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Reproduction of *Meloidogyne enterolobii* in lettuce cultivars of the American group

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ABSTRACT

Lettuce is the main vegetable cultivated in Brazil, in volume and in marketed value. There are ranges of diseases which may affect lettuce crops, including those caused by root-knot nematodes (*Meloidogyne* spp.). We evaluated the reproductive potential of *Meloidogyne enterolobii* in 22 lettuce cultivars. The plants were inoculated with 5,000 eggs and eventuales second stage juveniles of nematode per pot, two days after the transplanting. 'Rutgers' tomato was used as standard for inoculum viability of *M. enterolobii*. We evaluated, 60 days after inoculation, the gall index, egg mass index and reproduction factor (RF). The 'Ithaca', 'Raider Plus', 'RS-1397', 'L-104', 'Challenge', 'IP-11', 'Classic', 'Salinas 88', 'Vanguard 75', 'Calona' and 'Desert Queen' were resistant to *M. enterolobii* with RF varying from 0.22 to 0.9, and the cultivars Lady Winterset, Robinson, Sonoma, Raider, Lucy Brown, Bnondaga, Summer Time, Taina, Sundevil and L-109 were susceptible to this nematode, with RF ranging from 1.06 to 5.73.

Keywords: *Lactuca sativa*, root-knot nematodes, plant breeding.

RESUMO

Multiplicação de *Meloidogyne enterolobii* em cultivares de alface do grupo americano

A alface é a principal hortaliça folhosa cultivada no Brasil, tanto em volume como em valor comercializado. Em áreas de cultivo, a alface tem a sua produtividade comprometida por diversas enfermidades, incluindo as causadas pelos nematoídes-das-galhas (*Meloidogyne* spp.). Este trabalho teve como objetivo avaliar o potencial reprodutivo de *Meloidogyne enterolobii* em vinte e duas cultivares de alface do tipo americana. As plantas foram inoculadas com 5.000 ovos e eventuais juvenis de segundo estágio de *M. enterolobii* por vaso, dois dias após o transplante das cultivares. O tomateiro 'Rutgers' foi utilizado como padrão de viabilidade do inóculo de *M. enterolobii*. Foram avaliados o índice de galhas (IG), índice de massas de ovos (IMO) e fator de reprodução (FR) do nematoíde, aos 60 dias após a inoculação. As cultivares Ithaca, Raider Plus, RS-1397, L-104, Challenge, IP-11, Classic, Salinas 88, Vanguard 75, Calona e Desert Queen apresentaram resistência a *M. enterolobii*, com FR variando de 0,22 a 0,9, e as cultivares Lady, Winterset, Robinson, Sonoma, Raider, Lucy Brown, Bnondaga, Summer Time, Tainá, Sundevil e L-109 foram suscetíveis a esse nematoíde, com FR variando de 1,06 a 5,73.

Palavras-chave: *Lactuca sativa*, nematoíde-das-galhas, melhoramento genético.

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Lettuce (*Lactuca sativa*) is one of the most important vegetable crops grown worldwide (Sala & Costa, 2012). Originally from temperate climate, the adaptation to high temperature regions created obstacles to growth and development, preventing the crop express all its genetic potential. In these conditions, a reduction of the crop cycle can be noticed, compromising the production due to the acceleration of plant metabolism and, consequently, the anticipation of the reproductive phase (Grangeiro *et al.*, 2006).

However, along the years, through lettuce breeding programs, the development of cultivars adapted

to different climatic conditions was noticed. Therewith, it has become possible to grow and harvest good quality lettuce throughout the year in tropical and subtropical countries, such as Brazil (Filgueira, 2008).

Despite major advances achieved through plant breeding projects, with the generation of more lettuce cultivars adapted to tropical conditions, many problems persist and a constant search for more competitive genotypes is necessary (Carvalho Filho *et al.*, 2011). Among these problems is the parasitism in plants by nematodes genus *Meloidogyne*, particularly the species *M. incognita* and *M. javanica* (Charchar &

Moita, 2005; Sikora & Fernandez, 2005; Wilcken *et al.*, 2005).

