Document downloaded from:

http://hdl.handle.net/10251/155303

This paper must be cited as:

Garcia-Mendivil, HA.; Munera, M.; Giné, A.; Escudero, N.; Picó Sirvent, MB.; Gisbert Domenech, MC.; Sorribas, FJ. (2019). Response of two Citrullus amarus accessions to isolates of three species of Meloidogyne and their graft compatibility with watermelon. Crop Protection. 119:208-213. https://doi.org/10.1016/j.cropro.2019.02.005

The final publication is available at

https://doi.org/10.1016/j.cropro.2019.02.005

Copyright Elsevier

Additional Information

This article has been published in Crop Protection. Please cite as:

García-Mendívil HA, Munera M, Giné A, Escudero N, Picó MB, Gisbert C, Sorribas FJ. 2019. Crop Protection 119:208-2013. https://doi.org/10.1016/j.cropro.2019.02.005

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

Helio A. García-Mendívil[a], Mari[a] 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**

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 cm3 of sterile sand and maintained in a growth chamber at 25º 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 cm3 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.

Keywords:, *Meloidogyne spp., rootstock,*

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 (Gine et al., 2014; Vela et al., 2014; Lopez-Gomez

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 management 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 resistant rootstocks is an alternative method to control soilborne pathogens when no commercial resistant cultivars are available (Yetis ¸ir 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; Lopez-Gomez et al., 2016; Gine 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 (Huitron et al., 2007; Levi et al., 2017) and some RKN species in both greenhouse (Thies and Levi, 2003, 2007) and open field cultivation (Huitron 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 by maceration of the infected roots in 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 ^{o}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 (Lopez-Gomez 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 Exposito et al. (2018b). Seedlings were transplanted to 200 cm3 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 cm3 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% P2O5, 12% K2O, 2% MgO2, 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 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% <RI <10%), moderately resistant (10% <RI <25%), slightly resistant (25% <RI <50%), or susceptible (RI >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 m2 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 cm3 of soil using Baermann trays (Whitehead and Hemming, 1965) and incubated at 27 ±2 ºC for one week. Afterwards, the J2 were collected using a 25 μm aperture screen, counted, and expressed as J2 per 250 cm3 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 ¼complete and healthy root system and 10 ¼plants 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 Fundacion 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 (þ) or greenness ( ), and b value indicates yellowness (þ) 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 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.

3.Results

3.1. Pot experiments

The number of egg masses and eggs per plant was lower (P <0.05) in 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 cm3 of soil. Plant both *C. amarus* accessions than in the watermelon SB, irrespective of the RKN isolate. Both *C. amarus* accessions responded as resistant (1% <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 ^{o}C and 30.5 ^{o}C, respectively. The initial nematode densities at transplantation ranged from 46 to 1392 J2 per 250 cm3 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 accession and the rootstock RO performed as resistant (1% <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 ^{o}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 (Huitron 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 (Lopez-Gomez 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 (Huitron 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 (Exposito 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; Yetis ¸ir 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 (Lopez-Gomez et al., 2016; Giné e 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 (Garces 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.

4.Conclusion

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

5.Acknowledgments

The authors thank MINECO and FEDER for funding projects AGL2013-49040-C2-1-R, AGL2014-53398-C2-2-R-AR, AGL2017- 89785-R, and AGL2017-85563-C2-1-R-AR. H. A. García-Mendívil acknowledges CONACYT for suport through a pre-doctoral grant.

References

Cohen, R., Burger, Y., Horev, C., Koren, A., 2007. Introducing grafted cucurbits to modern agriculture: the Israeli experience. Plant Disease 91 (8), 916–923. 10.1094/PDIS-91-8-0916.

Colla G, Pérez-Alfocea F, Schwarz D. eds. 2017. Quality of Grafted Vegetables In: *Vegetable Grafting: Principles and Practices*. Oxfordshire, UK.CAB International 216-244.

Davis, A.R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J.V., Lee, S.-G., Huh, Y.-C., Sun, Z., Miguel, A., King, S.R., 2008. Cucurbit grafting. Critical Reviews in Plant Sciences 27 (1), 50–74. 10.1080/07352680802053940.

