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The possibility of transferring resistance genes *Mi1.2* and *Mi-3* by crossing between wild and susceptible tomato varieties

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Abstract

Eleven wild tomato (*Solanum* spp.) , obtained from Tomato Genetics Resource Center, Davis, Calif, and two cultivars were screened for resistance to Root-knot nematodes, *Meloidogyne* spp , by molecular marker analysis and nematode bioassay. DNA based test and nematode bioassay were used to determine the presence of the root-knot nematode resistance gene Mi in these wild species. Molecular markers Mi23 and TG180M were used to detect Mi-1.2 and Mi-3 respectively. The results of molecular markers were showed the presence of Mi1.2 genes in homozygous alleles (Mi/Mi), which indicated it's resistance to RKNs, in tomato wild species *Solanum pervianum* (accession numbers LA0153, LA0446), *S. arcanum* (accession numbers LA0441, LA1346) and *S. huaylasense* (accession number LA1360) when all these species gave single band (380bp). While TG180M marker was given single band 1124bp in all wild species and varieties which indicated homozygous resistance alleles (Mi-3/Mi-3). The results of molecular markers were agreed with gall index (GI) which not forming any galls in root systems in *Solanum pervianum* (accession numbers LA0153, LA0446), *S. arcanum* (accession numbers LA0441, LA1346) while GI was recorded 1.33 in *S. huaylasense* (accession number LA1360). The results of traditional hybridization between tomato wild species and susceptible tomato varieties were showed the success of hybridization with some species and their failed with other species. Molecular markers were showed inefficiency DNA extracted from hybrid seeds to detection Mi-1 genes in hybrids.

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Introduction

Root-knot nematodes (*Meloidogyne* spp.) endoparasites of many crops worldwide and most damaging nematodes pest in agriculture (Sasser, 1980), Tomato (*Solanum lycopersicum*) is highly susceptible host of *Meloidogyne* species and caused decreased in yield losses in warm temperature region to tropical and subtropical region as well as in greenhouse condition and other controlled environment production systems. The yield of susceptible tomato cultivars can be decreased by 50% or more in infected fields (Johnson, 1998). Resistance cultivars were carried *Mi.1* gene has proved to be highly effective such as a nematode management strategy (Roberts and May, 1986). This gene was first observed in wild species (*Solanum peruvianum* L. accession PI 128657) in the 1940 by Bailey (1941). Smith (1944) was used embryo rescue to introduce this trait into commercial cultivars. Gene *Mi-1* confers resistance to three root-knot nematode species, *M. incognita M. javanica* and *M. arenaria* (Roberts and Thomeson, 1986). It was found that, the resistance was caused by a dominant allele *Mi*, located in the short arm of the chromosome 6 (Vos *et al.,* 1998; Seah. *et al.,* 2004). Three homologs of this gene *Mi1.1, Mi1.2* and *Mi1.3* were identified at *Mi* locus however only *Mi1.2* gene is conferred resistance to RKN while *Mi1.1* and *Mi1.3* were indicted as pseudogenes (Kaloshian *et al.,* 1998; Milligan *et al.,* 1998).

The *Mi* gene was effected against RKN at soil temperature below 28°C but it was inactivated when soil temperature above 28°C (Holtzmann, 1965: Dropkin, 1969).

The role of Mi 1.2 gene is associated to as Mi hereafter. It shares many structural motifs with other R genes, including nucleotide- binding site (NBS) and Leucine-rich repeats (LRRs) domains, which are characteristic of a family of plant proteins that are required for resistance against several pathogens including nematodes (Chisholm *et al*., 2006). The mechanism of action of resistance genes stimulation localized hypersensitive reaction or tissue necrosis near the penetration zone therefore the nematode failed in such cases to establish feeding sites and then either die or leave the roots (Terrell *et al.,* 1983).

In addition to *Mi-1* gene there are other genes responsible for the resistance or have a complementary role in the expression of other genes such as *Mi-3* gene that mapped to the short arm of chromosome 12 (Yaghoobi *et al*., 1995; Huang *et al.* 2004). Mi-3 gene give resistance at temperatures above 30°C (Yaghoobi *et al*., 1995; Veremis and Roberts, 1996). Since *Mi-3* confers resistance to Mi-1-virulent nematode isolates and may also confer heat-stable resistance, combination of this trait into cultivated tomato is eligible and is expected to complement the qualities of *Mi-1*. Attempts to introduce these traits via traditional breeding schemes are ongoing.

Several methods have been used to detect the presence of these genes in commercial and wild tomato including the use of Molecular Markers by using specific primers which associated with specific sites of genomic DNA (Ho *et al.,* 1992; Williamson *et al.,* 1999; Chen *et al.,* 2015). These markers are linked to the sites of the resistant genes, in resistant plants which can be detected in the early stages of growth even in the absence of the nematodes (Devarn *et al*., 2016).

