

HOST STATUS OF CRISPY-LEAF LETTUCE CULTIVARS TO ROOT-KNOT NEMATODES

REAÇÃO DE CULTIVARES DE ALFACE DO GRUPO CRESPA AOS NEMATOIDES DE GALHAS

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ABSTRACT: Lettuce is the main leafy vegetable grown in the world, being the crispy-leaf lettuce type predominant. With consecutive cultivation in the same area, several factors may impair yield, highlighting the damage caused by root-knot nematodes, *Meloidogyne* spp. This study aimed at evaluating the reaction of twenty crispy-leaf lettuce cultivars to *Meloidogyne incognita* race 3, *M. javanica* and *M. enterolobii*. Three experiments were conducted, one for each nematode species. The experiments were carried out in a greenhouse, in pots with sterilized substrate. The design was completely randomized with five replications. Seedlings were inoculated with 1.000 eggs and second-stage juveniles of nematode per pot, on the day of transplantation of seedlings. The tomato 'Rutgers' was used as inoculum viability control for each specie tested. The variables evaluated were: reproduction factor (FR), total number of eggs and second-stage juveniles (NTOJ) and number of eggs and second-stage juveniles per gram of root (NOJGR), 60 days after inoculation. The results showed that the cultivars Veronica, Grand Rapids and Crespa para Verão are resistant to the three nematode species. The cultivars Thaís, SRV 2005 and Marisa are resistant to *M. incognita* race 3 and *M. javanica*. The cultivar Black Seed Simpson is resistant to *M. enterolobii*. The cultivars Vanda and Mônica SF 31 are resistant to *M. incognita* race 3. The cultivars Crespa, Rubia, Cinderela and Veneranda are resistant to *M. javanica*.

KEYWORDS: *Lactuca sativa* L. *Meloidogyne* spp. Reproduction factor. Plant resistance.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the most important leafy vegetable crop grown worldwide. In Brazil, the main types cultivated in order of economic importance are crispy-leaf, crisp-head, looseleaf and romaine (SALA & COSTA, 2012). Among the lettuce groups predominantly consumed in the country, crispy-leaf type has grown considerably in the last years and corresponded to 44.55% of the volume marketed in 2014 at Ceagesp (AGRIANUAL 2016).

Cropping lettuce consecutively in the same area has triggered several problems, among them, the increase of parasitic nematode populations, mainly root-knot nematodes (*Meloidogyne* spp.) (CARVALHO FILHO et al., 2011).

The root-knot nematodes are phytoparasites of the root system that cause serious damage to lettuce cultivation. Several studies report the parasitic action of these nematodes in the crop (CARVALHO FILHO et al., 2011; DIAS-ARIEIRA et al., 2012; PINHEIRO et al., 2014; ROSA et al., 2015; CORREA, et al., 2015).

Lettuce plants, when attacked by these nematodes, present intense debility caused by dense

formation of galls in the root system, resulting in restrictions on water and nutrients absorption and transport, becoming chlorotic, with reduced size, small leaf volume and no value for *in natura* consumption (CARVALHO FILHO et al., 2011).

The species *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood, stand out among the nematodes of importance for lettuce crop (WILCKEN et al., 2005). In the present days, *Meloidogyne enterolobii* Yang & Eisenback (syn. *Meloidogyne mayaguensis* Rammah & Hirschmann) has been of great concern because of its high aggressiveness in several plant species, including vegetables, fruits and ornamental crops (BRITO et al., 2007; MELO et al., 2011; ROSA et al., 2015).

The control of phytonematodes in lettuce growing areas has proved to be a challenging task. Crop rotation, a commonly recommended technique to control root-knot nematodes, is difficult to use due to wide host range and successive crops in the same areas (FERREIRA et al. 2010). Lettuce is a short-cycle crop that is predominantly consumed *in natura*, therefore, the use of chemical nematicides is not recommended as it may leave residues of the applied product (FERDANDEZ; KULCZYNSKI,

2009; FERREIRA et al., 2011). Controlling these parasites is indispensable for a good crop performance, since it can cause up to 100% of production losses, depending on the infestation intensity and the cultivar susceptibility (CHACHAR; MOITA, 1996).

The use of resistant cultivars is the ideal method of control of root-knot nematodes in lettuce, since in addition to the reduction of these phytoparasites, it does not add to the grower an additional cost (MOENS et al., 2009; FERREIRA et al., 2011). Thus, the present study had as objective to evaluate twenty crispy-leaf lettuce cultivars regarding the reaction to *M. incognita*, *M. javanica* and *M. enterolobii*.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at Sitio Santa Catarina, Marília-SP, located at the geographic coordinates, latitude 22°14'59.9"S, longitude 50°06'47.7"W and altitude of 449 meters, executed from November 2015 to January 2016.

