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RESEARCH ARTICLE

A new grafted rootstock against root-knot nematode for cucumber, melon, and watermelon

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Abstract Southern root-knot nematode causes dramatic galling on the roots of cucurbitaceous plants such as cucumber, melon, and watermelon. Even low nematode levels can cause high yield losses. Control of root-knot nematode is usually based on soil fumigation with toxic methyl bromide. However, since methyl bromide is now banned, growers are looking for alternative pest control. A potential solution is to graft susceptible scions onto nematode-resistant rootstocks. Here, we selected a *Meloidogyne incognita*-resistant rootstock suitable for cucumber, melon, and watermelon scions. First, we screened the resistance against nematode and *Fusarium*. Then, we tested a wild *Cucumis* species, *Cucumis pustulatus*, as a possible rootstock for cucumber, melon, and watermelon scions. We measured the survival rate, plant growth, yield, and fruit quality of grafted plants. Fifty-three accessions from 16 species were studied. Five accessions exhibited high resistance to southern root-knot nematode, and 12 accessions exhibited resistance to Fusarium wilt. This research is the first study to report that *C. pustulatus* is a suitable rootstock with simultaneous resistance to root-knot nematode and Fusarium wilt for cucumber, melon, and watermelon. *C. pustulatus* rootstocks are thus promising for low-input sustainable horticulture. They should benefit to home gardeners, especially to those in areas highly infested with southern root-knot nematode.

Keywords *Cucumis pustulatus* · *Meloidogyne incognita* · Fusarium wilt · Grafting · Rootstock

1 Introduction

Meloidogyne incognita (Kofoid and White 1919) Chitwood, 1949 is the predominant root-knot nematode species (*Meloidogyne* spp.) infecting cucurbitaceous plants such as cucumber (*Cucumis sativus*), melon (*Cucumis melo*), and watermelon (*Citrullus lanatus*). It causes dramatic galling on the roots of host plants, and low nematode levels can cause significant yield losses (Sasser et al. 1983). Root-knot nematodes can also increase the severity of soilborne diseases such as Fusarium wilt in cucurbit crops (Wang and Roberts 2006).

Control of root-knot nematode is generally based on methyl bromide soil fumigation, but with the withdrawal of methyl bromide, growers are looking for alternative non-chemical pest management approaches (Ristaino and Thomas 1997). In addition, the markets for organically grown fruit are increasing, where the use of chemicals is not allowed. Thus, the use of root-knot nematode-resistant varieties is one of the most economical, efficient, and environmentally friendly control measures. However, root-knot nematode resistance has not been identified in *C. melo* and *C. sativus* (Siguenza et al. 2005; Mukhtar et al. 2013). Resistance to root-knot nematodes was found in cucurbitaceous species such as *Cucumis metuliferus* (Wehner et al. 1991), but attempts to incorporate this resistance into cultivated species have not been successful yet (Walters and Wehner 2002). One method to solve this problem is to graft susceptible scions onto nematode-resistant rootstocks (Siguenza et al. 2005).

Bin Liu and Jiaojiao Ren contributed equally to this work

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Recently, *Cucurbita moschata* and *Cucurbita ficifolia* were evaluated as rootstocks for commercial production of cucurbitaceous cultivated species (Huang et al. 2010; Trionfetti Nisini et al. 2002). In China, where farming land is limited and farmers have to grow the same crop without rotation, soilborne diseases such as root-knot nematodes and Fusarium wilt have become a serious limitation for using only one *Cucurbita* species as rootstock. Screening root-knot nematode-resistant germplasm and developing resistant rootstocks would provide an economical and environmentally friendly method for disease management (Fig. 1).

In recent years, there were some studies evaluating *Cucumis* germplasm for resistance to root-knot nematodes and on their use for disease management (Siguenza et al. 2005; Wehner et al. 1991). However, suitable rootstocks of wild *Cucumis* species with simultaneous resistance to root-knot nematode and Fusarium wilt have not been reported in cucumber, melon, and watermelon The objective of this study was to screen wild *Cucumis* lines that may be used as suitable rootstocks resistant to southern root-knot nematodes and produce high yield and good fruit quality on the grafted cucumber, melon, and watermelon cultivars.

