Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Susceptibility of crop plants to the root-knot nematode *Meloidogyne luci*, a threat to agricultural productivity

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OPEN ACCESS

Citation: C. Maleita, A. Correia, I. Abrantes, I. Esteves (2022) Susceptibility of crop plants to the root-knot nematode *Meloidogyne luci*, a threat to agricultural productivity. *Phytopathologia Mediterranea* 61(1): 169-179. doi: 10.36253/phyto-13369

Accepted: March 9, 2022

Published: May 13, 2022

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Jean-Michel Savoie, INRA Villenave d'Ornon, France.

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Summary. The root-knot nematode (RKN) *Meloidogyne luci* is included in the Alert List of the European Plant Protection Organization, because it has potential negative impacts on economically important crops. Identification of plant species/cultivars resistant to *M. luci* is important for its management. Susceptibility of 35 commercial plant species/cultivars, from nine families to a *M. luci* isolate from Portugal was evaluated in pot assays, assessing root gall index (GI) and reproduction factor (Rf) 60 d after inoculation, with tomato 'Coração-de-Boi' used as the positive susceptible experimental control. Presence/absence of RKN resistance genes was also determined in the tomato and pepper cultivars. One cultivar of cabbage, three of lettuce, ten of pepper, one of sugar beet, and all the cultivars of Cucurbitaceae (five), Fabaceae (two) and Poaceae (one) were susceptible to *M. luci* (GI = 4-5; Rf = 2.1-152.3). One cultivar each of carrot, passion fruit, lettuce 'Cocktail', cabbage 'Bacalan', 'Coração' and 'Lombarda', and spinach 'Tayto' were resistant/hypersensitive (Rf < 1; GI > 2). The tomato 'Actimino', 'Briomino', 'Veinal' and 'Vimeiro', which carried at least one copy of the *Mi-1.2* gene, were resistant to the nematode (GI = 1-2; 0.0 < Rf < 0.1). These results indicate that the tomato cultivars have potential to contribute to reduction of *M. luci* populations in agro-ecosystems and improve the crop yields.

Keywords. Gall index, pathogenicity, plant-parasitic nematodes, reproduction factor.

INTRODUCTION

Root-knot nematodes (RKN, *Meloidogyne* spp.) are plant parasites responsible for significant economic crop losses (Nicol *et al*., 2011). *Meloidogyne* includes 98 described species, which are obligate parasites of almost all vascular plants (Jones *et al*., 2013; Subbotin *et al*., 2021). Although four species (*M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*) are considered the most common, many others have been gaining importance due as poten-

tial causes of damage to economically important crops (Elling, 2013).

Meloidogyne luci is a damaging and polyphagous RKN included in the European and Mediterranean Plant Protection Organization Alert List since 2017 (EPPO, 2017). This species has a wide host range and is a threat to agricultural productivity and ecosystem sustainability (Şen and Aydınlı, 2021). Additionally, *M. luci* shares some morphological and biochemical similarities with *M. ethiopica* and *M. inornata* (Gerič Stare *et al*., 2019), fact that has led to the misidentification of several *M. luci* populations in Europe (Gerič Stare *et al*., 2017b). In recent years, molecular information on *M. luci* has been attained, and molecular diagnostic methods for the accurate detection/discrimination of this RKN species were developed (Gerič Stare *et al*., 2019; Susič *et al*., 2020a; Maleita *et al*., 2021; Žibrat *et al*., 2021).

Meloidogyne luci was first described in 2014 from isolates originally collected from roots of lavender (*Lavandula spica* L.) in Brazil, and was maintained by periodically culturing on tomato (*Solanum lycopersicum* L. 'Santa Clara') (Carneiro *et al*., 2014). In this country, the nematode was found parasitizing broccoli (*Brassica oleracea* L.), common bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson], lettuce (*Lactuca sativa* L.), loofah [*Luffa cylindrica* (L.) Roem.], okra [*Abelmoschus esculentus* (L.) Moench], soybean [*Glycine max* (L.) Merr.], and yacón (*Polymnia sonchifolia* Poepp) (Carneiro *et al*., 2014; Machado *et al*., 2016; Bellé *et al*., 2016, 2019a, 2019b). Apart from Brazil, *M. luci* has been identified in Argentina, Bolivia, Chile, Ecuador, Greece, Guatemala, Iran, Italy, Slovenia, and Turkey, associated with economically important crops, ornamentals, herbs and weeds. The recorded host include aubergine (*Solanum melongena* L.), barley (*Hordeum vulgare* L.), beetroot (*Beta vulgaris* L.), broccoli, buckwheat (*Fagopyrum esculentum* Moench), carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.), celery (*Apium graveolens* L.), chicory (*Cichorium intybus* L.), common bean, endive (*Cichorium endivia* L.), Florence fennel (*Foeniculum vulgare* Mill.), grapevine (*Vitis vinifera* L.), groundsel (*Senecio vulgaris* L.), herb curled dock (*Rumex patientia* L.), kohlrabi (*Brassica oleracea* L.), lucerne (*Medicago sativa* L.), melon (*Cucumis melo* L.), morning glory (*Ipomoea* spp.), onion (*Allium cepa* L.), pea *(Pisum sativum* L.*),* peach [*Prunus persica* (L.) Batsch], pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), pumpkin (*Cucurbita moschata* Duchesne ex Poir.), radish (*Raphanus sativus* L.), rice (*Oryza sativa* L.), rose (*Rosa* sp. L.), sedum [*Hylotelephium spectabile* (Boreau) H. Ohba], snapdragon (*Antirrhinum majus* L.), spinach (*Spinacia oleracea* L.), sunflower (*Helianthus annuus* L.), sweet corn (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), tomato, and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) (Širca *et al*., 2004; Strajnar *et al*., 2009, 2011; Conceição *et al*., 2012; Aydınlı *et al*., 2013; Carneiro *et al*., 2014; Aydınlı and Mennan, 2016; EPPO, 2017; Gerič Stare *et al*., 2017a, 2017b; Aydınlı, 2018; Santos *et al*., 2019; Gonçalves *et al*., 2020; Susič *et al*., 2020b; Kaspary *et al*., 2021; Žibrat *et al*., 2021).

