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Reaction of lima bean accessions to *Meloidogyne javanica*[1]

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ABSTRACT

Lima bean is one of the most popular leguminous plants in northeastern Brazil, with a strong socioeconomic influence on the regional communities. However, the low yield related to biotic and abiotic factors limits the increase in production. Among the biological agents, root-knot nematodes compromise its vegetative and productive development. In order to select genotypes resistant to *Meloidogyne javanica*, an experiment was carried out in a greenhouse and in a laboratory, in a completely randomized design, with fifteen lima bean accessions and five replicates. The reaction of lima bean accessions was evaluated through the infectivity and reproduction of *M. javanica*, as well as the main agronomic traits of the crop. A variation was observed in the resistance level among the accessions, with Lavandeira preta, Orelha de vó and Fava cearense being rated as very resistant, while Lavandeira vermelha was slightly resistant and the remaining accessions were all rated as susceptible.

KEYWORDS: *Phaseolus lunatus*; root-knot nematodes; genetic control.

RESUMO

Reação de acessos de feijão-fava a *Meloidogyne javanica*

O feijão-fava é uma das leguminosas mais apreciadas na região Nordeste, exercendo forte influência socioeconômica sobre as comunidades dessa região. No entanto, a baixa produtividade relacionada a fatores bióticos e abióticos limita o aumento da produção. Dentre os agentes biológicos, os nematoides de galhas comprometem seu desenvolvimento vegetativo e produtivo. Com o objetivo de selecionar genótipos resistentes a *Meloidogyne javanica*, conduziu-se experimento em casa-de-vegetação e em laboratório, em delineamento inteiramente casualizado, com 15 acessos de feijão-fava e cinco repetições. Avaliou-se a reação dos acessos de feijão-fava por meio da infectividade e da reprodução de *M. javanica*, bem como as principais características agronômicas da cultura. Houve variação no nível de resistência entre os acessos avaliados, sendo que Lavandeira preta, Orelha de vó e Fava cearense foram considerados muito resistentes, enquanto Lavandeira vermelha mostrou-se levemente resistente e os demais foram suscetíveis.

PALAVRAS-CHAVE: *Phaseolus lunatus*; nematoides de galhas; controle genético.

INTRODUCTION

Phaseolus lunatus L., also known as lima bean, is a leguminous plant widely cultivated in the tropics, and also an alternative source of protein for human consumption. It may replace common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*), in Brazil (Azevedo et al. 2003).

Brazil has reached an average yield of 217 kg ha^{-1} and a production of 4,048 t year^{-1} of dry lima beans, in an area of 21,329 ha (IBGE 2015). Among the producing regions, the northeastern stands out with 98.24 % of the national production, represented mainly by the states of Ceará, Paraíba, Pernambuco and Piauí. However, Rio Grande do Sul also stands out in the national scenario, with a yield of 1,926 kg ha^{-1} year^{-1}, because of its better technology (IBGE 2015).

The low crop yield is the result of several factors, such as the lack of adequate technology (Gomes et al. 2016), absence of specific soil fertilization programs (Alves et al. 2008) and presence of pests and diseases (Garcia & Romeiro 2011, Boechat et al. 2014, Silva et al. 2014). Nematodes, especially the root-knot ones, are responsible for high production losses in several crops. The species *Meloidogyne incognita*, *M. javanica* and *M. enterolobii* are an important group of pathogens

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for a wide range of hosts, including lima bean (Ferreira 2010).

Due to the damages caused by the action of root-knot nematodes on lima bean, resistance is one of the best ways to control phytonematodes, since it is easily understood by farmers, without increasing production costs or environmental risks. Therefore, resistant accessions allow a more adequate nematode management, being accessible to all producers from different technological levels. However, there is little information on the use of nematode resistant varieties for this crop in Brazil. Studies conducted in the United States showed varieties of lima bean with different levels of resistance, such as White Ventura N, Maria, UC-90 and UC-92, which are resistant to *M. incognita*; Cariblanco N, resistant to *M. incognita* and *M. javanica*; and CB-50, resistant to *M. incognita* and tolerant to *M. javanica* (Helms et al. 2004, Sikora et al. 2004).

Thus, this study aimed to select genotypes resistant to *Meloidogyne javanica*, using fifteen lima bean accessions.

