Resistance of Dry Bean and Snap Bean Cultivars to Root-knot Nematodes

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Abstract. This study assessed the reaction of dry bean and snap bean cultivars to infection by *Meloidogyne incognita* (races 1, 3) and by *Meloidogyne javanica*, two species of root-knot nematodes. Three independent experiments were done, one for each species or race of *Meloidogyne*, using randomized complete block designs with four replications (plots) and four plants per plot. A plot with the susceptible tomato cultivar Santa Clara was used in each block to determine the efficiency of the inoculum and to calculate the reproduction rate for each of the genotypes assessed. The bean cultivars Aporé and Talismã were highly resistant to *Meloidogyne javanica*, slightly resistant to *Meloidogyne incognita* race 1, and moderately resistant and slightly resistant, respectively, to *Meloidogyne incognita* race 3. The snap bean cultivars Macarrão Atibaia and Macarrão Preferido were slightly resistant to *Meloidogyne javanica* and moderately resistant and susceptible, respectively, to *Meloidogyne incognita* race 1. The reactions of the latter cultivars against *Meloidogyne incognita* race 3 were very resistant and slightly resistant, respectively. The results of these experiments showed that the dry bean cultivars Aporé and Ouro Negro and the snap bean cultivar Macarrão Atibaia have potential to be used in breeding programs for a broader spectrum of root-knot nematode resistances.

Snap beans belong to the same botanical species as the dry beans (*Phaseolus vulgaris* L.) and are among the 10 most widely grown vegetable crops in Brazil, where their production is predominantly based on small farming (Peixoto et al., 2001).

Root-knot nematodes *Meloidogyne* spp. are considered major pathogens causing yield losses in *Phaseolus vulgaris* L., especially in regions with prevalent high temperatures, an environmental factor that increases stress and interferes with the expression of plant resistance to nematode parasitism (Pedrosa et al., 2000). These nematodes have a high reproduction rate, reaching large populations in the soils after successive crops of plants considered as good hosts (Campos et al., 2001).

Dry beans are considered good hosts for both *Meloidogyne incognita* and *M. javanica* with losses that can reduce pod numbers and seed weight per plant by 65%. Several studies report the occurrence of these root-knot nematodes in *P. vulgaris* L. cultivars (Simão et al., 2005; Walber et al., 2003). Studies with snap beans have shown that their susceptibility to *Meloidogyne* spp. is generally similar to that of dry beans. According to Charchar et al. (1995), losses in pod yield caused by *M. javanica* in snap beans can reach 31.5%.

Plant nematodes are hard to control, because their natural habitat is the soil (with great buffering effect) or within roots or other plant tissues. The usual control method for these pathogens is through cultural practices and/or with the use of chemical control with nematicides applied directly to the soil. These methods are not always effective, and chemical products can bring hazards to the environment such as water contamination and residues left in the soil. Depending on the crop and on the chemical safety interval, nematicide residues can be found in the commercial crop produce, bringing risks for human health.

Therefore, other control strategies are being sought, with the most viable and promising one being the use of resistant cultivars. Resistance sources to *Meloidogyne* spp. have been found in dry beans; however, they are not

widely used by breeding programs (Cameiro et al., 2003; Walber et al., 2003). Despite the great losses recorded on the dry bean and snap bean crops, little effort has been deployed toward obtaining resistant cultivars.

Some studies demonstrate the existence of genetic variability for root-knot nematode resistance among bean accessions (Mullin et al., 1991; Omwega and Roberts, 1992; Silva et al., 2005). Vieira (1964), evaluating 18 dry bean cultivars, found that they presented differential responses to damage caused by *M. incognita*, whereas Freire and Ferraz (1977), evaluating 31 cultivars, found that all of them were susceptible to *M. incognita*. Silva et al. (2005) found significant differences in resistance to *M. incognita* among F₃ families derived from a cross between bean cultivars Perola × Batatinha. Evaluation of root-knot nematode resistance of 16 dry bean cultivars (Moura and Regis, 1987) demonstrated that 18.75% of them were highly susceptible, 50% slightly resistant, and 31.25% moderately resistant to *M. javanica*. For *M. incognita*, 12.5% were highly susceptible, 12% susceptible, 25% slightly resistant, and 50% moderately resistant. None of the cultivars were described as resistant, highly resistant, or immune to both nematodes.

