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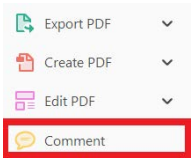

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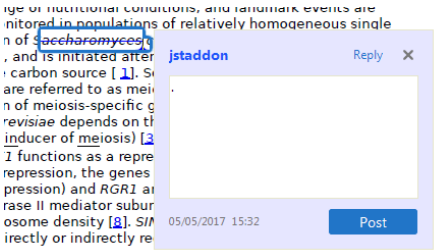

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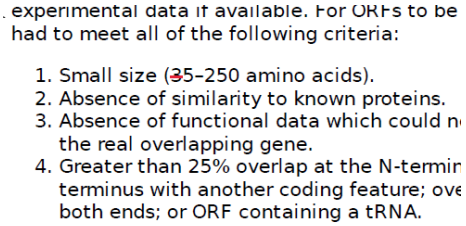

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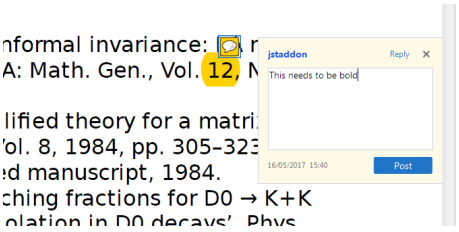

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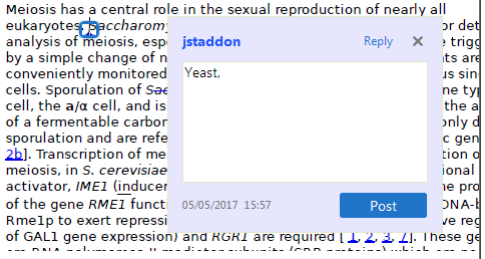

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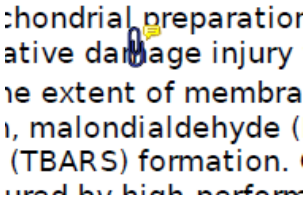

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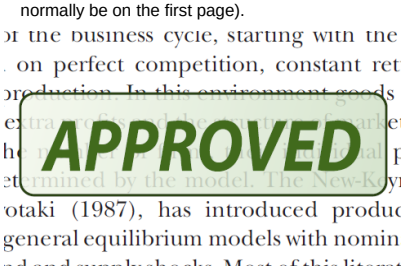

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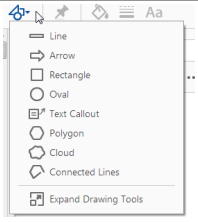

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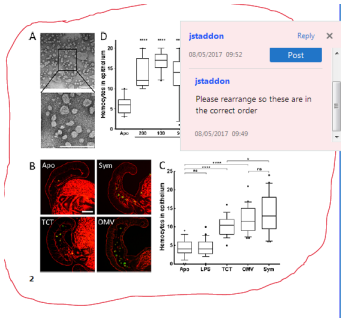

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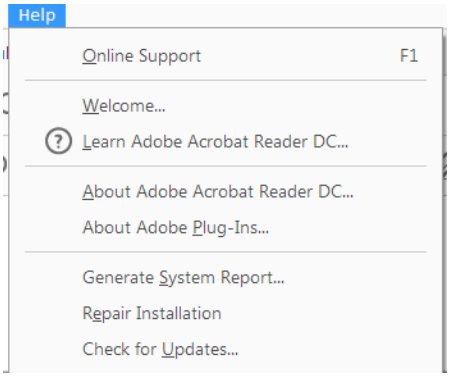

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Cucumis metuliferus is resistant to root-knot nematode *Mi1.2* gene (a)virulent isolates and a promising melon rootstock

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Pot experiments were carried out to characterize the response of two *Cucumis metuliferus* accessions (BGV11135 and BGV10762) against *Mi1.2* gene (a)virulent *Meloidogyne arenaria*, *M. incognita* and *M. javanica* isolates and to determine the compatibility and the effect on physicochemical properties of fruit melons. In addition, histopathological studies were conducted. One week after transplanting, plants were inoculated with one J2 cm^{-3} of sterilized sand (200 cm^3 pots) and maintained in a growth chamber at 25 °C for 40 days. The susceptible cucumber cv. Dasher II or melon cv. Paloma were included for comparison. The number of egg masses and number of eggs per plant were assessed, and the reproduction index (RI) was calculated as the percentage of eggs produced on the *C. metuliferus* accessions compared to those produced on the susceptible cultivars. The compatibility and fruit quality were assessed by grafting three scions, two of Charentais type and one of type *piel de sapo*, under commercial greenhouse conditions. The resistance level of both *C. metuliferus* accessions ranged from highly resistant (RI < 1%) to resistant (1% ≤ RI ≤ 10%) irrespective of *Meloidogyne* isolates. Melon plants grafted onto *C. metuliferus* accession BGV11135 grew as self-grafted plants without negatively impacting fruit quality traits. Giant cells induced by *Meloidogyne* spp. on *C. metuliferus* were in general poorly developed compared to those on cucumber. Furthermore, necrotic areas surrounding the nematode were observed. *Cucumis metuliferus* accession BGV11135 could be a promising melon rootstock to manage *Meloidogyne* spp., irrespective of their *Mi1.2* (a)virulence, without melon fruit quality reduction.

