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Screening and Evaluation of New Rootstocks from Local Mit-Ghamr Peach for Resistance to Root-Knot Nematodes (Meloidogyne incognita)

Elrefaey F. A. El-Dengawy[1]*; Galal, I. Eliwa[1]; Samir B. Gad[2]; Hanan H. A. Mohamed[1]
[1]Pomology Department, Faculty of Agriculture, Damietta University, Egypt.
[2]Zoology Department, Faculty of Agriculture, Mansoura University, Egypt.

Corresponding author*: *dengawy@du.edu.eg , Mobile no. 0201550860056*

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ABSTRACT

A greenhouse experiment was conducted to evaluate resistance behavior of six local Mit-Ghamr peach (*Prunus persica* L.) genotypes (SL = Sultani late, SM= Sultani medium, SE =Sultani early maturity, N= Neely, F = Fark and MA =Mawy) to root-knot nematode (*M. incognita*) infection, during two successive seasons (2017/18 and 2018/19). The evaluation was carried out by conducting several nematode assays on the root system of the tested peach seedlings, the most important of which was the total damage index (TDI), as well as vegetative and biochemical characteristics (phenols and proline contents in the leaves and roots of seedlings). Nematode and biochemical measurements were dominant in judging the resistance behavior of the tested genotypes, although the vegetative results (seedling length, leaf area increment and root system growth coefficient) showed differences between the tested genotypes. Three genotypes SL, SM and F recorded the highest number of galls, egg masses and second-stage juveniles on the roots of infected seedlings, while MA genotype recorded the lowest values in this regard. The MA genotype was rated as highly resistant (HR) to *M. incognita*. Also, SE and N genotypes were resistant (R), and all of them recorded TDI significantly lower than SL, SM and F genotypes, which obtained a medium, Susceptible or highly susceptible rank (HS, S or MS) to the nematode, respectively. The proline and phenolic contents of the seedling leaves and roots of resistant genotypes SE, N and MA were much higher than the corresponding values in the other non-resistant genotypes' SL and SM. A significant negative correlation was also observed between the proline content of leaves and roots and TDI.

INTRODUCTION

Peach (*Prunus persica* (L.) Batsch) is a member of the family Rosaceae. It is a deciduous fruit tree and is considered one of the most important fruit trees that are of great success in the recently reclaimed areas in Egypt according to the following reasons. Has self-pollination, a comparatively short juvenile period **(Arus *et al*., 2012)**. The fruit may be used fresh or after turning into jelly and jam, as well as it's high nutrients content and interesting flavor **(El-Dengawy *et al*., 2019)**. Local "Mit-Ghamr" peach is the principal cultivar grown under Dakahlia Governorate since a long time. It is included several genotypes namely, Sultani (early, medium, and late maturity), Mawy, Hegazy, Fark, and Neely. Such strains have greatly differed in growth habits, maturity date, yield and fruit characteristics within the same orchard and it was propagated by seeds (**Eliwa, 2005)**. Peach is susceptible to all abiotic stresses such as deficiency of water **(Wu and Cosgrove, 2000)**, and salinity **(Bernal-Vicente *et al.*, 2018)**. Plant nematodes are the most serious peach pests, especially the root-knot, which reduce crops yield and quality (**Mukhtar *et al*., 2013b; Hussain *et al*., 2015 and Kayani *et al*., 2016)**. Plant-parasitic nematodes are considered an important biotic stress in agriculture worldwide, causing economic losses estimated to be approximately US$ 75 -125 billion **(Chitwood, 2003)**, equaling 12 to 20% of the plant production annually **(Sasser and Freckman, 1987 and Koenning *et al*., 1999)**. The root-knot nematodes RKN (*Meloidogyne*) genus, includes over 100 identified species, which attack more than 3000 plant species, most of the RKN species are prevalent in the Mediterranean and hot climates regions, and are sedentary endoparasites (**Khan and Ahmad, 2000; Chitwood, 2003; Karssen and Moens, 2006 and Azeem *et al*., 2020)**.

In Egypt, previous studies showed that both *M. incognita* and *M. javanica* are widespread and adversely affect the growth and production of many crops, while M. *arenaria* is less common and of limited efficacy. *M. incognita* is the most dangerous of these species that cause severe plants root system damage **(Djian-Caporalino *et al.*, 2011; Wesemael *et al.*, 2011; Mukhtar *et al.*, 2014 and Zhou *et al.*, 2018).** Peach trees are one of the few crops that can die by damage resulting from nematode infection **(Ibrahim *et al.*, 2016)**. Not only are the productions of the diseased plants greatly affected, but also the quality is reduced, as in some crops like carrot, peach, peanut, potato and tomato **(Ibrahim and Rezk, 1988 and Mokble *et al.*, 2006)**. Root-knot nematode resistancet cultivars are a potent crop-protection, and strategy and it is destined to play a greater role in nematode management in sustainable agriculture. Most the effective nematicides have been banned in agriculture for their considerable risk to human health and the environment **(Veremis and Roberts, 1996)**. The root-knot nematodes as biotic stress cause measurable changes in the morphology and physiology of the tomato plant **(Williamson and Gleason, 2003)**. To minimize dependency on chemicals, the use of cultivars resistant or tolerant to nematodes can be one of the most efficient and economical approaches **(Mukhtar *et al.*, 2014; Hussain *et al.*, 2016 and Shigueoka *et al.*, 2016).** Hence, there is a need to develop commercially acceptable peach rootstocks with resistance/tolerance to this biotic stress.