Djian-Caporalino, C., Molinari, S., Palloix, A., Ciancio, A., Fazari, A., Marteu, N., Ris, N., Castagnone-Sereno, P., 2011. The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper. Eur J Plant Pathol 131 (3), 431–440. 10.1007/s10658-011-9820-4.

Ehwaeti, M.E., Fargette, M., Phillips, M.S., Trudgill, D.L., 1999. Host status differences and their relevance to damage by *Meloidogyne incognita*. Nematology 1 (4), 421–432. 10.1163/156854199508315.

Expósito, A., Munera, M., Giné, A., López-Gómez, M., Cáceres, A., Picó, B., Gisbert, C., Medina, V., Sorribas, F.J., 2018. *Cucumis metuliferus* is resistant to root-knot nematode Mi1. 2 gene (a) virulent isolates and a promising melon rootstock. Plant Pathology 67 (5), 1161–1167.

FAO (2016). FAOSTAT statistical database. Available at faostat.fao.org. Accessed August 2013.

Fourie, H., Mothata, T.S., Ntidi, K.N., Waele, D. de, 2012. Indications of variation in host suitability to root-knot nematode populations in commercial tomato varieties. African Journal of Agricultural Research 7 (15), 2344–2352. 10.5897/AJAR11.706.

Fredes, A., Roselló, S., Beltrán, J., Cebolla-Cornejo, J., Pérez-de-Castro, A., Gisbert, C., Picó, M.B., 2017. Fruit quality assessment of watermelons grafted onto citron melon rootstock. Journal of the Science of Food and Agriculture 97 (5), 1646–1655. 10.1002/jsfa.7915.

Giné, A., González, C., Serrano, L., Sorribas, F.J., 2017. Population dynamics of *Meloidogyne incognita* on cucumber grafted onto the Cucurbita hybrid RS841 or ungrafted and yield losses under protected cultivation. Eur J Plant Pathol 148 (4), 795–805. 10.1007/s10658-016-1135-z.

Giné, A., López-Gómez, M., Vela, M.D., Ornat, C., Talavera, M., Verdejo-Lucas, S., Sorribas, F.J., 2014. Thermal requirements and population dynamics of root-knot nematodes on cucumber and yield losses under protected cultivation. Plant Pathol 63 (6), 1446–1453. 10.1111/ppa.12217.

Giné, A., Sorribas, F.J., 2017. Effect of plant resistance and BioAct WG (*Purpureocillium lilacinum* strain 251) on Meloidogyne incognita in a tomato-cucumber rotation in a greenhouse. Pest management science 73 (5), 880–887. 10.1002/ps.4357.

Hadisoeganda, W.W., Sasser, J.N., 1982. Resistance of tomato, bean, southern pea, and garden pea cultivars to root-knot nematodes based on host suitability. Plant Disease 66 (2), 145–150.

Hassell, R.L., Memmott, F., Liere, D.G., 2008. Grafting methods for watermelon production. HortScience 43 (6), 1677–1679.

Huitrón, M.V., Diaz, M., Diánez, F., Camacho, F., 2007. The effect of various rootstocks on triploid watermelon yield and quality. Journal of Food Agriculture and Environment 5 (3/4), 344.

Hussey, R.S., 1973. A comparison of methods of collecting inocula of Meloidogyne spp., including a new technique. Plant Dis. Rep. 57, 1025–1028.

Kokalis-Burelle, N., Rosskopf, E.N., 2011. Microplot evaluation of rootstocks for control of Meloidogyne incognita on grafted tomato, muskmelon, and watermelon. Journal of nematology 43 (3-4), 166.

Kyriacou, M.C., Soteriou, G.A., Rouphael, Y., Siomos, A.S., Gerasopoulos, D., 2016. Configuration of watermelon fruit quality in response to rootstock-mediated harvest maturity and postharvest storage. Journal of the Science of Food and Agriculture 96 (7), 2400–2409. 10.1002/jsfa.7356.

