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REACTION OF SUGARCANE GENOTYPES TO PARASITISM OF *Meloidogyne javanica* AND *Pratylenchus zeae*[1]

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ABSTRACT - The objective of this work was to evaluate the response of sugarcane genotypes to *Meloidogyne javanica* and *Pratylenchus zeae*. Sugarcane seedlings of the genotypes RB985944, RB987932, RB877935, RB855156, RB935744, RB996961, RB925345, RB935581 and RB966928 obtained from tissue cultures and maintained in pots with sterilized soil in a greenhouse were inoculated with 5,000 eggs + second-stage juveniles of *M. javanica* or 1,600 of *P. zeae* per plant, using six replicates per treatment. Tomato (Rutgers) and sorghum (BRS506) plants were used as control and proof of viability of the inoculum for the *M. javanica* and *P. zeae*, respectively. The number of root-knot in the plants inoculated with *M. javanica*, final nematode population and reproduction factor (RF) of both nematodes were evaluated at 120 days after inoculation. The averages of the different variables were compared by the Scott-Knott's clustering test at 5%. The nematodes *M. javanica* and *P. zeae* showed RF>1.00 in all sugarcane genotypes assessed, however, different levels of susceptibility were found. The lowest reproduction of the nematodes was found in the genotypes RB008347, RB877935, RB975944 and RB987932 (*M. javanica*) and RB987932 and RB966928 (*P. zeae*).

Keywords: *Saccharum* spp.. Root-knot nematode. Lesion nematode. Susceptibility.

REAÇÃO DE GENÓTIPOS DE CANA-DE-AÇÚCAR AO PARASITISMO DE *Meloidogyne javanica* E *Pratylenchus zeae*

RESUMO - Teve-se por objetivo neste trabalho avaliar a reação de genótipos de cana-de-açúcar a *Meloidogyne javanica e Pratylenchus zeae.* Mudas de cada material genético (‘RB008347’, ‘RB975944’, ‘RB987932’, ‘RB877935’, ‘RB855156’, ‘RB935744’, ‘RB996961’, ‘RB925345’, ‘RB935581’ e ‘RB966928’), obtidas da cultura de tecidos e mantidas em vasos com solo esterilizado, em casa de vegetação, foram inoculados com 5.000 ovos + juvenis de segundo estádio de *M. javanica* ou 1.600 *P. zeae*/planta, utilizando-se seis repetições por tratamento. Para controle e a comprovação da viabilidade do inoculo, utilizaram-se plantas de tomate 'Rutgers' e sorgo ‘BRS 506’ para *M. javanica* ou *P. zeae*, respectivamente. Decorridos 120 dias avaliou-se o número de galhas nas plantas inoculadas com *M. javanica;* a população final de nematoide e o fator de reprodução (FR) de ambos nematoides. A seguir, as médias das diferentes variáveis foram comparadas entre si pelo teste de agrupamento de Scott-Knott a 5%. Verificou-se que embora *M. javanica* e *P. zeae* tenham apresentado FR>1,00 em todos os genótipos de cana-de-açúcar testados, forma observados diferentes níveis de suscetibilidade. Desse modo, a menor reprodução do nematoide das galhas foi observada em ‘RB008347’, ‘RB877935’, ‘RB975944’ e ‘RB987932’; e de *P. zeae* em ‘RB987932’ e ‘RB966928’.

Palavras-chave: *Saccharum* spp.. Nematoide-das-galhas. Nematoide das lesões. Suscetibilidade.

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INTRODUCTION

Sugarcane (*Saccharum* spp.) is one of the most socioeconomically important crops in Brazil and the world. The world's sugarcane production is approximately 2.5 billion Mg yr^{-1}. Brazil produced around 658.7 million Mg yr^{-1} over an area of more than 8.9 million hectares, accounting for about 25% of the world's production. The sugarcane raw material is used for production of sugar, ethanol, alcoholic beverages and animal feed (CONAB, 2015).

