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Reaction of common bean genotypes to the reproduction of *Meloidogyne javanica* and *Meloidogyne incognita*¹

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ABSTRACT

The production of common bean (*Phaseolus vulgaris* L.) may be compromised by a variety of pests and diseases, with root-knot nematodes standing out among the soil-borne pathogens that cause significant losses. It was assessed the reaction of common bean genotypes to the reproduction of these nematodes, with emphasis on the two species most frequently found parasitizing the crop: *Meloidogyne javanica* and *Meloidogyne incognita*. The experiments were conducted in a greenhouse, using a completely randomized design, with 26 treatments (genotypes), ten replications and plot consisting of a pot containing one plant. Each plot was inoculated with 1,000 eggs and second-stage juveniles and assessed at 62 days after the inoculation, when the final populations were estimated. The genotypic resistance was determined based on two criteria: reproduction factor and index. All the genotypes were susceptible to *M. incognita* according to both assessment criteria. The IAC Alvorada, IAC Imperador, BRS Esplendor and BRS Esteio cultivars, although may be classified as resistant to *M. javanica* by the reproduction factor, are classified only as moderately resistant if the assessment criterion is the reproduction index.

KEYWORDS: *Phaseolus vulgaris*, genetic resistance, root-knot nematode, integrated nematode management.

RESUMO

Reação de genótipos de feijoeiro à reprodução de *Meloidogyne javanica* e *Meloidogyne incognita*

A produção de feijão (*Phaseolus vulgaris* L.) pode ser prejudicada por diversas pragas e doenças, com destaque entre os patógenos de solo para os nematoides de galha, que causam perdas significativas. Objetivou-se avaliar a reação de genótipos de feijoeiro à reprodução desses nematoides, com ênfase nas duas espécies mais comumente encontradas parasitando a cultura: *Meloidogyne javanica* e *Meloidogyne incognita*. Os experimentos foram realizados em casa-de-vegetação, em delineamento inteiramente casualizado, com 26 tratamentos (genótipos), dez repetições e parcela composta por um vaso contendo uma planta. Cada parcela foi inoculada com 1.000 ovos e juvenis de segundo estágio e avaliada aos 62 dias após a inoculação, quando as populações finais foram estimadas. A resistência dos genótipos foi determinada com base em dois critérios: fator e índice de reprodução. Todos os genótipos foram suscetíveis a *M. incognita* por ambos os critérios. As cultivares IAC Alvorada, IAC Imperador, BRS Esplendor e BRS Esteio, embora possam ser classificadas como resistentes a *M. javanica* pelo fator de reprodução, são classificadas apenas como moderadamente resistentes se o critério de avaliação for o índice de reprodução.

PALAVRAS-CHAVE: *Phaseolus vulgaris*, resistência genética, nematoide de galha, manejo integrado de nematoides.

INTRODUCTION

Bean is a vital food source for the Brazilian population, especially in low-income groups, where it is the primary source of protein, in addition to being rich in iron, calcium, magnesium, zinc, vitamins, carbohydrates and fiber (Mesquita et al. 2007). The national production in the 2016/2017 growing season was 3.4 million metric tons, over an area of 3.2 million hectares, with an average yield of 1,070 kg ha⁻¹, and

the estimated production for the 2017/2018 season is approximately 3.37 million metric tons (Conab 2017).

The biotic stress caused by pests and diseases negatively affects bean crops, with the latter including root-knot nematodes (*Meloidogyne* spp.) (Bozbuga et al. 2015). According to Baida et al. (2011), this genus of nematode causes a significant damage and may lead to production losses of up to 90 %.

The *Meloidogyne* genus consists of 98 plant-parasitic species capable of infecting almost all

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vascular plants (Jones et al. 2013). The females deposit eggs in gelatinous masses, and first-stage juveniles (J1) develop while inside the egg, emerging and molting into infective second-stage juveniles (J2), which penetrate the root system of susceptible hosts and begin to feed, causing the formation of root galls. The males are vermiform and leave the plant, whereas the females exhibit distended pear-shaped bodies (Chitwood & Perry 2009).

