Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2012, 2 (4):931-939

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Screening of resistance genes to fusarium root rot and fusarium wilt diseases in tomato (*Lycopersicon esculentum*) cultivars using RAPD and CAPs markers

Bahar Morid[1]*, Shahab Hajmansoor[2], Nikoo Kakvan[1]

[1]*Department of Plant Protection, College of Agriculture, Takestan Branch, Islamic Azad University, Takestan, Iran*
[2]*Department of Plant Pathology, Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran*

ABSTRACT

Fusarium diseases inhabit most tomato-growing regions worldwide, causing tomato production yield losses. The best way to produce tomato is developing resistant cultivars against Fusarium species. Molecular marker linked to resistance gene would be useful for tomato improvement programmes. In this study RAPD and cleaved amplified polymorphic sequence (CAPS) markers were used to screen tomato (Lycopersicon esculentum) lines against resistance genes Frl and I-2, respectively. To understand better the genotypic structure whether they are homozygous or heterozygous, further analysis was carried out by digestion of the PCR products with Fok I and Rsa I restriction endonuclease. An Rsa I-digested fragment of 500 bp and two restriction fragments of 390 and 410 bp for Fok I digestion of $TAO1_{902}$ were revealed in the homozygous resistant plants Results showed that out of 27 tomato cultivars, 14 were resistant, and 13 were susceptible to fusarium wilt and 20 resistant and 7 susceptible to Fusarium root rot, respectively. These results were confirmed with pathgenesity test.

Key words: resistance gene, *frl* gene, *I-2* gene, marker assisted selection, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Fusarium oxysporum* f. sp. *lycopersici*

Introduction

Cultivated tomato (*Lycopersicon esculentum* Mill.) is one of the world's most important crops due to the high value of its fruits both for fresh market consumption and in numerous types of processed products [1]. One of the main constraints to tomato cultivation is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production [2,3]. Fungal phytopathogens are cause of many plant diseases and much loss of crop yields, especially in tropical and subtropical regions [4]**.** *Fusarium oxysporum* is major soilborne fungal pathogens of both greenhouse and field grown tomatoes in the warm vegetable growing areas of the world [5]. Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* causing damping off, cankers, root rots, fruit decay, foliage disease causes serious economic loss. *Fusarium oxysporum* penetrates the roots mainly through wounds and proceeds into and throughout the vascular system, leading to functional collapse, systemic wilting and often the death of the infected plant *Fusarium oxysporum* f. sp. *radicis-lycopersici (FORL)*

cause disease on hosts from several plant families, including tomato in the greenhouse [6]. Fusarium crown and root rot of tomato often referred to as 'crown rot' [7]. Crown rot develops primarily in cool climates in both field and greenhouse tomatoes. Substantial crop losses in infected fields have given the disease international attention. *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) causes disease only in plants of the genus *lycopersicon* [8] and inhabits most tomato growing regions worldwide, causing tomato production yield losses [9]. This fungus responsible for vascular wilt disease in tomato and infects the vascular system of roots, inhibiting water transport, which in turn results in rapid plant death [10-11]. The first symptom of fusarium wilt in gardens and fields is usually the golden yellowing of a single leaflet or shoot, or a slight wilting and drooping of the lower leaves on a single stem. Yellowed and wilted leaflets drop early. Affected plants turn to bright yellow, wilt, dry up, and usually die before maturity, producing few, if any, fruit. The control of the pathogen spread mainly in three strategies: husbandry practices, application of agrochemicals and use of resistant varieties [2]. Resistant varieties are mostly produced by crossing resistant wild types and existing cultivars developed for their properties like good taste, shape and color. A molecular marker linked to resistance would be useful for tomato improvement programmes [9]. *FORL* and *FOL* distinguished based on the following characteristics: 1) The *FORL* pathogen has distinctly different symptoms from those caused by *FOL.* Disease symptoms in mature crops caused by *FORL* are those of root and basal stalk rot rather than vascular wilt. 2) Crown and root rot disease occurs at cool (18^oC) soil temperatures [12] while that caused by *FOL* is most severe at soil temperatures of about 27^oC. 3) The host range of *FORL* is larger than *FOL* [8,6]. When Rowe tested pathogenicity to 17 plant species by inoculating different isolates of the crown rot organism, various species of the family Leguminosae, as well as *L. esculentum*, were infected. However, *F. oxysporum* f. sp. *lycopersici* is specific only to *Lycopersicon* spp. [8].

