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Evaluation of some Tomato Genotypes for Nematode Resistance and Detection of the *Mi-1.2* Resistance Gene

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ABSTRACT



Response of fourteen tomato genotypes from different geographical regions to root-knot nematodes (RKN; Meloidogyne spp.) was evaluated for two seasons under greenhouse conditions. Two PCR-based markers (Mi-23 and REX-1) were used for the detection of the RKN resistance gene (Mi-1.2) in the tested genotypes. The results showed a wide variation among tested tomato genotypes in their responses to RKNs. Highly significant differences were observed among tested genotypes for gall index and egg masses per root system. Based on nematode bioassay, the tomato genotype Strain B F_1 was highly resistant to RKN and ranked in top of resistant genotypes. Floradade and VF145-B were resistant, Castlerock, Peto86 and Qaha were moderately resistant, Super strain B, E115, Super Marmande, M82 and Edkawy were susceptible, and E37, E40 and Giza-80 were highly susceptible to RKN. Molecular analysis with Mi23 and REX-1 markers indicated a heterozygous state (Mi/mi) of Mi-1.2 gene for Strain B F1; meanwhile, the remaining genotypes displayed a homozygous recessive state (mi/mi). These finding suggested that Strain B F1 could be used to develop new tomato cultivars resistant to RKN through tomato breeding programs. In addition, the results suggested that tomato genotypes that were identified in the present study as homozygous recessive (mi/mi) for the Mi-1.2 gene and considered as resistant or moderately resistant may possess other genes controlling RKN resistance. However, further studies are still needed to determine these genes that control the resistance to RKNs in tomatoes.

Keywords: Root-knot nematode (RKN); *Meloidogyne spp.*; *Solanum lycopersicum; Mi-1.2* gene; *Mi-23* and *REX-1* markers.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide. Tomato is subjected to attack by various plant pathogens that threatening its production, including root-knot nematodes, RKN (*Meloidogyne spp.*). RKNs are responsible for huge economic yield losses, due to their short life cycles and high reproductive rates (Corbett *et al.*, 2011; Trudgill and Blok, 2001). Disease symptoms include formation of root galls, damaged root system, chlorosis, wilting, and stunted growth resulting in reduced production due to the nutritional stress created in the host plant during pathogenesis (Trudgill and Blok, 2001; Abad *et al.*, 2003).

Different strategies have been reported for nematode management, including crop rotation of resistant or less susceptible genotypes, tillage practices and nematicide treatments. However, not all managements and practices are effective in controlling plant nematodes. Breeding for tomato cultivars resistant to RKN is an alternative and efficient method for nematode management (Cook, 2000). Moreover, development of tomato breeding lines resistant to RKN is an effective tool to reduce levels of nematodes in the soil (El Mehrach, 2007).

Cultivated tomato plants are naturally susceptible to RKN (Banora and Almaghrabi, 2019). Resistance to RKN in tomatoes was first detected in the wild tomato species *Solanum peruvianum* (Bailey, 1941). Smith (1944) introduced RKN resistance into cultivated tomatoes, which was then found to be controlled by the *Mi-1* single dominant gene. The *Mi-1* gene became one of the well-known resistance genes against the most common Meloidogyne species, i.e. *M. javanica, M. arenaria* and *M. incognita* (Roberts and Thomason, 1986; Martinez de Ilarduya *et al.*, 2001; Nombela *et al.*, 2003).

The *Mi-1* gene was found to be located on chromosome 6 of tomato (Williamson *et al.*, 1994). Two homologs of *Mi-1* (*Mi-1.1* and *Mi-1.2*) were identified, but only the gene *Mi-1.2* conferred resistance (Milligan *et al.*, 1998). Response of different tomato genotypes with the *Mi-1.2* gene against different RKN isolates have been long reported (Molinari and Caradonna, 2003; López-Pérez *et al.*, 2006; Cortada *et al.*, 2008, Cortada *et al.*, 2009; Verdejo-Lucas *et al.*, 2013; Carvalho *et al.*, 2015). Recently, other nine resistance genes against RKN have been identified (El-Sappah *et al.*, 2019).

Numerous molecular markers have been developed to detect the presence of resistance genes in tomato, of which *Mi-23* (Seah *et al.*, 2007) and *REX-1* (Williamson *et al.*, 1994) are PCR-based markers widely used for the detection of *Mi* gene in cultivated and wild tomato species and to differentiate resistant from susceptible genotypes. These markers have also the potential to determine the allelic conditions *Mi/Mi* (dominant homozygous), *Mi/mi* (heterozygous) and *mi/mi* (recessive homozygous) at the gene locus (Cortada *et al.*, 2008; Devran *et al.*,

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2013). Moreover, molecular markers linked to resistance genes can be also used to identify novel sources of disease resistance at early stages in tomato breeding programs. Objectives of the study include evaluation of responses of fourteen tomato genotypes to RKN under greenhouse conditions through 2017 and 2018 seasons, and detection of the *Mi-1.2* resistance gene in the tested genotypes using *Mi23* and *REX-1* markers.