Therefore, the present study aims to evaluate, compare, and identify the local genotypes from Mit-ghamr peach, to determine their resistance rank to the root-knot nematodes (*Meloidogyne incognita*), This is of great importance, especially under Egypt's lack of peach resistant rootstocks, which necessitates importing them from abroad, raising the national economy's costs.

MATERIAL AND METHODS

2.1. Location and peach genotypes

To evaluate six strains (genotypes) of local "Mit-Ghamr" peach (*Prunus persica* L.) against root-knot nematode infection, namely Sultani (early, medium and late maturity), Mawy, Fark, and Neely (**Eliwa, 2005),** an experiment was conducted in greenhouse at Pomology department, Faculty of Agriculture, Damietta University, Damietta governorate, Egypt, for two seasons (2017/18 and 2018/19).

2.2. Preparation of nematode inoculum

A single egg mass was collected from roots of *Coleus* plants (*Coleus blumei* L.) heavily infected with *M. incognita* grown in a horticultural nursery then inoculated on coleus plants as a highly host for 3 months under greenhouse conditions. *Meloidogyne incognita* was previously identified according to **Eisenback (1985)**. A sodium hypochlorite (NaOCl) extraction technique **(Hussey and Barker, 1973b)** was undertaken to collect eggs of *M. incognita*. Infected roots free of soil were washed and cut into 2-3 cm segments. Root segments were vigorously shacked in 200ml of 1.0% NaOCl solution for 1 minute. The suspension was passed through two sieves 60 and 500 mesh to collect freed eggs. Residual NaOCL was removed by placing a 500-mesh sieve with eggs under a stream of tap water for few minutes and eggs were collected and counted by Hawksely slide under 100X magnification microscope.

2.3. Plant Material and Experimental Procedure

Seeds of the tested genotypes without endocarp were soaked in tap water for 24 hr, treated with fungicide (Topsin M 70% wp) at 1.5g/L for 3 min, and then subjected to moist chilling at 5±1ºC for 5 weeks to break dormancy. The moist-chilled seeds were sown in 25 x 35 cm perforated black polyethylene bags (One seed for each one) filled with 4 Kg soil of sand and peat moss mixture (2:1, v/v respectively) per bag. Three months after germination, the resulting seedlings of each genotype were classified into two similar groups (9 seedlings for each). Each group was arranged into three replicates (3 seedlings for each). The first group was inoculated with 4 thousand eggs of root-knot nematodes per seedling through 3 to 4 holes in the soil around the stem of the seedling, while the second group remained without infection (control). The bags were watered and fertilized regularly. The experiment was completed four months after the soil inoculation, and the following parameters were documented.

2.4. Nematode characteristics of the peach seedlings

2.4.1. Galls and egg masses indices (GI &EMI) of seedling root system

Galls index and egg masses index of peach seedling root system (1.0 g) were scored according to **Taylor and 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 than100 galls or egg masses.

2.٤.2. Number of Second stage Juveniles (J_2) per 250g soil

The root-knot nematode second-stage juveniles (J_2) were extracted by sieving modified technique and Baermann trays to obtain a clean nematode suspension according to **Goody (1957)** and counted by Hawksley counting slide under the light microscope.

2.4.3. Root system galled (RSG)

Each plant was thoroughly washed, visually examined, and scored on a 0 to 5 scale for the severity of root symptoms galling roots and gall formation according to **Barker (1985a)** as follows: 0 = no galling, 1 = 1 to 10% of the root system galled, 2 = 11 to 30%, 3 = 31 to 70%, 4 = 71 to 90%, and 5 = greater than 90%.

2.4.4. Reproductive factor (Rf)

The reproductive factor (Rf) was calculated according to the modified quantitative scheme of Canto-Sáenz **(Sasser *et al.*, 1984)** and **Banora and Almaghrabi (2019)** using the following equation: Rf = (Egg No. per root system + J_2. No. per root system)/ initial population (4 thousand eggs).

2.4.5. Total damage index (TDI)

Cultivars of various crops and vegetables are assessed for resistance to root-knot nematodes using root galling index (GI) as the only standard of peach damage, which is unreliable **(Florini, 1997 and Afolami, 2000)**. Therefore, the Total damage index includes four nematode measurements [(Galls index (GI), egg masses index (EMI), Root system galled (RSG) and Reproductive factor (Rf)] on the studied peach genotypes seedlings was computed by applying the following equation: TDI = [(GI + EMI + RSG + Rf))/4] and was used to evaluate genotype resistance or susceptibility based on all previous indexes by using scale from 1 to 4 as follows:

$$1 \leq HR < 2,\ 2 \leq R < 3,\ 3 \leq MR < 4,\ 4 \leq MS < 5,\ 5 \leq S < 6 \text{ and } HS > 6.$$

Where: HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible and HS = Highly susceptible.

2.5. Morphological characteristics of peach seedlings

2.5.1. Shoot length increase (cm)

Shoot length increase was measured as the following: Shoot length increase (cm) = seedling height at the end – seedling height at the start. The length decrease %compared to control (seedling length increase of the same genotype (strain) without infection) was calculated using the following formula:

Seedling length decrease % = [(control shoot length increase - shoot length increase of infected seedling (cm) / control shoot length increase) x 100].

2.5.2. leaf area increment

The length and width of the five complete upper leaves for the infected and uninfected seedlings were measured at the beginning and end of the experiment for each genotype, and then the leaf area was calculated using the equation **of Ahmed and Morsy (1999)** as follows: leaf area (cm^2) = 0.70 x (leaf length x leaf width) - 1.06. Then the increase in leaf area was calculated as follows: Leaf area increase% = [(leaf area at the end – leaf area at the beginning) / leaf area at the beginning] x 100].