In Portugal, *M. luci* was first detected in a potato field near Coimbra, in 2013 (Maleita *et al*., 2018). Since then, it was found parasitizing roots of ornamental cabbage trees [*Cordyline australis* (G.Forst.) Endl.], the weed yellow wood sorrel (*Oxalis corniculata* L.), and tomato in subsistence farms of Coimbra district, and potato in Pico Island, Azores (Santos *et al*., 2019; Rusinque *et al*., 2021).

Although the application of pesticides may be effective for RKN control, increasing environmental and human health concerns about pesticide use have stimulated development of alternative management strategies (Wesemael *et al*., 2011). Crop rotation and use of resistant host cultivars are known to be effective on managing RKN populations. Knowledge of host response of crops and cultivars to RKN is important for successful implementation of these nematode management methods (Nyczepir and Thomas, 2009; Rashidifard *et al*., 2021).

Presence of host plant genes conferring resistance to RKN constitutes an important strategy for integrated nematode pest management. Several resistance genes have been identified from various plant sources, including the tomato gene *Mi-1.2* (Williamson *et al*., 2009). This nematode resistance gene is a well characterized example, and is effective against various *Meloidogyne* species including *M. arenaria*, *M. ethiopica*, *M. incognita*, *M. javanica* and *M. luci* (Williamson, 1998; Aydınlı and Mennan, 2019; Santos *et al*., 2020). Several studies have also demonstrated that the *Mi-1.2* gene has greater effectiveness in homozygous (MiMi) host genotypes than on heterozygous (Mimi) genotypes (Jacquet *et al*., 2005; Maleita *et al*., 2012; Santos *et al*., 2020). However, when soil temperatures exceed 28°C, resistance conferred by this gene is overcome (Dropkin, 1969; Ammati *et al*., 1986; Tzortzakakis *et al*., 2014).

In pepper, the *Me* genes *Me1*, *Me3* and *Me7*, and the dominant *N* gene, were found to be effective against *M. arenaria*, *M. incognita* and *M. javanica* (Djian-Caporalino *et al*., 2011; Wang *et al*., 2009), but their effectiveness has been shown to decrease when used intensively (Djian-Caporalino *et al*., 2011). Laboratory assays showed that resistance conferred by *Me1* could not be overcome

by virulent isolates, although virulent variants overcame *Me3* gene resistance (Castagnone-Sereno *et al*., 2001).

The primary objective of the present study was to evaluate the ability of *M. luci* to reproduce on 35 cultivated plants which are commonly cropped on subsistence farms in Portugal, and some of which are grown in rotations with potato. In addition, the presence of RKN resistance genes was investigated in cultivars of tomato (*Mi-1.2* gene) and pepper (*Me*1, *Me*3, *Me7* and *N* genes), which were assessed for their host status to *M. luci*, to evaluate whether resistance in these plants was related to presence of these gene markers.

MATERIALS AND METHODS

Nematode isolate

An isolate of *M. luci*, originally obtained from a potato field in Coimbra, Portugal (Maleita *et al*., 2018), was maintained on tomato 'Coração-de-Boi', in a temperature-controlled growth chamber (23 ± 2°C) with 12 h daily light periods. The species identification of the isolate was confirmed by esterase phenotype analysis (Maleita *et al*., 2018).