MATERIALS AND METHODS

Source of root-knot nematodes

The RKNs (*Meloidogyne spp.*) inoculums used in the present study were obtained from infected tomato plants collected from farmer's fields in Assiut Governorate, Egypt. **Extraction and examination of RKNs**

Infected tomato roots collected from farmer's fields in Assiut Governorate were cut to pieces and placed into beakers (1000 ml) with 10% chlorine bleach solution to cover the roots (Hussey and Barker, 1973). The beakers were then agitated on a rotary shaker with 100 rpm for 4min. The roots were then removed from the beakers, and the 10% chlorine bleach-nematode extract was poured from the beaker and sieved through a 100 μ m sieve. The nematodes were then collected on a 20 μ m sieve. Nematodes were counted under the microscope at 40X magnification with the aid of tally counter.

Plant material and greenhouse experiment

The plant material used in the present study comprised of fourteen tomato genotypes (Table 1), including commercial cultivars and accessions from different geographical regions (two from Italy, one from Perù, one from France, seven from USA and three from Egypt). Seeds of tomato genotypes were sown under greenhouse conditions at Department of Plant Pathology, Faculty of Agriculture, Assiut University, Egypt. Tomato seedlings with 3-4 true leaves were then transplanted singly into sterilized pots (15cm in diameter) containing 2kg sterilized sandy-loam soil. Pots were inoculated seven days after transplanting. Infection was carried out on five plants for each genotype, leaving two healthy plants from each genotype as control. For nematode infection, 1ml of inoculums containing 1000 RKN eggs (*Meloidogyne spp.*) was used for each plant.

 Table 1. Names and origin of 14 tomato genotypes used in the study

| | in the study. | | |
|-----|-------------------------|--------|--|
| No. | Name | Origin | |
| 1 | E37 | Italy | |
| 2 | E40 | Italy | |
| 3 | E115 | Perù | |
| 4 | Super Marmande | France | |
| 5 | M82 | USA | |
| 6 | Castlerock | USA | |
| 7 | Floradade | USA | |
| 8 | Peto86 | USA | |
| 9 | VF145-B | USA | |
| 10 | Super strain B | USA | |
| 11 | Strain B F ₁ | USA | |
| 12 | Giza-80 | Egypt | |
| 13 | Edkawy | Egypt | |
| 14 | Qaha | Egypt | |

Three months after the inoculation, the root systems of tomato plants were removed from the pots, washed with tap water, and visually rated for gall index (GI). The roots were then macerated in a blender with 20% commercial bleach solution for 30-40 sec, the suspension poured onto 200 and 500 mesh sieves. The eggs were then collected for counting. Egg masses per plant root were recorded for each genotype. Tomato plants were then rated for number of galls using a 0-5 scale following Taylor and Sasser (1978) as: (0) = no galls, (1) = 1 to 2 galls,

| (0) = 10 gains, | |
|----------------------|--|
| (2) = 3 to 10 galls, | |

(3) = 11 to 30 galls, (5) = more than 100 galls.

 (4) = 31 to 100 galls,
 (5) = more than 100 galls. The nematode bioassay was repeated twice during 2017 and 2018 seasons and the phenotypic data were then averaged for statistical analysis. Levels of resistance/susceptibility of tomato genotypes were grouped based on gall index following Boiteux and Charchar (1996) and Silva

- *et al.* (2019) as follow:Gal index 1.0 1.6 = highly resistant (HR);
- Gal index 1.7 2.3 = resistant (R);
- Gal index 2.4 3.0 = moderately resistant (MR);
- Gal index 3.1 4.0 = susceptible (S);
- Gal index 4.1 5.0 = highly susceptible (HS).

Phenotypic data analysis Data of gal index and egg masses/root (two-year

average) of fourteen tomato genotypes were subjected to an analysis of variance (ANOVA) to test significance of differences among tested genotypes using IBM SPSS Statistics V21.0. Values of gal index were transformed to log10 (x+1) before analysis. Means were compared by Duncan's test. Pearson's correlation coefficient between gal index and egg masses/root was calculated.

Molecular marker analysis

Molecular analysis of the current study was conducted at Department of Genetics, Faculty of Agriculture, Assiut University, Egypt. DNA extraction from healthy and fresh leaves of tomato genotypes was carried out using the CTAB method (Murray and Thompson, 1980). Two PCR-based markers namely, *Mi23* (a SCAR marker, sequence characterized amplified region) and *REX-1* (a CAPS marker, cleaved amplified polymorphic sequence) were used to detect the *Mi-1.2* resistance gene in the tested genotypes (Table 2).

