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Sources of root-knot nematode (*Meloidogyne enterolobii*) resistance in sweetpotato genotypes

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ABSTRACT

The root-knot nematode (RKN) *Meloidogyne enterolobii* is gaining importance all around the world, including Brazil, damaging sweetpotato genotypes with known resistance to other RKN species, making it challenging to generate new resistant genes. This study aimed to assess the levels of resistance of 10 advanced sweetpotato cultivars to this nematode species. Two experiments were carried out in 2018/2019 and 2021/2022 seasons, under greenhouse conditions in Brasília-DF, Brazil. A completely randomized block design with six replicates of one plant/plot/treatment was used. The gall index (GI) and egg mass index (EMI) in the root system of each plant, the number of eggs and juveniles per gram of root with galls (NERG), and the nematode reproduction factor (RF) were determined. *M. enterolobii* resistance is present within three of the evaluated clones: BGBD 1399, MD 1609024, and MD 1610036, therefore demonstrating their potential as sources of resistance genes to assist breeding efforts to release cultivars, and help to reduce the impact and spread of this RKN.

Keywords: *Ipomoea batatas*, genetic resistance, integrated pest management, breeding.

RESUMO

Fontes de resistência ao nematoide-das-galhas (*Meloidogyne enterolobii*) em genótipos de batata-doce

A espécie de nematoide-das-galhas *Meloidogyne enterolobii* vem ganhando importância em todo o mundo, inclusive no Brasil, com áreas cada vez mais infestadas e causando danos a genótipos com resistência a outras espécies de nematoide-das-galhas prevalentes no país, inclusive em batata-doce, tornando desafiadora a busca por fontes de resistência. Este estudo teve como objetivo avaliar os níveis de resistência em 10 clones avançados do programa de melhoramento da Embrapa Hortaliças e cultivares de batata-doce a essa espécie de nematoide. Os experimentos foram conduzidos nas safras 2018/2019 e 2021/2022, em casa de vegetação em Brasília-DF, Brasil. Utilizou-se o delineamento em blocos casualizados com seis repetições de uma planta por parcela/tratamento. Foram determinados os índices de galhas (GI) e massa de ovos (EMI) no sistema radicular de cada planta, o número de ovos e juvenis por grama de raiz (NERG) e o fator de reprodução (RF) do nematoide. Três clones foram classificados como resistentes entre os avaliados: BGBD 1399, MD 1609024 e MD 1610036, sendo, portanto, potenciais fontes de resistência para programas de melhoramento genético visando lançar cultivares, auxiliando na diminuição do impacto e da expansão da ocorrência desse nematoide.

Palavras-chave: *Ipomoea batatas*, resistência genética, manejo integrado de pragas, melhoramento genético.

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Despite being considered a rustic plant species, sweetpotato (*Ipomoea batatas*) is a susceptible host to different root-knot nematodes (RKN) of the genus *Meloidogyne* (Villordon & Clark, 2018). These RKN may lead to major biotic stress in sweetpotato crop areas, resulting in yield losses and reduced quality of storaged roots (Villordon & Clark, 2018). The symptoms include stunted growth, leaf chlorosis, plant death, leaf wilting, poor shoot growth, and the development of root cracks in some cultivars or pimple-like bumps in others, presence of dark areas within the flesh, with the presence of enlarged females and their egg masses (Bernard *et al*., 2017; Wendimu, 2021). This causes great concern, especially in the tropics, subtropics and warm regions all around the world (Karuri *et al*., 2017; Rutter *et al*., 2019; Brito *et al*., 2020; Silva *et al*., 2021).

In Brazil, the most important RKN species in the main sweetpotato growing areas are *M. incognita* (Chaves *et al*., 2013; Carmona *et al*., 2020). However, *M. enterolobii* Yang & Eisenback (1983) (syn. *M. mayaguensis,* Ramah & Hirschmann, 1988) is gaining notoriety, due to its ability to infect resistant plants to other RKN species (Rutter *et al*., 2019; Carmona *et al*., 2020; Silva *et al*.,

2021, Wendimu, 2021).