Keywords: *Cucumis melo*, grafting, histopathology, horned cucumber, *Meloidogyne* spp., plant resistance

Introduction

Root-knot nematodes (RKN), *Meloidogyne* spp., are the most damaging plant parasitic nematodes for vegetable production worldwide (Sikora & Fernández, 2005). Nonetheless, the ability of RKN species to develop in a given plant species, to reproduce on it, and to affect its productivity differs according to the plant's host status. Regarding cucurbit crops, one of the most widely cultivated groups around the world, zucchini-squash and watermelon are a susceptible and a poor host, respectively, but both are tolerant (López-Gómez *et al.*, 2014, 2015). Melon and cucumber, on the other hand, are susceptible and get severely damaged by RKN (Di Vito *et al.*, 1983; Giné *et al.*, 2014, 2017). In Spain, crop rotation schemes including solanaceous and cucurbit crops are very common (Ornat *et al.*, 1997; Talavera *et al.*, 2012; Giné & Sorribas, 2016), but resistant cucurbit cultivars or rootstocks are not commercially available. According to the European directive 2009/128/CE, grafting onto resistant-tolerant rootstocks is a promising non-chemical way to suppress RKN populations and to reduce yield losses of the most susceptible-intolerant cucurbit crops. Plant resistance is an effective and profitable control method (Sorribas *et al.*, 2005) to reduce the RKN reproduction rate and the equilibrium density (Talavera *et al.*, 2009; Giné & Sorribas, 2017). This prevents subsequent yield losses on the following crop (Ornat *et al.*, 1997) that are directly related to nematode population densities in the soil at planting stage (Seinhorst, 1965). Grafting is also an effective tool for controlling other soilborne pathogens (Lee & Oda, 2010). In this sense, cucurbit crops are usually grafted onto *Cucurbita* hybrids, which are resistant to fusarium wilt but susceptible to *Meloidogyne* spp. (Thies *et al.*, 2010; López-Gómez *et al.*, 2016; Giné *et al.*, 2017). However, resistance to RKN has been found in wild *Cucumis* spp., including accessions of *C. africanus*, *C. anguria*, *C. ficifolius*, *C. metuliferus*, *C. myriocarpus*, *C. postulatus*, *C. subsericeus* and *C. zeyheri* (Fassuliotis, 1967; Sigüenza *et al.*, 2005; Kokalis-Burelle & Rosskopf, 2011; Pofu & Mashela, 2011; Guan *et al.*, 2014; Liu *et al.*, 2015). Moreover, some of these *Cucumis* species are resistant to pathogenic fungi, such as *Fusarium oxysporum* f. sp. *melonis* (Liu *et al.*, 2015) and *Monosporascus*

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cannonballus (Dias *et al.*, 2001). The inclusion of RKN resistant cucurbit rootstocks in the solanaceous–cucurbitaceous rotation sequence could be helpful to manage RKN, including the isolates that are virulent to the *Mi1.2* resistance gene of tomato. Such isolates have increased in the last years as a result of the reiterative use of resistant germplasm (Tzortzakakis *et al.*, 2005; Devran & Söğüt, 2010; Verdejo-Lucas *et al.*, 2012). Nonetheless, as far as is known, there is no information about the host suitability of *C. metuliferus* accessions to *Mi1.2* virulent RKN isolates.

Cucumis metuliferus is a compatible rootstock for melon but can affect fruit quality traits, such as the total soluble solids content (°Brix) and the flesh firmness, depending on melon type and agronomic conditions (Guan *et al.*, 2014). When testing for putative rootstocks, the evaluation of their impact on the scion's qualitative traits should be considered. The objectives of this study were to assess the host suitability of *C. metuliferus* against several RKN (a)virulent isolates, its compatibility as a rootstock to melon and the effects on fruit quality. Complementary, histopathological studies were conducted to identify resistance mechanisms of *C. metuliferus* against *M. javanica*.

