7.83 a	20.87 abc	4.1	3.6	12,666.7b	1,617.71	2.53	S
	<i>M. arenaria</i>	5.77 abc	20.78 bc	3.5	2.7	5,333.3b	924.32	1.07	S
	<i>M. enterolobii</i>	4.06 bc	23.86 a	3.5	1.6	266.6c	65.68	0.05	R
	<i>M. konaensis</i>	6.51 ab	20.20 c	4.8	4.1	15,733.3a	2,416.79	3.15	S

FW = fresh root; L = root length; EMI = egg mass index; GI = gall index; NE = number of eggs; EGR = Eggs per gram fresh root; RF = reproduction factor; Reaction: R = resistant; S = susceptible. (Oostenbrink, 1966). CV = coefficient of variation. The data were transformed into " $(x + k)^{1/2}$ " with $k = 0.01$. Averages followed by the same letter in the column for cultivar, do not differ by the Tukey test, at the level of 5% of significance.

Fonte: Própria.

Regarding FW, it was observed that in Pitiúba the values were similar for treatments with *M. incognita*, *M. arenaria* and, *M. enterolobii*, which did not differ from the control, however, it is lower for plants infected with *M. konaensis*. In Sempre Verde cultivar, the mean values of the FW did not differ significantly for *M. incognita*, *M. arenaria*, *M. konaensis* and, the control, differing only from *M. enterolobii*, whose average was the lowest of all treatments. The means of FW in João Paulo II were similar in cases of infection by *M. incognita*, *M. arenaria* and, *M. enterolobii*, being even higher than the means observed in plants with *M. konaensis* and with the control. In the Setentão cultivar, a higher mean of FW was observed in plants with *M. incognita*, *M. arenaria* and, *M. konaensis* and a lower FW in plants with *M. enterolobii* and those not inoculated.

Considering the L, Pitiúba presented higher averages in plants infected with *M. enterolobii* and, *M. konaensis*, differing significantly from the other treatments (Table 1). In Sempre Verde, the L was similar between the four treatments, not differing from the negative control. In João Paulo II, close L was observed in *M. incognita*, *M. arenaria* and, *M. enterolobii*, being superior to those observed for *M. konaensis* and negative control. In the Setentão cultivar, the L was higher in plants

with *M. enterolobii* and *M. incognita*, with averages close to the control, which differed from the cases in which *M. arenaria* and *M. konaensis* were parasitizing the roots.

The evaluation of the means referring to the IG and the EMI showed that João Paulo II presented the highest values for *M. incognita*, *M. arenaria* and *M. konaensis*, when compared to the other cultivars. In infections with *M. enterolobii*, however, the IG and EMI were higher in Pitiúba (Table 1). The lowest values of IG and EMI were observed in the cultivar Pitiúba infected with *M. arenaria*. In Sempre Verde, this *Meloidogyne* species was also the one that induced the smallest IG and EMI. For João Paulo and Setentão, the lowest values of IG and EMI were found for the species *M. enterolobii*.

According to the mean values of the cultivars for the RF and, according to the classification of Oostenbrink (1966), it was verified in this work that Pitiúba behaved as resistant (RF<1) to *M. incognita* and *M. arenaria* and showed susceptibility (RF>1) *M. konaensis* and *M. enterolobii*. Sempre Verde was resistant (RF<1.0) to all tested *Meloidogyne* species. João Paulo II and Setentão showed resistance to *M. enterolobii* but were susceptible to the other species of *Meloidogyne*. According to the classification by Sasser et al. (1984), Pitiúba proved to be a bad host for *M. incognita* and *M. arenaria* and a good host for the other two species. Sempre Verde was a bad host of all species evaluated with RF<0.9. João Paulo II and Setentão behaved very similarly, being bad hosts for *M. enterolobii*, however, good hosts for *M. incognita*, *M. arenaria* and *M. konaensis* (Table 1). All plants of tomato inoculated with the four species of *Meloidogyne* had the expected behavior with galls and egg masses in their roots, confirming the infectivity of the inoculum.

There are several studies evaluating cowpea varieties aiming for resistance to root-knot nematodes (SANTOS et al., 2018; SMITH et al., 2020). In quantitative studies of genetic inheritance in cowpea culture, resistance to *Meloidogyne* spp. it is attributed to different additive genes (EHLERS et al., 2000). In this trial, Pitiúba was the only variety that showed susceptibility to *M. enterolobii*, however, the susceptibility of cowpea to this pathogen was also observed by Guimarães et al. (2003) in the cultivar IPA-206 and common beans IPA -09. The resistance of the cultivar Pitiúba to the species *M. incognita* and *M. arenaria* observed in this work was previously reported by Oliveira et al. (2012). Villet et al. (2015), suggested that the resistance of Pitiúba in response to infection by *M. incognita* is possibly associated with oxidative stress, ubiquitination and, hydrolysis of glucosinolates, mechanisms that alter essential protein synthesis pathways. Torres Júnior et al. (2019) evaluating Sempre Verde and Setentão, reported the susceptibility of these two varieties to *M. incognita*, differing from what was observed in this trial in which Sempre Verde proved to be resistant to the nematode. Goulart et al. (2004) in a study conducted with 31 varieties of cowpea cultivated in Brazil, reported the predominance of susceptibility of cultivars to *M. incognita*. Different results were obtained by Harrison et al. (2006), who in a study carried out with 47 cowpea genotypes, observed susceptibility to *M. incognita* in only one of the accessions. In this trial, at

least two varieties behaved as susceptible to *M. incognita*, João Paulo II with FR=8.13 and Setentão with FR=2.53. In this study, João Paulo II showed resistance only to *M. enterolobii*.

In Brazil, there are few studies on the behavior of cowpea cultivars parasitized by *M. arenaria*. In studies by Kang et al. (2018), evaluating 40 varieties of cowpea, the authors did not identify any cultivar resistant to *M. arenaria*. In this study, Pitiúba and Sempre Verde reacted to resistance to *M. arenaria*, while João Paulo and Setentão showed susceptibility to the nematode.

Except for the Sempre Verde variety, the others tested in this work were susceptible to *M. konaensis*. The susceptibility to this species of *Meloidogyne* has been reported in two common bean varieties: Hawaiian Wonder and Manoa Wonder in Hawaii in an essay conducted by Zhang and Schmitt (1994), with no report of this nematode associated with cowpea in the world.

Given the several reports of susceptibility of the cowpea varieties to the root-knot nematodes, the control difficulties for *Meloidogyne* spp. in the field, and the occurrence in Ceará state of the four species of *Meloidogyne* evaluated in this study, it appears that the search for nematode-resistant cowpea material is one of the most viable alternatives for the exploration of the culture in naturally infested areas, mainly by small producers (KHAN et al., 2018). The results obtained in this work collaborate with information related to the reaction of four important varieties for the main species of *Meloidogyne* present in Ceará state.

Considering that Sempre Verde and Setentão are among the cowpea varieties recommended for cultivation in the Brazilian Northeast region (ROCHA et al., 2017) and that Sempre Verde showed resistance to three of the main species of *Meloidogyne* occurring in Brazil (*M. incognita*, *M. arenaria* and *M. enterolobii*) and, the only species of root-knot nematodes of restricted occurrence in Ceará state (*M. konaensis*), it is suggested that this variety will be less affected in field conditions infested with root-knot nematodes.

CONCLUSIONS

The information obtained in this work may be useful in future studies of cowpea control or breeding programs aiming to obtain cultivars resistant to root-knot nematodes in Brazil.

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