Lee, J.-M., ODA, M., 2002. Grafting of herbaceous vegetable and ornamental crops. Horticultural reviews 28, 61–124. 10.1002/9780470650851.ch2.

Lee JM, Oda M, 2010. Grafting of herbaceous vegetable and ornamental crops. *Horticultural Reviews* **28**, 61-124.

Levi, A., Jarret, R., Kousik, S., Wechter, W.P., Nimmakayala, P., Reddy, U.K., 2017. Genetic resources of watermelon, in: Genetics and Genomics of Cucurbitaceae. Springer, pp. 87–110. 10.1007/7397_2016_34

López-Gómez, M., Flor-Peregrín, E., Talavera, M., Sorribas, F.J., Verdejo-Lucas, S., 2015. Population dynamics of *Meloidogyne javanica* and its relationship with the leaf chlorophyll content in zucchini. Crop Protection 70, 8–14. 10.1016/j.cropro.2014.12.015.

López-Gómez, M., Gine, A., Vela, M.D., Ornat, C., Sorribas, F.J., Talavera, M., Verdejo-Lucas, S., 2014. Damage functions and thermal requirements of *Meloidogyne javanica* and *Meloidogyne incognita* on watermelon. Ann Appl Biol 165 (3), 466–473. 10.1111/aab.12154.

López-Gómez, M., Talavera, M., Verdejo-Lucas, S., 2016. Differential reproduction of *Meloidogyne incognita* and *M. javanica* in watermelon cultivars and cucurbit rootstocks. Plant Pathol 65 (1), 145–153. 10.1111/ppa.12394.

334 Miguel, A., Maroto, J.V., San Bautista, A., Baixauli, C., Cebolla, V., Pascual, B., López, S.,
335 Guardiola, J.L., 2004. The grafting of triploid watermelon is an advantageous alternative to soil
336 fumigation by methyl bromide for control of Fusarium wilt. Scientia Horticulturae 103 (1), 9–17.
337 10.1016/j.scienta.2004.04.007.
338 Morris, K.A., Langston, D.B., Dickson, D.W., Davis, R.F., Timper, P., Noe, J.P., 2015. Efficacy of
339 fluensulfone in a tomato–cucumber double cropping system. Journal of nematology 47 (4), 310.
340 Oda, M., 2002. Grafting of vegetable crops. Sci. Rep. Agric. Biol. Sci., Osaka Pref. Univ. 54: 49–72.
341 Omwega, C.O., Thomason, I.J., Roberts, P.A., 1988. A nondestructive technique for screening bean
342 germplasm for resistance to *Meloidogyne incognita*. Plant Disease 72, 970–972.
343 Ornat, C., Verdejo-Lucas, S., Sorribas, F.J., 1997. Effect of the previous crop on population densities
344 of *Meloidogyne javanica* and yield of cucumber. Nematrópica 27: 85-90.
345 Ornat, C., Verdejo-Lucas, S., Sorribas, F.J., 2001. A Population of Meloidogyne javanica in Spain
346 Virulent to the Mi Resistance Gene in Tomato. Plant Disease 85 (3), 271–276.
347 10.1094/PDIS.2001.85.3.271.
348 Sorribas, F.J., Ornat, C., Verdejo-Lucas, S., Galeano, M., Valero, J., 2005. Effectiveness and
349 profitability of the Mi-resistant tomatoes to control root-knot nematodes. Eur J Plant Pathol 111
350 (1), 29–38. 10.1007/s10658-004-1982-x.
351 Talavera, M., Verdejo-Lucas, S., Ornat, C., Torres, J., Vela, M.D., Macias, F.J., Cortada, L., Arias,
352 D.J., Valero, J., Sorribas, F.J., 2009. Crop rotations with Mi gene resistant and susceptible tomato
353 cultivars for management of root-knot nematodes in plastic houses. Crop Protection 28 (8), 662–
354 667. 10.1016/j.cropro.2009.03.015.
355 Thies, J.A., Ariss, J., Kousik, C.S., Hassell, R., 2008. Grafting–a tool for managing root-knot
356 nematodes in watermelon. Phytopathology 98, S156.
357 Thies, J.A., Ariss, J., Hassell, R., Olson, S., 2009. Grafting for Management of Root-Knot Nematodes
358 in Watermelon. HortScience 44 (3), 576.

