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Identification of *Mi1.2* Resistance gene in some Tomato Hybrids from diallel cross against root-knot nematode caused by *Meloidogyne spp*.

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ABSTRACT

Mi gene is an important resistance gene in tomato plant, which discovered in the wild tomato plant. This study was carried out to investigate the transferability of the Mi gene through completely diallel cross among eight tomato pure line which was different in their resistant to root-knot nematodes to obtained 56 hybrids (28 diallel and 28 reciprocal crosses). The hybrids were inoculated with *Meloidogyne spp* at rate of 5000 2ed stage juveniles / kg of soil. Genes for parents and hybrids resulting from the diallel crosses were diagnosed by using PCR and then DNA markers were applied through the use of the Mi23 marker to detect the Mi1.2 gene sites in their dominant, recessive and heterozygous forms alleles. The result showed that 6 and 8 parents gave a high resistance where it observed that zero galls on their roots. Also the 5 parent which show a single galls 60 days after inoculum. The parents 1,2 ,3,4 and 7 were showed different degree of susceptible which have 40- 100 galls/plant .The diallel cross(5x6,5x8) and reciprocal cross (6x5,8x5) were showed high resistant for the disease by forming single galls on their roots. While the hybrids 3x2, 4x2, 3x4 and 7x3 give high values for the number of galls (more than 30) which observed on their roots. The results indicated that using selection method or hybridization, followed by selection method could be used to improve tomato plants resistance to root-knot nematode.

Keywords: Tomato, Root-knot nematode-*Meloidogyne spp*, diallel and reciprocal hybrid; Mi23 marker.

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Introduction

Tomato(*Solanum lycopersicum L*.) is an important vegetable crop in Iraq and the world because it is rich in vitamins such as A and C , mineral elements such as iron and phosphorus. It is consumed fresh and enters in many food industries (Bhowmik,et al 2012).. In Iraq, the area cultivated with tomato crops was 22.532 thousand hectares with a production rate of 281,792 tons Central Statistical Organization, (CSO) (2013). Root-knot nematode *Meloidogyne spp* are common pathogens that attack vegetables like tomato and cause significant yield reductions worldwide (Sasser,1980). The loss causing by this pest may result in total loss of the crop, in case of minor injury, the loss is not less than 30-40%. (Charchar et al., 2003). Using resistant varieties is one of the most successful ways to control root knot nematode, Mi resistance gene was obtained from hybridization of wild tomato (*Lycopersicom. pervenium*) and commercial tomato *(Lycopersicom esculentum)* Smith (1944). The Mi gene can be resistance to many diseases as well as the root-knot nematode (Nombela,et al., 2003).

The Mi gene consist of a group of genes from Mi 1 to Mi 9 located on different chromosoms within the genome of a number of wild *Solanum* species (Yaghoobi *et al*., 1995; Veremis and Roberts,1996a,b; Ammiraju *et al*., 2003; Jablonska *et al*., 2007). Its work by encodes a group of proteins that work to prepare the plant to resistant this disease. Primers (Mi23 Forward and Revere) were preserved between *L. esculentum* and *L. peruvianum*-derived regions were choice with the assist of the program Primer3 by Williamson and(Seah et al 2007) . Mi23F is 5'-TGG AAA AAT GTT GAA TTT CTTTTG-3', and Mi23R is 5'- GCA TAC TAT ATG GCT TGT TTA CCC-3'.

The aim of this project was to investigate the transferability of the Mi gene through completely daillel cross among eight tomato pure liens and conducted this gene by Mi23 marker as a homoresistant, heteroresistant or susceptible .

MATERIAL AND METHODS

Eight local lines of tomato with different resistance to root-knot nematode was obtained from (Dr. Inad D.Abood) dept. of plant protection were used in this study(Table1). All the 8 pure lines were crossed in a full diallel method to obtain 28 daillel and 28 reciprocal crosses combinations.

Seeds of eight pure lines were sown in small pots in the greenhouse of Department of Plant Protection, college of agriculture, university of Baghdad to produce the F1 seedling. After one month these seedlings were transplanted in greenhouse in 15/2/2016.