The wild species of *Lycopersicon* have so far derived their importance mainly from the fact that they are a rich source of disease resistances. Of the greatest importance in this connection are the many botanical varieties of the *L. peruvianum* complex (Doolrrrle, 1954; Alexander and Hoover 1955; Hoover *et al*., 1955; Alexander, 1959; Holmes, 1960; Skrdla *et al*., 1968). In these, by far the most resistances were found and in many case also the highest level of resistance. Besides being interesting sources of disease resistances the wild species also appear to have unexpectedly great importance as sources of variation, e.g. in the form of 'novel variation' (Rick and Smith, 1953; Rick, 1967). This may concern morphological, physiological, floral biological and other characters (The genetics of species hybrids thus furnishes a good example of serendipity in research, Rick, 1967). Also as a source of variation in forms and adaptability, particularly the *L. peruvianum* complex is of importance (Rick, 1963).

Comparison with the previous section shows that exactly the most promising material for tomato breeding is the most strongly isolated from *L. esculentum*. This has led to a meagre exploitation of this material. Only in a few cases and with great difficulty was a successful hybridization realized, e.g. TMV-resistance (Alexander, 1963), nematode resistance (Rick, 1967), Cladosporium resistance (Kerr and Baily 1964) and corky root resistance (Szteyn, 1962) in which mostly only one resistance gene was transferred to tomato.

Therefore, the aim of this study is transferred genes resistance (*Mi*-genes) from wild tomato species to commercial cultivars through the process of traditional hybridization and investigated the possibility of transfer by using molecular technologies in the resulting hybrids.

Materials and methods

Plant material

In this study eleven wild tomatoes were used: *S. pimpinellifolium* LA0373, *Solanum peruvianum* (two accession numbers LA0153 and LA0446), *S. habrochaites* (two accession numbers LA0407 and LA1266), *S. huaylasense* (four accession numbers LA1360, LA1364, LA1365 and LA2808) and *S. arcanum* (two accession numbers LA0441 and LA1346). All accession numbers of wild tomatoes obtained from Tomato Genetics Resource Center, Davis, Calif. As well as two varieties were used Super Marmande and Rutgers which were expressed susceptible to root-knot nematode.

Nematode culture

Root knot-nematodes (*Meloidogyne* spp.) were obtained from tomato roots which infected with *Meloidogyne* spp grown in greenhouse of agriculture college/Baghdad University where nematode susceptible tomatoes are grown for several months, were used as source of the nematode inoculum. Tomato roots were cut to small pieces (2-3cm) and blended in 1% NaOCl solution to maintain eggs (Hussey and Barker, 1973). The contents of the solution passed directly through a 75µm, 50m and 25µm sieves respectably. Eggs were collected from 25µm sieves and used in experiment.

Testing sensitive of wild species to RKNs infection

Three-four true leaves old plants seedlings grown in river sand in 12 Styrofoam cups with drainage holes and every two weeks plants were watered with nutrients solutions. Three seedlings per genotype were infected with 5000 eggs, J_2/Kg. eight weeks after that roots were removed from the sands and wished to remove soil particles.

Then the degree of root knot nematode infection was evaluated by the gall index (GI) using scale Tyalor and Sasser (1978) as follows: 0= no galls , 1= 1-2 galls , 2= 3-10 galls, 3= 11-30 galls , 4= 31-100 galls, 5= more than 100 galls in per root system. Recorded plant response to infected within root-knot nematodes according this scale which was put by us: High resistance (H.R) = gall index 0-1, resistance (R) = gall index 2, moderately resistance (M.R)= moderately resistance (M.S)= gall index 2.1-2.6, Susceptible (S)= gall index 2.7-3.5, highly susceptible= gall index 3.6-5.

DNA extraction

Total DNA was extracted from young leaves according to Doyle and Doyle (1978). 200mg of young leaves were homogenate with 800µl of CTAB buffer (2% CTAB (hexadecyltrimethylammonium bromide), 1.4 M NaCl, 20mm EDTA, 100mm TrisHCl [pH=8], 0.2% β-mercaptoethanol). The extract was incubated in water bath at 60°C for 30 min then followed by centrifugation at 13000 rpm for 5 min at room temperature. The aqueous phase was transferred to another tube and 400µl chloroform: Isoamyl alcohol (24:1) was added and mixed gently by inverting for 20 sec, followed by DNA precipitated by added 500µl Ethanol absolute and 50µl sodium acetate (0.3M) and followed by 12000 rpm/min for 5 min. Genomic DNA was washed with 70% ethanol.

The tube that contained DNA left to dried at room temperature for 15 min. Finally DNA dissolved TE (Tris- EDTA) buffer and stored in 4c after used.