359 Thies, J.A., Ariss, J.J., Hassell, R.L., Olson, S., Kousik, C.S., Levi, A., 2010. Grafting for
360 management of southern root-knot nematode, *Meloidogyne incognita*, in watermelon. Plant
361 Disease 94 (10), 1195–1199. 10.1094/PDIS-09-09-0640.
362 Thies, J.A., Ariss, J.J., Hassell, R.L., Buckner, S., Levi, A., 2015a. Accessions of *Citrullus lanatus*
363 var. *citroides* are valuable rootstocks for grafted watermelon in fields infested with root-knot
364 nematodes. HortScience 50 (1), 4–8.
365 Thies, J.A., Ariss, J.J., Kousik, C.S., Hassell, R.L., Levi, A., 2016. Resistance to southern root-knot
366 nematode (*Meloidogyne incognita*) in wild watermelon (*Citrullus lanatus* var. *citroides*). Journal
367 of Nematology 48 (1), 14.
368 Thies, J.A., Buckner, S., Horry, M., Hassell, R., Levi, A., 2015b. Influence of *Citrullus lanatus* var.
369 *citroides* rootstocks and their F1 hybrids on yield and response to root-knot nematode,
370 *Meloidogyne incognita*, in grafted watermelon. HortScience 50 (1), 9–12.
371 Thies, J.A., Davis, R.F., Mueller, J.D., Fery, R.L., Langston, D.B., Miller, G., 2004. Double-cropping
372 cucumbers and squash after resistant bell pepper for root-knot nematode management. Plant
373 Disease 88 (6), 589–593. 10.1094/PDIS.2004.88.6.589.
374 Thies, J.A., Levi, A., 2003. Resistance of watermelon germplasm to the peanut root-knot nematode.
375 HortScience 38 (7), 1417–1421.
376 Thies, J.A., Levi, A., 2007. Characterization of watermelon (*Citrullus lanatus* var. *citroides*)
377 germplasm for resistance to root-knot nematodes. HortScience 42 (7), 1530–1533.
378 Thies, J.A., Levi, A., Ariss, J.J., Hassell, R.L., 2015c. RKVL-318, a root-knot nematode-resistant
379 watermelon line as rootstock for grafted watermelon. HortScience 50 (1), 141–142.
380 U.S. Environmental Protection Agency. 2012. Ozone layer protection. Available at
381 https://www.epa.gov/ozone-layer-protection. Accessed August 2018.
382 Vela, M.D., Giné, A., López-Gómez, M., Sorribas, F.J., Ornat, C., Verdejo-Lucas, S., Talavera, M.,
383 2014. Thermal time requirements of root-knot nematodes on zucchini-squash and population

dynamics with associated yield losses on spring and autumn cropping cycles. Eur J Plant Pathol 140 (3), 481–490. 10.1007/s10658-014-0482-x.

Verdejo-Lucas, S., Blanco, M., Cortada, L., Sorribas, F.J., 2013. Resistance of tomato rootstocks to *Meloidogyne arenaria* and *Meloidogyne javanica* under intermittent elevated soil temperatures above 28 C. Crop Protection 46, 57–62. 10.1016/j.cropro.2012.12.013.

Verdejo-Lucas, S., Cortada, L., Sorribas, F.J., Ornat, C., 2009. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of Mi resistance gene tomato rootstocks under field conditions. Plant Pathology 58 (5), 990–998. 10.1111/j.1365-3059.2009.02089.x.

Westphal, A., 2011. Sustainable approaches to the management of plant-parasitic nematodes and disease complexes. Journal of nematology 43 (2), 122.

Whitehead, A.G., Hemming, JR, 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann Appl Biol 55 (1), 25–38.