Mullin et al. (1991), assessing the reaction of more than 150 lines of dry beans to one or more root nematode *Meloidogyne* populations (*M. incognita* race 3; mixed *M. incognita* and *M. javanica*; *M. hapla*), collected in Colombia and in the United States, found only 30 lines that were moderately resistant or better. Among those that were highly resistant to the nematodes were A211, Carioca, Manoa Wonder, Nemasnap, and PI313709.

Characterization of the reaction of different cultivars to the infestation by specific *Meloidogyne* species, races, or isolates is needed to identify resistant genotypes that can be effectively used in breeding programs for root-knot nematode resistance in dry beans and snap beans. The objective of this study was to determine the resistance in dry bean and snap bean cultivars to *M. incognita* races 1 and 3 and *M. javanica*.

Materials and Methods

Three separate experiments were conducted in a greenhouse at HortiAgro Sementes Ltda in Ijaci, Minas Gerais, Brazil, from 6 Nov. 2007 through 7 Jan. 2008. Seven snap bean cultivars (Macarrão Atibaia, Macarrão Rasteiro Conquista, Macarrão Rasteiro, Macarrão Trepador, Macarrão Rasteiro Dourado, Macarrão Favorito, and Macarrão Preferido) and three dry bean cultivars (Aporé, Ouro Negro, and Talismã) were evaluated for resistance to *M. incognita* races 1 and 3 and *M. javanica* in separate experiments for each of the root-knot nematode tested. The dry bean cultivars were chosen based on several other preliminary studies that described the cultivars Aporé and Ouro Negro as resistant to the root-knot nematode *Meloidogyne incognita* and the cultivar Talismã as susceptible to this parasite (Cameiro et al., 2003; Silva et al., 2005). All

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dry bean and snap bean cultivars tested in the experiments are freely available for trial and breeding purposes.

Each of the experiments was set up in a randomized complete block design with four replications with four plants per plot grown in separate pots. Each block included a four-pot plot with tomato (*Solanum lycopersicum* L.) cultivar Santa Clara known to be susceptible to both *M. incognita* and *M. javanica* to confirm inoculum efficacy and to be a control for the determination of reproduction rates. Three seeds were sown in 3-L plastic pots containing a mixture of soil, sand, and compost (2:1:1 on a volume basis) as substrate, which were thinned to one plant per pot after germination and emergence. Fifteen days after sowing, the substrate was infested with eggs of *M. incognita* races 1 and 3 or *M. javanica* (for each respective experiment). The inocula were obtained from tomato plants of cv. Santa Clara infested with isolates of the nematode species/races under study and maintained in 10-L pots in a greenhouse at the experimental station of HortiAgro Sementes Ltda.

Inoculum was prepared according to Hussey and Barker's (1973) methodology modified by Bonetti and Ferraz (1981). Eggs were counted from a 1-mL aliquot in Peter's chamber (Southey, 1970) with the aid of a stereo microscope. In each experiment, 10,000 eggs (5 mL of a 2000 eggs/mL suspension) were used per 3-L pot, and that was considered the initial nematode population. The suspension was distributed with a syringe directly onto the substrate in two 3-cm deep × 0.5-cm wide holes placed beside the plant stem.

Forty-five days after inoculation (before completion of the second cycle of nematode reproduction), when intense gall formation and egg masses were noticeable on the tomato plants, the nematode reproduction was evaluated for all genotypes. The plants were removed from the pots, and the roots were gently rinsed to remove any adhered substrate. Subsequently, they were blotted dry and weighed to determine the fresh root mass (in grams). The root system was diced and the eggs were extracted as previously indicated (Bonetti and Ferraz, 1981). The number of eggs in the root system was counted with the aid of a stereo microscope, and the final number of nematode eggs per gram of fresh root was estimated both for bean plants (P_b) and susceptible tomato control plants (P_t). These values were used to determine the reproduction index (RI) for each individual plant tested. Reproduction indices were determined by dividing the number of eggs per gram of bean roots (P_b) of each plant by the mean number of eggs per gram of tomato roots (mean of P_t). Results were expressed as relative reproduction indices (RRI) relative to the susceptible tomato standard control (assumed as RRI = 100%). Analyses of variance were done for each experiment with data transformed by $\sqrt{x}$ using the statistical package Sisvar (Ferreira, 2003). Mean separation was according to the Scott and Knott (1974) procedure, a less ambiguous method than other commonly used mean sep-

aration tests (Tukey, Scheffé, Duncan, and others) that provides for a control of the Type I error.