Host status

The responses to *M. luci* of 35 commercial plant cultivars (Table 1), representing 15 species from nine botanical families, were evaluated under controlled conditions (23 ± 2ºC; 12 h daily light periods). Plants were grown from seeds in Petri dishes containing water-soaked filter paper, at 25ºC in the dark. After germination, seedlings were individually transplanted into 5 cm diam. plastic pots filled with a mixture of sterilised sandy loam soil, sand and substrate (1:1:2). After four weeks, the seedlings were transplanted into 10 cm diam. pots containing a mixture of sterilised sandy loam soil, sand and substrate (1:1:1).

Nematode inoculum was obtained from infected tomato 'Coração-de-Boi' roots, through extraction of eggs using a 0.52% sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). Five plants from each plant species/cultivar were inoculated with 5000 *M. luci* eggs (initial population density, Pi). Tomato 'Coração-de-Boi' was included as susceptible control of inoculum viability, and non-inoculated plants of each cultivar were included as negative controls. The pots were arranged in a completely randomized design in a growth chamber, which was set at 23 ± 2°C, 12 h daily photoperiod, and ±60% relative humidity, and the plants were watered each day.

Sixty days after inoculation, the plants were harvested and the root systems washed free of soil substrate. Numbers of galls/plant were recorded, and gall indices (GI) were assessed using a 0–5 scale (0 = no galls, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 ≥ 100 galls) (Taylor and Sasser, 1978). Nematode eggs were extracted from each plant, as described above (Hussey and Barker, 1973), to determine the final population density (Pf), and the reproduction factor (Rf = Pf/Pi) was calculated. Host susceptibility to *M. luci* was assessed based on GI and Rf (Sasser *et al*., 1984).

DNA analyses for the Mi-1.2*,* Me1*,* Me3*,* Me7 *and* N *genes*

Plant material

Roots of four tomato cultivars ('Actimino', 'Briomino', 'Veinal' and 'Vimeiro') and ten pepper cultivars ('Amarelo', 'Celta', 'Claudio', 'Rainbom', 'Rialto', 'Solero', 'Tauro', 'Torpedo', 'Vermelho' and 'Yoacali') were assessed for *Mi*-mediated resistance in tomato and *Me*-mediated resistance in pepper. The pepper accessions 'Yolo Wonder', lacking the RKN resistance genes *Me1*, *Me3*, *Me7* and *N*, and the double haploid pepper lines 'DH149' (with the *Me3* resistance gene) and 'DH330' (carrying the *Me1* resistance gene), were included in this analysis as experimental controls. No positive control for the *N* gene was included.

DNA extractions from plants

DNA from tomato and pepper plants was extracted using the kit DNeasy Plant Mini Kit (Qiagen) for purification of total DNA from plant tissues, with some modifications. Instead of using liquid nitrogen, plant roots were ground on ice, after being frozen overnight at -80°C. Genomic DNA concentration was determined in a Nanodrop 2000c spectrophotometer (ThermoScientific), and the samples were stored at -20°C until PCR analyses.

For the host accessions Yolo Wonder and DH149, plant DNA extraction was carried out from leaf tissue, and for the accession DH330 from seeds, for both tissue types using the protocol of Maleita *et al*. (2012).

Detection of the *Mi-1.2* gene

DNA amplification was carried out using the Mi23 marker to assess the present/absence of the *Mi* gene on tomato plants. DNA amplification was carried out as described by Seah *et al*. (2007), using the primers Mi23F

(5'-TGG AAA AAT GTT GAA TTT CTT TTG-3') and Mi23R (5'-GCA TAC TAT ATG GCT TGT TTA CCC-3'). PCR reactions were carried out as described in Maleita *et al.* (2012), and were analysed using 1.5% agarose gel electrophoresis with 1× TBE buffer and staining with GreenSafe (NZYTech).

Detection of *Me1*, *Me3*, *Me7* and *N* genes

Amplifications of the markers linked to the *Me1* and *Me7* genes, and the *Me3* gene as a SCAR, were carried out as described by Djian-Caporalino *et al.* (2007), using the primers CD-F/R (5'-GAA GCT TAT GTG GTA MCC-3' and 5'-GCA AAG TAA TTA TAT GCA AGA GT-3') for *Me1* and *Me7*, and B94-F/R (5'-GCT TAT CAT GGC TAG TAG GG-3' and 5'-CGG ACC ATA CTG GGA CGA TC-3') for *Me3*. Amplification of the marker linked to the *N* gene as a SCAR (forward 5'-AAT TCA GAA AAA GAC TTG GAA GG-3' and reverse 5'-TAA AGG GAT TCA TTT TAT GCA TAC-3') was carried out as described by Wang *et al.* (2009). The PCR products were analysed on 1.5-3% agarose gels for the *Me1*, *Me3* and *Me7* genes, or on a 15% polyacrylamide gel in 1× TBE buffer for the *N* gene, which were stained with GreenSafe.

Data analyses

Statistical significance between the different plant species/cultivars was obtained, for each parameter, using Analysis of Variance (ANOVA), after checking assumptions of normality (Shapiro–Wilk test) and equality (Levene's test). *Post-hoc* Fisher's Least Significant Differences (LSD) test was applied to test for differences between the plant species/cultivars. Statistical analyses were carried out using Statsoft Statistica version 7 for Windows.