Table.1 Tomato genotype used in the experiment

Name of genotype	No. of parents	NO. of parents in experiment	Reaction	No. of Disease Index
SH-17-170/D2	1	1	R* to S*	2
SH-17-170/G8	2	9	Midrate .S	3
SH-17-170/S4B51	3	17	High.S	5
SH-17-170/A3Z21	4	25	Midrate .S	3
SH-17-170/B7K3	5	33	Resistant	1
SH-17-170/C3P115	6	41	High.R	0
SH-17-170/F14J10	7	49	High.S	4
Wojdan F1 America hybrid	8	56	High.R	0

S*=Susceptible R*=Resistant

Seed collection

At harvest a sample of tomato fruits for each of crosses were collected in labeling polyethylene bags and taken to the laboratory for seed separation, to separate tomato

seeds by used electric mixture. Then impurities were removed and seeds dried on a drying paper at room temperature for two days and then collected in a special polyethylene bags until used.

DNA extraction and PCR methods

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 Nano drop and the purity and concentration was determined.

The DNA quality was estimated by the migration of samples on the Agarose gel (Maniatis et al., 1982).Polymerase chain reaction (PCR) mixture was set up in a total volume of 25 µl which included: 5 µl of PCR premix (contains: Taq DNA polymerase, MgCl2, dNTPs, KCl, stabilizer tracking dye and tris-HCl), from (Intron,Canada) ,1µl Forward and 1µl Revere of Mi23 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 where: 94°C for 5 minutes followed by 35cycles of 3sec at 94°C, 1 minutes at 55°C and 1 minutes at 72°C,Followed by 10 minutes at 72°C. Fragments of PCR amplified were separated by electrophoresis gel with 3% agarose.

Estimation of hybrids resistibility to root knot nematode

Hybrid seeds were sowing in sterile organic material and left to grown , irrigated by sterile water when it needed also add the fertilizer after 15 days of planting. Then seedlings transported to polystyrene pots (200 gm) with three replicates included 585 experimental units (72 parents plants + 504 diallel and reciprocal crosses and 9 plants as control) filled with sterilization soil (autoclave at 121c ° under pressure 1 bar for 20 minutes). When the seedling at 3 true leaf stage, its inoculated with 5000 juveniles/pot by making three holes around the seedling stem (Choudhurt, 1980). After that covered it by sterilize soil. Disease index for root–knot nematode were determined after 60 days on the following scale: 0=no knot, 1=1-2 knot, 2=3-10 knot,3=11-30 knot,4=31-100 knot, 5=more than of 100 knot according to(Taylor and Sasser, 1978).

Statistical and genetic analysis

The experiment design was compet randomized design (CRD). The analysis of the variance and the significance of differences within treatments was tested by using LSD Test at 0.05 probability level (Steel**,** R. G. D. and J. H. Torrie 1980). Heteross was estimated and diallel crosses in F1 generation were analyzed to obtain general (GCA) and specific (SCA) combining ability variances and effects for studied traits according to Model I (fixed effect) Method 1.(Griffing ,1956) also estimated type of gene action and degree of dominance .

RESULTS AND DISCUSSION

All possible crosses among the pure lines were made in a diallel crossing block to obtain the F1 seeds of 56 crosses from tomato as well as seeds of parents.

Table (2) showed significant genotypic effect on number of knot per plant, parents 6 and 8 showed zero of root knot after 60 days from inoculation . While in parent 5 there is only one root knot was formed. The parent 1 and 2 were formed less than 10, parents 4 gave (11-30) knot/plant. The highest root knots which reach more than 100 knot/plant were recorded on parent 7. These results indicated that some parents had a high resistance to the root-knot nematode including (5, 6, 8) and that some other parents such as parent 3,7 were high susceptible which gave more than 100 knot on its roots. Also show in same table different degrees of resistance to root-knot nematode for diallel and reciprocal cross.

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Table (2) Number of root-knot/ plant caused by *Meloidogyne. spp* in the purees line and its hybrids roots after 60 days of inoculation with juveniles.