Materials and methods

Nematode inoculum

RKN isolates belonging to *M. arenaria*, *M. incognita* and *M. javanica* were used in the experiments. The information on RKN species, code, origin and the (a)virulence status against tomato cultivars carrying the *Mi1.2* gene is presented in Table 1. The RKN isolates were maintained on the susceptible tomato cv. Durinta (Seminis Seeds). Second-stage juveniles (J2) were used as inoculum. Eggs were extracted from tomato roots by blender maceration in a 5% commercial bleach (40 g L^{-1} NaOCl) solution for 5 min (Hussey & Barker, 1973). The egg suspension was then passed through a 74 μm aperture sieve to remove root debris, and eggs were collected on a 25 μm sieve and placed on Baermann trays (Whitehead & Hemming, 1965) at 25 °C. Nematodes were collected daily using a 25 μm sieve over 7 days and stored at 9 °C until inoculation. *Meloidogyne* species identification was confirmed according to the morphology of the perineal pattern of the females, and by SCAR-PCR markers (Zijlstra *et al.*, 2000).

Table 1 *Meloidogyne* isolates from Spain, geographic origin, virulence status against tomato cultivars carrying the *Mi-1.2*, and reference.

Species	Isolate	Geographic origin	Virulence	Reference
M. arenaria	MA68	Barcelona	Avirulent	—
	MAAl06	Almería	Virulent	Verdejo-Lucas *et al.* (2012)
M. incognita	MIAl15	Almería	Partially virulent	Verdejo-Lucas *et al.* (2012)
	Agropolis	Barcelona	Avirulent	Giné & Sorribas (2017)
	Garriga	Barcelona	Avirulent	—
M. javanica	Bay	Murcia	Avirulent	—
	MJ05	Barcelona	Avirulent	Ornat *et al.* (2001)
	Tugues	Barcelona	Avirulent	—
	MJ27	Barcelona	Virulent	Ornat *et al.* (2001)
	MJLg	Almería	Virulent	—

Response of *C. metuliferus* accessions to RKN isolates

Three experiments were carried out to evaluate the response of *C. metuliferus* against (a)virulent RKN isolates. In the first experiment, accessions BGV11135 and BGV10762 of *C. metuliferus* from the Institute for Conservation and Improvement of Valencian Agrodiversity (COMAV-UPV) collection (Valencia, Spain) and cucumber cv. Dasher II (Seminis Seeds), used as susceptible control, were assessed against the Agropolis (*M. incognita*) and MJ05 (*M. javanica*) avirulent isolates. Each plant × RKN isolate combination was replicated 10 times. The experiment was carried out once. In the second experiment, the response of only the *C. metuliferus* accession BGV11135 against avirulent isolates of *M. arenaria* (MA68), *M. incognita* (Agropolis and Garriga) and *M. javanica* (Bay, MJ05 and Tugues) was assessed, because this accession showed the most consistent resistance response against the RKN isolates in the previous experiment. The susceptible standard cucumber cv. Dasher II was included for comparison. The experiment was repeated once. Each plant × RKN isolate combination was replicated seven and eight times in the first and second experiment repetition, respectively. In the third experiment, the response of the *C. metuliferus* accession BGV11135 and the susceptible melon cv. Paloma (Fitó) was assessed against four *Mi1.2* virulent RKN isolates belonging to *M. arenaria* (MAAl06), *M. incognita* (MIAl15) and *M. javanica* (MJ27 and MJLg). The avirulent *M. javanica* isolate MJ05 was included as standard for comparison. The experiment was repeated once. Each plant × RKN isolate combination was replicated eight times.

All experiments were conducted following the same procedure. Seeds of *C. metuliferus* were surface disinfested using a 20% commercial bleach solution (40 g L^{-1} NaOCl) for 2 min and washed twice in sterile distilled water. Seed germination was done on a cotton matrix saturated with sterile distilled water in Petri dishes and the seeds were incubated for 2 days at 37 °C. Afterwards, germinated seeds were sown in sterile vermiculite and maintained in a growth chamber at 25 ± 2 °C with a 16/8 h (light/dark) photoperiod programme for 1 week. Then, seedlings were individually transplanted into 200 cm^3 pots containing sterile river sand and inoculated with one J2 cm^{-3} of soil, a week after transplanting. Inoculated plants were maintained in a growth chamber for 40 days. Plants were watered as needed throughout the experiment and fertilized with a slow release fertilizer (15% N, 9% P_2O_5, 12% K_2O, 2% MgO_2, microelements; Osmocote Plus). Soil temperatures were recorded daily at 30 min intervals with a PT100 probe (Campbell Scientific Ltd) placed into the pots at 4 cm depth. At the end of the experiment, roots were carefully washed, weighed and immersed in a 0.01% solution of erioglaucine to assess the number of egg masses (Omwega *et al.*, 1988). RKN eggs were extracted from roots by maceration in a 10% commercial bleach solution (40 g L^{-1} NaOCl) (Hussey & Barker, 1973) and counted. The reproduction index (RI) was calculated as the percentage of eggs per plant in the experimental accessions compared to that on the susceptible cucumber cv. Dasher II or melon cv. Paloma. The response of the accessions was categorized according to the RI as highly resistant (RI < 1%), resistant (1% ≤ RI < 10%),

