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Response of tomato wild species, landraces and commercial cultivars to *Meloidogyne javanica* infection as revealed by molecular and conventional approaches

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Abstract

In present study, response of some commercial cultivars, landraces andwild species of tomato were evaluated against *Meloidogynejavanica*.Furthermore, presence of *Mi-1* alleles in the plant materials was traced using PMiF3/PMiR3, Mi23F/Mi23R and REXF1/REXR2 primer pairs. In greenhouse experiments, seven genotypes (ES1002, Super Beita, Ant 93-04, Samrudhi, Nun 6108, Pascal and *Solanumperuvianum*[LA0111])were less affectedby the nematode while the rest figured more susceptible. Using PMiF3/PMiR3 primer pair, two fragments of 350 and 550bp were amplified in the seven genotypes, whereas in the remaining genotypes only the allele of 350 bp was generated. Based on Mi23F/Mi23R primer pair, the seven genotypes were found heterozygote for 380bp and 430bp fragments and the others showed only a single allele of 430 bp. In all the genotypes except Super Strain B when were treated with REXF1/REXR2 primer pair, a 750bp fragment was realized. Digestion of the amplified fragments with *Taq*I restriction enzyme resulted in the appearance of 550bp and 150bp fragments in nine cultivars, the fore mentioned seven genotypes plus a landrace (Laleh) and *Solanumhabrochaites* (LA1223). It can be concluded that the Mi23 and PMi3 markers are more reliable for separating the resistant genotypes from susceptible ones.

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Introduction

Root-knot nematodes(*Meloidogyne* spp.) are economically important polyphagous group of highly adapted obligate plant parasites which distributed worldwide (Moens*et al.*,2009). With increased environmental concerns and restrictions imposed on nematicidesapplication, the importance of host resistance as a strategy for the management of the nematodesis being substantially grown nowadays (Williamson and Roberts, 2009).

In tomato breeding, identification of the resistance gene, *Mi,* againstroot-knot nematodes is mainly evaluated by conventional screening bioassays. Results from field assessments can be misleading due to variations in nematode populations and soil temperatures. When many recombinants or cultivars are to be screened, it will be time consuming and labor intensive (Danso*etal.*, 2011). The first root-knot nematode resistance gene was cloned from the *Mi-1* locus located on short arm of chromosome 6 of *Lycopersiconperuvianum*. In the locus, two genes (*Mi-1.1* and *Mi-1.2*) and one pseudo gene (*Mi-1.3*) were identified that are resistance gene homologues. The single dominant *Mi-1.2* gene is sufficient to confer resistance against root-knot nematodes (Bakker *et al.*, 2006).On the other hand, the gene*Mi-1* is very tightly linked to *Ty-1* gene that is derived from *Lycopersiconchilense* accession LA1969 and reveals resistance to TYLCV (Zamir*et al.*, 1994).

Different markers have been described for the detection of *Mi-1* gene (Gogginet *al.*,2004; DevranandElekçioglu, 2004; Bendezu, 2004; El Mehrach*et al.*, 2005).The REXCAPS marker is widely used to assay the *Mi-1* gene in tomato (Williamson *et al.*, 1994). However, El Mehrach*et al.* (2005) found that themarker cause false positive results for the presence of *Mi-1* in some of the begomovirus-resistant germplasms. Therefore, Mi23, which isco-dominant SCAR marker for the *Mi-1*, was subsequently developed.It can exclusively distinguish the presence of *Mi-1* gene in understudy plants from those containing*Ty-1* gene (Seah*et al.*, 2007). Recently, Devran*et al.* (2013), compared JB-1, REX, PMi12 and Mi23 markers for detection of *Mi-1* gene. The markers REX, PMi12, and Mi23 showed similar results in the plants lacking*Ty-1* gene. However, REX generated false positive results even in certain genotypes bearing *Ty-1* gene. The JB-1 yielded accurate results in all the genotypes, without any false positive results. It has been mentioned that Mi23 and PMi12 are good markers for screening the tomatoes in breeding programs.Cordata*et al.* (2012) compared some molecular markers of *Mi* gene and found that among the studied markers, REXwas effective to evaluate root-knot nematode resistance in tomato cultivars with only *S. peruvianum* introgressions in *S. lycopersicum* background. As the expression of resistance is affected by factors such as soil temperature, species and populations of *Meloidogyne*, Mi-dosage, and tomato genetic background,the efficient use of resistance to manage root knot nematodes must take into consideration these factors (Dropkin, 1969). On the other hand, some researchers stated that resistant tomatoes have a high level of resistance to populations of *M. incognita* and *M. arenaria*, but are less resistant to *M. javanica* (Ornat*et al.*,2001, SorribasandVerdejo-Lucas, 1994). So, the aim of the present study is to evaluate the response of some landraces, wild species and available commercial cultivars of tomato against infection with a native population of *Meloidogynejavanica*isolated from Khosroshah, East-Azarbaijan, Iran, under greenhouse condition. The genotypeswere also screened based on REX, PMi3 and Mi23 markers for monitoring the presence of*Mi-1* gene and its allelic condition.

