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Detection of Mi Genes Resistant To Meloidogynespp, Which Causes Root -Knot Nematode Disease on Hybrid Tomato Plants

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Abstract: *The study was carried out to detect the Migene alleles by conducting the completely diallel cross-process among eight pure lines of different resistance tomato plants to the disease of the root-knot nematode and obtained 56 hybrids .The hybrids were infected by Meloidogynespp larvae with 5000 juveniles/ kg of soil were diagnosed genes for parents and hybrids resulting from the process of multiplication using the technique of molecular PCR analysis and then the application of DNA indicators through the use of four specific primers(Intron, REX, C8B, and TG180) to detect the presence of 6 genetic sites in their forms of homoresistance, homosucestibile and heterozygous of resistance to the nematode of roots with tolerant to soil temperature conditions higher than 32 ° C effectiveness in detecting resistance genes through the configured DNA bands matching band sizes of what researchers reached by former users of these primers.*

Keywords*: Tomato Lycopersiconesculentum L, Root-knot nematode, Meloidogynespp,diallel and reciprocal hybrid,Mi gene, molecular marker.*

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I. Introduction

Many of the modern cultivars carry a gene resistant to nematodes called Mi. This gene gives resistance to more than three types of *Meloidogyne spp*. The gene has been introduced and activated in some varieties to reduce the use of pesticides to controlled nematodes Roberts et al. (1986). Work on the introduction of the gene Mi in the commercial species *Lycopersiconesculentum L.* from wild tomato species *L. peruvianum* since early 1940 Smith(1944)and enter the gene Mi in all resistant varieties currently exist Williamson *et al*..(1994). The resistance gene Mi is based on the genetic map of the tomato resistant on chromosome 6, and there are seven replicas of Mi-1 to Mi-7 and 650 kg base pairs discovered in wild type *S.perovanium*. These genes are distributed on two clusters, P1 and P2, Seah*et al*. (2007). *Mi-1.2, Mi-1.2, Mi-1.3* is the most active gene in the production of resistance proteins responsible for the resistance of the *Meloidogynespp*, located on the first cluster P1 and used to investigate to the resistance gene *Mi* Several primers are associated with it Seah*et al*. (2007). Gene-Mi is a gene organized by salicylic acid, where the high level of acid in the resistant plant leads to hypersensitivity and then the death of plant cells at the site of the infection, thus limiting the feeding and spread of nematodes to other roots cells Molinari andLoffredo(2006) Gene-*Mi*encodes proteins containing 1275 amino acid consisting of repeat rich leucine LRR (CC) coild coil-NBS (Nucleotide binding site) Milligan et al. (1998). Goggin*et al*..(2001) noted that the resistance given by the Mi-1.2 gene to the tomato plant is highly specialized. The resistance against the root-knot nematode is given by the Mi gene to the tomato plants early after two weeks of germination, as can investigate resistance gene Mi-1.2 in the leaves and roots of plants tomato resistance. Martinez *et al*.. (2001) .The study aimed to evaluate the effectiveness of gene Mi and investigating the genes involved in the resistance.

II. Material and Methods

The comparisons of the genotypes with the infection of the Root-knot nematodes of the hybrid seedlings.The hybrid seeds sowing in cinnamon plates containing autoclave sterilize soil and left to grow and were serviced by watering and fertilization. After 15 days of planting, they were transferred to 200 gm polystyrene pots filled with sterilize soil inautoclaved by one plant / pot, the seedling with three foil stage were inoculated by making 3 holes around the steam area,Choudhury. (1980). The inoculum was distributed on 1000 Juveniles second stage (J2) / 200 gm soil. After inoculation, the wells were covered with sterilize soil. The disease index of root knots nematode were calculated after 60 days of inoculation using the scale, Taylor and Sasser(1978).For the distinction between genotypes in terms of susceptibility and resistance to root - knotdisease. Four specific primers sets provided by (Macrogen Company. Korean) were used to detect the Mi genes resistant to root- knot nematode disease for all experimental plants explained in Table1. DNA was

extracted from plant tissues using Wizard genomic DNA Purification kit (Promega, US) according to the manufacture instruction. Quantification of DNA was conducted using Nanodrope and the purity and concentration was determined. The DNA quality was estimated by the migration of samples on the Agarose gel,Maniatiset al.(1982).Polymerase chain reaction (PCR) mixture was set up in a total volume of 20 µl which included: 2 µl of PCR premix (contains: Taq DNA polymerase, MgCl2, dNTPs, KCl, stabilizer tracking dye and tris-HCl), from (Intron,Canada) ,1 µl of each primer (final concentration was 10 picomol /µl) and 2 µl of DNA template then the volume was completed with sterile free nuclease D.W. PCR reaction tubes were mixed, vortexes and spin then finally were placed into conventional PCR instrument. Conditions of polymerase chain reactions was showed Table (2) were accomplished according to time through experimenting three gradient temperature for each primer set with different time till reach to the optimal amplification condition. Restriction enzymes Taq-1 is used with the REX-1 amplicons to diagnose the condition of the allele. Restriction enzymes (RS) Taq 1 is used with the REX-1 amplicons to diagnose the condition of the allele. Digestion was conducted in 20 µl of reaction mixture including 2µl of 10X Restriction enzyme buffer, 0.2µl of Acetylated BSA, 16.3 deionized distal water, 1µl of REX Amplicons, and 0.5µl of Taq1 RS .the mixture was mixed gently well, spin, and incubated at 60C for 15 min to reactivate the enzyme then incubated at room temperature for 30 min, and incubated at 95 for 10 min to inactivated the enzyme. The produced fragments electrophoresed on 3% Agarose for 2 hrs and visualized under UV light documentation.