Several management strategies have been used, but, in the last two decades, the vegetable industry has been addressing RKN control mainly with chemicals (Peiris *et al*., 2021). Although effective, the high cost and toxicity of chemical nematicides hampers their use. Therefore, plant genetic resistance, whenever available, is the most efficient control method in a strategy of integrated pest management, besides being economically sustainable and environmentally safe (Gomes, 2014; Gomes *et al*., 2015; Bernard *et al*., 2017; Wendimu, 2021).

Even though genetic resistance is an important component of an integrated control management program, there are few studies about the level of resistance of sweetpotato genotypes to *M. enterolobii* (Wendimu, 2021). Most of the evaluated accessions are considered susceptible (Carmona *et al*., 2020; Schwarz *et al*., 2021), including cultivars known to be resistant to other RKN (Brito *et al*., 2020). Thus, it is essential to evaluate and select novel sources of resistance in sweetpotato breeding programs and characterize advanced genotypes and cultivars that could be recommended to growers (Carmona *et al*., 2020). The aim of this study was to assess the levels of resistance to *M. enterolobii* on cultivars and advanced sweetpotato clones of Embrapa's breeding program.

MATERIAL AND METHODS

The experiments were conducted between November 26th 2018 and March 24th 2019, and September 22nd 2021 and January 19th 2022, in a greenhouse at Embrapa Hortaliças, Brasília-DF, Brazil (15º55'44"S, 48º08'29"W, 999 m altitude), where climatic classification is tropical Savannah, with concentrated rainy period during the summer and dry winter, according to Köppen.

All genotypes are derived from Embrapa's sweetpotato breeding program. In the first year, four advanced clones: BGBD 0080, BGBD 1399, BGBD 1402, and BGBD 1405, two purple-fleshed recently released cultivars BRS Anembé and BRS Cotinga and two cultivars with a known pattern of response to RNK Brazlândia Roxa and Beauregard were tested. The tomato cultivar Rutgers was used as susceptibility control. In the second year, seven advanced clones with different flesh colors (MD 1604002, MD 1609023, MD 1609024, MD 1609026, MD 1610036, MD 1611010 and BGBD 1399) and the same controls were evaluated for resistance to *M. enterolobii*.

The experiments were established using a completely randomized design with six replications. Each experimental plot consisted of one plant grown in a 2-L plastic pot containing substrate on the proportion 1:1:1:1 of subsurface soil (a clayey Oxisol, typically encountered in the Cerrado Biome region in Brazil), washed sand, cow manure and carbonized rice husk mix, autoclaved at 121°C for 60 min. To 30 kg of this soil mixture, 300 g N-P-K, 4-30-16 formulation, and 3 kg of calcined dolomitic lime were added. Four node cuttings were obtained from disease-free plants from each genotype.

The identification of *M. enterolobii* was accomplished by morphological examination of the perineal region of adult females and comparison with taxonomic descriptions and keys of Yang & Eisenback (1983); Rammah & Hirschmann (1988) and Eisenback & Hirschmann-Triantaphyllou (1991). Phenotypic analysis of esterase isozymes was performed with the technique proposed by Carneiro & Almeida (2001).

Thirty days after planting, eggs and second-stage juveniles (J_2) were extracted from tomato roots of cultivar Santa Cruz Kada Gigante, and inoculated according to the methodology described by Boneti & Ferraz (1981). After calibration, the inoculum was poured on the soil around the plants, with a concentration of approximately 5,000 eggs + eventual J_2 per plant.

Plants were managed according to the local recommendations for sweetpotato cultivation (Vendrame & Melo, 2021), and about one month after inoculation, top dressing was carried out using 3 g of Osmocote® (19-06-10 N-P-K) per liter of substrate.

Plants were harvested 90 days after inoculation. The gall index (GI) and egg mass index (EMI) in the root system of each plant were determined according to the scale proposed by Taylor & Sasser (1978) (0= roots without galls or egg masses; 1= presence of 1 to 2 galls or egg masses; 2= presence of 3 to 10 galls or egg masses; 3= presence of 11 to 30 galls or egg masses; 4= presence of 31 to 100 galls or egg masses and 5= presence of more than 100 galls or egg masses on the root system).

