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Research Article

Molecular identification of formae specialis and racial identity in Iranian strains of *Fusarium oxysporum* f. sp. *lycopersici*: detection of avirulence genes

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Abstract: *Fusarium oxysporum* f. sp. *lycopersici* (Fol) is the causal agent of vascular wilt in tomato, an important plant disease in Iran. Four monogenic resistance genes in tomato are used for identification of races of Fol and their corresponding avirulence genes *Avr1*, *Avr2* and *Avr3* were identified in pathogen one of which, *Avr2,* is f.sp. specific. Hence they can serve as reliable markers for racial identity and f.sp discrimination. These markers have been used for strains from other countries except Iran. Furthermore, a point mutation in *Avr3* can lead to enhanced virulence of Fol on a susceptible tomato cultivar. To identify forma specialis and racial identity, *Avr* genes were studied in a collection of Iranian strains. Results revealed that PCR assay is very efficient in distinguishing between non-pathogenic and low virulence strains and in the vast majority of strains, avirulence genotype was consistent with Fol race1. Furthermore, to determine whether allelic variation of *Avr3* could separate strains of different degrees of virulence, *Avr3* was sequenced in Fol strains with high and low virulence. The results revealed that allelic variation of *Avr3* was not correlated with degree of virulence in Iranian strains.

Keywords: Avirulence genotype, effector gene, tomato wilt, molecular detection

Introduction

During plant-pathogen co-evolution, plants employ an innate immune system. Adapted pathogens, however, confront this pressure by effector proteins (Jones and Dangl, 2006), which enable parasitic colonization (Hogenhout *et al*., 2009). On the other hand, in resistant cultivars, resistance genes encode specific receptors to recognize microbial effectors and trigger a second layer of defense. Therefore, genes that encode pathogen effectors can also be referred to as avirulence (*Avr*) genes. However, to avoid host defense responses, pathogens eliminate or modify their effectors. As it is clear, such adaptations will lead to the emergence of resistant varieties of plants and new races of pathogens in a pathosystem (Jones and Dangl, 2006). Therefore, studying effector genes has been considered as an important issue in phytopathology.

Vascular wilt disease of tomato, caused by Fol, is one of the most detrimental factors in tomato production that has been reported in at

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least 32 countries (Jones *et al*., 2014). Based on the ability of individual strains to overcome a specific immunity (*I*) gene (*I, I-1, I-2* or *I-3)*in host, Fol strains are divided into three races (Takken and Rep, 2010). So far, 14 (candidate) effector proteins have been identified in Fol (Schmidt *et al*., 2013), among which *SIX4* (*Avr1*), *SIX3* (*Avr2*) and *SIX1* (*Avr3*) have displayed avirulence function (Houterman *et al*., 2008; Houterman *et al*., 2009a; Rep *et al*., 2004). Elimination or modification of these avirulence genes enabled the pathogen to establish their races in its population; race2 arose from race1 by loss of *Avr1 (SIX4),* and race3 evolved from race 2 through point mutations in *Avr2 (SIX3)* (Hogenhout *et al*., 2009). Accordingly, studying avirulence genotypes could help distinguish Fol races (Lievens *et al*., 2009).

Fol belongs to *F. oxysporum* Schlechtend: Fr species complex. Since individual strains of *F. oxysporum* usually infect one or a few host species, they have been classified in more than 120 host-specific forms called formae speciales (f. spp.) (Michielse and Rep, 2009). Although greenhouse virulence test could be used to differentiate formae speciales of *F. oxysporum,* it is often associated with inherent problems (Correll *et al*., 1986), and requires analysis of large number of plant species (Fravel *et al*., 2003). Furthermore, replacing this method with molecular identification techniques is seriously complicated due to the polyphyletic nature of many formae speciales of *F. oxysporum* (Lievens *et al*., 2008). Previous studies showed that some of *Avr* genes are f.sp. specific, therefore these genes had high potential for molecular detection of f.sp. *lycopersic*i (Houterman *et al*., 2009a). Lievens *et al*. (2009) confirmed it in a worldwide collection of Fol strains but there are not any investigations on Iranian strains.

