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ORIGINAL ARTICLE

Differential response of some nematode-resistant and susceptible tomato genotypes to *Meloidogyne javanica* infection

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Abstract

Resistance genes in response to root-knot nematode (*Meloidogyne javanica*) infection suppress one or more of several critical steps in nematode parasitism and their reproduction rate. The reaction of seven commercial tomato genotypes to *M. javanica* infection was investigated under greenhouse conditions. Current results classified these genotypes as: three resistant (Jampakt, Malika and Nema Guard), one moderately resistant (Fayrouz), and three susceptible (Castle Rock, Super Marmande and Super Strain B). Except Nema Guard, nematode infection significantly reduced plant height, fresh and dry weights of shoots of the other tomato genotypes. Leaf area was significantly reduced for all examined tomato genotypes except Malika and Nema Guard. Total chlorophyll was reduced in all tested tomato genotypes except Jampakt. Infection parameters of *M. javanica* and their population were significantly reduced on all nematode-resistant tomato genotypes compared to the susceptible genotypes. Also, the maturation rate of *M. javanica* was suppressed in the resistant genotypes compared to the susceptible genotypes. These results were confirmed by histological study that illustrated a delay in nematode development and their maturation. Total phenolic content significantly increased in nematode infected roots of both resistant and susceptible genotypes except Malika. Among non-infected roots, Malika showed the highest level of total phenols while after *M. javanica* infection, Nema Guard revealed the highest level of total phenols. Among infected roots, the highest level of total phenols was recorded in Castle Rock. These results suggested that using nematode-resistant tomato genotypes could provide an efficient and nonpolluting method to control root-knot nematodes.

Keywords: histology, root-knot nematode, tomato, total phenols

Introduction

The main restrictive factor for tomato production is the root-knot nematode (*Meloidogyne* spp.). It is the most important soil borne pathogen in Mediterranean countries, where nematode growth is favored by climatic conditions (Ornat *et al.* 2001). The most common root-knot nematode species in Egypt is *Meloidogyne javanica* (Taylor and Sasser 1978; Banora 2015). In Egypt, the total loss of tomato yield caused by these nematodes ranges from 20 to 80% (Abd-Elgawad and Askary 2015).

Root-knot nematodes (*Meloidogyne* spp.) are obligate sedentary endoparasites, parasitizing healthy plants to support their development and reproduction (Hussey 1985). In the course of a compatible interaction, these nematodes can alter the host plant metabolic pathways to their own benefit (Jansky *et al.* 2008). In tomato plants, these nematodes reduce the photosynthetic rates (Loveys and Bird 1973; Bali *et al.* 2018), and the growth parameters of plants correlate negatively with the initial population density of

M. javanica (Mekete *et al.* 2003; Schomaker *et al.* 2006). In addition, synthesis of phenolic compounds is associated with nematode infection (Sharma *et al.* 1990; Patel *et al.* 2017).

Among plant-parasitic nematode management strategies, chemical nematicides are the most frequently used. However, their potential negative impact on the environment and human health has led to a restricted use of most nematicides. The use of root-knot nematode-resistant genotypes is an effective alternative strategy for nematode management that reduces nematode populations in soil (Molinari 2011).

Cultivated tomato plants are naturally susceptible to root-knot nematodes. Some accessions of the related tomato species, *Solanum peruvianum* possess a single dominant gene called *Mi-1* that confers resistance to the most damaging species of root-knot nematodes: *M. incognita*, *M. javanica* and *M. arenaria* (Roberts and Thomason 1986; Messeguer *et al.* 1991). Genetic and physical mapping localized *Mi-1* gene to the short arm of tomato chromosome 6 (Kaloshian *et al.* 1998). Two homologs of this gene *Mi-1.1* and *Mi-1.2* were identified at the *Mi* locus. Only *Mi-1.2* conferred resistance to root-knot nematodes in tomato plants (Milligan *et al.* 1998).

This study compared the variability response of some commercial *Mi-1* gene-resistant tomato genotypes to *M. javanica* with some susceptible cultivars. In addition, this investigation compared the efficiency of nematode infection on the quantity of total phenols and chlorophyll content with non-infected plants. Also, the histological response of nematode-resistant tomato genotypes to *M. javanica* infection was compared with susceptible cultivars.

Materials and Methods

Plant material

All experiments were performed using commercial seeds of tomato genotypes. The nematode-resistant tomato genotypes: Fayrouz®, Malika®, Nema Guard® (Namdhari Seeds, India) and Jampakt® (Sakata Seed, South Africa) which possess the *Mi-1.2* gene (Heikal *et al.* 2008), were evaluated under greenhouse conditions for *M. javanica* infestation and compared with the susceptible cultivars Castle Rock®, Super Marmande® and Super Strain B® (Samyer Inc., USA). Increasing inoculum levels of nematode were also observed. Both resistant and susceptible tomato seeds were sown in multi-well foam trays (84 wells) filled with fertilized peat moss.

Five-week old seedlings of each genotype were transplanted into 25 cm-diameter pottery pots containing 1.5 kg sterilized sandy loam soil (1 : 1 v/v), watered every 2 days, and fertilized with nutrient solution; Super Vit® (N : P : K, 19 : 19 : 19).