2.5.3. Root system growth coefficient

Root system growth coefficient (RSGC) was calculated by using root parameters include length, width and numbers of lateral and secondary roots according to the equation of **El-Dengawy *et al*., (2021)**: RSGC = [RL * RW * (LR + SR)] for treatment / [RL * RW * (LR + SR)] for control. Where: RSGC: Root system growth coefficient, RL: root length (cm) and RW: root width (cm), LR: number of lateral roots, and SR: number of secondary roots.

2.6. Biochemical characteristics of peach seedlings

2.6.1. Total phenolic content (mg/g DW)

The content of poly phenols was determined in dried leave samples and roots according to **Stabell *et al*. (1996**) and **Li *et al*. (2007)**. Total phenol contents were expressed as µg gallic acid equivalent (µg GAE)/g DW.

2.6.2. Proline content (mg/g DW)

Proline concentration was determined according the method of **Bates *et al*. (1973)**. Leaf samples were collected at the end of the experiment. A 0.5 g of fresh weight was mixed with 5 ml aliquot of 3% (w/v) sulfosalicylic acid in glass tubes covered at the top and boiled in a water bath at 100°C. The mixture was centrifuged at 3000 g for 4min at 25°C. A 300 µl aliquot of the extract was mixed with 700 µl distilled water and 14 ml of the reagent mixture (30ml glacial acetic acid, 20ml distilled water and 0.5 g of ninhydrin) and boiled at 100°C for 60 min. After cooling the mixture, we added 5.0ml of toluene. The chromophore containing toluene was separated and absorption was read at 520 nm, using toluene as a blank. Proline concentration was calculated using L-proline for the standard curve and calculated as mg/g DW.

2.7. Statistical analyses

The experiment was carried out using a completely randomized design with three replicates, and the differences between means were compared using Duncan's Multiple Range Test at a 5% level of probability using SPSS statistical software **(Duncan, 1955).**

RESULTS AND DISCUSSION

Based on the nematode's ability to produce galls on the roots and its reproductive potential, all peach genotypes exhibited a wide range of responses to root-knot nematodes, ranging from resistant to susceptible. The primary symptom of root-knot nematode infection is developing galls in the infected plants. **Fassuliotis (1979)** mentioned that this can often be the only measure of resistance during screening. Moreover, significant disparities in the number of galls present on roots, suggest varying degrees of susceptibility **(Jaiteh *et al*., 2012**). Therefore, the results of the infestation effect of *M. incognita* on the tested peach genotypes in the current study were shown and discussed as follows:

3.1. Numbers of galls, egg masses and the second stage Juvenile on tested genotypes of peach seedlings roots

Data in **Table 1** show nematode galls and egg masses numbers on the root system as well as the number of the second-stage juveniles (J_2) of nematodes in 250 g of soil in seedlings of six peach genotypes. The results proved that SL, SM, and F genotypes had the highest number of galls and egg masses on the root system, and the same trend was observed for the second-stage juveniles (J_2), while MA, N, and SE genotypes exhibited the lowest numbers of galls, egg masses and the second-stage juveniles (J_2) in both seasons. Data also evidenced that the MA genotype recorded the lowest values in the numbers of gall and egg mass as well as the second stage juveniles (J_2). The differences were significant compared to the other genotypes and also recorded the gall index (GI) = 3 while egg mass index (EMI) = 2 in both seasons, followed by the N genotype. While the SL genotype recorded the highest values significantly for the numbers of galls, egg masses and the second stage Juveniles (J_2) compared to their corresponding in the other genotypes which recorded gall index (GI) = 5 while egg mass index (EMI) = 5 in both seasons, followed by the SM genotype.

A similar tendency was obtained by **Khan *et al*. (2011), Mukhtar *et al.* (2014), Özarslandan and tanriver (2018) and Ibrahim *et al*. (2019)**. According to **Karssen and Moens (2006),** highly vulnerable host plants allowed juveniles to penetrate the roots, mature, and generate many egg numbers, whereas resistant plants restricted their growth and hence prevented reproduction. Also, **Khan (1994)** mentioned that the development of galls on plant roots increased significantly in the susceptible genotypes compared with resistant genotypes and thus affecting plant performance. The same author added that nematode resistance in host plants was manifested by reduced rates of egg masses, nematode reproduction, and consequently, low nematode population densities than that of a susceptible one. **Sasser (1954)** described infection as the incursion of the plant by nematode juveniles and immunity as the ability to prevent infection with the result of no disease development (resistance). Based on Table 2 that depicts the use of various scales to confirm the judgment on peach genotypes for resistance or susceptibility to nematode, some of the classification scales are a modification of a system used by many researchers to classify plant reactions to root-knot nematode (**Kinloch and Hinson, 1973; Williams *et al*., 1973; Amosu and Franckowiak, 1974 and Sharma et al., 1994)** to harmonize the many scales that were utilized to confirm the judgment.

3.2. Root system galled (RSG), reproductive factor (Rf) and total damage index (TDI) on seedlings roots of tested peach genotypes

Data in **Table 2** show the effect of infection with the root-knot nematode *M. incognita* on root system galled (RSG), reproductive factor (Rf), and total damage index (TDI) in seedlings of six peach genotypes. The genotypes were also compared for damage assessment by the nematode.