Fusarium wilt is an important tomato plant disease in Iran, which was first reported in Hormozgan province in 1985, and later in other locations (Amini, 2009; Amini *et al*., 2013; Etebarian, 1992; Fassihiani, 1985; Heidarzadeh, 2006; Manafi Dizaji *et al*., 2012). Despite several reports on Iranian Fol strains, no study has molecularly indicated forma specialis and avirulence genotype of races. Therefore, the aim of present research was to study avirulence genes in a collection of Iranian Fol strains, and clarify their forma specialis, racial identity and avirulence genotype. Finally the allelic variation of *Avr3 (SIX1)* was determined and the ability of this gene in the separation of strains with different degree of virulence studied.

Materials and Methods

Fungal strains

The fungal strains used for this study are showed in Table 1. This is a collection of 21 *F. oxysporum* strains, collected from 5 provinces of Iran including 18 FOL strains and 3 avirulent strains that were obtained from tomato roots (Table 1). Previously, races of Fol strains were determined by inoculation assays on the differential cultivars (Amini, 2009; Amini *et al*., 2013; Etebarian, 1992; Fassihiani, 1985; Heidarzadeh, 2006; Manafi Dizaji *et al*., 2012). Strains were cultured on potato dextrose agar (PDA; Merck, Darmstadt, Germany) containing 0.1 mg/mL streptomycin sulfate in the dark at 22 °C and then stored on sterile sand at 4 °C.

Polymerase chain reaction and sequencing

For polymerase chain reaction (PCR) analysis, genomic DNA extraction from mycelium was performed using Qiagen DNeasy Plant Mini Kit (Qiagen Co., Germany) and the DNA quantity and quality was spectrophotometrically determined. To confirm the quality of DNA extracts, all samples were subjected to a PCR program using the primers TOM2 and TOM3, which anneal to *FoToml* gene that encodes tomatinase in *F. oxysporum* (Ito *et al*., 2004) (table 2). Afterwards, the entire open reading frames of *Avr1 (SIX4)*, *Avr2 (SIX3)* and *Avr3 (SIX1)* were amplified by specific primers (Table 2). PCR amplification was performed in a reaction volume of 20 µL containing 200µM of dNTPs (GeNetBio Co., South Korea), 5µM

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of each primer (GeNetBio Co., South Korea) , 2mM $MgCl_2$, 0.02 U/µL Taq DNA polymerase (GeNetBio Co., South Korea), 2µL 10X PCR buffer (GeNetBio Co., South Korea) and 20 ng/µL genomic DNA. Thermal cycling conditions consisted of 3 min at 93 °C, Followed by 35 cycles of 45 s at 92 °C, annealing at appropriate temperature for 45 s, and 1 min at 72 °C, with a final elongation step at 72 °C for 5 min (Table 2). Amplified products were then loaded on 1% agarose gel for electrophoresis with 1X TAE buffer at 5Vcm. All reactions were performed four times. Following PCR amplification of *Avr3 (SIX1)*, a number of amplicons were sequenced. To do so, suitable size bands were excised from gels and purified using a Qiagen Gel Purification kit (Qiagen Co., Germany) according to the manufacturer's instructions. Then, 50-200 ng of PCR products was sequenced (Macrogen Co., Seoul, Korea), and finally analyzed by Clustal w version 2.1 (19 reference??). Also the PCR product of *Avr2 (SIX3)* was purified using the PCR purification kit and then ligated into the pTG19-T by T4 DNA ligase (Fermentas Co., Germany). The ligation mixture was transformed into *Escherichia* coli DH5 alpha. After plasmid present in colonies was verified by PCR specific primers Psh10-2F/R (Table 2), the positive colonies were cultured on liquid LB and plasmid was extracted by Plasmid Extraction kit (GenetBbio Co., South Korea) according to the manufacturer's instructions and verified by PCR specific primers SIX3 F/R and sequenced by specific primers Psh10-2F/R.