The most common, frequent and damaging species of root-knot nematodes in bean crops are *Meloidogyne incognita* and *Meloidogyne javanica* (Freire & Ferraz 1977). The root damage caused by these pathogens affects the water and nutrient absorption of infected plants, with symptoms including dwarfism and small, yellowish leaves, thus compromising production (Santos et al. 2012). Additionally, according to Mota et al. (2013), these damaged roots are more prone to secondary infection by fungi and bacteria.

Nematodes are difficult to control, and crop control practices and chemical applications are often inefficient, with the latter may having serious consequences for both the ecosystems and humans (Ferreira et al. 2012). The use of resistant cultivars, when available, is the most promising and viable practice for nematode control (Ferreira et al. 2010). According to Trudgill (1991), genetic resistance is defined as a plant's ability to prevent or reduce nematode multiplication.

In Brazil, there have been no reports of *Phaseolus* spp. cultivars that exhibit effective resistance, but a few moderately resistant cultivars have been identified, although none are sufficiently capable of reducing nematode populations (Pedrosa et al. 2000). Therefore, further researches are needed to identify nematode-resistant materials able to reduce the populations of these parasites to levels that do not damage crops, enabling the bean production in infected areas (Baida et al. 2011). Hence, in the present study, it was assessed the reaction of common bean genotypes to the reproduction of *M. javanica* and *M. incognita* nematodes, which are most frequently found parasitizing this crop.

MATERIAL AND METHODS

The experiments were conducted in a greenhouse, without controlling the weather conditions, at the Universidade Estadual Paulista,

in São Paulo, São Paulo state, Brazil, in February 2016.

Twenty-six common bean genotypes were assessed: BRS Esteio, BRS Notável, 49/61-1/2, BRS Campeiro, BRS Esplendor, BRS MG Majestoso, IAC Imperador, IAC Alvorada, BRS Madre Pérola, BRS Sublime, BRS Estilo, IAC Tybatã, IAC Jabola, IAC Milênio, BRS Pérola, C10-2-4/41, C10-2-16/8, BRS Ametista, IAC Eté, FAP-F3-2, 45/57-4-2-1/4, IPR Curió, IPR Tangará, IPR Campos Gerais, IPR Andorinha and IPR Quero Quero. BRS Pérola and *Crotalaria spectabilis* were also assessed as reference for susceptibility and resistance, respectively. In addition, the viability of the inoculum was evaluated on the Santa Cruz Kada (tomato) and Santa Cruz 47 (okra) cultivars.

Inocula of *M. javanica* and *M. incognita* race 3 were obtained from roots of a Santa Cruz Kada tomato plant. Populations were previously identified based on the morphology of the perineal pattern (Taylor & Netscher 1974) and of the labial region in males (Eisenback 1981), in a photonic force microscope, as well as the isoenzymatic phenotype for esterase (Esbenshade & Triantaphyllou 1990), in a traditional vertical electrophoresis system (BIO-RAD Mini Protean II).

Two experiments were carried out, one for assessing each nematode species. The experimental design was completely randomized, with 26 treatments (genotypes) and ten repetitions. The plot consisted of a pot (2.0 L) filled with a previously autoclaved (120 °C, 1 atm, 1 h) mixture of soil and sand (1:4). Three seeds were sown per pot, and one thinning was performed at 10 days after sowing, leaving only the most vigorous plant in each pot. The root systems of each plant were then inoculated with 10 mL of suspension containing a total of 1,000 eggs and second-stage juveniles (J2), representing the initial population.

The roots of each plant were identified and submitted to extraction at 62 days after the inoculation, using the method proposed by Hussey & Barker (1973) and modified by Bonetti & Ferraz (1981). Populations were then estimated by counting under a photonic force microscopic, using a Peters counting chamber to obtain the total number of eggs and second-stage juveniles, which constituted the final population for each plot.

Cultivar resistance was assessed based on the classification criteria for the reproduction factor and

index, and for statistical grouping in the variable total number of eggs and second-stage juveniles. The highest total number of eggs and second-stage juveniles value among all the treatments in a statistical group was used to define the class within that group.

