



Growth and defense enzyme responses of grafted cucurbit crops to varying initial populations of *Meloidogyne incognita*

Research Article

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Abstract: Root-knot nematode (*Meloidogyne incognita*) is among the most serious constraints to cucurbit crop production worldwide. This study evaluated the effects of different initial population densities (0, 1,000, 2,000, 4,000, and 8,000 second-stage juveniles [J_2]/plant) on nematode development, reproduction, plant growth, and defense-related enzyme activities in watermelon (*Citrullus lanatus*, cv. Aswan F1) and sweet melon (*Cucumis melo*, cv. Faransawy) hybrids, their grafted combinations, and the resistant rootstocks 6001 F1 and BS F1. Plants were grown in sterilized sandy-loam soil under greenhouse conditions, and nematode parameters, including gall index, females per root system, egg masses per root, final population (Pf), reproduction factor, penetration rate, and reproduction rate were assessed 60 days after inoculation. Both host genotype and inoculum density significantly ($P < 0.001$) affected nematode reproduction and plant growth traits. Resistant rootstocks and their grafted scions consistently exhibited lower Pf/Pi ratios, fewer galls, reduced shoot weight losses, and significantly higher peroxidase and polyphenol oxidase activities compared to susceptible cultivars. These findings demonstrate that combining resistant rootstocks with grafting can effectively suppress *M. incognita* multiplication and associated growth losses, while enzyme activity profiling provides a useful biochemical indicator of partial resistance in cucurbit breeding and management programs.

Keywords: Root-knot nematode • Grafted cucurbits • Rootstock resistance • Defense enzymes • Reproduction factor • Inoculum density

1. Introduction

Root-knot nematodes (*Meloidogyne* spp.) are among the most devastating plant-parasitic nematodes worldwide, causing substantial economic losses in cucurbit cultivation (Jones et al., 2017; Sikora et al., 2022). These nematodes infect plant roots, inducing gall formation that disrupts water and nutrient uptake, leading to stunted growth, diminished yields, and poor fruit quality (Khan and Tanaka, 2023). *Meloidogyne incognita* is notably prevalent globally and highly polyphagous, posing a significant challenge to sustainable cucurbit production (Bui and Desaegeer, 2022).

Watermelon (*Citrullus lanatus*) and sweet melon (*Cucumis melo*) are economically important cucurbit crops highly susceptible to *M. incognita* infestations. Even low nematode population densities can markedly impair plant growth and productivity (Chahar et al., 2021; Patel et al., 2023). Notably, inoculum levels as low as 10 second-stage juveniles (J_2) per plant reduce vine length and biomass in cucumbers, underlining the critical need for effective nematode management strategies.

Grafting susceptible scions onto resistant rootstocks represents a promising and eco-friendly approach to mitigate root-knot nematode damage. Resistant rootstocks confer enhanced tolerance by restricting nematode penetration and reproduction through both physical barriers and induced biochemical defenses (García-Mendivil and Sorribas, 2021; Mukhtar and Hussain, 2023; McKenry and

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Anwar, 2006). However, grafting success depends on rootstock-scion compatibility and the rootstock's capacity to withstand biotic and abiotic stresses (Jayswal and Lal, 2020).

Biochemical defense responses, mediated by key enzymes, such as peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase, are integral to plant resistance against nematodes. These enzymes facilitate cell wall reinforcement via lignin and phenolic synthesis, generate reactive oxygen species, and produce antimicrobial compounds, thereby limiting pathogen progression (Ngadze et al., 2012). Increased activities of POD and PPO have been correlated with reduced *M. incognita* reproduction and enhanced host tolerance in cucurbits (Chandrawat et al., 2020; Khalifa et al., 2024).

Despite these advances, a comprehensive understanding of how defense enzyme activities respond to varying *M. incognita* initial population densities in grafted cucurbit hybrids remains incomplete. Investigating these biochemical responses in conjunction with nematode reproduction and plant growth under differing inoculum pressures is essential to optimize grafting strategies and refine integrated pest management practices.

While the relationship between inoculum level density and disease severity is well established, the integrated physiological, biochemical, and nematological responses of grafted cucurbit systems under varying inoculum pressure remain less explored. This study uniquely evaluates how grafting onto resistant root stocks influences not only *M. incognita* reproduction and plant growth but also key defense enzyme activities across a gradient of nematode densities, providing a mechanistic insight into rootstock-mediated resistance.

2. Materials and methods

2.1 Propagation and maintenance of Root-knot nematode

A pure culture of *M. incognita* was maintained on tomato plants (*Solanum lycopersicum* Mill.) cv. Super Strain B, following the method of Abdel-Rahman et al. (2023). The culture was sustained under greenhouse conditions at the Department of Zoology and Agricultural Nematology (Nematology Division), Faculty of Agriculture, Cairo University. Species identification was confirmed by morphological examination of perineal patterns from adult females (Khalifa et al., 2024), and race determination was conducted according to the differential host test of Taylor and Sasser (1978). Tomato cultivar susceptibility profiles confirmed the population as *M. incognita* race 1.

For inoculum preparation, heavily galled roots were collected from infested tomato plants. Egg masses were isolated using the sodium hypochlorite method as described by Khalifa et al. (2024), a modification of the Hussey and Barker (1973) protocol using 1% NaOCl with gentle shaking for 4 min, and incubated in water at room temperature to obtain freshly hatched second-stage juveniles (J_2). Juvenile counts were made under a stereomicroscope to standardize inoculum density.