Materials and methods

Plant Materials

Sixteen commercial cultivars, five landraces of tomato (*Solanumlycopersicum*) and four accessions of wild tomato species used in this study are listed in Table 1. Tomato seeds of commercial cultivars were provided by local suppliers in Tabriz and Tehran cities and the landraces collected from Agriculture Research Centers of Tabriz, Urmia and Abadeh cities, Iran. The

wild speciesaccessions provided by the Tomato Genetic Research Center (TGRC), Davis, California, USA. Unfortunately, sufficient information was not available for the most of the cultivars that are imported to the country. The cultivars ES1002 and Pascal are considered as resistant to the root-knot nematodes buttheir genetic identity are not clear. The cultivar Samrudhihas heterozygote resistance condition (*Mi/mi*)to root-knot nematode and the cultivar CH Falat(*mi/mi*) was chosen as susceptible control.

Nematode culture

The original population of root-knot nematode was collected from infected roots of cucumber grown in a commercial greenhouse in Khosroshah, East-Azarbaijan province, Iran. Nematode culture was raised from a single egg mass and propagated continuously on a susceptible tomato cultivar, Super Chief grown in pots containing 1kg sterilized soil, sand and peat moss (2:1:1 ratios) in the greenhouse at 25±2°Cand 16:8 hours photoperiod. The species was primarily identified as *Meloidogynejavanica* based on perineal patterns of females and other morphological and morphometrical characters. Diagnosis of the population subsequently confirmed based on species-specific primers.

DNA isolationand PCR analyses

Genomic DNA was extracted from fresh leaves of plants grown in the greenhouse using CTAB according to modified method of Lodhi*et al.* (1994). The quantity and quality of DNA samples were assessed using spectrophotometer and electrophoresis in 0.8% agarose gel.PCR for PMiF3/PMiR3 and Mi23F/Mi23R primer pairs were carried out in 10 µl solution consists of 4 µl of Ampliqon® 2X Master mix, 0.5 µM of each primer, 50 ng template DNA and ddH_2O. The reactions were done using Biorad® My Cycler™Thermal Cycler as the following profile: one cycle of 3 minutes at 94°C, 35 cycles of (94°C, 30s; 56°C, 30s; 72°C, 60s) and one cycle for 7 minutes at 72°C with Mi23F(5'-TGGAAAAATGTTGAATTTCTTTTG-3'), Mi23R(5'-GCATACTATATGGCTTGTTTACCC-3') primers (Seah*et al.*, 2007) and same cycles with 58°Ctemperature for annealing step with PMiF3(5'GGTATGAGCATGCTTAATCAGAGCTCTC3') ,.PMiR3(5'CCTACAAGAAATTATTGTGCGTGTGAAT G3') primers (El Mehrach*et al.*, 2005). The same PCR reactions in 20 µl were performed using REXF1. (5´-TCGGAGCCTTGGTCTGAATT-3´),REXR2.(5´-GCCAGAGATGATTCGTGAGA-3´) primers (Williamson *et al.*, 1994) and the amplification profile was similar to that of followed in the first primer pairs. The PCR products resulted from theREX primer pair was digested with *Taq*I Fermentas® according to the manufactureinstruction.All the products were run on 1.5% agarose gel stained with Ethidium Bromide and illuminated under UV light.