YETİŞİR, H., Kurt, Ş., Sari, N., TOK, F.M., 2007. Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: plant growth, graft compatibility, and resistance to Fusarium. Turkish Journal of Agriculture and Forestry 31 (6), 381–388.

Yetışır, H., Sari, N., Yücel, S., 2003. Rootstock resistance to Fusarium wilt and effect on watermelon fruit yield and quality. Phytoparasitica 31 (2), 163–169. 10.1007/BF02980786.

Zeck, W.M., 1971. Rating scheme for field evaluation of root-knot nematode infestations. Pflanzenschutz nachrichten.

Zijlstra, C., Donkers-Venne, D.T., Fargette, M., 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematology 2 (8), 847–853. 10.1163/156854100750112798.

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	Exposito *et al.*, 2018
M. incognita	Agrópolis	Barcelona	Avirulent	Giné & Sorribas, 2017
	Garriga	Barcelona	Avirulent	Exposito *et al.*, 2018
	MiMan	Almería	Virulent	García-Mendívil *et al.*, 2018 (Not published, yet)
M. javanica	MJ05	Barcelona	Avirulent	Ornat *et al.*, 2001
	Tugues	Barcelona	Avirulent	Exposito *et al.*, 2018
	Bay	Murcia	Avirulent	Exposito *et al.*, 2018

406

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 *Citrullus lanatus* var. *citroides* accessions BGV5167 and BGV5264 in experiments 1 and experiment 2, and the watermelon commercial rootstock *C. lanatus* var. *lanatus* cv. Robusta on experiment 3, and on the watermelon cv. Sugar baby.

			Egg masses per plant			Eggs per plant x 100				RI (%) [a]		
			C. lanatus var. *citroides*		*C. lanatus* var. *lanatus*	*C. lanatus* var. *citroides*		*C. lanatus* var. *lanatus*		*C. lanatus* var. *citroides*		*C. lanatus* var. *lanatus*
Experiment	Species	Isolate	CIBVB5167	CIBGV5264	Sugar Baby	CIBVB5167	CIBGV5264	Robusta	Sugar baby	CIBVB5167	CIBGV5264	Robusta
Experiment 1	*M. incognita*	Agrópolis	1 ± 0.7 b	1 ± 0.2 b	17 ± 3.7 a	0.46 ± 0.32 b	0.3 ± 0.1 b	-	126.9 ± 26.7 a	4 ± 3	3 ± 1	-
	M. javanica	MJ05	2 ± 0.7 b	1 ± 0.6 b	25 ± 0.20 a	1.27 ± 1.05 b	877 ± 818 b	-	180.5 ± 32.5 a	7 ± 6	5 ± 5	-
Experiment 2	*M. arenaria*	MA68	0.5 ± 0.2 b AB	0.8 ± 0.2 b A	5 ± 1.2 a A	1.1 ± 0.6 b	2.1 ± 0.8 b AB	-	39.8 ± 11.4 a A	3 ± 1	4 ± 1	-
	M. incognita	Agropolis	0.1 ± 0.1 b B	0.5 ± 0.4 b AB	5 ± 1.6 a A	0.1 ± 0.1 b	0.4 ± 0.4 b AB	-	23.9 ± 13.7 a AB	0.5 ± 0.5	2 ± 2	-
		Garriga	0.2 ± 0.1 b AB	0.3 ± 0.1 b AB	4 ± 0.9 a AB	0.3 ± 0.2 b	0.1 ± 0 b B	-	12.2 ± 3.5 a AB	2 ± 2	0.5 ± 0.3	-
	M. javanica	Bay	0.6 ± 0.2 b B	0.3 ± 0.1 b AB	6 ± 1.7 a A	0.3 ± 0.1 b	0.2 ± 0.1 b B	-	33.8 ± 12 a A	0.9 ± 0.4	0.3 ± 0.1	-
		MJ05	0.4 ± 0.2 b AB	0.7 ± 0.2 b AB	5 ± 1 a A	1.0 ± 0.8 b	1.2 ± 0.8 b A	-	30 ± 9.1 a AB	3 ± 3	6 ± 3	-
		Tugues	0.4 ± 0.2 b AB	0.1 ± 0.1 b B	1 ± 0.1 a B	0.3 ± 0.1 b	0.5 ± 0.5 b AB	-	3.7 ± 1.7 a B	5 ± 3	14 ± 13	-
Experiment 3	*M. incognita*	MiMan	-	-	-	0.7 ± 0.6 b	0.3 ± 0.3 b	1.2± 0.5 ab	6.6 ± 2.4 a	10.4 ± 8.8	5.0 ± 5.0	17.5 ± 8.1

