



Screening for Tolerance-Resistance to Root-knot Nematode *Meloidogyne incognita* in Grafted and Ungrafted Cucurbit Rootstocks



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THE CURRENT study aimed to screen eight ungrafted and grafted cucurbit rootstocks as well as melon Hybrid London genotype against root-knot nematode infection. Ungrafted and grafted rootstocks i.e. Nun 6001, Squash No3, Super Shintosa, Flixel Fort, Ferro RZ, Coplt, Bottle gourd were classified as tolerant to *M. incognita* infection whereas melon genotype Hybrid London and Pakistani Loof rootstock ranked as susceptible hosts. Grafted cucurbit rootstocks i.e. Nun 6001 and Ferro RZ, gave the higher plant survival rate and growth parameters compared to ungrafted ones and the rest of rootstocks and melon genotype. Number of galls, females, and egg masses of *M. incognita* were significantly suppressed in ungrafted and grafted rootstocks compared to melon plant Hybrid London. All the rootstocks differed in their ability to stimulate peroxidase (PO), polyphenol oxidase (PPO) activities, and phenol content. The study revealed that grafting melon plants onto all the previous tolerant rootstocks is an effective tool that may enable the susceptible melon scion to tolerate *M. incognita* which leads to an increase in plant survival rate and growth parameters.

Keywords: Melon, Peroxidase, Root-knot nematode, Rootstocks, Survival rate.

Introduction

Root-knot nematode (*Meloidogyne incognita*) is a critical pest of cultivated melon (*Cucumis melo*) in Egypt and severe crop damage especially in light soils (Abd-Elgawad & Aboul-Eid, 2001; Abd-Elgawad, 2008; EL-Mesalamy et al., 2020). Root-knot nematodes cause dramatic galling on the roots of host plants, and even low nematode levels can cause high yield losses (Sasser et al., 1983; Amin, 1993). Root-knot nematodes can also enhance the severity of soil-borne diseases for example fungal wilt in cucurbit crops caused by *Fusarium* (Wang & Roberts, 2006; Talavera et al., 2012; López-Gómez et al., 2016). *M. incognita* is the most predominant species of those infecting cucurbitaceous plants such as melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), and cucumber (*C. sativus*) in

Egypt. No commercial melon cultivars are known to be resistant to root-knot nematodes (Siguenza et al., 2005; López-Gómez et al., 2016; Ayala-Doñas et al., 2020).

The major method for controlling root-knot nematodes in melon and other cucurbit crops is soil fumigation and other nematicides. However, since methyl bromide is banned, farmers are searching for alternative control methods. A potential solution is grafting melon sections onto tolerant or resistant rootstocks (Siguenza et al., 2005; Liu et al., 2020).

Most grafting literature on soil-borne diseases reported that grafting can induce resistance or tolerance to various soil-borne pathogens as fungal, bacterial, and nematodes (Penelope, 2008; Aslam, et al., 2020, Ayala-Doñas et al., 2020, Thakur &

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Savita, 2020). Therefore, the primary motive for grafting cucurbits is improving vigor and tolerance to abiotic stress and resistance to soil-borne pests and pathogens when genetic or chemical approaches for disease management are not available (Oda, 2002a & b; Thakur et al., 2020). Moreover, identifying root-knot-resistant rootstocks would introduce an economical and eco-friendly approach for managing root-knot nematodes in melon (Thies et al., 2010).

Grafting a susceptible scion onto a resistant rootstock can create resistance to a cultivar without prolonged screening as well as selection required to breed for resistance into a cultivar (Liu et al., 2020). Grafting melon on resistant or tolerant cucurbits rootstocks led to reduced nematode gall formation (Siguenza et al., 2005; Kokalis-Burelle & Roskopf, 2011).

In some cases, the rootstocks induce tolerance contingent on the extensive root system and vigor (Giannakou & Karpouzas, 2003; Miguel et al., 2005), but some rootstocks possess genetic resistance that is appeared in the grafted plants (Hagitani & Toki, 1978; Siguenza et al., 2005; Gu et al., 2006). Moreover, Biles et al. (1989) reported that substances related to soil-borne pathogen tolerance are formed in the roots of the rootstocks then translocated to the scion through the xylem in grafted cucurbits plants. Grafted plants showed more leaves, larger leaf area, thicker main stem diameter (Lee & Oda, 2003; Yetisir & Sari, 2003) comparing with the control. Grafted plants were more vigorous with remarkably higher yields compared to non-grafted plants (Paroussi et al., 2007).

Therefore this study aimed to screen several

commercial and wild cucurbit rootstocks used in grafting as suitable rootstocks to provide resistance or tolerance to *Meloidogyne incognita* and improve the productivity of melon.

Materials and Methods

This study was conducted in a greenhouse at the Department of Nematodes Plant Diseases, Agricultural Research Center (ARC), Giza during the two successive seasons i.e., 20/8/2015 and 22/8/2016. This study aimed to screen eight cucurbit rootstocks, and melon (*Cucumis melo*) “Hybrid London (Galia type)” genotypes for tolerance against the root-knot nematode *Meloidogyne incognita*. Melon cultivar, as a scion, and the tested rootstocks were listed (Table 1).