moderately resistant (10% ≤ RI < 25%), slightly resistant (25% ≤ RI < 50%) or susceptible (RI ≥ 50%) (Hadisoeganda & Sasser, 1982).

Histopathology

Seeds of *C. metuliferus* BGV11135 and cucumber cv. Dasher II were germinated and transferred to growth pouches as reported by Atamian *et al.* (2012). Plantlets were placed in a growth chamber at 25 ± 2 °C with a 16/8 h (light/dark) photoperiod programme, and inoculated at the 2-true-leaf expanded stage with 2500 J2 of the *M. javanica* MJ05 isolate. After 12 days, roots were carefully washed and cut into 10 mm pieces. Then, roots containing galls were selected and fixed in 2.5% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) overnight at 4 °C and washed three times with the same buffer. Afterwards, root pieces were post-fixed in 1% (w/v) osmium tetroxide in 0.1 M sodium phosphate buffer (pH 7.2) for 1 h and washed three times with the same buffer and dehydrated in an acetonitrile series (30–100%) before embedding in epoxy resin (Embed 812; Aname) and polymerizing at 60 °C for 48 h. Semithin (2 µm) sections of samples were obtained in a Reichert-Jung Ultracut E Ultra microtome EM UC6 (Leica Microsysteme GmbH) and left to dry on a slide previously stained with Richardson's blue (azure II in dH_2O:methylene blue in 1% sodium borate, 1:1; v/v). The sections were mounted in a DPX mountant for histology and observed under a DM4000 B microscope (Leica Microsystems). Sections were photographed using a Leica DFC300 FX 1.4-megapixel digital colour camera equipped with the software application suite LAS v. 3.8 (Leica Microsystems).

Compatibility and fruit quality assessment

The performance of *C. metuliferus* BGV11135 as a potential rootstock was evaluated using cultivars Vedrantais (COMAV-UPV) and Paloma (Fitó Seeds) of Charentais melon (*Cucumis melo* var. *cantalupensis*) and cv. Finura (Rijk Zwaan) of *piel de sapo* melon (*Cucumis melo* var. *inodorus*) as scions. Plants were self-grafted (used as control treatment) and grafted onto *C. metuliferus* BGV11135 using the cleft procedure (Lee & Oda, 2010). Plants were grown under hydroponic conditions in a commercial greenhouse at Fundación Cajamar (Paiporta, València, Spain) during the spring–summer of 2017. Plant vigour was evaluated at 30 and 60 days after transplanting using a visual scale of 0 (low) to 4 (high). The flowering time was recorded as the number of days after transplanting at which the first female flower appeared. In order to evaluate the impact of grafting on fruit quality, each fruit (eight per treatment) was characterized for the following fruit traits: weight (g), length and width (cm), rind (mm), flesh thickness (cm) and firmness (kg cm^{-2}) (penetrometer (8 mm) FHT-803; Melrose), pH (pH indicator paper pH 1–14; Merck), total soluble solids (digital rephractometer; Atago), and flesh colour (CR-400 colourimeter; Minolta) using the colour parameters Hunter *L*, *a* and *b*, where the *L* value indicates lightness (from 0 to 100), the *a* value redness (+) or greenness (−), and the *b* value yellowness (+) or blueness (−).

Statistical analysis

Statistical analysis was performed using SAS system v. 9 (SAS Institute, Inc.). Data on number of egg masses and eggs per plant were submitted to nonparametric analysis by the NPAR1WAY procedure to compare between replications of the same experiment, and considered as the same experiment if no differences were found ($P \geq 0.05$) by the Kruskal–Wallis test. Comparisons were made between the number of eggs masses and eggs per plant produced on each *C. metuliferus* accession and those on the susceptible cucumber or melon cultivars, as well as between *C. metuliferus* accessions in the first experiment. Moreover, a comparison was made between RKN isolates for each plant material. Paired comparisons of fruit quality traits between all grafted and self-grafted cultivars were performed by Student's *t*-test because data were normally distributed.