GreenhouseExperiments

Seeds of all the genotypes were incubated in sterile Petri dishes covered with filter paper and thegerminated seedlings transplanted to experimental pots containing 750 ml of sterilized soil, sand and peat moss (with 2:1:1 ratios) mixture. The egg masses were collected from infected roots and dissolved in 0.5% NaOCl to remove the gelatinous sacs (Hussey and Barker 1973). The extracted eggs were transferred into sterile distilled water and incubated in 25±2°C for 48 hours. The suspension of eggs and freshly hatched J_2s were used as inoculum of the nematode.The seedlings having true 5-6 leaves were inoculated with three eggs and J_2s per ml of soil mixture and the control plants received only water. Inoculated and control treatments were arranged in a completely randomized design with three replicates and kept in the greenhouse with 25±2°C temperature and 16:8 hours photoperiod. The experiment replicated twice.

Datacollection andanalyses

The plants were harvested 60 days after inoculation. The infection rates of the genotypes with root-knot nematode were indexed based on the number of galls and egg-masses in root systems, number of eggs per egg-mass and number of J_2s in soil of the pots. Final

population (Pf) and reproduction factor (RF) of the nematode were also calculated. The gall and egg-mass indices were assessed according to Taylor andSasser (1978).Data analysis and drawing of graphs were performed using SAS, Statistica and Microsoft Excel softwares. The transformed data were exposed to analysis variance (ANOVA) and significant differences of the genotypes were realized using Duncan's test. Cluster analysis using Ward's algorithm and Euclidean distance was finally performed for grouping of the genotypes.It has to be mentioned that since the nematode reproductive factors in uninfected control plants were zero and not measurable, only the genotypes that were treated with the nematode compared with each other.

Results and discussion

Molecularanalyses

Based onMi23F/Mi23R primers, PCR products of ES1002, Super Beita, Ant 93-04, Samrudhi, Nun 6108, Pascal and LA0111 accession showed two fragments of 380bp and 430bp as heterozygous pattern (*Mi/mi*). In the rest of the genotypes only a 430bp fragment (*mi/mi* state) was observed (Fig. 1A.).UsingPMiF3/PMiR3primer pair, two fragments of 350 and 550 bp were amplified in ES1002, Super Beita, Ant 93-04, Samrudhi, Nun 6108 and Pascal commercial cultivars as well as *S.peruvianum* (LA0111). The other genotypes were homozygote for 350 bp allele (Fig. 1B.).

TheREXF1/REXR2 primer pairgenerateda single 750 bp fragment in all the genotypes except Super Strain B cultivar (Fig. 2A.). Digestion of PCR products with *Taq*I enzyme yielded in three fragments (750, ~170 and 550 bp) in nine genotypes. In the rest genotypes, restriction enzyme did not digest the product(Fig. 2B.).

These nine genotypes include seven heterozygous genotypes that were identified based onthe two former primer pairs (Mi23F/Mi23R and PMiF3/PMiR3), a landrace (Laleh) and LA1223 accession of wild species. Replication of digestion with different amounts of the *Taq*I,indicated the same results.

Greenhouse Experiments

Number of galls and egg-masses per root system, J_2s in each pot, final population and RFweresignificantly different ($p<0.01$) between the genotypes. However, differences of the genotypes for number of eggs per egg-mass were not revealed significant (Table 2).

Table 1. The list of the studied tomato materials.