Meloidogyne incognita inoculum

Second stage juveniles of *M. incognita* were obtained from a single egg mass propagated on roots of coleus plant (*Coleus blumei* L.) for three months under the greenhouse conditions at Nematological Research Unit (NERU), Plant Pathology Research Institute, Giza, Egypt. A sodium hypochlorite (NaOCl) extraction technique (Hussey & Barker, 1973) was used for the collection of eggs of *M. incognita*. Infected roots were gently washed with tap water and cut into small segments. Root segments were then shacked in 200mL of 1.0% NaOCl solution for 1-2min. NaOCl solution was passed through a 60-mesh sieve nested over a 400-mesh sieve to separate freed eggs. A 400-mesh sieve with eggs was quickly placed under a stream of tap water for removing residual NaOCl and collected. The remaining roots were rinsed using water to remove additional eggs and then collected. Eggs were then placed in a Petri dish with a sufficient amount of water and left for incubation for 3-4 days.

TABLE 1. Melon cultivar and cucurbit rootstocks used in this experiment

Plants	Genotypes	Species	Seed production company
Melon (Scion)	Hybrid London ME	<i>Cucumis melo</i> L.	Rijk Zwaan
	Squash No3	<i>Cucurbita maxima</i>	Sakata
	Super Shintoza	<i>Cucurbita maxima</i> × <i>C. moschata</i>	G.S.I
	FliexFort	<i>Cucurbita maxima</i> × <i>C. moschata</i>	Enza Zaaen
	Ferro RZ	<i>Cucurbita maxima</i> × <i>C. moschata</i>	Rijk Zwaan
	Nun 6001	<i>Cucurbita maxima</i> × <i>C. moschata</i>	Nunhium
	Coplt	<i>Cucurbita maxima</i> × <i>C. moschata</i>	Rijk Zwaan
	Bottle gourd	<i>Lagenaria siceraria</i>	*HRI
Rootstocks	Pakistan luffa	<i>Luffa cylindrical</i>	Pakistan

*HRI: Horticultural Research Institute

Experimental design

Grafted rootstocks

Eight cucurbit rootstocks and melon 'Hybrid London'© seeds were sown in plug trays (cell volume: 50mL) contained peat moss and vermiculite mixture in a ratio of 1:1 (v/v) under greenhouse to produce seedlings. Melon seedlings (17 days old) were then grafted onto the previous rootstocks by splice method according to Hassell et al. (2008). Grafted rootstocks (25 days old), as well as melon seedlings, were separately transplanted in plastic pots (25cm-d) filled with 2kg sterilized clay-sand soil (1:1) (v:v).

Ungrafted rootstocks

Eight cucurbit rootstocks genotypes and melon 'Hybrid London'© seeds (2 seeds/pot) were sown in sterilized clay-sand soil (1:1) (v:v) as previously mentioned.

Grafted and ungrafted rootstocks, as well as melon seedlings, were inoculated with 1000 J2 *M. incognita* after four and seven days of planting, respectively. Eight pots were used for each plant cultivar genotype, and half of them were inoculated with nematode. While the other eight pots for each cultivar were left without nematode inoculum and served as control. All pots were arranged in a Completely Randomised Block Design system and horticultural treated the same under greenhouse conditions for two seasons 2015 and 2016.

Recorded data

After fifty-five days from nematode inoculation and seventy-five days from planting grafted melon seedlings, plant survival rates were measured by dividing the numbers of the successful plants by the total number of the plants. Moreover, plants were uprooted and root systems were washed from the soil. Length and fresh weight of shoot and root and shoot dry weight was measured and percent reduction in such growth parameters was calculated concerning healthy plants. The number of juveniles (J_2) in soil /pot, galls, egg masses, and females per root system were counted and recorded. The infected roots were stained using acid fuchsin (Byrd et al., 1983), washed with tap water, and placed in pure cold glycerin (Goodey, 1957) to facilitate the counting.

After clearing, numbers of endo-parasitic

forms were determined with the aid of a stereomicroscope. The nematode reproduction (R factor) for each cultivar or rootstocks was calculated by dividing the final nematode population (P_f) by the initial nematode population (P_i). Root gall index (RGI) and egg mass index (EI) were determined and recorded as follows: 0= no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10 galls or egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses and 5= more than 100 galls or egg masses, according to the described scale by Taylor & Sasser (1978).

Host suitability was measured based on the relation between root gall index (RGI) and nematode reproduction (RF) according to Canto- Saenz (1983) as follows: ($RGI \leq 2 \& R < 1$) resistant (R), ($RGI \leq 2 \& R \geq 1$) tolerant (T), and ($RGI \geq 2 \& R \geq 1$) susceptible (S).