Molecular markers that used to detect resistance genes

Mi1.2 gene was detected by co-dominant SCAR marker, Mi23 primer, Mi23 F 5- TGG AAA AAT GTT GAA TTT CTTTTG-3', and Mi23R is 5'- GCA TAC TAT ATG GCT TGT TTA CCC-3' (Seah *et. al.*, 2007) and used TG180

primer for detection Mi-3 gene that designed by Yaghoobi *et. al.* (2005) with re- designed prime, Bioinformatics analyses were performed to identify the location of markers linked to *Mi-3* locus. The primer 3-plus software (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) was used for in silico analyses considering Select primer pairs to detect the given template sequence.

Optionally targets and included/excluded regions can be specified, because primer self-compatibility and containing hair-pine, there for replace one base from forward primer ATACTTCTTTRCAGGAACAGCTCAC using modified base R which is represent A or G base and remove the first base from reverse primer ACATTAGTGATCATAAAGTACCAG to avoid being hair-pine that cause low yield in PCR production as showed in Table 1).

Table 1. Marker sets designed to detection *Mi*- genes for resistance to root-knot nematode (*Meloidogyne* spp) in *Solanum* spp.

Marker	Sequence of marker 5'to 3'	Amplified region	Amplified sizes (bp)		Reference
			Ristance	Susceptible	
Mi23	F-TGG AAA AAT GTT GAA TTT CTTTTG R-GCA TAC TAT ATG GCT TGT TTA CCC	*Mi1.2* gene	380	430	Seah *et.al.*, 2007
TG180	F- ATACTTCTTTGCAGGAACAGCTCAC R-C ACATTAGTGATCATAAAGTACCAG	*Mi-3* gene	1200	900	Yaghoobi *et.al.*, 2005
TG180M	F- ATACTTCTTTRCAGGAACAGCTCAC R- ACATTAGTGATCATAAAGTACCAG	*Mi-3* gene	1124	-	In present study

*R= G/A ; removed base C from reverse primer , TG180M is modify marker replaced G within A and removed first base (C) from reverse primer to avoiding primer self-compatibility and containing hair-pine.

The PCR amplifications were done in 25µl reaction volume for both primers containing 5µl *Taq* PCR PreMix (INTRON company/Korea), 1µl (10 pmol) of each primers, 1µl (100ng) DNA template and 17µl Deionized water. Amplification condition for Mi23 markers were 94˚C for 5min followed by 35 cycles of 30 sec at 94˚C , 1 min at 57 ºC and 1 min at 72˚C , followed by 10 min at 72˚C while amplification conditions for TG180 modified were: 5 min initial denaturing at 94˚C and 10 min final extension at 72˚C with the intervening 35 cycles of 30 sec at 94˚C, 1 min primer annealing at 58˚C and 1 min primer extension at 72˚C.

Hybridization wild tomato species within commercial cultivars

Experiment two was carried from 20 February to 20 May 2017 in greenhouse of agriculture college/Baghdad University. The same wild species were crossing with tomato cultivars (Super Marmande, Rutgers). F1 progenies were produce by crossing all genotypes of wild species with tomato cultivars (Super Marmande, Rutgers). Crosses with emasculated flowers were carried on greenhouse conditions between 8 a.m to 11 a.m.

Pollinated ovaries were covered with paper bags to inhabited any cross-pollination from other strange tomato pollen. Seeds collection from mature fruit for obtaining F1 seeds. some crosses gave fruit, but not all fruit produced mature seeds.

DNA extraction from seed hybrids and PCR amplification

DNA was extracted from 20mg of seed hybrids according method Doyle and Doyle (1978) similar to those described previously. Similar PCR conditions were applied as that mention in first experiment to detect the possibility of transferring resistance genes (*Mi* 1.2 and *Mi-3*) from wild species to commercial cultivars.

Hybrids response to Meloidogyne spp. infection.

Hybrid seedlings, five wild species and two commercial cultivars (Super Marmande and Rutgers) were grown at the third true leaves stage in 12 Styrofoam cups contain soil sand with drainage holes. All seedlings after one week were inculcated with 5000 eggs, J_2/Kg as mention in the first experiment. Roots were harvested after two months from nematode inoculation.

Each hybrid was replicated three times according to a complete randomized design. Experimental conditions and assessments of nematode infectivity and root gall index were similar to those described for the first experiment.

Data analysis

Statistical analysis of the research data was done by using the program (SAS) (2012) used complete randomized design (CRD). The differences between root gall index for two experiments into this study by using L.S.D (P≥0.05).