III. Results and discussion

The infection of the roots tomato plants appear after 60 days of the inoculation. Genotypes and different proportions were conducted by observing the number of root-knot, which exceeded 100 knots / plant of some structures to the absence of contract of disease on the roots of other genetic compositions. The comparison results of the experiment between the genotypes by the infection of the root-knot nematode of the hybrid seedlings and amplification their DNA using DNA markers. The PCR products of tested cultivar using four marker genes (REX, Intron, TG180,and C8B). REX primers flanked region 750 bp indicated the presence of Mi 1.2 locus to identify susceptible and resistant pure lane of root-knot nematode disease the parents 1, 9, 17, 25 and 49 were susceptibility were crosses with the resistant parents 33, 41 and 57 allele involves the RFLP PCR product which yields in the production750 bp fragment of various numbers of fragments after restriction with Taq1 restriction enzyme , to represent the resistance and susceptible of the disease against the root-knot nematodes. For the absence of an enzyme-cut site in susceptible plants similar to the mi / mi. A single band with a molecular size of 750 pairs appeared in pure line 1, 2, 3, 4, 6, 7, 11, 12, 15, 17, 18, 19, 20, 23, 26, 27, 28, 49, 50, 51, 52, 53 And 65, while there is one cut-off site for this enzyme in the gene that is amplified in the resistant plants that have a homozygous Mi / Mi, resulting in the emergence of two bands of 570 and 160 pairs in breeds 33, 38, 41, 42, 46, 48, 58 and 63, The amplified gene in the resistant plants is differentiated by the marriage. Three bands of 750, 570 and 160 bp appeared respectively. 2, 5, 8, 10, 13, 14, 16, 21, 22, 24, 29, 30, 32, 34, 35, 36, 37, 39, 40, 43, 44, 45, 47, 54, 55, 56, 57, 59, 60, 61, 62 and 64 The sensitive strain 65 was used as control, As shown in Figs. 1,2 and 3. These primers were also used byEl Mehrach*et al*. (2005) in a similar study on the tomato plant in Morocco, Chen *et al*. (2012) also found similar results using REX with Taq1 restriction enzyme. The TG180 marker as shown in Figs. 4 and 5 was used to detect the Mi-9 gene, which gives resistance to tomato plants against nematode root disease at soil temperatures above 32°C, giving a gene band sized 1200bp DNA bp in allele 1, which is associated with the appearance of resistance, according to Yaghoobi*et al*. (2005)the package size 1200 base pairs has been observed in all breeds of the subject in these study, which indicates that all of them have allele 1, which encodes the resistance to temperatures of soil above 32°C Yaghoobi*et al*. (2005). Marker C8B as shown in Figs. 6 and 7 revealed the resistance of tomato plants to the root-knot nematodes at soil temperature higher than 28 °C this primers detected the presence of Mi-9 gene located on chromosome 6 the gene that is amplified in the resistant plants that have a homozygous Mi / Mi appearance of band size 400bp and gene that is amplified in the susceptible plants that have a homozygous mi / mi appearance of band size 360bp,while showed the appearance of two bands size 400 and 360 pairs in all breeds represented the resistant heterozygote genotype. Our result is similar to gene Mi, as Ammiraju*et al*. (2003) the resistance to the disease of root-knot nematodes at temperatures above 28 °C and is very suitable for the Iraqi climate, which is characterized by high soil temperatures .

The results of the use of Marker Mint primers for the detection of Mi resistance genes in the tomato plants as shown in Figs. 8 and 9, which are resistant to the disease of root-knot nematode. bands of 1410, 1186,1372, and 622 base pairs were shown to indicate the presence of the Mi1.6, Mi1.4, Mi1.2 ,and Mi1.1 gene resistance ,pattern respectively and 1353, 981, and 1137 bp indicate the presence of susceptible type genes Mi-1.C, Mi-1.G and Mi-1.F, respectively. Bands 1186 and 981 bp when detecting the Mi-1.4 gene, the plant is a heterozygous resistance. Thecase for the Mi-1.2 gene. If bands sized1372pb are shown to be resistant, 1137 band for the gene of susceptibility while the tow bands appear is a heterozygous resistance. The pure line and hybrids that did not have band 622 bp were susceptible for Mi1.1 gene to the disease of the root-knot