Total phenol content in leaves of rootstocks infested with *M. incognita* was determined and compared to uninfected control plants. Also, enzyme peroxidase (PO) and polyphenol oxidase (PPO) were determined in the fresh leaves of cucurbit rootstocks infected with *M. incognita* compared to control plants.

Statistical analysis

All obtained data were statistically analyzed according to the Randomized Complete Block Design in factorial arrangement using Duncan's Multiple Range Test at 5% level to compare between treatment means as described by Gomez & Gomez (1984).

Results

Plant survival rate

Results in Tables 2 show the plants' survival rate for ungrafted and grafted rootstocks and melon genotypes in soil inoculated with *M. incognita*. Nun 6001, Squash No3, Super Shintosa, Flixel Fort, Ferro RZ, Coplt, and Bottle gourd rootstocks showed a higher plant survival rate than the Pakistani Loof rootstock and melon genotype during the two studied seasons (Table 2). Grafted melon onto all the previous rootstocks showed also a higher plant survival rate than the grafted melon plant onto Pakistani Loof rootstock or un-grafted melon plants during the two studied seasons (Table 2).

TABLE 2. Plant growth response of eight ungrafted and grafted cucurbit rootstocks to *Meloidogyne incognita* under greenhouse conditions (25±3°C)

Genotypes	Ungrafted rootstocks																							
	Plant survival rate %			Plant fresh wt.(gm)			Shoot dry wt. (gm)			Plant survival rate %			Plant fresh wt.(gm)			Shoot Dry wt. (gm)			Inc.%					
	Shoot	Root	Total	Inc.%	Shoot	Root	Total	Inc.%	Shoot	Root	Total	Inc.%	Shoot	Root	Total	Inc.%	Shoot	Root	Total	Inc.%	Shoot	Root	Total	Inc.%
	First season (2015)																							
Nun 6001	87.0 ^a	7.1 ^b	0.87 ^b	7.97	104.4	0.90 ^b	104.5	88.6 ^a	12.3 ^b	0.75 ^d	13.1	21.3	0.72 ^b	44.0										
Squash No3	83.1 ^a	5.6 ^c	0.76 ^c	6.4	64.0	0.80 ^c	100.0	84.7 ^a	11.9 ^d	0.70 ^f	12.6	16.7	0.70 ^c	40.0										
Super Shintosa	82.4 ^a	5.0 ^d	1.8 ^a	6.8	74.4	0.80 ^c	100.0	88.4 ^a	10.9 ^e	0.71 ^e	11.6	7.4	0.67 ^d	34.0										
Flixel Fort	83.2 ^a	4.2 ^f	1.8 ^a	6.0	53.8	0.59 ^d	34.1	87.2 ^a	11.0 ^f	0.90 ^b	11.9	10.2	0.70 ^c	40.0										
Ferro RZ	91.2 ^a	9.0 ^a	0.95 ^a	9.95	155.1	1.1 ^a	150.0	93.9 ^a	13.7 ^a	0.77 ^e	14.5	34.3	0.74 ^a	48.0										
Coplt	86.3 ^a	4.9 ^e	0.8 ^d	5.7	46.2	0.56 ^e	27.3	88.5 ^a	12.0 ^c	0.67 ^e	12.7	17.1	0.60 ^e	20.0										
Bottle gourd	85.3 ^a	3.1 ^e	0.8 ^d	3.9	0.0	0.48 ^e	9.1	87.4 ^a	11.7 ^c	0.95 ^a	12.7	17.1	0.60 ^e	20.0										
Pakistani Loof	63.0 ^b	4.9 ^e	0.59 ^f	5.5	41.0	0.54 ^f	22.7	64.8 ^b	12.0 ^c	0.90 ^b	12.9	19.4	0.70 ^c	40.0										
Hybrid London	70.2 ^b	3.1 ^b	0.8 ^d	3.9	0.0	0.44 ^e	0.0	74.1 ^b	10.3 ^b	0.50 ^b	10.8	0.0	0.50 ^f	0.0										
	Grafted rootstocks																							
Nun 6001	89.9 ^a	18.9 ^d	4.6 ^c	23.5	60.9	2.3 ^c	21.1	89.9 ^a	20.1 ^d	9.8 ^a	29.9	54.9	3.2 ^d	28.0										
Squash No3	87.5 ^a	22.9 ^a	4.2 ^d	27.1	85.6	2.8 ^a	47.4	90.5 ^a	23.5 ^b	7.0 ^c	30.5	58.0	3.3 ^c	32.0										
Super Shintosa	89.7 ^a	14.8 ^c	5.5 ^b	20.3	39.0	2.5 ^b	31.6	90.5 ^a	20.0 ^e	4.0 ^f	24.0	24.4	2.7 ^e	8.0										
Flixel Fort	88.4 ^a	12.8 ⁱ	9.6 ^a	22.4	53.4	2.8 ^a	47.4	89.8 ^a	19.4 ^c	8.8 ^b	28.2	46.1	3.4 ^b	36.0										
Ferro RZ	92.0 ^a	21.8 ^b	4.7 ^a	26.5	81.5	2.2 ^c	15.8	92.6 ^a	25.0 ^a	6.8 ^d	31.8	64.8	3.6 ^a	44.0										
Coplt	87.1 ^a	20.0 ^c	2.8 ^c	22.8	56.2	2.1 ^d	10.5	89.4 ^a	17.7 ^f	6.7 ^e	24.4	26.4	3.1 ^e	24.0										
Bottle gourd	89.2 ^a	15.3 ^e	2.2 ^f	17.5	19.9	2.1 ^d	10.5	89.2 ^a	21.8 ^e	6.2 ^f	28.0	45.1	2.6 ^b	4.0										
Pakistani Loof	69.8 ^b	14.0 ^f	2.0 ^e	16.0	9.6	2.1 ^d	10.5	70.6 ^b	15.7 ^h	5.0 ^e	20.7	7.3	2.5 ⁱ	0.0										
Hybrid London	76.9 ^b	13.7	1.8 ^h	14.6	0.0	1.9 ^e	0.0	76.7 ^b	15.0 ⁱ	4.3 ^h	19.3	0.0	2.5 ⁱ	0.0										

