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Original paper

Resistance of common bean to *Meloidogyne javan*ica under elevated temperatures

Rodrigo R. Fidelis[1], Danilo P. Ramos[1], Paulo R. Pereira[1], Ildon R. do Nascimento[1], Vitor de L. Nascimento[1] and Karen C. L. Silva[1]

1 Faculty of Agronomy, Department of plant production, Federal University of Tocantins, Gurupi-TO, Brazil

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Abstract

The aim of this work was to assess the resistance of seven commercial cultivars of common bean, black typ, (Xamego, BRS-Grafite, IAC-Diplomata, BRS-Esplendor, IPR-Uirapuru, IPR-Tiziu, and IAC-Una) to *M. javanica* under elevated temperature condition. The greenhouse experiments were carried out in a complete randomized design, with four replications and five plants per plot. The susceptible tomato cultivar Santa Clara was used to evaluate inoculation efficiency and reproduction index. The classification of resistance levels was estimated according to galls index, size of galls, position of galls, and reproduction index relative to tomato plants. In relation to common beans, there was no significant difference among cultivars in the evaluated traits, except for size of galls, indicating which there is no big genetic variability about *M. javanica* resistance. The elevated temperatures probably contributed to the non-observation of greater resistance degrees among the evaluated cultivars. Regarding the reproduction index, the cultivar IAC-Una was classified as moderately resistant to *M. javanica*.

Key-words: *Phaseolus vulgaris* L., Nematode, Black Type, Genetic Variability

Introduction

Common beans (*Phaseolus vulgaris* L.) are the most widespread and cultivated specie of the Fabaceae family, being one of the main sources of protein and calories for more than half billion people, especially in the less developed regions of the world (FAO, 2012). This crop can be produced on all continents from sea level to altitudes ranging from 3000 m and it is cultivated in approximately 100 countries (Aidar et al., 2002; Didonet, 2005).

In Brazil, common bean is cultivated by small farmers, targeting at subsistence, on the most varied soil and climatic conditions, however, in some places, also occur production with a higher technological level (Aidar et al., 2002; Barbosa, 2007). Since the 1970s, Tocantins state has been participating in the production of beans only in the harvest season (CONAB, 2011). Elevated temperatures increase the stress of plants, affecting the resistance to nematode parasitism, and under these conditions, the gall-forming nematodes, especially *Meloidogyne javanica*, are one of the main factors responsible for the low productivity of the common bean crop, which can cause yield losses of up to 90% and in extreme conditions, it may lead the plants to death (Simão et al., 2005; Baida et al., 2011).

Despite that, elevated temperatures affect nematode reproduction rate, increasing the proportion of females to males, and these conditions are also directly related to size of galls (Campos et al., 2011). The use of cultivars without resistance or tolerance to nematodes associated with the presence of host plants is another factor that may increase infestation and dissemination of *Meloidogyne* spp (Baida et al., 2011). Associated to the infection of the root system there is reduction of the absorbent roots, which result in alterations in the whole physiology of the plant (Carneiro et al., 2002).

Rodrigo Ribeiro Fidelis
E-mail: fidelisrr@mail.uft.edu.br

In Brazil, sources of resistance to *Meloidogyne* spp. have already been described in the genus *Phaseolus*, however, they are still few explored (Silva et al., 2003; Carneiro et al., 2006). Simão et al. (2005) demonstrate that the common bean cultivars Pérola and Iapar 81 were considered tolerant because there was no reduction in yield, although the reproduction of *M. javanica* was detected in the roots. Silva et al. (2005), studying the genetic control of resistance to *Meloidogyne incognita* in $F_{2:3}$ progenies (Pérola x Batatinha), detected a significant difference between the progenies, demonstrating the existence of genetic variability for this trait.

Due to the difficulty to control *Meloidogyne* spp in the field, the identification of sources of resistance in the genus *Phaseolus* to the gall-forming nematodes in plant breeding programs that seek the development of tolerant or resistant cultivars stands out as the most economical and viable way to control this disease. The aim of this work was to assess the resistance of seven commercial cultivars of common bean to *M. javanica* under elevated temperature condition.