 Table 2. SCAR and CAPS markers used for detecting the presence of *Mi-1.2* resistance gene.

| Marker | Sequence of forward (F) and reverse (R) primers (5' - 3') | Annealing temperature (°C) | *Size of amplified bands (bp) | Size after digestion with TaqI (bp) |
|-----------------|---|-------------------------------|----------------------------------|--|
| Mi23 (SCAR) | F: TGGAAAAATGTTGAATTTCTTTTG R: GCATACTATATGGCTTGTTTACCC | 57 | 430 380 | - |
| REX-1 (CAPS) | F: TCGGAGCCTTGGTCTGAATT R: GCCAGAGATGATTCGTGAGA | 55 | 750 | 750 570 160 |

SCAR: sequence characterized amplified region, CAPS: cleaved amplified polymorphic sequence.

Sizes of expected DNA fragments of *Mi23* marker were determined following Seah *et al.* (2007).

Sizes of expected DNA fragments of *REX-1* were determined following Williamson *et al.* (1994).

PCR conditions were carried out following Seah *et al.* (2007) for *Mi23* and Williamson *et al.* (1994) for *REX-1*. PCR products of *REX-1* were digested with the restriction enzyme *Taq I* to indicate the allelic conditions at the gene locus. PCR and digested products were separated on 2% agarose gels in TBE buffer (0.5X) using horizontal gel electrophoresis and photographed using a gel documentation system. Sizes of expected DNA fragments were then estimated.

RESULTS AND DISCUSSION

Nematodes bioassay

Analysis of variance of gall index and egg masses/root (Table 3) revealed highly significant differences (P < 0.01) among tomato genotypes. The tested tomato genotypes exhibited wide variations in their responses to RKNs (Table 4). The gall index (0-5) scale ranged from 1.3 in Strain B F_1 to 5.0 in E40 genotype (averaged 3.2). Egg masses/root ranged from 15.8 in Strain B F1 to 71.8 in E40 (averaged 44.0). The gal index was positively correlated with egg masses/root (r= 0.89, P < 0.01) as shown in Table 4. It has been reported that the primary symptom of the infection on susceptible plants is the formation of galls on the roots. As a result, the galls disrupt the normal function of the xylem tissue of the plant (Wubie and Temesgen, 2019). In addition, the nutrient and water uptake of the plant reduced due to damaged roots, resulting in yield reduction (Abad et al., 2003). The gall index and egg masses/root were proven as practical and nondestructive criteria to evaluate plant responses to RKNs. The gall index was efficient to discriminate levels of resistance in tomatoes and allowed the identification of a wide range of plant responses against RKNs, which vary from highly susceptible to highly resistant (Silva et al., 2019). The low number of root galls observed in resistant genotypes were due to low number of nematodes which penetrated the plant root (Wubie and Temesgen, 2019). In accordance, root galls and egg masses were significantly lower in tomato cultivars resistant to RKNs (Rumbos et al., 2011). In addition, lower number of galls was observed on tomato cultivar grafted onto rootstock carrying Mi gene (Wubie and Temesgen, 2019).

In the present study, levels of resistance/ susceptibility of fourteen tomato genotypes to RKN were classified based on gal index into five groups (Table 4), of which only Strain B F₁ was classified as highly resistant (HR) to RKN and ranked in top of the resistant genotypes. Two genotypes (Floradade and VF145-B) were classified as resistant (R) to RKN. Three genotypes (Castlerock, Peto86 and Qaha) were moderately resistant (MR), five genotypes (Super strain B, E115, Super Marmande, M82 and Edkawy) responded as susceptible (S) and three genotypes (E37, E40 and Giza-80) were highly susceptible (HS) to RKN. These results indicated the occurrence of remarkable genetic diversity among tested genotypes for RKN resistance. In the present study, the tested tomato genotypes belong to different geographical regions. Accordingly, it has been reported that the genetic background of plant has a major effect in variability of their resistance to nematodes (Jacquet et al., 2005; Cortada et al., 2009; Verdejo-Lucas et al., 2009). Evidently, the use of resistant tomatoes would provide an efficient alternative and non-polluting method for nematode management (Molinari, 2011; Banora and Almaghrabi, 2019).

