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Host status of soybean genotypes to *Meloidogyne arenaria* and *Meloidogyne morocciensis*

Hospedabilidade de genótipos de soja a Meloidogyne arenaria e Meloidogyne morocciensis

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ABSTRACT

Soybean crop productivity is limited by several biotic factors, particularly plant-parasitic nematodes. Several species have been reported to cause crop damage, especially those of the genus *Meloidogyne*. Therefore, the aim of this study was to evaluate, the reaction of 28 soybean genotypes to *Meloidogyne arenaria* and *M. morocciensis* in a greenhouse. The soybean genotypes were the same for experiments with different species of plant-parasitic nematodes and were individually inoculated with 5,000 eggs + second-stage juveniles (J2) of *Meloidogyne* and kept in a greenhouse. After 60 days of inoculation, the roots of each plant were assessed for the number of galls, final population, and reproduction factor (RF = final population/initial population). The averages of the different variables were then compared to each other by the Scott-Knott cluster analysis at a significance level of 5%. All of the soybean genotypes in the study were susceptible to both nematodes, with RF ranging from 3.5 to 24.1 for *M. arenaria* and 5.3 to 37.5 for *M. morocciensis*.

KEYWORDS: *Glycine max,* root-knot nematode, reproduction, susceptibility.

RESUMO

A cultura da soja tem sua produtividade limitada por diversos fatores bióticos, dentre estes destacam-se os fitonematoides. Várias espécies já foram relatadas causando danos à cultura, especialmente as do gênero *Meloidogyne*. Diante disso, o objetivo do trabalho foi avaliar, em casa de vegetação, a reação de 28 cultivares de soja a *M. arenaria* e *M. morocciensis*. As cultivares de soja testadas foram as mesmas para os dois ensaios, sendo individualmente inoculadas com 5.000 ovos + juvenis do segundo estádio (J2) de *Meloidogyne* mantidas em casa de vegetação. Decorridos 60 dias da inoculação, as raízes de cada planta foram avaliadas quanto ao número de galhas, população final e fator de reprodução (FR = população final/população inicial). A seguir, as médias das diferentes variáveis foram comparadas entre si pelo teste de agrupamento de Scott e Knott a 5%. Todas as cultivares de soja avaliadas comportaram-se como suscetíveis a ambos nematoides, com FR variando 3,5 a 24,1 para *M. arenaria*, e de 5,3 a 37,5 para o *M. morocciensis*.

PALAVRAS-CHAVE: *Glycine max,* nematoide-das-galhas, reprodução, suscetibilidade.

INTRODUCTION

Soybean (*Glycine max*) is one of the most socioeconomically important crops for the world and Brazil. The world's soybean production is approximately 320 million tons, while Brazil produces about 96 million tons in an area of about 36 million hectares (CONAB 2019). However, this production is affected by the onset of diseases, which may be caused by fungi, bacteria, viruses, and nematodes, capable of drastically decreasing productivity.

In the various soybean-producing regions around the world, over 100 species of plant-parasitic nematodes have been reported for causing infection and crop damage (DIAS et al. 2010). These species are distributed across 50 genera and cause an estimated 30% in average losses when associated with the soybean crop, making crop production completely unfeasible in extreme cases (DIAS et al. 2010). Nevertheless, losses vary depending on the plant-parasitic nematode species, population levels, susceptibility of the cultivar or genotype, and the growing season of the year (ASMUS 2001, BELLÉ et al. 2017).

In Brazil, root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) are the most common nematodes related to soybean crop damage (DIAS et al. 2010). Symptoms of damage in the soybean crop by plant-parasitic nematodes are generally observed in patches where the plants are stunted and yellowish and in the roots where there are twigs or lesions of varying number and size, depending on the susceptibility of the cultivar or genotype, and the nematode population density in the soil (DIAS et al. 2010). In this context, using genotypes that are resistant to plant-parasitic nematodes is advantageous: they reduce nematode reproduction and the risk of environmental contamination; they are easy to use and do not require special equipment; and their cost of acquisition is similar to the cost of seeds of susceptible cultivars (ARAUJO et al. 2012).

