Pakistan J. Zool., vol. 55(2), pp 723-733, 2023 DOI: https://dx.doi.org/10.17582/journal.pjz/20211226141226

Effect of Grafting on Resistance of Cucurbit Hybrids against *Meloidogyne incognita* Infection under Greenhouse Conditions

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ABSTRACT

The host response of cucurbit hybrids (6001 F1, BS F1, Ohbkatosa F1, and RG F1) as rootstocks, watermelon hybrids (Aswan F1 and Nems) as scions, and their accessions to *Meloidogyne incognita* was detected. Nematode penetration, development, and potential reproduction were estimated. The rootstocks (6001 F1, Ohbkatosa F1, and RG F1) were considered highly resistant and BS F1 resistant. Scions (Aswan F1, and Nems) were moderately susceptible. Grafted watermelon hybrids varied in their reaction to nematode infection. Rootstock, 6001 F1 reduced significantly nematode parameters meanwhile BS F1 was insignificant. Levels of *ClabZIP59* and *ClabZIP52* genes expression were upregulated significantly in nematode infected treatments compared to the check. Root exudates of Aswan F1/6001 F1 (HR) and Aswan F1/BS F1 (MS) cultivars were analyzed by GC/MS/MS and revealed that the most abundant components in the root exudates were fatty acids, terpenoids, alkaloids, alcohols, and esters with different concentrations. Terpenoids were the major compounds in root exudates of HR cultivar and fatty acids in MS ones. Such phytochemicals' high ratios were proportionally connected with the host resistance reaction.

Article Information
Received 26 February 2021
Revised 18 January 2022
Accepted 11 February 2022
Available online 22 March 2022 (early access)
Published 21 December 2022

Authors' Contribution
Methodology all authors. Formal analysis AA and HK. Investigation all authors. Data curation NM and AA. Writing original draft preparation all authors. Writing review and editing AA and HK.

Key words
***Meloidogyne incognita*, Resistance, bZIP transcription factor, Root exudates**

INTRODUCTION

The gall-forming nematodes, *Meloidogyne* spp. are serious pests infecting cucurbit crops and play an effective role as a pathogenic nematode in growth deterioration and yield reduction (Farahat *et al*., 2012; Amin and El-Wanis, 2014). Grafting of cucurbit scions on resistant rootstocks is a means of controlling soil-borne and root-knot nematode diseases in different soil types especially the intensive ones (Amin *et al*., 2012; Byeon *et al*., 2014; Aydinli *et al*., 2019; El-Mesalamy *et al*., 2020; García-Mendívil and Sorribas, 2021).

The basic group of leucine zipper (*bZIP*) is the largest transcription factor that plays a vital role in the defense mechanism of plants to *M. incognita* (Yang *et al*., 2015; 2018). The functional of *ClabZIP* genes are responsible for plant defense against different biotic and abiotic stresses (Yang *et al*., 2019). They also provided new insights into the functions of different *ClabZIP* genes in watermelon and their roles in response to cold stress and nematode infection.

Resistant plants produce compounds that improve the physiological defense mechanism e.g. enzymatic and non-enzymatic compounds against parasitic nematodes (Kesba and El-Beltagi, 2012) jasmonic acids (Reymond and Farmer, 1998), polyphenols (Metodiewa *et al*., 1999). Yu and Matsui (1994) found that the exudates contained phytotoxic substances such as benzoic, p-hydroxybenzoic, 2,5-dihydroxybenzoic, 3-phenyl propionic, cinnamic, p-hydroxycinnamic, myristic, palmitic, and stearic acids, as well as p-thiocyanatophenol and 2-hydroxybenzothiazolein root exudates of cucumber (*Cucumis sativus* L). Some of these compounds have nematistatic and/or nematicidal activity of some phytonematodes (Yang *et al*., 2016; Danahap and Wonang, 2016; Abdel-Rahman *et al*., 2019).

This study was aimed to (1) screen commercially unknown ancestor's rootstocks and local watermelon hybrids for their resistance to the root knot nematode, *M. incognita* in Egypt, (2) to study the effect of grafting on nematode reproduction and (3) to study the molecular and biochemical reactions of rootstocks to nematode infection.

MATERIALS AND METHODS

Meloidogyne incognita *propagation*

A pure stock culture of *M. incognita* (Taylor *et al*., 1955) population was obtained from an isolate belonging

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0030-9923/2023/0002-723 $ 9.00/0

to Nematology Division, Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University, Giza, Egypt and propagated on tomato plants (*Solanum lycopersicum* Mill) cv. Super Strain B.