Nematode cultures and inoculation

The nematode inoculums (second stage juveniles J2) were obtained from a pure culture of *M. javanica* that was previously initiated by a single egg mass and propagated on tomato cv. Super Marmande® plants in the greenhouse of the Plant Pathology Department, Faculty of Agriculture, Ain Shams University at an average ambient temperature of 20 ± 5°C. The infective stages (J2s) were extracted from the galled tomato roots by mist chamber technique (Reddy 1983). All seedlings of tomato genotypes were inoculated with 1,000 J2 of *M. javanica* except the un-inoculated plants. The pots were arranged in a completely randomized design with 15 replicates for each genotype and 15 un-inoculated replicates as a check for each genotype.

Data collection

Effect of *M. javanica* infection on growth parameters of evaluated tomato genotypes under greenhouse conditions

During all the experiments in this study, plant height and fresh weight of shoots were measured for inoculated and uninoculated plants. Shoots were placed in paper bags, dried in an oven at 60°C for 3 days, and then dry weight was measured. Total chlorophyll content was measured weekly on the uppermost fully expanded leaf using a Minolta SPAD-502 chlorophyll meter (Konica Minolta, Ramsey, NJ, USA); three measurements were taken, and the mean was recorded. Leaves were removed from the plants and total leaf area was measured using a LI-3100 Area Meter (LI-COR Biosciences, Lincoln, NE, USA). The percent of reduction in growth parameters (plant height, shoot fresh and dry weight, leaf area and total chlorophyll content) was calculated over controls (Irshad *et al.* 2012) as follows:

$$\text{Percentage of reduction} = \frac{\text{uninoculated} - \text{inoculated}}{\text{uninoculated}} \times 100\ [\%].$$

Nematode developmental stages on tomato genotypes under greenhouse conditions

Seven weeks after nematode inoculation, the plants were removed from the pots and the root systems were carefully washed under tap water. The galling severity caused by *M. javanica* for each root system was rated. In addition, to observe the computability of tomato genotypes with *M. javanica* and their development, the number of galls, egg-masses, premature stages and

total females per root system for each genotype were recorded. Also, the number of egg-masses per gall and the number of eggs per egg-mass were recorded in 1 g of randomly dissected galls for each tomato genotype. To determine the reproduction factor (*Rf*) of *M. javanica* on tomato genotypes, the number of juveniles per pot were counted at the end of the experiments for each genotype and *Rf* was calculated according to the following formula: *Rf* = number of eggs and J2 in roots and J2 in soil as a final population (P_f)/ initial population (P_i). Reproduction factor is an indicator of nematode reproduction or host efficiency, according to the modified quantitative scheme of Canto-Sáenz (Sasser *et al.* 1984). Each root system was stained by lactophenol acid fuchsine to determine the total count of different stages within the infected root tissue. Premature stages (spike-tailed and young females) and mature stages (mature females) were counted and the percentage of each stage was calculated according to the following formula:

$$\text{Percentage of stage} = \frac{\text{number of stage}}{\text{total number of all stages}} \times 100\ [\%].$$

Histological processes

To observe the histological response for nematode-resistant and susceptible tomato genotypes to *M. javanica* infection, 30 days after nematode inoculation (30 DAI), nematode feeding sites (galls) on infected roots were dissected using stereomicroscopy. Dissected galls for each genotype were individually collected and fixed in 2% glutaraldehyde in 50 mM PIPES buffer, pH 6.9, and then dehydrated and embedded in Technovit 7100® (Heraeus Kulzer). Embedded gall tissues were sectioned (3 mm) and stained in 0.05% toluidine blue and mounted in Depex (Sigma-Aldrich). Microscope observations were performed using bright-field optics and images were performed with a digital camera (Axiocam, Zeiss) as described by Banora *et al.* (2011).

Determination of total phenolic content

Colorimetric protocol was used to determine total phenolic content of methanolic extract of infected and non-infected roots of tomato genotypes using the method of Singleton *et al.* (1999). To 0.5 ml of test sample, 1 ml (1 : 10 v/v diluted with distilled water) Folin-Ciocalteau reagent was added and allowed to stand for 5 min at 22°C. After 5 min, 1 ml of saturated sodium carbonate was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation a blue color was observed. Finally, the absorbance of blue in different samples was measured at 725 nm using a colorimeter. The phenolic content was calculated as gallic acid equivalents (GAE) · g^{-1} based on the standard curve of gallic acid. The results were expressed as mg of GAE · g^{-1} of the plant material. All the determinations were carried out three times.

Statistical analysis

Collected data were analyzed using the SAS ANOVA (SAS Institute, 1992). Where ANOVA indicated significant treatment differences, the Least Significant Difference (LSD) at 5% was used for comparing means.

Results

Effect of *M. javanica* infection on growth parameters of evaluated tomato genotypes under greenhouse conditions

Infection of *M. javanica* significantly reduced plant height of both verified nematode-resistant and susceptible tomato genotypes except Fayrouz® (Fig. 1). Also, fresh and dry shoot weights of all tomato genotypes were significantly reduced except Fayrouz® (Fig. 2A and B). The leaf area was significantly reduced for all tomato genotypes except Malika® and Nema Guard® (Fig. 3A). The chlorophyll concentration of all tomato genotypes was significantly reduced except Jampakt® (Fig. 3B). All tested susceptible genotypes revealed a high percentage of growth parameters reduction particularly Super Marmande® which had the highest percentage of reduction for all growth parameters (Figs. 1, 2A–B, 3A–B).