In recent years, the expansion of soybean cultivation areas has evidenced the exposure of the crop to other species of the genus *Meloidogyne*, such as *M. arenaria* and *M. morocciensis* (KIRSCH et al. 2016, MATTOS et al. 2016). The literature has few studies related to these species' reaction to the soybean crop. Given the occurrence of these species in soybean plants, studies on the resistance of soybean materials to root-knot nematodes are important for better crop management planning. Therefore, the aim of this study was to evaluate the reaction of soybean genotypes to *M. arenaria* and *M. morocciensis* under greenhouse conditions.

MATERIAL AND METHODS

In 2018, two greenhouse trials were conducted to observe the reaction of 28 soybean genotypes (Table 1) to the nematodes *M. arenaria* (January to April) and *M. morocciensis* (February to June). A completely randomized design with ten replications was adopted for all trials. The initial inocula of *M. arenaria* (Est. A2) and *M. morocciensis* (Est. A3) were taken from pure populations of their respective species that had multiplied in tomato plants (*Solanum lycopersicum*) of the Santa Clara cultivar. As proposed by CARNEIRO & ALMEIDA (2001), the isozyme electrophoresis technique was performed to confirm the species.

Three days after emergence, individual soybean plants of different genotypes were transplanted to pots (2,000 dm^3) containing sterilized substrate (mixture of sand and soil at a 2:1 ratio). Five days after transplantation, the plants were inoculated separately with different species of *Meloidogyne* using a suspension of 5,000 eggs + second-stage juveniles (J2) (initial population) based on the method of HUSSEY & BARKER (1973) and modified by BONETI & FERRAZ (1981). Santa Clara tomatoes were used for viability testing of the inoculum.

The soil used in the experiment is classified as dystrophic Red Oxisol and has the following physicochemical properties: clay = 61%; water pH value = 6.2; SMP index = 6.5; organic matter = 3.1%; phosphorus = 10.9 mg dm^{-3}; potassium = 88 mg dm^{-3}; calcium = 5.3 $cmol_c$ dm^{-3}; magnesium = 5.0 $cmol_c$ dm^{-3}; and sulfur = 10 $cmol_c$ dm^{-3}.

Sixty days after inoculation, the root system was washed individually under tap water. After removing the excess water with paper towels, it was weighed, and the number of galls were counted. Following the method of HUSSEY & BARKER (1973), the root systems were processed using a 0.5% sodium hypochlorite solution to triturate the roots in a blender to obtain the final suspension and quantify the nematode population. Additionally, the number of nematodes per gram of root was estimated, which is determined by the ratio between the total number of nematodes and the total mass of the roots in grams in each replicate.

The experiment was conducted twice, and the similarity between the experiments was tested by a preliminary analysis of variance (ANOVA) using experimental runs as factors in order to determine the interaction between experiment and treatment. This interaction was not significant, thereby allowing data to be combined for ANOVA. Hartley's test (homoscedasticity) was used to analyze data sets (NG, NNGR, and RF) prior to the one-way analysis of variance (ANOVA). Subsequently, treatments were compared with the Scott-Knott cluster analysis at a 95% confidence level using the GENES software. Furthermore, the soybean genotype's reaction was classified according to the reproduction factor (RF) values for each of the nematodes tested (RF = final population (Pf)/initial population (Pi)), a method proposed by OOSTENBRINK (1966), where rice cultivars were classified as resistant when RF<1.00 and susceptible when RF>1.00.

Table 1. Description of commercial soybean genotypes and their respective characteristics.