Table 1 Strains of *Fusarium oxysporum* used in current study.

Avirulence genotype	Avirulence gene PCR			Geographical region		Original code (Source)	Strain	Species
	Avr3 (SIX1)	*Avr2 (SIX3)*	*Avr1 (SIX4)*	Province	Country			
Avr1 – Avr2- ---	-	+	+	Khorasan	Iran	F24[a]	FOL001	*F.oxysporum* f.sp. *lycopersici*
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F10[a]	FOL002	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F20[a]	FOL003	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F15[a]	FOL004	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F7[a]	FOL006	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F22[a]	FOL007	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F1[a]	FOL013	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F11[a]	FOL015	
Avr1 – Avr2- ---	-	+	+	Khorasan	Iran	F18	FOL016	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F28[a]	FOL018	
Avr1 – Avr2 – Avr3	+	+	+	Hormozgan	Iran	23[a]	FOL021	
Avr1 – Avr2 – Avr3	+	+	+	Hormozgan	Iran	25(F-6-1)[b]	FOL022	
Avr1 – Avr2 – Avr3	+	+	+	Hormozgan	Iran	27(F-4-1)[b]	FOL023	
Avr1 – Avr2 – Avr3	+	+	+	-	USA	F-026-B[b]	FOL025	
Avr1 – Avr2 - ---	-	+	+	Kordestan	Iran	F30[c]	FOL028	
Avr1 – Avr2 - ---	-	+	+	Kordestan	Iran	F31[c]	FOL029	
Avr1 – Avr2 – Avr3	+	+	+	Kordestan	Iran	F58[c]	FOL030	
Avr1 – Avr2 - ---	-	+	+	Golestan	Iran	F75[c]	FOL032	
-	-	-	-	Khorasan	Iran	Fg1[d]	FO-039	*F. oxysporum*
-	-	-	-	Khorasan	Iran	F26	FO-011	
Avr1 – Avr2 – Avr3	+	+	+	Khorasan	Iran	F2[a]	FOL012	

a: Heidarzadeh *et al*. (2011).
b: Plant Protection Dept., College of Agriculture, Shiraz University, Shiraz, Iran.
c: Amini (2009).
d: Plant Protection Dept., College of Agriculture, Ferdowsi University, Mashhad, Iran.

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Virulence determination

To test the virulence of the strains on tomato cultivar Bonny Best, with no resistance to *Fusarium* wilt, the root dip method was used on 10-day-old seedlings (Wellman, 1939). Briefly, spores were collected from 5-day-old cultures in potato-dextrose broth (PDB; Merck, Darmstadt, Germany) and used for root inoculation of 10-day-old plants at a spore density of 10^7mL. For control plants inoculation was performed using distilled water. The plants were then potted individually and grown at 25 °C in a greenhouse. Disease index was scored 8 weeks after inoculation on the Following scale (Marlatt *et al*., 1996): 1 = no symptoms; 2 = slight chlorosis, stunting, or wilting; 3 = moderate chlorosis, stunting, or wilting; 4 = severe chlorosis, stunting, or wilting; 5 = death. The pathogenicity test was performed three times. Finally, for individual plants, sections of stem were cultured on PDA in the dark at 25 °C. The key of methods and identification of *F. oxysporum* were used to identify them (Leslie and Summerell, 2006).

