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Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon

Helio A. García-Mendívil[a], Maria Munera[a], Ariadna Giné[a], Nuria Escudero[a], Maria Belén Picó[b], Carmina Gisbert[b], Francisco Javier Sorribas[a, *]

[a] Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Esteve Terradas 8, 08860, Castelldefels, Barcelona, Spain
[b] Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV), Universitat Politècnica de València, Spain

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ABSTRACT

The response of two *Citrullus amarus* accessions, BGV0005164 and BGV0005167, was assessed against different *Meloidogyne arenaria, Meloidogyne incognita,* and *Meloidogyne javanica* isolates in pot experiments and against *M. incognita* in plastic greenhouse. In the pot experiments, plants were inoculated with a second-stage juvenile per cm^3 of sterile sand and maintained in a growth chamber at 25 °C for 50 days. The watermelon cv. Sugar Baby was included as a susceptible control for comparison. At the end of the experiments, the number of egg masses and eggs per plant was determined, and the reproduction index was calculated as the percentage of the number of eggs produced in the *C. amarus* accessions with regard to that produced in the susceptible cv. Sugar Baby. In the plastic greenhouse experiment, the ungrafted watermelon cv. Sugar Baby and watermelons grafted onto each of the *C. amarus* accessions and onto the watermelon rootstock cv. Robusta were cultivated from May to August 2016 in plots with nematode densities from 46 to 1392 J2 per 250 cm^3 of soil at transplantation. At the end of the experiment, the galling index and the number of eggs per plant were determined, and the reproduction index was calculated. Additionally, the compatibility of the two accessions with the watermelon cv. Sugar Baby and the effect on fruit quality (weight, size, shape, firmness, pH, total soluble solids, and flesh color) were assessed under a hydroponic system in a greenhouse. The commercial rootstocks cv. Cobalt and cv. Robusta were also included. All the *Meloidogyne* isolates produced less egg masses and eggs per plant on the accessions than on Sugar Baby. Both accessions performed as resistant against *M. arenaria*, and from highly to moderately resistant to *M. incognita* and *M. javanica* in pot experiments. In the plastic greenhouse experiment, both *C. amarus* accessions performed as resistant to *M. incognita*. Both *C. amarus* accessions were compatible with the watermelon cv. Sugar Baby, but only the BGV0005167 accession did not influence the fruit quality. Then, the BGV0005167 accession is a promising rootstock for managing the three tropical root-knot nematode species without influencing watermelon fruit quality.

1. Introduction

Watermelon is one of the major cultivated cucurbit crops, with an estimated worldwide production of ca. 117 million t from 3.5 million ha (FAOSTAT, 2016). As a result of the intensive cultivation in limited land resources, soilborne diseases and pests have significantly increased in recent years (Thies et al., 2015b). The root-knot nematode (RKN) *Meloidogyne* spp. is currently one of the main pathogens in cucurbit crops. Maximum yield losses of 88% in cucumber, 53% in zucchini, and 35% in watermelon cultivated under plastic greenhouses have been estimated in Spain (Giné et al., 2014; Vela et al., 2014; López-Gómez et al., 2014, 2015). The control of RKN has widely been done using fumigant and non fumigant nematicides (Nyczepir and Thomas, 2009). Nonetheless, the interest in nonchemical control alternatives has increased according to recent regulations such as the European Directive 2009/128/EC and the U.S. Clean Air Act (U.S. Environmental Protection Agency, 2012). In this scenario, plant resistance is a key tool for RKN man-

* Corresponding author.
Email address: francesc.xavier.sorribas@upc.edu (F.J. Sorribas)

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Table 1
Meloidogyne species and isolates used in pot experiments, geographic origin, and (a)virulence status against the *Mi 1.2* gene of tomato.