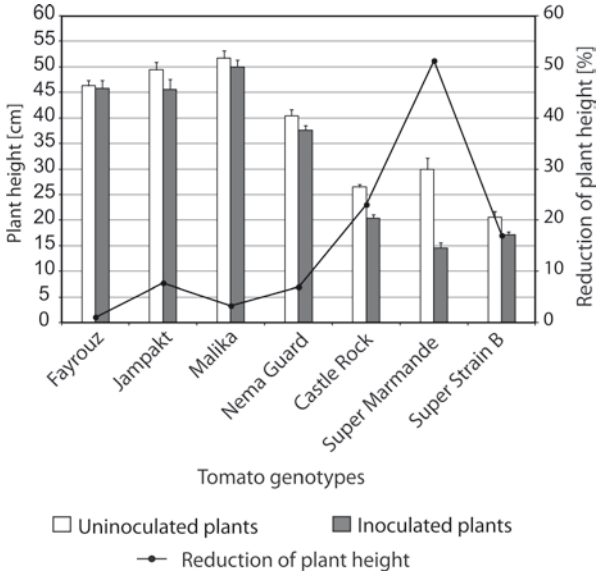

Fig. 1. Effect of *Meloidogyne javanica* infection on plant height of tested resistant and susceptible tomato genotypes

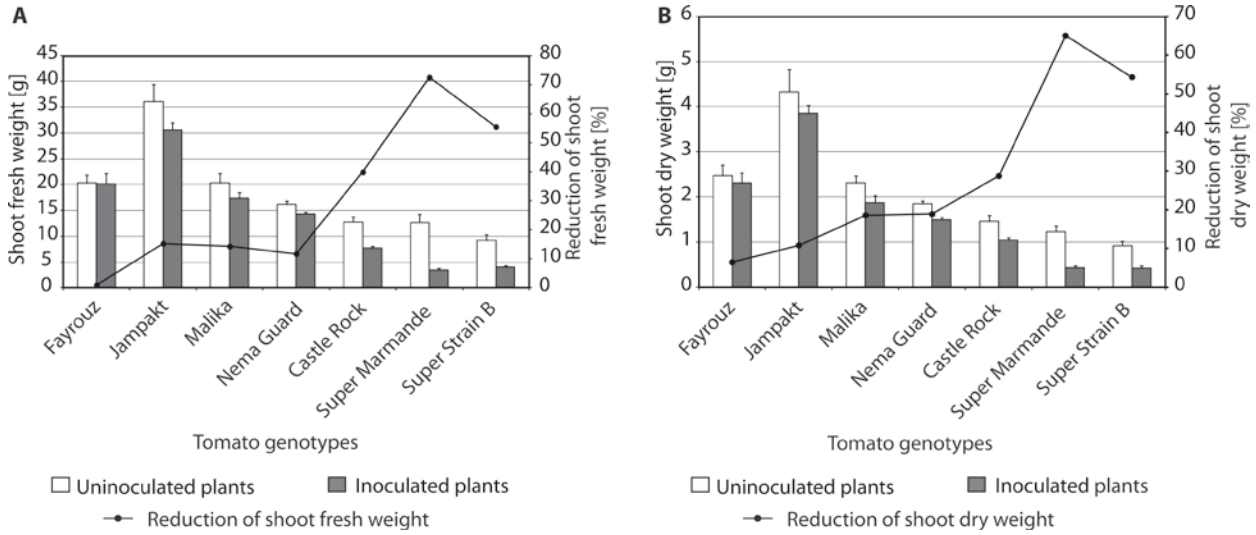

Fig. 2. Effect of *Meloidogyne javanica* infection on fresh (A) and dry (B) shoot weight of tested resistant and susceptible tomato genotypes

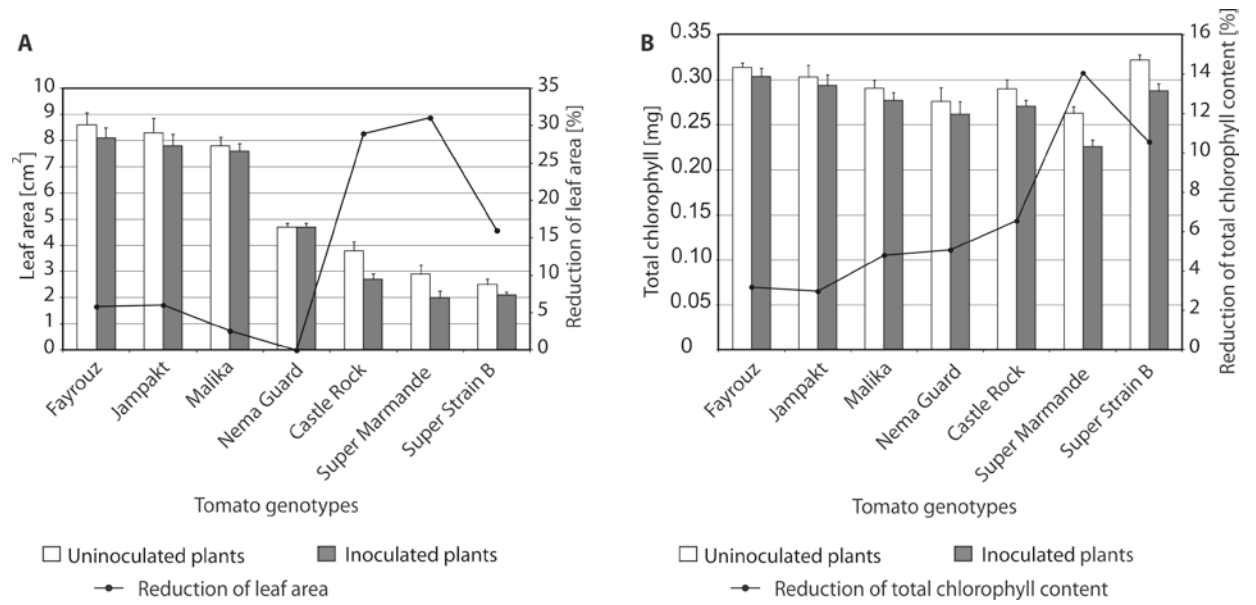

Fig. 3. Effect of *Meloidogyne javanica* infection on leaf area (A) and chlorophyll concentration (B) of tested resistant and susceptible tomato genotypes