Results

Response of *C. metuliferus* accessions against *Meloidogyne* spp. isolates

The number of egg masses and eggs per plant on both *C. metuliferus* accessions (BGV11135 and BGV10762) were significantly lower ($P < 0.05$) than on the susceptible cucumber cv. Dasher II, irrespective of the *Meloidogyne* isolates (Table 2). Both *C. metuliferus* accessions responded as highly resistant (RI < 1%) or resistant (1% ≤ RI ≤ 10%) to RKN depending on the nematode isolate. The MJ05 isolate produced more ($P < 0.05$) egg masses and eggs per plant on BGV10762 than BGV11135 accessions.

The infective and reproductive ability of the *Meloidogyne* isolates differed ($P < 0.05$) on both *C. metuliferus* BGV11135 and the cucumber cv. Dasher II. The nematode isolates Agropolis and Garriga of *M. incognita*, and MJ05 of *M. javanica* produced the highest number of egg masses and eggs per plant ($P < 0.05$) compared to the remaining RKN isolates on *C. metuliferus*. *Meloidogyne arenaria* isolate MA68 produced the highest amount of egg masses on cucumber, although reproduction was higher in the Agropolis and Garriga isolates of *M. incognita* ($P < 0.05$). The accession BGV11135 of *C. metuliferus* was classified as resistant against most RKN isolates assessed.

Regarding the *Mi1.2* gene virulent isolates, the BGV11135 accession responded as highly resistant (RI < 1%), resistant (1% ≤ RI ≤ 10%) or moderately resistant (10% ≤ RI < 25%; Table 3).

Histopathology

Meloidogyne javanica isolate MJ05 induced giant cells in both *Cucumis* species (Fig. 1), but those produced in *C. metuliferus* were in general poorly developed with multiple vacuoles compared to those on cucumber. Furthermore, giant cells without cytoplasm and necrotic areas surrounding the nematode were observed.

Compatibility and fruit quality assessment

Cucumis metuliferus used as rootstock did not affect the plant growth of Charentais and *piel de sapo* melons. Grafted plants of each cultivar showed similar vine

Table 2 Number of egg masses per plant, eggs per plant and reproduction index (RI) of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* isolates on the *Cucumis metuliferus* accessions BGV11135 and BGV10762 in experiment 1 and BGV11135 in experiment 2, and on the cucumber cv. Dasher II.

			Egg masses per plant			Eggs per plant (×100)				
			C. metuliferus		Cucumber	*C. metuliferus*		Cucumber	RI (%)[b]	
Experiment	Species	Isolate	BGV10762	BGV11135	Dasher II	BGV10762	BGV11135	Dasher II	BGV10762	BGV11135
Experiment 1	*M. incognita*	Agropolis	1.0 ± 0.2 b*[a]	2.0 ± 0.5 a*	78 ± 9.7 a	2.1 ± 0.9 b*	3.7 ± 1.1 a*	526 ± 72 a	0.4 ± 0.2	1 ± 2
	M. javanica	MJ05	4.0 ± 0.6 a*	1.0 ± 0.3 a*	44 ± 13.6 b	16.0 ± 4.1 a*	4.3 ± 1.3 a*	407 ± 118 a	4.0 ± 1.0	1 ± 3
Experiment 2	*M. arenaria*	MA68	—	1.0 ± 0.3 b*	58 ± 3.2 a	—	0.3 ± 0.1 b*	3.9 ± 1.3 d	—	8 ± 4
	M. incognita	Agropolis	—	2.0 ± 0.3 a*	35 ± 4.9 b	—	4.7 ± 1.1 a*	178 ± 31 a	—	1 ± 1
		Garriga	—	4.0 ± 0.7 a*	32 ± 0.3 b	—	8.6 ± 2.2 a*	157 ± 18 a	—	4 ± 2
	M. javanica	Bay	—	0.4 ± 0.2 b*	11 ± 1.2 d	—	0.1 ± 0.05 b*	32 ± 6.8 c	—	<1
		MJ05	—	3.0 ± 0.6 a*	33 ± 1.2 b	—	3.6 ± 0.9 a*	51 ± 14 bc	—	7 ± 2
		Tugues	—	0.3 ± 0.2 b*	19 ± 2.4 c	—	0.6 ± 34 b*	68 ± 972 b	—	3 ± 2

[a]Data are mean ± standard error of 10 and 15 replicates in experiments 1 and 2, respectively. Data within the same column and experiment followed by the same letter did not differ ($P < 0.05$) according to the Kruskal–Wallis test. Data of egg masses per plant or eggs per plant within the same row followed by * indicate differences ($P < 0.05$) between each *C. metuliferus* accessions and cucumber according to the Kruskal–Wallis test.

