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Pathogenic potential, parasitic success and host efficiency of *Meloidogyne incognita* and *M. javanica* on cucurbitaceous plant genotypes

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Abstract The response to *Meloidogyne incognita* and *M. javanica* of 29 cucurbitaceous genotypes belonging to the genera *Cucurbita, Lagenaria* and *Luffa* was evaluated to determine their relative host suitability and utility as rotational crops in sustainable agriculture. The pathogenic potential, parasitic success and host efficiency were estimated based on the ability of the nematode to form galls and generate egg masses on selected genotypes. *Meloidogyne incognita* showed higher pathogenic potential (gall formation) than *M. javanica* across all genotypes except for *C. argyrosperma*. However, *M. javanica* had higher parasitic success (egg mass production) than *M. incognita* on the *Cucurbita* genotypes. By contrast, on *Lagenaria* and *Luffa, M. incognita* had higher pathogenic potential and parasitic success than *M. javanica*. All genotypes except *Luffa* spp., showed higher host efficiency (rate of egg masses per gall) for *M. javanica* than for *M. incognita*. The genotypes of *Cucurbita pepo* ssp. *pepo* will be useful for *M. incognita* management because they reduce population build-up, but root damage would be more severe due to abundant root galling. The *Cucurbita* genotypes would be more tolerant to higher Pi when infected by *M. javanica* as they suffer less root damage, but the residual populations may affect the subsequent crop in the rotation. *Lagenaria siceraria* showed a good level of resistance to *M. javanica* and will be useful by itself or for grafting other cucurbitaceous crops. The species of *Luffa* were poor hosts of *M. javanica* and can be used also for grafting.

Keywords Cucurbita · Lagenaria · Luffa · Parasitism · Pathogenicity · Root-knot nematodes

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Introduction

The botanical family Cucurbitaceae consists of 118 genera and 825 species, including vegetable crops that are important sources of food and fresh products worldwide, ornamentals and weeds (Ojo 2016). There is a remarkable genetic diversity within this family that extends to both vegetative and reproductive characteristics as well as a range of adaptations to most climatic conditions. The genus *Cucurbita* includes economically important species such as *C. pepo*, *C. maxima, C. moschata*, and *C. argyrosperma*. In addition, *C. pepo* is divided taxonomically, into three subspecies, *C. pepo* ssp. *pepo*, *C. pepo* ssp. *ovifera* (also known as ssp. *texana*), both including cultivated varieties, and *C. pepo* ssp. *fraterna* which includes wild types used mainly for ornamental purposes. According to the morphology of the fruits, four types are distinguished within *C. pepo* ssp. *pepo*: zucchini, vegetable marrow, pumpkin and cococelle, and four types within *C. pepo* ssp. *ovifera*: scallop, acorn, straightneck and crookneck.

Lagenaria siceraria is an annual crop, which is cultivated in tropical regions of Africa and America and used as a vegetable when fruits are harvested green or as a recipient when mature fruits are desiccated. The genus *Luffa* comprises five species mostly used as vegetable sponges although some species such as *L. acutangula* is consumed as a vegetable when the fruits are small.

Root-knot nematodes (RKN), particularly *Meloidogyne arenaria, M. incognita* and *M. javanica* cause damage and reduce yield in most cucurbit crops (Sikora and Fernandez 2005; Talavera et al. 2012). In addition, zucchini is a good host for *M. hispanica* (Carneiro et al. 2004) and *M. enterolobii* (Brito et al. 2007) and *C. moschata* for *M. floridensis* (Kokalis-Burelle and Nyczepir 2004). Although genetic resistance would be the preferred strategy for RKN management, resistance genes have not been identified so far in the genera *Cucurbita, Lagenaria* or *Luffa*. Nonetheless, partial resistance to *M. incognita* has been recently described in *C. pepo* ssp. *pepo* 'Amalthee` (Talavera et al. 2018a). Host range studies have shown large variation in host status within RKN species, and among genotypes which may provide tolerance to the nematode (Fourie et al. 2012; Maleita et al. 2012; López-Gómez et al. 2016; Hallmann and Kievnick 2018). However, such studies are limited in the case of *Cucurbita* and gourds (de Souza et al. 2013; Tamilselvi et al. 2017). Sustainable agricultural practices and organic farming demand a major reduction in the use of chemical pesticides to control pathogens including RKN. Therefore, it is useful to know the relative host potential to different RKN of cucurbitaceous genotypes most frequently grown in a region because differences in host status could be exploited to regulate nematode population increase in the absence of resistance genes.