However, currently, *M. enterolobii* has caused great concern due to its high aggressiveness on several plant species, including vegetables, fruits and ornamentals (Guimarães *et al.*, 2003; Brito *et al.*, 2007; Kaur *et al.*, 2007). In general, plants which show resistance to *M. incognita*, *M. javanica* and *M. arenaria* species, such as tomato and sweet pepper genotypes which carry *Mi-1* and *N* gene, are susceptible to *M. enterolobii* (Moens *et al.*, 2009; Bitencourt & Silva, 2010).

Isolates of *M. enterolobii* from Florida were able to parasitize tomato and

sweet pepper which showed resistance genes *Mi-1* and *N*, respectively (Brito *et al.*, 2007). The same situation was observed in Switzerland with other tomato and sweet pepper cultivars which show these resistance genes (Kiewnick *et al.*, 2009). In Brazil, this capacity of *M. enterolobii* to develop on a tomato carrying the *Mi-1* gene was detected in eight rootstocks (Cantu *et al.*, 2009). Biological and histopathological studies carried out in tomato resistant ('Magnet') and susceptible ('Rutgers') to *M. incognita*, *M. javanica* and *M. arenaria* showed the capacity of *M. enterolobii* to develop nurse cells and multiply in both tomato, whereas *M. javanica* only produced nurse cells and reproduced in tomato without the *Mi* gene (Westerich *et al.*, 2011, 2012).

Studies carried out in Cuba aiming to verify the response of different vegetable species to *M. enterolobii*, based on reproduction factor (RF), showed the resistance of 'Black Seed Simpson' lettuce to this nematode (Rodriguez *et al.*, 2003). In Brazil, head lettuce cultivars, 'Grande Lagos 659', and the crisphead lettuce 'Mônica', were also considered resistant to this nematode, although they provide an RF greater than 1.0, as this one did not differ significantly from the reproduction factor below 1.0 of other studied cultivars (Bittencourt & Silva, 2010).

Based on infection rate, 'Elisa', 'Luisa', 'Mirella', 'Vera' and 'Salinas 88' lettuce were considered moderately resistant to *M. enterolobii*, whereas 'Júlia', 'Hortência', 'Verônica', 'Grand Rapids' and 'Babá de Verão' were considered highly resistant (Melo *et al.*, 2011). Thus, heretofore, susceptibility of lettuce cultivars to *M. enterolobii* was not verified.

The objective of this work was to determine RF for *M. enterolobii* in different head lettuce cultivars, aiming to identify sources of resistance to be used in breeding programs and options of cultivars to be used in areas infested with this nematode species.

MATERIAL AND METHODS

The experiment was carried out in

a greenhouse, adjusted to not exceed 30°C, at Universidade Estadual Júlio de Mesquita Filho, in Botucatu, São Paulo state.

The twenty two head lettuce cultivars used in this study were Lucy Brown, Robinson, Calona, Classic, Vanguard 75, Winterset, Salinas 88, Bnondaga, Ithaca, Raider Plus, Desert Queen, Sonoma, IP-11, Sundevil, Challenge, Summer Time, L-104, L-109, Tainá, Raider, Lady and RS-1397. The cultivars were provided by the Plant Breeding Program of Universidade Estadual Júlio de Mesquita Filho.

Seeds were sown in expanded polystyrene trays of 128 cells, with sterilized substrate. At 25 days after sowing, the seedlings were transplanted into definitive pots, with volumetric capacity of two liters, containing substrate {earth, sand and organic matter (1:2:1)}, previously autoclaved.

The population of *M. enterolobii* was obtained from the isolation of green pepper 'Silver' originated from Campos Novos Paulista. São Paulo state, and identified by the perineal pattern of females and the electrophoretic pattern of isoenzymes, according to Oliveira *et al.* (2012).

The multiplication of the pure population was performed in tomato cultivar 'Rutgers' in 1-liter volumetric pots, containing soil previously autoclaved. The plants were kept in greenhouse and after 60 days the roots were processed, according to Hussey & Barker (1973) method, modified by Bonetti & Ferraz (1981), for the extraction of eggs.