Three physiologic races of *FOL*, named 1, 2 and 3 in order of their discovery [13,14], are traditionally distinguished by each having a specific pathogenicity to tomato cultivars. Species of *Fusarium* are traditionally differentiated by their morphological characteristics on selective media [15,16]. It is almost impossible, however, to identify pathogenic types, or formae speciales, and races of *Fusarium oxysporum*, using morphological features. More recently molecular markers have become popular for this purpose. Some of the techniques that have been reported are: DNA fingerprinting with nuclear repetitive DNA sequences [17], amplified fragment length polymorphism (AFLP) [18], random amplified polymorphic DNA (RAPD) [19,20], restriction fragment length polymorphisms (RFLP) [21-5], direct amplification of length polymorphism [22] and microsatellite markers [23]. The PCR-based differentiation of the various races of the vascular wilt fungi of tomato, *FOL* and *FORL* using specific primer sets [24]. Control of the pathogen spread mainly in three strategies: husbandry practices, application of agrochemicals and use of resistant varieties [2]. Resistant varieties are mostly produced by crossing resistant wild types and existing cultivars developed for their properties like good taste, shape and color. A molecular marker linked to resistance would be useful for tomato improvement programmes [9]. Breeding of resistant cultivars is an alternative approach to chemical treatments, limiting environmental and consumer risks. Four race-specific R genes for resistance to this pathogen have been genetically mapped in tomato and introgressed into commercial tomato cultivars from wild tomato species [2-25]. The genes *I*-1 and *I*-3 are located on chromosome 7, whereas *I* and *I*-2 are known to be on the short and long arms of chromosome 11, respectively. The gene *I*-2 confers resistance to FOL race 2 [26]. A CAPS marker, designated $TAO1_{902}$, linked to the *I*-2 gene, which useful for marker-assisted selection in tomato. Molecular marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers. In the context of MAS, DNA-based markers can be effectively utilized for tracing favorable allele(s) (dominant or recessive) across generations and identifying the most suitable individual(s) among the segregating progeny, based on allelic composition across a part or the entire genome [27].

MATERIALS AND METHODS

Isolation and storage of isolates

A total of 35 isolates of *F. oxysporum* were used in this study. The isolates were collected from wilting tomato plants in greenhouses in Iran. Stems and roots from symptomatic plants were washed with tap water. After removal of the stem and root cortex, small pieces of chocolate brown vascular tissue were surface-sterilized in 0.5% NaOCl for 30-60 sec, then placed in petri plates containing 2% water agar or acidified potato-dextrose agar (APDA) containing 2 ml of 25% lactic acid per liter. Plates were incubated in a laboratory incubator at 25 ± 1^oC. After 2-3 days, colonies of *F. oxysporum* were sub-cultured onto nonacidified PDA. Monoconidial isolates were prepared. Then microspore suspensions of these were added to sterile soil in small vials and incubated at 25^oC for 10-14 days. For long-term storage, soil cultures were kept at 4^oC**.**

Disease test
Disease test was down two times. To compare the pathogenic reactions of *F. oxysporum* isolates, Early Urbana 111 cultivar was selected that susceptible to FORL and FOL racees. Tomato seedlings were inoculated at the two-leaf stage. Plants were inoculated by soaking their roots in a freshly prepared spore suspension, (or, in the case of control seedlings, water) for 1 min and replanted to pots (9 cm in diameter). Seedlings were maintained at 25^{o}C under 12 h of fluorescent light per day. After 3 weeks, they were uprooted and the lower stem and tap root were longitudinally sectioned for examination of internal tissues. Each plant was rated on a scale of 0-4 as follows: 0 = healthy plants; 1 = < 25% vascular discoloration; 2 = 26-50% vascular discoloration; 3 = wilting with 51-75% vascular discoloration; and 4 = 76-100% vascular discoluration or death [28].