 Table 3. Analysis of variance of gall index and egg masses per root system.

| | | Mean square | | |
|-------|-----------|--|--|--|
| d.f - | Gal index | Egg masses | | |
| 13 | 0.066** | 1033.269** | | |
| 42 | 0.003 | 14.131 | | |
| 55 | | | | |
| | 13 42 | Gal index 13 0.066** 42 0.003 55 | | |

Gal index values were transformed to log₁₀(x+1) before analysis. ** stand for significant differences at 0.01 probability level.

Table 4. Responses of 14 tomato genotypes against RKNs (data are average of 2017 and 2018 seasons).

| No. | Genotypes | Gall index (0-5 scale) | Egg masses per plant root | Response to RKN | Resistance Ranking |
|------|-------------------------|---------------------------|---------------------------------|--------------------|-------------------------------------|
| 1 | E37 | 4.5 ab | 52.0 b | HS | 12 |
| 2 | E40 | 5.0 a | 71.8 a | HS | 14 |
| 3 | E115 | 3.3 cde | 49.3 bcd | S | 8 |
| 4 | Super Marmande | 3.0 def | 44.0 def | S | 7 |
| 5 | M82 | 3.5 cde | 52.8 b | S | 9 |
| 6 | Castlerock | 2.5 fgh | 39.8 f | MR | 5 |
| 7 | Floradade | 2.0 hi | 24.3 h | R | 3 |
| 8 | Peto86 | 2.8 efg | 41.0 ef | MR | 6 |
| 9 | VF145-B | 1.8 i | 24.3 h | R | 2 |
| 10 | Super strain B | 3.8 bcd | 46.0 cde | S | 10 |
| 11 | Strain B F ₁ | 1.3 j | 15.8 i | HR | 1 |
| 12 | Giza-80 | 4.8 a | 69.8 a | HS | 13 |
| 13 | Edkawy | 4.0 abc | 51.3 bc | S | 11 |
| 14 | Qaha | 2.3 ghi | 34.3 g | MR | 4 |
| Mean | n | 3.2 | 44.0 | | |
| CV 9 | % | 37.6 | 36.3 | | |

Correlation between gal index and egg masses per root: r = 0.89, P < 0.01 Gal index and egg masses values in the same column followed by different letters are significantly different according to Duncan's test (P < 0.05). Levels of resistance/susceptibility of different genotypes were classified based on gal index as: highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S), and highly susceptible (HS).

Molecular marker analysis

In the present study, fourteen tomato genotypes were screened with the Mi23 SCAR and REX-1 CAPS markers to detect the Mi-1.2 gene in the tested genotypes. PCR amplifications with the Mi23 (Fig. 1) revealed a single amplified product (430 bp) in thirteen genotypes, indicating a homozygous recessive state (mi/mi). Meanwhile, the genotype Strain B F1 showed two DNA fragments (430 and 380 bp), indicating a heterozygous state (Mi/mi). Meantime, PCR amplifications with REX-1 (Fig. 2) revealed a single amplified product (750 bp) in all genotypes. Digestion of PCR products of REX-1 with the Taq I (Fig. 3) resulted in three DNA fragments (750, 570 and 160 bp) only in Strain B F₁, confirming a heterozygous state (*Mi/mi*). However, each of the remaining thirteen genotypes exhibited only a single fragment of 750 bp (digestion doesn't occur), indicating a homozygous state (mi/mi). These results are in agreement with the results of Seah et al. (2007) for Mi23 and Williamson et al. (1994) for REX-1. Obviously, in the case of homozygous tomato genotypes (mi/mi), the PCR product of REX-1 doesn't contain a cleavage site of Taq I enzyme and thereby Taq I failed to cleave the target DNA fragment, which resulted in a single band (750 bp).

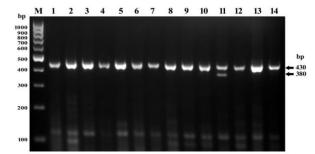


Fig. 1. PCR amplicons detected in fourteen tomato genotypes using the *Mi23* SCAR marker for detection of *M-1.2* resistance gene. M: 100bp DNA ladder. 1: E37 (*mi/mi*), 2: E40 (*mi/mi*), 3: E115 (*mi/mi*), 4: Super Marmande (*mi/mi*), 5: M82 (*mi/mi*), 6: Castlerock (*mi/mi*), 7: Floradade (*mi/mi*), 8: Peto86 (*mi/mi*), 9: VF145-B (*mi/mi*), 10: Super strain B (*mi/mi*), 11: Strain B F₁ (*Mi/mi*), 12: Giza-80 (*mi/mi*), 13: Edkawy (*mi/mi*) and 14: Qaha (*mi/mi*). Arrows indicate sizes of expected DNA fragments (430 and 380 bp) according to Seah *et al.* (2007).