Determination of the number of eggs, and any newly hatched juveniles in suspension, was performed with the aid of Peter's blade, under light microscope. Then, lettuce was inoculated individually with 5,000 eggs and infective second-stage juveniles of the nematode studied, two days after the transplanting. The inoculation was performed placing 2 mL of the inoculum suspension in two holes of 3-centimeter depth in the rhizosphere of each plant. The tomato 'Rutgers' was used as inoculum viability standard in the experiment.

The completely randomized

experimental design was used, with five replications, each plot consisting of one plant.

Evaluations were carried out at 60 days after inoculation. For this purpose, the root systems of cultivars were washed individually under running water, weighed after removing excess of water with a paper towel and then submitted to staining with Floxine B, to facilitate the count of egg masses (Taylor & Sasser, 1978).

Gall index (GI) and egg masses index (EMI) found were related according to the rating scale: 0 (no galls or no egg masses); 1 (1-2 galls or egg masses); 2 (3-10 galls or egg masses); 3 (11-30 galls or egg masses); 4 (31-100 galls or egg masses) and 5 (more than 100 galls or egg masses per root) (Taylor & Sasser, 1978). Then, root systems were processed according to the methodology proposed by Coolen & D'Herde (1972) using 0.5% sodium hypochlorite solution instead of water, to grind the roots in a blender. The determination of the final number of eggs and newly hatched juveniles in the final suspension was performed with the aid of a Peter's blade, under a light microscope. This number (final population, FP) was used for obtaining the reproduction factor (RF) (final population of the nematode (FP) / initial population {number of eggs used in the nematode inoculation (IP)}), according to Oostenbrink (1966), that means, RF {RF = FP/IP} equal or higher than 1.0, susceptible (S) and lower than 1.0, resistant (R).

RESULTS AND DISCUSSION

According to data obtained (Table 1), a difference in response to *M. enterolobii*, among head lettuce cultivars, was noticed, using as basis the nematode reproductive factor. The tomato 'Rutgers', used as susceptibility pattern, presented RF of 13.95, proving the viability of the inoculum.

Cultivars Ithaca, Raider Plus, RS-1397, L-104, Challenge, IP-11, Classic, Salinas 88, Vanguard 75, Calona and Desert Queen were unfavorable for *M. enterolobii* multiplication with reproductive factors ranging from 0.22

Table 1. Average values of the gall index (GI), egg mass index (MI) and reproduction factor (RF) of *Meloidogyne enterolobii* in twenty-two lettuce cultivars {valores médios do índice de galhas (GI), índice de massas de ovos (MI) e do fator de reprodução (RF) de *Meloidogyne enterolobii* em vinte e duas cultivares de alface do grupo americano}. Botucatu, UNESP, 2012.

Cultivar	GI ¹	MI ¹	RF ¹	Reaction ²
Ithaca	3.2 a	2.2 a	0.22 a	R
Raider Plus	3.2 a	2.6 a	0.31 a	R
RS-1397	3.8 b	3.6 b	0.34 a	R
L-104	3.4 a	3.0 b	0.62 b	R
Challenge	2.6 a	2.2 a	0.71 b	R
IP-11	3.6 b	3.4 b	0.75 b	R
Classic	3.4 a	2.6 a	0.79 b	R
Salinas 88	3.0 a	2.0 a	0.79 b	R
Vanguard 75	3.2 a	2.0 a	0.88 c	R
Calona	4.0 b	3.0 b	0.9 c	R
Desert Queen	3.0 a	2.2 a	0.9 c	R
Lady	3.8 b	3.6 b	1.06 c	S
Winterset	3.4 a	1.6 a	1.22 c	S
Robinson	3.8 b	2.8 b	1.28 c	S
Sonoma	3.0 a	2.0 a	1.99 d	S
Raider	3.2 a	3.2 b	3.14 e	S
Lucy Brown	3.6 b	2.6 a	3.16 e	S
Bnondaga	4.0 b	3.2 b	3.53 e	S
Summer Time	3.2 a	2.6 a	4.47 f	S
Tainá	3.4 a	2.8 b	4.75 f	S
Sundevil	3.4 a	2.4 a	4.91 f	S
L-109	3.8 b	3.8 b	5.73 g	S
CV (%)	7.62	10.95	10.19	