Commercial cultivars			Landraces	Wild species (accessions)
Polaris	Ant 93-04	Korall	Urmia 1 (West-Azarbaijan province)	*Solanumpimpinellifolium*(*Lycopersiconpimpinellifolium*) (LA2184)
ES1002	Mobil	Cal. J. N3	Urmia 2 (West-Azarbaijan province)	*Solanumperuvianum*(*Lycopersiconperuvianum*) (LA0111)
Super Strain B	Nun6108	Rio grande	Laleh (East-Azarbaijan province)	*Solanumpimpinellifolium*(*Lycopersiconpimpinellifolium*) (LA0722)
Super Beita	Samrudhi	Pascal	Sardasht (West-Azarbaijan province)	*Solanumhabrochaites*(*Lycopersiconhirsutum* f. glabratum)(LA1223)
Super Chief	CH Falat		Vakil Abad (Fars province)	
NDM-447	Cherry			

Table 2. Analysis of variance for nematode infection factors in tomato cultivars, landraces and wild species.

		Mean squares							
Source of variations	df	galls in root system	galls per gram of root	egg-masses in root system	egg-masses per gram of root	eggs per egg-mass	J_2s in each pot	Pf	RF[1]
genotype	24	[2]1.5×10^{5}**	3.4×10^{3}**	1.8×10^{3}**	3.3×10^{3}**	3.2×10^{3} ns	1.2×10^{7}**	1.8×10^{10}**	3.7×10^{3}**
error	50	8.9×10^{3}	13.6×10^{2}	2.2×10^{3}	94.4	1.9×10^{4}	2.8×10^{6}	2.7×10^{8}	4.7×10^{2}

[1]RF= Pf/ Pi (Final population/ Initial population of nematode).

ns and**: no significant and significant at the 0.01 probability level, respectivel.

Amongst the genotypes, ES1002, Pascal and Samrudhi commercial cultivars had fewer numbers of gallsper root system and other reproductive factors of nematode as well,followed by Super Beita, Ant93-04, Nun 6108 and *S.peruvianum* (LA0111). The other ten cultivars, five landraces and three wild species were foundmore susceptible against the nematode infection (Table 3). The gall and egg-mass indices were also found lower in the mentioned seven genotypes (Table 4).

Table 3. Mean comparison for different factors in the selected genotypes.

Genotypes	Galls per root		Galls per gram of root		Egg-masses per root		Egg-masses per gramof root		J2 in soil		Pf		RF	
Polaris	[1]400	cbd	57	de	159	bcd	21.3	cd	3150	bcdefg	155660	bcde	69	bcde
ES1002	4	i	0.6	g	2	h	0.2	f	265	fg	2635	i	1.1	i
Super Strain B	439	cb	71	bcd	187	bc	31	cd	5265	ab	171159	bcd	76	bcde
Super Beita	79	ghi	19	gf	55	efgh	13	def	1212	defg	55856	efghi	24.8	fghi
Super Chief	546	b	62	de	189	bc	21	cd	3447	bcdef	141958	bcdef	63	cdefg
NDM-447	543	b	51	de	218	b	20	cd	1382	defg	228032	ab	101	abc
Ant93	21	hi	4	gf	10	gh	2	f	550	efg	10324	hi	4.5	i
Mobil	416	bc	47	e	156	bcd	18	cdef	4000	bcd	159971	bcd	71	bcde
Urmia 1	156	fghi	45	e	103	cdef	30	cd	2007	cdefg	103773	defgh	46	efgh
Urmia 2	314	cdef	44	e	122	cdef	23	cdef	1182	defg	131633	bcdef	58	defg
Laleh	416	bc	58	de	153	bcd	20	cde	3825	bcde	148880	bcdef	65.8	bcdef
Sardasht	321	cdef	53	de	133	bcde	22	cd	330	fg	153950	bcdef	68.1	cdefg
Vakilabad	384	bcd	63	cde	151	bcd	25	cd	2832	bcdefg	140899	bcdef	62.5	cdefg
Nun6108	109	ghi	23	de	55	efgh	12	def	547	efg	52466	fghi	23.2	ghi
Samrudhi	2.5	i	0.4	g	2.5	h	0.4	f	15	g	2938	i	1.2	i
CH Falat	725	a	64	bcd	222	b	19	cde	5912	ab	215984	abc	95.9	bcd
Cherry	231	defg	53	gf	131	bcde	34	c	7482	a	122300	cdefg	54.3	defg
Korall	316	cdef	53	de	122	cdef	21	cd	5047	abc	127061	bcdef	56.4	defg
Cal. J. N3	356	cde	58	de	149	bcd	24	cd	1800	defg	136751	bcdef	60.7	cdefg
Riogrande	776	a	57	gf	307	a	23	cd	1097	defg	305305	a	135.3	a
LA2184	193	efgh	84	e	177	bcd	78	b	4300	bcd	211733	bc	105.9	ab
Pascal	2	i	0.9	e	0.6	h	0.2	f	283	fg	300	i	0.2	i
LA0111	36	hi	20	e	33	fgh	18	cdef	274	fg	27692	ghi	12.2	hi
LA0722	105	ghi	91	de	105	cdef	90	b	2700	bcdefg	92883	defghi	41.2	efghi
LA1223	91	ghi	156	de	91	defg	156	a	3550	bcdef	76650	defghi	34	efghi