Greenhouse screening tests

Seeds of certified local and imported cucurbit hybrids, 4 rootstocks (6001F1, BSF1, OHBKATOSAF1, RGF1), 2 watermelon cultivars (Aswan F1, Nems) and their grafting were obtained from the Roots Nurseries located at the 75th kilometer of the Cairo-Alexandria Desert Road.

Seeds were germinated and nursed separately in foam seed trays containing peat moss under controlled environmental conditions (24 ± 3°C and 70% humidity). Three weeks after germination, the most uniform seedlings were transplanted into 20cm diameter earthen pots containing steam-sterilized sandy loam soil (1:1, v/v) for assessing their reactions to the root-knot nematode, *M. incognita*.

Reactions of the testing cucurbit rootstocks, cultivars, and their grafting to the infection of *M. incognita* were studied under greenhouse conditions at 35 ± 5°C early summer (May to June, 2021). Inocula were introduced to pots after one week of establishing seedlings. Each seedling received 1000 newly hatched J_2 of *M. incognita* by pouring nematode water suspension in 3-4 pores made around the plant root, then covered with sterilized soil, and immediately watered. The pots were arranged on a clean bench in a completely randomized design with six replications for each hybrid and watered regularly. Non-inoculated seedlings were served as a check.

Forty-five days after nematode inoculation, plants were uprooted gently, and measurements were performed on nematode parameters, numbers of galls, females, egg masses per root after staining with hot acid fucine for 3 minutes, final population, the build-up (Pf/Pi), and the number of eggs per egg mass (Hooper *et al*., 2005). Also, rate of penetration (R.P.) = (root population ÷ initial population) × 100, rate of reproduction (R.R.) = (number of egg masses ÷ root population) × 100, potential reproductive index (P.R.I.) = (nematode final population on the hybrid ÷ nematode final population on the highest hybrid) × 100 were calculated (Norton, 1979).

Expression of ClbZIP59 *and* ClbZIP52 *genes*

Total RNA was isolated from leaves of Aswan F1 grafted on 6001 F1 or BS F1 by RNeasy Plant Mini Kit (QIAGEN, Germany).

The purity of total RNA was assessed by the 260/280 nm ratio (between 1.8 and 2.1). Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots of total RNA were used immediately for reverse transcription (RT), otherwise stored at -80 °C.

The complete Poly(A)$^+$ RNA isolated from leaf tissues was reverse transcribed into cDNA in a total volume of 20 µl using RevertAid^{TM} First Strand cDNA Synthesis Kit (MBI Fermentas, Germany).

StepOne™ Real-Time PCR System from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA USA) was used to determine leaf's cDNA copy number of Aswan F1 grafted on both 6001 F1 and BS F1. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex Taq TM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction program was allocated to 3 steps. The first step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. At the end of each sqRT-PCR, a melting curve analysis was performed at 95.0°C to check the quality of the used primers. Each experiment included a distilled water control. At the end of each qPCR, a melting curve analysis was performed at 95.0°C to check the quality of the used primers (*ClbZIP59* and *ClbZIP52* genes) shown in Table I. The relative quantification of the target to the reference was determined by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Table I. Primers sequence used for *RT-qPCR*.

Gene	Sequences (5`-3`)	NCBI reference
ClbZIP59	F:CCA TGTA ATG TTG CCA TCC AG	CIa007792
	R:CCG GGA AGA ACA ATG AGCTG	
ClbZIP52	F:CGC GAG GAG ATC GAG AAT GA	CIa016581
	R:ACG GT GAG ATT AGC GAC GAT	
β-actin	F: GTT CCG CTG TCA AAC AAG GT	CIa017709
	R: TGG TTT GTG CCT TCT TTC CG	

GC/MS/MS analysis of the phytochemicals in root exudates

Using 6 subsamples of root exudates of Aswan F1/6001 F1 (HR) and Aswan F1/BS F1 (MS) were analyzed by GC/MS/MS (Gas Chromatography-Tandem Mass Spectrometry) . The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with a linear velocity of 1ml/min. The injector and detector temperatures were 200ºC and 250ºC, respectively. The volume injected 1µl of the sample. The MS operating parameters were as follows: Ionization potential 70 eV, interface temperature 250ºC, and acquisition mass range 50–800.