Material and methods

The work was conducted in a greenhouse at the Experimental Station of the Universidade Federal do Tocantins (UFT) - Gurupi Campus (11°44'S, 49°02'W, 278 m of elevetion) and the seven commercial cultivars of common bean (black type) evaluated for nematode resistance were: Xamego, BRS-Grafite, IAC-Diplomata, BRS-Esplendor, IPR-Uirapuru, IPR-Tiziu, and IAC-Una. Santa Clara tomato cultivar was used as the standard nematode host (Taylor, 1967).

The isolate of *Meloidogyne javanica* used was assigned and characterized by Professor Dr. Luiz Antônio Augusto Gomes, Universidade Federal de Lavras (UFLA). This isolate was multiplied in tomato plants maintained in pots of soil, sand and manure (all previously sterilized), in proportion 2:1:1, and kept on suspended benches at approximately 90 cm of the soil level and 100 cm from each other, avoiding possible contaminations.

The experimental design was completely randomized, with four replicates for each treatment and five plants per plot. The experiment was conducted between April and June 2011 and the average of ambient temperature was 25 °C (data provided by the meteorological station of the UFT - Gurupi Campus). Irrigation was performed twice a day, morning and afternoon until field capacity. The sowing of the cultivars was done in plastic pots of 250 mL, containing as substrate a sterilized mixture of ravine and sand soil, manually mixed, in a ratio of 1:1. The average temperature of the soil in the cups was 34.5º C, measured daily with a digital soil thermometer, in a period of 22 days, being eight days after inoculation of the soil with the isolate and eight days before the evaluation, considered the maximum period of development and reproduction of nematodes.

At the time of sowing, basal fertilization was carried out with 1.07 g of NPK granulated formulation 4-14-8 per pot, homogeneously mixed with the soil, and 22 days after sowing, cover fertilization was carried out with 0.35 g of the NPK formulation 20-20-20 per pot, diluted in 50 mL of water.

Soil inoculation with the *Meloidogyne javanica* isolate was performed 12 days after planting, in stage V3 of the common bean plant development. The eggs of *M. javanica* were extracted from susceptible tomato (Santa Cruz cultivar) previously inoculated, according to a technique developed by Hussey & Baker (1973) described by Silva et al. (2004). In summary, after washing, the tomato roots were minced and processed in a blender for 30 s in 0.5% sodium hypochlorite solution. After grinding, the solution was passed through a 0.074 mm sieve and placed on a 0.028 mm sieve, together with abundant pure water. In the 0.074 mm sieve, the root remains were retained, while in the 0.028 mm the eggs and eventual juveniles of the second stage (J2) of *M. javanica* were collected and transferred to a beaker obtaining a suspension adjusted for the average concentration of 1,000 eggs per mL.

For the inoculation of common bean plants, was used the amount of 5 mL of the suspension in the basis of each plant, totaling 5,000 eggs and eventual juveniles (J2) of the respective nematode, applied with the use of a plastic syringe with a volume of 10 mL. Fifty days after inoculation, the plants were carefully removed from the pots and their root systems were washed in running water and submitted to the evaluation of the following traits: gall index (GI), size of galls (SG), position of galls (PG), reproduction index (RI) relative to Santa Clara cultivar of tomato, fresh mass of the roots (FMR) and dry mass of the roots (DMR).

The gall index (GI) was determined according to the International Meloidogyne Project (IMP) scale grades according Taylor & Sasser (1978), where scores between 1 and 5 were assigned to quantify the number of galls throughout the root system of each plant: grade 0 - roots with no galls; grade 1 - roots containing 1 to 2 galls; grade 2 - roots containing 3 to 10 galls; grade 3 - roots containing between 11 and 30 galls; grade 4 - roots containing between 31 and 100 galls; grade 5 - roots containing more than 100 galls.