Data are mean ± the standard error of 15 repetitions on experiments 1 and 2, and 10 on the experiment 3. Data within the same column and experiment followed by the same letter did not differ ($P \leq 0.05$) according to the Kruskal-Wallis test. Different capital letter on the same column and experiment indicate significant differences ($P \leq 0.05$) between nematode species on the same germplasm according to the Kruskal-Wallis test.

[a]RI (reproduction index) calculated as the number of eggs per plant in the CI accessions by the number of eggs per plant on the susceptible cv. Sugar baby × 100.

407

Table 3. Gall index, eggs per plant, reproduction factor (*Rf*), reproduction index (*RI*) and fruit biomass per plant (kg) of *M. incognita* on the watermelon cv. *Sugar baby*, the watermelon rootstock cv. Robusta and the *C. lanatus* var. *citroides* (CI) accessions conducted in a nematode-infested plastic house (*Pi:* 46-1392 J2 250 cm^{-3}) after 93 d of culture from May to August in 2016.

Germplasm	*GI*[a]	Eggs per plant (× 1000)	*RI*[b]
Sugar Baby	5.0 ± 0.59 a	103.1 ± 48.4 a	
Robusta	2.8 ± 0.38 b	5.0 ± 1.1 b	39.7 ± 23.1 a
CIBGV5167	1.5 ± 0.52 b	1.5 ± 1.1 c	11.3 ± 7.8 b
CIBGV5264	2.5 ± 0.52 b	1.6 ± 0.9 c	4.1 ± 2.1 b

Data are mean ± the standard error of 10 repetitions. Different letter on the same column and nematode specie indicate significant differences ($P \leq 0.05$) between germplasms according to the Kruskal-Wallis non-parametric test.
[a]*GI* (Gall index) on scale from 0 to 10, where 0 = complete and healthy root system and 10 = plant and roots dead (Zeck 1971).
[b]*RI* (reproduction index) calculated as the number of eggs per plant in the resistant cv. Robusta and the CI accessions by the number of eggs per plant on the susceptible cv. Sugar baby × 100.

408

409

410 **Table 4.** Fruit quality parameters of the 'Sugar baby' watermelon (SB) from plants un-grafted, selfgrafted (SB-SB) and grafted onto the
411 commercial rootstocks Cobalt (CO) and Robusta (RO) and the experimental *C. citrullus* var. *citroides* rootstocks BGV5167 (CI67) and
412 BGV5264 (CI64) grown under hydroponic greenhouse conditions.

eRootstock-scion	Fuit size			Rind thickness (mm)	Flesh thickness (cm)	Flesh firmness ($kg.cm^{-2}$)	*Brix*[b]	*pH*	Colour[c]		
	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)						*L*	*a*	*b*
SB	4732.33 ± 349.40[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	5041.5 ±247.06	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	5482.51 ± 202.59*	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	5060.53 ± 247.06	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	5221.67 ± 237.06	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	4961.11 ± 201.73	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	32.8 ±0.88	19.03± 0.71	12.18± 0.36

[a]Data are mean ± SE of ten replicates. Asterisks indicate significant differences ($P \leq 0.05$) as determined by Student's t-test compared with the ungrafted control.

[b]Brix: soluble solid content measured in fruit flesh and Brix degrees.

[c]Colour parameters measured in fruit flesh: Hunter L, lightness (from 0 to 100); a, red (+); b, yellow (+) or blue (-)