We carried out three experiments with 20 cultivars of crispy-leaf lettuce for the following root-knot nematodes: I) *M. enterolobii*, II) *M. incognita* and III) *M. javanica*. The tests were performed in complete randomized design, with five replicates, each replicate being one pot containing one plant. The lettuce cultivars used in this study were: Cinderela (Feltrin), Crocanela (Feltrin), Mônica SF 31 (Feltrin), Rubinela (Feltrin), Veneranda (Feltrin), Ariel (Horticeres), Marisa (Horticeres), Rubia (Horticeres), Vanda (Horticeres), Black Seed Simpson (Topseed), Crespa para Verão (Topseed), Elba (Topseed), Grand Rapids (Topseed), Thaís (Sakata), Vera (Sakata), Verônica (Sakata), Banchu (Takii Seed), Crespa (Hortec), Solaris (Hortibras), and SRV 2005 (Semini). We used the tomato 'Rutgers' as control of susceptibility.

The seeds of lettuce and tomato cultivars were sown in 200 cells expanded polystyrene trays filled with autoclaved Bio Plant® substrate. The seedlings were transplanted to 2 liters pots at 25 days after sowing. The pots were filled with a previously autoclaved mixture composed of soil, sand and tanned cattle manure, in a proportion of 1:2:1.

The inoculum of each nematode species was obtained from tomato plants kept as sources of inoculum in greenhouse for 60 days. The species were identified by the perineal pattern of the females (TAYLOR; NETSCHER, 1974) and the

male lip morphology (EISENBACK et al., 1991). The inoculum was multiplied in tomato 'Rutgers' plants in 2 L pots containing a previously autoclaved mixture composed of soil, sand and tanned cattle manure, in a proportion of 1:2:1. Highly infected tomato roots were processed according to Hussey & Baker (1973), for the extraction of eggs and second-stage juveniles that constituted the initial inoculum. The determination of the number of eggs and second-stage juveniles in the suspension was performed with the aid of Peters chamber (SOUTHEY, 1970), under light microscope.

The lettuce seedlings were inoculated individually with 1.000 eggs and second-stage juveniles (Initial population - Ip) of the nematode population under test, on the day of transplanting. The inoculation was carried out by placing 10 mL of the inoculum suspension in two holes of 3 cm deep, near the rhizosphere of each plant. The plants were fertilized as proposed by Hoagland & Arnon (1950).

The evaluations were performed 60 days after inoculation. The aerial part and the root system of the plants were separated. The roots were processed according to Hussey & Baker (1973).

The final number of eggs and second-stage juveniles (Final population - Fp) in the suspension was determined with the aid of Peters chamber (SOUTHEY, 1970), under light microscope. The final and initial population was used to obtain the reproduction factor ($RF = Fp/Ip$). The cultivars were classified according to Oostenbrink (1996), where RF equal to or greater than 1 the cultivar is rated as susceptible (S), and less than 1, as resistant (R).

The data were transformed to $\log(x+1)$. The analyzes were performed by the statistical program AgroEstat (BARBOSA; MALDONADO JÚNIOR, 2015). The means were grouped by the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

According to the data obtained, there was a significant difference for the variables total number of eggs and second-stage juveniles (NTOJ) and number of eggs and second-stage juveniles per gram of root (NOJGR) between crispy-leaf lettuce cultivars for the three experiments (Table 1). The inoculation carried out in the experiment was successful, since there was excellent multiplication of the three species of root-knot nematodes in 'Rutgers' tomato, used as a susceptibility control for *M. enterolobii*, *M. incognita* race 3 and *M. javanica*, with FR 60.48, 12.17 and 28.44, respectively, confirming the viability of the inoculum

The cultivars Verônica, Black Seed Simpson, Crespa para Verão, Grand Rapids, Vanda and Veneranda presented the lowest NTOJ values for *M. enterolobii*, differing significantly from the other cultivars. Among these, 'Veronica', 'Black Seed Simpson', 'Crespa para Verão' and 'Grand Rapids' obtained resistance reaction, with reproduction factors ranging from 0.36 to 0.72 (Table 1). The resistance of Verônica and Grand Rapids cultivars to *M. enterolobii* was also reported by Melo et al., 2011 and Rosa et al., 2015.