[1] Means with the same letter are not significantly different according to Duncan's test (P ≤ 0.05).

Table 4. Average of gall and egg-mass indices[1] in the selected genotypes.

Genotypes	Gall index	Mass index	Genotypes	Gall index	Mass index
Polaris	5	4.6	Nun6108	4.3	3.6
ES1002	2	1	Samrudhi	1	1
Super Strain B	5	5	CH Falat	5	5
Super Beita	4.3	3.6	Cherry	5	4.6
Super Chief	5	5	Korall	5	5
NDM-447	5	5	Cal. J. N3	5	5
Ant93-04	3.3	2.3	Rio grande	5	5
Mobil	5	4.6	LA2184	4.6	4.6
Urmia 1	5	4.6	Pascal	1	0.6
Urmia 2	5	5	LA0111	3	3
Laleh	5	4.6	LA0722	5	5
Sardasht	5	4	LA1223	4	4
Vakilabad	5	5			

[1]Accoridng to Taylor and Sasser (1987), (0 = no gall/egg-masses on the roots, 1 = 1-2 galls/egg-masses; 2 = 3-10; 3 = 11-30; 4 = 31-100 and 5 = more than 100 galls/egg-masses per root).

All the genotypes were divided into two groups whenWard's algorithm and Euclidean distance followed (Fig.3.). The genotypes grouped in cluster I were susceptible and homozygous (*mi/mi*) in molecular screenings.Group II consisted of ES1002, Super Beita, Ant 93-04, Samrudhi, Nun 6108 and Pascal commercial cultivars as well as wild species LA0111, were resistant and showed heterozygote (*Mi/mi*) pattern for *Mi-1.2* gene. It can be concluded that, all the landraces and wild species except LA0111 and most of the cultivars except ES1002, Super Beita, Ant 93-04, Samrudhi, Nun 6108 and Pascal were susceptible against infection with the root-knot nematode.