The sugarcane area and yield is increasing in recent years; however, several factors have been limiting the production, such as the phytonematodes. Different species of these pathogens can negatively affect sugarcane crops in virtually all regions of the world where it is cultivated. According to Cadet and Spaull (2005), more than 300 phytonematode species, distributed in 48 genera, have been found in sugarcane crops, with have caused estimated average losses of 20%. However, these losses may vary depending on the phytonematode species, their population levels, susceptibility of the sugarcane variety, and growing period (CHAVES et al., 2009a).

There are many species of phytonematodes that attach the roots of sugarcane plants, therefore the root-knot (*Meloidogyne* spp.) and root lesion (*Pratylenchus* spp.) nematodes are the most common pests related to damage in Brazil (DINARDO-MIRANDA et al., 2008; SEVERINO et al., 2010; BELLÉ et al., 2014). According to Severino et al. (2008) *Meloidogyne javanica* and *M. incognita* are the most common nematodes associated to sugarcane in Paraná state. In a recent survey conducted by Bellé (2014) in the northwest region of the State of Rio Grande do Sul (RS), *M. javanica* was the predominant species of root-knot nematode. Besides that these authors found *Pratylenchus zeae* and *P. brachyurus* in sugarcane crops in RS and, similar to other Brazilian regions, *P. zeae* was the predominant root-lesion specie.

The importance of root-knot nematodes in sugarcane crops can be noticed by their yield decrease due to the difficulties to control these pathogens (BARROS et al., 2005; DINARDO-MIRANDA et al., 2008). Several methods of control of nematodes have been studied, aiming to decrease phytonematode populations in sugarcane crops to levels below the economic damage threshold. Among these methods, the use of nematicides, crop rotation, soil tillage during the hottest periods of the year, resistant or tolerant varieties using and incorporation of organic matter are the most common strategies (BARROS et al., 2000). The use of genetic resistance is considered one of the most desirable control practice because it is economically feasible, accessible to producers and has no risk to human health or the environment. However, no resistant varieties of sugarcane to root-knot and lesion nematodes are available in the Brazilian market (DIAS-ARIEIRA et al., 2010; SANTOS et al., 2012).

Considering new sugarcane genotypes have been recomended

for planting in commercial areas, especially in Rio Grande do Sul, thus, to evaluate the response of these new varieties of sugarcane to root-knot and lesion nematodes is important. Therefore, the objective of this work was to evaluate the reaction of different sugarcane genotypes to *M. javanica* and *P. zeae*.

MATERIAL AND METHODS

The experiment was conducted from December 2012 to April 2013 in a greenhouse (25±3°C) at Embrapa Temperate Agriculture, Pelotas-RS. Ten adapted sugarcane genotypes (RB008347, RB975944, RB987932, RB877935, RB855156, RB935744, RB996961, RB925345, RB935581, RB966928) to the edaphoclimatic conditions of RS state and come from to the plant breeding program of the RIDESA (Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético) in partnership with Embrapa, were evaluated. Sugarcane seedlings were obtained from the genotypes through tissue culture. A completely randomized experimental design with six replicates per treatment was used.

Pure populations of *M. javanica* (Est J3) and *P. zeae* from sugarcane fields were multiplied and maintained in tomato (*Solanum lycopersicum* L. cv. Rutgers) and sorghum (*Sorghum bicolor* L. cv. BRS506) plants, respectively at greenhouse conditions (25±2ºC) for using as inoculum. Both nematodes were obtained from sugarcane fields in Pelotas RS, Brazil. The *M. javanica* population was characterized by electrophoresis technique with the isozyme esterase (CARNEIRO; ALMEIDA, 2001), and the *P. zeae* population was identified by morphological and morphometric characterization (LOOF, 1991).

Plants of sugarcane from the different genotypes were maintained in 2000 cm^3 pots (one plant per pot) with vermiculite and sterilized soil (1:3) and inoculated with a suspension of 5,000 eggs + juveniles of second stage (J_2) of *M. javanica* or 1,600 *P. zeae* (initial population). *Meloidogyne* specimens were obtained through the blender grinding method with the addition of 0.5% of sodium hypochlorite followed by sieving and centrifugation with a sucrose solution (HUSSEY; BARKER, 1973; BONETTI; FERRAZ 1981) and *Pratylenchus* specimens were obtained through the same method without adding sodium hypochlorite (COOLEN; D`HERDE, 1972). The inoculation was carried out in three 4 cm deep holes around each sugarcane

plant. The viability of the inocula was assessed by inoculate tomato Rutgers (*M. javanica*) and sorghum BRS506 (*P. zeae*) plants with the same amount of inoculum at the same time as the inoculation of sugarcane seedlings.