The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Dong *et al*., 2014).

Statistical analysis

The obtained data were statistically analyzed by one-way ANOVA with SPSS software package version 12 (SPSS, 2003). The differences between means were compared by multiple ranges Duncan test at the 5% significance level.

RESULTS

Screening of cucurbit hybrids against the root-knot nematode, Meloidogyne incognita

Four imported cucurbit hybrids (6001 F1, BS F1, Ohbkatosa F1, and RG F1) rootstocks, 2 local hybrids of watermelon (Aswan F1 and Nems) unknown in their parentage, and their grafting were tested for their resistance to the infection with the root-knot nematode, *M incognita*. Nematode penetration, development, and potential reproduction were estimated.

Data extracted after 45 days of cucurbits exposure to *M. incognita* are presented in Table II. Nematode penetration, development, fecundity, and reproduction were variable on hosted cucurbits. Almost equal very low rates of penetration were achieved on rootstocks. 6001 F1, BS F1, Ohbkatosa F1, and RG F1 showed very low penetration, fewer females, and egg masses, and prohibited *M. incognita* from folding. It was also noticed that a negative correlation between rates of penetration, and reproduction on rootstocks. The highest values of reproduction are corresponding with the lowest rates of penetration. Watermelon hybrids, Aswan F1 and Nems cultivars seemed to be the most favorable to the nematode infection. On such cultivars, the nematode gained the highest nematode final population, nematode build-up, and the number of galls with significant differences amongst the tested hybrids. Also, they sustained the highest values of females and egg masses per root which achieved the highest numbers of deposited eggs per egg mass, and consequently, the nematode was able to fold more than once with no significant differences and performed to a great extent higher rates of penetration. The highest rates of penetration resulted in moderate reproduction. Yet achieved maximum percentages of potential reproductive indices. Adverse results were recorded in nematode parameters when the cultivars were grafted on rootstocks where the results showed that grafting turned the tested rootstocks reactions to be more infectious to the nematode. By grafting Aswan F1 on the four rootstocks, it has been found that Aswan F1/6001 F1 achieved the lowest values of nematode parameters compared with the other accessions; meanwhile, Aswan F1/BS F1 recorded significant differences with the highest values of numbers of galls, females, egg masses as well as final population, build-up and eggs/egg mass. Also, the maximum rate of penetration was recorded on Aswan F1/BS F1, followed by Aswan F1/ Ohbkatosa F1 and Aswan F1/RG F1 then Aswan F1/6001 F1. Concerning the rate of reproduction (% R.R.), Aswan F1/6001 F1 exhibited the high value followed by Aswan F1/RG F1, Aswan F1/BS F1, and Aswan F1/ Ohbkatosa F1.

On the other hand, grafted cv. Nems on such rootstocks achieved the opposite results on nematode parameters with more or less significance when grafted on 6001 F1 which recorded the higher values of penetration, numbers of galls, females, and egg masses more than the other rootstocks as well as nematode final population and eggs/egg mass. Accordingly, the rate of reproduction was recorded as follows, Nems/RG F1 (36%), Nems/ Ohbkatosa F1 (24%), and finally Nems/BS F1 (17%).

The potential reproductive indexes were getting along with the results of the other nematode parameters which showed the lowest values on rootstocks. The highest values were obvious on CVs. Aswan F1 and Nems (100 and 98%) respectively, and the lowest recorded with the rootstock, 6001 F1 (16%). Grafting either Aswan F1 or Nems on four rootstocks increased the nematode potential indexes with moderate to high values and even turned BS F1 ranking from resistant to moderately susceptible and lowered the others to be resistant instead of being highly resistant except 6001 F1. Ranking the host status is shown in Table II.

Table II. Reproductivity of *M. incognita* on cucurbitaceous hybrids (watermelon).