Meloidogyne spp. cause damage to plants by forming galls in the roots which impair water and nutrient uptake. Root galling has been considered as an indicator of successful establishment of the feeding site that will allow further nematode development and reproduction. Rating the degree of root galling is frequently used by Nematologists and Pest advisors to assess disease severity but counting the number of galls is less frequent (Tamilselvi et al. 2017; Talavera et al. 2018a). In most host crops, there is a strong relationship between the number of galls and egg masses (EM) (Mukhtar et al. 2013; López-Gómez et al. 2015a) but gall formation is not always followed by successful nematode development and EM production (Fourie et al. 2012; Maleita et al. 2012; Talavera et al. 2018a). Therefore, the competence to form galls but also EM, should be used as indicators of the pathogenic potential and parasitic and reproductive abilities of RKN in a host plant. Both parameters are valuable for assessing host responses as they offer information on two different functional traits, pathogenicity and parasitism.

The objectives of this study were to evaluate the responses of a wide range of cucurbitaceous crops to *M. incognita* and *M. javanica*, to determine their relative host suitability, and their usefulness as rotational crops in sustainable agriculture. The pathogenic potential, parasitic success and host efficiency were estimated based on the ability of *M. incognita* and *M. javanica* to form galls and generate egg masses on selected genotypes of *Cucurbita* spp., *Lagenaria siceraria* and *Luffa* spp.

Materials and methods

Nematodes and inoculum preparation

The RKN species *Meloidogyne incognita* (code Mi-PM26) and *M. javanica* (code Mj-05) were used for the experiments. They were originally collected from infected tomato roots and started from the progeny of one female. The RKN were multiplied on susceptible tomato 'Roma' to produce the second stage juveniles (J2) inoculum for the experiments. Tomato roots infected by the respective nematodes were macerated in a 0.5% sodium hypochlorite solution for 5 min in a blender (Hussey and Barker 1973), and the resulting egg suspension concentrated on a 25 μm sieve and placed in Baermann trays. Second-stage juveniles hatching within 72 h were used as the inoculum.

Plant materials

The 29 cucurbitaceous plant genotypes tested are listed in Table 1 and they included cultivars of *Cucurbita pepo* ssp. *pepo*, *C. pepo* ssp. *ovifera*, *C. argyrosperma*, *C. maxima*, *C. moschata*, *C. maxima* × *C. moschata*, *Cucurbita* sp., *Lagenaria siceraria*, *Luffa acutangula* and *Luffa cylindrica*.

Experimental design and conditions

Experiments were arranged as a factorial design in which the main factors were the RKN species

Table 1 Genotypes of *Cucurbita* spp., *Lagenaria siceraria* and *Luffa* spp. evaluated to determine their response to *Meloidogyne incognita* and *M. javanica* in pot tests conducted in a growth chamber

Botanical names and genotypes	Common names and source	Use
Cucurbita pepo ssp. *pepo*	Zucchini	
'Amalthee'	Gautier	Cultivar
'Victoria'	Clause Ibérica	Cultivar
'Musa'	Clause Ibérica	Cultivar
'Natura'	Clause Ibérica	Cultivar
'Jedida'	Clause Ibérica	Cultivar
'Sinatra'	Clause Ibérica	Cultivar
'Cavili'	P. Gómez, IFAPA La Mojonera	Cultivar
'Whitetaker'	P. Gómez, IFAPA La Mojonera	Cultivar
Cucurbita pepo	Vegetable marrow	
'Zebra Cross'	Tozer Ibérica	Cultivar
'Picolo'	Tozer Ibérica	Cultivar
Cucurbita pepo ssp. *ovifera*	Scallop	
BGV005382	Banco Germoplasma Valencia	Accession
Cucurbita pepo ssp. *ovifera*	Acorn	
BGV005329	Banco Germoplasma Valencia	Accession
Cucurbita pepo ssp. *ovifera*	Crookneck	
BGV009476	Banco Germoplasma Valencia	Accession
Cucurbita argyrosperma BGV13026	Pumpkin Banco Germoplasma Valencia	Accession
Cucurbita maxima	Pumpkin Hallowing	
'Mars'	Tozer Ibérica	Cultivar
Cucurbita moschata	Pumpkin	
64–063 RZ [1]	Rijk Zwaan	Rootstock
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Cucurbita spp.	Winter squash	
Butternut Sprinter	Tozer Ibérica	Cultivar
TZ3380	Tozer Ibérica	Cultivar
C. maxima × *C. moschata*	Hybrid rootstocks	
'Azman'	Rijk Zwaan	Rootstock
'Shintoza Camelforce'	Nuhens	Rootstock
'Hercules'	Ramiro Arnedo	Rootstock
'Routpower'	Sakata	Rootstock
'Shintoza F-90'	Semillas Fitó	Rootstock
'Carnivor'	Syngenta	Rootstock
Lagenaria siceraria	Bottle gourd	
'Pelops'	Rijk Zwaan	Rootstock
BGV010336	Banco Germoplasma Valencia	Accession
BGV008508	Banco Germoplasma Valencia	Accession
Luffa acutangula	Ridge gourd M.D. Vela, IFAPA Chipiona	Cultivar
Luffa cylindrica	Sponge gourd M.D. Vela, IFAPA Chipiona	Cultivar