nematodes. The parents used in crosses varied in their susceptibility to infected by nematodes especially 9,17,25,49 , depending on the high rate of disease index 3,4,3,5 .Upon molecular detection of resistance genes, that found genes Mi1-6, Mi1-4, Mi1-2, Mi1-1 to be allele of genetic for all sites except the parent 49 the gene site Mi1-1 was heterozygous and was not effective in making the pure line resistant, indicating the main role of the Mi1-2 gene in resistance , and that the presence of the gene pattern Mi1-2 as a susceptible form make the parents essay to infecting by nematode while the parents 33,41,57 were resistance because the genes mentioned were homozygous or heterozygous except parent 57 the gene Mi1-4 for it was susceptible but did not affect the degree of resistance , which shows the weakness of the role of this gene in resistance compared to 41 parent the resistance was not affected by the decrease of gene action when the gene Mi1-4 was susceptible state .However, the resistance was 0.0 for both parents, that indicating the weak role of the gene Mi1-4 in the resistance to the nematode infection. It should be noted the genetic sites of all these genes of parent 1 were susceptible but this parent was resistant, confirming that there were genes that had an active role in the resistance but not yet discovered. In Table (3), we find that the results indicate the crosses (1 × 6, 6 × 1, 1 × 8, 8 × 1, 2× 5, 5 × 2, 2 × 6, 6 × 2, 3 × 6, 6 × 3 , 4 × 5, 5 × 4, 4 × 6, 6 ×4, 4 × 8, 8 × 4, 5 × 6, 6 × 5, 5 × 7, 7 × 5, 5 × 8, 8 × 5, 6×7, 7 × 6, 7 × 8, 8 × 7) The common factor in these combinations is parents 5, 6 and 8 that carry allele-resistant to genes Mi1-1, Mi1-2, whether in Homozygosity or Heterozygosity although to crosses with susceptible parents such as 9,17,25,49 the resultant of hybrid is resistant to root-knot disease because the genetic sites of the Mi1-1, Mi1-2 gene remained dominant despite the presence of individuals carrying susceptible allele of one of the sites of these two genes. However, the dominant site of the second gene plays its role in the resistance. Therefore, the average disease index of the number of knot of each of these communities of crosses is low and resistant, confirming that these two genes have an effort to resist as main action. Genetic sites such as Mi1-4, Mi1-6 they have little role and secondary action effort in resisting the disease. The 1 × 2 and 2 × 1 crosses were differentiated in the disease index of the number of knot in the roots. The cross 2 x 1 was resistor while the 1 × 2 was susceptible to nematode, although the two subunits did not contain the gene sites Mi1-1 , Mi1-2, Mi1-4 , Mi1-6 This resistance is due to genes found in the cytoplasm or genes that have a role in the resistance that did not discover yet or indicating that gene undergone considerable during evolution and this finding is agree with Seah et al. (2007) rearrangement. In the diallel 1 × 5, 3 × 5, 3 × 8, 6 × 8 interconnections, the reciprocal cross of 5 × 1, 5 × 3, 8 × 3, 8 × 6 the rate of disease index to number of roots-knot to diallel cross 2,2,2, 3 sequentially, while the disease index of the number of knot in reciprocal was 4.4,4,1, reflecting the mother's role in resistance to parents 5, 6, 8 as they were genetic sites of mother 5 has a pure predominant gene for Mi1-2, Mi1-1 and heterozygous of the Mi1-6 and Mi1-4 gene while the mother 6 differed from the genetic sites of the genes mentioned except the genetic site Mi1-2, it was predominant, giving it a stronger gene action more than the mother 8, while it had a migratory site of the gene Mi1-4.

Although the role of this gene in the resistance is almost a little, but the individuals resulting from the above crosses included individuals carrying susceptible genes for all sites. The role of cytoplasmic genetics in its interaction with the disease, especially for those found in mitochondria and plastids, is emphasized because it is contained on its own cyclic DNA. Some plant physiologists have considered them to be cell-colonizing organisms and have a role in resistance and rapidly multiply in response to the requirements of the cell.

IV. Conclusion

The results showed that the resistance in the tomato plants was subjected to the cooperation of a number of genes to appear this status and not to one gene. The main advantages of DNA molecular markers are that, they areefficient, faster, free of environmental effects on the phenotype, less subjective, non-destructive, and low expenses to run than standard bioassays. Molecular markers can provide data that can be analyzed objectively also have low negative selection pressure in populations .The results showed that the Mi1.1 gene had an important role in showing the resistance to its presence in all the plants tested which showed resistance to the disease of root-knot nematode whilethe previous researches was referring to the main role of the Mi1.2 gene in the resistance as well as the emergence of resistance to some of the hybrid for the disease of the root-knot nematode, although there are resistance genes that tested as a mi / mi recessive which indicate there are other genes have not been detected had a role in resistance . Molecular tests revealed that the pure line used in the research were all carrying the predominant alleles of Mi-9 and the differentiated alleles resistant to Mi-3, which gave the plants the ability to cannot break resistance when soil temperature above 28 °

Table. (1) Primers used in experiments to detect resistance genes of Mi-genes in commercial and commercial strains.

Primer	Gene	Applications size	Oligonucleotide	reference
C8B	Mi-9	400 ,360	F: TACCCACGCCCCATCAATG R:TGCAAGAGGGTGAATATTGAGTGC	Kaloshian et al., 1998
Intron	Mi-1.1,1.2,1.4, 1.6	1,353, 981, 1137 1,186, 1372, 622, 1410	F-TTCTCTAGCTAAACTTCAGCC R-TTTTCGTTTTTCCATGATTCTAC	Jablonska et a.,2007l
REX	Mi-1.2	750 570* 160*	F: TCGGAGCCTTGGTCTGAATT R: GCCAGAGATGATTCGTGAGA	Williamson et al.,1994
TG180	Mi-3	1200	F: ATACTTCTTTRCAGGAACAGCTCA R: ACATTAGTGATCATAAAGTACCA	Yagoobi et al .,2005

* The gene fragments 570, 160 represent fragment size after using Taq-1 restriction enzyme

Table (2). The amplification program cycles in PCR

Gene	Pre-denaturation	Second denaturation	Annealing	Extension	Final extension
Intron	95◦C (3 min)	95◦C(30 sec)	58 ◦C (1 min)	72◦C (30 sec)	72◦C (5 min)
C8B	94 ◦C (4 min)	95◦C(30 sec)	58 ◦C (1 min)		
TG180	94 ◦C (3 min)	94 c (1 min)	55 ◦C (2 min)	72◦C (2 min)	
REX	94 ◦C (5min)	94 c (1min)	55◦C (2min)	72 ◦C (2min)	

Table (3): The rate of the pathological index of the number of nodes and the genetic sites of pure line and their crosses (ɬ represents the apparent result, + represents the expected results)