The final population (Fp) of the nematode in the root system and in the portions of the tuberous roots with galls were also quantified, extracting the eggs and nematodes using the method of Boneti & Ferraz (1981). The Fp was quantified, counting the eggs and J_2 under an optic microscope. The results were divided by the fresh weight of the root system and the part of the storage roots with galls and expressed as eggs + J_2 per gram of root (NERG). The nematode reproduction factor (RF = Fp/Ip) was calculated by dividing the final and initial populations (inoculated). Genotypes showing a RF value less than 1 were considered resistant, and those showing RF greater or equal to the unit were considered susceptible (Oostenbrink, 1966).

The data were transformed to $\sqrt{x + 0.5}$, to meet the assumptions of normal distribution and homoscedasticity, presenting the original values, and subjected to a one-way analysis of variance (ANOVA), for each trait, with means grouped by the Scott-Knott clustering test at a 0.05 significance level, using the Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

The coefficients of variation (CV) of the reproduction factor (RF) and the number of eggs per root gram (NERG) were higher when compared to other variables, though the genotypic coefficient of variation (CVg), or the rate amongst the CVg and CV, shows the predominance of variation of genetic order over the environmental variation, higher than its unit (1.0),

Table 1. Reaction of sweetpotato genotypes to the infection by *Meloidogyne enterolobii*. Brasília, Embrapa, 2019.

Genotypes	2019					
	Root flesh color	GI	EMI	NERG	RF	Reaction
BGBD 080	Purple	4.50 b	4.50 a	943.17 b	12.82 b	S
BGBD 1399	Purple	2.00 c	2.00 c	16.67 c	0.53 c	R
BGBD 1402	Purple	4.67 a	4.67 a	1205.33 a	19.74 b	S
BGBD 1405	Purple	4.20 b	4.20 b	572.83 b	3.17 c	S
BRS Anembé	Purple	4.83 a	4.83 a	3285.67 a	34.80 a	S
BRS Cotinga	Purple	4.67 a	4.67 a	1525.50 a	30.80 a	S
Brazlândia Roxa	Cream	4.33 b	4.33 b	640.67 b	18.72 b	S
Beauregard	Orange	4.83 a	4.83 a	1498.00 a	17.60 b	S
General mean	-	4.25	4.25	1210.98	17.27	-
CV (%)	-	9.88	11.35	35.06	26.18	-
CVg/CV	-	1.97	1.72	1.08	1.35	-
			2021			
MD 1604002	Cream	3.83 a	3.83 a	382.45 b	6.63 b	S
MD 1609023	Cream	3.67 a	3.67 a	436.55 b	4.34 b	S
MD 1609024	Orange	1.17 b	1.17 b	0.57 c	0.01 c	R
MD 1609026	Yellow	4.33 a	4.33 a	572.75 b	7.13 b	S
MD 1610036	Orange	1.17 b	1.17 b	8.57 c	0.10 c	R
MD 1611010	Orange	3.50 a	3.50 a	3311.86 a	14.26 a	S
BGBD 1399	Purple	1.17 b	1.17 b	1.63 c	0.02 c	R
Brazlândia Roxa	Cream	4.00 a	4.00 a	286.94 b	5.29 b	S
Beauregard	Orange	4.17 a	4.17 a	448.16 b	7.74 b	S
General mean	-	3.00	3.00	605.50	5.06	-
CV (%)	-	18.39	17.43	68.56	39.35	-
CVg/CV	-	2.37	2.47	1.20	1.00	-

GI= Gall index; EMI= Egg mass index; NERG= J_2 per root gram part of tuberous root with galls; RF= reproduction factor; Reaction: genotype reaction (R= resistant; S= susceptible). Means followed by same letters in the columns do not differ by Scott-Knott hierarchical clustering algorithm, at 0.05 significance level for the means/grouping test. CV= environmental coefficient. CVg/CV= genotypic and environmental coefficients rate.

indicating a favorable situation for the characterization of the resistance levels of the evaluated genotypes (Table 1).