The reproduction factor (RF) was determined based on the ratio between the final (Fp) and initial (Ip) population of each plot: $RF = Fp/Ip$. The cultivars were classified as resistant when $RF < 1$, and susceptible when $RF \geq 1$ (Oostenbrink 1966).

The reproduction index (RI) was calculated using the total number of eggs and second-stage juveniles value of the susceptible control plant (BRS Pérola cultivar), based on the following formula: $RI\% = [(Fp \text{ of the treatment}) / (Fp \text{ of the susceptible control})] \times 100$.

Based on the RI% grade scale proposed by Taylor (1967), the cultivars were classified as it follows: susceptible when the RI was greater than 50 % of the value obtained for the susceptible control; slightly resistant - RI of 26-50 %; moderately resistant - RI of 11-25 %; very resistant - RI of 1-10 %; extremely resistant - RI of less than 1 %; and immune - RI equal to 0.

The total number of eggs and second-stage juveniles data were log transformed ($x + 5$) and submitted to analysis of variance, but means without transformation are showed in the tables to facilitate interpretation. The means were compared using the Scott-Knott test at 5 % of probability and the AgroEstat statistical software (Barbosa & Maldonado Júnior 2015).

RESULTS AND DISCUSSION

The cultivar reactions to *M. incognita* and *M. javanica* for total number of eggs and juveniles, reproduction factor and reproduction index are presented in Tables 1 and 2. The reproduction of both root-knot nematode species was high in the two inoculum viability controls [Santa Cruz Kada (tomato) and Santa Cruz 47 (okra)], indicating adequate environmental, experimental and inoculum conditions. There was no reproduction for the *Crotalaria spectabilis* resistance pattern.

All the cultivars were susceptible to *M. incognita*, according to the reproduction factor and index, but they exhibited statistical differences in the total number of eggs and juveniles. BRS Pérola,

IAC Imperador, BRS Esplendor, IPR Curió and IAC Eté showed a lower multiplication and greater reproduction suppression of the pathogen, in relation to the other genotypes.

Similar results were reported by Santos et al. (2012), where all the materials studied had a reproduction factor higher unit ($RF > 1$) for *M. incognita* race 3, and were therefore considered susceptible to the species. Ferreira et al. (2010) used

Table 1. Total number of eggs and juveniles (TNEJ), reproduction factor (RF), response (R) and reproduction index (RI) for common bean genotypes, two susceptible controls (Cs) and one resistant control (Cr), inoculated with *Meloidogyne incognita*.

Treatment	TNEJ ⁽¹⁾	RF	R ⁽²⁾⁽⁴⁾	RI (%)	R ⁽³⁾⁽⁴⁾
Tomato - Cs	20,247 a	20.24	S	786.60	S
Okra - Cs	9,489 a	9.48	S	368.65	S
Crotalaria - Cr	33 d	0.03	R	1.28	VR
45/57-4-2-1-/4	7,924 b	7.92	S	307.85	S
49/61-1/2	7,911 b	7.91	S	307.34	S
BRS Ametista	6,268 b	6.26	S	243.51	S
BRS Campeiro	11,342 b	11.34	S	440.64	S
BRS Esplendor	1,250 c	1.25	S	48.56	S
BRS Esteio	12,390 a	12.39	S	481.35	S
BRS Estilo	33,750 a	33.75	S	1,311.19	S
BRS Madre Pérola	9,852 a	9.85	S	382.75	S
BRS Mg Majestoso	3,287 b	3.28	S	127.70	S
BRS Notável	13,490 b	13.49	S	524.09	S
BRS Pérola	2,574 c	2.57	S	100.00	S
BRS Sublime	26,560 a	26.56	S	1,031.86	S
C10-2-16/8	25,025 a	25.02	S	972.22	S
C10-2-4-/41	15,591 a	15.59	S	605.71	S
FAP-F3-2	2,704 b	2.70	S	105.05	S
IAC Alvorada	9,274 b	9.27	S	360.30	S
IAC Eté	1,110 c	1.11	S	43.12	S
IAC Imperador	1,357 c	1.35	S	52.72	S
IAC Jabola	14,757 a	14.75	S	573.31	S
IAC Milênio	3,576 b	3.57	S	138.93	S
IAC Tybatã	4,818 b	4.81	S	187.18	S
IPR Andorinha	3,080 b	3.08	S	119.66	S
IPR Campos Gerais	22,891 a	22.89	S	889.32	S
IPR Curió	628 c	0.62	S	24.40	S
IPR Quero Quero	9,815 a	9.81	S	381.31	S
IPR Tangará	17,882 a	17.88	S	694.72	S
F Test	13.85**	7.95**	-	11.05**	-
CV(%)	15.62	22.72	-	22.46	-