2.2 Plant material and experimental design

Certified seeds of watermelon (*Citrullus lanatus*) cv. Aswan F1, sweet melon (*Cucumis melo*) cv. Faransawy, two commercial rootstocks (6001 F1 and BS F1), and their grafted combinations (Aswan F1/6001 F1, Aswan F1/BS F1) were obtained from Roots Nurseries, km 75 Cairo–Alexandria Desert Road, Egypt. Resistance categories highly resistant (HR), resistant (R), or moderately susceptible (MS) were assigned according to preliminary screening data (Hassnin et al., 2023).

Seedlings were transplanted into 20 cm diameter clay pots containing approximately 2 kg sterilized sandy-loam soil mixture (1:1, v/v). Four inoculum levels of freshly hatched *M. incognita* J_2 1,000, 2,000, 4,000, and 8,000 juveniles/plant were applied in a 25 mL water suspension. The suspension was pipetted into four evenly spaced holes around the root zone. Control plants received no nematode inoculation. Treatments were arranged in a completely randomized design with four replicate pots per treatment. Plants were grown on sterilized benches in a greenhouse ($27 \pm 2^\circ\text{C}$; 60–70% relative humidity (RH)) under uniform horticultural care. The experiment was conducted twice under identical greenhouse conditions with consistent results; data presented are from one representative trial with four replicate pots per treatment.

2.3 Growth and nematode assessments

Forty-five days after inoculation, shoot and root lengths and fresh weights were recorded. Infected root systems were washed free of soil and fixed in formalin–acetic acid–alcohol. Nematode parameters were evaluated as follows:

- Number of galls, females, and egg masses per root (Hussey and Barker, 1973)
- Number of J_2 in 250 g soil (extracted by the modified Baermann funnel method; Hooper et al., 2005)
- Final population (Pf) = nematodes in roots + soil. Note: Pf included only females/ egg masses in roots + hatched J_2 from

soil. Unhatched eggs within egg masses were not counted separately as they were considered part of the reproductive potential already accounted for in root assessments.

- Reproduction factor (Rf) = Pf/Pi.
- Penetration rate (%R.P.) = (No. of developmental stages and females in root/Pi) $\times$ 100. Roots were stained with 0.1% acid fuchsin to visualize and count developmental stages and females prior to assessment.
- Reproduction rate (%R.R.) = (egg masses/root population) $\times$ 100.

2.4 Enzyme activity assays

Defense-related enzyme activities were assayed in fresh shoot and root tissues (1 g/sample). Samples were homogenized in 10 mL of ice-cold 0.1 M phosphate buffer and centrifuged at 10,000 $\times g$ for 15 min at 4°C.

The resulting supernatants were used for spectrophotometric assays.

POD activity was determined following the method by Hammerschmidt et al. (1982) in a reaction mixture containing phosphate buffer, 0.3% H₂O₂, and 0.1% guaiacol; absorbance was measured at 470 nm after 5 min incubation at room temperature.

PPO activity was measured according to Mayer and Harel (1965) using catechol (0.1%) as a substrate; absorbance was read at 420 nm after 5 min incubation.

Enzyme activities were expressed as μg substrate converted $\text{min}^{-1} \text{g}^{-1}$ fresh weight ($\mu\text{g min}^{-1} \text{g}^{-1} \text{FW}$).

2.5 Statistical analysis

Data were analyzed using SPSS version 12 (SPSS Inc., 2003). A two-way ANOVA tested the main effects of genotype and

Table 1. *Meloidogyne incognita* reproduction parameters on watermelon, rootstock hybrids, and grafted combinations at different inoculum levels

Hybrids	Inoculum level	Galls/root	Females/root	Egg-masses/root	Pf	Pf/Pi	%R.P.	%R.R.
6001 F1 (HR)	1,000	20 K	12 O	18 N	252 Q	0.25 M	3.0	60.0
	2,000	91 J	71 N	81 L	1,360 NO	0.68 JK	7.6	53.3
	4,000	171 H	128 KL	150 I	2,520 LM	0.63 K	7.0	54.0
	8,000	272 G	154 JK	163 I	3,261 K	0.41 L	4.0	51.4
BS F1 (R)	1,000	51 K	16 O	39 M	706 P	0.71 JK	5.5	70.9
	2,000	181 H	164 JK	73 L	2,743 L	1.37 H	11.9	30.8
	4,000	261 G	258 H	185 H	4,662 I	1.17 I	11.1	41.8
	8,000	392 F	319 F	181 H	5,850 H	0.73 J	6.3	36.2
ASWAN F1 (MS)	1,000	709 E	405 E	210 G	1,550 N	1.55 G	61.5	34.1
	2,000	827 D	685 D	469 D	15,012 D	7.51 A	57.7	40.6
	4,000	983 C	815 C	688 C	18,903 C	4.73 B	37.6	45.8
	8,000	1325 A	994 A	1,147 A	21,421 A	2.68 C	26.8	53.6
ASWAN F1/6001 F1 (HR)	1,000	107 J	79 MN	41 M	382 Q	0.38 L	12.0	34.2
	2,000	127 IJ	95 LMN	111 K	2,402 M	1.20 I	10.3	53.9
	4,000	152 HI	114 LM	134 J	6,362 G	1.59 G	6.2	54.0
	8,000	395 F	297 FG	346 E	11,248 E	1.41 H	8.0	53.8
ASWAN F1/BS F1 (MS)	1,000	391 F	223 I	79 L	1,142 O	1.14 I	30.2	26.2
	2,000	246 G	185 J	216 J	3,870 J	1.94 E	20.1	53.9
	4,000	359 F	269 GH	314 F	7,287 F	1.82 F	14.6	53.9
	8,000	1,039 B	933 B	836 B	19,466 B	2.43 D	22.1	47.3
SE $\pm$		40.867	33.245	32.653	754.085	0.186		