The degree of resistance of each cultivar to *M. incognita* races 1 and 3 and to *M. javanica* was determined based on the RRI according to the criteria established by Taylor (1967). The resistance degrees were considered as follows: S = susceptible (normal reproduction) = reproductive index (RRI) of 50% or more; SLR = slightly resistant = RRI between 25% and 50%; MoR = moderately resistant = RRI between 10% and 25%; VR = very resistant = RRI between 1% and 10%; HR = highly resistant = RRI between 0% and 1%; and I = immune = no reproduction = RRI = 0%.

Results and Discussion

The analyses of variance in all three experiments indicated significant differences in nematode resistance among genotypes, confirming the existence of genetic variability for resistance against *M. javanica* and against *M. incognita* races 1 and 3 in dry bean and snap bean cultivars. Differences among the bean cultivars tested were detected by the Scott-Knott test at 5% with respect to root-knot nematode reproduction (Table 1).

According to the reproduction criterion established by Taylor (1967), the dry bean cultivars Aporé and Talismã were considered as very resistant (VR) against *M. javanica* (Table 1). The dry bean cv. Ouro Negro and the snap beans 'Macarrão Preferido', 'Macarrão Atibaia', and 'Macarrão Trepador' were considered slightly resistant (SLR) to this nematode (Table 1). In contrast, the snap bean cultivars Macarrão Favorito, Macarrão Rasteiro Conquista, Macarrão Rasteiro, and Macarrão Rasteiro Dourado were susceptible (S) to *M. javanica* (Table 1).

Other authors reported sources of resistance to *M. javanica* in *Phaseolus vulgaris*.

Perre and dos Santos (2003) reported eight cultivars considered resistant to *M. javanica* and three ('Diamante Negro', 'FT-Nobre', and 'Iapar-80') susceptible (with relative RIs greater than 1.00) that were considered good nematode hosts. Pedrosa et al. (2000) reported only five *M. javanica* resistant genotypes ('A 521', 'A 790', 'L 611002', 'L 634005', and 'L 639012') among 162 tested based on egg mass and RIs relative to the susceptible dry bean cultivar control IPA-9. Despite different methodologies deployed for assessment of *M. javanica* resistance, Perre and dos Santos (2003) and Pedrosa et al. (2000) as well as our present results indicate variability for resistance to this nematode species among *Phaseolus vulgaris* genotypes.

Snap bean cultivar Macarrão Atibaia presented the greatest resistance level against *M. incognita* race 1 and was classified as moderately resistant (MoR) (Table 1). The dry bean cultivars Ouro Negro, Talismã, and Aporé and the snap bean cultivar Macarrão Favorito were considered slightly resistant (SLR) (Taylor, 1967), although the Scott-Knott test did not indicate significant differences among these cultivars and 'Macarrão Atibaia' (Table 1). In contrast, snap bean cultivars Macarrão Trepador, Macarrão Preferido, Macarrão Rasteiro, Macarrão Rasteiro Conquista, and Macarrão Rasteiro Dourado were susceptible (S) (Table 1). In contrast with these results, Pedrosa et al. (2000), using the dry bean cultivar IPA-9 as a susceptible control, could not identify genotypes with resistance to *M. incognita* race 1 among the 162 accessions tested.

Similar to the *M. incognita* race 1 trial, snap bean cultivar Macarrão Atibaia presented the highest level of resistance against *M. incognita* race 3 and was classified as very resistant (VR) (Table 1). The dry bean cultivars Ouro Negro and Aporé and the snap bean cultivar Macarrão Favorito were moderately resistant (MoR), whereas dry bean cultivar Talismã and

Table 1. Relative reproduction index (RRI) of *Meloidogyne javanica* and *Meloidogyne incognita* races 1 and 3 and resistance degree (RD) of three dry bean cultivars, seven snap bean cultivars, and the tomato cultivar Santa Clara (standard susceptible control); Ijaci, MG, Brazil; 2008.