No. Disease index	Genotypes	0	1-2	3--10	11-30	31-100	100>	No. Disease index	Genotypes	0	1-2	3-10	11-30	31-100	>100
1	p1			*				34	5x1			*			
2	1x2			*				35	5x2			*			
3	1x3				*			36	5x3			*			
4	1x4				*			37	5x4			*			
5	1x5			*				38	5x6		*				
6	1x6			*				39	5x7		*				
7	1x7				*			40	5x8		*				
8	1x8			*				41	p6	*					
9	p2				*			42	6x1	*					
10	2x1				*			43	6x2	*					
11	2x3					*		44	6x3		*				
12	2x4					*		45	6x4			*			
13	2x5			*				46	6x5		*				
14	2x6		*					47	6x7			*			
15	2x7			*				48	6x8		*				
16	2x8				*			49	p7					*	
17	p3						*	50	7x1					*	
18	3x1					*		51	7x2					*	
19	3x2					*		52	7x3						*
20	3x4				*			53	7x4				*		
21	3x5			*				54	7x5			*			
22	3x6			*				55	7x6			*			
23	3x7				*			56	7x8		*				
24	3x8			*				57	p8	*					
25	p4				*			58	8x1			*			
26	4x1				*			59	8x2				*		
27	4x2				*			60	8x3					*	
28	4x3				*			61	8x4			*			
29	4x5			*				62	8x5			*			
30	4x6			*				63	8x6		*				
31	4x7				*			64	8x7			*			
32	4x8			*				65	**superm arim and**						*
33	p5		*												

In order to confirm the results obtained, it was used Mi23 marker to identify the resistance and susceptible genes in tested plants. The amplification results of DNA fragment Fig 1,2 after immigration on the agarose gel showed a bundle it size 430 bp as a susceptible in plants (1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 15, 17, 18, 19, 20, 23, 25, 26, 27, 28, 31, 49,50.51,52,53and 65 also bandle at 380 bp as a homoresistant plants(33,38, 41,46,48,63) While the two bands appeared in resistant pure line in heteroresistant plants(5,8,13,14,16,19.21,22,24, 29 ,30,32 ,34 ,35 36,37,39,40.42,43,44,45,47,55,56,57,58,59,60,61,62,64) .

Table (3) revealed there was a difference in the mean of disease index associated with the number of root-knots among the parents and their hybrids .Crosses 2x3 ,4x2,4x3 and 7x3 showed high rates in the

disease index number of root-knots (3.67, 3.33, 3.00and3.00) respectively, which indicated their susceptibility to root-knot nematodes, while the lower values were shown by the crosses 6x5 and 8x5 were recorded(0.67) knot which refers it were resistant to the disease. The results revealed a significant decrease for knot-roots of (19) diallel crosses which gave low number of knot on its roots. The parent 5 was characterized when used as a female, where the mean of its hybrids decreased in knot number and show the resistance to the disease that indicated the role of cytoplasm effect in the inheritance of the resistant to root-knot nematodes. The data in table (4) showed the parents (5, 6 and 8) the inherit in the direction of increased resistance to root-knot nematodes, which gave a negative effects of General Combining Ability (GCA) and other pure line have given a positive for GCA values indicating that the inheritance of genes were in the direction of susceptibility to root-knot nematodes. The results of specific Combining Ability (SCA) effects analysis showed that (15) daillel crosses were Inheritance to increase the resistance to this disease, and the ether daillel crosses were inherited to increase susceptibility in it. In reciprocal crosses the GCA was a negative values in 13 crosses and the crosses, 5×1, 4×3, 7×4 and 7×6, shown equal value (0.00), these results indicate an alliance among parents to increase disease resistance in its crosses. Also the results indicated the significance effect of GCA and SCA which an importance of both additive and dominance genes action to root- knots nematodes. The additive gene action was more evident to inheritance of number of knots in tomato roots where an increase in the value of the additive variance(1.02) comparing with the dominance variance(0.25) as well as the high values of narrow sense for the diallel (75.3%) and reciprocal crosses (79.85%) also the degree of dominance was a less than one. The results indicated in table 5 that the value of GCA variance was (0.511) while it was (0.25) for SCA and (0.173) for RCA, which indicated that the additive gene action is an effective in root-knot nematode in tomato followed by dominance gene action than cytoplasm effect in inheritance in this treat, and there is non-effect of environment in it because the experiment was controlled inside the green house. These results indicate the importance of genetic variation in the control of inheritance resistance to root-knot nematodes in tomato .The significant differences among the parents well be reflected to the hybrids in heterosis values which calculated by deviation of the mean for the F1 generation to best pure line . The data in table 6 shows that the diallel and reciprocal crosses have given negative values for heterosis especially in the crosses in which the parent (6) was participated in it as male and the parents(1,2,3and 4) as female lines , which indicates increased the resistance in this hybrids. The pure line (6) negative values for all diallil and reciprocal crosses in heterosis, thus can be adopted the 6 and 5 pure lines to raise the degree of resistance in the combinations which it's are inserted it. The cross (3 × 8) showed negative value of heterosis (-71.43%). while the crosses (5 × 6) and (5 × 8) gave the highest negative value of heterosis towards the resistance of this disease reaching (-100%). The reciprocal crosses (6×1) and (6× 2) showed a high percentage (-100%) in the resistance of the disease, were no significant differences between them, and there are 17 reciprocal crosses showed resistance to the disease with varying values between (-16.67% and -100.00%). The data in table 5 shows that diallil cross (6×8) and reciprocal crosses (4×1),(4×2) and(8×7) were gave a heterosis value equal to (0.00%) which indicating that the hybrids is equal with the best of parent for resistance of root-knot nematodes.