2 Materials and methods

2.1 Study location

The study was conducted in the greenhouse of Jinliuhuan experimental station, China Agricultural University in Beijing, China (40° 02′ N and 116° 02′ E). The temperature was maintained at 26–30 °C (day) and 18–22 °C (night). The soil pH was 6.5–7.5.

2.2 Nematode resistance screening assay

Fifty-three accessions of wild *Cucumis* germplasm from the National Plant Germplasm System, US Department of Agriculture, were evaluated for resistance to *M. incognita* in a greenhouse study. The experimental design was a randomized complete block with 56 *Cucumis* genotypes × four replicates × five plants per replicate (n=20) in the first test in spring and four replicates × ten plants per replicate (n=40) in the second test in autumn.

Seed from each *Cucumis* accession was sown in a 15-cm-diameter plastic pot filled with autoclaved soil (50 % nutritive soil and 50 % vermiculite) and placed in a greenhouse with temperature maintained between 25 and 30 °C. When seedlings were at the first true leaf stage, approximately 2,000 freshly hatched J2 of *M. incognita* race 3 (from Institute of Plant Protection, China Academy of Agricultural Sciences) suspended in 20 mL of water were pipetted into five equidistant 3-cm-deep holes around each plant. The inoculation holes were refilled with autoclaved soil, and pots were watered immediately to moisten the soil. Pots were moved into a greenhouse, which was maintained at 26–30 °C (day) and 18–22 °C (night).

Eight weeks later, the roots of all plants were removed from the soil, carefully rinsed in running tap water and evaluated for galling severity using a 1 to 9 scale in which 1=0, 2=1 to 3 %,

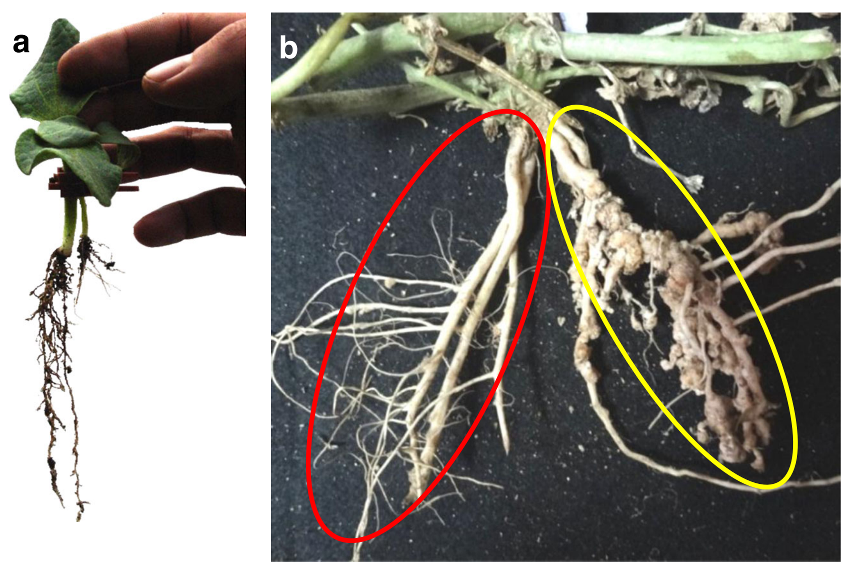

Fig. 1 Comparison of nematode resistance between *Cucumis pustulatus* and *Cucumis sativus* by grafting roots together. **a** Graft was made by approach - method. **b** In order to compare the nematode resistance between *Cucumis pustulatus* and *Cucumis sativus* under the same soil condition, the scion (*Cucumis sativus*) root system was left in place after grafting. *Cucumis pustulatus* root (*left red loop*) of grafting seedlings exhibited high resistance, while *Cucumis sativus* (*right yellow loop*) root of the same seedlings showed susceptible to *Meloidogyne incognita.* However, This experiment was only performed for comparing resistance to nematode, when crops were grew for production, the scion root system would not be left in place

3=4 to 12 %, 4=13 to 25 %, 5=26 to 38 %, 6=39 to 50 %, 7=51 to 65 %, 8=66 to 80 %, and 9=81 to 100 % of root system galled or covered with egg masses, respectively (Thies and Fery 1998). Ratings were categorized as 1.0 to 2.9=high resistance, 3.0 to 3.9=moderate resistance, 4.0 to 4.9=low resistance, 5.0 to 6.9=susceptible, and 7.0 to 9.0=highly susceptible (Thies and Levi 2003).