*Each value presented the mean of four replicates.

Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan's multiple range tests

The influence of M. incognita infection on growth parameters

Results reported in Table 2 indicated that the influence of *M. incognita* infection on growth parameters of ungrafted and grafted rootstocks and melon, Hybrid London under greenhouse conditions. Growth parameters of ungrafted and grafted cucurbit rootstocks were significantly increased compared to melon genotype grown in soil inoculated with *M. incognita* to a various extent (Table 2). Shoot dry weights of ungrafted rootstocks of Nun 6001 and Ferro RZ showed better improvement than those of grafted ones for the two seasons. Whereas, plant fresh weights of such rootstocks were significantly increased in the first season.

The highest values of growth parameters (total plant fresh weight and shoot dry weight) were recorded with grafted melon seedlings onto Ferro and Squash No3 at the first and second seasons by 81.5, 85.6; 15.8, 47.4 and 64.8, 58.0; 44.0, 32.0, respectively (Table 2). However, the lowest percentage values on growth parameters (total plant fresh weight and shoot dry weight) were recorded with grafted melon seedlings onto Pakistan Loof rootstock at the first and second seasons by 9.6 & 10.5% and 7.3 & 0.0%, respectively. Also, the tested melon genotype (Hybrid London) showed the lowest percentage value of most parameters of total plant fresh weight and shoot dry weight at the first and second seasons.

Host suitability of the tested rootstocks to M. incognita infection

Most of the studied rootstocks either grafted or ungrafted i.e. Nun 6001, Squash No3, Super Shintosa, Flixel Fort, Ferro RZ, Coplt, and Bottle gourd significantly ($P \geq 0.05$) decreased the number of galls, females, and egg masses during the first and second season (Tables 3, 4) compared with melon "Hybrid London".

According to the scale described by Canto-Saenz (1983) based on the relationships between root gall index (RGI) and R factor, all ungrafted rootstocks except Pakistani Loof and melon Hybrid London were rated as tolerant hosts at the first and second season. Root gall index ranged from 2.0 to 3.0 for both seasons while the R factor ranged from 0.39 to 0.66 and from 0.34 to 0.78 for the first and second seasons, respectively (Table 3). Conversely grafted or ungrafted Pakistan loof rootstock was recorded as a susceptible host to *M. incognita* at two studied seasons (Tables 3, 4). From the same tables, the tested

melon (Hybrid London) was highly infected with *M. incognita* and ranked as a susceptible host with no significant differences in the two studied seasons.

Enzyme peroxidase and polyphenol oxidase contents

Data presented in Table 5 revealed that all rootstocks infected with *M. incognita* differed in their ability to stimulate peroxidase (PO) and polyphenol oxidase (PPO) activities. Both PO and PPO activities were lower in grafted and ungrafted Pakistani Loof rootstock infected with *M. incognita* in the first and second seasons.

Phenol content

Total phenol evaluated in leaves of rootstocks and grafted melon grown in soil infested with *M. incognita* revealed a moderate enhancement compared to melon plants (Table 5). All rootstocks showed a noticeable reduction in total phenol to various extents compared to non-inoculated plants. However, the least reduction in total phenol was recorded with ungrafted Pakistani Loof rootstocks (29.4 %) in the first season. Meanwhile, Flixel Fort rootstock (6.3%) induced the least reduction in the second season compared with melon plants. On the other hand, grafted Pakistani Loof (13.8, 5.0%) recorded the lowest rank in total phenol at the first and second seasons comparing with melon, Hybrid London respectively.

Discussion

The present study showed that the commercial cultivar melons (Hybrid London) ranked as susceptible host to root-knot nematode, *M. incognita* that leads to a decrease in plant survival rate, growth parameters, activities of PO and PPO, total phenol, and increased the number of galls, females, and egg masses. These results agree with Siguenza et al. (2005), Mukhtar et al. (2013), and López-Gómez et al. (2016) who reported that no commercial cultivation melons are known to be resistant to root-knot nematode.