Table 1. Evaluation of tested resistant and susceptible tomato genotypes for *Meloidogyne javanica* infection under greenhouse conditions at an average ambient temperature of 20 ± 5°C

Tomato genotypes	No. of galls	No. of egg-mass	No. of egg-masses/gall	No. of eggs/egg-mass	*RF*[a]	Resistance[b]
Fayrouz	17	26.1	1.3	23.1	0.59	MR
Jampakt	7	17.3	1.7	110.5	1.96	R
Malika	7	15.3	1.8	54.3	0.64	R
Nema Guard	4	6.5	1.1	36.8	0.28	R
Castle Rock	128	143.9	2.9	232.9	33.1	S
Super Marmande	194	329.3	3.4	129.3	44.3	S
Super Strain B	152	314.7	2.8	206.8	69.1	S
LSD (0.05)	4.67	15.27	0.25	13.27	4.14	

[a]*RF* = Reproduction Factor (P_f/P_i)
[b]*R* = Resistant (<20 egg masses found)
MR = Moderately Resistant (> 20 < 90 egg masses found); S = Susceptible (> 90 egg masses found) (Yaghoobi *et al.* 1995)

Table 1 shows the responses of tested resistant and susceptible tomato genotypes to infection with root-knot nematode (*M. javanica*). Infection parameters and resistance rates were recorded for each genotype. All parameters significantly decreased on resistant genotypes compared with the susceptible genotypes. Fewer galls, egg-masses, egg-masses per gall and the reproduction factor of *M. javanica* were recorded on Nema Guard as a nematode-resistant tomato genotype. The response of both Malika and Jampakt to *M. javanica* was slightly higher than Nema Guard but not significantly and were also recorded as resistant genotypes.

Concerning the response of Fayrouz to infection by *M. javanica*, the number of galls and egg-masses were the highest and significantly different from the other resistant genotypes. Therefore, Fayrouz was recorded as a moderately resistant genotype to *M. javanica*. Although Jampakt was classified as a resistant genotype, the number of eggs per egg-mass and the reproduction factor of *M. javanica* were the highest compared with the other infected nematode-resistant tomato genotypes. Also, the number of egg-masses per gall was significantly higher on Malika and Jampakt, respectively, than on the other resistant genotypes. The infected nematode-susceptible tomato genotypes had the highest response to *M. javanica* infection. Super Marmande®, Super Strain B and Castle Rock, respectively, showed the highest level of all infection parameters which were significantly different.

Maturation rates of *M. javanica* in nematode--resistant and susceptible tomato genotypes

Figure 4A shows the developing frequency of premature and mature endo-parasitic stages of *M. javanica* within the root tissue of tested nematode-resistant and susceptible tomato genotypes. The highest number of