2.3 Fusarium wilt screening assay

Fusarium wilt pathogen (*Fusarium oxysporum* f. sp. *melonis* Snycler and Hansen) from Department of Plant Protection, China Agricultural University was used to screen 53 accessions of *Cucumis* species from the U.S. PI *Cucumis* germplasm collection. The experimental design was a randomized complete block with 53 accessions×three replicates×20 plants per replicate (n=60). A root-cut dip method was performed for the *Fusarium oxysporum* f. *melonis* pathogenicity tests. Roots of 1-week-old seedlings from each line were cut and dipped into liquid suspension of 5×10^6 spores/mL for 5 min. After inoculation, the plants were placed in a greenhouse and symptoms were examined on the seventh day and then once every 2 days until 4 weeks. Individual plant was rated on a scale of 1 to 5 based on Chikh-Rouhou et al. (2010) with 1=healthy, no symptoms over a 4-week period, 2=beginning of wilting or yellowing on leaves, 3=slightly wilted and stunted plants, 4=all leaves completely wilted, stem standing, and 5=heavily wilted and death of the seedlings. Disease ratings were conducted over 4 weeks, which were 1.0 to 1.9=resistant, 2.0 to 3.9=intermediate, and 4.0 to 5=susceptible. Crown (stem-root junction) sections of selected plants, both susceptible and resistant, were surface sterilized and plated on quarter-strength potato dextrose agar to verify *Fusarium* infection (Wechter et al. 1995).

2.4 Grafting experimental design

One wild *Cucumis* species (*Cucumis pustulatus*) that was selected with resistance to both southern root-knot nematodes and Fusarium wilt and three cultivated species *Zhong Nong No.21 Cucumber* (*C. sativus*), *Elizabeth Melon* (*C. melo*) and *Beyond Dreams Watermelon* (*C. lanatus*) were used in this study. Seeds from the four species were sown directly in soil (50 % nutritive soil and 50 % vermiculite) and grown for 7 days in a non-heated greenhouse under 25/20 °C in day (8:00–20:00)/night (20:00–8:00). Then, the plants were grafted together (approach grafting), using the four species as the rootstocks and cucumber, melon, and watermelon as the scions. The resulting six groups of seedlings, designated as *C. sativus*/*C. pustulatus*, *C. melo*/*C. pustulatus*, *C. lanatus*/*C. pustulatus*, *C. sativus*/*C. sativus*, *C. melo*/*C. melo*, and *C. lanatus*/*C. lanatus*, respectively, were then placed in a mist chamber in a greenhouse, delivering a mist for 10 s/min to maintain at a constant humidity of 95–100 %. Seedlings were kept in the mist chamber at 25–30 °C for 6 days with dim light, and the misting interval was changed from 10 s/min to 10 s/10 min over this period. Seedlings were then transplanted into a greenhouse randomly with a high soil infestation (approximately 5,100 nematodes per liter of soil) of *M. incognita* under 25/18 °C in day (8:00–20:00)/night (20:00–8:00). For each scion-rootstock combination, 54 of them were transplanted (three replicates and 18 plants per replicate). These 18 plants were planted together as a “block.”

Plant growth was measured once a week after transplanting in the first 5 weeks. Photosynthetic analysis was carried out on the second flag leaf from the top of each plant by using LI-6400 photosynthesis equipment (LI-COR Company, USA) a month later. Yield and fruit quality were studied when fruits were ripe. Soluble sugar measured as described by Gomez et al. (2002), protein measured as described by Smith et al. (1985), and ascorbic acid measured as described by Roe and Kuether (1943). A total of 30 people were randomly selected to taste the fruits and according to their feelings of mouth to divide into four ranks (better, good, general, bad) with the value of 3.1–4.0, 2.1–3.0, 1.1–2.0, 0–1.0. At the late growth stage (About 3 months after transplanted), plants were carefully removed from the soil, and the roots were examined.

2.5 Statistical analysis

Treatment effects were analyzed using ANOVA procedures, and the significance of differences between treatments was tested using Duncan’s test. All analyses were carried out using the Statistics Analysis System (SAS) 9.0 for Windows.