CONCLUSION

Concluded that the use ofthe selection method or hybridization followed by selection method to improve the plant resistant to root- knot nematodes, with taking into account to use of more resistant parents in these methods. Also it is possible to rely on the PCR method through the use of the Mi23 primer initiator to accurately detect of the presence of a Mi1.2 gene in tomato plants resistant to disease of the root knot.

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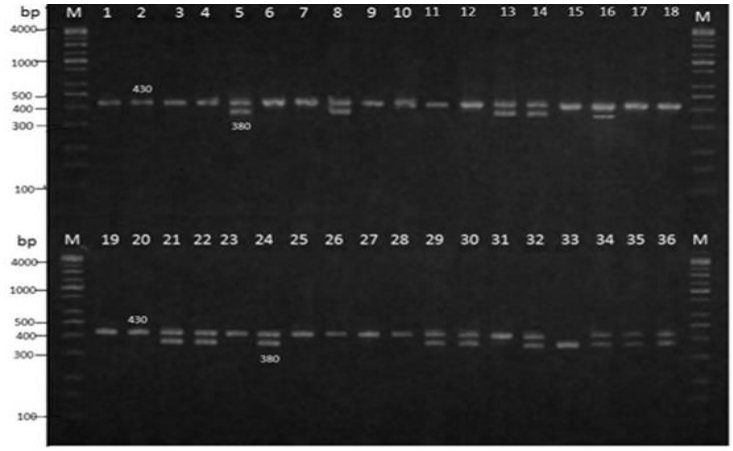

Figure(1).electrophoresis to the PCR products of Mi 1.2 gene flanked region to Mi23 primer on 3%agaros , 3vol/cm^2 stained with red safe dye visualized under UV light. LineM represented 100bp DNA marker.lines 1, 2, 3, 4, 6, 7, 9, 10, 11, 12, 15, 17, 18, 19, 20, 23, 25, 26, 27, 28, 31. were shown three DNA bands siz(430)bp indicated the susceptible homozygote genotypes,lines 5,8, 13,14,16,21,22,24,29,30,32,34,35,36 were shown DNA band sized(380,430)bp indicated resistance heterozygote genotype and Lines **33 were shown DNA band sized 380 bp represented resistance genotype**

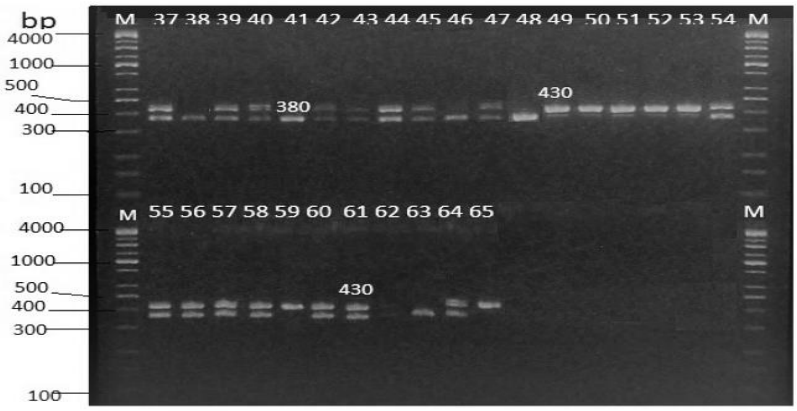

Figure (2).electrophoresis to the PCR products of Mi 1.2 gene flanked region to Mi23 primer on 3%agaros, 3vol/cm^2 stained with red safe dye visualized under UV light. Line M represented 100bp DNA marker. Lines 49,50.51,52,53 and 65were shown two DNA bands siz(430)bp indicated the susceptible homozygote genotypes ,lines 37,39,40,42,43,44,45,47,55,56,57,58,60,61,64 were shown DNA band sized(380,430)bp indicated resistance heterozygote genotype andLines 38, 41,46,48,63were shown DNA band sized 380 bp represented resistance genotype