Genotype	Breeder	Growth habit	Maturation Group
BMX Alvo RR	Brasmax	Undetermined	5.9
BMX Apolo RR	Brasmax	Undetermined	5.5
BMX Garra IPRO	Brasmax	Undetermined	6.3
BMX Ícone IPRO	Brasmax	Undetermined	6.8
BMX Lança IPRO	Brasmax	Determined	5.6
BMX Ponta IPRO	Brasmax	Undetermined	6.6
BMX Potencia RR	Brasmax	Undetermined	6.7
BMX Raio IPRO	Brasmax	Undetermined	5.0
BMX Tornado RR	Brasmax	Undetermined	6.2
BMX Turbo RR	Brasmax	Undetermined	5.8
BMX Veloz RR	Brasmax	Undetermined	5.0
BMX Zeus IPRO	Brasmax	Undetermined	5.5
BRS 246 RR	Embrapa	Determined	7.0
BRSMT Pintado	Embrapa	Undetermined	8.4
CD 235 RR	Coodetec	Semi-determined	6.4
FPS Atalanta IPRO	Fundação Pró-sementes	Undetermined	5.7
FPS Urano RR	Fundação Pró-sementes	Determined	6.3
FPS Solar IPRO	Fundação Pró-sementes	Undetermined	6.3
Fundacep 58 RR	Fundacep	Determined	6.8
GMX Redomão RR	Gmax Genética	Undetermined	6.2
GMX Xiru RR	Gmax Genética	Undetermined	6.4
HO Amambay IPRO	Ho Genetica	Undetermined	5.8
HO Arinos RR	Ho Genetica	Undetermined	7.1
HO Iriri RR	Ho Genetica	Undetermined	7.1
NA 5909 RR	Nidera	Undetermined	6.2
NS 5959 RR	Nidera	Undetermined	6.9
TEC 5936 IPRO	CCGL TEC	Undetermined	6.1
TMG 7067 INOX	Tropical Melhoramento e Genética	Semi-determined	6.7

RESULTS AND DISCUSSION

Based on the results, all the soybean genotypes in the study were susceptible (RF>1.00) to *M. arenaria* and *M. morocciensis* (Tables 2 and 3). However, different levels of susceptibility were observed between the genotypes. In the tomato plants used to verify the viability of the *M. arenaria* and *M. morocciensis* inocula, the values for RF were 36 and 54, respectively. Therefore, viability of the inocula used in the experiments was confirmed.

The highest incidence of galls caused by *M. arenaria* was observed for soybean genotypes BMX Icone IPRO, BMX Lança IPRO, and BRSMT Pintado, with 556 to 626 galls per plant (Table 2). While the genotypes HO Arinos RR, HO Amambay IPRO, CD 235 RR, HO Iriri RR, BMX Raio IPRO, BMX Zeus IPRO, BMX Turbo RR, Fundacep 58, GMX Redomão RR, NS 5959 RR, BMX Apolo RR, and FPS Atalanta IPRO presented the lowest number of galls caused by *M. arenaria*, differing statistically from the other genotypes (Table 2). As for the number of galls caused by *M. morocciensis* in each root system, the genotypes with the lowest values were HO Arinos RR and HO Amambay IPRO at 281 and 298 galls, respectively (Table 3). The TEC 5936 IPRO, TMG 7067 INOX, BMX Icone IPRO, and BRS 246 RR genotypes presented the highest incidence of galls from this nematode, with 557 to 689 galls per root system.

The number of eggs and J2 *Meloidogyne* per gram of soybean root showed varying responses from the genotypes. HO Amambay IPRO, HO Arinos RR, BMX Ray IPRO, HO Iriri RR, CD 235 RR, and BMX

Zeus IPRO registered the lowest values for *M. arenaria*, while BMX Icone IPRO, BMX Launches IPRO, and TMG 7067 INOX presented the highest values. (Table 2). The genotypes BRS 246 RR, BMX Zeus IPRO, TMG 7067 INOX, and BMX Icone IPRO exhibited the highest population densities of *M. morocciensis* per gram of root, while HO Amambay IPRO, HO Arinos RR, and BMX Potencia RR had the lowest, thus differing significantly from the other genotypes (Table 3).

Table 2. Number of galls (NG), number of nematodes per gram of root (NNGR), and reproduction factor (RF) of *Meloidogyne arenaria* in different soybean genotypes.