3 Results and discussion

3.1 Germplasm screening for resistance to *M. incognita*

Of the 53 accessions, five exhibited high resistant and 33 were moderately resistant (Table 1). The root systems of these resistance accessions were sturdier and had fewer and smaller galls than the seven susceptible accessions. Some accessions in *Cucumis africanus*, *C. metulifer*, *Cucumis ficifolius*, *Cucumis anguria*, and *Cucumis myriocarpus* exhibited different level of

Table 1 The number of *Cucumis* germplasm response to root-knot nematode and Fusarium wilt

Germplasm	Number of accession	Response to root-knot nematode					Response to Fusarium wilt		
		High resistance	Moderate resistance	Low resistance	Susceptible	High susceptible	Resistant	Intermediate	Susceptible
Cucumis africanus	1	1	–	–	–	–	1	–	–
Cucumis anguria	4	–	4	–	–	–	4	–	–
Cucumis anguria var. *anguria*	3	–	3	–	–	–	2	1	–
Cucumis anguria var. *longaculeatus*	2	–	1	1	–	–	–	–	2
Cucumis dipsaceus	5	–	2	1	2	–	–	–	5
Cucumis ficifolius	7	1	6	–	–	–	–	–	7
Cucumis metuliferus	19	–	12	6	1		4	8	7
Cucumis myriocarpus	1	–	1	–	–	–	–	–	1
Cucumis myriocarpus subsp. *leptodermis*	1	–	–	1	–	–	–	–	1
Cucumis prophetarum	1	–	1	–	–	–	–	–	1
Cucumis pustulatus	1	1	–	–	–	–	1	–	–
Cucumis subsericeus	1	1	–	–	–	–	–	–	1
Cucumis zambianus	1	–	1	–	–	–	–	–	1
Cucumis zeyheri	3	1	2	–	–	–	–	–	3
Cucumis sativus var. *hardwickii*	2	–	–	–	1	1	–	–	2
Cucumis sativus var. *sikkimensis*	1	–	–	–	–	1	–	–	1

resistance to *M. incognita* which was consistent with previous studies (Pofu et al. 2010; Wehner et al. 1991; Siguenza et al. 2005; Fassuliotis 1970). This is the first report of resistance to root-knot nematodes in *Cucumis dipsaceus* (PI 193498), *Cucumis prophetarum* (PI 274036), *C. pustulatus* (PI 532673), *Cucumis subsericeus* (PI 273650), *Cucumis zambianus* (PI 505597), and *Cucumis zeyheri* (PI 532629). Of these resistant varieties, *C. pustulatus* (PI 532673) was the most resistant with average gall indexes of 1 for *M. incognita*.

Wild species of *Cucumis* are important as potential multigenic sources for resistance to nematodes (Fassuliotis 1970). Our results demonstrate that there is significant genetic variability within *Cucumis* for response to *M. incognita* race 3, and several *Cucumis* PIs may provide genetic sources of resistance to root-knot nematodes for the development of resistant cultivars.

3.2 Germplasm screening resistant to Fusarium wilt

Of the 53 accessions, 12 were resistant, nine were intermediate, and 32 were susceptible (Table 1). The 12 resistant accessions were from *C. africanus*, *C. anguria*, *C. metulifer*, and *C. pustulatus*, which were fully resistant to the pathogen and grew normally. Major differences in disease incidence among the 32 susceptible accessions tested were observed. The first symptoms of Fusarium wilt were observed in the susceptible seedlings as early as 7 days after inoculation, and plants died within 3 weeks. A correlation between wilt severity and inoculum concentration has been reported before (Martyn and Mclaughlin 1983), so we used higher spore concentration (5×10^6 spores/mL) rather than those used by previous studies (1×10^6 spores/mL) (Alvarez et al. 2005) to make the susceptible seedlings wilt rapidly. Wang and Roberts (2006) reported that root-knot nematodes also increase the severity of Fusarium wilt in cucurbit crops, so it is necessary to screen a rootstock that is resistant to both root-knot nematodes and Fusarium wilt.

3.3 *C. pustulatus* (PI 532673) as a grafting rootstock of *C. sativus*, *C. melo*, and *C. lanatus*

According to resistance screen assays, *C. pustulatus* (PI 532673) exhibited high resistance to both *M. incognita* and Fusarium wilt, and it may be used as a rootstock to manage soilborne disease.