(*M. incognita* vs. *M. javanica*) and the cucurbitaceous taxon. Seeds were soaked in water for 24 h and transferred to trays filled with vermiculite for germination. After 48 h, pre-germinated seeds were transplanted singly to 325-cm^3 styrofoam pots filled with vermiculite no. 2. Seedlings were allowed to grow for one week

before nematode inoculation. Plants were inoculated with 250 J2 in c. 2 ml of water delivered into two holes made in the vermiculite. Experiments were conducted in a climatic growth chamber maintained at 24 ± 2 °C with a photoperiod 16 h light, 50% relative humidity. Plants were fertilized with a slow-release fertilizer (Osmocote ® Scotts Company, Netherlands, 15% N + 10% P_2O_5 + 12% K_2O + 2% MgO_2 + microelements) by adding approximately 2 g onto the surface of each pot just after transplanting.

Plants were evaluated after completion of a nematode reproduction cycle, 35 days after inoculation (dpi) (Vela et al. 2014). At harvest, tops were cut at ground level and their fresh and dry weight determined. The dry top weight was determined after 48 h desiccation in an oven at 60 °C. Roots were separated from soil, washed, and weighed. The EM were stained by immersion of the entire root system into a 0.1 g/L erioglaucine solution (Aldrich Chemical Company, St Louis, Mo, USA) for two hours (Omwega et al. 1988). Roots were de-stained by washing in tap water and the total number of galls per root system was recorded by counting separately the number of galls with and without EM under a stereo microscope. Galls with no EM were dissected to confirm the absence of EM inside the galls. Based on these parameters, the following indicators were used to compare the cucurbitaceous genotypes. The pathogenic potential (ability to cause disease) is an indicator of plant damage and represents the fraction of the J2 inoculum that induces galls and was calculated by dividing total number of galls per root system by the J2 inoculum × 100. The parasitic success measures the successful development of the J2 inoculum until the reproductive stage and was calculated by dividing EM per root system by the J2 inoculum ×100 (Djian-Caporlino et al. 2011). The host efficiency refers to nematodes generating EM from those that formed galls and was calculated by dividing the number of EM by the total number of galls per root system ×100. The experiments were repeated once following the same experimental procedure. Each treatment (RKN × genotype) was repeated five or six times depending on the experiment.

Statistical analyses

Data are expressed as mean ± standard deviation. The IBM® SPSS® Statistics package version 21 was used for the statistical analyses. Data from repeated experiments were combined in a single set of data because there were no differences ($P < 0.05$) between them. Analyses of variance (ANOVA) were performed to determine main effects of RKN species and cucurbitaceous genotype, and their interactions on total galls, and galls with and without EM per root system and per g of roots. When the nematode × genotype interaction was significant, a new analysis was conducted to determine simple effects. Means separation was done when the F values were significant according to the Bonferroni test ($P < 0.05$). Linear correlation analysis was performed across all RKN species and genotypes to determine the relationship between pathogenic potential and parasitic success and pathogenic potential and host efficiency ($P < 0.05$). The correlation coefficients were compared to assess if there were differences between the RKN species using the Fisher's Z transformation ($P < 0.05$) corrected by Bonferroni test.

Results

The response to RKN of the cucurbitaceous plants based on their pathogenic and parasitic abilities ranked the genotypes in opposite ways because *M. incognita* formed more galls but produced fewer EM whereas *M. javanica* formed fewer galls but produced more EM. Overall, galls without EM discriminate the *Cucurbita* genotypes whereas galls with EM did the *L. siceraria* genotypes.

The genotypes of *C. pepo* ssp. *pepo* differed ($P < 0.05$) in total galls per root system and galls without EM when infected by *M. incognita* but they did not when infected by *M. javanica* (Table 2). The RKN species did not differ in the average total number of galls and galls with EM. *Meloidogyne incognita* induced more ($P < 0.05$) galls without EM than *M. javanica*, in fact, only 30% of the *M. incognita* galls formed EM in comparison with 90% of the *M. javanica*.

The *C. pepo* ssp. *ovifera* genotypes had similar numbers of galls with and without EM per root system when infected by *M. incognita* (Table 3). However, the number of galls with EM were higher ($P < 0.05$) on 'Acorn' than 'Scallop' and 'Crookneck' but 'Scallop' formed more ($P < 0.05$) galls without EM than the other two genotypes, when infected by *M. javanica* (Table 3). The average number of galls without EM on these genotypes was higher ($P < 0.05$) for *M. incognita* than for *M. javanica*-infected plants.