Among the tested genotypes, it was found that the SL genotype was the most damaging, as it scored the highest values for TDI, RSG, and Rf where the values were 7.88, 59.9, and 18.5, respectively in the first season while they were 8.20, 60, and 19.8, respectively in the second season. Therefore, it recorded a high susceptibility (HS) rank. The SM genotype ranked second in the severity of damage under the influence of nematode infection, as tabulated values for TDI, RSG, and Rf were 5.33, 43.5, and 9.3, respectively in the first season and 5.25, 32.5, and 9, respectively in the second season. On the other hand, it was noted that the MA genotype gave the lowest values for TDI, RSG, and Rf in the two tested seasons, and accordingly, it received the high resistance (HR) rank. This genotype gave values of 1.68, 3.4, and 1.2 for TDI, RSG, and Rf, respectively in the first season while they were 1.68, 3.2 and 1.2, respectively in the second season. It could be reported that resistance and susceptibility to phytopathogenic nematode manifest the effects of hosts on the reproductive ability of nematode.

The SE and N genotypes (**Table 2)** obtained the resistance rank (R) where they recorded total damage index (TDI) values ranging from 2.50 to 2.85 in the two seasons of the study which were significantly lower than those for the SL, SM and F genotypes. It was also observed that genotype F recorded total damage index (TDI) values equal to 4.28 and 4.33, respectively in the two seasons, and the values in each of the other measurements (RSG and Rf) were also significantly lower than those of genotypes SL and SM but significantly higher than those of genotypes SE, N and MA with values 19.6 and 7.1, respectively in the first season and 19.5 and 7.3, consecutively in the second season which resulted in its attaining the rank of moderate susceptible (MS). From the above results, it could be concluded that the tested peach genotypes showed significant variations in total damage index (TDI) in response to *M. incognita*. The HR and R genotypes suffered minimum damage by the nematode while the HS and S genotypes showed maximum damage parameters. Similarly, the damage in F genotypes was comparatively less as compared to genotypes showing other susceptible reactions. The reductions in total damage index (TDI) were in the order: HR < R <MR< MS< S < HS.

3.3. Vegetative parameters of seedlings root of the tested peach genotypes

Data documented in T**able 3** about vegetative characteristics note that there was a decrease in the shoot length of infected seedlings compared to uninfected ones in all the genotypes under the present study. These findings are consistent with the results of **Hussain *et al*., (2011); Khan *et al*., (2011); Ansari *et al*., (2012); Mukhtar *et al*., (2013a) and Hussain *et al*., (2014)** on okra, tobacco, tomato, cucumber, and okra cultivars, respectively. The highest percentage of length decrease (47.3%) was found in the SL genotype, which is highly sensitive to nematode infection, also the length decrease percentage in the MA genotype which is highly resistant to nematodes was 54.1%, while the SE and N genotypes which are resistant to nematodes, had a length deficiency rate ranging between 24.5 and 27%. The same trend was observed in the two study seasons. However, the results for some of the Vitis rootstocks (i.e., 161-49C, 41B, 110R, and SO4) failed to reveal a significant correlation between the three *Meloidogyne spp.* numbers and the shoot height, this are in agreement with **Gutie´rrez-Gutie´rreza *et al*. (2011)**. Unlike, **Mukhtar *et al.* (2017) and Mukhtar and Kayani (2019)** on green gram and cucumber, respectively found no effect of the nematode infection on resistant genotypes. Moreover, **Montasser *et al*. (2019)** on certain vegetable genotypes against *M. incognita* reported growth parameter reductions, however, the differences between infected and healthy plants were often insignificant. Among the current tested genotypes of peach rootstocks, the MA genotype exhibited the highest decrease in shoot length and this result may be because the genotype plants were dwarfed as behavior mechanism of its resistance to nematode stress, whereas the severity of the shoot length decrease in the SL genotype is due to the severity of nematode infection on roots. Similar results were found by **Mukhtar *et al*. (2017) and Mukhtar and Kayani (2019)** who indicated that the highly susceptible genotypes of corn for nematode infection achieved the greatest reduction in growth parameters.

In terms of the increase % in leaf area **(Table 3)**, the results indicated that there are no significant differences between the studied genotypes, except for the N genotype, which decreases significantly compared to other genotypes in this trait. The exposure of most genotypes to nematode infection leads to a significant decrease in the percentage of increase in leaf area, and the decrease was more pronounced in the MA genotype. These findings are consistent with **Amin and Abd El-Wanis (2014)** who reported that infected cucumber plants have less leaf area. On the other hand, it was observed that nematode infection to the seedlings of the peach SE genotype led to a non-significant increase in leaf area compared to the corresponding seedlings without infection. Moreover, Regarding the Root system growth coefficient (RSGC) in **Table (3)**, the results show that the peach genotypes differ significantly in the RSGC, and this difference is somewhat related to degree of resistance to root-knot nematode. Where the resistant (SE) and highly resistant (MA) genotypes gave the lowest values of the RSGC in the two seasons. The values were 0.57 and 0.53 in the first season, while they were 0.49 and 0.33 in the second season. However, the root-knot nematode-sensitive genotype (SM) gave the highest values of the RSGC in the two tested seasons (1.96 and 1.31, respectively). Also, it was observed that highly sensitive (SL), sensitive (SM) and moderately sensitive (F) genotypes to root-knot nematode were significantly higher in the values of the RSGC compared to the resistant genotypes, except for the genotype (N).

Therefore, our results suggest that more research is needed to determine the relationship between vegetative growth and nematode infection, as there is no clear trend of the harmful effect of nematode infection on vegetative growth. This could be due to the severe effect of the damage that needs a longer time to appear on the peach seedlings, as they are perennial seedlings rather than annuals, or it could be due to differences in behavior between genotypes. In addition, **Minton (1962)** described the factors that influence the cotton plant's response to root-knot nematodes and found that resistance was caused by root tip hypersensitivity to juveniles penetrating and root cell failure to respond to nematode, rather than morphological differences or toot barriers that prevented penetration.