The suitable rootstock requires strong affinity with the scion in order to guarantee the survival rate of graft (Traka-Mavrona et al. 2000). Our result showed that *C. pustulatus* had a good scion-rootstock compatibility with cucumber, melon, and watermelon. The survival rate of *C. sativus*/*C. pustulatus*, *C. melo*/*C. pustulatus*, and *C. lanatus*/*C. pustulatus* was 91, 91, and 87 %, respectively, which had no significant difference to self-rooted counterparts (Table 2). Salehi-Mohammadi et al.

Table 2 Survival rate and photosynthetic analysis of grafted seedling after infected *Meloidogyne incognita*

Treatment	Grafted seedling number	Survival rate (%)	Pn (umol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	Gs (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	Ci (umol $CO_2 \cdot mol^{-1}$)	Tr (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)
CS/CP	100.0	91.0a	17.9a	425.2a	292.6a	7.8a
CS/CS	100.0	92.0a	14.8b	365.4b	264.3b	6.6b
CM/CP	100.0	91.0a	20.4a	488.3a	291.5a	9.0a
CM/CM	100.0	90.0a	15.8b	392.8b	268.5b	6.5b
CL/CP	100.0	87.0a	16.6a	358.7a	288.3a	7.4a
CL/CL	100.0	86.0a	14.5b	245.2b	261.8b	6.3b

Different letters (a, b) in the same column indicate significant differences ($p<0.05$) between two graft treatments according to least significant difference comparison

CS Cucumis sativus, *CM Cucumis melo*, *CL Citrullus lanatus*, *CP Cucumis pustulatus*, *Pn* photosynthetic rate, *Gs* cond, *Ci* intercellular CO_2 concentration, *Tr* transpiration rate

(2009) suggested that several factors such as the numbers of connecting sieve tubes and pith cavity, other than stem diameter of rootstock, affected survival rate after grafting. *C. pustulatus* is probably similar to cucumber, melon, and watermelon on these factors. Thus, it was to be expected that grafting success rates were less than 100 %; the high survival rate in our study indicated the potential scale-up use of grafting in commercial production.

Figure 2 shows that the growth of grafted plants after transplanting into a field infested with *M. incognita*. Seedlings of *C. sativus*/*C. pustulatus*, *C. melo*/