Results and discussion

Testing sensitive of wild species to RKNs infection

The results showed significant differences in the means of gall index (GI) between wild and commercial species. the lowest means of GI were 0.0 in tomato wilds *S. peruvanium* (LA0153, LA0446) and *S. arcanum* (LA0441, LA1346) compared with Super Marmande and Rutgers were 3.66 and 4.66 means of GI (table. 2) . This is due to forming of small and immature females in these species, as they stimulate the defense mechanisms in the roots when penetrating second juveniles (J_2) to root, which in turn excreted some of the enzymes stimulated to form giant cells (GCs) of the esophagus glands that help to break cell walls and so plant stimulates defense mechanisms in the roots as stimulating the production of the enzyme Peroxidase, chitinase and proteinase. As well as forming of physical barriers in the walls of root cells including: increase the production of lignin and callose as a physical barrier to prevent the penetration of the juvenile style roots (Egclund *et al.*, 2004). In addition hypersenesitive reactions (HR) are stimulated at the injury sites, which it prevents the formation of feeding sites (GCs) and thus does not form a galls contract resulting in no reproduction or little happening (Heath, 2000; Molinari, 2008).

It is believed that hypersensetive reaction (HR) occurs as a result of the rapid formation of the reaction of oxidative explosion after the penetration of juveniles at the sites of infection (Melillo *et al.,* 2006). Bleve-Zacheo and Melillo (1997) indicted to Physiologyical and molecular changes associated with the maintenance of giant cells during compatible interaction.

Table. 2. Screening of some tomato species for nematode resistance.

Plant	Mean of Gall index	Response
Super Marmande	3.66	H.S
Solanum pimpinellifolium (LA0373)	1.00	H.R
Solanum peruvianum (LA0153)	0.0	H.R
Solanum peruvianum (LA0446)	0.0	H.R
Solanum habrochaites (LA0407)	2.66	M.R-> S
Solanum habrochaites (LA1266)	2.33	M.R-> S
Solanum huaylasense (LA1360)	1.33	H.R
Solanum huaylasense (LA1364)	2.00	R
Solanum huaylasense (LA1365)	2.00	R
Solanum huaylasense (LA2808)	1.00	H.R
Solanum arcanum (LA0441)	0.0	H.R
Solanum arcanum (LA1346)	0.0	H.R
Rutgers	4.33	H.S
L.S.D.(P≥0.05)	0.600	

*recorded plant response to infected by nematode according this scale: High resistance (H.R)= gall index 0-1, resistance (R)= gall index 2, moderately resistance (M.R)= moderately resistance (M.S)= gall index 2.1-2.6, Susceptible (S)= gall index 2.7-3.5, highly susceptible= gall index 3.6-5.

In molecular screening, Super Marmande, Rutgers and eleven wild species were examined to detect the presence of *Mi1.2* and Mi-3 genes by used, specific markers, Mi23 and TG180 respectively.

Molecular results were coincided with the results of nematode screening, gall index (Gi), that confirmed the presence of *Mi1.2* gene resistance in *S. peruvanum* (accession LA0153, LA0446), S. *arcanum* (accession LA0441, LA1346) and

S. huaylasense (LA1360, LA1365) as that were recorded Which did not notice galls formed of in root systems (GI=0.0) compared with cultivars Super Marmande and Rutgers that recorded the highest root index were 3.66 and 4.66 respectively.

Similar results were reported by Peacock (1959] who also reported that the larvae of *M. incognita* were attracted to excised root tips of *Lycopersicum peruvanum* a little less strongly compared to *L. esculentum*. He also noticed either a slower rate of development or little or no development of the juveniles and swelling in *L. peruvanum* compared to susceptible tomato, *L. esculentum*. Gowen *et al.* (1969) reported that significantly fewer juveniles entered the roots of Nemared compared to other tested varieties.

He found no galls in the resistant Nemared 28 days after inoculation and the hybrids had significantly fewer galls than the susceptible tomato varieties tested. These cultivars were given single band (430 bp) when used Mi23 primer to detected *Mi1.2* gene indicating that they were homozygous susceptible for this locus, while *S. peruvanum* (accession LA0153, LA0446), *S. arcanum* (accession LA0441, LA1346) and *S. huaylasense* (LA1360, LA1365) were given only single band 380 bp indicting that homozygous resistance. While *S. huaylasense* LA1365 gave two bands 430bp and 380bp, indicating that it was heterozygous (*Mi1.2 /mi1.2)* in this species Fig. 1.

These results were consisted with the results of EI-Mehrach *et al.* (2005), Seah *et al.* (2007), Devran *et al.* (2013, 2016). In another study, the Mi23 initiator gave three types of DNA bundles when PCR reaction (380, 430, 500 bp) was carried out in some of the tomato lines, which showed different heterozygous (Mi1.2/mi1.2) alleles and two types of (380 and 430 bp), which is also referred to as heterozygous.

This heterogeneity in band sizes indicates a presence differences in the genome of the parents (Garcia *et al.*, 2007). Reddy *et al.* (2016) identified the efficiency of Mi23 marker in detecting the presence of *Mi1.2* resistance gene in the tomato cultivar compared to REX-1 and CT119 because it did not require restriction enzymes to determine the resistance or susceptibility of species to root-knot nematodes, Is located at the Mi.1.2 gene site, which is primarily responsible for resistance in the tomato against *Meloidogyne* spp (Williamson *et al.*, 1999).