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Table (3) number of root knot/ Plant of tomato per parents (diagonal values),daillel cross (above diagonal)and reciprocal cross (under diagonal).

Parents	p1	p2	p3	p4	p5	p6	p7	p8
p1	2.00	1.67	2.33	2.67	1.67	1.67	2.67	1.33
p2	2.67	2.67	3.67	3.33	1.33	1.00	2.33	1.33
p3	3.33	4.00	4.67	3.00	2.00	2.00	3.00	1.33
p4	3.00	3.00	3.00	3.00	2.00	1.67	2.67	1.33
p5	1.67	1.67	1.33	1.67	0.33	0.67	1,00	0.67
p6	0.00	0.00	1.00	1.33	0.33	0.00	1.33	1.67
p7	4.00	4.00	4.33	2.67	2.00	1.33	3.33	2.00
p8	1.67	2.67	3.33	2.00	1.67	0.67	1.33	0.00
LSD 0.05	0.814							

Table(4) Estimate of general combining ability (GCA)(diagonal values) ,specific combining ability (SCA)(above diagonal) and reciprocal combining ability (RCA)(under diagonal) for number of root knot/ Plant of tomato .

parents	p1	p2	p3	p4	p5	p6	p7	p8
p1	0.13	-0.34	-0.23	0.24	0.27	-0.21	0.62	-0.07
p2	-0.5	0.36	0.54	0.35	-0.13	-0.78	0.22	0.2
p3	-0.5	-0.17	0.92	-0.38	-0.53	-0.34	0.16	-0.03
p4	-0.17	0.17	0.00	0.44	0.12	0.14	-0.36	-0.21
p5	0.00	0.67	0.33	0.17	-0.74	0.33	-0.34	0.47
p6	0.83	-0.5	-1.5	0.17	0.17	-1.1	-0.15	0.83
p7	-0.67	-0.83	-0.67	0.00	-0.5	0.00	0.57	-0.34
p8	-0.17	-0.67	-1.00	-0.33	-0.5	0.5	0.33	-0.58
	Se	GCA	0.511	SCA	0.578	RCA	0.206	

Table(5) Estimated of genetic parameters for number of root knot/ Plant of tomato.

GCA	ScA	RCA	e-	σ^2gca \$\sigma^2$rca	σ^2gca/σ^2sca	σ^2gca	σ^2A
8.77	0.33	0.43	0.085	2.039	2.951	0.51	1.02
Daillil crosses				Reciprocal crosses			
σ^2D	a-	h^2b.s	h^2n.s	σ^2D-r	a-r	h^2b.s-r	h^2n.s-r
0.25	0.7	93.76	75.3	0.17	0.58	93.38	79.85

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Table (6) Estimated of heterosis (%) for daillel cross (above diagonal)and reciprocal cross (under diagonal) for number of root knot/ plant of tomato.

parents	p1	p2	p3	p4	p5	p6	p7	p8
p1		-37.5.0	-50.00	-11.11	-16.67	-16.67	-20.00	-33.33
p2	0.00		-21.43	11.11	-50.00	-62.50	-30.00	-50.00
p3	-28.57	-14.29		-35.71	-57.14	-57.14	-35.71	-71.43
p4	0.00	0.00	-35.71		-33.33	-44.44	-20.00	-55.56
p5	-16.67	-37.50	-71.43	-44.44		-100.00	-70.00	-100.00
p6	-100.00	-100.00	-78.57	-55.56	0.00		-60.00	-
p7	20.00	20.00	-7.14	-20.00	-40.00	-60.00		-40.00
p8	-16.67	0.00	-28.57	-33.33	400.00	-	-60.00	
Se for daillil crosses cross			7.82	Se for reciprocal crosses				16.45

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