One hundred e twenty days after inoculation, the roots of each sugarcane seedling inoculated with *M. javanica* were separated from the shoots to evaluate the number of galls. Subsequently, the eggs + J_2 were extracted from the roots (final population) to quantify the nematodes and determine the nematode reproduction factor (RF = final population/initial population) (OOSTENBRINK, 1966). For evaluating the plants inoculated with *P. zeae,* each root system was processed for nematode extraction and counting the number of specimens to determinate the RF nematode.

The data of the different variables were submitted to analysis of variance, and the means of each treatment were compared by the Scott-Knott clustering test at 5%, using the software SISVAR (FERREIRA, 2011). The responses of the sugarcane genotypes were classified according to the RF values of each nematode species evaluated, considering as resistant the plants whose nematode had RF<1.00 and as susceptible those with RF≥1.00.

RESULTS AND DISCUSSION

All sugarcane genotypes evaluated were susceptible (RF>1.00) to *M. javanica* and *P. zeae* (Tables 1 and 2), however, the evaluated materials presented different levels of susceptibility. The mean values of RF *M. javanica* and *P. zea* in the tomato (12.75) and sorghum (15.20) plants, respectively, confirmed the viability of the inoculum used in this experiment.

Among the genotypes studied, RB008347, RB877935, RB975944 and RB987932 presented the lowest number of galls, final population and reproduction factor for *M. javanica*, thus showing a lower susceptibility. The genotypes RB996961, RB935744 and RB855156 were classified as susceptible, and the RB966928, RB925345 and RB935581 were the most susceptible genotypes to *M. javanica* (Table 1).

Table 1. Number of galls (NRK), final population (FP) reproduction factor (RF) of *Meloidogyne javanica* and susceptibility (SC) of different sugarcane genotypes to the nematode.

Genotype	NRK	FP	RF*	SC
RB966928	1,906.00 a	183,844.45 a	36.77 a	S
RB935581	1,882.17 a	157,961.11 a	31.59 b	S
RB925345	1,412.33 b	125,133.33 b	25.03 c	S
RB996961	854.67 d	105,705.56 b	21.14 d	S
RB935744	1,060.50 c	104,761.11 b	20.95 d	S
RB855156	1,278.00 b	68,111.11 c	15.14 e	S
RB877935	1,105.33 c	56,094.45 c	11.79 f	S
RB987932	797.83 d	55,461.11 c	11.46 f	S
RB975944	525.83 e	47,088.89 d	10.44 f	S
RB008347	625.17 e	44,305.56 d	7.93 f	S
CV	16.42	16.39	13.92	-

Averages followed by the same letter in the column do not differ by the Scott-Knott's test at 5%; RF = final population/initial population (Pi = 5,000); S = Susceptible (RF>1).

The susceptibility of sugarcane varieties to *M. javanica* was also observed by other researchers. Dinardo-Miranda et al. (1995) assessed the response of twelve sugarcane varieties in areas naturally infested with this species and found susceptibility to this nematode in all genotypes, however, the genotypes RB735275, SP71-1632 and SP72-1861 were tolerant. Dias-Arieira et al. (2010) evaluated the response of varieties of the group RB to *M. javanica*, including four genotypes also evaluated in the present study (RB975942, RB855156, RB925345 and RB966928) and verified RF with values 3 to 10 times lower than those found in the present study. This result was probably due to their shorter time from inoculation to evaluation, which may have affected the number of generations of the nematode and consequently, the multiplying of the pathogen in the sugarcane plants.