Table 2 Total number of galls with and without egg masses (EM) of *Meloidogyne incognita* (Mi) and *M. javanica* (Mj) on eight genotypes of *Cucurbita pepo* ssp. *pepo* (zucchini) 35 days after the inoculation of 250 second-stage juveniles per plant in pot experiments conducted in a growth chamber

Genotype	Galls					
	Total galls		with EM		without EM	
	Mi	Mj	Mi	Mj	Mi	Mj
'Amalthee'	91 ± 35 b	62 ± 27 a	30 ± 14 a	53 ± 20 a	61 ± 24 b	9 ± 10 a
'Cavili'	115 ± 19 a	53 ± 18 a	39 ± 19 a	49 ± 18 a	76 ± 5 b	2 ± 4 a
'Jedida'	106 ± 36 b	64 ± 32 a	31 ± 16 a	63 ± 28 a	75 ± 23 b	1 ± 2 a
'Musa'	56 ± 50 b	42 ± 15 a	14 ± 13 a	42 ± 16 a	42 ± 37 b	0.4 ± 1 a
'Natura'	82 ± 28 b	47 ± 18 a	21 ± 11 a	43 ± 18 a	61 ± 22 b	4 ± 4 a
'Sinatra'	119 ± 24 a	53 ± 11 a	42 ± 20 a	44 ± 9 a	77 ± 17 b	8 ± 5 a
'Vitoria'	42 ± 23 b	64 ± 17 a	25 ± 13 a	63 ± 16 a	17 ± 10 c	1 ± 2 a
'Whitetaker'	180 ± 53 a	33 ± 10 a	44 ± 25 a	29 ± 6 a	136 ± 34 a	4 ± 6 a
Mean	95 ± 44	53 ± 22	29 ± 17	48 ± 18	66 ± 33 *	5 ± 7

Values are mean ± standard deviation of two experiments (5 replicated plants/ genotype per experiment). Mean separation among genotypes within nematode species according to Bonferroni Test ($P < 0.05$)

*Statistical differences ($P<0.05$) between *Meloidogyne* species

Total galls per root system on the hybrids of *C. maxima* × *C. moschata* were similar for both RKN species, but most of the galls formed by *M. javanica* produced EM (96%) and only 56% of the *M. incognita* galls did (Table 4). The hybrids differed in the number of galls with EM; *M. incognita* produced more ($P < 0.05$) galls with EM on 'Shintoza Camelforce' and 'Shintoza F-90' than on the remaining hybrids, and *M. javanica* did on 'Azman', 'Hercules' and 'Shintoza F-90' than on 'Carnivor. Galls without EM were higher ($P < 0.05$) for *M. incognita* than *M. javanica.*

On *L. siceraria*, total galls per root system and galls with EM were lower ($P < 0.05$) on *M. javanica* than *M. incognita*-infected plants (Table 5). The genotypes of *L. siceraria* infected by *M. javanica* differed in total galls and galls without EM. The commercial rootstock 'Pelops' showed values somehow closer to genotype BGV010336 than BGV008508, except for galls with EM in the *M. incognita* plants, which were similar to BGV008508.

Table 3 Total number of galls with and without egg masses (EM) of *Meloidogyne incognita* (Mi) and *M. javanica* (Mj) on three genotypes of *Cucurbita pepo* ssp. *ovifera* 35 days after the inoculation of 250 second-stage juveniles per plant in pot experiments conducted in a growth chamber

Genotype	Galls					
	Total galls		with EM		without EM	
	Mi	Mj	Mi	Mj	Mi	Mj
Scallop	85 ± 25 a	51 ± 15 ab	29 ± 14 a	32 ± 11b	56 ± 14 a	19 ± 5 a
Acorn	70 ± 22 a	66 ± 6 a	17 ± 6 a	59 ± 4 a	53 ± 17 a	7 ± 5 b
Crookneck	78 ± 35 a	39 ± 12 b	29 ± 18 a	35 ± 11 b	49 ± 21 a	4 ± 3 b
Mean	79 ± 29	49 ± 15	27 ± 17	38 ± 14	52 ± 15 *	11 ± 8

Values are mean ± standard deviation of two experiments (5 replicated plants/ genotype per experiment). Mean separation among genotypes within nematode species according to Bonferroni Test ($P < 0.05$)

*Statistical differences ($P<0.05$) between *Meloidogyne* species

Table 4 Total number of galls with and without egg masses (EM) of *Meloidogyne incognita* (Mi) and *M. javanica* (Mj) on six hybrid rootstocks of *Cucurbita maxima* × *Cucurbita moschata* 35 days after the inoculation of 250 second-stage juveniles per plant in pot experiments conducted in a growth chamber