3.4. Total phenolic content in seedlings at tested six peach progenies

Phenolic compounds played a significant role in plant defense mechanisms against various infectious organisms. Plant responses to parasites are determined not only by the quantitative and qualitative composition of nematode secretion and excretion but also by the chemical composition of the plants or tissues attacked **(Nayak, 2015)**; phenolic compounds are the best-known infection factors responses. In nematode inoculated samples, there is a clear correlation between the degree of plant resistance and phenolic compounds **(Giebel, 1974).** The present results in **Table 4** showed the relationship between the effect of root-knot nematode infection on the content of phenols in the leaves and roots of seedlings of six peach genotypes studied over two years. The results demonstrated that the genotypes of the peach seedlings studied can be classified into three groups based on their phenol content after nematode infection: The first group is a group of genotypes susceptible to nematode such as SL, SM and F whose phenol content ranges between 3.64 and 14.14 mg/g dry weight in its leaves and ranges from 3.68 to 13.54 mg/g dry weight in its roots of seedlings. The second one is a group of resistant genotypes that includes SE and N, with phenol content in leaves ranging from 14.68 to 19.63 mg/g dry weight, and phenol content in seedling roots ranging from 18.18 to 29.87 mg/g dry weight. The third group is a highly resistant genotype (MA) where the leaf phenols content of its seedlings ranges between 34.09 and 39.29 mg/g dry weight, while the root phenol content ranges from 30.17 to 45.63 mg/g dry weight. From the previous results, it could be concluded that the screened genotypes of peach seedlings as susceptible genotypes to nematode (SL, SM and F) their leaves and roots contained phenols amounts significantly lower compared to their corresponding genotypes resistant and highly resistant to nematode. While MA genotype was significantly superior in the phenols content of leaves and roots compared to other resistant and susceptible genotypes SL, SM, F, SE, and N, these conclusions were bolstered by the findings of **Korayem *et al.* (2012), Choudhary *et al.* (2013), Shobha *et al. (*2017) and Kavya *et al.* (2019)** on tobacco, sugar beet, tomato, brinjal, tuberose, ridge gourd, and guava varieties, respectively.

The statistical study revealed that there was a significant positive correlation coefficient between the content of phenols in leaves and roots, and it was equivalent to 0.987** in the first season and 0.939** in the second season. It was also found that there was a significant negative correlation between the content of phenols and the total damage index (TDI), which was 0.729 and 0.772 in the first season and 0.678 and 0.825 in the second season for leaves and roots, respectively. Previously, researchers discovered that increased phenolic content was a contributing factor in resistance to various nematode infections **(Narayana and Reddy, 1980 and Chitwood, 2002)** and this is also confirmed by **Ganguly and Dasgupta (1982)** found that nematode-resistant tomato cultivars had a higher phenolics content. **Acedo and Rohde (1971)** also reported that phenol contents play a role in the resistance mechanism against various nematode infections. The accumulation of phenolic compounds in the injured area, as well as the activation of associated oxidative enzymes, were demonstrated by **Balasubramanian and Purushothaman (1972) and Bajaj *et al*. (1983)**, respectively. Also, Phenolic compounds act as a substrate for many antioxidant enzymes so it mitigates stress injuries **(Khattab, 2007)**.

Potentially, the quick release of conjugated phenols with glycosidic substances was generated by the action of the hydrolysis enzymes. During the feeding process, the non-toxic phenolic glycosides have been proved to be hydrolyzed by the worm's β-glycosidase enzyme, and the resulting compound could prevent localized parasitization or even kill the nematode **(Star, 198; Hussey and Williamson, 1998)**. Furthermore, the quick decomposition of phenols or turning of phenols for different paths leading to the creation of diverse compounds such as polymer and lignin, which plays a crucial part in the resistive reaction, could be related to the increase in phenolic compounds during infection **(Mahapatra and Nayak, 2019 and Nayak, 2015)**. It's also likely that higher β-glycosidases activity liberated active phenols from glycosides, which were then oxidized in resistant genotypes.

3.5. Proline content in seedlings of the tested peach progenies

Some substances are synthesized by the plant in response to stress conditions, including osmoprotectant amino acids **(Hassan *et al*., 1994)** such as proline, which may increase the plant's stress tolerance **(Shulaev *et al*., 2008).** Because the roles of proline in biology are complex and affect a wide range of cellular processes, proline content could be a useful parameter for assessing the impact of microorganisms on plants. The presented results in **Table 5** showed the effect of root-knot nematode *M. incognita* infection on proline content in leaves and roots of seedlings of 6 genotypes of peach during the two seasons of the experiment. It was pointed out that the content of proline increases significantly in both leaves and roots of seedlings of infected genotypes compared to uninfected ones. These results are in accordance with results obtained by **(Nayak and Mohanty, 2010; Patel *et al.*, 2018; Mahapatra and Nayak, 2019 and Pandey, 2020),** on brinjal, tomato, and bitter gourd and rice varieties respectively.