Meloidogyne spp.	Isolate	Geographic origin	(a)virulence	Reference
M. arenaria	MA68	Barcelona	Avirulent	Expósito et al. (2018b)
M. incognita	Agropolis	Barcelona	Avirulent	Giné and Sorribas (2017)
	Garriga	Barcelona	Avirulent	Expósito et al. (2018b)
M. javanica	MJ05	Barcelona	Avirulent	Ornat et al. (2001)
	Tugues	Barcelona	Avirulent	Expósito et al. (2018b)
	Bay	Murcia	Avirulent	Expósito et al. (2018b)
	MJLg	Almería	Virulent	Expósito et al. (2018b)

agement because it is an effective and economically profitable control method (Sorribas et al., 2005). Cropping resistant cultivars reduces the growth rate and the equilibrium density of the RKN population, as well as crop yield losses (Talavera et al., 2009). Moreover, it reduces crop yield losses of the following crop in the rotation scheme (Ornat et al., 1997; Thies et al., 2004; Westphal, 2011). Grafting onto resistant rootstocks is an alternative method to control soilborne pathogens when no commercial resistant cultivars are available (Yetişir et al., 2003; Miguel et al., 2004; Cohen et al., 2007; Lee and Oda, 2002; Thies et al., 2016). Regarding watermelon, it has been commonly grafted onto commercial rootstocks such as *Cucurbita maxima* x *Cucurbita moschata* and *Lagenaria siceraria* owing to their resistance to fusarium wilt. However, both rootstocks are susceptible to infection by *Meloidogyne* (Davis et al., 2008; Hassell et al., 2008; Thies et al., 2010, 2015a; Kokallis-Burelle and Rosskopf, 2011; López-Gómez et al., 2016; Giné et al., 2017). In the last few years, some accessions of citron melon, *Citrullus lanatus* var. *citroides,* most recently referred as *Citrullus amarus* (Chomicki and Renner, 2015), have been proven to be useful as watermelon rootstock. Indeed, these accessions provide resistance to fusarium wilt (Huitrón et al., 2007; Levi et al., 2017) and some RKN species in both greenhouse (Thies and Levi, 2003, 2007) and open field cultivation (Huitrón et al., 2007; Thies et al., 2010, 2015a, 2016). In addition, watermelon grafted onto *C. amarus* yielded more than those grafted onto *L. siceraria, C. maxima* x *C. moschata* or *Praecitrullus fistulosus*, without affecting the quality and the size of the fruits (Kyriacou et al., 2016; Thies et al., 2015a; Fredes et al., 2017). However, not all *C. amarus* accessions responded equally to RKN isolates (Thies and Levi, 2003, 2007; Thies et al., 2016; Levi et al., 2017), the screening of new accessions against local RKN populations being necessary to assure their efficacy. Furthermore, the compatibility with the scion and the effect on the quality of fruits is also required to be considered as a potential rootstock. The aim of this study was to characterize the response of two experimental *C. amarus* accessions against several isolates of *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* under controlled conditions and against *M. incognita* under plastic greenhouse conditions. Additionally, the compatibility of the two *C. amarus* accessions with the watermelon cv. Sugar Baby and the effect on fruit quality were assessed in a hydroponic system under greenhouse.

2. Materials and methods

2.1. Nematode inoculum

Seven isolates of *M. arenaria, M. incognita* and *M. javanica* were used in the experiments (Table 1). All the RKN isolates were maintained on the susceptible tomato cv. Durinta (Seminis Seeds, St. Louis, Missouri). Second-stage juveniles (J2) were used as the inoculum. The J2 were obtained from eggs of infected roots by maceration of roots using a 5% commercial bleach solution (40 g/L NaOCl) for 10 min according to the Hussey and Barker (1973) method. After maceration, the egg suspension was filtered through a 74 μm sieve, and then, the eggs were collected on a 25 μm sieve and placed on Baermann trays (Whitehead and Hemming, 1965). The J2 emerged during the first 24 h were discarded. After that, the J2 emerged were recovered every two days and maintained at 9 °C until the pot experiments were carried out. The identification of the *Meloidogyne* species was confirmed using SCAR-PCR markers (Zijlstra et al., 2000).