No. of hybrid	crocss		Resistance and susceptible design of Mi gene																		Rate of disease index
			Mi1.1			Mi1.2			Mi1.4			Mi1.6			Mi-3			Mi -9			
			Mi /Mi	Mi/ mi	mi/ mi	Mi/ Mi	Mi/ mi	mi/ mi	Mi/ Mi	Mi/ mi	mi/ mi	Mi/ Mi	Mi/ mi	mi/m i	Mi/M i	Mi/ mi	mi/ mi	Mi/M i	Mi/ mi	mi/ mi	
1	1×1	1×1			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	2
10,2	1×2	2×1			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	2,3
18,3	1×3	3×1			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	3,4
26,4	1×4	4×1			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	3,4
34,5	1×5	5×1		ɬ			ɬ			ɬ	+		ɬ	+	ɬ			+	ɬ	+	2,4
42,6	1×6	6×1		+	ɬ		ɬ			ɬ	+		ɬ	+	ɬ			+	ɬ	+	2,0
50,7	1×7	7×1		+	ɬ			ɬ		+	ɬ			ɬ	ɬ			+	ɬ	+	3,4
58,8	1×8	8×1		ɬ	+		ɬ	+		ɬ	+		+	ɬ	ɬ			+	ɬ	+	2,2
9	2×2	2×2			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	3
11,19	2×3	3×2			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	4,4
12,27	2×4	4×2			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	4,3
13,35	2×5	5×2		ɬ			ɬ			+	ɬ		ɬ	+	ɬ			+	ɬ	+	2,2
14,43	2×6	6×2		ɬ	+		ɬ			+	ɬ		ɬ	+	ɬ			+	ɬ	+	1,0
15,51	2×7	7×2		+	ɬ		+	ɬ			ɬ		ɬ	+	ɬ			+	ɬ	+	3,4
16,59	2×8	8×2		ɬ	+		ɬ	+		ɬ	+		+	ɬ	ɬ			+	ɬ	+	2,3
17	3×3	3×3			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	5
20,28	3×4	4×3			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	3,4
21,36	3×5	5×3		+			ɬ			+	ɬ		+	ɬ	ɬ			+	ɬ	+	2,4
22,44	3×6	6×3		ɬ	+		ɬ			ɬ	ɬ		ɬ	ɬ	ɬ			+	ɬ	+	2,1
23,52	3×7	7×3		+	ɬ			ɬ			ɬ		+	ɬ	ɬ			+	ɬ	+	3,5
24,60	3×8	8×3		ɬ	+		ɬ	+			ɬ		ɬ	ɬ	ɬ			+	ɬ	+	2,4
25	4×4	4×4			ɬ			ɬ			ɬ			ɬ	ɬ			+	ɬ	+	3
29,37	4×5	5×4		ɬ	+		ɬ	+		ɬ	+		+	ɬ	ɬ			+	ɬ	+	2,2
30,45	4×6	6×4		ɬ	+		ɬ	+		ɬ	+		ɬ	+	ɬ			+	ɬ	+	2,2
31,53	4×7	7×4		+	ɬ			ɬ			ɬ		+	ɬ	ɬ			+	ɬ	+	3,3
32,61	4×8	8×4		ɬ	+		ɬ	+			ɬ		+	ɬ	ɬ			+	ɬ	+	2,2
33	5×5	5×5	ɬ			ɬ			ɬ				ɬ		ɬ			+	ɬ	+	1
46,38	5×6	6×5	ɬ	+		ɬ			+	+	ɬ	+	+	ɬ	ɬ			+	ɬ	+	1,1
54,39	5×7	7×5	+	ɬ			ɬ			ɬ	+		+	ɬ	ɬ			+	ɬ	+	1,2
62,40	5×8	8×5	+	ɬ		+	ɬ			ɬ	+	+	ɬ	+	ɬ			+	ɬ	+	1,2
41	6×6	6×6		ɬ		ɬ				ɬ			ɬ		ɬ			+	ɬ	+	0
55,47	6×7	7×6	+	ɬ	+		ɬ			+	ɬ	+	+	ɬ	ɬ			+	ɬ	+	2,2
63,48	6×8	8×6	ɬ	+	+	ɬ			ɬ	+	ɬ	+	+	ɬ	ɬ			+	ɬ	+	3,1
49	7×7	7×7		+				ɬ			ɬ		ɬ		ɬ			+	ɬ	+	4
64,56	7×8	8×7	+	ɬ	+		ɬ	+		+	ɬ	+	+	ɬ	ɬ			+	ɬ	+	2,2
57	8×8	8×8		ɬ			ɬ				ɬ		ɬ		ɬ			+	ɬ	+	0

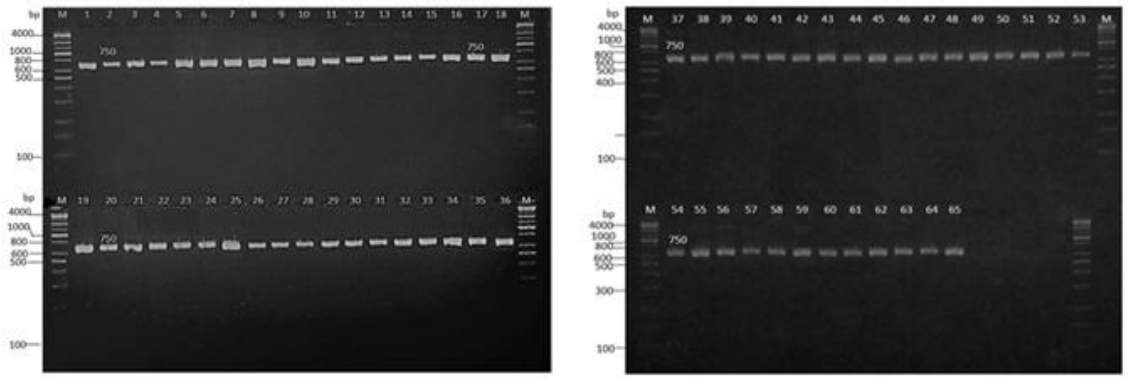

Figure (1) Electrophoresis to the PCR products of Mi 1.2 gene flanked region to REX-1 primer, the product digested with Taq1 RS enzyme on 3% Agarose stained with red safe dye and with TBA buffer at 3V/cm for 2 hsr..Lan M represented 100bp DNA marker, lane 1-64 the REX-1 amplicons sized 750bp.