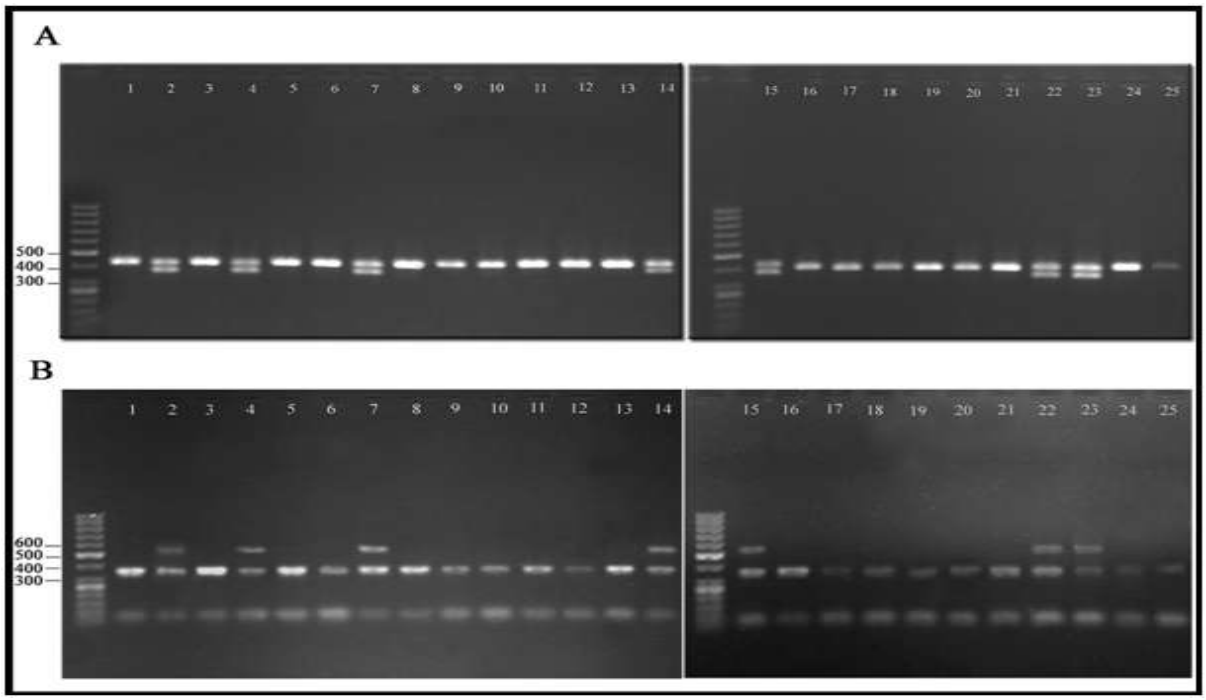

Fig. 1. PCR product patterns of tomato cultivars, landraces and wild species for A, Mi23F/Mi23R primer pair and B, PMiF3/PMiR3 primer pairs 1 to 25: Polaris, ES1002, Super Strain B, Super Beita, Super Chief, NDM-447, Ant 93-04, Mobil, Urmia1,Urmia 2, Laleh, Sardasht, Vakil Abad, Nun 6108, Samrudhi (*Mi/mi*), CH Falat (*mi/mi*), Cherry, Korall, Cal. J. N3, Rio grande, LA2184, Pascal, LA0111, LA0722, LA1223, respectively. (Marker: Gene Ruler ™ 50 bp DNA ladder, Fermentas®).

As some indices of infection by the nematode, are concerned, significant differences among the susceptible genotypes as well as resistant genotypes, especially the genotypes containing an allele of *Mi-1.2* gene were recognized (Table 3).

The genetic profiles of the fragments obtained from the under study genotypes showed complete similarity with the patterns reported by El Mehrach*et al.* (2005). They used PMiF3/PMiR3 primer pair and reported that susceptible (*mi/mi*) and resistant (*Mi/Mi*) cultivars were homozygote for 350 and 550 bp fragments, respectively.However, some resistant cultivars were heterozygote (*Mi/mi*) for the fragments (350 and 550 bp).

Garcia *et al.* (2007)screening some known susceptible (*mi/mi*) and homozygous resistant (*Mi/Mi*) genotypes,also recorded430 bp and 380 bp fragments,respectively using Mi23 marker,whereas heterozygous resistant (*Mi/mi*) cultivarsgeneratedthree fragments of 380, 430 and 500 bpprofile. The third, slower moving PCR-fragment from the heterozygous plant materials was shown to be a heteroduplex between the two

fragments (380 and 430 bp.The rest ofthe commercial resistant hybrids in their study showed the heterozygous pattern (*Mi/mi*). *S.peruvianum* (LA0111) was identifiedas a heterozygous genotypecontaining the three above mentioned fragments that was also obtained in our results but with two 380 and 430 bp fragments.Cordata*et al.* (2012) and Devran*et al.* (2013) reported similar results with the same marker.