Hybrids		Galls/ root	Females/ root	Egg masses/ Root	Final population	Pf/Pi	Eggs/ Egg-mass	% R.P.	% R.R.	P.R.I.	Host status
Rootstock	6001 F1	20 J	12 I	18 F	253 J	0.25 K	56 G	3	60	16	HR
	BS F1	51 I	16 H	39 D	705 E	0.71 E	163 C	6	71	45	R
	Ohbkatosa F1	21 J	16 H	20 F	322 I	0.32 J	72 F	4	56	21	HR
	RG F1	30 J	27 G	26 E	442 H	0.44 H	101 E	5	49	29	HR
Cultivar	Aswan F1	709 A	405 A	210 B	1550 A	1.55 A	234 A	62	34	100	MS
	Nems	459 B	372 B	216 A	1524 A	1.52 A	237 A	59	37	98	MS
Acces-sions	Aswan F1 / 6001 F1	107 H	79 F	41 D	383 I	0.38 I	66 FG	12	34	25	HR
	Aswan F1 / BS F1	391 C	223 C	79 C	1143 B	1.14 B	211 B	30	26	74	MS
	Aswan F1 / Ohbkatosa F1	387 C	214 C	36 D	790 D	0.79 D	135 D	25	14	51	R
	Aswan F1 / RG F1	161 F	104 G	39 D	597 FG	0.60 FG	114 E	14	27	39	R
	Nems / 6001 F1	284 D	155 D	40 D	888 C	0.89 C	173 C	20	21	57	R
	Nems / BS F1	206 E	111 E	22 EF	566 G	0.57 G	108 E	13	17	37	R
	Nems / Ohbkatosa F1	137 G	75 F	23 EF	632 F	0.63 F	134 D	10	24	41	R
	Nems / RG F1	125 G	68 F	39 D	623 FG	0.62 F	129 D	11	36	40	R
SE±		18.216	16.002	8.320	49.265	0.005	7.096				

Means followed by the same letter(s) within a column are not significantly different ($P \leq 0.05$) according to Duncans' multiple range tests. Final population, Embedded stages + Soil population. R.P., Rate of Penetration = (Root population / Initial population) × 100. % R.R., Rate of Reproduction = (Number of egg masses / Root population) × 100. P.R.I., Potential Reproductive Index = (Nematode final population on a hybrid/Nematode final population on a highest hybrid) × 100. SE±, Standard error.

Genome-wide identification of bZIP family genes in watermelon

To investigate the response of grafted watermelon cultivar (Aswan F1) on two rootstocks (6001 F1 and BS F1) to nematode infection, the expression levels of the genes *ClabZIP59* and *ClabZIP52* in two different Aswan F1/6001 F1 (HR) and Aswan F1/BS F1 (MS) accessions were measured using quantitative real-time PCR (qRT-PCR) analysis. The transcript abundance of these two genes in uninfected and nematode-infected plants was examined. It was noticed that *ClabZIP59* was significantly upregulated in nematode infected treatments of the HR accession (Aswan F1/6001 F1) compared to the check (uninfected) treatments. It was also found that *ClabZIP59* was significantly downregulated in nematode treatments of the MS accession (Aswan F1/BS F1) compared to nematode treatments of the HR ones. Levels of *ClabZIP59* expression were upregulated significantly in nematode infected treatments of Aswan F1/BS F1compared to the check treatment of the same accession (Figs. 1 and 2). For *ClabZIP52* the same expression manner was followed. *ClabZIP52* expression levels were significantly upregulated in nematode infected treatments compared to check treatments of both, HR and MS accessions, while *ClabZIP52* levels of expression were significantly down-regulated in nematode infected plants of the MS accession when compared to nematode infected plants of the HR accession (Figs. 3 and 4).

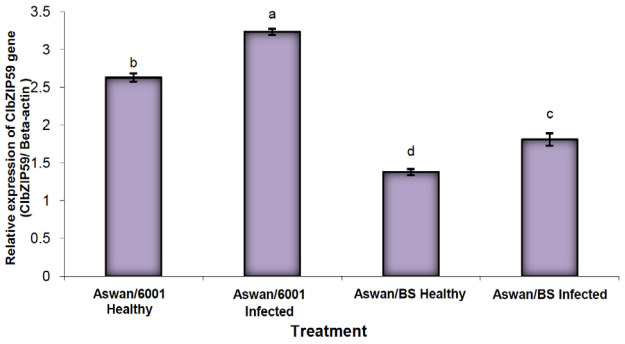

Fig. 1. Expression of C1bZIP59 gene in watermelon plant samples. Data are presented as Mean±SD.

The phytochemical contents of root exudates

Components of Aswan F1/6001 F1 (HR) and Aswan F1/BS F1 (MS) root exudates were analyzed by GC/MS/MS (Tables III and IV). The exudates comprised different chemical groups of components. Fatty acids, Terpenoids, alkaloids, alcohols, and esters were highly present with different concentrations. Fatty acids were the major compounds in root exudates, except the highly resistant

Table III. The main classes of phytochemical compounds of root exudates of Aswan F1/6001 F1 (HR) identified by GC/MS/MS.