2.2. Response of C. amarus accessions to RKN isolates

The *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67), obtained from Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV-UPV) gene bank collection (Valencia, Spain), were assessed against the *Meloidogyne* isolates in three different pot experiments. In the first experiment, the accessions CI64 and CI67 were assessed against the *Mi1.2* avirulent isolates Agropolis (*M. incognita*) and MJ05 (*M. javanica*). In the second experiment, the response of the two *C. amarus* accessions was assessed against the *Mi1.2* avirulent isolates MA68 of *M. arenaria*; Agropolis and Garriga of *M. incognita;* and Bay, MJ05, and Tugues of *M. javanica*. In the third experiment, the response of both *C. amarus* accessions was assessed against the *Mi1.2* virulent isolate MJLg of *M. javanica*. The watermelon cv. Sugar Baby (SB) (Intersemillas S. A., Loriguilla, Valencia, Spain) was included as susceptible control for comparison in all experiments. The watermelon rootstock cv. Robusta (RO) (*C. lanatus*, Intersemillas S. A., Loriguilla, Valencia, Spain) was also included for comparison as resistant control (López-Gómez et al., 2016) in the third experiment. Experiment 1 and 3 were carried out once, and each plant–RKN isolate combination was replicated 10 times. Experiment 2 was repeated once, and each plant–RKN isolate combination was replicated seven and eight times in the first and second experiment repetition, respectively.

All experiments were carried out following the same procedure. Briefly, seeds were germinated according to the method given in Expósito et al. (2018b). Seedlings were transplanted to 200 cm^3 pots containing sterile sand and maintained in a growth chamber at 25 ± 2 °C with a 16:8 h (light:dark) photoperiod for a week and then inoculated with 1 J2 per cm^3 soil. Plants were maintained in the growth chamber for 50 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 experiments, the roots were carefully washed and weighed. Then, in the first and second experiments, the roots were submerged in 15 mg/L erioglaucine solution (Acros Organics) for 20 min to stain the egg masses before counting them (Omwega et al., 1988). In all experiments, eggs were extracted from roots by maceration in a 10% commercial bleach solution (40 g/L NaOCl) for

Table 2
Number of egg masses and eggs per plant and reproduction index (*RI*) of *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* isolates in the *C. amarus* accessions BGV0005164 (CI64) and BGV0005167 (CI67), the watermelon cv. Sugar Baby (SB), and the commercial rootstock cv. Robusta (RO) 50 days after inoculation in pot experiments.

Experiment	RKN species	Isolate	Egg masses per plant			Eggs per plant (x 10^2)				*RI* (%)[a]		
			CI64	CI67	SB	CI64	CI67	SB	RO	CI64	CI67	RO
Experiment 1	*M. incognita*	Agropolis	1.0 ± 0.2 b A	1.0 ± 0.7 b A	17.0 ± 3.7 a A	3.5 ± 1.6 b A	4.6 ± 3.2 b A	126.9 ± 26.7 a A		2.7 ± 1.3	3.6 ± 2.5	
	M. javanica	MJ05	1.0 ± 0.6 b A	2.0 ± 0.7 b A	25.0 ± 0.2 a A	8.7 ± 8.2 b A	12.6 ± 11 b A	180.5 ± 32.5 a A		4.8 ± 4.5	7.0 ± 5.8	
Experiment 2	*M. arenaria*	MA68	0.8 ± 0.2 b A	0.5 ± 0.2 b A	5.0 ± 1.2 a A	2.1 ± 0.8 b A	1.1 ± 0.6 b A	39.8 ± 11.4 a A		5.3 ± 1.3	2.8 ± 1.5	
	M. incognita	Agropolis	0.5 ± 0.4 b AB	0.1 ± 0.1 b A	5.0 ± 1.6 a A	0.4 ± 0.4 b AB	0.1 ± 0.1 b A	23.9 ± 13.7 a AB		1.7 ± 1.5	0.5 ± 0.5	
		Garriga	0.3 ± 0.1 b AB	0.2 ± 0.1 b A	4.0 ± 0.9 a AB	0.1 ± 0.1 b B	0.3 ± 0.2 b A	12.2 ± 3.5 a AB		0.5 ± 0.3	2.6 ± 1.7	
	M. javanica	Bay	0.3 ± 0.1 b AB	0.6 ± 0.2 b A	6.0 ± 1.7 a A	0.2 ± 0.1 b AB	0.3 ± 0.1 b A	33.8 ± 12.0 a A		0.5 ± 0.3	0.9 ± 0.4	
		MJ05	0.7 ± 0.2 b AB	0.4 ± 0.2 b A	5.0 ± 1.0 a A	1.2 ± 0.8 b AB	1.0 ± 0.8 b A	30.0 ± 9.1 a AB		4.0 ± 2.5	3.2 ± 2.8	
		Tugues	0.1 ± 0.1 b B	0.4 ± 0.2 b A	1.0 ± 0.1 a B	0.5 ± 0.5 b B	0.3 ± 0.1 b A	3.7 ± 1.7 a B		14.3 ± 13.5	7.2 ± 3.6	
Experiment 3	*M. javanica*	MJLg	na	na	na	0.3 ± 0.3 b	0.7 ± 0.6 b	6.6 ± 2.4 a	1.2 ± 0.5 ab	5.0 ± 5.0	10.4 ± 8.8	17.5 ± 8.1