The reactions of the treatments were classified according to the criteria established by Sasser (1980) that susceptibility reactions are obtained when the average number of galls is equal to or greater than three ($GI \geq 3.00$). The size of galls (SG) was determined according to the average size of the galls (mm of diameter), according to Rios (1990), where: grade 1 ($SG \leq 1$ mm); grade 2 (1 mm $< SG \leq$ 2 mm); grade 3 (2 mm $< SG \leq$ 3 mm); grade 4 (3 mm $< SG \leq$ 4 mm); and grade 5 ($SG >$ 4 mm); were the highest susceptibility equals the greater average size of galls.

Position of galls was determined according to the method of Ponte (1991), which considers only the position of the galls in the root system and is quantified in a scale of five grades, according to the following criteria : grade 0 - total absence of galls; grade 1 - presence of galls only at the main root; grade 2 - galls present in the main and secondary roots; grade 3 - galls present in primary, secondary and tertiary roots; grade 4 - galls present in the primary, secondary, tertiary and quaternary roots, according to Figure 1. The order of hypersensitivity (understood as extremely rapid death of the cells of the root epidermis at the site of nematode penetration) is more frequent in the quaternary and tertiary roots, less frequent in the secondary roots and rare in the pivotal or main.

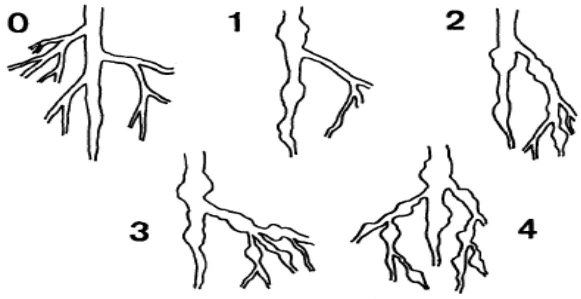

Fig 1. Representation of the grades used to define the positioning of gall in roots of common bean plants, according to Ponte (1991).

The reproduction index (RI) of and resistance to *M. javanica* was determined using the nematode reproduction in tomato as a standard control (100%) in comparison to commercial bean cultivars, according to the methodology established by Taylor (1967) and used by Marchese et al. (2010).

The final population found in common bean cultivars was divided by that found in the tomato, to define the reproduction indexes. Based on these values, the resistance levels of each common bean cultivar to *M. javanica* were defined according to the following reproduction criteria established by Taylor (1967): susceptible plant (S), normal reproduction, RI above of 51%; slightly resistant (SR), RI of 26 to 50%; moderately resistant (MR) with RI of 11 to 25%; very resistant (VR), RI of 1 to 10%; highly resistant/immune (HR/I), RI below 1%.

To determine the fresh and dry mass of roots initially the root systems were weighed to obtain the fresh mass, then the properly identified samples were placed in paper bags in a forced circulation oven with an average temperature of 60-70ºC, until constant mass. Subsequently the samples were again weighed by determining the dry mass of the roots.

The means of each cultivar were submitted to analysis of variance followed by Tukey's test ($p = 0.05$), using SISVAR software (Ferreira, 2008).

Results and discussion

By analyzing variance (Table 1), it was observed that there was no significant difference between the common bean cultivars of the black type for the traits evaluated, except for the size of galls, indicating that there should be no great genetic variability among them the resistance to *M. javanica*.

The higher coefficients of variation are common in experiments that use scale of notes and percentage (Junior et al., 1997). Studying the resistance of sweet potato (*Ipomoea batatas*) clones to gall-forming nematodes, Marchese et al. (2010) found coefficient of variation of 132.33% and 128.33% for the reproduction factor and reproduction index characteristics, respectively, the first one using a scale of grades and the second percentage.

Table 1. Analysis of variance of the traits gall index (GI), size of galls (SG), position of galls (PG), reproduction index (RI), fresh mass of the roots (FMR) and dry mass of the roots (DMR) in seven commercial common bean cultivars (Gurupi, TO, 2011).