MATERIAL AND METHODS

The experiment was performed in a greenhouse and a laboratory of the Universidade Federal de Campina Grande (06º46'13'S, 37º48'06'W and altitude of 242 m), in Pombal, Paraíba state, Brazil, from 20 September 2016 to 20 February 2017. The maximum and minimum temperatures during the experiment were 33.3 ºC and 24.5 ºC, respectively, and the relative air humidity was 41 % (Brasil 2017).

The experiment was conducted in a completely randomized design, with fifteen lima bean accessions and five replicates. The following accessions were tested: Lavandeira preta, Orelha de vó, Rajada 01, Rajada 02, Coquinho, Fava branca, Lavandeira vermelha, Raio de sol, Boca de moça, Paulistinha, Rajadinha, Fava cinza, Mulatinho, Lavandeira and Fava cearense. Lima bean seeds were collected from the main producers in several municipalities of the Paraíba state, including Cajazeiras, Sapé, Picuí, Santa Cruz and Queimadas.

The seeds of each accession were allocated in plastic pots with capacity of 4,000 dm^3, containing a mixture of soil-sand-manure in the proportion of 3:2:1, respectively. The whole substrate was pre-autoclaved at 120 ºC and pressure of 1.05 kg cm^{-2}, for 2 h. The chemical and physical characteristics of the substrate were previously evaluated and presented the following results: pH(H_2O): 8.48; N: 0.5 %; P: 60 mg dm^{-3}; K: 6.64 $cmol_c$ dm^{-3}; Ca: 3.65 $cmol_c$ dm^{-3}; Mg: 4.25 $cmol_c$ dm^{-3}; H + Al: 0.00 $cmol_c$ dm^{-3}; OM: 25.92 g kg^{-1}; sand: 86.96 %; clay: 7.46 %; silt: 5.58 %. Based on these results, the fertilization was carried out following the recommendation of the Embrapa for bean crops (Embrapa 1993).

The *M. javanica* inoculum was obtained from tomato roots provided by the Universidade Federal Rural de Pernambuco, in Recife, Pernambuco state, Brazil. The extraction of nematodes from the roots was performed using the methodology of Hussey & Barker (1973), modified by Bonetti & Ferraz (1981). Nematodes were then inoculated in tomato cv. Santa Clara (*Solanum lycopersicum L.*) for multiplication, under greenhouse conditions. The species identification was previously performed with the use of temporary (formalin) and permanent (glycerine) slides, examined under a microscope, through identification based on morphological and morphometric characteristics and the use of genomic taxonomic keys (Handoo & Golden 1989). The identification was also made by examining the perineal configuration and was confirmed by the isoenzyme electrophoresis technique, in analysis of esterase phenotypes (Carneiro & Almeida 2001).

After the nematodes multiplication, three seeds of lima bean accessions were sown in plastic pots (4.5 dm^3 capacity) and thinned at 10 days after emergence, leaving one plant per pot, which comprised the experimental unit. These plants were inoculated with 10 mL of a suspension containing 4,000 eggs and/or J2 of *M. javanica*, distributed in three holes of approximately 3.0 cm of depth, spaced 2.0 cm apart from each other and from the hypocotyl.

The inoculated plants were kept in a greenhouse under a daily watering regime, divided into two shifts (morning and afternoon). Nematodes remained under observation with the different lima bean accessions.

At 60 days after inoculation, the evaluations were performed for the parasitism and agronomic traits. The first variables related to the parasitism consisted of estimating the number of second-stage juveniles (J2) and eggs in the soil of each treatment. So, the substrate was homogenized and a 300 cm^3 sample was taken for extraction (Jenkins 1964). Then, the nematodes were quantified under an optical microscope (40x magnification), using a Peter's counting slide.

The roots of each plant were washed and dried on absorbent paper, and then the root-knots were counted with the help of a Hansor's magnifying glass. The root system was then cut into 1 cm fragments and mixed to obtain 10 g of root, for estimating the number of juveniles and eggs, using the Hussey & Barker methodology (1973), modified by Bonetti & Ferraz (1981). At the end, the number of eggs and J2 per root system were determined using a Peter's counting slide under microscope (40x magnification).