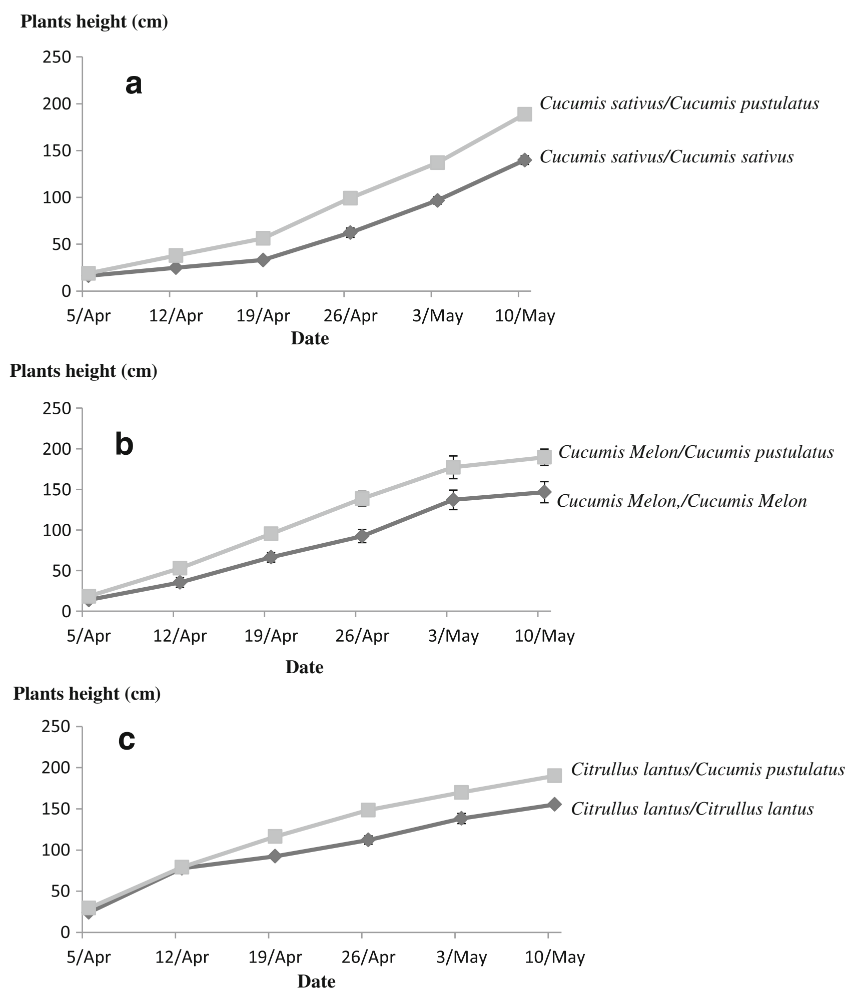

Fig. 2 The growth of grafted plants after transplanting into a nematode-infested greenhouse. **a** *Cucumis sativus/Cucumis pustulatus* and *Cucumis sativus/Cucumis sativus.* **b** *Cucumis melo/Cucumis pustulatus* and *Cucumis melo/Cucumis melo.* **c** *Citrullus lanatus/Cucumis pustulatus* and *Citrullus lanatus/Citrullus lanatus*

C. pustulatus, and *C. lanatus*/*C. pustulatus* grew faster than the control plants, *C. sativus*/*C. sativus*, *C. melo*/*C. melo*, and *C. lanatus*/*C. lanatus*, respectively. In 10 May 2012, *C. sativus*/*C. pustulatus*, *C. melo*/*C. pustulatus*, and *C. lanatus*/*C. pustulatus* were about 50, 45, and 43 cm taller than control for *C. sativus*/*C. sativus*, *C. melo*/*C. melo*, and *C. lanatus*/*C. lanatus*, respectively. Lee and Oda (2003) reported different responses of vegetative growth for the grafted combinations related to vigor of rootstocks and compatibility of rootstocks and scions, which was similar to our results.

In the six groups of grafted plants, *C. sativus*/*C. pustulatus*, *C. melo*/*C. pustulatus*, and *C. lanatus*/*C. pustulatus* had higher yields than the other three self-rooted plants, *C. sativus*/*C. sativus*, *C. melo*/*C. melo*, and *C. lanatus*/*C. lanatus*, respectively (Table 3). *C. sativus*/*C. pustulatus* yielded approximately 800 g more per plant than *C. sativus*/*C. sativus*; *C. melo*/*C. pustulatus* out-yielded *C. melo*/*C. melo* by more than 600 g and *C. lanatus*/*C. pustulatus* output about 600 g above than *C. lanatus*/*C. lanatus*. Grafting onto *C. pustulatus* also resulted in heavier fruit, approximately 60, 66, and 250 g per fruit in the case of cucumber, melon, and watermelon, respectively. *C. pustulatus* with sturdier root system and high resistance to *M. incognita* enhanced the outputs of cucumber, melon, and watermelon. A higher fruit yield was obtained when plants were grafted onto different *Cucurbita* rootstocks. This may have resulted from different factors such as increase in uptake of water and nutrient resulting from the larger root systems (Salehi-Mohammadi et al. 2009) and increased diseases tolerance (Miguel et al. 2004).

The photosynthetic rate, intercellular CO_2 concentration, and transpiration rate of *C. sativus*/*C. pustulatus*, *C. melo*/*C. pustulatus*, and *C. lanatus*/*C. pustulatus* were significantly higher than those of self-rooted plants, *C. sativus*/*C. sativus*, *C. melo*/*C. melo*, and *C. lanatus*/*C. lanatus*, respectively, after being infected by *M. incognita* (Table 2). The result indicated that seedlings grafted onto *C. pustulatus* had stronger assimilation than self-rooted ones and thus led to more photosynthetic product for the plants growth which is consistent with previous studies (Zhou et al. 2009).

Furthermore, *C. sativus*, *C. melo*, and *C. lanatus* rootstocks (Fig. 3a–c) had severe root galls as a result of severe root-knot nematode infection, while the root systems of *C. pustulatus* rootstocks with few root galls were much stronger and healthier than the above three rootstocks under the same soil conditions (Fig. 3d).