RESULTS

Nematode reproduction

Meloidogyne luci reproduced (Rf > 1) on 24 of 35 the plant species/cultivars, although considerable variation was found among replicates (Table 1). Cabbage 'Kale', faba bean, maize, lettuce ('Batavia', 'Butterhead' and 'Folha-de-Carvalho'), pea 'Maravilha D'América', pepper ('Amarelo', 'Celta', 'Cláudio', 'Rainbom', 'Rialto', 'Solero', 'Tauro', 'Torpedo', 'Vermelho' and 'Yoacali'), pumpkin 'Havana F1', beetroot, sweet melon 'Galia F1', tomato 'Coração-de-Boi', watermelon 'Sugar Baby', and zucchini ('Black Beauty' and 'Nova Zelândia') were classified as susceptible hosts, with $2.1 \leq Rf \leq 152.3$ and $GI \geq 4$ (Table 1). Additionally, seven cultivars were classified as resistant/hypersensitive, including cabbage ('Bacalan', 'Coração' and 'Lombarda'), carrot, lettuce 'Cocktail', spinach 'Tayto', and passion fruit, with $0.0 \leq Rf \leq 0.9$ and $GI \geq 4$ (Table 1). Four tomato cultivars ('Actimino', 'Briomino, 'Veinal' and 'Vimeiro') were classified as resistant ($0.0 \leq Rf \leq 0.1$ and $1 \leq GI \leq 2$; Table 1).

Statistically significant differences in *M. luci* reproduction were detected between the different plant species/cultivars ($P \leq 0.05$, Fisher's LSD test). Calculation of Rf across species/cultivars showed that the most susceptible hosts were (in order of susceptibility; Table 1): tomato 'Coração-de-Boi' > zucchini 'Nova Zelândia' > sweet melon 'Galia F1' > pepper 'Cláudio' > pepper 'Torpedo' > Pepper 'Vermelho' > Zucchini 'Black Beauty' > Pepper 'Tauro' > Pepper 'Amarelo' > Pea 'Maravilha d'América' > Lettuce 'Flor-de-Carvalho' > Pumpkin 'Havana F1' > Pepper 'Solero' > Pepper 'Rialto' > Faba bean > Pepper 'Celta' > Sugarbeet > Pepper 'Raimbom' > Lettuce 'Butterhead' > Maize > Lettuce 'Batavia' > Watermelon 'Sugar Baby' > Pepper 'Yoacali' > Cabbage 'Kale' > Lettuce 'Cocktail' > Spinach 'Tayto' > Cabbage 'Lombarda' > Cabbage 'Coração' > Carrot > Passion Fruit > Tomato 'Actimino' > Cabbage 'Bacalan' > Tomato 'Briomino' > Tomato 'Vimeiro' > Tomato 'Veinal'.

Detection of Mi-1.2 *gene in tomato*

Amplification of the Mi23 marker was carried out using DNA from the tomato genotypes, resulting in one band of approx. 380 bp, associated with homozygous resistant genotypes (MiMi), for 'Actimino' and 'Briomino'. This confirmed the presence of the *Mi-1.2* gene. Two bands of 430 bp and 380 bp were associated with the heterozygous Mimi genotypes in 'Veinal' and 'Vimeiro' (Mimi) (Figure 1).

Detection of Me1, Me3, Me7 *and* N *genes in pepper*

Amplification of the SCAR_B94 marker using DNA from pepper cultivars resulted in a single DNA band of approx. 240 bp for the pepper accession 'DH149', indicating the presence of the *Me3* gene, and a band of approx. 220 bp for the remaining pepper cultivars ('Amarelo', 'Celta', 'Cláudio', 'Raimbom', 'Rialto', 'Solero', 'Tauro', 'Torpedo', 'Vermelho', 'Yoacali' and 'Yolo Wonder'), representing the absence of this gene in these cultivars (Figure 2).

Table 1. Means of GI, Pf and Rf (see footnote) for different cultivated plants inoculated with the root-knot nematode *Meloidogyne luci,* 60 d after inoculation with 5000 eggs/plant in a pot assay conducted in a growth chamber (23 ± 2°C, 12 h daily photoperiod, ±60% relative humidity).