Compound	Sum area %	Chemical group	%
N_(4_Methylphenyl)_N_butyllurea	1.14	Amides, Imides	1.14
2-(4-(Dimethylamino)phenyl)ethanol	7.16	Alcohols	8.33
3_ Methyl pyrazolobis(diethyl boryl) hydroxide	1.17		
Sec-butyl 4-ethylbenzoate	1.05	Esters	6.00
Octyl 4-methyl benzoate	4.95		
1,2,3,4-tetrahydro-6,7,8-trimethoxy(Anhalinine)	1.07	Alkaloids	14.46
Carnegie	5.94		
Benzenammine	0.58		
1-Methyl-2-phenyl-Indol	1.25		
Benzoic acid(4-dimethylamino methyl ester)	1.17		
Hydrazine carboxamide	0.61		
Formamide	0.47		
4-Amino-2-nitro-benzaldeehyde oxime	0.59		
Benzene(1,2-bis(2,5-dimethyl phenylamino methyl)	0.99		
Pyridine(1,2,3,6-tetrahydro-1-methyl-4-(4-chlorophenyl))	1.06		
Dodecahydropyrido	0.73		
Hexadecanoic acid, methyl ester	6.89	Fatty acids	
Methyl 6-octadecenoate	9.62		
Heptadecanoic acid, methyl-, methyl ester	2.15		30.34
Hexadecanoic acid(palmitic acid)	3.61		
9-Octadecenoic acid (Oleic Acid)	8.07		
4-(Anisylideneamino) cinnamic acid	1.11	Flavonoids	1.11
L_Aspertyl_L_phenlalanine methyl ester (Aspartame)	0.52	Dipeptides	0.52
Corlumine	16.79	Terpenoides	
4-cyclophenxene-1,2-dicarboxylic acid	0.42		
1,2,3-Trimethoxy-5-(1-propenyl benzene	0.73		
Tert-Butyl(Dimethyl)(10-undecynyloxy)silane	0.78		
Tert-butyl dimethyl silyl ether	0.56		23.52
10-methyl-E-11-tridecen-1-ol propionate	0.46		
cyclotrisiloxane	2.33		
d-Glycerol-gluco-heptose	0.92		
4-Hydroxy-9-vinyladamantane-2,6-dione	0.53		
3-(3-carboxy-4-hydroxyphenyl)D-alanine	0.4	Mixed phenolic compounds (Alkaloid and phenolic)	1.72
Hexahydropyridine(1-methyl-4-(4,5-dihydroxyphenyl)	0.58		
3,4-Dihydroisquinolin-7-ol)	0.74		
13 octadedecenoate	1.27	Aldehyde	1.27

(Aswan F1/6001 F1) in which the major group was Terpenoids. In Aswan F1/6001 F1 (HR) root exudates, the maximum percentage recorded was Fatty acids (30.34%) followed by Terpenoids (23.52%) and Alkaloids (14.46%) then Alcohols (8.33%) and Esters (6.00%), while the minimum was a mix of phenolic compounds (1.72%) followed by Aldehyde, Amides, Flavonoids, and Dipeptides (1.27, 1.14, 1.11, and 0.52%), respectively. Fatty

acids also were the maximum chemical groups observed in root exudates of Aswan F1/BS F1 (43.01%) followed by Dipepptides, Alkaloids, Terpenoids, and Aldehydes (12.54, 5.91, 4.27, and 4.25%), respectively; meanwhile, the minimum groups observed were Monoglycerides, Unsaturated hydrocarbon, Alcohols, and non-essential amino acid (2.54, 1.36, 1.21, and 1.09%), respectively. In general, by comparing between the percentages of root exudates contents of the 2 grafted HS and Ms plants, the toxic compounds with significant suppressive effects on nematode (Terpenoids, Alkaloids, Alcohols, and Esters) were much higher in HR than MS ones which showed the role of these compounds on resistance to RKN (Fig. 5).

Table IV. The main classes of phytochemical compounds of root exudates of Aswan F1/BS F1 (MS) identified by GC/MS/MS.