[b]RI% (reproduction index) = 100 × (no. eggs on the *C. metuliferus* accession)/(no. eggs on the cucumber cv. Dasher II).

vigour and flowering time than the corresponding self-grafted plants. There were no significant effects of the rootstock on fruit's external and internal quality in the two Charentais melons cultivars, except for a slight increase in flesh thickness for cv. Paloma (Table 4). Each grafted Charentais melon cultivar maintained its fruit size, rind and flesh firmness, and flesh quality (°Brix, pH and colour). Grafting the *piel de sapo* melon cv. Finura onto *C. metuliferus* increased both fruit weight and length, although they were softer, sweeter and the flesh presented a lighter colour compared to the self-grafted plants (Table 4).

Discussion

The *C. metuliferus* accessions assessed in this study were highly resistant (RI < 1%) or resistant (1-% ≤ RI ≤ 10%) to most RKN isolates tested. This is in agreement with previous reports by other authors (Fassuliotis, 1967, 1970; Sigüenza *et al.*, 2005; Walters *et al.*, 2006; Guan *et al.*, 2014; Ye *et al.*, 2017). The host suitability of *C. metuliferus* was not affected by the *Mi1.2* (a)virulence of the nematode isolate. The frequency of detection of virulent *Mi1.2* populations of *Meloidogyne* in commercial growing areas has increased since the last century (Tzortzakakis *et al.*, 2005; Devran & Söğüt, 2010; Verdejo-Lucas *et al.*, 2012), which is a serious problem that needs to be solved. Verdejo-Lucas *et al.* (2012) reported for example that 48% of the RKN populations from 29 fields sampled in Almería (Spain), the most important tomato growing area under protected cultivation in Europe, were virulent. Selection of virulence to the *Mi1.2* gene in field conditions can be progressive (Verdejo-Lucas *et al.*, 2009; Giné & Sorribas, 2017) or can occur suddenly (Ornat *et al.*, 2001) depending on the genetic background of the plant and/or the nematode population (Ornat *et al.*, 2001; Cortada *et al.*, 2008). Different strategies for managing the selection for virulence on solanaceous crops have been assessed. Such strategies were mainly based on the rotation of tomato germplasm carrying the *Mi1.2* resistance gene with susceptible cultivars (Talavera *et al.*, 2009; Giné & Sorribas, 2017) or on pyramiding multiple *R* genes in pepper (Djian-Caporalino *et al.*, 2014). Until now, no virulent RKN populations to *C. metuliferus* have been reported. Including new sources of resistance to RKN, as such on *C. metuliferus*, could thus be a useful tool for managing RKN, irrespective of their (a) virulence. Moreover, it could be difficult to select for virulence to resistance genes on solanaceous crops in rotation schemes with susceptible cucurbits grafted onto resistant rootstocks. In addition, the RKN population able to reproduce on both resistant solanaceous crops and *C. metuliferus* could be an indicator of the durability of the resistance due the high specificity of resistance genes. This hypothesis should be verified in long-term experiments.

Fassuliotis (1967, 1970) reported the resistance response of *C. metuliferus* accession C-701 to *M.*

Table 3 Number of eggs per plant of avirulent (MJ05), partially virulent (MIAI15) and virulent (MAAI06, MJLg and MJ27) isolates to the *Mi1.2* gene on *Cucumis metuliferus* accession BGV11135 and melon cantaloupe cv. Paloma and reproduction index (RI) in experiment 3.

		Eggs per plant (×100)		
Meloidogyne species	Isolate	BGV11135	Paloma	RI (%)[b]
M. arenaria	MAAI06	0.6 ± 0.2 b*[a]	4.4 ± 2.4 b	13.4 ± 4.7
M. incognita	MIAI15	10.0 ± 3.8*	133.0 ± 25.0 a	7.5 ± 2.8
M. javanica	MJLg	11.0 ± 5.0 a*	88.0 ± 35.0 a	13.0 ± 6.0
	MJ27	0	6.1 ± 2.1 b	0
	MJ05	3.9 ± 2.2 ab*	159.0 ± 17.0 a	2.4 ± 1.3

[a]Data are mean ± standard error of 16 replicates. Data within the same column followed by the same letter did not differ ($P < 0.05$) according to the Kruskal–Wallis test. Data of eggs per plant followed by * indicate differences ($P < 0.05$) between the *C. metuliferus* accession and melon cv. Paloma according to the Kruskal–Wallis test.
[b]RI% (reproduction index): 100 × (no. eggs on the *C. metuliferus* accession)/(no. eggs on the melon cv. Paloma).