Source	DF	Mean Square					
		GI	SG	PG	RI	FMR	DMR
Cultivar	6	0.3032^{ns}	0.3221**	0.5983^{ns}	161.5788^{ns}	0.0749^{ns}	0.0054^{ns}
Residual	18	0.4007	0.0713	0.2810	1.431.485	0.1385	0.0067
Total	27	125.836	33.866	10.105	49.556.308	48.068	0.1924
Average		3.39	1.56	1.76	310.878	3.05	0.58
CV%		18.66	17.03	30.00	38.49	12.18	13.98

^{ns} non-significant; ** significant by F test (p-value < 0.01).

Regarding the characteristic gall index, no genetic variability was observed among the evaluated common bean cultivars, and therefore all were considered susceptible (GI ≥ 3.0) to *M. javanica* (Table 2). These results are different from those found by Baida et al. (2011), where all genotypes of common bean evaluated were resistant (GI ≤ 2.55) to *M. javanica* in studies carried out in the Paraná state and this was probably because the temperatures were lower in relation to the Tocantins state, resulting in less reproduction of females of nematodes in relation to males, and consequently in lower rates of galls.

Table 2. Average of the traits gall index (GI), size of galls (SG), position of galls (PG), reproduction index (RI), fresh mass of the roots (FMR) and dry mass of the roots (DMR) in seven commercial common bean cultivars (Gurupi, TO, 2011).

Cultivars	GI	SG	PG	RI	FMR	DMR
	grades	mm	grades	%	g	g
Xamego	3.68 a	2.00 b	1.69 a	31.03 a	3.01 a	0.55 a
IPR-Grafite	3.22 a	1.44 ab	1.97 a	28.37 a	3.08 a	0.57 a
IAC-Diplomata	3.70 a	1.82 b	2.26 a	35.35 a	2.85 a	0.53 a
BRS-Esplendor	3.50 a	1.50 ab	1.75 a	37.11 a	3.12 a	0.64 a
IPR-Uirapuru	3.13 a	1.55 ab	2.08 a	34.97 a	3.29 a	0.60 a
IPR-Tiziu	3.50 a	1.55 ab	1.50 a	32.46 a	3.06 a	0.59 a
IAC-Una	3.00 a	1.11 a	1.11 a	18.30 a	2.96 a	0.60 a

Same letters in column do not differ statically by Tukey test (p = 0.05).

In relation to size of galls, even though the averages were not high (1 mm <SG ≤ 2 mm), Xamego (SG = 2.00) and IAC-Diplomata (SG = 1.82) present higher values but did not differ from the cultivars that presented average above 1.44 (Table 2). The cultivar IAC-Una showed lower values (SG = 1.11) although it did not differ from cultivars with an average of less than 1,55. This was probably because Xamego and IAC-Diplomat presented a lower degree of resistance to the aggressiveness of M. javannica, allowing the accumulation of larger numbers of females feeding on the same gall. This indicates the potential of the cultivar IAC-Una to be used in breeding programs for resistance to *M. javanica*, since it provides a lower accumulation of larger numbers of females feeding on the same gall, showing a moderate degree of resistance to the aggressiveness of the greenhouse nematodes.

There was no difference between the evaluated cultivars in relation to the position of galls, although there was an amplitude of 1.15 between the means (Table 2). According to the scale of the grades, the cultivars IPR-Uirapuru and IAC-Diplomata presented the highest values (PG ≥ 2), thus, the galls located in the main root and the secondary roots and were therefore considered more hypersensitive. Secondary roots are thinner, and therefore the faster death of the cells of the root epidermis at the site of nematode penetration becomes more sensitive. The cultivar IAC-Una (PG = 1.11) has galls located only in the main root, and is therefore considered to be less hypersensitive, since in thicker roots, the death of the cells of the root epidermis occurs more slowly in the site of nematode penetration. According to Ponte (1991), the order of hypersensitivity (understood as extremely rapid death of cells of the root epidermis at the site of nematode penetration) is, in order, more frequent in the quaternary and tertiary roots, less frequent in the secondary roots and rare in the pivotal or main.