Results

Confirmation of forma specialis lycopersici by *Avr2 (SIX3)*

Until now, *Avr2* (*SIX3*) has exclusively been found in Fol, indicating its importance as a Fol-specific marker (Lievens *et al*., 2009). Therefore, in an attempt to molecularly confirm the forma specialis *lycopersici* in Iran, we studied the presence of *Avr2 (SIX3)* using PCR technique. In this regard, specific primers SIX3-F/R (Table 2) that anneal just outside the ORF were used to screen DNA in 20 strains of Iranian *F. oxysporum.* Among our samples, 17 strains were previously classified as forma specialis *lycopersici* and 3 were non-pathogenic strains from tomato root that we used as negative control. To mention, the quality of extracted DNA was confirmed by PCR using primers for *FoToml* gene, and all strains showed one specific 1047 bp band. This result demonstrated that the extracted DNA had high quality for PCR reaction (Fig. 1-a). In Fig. 1-b PCR results for *Avr2* (*SIX3*) on the representative set of strains was shown. *Avr2* (*SIX3*) was amplified from all Fol strains in our collection. No specific band on approximately 600 bp (expected band size, 608 bp) was observed in non-pathogenic strains Fo-039 and Fo-011, as expected. These observations confirmed the results of previous pathogenicity test (Amini, 2009; Fassihiani, 1985; Heidarzadeh, 2006). Unexpectedly, such band was detected in non-pathogenic strain FOL012. This result was contradictory with the fact that known PCR detectable *SIX* genes (*SIX1* to *SIX7*), including *Avr2* (*SIX3*), are not present in non-pathogenic strains of *F. oxysporum* (Lievens *et al*., 2009).

Table 2 List of primers, their sequence, and product length used in the current study.

Code	Sequence (5′ to 3′)	Target	Amplicon length (bp)	Annealing temp. (C°)	Reference
P12-F2B	GTATCCCTCCGGATTTTGAGC	*Avr3(SIX1)*	992	53	Vander Doesetal. (2008)
P12-R1	AATAGAGCCTGCAAAGCATG				
SIX4-F1	TCAGGCTTCACTTAGCATAC	*Avr1(SIX4)*	967	53	Lievens *et al*. (2009)
SIX4-R1	GCCGACCGAAAAACCCTAA				
SIX3-F1	GGCAATTAACCACTCTGCC	*Avr2(SIX3)*	608	53	Hortman *et al*. (2009)
SIX3-R1	CCAGCCAGAAGGCCAGTTT				
Tom2	TGGAGTCAAGACGCCCGAACAAAGT	*FoToml*	1067	56	Ito *et al*. (2004)
Tom3	CATGAAGGCCACTATCATCATCGG				
Psh10-2-F	AGGGTTTTCCCAGTCACGA	pTG19-T	169	56	Abaspour *et al.* (2012)
Psh10-2-R	GAGCGGATAACAATTTCACAC				