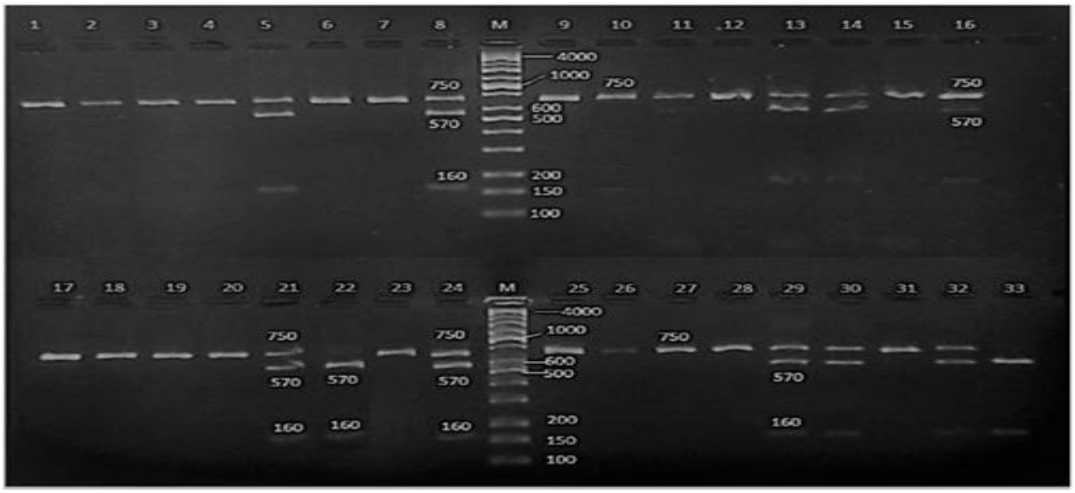

Figure (2). Electrophoresis to the PCR products of Mi 1.2 gene flanked region to REX-1 primer, the product digested with Taq1 RS enzyme on 3% Agarose stained with red safe dye and with TBA buffer at 3V/cm for 2 hsr. Lane M represented 100bp DNA marker, lane 5, **8**,**13** ,**14**,**16** , **21**,**22**,24,,**29**, **30** ,32 Shown three bands(160,570,750)bpindicated the resistant heterozygote genotype Line1,2,3,4,6,7,9,10,11,12,15,17,18,19,20, 23,26,27,28,31) Shown bands sized 750 bp indicated susceptible homozygote genotype, and lane 33 shown two bands(570,160) bp indicated the resistant homozygote genotype

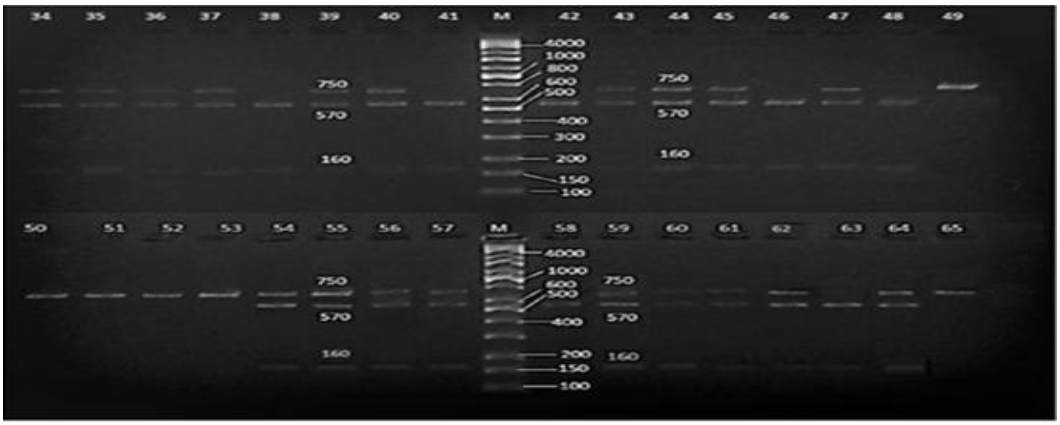

Figure (3) . electrophoresis to the PCR products of Mi -9 gene flanked region to REX-1 primer, the product digested with Taq1 RS enzyme on 3% Agarose stained with red safe dye and with TBA buffer at 3V/Cm for 2 hr. Lane M represented 100bp DNA marker, line 34, 35,36,37**39,40,و**43,**44**,**45**,54,55,57,59,60,61,62,64 Shown three bands(160,570,750)bp indicated the resistant heterozygote genotype, line 49,50,51,52,53,65) Shown bands size 750 bp indicated susceptible homozygote genotype, and line 38,41,42,44.48,58,63 shown two bands(570,160) bp indicated the resistant homozygouse genotype.