Noticed that by comparing the proline content in the leaves of infected seedlings to the uninfected seedlings "as the percentage of increase in proline compared to the control",**(Table 5)** the following was found: The proline content of the seedlings leaves of the genotypes SE, N and MA was significantly higher than the corresponding values of proline in the other genotypes SL, SM, and F. Genotype SE had the highest percentage rise in leaves proline content following infection compared to uninfected seedlings, where the increase percentage was higher than control and recorded 190.17 and 191.16 in the two seasons of the study, respectively. It was found that the three genotypes SE, N, and MA outperformed the other genotypes in terms of proline content in both leaves and roots, and this result could confirm nematode resistance. These three genotypes achieved the highest percentage of increasing proline content in the leaves (35.47-191.16) and roots (98.67-420.0) during the two seasons of the experiment, compared to the other genotypes of SL, SM, and F, which recorded the lowest values of proline content that ranged between (22.18 and 27.38) in leaves and from (10.28 to 77.0) in roots. On the other hand, the three genotypes SE, N and MA recorded the lowest values (1.68- 2.85) of total damage index (TDI) compared to the other genotypes which tabulated the highest values of TDI (4.28 – 8.20). This results in harmony with the results of **El-Hady *et al.* (2015) and Pandey *et al.* (2016)** on grapevine and green gramn varieties, respectively. From our results, it was also found that there was a negative correlation between the proline content of both leaves and roots and the total damage index, and its value was 0.550 and 0.420 in the leaves, while in the roots it was 0.535 and 0.571 in the two seasons, respectively.

In addition, the N genotype had the highest increase in the proline content of infected seedlings roots compared to uninfected seedlings, where it recorded 420 and 393.10 mg/g, respectively, while the other genotypes (SL, SM, SE, F. and MA) recorded increase rates ranging between 129.89 mg/g and 10.58 mg/g in the first season, 122.83 mg/g and 10.28 mg/g in the second season. The amount of proline content was greater in both the leaves and the roots of resistant genotypes than in susceptible genotypes, which confirmed the results of **Mahapatra and Nayak (2019)** on bitter gourd cultivars. Moreover, the proline content of seedling roots was significantly lower than that of seedling leaves. The reason for such a state may be due according to **Nayak and Mohanty (2010)**, who demonstrated in their study that the stress conditions increased the accumulation of these amino acids at the site of nematode activity, which may meet the nematode's nutritional or reproductive needs. Another explanation, the quantitative increase in various amino acids during the post-infection period could be attributed to the proteolysis of existing tissue protein or the synthesis of new compounds via various metabolic pathways during plant-nematode interactions.

CONCLUSION

Root-knot nematodes are obligate parasites. Peach trees are one of the few crops that can die by damage resulting from nematode infection. Given the importance of the subject, the current study was conducted to determine several changes in morphological, biochemical, and nematode characteristics in peach seedlings that inoculated with root-knot nematode, *M. incognita*. Depending on all of the present results, the total damage index (TDI) is the most important measure to judging on resistant behavior of peach rootstocks. Our results revealed significant differences in response for six genotypes rootstocks of the local Mit-Ghamr peach to *M. incognita* infection. The highly resistant and resistant genotypes (MA, N and SE) suffered minimum total damage by the nematode, while the highly susceptible, susceptible and moderately susceptible genotypes (SL, SM and F) showed maximum total damage parameters. Finally, we recommend using the MA, N and SE genotypes as resistance rootstocks to *M. incognita* infection for production of the grafted peach seedlings. Thus, cultivating resistant genotypes in *M. incognita*-infested fields would help reduce nematode reproduction while also minimizing environmental pollution, preserving agro-ecosystems and biodiversity, and making management processes more cost-effective.

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The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

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Table 1. Number of the rook knot nematode galls, egg masses and second stage juveniles as affected by *M. incognita* infestation on seedlings roots of six peach genotypes at two growing seasons

Peach genotypes	Galls of the root system		Egg masses of the root system		J_2 / 250g.soil
	Number	Gall index (GI)	Number	Egg mass index (EMI)	Infected
2017/2018 season					
SL	248^{a}	5	119^{a}	5	2976^{a}
SM	159^{b}	5	88^{b}	4	1140^{b}
SE	92^{d}	4	29^{c}	3	448^{cd}
N	78^{e}	4	15^{d}	3	244^{d}
F	105^{c}	5	31^{c}	3	1244^{b}
MA	13^{f}	3	8^{e}	2	382^{cd}
2018/2019 season					
SL	201a	5	113.5^{a}	5	3183^{a}
SM	148b	5	90^{b}	4	1586^{b}
SE	91c	4	31^{c}	4	453^{d}
N	76d	4	17^{d}	3	575^{d}
F	95c	4	34^{c}	4	1178^{c}
MA	18e	3	6^{e}	2	522^{d}

Means followed by the same letter (s) in the same column don't significantly differ at 0.05 of probability according to Duncan's Multiple Range Test.[SL=Sultani late, SM= Sultani medium, SE =Sultani early maturity, N= Neely, F= Fark and MA =Mawy].

Table 2. Infestation effect of M. incognita nematode on root system galled (RSG), reproductive factor (Rf) and total damage index (TDI) in seedlings roots of six peach genotypes at two growing seasons

Peach genotypes	Root system galled (RSG)		Reproductive factor (Rf)	Total damage index	
	RSG%	Scale		TDI	Host suitability (Hs)
2017/2018 season					
SL	59.9^{a}	3	18.5^{a}	7.88^{a}	HS
SM	43.5^{b}	3	9.3^{b}	5.33^{b}	S
SE	6.1^{e}	0.5	2.7^{d}	2.55^{d}	R
N	10.6^{d}	1	2.0^{de}	2.50^{d}	R
F	19.6^{c}	2	7.1^{c}	4.28^{c}	MS
MA	3.4^{e}	0.5	1.2^{e}	1.68^{e}	HR
2018/2019 season					
SL	60.0^{a}	3	19.8^{a}	8.20^{a}	HS
SM	32.5^{b}	3	9.0^{b}	5.25^{b}	S
SE	5.9^{e}	0.5	2.9^{d}	2.85^{d}	R
N	10.6^{d}	1	2.5^{d}	2.63^{d}	R
F	19.5^{c}	2	7.3^{c}	4.33^{c}	MS
MA	3.2^{e}	0.5	1.2^{e}	1.68^{e}	HR

Means followed by the same letter(s) in the same column don't significantly differ at 0.05 of probability according to Duncan's Multiple Range Test.[SL=Sultani late, SM= Sultani medium, SE =Sultani early maturity, N= Neely, F= Fark and MA =Mawy].