Compound	Sum area %	Chemical groups	%
L_ Serine, o_(phenylmethyl)	1.09	Non-essential amino acid	1.09
Hexadecanoic acid, 2,3-dihydroxypropyl ester(Glycerylpalmitate)	2.54	Monoglycerides	2.54
L_Aspertyl_L_phenlalanine methyl ester (Aspartame)	12.54	Dipeptides	12.54
Hexadecanoic acid, methyl ester (Methyl palmitate)	10.44	Fatty acids	43.01
6-Octadecenoic acid	10.46		
9-Octadecenoic acid (Oleic Acid)	22.11		
1,19-Eicosadiene	1.36	Unsaturated Hydrocarbon	1.36
2, 3-Dihydroxypropyl elaidate (Monoelaidin)	1.21	Alcohols	1.21
2-(4-(Dimethylamino) phenyl) ethanol	0.96	Alkaloids	5.91
Methyl 2-amino-3-(1H-imidazol-4-yl)propanoate	1.43		
5-Amino-1-benzoyl-1H-pyrazole-3,4-dicarbonitrile	0.54		
Benzoic acid	1.34		
Benzeneethanamine	0.53		
2,4-Dinitrophenyl) hydrazone	1.11		
Nonanal	2.63	Aldehyde	4.25
Heptanal	0.72		
Octanal	0.9		
Corlumine	1.45	Terpenoides	4.27
Dimethyl acetal	0.56		
7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	0.83		
Di-(9-Octadecenyl)glycerol	1.43		

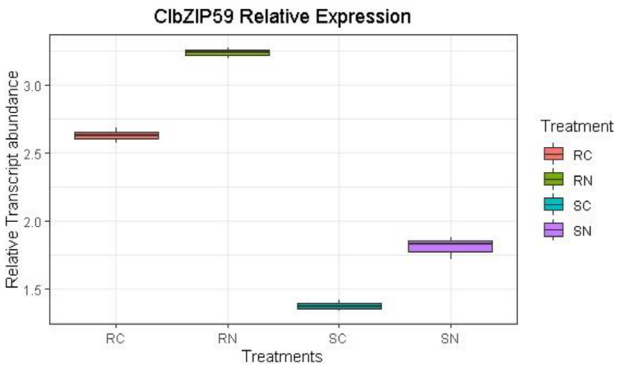

Fig. 2. The relative expression of C1bZIP59 gene in watermelon plant samples (RC) Aswan F1/6001 F1 health, (RN) Aswan F1/6001 F1 F1/BS F1 infected with the nematode.

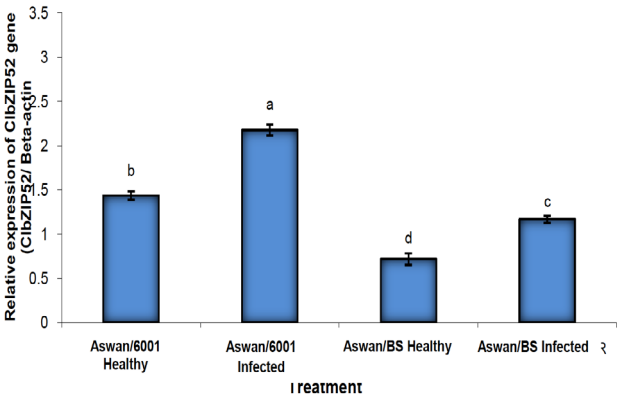

Fig. 3. Expression of C1bZIP62 gene in watermelon plant samples. Data are presented as Mean±SD.

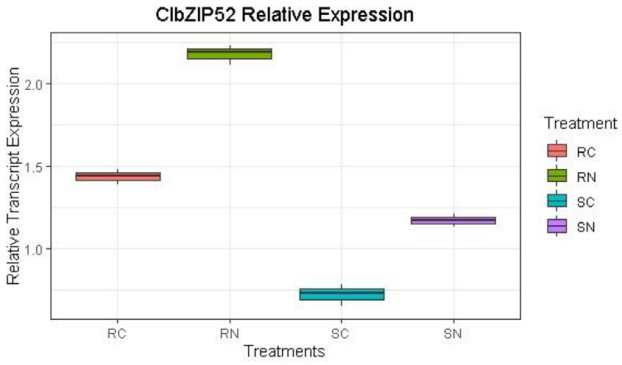

Fig. 4. The relative expression of C1bZIP52 gene in watermelon plant samples, (RC) Aswan F1/6001 F1 healthy, (RN) Aswan F1/6001 F1 infected with nematode, (SC) Aswan F1/BS F1 healthy, (SN) Aswan F1/BS F1 infected with the nematode.