The reaction of lima bean accessions to *M. javanica* was evaluated by the reproduction factor and reproduction index. The reproduction factor (RF) was calculated by the ratio between the total number of eggs and J2 in the soil and in the root system (final population - Fp) and the number of eggs used in the inoculum (initial population - Ip) (RF = Fp/Ip). Accessions that presented RF equal to or lower than one were considered resistant, and those with a value greater than one were considered susceptible (Oostenbrink 1966).

The Santa Clara cultivar was used as a standard control (100 %), in comparison with the accessions of lima beans, to obtain the reproduction index (RI) of the species. The final population in the accessions was divided by the population found in the tomato, defining the reproduction indexes. The resistance levels of the lima beans were obtained according to the breeding criterion established by Taylor (1967), in which: S - accessions with susceptible plant, normal reproduction (RI above 51 %); LR - accessions with slightly resistant plants (RI from 26 % to 50 %); MoR - accessions with moderately resistant plants (RI from 11 % to 25 %); VR - accessions with very resistant plants (RI from 1 % to 10 %); HR/I - accessions with highly resistant/immune plants (RI below 1 %).

Regarding the agronomic traits, the following variables were analyzed: fresh root biomass (after washing in running water), obtained with the aid of a semi-analytical scale; root length, determined by measuring the distance from the plant stem to the apex of the root, using a graduated ruler; and root volume, obtained by submerging the roots in a pot partially filled with water and recording the difference between the initial and final values, expressed in cm^3.

Data were submitted to variance analysis to diagnose significant effects through the F test and the means were compared by the Scott-Knott test at 1 %.

RESULTS AND DISCUSSION

The results for variance analysis are shown in Table 1. All the parasitism variables presented significant differences ($p \leq 0.01$), while no agronomic traits differed significantly among the treatments ($p > 0.01$; Table 1).

It was observed that lima bean accessions presented different reactions in the presence of *M. javanica* (Table 2). However, no immune material or a high degree of resistance to the pathogen was observed.

The accessions Rajada 02, Boca de moça, Paulistinha, Rajadinha and Mulatinho presented the highest means for number of root-knots, not differing significantly from the tomato (control). However, the other accessions promoted reductions of 49.72 % in the number of root-knots, corresponding to more than 66.0 % of the evaluated accessions, with no statistical difference among them. These results suggest a wide genetic variability among the studied materials, which may promote divergence among resistance levels to the pathogens (Guimarães et al. 2007).

M. javanica parasitized all the lima bean accessions, what was demonstrated by the species

Table 1. Variance analysis for parasitism and agronomic traits [number of root-knots (NRK); eggs in the soil (ES); eggs in the root (ER), juveniles in the soil (JS); juveniles in the root (JR); reproduction index (RI); reproduction factor (RF); fresh root biomass (FRB); root length (RL); root volume (RV)].

Variation source	Df	Mean square of traits									
		Parasitism							Agronomic traits		
		NRK	ES	ER	JS	JR	RI	RF	FRB	RL	RV
		root/system	300 g/soil	root/system	300 g/soil	root/system	%	-	g	cm	mL
Treatments	15	0.46**	4.71**	6.12**	3.09**	4.49**	6.86**	0.47**	3.03^{ns}	2.03^{ns}	2.61^{ns}
Residue	64	0.17	0.25	0.58	0.65	0.70	0.52	0.15	3.00	1.56	2.56
Total	79										
CV (%)	-	7.74	15.59	9.60	26.51	12.44	19.40	24.68	20.29	13.99	19.78

^{ns} and **: not significant and significant at 1 %, by the F test; Df: degree of freedom.

ability to complete the biological cycle expressed by infectivity and reproduction (Table 2). Mean values for eggs in the soil ranged from 10.80 to 52.20 among the accessions. Lavandeira preta, Orelha de vó, Fava branca, Paulistinha and Rajadinha presented higher means, statistically differing from the control and significantly reducing the *M. javanica* reproduction from 95.38 % to 98.35 %, when compared to tomato. Campos et al. (2001) emphasized that this nematode species has a high reproductive capacity, with direct dispersion of eggs in the soil, which favors the quick dissemination and infection of plants. Therefore, it is assumed that some accessions of this study retard the infection action and, consequently, reduce the reproductive process in the roots.