Family Species (Common name)	Cultivar	GI[a]	Pf[b]	Rf[c]	Host status[d]
Apiaceae					
Daucus carota L. (Carrot)	-	4	1740 ± 3098	0.4^{p,q}	RH
Asteraceae					
Lactuca sativa L. (Lettuce)	Batavia	5	28867 ± 10753	5.8^{l,m}	S
	Butterhead	5	45720 ± 3293	9.1^{l}	S
	Cocktail	5	4531 ± 2075	0.9^{n,o,p}	RH
	Folha-de-Carvalho	5	167067 ± 44537	33.4^{f,g,h}	S
Brassicaceae					
Brassica oleracea L. (Cabbage)	Bacalan	5	82 ± 168	0.1^{p,q}	RH
	Coração	5	805 ± 683	0.1^{p,q}	RH
	Lombarda	5	713 ± 160	0.2^{p,q}	RH
	Kale	5	10587 ± 7377	2.1^{n,o}	S
Chenopodiaceae					
Beta vulgaris L. (Beetroot)	-	5	77680 ± 21389	15.5^{j,k}	S
Spinacia oleracea L. (Spinach)	Tayto	5	2437 ± 3646	0.5^{o,p,q}	RH
Cucurbitaceae					
Citrullus lanatus (Thunb.) Matsum & Nakai (Watermelon)	Sugar Baby	5	13387 ± 3530	2.7^{m,n}	S
Cucumis melo L. (Sweet melon)	Galia F1	5	313080 ± 50659	62.6^{c}	S
Cucurbita moschata Duchesne ex Poir. (Pumpkin)	Havana F1	5	162667 ± 24057	32.5^{f,g,h}	S
C. pepo L. (Zucchini)	Black Beauty	5	214320 ± 20273	42.9^{d,e,f}	S
	Nova Zelândia	5	425520 ± 51288	85.1^{b}	S
Fabaceae					
Pisum sativum L. (Pea)	Maravilha D'América	5	172267 ± 47378	34.5^{f,g,h}	S
Vicia faba L. (Faba bean)	-	4	155296 ± 93023	31.1^{h,i}	S
Passifloraceae					
Passiflora edulis Sims (Passion Fruit)	-	4	4 ± 4	0.0^{p,q}	RH
Poaceae					
Zea mays L. (Maize)	-	4	42400 ± 9879	8.5^{l}	S
Solanaceae					
Capsicum annuum L. (Pepper)	Amarelo	5	175680 ± 41311	35.1^{f,g}	S
	Celta	5	94720 ± 9545	18.9^{i,j}	S
	Cláudio	5	272147 ± 37839	54.4^{c,d}	S
	Raimbom	5	55527 ± 49571	11.1^{k,l}	S
	Rialto	5	160960 ± 26478	32.2^{g,h}	S
	Solero	5	161920 ± 25911	32.4^{f,g,h}	S
	Tauro	5	194480 ± 63049	38.9^{e,f,g}	S
	Torpedo	5	260160 ± 30145	52.0^{c,d}	S
	Vermelho	5	240533 ± 57283	48.1^{d,e}	S
	Yoacali	5	11653 ± 2699	2.3^{m,n}	S
Solanum lycopersicum L. (Tomato)	Actimino	2	197 ± 107	0.1^{p,q}	R
	Briomino	1	19 ± 29	0.0^{q}	R
	Coração-de-Boi	5	761616 ± 86004	152.3^{a}	S
	Veinal	1	0 ± 0	0.0^{q}	R
	Vimeiro	1	20 ± 44	0.0^{q}	R

[a] GI = Gall Index (0-5): 0 = no galls, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 ≥ 100 galls/root system.
[b] Pf = final population density. Data are means of five replicates (except for *Passiflora edulis*, four replicates) ± standard deviation. Means in this column followed by the same combination of letters do not differ ($P > 0.05$), according to the *Post hoc* Fisher's Least Significant Differences test.
[c] Rf (Reproduction factor) = Pf/initial population density (5000 eggs).
[d] Host status categories: S = susceptible (GI > 2, Rf > 1), RH = resistant/hypersensitive (GI > 2, Rf ≤ 1), R = resistant (GI ≤ 2, Rf ≤ 1) (Sasser *et al*., 1984).

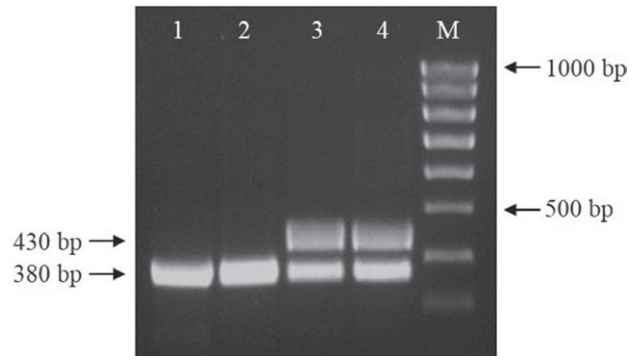

Figure 1. DNA amplification products of tomato (*Solanum lycopersicum*) using the Mi23 markers linked to the *Mi-1.2* gene. Bands 1, 'Actimino'; 2, 'Briomino'; 3, 'Veinal'; 4, 'Vimeiro'; M, DNA marker; (Hyper-Ladder IV, Bioline).