To compare the reactions of tomato varieties to FOL races and FORL isolates, one isolate was selected from each of formae specials and races with the highest pathogenicity. Plants were inoculated with method that described previously. At 3 weeks post-inoculation, plants were visually rated for disease symptom development on vertical and horizontal sections of roots and hypocotyls. Plants were classified as resistant (with no symptoms) and susceptible (with symptoms)**.**

Extraction genomic DNAs from fungal mycelium
Genomic DNA of *F. oxysporum* was extracted from fungal mycelia using CTAB method with some modifications [29].

Polymerase chain reaction (PCR) to identify FORL and races of FOL
To amplify the fragment of the endo and exo polygalacturonase using the primer sets designed by Hirano and Arie [24]. that the nucleotide sequence for these primers has showed in table1. Amplification was performed using a Eppendorf Thermal Cycler Mastercycler (Gradient). The PCR reaction mixture (25µl) consisted of 0.4 mM dNTPs, 0.4 mM of primer, 2 mM $MgSO_4$, 1X PCR buffer, 1 units of *Taq* polymerase with template DNA 40 ng. The thermal conditions were set as follows: initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 60 s, annealing at an appropriate temperature for 60 s, and elongation at 72°C for 2 min; final extension at 72°C for 7 min. Amplification products were resolved using 1.2% agarose gel under 3 V/cm potential and 90 mA currency in TBE buffer for 2 h. Gels were stained with ethidium bromide for 30 min and visualized under UV light using UVP Transillaminator.

Table1: Primers that used in this study

primers	Melting Point (°c)	Sequence	Ampelicons size (bp)
Uni f	55.9	5'-ATCATCTTGTGCCAACTTCAG-3'	670-672
Uni r	56.5	5'-GTTTGTGATCTTTGAGTTGCCA-3'	
SP 13f	59.4	5'-GTCAGTCCATTGGCTCTCTC-3'	445
SP 13r	57.3	5'-TCCTTGACACCATCACAGAG-3'	
SP 23 f	57.9	5'-CCTCTTGTCTTTGTCTCACGA-3'	518
SP 23 r	59.4	5'-GCAACAGGTCGTGGGGAAAA-3'	
TAO1 f	61.8	5'-GGGCTCCTAATCCGTGCTTCA-3'	902
TAO1 r	62.1	5'-GGTGGAGGATCGGGTTTGTTTC-3'	
UBC194	34	5'-AGGACGTGCC-3'	590

Extraction of total genomic DNA from plant tissues
Total genomic DNA of the 27 plants (Table3), were used for DNA extraction. Fresh young leaves of the lines were subjected to extraction, in 200 mg of fresh tissue, using the CTAB procedure described by Ausubel [1].

PCR conditions
Extracted DNA was diluted as 5 ng per 1 µL. RAPD analysis was performed according to Fazio [7]. The nucleotide sequence for UBC194 primer has showed in table1. The marker was amplified in 25 µL reaction volume containing 0.4 mM dNTPs, 0.4 mM of primer, 2 mM $MgSO_4$, 1X PCR buffer, 1 units of *Taq* polymerase with 40 ng template

DNA. The PCR parameters were: 94°C for 45 s followed by 36 cycles of 94°C for 35 s, 31°C for 60 s, 72°C for 70 s and a final extension time of 7 min at 72°C, using an Eppendorf Thermal Cycler Mastercycler (Gradient).