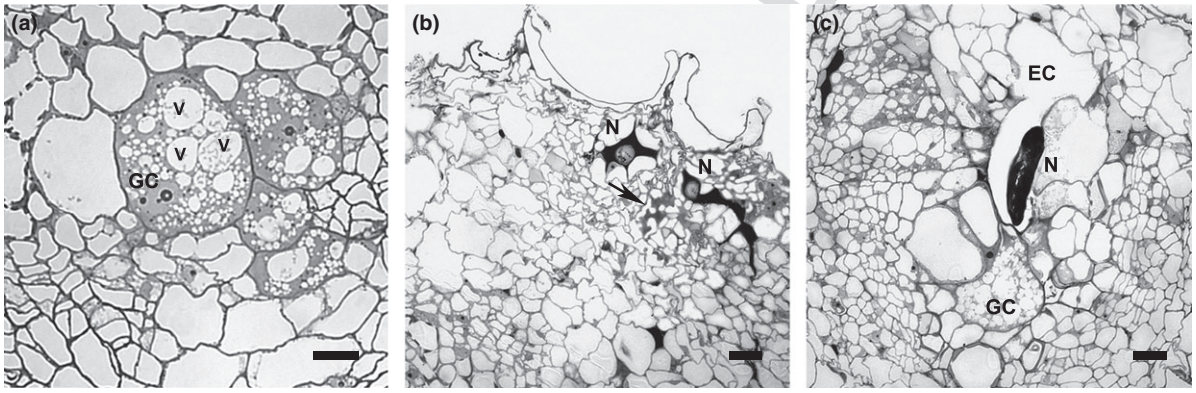

Figure 1 Light microscope images of 2 μm tranversal sections of cucumber cv. Dasher II (a) and *Cucumis metuliferus* accession BGV11135 (b, c) infected roots by *Meloidogyne javanica* (MJ05) 12 days after inoculation. GC, giant cells; V, vacuole; N, nematode; EC, empty cell; arrows indicates the nematode-necrosed area around the nematode. Bars = 20 μm.

incognita. They conducted histopathological studies, and observed small giant cells affecting nematode development and increasing the proportion of males. However, no hypersensitive response was observed. Similar results were found by Walters *et al.* (2006) in the accession PI 482454 inoculated with *M. arenaria*, *M. hapla*, *M. incognita* or *M. javanica*. Recent studies (Ye *et al.*, 2017) have reported a reduction of the number of *M. incognita* J2 in roots of the *C. metuliferus* accession PI 482443 at 7 days post-inoculation (dpi) compared to at 4 dpi, indicating death or emigration from roots and a delayed development of the nematodes remaining in the roots. Empty or poorly developed giant cells with multiple vacuoles were observed at 7 and 14 dpi, with giant cells appearing to be collapsed or without cytoplasm. In addition, several genes related to plant defence mechanisms were significantly modified and, in contrast with previous reports (Fassuliotis, 1970; Walters *et al.*, 2006), hypersensitive necrosis was observed (Ye *et al.*, 2017). The results of this study are consistent with those previously reported, in which giant cells were multivacuolated or appeared collapsed without cytoplasm. Furthermore, necrotic areas were observed. These results indicate that the *C. metuliferus* genetic background could play an important role in the interaction with *Meloidogyne* spp.

Grafting can affect fruit quality depending on the rootstock × scion interactions, climatic and agronomic conditions (Leonardi *et al.*, 2017). For instance, fruit melons of cultivars Supermarket or Proteo grafted onto *C. metuliferus* contained less °Brix than the ungrafted plants in one out of two cropping seasons (Trionfetti-Nisini *et al.*, 2002). Guan *et al.* (2014) reported less °Brix content and flesh firmness in galia but not in honeydew melons grafted onto *C. metuliferus* conducted in a conventional manner. However, no differences were found when plants were conducted under organic farming. In this study, no differences were found on growth or fruit quality between self-grafted cantaloupe melon cv. Vedrantais and cv. Paloma and those grafted onto *C. metuliferus*. These results are in agreement with those reported by Gisbert *et al.* (2017) who did not find differences among fruit quality from ungrafted, self-grafted or grafted cv. Vedrantais onto *C. metuliferus*. Conversely, grafted melon *piel de sapo* cv. Finura onto *C. metuliferus* affected fruit weight and length. Nonetheless, these changes do not reduce the commercial value of the fruits

Table 4 Quality parameters of fruit of the Charentais melon cv. Vedrantais (VED) and cv. Paloma (PAL) and the *piel de sapo* melon cv. Finura (FIN) from plants self-grafted and grafted onto *Cucumis metuliferus* BGV11135.