Mean values followed by the same letter(s) within a column in each block are not significantly different ($P \leq 0.05$) according to Duncan's multiple range tests. Pf = Final population = Embedded stages + Soil population. %R.P. = Rate of Penetration = (Root population/Initial population) $\times$ 100. %R.R. = Rate of Reproduction = (Number of egg masses/Root population) $\times$ 100. SE $\pm$ = Standard error.

inoculum level, and their interaction (genotype $\times$ inoculum). Mean comparisons were performed using Duncan's multiple range test (Duncan, 1955) at $P \leq 0.05$.

3. Results

3.1 Impact of *M. incognita* inoculum levels on reproduction in watermelon hybrids and grafted combinations

Based on previous screening results, watermelon cv. Aswan F1 (MS), and the rootstocks 6001 F1 (HR) and BS F1 (R), together with their grafted combinations, were evaluated under five *M. incognita* inoculum levels (0, 1,000, 2,000, 4,000, and 8,000 J_2 /plant).

Across all host genotypes, nematode reproduction parameters increased significantly ($P < 0.001$) with rising inoculum density (Table 1). The susceptible watermelon cultivar Aswan F1 showed the highest Pf/Pi ratios at 2,000 J_2 /plant (7.51) and 4,000 J_2 /plant (4.73). In contrast, the resistant rootstocks 6001 F1, BS F1, along with their grafted combinations maintained significantly lower Pf/Pi values. For instance, grafting Aswan F1 onto BS F1 reduced Pf/Pi by 68% compared to non-grafted Aswan F1 at 8,000 J_2 /plant ($P < 0.001$). Similar trends were observed for gall, female, and egg mass counts.

Across all genotypes, female and egg-mass counts rose significantly with increasing inoculum pressure, with the largest values occurring at 8,000 J_2 /plant in Aswan F1. Penetration rate (%R.P.) generally increased with

inoculum level but was lowest in resistant hosts and highest in Aswan F1 at 8,000 J_2 /plant. Reproduction rate (%R.R.) gradually declined with higher inoculum loads in rootstocks, but in Aswan F1 and its grafts, %R.R. tended to remain higher or decline less sharply.

Regression analysis indicated no consistent relationship between inoculum level and Pf/Pi in the rootstocks alone but a positive association in the grafted accessions (Aswan F1/6001 F1 and Aswan F1/BS F1) (Fig. 1).

3.2 Impact on growth of watermelon hybrids and grafted combinations

All inoculum levels significantly suppressed growth parameters, with maximum inhibition observed at 8,000 J_2 /plant (Figs 2 and 3). Shoot and root FWs decreased progressively with increasing inoculum in all genotypes except Aswan F1/BS F1, which maintained relatively higher shoot weights, even at higher nematode pressures. Root weight reductions were generally more pronounced than shoot weight reductions.

At each inoculum level, non-grafted 6001 F1, BS F1, and Aswan F1 suffered greater percentage losses in root weight compared with grafted combinations. Aswan F1/BS F1 exhibited the smallest reductions in both shoot and root biomass, followed by Aswan F1/6001 F1. Across hybrids, the greatest shoot-weight loss was at 8,000 J_2 /plant, while the least occurred at 1,000 J_2 /plant. The partial resistance observed in grafted combinations is characterized by reduced nematode penetration, impaired development, and enhanced biochemical defenses, consistent

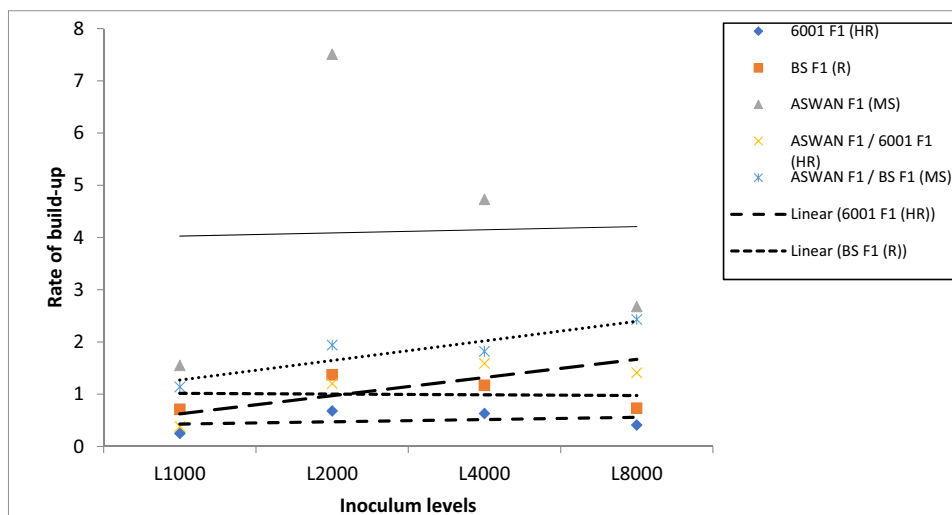


Figure 1. Correlation between initial inoculum levels and population build-up of *Meloidogyne incognita* on watermelon, rootstock hybrids, and grafted combinations.