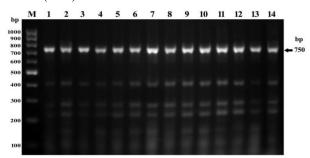


Fig. 2. PCR amplicons detected in fourteen tomato genotypes using the *REX-1* CAPS marker for detection of *M-1.2* resistance gene. M: 100bp DNA ladder. 1: E37, 2: E40, 3: E115, 4: Super Marmande, 5: M82, 6: Castlerock, 7: Floradade, 8: Peto86, 9: VF145-B, 10: Super strain B, 11: Strain B F₁, 12: Giza-80, 13: Edkawy and 14: Qaha. Arrow indicates the size of expected DNA fragment (750 bp) according to Williamson *et al.* (1994).

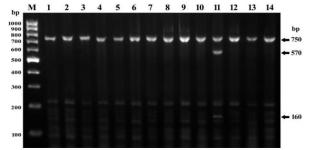


Fig. 3. Digestion of PCR product of *REX-1* with *Taq* I restriction enzyme. M: 100bp DNA ladder, 1: E37 (*mi/mi*), 2: E40 (*mi/mi*), 3: E115 (*mi/mi*), 4: Super Marmande (*mi/mi*), 5: M82 (*mi/mi*), 6: Castlerock (*mi/mi*), 7: Floradade (*mi/mi*), 8: Peto86 (*mi/mi*), 9: VF145-B (*mi/mi*), 10: Super strain B (*mi/mi*), 11: Strain B F₁ (*Mi/mi*), 12: Giza-80 (*mi/mi*), 13: Edkawy (*mi/mi*) and 14: Qaha (*mi/mi*). Arrows indicate sizes of expected DNA fragments after digestion (750, 570 and 160 bp) according to Williamson *et al.* (1994).

It has been reported that the Mi23 and REX-1 are codominant markers, which have been developed to detect the Mi-1.2 gene and differentiate RKN resistant and susceptible tomato genotypes. However, it has been reported that REX-1 gave false positives results for the presence of Mi-1 gene in some begomovirus-resistant tomato genotypes (El Mehrach et al., 2005). Consequently, REX-1 has proven relatively reliable. Unlike, the Mi23 are more reliable where it is tightly linked to the Mi-1.2 gene, the digestion with a restriction enzyme is not required and allows the potential to differentiate homozygous from heterozygous genotypes with decreased false positives results (Seah et al., 2007; Devran et al., 2013). However, Mahfouze and Mahfouze (2019) found that REX marker was more accurate than Mi23 marker, as some accessions gave results with REX and no results was recorded with Mi23. Numerous tomato genotypes were subsequently evaluated for nematodes resistance by the detection of Mi-1.2 gene and determine its allelic conditions using Mi23 (Cortada et al., 2009; Danso et al., 2011; Bhavana et al., 2019; Mahfouze and Mahfouze, 2019; Santos et al., 2020) and REX-1 (Cortada et al., 2009; Bhavana et al., 2019; Mahfouze and Mahfouze, 2019). In the present study, the Mi23 and REX-1 markers were successfully able to differentiate between the heterozygous (Mi/mi) tomato genotype (Strain B F_1) and the remaining homozygous recessive (mi/mi) genotypes.

In the present study, Strain B F1 exhibited the lowest gall index and egg masses/root and was ranked in the top of resistance tomato genotypes (highly resistant to RKN). This finding can be explained as the impact of the Mi-1.2 which was present in a heterozygous state (Mi/mi). It has been reported that the Mi-1.2 gene was equally effective in the homozygous (Mi/Mi) and heterozygous (Mi/mi) tomato genotypes (Cap et al., 1993). However, it has been also reported that Mi-1.2 gene was more effective in the homozygous (Mi/Mi) than heterozygous (Mi/mi) state (Jacquet et al., 2005). Recently, the effect of the Mi-1.2 gens on tomato genotypes was determined using molecular and screening assays (Bozbuga et al., 2020). In addition, responses of tomato genoptypes carrying the Mi-1.2 gene against different Meloidogyne species have been widely reported (Molinari and Caradonna, 2003; López-Pérez et al., 2006; Cortada et al., 2008; Cortada et al., 2009; Verdejo-Lucas et al., 2013; Carvalho et al., 2015). It has been reported that the Mi-1.2 gene in tomato confers resistance not only to the major Meloidogyne species, namely M. javanica, M. arenaria and M. incognita (Roberts and Thomason, 1986; Martinez de Ilarduya et al., 2001; Nombela et al., 2003), but also to minor species such as M. hispanica, M. luci and M. ethiopica (Maleita et al., 2011; Santos et al., 2020). Recently, the Mi-1.2 gene was reported to be effective against a wide range of Meloidogyne spp (Gabriel et al., 2020). However, they concluded that further studies using different tomato genotypes and other nematode populations are still required to confirm these results.