Out of eight rootstocks, melon grafted onto Ferro rootstock seedlings gave the highest percentage values of total plant fresh weight and shoot dry weight of cucurbits in the first season and second season. The results also showed that none of the eight tested rootstocks were immune or highly resistant to *M. incognita* infection. These results agree with Giné et al. (2017) who reported that the rootstock (*Cucurbita maxima* x *C. moschata*) was not resistant to *M. incognita*.

TABLE 3. Development and reproduction of *Meloidogyne incognita* and relative susceptibility of ungrafted rootstocks under greenhouse conditions (25±3°C)

Genotypes	Ungrafted rootstocks										
	Nematode population *in			Final population (Pf)	RF**	No. of galls	***RGI	No. of Egg masses	***EI	****Host category	
	Soil	Root									
	Developmental stages	Females									
First season (2015)											
Rootstocks	Nun 6001	459.0 ^b	2.0 ^f	9.67 ^h	487.0	0.49	11.3 ⁱ	3.0	5.0 ^f	2.0	T
	Squash No3	460.0 ^e	2.0 ^f	9.0 ⁱ	488.0	0.49	10.0 ^j	2.0	7.0 ^e	2.0	T
	Super Shintosa	568.0 ^c	3.66 ^c	10.0 ^g	601.4	0.60	11.7 ^h	3.0	8.0 ^d	2.0	T
	Flixel Fort	463.0 ^f	2.0 ^f	12.30 ^e	497.0	0.50	11.7 ^h	3.0	8.0 ^d	2.0	T
	Ferro RZ	360.0 ⁱ	0.0 ^g	12.0 ^f	393.0	0.39	12.0 ^e	3.0	9.0 ^c	2.0	T
	Coplt	626.0 ^d	2.30 ^d	12.67 ^d	662.97	0.66	14.0 ^d	3.0	8.0 ^d	2.3	T
	Bottle gourd	430.0 ⁱ	2.0 ^f	9.0 ⁱ	456.0	0.46	10.0 ⁱ	2.0	5.0 ^f	2.0	T
	Pakistani Loof	887.0 ^c	20.25 ^a	50.0 ^a	1024.25	1.0	42.0 ^a	4.0	25.0 ^a	3.0	S
Melon (Scion)	Hybrid London	1502.0 ^a	5.30 ^b	33.00 ^b	1602.0	1.6	36.7 ^b	4.0	25.0 ^a	3.0	S
Second season (2016)											
Rootstocks	Nun 6001	306.0 ^j	12.75 ^g	19.30 ^b	360.1	0.36	11.0 ^h	3.0	11.0 ^h	3.0	T
	Squash No3	318.0 ^b	13.50 ^f	25.0 ^f	386.9	0.39	19.7 ^f	3.0	10.7 ^j	3.0	T
	Super Shintosa	370.0 ^c	9.25 ^d	23.30 ^g	436.6	0.78	17.0 ^g	4.0	17.0 ^f	3.0	T
	Flixel Fort	349.0 ^f	17.0 ^d	8.66 ^j	396.1	0.40	6.7 ^j	3.0	14.7 ^g	3.0	T
	Ferro RZ	309.0 ⁱ	3.0 ^j	12.0 ⁱ	343.0	0.34	10.0 ⁱ	2.0	9.0 ^j	2.0	T
	Coplt	348.0 ^e	7.0 ⁱ	30.0 ^e	426.0	0.43	26.0 ^e	3.0	15.0 ^e	3.0	T
	Bottle gourd	398.0 ^d	9.0 ^h	35.0 ^d	488.0	0.49	28.0 ^d	3.0	18.0 ^d	3.0	T
	Pakistani Loof	877.0 ^c	18.25 ^c	56.66 ^b	1015.9	1.0	42.0 ^c	4.0	22.0 ^c	3.0	S
Melon (Scion)	Hybrid London	1076.0 ^b	45.0 ^b	50.0 ^c	1246.0	1.2	45.0 ^b	3.0	30.0 ^b	3.0	S

*Each value presented the mean of four replicates

M. incognita (1000 J₂/ plant)

** Reproduction Factor (RF) = $\frac{\text{Nematode population in soil} + \text{No. of developmental stages} + \text{No. of females} + \text{No. of egg masses}}{\text{No. of juveniles inocula}}$

*** Root gall index (RGI) or egg masses index (EI) was determined according to the scale given by Taylor & Sasser (1978) as follows: 0= no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10 galls egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses and 5= more than 100 galls or egg masses.

**** Host category based on a relationship between RGI & R Factor according to Canto-Saenz (1983) as follows : (RGI ≤ 2 & R ≤ 1) resistant (R), (RGI ≤ 2 & R ≥ 1) tolerant (T) and (RGI ≥ 2 & R ≥ 1) susceptible (S).

Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan's multiple range tests.