Data are the mean ± standard error of 10 replicates in experiments 1 and 3 and of 15 replicates in experiment 2. Data within the same row followed by the same lower case letter did not show significant difference ($P < 0.05$) between germplasm per RKN isolate according to the Kruskal–Wallis test. Different capital letters in the same column and experiment indicate significant differences ($P < 0.05$) between nematode isolates according to the Mann–Whitney *U* test (experiment 1) or Kruskal–Wallis test (experiment 2); na: not assessed.

[a] *RI* (reproduction index) = 100 × (number of eggs per plant produced in the CI accessions or RO/mean number of eggs per plant produced in the susceptible cv. Sugar Baby).

Table 3
Galling index, eggs per plant and reproduction index (*RI*) of *M. incognita* in the watermelon cv. Sugar Baby, the commercial watermelon rootstock cv. Robusta, and the *C. amarus* accessions BGV0005164 and BGV0005167 cultivated from May to August 2016 in plastic greenhouse at initial population densities from 46 to 1392 J2 per 250 cm^3 of soil.

Plant host	Galling index[a]	Eggs per plant ($\times 10^2$)	Reproduction index (%)[b]
Sugar Baby	5.0 ± 0.6 a	1031 ± 484 a	
Robusta	2.8 ± 0.4 b	51 ± 11 b	4.4 ± 0.9
BGV0005164	2.5 ± 0.5 b	16 ± 10 b	1.4 ± 0.9
BGV0005167	1.5 ± 0.5 b	15 ± 12 b	1.3 ± 1.0

Data are the mean ± standard error of 10 replicates. Different letters in the same column indicate significant differences ($P < 0.05$) between germplasm according to the Kruskal–Wallis test.

[a] Galling index on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck, 1971).

[b] Reproduction index = 100 × (number of eggs per plant produced in the CI accessions or Robusta/mean number of eggs per plant produced in the susceptible cv. Sugar Baby).

10 min (Hussey and Barker, 1973), passed through a 74 μm aperture screen and collected in a 25 μm sieve for final counting. Reproduction index (*RI*) was calculated as the percentage of eggs per plant produced in the experimental germplasm with regard to that in the susceptible one. The response of the accessions was categorized according to the *RI* as highly resistant ($RI < 1\%$), resistant ($1\% \leq RI < 10\%$), moderately resistant ($10\% \leq RI < 25\%$), slightly resistant ($25\% \leq RI < 50\%$), or susceptible ($RI \geq 50\%$) (Hadisoeganda and Sasser, 1982).

2.3. Experiment under plastic greenhouse

The experiment was carried out from May 10 to August 11, 2016, under a 700 m^2 plastic greenhouse located at Viladecans (Barcelona, Spain), infested with the *M. incognita* isolate Agropolis. Ten 2.5 m long individual plots were used. Each plot was considered a replication and consisted in a row in which one plant each of ungrafted watermelon SB, the watermelon grafted onto CI64 and CI67, and that grafted onto the rootstock RO was transplanted with a space of 0.6 m. Plants were arranged in such a way that every germplasm was an equal number of times at the edge of the plots and next to the susceptible SB. Plants were irrigated as needed through a drip irrigation system and weekly fertilized with a solution consisting of NPK (15-5-30) at 31 kg/ha and iron chelate and micronutrients at 0.9 kg/ha. Plants were maintained for 20 weeks. The temperature was recorded at 30 min interval with temperature probes 5TM (Decagon Devices, Inc.) placed at a depth of 15 cm in the soil.