Interestingly, although thirteen tomato genotypes tested in the present study exhibited a homozygous recessive state (*mi/mi*) for the *Mi-1.2* gene (susceptible banding patterns), two out of them responded as resistant to RKNs and three genotypes were moderately resistant. These results

suggested that these genotypes could possess other genes different from the *Mi-1.2* which may be control RKN resistance. In accordance, several RKNs resistance genes have been reported within *Solanaceae* (Wubie and Temesgen, 2019). In addition to *Mi-1*, other RKNs resistance genes have also been identified (El-Sappah *et al.*, 2019).

In conclusion, the present study reported the resistance potential of some tomato genotypes from different geographical regions against RKNs, with different levels of resistance. The *Mi23* and *REX-1* markers were successfully able to distinguish homozygous (*mi/mi*) from heterozygous (*Mi/mi*) tomato genotypes at the *Mi-1.2* locus. The genotype Strain B F_1 which was identified as heterozygous (*Mi/mi*) for *Mi-1.2* gene can be used for developing tomato cultivars resistant to RKN in tomato genotypes which identified as homozygous recessive (*mi/mi*) and responded as resistant or moderately resistant may possess other RKN resistance genes. However, further studies are still needed to identify these genes.

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REFERENCES

- Abad, P., Favery, B., Rosso, M-N. and Castagnone-Serena, P. 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Mol. Plant Pathol. 4:217-224.
- Bailey, D.M. 1941. The seedling test method for root-knot nematode resistance. Proc. Am. Soc. Hortic. Sci. 38:573-575.
- Banora, M.Y., and Almaghrabi, O.A.A. 2019. Differential response of some nematode-resistant and susceptible tomato genotypes to *Meloidogyne javanica* infection. J. Plant Prot. Res. 59:113-123.
- Bhavana, P., Singh, A.K., Kumar, R., Prajapati G.K., Thamilarasi K., Manickam R., Maurya S. and Choudhary J.S. 2019. Identification of resistance in tomato against root knot nematode (*Meloidogyne incognita*) and comparison of molecular markers for *Mi* gene. Australasian Plant Pathol. 48:93-100.
- Boiteux, L.S. and Charchar, J.M. 1996. Genetic resistance to root-knot nematode (*Meloidogyne javanica*) in eggplant (*Solanum melongena*). Plant Breed. 115:198-200.
- Bozbuga, R., Dasgan, H.Y., Akhoundnejad, Y. Imrenc M., Günayd O.C., and Toktaye H. 2020. Effect of *Mi* gene and nematode resistance on tomato genotypes using molecular and screening assay. Cytol. Genet. 54: 154– 164.
- Cap, G.B., Roberts P.A., Thomason I.J., 1993. Inheritance of heat-stable resistance to *Meloidogyne incognita* in *Lycopersicon peruvianum* and its relationship to the *Mi* gene. Theo. App. Genet. 85:777–783.

- Carvalho, L.M.D., Benda, N.D., Vaughan, M.M., Cabrera, A.R., Hung, K., Cox, T., Abdo, Z., Allen, L.H. and Teal, P.E.A. 2015. *Mi-1*-mediated nematode resistance in tomatoes is broken by short-term heat stress but recovers over time. J. Nematol. 47:133-140
- Cook, R.J. 2000. Advances in plant health management in the twentieth century. Annu. Rev. Phytopathol.38:95-116.
- Corbett, B., Jia, L., Sayler, R., Arevalo-Soliz, L. and Goggin, L. 2011. The Effects of Root-knot Nematode Infection and *Mi*-mediated Nematode Resistance in Tomato on Plant Fitness. J. Nematol. 43:82-89.
- Cortada, L., Sorribas, F.J., Ornat, C., Andres, M.F. and Verdejo-Lucas, S. 2009. Response of tomato rootstocks carrying the *Mi*-resistance gene to populations of *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. Eur. J. Plant Pathol. 124:337-343.
- Cortada, L., Sorribas, F.J., Ornat, C., Kaloshian, I. and Verdejo-Lucas, S. 2008. Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. Plant Pathol. 57:1125-1135.
- Danso, Y., Akromah, R. and Osei, K. 2011. Molecular marker screening of tomato, (*Solanum lycopersicum* L.) germplasm for root-knot nematodes (*Meloidogyne species*) resistance. Afr. J. Biotechnol. 10:1511-15.
- Devran, Z., Başköylü, B., Taner, A. and Doğan, F. 2013. Comparison of PCR-based molecular markers for identification of *Mi* gene. Acta Agr. Scand. B-S P 63:395-402.
- El Mehrach, K., Gharsallah Chouchane, S., Mejia, L., Williamson, V.M., Vidavsky, F., Hatimi, A., Salus, M.S., Martin, C.T. and Maxwell, D.P. 2005. PCR based methods for tagging the *Mi-1* locus for resistance to root-knot nematode in begomovirus resistant tomato germplasm. Acta Hortic.695:263-270.
- El Mehrach, K., Sedegui, M., Hatimi, A., Tahrouch, S., Arifi, A., Czosnek, H. and Maxwell, D.P. 2007. Detection of *Mi-1.2* gene for resistance to root-knot nematode in tomato-breeding lines developed for resistance to begomovirus in Morocco. Acta Bot. Gallica 154: 495-501.
- El-Sappah, A.H., Islam M.M., El-Awady, H.H., Yan, S., Qi, S., Liu, J., Cheng, G.T. and Liang, Y. 2019. Tomato natural resistance genes in controlling the root-knot nematode. Genes, 10:925. https://doi.org/10.3390/ genes1 0110925.
- Gabriel, M., Kulczynski, S.M., Muniz, M.F., Boiteux, L.S. and Carneiro, R.M. 2020. Reaction of a heterozygous tomato hybrid bearing the *Mi-1.2* gene to 15 *Meloidogyne species*. Plant Pathol. 69: 944-952.
- Hussey, R.S. and Barker, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne spp.*, including a new technique. Plant Dis. Rep. 57:1025-1028.
- Jacquet, M., Bongiovanni, M., Martinez, M., Verschave, P., Wajnberg, E. and Castagnone-Sereno, P. 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. Plant Pathol. 54:93-99.