Table 3. **Infestation effect of *M. incognita* on shoot length, leaf area and Root system growth coefficient of seedlings in six peach genotypes at two growing seasons**

Peach genotypes	**Vegetative parameters of seedling**					
	Shoot length			**Leaf area Increase %**		**Root system growth coefficient (RSGC)**
	Increase (cm)		**Decrease% compared to control***			
	Uninfected (control)	Infected	Infected	uninfected	Infected	Infected
	2017/2018 season					
SL	23.3^{ab}	13.0d^{e}	47.3^{b}	127.6^{bcd}	94.8^{e}	0.59^{d}
SM	20.7^{bc}	15.5^{d}	23.4^{d}	131.5^{abc}	111.1^{d}	1.31^{a}
SE	25.7^{a}	18.8^{c}	24.5^{d}	136.7^{ab}	148.2^{a}	0.53^{d}
N	19.0^{c}	13.7^{de}	27.0^{cd}	52.8^{f}	116.0^{cd}	1.09^{b}
F	20.3^{c}	14.5^{d}	32.5^{c}	128.8^{bc}	88.0^{e}	0.79^{c}
MA	24.7^{a}	11.3^{e}	54.1^{a}	136.0^{ab}	53.5^{f}	0.57^{d}
	2018/2019 season					
SL	24.3^{a}	12.5^{de}	45.2^{a}	127.2^{abc}	98.3^{d}	0.69^{c}
SM	19.3^{b}	15.2^{cd}	21.7^{c}	127.5^{abc}	112.2^{cd}	1.96^{a}
SE	23.3^{a}	18.7^{b}	22.5^{c}	138.4^{a}	144.7^{a}	0.33^{e}
N	16.7^{bc}	13.3^{de}	21.4^{c}	43.7^{f}	113.3^{cd}	1.01^{b}
F	19.7^{b}	13.2^{de}	29.2^{b}	118.6^{bc}	81.0^{e}	0.75^{c}
MA	22.7^{a}	11.2^{e}	50.6^{a}	136.1^{ab}	55.5^{f}	0.49^{d}

Means followed by the same letter(s) in the same column don't significantly differ at 0.05 of probability according to Duncan's Multiple Range Test. *Control represents seedling length increase of the same genotypes without infection [SL=Sultani late, SM= Sultani medium, SE =Sultani early maturity, N= Neely, F= Fark and MA =Mawy].

Table 4. Estimation of total phenolic content (mg/g) in the seedlings of the tested six peach genotypes influenced by root knot nematode *M. incognita*

Peach genotypes	**Total phenolic content (mg/g) of dry wt.**					
	2017/2018			***2018/2019***		
	Leaves					
	Uninfected (control)	Infected	Over control%*	Uninfected (control)	Infected	Over control%*
SL	1.01^{bcd}	1.09^{abcd}	7.92^{d}	0.96^{de}	1.09^{bcd}	13.54^{c}
SM	1.08^{abcd}	1.14^{abc}	5.56^{d}	1.10^{bc}	1.14^{b}	3.64^{d}
SE	1.00^{cde}	1.16^{abc}	16.0^{b}	0.97^{cde}	1.14^{b}	17.53^{b}
N	1.09^{abcd}	1.25^{a}	14.68^{bc}	1.07^{bcd}	1.28^{a}	19.63^{b}
F	0.96^{de}	1.07^{bcd}	11.46^{c}	0.99^{cde}	1.13^{b}	14.14^{c}
MA	0.84^{e}	1.17^{ab}	39.29^{a}	0.88^{e}	1.18^{ab}	34.09^{a}
	Root					
SL	1.17^{ef}	1.28^{de}	9.40^{d}	1.04^{ef}	1.13^{de}	8.65^{c}
SM	1.25^{e}	1.39^{cd}	11.24^{cd}	1.36^{c}	1.41^{bc}	3.68^{d}
SE	1.43^{bc}	1.69^{a}	18.18^{b}	1.23^{d}	1.57a	27.64^{b}
N	1.30^{de}	1.55^{b}	19.23^{b}	0.77^{g}	1.00^{f}	29.87^{b}
F	0.96^{g}	1.09^{f}	13.54^{c}	1.42^{bc}	1.54^{ab}	8.45^{c}
MA	1.16^{ef}	1.51^{bc}	30.17^{a}	1.03^{ef}	1.50^{ab}	45.63^{a}

Means followed by the same letter(s) in the same column don't significantly differ at 0.05 of probability according to Duncan's Multiple Range Test. Over control %* = [100 * (infected - uninfected)/uninfected]. [SL=Sultani late, SM= Sultani medium, SE =Sultani early maturity, N= Neely, F= Fark and MA =Mawy].