The marker $TAO1_{902}$ was amplified in 25 µL reaction volume of 0.2 mM dNTPs, 0.2 mM of each primer, 2.67 mM $MgSO_4$, 1X PCR buffer, 1 units of *Taq* polymerase with 40 ng template DNA. The PCR parameters were: 94°C for 60 s followed by 40 cycles of 93°C for 15 s, 63°C for 20 s, 72°C for 60 s and a final extension time of 5 min at 72°C, using an Eppendorf Thermal Cycler Mastercycler (Gradient). The sequences of primers for TAO1 and UBC 194 (Germany, MWG) have showed in table1. PCR products were digested with 5 units of *Fok*I restriction endonuclease fastDigest (Germany, Fermentas) in a 15 µL reaction volume containing recommended buffer (FDBuffer 2144) for 5 M at 37°C and an enzyme inactivation step was performed for 5 min at 65°C. Amplification products were resolved using 2% agarose gel under 3 V/cm potential and 90 mA currency in TBE buffer for 3 h. Gels were stained with ethidium bromide for 30 min and visualized under UV light using UVP Transillaminator.

RESULTS

PCR based identification of FOL, FORL and races in FOL

Formae specials and races were determined according to the primer sets designed by Hirano and Arie [24]. Primer set Uni was used to differentiate *FOL* and *FORL* from other formae specials of *F. oxysporum*. The Uni primer set amplified a 670-672 bp fragment from isolates of *FOL* and *FORL*. Primer Sp23 was used to differentiate *FOL* race 2 and 3 from *FOL* race 1 and *FORL*. The Sp23 primer set amplified a 518 bp fragment from *FOL* race 2 and 3 but did not amplify any fragment from *FOL* race 1 and *FORL*. The Sp13 primer set amplified a 445 bp fragment from *FOL* race 1 and 3 but did not amplify any fragment from race 2 and *FORL*. In this study a total of 44 isolates including 7 isolates of *FOL* race 1, 12 isolates of *FOL* race 2, 11 isolates of *FOL* race 3, 14 isolates of *FORL* (Table 2).

Table 2: Determination of formae specials, races and disease index (DI) in *Fusarium oxysporum* isolates.

Isolate	Uni	Sp13	Sp23		DI	Isolate	Uni	Sp13	Sp23		DI
F1	+	-	+	Race2	3.6	F23	+	+	+	Race3	4
F2	+	-	-	*Forl*	3	F24	+	+	-	Race1	3.3
F3	+	-	-	*Forl*	2.6	F25	+	+	+	Race3	2
F4	+	-	+	Race2	3	F26	+	-	-	*Forl*	3.3
F5	+	-	+	Race2	3.3	F27	+	+	-	Race1	4
F6	+	+	+	Race3	4	F28	+	+	+	Race3	2.6
F7	+	+	+	Race3	2.6	F29	+	-	-	*Forl*	2
F8	+	-	-	*Forl*	1.3	F30	+	+	+	Race3	3.6
F9	+	-	-	*Forl*	2.6	F31	+	-	-	*Forl*	3
F10	+	+	+	Race3	3	F32	+	-	-	*Forl*	3
F11	+	-	-	*Forl*	2	F33	+	-	+	Race2	3.3
F12	+	+	-	Race1	2.6	F34	+	-	+	Race2	4
F13	+	+	+	Race3	2	F35	+	-	+	Race2	1
F14	+	-	+	Race2	1.3	F36	+	-	+	Race2	3
F15	+	+	-	Race1	3	F37	+	-	-	*Forl*	2.6
F16	+	+	+	Race3	2.6	F38	+	-	-	*Forl*	3.3
F17	+	-	-	*Forl*	3.3	F39	+	-	-	*Forl*	3
F18	+	-	+	Race2	4	F40	+	-	+	Race2	4
F19	+	+	+	Race3	3	F41	+	-	+	Race2	3.6
F20	+	+	-	Race1	2	F42	+	+	-	Race1	4
F21	+	+	+	Race3	1	F43	+	+	-	Race1	1.3
F22	+	-	-	*Forl*	2.6	F44	+	-	+	Race2	3

Amplification with RAPD primer

According to Fazio [7], amplification with UBC 194 primer gives a marker (590 bp) linked to *Frl* gene. In this study, 20 of the 27 samples revealed the 590 bp marker (Fig. 1) so they were determined to be *Frl* positive (Table 3).