Similarly, amplification of the SCAR_CD produced a single DNA fragment of approx. 160 bp, implying absence of the *Me1* and *Me7* genes in all the pepper cultivars, with the exception of pepper 'DH330'. This accession was used as a positive control of the *Me1* gene, and displayed a band of approx. 100 bp, confirming the presence of the gene (Figure 2).

Amplification of the SCAR marker linked to the *N* gene resulted in a band of approx. 330 bp similar to that expected in the negative control 'Yolo Wonder'. This indicated absence of the *N* gene in all the assessed pepper cultivars (data not shown for 'Yoacali' and 'Raimbom'; Figure 3).

DISCUSSION

In this study, the *M. luci* isolate reproduced (Rf > 1) on 24 (69%) of the 35 plant species/cultivars assessed. *Cucurbitaceae* and *Fabaceae* species/cultivars were susceptible to *M. luci*. However, watermelon 'Sugar Baby' displayed a lower reproduction factor (Rf = 2.7) that was less than the other cucurbitaceous cultivars. Aydınlı *et al.* (2019) assessed the susceptibility of five pumpkin genotypes to *M. luci*, measuring nematode reproduction as a percentage of the most susceptible genotype, and found that two genotypes were moderately resistant. All genotypes allowed significant egg production, which was 3.1 to 6.9 fold greater than the initial population density (5000 eggs), contributing to the build-up of the *M. luci* populations (Aydınlı *et al.*, 2019). Sen and Aydınlı (2021) showed that watermelon 'Charleston Gray' was a good host for *M. luci* reproduction (Rf = 2.5), while 'Crimson Sweet' was a poor host (Rf = 0.52). Gerič Stare *et al.* (2017b) reported that watermelon 'Charleston Gray' was

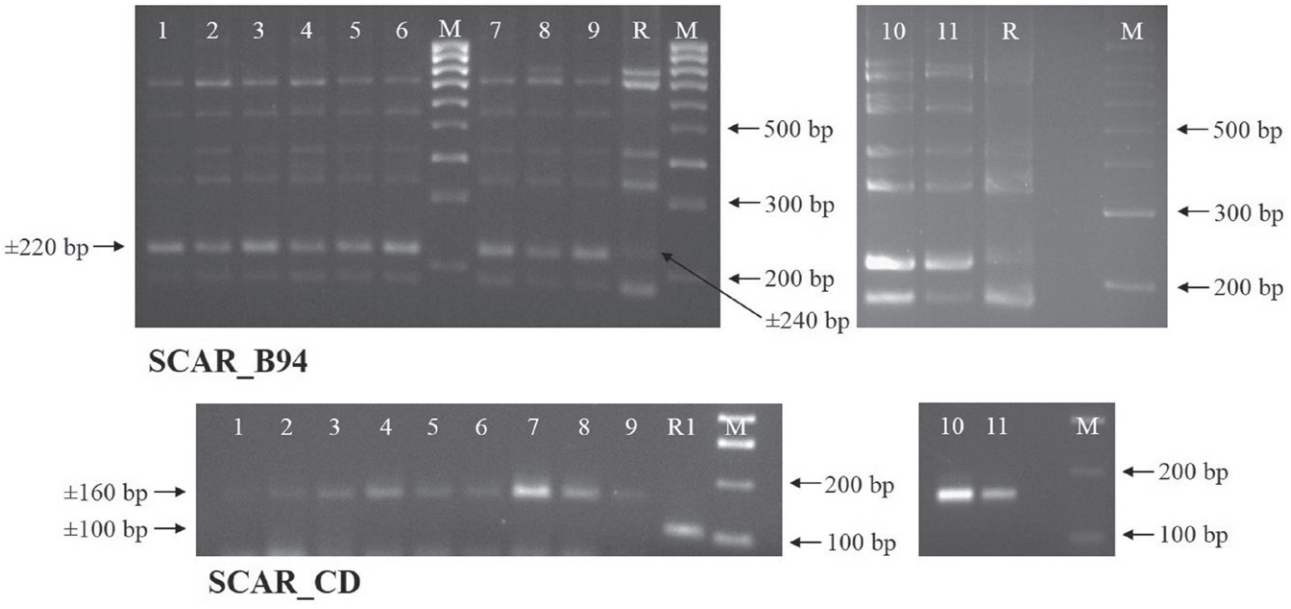

Figure 2. DNA amplification products from pepper cultivars (*Capsicum annuum*), using SCAR_B94 linked to the *Me3* gene and SCAR_CD linked to *Me1* and *Me7*. Band 1, 'Yolo Wonder'; 2, 'Amarelo'; 3, 'Vermelho'; 4, 'Celta'; 5, 'Cláudio'; 6, 'Rialto'; 7, 'Solero'; 8, 'Tauro'; 9, 'Torpedo'; 10, 'Yoacali'; 11, 'Raimbom'; R, 'DH149'; R1, 'DH330'; M, DNA marker (HyperLadder IV, Bioline).