Crop	Cultivar	<i>Meloidogyne incognita</i>					
		<i>Meloidogyne javanica</i>		Race 1		Race 3	
		RRI(%) ^{z,x}	RD ^{y,w}	RRI(%) ^{z,x}	RD ^{y,w}	RRI(%) ^{z,x}	RD ^{y,w}
Tomato	Santa Clara	100 c	S	100 b	S	100 b	S
Dry bean	Aporé	3 a	VR	42 a	SLR	12 a	MoR
	Talismã	9 a	VR	40 a	SLR	27 a	SLR
	Ouro Negro	41 b	SLR	29 a	SLR	11 a	MoR
Snap bean	Macarrão Atibaia	48 b	SLR	15 a	MoR	6 a	VR
	Macarrão Preferido	36 b	SLR	65 b	S	36 a	SLR
	Macarrão Trepador	49 b	SLR	52 b	S	29 a	SLR
	Macarrão Favorito	66 b	S	32 a	SLR	15 a	MoR
	Macarrão Rasteiro	125 c	S	157 c	S	146 b	S
	Conquista						
	Macarrão Rasteiro	155 c	S	151 c	S	149 b	S
	Macarrão Rasteiro Dourado	341 d	S	400 d	S	311 c	S

^zRRI = reproduction index relative to susceptible tomato.

^yMeans followed by the same letter do not differ from each other by the Scott and Knott (1974) test at 5% probability.

^wRD = resistance degrees, as defined by Taylor (1967).

^xS = susceptible (more than 50%); SLR = slightly resistant (25% to 50%); MoR = moderately resistant (10% to 25%); VR = very resistant (1% to 10%); HR = highly resistant (less than 1%); I = 0% = immune, according to Taylor (1967).

snap bean cultivars Macarrão Trepador and Macarrão Preferido were slightly resistant (SLR) by Taylor's (1967) criterion, although their RRI's did not differ significantly from that of 'Macarrão Atibaia' by the Scott-Knott test (Table 1). The snap bean cultivars Macarrão Rasteiro Conquista, Macarrão Rasteiro, and Macarrão Rasteiro Dourado were susceptible (S) to this nematode (Table 1). In contrast with our results, a study by Carneiro et al. (1992) found that all 111 dry bean accessions tested were found to be susceptible to *M. incognita* race 3. However, their results may not be strictly comparable to our results, because the set of accessions tested, the control dry bean cultivar (Rico 23), and the trait assessed (gall index) were different.

The present study indicated that there is genetic variability for resistance against root-knot nematodes among the *Phaseolus vulgaris* cultivars of both dry beans and snap beans. The resistance reaction tends to be different, in some genotypes, when challenged with different nematode species or races, a conclusion inferred from Pedrosa et al. (2000). This was the case with dry bean cultivars Aporé and Talismã, which were very resistant to *M. javanica*, but only slightly resistant to *M. incognita* race 1, whereas their resistance to race 3 of *M. incognita* was moderate and slight, respectively. Among the snap beans, the cultivars Macarrão Atibaia and Macarrão Preferido were slightly resistant to *M. javanica*, but, when challenged with *M. incognita* race 1, they were classified as moderately resistant and susceptible, respectively. When race 3 of *M. incognita* was used, their reactions were very resistant and slightly resistant, respectively.

These results indicate that resistance against root-knot nematodes in *Phaseolus vulgaris* may be controlled by different genes depending on the *Meloidogyne* species or races considered. Crosses between cultivars with the greatest resistance level for different root-knot nematode species or races should be feasible alternatives to obtain new cultivars with a resistance to a broader range of root-knot nematode species or races. Among the genotypes

tested, the snap bean cultivar Macarrão Atibaia appears to be the best source of resistance to *M. incognita* races 1 and 3 according to Taylor's (1967) criterion, whereas the dry bean cultivars Aporé and Talismã are the best sources of resistance *M. javanica*. Attempts to breed new dry bean or snap bean cultivars with resistance to a broad range of root nematode species or races should include crosses with snap bean cultivar Macarrão Atibaia and either dry bean cultivar Aporé or Talismã.

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