Genotype	Galls					
	Total galls		with EM		without EM	
	Mi	Mj	Mi	Mj	Mi	Mj
'Azman'	98 ± 32 a	88 ± 25 a	48 ± 16 ab	87 ± 26 a	50 ± 18 a	1 ± 1 c
'Camelforce'	114 ± 40 a	61 ± 14 ab	80 ± 31 a	60 ± 14 ab	34 ± 13 a	1 ± 1 c
'Hercules'	73 ± 10 a	83 ± 18 a	44 ± 5 b	83 ± 17 a	29 ± 8 a	1 ± 1 c
'Routpower'	69 ± 25 a	67 ± 5 ab	31 ± 5 b	57 ± 7 ab	38 ± 21 a	10 ± 2 a
'Shitonza F-90'	116 ± 38 a	87 ± 30 a	73 ± 21 a	86 ± 30 a	43 ± 21 a	1 ± 1 c
'Carnivor'	75 ± 22 a	29 ± 15 b	42 ± 15 b	24 ± 14 b	33 ± 9 a	5 ± 3 b
Mean	89 ± 32	70 ± 20	51 ± 23	67 ± 22	38 ± 16 *	3 ± 3

Values are mean ± standard deviation of two experiments (5 replicated plants/ genotype per experiment). Mean separation among genotypes within nematode species variables according to Bonferroni Test ($P < 0.05$)

*Statistical differences ($P < 0.05$) between *Meloidogyne* species

On *Luffa* spp., total galls per root system, galls with EM and galls without EM were lower ($P < 0.05$) on the *M. javanica* than *M. incognita*-infected plants (Table 6). *Luffa acutangula* infected by *M. incognita* supported lower numbers of galls with EM but higher numbers of galls without EM than *L. cylindrica* but the *Luffa* species did not differ when infected by *M. javanica*.

When comparing RKN infection on other cucurbit species, winter squash (*Cucurbita* sp.), *C. moschata,* vegetable marrow (*C. pepo*), *C. maxima,* and *C. argyrosperma* did not differ in total galls, and galls with EM per root system (Table 7). Galls without EM was the only parameter differentiating the RKN species and was higher ($P < 0.05$) for *M. incognita* than *M. javanica* on all these cucurbit genotypes except for *C. argyrosperma* on which galls without EM were similar for both RKN species (Table 7).

No significant differences in top fresh and dry weights were observed between RKN species probably due to the short duration of the experiments that involved only one nematode reproduction cycle. However, fresh weight was reduced by 10% when zucchini plants had been inoculated with *M. incognita* compared with *M. javanica* (data not shown). Plants infected by *M. incognita* and *M. javanica* had similar root weight except for *C. argyrosperma* (Table 8).

Table 5 Total number of galls with and without egg masses (EM) of *Meloidogyne incognita* (Mi) and *M. javanica* (Mj) on three genotypes of *Lagenaria siceraria* 35 days after the inoculation of 250 second-stage juveniles per plant in pot experiments conducted in a growth chamber

Genotype	Galls					
	Total galls		with EM		without EM	
	Mi	Mj	Mi	Mj	Mi	Mj
BGV010336	100 ± 28 a	18 ± 8 b	73 ± 24 a	5 ± 6 a	27 ± 10 b	13 ± 8 b
BGV008508	107 ± 23 a	40 ± 18 a	56 ± 18 b	5 ± 4 a	51 ± 15 a	35 ± 15 a
'Pelops'	88 ± 29 a	21 ± 10 b	50 ± 10 b	5 ± 4 a	38 ± 21 ab	16 ± 10 b
Mean	98 ± 28 *	27 ± 16	59 ± 20 *	5 ± 5	39 ± 19	22 ± 15

Values are mean ± standard deviation of two experiments (5 and 6 replicated plants/genotype experiment 1 and 2, respectively). Mean separation among genotypes within nematode species according to Bonferroni Test ($P < 0.05$)

*Statistical differences ($P<0.05$) between *Meloidogyne* species

Table 6 Total number of galls with and without egg masses (EM) of *Meloidogyne incognita* and *M. javanica* on *Luffa acutangula* and *L. cylindrica* 35 days after the inoculation of 250 second-stage juveniles per plant

Species	Galls					
	Total galls		with EM		without EM	
	Mi	Mj	Mi	Mj	Mi	Mj
L. acutangula	79 ± 15	22 ± 23	26 ± 9 b	18 ± 21	53 ± 11 a	4 ± 3
L. cylindrica	73 ± 21	16 ± 15	43 ± 13 a	6 ± 7	30 ± 11 b	10 ± 8
Mean	76 ± 18*	19 ± 19	35 ± 14 *	12 ± 17	42 ± 16 *	7 ± 7

Values are mean ± standard deviation of two experiments (5 and 6 replicated plants/ genotype experiment 1 and 2, respectively). Values for the *Luffa* species on the same column sharing different letters are statistically significant ($P < 0.05$)

*Statistical differences ($P<0.05$) between *Meloidogyne* species

Correlation analyses across all genotypes indicated a positive correlation between pathogenic potential and parasitic success ($R^2 = 0.631$, $P < 0.001$) and parasitic success and host efficiency ($R^2 = 0.638$, $P < 0.001$) but pathogenic potential and host efficiency were not correlated ($R^2 = -0.06$, $P = 0.223$). The correlation analyses done separately by RKN species indicated that the lack of correlation between pathogenic potential and host efficiency only occurred in the *M. incognita*-infected plants (Table 9).