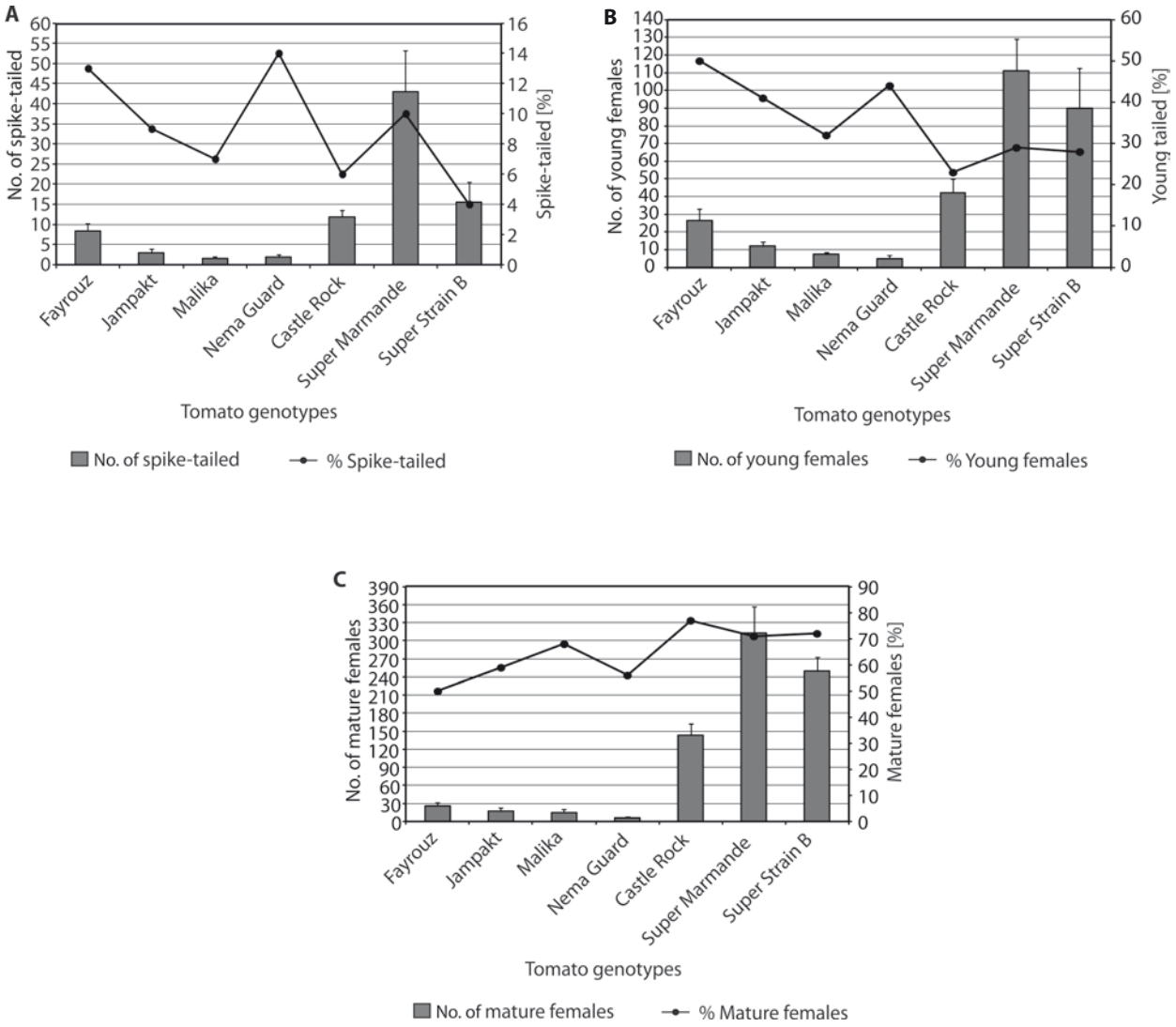

Fig. 4. Maturation rate of *Meloidogyne javanica* in resistant and susceptible tomato genotypes: (A) number and percentage of spike-tailed stages, (B) number and percentage of young females and (C) number and percentage of mature females

premature stages (spike-tailed and young females) and mature stages (mature females) significantly resulted from the response of the Fayrouz genotype to *M. javanica* infection compared with the other infected nematode-resistant tomato genotypes (Figs. 4A, B and C). The second significant response to *M. javanica* infection among resistant genotypes was Jampakt which had a higher number of young and mature females than Malika and Nema Guard, respectively (Figs. 4B and C). Nema Guard inhibited the developmental rate of *M. javanica* and revealed the lowest number of premature and mature stages (Figs. 4A, B and C). In addition, Nema Guard had the highest percentage of spike-tailed premature stages and the lowest percentage of mature females compared with the susceptible genotypes (Figs. 4A and C). In contrast, the tested nematode-susceptible tomato genotypes responded easily to *M. javanica* infection and supported the maturation rate of the nematodes. The highest numbers of premature and mature stages were recorded within infected root tissue of Super Marmande, Super Strain B and Castle Rock, respectively (Figs. 4A, B and C). Also, the percentage of mature females in all susceptible genotypes was higher than in tested resistant genotypes (Fig. 4C), and the percentage of young females was less in tested resistant genotypes (Fig. 4B).

Histological analysis of galls induced by *M. javanica* in nematode-resistant and susceptible tomato genotypes

Figure 5 illustrates the histological analysis of nematode feeding sites induced by *M. javanica* in infected root tissue of both nematode-resistant and susceptible tomato genotypes 30 days after inoculation. Females of *M. javanica* observed in the tissue of tested nematode--resistant tomato genotypes were young and therefore laying egg-masses was delayed (Figs. 5A, B, C and D)