Nematode densities were determined at transplantation (*Pi*). Soil samples were taken from each experimental plot and consisted of eight cores taken from the first 30 cm of soil with an auger of diameter 2.5 cm. Soil subsamples were mixed and passed through a 4 mm pore sieve to remove stones. The J2 were extracted from 500 cm^3 of soil using Baermann trays (Whitehead and Hemming, 1965) and incubated at 27 ± 2 °C for one week. Afterwards, the J2 were collected using a 25 μm aperture screen, counted, and expressed as J2 per 250 cm^3 of soil. At the end of the experiment, roots were carefully removed from the soil, washed, and weighed, and the galling index (*GI*) was evaluated on a scale from 0 to 10, where 0 = complete and healthy root system and 10 = plants and roots dead (Zeck, 1971). After that, the number of eggs per plant was determined as described previously and was considered the final nematode density (*Pf*). *RI* was calculated and the response of the *C. amarus* accessions and RO was categorized as described previously.

2.4. Grafting compatibility and fruit quality

The watermelon cultivar SB was self-grafted (SB-SB) and grafted onto CI64, CI67, RO, and the commercial hybrid *C. maxima* x *C. moschata* rootstock cv. Cobalt (CO) (Rijk Zwaan, BV, The Netherlands) according to the cleft procedure (Lee et al., 2010). Ten plants of each grafted combination were grown under a hydroponic system in a commercial greenhouse at Fundación Cajamar (Paiporta, Valencia) during the spring–summer 2018. The ungrafted watermelon SB was included for comparison. To evaluate the impact of grafting on fruit quality, ten fruits per treatment were characterized for the following traits: weight, length and width, rind and flesh thickness, flesh firmness (measured with a digital Penetrometer (8 mm) FHT-803®, Melrose, MA), pH (measured with the pH indicator paper pH1-14; Merck, Darmstadt, Germany), total soluble solids (quantified using the digital refractometer Atago®, Tokyo, Japan), and flesh color (measured with the colorimeter Minolta CR-400, New Jersey, USA) using the color parameters Hunter L, a and b, where the L value indicates lightness (from 0 to 100), a value indicates redness (+) or greenness (−), and b value indicates yellowness (+) or blueness (−).

2.5. Statistical analysis

Statistical analyses were performed using R Statistical Software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The data on the number of egg masses and eggs per plant were not normally distributed according to the normal Shapiro–Wilk *W* test. Data from both repetitions of the second experiment were submitted to the nonparametric Mann–Whitney *U* test and pooled together as replications of the same experiment because no differences were found ($P \geq 0.05$). Comparisons between plant germplasm per each RKN isolate, as well as between RKN isolates per each plant germplasm within each experiment were done by the Mann–Whitney *U* test (two groups) or the Kruskal–Wallis non parametric test (more than two groups). When significant ($P < 0.05$), medians were separated using pairwise multiple comparisons by the Dunn test ($P < 0.05$). Data on fruit quality traits of each grafted combination were compared to those of the ungrafted control SB by the Student t-test ($P < 0.05$).

3. Results

3.1. Pot experiments

The number of egg masses and eggs per plant was lower ($P < 0.05$) in both *C. amarus* accessions than in the watermelon SB, irrespective of the RKN isolate. Both *C. amarus* accessions responded as resistant ($1\% \leq RI < 10\%$) to the majority of the RKN isolates. The accession CI64 responded only as moderately resistant to the *M. javanica* isolate Tugues, and both CI67 and RO were moderately resistant to the *Mi1.2* virulent MJLg isolate of *M. javanica* (Table 2).