The pathogenic potential, parasitic success and host efficiency of the RKN species across all cucurbitaceous genotypes are presented in Fig. 1. The general trend was greater pathogenic potential for *M. incognita* than *M. javanica* (Fig. 1a) except for *C. argyrosperma*. In contrast, the parasitic success was higher for *M. javanica* than *M. incognita* (Fig. 1b) except for *Lagenaria* and *Luffa* species. A similar trend was observed for the host efficiency, which was higher for *M. javanica* than *M. incognita* (83% and 44%, respectively) in all genotypes but *L. siceraria* (18% and 60%, respectively) (Fig. 1c). Genus *Cucurbita*, which included 24 genotypes, showed a host efficiency ≥67% for *M. javanica* with no difference among the species of *Cucurbita*.

ANOVA analyses performed on galls with and without EM per gram of root showed similar results (data not shown) to those performed on data per root system, and differences between plant genotypes and nematode species were comparable, indicating that the root weight of the different plant species did not affect the outcome of the results under the experimental conditions of this study.

Discussion

Meloidogyne incognita showed higher pathogenic potential than *M. javanica* across all genotypes except for

Table 7 Total number of galls with and without egg masses (EM) of *Meloidogyne incognita* (Mi) and *M. javanica* (Mj) on cucurbitaceous genotypes 35 days after the inoculation of 250 second-stage juveniles per plant in pot experiments conducted in a growth chamber

Genotype	Galls					
	Total galls		with EM		without EM	
	Mi	Mj	Mi	Mj	Mi	Mj
Winter squash (*Cucurbita* sp.)	93 ± 25	84 ± 20	66 ± 13	79 ± 19	27 ± 14 *	5 ± 6
Cucurbita moschata	114 ± 28	101 ± 15	73 ± 20	96 ± 21	42 ± 15 *	5 ± 4
Vegetable marrow (*C. pepo*)	104 ± 24	75 ± 23	41 ± 19	67 ± 22	62 ± 18 *	8 ± 10
Cucurbita maxima	143 ± 85	114 ± 21	36 ± 32	87 ± 22	107 ± 54 *	27 ± 11
Cucurbita argyrosperma	32 ± 6	58 ± 19	17 ± 5	39 ± 12	15 ± 8	18 ± 7

Values are mean ± standard deviation of 10 replicated plants, two genotypes per *Cucurbita* five plants of each genotype

* Statistical differences ($P < 0.05$) between *Meloidogyne* species

Table 8 Root weight (gram) of genotypes of *Cucurbita* spp., *Lagenaria siceraria* and *Luffa* spp. 35 days after the inoculation of 250 second-stage juveniles per plant of *Meloidogyne incognita* and *M. javanica* in pot tests conducted in a growth chamber

Genotype	*M. incognita*	*M. javanica*
Cucurbita pepo pepo	3.12 ± 1.19	2.82 ± 1.32
Cucurbita pepo ovifera	3.42 ± 1.80	3.45 ± 0.80
C. máxima x C. moschata	3.95 ± 1.57	4.32 ± 0.69
Winter squash (*Cucurbita* sp.)	3.92 ± 0.47	3.61 ± 0.30
Cucurbita moschata	4.63 ± 0.85	4.93 ± 0.91
Vegetable marrow (*C. pepo*)	5.14 ± 0.73	4.27 ± 0.49
Cucurbita maxima	4.43 ± 0.74	4.05 ± 0.48
Cucurbita argyrosperma	6.10 ± 1.07	10.08 ± 1.66
Lagenaria siceraria	7.87 ± 1.57	7.05 ± 1.95
Luffa acutangula	7.06 ± 1.54	7.96 ± 0.58
Luffa cylindrica	4.82 ± 0.67	4.21 ± 0.79

C. argyrosperma (Fig. 1). Independent observations support the higher pathogenic potential (ability to cause disease) of *M. incognita* over *M. javanica* on various cucurbitaceous crops including melon, cucumber, zucchini, and several gourds in response to RKN populations from different origins (Edelstein et al. 2010; López-Gómez et al. 2015a; Tamilselvi et al. 2017). More galls would cause greater hyperplasia and hypertrophy of root tissues that would impair plant growth and yield. Talavera et al. (2018a, b) reported larger ($P < 0.05$) gall size for *M. incognita* than *M. javanica* and similar root galling indices on zucchini plants with 1000 *M. incognita* and 10,000 *M. javanica* (4.3 and 4.6 respectively, scale 1 to 10, Zeck 1971). In addition, the initial densities of the nematode (Pi) affect plant damage. Progressively higher Pi decreased top biomass, leaf chlorophyll content, and yield on zucchini (Johnson and Leonard 1995; López-Gómez et al. 2015b; Talavera et al. 2018b) but both RKN species caused a similar reduction in top biomass at Pi = 10,000 J2 per plant (Talavera et al. 2018b). Root galling severity and host efficiency, measured as the reproduction factor (Rf¸ final population/initial population densities), were inversely related on four cucurbits (Chandra et al. 2010). Galling was most severe on *C. pepo*, followed by *Cucumis sativum*, *Momordica charantia* and *L. siceraria* whereas Rf was highest on *L. siceraria*, followed by *C. sativum*, *M. charantia* and *C. pepo*.