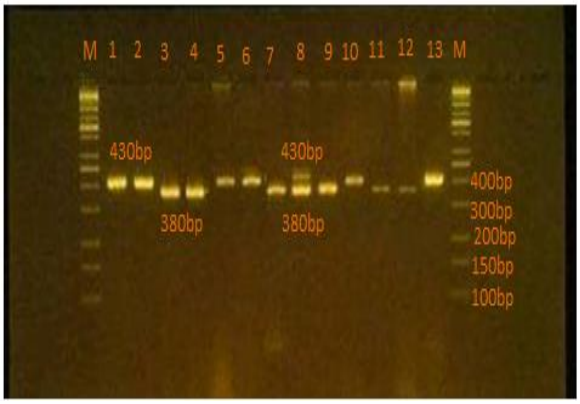

Fig. 1. Mi-1.2 gene amplification products obtained using Mi23 marker. The test plants used were 1: Super Marmande, 2. *Solanum pimpinellifolium* (LA0373), 3: *Solanum peruvianum* (LA0153), 4: *Solanum peruvianum* (LA0446), 5: *Solanum habrochaites* (LAO407), 6: *Solanum habrochaites* (LA1266), 7: *Solanum huaylasense* (LA1360), 8: *Solanum huaylasense* (LA1364), 9: *Solanum huaylasense* (LA1364), 10: *Solanum huaylasense* (LA2808), 11: *Solanum Arcanum* (LA0441), 12: *Solanum arcanum* (LA1346), 13: Rutgers. The line marked M contained a 10kb ladder DNA marker (KAPA Biosystem).

TG180 modify marker was used to detect the presence of *Mi-3* on chromosome 12, the results of this primer were given single band (1124bp) in all wild species and commercial cultivars Fig. 2.

While Yaghoobi *et al.* (2005) pointed out that TG180 (before modify) gives a single band 1200 bp in the plants that carry *Mi-3* homozygous resistance gene allele *Mi3/Mi3* while gave 900 bp in the susceptible species and indicated that they are identical to *mi-3/mi-3* indicates that a single locus, *Mi-3*, confers resistance to *Mi-1*-virulent nematodes at 27°C and to *Mi-1*-avirulent nematode strains at 32°C (heat-stable resistance).

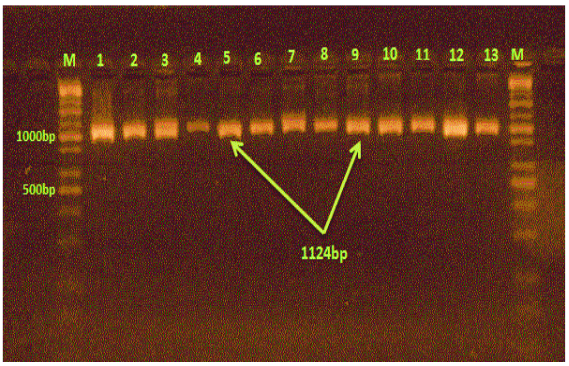

Fig. 2. Mi-3 gene amplification products obtained using modify marker TG180. The test plants used were 1: Super Marmande, 2: *Solanum pimpinellifolium* (LA0373), *3: Solanum peruvianum* (LA0153), *4: Solanum peruvianum* (LA0446), *5: Solanum habrochaites* (LAO407), *6: Solanum habrochaites* (LA1266), *7: Solanum huaylasense* (LA1360), *8: Solanum huaylasense* (LA1364), *9: Solanum huaylasense (LA1364), 10: Solanum huaylasense* (LA2808), *11: Solanum arcanum* (LA0441), *12: Solanum arcanum* (LA1346), *13: Rutgers*. The line marked M contained a 10 kb ladder DNA marker (KAPA Biosystem).

Hybridization wild tomato species within commercial cultivars

The results of hybridization showed the success of hybridization in a number of species and failed in the other when the adoption of wild species as parents and commercial varieties as mothers, as shown in Table 3. The main purpose of hybridization between commercial species and wild species is to transfer *Mi*-genes or some of these genes to commercial tomatoes because commercial varieties have a large production but are sensitive to infect by root knot nematode and hence the goal to transfer *Mi*-gene from wild species to commercial cultivars for obtaining high-yield and resistance to RKNs.