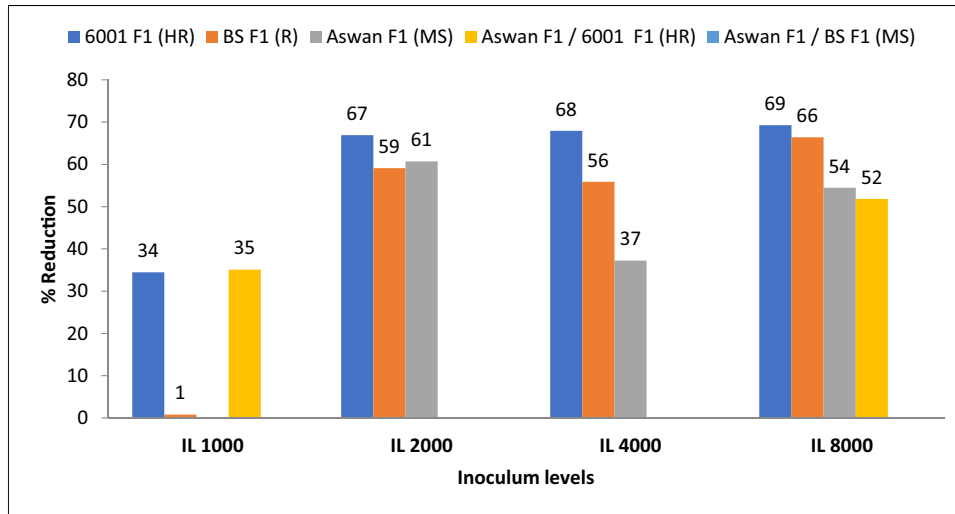


Figure 2. Shoot FW reduction (%) in watermelon and rootstock hybrids at different inoculum levels of *Meloidogyne incognita*.

with rootstock-mediated resistance mechanisms. Even resistant rootstocks showed mild growth reduction under high inoculum pressure, indicating that resistance does not confer complete immunity but rather reduces damage through physiological and biochemical tolerance mechanisms.

3.3 Impact of *M. incognita* inoculum levels on reproduction in sweet melon hybrids and grafted combinations

For sweet melon cv. Faransawy (MS), rootstocks 6001 F1 (HR) and BS F1 (R), and their grafted accessions,

reproductive criteria also increased with inoculum density (Table 2). Faransawy supported the highest nematode reproduction and Pf/Pi, followed by Faransawy/BS F1 and Faransawy/6001 F1. At inoculum levels $\geq 2,000$ J_2 /plant, Faransawy exhibited markedly elevated nematode counts in both roots and soil. The 6001 F1 rootstock maintained the lowest Pf at all inoculum levels.

Faransawy recorded its maximum Pf/Pi values at 2,000 J_2 /plant (8.69), 4,000 J_2 /plant (5.29), and 8,000 J_2 /plant (2.96). Female and egg mass numbers peaked at 4,000 and 8,000 J_2 /plant for Faransawy, and at 8,000 J_2 /plant for Faransawy/BS F1.

Correlation analyses showed no relationship between inoculum level and Pf/Pi in 6001 F1, BS F1, or Faransawy/BS

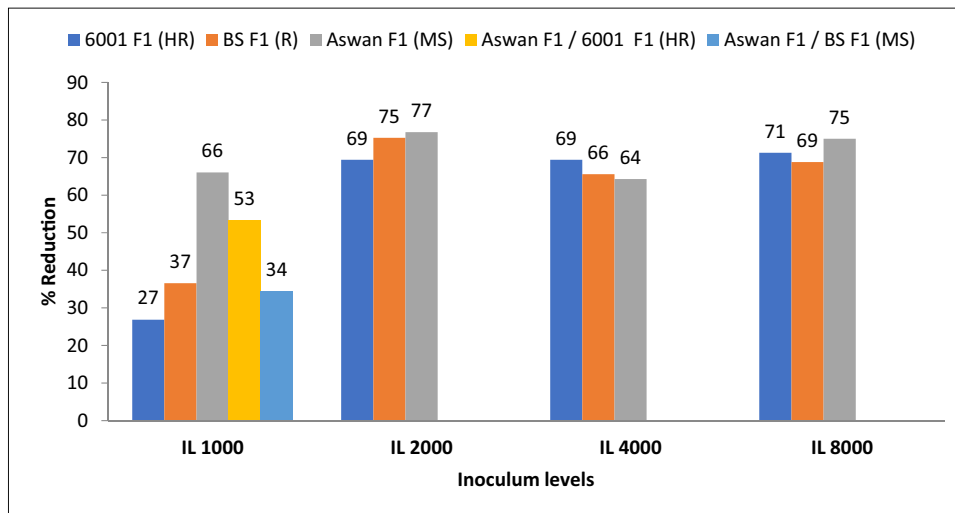


Figure 3. Root FW reduction (%) in watermelon and rootstock hybrids at different inoculum levels of *Meloidogyne incognita*.