As obligate parasites, *M. javanica* had higher parasitic success than *M. incognita* on the *Cucurbita* genotypes despite inducing fewer galls per root system. The high value of the correlation between pathogenic potential and parasitic success was explained by the high correspondence between total galls per root system and EM (> 90%) on zucchini, winter squash, *C. moschata*, and the hybrids of *C. maxima x C. moschata* which represented 17 of the 29 genotypes tested. The elevated efficiency of these hosts for *M. javanica* points to a great parasitic fitness, defined as the ability of an organism to survive and reproduce, i.e., to pass its genes to the next generation (Holliday 2001) since most of the *M. javanica* individuals inducing galls generated EM. In contrast, the pathogenic potential of *M. incognita* was not correlated with the parasitic success owing to the large number of galls without EM. Consequently, the host efficiency of the *M. incognita* plants was <34% on *C. pepo* ssp. *pepo*, *C. pepo* ssp. *ovifera*, *C. maxima*, and *C. argyrosperma* and confirms previous observations on zucchini (López-Gómez et al. 2015a; Talavera et al. 2018a). Galls

Table 9 Pearson correlation analysis to determine the relationship between pathogenic potential, parasitic success and host efficiency on cucurbitaceous genotypes infected by *Meloidogyne incognita* and *M. javanica*

Nematode		Parasitic success		Host efficiency	
		R^2	*P* value	R^2	*P* value
Meloidogyne	Pathogenic potential ($n = 419$)	0.631	0.0001	−0.060	0.223
	Parasitic success ($n = 415$)			0.638	0.0001
M. incognita	Pathogenic potential ($n = 210$)	0.607	0.0001	- 0.071	0.304
	Parasitic success (n = 210)			0.681	0.0001
M. javanica	Pathogenic potential ($n = 209$)	0.943	0.0001	0.514	0.0001
	Parasitic success ($n = 205$)			0.710	0.0001

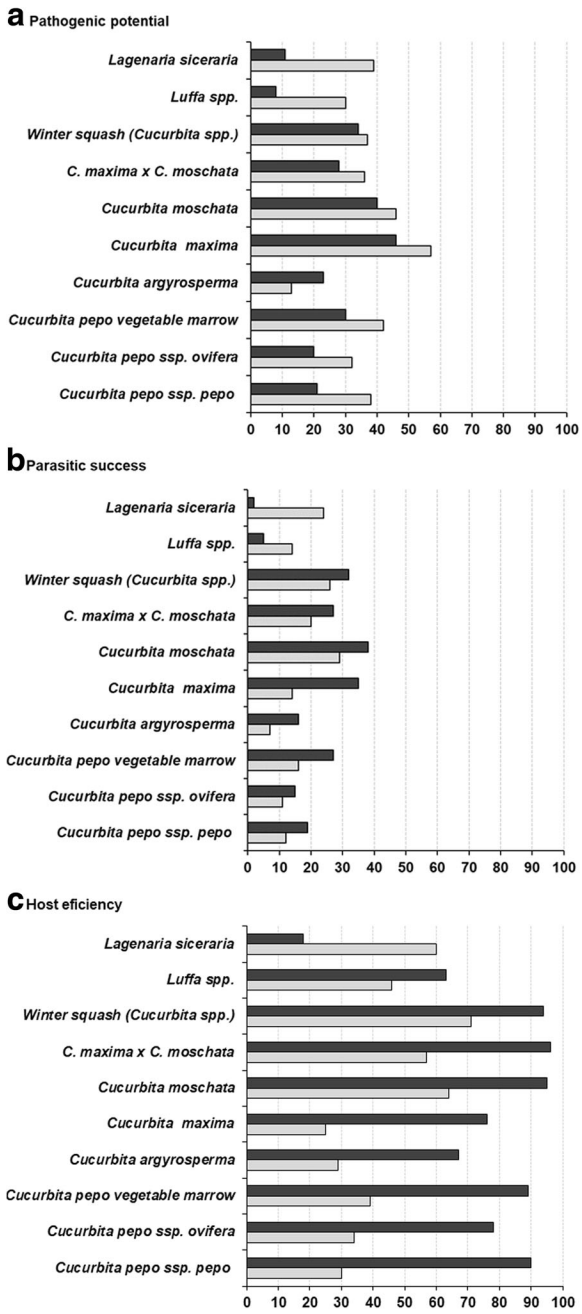

Fig. 1 Pathogenic potential (total galls × 100 /250 J2 inoculum), parasitic success (egg masses × 100/ 250 J2 inoculum) and host efficiency (egg masses × 100/ total galls) of cucurbitaceous genotypes to *Meloidogyne incognita* (light grey) and *M. javanica* (dark grey)