The results showed the success of traditional hybridization with *S. pimpinellifolium* (LA0373), *S. habrochaite* (LA0407, LA1266), *S. huaylasense* (LA1360) and *S. arcanum* (LA0441), but its failed in *S. peruvianum* (LA0153, LA0446), *S. huaylasense* (LA1364, LA2808). hybridization was successful when cross with Rutgers and failed when cross with Super Marmande this is may be due to compatibility of some of the genetic sites of the parents with each other, which led to the success of hybridization while explaining the lack of successful hybridization in the above species to several factors may be due to genetic factors and incompatibility of some genetic sites of parents together, resulting in the formation of fruits mature but empty of seeds. Previously studies showed that *Lycopersicon* presents widely different breeding barriers within a limited amount of material and mention these barriers sometimes only occurs in part of the material (Smith, 1944; Lamm, 1950; Bohn, 1951; De Zerpa, 1952; Rick, 1953, 1961, 1963; MCGuire and Rick, 1954; Rick and Lamm, 1955; Rick and Buter, 1956; Lewis and Crowe, 1958; Martin, 1961a,b, 1966; Chmtelewski, 1962, 1966, 1968a,b). Hogenboom (1972) was indicated to No barriers exist between *L. pimpinellifolium* and *L. esculentum*. This explains why successed of hybridization between *L. pimpinellifolium* and commercial cultivars and and he mentioned reasons that be caused failed hybridization between *S. peruvianum* and commercial cultivars once of that almost species of *S. peruvianum* very strict form of self-incompatibility occurs. Further, this group shows unilateral incompatibility with *L. esculentum* while in crosses in the compatible combination the embryos abort.

This embryo abortion is a very serious barrier, which may in some measure be avoided with the aid of embryo culture (Smith, 1944), but there are many instances on record where, in spite of this possibility, interspecific hybrids were hardly obtained. Veremis and Roberts (2000), Ammiraju *et al.* (2003) were indicated the main obstacle to obtain new resistant hybrids is the incompatibility between the germplasms of wild *Solanum* species and cultivated tomatoes, therefor transference of *Mi*-resistance gene to the susceptible tomato plants has been achieved using transgenic technique (Williamson, 1998; Goggin *et al.,* 2006; Willamson and Kumar, 2006) and through bridge line or embryo rescue (Willamson and Hussey, 1996). Therefor the most feasible alternative to overcome germplasm incompatibility is the use of tomato hybrid rootstocks (*S. lycopersicum* × *Solanum* spp.) (Santos *et. al.,* 2004).

Table 3. Results of hybridization between wild species and tomato cultivars.

Female Male	LA0153	LA0446	LA0373	LA0407	LA1266	LA1360	LA1364	LA1365	LA2808	LA0441	LA1346
Super Marmande	-	-	+	+	+	+	-	-	-	+	-
Rutgers	-	-	+	+	+	+	-	-	+	+	-

+ indicates that there is succeed hybridization between wild species and tomato cultivated.
-indicated that there is failed hybridization between wild species and tomato cultivated.

The results of molecular bioassay to detection possible transfer of resistance genes in hybrid seeds

The same markers which mentioned previously were used to detect the existence of *Mi1.2*-gene resistance in genomic DNA that extracted from hybrid seeds that obtained through conventional hybridization and commercial cultivars. The results of using Mi23 marker showed that all hybrids were given a single band 430 bp Fig. 3. Which indicate that they are homozygous susceptible alleles (*mi1.2/mi1.2*) and that *Mi1.2* resistance gene may be cannot be transferred this gene to hybrids from some wild species. This results consistent with the results of previous studies (Rick, 1963; Ho *et.al.*, 1992).

