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CROP SCIENCE

Reproduction of *Meloidogyne javanica* in soybean genotypes

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Abstract: *Meloidogyne javanica* is among the most important nematodes that damage soybean, and although genetic resistance is the ideal control measure, there are few cultivars described as resistant among those recommended for southern Brazil. The objective of this work was to evaluate the reaction of soybean cultivars to *M. javanica*. The inoculum of nematodes (Est. J3) was obtained from soybean plants and inoculated into tomato plants cultivar “Santa Cruz”. Thirty-seven soybean cultivars widely used in the South, Southeast and Midwest of Brazil were used in the experiment. For each plant a suspension of 5,000 eggs + juveniles of second stage of *M. javanica* was inoculated into a sterile soil hole in 2-liter pots with six replications. The evaluation of root weight, number of galls, number of nematodes was 60 days after *M. javanica* inoculation. The results were subjected to analysis of variance, and the averages of each treatment were compared to each other by the Scott-Knott cluster test at 5% probability. Even though *M. javanica* presented RF> 1.00 in all soybean genotypes tested, different levels of susceptibility were observed. Thus, the lowest reproduction of the root-knot nematode was observed in M 5947 IPRO, HO AMAMBAY IPRO, BMX GARRA IPRO and FPS ATALANTA.

Key words: Root-knot nematode, *Glycine max*, Genetic control, Integrated management, Resistance.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) had about 35.82 million hectares cultivated in the 2018/19 crop in Brazil, producing 115 million tons (CONAB 2019). Soybean crop yield can be decreased with pests, diseases, weeds and other factors. Among the biotic factors, plant-parasitic nematode can generate losses above 30% in soybean crop (Agrios 2005, Araujo et al. 2012). There are approximately 100 species of nematodes that can parasitize soybean in Brazil and worldwide (Dias et al. 2010).

The plant-parasitic nematode genera reported as the most frequent in soybean are Meloidogyne Göldi 1887, Heterodera Ichinohe 1952, Pratylenchus Filipjev 1936 and, Rotylenchus Linford; Oliveira, 1940, being the root-knot nematode the most harmful to the culture (Antonio 1992, Gomes et al. 2003, Kirsch et al. 2016). In the genus Meloidogyne, the species *Meloidogyne javanica* (Treub, 1885) Chitwood 1949 for presenting a wide geographical distribution and wide host range (Soares 2006), which includes weeds (Balardin et al. 2019, Ramos et al. 2019), may lead to losses of 10 to 40% in soybean crop (Almeida et al. 2005, Miranda et al. 2011). Symptoms in soybean-parasitized crops are generally observed in patches, where plants are stunted and yellowish, and in roots there are galls of varying number and size, depending on cultivar susceptibility and nematode population density in the soil (Dias et al. 2010).

To minimize losses caused by nematodes, it is necessary to use a set of strategies to control this pest. Soares et al. (2016) explain that there are many ways to control nematodes, but most effective modes of management have limiting factors caused by nematode-like abilities, such as the ability to penetrate host plant roots that have thin cuticles (or absent) that are not resistant to the penetration of these pests. Therefore, host plants that do not have basic plant-parasitic nematode defense characteristics should not be used in the integrated management of this pest.

Integrated management brings a combination of strategies to overcome or reduce the parasitic capacity of nematodes to plants. This type of management integrates biological control, crop rotation, chemical nematicides and resistant cultivars (Almeida et al. 2005, Lima et al. 2017). Thus, genetic management is one of the best ways to control nematodes, as it does not increase the cost of production for farmers and also helps to reduce the use of chemical nematicides, which benefits the environment (Teixeira 2013).

However, several soybean cultivars have been described as resistant or moderately resistant to *M. javanica*, although presenting low levels of resistance to *M. javanica*, under conditions of high soil nematode populations, this resistance may be overcome (Dias et al. 2010). Another reason for overcoming resistance is that most resistant cultivars are descended from the same source of resistance, the North American cultivar 'Bragg'. In addition to Bragg there are other cultivars that are used in breeding programs, such as the Cordell, Hartwig, Kirby and Leflore cultivars, but these cultivars are used less than 'Bragg' because of their difficulty in transmitting the resistance gene (Silva, 2001, Dias et al. 2010, Schmitt and Belle 2016).