Table 5. Estimation of proline content (mg/g) in the seedlings of the tested six peach genotypes influenced by root knot nematode *M. incognita*

Peach genotypes	***Proline (mg/g) of dry wt.***					
	2017/2018			***2018/2019***		
	Leaves					
	Uninfected (control)	Infected	Over control%*	Uninfected (control)	Infected	Over control%*
SL	5.86^{cd}	7.16^{b}	22.18^{d}	5.80^{d}	7.21^{b}	24.31^{d}
SM	5.26^{d}	6.70^{bc}	27.38^{cd}	5.29^{d}	6.62^{c}	25.14^{d}
SE	2.94^{f}	8.56^{a}	191.16^{a}	2.95^{gh}	8.56^{a}	190.17^{a}
N	2.78^{f}	4.17^{e}	50.0^{b}	2.77^{hi}	4.15^{e}	49.82^{c}
F	3.44^{ef}	4.31^{e}	25.29^{cd}	3.51^{fg}	4.36^{e}	24.22^{d}
MA	2.96^{f}	4.01^{e}	35.47^{c}	2.26^{i}	4.03^{ef}	78.32^{b}
	Root					
SL	1.37^{e}	1.68^{de}	22.63^{c}	1.40^{de}	1.61d	15.0^{e}
SM	2.74^{b}	3.03^{b}	10.58^{c}	2.82^{b}	3.11b	10.28^{e}
SE	2.25^{c}	4.47^{a}	98.67^{b}	2.26^{c}	4.62a	104.42^{c}
N	0.58^{g}	2.86^{b}	393.10^{a}	0.55^{g}	2.86b	420.0^{a}
F	1.29^{ef}	1.61^{de}	24.81^{c}	1.13^{ef}	2.01c	77.0^{d}
MA	0.87^{fg}	2.00^{cd}	129.89^{b}	0.92^{f}	2.05c	122.83^{b}

Means followed by the same letter(s) in the same column don't significantly differ at 0.05 of probability according to Duncan's Multiple Range Test. Over control %*= [100 * (infected - uninfected)/uninfected]. [SL=Sultani late, SM= Sultani medium, SE =Sultani early maturity, N= Neely, F= Fark and MA =Mawy].

فحص وتقييم أصول جديدة من خوخ "ميت غمر" المحلي لمقاومة نيماتودا تعقد الجذور (*Meloidogyne incognita*)

الرفاعى فؤاد الدنجاوى[١]، جلال إسماعيل عليوة[١]،سمير برهام جاد[٢]، حنان حنفى عبد العاطى محمد[١]
[١] قسم الفاكهة – كليةالزراعة – جامعة دمياط – دمياط - مصر
[٢]قسم الحيوان الزراعي – كلية الزراعة – جامعة المنصورة - مصر

تم إجراء تجربة صوبة زجاجية لتقييم سلوك المقاومة لستة طرز وراثية من الخوخ (SL = سلطانى متأخر ، SM = سلطاني وسط ، SE = سلطاني مبكر ، N = Neely، F = Fark وMA = Mawy) مختارة من خوخ ميت غمر المحلي (*Prunuspersica* L.) لعدوى نيماتودا تعقد الجذور (*M. incognita*) خلال موسمين متتاليين (2018/17) و (2019/18). وتم إجراء التقييم من خلال إجراء عدة فحوصات نيماتودية على المجموع الجذربلشتلات الخوخ المختبرة ، وكان أهمها مؤشر الضرر الكلي (TDI) ، بالإضافة إلى الخصائص الخضرية والبيوكيميائية (محتويات الفينولات والبرولين في أوراق و جذور الشتلات المختبرة). **وقد اوضحت النتائج ما يلي:**

١. كانت القياسات النيماتودية والبيوكيميائية هي السائدة في الحكم على سلوك المقاومة لأصول الخوخ الستة ، على الرغم من أن القياسات الخضرية (طول الشتلة ، مساحة الورقة ومعامل نمو المجموع الجذرى) أظهرت اختلافات بين الطرز الوراثية المختبرة.
٢. الطرز الوراثية الثلاثة SL، SM ، F سجلت أكبر عدد من كتل البيض ، والعقد النيماتودية والطور اليرقي الثاني لنيماتودا تعقد الجذور على جذور شتلات الخوخ المصابة ، في حين سجل التركيب الوراثي MA القيم الأقل في هذا الصدد.
٣. الطراز الوراثى MA حصل على درجة عالى المقاومة (HR) والطرز الوراثية E, N حققت درجة مقاومة (R) وجميعها سجلت مؤشر ضرر كلي TDI للاصابة بالنيماتودا *M. incognita* أقل معنويا من الطرز الوراثية SL وSM و F، والتى حصلت على درجة عالى الحساسية (HS) وحساس (S) ومتوسط الحساسية (MS) للنيماتودا على التوالى.
٤. سجلت الطرز الوراثية SL و SM و F كميات أقل معنويا من الفينولات في أوراقها وجذورها مقارنة بالأنماط الوراثيةالاخري المقاومة وعالية المقاومة للاصابة بالنيماتودا. في حين تميز الطراز الوراثي MA بمحتوى عالي معنويا من الفينولات في الأوراق والجذور مقارنة بالأنماط الجينية الأخرى SL و SM وF وSE و N.
٥. كان محتوى البرولين لأوراق الشتلات من الأنماط الجينية المقاومة للنيماتودا SE و N و MA أعلى بكثير من القيم المقابلة للبرولين في الأنماط الجينية الأخرى الغير مقاومة SL و SM وF .
٦. كما لوحظ وجود علاقة ارتباط سلبية بين محتوى البرولين لكل من الأوراق والجذور ومؤشر التلف الكلي.


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