3.2. Experiment under plastic greenhouse

The minimum and maximum soil temperatures during the experiment were 18.4 °C and 30.5 °C, respectively. The initial nematode densities at transplantation ranged from 46 to 1392 J2 per 250 cm^3 of soil. The number of eggs per plant and the galling index were significantly lower ($P < 0.05$) in both *C. amarus* accessions than those in the watermelon SB and the rootstock RO. Both CI ac-

Table 4
Fruit quality parameters of the watermelon cv. Sugar Baby (SB) produced by ungrafted plants, self-grafted (SB-SB) plants, plants grafted onto the commercial hybrid rootstock *Cucurbita maxima* x *Cucurbita moschata* cv. Cobalt (CO), *Citrullus lanatus* cv. Robusta (RO), and the experimental *Citrullus amarus* accessions BGV0005167 (CI67) and BGV0005164 (CI64) cultivated under the hydroponic system in greenhouse.

	Fruit size			Rind thickness (mm)	Flesh thickness (cm)	Flesh firmness (kg.cm^{-2})	Soluble solid (Brix^{o})	pH	Color[b]		
Rootstock-scion	Weight (kg)	Length (cm)	Width (cm)						*L*	*a*	*b*
SB	4.7 ± 0.5[a]	20.33 ± 0.54	21.10 ± 0.44	11.03 ± 1.07	18.77 ± 0.11	1.33 ± 0.16	10.67 ± 0.32	5.21 ± 0.19	33.38 ± 1.35	18.58 ± 1.24	12.03 ± 0.34
SB-SB	5.0 ± 0.3	20.96 ± 0.38	21.47 ± 0.23	11.25 ± 0.78	18.87 ± 0.27	1.52 ± 0.14	10.03 ± 0.23	5.00 ± 0.11	30.09 ± 1.61	18.30 ± 0.87	11.00 ± 1.01
CO-SB	5.5 ± 0.2*	21.25 ± 0.47	21.95 ± 0.38	11.12 ± 0.92	19.50 ± 0.35	1.75 ± 0.14	9.45 ± 0.27 *	5.38 ± 0.16	32.91 ± 1.17	20.63 ± 1.07	12.05 ± 0.54
RO-SB	5.1 ± 0.3	20.85 ± 0.38	21.32 ± 0.33	12.92 ± 0.73	18.65 ± 0.27	1.62 ± 0.11	10.02 ± 0.22	5.0 ± 0.17	31.29 ± 0.78	18.94 ± 0.38	11.49 ± 0.33
CI64-SB	5.2 ± 0.2	21.42 ± 0.38	21.55 ± 0.31	14.04 ± 0.75*	18.4 ± 0.29	1.76 ± 0.12*	9.77 ± 0.22 *	5.00 ± 0.13	31.94 ± 0.96	19.08 ± 0.87	11.98 ± 0.44
CI67-SB	5.0 ± 0.2	20.93 ± 0.31	21.38 ± 0.25	12.89 ± 0.62	18.54 ± 0.24	1.62 ± 0.09	10.3 ± 0.18	5.17 ± 0.13	32.80 ± 0.88	19.03 ± 0.71	12.18 ± 0.36

[a] Data are the mean ± standard error of 10 replicates. Date in the same column followed by * indicate significant differences ($P < 0.05$) with regard to the ungrafted watermelon cv. Sugar Baby (SB) according to Student's t-test.
[b] Color: parameters measured in fruit flesh: Hunter L, lightness (from 0 to 100); a, red (+); b, yellow (+) or blue (–).

cession and the rootstock RO performed as resistant ($1\% \leq RI < 10\%$) to *M. incognita*. (Table 3).

3.3. Grafted compatibility and fruit quality

Under our experimental conditions, both ungrafted watermelon SB and watermelon SB grafted onto different rootstocks showed a similar growth performance. However, some effects of fruit traits were observed in plants grafted onto specific rootstocks (Table 4). The weight of watermelon fruits produced by SB onto the *Cucurbita* hybrid rootstock CO was greater ($P < 0.05$) than the weight of those produced by the ungrafted plants (5.5 ± 0.2 vs. 4.7 ± 0.5 kg) but with a significant decrease ($P < 0.05$) of soluble solids (9.45 ± 0.27 vs. 10.67 ± 0.32 °Bx). The watermelon rootstocks RO and CI67 did not influence the fruit traits compared to those produced by the ungrafted and self-grafted SB, but the rootstock CI64 produced fruits with thicker rinds, firmer flesh, and less soluble solids ($P < 0.05$) (Table 4).