The susceptibility reaction to *M. enterolobii* was observed in cultivars Vanda, Veneranda, Mônica SF 31, Cinderela, Thaís, SRV 2005, Solaris, Crespa, Ariel, Elba, Banchu, Crocantela, Vera, Marisa, Rubinela and Rubia. These cultivars provided an increase in the initial nematode population, with FR ranging from 2.16 to 31.68. The lettuce cultivar Rubia and Rubinela presented the highest values of NTOJ for *M. enterolobii*, followed by cultivars Marisa, Vera and Crocantela. For NOJGR, the cultivar Rubia obtained the highest value, followed by the cultivars Rubinela and Marisa. The high susceptibility of lettuce genotypes to *M. enterolobii* is reported in several studies (RODRIGUEZ et al., 2003, BITENCOURT; SILVA, 2010, MELO et al., 2011, ROSA et al., 2015).

For the reaction to *M. incognita* race 3, the cultivars Grand Rapids, Veronica, Vanda, Marisa, Monica SF 31, Crespa para Verão, SRV 2005, Thaís, Ariel, Crocantela, Elba, Rubia and Crespa were the ones that provided the lowest values of NTOJ, ranging from 0 to 1800. Among these, the cultivars Grand Rapids, Veronica, Vanda, Marisa, Monica SF 31, Crespa para Verão, SRV 2005 and Thaís were considered resistant to *M. incognita*, with reproduction factors ranging from 0 to 0.72. In soil infested with *M. incognita*, the cultivar Grand Rapids was also highly resistant in the study conducted by Charchar & Moita (1996).

We verified that the cultivars Vera, Banchu, Veneranda, Solaris, Cinderella, Rubinela, and Black Seed Simpson presented the highest NTOJ for *M. incognita* race 3, varying from 2880 to 6120. The cultivars Ariel, Crocantela, Elba, Rubia, Crespa, Vera, Banchu, Veneranda, Solaris, Cinderella, Rubinela and Black Seed Simpson presented susceptibility reaction to *M. incognita* race 3, with reproduction factor ranging from 1.08 to 6.12.

For NOJGR, there were significant differences between cultivars. The cultivars Grand Rapids, Veronica, Vanda, Monica SF 31, Thaís, Crespa para Verão, Marisa, Crocantela, SRV 2005, Ariel, Elba and Crespa were the ones with the

lowest NOJGR values ranging from 0 to 37.20. The cultivar Rubinela (NOJGR = 238.60) presented the highest number of eggs and second-stage juveniles per gram of root.

Gomes (2000) reports that the resistance of the cultivar Grand Rapids to races 1, 2, 3 and 4 of *M. incognita* is controlled by a single gene locus, with predominantly additive effect, with relatively high heritability, being an important indicative that this cultivar can be used in breeding programs aiming at resistance of lettuce to *M. incognita*. Carvalho Filho et al. (2011) reported that the gene *Me* confers resistance to *M. incognita* in the cultivar Grand Rapids.

The cultivars Verônica, Rubia, Cinderella, Grand Rapids, SRV 2005, Marisa, Crespa para Verão, Crespa, Thaís, Veneranda, Rubinela, Vanda, Ariel, Elba, Monica SF 31 and Solaris presented the lowest NTOJ values, ranging from 0 to 2160 for *M. javanica*. Among them, the cultivars Verônica, Rubia, Cinderela, Grand Rapids, SRV 2005, Marisa, Crespa para Verão, Crespa, Thaís and Veneranda presented resistance reaction to *M. javanica* species, with reproduction factor varying from 0.00 to 0.72.

The cultivars Rubinela, Vanda, Ariel, Elba, Mônica SF 31, Solaris, Crocantela, Vera, Black Seed Simpson and Banchu showed susceptibility reaction to *M. javanica*, with reproduction factors varying from 1.08 to 5.76. Among these cultivars, Crocantela, Vera, Black Seed Simpson and Banchu presented higher NTOJ, ranging from 3240 to 5760.

Table 1. Mean values of total number of eggs and second-stage juveniles (NTOJ), reproduction factor (FR), number of eggs and second-stage juveniles per gram of root (NOJGR), reproduction index (IR), reaction and degree of resistance (GR) of twenty cultivars of lettuce of the crispy group to *Meloidogyne enterolobii*, *Meloidogyne incognita* race 3 and *Meloidogyne javanica*.