The number of eggs in the root showed a great variation among the accessions Fava branca, Boca de moça, Paulistinha, Rajadinha, Fava cinza and Mulatinho, indicating that there was susceptibility of these accessions to *M. javanica*. Similar results were obtained by Bitencourt & Silva (2010), who observed a different behavior among the materials tested in the evaluation of different lima bean genotypes in the presence of two *Meloidogyne* species. According to the authors, the genotypes UFPI-464, Sunbeam and UFPI-463 were very resistant to *M. incognita*, whereas the other genotypes were moderately resistant or susceptible. For *M. enterolobii*, the reaction varied from mildly resistant to susceptible.

The comparison between the number of eggs and the number of root-knots demonstrates that the largest number of root-knots observed in a given accession will not always correspond to a higher egg production, a situation observed in both the control and the Rajada 02. Zanella et al. (2005) had already found a similar situation, in which they affirm that the amount of root-knots has no direct relation with the nematodes reproduction. This demonstrates the importance of evaluating the resistance to nematodes using the maximum number of variables, in order to avoid misunderstandings in the interpretation.

Regarding the number of juveniles in the soil, the control presented the highest mean, with a significant difference for all lima bean accessions, indicating that there was inhibition to parasitism for this variable. Orelha de vó, Rajada 01, Boca de moça, Fava cinza and Fava cearense stood out among the lima bean accessions that showed the lowest means, presenting a reduction greater than 99.17 %, when compared to tomato.

Lavandeira preta presented higher means for juveniles in the root than the other accessions, with a reduction of 94.75 %, followed by Orelha de vó and Fava cearense, which did not differ statistically

Table 2. Number of root-knots (NRK), eggs in the soil (ES), eggs in the root (ER), juveniles in the soil (JS) and juveniles in the root (JR) in lima bean accessions, at 60 days after inoculation.

Accession	*Meloidogyne javanica* infectivity and reproduction				
	NRK	ES	ER	JS	JR
	root/system	300 g/soil	root/system	300 g/soil	root/system
Tomato	287.6 a[1]	656.00 a	1,620 b	664.0 a	1,640 a
Lavandeira preta	165.2 b	48.00 b	216 b	23.1 b	86 c
Orelha de vó	144.6 b	39.30 b	723 b	13.9 c	296 b
Rajada 01	157.0 b	10.80 c	3,959 b	17.7 c	822 a
Rajada 02	280.0 a	20.40 c	3,832 b	24.5 b	1,289 a
Coquinho	166.6 b	16.20 c	3,652 b	24.3 b	842 a
Fava branca	152.0 b	30.30 b	8,173 a	36.0 b	3,187 a
Lavandeira vermelha	201.6 b	18.90 c	3,737 b	25.3 b	1,237 a
Raio de sol	200.4 b	15.00 c	5,960 b	28.8 b	1,439 a
Boca de moça	396.6 a	17.70 c	14,348 a	5.50 c	2,738 a
Paulistinha	323.6 a	40.13 b	12,179 a	46.1 b	4,227 a
Rajadinha	286.2 a	52.20 b	7,345 a	36.6 b	1,609 a
Fava cinza	194.2 b	18.90 c	7,449 a	12.6 c	2,042 a
Mulatinho	272.2 a	17.70 c	8,417 a	29.4 b	1,463 a
Lavandeira	156.2 b	19.20 c	4,488 b	36.6 b	1,987 a
Fava cearense	213.8 b	15.60 c	698 b	12.0 c	286 b
CV (%)	7.74	15.59	9.60	26.51	12.44

[1] Means followed by the same lowercase letter did not differ among themselves by the Scott-Knott test at 1 %. In the analysis, the values were transformed into log (x + 1). Original data on the table.

among themselves and presented a reduction in the number of juveniles in the root of 82.5 %, in relation to the control. The other accessions did not differ from the tomato.

Significant differences were observed among lima bean accessions, in relation to reproduction indexes and reproduction factor (Table 3), which express the existence of genetic variability for resistance to *M. javanica*. Nevertheless, only 26.70 % of the accessions were classified with some degree of resistance. Lavandeira preta, Orelha de vó and Fava cearense were considered as very resistant, followed by Lavandeira vermelha, which was considered slightly resistant. The other accessions were considered susceptible, with reproduction rates above 50 %, according to the scale adopted by Taylor (1967).