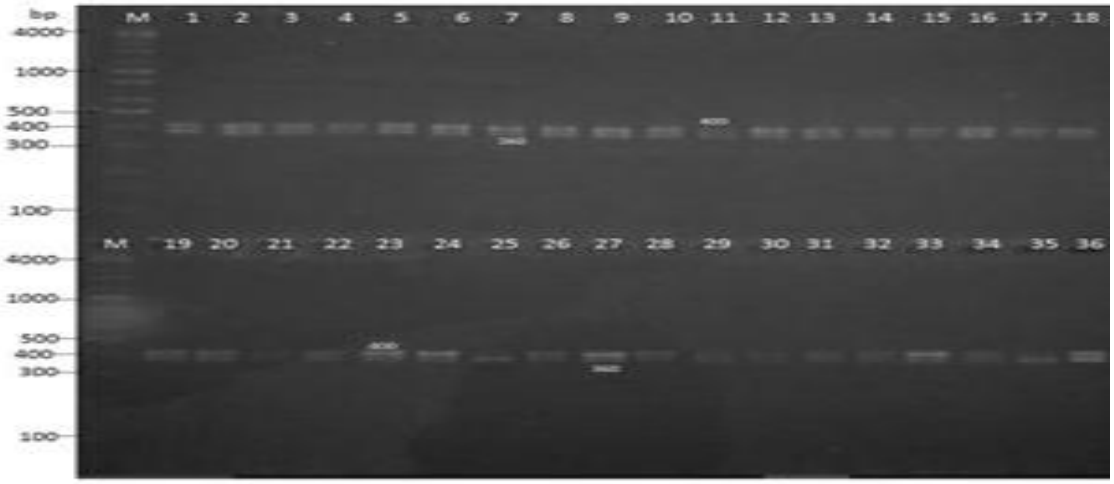

Figure (4). Electrophoresis to the PCR products of Mi -9 gene flanked region to C8B primer on 3% Agarose stained with red safe dye and with TBA buffer at 3V/Cm for 2 hsr. Lane M represented 100bp DNA marker, lane (1- 36) shown two bands (360,and 400) bp indicated the resistant heterozygote genotype above 28 ◦C.

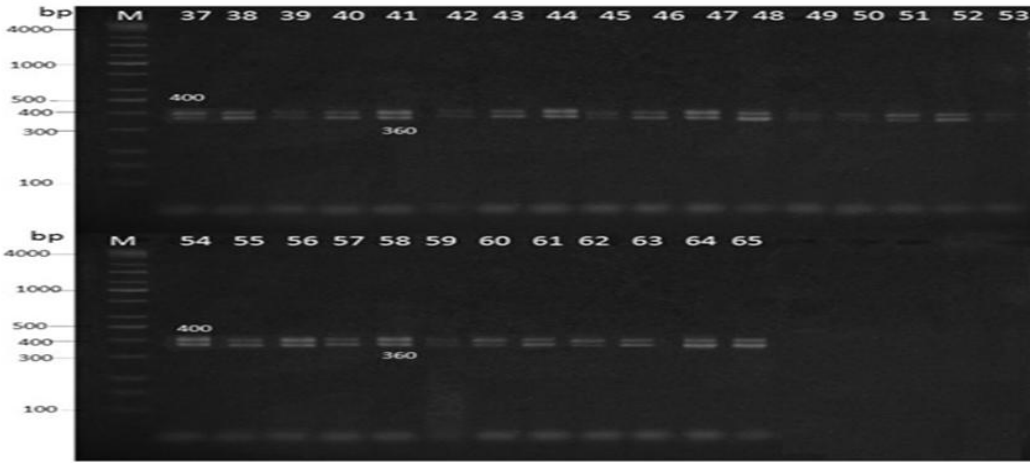

Figure (5) Electrophoresis to the PCR products of Mi -9 gene flanked region to C8B primer on 3% Agarose stained with red safe dye and with TBA buffer at 3V/Cm for 2 hsr. Line M represented 100bp DNA marker, lanes (37- 65) shown two bands (360,and 400) bp indicated the resistant heterozygote genotype above 28 ◦C.

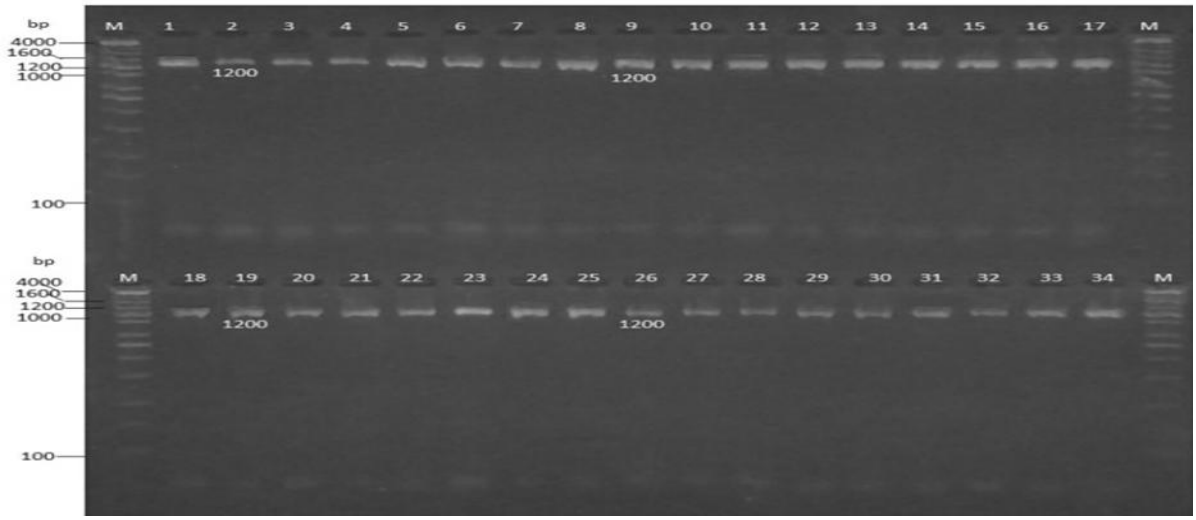

Figure (6) Electrophoresis to the PCR products of TG180 flanked region on 3% Agarose stained with red safe dye and with TBA buffer at 3V/Cm for 2 hsr. Line M represented 100bp DNA marker, lanes (1- 34) shown one band (1200) bp indicated the resistant genotype to the temperature above 32 ◦C