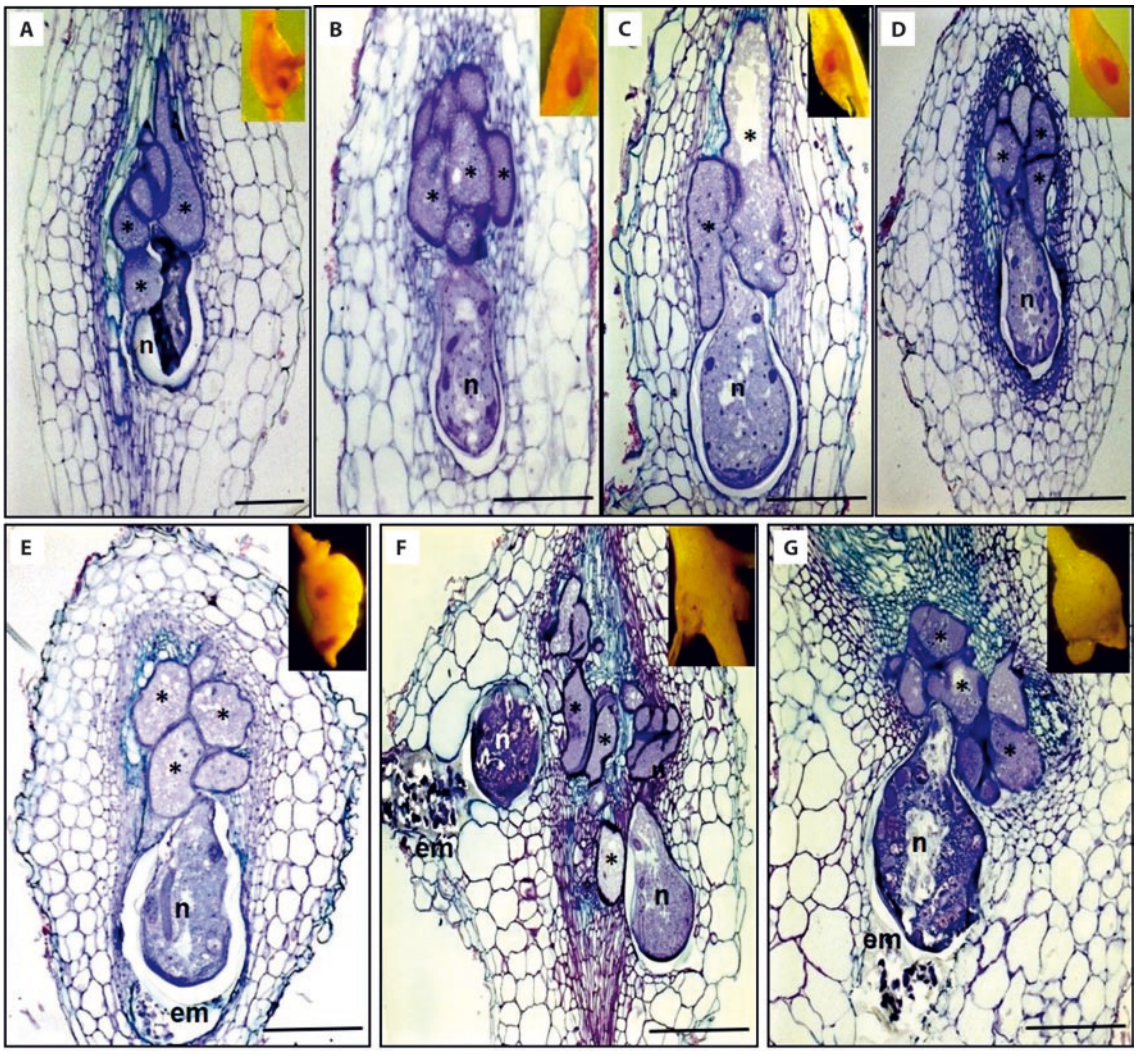

Fig. 5. Histological analysis of galls in nematode-resistant and susceptible tomato genotypes 30 days after *Meloidogyne javanica* inoculation. Bright-field images of sections stained with toluidine blue. Gall in: (A) Fayrouz roots, (B) Jampakt roots, (C) Malika roots, (D) Nema Guard roots, (E) Castle Rock roots, (F) Super Marmande roots and (G) Super Strain B roots. (*) giant cells, (em) egg-mass and (n) nematode. Bars = 100 µm (A) to (C); 200 µm (D) to (G)

compared with the sections of tested nematode-susceptible tomato genotypes that had mature females with egg-masses (Figs. 5E, F and G). In addition, almost all dissected galls formed on infected resistant genotypes contained single females (Figs. 5A, B, C and D). Some dissected galls formed on infected susceptible genotypes contained more than one mature female as observed in a galls of Super Marmande (Fig. 5F).

Total phenol analysis in roots of tested nematode-resistant and susceptible genotypes to *M. javanica*

Total phenol compounds in non-infected and infected roots of tested nematode-resistant and susceptible tomato genotypes to *M. javanica* was measured 30 days after inoculation. The results (Fig. 6) showed that the quantity of phenolic compounds significantly increased in infected roots of both tested nematode-resistant and susceptible tomato genotypes compared with non-infected roots. Among non-infected roots of tested tomato genotypes, the highest level of phenolic compounds was recorded in Malika, Fayrouz and Jampakt, respectively. Both Nema Guard and Castle Rock genotypes had the smallest quantity of phenolic compounds. In contrast, among infected roots of tested tomato genotypes, Nema Guard had the largest quantity of phenolic compounds, followed by Castle Rock, Super Marmande, Jampakt, Malika, Super Strain B and Fayrouz, respectively. Interestingly, Malika genotype had the same quantity of phenolic compounds in both non-infected and infected roots compared with tested nematode-resistant and susceptible tomato genotypes.

Similarly, the percentage of phenolic compounds showed that the quantity of total phenols increased more than five times in infected roots of Nema Guard, four times in infected roots of Castle Rock, around three times in infected roots of both Super Marmande and Jampakt, two times in infected roots of Super Strain B and more than one time in infected roots of Fayrouz. The quantity of total phenols did not increase in infected roots of Malika.

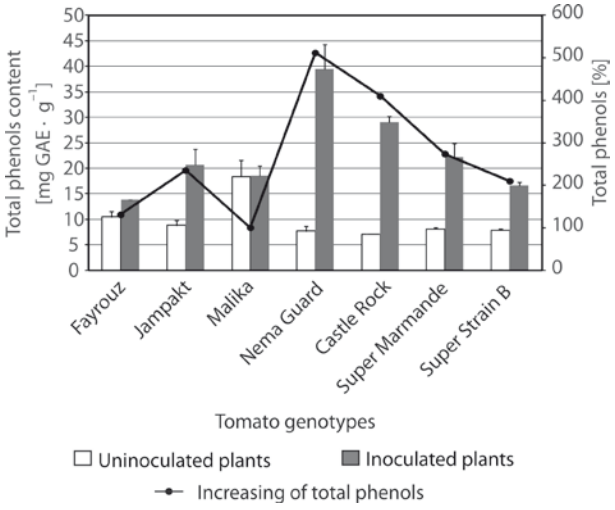

Fig. 6. Effect of *M. javanica* infection on quantity and percentage of total phenols in nematode-resistant and susceptible tomato genotypes

Discussion

Plant growth parameters were significantly affected by *M. javanica* infection in relation to shoot length, fresh and dry shoot weights for tested nematode-resistant and susceptible tomato genotypes except Fayrouz. Leaf area was significantly reduced for all experimented tomato genotypes except Malika and Nema Guard. Levels of total chlorophyll were significantly reduced in all investigated tomato genotypes except Jampakt. Generally, all growth parameters of nematode-susceptible tomato genotypes were severely reduced and revealed a high percentage of growth parameters reduction.