F1. In contrast, a negative correlation was found for Faransawy and Faransawy/6001 F1, where increasing inoculum levels reduced Pf/Pi (Fig. 4).

3.4 Impact on growth of sweet melon hybrids and grafted combinations

All inoculation levels $\geq 2,000$ J_2 /plant significantly reduced shoot and root biomass in sweet melon hybrids (Figs 5 and 6). Losses increased with inoculum level but were less severe in grafted plants. Notably, Faransawy/6001 F1 exhibited increases in root FW at the lower inoculum levels (1,000–4,000 J_2 /plant), while Faransawy/BS F1 showed similar increases at 1,000–2,000 J_2 /plant. In most cases, root weight reductions exceeded shoot reductions, and the magnitude of reduction was genotype-dependent. The observed partial resistance in grafted sweet melon combinations similarly

reflects rootstock-mediated defense mechanisms that limit nematode success while maintaining plant growth.

The largest biomass decreases occurred at 8,000 J_2 /plant in all hybrids, whereas 1,000 J_2 /plant caused the smallest losses. The Faransawy cultivar had the highest percentage reductions in root biomass, followed by BS F1, while Faransawy/6001 F1 exhibited the smallest losses. As observed in watermelon, even resistant sweet melon rootstocks displayed some growth reduction at the highest inoculum level, reinforcing that resistance represents damage mitigation rather than complete immunity.

3.5 Comparative ANOVA findings

ANOVA (Table 3) confirmed significant effects ($P < 0.001$) of hybrid genotype, inoculum level, and their interaction on nematode reproduction and plant growth parameters. Resistant

Table 2. *Meloidogyne incognita* reproduction parameters on sweet melon, rootstock hybrids, and grafted combinations at different inoculum levels

Hybrids	Inoculum level	Galls/root	Females/root	Egg-masses/root	Pf	Pf/Pi	%R.P.	%R.R.
6001 F1 (HR)	1,000	20 M	12 J	18 O	252 Q	0.25 O	3.0	60.0
	2,000	91 K	71 I	81 M	1,360 P	0.68 LM	7.6	53.3
	4,000	171 J	128 H	150 L	2,520 L	0.63 M	7.0	54.0
	8,000	272 I	154 H	163 KL	3,261 K	0.41 N	4.0	51.4
BS F1 (R)	1,000	51 L	16 J	39 N	343 Q	0.34 NO	5.5	70.9
	2,000	181 J	164 H	73 M	2,743 L	1.37 J	11.9	30.8
	4,000	261 I	258 G	185 J	4,662 I	1.17 K	11.1	41.8
	8,000	392 G	319 F	181 JK	5,850 H	0.73 L	6.3	36.2
Faransawy (MS)	1,000	777 E	485 D	213 I	2,219 M	2.22 D	69.8	30.5
	2,000	942 D	763 C	586 D	17,377 C	8.69 A	67.5	43.4
	4,000	1,327 B	937 B	860 B	21,165 B	5.29 B	44.9	47.9
	8,000	1,507 A	1,072 A	945 A	23,686 A	2.96 C	25.2	46.9
Faransawy/6001 F1 (R)	1,000	561 F	447 E	348 E	1,642 O	1.64 I	79.5	43.8
	2,000	566 F	322 F	305 G	2,628 L	1.31 J	31.4	48.6
	4,000	338 H	237 G	227 I	6,578 G	1.64 I	11.6	48.9
	8,000	328 H	356 F	329 F	11,290 E	1.41 J	8.6	48.0
Faransawy/BS F1 (MS)	1,000	260 I	472 DE	256 H	1,890 N	1.89 G	72.8	35.2
	2,000	271 I	319 F	228 I	4,211 J	2.11 E	27.4	41.7
	4,000	388 G	236 G	261 H	7,202 F	1.80 H	12.4	52.5
	8,000	1,053 C	943 B	837 C	15,937 D	1.99 F	22.3	47.0
SE±		46.348	34.472	30.178	783.694	0.216		

Mean values followed by the same letter(s) within a column in each block are not significantly different ($P \leq 0.05$) according to Duncans' multiple range tests. Pf = Final population = Embedded stages + Soil population. %R.P. = Rate of Penetration = (Root population/Initial population) $\times$ 100. %R.R. = Rate of Reproduction = (Number of egg masses/Root population) $\times$ 100. SE± = Standard error.

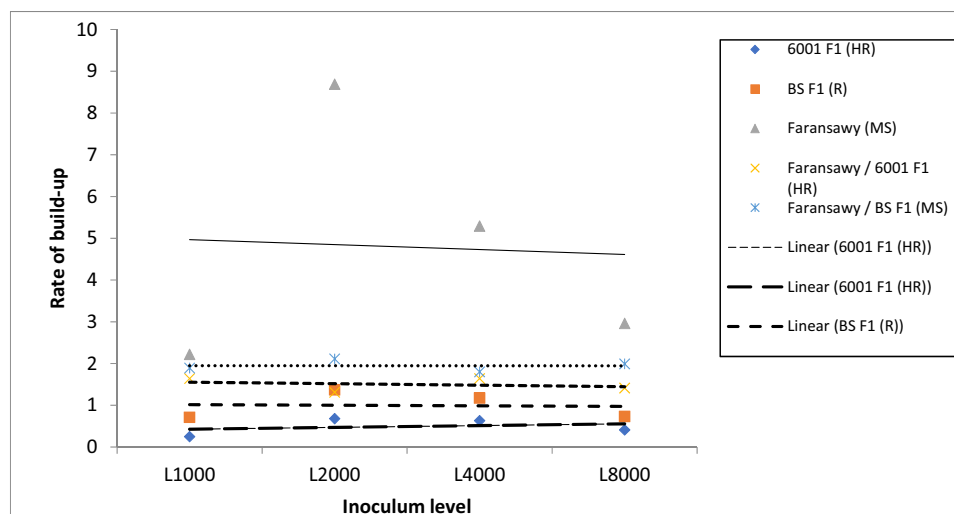


Figure 4. Correlation between initial inoculum levels and population build-up of *Meloidogyne incognita* on sweet melon, rootstock hybrids, and grafted combinations.