- López-Pérez, J.A., Strange, M.L., Kaloshian, I. and Ploeg, A.T. 2006. Differential response of Mi gene-resistant rootstocks to root-knot tomato nematodes (Meloidogyne incognita). Crop Prot. 25:382-388.
- Mahfouze, S.A. and Mahfouze, H.A. 2019. A Comparison between CAPS and SCAR Markers in the detection of resistance genes in some tomato genotypes against tomato yellow leaf curl virus and whitefly. Jordan J. Biol. Sci. 12:123-133.
- Maleita, C.M., Santos, M.C.V., Curtis, R.H.C., Powers, S.J. and Abrantes, I.M.O. 2011. Effect of the Mi gene on reproduction of Meloidogyne hispanica on tomato genotypes. Nematology 13:939-949.
- Martinez de Ilarduya, O., Moore, A.E. and Kaloshian, I. 2001. The tomato Rme1 locus is required for Mi-1-mediated resistance to root-knot nematodes and the potato aphid. Plant J. 27:417-425.
- Milligan, S.B., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P. and Williamson, V.M., 1998. The root-knot resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307-1321.
- Molinari, S. 2011. Natural genetic and induced plant resistance, as a control strategy to plant-parasitic nematodes alternative to pesticides. Plant Cell Rep. 30:311-323.
- Molinari, S. and Caradonna, S. 2003. Reproduction of selected natural and resistancebreaking Meloidogyne population on near-isogenic tomato lines. Nematol. Medit. 31:181-185.
- Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321-4325.
- Nombela, G., Williamson, V.M. and Muniz, M. 2003. The root-knot nematode resistance gene Mi-1.2 of tomato is responsible for resistance against the whitefly Bemisia tabaci. Mol. Plant Microbe Int. 16:645-649.
- Roberts, P.A. and Thomason, I.J. 1986. Variability in reproduction of isolates of Meloidogyne incognita and M. javanica on resistant tomato genotypes. Plant Dis. 70:547-551
- Rumbos, C.I., Khah, E.M. and Sabir, N. 2011. Response of local and commercial tomato cultivars and rootstocks to Meloidogyne javanica infestation. Aust. J. Crop Sci. 5:1388-1395.