TABLE 4. Development, reproduction of *Meloidogyne incognita*, and relative susceptibility of grafted rootstocks under greenhouse conditions (25±3°C)

Genotypes	Grafted rootstocks										
	Nematode population in*										Host category
	Soil	Root			Final population (Pf)	RF**	No. of galls	***RGI	No. of Eggmasses	***EI	
		Developmental stages	Females								
First season (2015)											
Rootstocks	Nun 6001	361.2 ^h	0.0 ^c	19.5 ^h	411.0	0.41	18.0 ^e	3.0	13.0 ^f	3.0	T
	Squash No3	380.0 ^g	0.0 ^c	14.0 ^k	414.0	0.41	12.0 ^h	3.0	8.0 ^j	2.0	T
	Super Shintosa	372.5 ^d	0.0 ^c	20.5 ^e	426.6	0.43	19.3 ^d	3.0	14.3 ^c	3.0	T
	Flixel Fort	388.5 ^e	0.0 ^c	21.3 ^d	444.3	0.44	20.0 ^c	3.0	14.5 ^d	3.0	T
	Ferro RZ	299.5 ⁱ	0.0 ^c	15.8 ^j	339.9	0.34	13.8 ^g	3.0	11.3 ^h	3.0	T
	Coplt	385.0 ^f	0.0 ^c	16.0 ⁱ	525.6	0.53	14.0 ^f	3.0	11.0 ⁱ	3.0	T
	Bottle gourd	390.0 ^d	0.0 ^c	20.0 ^f	442.0	0.44	20.0 ^c	3.0	12.0 ^g	3.0	T
	Pakistani Loof	399.5 ^e	1.3 ^a	22.5 ^c	459.5	0.46	20.5 ^b	3.0	17.5 ^b	3.0	S
Melon (Scion)	Hybrid London	447.0 ^a	1.0 ^b	33.7 ^a	525.3	0.53	25.3 ^a	3.0	18.3 ^a	3.0	S
Second season (2016)											
Rootstocks	Nun 6001	301.0 ^h	0.0 ^a	20.0 ^f	356.0	0.36	20.0	3.0	15.0 ^c	3.0	T
	Squash No3	330.0 ^g	0.0 ^a	25.0 ^a	385.0	0.39	20.0	3.0	10.0 ^h	3.0	T
	Super Shintosa	347.5 ^f	0.0 ^a	21.0 ^c	403.1	0.40	26.3 ^d	3.0	11.8 ^f	3.0	T
	Flixel Fort	349.0 ^e	0.0 ^a	20.0 ^g	399.0	0.40	20.0	3.0	10.0 ^h	3.0	T
	Ferro RZ	279.5 ^f	0.0 ^a	15.0 ^h	319.0	0.32	14.3 ^e	3.0	11.5 ^g	3.0	T
	Coplt	362.5 ^d	0.0 ^a	21.3 ^e	424.9	0.42	28.3 ^c	3.0	12.8 ^f	3.0	T
	Bottle gourd	360.5 ^e	0.0 ^a	12.5 ⁱ	425.5	0.43	31.5 ^f	3.0	21.0 ^b	3.0	T
	Pakistani Loof	400.5 ^a	0.0 ^a	24.8 ^b	471.4	0.47	23.8 ^a	3.0	22.3 ^a	3.0	S
Melon (Scion)	Hybrid London	384.5 ^b	0.0 ^a	21.8 ^d	449.3	0.45	28.5 ^b	3.0	14.5 ^d	3.0	S

*Each value presented the mean of four replicates

M. incognita (1000 J₂/ plant)** Reproduction Factor (RF) = $\frac{\text{Nematode population in soil} + \text{No. of developmental stages} + \text{No. of females} + \text{No. of egg masses}}{\text{No. of juveniles inocula}}$

*** Root gall index (RGI) or egg masses index (EI) was determined according to the scale given by Taylor & Sasser (1978) as follows: 0= no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10 galls or egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses and 5= more than 100 galls or egg masses.

**** Host category based on a relationship between RGI & R Factor according to Canto-Saenz (1983) as follows: (RGI ≤ 2 & R ≤ 1) resistant (R), (RGI ≤ 2 & R ≥ 1) tolerant (T) and (RGI ≥ 2 & R ≥ 1) susceptible (S).

Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan's multiple range tests.