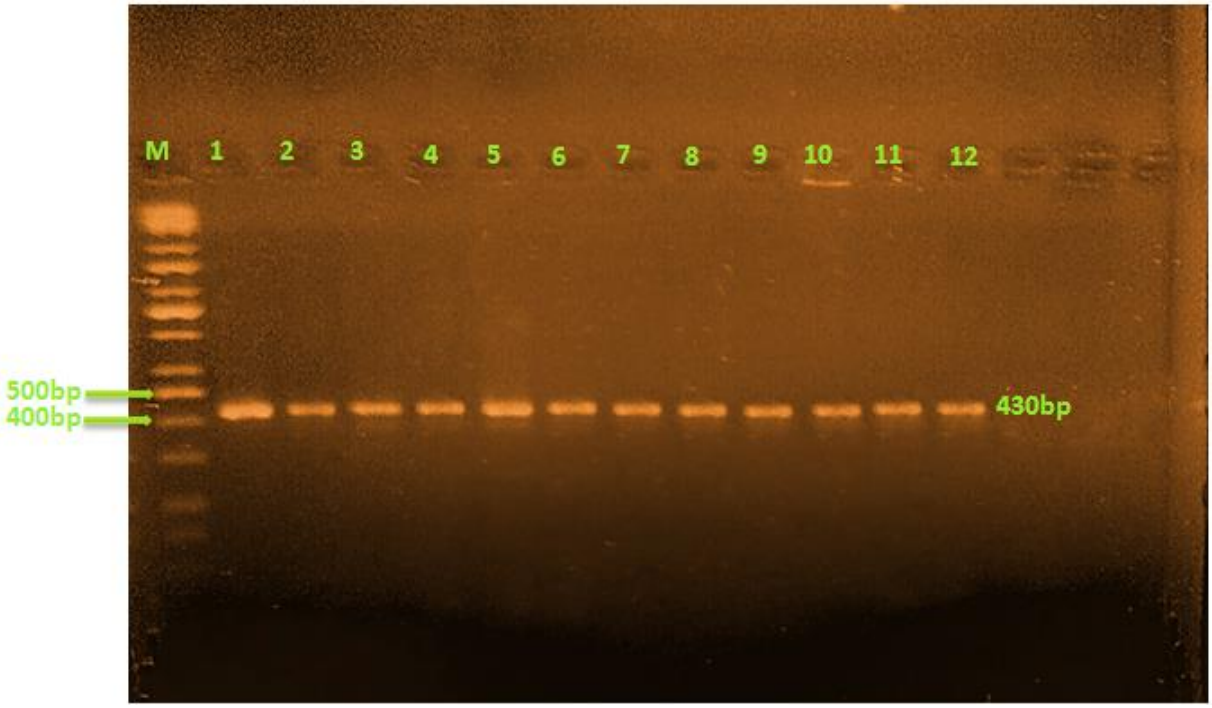

Fig. 3. Mi-1.2 gene amplification products obtained using Mi23 marker. The test plants used were 1: Super Marmande × *Solanum pimpinellifolium* (LA0373), 2: Super Marmande, 3: *Rutgers* × *Solanum pimpinellifolium* (LA0373), 4: *Rutgers*, 5: *Rutgers* × *Solanum habrochaites* (LAO407), 6: *Super Marmande* × *Solanum habrochaites* (LA1266), 7: *Rutgers* × *Solanum habrochaites* (LA1266), 8: *Super Marmande* × *Solanum huaylasense* (LA1360), 9: *Rutgers* × *Solanum huaylasense* (LA1360), 10: *Super Marmande* × *Solanum arcanum* (LA0441), 11: *Super Marmande* × *Solanum huaylasense* (LA2808), 12: *Solanum rutgers* × *arcanum* (LA0441). The line marked M contained a 10kb ladder DNA marker (KAPA Biosystem).

The results of modify TG180 marker indicated that all hybrids were given a single band 1124 bp Fig. 4. indicating presence of the dominant homozygous resistance (*Mi3/Mi3*), while results of study Yaghoobi *et al.* (2005), which demonstrated the efficiency of this marker in the distinction between resistant and sensitive plants for temperatures above 32°C where 1200 bp was given in similar resistant plants.

Heat-sensitive plants are referred to as mi-3/mi-3. *Mi-3* gene thought heat- stable genes and their presence helps complete the work of *Mi-1* resistance genes. Therefore, plant breeders have sought to transfer this gene to the cultivar species (Rashid *et al.,* 2017). Gene *Mi-3* is co-dominant gene, since the presence of a *Mi-3* gene in combination with *Mi-5* gene provides resistance against the occurrence of *M. incognita* and *M. javanica* when soil temperature is 30°C (Yaghoobi *et al.,* 2005) We conclude from molecular results inefficiency DNA extracted from seeds to detection *Mi-1* genes in hybrids. Although molecular analysis confirmed the absent of *Mi-1.2* genes in the studied tomato hybrids while root-knot nematode bioassays after 60 days from infection showed that there were root index no significant differences in hybrids Super Marmmande X *S. pimpinellifolium* LA0373, Rutgers X *S. habrochaites* LA1266, Super Marmmande X *S. arcanum* LA0441, Super *Marmmande* X *S. huaylasense* LA1360 and Rutgers X *S. huaylasense* LA1360 were recorded the lowest root index (GI) 1.33, 1.33, 1.00, 1.00 and 1.00 respectively which indicates its high resistance (H.R) as well as they not different with *S. pimpinellifolium* LA0373 and *S. huaylasense* LA1360 which were carried the *Mi1.2* resistance gene in the form of susceptible dominant alleles (*mi1.2/mi1.2*) Table 4.

This may be due to the presence of genetic sites in wild species that have not been detected by molecular markers but they transferred through the hybridization process or due to stimulate the work of silent genes that were not stimulating in the first environment in theirs.