rootstocks (6001 F1, BS F1) exhibited consistently lower Pf and % R.P., higher %R.R. stability, and reduced growth suppression compared with susceptible cultivars (Aswan F1, Faransawy). Susceptible cultivars showed steep increases in Pf and pronounced growth losses under high nematode pressure.

3.6 Enzyme activity in watermelon genotypes and grafted combinations

In watermelon cv. Aswan F1, POD and PPO activities increased progressively with inoculum density (Fig. 7). At 8,000 J_2 /plant, POD and PPO activities were 10.2 ± 0.7 U g^{-1} FW and 6.1 ± 0.6 U g^{-1} FW, respectively ($P < 0.01$).

Grafted combinations (Aswan F1/6001 F1, Aswan F1/BS F1) exhibited significantly higher baseline and induced enzyme activities than non-grafted Aswan F1. At 8,000 J_2 /plant, Aswan F1/6001 F1 recorded POD activity of 24.5 ± 2.3 μg H_2O_2 min^{-1} g^{-1} FW and PPO activity of 16.8 ± 1.2 μg catechol min^{-1} g^{-1} FW ($P < 0.01$), with slightly lower but still elevated values in Aswan F1/BS F1.

3.7 Enzyme activity in sweet melon genotypes and grafted combinations

Similar patterns were observed in sweet melon cv. Faransawy (Fig. 8). POD and PPO activities increased

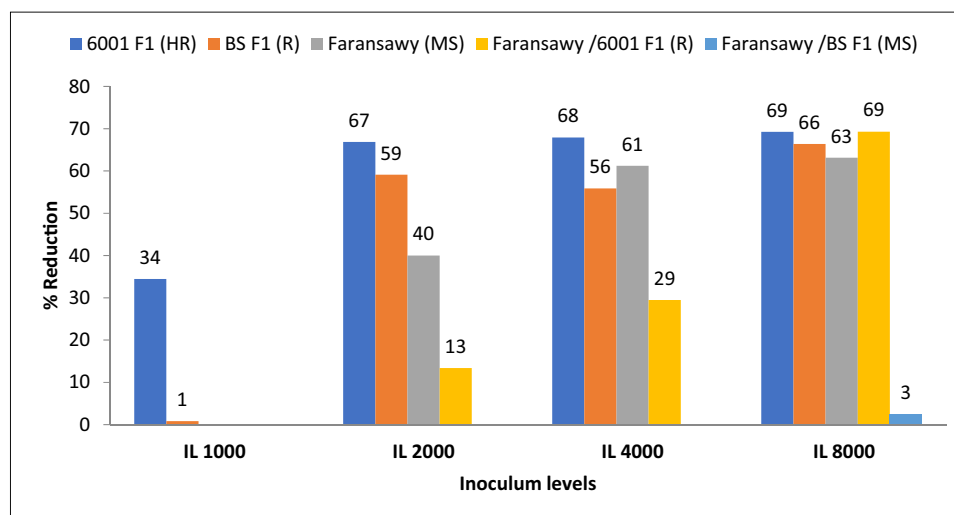


Figure 5. Shoot FW reduction (%) in sweet melon and rootstock hybrids at different inoculum levels of *Meloidogyne incognita*.

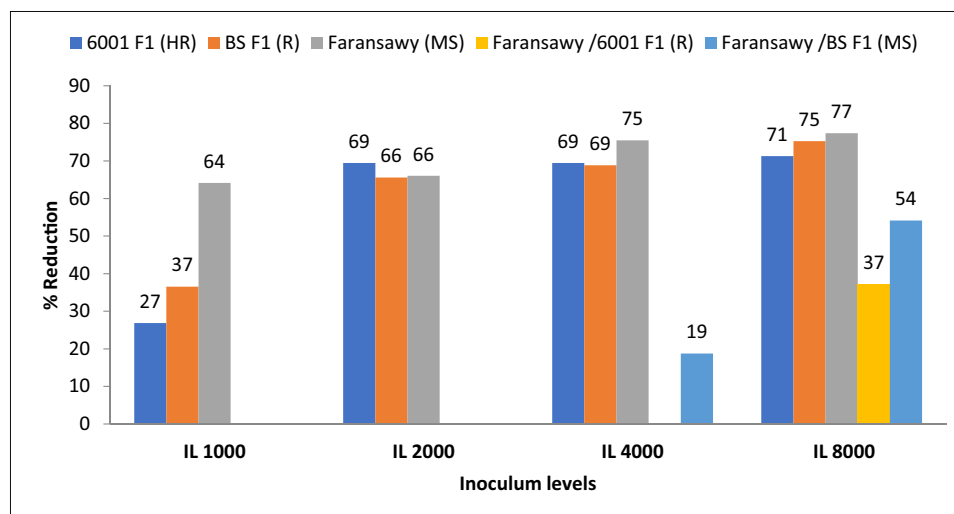


Figure 6. Root FW reduction (%) in sweet melon and rootstock hybrids at different inoculum levels of *Meloidogyne incognita*.