TABLE 5. Enzyme activities and total phenol in fresh leaves of rootstocks grown in soil infected with *Meloidogyne incognita* under greenhouse conditions

Cultivars		Ungrafted rootstocks						Grafted rootstocks					
		PO	% Dec.	PPO	% Dec.	T. Phenol	% Dec.	PO	% Dec.	PPO	% Dec.	T. Phenol	% Dec.
		First season (2015)						First season (2016)					
		Second season (2015)						Second season (2016)					
Rootstocks	Nun 6001	0.506 ⁱ	15.4	0.029 ⁱ	73.0	0.420 ⁱ	53.2	0.537 ⁱ	9.6	0.021 ^g	53.3	0.423 ⁱ	37.3
	Squash No3	0.509 ^h	14.9	0.037 ^h	66.7	0.425 ^h	52.6	0.546 ^h	8.1	0.023 ^f	48.9	0.446 ^h	33.9
	Super Shintosa	0.546 ^d	8.7	0.065 ^g	41.4	0.564 ^d	37.1	0.567 ^f	4.5	0.031 ^c	31.0	0.457 ^g	32.3
	Flixel Fort	0.511 ^g	14.5	0.073 ^f	34.2	0.458 ^f	48.9	0.565 ^g	4.9	0.031 ^c	31.0	0.465 ^f	31.1
	Ferro RZ	0.499 ^j	16.6	0.024 ^j	78.4	0.413 ^j	54.0	0.524 ^j	11.8	0.015 ^h	<u>66.7</u>	0.417 ^j	38.2
	Copl ^t	0.523 ^f	12.5	0.089 ^d	19.8	0.470 ^e	47.6	0.577 ^d	2.9	0.032 ^d	<u>28.9</u>	0.566 ^e	16.1
	Bottle gourd	0.534 ^e	10.7	0.087 ^c	21.6	0.446 ^g	50.3	0.576 ^c	3.0	0.038 ^c	<u>15.6</u>	0.571 ^d	15.4
	Pakistani Loof	0.578 ^c	3.3	0.097 ^c	12.6	0.633 ^c	29.4	0.580 ^c	2.4	0.042 ^b	<u>6.7</u>	0.582 ^c	13.8
Melon (Scion)	Hybrid London	0.598 ^a	0.0	0.111 ^a	0.0	0.897 ^a	0.0	0.594 ^a	0.0	0.045 ^a	<u>0.0</u>	0.675 ^a	0.0
Rootstocks	Nun 6001	0.569 ^h	6.3	0.067 ⁱ	63.8	0.340 ^h	40.0	0.544 ^h	7.2	0.033 ^g	36.5	0.423 ^d	7.6
	Squash No3	0.578 ^g	4.8	0.077 ^h	58.4	0.357 ^e	37.0	0.554 ^h	5.5	0.036 ^f	30.8	0.345 ⁱ	24.7
	Super Shintosa	0.588 ^d	3.1	0.112 ^g	39.5	0.432 ^d	23.8	0.555 ^g	5.3	0.044 ^c	15.4	0.350 ^h	23.6
	Flixel Fort	0.597 ^c	1.6	0.152 ^c	17.8	0.531 ^c	6.3	0.561 ^f	4.3	0.036 ^f	30.8	0.398 ^g	13.1
	Ferro RZ	0.560 ⁱ	7.7	0.065 ^j	64.9	0.312 ^j	45.0	0.522 ⁱ	10.9	0.026 ^h	50.0	<u>0.320ⁱ</u>	30.1
	Copl ^t	0.579 ^f	4.6	0.115 ^f	37.8	0.356 ^f	37.2	0.565 ^e	3.5	0.048 ^d	7.7	<u>0.399^f</u>	12.9
	Bottle gourd	0.589 ^d	3.0	0.165 ^d	10.8	0.342 ^g	39.7	0.569 ^d	2.9	0.048 ^d	7.7	<u>0.412^g</u>	10.0
	Pakistani Loof	0.597 ^c	1.6	0.166 ^c	10.3	0.327 ⁱ	42.3	0.578 ^c	1.4	0.049 ^c	5.8	<u>0.435^g</u>	5.0
Melon (Scion)	Hybrid London	0.607 ^a	0.0	0.185 ^a	0.0	0.567 ^a	0.0	0.586 ^a	0.0	0.052 ^a	0.0	<u>0.458^g</u>	0.0

*Each value presented the mean of four replicates

Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan's multiple range tests

Grafted or ungrafted rootstocks i.e. Ferro RZ and Nun 6001 Squash No3, Flixel Fort, Super Shintosa, Bottle gourd, and Coplt were classified as tolerant hosts to *M. incognita* infection. Meanwhile, Pakistan loof was rated as a susceptible host. These results indicated that the use of tolerant rootstocks has great potential for improving nematode control in the absence of soil fumigants due to reduced nematode gall formation as reported by Siguenza et al. (2005), Kokalis-Burelle & Roskopf (2011), and Ayala-Doñas et al. (2020).

Data also indicate that grafting melon plants onto all the previous rootstocks gave the higher plant survival rate than the grafted melon plant onto Pakistani Loof rootstock or un-grafted melon plants during the two studied seasons. These

results indicate that grafting melon plants onto all the previous tolerant rootstocks is an effective tool that may enable the susceptible melon scion to tolerate *M. incognita* which leads to an increase in the plant's survival rate compared to the un-grafted melon seedlings. This investigation is in the line with (Aslam et al., 2020; Guan & Haseman, 2020).