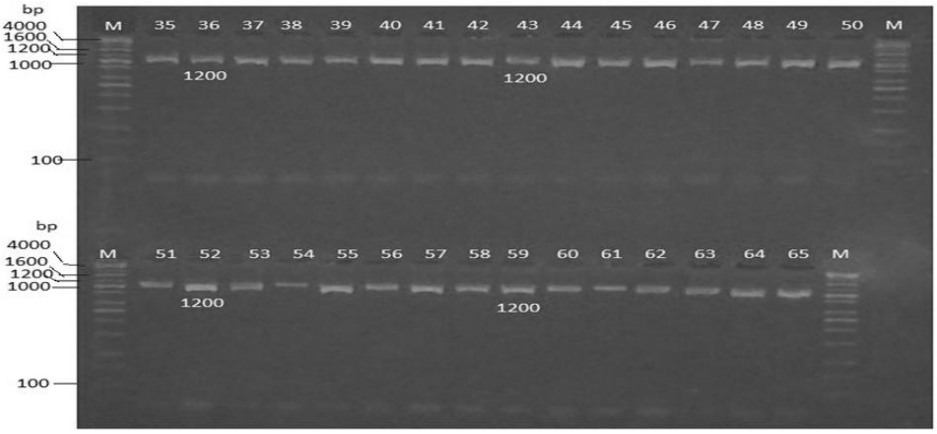

Figure (7) Electrophoresis to the PCR products of TG180 flanked region on 3% Agarose stained with red safe dye and with TBA buffer at 3V/Cm for 2 hsr. Line M represented 100bp DNA marker, lanes (37- 65) shown one band (1200) bp indicated the resistant genotype to the temperature above 32 ◦C

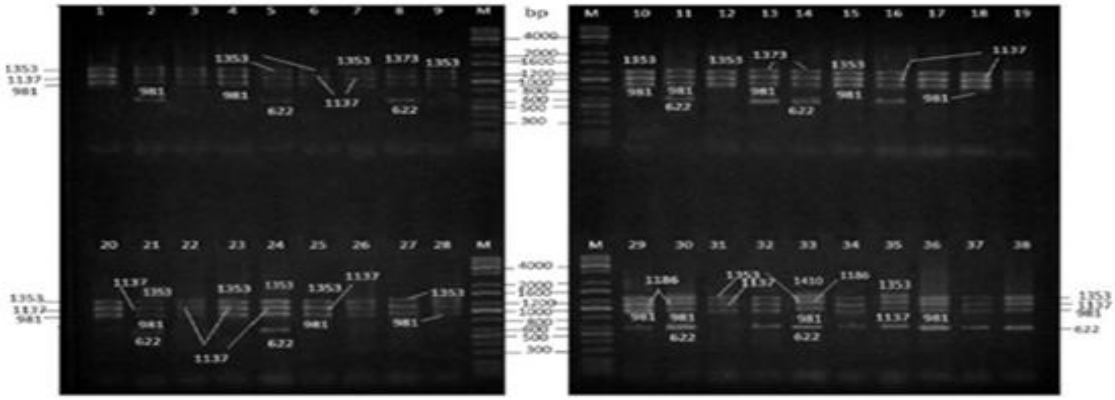

Figure (8). Electrophoresis to the PCR products of Mi 1.1,1.4,1.6 gene flanked region to intron primer on 3%agaros , 3vol/cm^2 stained with red safe dye visualized under UV light. Line M represented 100bp DNA marker.Line1, 3, 6, 10, 11, 12, 15, 17, 18, 19,20,21 ,22,24,25,26,27,28,31,32 were shown three DNA bands sized (981, 1137, 1353) bp indicated the susceptible homozygote genotypes for the Mi1.1,1.6, 1.4, 1.2genes, lines 2,5,8,11,13,14,16,21,24,29,30,32,33,34,35,36,37,38 were shown DNA band sized 622bp indicated resistance heterozygote genotype for Mi 1.1 gene. Lines 2, 33, 35, 36 ,37,38 were shown DNA band sized 1410 bp represented resistance heterozygote genotype for Mi 1.6 ,lines 24,29,30 were shown two bands sized 1189 and 981 bp represented the resistant heterozygous.