Genetic diversity is more evident when we compare the divergence of the results found in this experiment and those observed by Bitencourt & Silva (2010), with the accessions Orelha de vó and Fava cearense pointed out as susceptible, in a study carried out in the Maranhão state, Brazil.

According to Inomoto et al. (2007), the most relevant variable in the evaluation of genetic resistance is the reproduction factor, because it represents the effect of the species (cultivar) on the nematode population. All the accessions, except for Boca de moça and Paulistinha, did not differ statistically from each other. However, only the accessions Lavandeira preta, Orelha de vó and Fava cearense presented low values of reproduction factor (0.09, 0.46 and 0.25, respectively), being considered resistant (RF < 1; Table 3). Thus, they could be studied as promising sources of *M. javanica* resistance in breeding programs.

In Brazil, there is little information about lima bean genotypes with some type of resistance to root-knot nematodes or losses caused by the pathogen, unlike what happens with common bean (*P. vulgaris* L.), with consolidated researches and data about losses of over 65 % in areas with the pathogen presence. However, some bean genotypes demonstrate resistance to nematodes, for example, Aporé is considered very resistant to *M. javanica*, slightly resistant to *M. incognita* 1 and moderately resistant to *M. incognita* 3, like the cultivars Macarrão Atibaia, Macarrão favorito, Talisman and Ouro Preto, with moderate levels of resistance to the same species (Chen & Roberts 2003, Ferreira et al. 2010).

In the United States, even using only *in natura* lima beans for consumption, the nematode damage

Table 3. Means for reproduction index (RI), reproduction factor (RF) and classification of 15 lima bean accessions and the Santa Cruz tomato cultivar for *Meloidogyne javanica*.

Accession	*Meloidogyne javanica*			
	Reproduction index		Reproduction factor	
	RI**	Class	RF**	Class
Tomato	100.00 a[1]	S[2]	1.14 b	S[3]
Lavandeira preta	2.08 d	MR	0.09 b	R
Orelha de vó	9.30 c	MR	0.46 b	R
Rajada 01	60.87 b	S	1.21 b	S
Rajada 02	50.62 b	S	1.12 b	S
Coquinho	56.47 b	S	1.15 b	S
Fava branca	78.54 b	S	2.85 b	S
Lavandeira vermelha	47.97 b	LR	1.25 b	S
Raio de sol	183.87 a	S	1.86 b	S
Boca de moça	135.25 a	S	4.29 a	S
Paulistinha	154.62 a	S	3.30 a	S
Rajadinha	103.17 a	S	1.73 b	S
Fava cinza	117.06 a	S	1.90 b	S
Mulatinho	71.15 b	S	1.99 b	S
Lavandeira	52.35 b	S	1.31 b	S
Fava cearense	9.15 c	MR	0.25 b	R
CV (%)	19.40	-	24.68	-

[1] Means followed by the same lowercase letter are not different by the Scott Knott test at 1 %. Original means. [2] S: susceptible crop (above 50 %, in relation to tomato); SR: slightly resistant (26-50 %); MoR: moderately resistant (11-25 %); VR: very resistant (1-10 %); HR: highly resistant (less than 1 %); I: immune (when there was no reproduction). [3] RF = resistant (RF < 1); S = susceptible (RF ≥ 1).

is easily attenuated by the use of different varieties with different levels of resistance, such as White Ventura N, Maria, UC-90 and UC-92, which are resistant to *M. incognita*; Cariblanco N, resistant to *M. incognita* and *M. javanica*; and CB-50, resistant to *M. incognita* and tolerant to *M. javanica* (Helms et al. 2004, Sikora et al. 2004). Therefore, it is evident the need for researches in the field of genetic resistance to different species of root-knot nematodes in bean crops in Brazil (Freire & Ponte 1976). Research groups should give a greater attention to lima bean crops, considering that they represent a great social and economic relevance for the northern and northeastern regions of Brazil.

CONCLUSION

There is variation in the resistance behavior of lima bean accessions to *M. javanica*. Only three accessions (Lavandeira preta, Orelha de vó and Fava cearense), among the fifteen accessions evaluated, presented resistance with reduction in the nematode population density.

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