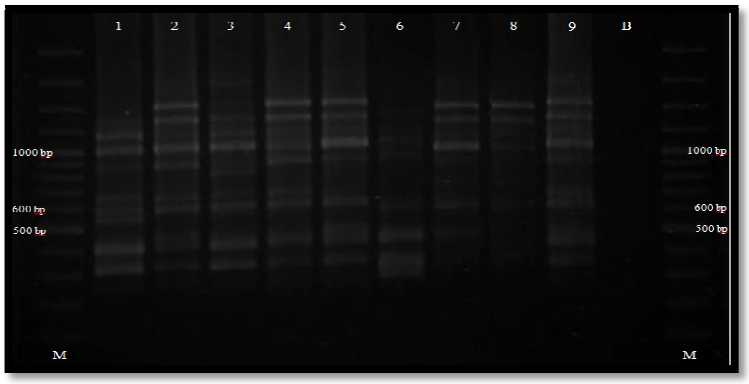

Figure 1: PCR products amplified with UBC 194 primer. 1-5 and 9 are resistance varieties and 6-8 are susceptible varieties. 1: Peto Rock, 2: Queenty, 3: Eden, 4: Caligen, 5: Firenze, 6: Speedy, 7: Super 2270, 8: Super Queen, 9: Fani, M: Marker

Table 3: Distribution of the restriction fragments of the CAPS marker $TAO1_{902}$ and USB 194 primer in tomato cultivars, and F1 hybrids

Cultivars or F1 hybrids	Response to *FOL*	TAO1 restriction fragments *Rsa* I		*Fok* I		Response to *FORL*
		A	B	C	D	
Peto Rock	**R**	+	+	+	+	**R**
Sunseed	**R**	+	+	+	+	**R**
Cal JN	**R**	+	+	+	+	**R**
Cal JN 3	**R**	+	+	+	+	**R**
Caligen	**R**	+	+	+	+	**R**
Hyb. Petopride 5	**R**	+	+	+	+	**S**
Hyb. Queenty	**R**	+	+	+	+	**R**
Hyb. Comodoro	**R**	+	+	+	+	**S**
Matin	**R**	+	+	+	+	**S**
Hyb. Petopride II	**R**	+	+	+	+	**R**
Hyb. Speedy	**R**	+	+	+	+	**S**
Navid-3(N.3)	**R**	+	+	+	+	**R**
Hyb. Fani	**R**	+	+	+	+	**R**
Hyb. Firenze (PS8094)	**R**	+	+	+	+	**R**
Chef	**S**	-	+	-	+	**R**
Falat Y	**S**	-	+	-	+	**R**
Peto Early 84	**S**	-	+	-	+	**R**
CH-Falat	**S**	-	+	-	+	**R**
Super Queen	**S**	-	+	-	+	**S**
Hyb. PS 6515	**S**	-	+	-	+	**R**
Karoon	**S**	-	+	-	+	**R**
Hyb. Eden F1	**S**	-	+	-	+	**R**
Hyb. Pulad	**S**	-	+	-	+	**R**
Hyb. Petopride 6	**S**	-	+	-	+	**R**
Early Urbana 111	**S**	-	+	-	+	**S**
King Stone	**S**	-	+	-	+	**R**
Super 2270	**S**	-	+	-	+	**S**

FOL, Fusarium oxysporum f. sp. lycopersici; FORL, Fusarium oxysporum f. sp. radicis-lycopersici, A, Rsa I–restriction fragment of 500 bp; B, Rsa I–restriction fragment of 220 bp; C, Fok I restriction fragments of 390 and 410 bp; D, Fok I-restriction fragment of 800 bp; +, presence of marker;), - absence of marker; R, resistant; S, susceptible.

Amplification with CAPS primers and restriction with Fok I and Rsa I

Amplification with TAO1 primers revealed the 902 bp fragment for 27 varieties (Fig. 2). A size of 902-bp-long fragment of the TAO1 marker was found to be polymorphic in tomato lines [9]. The DNA polymorphism was revealed after digestion of the amplicons with restriction enzymes *Rsa*I and *Fok*I. But, to understand better the

genotypic structure whether they are homozygous or heterozygous, further analysis was carried out by digestion of the PCR products with *Fok* I and *Rsa* I restriction endonuclease.