- Santos, D.Z., da Silva, P.M., Abrantes, I. and Maleita, C. 2020. Tomato *Mi-1.2* gene confers resistance to Meloidogyne luci and M. ethiopica. Eur. J. Plant Pathol. 156:571-580.
- Seah, S., Williamson, V.M., Garcia, B.E., Mejia, L., Salus, M.S., Martin, C.T. and Maxwell, D.P. 2007. Evaluation of a co-dominant SCAR marker for detection of the Mi-1 locus for resistance to root-knot nematode in tomato germplasm. Tomato Genet. Coop. Rep. 57:37-40.
- Silva, A.J., Oliveira, G.H.F., Pastoriza, R.J.G., Maranhão, E.H.A., Pedrosa, E.M.R., Maranhão, S.R.V.L., Boiteux, L.S., Pinheiro, J.B. and Carvalho Filho, J.L.S. 2019. Search for sources of resistance to Meloidogyne enterolobii in commercial and wild tomatoes. Hortic. Bras. 37:188-198.
- Smith, P.G. 1944. Embryo culture of a tomato species hybrid. Proc. Am. Soc. Hortic. Sci. 44:413-416.
- Taylor, A.L. and Sasser, J.N., 1978. Biology, identification and control of root knot nematodes (Meloidogyne Species). A cooperative publication of North Carolina State University, Dept. of Plant Pathology, and USAID, Raleigh, NC, USA.
- Trudgill, D.L. and Blok, V.C. 2001. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Annu. Rev. Phytopathol. 39:53-77.
- Verdejo-Lucas, S., Blanco, M., Cortada, L. and Sorribas, F.J. 2013. Resistance of tomato rootstocks to Meloidogyne arenaria and Meloidogyne javanica under intermittent elevated soil temperatures above 28°C. Crop Prot. 46:57-62.
- Verdejo-Lucas, S., Cortada, L., Sorribas, F.J. and Ornat, C. 2009. Selection of virulent populations of Meloidogyne javanica by repeated cultivation of Mi resistance gene tomato rootstocks under field conditions. Plant Pathol. 58:990-998.
- Williamson, V.M., Ho, J.Y., Wu, F.F., Miller, N. and Kaloshian, I. 1994. A PCR-based marker tightly linked to the nematode resistance gene, Mi, in tomato. Theor. Appl. Genet. 87:757-763.
- Wubie, M. and Temesgen, Z. 2019. Resistance mechanisms of tomato (Solanum lycopersicum) to root-knot nematodes (Meloidogyne species). J. Plant Breed. Crop Sci. 11:33-40.

تقييم بعض الطرز الوراثية للطماطم لمقاومة النيماتودا والكشف عن جين المقاومة Mi-1.2 محمد إبراهيم محمد حسن 1 و عامر فايز أحمد محمود 2 ¹ قسم الُوراثة – كلية الزراعة – جامعة أسيوط – جمهورية مصر العربية 2قسم أمراض النبات – كلية الزراعة – جامعة أسيوط – جمهورية مصر العربية

في الدراسة الحالية، تم تقييم استجابة أربعة عشر طرازاً وراثياً من الطماطم من مناطق جغرافية مختلفة لنيماتودا تعقد الجذور لمدة موسمين منتاليين تحت ظروف الصوبة الزجاجية. تم استخدام اثنين من الواسمات الجزيئية المعتمدة على تفاعل البلمرة المتسلسل وهما 13-M و I EEX للكشف عن جين مقاومة نيماتودا تعقد الجذور Mi-1.2 في الطرز الوراثية المختبرة. أظهرت النتائج تبايناً كبيراً بين الطرز الوراثية المختبرة في استجاباتها لنيماتودا تعقد الجنور. لوحظت فروق معنوية جداً بين الطرز الوراثية المختبرة بالنسبة لدليل التعدُّوكتل البيض لكل نظام جذري. أعتماداً على التقييم الحيوي للنيماتودا، كان الطر از الور اتي Strain B F شديد المقاومة للنيماتودا كما احتل المرتبة الأولى من بين الطرز الورائية المقاومة. كانت الطرز الوراثية Floradade و Florads مقاومة، والطرز الوراثية Castlerock و Peto86 متوسطة المقاومة، والطرز الوراثية Super strain B و E115 و Super Marmande و M82 و M82 حساسة، كما كانت الطرز الورائية E37 و E40 و Giza-80 شديدة الحساسية لمنيماتودا تعقد الجذور. أظهر التحليل الجزيئي باستخدام الواسمات M23 و 10.02 و الطراز الوراثي Strain B F خليط (*Mi/mi*) لجين المقاومة *Mi-1.2 في حين* أظهرت الطرز الوراثية المتبقية الحالة المنتدية الأصيلة (*mi/mi*). تشير تلك النتائج إلى أنه يمكن استخدام الطراز الوراثي Strain B F تلوير أصناف طماطم جديدة مقاومة لنيماتودا تعقد الجنور في برامج تربية الطماطم. كما تشير النتائج أيضاً إلى أن الطرز الوراثية للطماطم التي تم تحديدها في الدراسة الحالية على أنها منتجية أصيلة (*mi/mi*) لجين المقاومة (*mi/mi*) في حين أظهرت الطرز الوراثية المنتعية الطماطم. كما المقاومة قد تمتلك جُينات أخرى تتحكم في مقاومة نيماتودا تعقد الجذور . ومع ذلك ، لا تزال هناك حاجة إلى مزيد من الدر اسات لتحديد تلك الجينات التي تتحكم في مقاومة النيماتودا في الطماطم.