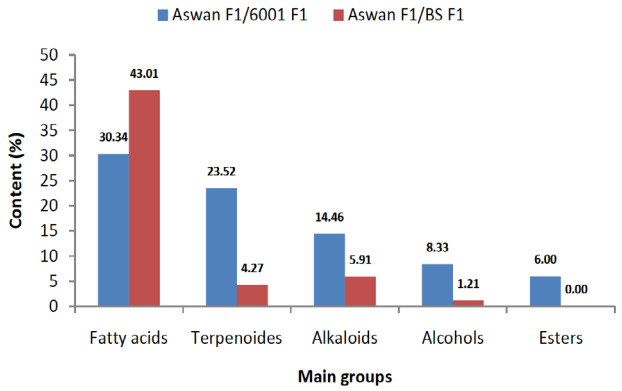

Fig. 5. Comparsion between the high contents (%) of phytochemical groups in root exudates of Aswan F1/6001 F1 (HR), and Aswan F1/BS F1 (MS).

DISCUSSION

Results proved that 6001 F1, Ohbkatosa F1, and RG F1 achieved acceptable scion-rootstock compatibility with watermelon. Out of the 8 accessions, one exhibited high resistance (Aswan F1/6001 F1), 6 were categorized resistant and 1 was considered moderately resistant. Few but small galls were found on the root systems of these resistance accessions.

The grafting results indicated that reduction in the number of galls, females, and egg masses confirm reports of (Amin *et al*., 2012; El-Wanis *et al*., 2013) who reported that grafting of cucumber onto resistant rootstocks seems to be effective against *M. incognita*. Also, grafting changed the plant susceptible reaction to being tolerant but not resistant (Sigüenza *et al*., 2005).

Grafting commercial cultivars of watermelon on resistant rootstocks is considered a promising approach to control pathogens causing plant diseases has become an acceptable practice (Lopez- Gomez *et al*., 2016; Giné *et al*., 2017; Leonardi *et al*., 2017).

Two watermelon cultivars (Aswan F1 and Nems) proved to be excellent hosts for nematode reproduction due to the high numbers of galls and egg masses (Anwar *et al*., 2007; Zhang and Schmitt, 1994). With the low build-up, rate of penetration, and potential reproductive indexes, the other treatments (rootstocks and their assessions) were ranked as highly resistant and resistant to *M. incognita*. This suggests that J2 penetration, development to egg-laying females were impaired and their egg hatching might be reduced by some root factors (Edelstein *et al*., 2010; Mukhtar *et al*., 2013; Liu *et al*., 2014).

The resistant accessions identified are used in genetic studies to ascertain the mode of inheritance for defense response to root-knot nematodes in watermelon (Thies *et al*., 2010). Hybrids of cucurbits are considered potential multigenic banks for resistance to nematode infection. Our results proved that there is wide significant genetic variability within hybrids against the root-knot nematode, *M. incognita*.

To cope with the surrounding biotic, abiotic stresses and pathogens, plants have a variety of defense mechanisms against such stresses. One of those mechanisms is the expression of transcription factors regulating the expression of stress-related genes (Jin *et al*., 2017). The basic leucine zipper (bZIP) family members have been recorded to regulate the plant defense mechanisms against bacterial pathogens (Li *et al*., 2017; Lim *et al*., 2018). Other members of the bZIP transcription factors family such as *ClabZIP52* and *ClabZIP59* were found to be upregulated in the roots of watermelon in response to root-knot nematode infection (Yang *et al*., 2019). Our results indicate that levels of both genes were found to be upregulated significantly in the leaves of both the highly resistant and moderately susceptible grafted watermelon leaves. This gives an idea that the grafting process enhanced the resistance ability of the susceptible watermelon cultivar and affected the degree of resistance in the highly resistant rootstocks.

Regarding the chromatographic analysis of root exudates, a lot of compounds with nematicidal effects existed in both highly resistant (HR) and moderately susceptible (MS) cultivars. Baetz and Martinoia (2014) stated that the profile defense of root exudates is diverse and strikingly dynamic. Also, the importance of root exudates in the plant resistance mechanism against plant-parasitic nematodes and their nematicidal action was reported (Salako, 2002; Abdel-Rahman *et al*., 2019). Such action is greatly affected by the active ingredient

that can kill or inhibit the nematode growth and/ or suppress nematode development after a host was planted (Sharma and Scolari, 1984). The mortality of juveniles may be attributed to several compounds exuding from plant roots in different ratios, resulting in variable contact nematicidal effectiveness. These nematicidal properties might restrict nematode penetration and inhibit metabolic reactions such as those of respiratory enzymes, acetylcholinesterase enzyme, hydrolysis of acetylcholine by acetylcholinesterase, and esterases that function in various metabolic systems (Atkinson and Fowler, 1990; Nile *et al*., 2017). Toxic substances of root exudates of certain plants may include; spectine, autofine, phenols, saponins, alkaloids, tylophonine, and glucosinolates (Monfort *et al*., 2007; Jada *et al*., 2013) which have a negative effect on nematode reproduction.