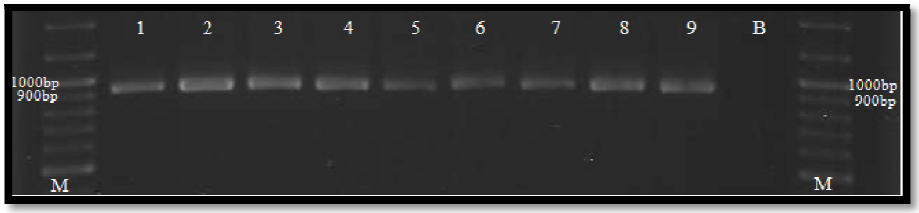

Figure 2: PCR products amplified with TAO1 primer. All of the samples show 902 bp band. 1: Caligen, 2: Queenty, 3: Matin, 4: Fani, 5: CH-Falat, 6: Eden, 7: Pulad, 8: Early Urbana 111, 9: Super 2270, M: Marker

After digestion with *Fok* I, some of the samples revealed 390 and 410 bp fragments as reported by Staniazsek [9]. These fragments show that both alleles from parents related to the *I*-2 gene are present in the sample, thus the lines were considered to be from a homozygous resistant plant. Fourteen (14) tomato varieties showed these restriction fragments. Full list of the plants was given in Table 3 showing the positive and negative results.

The DNA polymorphism was revealed after digestion of the amplicons with restriction enzymes *Rsa* I and *Fok* I. An *Rsa* I-digested fragment of 500 bp and two restriction fragments of 390 and 410 bp for *Fok* I digestion of $TAO1_{902}$ were revealed in the homozygous-resistant plants (Fig. 3) Restriction products were analysed in 27 plants and the *I-2*-specific restriction fragments were detected in all 14 resistant but not in the 13 susceptible plants. The genotyping of 9 plants using the *Fok* I-digested fragments is shown in Fig. 3 and the genotyping of 9 plants using the *Rsa* I digested fragments is shown in Fig. 4. 27 genetically diverse tomato cultivars, lines and F1 hybrids were scored using $TAO1_{902}$ (Table 3).

Figure 3: PCR products digested with *Fok* I restriction endonuclease of samples. 1 – 5 are resistance varieties and 6-10 are susceptible varieties. 1: Sunseed, 2: Matin, 3: Fani, 4: Caligen, 5: Petopride II, 6: Karoon, 7: Chef, 8: pulad, 9: Eden, M: Marker

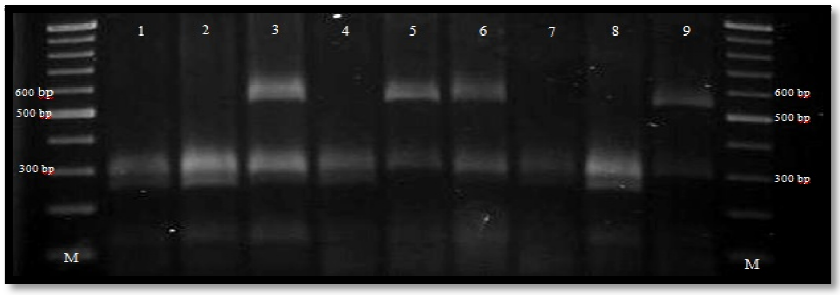

Figure 4: PCR products digested with *Rsa*I restriction endonuclease of samples. 1-4 and 8 are resistance varieties and 5-7 and 9 are susceptible varieties. 1: Sunseed, 2: Matin, 3: Fani, 4: Caligen, 5: Pulad, 6: Karoon, 7: Chef, 8: queenty, 9: Eden, M: Marker

The codominant marker $TAO1_{902}$ directly recognizes the homozygote and heterozygote classes in F2 progeny, making the selection process for *F. oxysporum* f. sp. *lycopersici* resistance more precise compared to phenotypic selection in segregating populations [9].