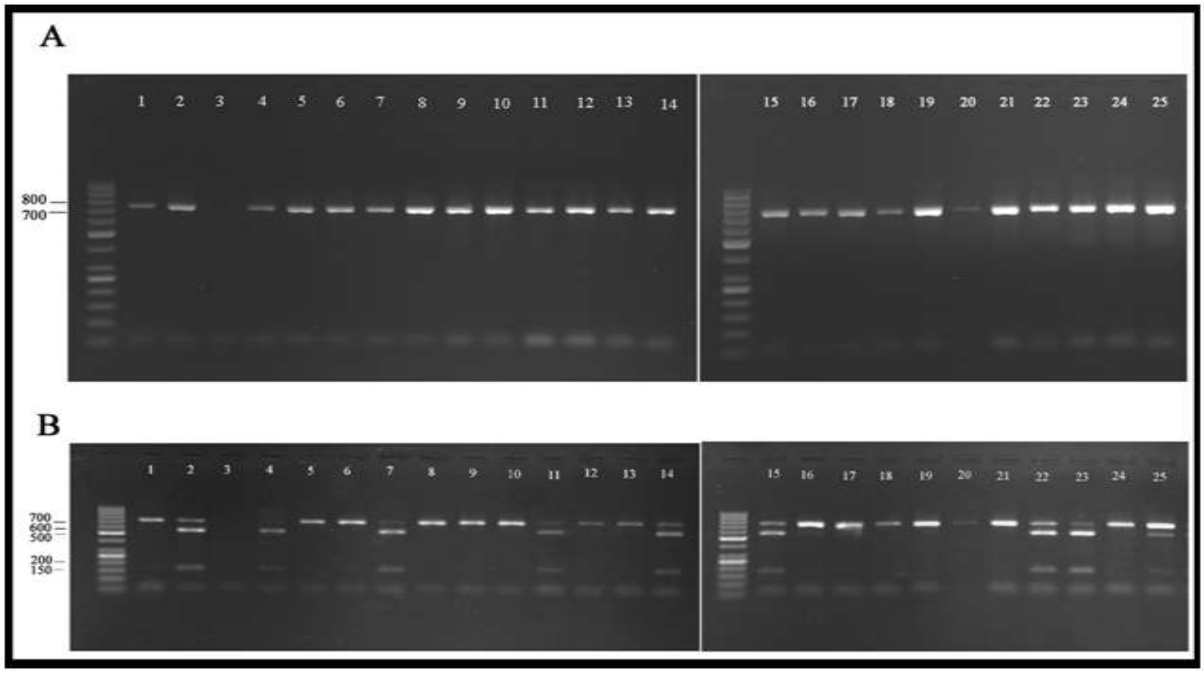

Fig. 2. PCR product patterns of tomato cultivars, landraces and wild species for. A, REXF1/REXR2 primer pairs before restriction with *Taq* I. B, The pattern of digestion of PCR product with *Taq* I. 1 to 25: Polaris, ES1002, Super Strain B, Super Beita, Super Chief, NDM-447, Ant 93-04, Mobil, Urmia1,Urmia 2, Laleh, Sardasht, Vakil Abad, Nun 6108, Samrudhi (*Mi/mi*), CH Falat (*mi/mi*), Cherry, Korall, Cal. J. N3, Rio grande, LA2184, Pascal, LA0111, LA0722, LA1223, respectively. (Marker: Gene Ruler ™ 50 bp DNA ladder, Fermentas®).

Using REX marker, the results of present study are consistent with results of some other researches. But the digested PCR product of a landrace (Laleh) and a wild species (LA1223 accession) which were homozygous susceptible as treated with two other markers, showed three fragments as expected for heterozygous resistant genotypes. Skupinova*et al.* (2004)obtained a 750 bp fragment in all tested genotypes using REX marker. While homozygous susceptible genotypes (*mi/mi*) were not digested with *Taq*I enzyme the heterozygous (*Mi/mi*) genotypes yielded three fragments (750, 570 and 160 bp) using the enzyme and two fragments (570 and 160 bp) appeared by the homozygous resistant genotypes (*Mi/Mi*).Devran*et al.* (2010) used similar marker and reported a fragment of nearly 700 bp in their genotypes and after digestion by *Taq*I, two fragments (550 and 150 bp) were shownfor homozygous resistant and nothing for susceptible genotypes. Meanwhile, in a single genotype(Astona F1), three fragments (700, 550 and 150 bp) were observed that indicates its heterozygous identity. In another study that carried out by Devran*et al.* (2013) with the marker, they had gained the same response.