¹Means followed by same letters do not differ among themselves according to the Scott-Knott test at 5% probability (analysis from data transformed into $\sqrt{x+0.5}$) ²R= resistant (RF<1.0) and S= susceptible (RF>1.0), according to Oostenbrink (1966) {médias seguidas pela mesma letra não diferem entre si pelo teste Scott-Knott a 5% de probabilidade (análise realizada a partir de dados transformados em $\sqrt{x+0.5}$) ²R= resistente (FR<1,0) e S= suscetível (FR>1,0), segundo Oostenbrink (1966)}.

to 0.9; being considered resistant to this species of gall nematodes. The reproduction factor of *M. enterolobii* in cultivars Ithaca, Raider Plus and RS-1397 were the most nearly to zero and did not differ statistically among each other, with RF of 0.22, 0.31 and 0.34, respectively.

Susceptibility reaction to *M. enterolobii* was noticed in lettuce cultivars Lady, Winterset, Robinson, Sonoma, Raider, Lucy Brown, Bnondaga, Summer Time, Tainá, Sundevil and L-109. These cultivars provided an increase in the initial nematode population, with RF ranging

from 1.06 to 5.73. Among these cultivars, Lady (RF= 1.06), Winterset (RF= 1.22) and Robinson (RF= 1.28), despite presenting RF higher than 1.0, were not statistically different from resistant cultivars Vanguard 75 (RF= 0.88), Calona (RF= 0.9), and Desert Queen (RF= 0.9). Lettuce 'L-109' (RF= 5.73) presented the highest reproductive factor of *M. enterolobii*, concerning all the studied cultivars, followed by 'Sundevil' (RF= 4.91), 'Tainá' (RF= 4.75) and 'Summer Time' (RF= 4.47).

Resistance of cultivars Classic, Vanguard 75, Salinas 88 and Challenge, to *M. incognita* race 2, was verified

(Wilcken *et al.*, 2005). These cultivars also behaved as resistant to *M. enterolobii* in this study. The resistance of 'Salinas 88' to this nematode had been reported by Melo *et al.* (2011).

Until then, studies of response of lettuce cultivars to *M. enterolobii* had only detected resistance to the nematode. The cultivar Black Seed Simpson, as an example, was considered resistant with RF of 0.3 and the cultivars Grande Lagos 659, head lettuce, and Mônica crisphead lettuce were considered very resistant to this nematode (Rodríguez *et al.*, 2003; Bitencourt & Silva, 2010). In another study, 'Elisa', 'Luisa', 'Mirella', 'Vera' and 'Salinas 88' were considered moderately resistant when the variable infection rate was used, whereas 'Júlia', 'Hortência', 'Verônica', 'Grand Rapids' and 'Babá de Verão' were considered highly resistant to *M. enterolobii* (Melo *et al.*, 2011).

Although IG, determined by the scale of infection (grades from 1 to 5), had been used only as an auxiliary parameter to indicate the response of symptoms in plants, the authors observed that most of the tested genotypes showed relatively high GI, ranging from 2.6 to 4.0, even those unfavorable to nematode multiplication, as an example of cultivar Calona which presented the highest average of GI (4.0), however, this cultivar was considered resistant as it did not allow nematode multiplication (RF= 0.9). This fact demonstrates that part of the population of *M. enterolobii* present in the substrate penetrated and induced parasitism, although it has not been able to complete its life cycle, the same was observed in studies with *M. incognita* race 2 in lettuce (Wilcken *et al.*, 2005). This fact shows that GI should not be used as the main parameter for evaluating resistance of this crop; it is possible to be used only as aid in the interpretation of the final data.

Even EMI should be carefully used, since all cultivars studied as susceptible, using RF as pattern, showed EMI relatively low as it was the case mainly for cultivars Summer Time, Tainá and Sundevil which showed EMI of 2.6, 2.8 and 2.4 and RF of 4.47, 4.75 and 4.91, respectively. This fact is due to internal egg masses which were not recorded

during EMI evaluations.

Although in literature only information on lettuce cultivars with resistance to *M. enterolobii* can be found, in this work the authors could observe that some behavior variations of head lettuce were noticed, studies on responses of different cultivars to be indicated safely being important in order to be used in fields infested with this nematode or in breeding programs.

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