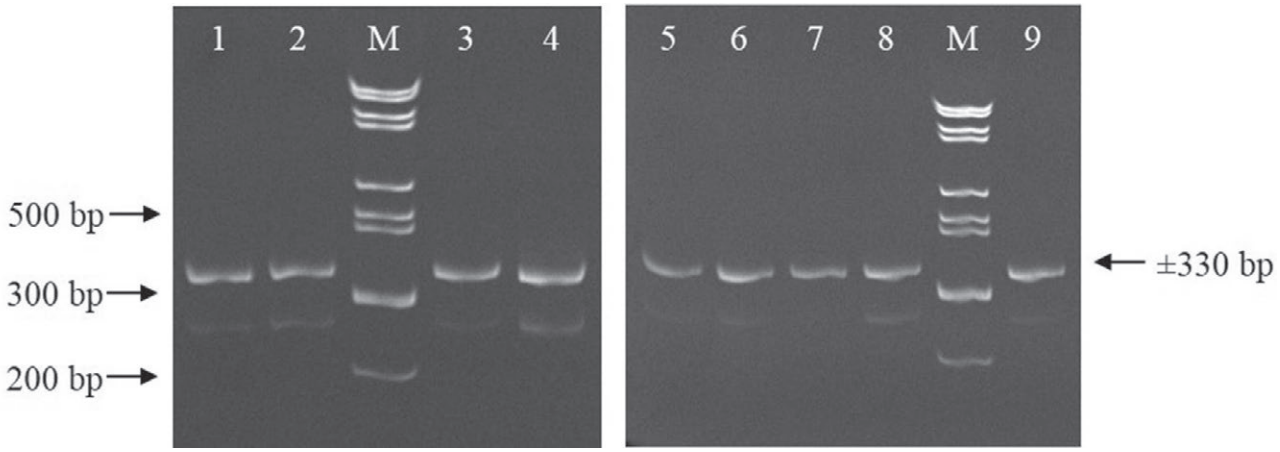

Figure 3. DNA amplification products from pepper cultivars (*Capsicum annuum*), using SCAR linked to the *N* gene. Band 1, 'Cláudio'; 2, 'Celta'; 3, 'Amarelo'; 4, 'Yolo Wonder'; 5, 'Tauro'; 6, 'Solero'; 7, 'Vermelho'; 8, 'Rialto'; 9, 'Torpedo'; M, DNA marker (HyperLadder IV, Bioline).

a poor host for two populations of *M. ethiopica* respectively from Brazil and Africa, and a Slovenian population of *M. luci* (Rf < 1), while Carneiro *et al*. (2003) reported that watermelon 'Charleston Gray' was a good host for *M. ethiopica* and a non-host of *M. luci*. Watermelon cultivars have been described as poorer hosts of RKN, such as *M. incognita* and *M. javanica*, than their Cucurbitaceae counterparts (López-Gómez *et al*., 2016). On the other hand, plant species such as *Luffa cylindrica* (L.) Roem. (Cucurbitaceae) (Bellé *et al*., 2019a) or *Phaseolus vulgaris* L. (Fabaceae) (Bellé *et al*., 2016; 2019b; Sen and Aydınlı, 2021) have been described as highly susceptible to *M. luci*, and displayed considerably high Rf values. Other *Meloidogyne* spp., such as *M. arenaria*, *M. hispanica*, *M. incognita* and *M. javanica,* were also found parasitizing cucurbitaceous and fabaceous plants, so these plants can be generally classified as good hosts of RKN (Hillocks *et al*., 1995; Anwar and McKenry, 2010; Maleita *et al*., 2012; López-Gómez and Verdejo-Lucas, 2014; Aydınlı *et al*., 2019). Conceição *et al*. (2012) demonstrated that maize (Poaceae) was susceptible to *M. luci* (Rf = 8.5), although the cultivars 'Apex' and 'Merit' were considered poor hosts (Rf = 0.44 and 0.09, respectively), whereas 'Otello' and 'Sy Lucroso' were found to be good hosts (Sen and Aydınlı, 2021). Other poaceous crops have also been reported as suitable hosts for other *Meloidogyne* spp., including *M. graminicola, M. hispanica* and *M. kikuyensis* (Maleita *et al*., 2012; Onkendi *et al*., 2014).