Jacquet*et al.* (2005) evaluated the response of six homozygous resistant (*Mi/Mi*) lines, eight heterozygous (*Mi/mi*) hybrids and two standard homozygous susceptible (*mi/mi)* and resistant (*Mi/Mi*) controls of tomatoes against infection with virulent and avirulent populations of *Meloidogyneincognita*

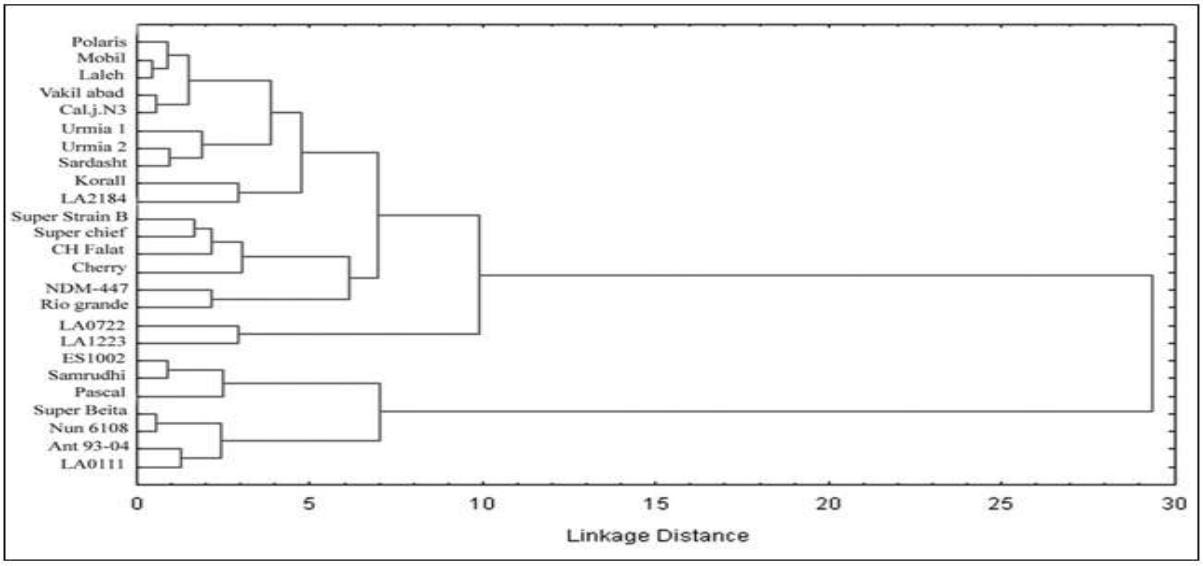

Fig. 3. Grouping of tomato cultivars, landraces and wild species as revealed by Ward's algorithm and Euclidean distance using reproductive factors of *Meloidogynejavanica*.

They indicated that the reproduction of nematode on heterozygous genotypes was significantly more than that of homozygous resistant ones. In addition to gene dosage, the genetic background of the tomato had a significant effect on reproduction of the nematode especially when the tomato genotype was in a heterozygous allelic condition of *Mi* gene.

Conclusion

Considering the outputs of both molecular and greenhouse experiments in present study, it can be concluded that the Mi23 and PMi3 markers have an equal capability for separating the resistant genotypes from susceptible ones. The two former markers andREX gave similar results, but some exceptions are deserved to be realized. When these markers, especially Mi23 and PMi3 are used, separation of the resistant and susceptible cultivars against infection with the root-knot nematode is easily possible, at least as a preliminary test before commence of greenhouse experiments. However, it should be noted that since in this study only the usual and available cultivars of tomatoes that are commonly grown in Iran were evaluated based on molecular and conventional approaches, the results could be completed and promoted by utilizing more different genotypes and markers as well.

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