⁽¹⁾ Means followed by the same letter do not differ according to the Scott-Knott test at 5 % of significance. ** Significant at 1 % of probability. The original means of log-transformed data are presented ($x + 5$). ⁽²⁾ R = resistant, $RF < 1$; S = susceptible, $RF \geq 1$. ⁽³⁾ S = susceptible, $RI > 51$ %; SR = slightly resistant, $26 < RI < 50$ %; MoR = moderately resistant, $11 < RI < 25$ %; VR = very resistant, $1 < RI < 10$ %; ER = extremely resistant, $0 < RI < 1$ %; I = immune, $RI = 0$. ⁽⁴⁾ Classifications were corrected based on the statistical grouping of TNEJ, using the highest TNEJ value among the treatments, for each group, to define the RF and RI class within it.

Table 2. Total number of eggs and juveniles (TNEJ), reproduction factor (RF), response (R) and reproduction index (RI) for common bean genotypes, two susceptible controls (Cs) and one resistant control (Cr), inoculated with *Meloidogyne javanica*.

Treatment	TNEJ ⁽¹⁾	RF	R ⁽²⁾⁽⁴⁾	RI (%)	R ⁽³⁾⁽⁴⁾
Tomato - Ts	17,565 b	17.57	S	243.96	S
Okra - Ts	40,695 a	40.70	S	565.21	S
Crotalaria - Tr	0 d	0.00	R	0.00	MoR
45/57-4-2-1/4	19,335 b	19.34	S	268.54	S
49/61-1/2	7,875 b	7.88	S	109.38	S
BRS Ametista	25,983 b	25.98	S	360.88	S
BRS Campeiro	9,900 b	9.90	S	137.50	S
BRS Esplendor	450 d	0.45	R	6.25	MoR
BRS Estilo	21,450 b	21.45	S	297.92	S
BRS Esteio	656 d	0.66	R	9.11	MoR
BRS Madre Pérola	24,975 b	24.98	S	346.88	S
BRS Mg Majestoso	5,133 c	5.13	S	71.29	S
BRS Notável	4,293 c	4.29	S	59.63	S
BRS Pérola	7,200 b	7.20	S	100.00	S
BRS Sublime	76,000 a	76.00	S	1,055.56	S
C10-2-16/8	35,700 a	35.70	S	495.83	S
C10-2-4/41	16,800 b	16.80	S	233.33	S
FAP-F3-2	7,687 c	7.69	S	106.76	S
IAC Alvorada	801 d	0.80	R	11.13	MoR
IAC Eté	13,500 c	13.50	S	187.50	S
IAC Imperador	525 d	0.53	R	7.29	MoR
IAC Jabola	17,475 b	17.48	S	242.71	S
IAC Milênio	9,525 b	9.53	S	132.29	S
IAC Tybatã	6,060 c	6.06	S	84.17	S
IPR Andorinha	9,960 b	9.96	S	138.33	S
IPR Campos Gerais	32,366 a	32.37	S	449.53	S
IPR Curió	14,828 b	14.83	S	205.94	S
IPR Quero Quero	10,387 b	10.39	S	144.26	S
IPR Tangará	13,230 b	13.23	S	183.75	S
F-Test	8.49**	7.29**	-	9.16**	-
CV(%)	29.03	26.04	-	28.09	-