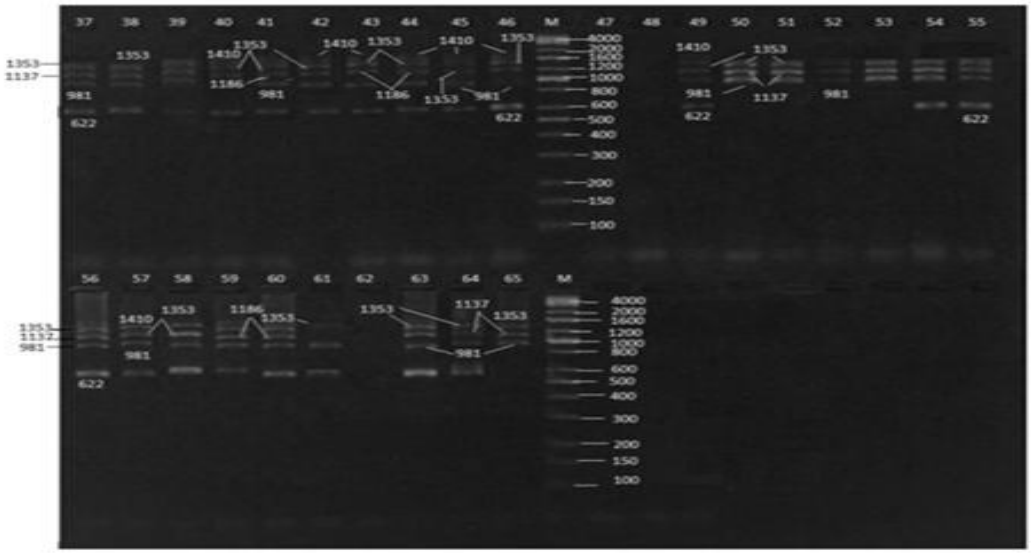

Figure (9). Electrophoresis to the PCR products of Mi 1.1,1.4,1.6 gene flanked region to intron primer on 3%agaros , 3vol/cm2 stained with red safe dye visualized under UV light. Line M represented 100bp DNA markerLine 50, 51, 52, 53, 54, 55, 56,63, 64were shown three DNA bands sized (981, 1137, 1353) bp, representedsusceptible homozygote genotypes for the Mi1.1,1.6, 1.4, 1.2 gene .Lines 40,41, 44,45,46,63shown four DNA bands sized (1186, 1353, 981, 1410) bp represented theresistance heterozygote genotype for Mi 1.1 gene. Lines 37, 38 , 39 , 40 ,41 ,42 ,43 ,44 ,45 ,46 ,49 ,54 ,55 ,56 ,57 ,59 ,60 ,61 ,63 ,64were shown DNA band sized 622bp indicated resistance heterozygote genotype for Mi 1.1 gene.

References

[1] Ammiraju,J.S.S., J.Veremis, X.Huang, P.A.Roberts andI.Kaloshian .2003. The heat-stable, root-knot nematode resistance geneMi-9from *Lycopersiconperuvianum*is localized on the short arm of chromosome 6. TheorAppl Genet 106:478-484.

[2] Chen,H.,J.N.Du, L.N.Hao,C.Y.Wang, Q. Chen and Y.X. Chang.2012. Identification of markers tightly linked to tomato yellow leaf curl disease and root-knot nematode resistance by multiplex PCR Res. 11: 2917-2928 .

[3] Choudhury, B.C. (1980). Effect of different inoculum levels of *Meloidogyne incognita* on two tomato varienties.Bangladesh journal of Agricultural research. 5: 13 – 16.

[4] Goggin, F.L., V.M. Williamson and D.E. Ullman.(2001). Variability in the response of *Macrosiphumeuphorbiae* and *Myzuspersicae* (Hemiptera: Aphididae) to the tomato resistance gene Mi. Environ. Entomol. 30: 101 – 106.

[5] El Mehrach K., S. GharsallahChouchane, L. Mejia, V.M.Williamson, F. Vidavski, A. Hatimi, M.S. Salus, C.T.Martinand D.P. Maxwell.(2005).- PCR-based methodsfor tagging the *Mi-1* locus for resistance to root-knot nematode in begomovirus-resistant tomato germplasm.ActaHorticulturae, 695: 263-270.

[6] Jablonska, B., J.S.S. Ammiraju, K.K. Bhattarai, S. Mantelin, O. Martinez de Ilarduya , P.A. Roberts and I. Kaloshian. (2007). The Mi-9gene from *Solanumarcanum*conferring heat-stable resistance to root-knot nematodes is a homolog of Mi-1[W][OA]. Plant Physiology143: 1044-1054.

[7] Kaloshian, I., J. Yaghoobi, T. Liharska, J. Hontelez, D. Hanson, P. Hogan, T. Jesse, J. Wijbrandi, G. Simons and G. Vos. (1998). Genetic and physical localization of the rootknot nematode resistance locus Miin tomato. Molecular and General Genetics257: 376-385.

[8] Martinez, D., A.E. Moore and I. Kaloshian.(2001). The tomato Rmel locus is required for Mi-1 mediated resistance to root- knot nematodes and the potato aphid. Plant J. 27: 415 – 425.

[9] Martinez, D. and I. Kaloshian.(2001). Mi-1.2 transcripts accumulate ubiquitously in resistant *lycopersiconeesculentum*. J. Nematol. 33: 116 – 120.

[10] Milligan, S. B., J. Bodeau, J. yaghoobi, I. Kaloshian, P. Zabel and V. M. Williamson. (1998). The root knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine – rich repeat family of plant genes. The plant cell. 10: 1307 – 1319.

[11] Maniatis, T., Fritsch.E.F and Sambrook.J. (1982). Molecular cloning : A laboratory manual. Cold Spring Harbor laboratory. New York

[12] Molinari, S. and E.Loffredo .(2006). The role of salicylic acid in defenseresponse of tomato to root-knot nematodes.PhysiolMolPlantPathol 68:69–78

[13] Molinari, S. 2007. New developments in understanding the role of salicylic acid in plant defence. CAB.Rev. 67: 1-10.

[14] Roberts,P.A., D.Mayand W.C.Matthews .(1986). Root-knot nematode resistance in processing tomatoes. Calif. Agric. 40: 24–26.

[15] Smith, P.G. (1944). Embryo culture of a tomato species hybrid.Proc. Am. Soc. Hortic. Sci. 44: 413–416.

[16] Seah, S., V. M. Williamson and B. E. Garcia. (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 genetic cooperative report. 57:37-40

[17] Taylor, A. L. and J.N. Sasser .(1978). Biology, Identification and control of root-Knot nematodes (*Meloidogyne* species).Dep.Of plant pathol. N.C. state Univ. Raleigh, NC 27650, US. III PP.

[18] Williamson, V.M., H.Y. Miller and I.Kaloshian .(1994). A PCR-based marker tightly linked to nematode resistance gene Mi, in tomato. Theor. Appl. Genet. 87: 757-763.

[19] Yaghoobi, J.J., L. Yates and V. M. Williamson.(2005) .Finemapping of the nematode resistance gene Mi-3 in Solanumperuvianum and construction of a S. lycopersicum DNA contig spanning the locus.Mol Gen Genomics) 274: 60–69.

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