Cultivars	<i>Meloidogyne enterolobii</i>				<i>Meloidogyne incognita</i> raça 3				<i>Meloidogyne javanica</i>			
	NTOJ	FR	Reaction ⁽²⁾	NOJGR	NTOJ	FR	Reaction ⁽²⁾	NOJGR	NTOJ	FR	Reaction ⁽²⁾	NOJGR
Verônica	360 a ⁽¹⁾	0,36	R	12,43 a ⁽¹⁾	0 a ⁽¹⁾	0,00	R	0,00 a ⁽¹⁾	0 a ⁽¹⁾	0,00	R	0,00 a ⁽¹⁾
Grand Rapids	720 a	0,72	R	21,45 a	0 a	0,00	R	0,00 a	0 a	0,00	R	0,00 a
Crespa para Verão	720 a	0,72	R	6,15 a	360 a	0,36	R	6,00 a	360 a	0,36	R	3,40 a
Black Seed Simpson	360 a	0,36	R	5,26 a	6120 b	6,12	S	84,60 b	5400 b	5,40	S	103,60 b
Vanda	2160 a	2,16	S	19,61 a	0 a	0,00	R	0,00 a	1080 a	1,08	S	12,00 a
Veneranda	3240 a	3,24	S	45,47 a	3960 b	3,96	S	49,00 b	720 a	0,72	R	14,00 a
Mônica SF 31	5040 b	5,04	S	114,68 a	360 a	0,36	R	2,80 a	1440 a	1,44	S	24,60 a
Cinderela	5760 b	5,76	S	79,97 a	5040 b	5,04	S	66,20 b	0 a	0,00	R	0,00 a
Thaís	6480 b	6,48	S	146,49 a	720 a	0,72	R	13,80 a	720 a	0,72	R	13,60 a
SRV 2005	6840 b	6,84	S	62,93 a	720 a	0,72	R	12,40 a	360 a	0,36	R	19,00 a
Solaris	7560 b	7,56	S	125,16 a	4320 b	4,32	S	86,80 b	2160 a	2,16	S	14,40 a
Crespa	7560 b	7,56	S	91,52 a	1800 a	1,80	S	37,20 a	360 a	0,36	R	8,40 a
Ariel	9720 b	9,72	S	195,11 a	1080 a	1,08	S	25,60 a	1080 a	1,08	S	19,20 a
Elba	10080 b	10,08	S	112,56 a	1080 a	1,08	S	10,40 a	1440 a	1,44	S	14,00 a
Banchu	10080 b	10,08	S	193,93 a	3960 b	3,96	S	84,00 b	5760 b	5,76	S	133,20 b
Crocantela	15840 c	15,84	S	130,10 a	1080 a	1,08	S	13,80 a	3240 b	3,24	S	35,80 a
Vera	19800 c	19,80	S	460,54 a	2880 b	2,88	S	66,20 b	3960 b	3,96	S	158,00 b
Marisa	21960 c	21,96	S	829,64 b	360 a	0,36	R	8,80 a	360 a	0,36	R	7,00 a
Rubanela	27000 d	27,00	S	792,94 b	5400 b	5,40	S	238,60 c	1080 a	1,08	S	30,20 a
Rubia	31680 d	31,68	S	1127,95 c	1800 a	1,80	S	62,60 b	0 a	0,00	R	0,00 a
CV (%)	15,25			19,5	27,51			26,68	32,45			31,37

⁽¹⁾ Means followed by the same letter do not differ by Scott-Knott test at 5% probability. Analysis performed from data transformed into log (x+1). ⁽²⁾ Reaction, according to Oostenbrink (1966), that is, equal to or greater than 1.0, susceptible (S) and less than 1.0, resistant (R).

For the variable NOJGR, the cultivars Verônica, Rubia, Cinderella, Grand Rapids, Crespa para Verão, Marisa, Crespa, Vanda, Thaís, Veneranda, Elba, Solaris, SRV 2005, Ariel, Monica SF 31, Rubinela and Crocanela presented the lowest NOJGR, ranging from 0.0 to 35.80. The Black Seed Simpson, Banchu and Vera cultivars obtained the highest NOJGR, ranging from 103.60 to 158.00.

The cultivars of the crispy group had higher levels of resistance to *M. javanica*. Charchar & Moita (1996) also reported higher levels of resistance to *M. javanica* for the crispy group, highlighting the cultivar Grand Rapids.

The crispy lettuce cultivars Veronica, Grand Rapids and Crespa para Verão showed resistance to *M. enterolobii*, *M. incognita* race 3 and *M. javanica*, simultaneously, presenting good potential for future lettuce breeding programs. These results are in agreement with those obtained in other studies (CHARCHAR; MOITA, 1996; GOMES, 2000; MALUF et al., 2002; PINHEIRO et al., 2014), who observed the potential of crispy-leaf cultivars for resistance to root-knot nematodes.