⁽¹⁾ Means followed by the same letter do not differ according to the Scott-Knott test at 5 % of significance. ** Significant at 1 % of probability. The original means of log-transformed data are presented (x + 5). ⁽²⁾ R = resistant, RF < 1; S = susceptible, RF > 1. ⁽³⁾ S = susceptible, RI > 51 %; SR = slightly resistant, 26 % < RI < 50 %; MoR = moderately resistant, 11 % < RI < 25 %; VR = very resistant, 1 % < RI < 10 %; ER = extremely resistant, 0 < RI < 1 %; I = immune, RI = 0. ⁽⁴⁾ Classifications were corrected based on the statistical grouping for TNEJ, using the highest TNEJ value among the treatments, for each group, to define the RF and RI class within it.

the reproduction index proposed by Taylor (1967) to study the resistance of common and green beans to *M. incognita* races 1 and 3, and found that some were moderately resistant, while the Macarrão Atibaia cultivar of green bean was very resistant to race 3. However, it is important to note that the reproduction index in the latter study was based on the reproduction of the Santa Clara tomato cultivar, whereas, in the present study, the BRS Pérola cultivar was used.

Indeed, the fact that the reproduction index is a comparison between the materials assessed and a susceptible material makes it a relative measure. Moreover, its application based on susceptible reference from other species, such as tomato, could easily lead to the overestimation of the bean resistance, since tomato is considered the ideal host for *Meloidogyne* spp. Soares et al. (2018) reported that the reproduction factor is more reliable in selecting resistant genotypes than the reproduction index, because it only takes into account the final and initial populations.

For *M. javanica*, the IAC Alvorada, BRS Esteio, IAC Imperador and BRS Esplendor cultivars were classified as resistant for the reproduction factor, and as moderately resistant for the reproduction index. The remaining cultivars were susceptible according to both criteria, and totaled 84 % of the genotypes assessed, although with differences in the total number of eggs and juveniles. Santos et al. (2012) obtained a reproduction factor of *M. javanica* equal to 0.9, for the Fort-19 bean genotype, while Baida et al. (2011) observed resistance in all the green bean genotypes assessed. The ability to suppress the nematode reproduction is associated to the genetic constitution of resistant genotypes, and thus can be exploited by breeding programs. It is worth noting that the genotypes identified as resistant in this study are commercial cultivars and already have desirable agronomic characteristics, being readily available to producers.

Simão et al. (2005) evaluated BRS Pérola and Iapar 81 cultivars with different initial *M. javanica* populations and found that both genotypes were good hosts for the species. However, despite allowing the nematode reproduction, the grain yield did not decline, when compared to the non-inoculated controls, indicating the tolerance of the nematode. Trudgill (1991) defines tolerance as the host plant's ability to compensate the harmful effects of the pathogen, or recover itself from these damages, and yield well even in its presence.

In a study by Santos et al. (2012), the BRS Pérola cultivar allowed the multiplication of *M. incognita* race 3 and *M. javanica*. The authors also observed a decline in height, number of nodes, number of trifoliate leaves, fresh and dry weight, when compared to plants not inoculated with the nematode, revealing a possible intolerance of the cultivar to both species.

Cultivars used in areas infested with *Meloidogyne* spp. should be chosen carefully, in order to prevent the nematode reproduction and benefit subsequent crops by reducing its population (Ferreira et al. 2010). In light of the results, only the IAC Alvorada, BRS Esteio, IAC Imperador and BRS Esplendor cultivars can be recommended for planting in *M. javanica*-infected areas, to suppress its reproduction and reduce populations. Although agronomic traits related to plant architecture and grain yield were not assessed in this study, such genotypes have already supplanted this type of evaluation, since they are commercial varieties recommended and widely cultivated in Brazil.

CONCLUSIONS

1. All the cultivars assessed in this study are susceptible to *Meloidogyne incognita* race 3, based on both the classification criteria adopted: reproduction factor and index. BRS Pérola, IAC Imperador, BRS Esplendor, IPR Curió and IAC Eté showed the lowest reproduction factor when inoculated with this species;
2. Only IAC Alvorada, BRS Esteio, IAC Imperador and BRS Esplendor were classified as resistant to *Meloidogyne javanica*, based on the reproduction factor (and moderately resistant based on the reproduction index). Thus, these genotypes can be cultivated in infected areas, in order to reduce the population of this pathogen.

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