The differences in plant survival rate may be attributed to rootstock vigor (Andrews & Marquez, 1993; Lee, 1994; Lee et al., 1998; Oda, 1999; Trakamavrona et al., 2000; Edelman et al., 2004; Aslam et al., 2020). Also, these findings are in par with those of Tamil Selvi et al. (2013) who reported that the plant growth response to nematode infection varied according to the cultivated cucurbitaceous species and use of resistant rootstocks for grafting.

They also added that the reduction in tested cultivars growth criteria due to *M. incognita* infection was obviously in fresh weights of either plant shoots or roots. Also, the uses of tolerant rootstocks to root-knot nematode for grafted melon plants are favorable for low-input sustainable horticulture. Therefore it should be a benefit to grow in areas with high root-knot nematode infestation (Jansea & Van der Wurff, 2010; Thies et al., 2010; Al-Debei et al., 2012; Wilcken et al., 2013; Guan et al., 2014; Ito et al., 2014; Liu et al., 2015; Punithaveni et al., 2015; López-Gómez et al., 2016; Yankova et al., 2017; EL-Mesalamy et al., 2020). In this connection, Giné et al. (2017) reported that the rootstock (*Cucurbita maxima* x *C. moschata*) was no resistant to *M. incognita*.

A high reduction in the number of galls, females, and egg masses of *M. incognita* was indicated in tolerant rootstocks that agreed with results obtained by Youssef & Amin (1997), Rather & Siddiqui (2007), Amin et al. (2013), EL-Mesalamy et al. (2020) in using effective cucurbit rootstocks against *M. incognita*.

Tolerant rootstocks infected with *M. incognita* showed differences in their ability to stimulate peroxidase (PO) and polyphenol oxidase (PPO) activities, while, the susceptible Pakistani Loof rootstock showed the lowest activity of both PO and PPO. These results confirmed the findings of Marin et al. (2017) concerning melon rootstocks. Also, Ayala-Doñas et al. (2020) indicated the activity of peroxidases is increased after root-knot nematodes infection higher in resistant than susceptible cucurbits. Data also indicated the activity increase of total phenol which is at least in part responsible for defense mechanisms of the plant to nematodes.

Finally, it can be concluded that most of grafted and ungrafted rootstocks are tolerant of *M. incognita* infection compared to the highly susceptible melon cultivar. Thus grafting is an effective tool that may enable the susceptible melon scion to tolerate *M. incognita* which leads to an increase in the plant's survival rate compared to scion plants or un-grafted melon seedlings.

Conclusions

Grafting melon plants onto the previous tolerant cucurbit rootstocks has the potential to be an additional management tool for tolerance to root-

knot nematode *M. incognita* diseases, improvement in plant growth parameters as well as increase peroxidase, polyphenol oxidase, and total phenol activities in plants.

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Conflict of interests: The authors declare no conflict of interest.

Authors contribution: DI and MZ suggested the plan of study and followed up on the carried-out work in addition to scripting the manuscript. DI executed the plan of work, analyzed the output data, and contributed in writing the manuscript.

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استجابة أصول القرعيات لمقاومة نيماتودا تعقد الجذور ميليدوجيني اوجنيتا *Meloidogyne incognita*

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هدفت الدراسة الحالية إلى قياس مدى حساسية ثمانية أصول من القرعيات وصنف واحد من الشام (Hybrid London) للإصابة نيماتودا تعقد الجذور ميليدوجيني اوجنيتا. وجد أن صنف الشام (Hybrid London) حساس للإصابة بـ ميليدوجيني اوجنيتا، كذلك تم تصنيف Nun 6001، Squash No3، Super Shintosa، Coptl، Ferro RZ، Flixel Fort و Bottle gourd على أنها متحملة للإصابة وصنف أصل Pakistani Loof كصنف حساس للإصابة. ومن بين الأصول التي تم اختبارها أثناء زراعة شتلات الشام المطعومة في التربة المصابة، أعطى كل من أصل Nun 6001 و Ferro RZ معدل بقاء ومعايير نمو أعلى للنباتات مقارنة بالأصول الأخرى وصنف الشام المقارن. أسفرت النتائج أن عدد العقد الجذرية والإناث وكتل البيض لنيماتودا تعقد الجذور ميليدوجيني اوجنيتا تم تثبيطها معنويًا في جذور الأصول محل الدراسة مقارنة بالصنف غير المطعوم من الشام. اختلفت جميع الطعوم من أصناف القرعيات في قدرتها على تحفيز النشاط الانزيمي لكلا من انزيم البيروكسيداز (PO) وانزيم البوليفينول أوكسيداز (PPO) وأيضا محتوى الفينولات بالنبات. أوضحت الدراسة أن تطعيم نباتات الشام على جميع الأصول المتحملة السابقة هي أداة فعالة قد تمكن طعم الشام القابل للإصابة بميليدوجيني اوجنيتا في التربة المصابة إلى أن تكون متحملة مما يؤدي إلى زيادة معدل بقاء النباتات ومعايير النمو، مقارنة بزراعة بذور الشام أو زراعة شتلات الشام الغير مطعومة.