Disease test

Tomato varieties that showed resistance amplicons with RAPD and CAPS primers, no symptoms produced on tomato plants while the varieties did not show resistance band produced disease symptoms with different disease index (Table 4).

Table 4: Determination of disease index (DI) in tomato cultivars, and F1 hybrids to *FOL* races and *FORL* isolates

Cultivars or F1 hybrids	Race 1	Race 2	Race 3	*FORL*
Peto Rock	0	0	0	0
Sunseed	0	0	0	0
Cal JN	0	0	0	0
Cal JN 3	2	0	1	0
Caligen	2	0	2	0
Hyb. Petopride 5	0	0	0	2
Hyb. Queenty	2	0	0	0
Hyb. Comodoro	0	0	0	3
Matin	0	0	4	1
Hyb. Petopride II	0	0	0	0
Hyb. Speedy	0	0	0	2
Navid-3(N.3)	1	0	1	0
Hyb. Fani	0	0	0	0
Hyb. Firenze	0	0	0	0
Chef	0	3	1	0
Falat Y	1	2	1	0
Peto Early 84	3	2	2	0
CH-Falat	3	4	2	0
Super Queen	0	2	1	3
Hyb. PS 6515	0	1	1	0
Karoon	1	3	2	0
Hyb. Eden F1	0	2	1	0
Hyb. Pulad	2	2	1	0
Hyb. Petopride 6	2	3	2	0
Early Urbana 111	3	4	3	4
King Stone	4	3	3	0
Super 2270	3	3	4	4

DISCUSSION

Tomato is one of the most widely grown vegetable crops in the world. [2]. One of the most important diseases of tomato, is fusarium wilt, caused by three races of *F. oxysporum* f. sp. *lycopersici*. Races 1 and 2 are distributed worldwide, whereas race 3 has a more limited geographic distribution. In Iran race 3 was reported. *F. oxysporum* f. sp. *radicis-lycopersici* (*FORL*) causes fusarium crown and root rot of tomato often referred to as 'crown rot' [7] which also gives a substantial damage to crops. Conventionally, genetic diversity is estimated on the basis of

morphological and phenotypic characters. Molecular tools provide more accurate methods for identification than the few characters afforded by traditional morphological features [30]. Assessment of crop germplasm diversity phenotypically and morphologically is usually devoid of the resolving power needed to identify an individual genotype. Identification based on morphological characters is time consuming and requires extensive field trials and evaluation [31]. A phenotypic selection for fusarium wilt resistance is a complex and time-consuming process in tomato [7]. DNA marker technology has been used in commercial plant breeding programmes has proved helpful for the rapid and efficient transfer of useful agronomically important traits into desirable varieties and hybrids [7].

In Conclusion 27 varieties of tomato were screened for *I*-2 resistance gene by $TAO1_{902}$ CAPS marker and for *Frl* resistance gene by UBC 194 RAPD marker. These analyses revealed that twenty of the lines screened were resistant to the soil-borne fungus *F. oxysporum* f. sp. *radicis-lycopersici* (*FORL*) and fourteen of them had the *I*-2 gene which confers resistance to *F. lycopersici* race 2. Peto rock, Sunseed and Cal JN tomato caltivars and Petpopride II, Fani and Firenze hybrids were resistance to *FORL* and all races of *FOL* so they are recommended for planting in all infected areas. Super 2270, Early Urbana 111 and Super Queen tamato caltivars and Comodoro hybrid were susceptible to *FORL*. CH-Falat, Karoon, Early Urbana 111 and King Stone were susceptible to three races of *FOL*. Early Urbana 111 and Super 2270 caltivars so planting them should be avoided.

Acknowledgement
This work was financially supported by the Research Deputy of Takestan Branch, Qazvin-Iran.