In the first year of experiment (Table 1), 'BGBD 1399' was the only clone resistant to *M. enterolobii*, with lower values of gall GI, EMI, NERG, and a RF value lower than one, namely a population of nematodes lower than the population inoculated, demonstrating to be a poor host, thus not providing conditions for the reproduction and population increase of this nematode. The clone BGBD 1405 was not significantly different from 'BGBD 1399' for RF, with the nematode population increasing threefold, being susceptible according to Oostenbrink (1966), but with lower susceptibility compared to other genotypes. The cultivars BRS Anembé and BRS Cotinga were the most susceptible genotypes, with RF values similar to the susceptible control, tomato cultivar Rutgers (31.80). Therefore, they are not recomended for growing areas with the presence of this nematode.

Carmona *et al*. (2020) also verified the susceptibility of 'BRS Anembé' (formerly identified using the code CNPH 0005) with a very similar RF value (34.27) to this nematode. According to the same authors, this cultivar is resistant to *M. incognita* races 1 and *M. javanica*, reiterating the assumption that *M. enterolobii* is more virulent to sweetpotato than other RKN, even to cultivars that are resistant to other species. Melo *et al*. (2020), evaluating the aforementioned cultivars (identified by the codes 0005 and 1261), stated that the high yields associated with quality traits and pest resistance were an indicator of their potential. However, considering their degree of susceptibility, cultivars that combine RKN resistance with horticulturally favorable traits are urgently needed (Rutter *et al*., 2021).

The clones BGBD 0080, BGBD 1402 and the cultivars Beauregard and Brazlândia Roxa, despite being susceptible, with higher RF values, presented a degree of susceptibility

lower than 'BRS Cotinga' and 'BRS Anembé' (Table 1). Carmona *et al*. (2020) evaluated the clone BGBD 0080, as well as the cultivars Beauregard and Brazlândia Roxa, and also classified them as susceptible to *M. enterolobii*. In that study, 'BGBD 0080' and 'Brazlândia Roxa' were resistant to *M. javanica* and *M. incognita* race 1, and 'Beauregard' was susceptible to all species.

In the second experiment (Table 1), the resistance of the clone BGBD 1399 was confirmed, and two advanced orange-fleshed clones were also classified as resistant ('MD 1609024' and 'MD 1610036'), displaying the lowest GI, EMI, NERG and RF values. The control cultivars Brazlândia Roxa and Beauregard were classified as susceptible presenting a higher number of eggs, egg mass and galls. The lower RF value for 'Rutgers' in this experiment (2.45), compared with the first experiment, may have occurred because of the starkly damage level in the roots, reflecting in lower feeding extensions.

'Beauregard' is susceptible to *M. enterolobii* (Brito *et al*., 2020; Schwarz *et al*., 2021), *M. incognita* and *M. javanica* (Mello *et al*., 2022). Melo *et al*. (2011), Gonçalves *et al*. (2019) and Mello *et al*. (2022) also characterized 'Brazlândia Roxa', which is resistant to *M. javanica* and *M. incognita* race 1, as susceptible to *M. enterolobii*. Mello *et al*. (2022) also found 'Canadense' and 'CIPBRS Nuti' as resistant to these tree nematode species. The existence of susceptible genotypes to *M. enterolobii* that are resistant to other RKN species, as 'Brazlândia Roxa' indicate that distinct genes confer resistance to different RKN species.

Therefore, considering the expansion of infected areas, the damage of this species all around the world, and that genetic data are not yet available (Rutter *et al*., 2021), these results indicate that resistance to *M. enterolobii* is present within the evaluated sweetpotato clones BGBD 1399, MD 1609024, and MD 1610036, requiring future research to characterize the basis of their resistant genes to assist breeding efforts to select resistant clones and release cultivars to help diminish the impacts and spread of *M. enterolobii* .

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