For Mazzetti et al. (2019), there is not enough information on the reaction of currently used commercial soybean cultivars, which makes it difficult to choose cultivars for infested areas. Therefore, this study aimed to study the reaction of soybean cultivars to *Meloidogyne javanica*.

MATERIALS AND METHODS

Thirty-seven soybean genotypes were used (Table I). In these genotypes the reaction to *Meloidogyne javanica* was evaluated in greenhouse under controlled temperature of 25°C ± 2°C.

The population of *M. javanica* (Est.J3) used was obtained from soybean plants (BMX Ativa RR cultivar) from Julio de Castilhos municipality, Rio Grande do Sul, Brazil, and multiplied in tomato 'Santa Cruz' (*Solanum lycopersicum*). The identification of root-knot nematode species was performed by electrophoresis using isoenzyme esterase (Est) in 7% polyacrylamide gel, according to Carneiro & Almeida (2001).

Individual soybean plants of different genotypes (Table I) were kept in 2000 dm^3 pots with sterilized soil and inoculated with a suspension of 5,000 eggs + second stage (J2) of *M. javanica*, obtained according to Hussey & Barker (1973) method, modified by Bonetti & Ferraz (1981). The method consists of grinding in a blender with the addition of 0.5% sodium hypochlorite followed by sieving and centrifugation with sucrose solution. Inoculation was performed in three 4 cm deep holes around each soybean plant, eight days after emergence. As a positive control of the treatments, and to confirm the viability of the inoculum, tomato plants "Santa Cruz" were used, which were inoculated with the same amount of inoculum *M. javanica* inoculated at the same time as soybean seedlings. Fertilization was carried out according to the recommendation described

Table I. **Description of commercial soybean genotypes with their respective agronomic characteristics.**

Cultivars	Company	Growth habit	Maturation group
98Y52	Pioneer	Undetermined	8.5
BMX Raio IPRO	Brasmax	Undetermined	5.0
BMX Alvo RR	Brasmax	Determined	5.9
BMX Ativa RR	Brasmax	Determined	5.6
BMX Desafio RR	Brasmax	Undetermined	7.4
BMX Elite IPRO	Brasmax	Undetermined	5.5
BMX Foco IPRO	Brasmax	Undetermined	7.2
BMX Garra IPRO	Brasmax	Undetermined	6.3
BMX Lança IPRO	Brasmax	Undetermined	5.8
BMX Vanguarda IPRO	Brasmax	Undetermined	6.0
BMX Veloz RR	Brasmax	Undetermined	5.0
BMX Zeus IPRO	Brasmax	Undetermined	5.5
BRS 7380 RR	Embrapa	Undetermined	7.3
CD 2728 IPRO	Coodetec	Undetermined	7.2
DM 4309 IPRO	Dom Mario	Undetermined	6.1
DM 53I54 IPRO	Dom Mario	Undetermined	5.4
FPS Atalanta IPRO	Fundação Pró-Sementes	Undetermined	5.8
FPS Urano RR	Fundação Pró-Sementes	Determined	6.2
FTR 2155 RR	FT Sementes	Undetermined	5.8
GMX Cancheiro RR	Gmax Genética Gaúcha	Undetermined	6.2
HO Amambay IPRO	HO Genética	Undetermined	5.8
HO Arinos RR	HO Genética	Undetermined	7.1
M 5947 IPRO	Monsoy	Undetermined	5.9
M 8210 IPRO	Monsoy	Determined	8.2
NS 4823 RR	Nidera Sementes	Undetermined	4.8
NS 5000 IPRO	Nidera Sementes	Undetermined	5.0
NS 5106 IPRO	Nidera Sementes	Undetermined	5.2
NS 5160 IPRO	Nidera Sementes	Undetermined	5.3
NS 5258 RR	Nidera Sementes	No information	-
NS 5445 IPRO	Nidera Sementes	Undetermined	5.4
P95R51	Pioneer	Undetermined	5.7
P95Y72	Pioneer	Undetermined	5.5
Produza IPRO	FAPA	Semi-determined	6.0
Rota 54 IPRO	Sementes Roos	Undetermined	5.4
SYN 13671 IPRO	Syngenta	Undetermined	7.3
AMS Tibagi RR	Melhoramento Agropastoril Ltda	Semi-determined	5.0
TMG 1180 RR	TMG	Semi-determined	8.0

FAPA - Agricultural Foundation for Agricultural Research; TMG - Tropical Breeding & Genetics.

for soybean culture by the Soil Fertility and Chemistry Commission of the states of Rio Grande do Sul and Santa Catarina (2016).