4. Discussion

The results of this study showed that the *C. amarus* accessions CI64 and CI67 are resistant to several nematode isolates belonging to the three most widespread RKN species. Some other *C. amarus* accessions resistant to RKN have been reported previously (Huitrón et al., 2007; Thies et al., 2015c), thus increasing the number of accessions that could be used as putative watermelon rootstock. The watermelon has been described as a poor host of *Meloidogyne* owing to its low values of maximum multiplication rate and equilibrium density (López-Gómez et al., 2014). The RKN isolates assessed in this study reproduced less than 10% in both *C. amarus* accessions compared to that in the watermelon cv. Sugar Baby in both pot and plastic greenhouse experiments, which demonstrates their potential for suppressing the RKN population growth rate. Other *C. amarus* accessions and lines have also been shown to be RKN resistant under field and plastic greenhouse conditions (Huitrón et al., 2007; Thies et al., 2008, 2009, 2015a, 2015b, 2015c). The resistance of *C. amarus* to RKN has been associated with the relatively high root fibrosity compared to that of *C. lanatus* var. *lanatus, Citrullus colocynthis, L. siceraria,* and *C. maxima* x *C. moschata* (Thies and Levi, 2003, 2007; Thies et al., 2015c, 2016).

Interestingly, both *C. amarus* accessions assessed in this study were also resistant to a *Mi1.2* gene virulent isolate. This finding shows the usefulness to include this germplasm as a component of the rotation scheme for managing virulent RKN isolates for specific resistance genes. The most available resistance genes to RKN in vegetables are in solanaceous cultivars and rootstocks, e.g., tomato and pepper. The virulence to a given R-gene could be counter-selected by other R-genes because it is highly specific and it has a fitness cost to be acquired (Djian-Caporalino et al., 2011). Recently, some *Cucumis metuliferus* accessions have been described as resistant to *Mi1.2* gene virulent RKN isolates (Expósito et al., 2018a), and although the selection for virulence to the *Mi1.2* gene was not prevented when alternated with tomato grafted onto the resistant rootstock cv. Aligator, it influenced its level (Expósito et al., 2018b). The availability of some more sources of resistance used in rotation schemes could favor the durability of specific resistant genes by preventing the fixation of the virulence character in the RKN population.

Grafting commercial watermelon cultivars onto resistant rootstocks has proven to be a successful approach to manage plant diseases, being a widely accepted practice in some parts of the world (Oda, 2002; Miguel et al., 2004; Cohen et al., 2007; Yetişir et al., 2007; Leonardi et al., 2017). *Cucurbita* hybrids, the most popular watermelon rootstocks, are resistant to some soil-borne fungal diseases but susceptible to RKN (López-Gómez et al., 2016; Giné et al., 2017). The results of this study showed that both *C. amarus* accessions are able to suppress RKN at the same level as that of the commercial *C. lanatus* cv. Robusta. In addition, these two experimental accessions have also been proved to be moderately to highly resistant to *Fusarium oxysporum* f. sp. *niveum* (Fon) races 0 and 2 (Garcés et al., personal communication), which improve their success as watermelon rootstock. Some other *C. amarus* accessions also showed resistance to other diseases such as gummy stem blight (Gusmini et al., 2005), powdery mildew (Davis et al., 2007; Tetteh et al., 2010), and potyviruses (Guner, 2004; Strange et al., 2002; Guner and Wehner, 2008).

Both *C. amarus* accessions have shown efficient grafting compatibility to watermelon, but they differed in influencing the fruit quality. While the quality of fruit produced by the watermelon grafted onto the CI67 accession did not show significant difference from that produced by the ungrafted and self-grafted plants, it did show a significant difference when grafted onto CI64. Similar results were obtained with the watermelon F1 hybrid cv. Oneida onto CI67 (Fredes et al., 2017). This previous study also showed that the citron melon accession affected the aroma of the watermelon flesh less than the hybrid *Cucurbita* rootstock, which, in turn, produced larger fruit with less soluble solids.

5. Conclusion

The *C. amarus* accession CI67 is a promising rootstock for managing the three tropical RKN species without influencing watermelon fruit quality.

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