Table 3. ANOVA summary for effects of genotype, inoculum level, and interaction on nematode and plant growth traits

Source of variation	Galls/root	Females/root	Egg-masses/root	Pf	P/Pi	%R.P.	%R.R.
Hybrids	45.23 (<0.001)	38.76 (<0.001)	42.15 (<0.001)	50.12 (<0.001)	12.34 (<0.001)	25.67 (<0.001)	18.45 (<0.001)
Inoculum levels	67.89 (<0.001)	55.43 (<0.001)	60.78 (<0.001)	72.34 (<0.001)	15.67 (<0.001)	30.12 (<0.001)	22.34 (<0.001)
Hybrids × inoculum	12.34 (<0.001)	10.56 (<0.001)	11.23 (<0.001)	14.56 (<0.001)	5.67 (<0.001)	8.45 (<0.001)	7.89 (<0.001)

The *F*-values and *P*-values indicate significant interactions between hybrids and inoculum levels for all measured parameters ($P < 0.001$).

This suggests that both the hybrid type and inoculum level significantly influence the development and reproduction of *M. incognita*.

significantly with inoculum level, peaking at $9.0 \pm 0.6 \text{ U g}^{-1}$ FW and $5.5 \pm 0.5 \text{ U g}^{-1}$ FW, respectively, in non-grafted plants at 8,000 J_2 /plant.

Grafted combinations showed substantially higher constitutive and induced enzyme levels. At 8,000 J_2 /plant, Faransawy/6001 F1 reached $21.5 \pm 2.1 \mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1}$ FW

(POD) and $14.5 \pm 1.0 \mu\text{g catechol min}^{-1} \text{ g}^{-1}$ FW (PPO), outperforming their non-grafted counterpart ($P < 0.01$).

Across both cucurbit crops, increasing *M. incognita* inoculum levels generally enhanced nematode reproduction and enzyme activity, while suppressing plant growth. Resistant rootstocks and grafted combinations consistently

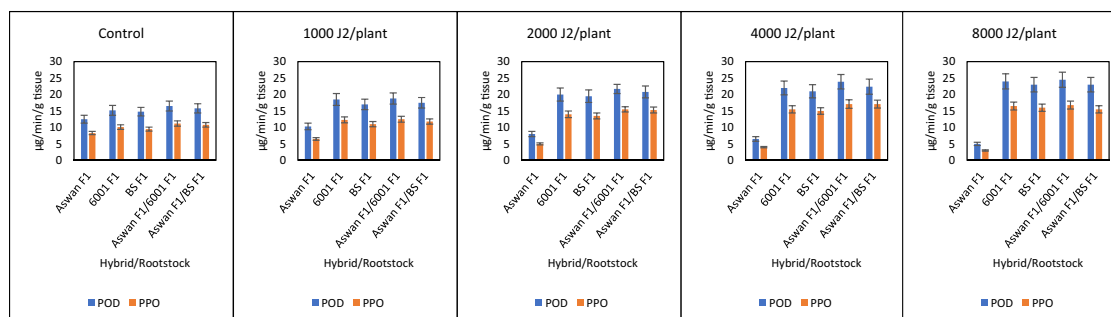


Figure 7. POD and PPO activities in watermelon and rootstock hybrids at different inoculum levels of *Meloidogyne incognita* (mean value $\pm$ SD, $n = 4$).

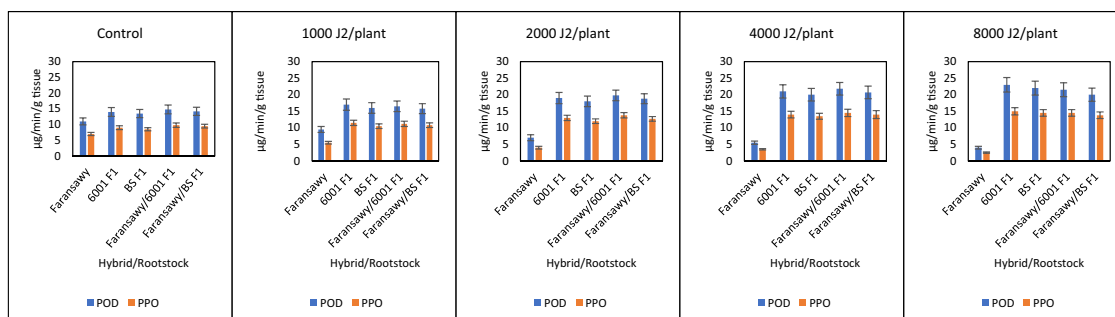


Figure 8. POD and PPO activities in sweet melon, and rootstock hybrids at different inoculum levels of *Meloidogyne incognita* (mean value $\pm$ SD, $n = 4$).

moderated nematode multiplication, limited growth reductions, and boosted defense enzyme activities compared to susceptible cultivars, even under high nematode pressure.