After 60 days of the inoculation of *M. javanica*, the roots of each soybean plant were separated from the shoot to evaluate the number of galls. Next, eggs + J2 were extracted from the roots of each plant, from each genotype, according to the methodology of Hussey & Barker (1973), modified by Bonetti & Ferraz (1981), to quantify and determine the reproduction factor (RF = final population / initial population) of *M. javanica* (Oostenbrink 1966). The RF was determined in each of the repetitions.

First, the roots of each plant were cleaned and weighed to obtain the weight of the root system, then processed to extract the nematodes, according to the methodology cited specifically for *Meloidogyne*. Subsequently, we counted the number of nematodes / roots to determine the reproduction factor (RF), using the methodology described above. Additionally, the number of nematodes per gram of root was estimated, defined by the ratio between the total number of nematodes and the total root mass, in grams, for each repetition.

The experimental design used in the experiment was completely randomized with six replications. Treatments with values of the different variables obtained in each repetition were subjected to analysis of variance, and the averages of each treatment were compared with each other by the Scott-Knott clustering test (1974) at 5% probability, using the software SISVAR (Ferreira 2011). In addition, the reaction of soybean genotypes was classified according to the RF values of each treatment, considering as resistant those whose nematode had RF <1.00 and susceptible those with RF>1.00.

RESULTS

All soybean cultivars tested were susceptible (RF> 1.0) to *M. javanica* (Table II). However, different levels of susceptibility were observed among soybean cultivars. In tomato plants used to evaluate the viability of the inoculum of *M. javanica* the highest FR= 53.3 values were obtained, thus confirming the viability of the inoculum of the assay.

Regarding the damage to the root system caused by *M. javanica*, the cultivars with the highest gall numbers, ranging from 531 to 650 galls were BMX RAIO IPRO, BMX VANGUARDA IPRO, DM 53I54 IPRO, HO ARINOS RR, NS 5000 IPRO, NS 5106 IPRO, NS 5258 RR, PRODUZA IPRO and, AMS TIBAGI RR, not differing statistically from each other. The cultivars that presented the lowest gall number values, ranging from 188 to 293 galls were BMX Elite IPRO, BMX Garra IPRO, BMX Lança IPRO, CD 2728 IPRO, FPS Atalanta IPRO, M 5947 IPRO, NS 4823 RR, NS 5445 IPRO, P95Y51, P95Y72, ROTA 54 IPRO and SYN 13671 IPRO, not statistically different (Table II)

Regarding the number of eggs and *M. javanica* J2 per gram of soybean roots, the cultivars with the lowest values were BMX Raio IPRO, BMX Alvo RR, BMX Foco IPRO, BMX Garra IPRO, BMX Veloz RR, BMX Zeus IPRO, BRS 7380 RR, FPS Atalanta IPRO, FT 2155 RR, HO Amambay IPRO, HO Arinos RR, M 8210 IPRO, NS 5160 IPRO, P95Y72 and TMG 1180 RR, ranging from 293 to 1831 eggs or J2 per gram of roots and differing statistically from the others. The highest values were observed in the cultivars CD 2728 IPRO, DM 53I54 IPRO and BMX Vanguarda IPRO, ranging from 7804 to 9745 eggs and J2 per gram of roots and statistically differing from the others (Table II).