Genotypes	NG		NNGR[1]		FR[2]		Reaction[3]
BMX Alvo RR	345	C[4]	2361	C	14,0	C	S
BMX Apolo RR	299	D	1811	C	11,5	D	S
BMX Garra IPRO	344	C	2372	C	13,2	C	S
BMX Ícone IPRO	556	A	4021	A	21,1	A	S
BMX Lança IPRO	572	A	4198	A	22,0	A	S
BMX Ponta IPRO	432	B	2943	B	16,6	B	S
BMX Potência RR	431	B	2570	C	16,6	B	S
BMX Raio IPRO	161	D	796	D	6,2	E	S
BMX Tornado RR	445	D	2541	C	16,4	C	S
BMX Turbo RR	213	D	1796	C	8,2	D	S
BMX Veloz RR	366	C	2479	C	14,1	C	S
BMX Zeus IPRO	165	D	1105	D	6,4	E	S
BRS 246 RR	393	C	3222	B	15,1	C	S
BRSMT Pintado	626	A	3186	B	24,1	A	S
CD 235 RR	147	D	1052	D	5,7	E	S
FPS Atalanta IPRO	301	D	2280	C	11,6	D	S
FPS Urano RR	340	C	2174	C	13,1	C	S
FPS Solar IPRO	385	C	2547	C	11,8	D	S
Fundacep 58	272	D	2432	C	10,5	D	S
GMX Redomão RR	281	D	2057	C	10,8	D	S
GMX Xiru RR	452	B	2607	C	17,4	B	S
HO Amambay IPRO	96	D	554	D	3,7	E	S
HO Arinos RR	91	D	580	D	3,5	E	S
HO Iriri RR	156	D	1030	D	6,0	E	S
NA 5909 RR	359	C	2066	C	13,8	C	S
NS 5959 RR	282	D	1975	C	10,8	D	S
TEC 5936 IPRO	379	C	2129	C	17,4	B	S
TMG 7067 INOX	420	B	4210	A	23,2	A	S
Tomato (control)	920	-	5515	-	36	-	S
CV (%)	21,7	-	27,78	-	24,8	-	-

[1]Number of nematodes per gram of root: ratio between total nematodes and total root mass. [2]RF = Final population/Initial population. [3]Reaction based on OOSTENBRINK (1966): resistant (R) (RF<1.0) and susceptible (S) (RF≥1.0). [4]Means followed by the same letter in each column do not differ significantly by the Scott-Knott test at 5 % probability.

Reproduction of root-knot nematodes in the different soybean genotypes showed the highest reproduction factor values for *M. arenaria* in BMX Icone IPRO, BMX Lance IPRO, TMG 7067 INOX, and BRSMT Pintado presented, ranging from 21.1 to 24.1 (Table 2). The genotypes HO Arinos RR, HO Amambay IPRO, and CD 235 RR had the lowest reproduction factor values at 3.5, 3.7, and 5.7, respectively. The highest reproduction factor values for *M. morocciensis* was observed in the genotypes BMX Icone IPRO and BRS 246 RR, at 32.7 and 37.5, respectively (Table 3). However, the genotypes HO Arinos RR and HO Amambay IPRO demonstrated the lowest reproduction factor values, ranging between 5.3 and 5.9, for this nematode (Table 2).

Table 3. Number of galls (NG), number of nematodes per gram of root (NNGR), and reproduction factor (FR) of *Meloidogyne morocciensis* in different soybean genotypes.