Due to root-knot nematodes which induce giant cells in nematode feeding sites within the root vascular system, galls are formed on the root system. This disturbance in the root structure reduces the uptake of water and nutrients and their transport from the roots to the shoots (Abad *et al.* 2003; Roduic *et al.* 2014). In addition, these nematodes regulate greater translocation in the output of photosynthesis toward infected root tissue while depriving the foliage parts (Di Vito *et al.* 2004). Plant response to nematode parasitism thus causes morphological and physiological changes that affect the photosynthetic processes (Hussey and Williamson 1998; Strajnar *et al.* 2012). These effects increase during nematode infection (Melakeberhan *et al.* 1987) which was clearly seen on all susceptible genotypes. In a recent study, nematode-resistant genotypes infected by *M. javanica* had a slight reduction in shoot length, and fresh and dry shoot weights, except Fayrouz which was not affected, while nematode-resistant and susceptible genotypes had severe reduction. This reaction of Fayrouz may be due to it carrying *Mi-1.1* and *Mi-1.2* genes (Heikal *et al.* 2008). Therefore, as a result of an irregular supply of water, nutrients, photosynthates and energy, the growth and development of leaf tissue and its constituents especially chlorophyll pigments are severely affected (Khan and Khan 1997; Strajnar *et al.* 2012; Ahmad *et al.* 2017). These effects were clearly seen on all susceptible genotypes compared to resistant genotypes. Except Malika and Nema Guard, leaf area was slightly affected in the other resistant genotypes. The total chlorophyll affected both infected resistant and susceptible genotypes except Jampakt. A reduction of total chlorophyll has also been reported in tomato (Loveys and Bird 1973;

Bali *et al.* 2018), French bean (Melakeberhan *et al.* 1986), rice (Swain and Parasad 1988) and cucumber (Giné 2014) infected with *M. javanica*. Similarly, total chlorophyll was decreased in infected tomato with *Meloidogyne ethiopica* (Strajnar *et al.* 2012). Also, *M. incognita* infection reduced chlorophyll content and photosynthesis of black henbane (*Hyoscyamus niger*), cotton plants (Haseeb *et al.* 1990; Lu *et al.* 2014), and patchouli (*Pogostemon cablin*) plants (Bhau *et al.* 2016). Previous studies have discussed these reactions and indicate that leaf pigment composition is sensitive to plant stress and nematode infection causes a loss of photosynthetic pigments (e.g. chlorophyll) (Demming--Adams and Adams 1992; Strajnar *et al.* 2012). Many abiotic and biotic stresses damage plant leaf tissue and the chloroplasts (Karpinski *et al.* 2003). In addition, the previous study showed that nematode-resistant tomato genotypes that carry the *Mi-1.2* resistant gene had significantly greater foliar biomass and root mass than infected susceptible plants (Corbett *et al.* 2011). Recently, these characters were seen on experimental resistant genotypes. The growth responses of tomato resistant genotypes can be related to the presence of the *Mi-1.2* gene (Heikal *et al.* 2008).

Infection parameters of *M. javanica* in a recent study significantly decreased on resistant genotypes compared with the susceptible genotypes. Fewer galls, egg-masses, egg-masses per gall and the reproduction factor of *M. javanica* were recorded on nematode-resistant tomato genotypes than on nematode-susceptible tomato genotypes. The response of Fayrouz against *M. javanica* infection was significantly different than the other resistant genotypes. According to Yaghoobi *et al.* (1995), Fayrouz is classified as moderately resistant while Jampakt, Malika and Nema Guard are identified as resistant genotypes to *M. javanica*. The infected nematode-susceptible tomato genotypes had the highest response to *M. javanica* infection. Super Marmande, Super Strain B and Castle Rock, respectively, showed the highest levels of all infection parameters and were significantly different. Generally, the developing rate of *M. javanica* within the infected root tissue showed that the nematode-susceptible tomato genotypes support the maturation rate of nematodes compared with nematode-resistant tomato genotypes. Among susceptible genotypes, Super Marmande showed the highest count of premature and mature stages. Castle Rock revealed the lowest number of premature and mature stages. The percentage of mature females developed in tested nematode-susceptible tomato genotypes was ≥70% and premature females was between 20 to 30% while the spike-tailed stages were very few (4–9%). In contrast, resistant genotypes suppressed the developing rate of *M. javanica.* Among resistant genotypes, the moderately resistant Fayrouz had the highest number of premature stages and mature females while the other resistant genotypes, Jampakt, Malika and Nema Guard, respectively, had the lowest number of premature stages and mature females. Particularly Nema Guard had the highest percentage of spike-tailed stages and premature females. Therefore, the developing rate of *M. javanica* within the infected root tissue was more seriously developed on nematode-susceptible tomato genotypes than on the other resistant genotypes. These results suggested that the susceptible tomato genotypes respond positively to *M. javanica* infection and support the maturation rate of nematodes. In contrast, resistant genotypes suppressed the developing rate of *M. javanica.* The histological results of recent study confirm that the development of *M. javanica* was delayed in resistant genotypes and well developed in infected roots of susceptible genotypes. The images of dissected galls and their longitudinal sections illustrated the egg-mass associated with the mature females on nematode-susceptible tomato genotypes. Various stages during the life cycle of root-knot nematodes could be affected by host response (Mukhtar *et al.* 2014). In addition, the level of susceptibility of tomato to root-knot nematodes is controlled by the presence of resistant genes such as the *Mi* gene (Jacquet *et al.* 2005). On the other hand, susceptible host plants allowed the juveniles of root-knot nematodes to mature and produce many eggs (Karssen and Moens 2006). It has been shown that the *Mi*-gene provides partial protection against the development of *M. javanica* (Tzortzakakis *et al.* 1998), *M. incognita* (Jacquet *et al.* 2005) and *M. hispanica* (Maleita *et al.* 2011) on tomato. These results suggest that nematode reproduction is influenced by the genetic background of the plant host, which agrees with recent results. Also, Talavera *et al.* (2009) recorded that the *Mi* resistant tomato cultivar effectively suppressed the population densities of *M. javanica*, *M. arenaria* and *M. incognita* in three different localities.