Genotype	Fruit size			Rind thickness (mm)	Flesh thickness (cm)	Cavity thickness (cm)	Rind firmness (kg cm^{-2})	Flesh firmness (kg cm^{-2})	°Brix[b]	pH	Colour[c]		
	Weight (g)	Length (cm)	Width (cm)								*L*	*a*	*b*
VED–VED	723.4 ± 26.5[a]	10.6 ± 0.2	11.3 ± 0.1	3.3 ± 0.3	26.4 ± 1.3	47.4 ± 1.2	11.7 ± 0.7	1.0 ± 0.3	14.4 ± 0.5	6.2 ± 0.1	53.4 ± 1.1	11.1 ± 0.2*	23.9 ± 1.2
C. metuliferus–VED	758.1 ± 49.8	10.6 ± 0.2	11.7 ± 0.3	3.5 ± 0.2	27.1 ± 0.7	46.6 ± 1.8	11.4 ± 0.8	1.3 ± 0.2	14.2 ± 0.4	6.1 ± 0.1	55.5 ± 0.4	13.1 ± 0.6	24.9 ± 0.5
PAL–PAL	811.9 ± 48.5	11.8 ± 0.2	11.6 ± 0.3	3.0 ± 0.1	24.5 ± 0.6*	54.5 ± 1.5	13.0 ± 0.0	3.6 ± 0.1	15.9 ± 0.2	6.0 ± 0.0	62.3 ± 0.8	14.1 ± 0.4*	27.8 ± 0.1
C. metuliferus–PAL	907.1 ± 24.4	12.3 ± 0.1	11.9 ± 0.1	2.9 ± 0.2	27.7 ± 0.9	53.4 ± 1.9	12.8 ± 0.2	3.2 ± 0.2	15.3 ± 0.3	6.1 ± 0.1	62.0 ± 1.2	12.9 ± 0.3	27.5 ± 0.4
FIN–FIN	1340.5 ± 48.3*	16.4 ± 0.2*	12.8 ± 0.2	4.1 ± 0.1*	34.9 ± 0.2	53.6 ± 0.7	13.0 ± 0.0	2.3 ± 0.1*	14.5 ± 0.2*	6.0 ± 0. 0	58.3 ± 0.3*	−2.5 ± 0.1	8.4 ± 0.2
C. metuliferus–FIN	1552.6 ± 85.9	17.4 ± 0.2	13.3 ± 0.2	3.2 ± 0.1	33.4 ± 1.0	56.9 ± 1.3	13.0 ± 0.0	1.8 ± 0.1	15.5 ± 0.2	6.0 ± 0.0	64.3 ± 0.9	−2.6 ± 0.1	8.9 ± 0.1

[a]Data is mean ± standard error of eight replicates. Values of each parameter in the same cultivar followed by * are significantly different according to Student's *t*-test ($P < 0.05$).
[b]°Brix: soluble solid content measured in fruit flesh as Brix degrees.
[c]Colour parameters measured in fruit flesh: Hunter *L*, lightness (from 0 to 100); *a*, red (+) or green (−); *b*, yellow (+) or blue (−).

as the market of *piel de sapo* melons accepts a wide range of fruit sizes and variability in shapes. The changes in parameters associated with flesh quality (higher °Brix, lower flesh firmness and lighter flesh colour) might be associated with a more advanced ripening state of the melons grafted onto *C. metuliferus*. Effects on fruit quality in grafted plants due to growing cycle alterations have been reported previously (Davis *et al.*, 2008; Soteriou *et al.*, 2014). Therefore, these effects could be reduced by adapting the harvesting period for each rootstock × scion combination.

In conclusion, the *C. metuliferus* accession BGV11135 could be a promising melon rootstock to manage *Meloidogyne* spp. irrespective of their *Mi1.2* (a)virulence, without reducing melon fruit quality. In addition, the *C. metuliferus* accessions assessed in this study are highly resistant to fusarium wilt (Gisbert *et al.*, 2014), and tolerant to *Monosporascus cannonballus* in field conditions (G. Perpiñà *et al.*, Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV), Valencia, Spain, personal communication).

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