Our results indicated the presence of fatty acids with high ratios in both highly resistant and moderately susceptible cultivars. This could be expected in the resistant one referring to Zhang *et al*. (2012) findings, who reported an inhibitory effect of nine tested fatty acids (butyric, caprylic, capric, lauric, myristic, palmitic, and oleic acids) on *M.incognita* reproduction and egg hatching. Also, acetic and caprylic acids reduced hatching most by impairing embryogenesis at 'static-vermiform' and 'gastrula' stages respectively (Bansage and Visser, 1965). On the other hand, the presence of high concentrations of fatty acids in the root exudates of the susceptible cultivar did not suppress RKN. According to Oka (2021), this may be interpreted by their final concentration which did not reach the dose to be nematicidal in the soil; he tested 60 aromatic compounds mainly carboxylic acids, alcohols, aldehydes, and phenols against *Meloidogyne* spp., 35 compounds were not only attractants but also nematicidal to J2 of all nematode species. In addition, the reason for this suicidal behavior is not known, suggesting that these attractants act as cues for nematodes orientation, but have never found them at nematicidal concentrations in the rhizosphere. Iannucci *et al*. (2013) found that the peak concentrations of vanillin and 4-hydroxybenzoic acid secreted from *Avena fatua* roots to the rhizosphere soil were approximately 0.16 µg/ kg soil, a very much lower than that used in the Oka (2021) study or even other studies which used concentrations measured with milligrams (1mg = 1000 µg).

The high ratios of fatty acids in both HR and Ms cultivars are close to each other, so we should not expect opposite effects due to the fatty acids, i.e. the effect of fatty acids in both cultivars are almost the same, and did not affect *Meloidogyne* adversely.

In the HR cultivar, the presence of Terpenes (23%) and alkaloids (14 %) with such amounts explain the reductions in gall numbers, as terpenes were found to be nematicidal as reviewed by (Ohri and Pannu, 2009; Wuyts *et al*., 2006) who reported that alkaloids and terpenoids were nematicidal and/or inhibitory for egg hatching. Oppositely, the ratios of terpenes and alkaloids in the susceptible cultivar were very much lower than that of the resistant one, which did not achieve the nematicidal effect, leading to more successful infections.

Esters amount in root exudates from HR and MR tomato rootstocks increased obviously after inoculation (Yang *et al*., 2016), this may indicate to the role of esters as resistance inducers in the root exudates of the resistant cultivar. Also, Abdel-Rahman *et al*. (2019) reported that the most abundant components in the root exudates of marjoram and rosemary were flavonoids, phenolics, and terpenoids which were suppressive to nematode survival, development, and reproduction.

Concerning the MS cultivar, the presence of alcohols and aldehydes does not necessarily associate with inducing resistance in plants. Although some studies referred to them as nematicidal phytochemicals, such chemicals play a role as nematode attractants as well. Also, Oka (2021) found that some aromatic acids, alcohols, and aldehydes attract the juveniles of RKN. It seems that the interaction between the diverse compounds in the root exudates of plants and nematodes depends on several factors; the kind of compound, concentration, and nematode species. Any change in any factor could alter or promote the plant-nematode interaction to make the host compatible or incompatible and vice versa.

CONCLUSION

Of these rootstocks, 6001 F1 proved to be highly resistant to *M. incognita* and it may be considered as a promising rootstock to control soilborne diseases especially under organic farming conditions and sustainable agricultural systems. In any screening test seeking for resistance in plant species should be confirmed with the genetic analysis. Also, these results indicate that more experimental studies are needed either in the greenhouse or the field to ensure that the biochemical and molecular contribution plays an essential role in crop resistance against the root-knot nematode.

ACKNOWLEDGMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Also, we would like to thank Esraa M. Ahmad for her help with molecular analysis.

Statement of conflict of interest

The authors have declared no conflict of interest.

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