4. Discussion

The study clearly demonstrates that resistant rootstocks, such as 6001 F1 (HR) and BS F1 (R), effectively suppress *M. incognita* multiplication across a range of inoculum levels, maintaining their resistance even at high nematode pressures (up to 8,000 J_2 /plant). This suggests strong potential for practical use in heavily infested fields. In contrast, MS cultivars like Aswan F1 and Faransawy, as well as their grafted versions, suffer greater reductions in plant growth, particularly in shoot and root fresh weights, due to extensive root damage from high nematode populations. This damage disrupts key physiological processes, including photosynthesis, carbon metabolism, and nutrient uptake, in line with previous findings (Mateille et al., 1995; Khan et al., 2019; Chahar et al., 2021).

Nematode population dynamics show that susceptible plants support successful juvenile parasitism through the formation of giant cells in vascular tissues, which are essential for nematode development. Conversely, resistant hybrids inhibit giant cell formation, leading to juvenile mortality or root abandonment. Differences in nematode reproduction metrics, including egg mass counts and reproduction factors, between resistant and susceptible types reflect underlying genetic variation in host resistance and nematode adaptability (Griffin, 1982; Castagnone-Sereno, 2006). MS hybrids typically had elevated egg masses and reproduction rates, while resistant cultivars showed limited nematode development.

The resistance mechanisms described including hypersensitive response, and giant cell inhibition, which effectively

suppressed nematode multiplication and maintained growth under high inoculum pressure (Dropkin, 1969; Nelson et al., 1990). Notably, nematode multiplication rates were paradoxically higher at lower inoculum densities, probably due to reduced intraspecific competition and more abundant resources in less damaged roots (Haynes and Jones, 1976; Bendezu and Starr, 2003). High nematode populations restrict subsequent juvenile infection sites, limiting reproduction (Ogunfowora, 1977; Oostenbrink, 1966).

This study confirms the complex interplay among host resistance, nematode genetics, and environmental conditions in determining nematode population dynamics and plant health outcomes. Importantly, growth parameters were significantly influenced by final nematode populations and gall severity, corroborating earlier research on nematode pathogenicity thresholds.

On the biochemical defense front, key enzymes POD and PPO showed significant positive correlations with inoculum density in both watermelon cv. Aswan F1 and sweet melon cv. Faransawy. These enzymes are integral to plant defense, contributing to reactive oxygen species generation, cell wall fortification, and antimicrobial compound synthesis (Almagro et al., 2009; Constabel and Barbehenn, 2008). The increased enzyme activities at higher nematode inoculum levels indicate an inducible defense response aligned with previous reports linking elevated POD and PPO to nematode resistance and better plant health (Elsharkawy et al., 2022; Chandra et al., 2010).

Differences in enzyme activity between the two cucurbit crops suggest that watermelon possesses more robust biochemical defense mechanisms compared to sweet melon, consistent with their relative resistance profiles (López-Gómez et al., 2016). The clear statistical distinctions in enzyme responses across inoculum levels underscore their potential as biomarkers for plant health and resistance, supporting their use in integrated pest management strategies that harness natural defense

pathways. While this study was conducted under controlled greenhouse conditions, the findings have important implications for field management. In our pot system (2 kg soil), 8,000 J_2 /plant corresponded approximately to 4,000 J_2 /250 g soil. However, field equivalency depends on root volume, soil type, and environmental factors. We recommended that future field trials establish soil-density-based thresholds for these rootstocks. For growers, pre-plant soil sampling is advised to estimate nematode pressure, with grafted plants recommended for fields with known *M. incognita* history, particularly at densities exceeding 2,000 J_2 /250 g soil.

Together, these findings confirm the efficacy of resistant rootstocks and grafting in mitigating root-knot nematode damage, highlight the importance of initial nematode population management, and emphasize the valuable role of defense enzymes in cucurbit resistance. The results also reinforce the need for rootstock specificity when selecting for nematode resistance, echoing studies such as García-Mendivil and Sorribas (2021) while accounting for some contradictory findings (Giné *et al.*, 2017).

Overall, the study advances understanding of nematode-host interactions in grafted cucurbits and provides practical insights for improving nematode management through resistant rootstock deployment and biochemical defense monitoring.

5. Conclusion

This study demonstrates that resistant cucurbit rootstocks (6001 F1 and BS F1) significantly suppress *M. incognita* reproduction and mitigate growth damage compared to susceptible cultivars. Grafting MS cultivars onto resistant rootstocks enhances tolerance and reduces nematode population build-up. Increased POD and PPO activities in resistant and grafted plants suggest key roles for biochemical defenses in nematode resistance. These findings support integrating genetic resistance, grafting, and biochemical monitoring in sustainable root-knot nematode management for cucurbit production. Future research should include multi-location field trials with higher replication to establish economic thresholds and validate the practical efficacy of tested rootstocks under varying environmental conditions.

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Author contributions

Hosny Kesba and Ali Ali conceived and designed the study; Sherif El-Ganainy and Nourhan Hassnin performed data analysis, with Nourhan Hassnin conducting the investigation and data validation; Hosny Kesba provided resources, secured funding, and supervised the project, while Ali Ali managed project administration; Sherif El-Ganainy developed the methodology and handled software tasks. All authors contributed to drafting the manuscript, with Nourhan Hassnin and Sherif El-Ganainy preparing the original draft, and Hosny Kesba and Ali Ali reviewing and editing the final version.

Conflict of interest statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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