Besides that, there was no difference between the cultivars evaluated for the reproduction index (Table 2). According to the reproduction criteria established by Taylor (1967), BRS-Esplendor, IAC-Diplomata, IPR-Uirapuru, IPR-Tiziu, Xamego and BRS-Grafite were considered slightly resistant and cultivar IAC-Una was moderately resistant to *M. javanica*. Ferreira et al. (2010) found resistance among common bean genotypes evaluated for *M.*

javanica in the region of Lavras, Minas Gerais, probably due to the presence of milder temperatures, thus depressing the higher reproduction of females in relation to males, resulting in a lower reproduction index.

The cultivar IAC-Una was characterized by lower gall index and size of galls, because it was affected only in the main root and was considered moderately resistant due to its reproduction index, showing the potential for the development of new sources of resistance in in plant breeding programs, that seeks tolerant or resistant cultivars to the greenhouse nematode. Simão et al. (2010) did not find sources of resistance to *M. javanica* in the bean genotypes evaluated. Pedrosa et al. (2000) observed several behaviors among the 162 genotypes studied for resistance to *M. javanica*.

Regarding the fresh and dry root mass, it is observed that although there is no difference between the cultivars evaluated, however there is a response of the cultivar IAC-Diplomata to the most severe nematode attack in relation to all other characteristics analyzed, resulting in lesser development and root mass. Baida et al. (2011) working with lineages of *Phaseolus vulgaris* L. also found responses to the most severe nematode attack, resulting in lower mass of roots.

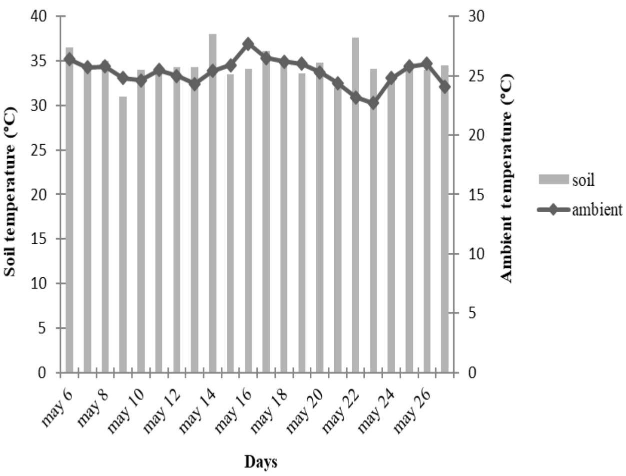

Fig 2. Average temperature (°C) of the ambient and the soil during the conduction of the experiment with greenhouse nematodes in common bean, registered in May 2011 in the critical period of nematode development and reproduction (Gurupi, TO, 2011).

The temperature data, obtained during the experiment, are shown in Figure 2. The maximum ambient temperature was around 28ºC, the minimum of 23ºC, with an average of 25ºC, and the maximum soil temperature, conditioned in the pots, was around 38 ºC, the minimum of 31ºC, with an average of 34ºC. Both the ambient and soil temperatures were considered high, thus favoring the development of gall nematodes.

According to Campos et al. (2011), nematode reproduction rate is affected by elevated temperatures, which increases the stress level of the plant, increasing the proportion of females to males. Elevated temperatures also have direct correlation with the size of galls, resulting in lower resistance in the cultivars used in these environments. Studies with soybean (Campos et al., 2011) and common bean (Owega et al., 1990) showed lower resistance of cultivars or greater reproduction of *Meloidogyne* spp at soil temperatures above 28 ° C. These elevated temperatures probably contributed to the lack of evidence of higher degrees of resistance in the evaluated cultivars.

In the state of Tocantins, where the common bean cultivation is carried out inevitably under elevated temperatures, the identification of genotypes that have some resistance capacity to the greenhouse nematode is a good method to increase and promote the production of this crop in this region.

Conclusion

The seven commercial cultivars of common bean (black type) did not differ in relation to gall index, position of gall, fresh and dry mass of the roots. However, regarding the reproduction index, the cultivar IAC-Una was classified as moderately resistant to *Meloidogyne javanica*, displaying potential for the development of new sources of resistance in in plant breeding programs.

Conflict of interest: All authors declare no conflict of interest

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