Our results show that the improved plant growth and increased yield of plants grafted onto *C. pustulatus* was due to several factors such as the compatibility between rootstocks and scions, increased photosynthesis, a larger root system, and an increased resistance to soilborne diseases. Our results were consistent with previous studies (Zhou et al. 2009; Lee and Oda 2003; Salehi-Mohammadi et al. 2009; YetıŞır et al. 2003).

3.4 Grafting onto *C. pustulatus* resulted in similar fruit quality

Our data suggest no significant differences in fruit soluble sugar, amino acids, and ascorbic acid between *C. sativus*/*C. pustulatus*, and *C. sativus*/*C. sativus*, *C. melo*/*C. pustulatus*, and *C. melo*/*C. melo*, *C. lanatus*/*C. pustulatus*, and *C. lanatus*/*C. lanatus*, respectively (Table 3). In addition, fruits of *C. sativus*/*C. pustulatus*, *C. melo*/*C. pustulatus*, and *C. lanatus*/*C. pustulatus* had a similar good taste as their counterparts (Table 3).

Abnormal fruit quality issues were reported previously for Cucurbitaceae grafting, including reduced fruit soluble solids content, insipid taste, and more fibers (Lee and Oda 2003; Koutsika-Sotiriou and Traka-Mavrona 2000). However, some researchers reported positive effects of grafting Cucurbitaceae, including an increase in soluble sugar, amino acids, and vitamin C content (Davis and Perkins-Veazie 2005; Salam et al. 2002; YetıŞır et al. 2003). Whereas our results showed nutritional qualities of grafted plants were similar to self-rooted controls, which were consistent with other studies (Miguel et al. 2004; Bianco et al. 2007).

Table 3 The yield and fruits quality of grafted plants

Treatment	Total yield per plant (g)	Single fruit weight (g)	Soluble sugar (%)	Amino acids (mg/g)	Ascorbic acid (mg/100 g)	Taste
CS/CP	2032.5a	261.3a	2.4a	8.9a	9.1a	Good (2.8)
CS/CS	1234.6b	202.4b	2.3a	8.4a	9.3a	Good (2.6)
CM/CP	2276.1a	469.0a	10.8a	10.1a	9.1a	Good (2.6)
CM/CM	1612.4b	403.0b	10.5a	10.4a	8.9a	Good (2.8)
CL/CP	3901.8a	1900.5a	12.1a	8.4a	9.3a	Good (2.7)
CL/CL	3304.2b	1650.8b	11.9a	8.5a	9.4a	Good (2.6)

Different letters (a, b) in the same column indicate significant differences ($p<0.05$) between two graft treatments according to least significant difference comparison. Taste divide into four ranks (better, good, general, bad) with the value of 3.1–4.0, 2.1–3.0, 1.1–2.0, 0–1.0

CS Cucumis sativus, *CM Cucumis melo*, *CL Citrullus lanatus*, *CP Cucumis pustulatus*

Fig. 3 Different rootstocks grown in a field infested with southern root-knot nematode, *Meloidogyne incognita. Cucumis sativus* (**a**), *Cucumis melo* (**b**), and *Citrullus lanatus* (**c**) rootstock exhibiting severe root galling caused by root-knot nematodes, and *Cucumis pustulatus* (**d**) rootstock resistant to root-knot nematodes

Therefore, the use of *C. pustulatus* as a rootstock would provide a potential strategy to increase total yield without having remarkable deterioration in the quality and taste of fruit.

4 Conclusions

Screening of root-knot resistant germplasm and developing resistant rootstocks can provide an environmentally friendly method for managing soilborne diseases in cucurbitaceous species. In our study, of 53 wild *Cucumis* accessions screened, five varieties exhibited high resistance to southern root-knot nematode and 12 resistant to Fusarium wilt. Theses varieties may provide genetic resources of resistance to root-knot nematodes and Fusarium wilt to develop resistant cultivars. We demonstrated that *C. pustulatus* was a suitable rootstock for cucumber, melon, and watermelon to manage *M. incognita* by producing high yield and good fruits quality. With the rising cost of nematicides and their restrictions on commercial use, grafting may become an economically feasible method. *C. pustulatus* rootstocks may be useful for low-input sustainable horticulture and benefit to home gardeners, especially to those in areas with highly infested south root-know nematode.

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