Regarding the reproductive capacity of *M. javanica* in the tested cultivars, it was observed that the cultivar FPS Atalanta IPRO presented the lowest RF = 1.2, statistically differing from the other cultivars, followed by BMX GARRA IPRO, HO

Table II. **Root system weight (RST), gall number (GN), number of nematodes per root gram (NNRG), reproduction factor (RF) and reaction of *Meloidogyne javanica* in soybean cultivars.**

CULTIVARS	RST		GN		NNRG[1]		RF[2]		Reaction[3]
98Y52	19,0		396	c[4]	2531	c	9,9	d	S
BMX Raio IPRO	23,0		546	a	1318	d	5,8	e	S
BMX Alvo RR	20,2		356	c	1587	d	6,2	e	S
BMX Ativa RR	20,6		376	c	3546	c	9,2	d	S
BMX Desafio RR	18,7		303	c	1994	c	6,1	e	S
BMX Elite IPRO	11,2		193	d	4513	b	8,3	d	S
BMX Foco IPRO	24,5		346	c	1831	d	8,0	d	S
BMX Garra IPRO	28,9		291	d	694	d	3,6	f	S
BMX Lança IPRO	20,7		275	d	1958	c	7,7	d	S
BMX Vanguarda IPRO	15,6		600	a	9745	a	29,0	a	S
BMX Veloz RR	26,9		311	c	1075	d	5,4	e	S
BMX Zeus IPRO	27,3		311	c	1766	d	8,7	d	S
BRS 7380 RR	27,1		460	b	1045	d	5,3	e	S
CD 2728 IPRO	14,3		188	d	7804	a	22,2	b	S
DM 4309 IPRO	14,3		348	c	2368	c	6,2	e	S
DM 53I54 IPRO	7,0		531	a	9451	a	14,4	c	S
FPS Atalanta IPRO	20,7		203	d	293	d	1,2	g	S
FPS Urano RR	22,6		438	b	2195	c	8,8	d	S
FT 2155 RR	20,9		308	c	1695	d	6,9	d	S
GMX Cancheiro RR	17,8		408	c	6494	b	11,1	d	S
HO Amambay IPRO	27,7		319	c	925	d	4,4	f	S
HO Arinos RR	21,6		533	a	1652	d	6,9	d	S
M 5947 IPRO	13,7		225	d	2158	c	4,9	f	S
M 8210 IPRO	26,8		351	c	1217	d	6,3	d	S
NS 4823 RR	14,9		243	d	5375	b	14,2	c	S
NS 5000 IPRO	23,6		650	a	2495	c	9,2	d	S
NS 5106 RR	25,0		575	a	3789	c	14,9	c	S
NS 5160 IPRO	26,0		460	b	1539	d	7,3	d	S
NS 5258 RR	25,5		555	a	2716	c	12,0	d	S
NS 5445 IPRO	19,9		286	d	2536	c	9,5	d	S
P95R51	19,5		268	d	3301	c	10,3	d	S
P95Y72	23,2		275	d	1622	d	7,6	d	S
Produza IPRO	26,0		541	a	3625	c	18,1	c	S
Rota 54 IPRO	10,1		293	d	3582	c	5,8	e	S
SYN 13671 IPRO	21,8		281	d	1979	c	7,5	d	S
AMS Tibagi RR	20,7		591	a	4414	b	15,0	c	S
TMG 1180 RR	22,1		380	c	1305	d	5,4	e	S
Tomato	-		720		6667		53,3		S
CV (%)	-		23		33,4		18,7		

[1]Number of nematodes per gram of root: Ratio between the total number of nematodes and the total root mass. [2]Reproduction factor (RF) = Final Population / Initial Population. [3]Oostenbrink-based reaction (1966): Resistant (R) (RF <1.0) and susceptible (S) (RF ≥ 1.0). [4]Means followed by the same letter in the column do not differ significantly by the Scott-Knott test at 5% probability of error.

Amambay IPRO and M 5947 IPRO, with RF of, 3.6, 4.4 and 4.9, respectively. For the highest RF value, it was observed in the cultivar BMX Vanguarda, with RF = 29.0, differing statistically from the other cultivars. Following the cultivar with the highest RF value is CD 2728, with RF = 22.2, also differing statistically from other cultivars (Table II).

DISCUSSION

The use of resistant soybean cultivars can be effective for reducing plant-parasitic nematode populations in the soil (Soares & dos Santos 2009, Araújo et al. 2012). However, according to Carneiro et al. (2019), few cultivars are reported as resistant. Thus, it is decided to use cultivars in which the reproduction factor is the closest to 1, which allows the population to be reduced when combined with other integrated nematode management techniques.