without EM did not derive from late invasions or overlook of the EM in their interior because J2 inoculum was used to synchronize the nematode life cycle, and galls without EM were dissected to confirm their absence inside the galls. According to their thermal time requirements at 24.3 °C, 21 and 23 days were needed for *M. incognita* and *M. javanica* on zucchini to develop to the egg laying female stage (eggs already deposited within the gelatinous matrix) (Vela et al. 2014). Talavera et al. (2018a) demonstrated that *M. incognita* galls without EM on zucchini were due to the failure of the fourth stage juveniles to develop into females which affected to 74% of the *M. incognita* galls. Overall, galls without EM contained undersized adult immature females (López-Gómez et al. 2015a). Zucchini was moderately susceptible to Brazilian populations of *M. incognita*, *M. arenaria*, and *M. javanica* but resistant to *M. hapla* (Carneiro et al. 2000).

Hybrids of *C. maxima* × *C. moschata* are used as rootstocks mainly for grafting watermelon but also melon, and cucumber, and more recently, zucchini. Generally, rootstocks counteract damage caused by pathogens because they provide superior vigour, extensive root systems and tolerance to environmental stresses (i.e. salinity) (Davies et al. 2008). All the tested hybrids of *C. maxima* × *C. moschata* were susceptible to both RKN species but had variable ability to support the nematode. Plants with similar root galling may differ in their effect on yield and so provide tolerance to RKN infection. Thus, watermelon grafted onto *C. maxima* × *C. moschata* 'Titan' was tolerant to *M. javanica* but suffered yield losses when grafted onto 'RS841' (López-Gómez et al. 2016). Similarly, grafting melon onto *C. moschata* made the plant tolerant to *M. incognita* (Sigüenza et al. 2005).

Lagenaria siceraria had a good level of resistance to *M. javanica* shown consistently on the three genotypes tested, 3.6 and 12 times fewer galls and EM were produced by *M. javanica* than by *M. incognita*. These findings suggest pre- and post- infection mechanisms involved in the resistance of *L. siceraria* which resulted in reduced feeding site establishment and malfunction of the established feeding sites that reduced the generation of EM. However, the susceptibility of *L. siceraria* to *M. incognita* was similar to that of other cucurbits which confirms previous reports (Chandra et al. 2010; Levi et al. 2009; Tamilselvi et al. 2017). *Lagenaria siceraria* is used as a rootstock for grafting watermelon (Davies et al. 2008) and had a lower ability to support the nematode than some *C. maxima* x *C. moschata* rootstocks (Thies et al. 2010; López-Gómez et al. 2016).

The response of *Luffa* spp. to RKN was similar to that of *Lagenaria*; that is, both *Luffa* species were more effective in reducing gall formation and EM production by *M. javanica* than by *M. incognita*. *Luffa* species could be used as rootstocks for grafting susceptible cucurbit scions as a tool for nematode management (de Souza et al. 2013; Tamilselvi et al. 2017). However, the grafting compatibility and adaptation to regional conditions need to be determined (de Souza et al. 2013). *Cucurbita argyrosperma* and *Luffa cylindrica* supported low to moderate population increases of *M. incognita* (Anwar and McKenry 2010; de Souza et al. 2013; Tamilselvi et al. 2017).

In summary, the variation in pathogenic potential and parasitism between RKN species on cucurbitaceous genotypes warrant further investigations to explore the most effective host-parasite combinations for nematode management, depending on the species of RKN involved. The competence of RKN to form galls and EM should be considered when assessing host responses as valuable complementary indicators of pathogenicity and parasitism. The *Cucurbita* genotypes will be useful for *M. incognita* management because they reduce population build-up, but root damage would be more severe due to abundant root galling. The consistency of poorer susceptibility of all zucchini genotypes to *M. incognita* indicates that an ample choice of genotypes is available to growers for nematode management. The *Cucurbita* genotypes will be more tolerant to higher Pi when infected by *M. javanica* as they suffer less root damage, but the residual populations may affect the subsequent crop in the rotation. *Lagenaria siceraria* and *Luffa* spp. can be useful by itself or for grafting cucurbitaceous crops, and more effective for nematode management on *M. javanica*-infested soils.

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Compliance with ethical standards

Conflict of interests The Banco de Germoplasma de Valencia is acknowledged for providing the accessions used in this study. Thanks are given to, Technobioplant, Tozer Ibérica, Rijk Zwaan Ibérica and Claus Ibérica for kindly providing seeds for the experiments. The authors declare that they have no conflict of interest and give their consent for publishing the results of the present study.

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