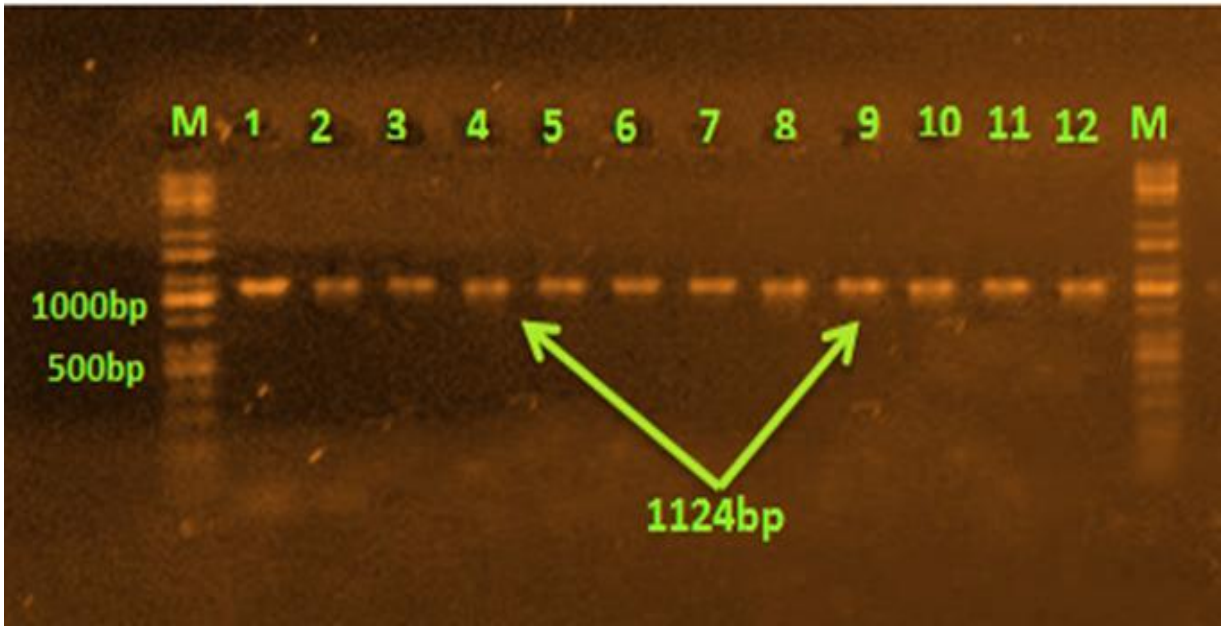

Fig. 4. Mi-3 gene amplification products obtained using modify marker TG180 in seed plants which they were obtained from hybridation, The test plants used were 1: *Super Marmande* × *Solanum pimpinellifolium* (LA0373), 2*: Super Marmande*, 3: *Rutgers* × *Solanum pimpinellifolium* (LA0373), 4: *Rutgers*, 5: *Rutgers* × *Solanum habrochaites* (LAO407), 6: *Super Marmande* × *Solanum habrochaites* (LA1266), 7: *Rutgers* × *Solanum habrochaites* (LA1266), 8: *Super Marmande* × *Solanum huaylasense* (LA1360), 9: *Rutgers* × *Solanum huaylasense* (LA1360), 10: *Super Marmande* × *Solanum arcanum* (LA0441), 11: *Super Marmande* × *Solanum huaylasense* (LA2808), 12: *Solanum Rutgers* × *arcanum* (LA0441),. The line marked M contained a 10 kb ladder DNA marker (KAPA Biosystem).

Table. 4. The means of gall index of *root –knot nematodes (Meloidogyne* spp.) on parents and F1hybrids.

Plant	Mean of Gall index	Response
Super Marmande × *Solanum pimpinellifolium* (LA0373)	1.33	H.R
Super Marmande	3.33	S
Rutgers × *Solanum pimpinellifolium* (LA0373)	2.00	R
Rutgers	4.00	H.S
Rutgers × *Solanum habrochaites* (LA0407)	2.67	M.R -> S
Super Marmande × *Solanum habrochaites* (LA1266)	2.67	M.R -> S
Rutgers × *Solanum habrochaites* (LA1266)	1.33	H.R
Super Marmande × *Solanum huaylasense* (LA1360)	1.00	H.R
Rutgers × *Solanum huaylasense* (LA1360)	1.00	H.R
Super Marmande × *Solanum arcanum* (LA0441)	2.67	M.R -> S
Super Marmande × *Solanum huaylasense* (LA2808)	3.00	S
Rutgers ×*Solanum arcanum* (LA0441)	1.00	H.R
Solanum pimpinellifolium (LA0373)	1.00	H.R
S. peruvianum LA0446	0.00	H.R
S. habrochaites LA1266	2.33	S
S. huaylasense LA1360	1.33	H.R
S. arcanum LA1346	0.00	H.R
L. S. D (P≥0.05)	0.7706	

* recorded plant response to infected by nematode according this scale: High resistance H.R)= gall index 0-1 , resistance (R) = gall index 2 , moderately resistance(M.R)= moderately resistance (M.S)= gall index 2.1-2.6, Susceptible(S)= gall index 2.7-3.5, highly susceptible= gall index 3.6-5.

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