The response of the genotypes to *P. zeae* showed RF>1.00 in all genotypes, denoting the susceptibility of the tested materials to this root-lesion nematode species. However, as observed for *M. javanica*, the sugarcane genotypes presented different levels of susceptibility to *P. zeae*. The genotypes RB935744, RB008347, RB925345, RB877935 and RB975944 presented intermediate susceptibility (RF of 15.23 to 20.91), RB996961, RB855156 and RB935581 presented more

susceptible, and RB987932 and RB966928 presented less susceptibility, as shown by their lower final population and reproduction factor nematode presented in the Table 2. Santos et al. (2012) evaluating the reaction of genotypes RB975942, RB855156, RB925345 and RB966928 to *P. zeae* observed RF values 3 to 10 times lower than those verified in the present study.

Table 2. Final population (FP) reproduction factor (RF) of *Pratylenchus zeae* and susceptibility (SC) of different sugarcane genotypes to the nematode.

Genotype	FP	RF*	SR
RB935581	42,083.33 a	26.31 a	S
RB855156	38,227.78 a	25.31 a	S
RB996961	36,600.00 a	22.87 a	S
RB975944	33,461.11 a	20.91 b	S
RB877935	28,250.00 b	19.25 b	S
RB925345	28,472.22 b	17.80 b	S
RB008347	24,150.00 b	13.77 c	S
RB935744	24,372.22 b	15.23 c	S
RB987932	17,088.89 c	10.68 d	S
RB966928	13,655.56 c	8.54 d	S
CV (%)	20.37	15.94	-

Averages followed by the same letter in the column do not differ by the Scott-Knott's test at 5%; RF = final population/initial population (Pi = 5,000); S = Susceptible (RF>1).

According to Cadet and Spaull (2005), losses caused by *M. javanica* in production areas of sugarcane can reach 15 Mg ha^{-1}. This species has been reported as one of the most frequent in Brazilian sugarcane crops (DINARDO-MIRANDA et al., 2003; SEVERINO et al., 2008; BELLÉ et al., 2014). Thus, studies on this pathogen may contribute to minimize losses in crops due to its attack.

Studies on genetic resistance are important from the point of view of phytonematode control, since responses to these pathogens are assessed through their reproduction in the hosts. Therefore, *P. zeae* multiplication in sugarcane, in general, is rapid, reaching high levels over a short period of time.

According to the *P. zeae and M. javanica* RF values presented in Tables 1 and 2, the genotype RB987932 presented the lowest levels of susceptibility. This genotype has a medium-late cycle and its main characteristic is the resistance to frost (SILVA et al., 2012), thus, it is an option for subtropical regions such as southern Brazil. Therefore is important to note that the response of the different sugarcane varieties is variable in the field and not always the largest number of nematodes means a greater reduction in the vegetative parameters of the plant. For example, Barros et al. (2005) found the highest number of nematodes (*Meloidogyne* + *Pratylenchus*) in the variety SP79-1011, however, its yield was not compromised compared with the varieties RB855156 and RB966928.

In this sense, the aggressiveness evaluation form nematode populations of the same species in distinct sugarcane genotypes are relevant for studies on genetic resistance. The susceptibility of the varieties evaluated to *M. javanica* and *P. zeae* is an important indicator of the need to adopt different control measures, since these species are widely distributed in sugarcane areas. Several management strategies are used to increase the yield of sugarcane crops (DINARDO-MIRANDA et al., 2008; CHAVES et al., 2009b; NOVARETTI; REIS, 2009), however, none have been fully effective in maintaining nematodes populations at levels below the economic damage threshold.

The results found in the present work show that all evaluated genotypes present susceptibility to *M. javanica* and *P. zeae*, however, the use of genetic materials with lower susceptibility, combined with other management strategies, may increase the viability of sugarcane crops. These measures include the incorporation of organic matter in the soil, use of antagonistic plants, crop rotation with non-host plants and reducing number of systemic nematicides. The combination of these practices can contribute to reduce phytonematode populations in sugarcane crop areas, minimizing the problems caused by them and consequently, increasing the crop yield.

CONCLUSIONS

The evaluated sugarcane genotypes present different suceptibility levels to *M. javanica* and *P. zeae*.

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