REFERENCES

[1] Giovanni CD, Orco PD, Bruno A, Ciccarese F, Lotti C, Ricciardi L, *Plant Sci*., **2004**, 166, 41-48.
[2] Barone A, Frusciante L, *Marker-Assisted selection, Current status and future perspectives in crops, livestock, forestry and fish*, **2007**, 153-164.
[3] Sasirekha B, Shivakumar A, *Asian J. Plant Sci. and Res*., **2012**, 2 (2), 180-186.
[4] Cirumalla RG, Sharan M, Sharon, M, *Euro. J. Exp. Bio.*, **2011**, 1(2), 17-22.
[5] Rosewich UL, Pettway RE, Katan T, Kistler HC, *Phytopathol*., **1999**, 89, 623-630**.**
[6] Menzies JG Koch C, Seywerd F, *Plant Dis*., **1990**, 74, 569-572.
[7] Fazio G, Stevens MR, Scott JW, *Euphyt*, **1998,** 105, 205-210.
[8] Rowe RC, *Phytopathol*., **1980**, 70, 1143-1148.
[9] Staniazsek M, Kozik EU, Marczewski W, *Plant Breed*., **2007**, 126(3), 331-333.
[10] Malhotra SK, Vashistha RN, *Indian J. Agric. Sci*., **1993**, 63, 246-347.
[11] McGrath DJ, Gillespie G, Vawdrey L, *Aust. J. Agric. Res*., **1987**, 38, 729-733.
[12] Jarvis WR, Thorpe HJ, *Plant Dis*., **1976**, 60, 1027-1031.
[13] Booth C, *The genus Fusarium*, Commonwealth Mycological Institute, Kew, Surrey, England, **1971**.
[14] Grattidge R, Obrien RG, *Plant Dis.*, **1982**, 66, 165-166.
[15] Nelson PE, Toussoun TA, Marasas WFO, *Fusarium Species: an illustrated annual for Identification*, Pennsylvania State University Press, **1983**.
[16] Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D, *Laboratory Manual for Fusarium Research*, University of Sydney, Sydney, Australia, **1994**, pp 783.
[17] Namiki F, Shiomi T, Kayamura T, Tsuge T, *Appl. Environ. Microbio.*, **1994**, 60, 2684-2691.
[18] Vos P, Hogers R, Bleeker M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M, *Nuc. Acids Res*., **1995**, 23, 4407-4414.
[19] Kalc GF, Wright DI, Guest D, Wimalajeewa R, *Europ. J. Plant Pathol*., **1996**, 102, 451-457.
[20] Balmas V, Scherm B, Primo PD, Rau D, Marcello A, Migheli Q, *Europ. J. Plant Pathol*., **2005**, 111, 1-8.
[21] Baayen RP, van Dreven F, Krijger MC, Waalwijk C, *Europ. J. Plant. Pathol*., **1997**, 103, 395-408.
[22] Desmarais E, Lanneluc I, Langel J, *Nuc. Acids Res*., **1998**, 26, 1458-1465.
[23] Huang CCH, Lindhout P, *Euphyt.*, **1997,** 93, 145-153.
[24] Hirano Y, Arie T, *Jap. J. Gen. Plant Pathol*., **2006**, 72, 273-283.
[25] Frary A, Tanksley SD, *The molecular map of tomato*. Phillips RL, Vasil IK, (Eds.). Kluwer Academic Publishers, Dordrecht/Boston/London, **2001**, 125-183.
[26] Stall RE, Walter JM, *Phytopathol*., **1965**, 55, 1213-1215.
[27] Kamali M, Ahmadikhah A, Pahlavani MH, Dehghan MA, Sheikh F, *Adv. Appl. Sci. Res.*, **2010,** 1 (3),180-186.
[28] Validov SZ, PhD thesis (Netherland, **2007**).

[29] Ausubel FM, Brent R, Kingston RE Moore DD, Seidman JG, Smith JA, Struhl K, *Current Protocols in Molecular Biology*, John Wiley & Sons Inc, **1994**, pp 426.
[30] Sasidhara R, Thirunalasundari T, *Euro. J. Exp. Bio.*, **2012**, 2 (2), 369-373.
[31] Noori A, Ahmadikhah A, Soughi H, Dehghan M, *Adv. Appl. Sci. Res.*, **2010**, 1 (3),153-159.