Tihohod et al. (1988) and Mendes et al. (2001) evaluated in a greenhouse study the behavior of 24 cultivars and 73 soybean genotypes, all of which were classified as susceptible to *M. javanica*. Corroborating these data, Bruinsma & Antoniolli (2015) evaluated 14 cultivars where all were classified as susceptible to *M. javanica*. As well as Kirsch (2016), who evaluated six soybean cultivars and all presented RF higher than 1, classifying them as susceptible to *M. javanica*.

However, Soares & Santos (2009) explain in a study that even considered susceptible to a cultivar, if the evaluated RF is close to 1, it can be considered less susceptible compared to high RF values. Thus, it is suggested that when there are no cultivars considered resistant to gall nematode available, it is preferred to cultivate with the lowest RF values. Sharma (1993) evaluated the reaction of 60 soybean genotypes to root-knot nematodes, where 12 were considered resistant. Corroborating this, Mazzetti et al. (2019) concluded in a reaction assay of 27 cultivars that 15 were classified as resistant with RF lower than 1.

Thus, the cultivars that were classified by Mazzetti et al. (2019) as resistant and contrary to the results obtained in the present work were the cultivars BMX Elite, GMX Cancheiro and M5947 IPRO. This may be linked to a number of factors, such as the aggressiveness of the pathogen used in the study, as Mattos et al. (2016) considers that aggressiveness, virulence and host ability as factors that interfere with plant-pathogen interaction, as well as climatic conditions that the plant was submitted. However, corroborating the results found in the present work, the cultivars BMX Ativa RR, BMX Lança IPRO, BMX Vanguarda IPRO, NS 5445 IPRO and, AMS Tibagi RR were classified as susceptible to *M. javanica* in both studies.

Alves et al. (2011) reports that cultivars with high reproductive factor, i.e., RF greater than 1, should be avoided in areas with nematode presence, especially *M. javanica*. In addition, caution should be taken in the use of susceptible cultivars, as the degree of susceptibility may differ according to the species and population present in the soil and the climatic conditions of the crop (Li & Chen 2005).

As *M. javanica* is an aggressive species with wide territorial distribution, the monoculture of susceptible hosts favors its multiplication (Bruinsma & Antoniolli 2015). Thus, a strategy for soil nematode control is rotation / succession with low potential host crops and the use of resistant soybean cultivars when commercially available (Dias-Arieira & Chiamolera 2011, Kirsch et al. 2019), or the use of cultivars with low susceptibility that were presented in this study, being the cultivar FPS Atalanta IPRO the only evaluated cultivar that presented low levels of susceptibility with RF = 1,2. Thus, being the only cultivar suitable for use in fields with high populations of *M. javanica*.

The data obtained with the present work showed that all evaluated cultivars presented susceptibility reaction to *M. javanica*, but at different levels. In this sense, the susceptibility of soybean to root-knot nematode is an important indicator of the need for other control measures, since this species is widespread in cultivated areas. Although several management strategies are used to increase soybean crop productivity, none of them in isolation have been fully effective in keeping populations below the level of economic damage. This condition reflects the need to adopt other control measures jointly in order to enable faster and, to a greater degree, the reduction of the initial nematode population in soybean cultivation areas, thus minimizing the problems caused by such pathogens.

Although the data obtained with the present work demonstrate that all evaluated genotypes have *M. javanica* susceptibility, the use of less susceptible genetic materials, associated with other management strategies, as previously discussed, may contribute to the increase of grain yield in areas contaminated with such a nematode. These measures include the incorporation of organic matter into the system, the use of antagonistic plants, crop rotation with non-host plants (Ferraz 2006, Santana-Gomes et al. 2014) and the use of systemic nematicides in culture (Agrofit 2019), resistance inducers and use of biological nematicides. Thus, the use of these techniques together can contribute decisively to the reduction of plant-parasitic nematode populations in soybean areas in order to minimize the problems caused by such pathogens and, consequently, increase crop productivity.

CONCLUSIONS

All soybean cultivars presented RF> 1.00, being classified as susceptible, however, the soybean cultivars with the lowest susceptibility levels to *M. javanica* are the M 5947 IPRO, HO Amambay IPRO, BMX Garra IPRO and FPS Atalanta IPRO cultivars.

However, it is very important that work of this nature continues, as many more soybean cultivars are released every year, and knowledge of their reaction to all plant-parasitic nematodes is extremely important to help increase productivity.

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