Carrot (Apiaceae) and passion fruit (Passifloraceae) have been referred to as good hosts for RKN reproduction, including for *M. incognita* (Anwar and McKenry, 2010; Khan *et al*., 2017). In the present study, however, low reproduction of *M. luci* was obtained in carrot (Rf = 0.4) and passion fruit (Rf = 0.0). Similarly, carrot and parsley (Apiaceae) were classified as poor hosts by Sen and Aydınlı (2021), because they supported low levels of *M. luci*. The susceptibility of a greater number of plants within these two families should be evaluated, to further elucidate host suitability of Apiaceae and Passifloraceae plants to *M. luci*. In addition, brassicas are either poor or average hosts for *Meloidogyne* spp. (Anwar and McKenry, 2010; Carneiro *et al*., 2000; Maleita *et al*., 2012; Sen and Aydınlı, 2021), including broccoli (*Brassica oleracea* L. 'Italica'), which was previously identified as a host for *M. luci* (Carneiro *et al*., 2014). In the present study, Brassicaceae cultivars, except for 'Kale', were resistant/hypersensitive, indicating that they are non-efficient hosts that endure significant nematode damage (GI > 2) despite the nematode not actually reproduced (Rf<1) (Canto-Sáenz, 1985). Although some brassicas may not be good hosts for *M. luci*, caution should be taken when using cabbage as cover crops, as some cultivars may support nematode reproduction, and their use must be limited to non-infested soils or soils with low nematode population densities (Sen and Aydınlı, 2021).

Substantial Rf values variation occurred among hosts in the Chenopodiaceae, Asteraceae and Solanaceae. Solanaceous plants, including pepper, potato and tomato, are considered good or very good *M. luci* hosts (Širca *et al*., 2004; Carneiro *et al*., 2014; Maleita *et al*., 2018; Santos *et al*., 2019; Sen and Aydınlı, 2021). In the present study, the assessed pepper cultivars displayed mostly high Rf values, and were all classified as susceptible to *M. luci*, which is in accordance with the results obtained with SCAR_CD and SCAR_B94 markers and the marker linked to the *N* gene. These results indicated that the *Me1*, *Me3*, *Me7* and *N* genes were absent from these pepper cultivars.

The tomato 'Coração-de-Boi', used as the susceptible control, displayed the greatest Rf (152.3) among all the plant species/cultivars assessed. In contrast, the other tomato cultivars ('Actimino', 'Briomino', 'Veinal' and 'Vimeiro') exhibited low Rf values, and were classified as resistant (GI ≤ 2, Rf ≤ 1). The molecular assays indicated that these plants carried at least one copy of the *Mi-1.2* gene. Although this resistance has been described as more efficient in the presence of two copies of the gene (MiMi), as opposed to one copy (Mimi) (Jacquet *et al*., 2005; Maleita *et al*., 2012; Santos *et al*., 2020), the Rf values did not vary significantly according to the genotype displayed (Rf = 0.0 to 0.1). These resistant tomato cultivars could be used to inhibit nematode population increase and reduce losses due to *M. luci*. The cultivars may have potential for inclusion in integrated nematode management programme. However, the duration of resistance can be limited due to selection to virulence in nematode populations by continuous exposure to resistant plants or changes in the environmental conditions (Dropkin, 1969). Additionally, assessment of *Mi*-tomato plants for susceptibility to local populations before their field use is advisable, because natural virulent nematode population may be present (Maleita *et al*., 2012; Aydınlı and Mennan, 2019).

The present study has confirmed that *M. luci* is a polyphagous species with a wide host range which includes plants from different families. This indicates that control strategies based on crop rotations could be ineffective against this RKN, if susceptible plants are among rotation candidates. Although the pot experiment in this study was not repeated, due to the high number of plant species/cultivars assessed, the results show that most of the plants were susceptible to *M. luci*, while 11 were resistant or resistant/hypersensitive. Variability was observed among replicates of each cultivar, but host status was consistent among these replicates. Inoculum viability was also confirmed by the high Rf values obtained on tomato 'Coração de Boi'.

Brassicas prevented nematode reproduction in three of four cultivars, with low Rf values. Cabbage 'Bacalan', 'Coração' and 'Lombarda' may be suitable as rotation crops since they are widely used in Portugal. Likewise, the resistant tomato 'Actimino, 'Briomino', 'Vimeiro' and 'Veinal' can also be recommended for the management of *M. luci* populations. Knowledge on the susceptibility of local cultivars to RKN, along with the use of these resistant cultivars in fields where susceptible hosts are grown, could reduce *M. luci* population densities and increase crop yields.

This study has highlighted the importance of identification of *Meloidogyne* resistant local cultivars, to be used in crop rotations as an efficient strategy to maintain agricultural sustainability.

ACKNOWLEDGMENTS

This research was supported by CFE, Department of Life Sciences, UC, and CIEPQPF, Department of Chemical Engineering, University of Coimbra, FEDER funds through the Portugal 2020 (PT 2020), "Programa Operacional Factores de Competitividade 2020"(COMPETE2020), and by "Fundação para a Ciência e a Tecnologia" (FCT, Portugal), under contracts UIDB/04004/2020, UIDB/00102/2020, UIDP/00102/2020, POCI-01-0145-FEDER-031946 (Ref. PTDC/ASP-PLA/31946/2017), CEECIND/02082/2017 (to I. Esteves), Project ReNATURE—Valorization of the Natural Endogenous Resources of the Centro Region (Centro-01-0145-FEDER-000007) and by "Instituto do Ambiente, Tecnologia e Vida".

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