Meloidogyne javanica infection significantly increased the content of total phenols in infected roots of both tested nematode-resistant and susceptible tomato genotypes compared with non-infected roots, except the resistant genotype, Malika. Remarkably, the highest level of phenolic compounds in non-infected roots of tested tomato genotype was recorded in Malika. Except the resistant genotype Nema Guard, the quantity of phenols in non-infected roots of nematode-resistant tomato genotypes was significantly more than in all the susceptible genotypes. Nema Guard had the greatest quantity of phenolic compounds in infected roots (more than five times) compared with the resistant and susceptible genotypes. Also, phenols increased approximately three times more after nematode infection in roots of Jampakt. Among susceptible genotypes, Castle Rock genotypes had the greatest quantity of phenolic compounds (approximately more than four times).

Commonly, all pathogen elicitors stimulate the phenylpropanoid pathway that leads to biosynthesis of flavonoids as well as lignin and phenolic compounds (Bleve-Zacheo *et al.* 2007). An increased rate of phenol synthesis induced by pathogen invasion triggered the transcription of messenger RNA that codes for phenylalanine ammonia lyase (PAL) (Taiz and Zeiger 2002). Phenolic compounds play a major role in the defense mechanisms of plants against pathogens. As in our study, it has been shown that nematode-resistance in tomatoes to *M. incognita* is attributed to high concentrations of phenols in infected roots (Bajaj and Mahajan 1977; Patel *et al.* 2017). The recent results revealed the same reaction on Nema Guard, Jampakt and Fayrouz, respectively. The total phenols in Malika revealed stability during nematode infection. Consequently, probably Malika genotype has a pre-infection nematode resistance mechanism, whereas the presence of phenolic compounds in plant roots prevents or obstructs penetration of J2s (Bendezu and Starr 2003). Also, the amount of phenolic compounds in root tissue can suppress the development of nematode feeding sites and thus the developing rate of nematodes (Chin *et al.* 2018). Also, chlorogenic acid was identified as the major phenolic compound in the roots before or after infection of plant parasitic nematodes (Ohri and Pannu 2010). It has been proposed that phenol accumulation is related to resistance in tomato to root-knot nematodes (Hung and Rohde 1973). Thus, the resistance mechanism of Fayrouz, Jampakt and Nema Guard probably classifies as post-infection resistance (Anwar and McKenry 2010).

According to Korves and Bergelson (2004), the *Mi*-gene in tomato confers resistance to the three most common warm climate root-knot nematodes, *M. arenaria*, *M. incognita* and *M. javanica* (Williamson 1999), but not immunity. To date, *Mi-1* is the only commercially available resistant gene for root-knot nematodes (Mantelin *et al.* 2013). Remarkably, the *Mi-1.2* gene but not the *Mi-1.1*gene was sufficient to confer resistance to *M. javanica* (Hwang *et al.* 2000). According to Heikal *et al.* (2008), in addition to the *Mi-1.1*gene, the nematode-resistant tomato genotypes, Fayrouz, Jampakt, Malika and Nema Guard, carry the *Mi-1.2* gene while the nematode-susceptible genotypes investigated in this study, Castle Rock, Super Marmand and Super Strain B, possess only the *Mi-1.1*gene. In addition, 83 WRKY genes have recently been identified in tomato plants (Karkute *et al.* 2018). One or more members of this gene family such as *SlWRKY72*, *SlWRKY73*, or *SlWRKY74* have been investigated as contributing positively to both PAMP-triggered immunity (PTI) and *Mi-1*-mediated effector-triggered immunity (ETI) against *M. javanica* (Bhattarai *et al.* 2010). Also, the *SlWRKY80* gene was required for *Mi-1*-mediated resistance against root-knot nematodes (Atamian *et al.* 2012; Bai *et al.* 2018). Thus, these genes could play an important role during nematode infection in investigated resistant tomato genotypes as *Mi-1*-mediated effector-triggered immunity.

The different responses of the investigated tomato genotypes to *M. javanica* infection indicated that all the resistant genotypes that possess resistant gene (*Mi1.2*) have greater foliar biomass, larger amounts of phenols and can delay or suppress the development and reproduction of nematodes. The susceptible genotypes that possess only the *Mi1.1* gene but lack the *Mi1.2* gene were highly compatible with *M. javanica* infection. This suggests that cultivating the nematode-resistant tomato genotypes was highly effective for decreasing the population of *M. javanica.* Therefore, the careful integration of resistant genotypes in the cropping rotation system is essential to reduce both the root-knot nematode population and crop losses. The approach will also help to minimize environmental pollution, preserve agro-ecosystems and biodiversity and help keep management processes more economical.

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