Genotypes	NG		NNGR[1]		FR[2]		Reaction[3]
BMX Alvo RR	509	C[4]	2773	D	16,9	C	S
BMX Apolo RR	553	C	3548	C	20,0	C	S
BMX Garra IPRO	432	D	2459	D	11,9	D	S
BMX Ícone IPRO	707	A	6122	A	32,7	A	S
BMX Lança IPRO	616	B	4251	B	24,7	B	S
BMX Ponta IPRO	618	B	3923	B	24,9	B	S
BMX Potencia RR	375	D	1156	E	9,3	D	S
BMX Raio IPRO	330	D	1677	D	8,5	D	S
BMX Tornado RR	426	D	2526	D	11,8	D	S
BMX Turbo RR	541	C	3618	B	19,8	C	S
BMX Veloz RR	388	D	1671	D	9,8	D	S
BMX Zeus IPRO	610	B	5234	A	24,4	B	S
BRS 246 RR	757	A	5022	A	37,5	A	S
BRSMT Pintado	358	D	1619	D	8,4	D	S
CD 235 RR	534	C	3648	B	18,5	C	S
FPS Atalanta IPRO	553	C	3304	C	19,9	C	S
FPS Urano RR	601	B	3401	C	20,1	C	S
FPS Solar IPRO	503	C	3936	B	16,7	C	S
Fundacep 58	507	C	3223	C	16,8	C	S
GMX Redomão RR	644	B	4071	B	27,0	B	S
GMX Xiru RR	540	C	4503	B	26,5	B	S
HO Amambay IPRO	298	E	835	E	5,9	E	S
HO Arinos RR	281	E	849	E	5,3	E	S
HO Iriri RR	378	D	1628	D	9,3	D	S
NA 5909 RR	581	C	3240	C	22,2	C	S
NS 5959 RR	521	C	3128	C	17,8	C	S
TEC 5936 IPRO	689	A	4732	B	28,4	B	S
TMG 7067 INOX	690	A	5740	A	27,8	B	S
Tomato (control)	902	-	8399	-	54,0	-	S
CV (%)	20,9	-	26,8	-	25,11	-	-

[1]Number of nematodes per gram of root: ratio between total nematodes and total root mass. [2]RF = Final population/Initial population. [3]Reaction based on OOSTENBRINK (1966): resistant (R) (RF<1.0) and susceptible (S) (RF≥1.0). [4]Means followed by the same letter in each column do not differ significantly by the Scott-Knott test at 5% probability.

The results show that all the soybean genotypes in the study are susceptible to *M. arenaria* and *M. morocciensis*. Unfortunately, sources of resistance to these and other species of root-knot nematodes of soybean are scarce. Most of the cultivars considered to be resistant to these nematodes descend from a single source of resistance, the "Bragg" genotype. In addition to the Bragg genotype, there are other sources of resistance that are used to a lesser extent in breeding programs, such as the Hartwig, Kirby, Cordell, and Leflore genotypes, which, in addition to the resistance genes for *Meloidogyne,* also have resistance genes for *H. glycines* (SILVA 2001).

The susceptibility of soybean genotypes to *M. arenaria* and *M. morocciensis* is an important indication of the need for other control measures for this plant parasite, since these species are widespread in cultivated areas. Although several management strategies are applied to increase crop yield, none have been fully effective in keeping populations below the level of economic loss (DIAS-ARIEIRA et al. 2018). Research on genetic resistance is important from the perspective of controlling plant-parasitic nematodes since the responses to these pathogens are reproduced in these hosts. In this sense, multiplication of *M. arenaria* and *M. morocciensis* in soybean generally occurs rapidly and reaches high levels within a short period of time.

The most widely used strategy for controlling nematodes in the soil is rotation/succession with non-host or poor-host crops and the use of resistant soybean genotypes when commercially available (DIAS-ARIEIRA & CHIAMOLERA 2011, SCHMITT & BELLÉ 2016). Genetic materials with lower susceptibility can also be used, as observed in this study, and can serve as a tool to contribute to proper management. However, when selecting soybean genotypes, in addition to nematode resistance, their adaptation and yield potential in the region should also be considered.

Other control measures should also be implemented for the integrated management of these nematodes, such as recovering organic matter and soil microbial activity, aiding the population growth of the natural enemies of nematodes, using antagonistic or non-host plants, and applying systemic nematicides (SANTANA-GOMES et al. 2014). The combined use of these management techniques will help decrease the initial nematode population in soybean-producing areas, thereby resulting in fewer problems and higher crop yields.

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