In lettuce, the multiple resistance to the three species of *Meloidogyne* is probably provided by the same gene or by nearby gene loci. Unlike what occurs in other vegetables, where genes that confer resistance to *M. incognita* and *M. javanica* apparently have no effect on *M. enterolobii* (CANTU et al., 2009; BITENCOURT; SILVA, 2010; ROSA, 2010; MELO et al., 2011; ROSA et al., 2014). Good examples are tomato that possesses the *Mi* gene, conferring resistance to *M. incognita*, *M. javanica* and *M. arenaria* (BOITEUX et al., 2012); *Capsicum*, *Me* gene series, conferring resistance to *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria* (PINHEIRO et al., 2015).

Besides the possibility of using the resistant cultivars in lettuce breeding aiming at resistance to root-knot nematodes, the resistant crispy lettuce

cultivars can be used as alternatives in planting or crop rotation in areas infested by these pathogens as they are commercial and present good productive performance.

In order to manage the root-knot nematodes, the ideal is to identify the species that are occurring in the area (CUNHA et al., 2018). In an area that only occurs *M. enterolobii*, for example, in addition to the cultivars resistant to the three species evaluated, we can recommend the planting of the cultivar Black Seed Simpson.

The identification of a nematode species requires taxonomic analysis and should be carried out by a specialized laboratory, resulting in the fact that growers still do not have the habit of sending infested root systems to identify the species present in the area, thus hindering the efficient management of this parasite (COLLANGE et al., 2011).

Thus, it is important that cultivars are resistant to as many root-knot nematode species as possible. The three species evaluated in this study are those of greater socioeconomic importance in Brazil, either by distribution in the territory or by aggressiveness. In this way, it is important to have resistant cultivars to the three species, since it can be recommended for cropping in infested areas where the occurring species is yet to be identified.

CONCLUSIONS

Based on the reproduction factor, the cultivars Thaís, SRV 2005 and Marisa are resistant to the *M. incognita* race 3 and *M. javanica*; The cultivar Black Seed Simpson is resistant to *M. enterolobii*; The cultivars Vanda and Monica SF 31 is resistant to *M. incognita* race 3; The cultivars Crespa, Rubia, Cinderella and Veneranda are resistant to *M. javanica*.

The cultivars Verônica, Grand Rapids and Crespa para Verão are resistant to *M. incognita* race 3, *M. javanica* and *M. enterolobii*

RESUMO: A alface é a principal hortaliça folhosa cultivada no mundo, sendo o tipo crespa mais comercializada. Com o cultivo consecutivo na mesma área, vários fatores podem prejudicar a produtividade, se destacando os danos causados pelos nematoides de galha, *Meloidogyne* spp. Este trabalho teve como objetivo avaliar vinte cultivares de alface, do grupo crespa, quanto a reação à *Meloidogyne incognita*, *Meloidogyne javanica* e *Meloidogyne enterolobii*. Foram realizados três ensaios, um para cada espécie de nematoide. Os experimentos foram conduzidos em casa de vegetação, em vaso com substrato autoclavado. O delineamento experimental foi em blocos casualizados, com cinco repetições. As plantas foram inoculadas com 1.000 ovos e eventuais juvenis de segundo estágio do nematoide, no dia do transplântio das cultivares. O tomateiro 'Rutgers' foi utilizado como padrão de viabilidade do inóculo de cada espécie de nematoide. As variáveis avaliadas foram: número total de ovos e juvenis do segundo estágio (NTOJ), fator de reprodução (FR) e número de ovos e juvenis de segundo estágio por grama de raízes (NOJGR) avaliadas 60 dias após a inoculação. Os resultados obtidos mostraram que as cultivares Verônica, Grand Rapids e Crespa para Verão foram resistentes as espécies *M. enterolobii*, *M. incognita*, *M. javanica*. As cultivares Thaís, SRV 2005 e Marisa foram resistentes as espécies *M.*

incognita e *M. javanica*. A cultivar Black Seed Simpson foi resistente à *M. enterolobii*. Vanda e Mônica SF 31 foram resistentes à *M. incognita*. As cultivares Crespa, Rubia, Cinderela e Veneranda foram resistentes à *M. javanica*.

Palavras-chave: *Lactuca sativa* L.. *Meloidogyne*. Fator de reprodução. Resistência de plantas.

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