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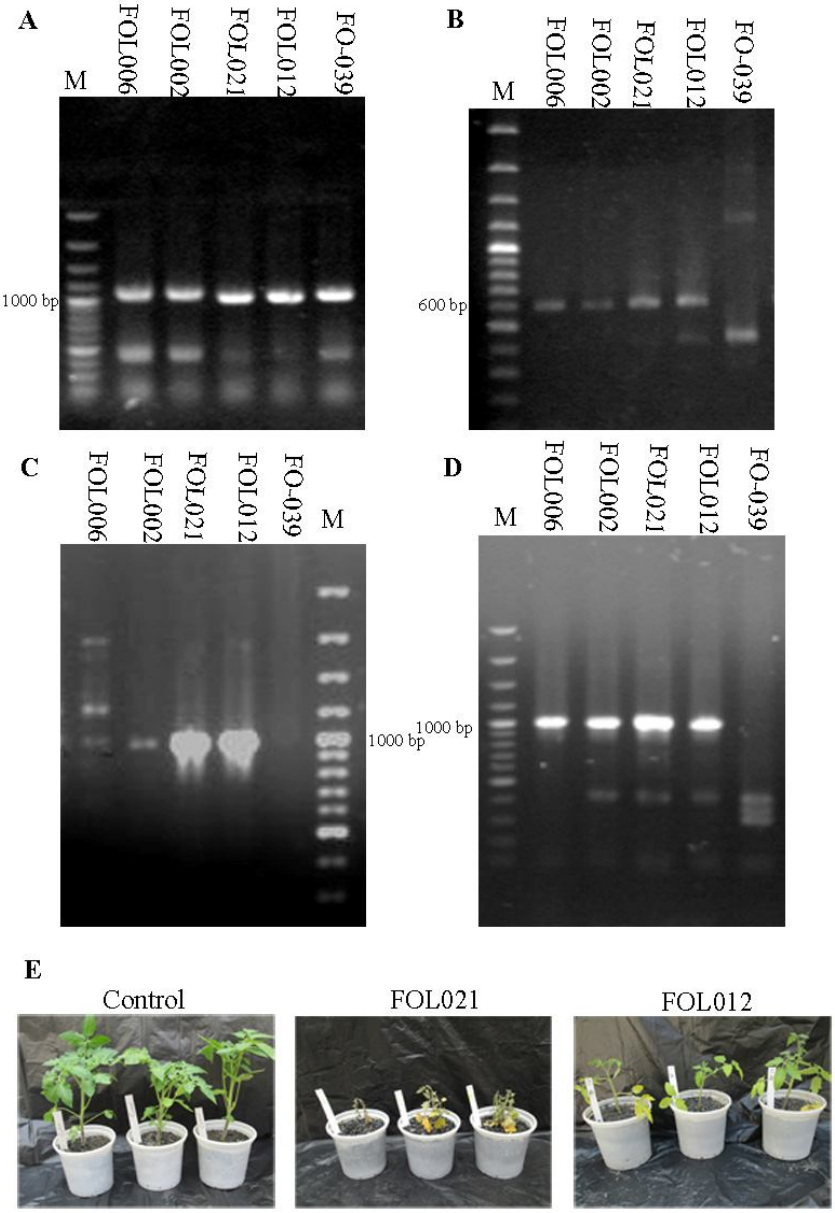

Figure 1 Agarose gel analysis of **A**) *Tomatinase,* **B**) *Avr2 (SIX3)* **C**) *Avr1 (SIX4)* **D**) *Avr3 (SIX1)* amplified from genomic DNA of a selection of *F. oxysporum* f.sp. *lycopersici* (FOL) and non-pathogenic strains. Details of strains are given in table 1. **E**) FOL012 caused low virulence on tomato plant. Three 10-day-old seedlings of cultivar Bonny Best (no resistance to Fusarium wilt) were inoculated with Fol021 (positive control), FOL012 (race 1 that was previously misidentified as non-pathogenic strain) FO-039 (non-pathogenic strain).

Confirmation of race 1 by *Avr1* (*SIX4*)

Previously, avirulence genotypes of Fol races were demonstrated; Race 1 contains all three *AVR* genes but *Avr1* (*SIX4*) is absent in races 2 and 3 (Houterman *et al*., 2009b). Therefore, *Avr1 (SIX4)* has been reported as an exclusive marker for race 1 in Fol strains (Lievens *et al*., 2009). Accordingly, detection of *Avr1*

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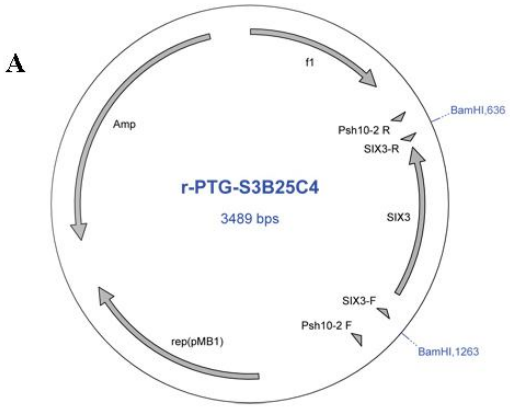
(SIX4) effector gene in Iranian FOL strains was performed using PCR specific primers that anneal outside the ORF. In all studied strains, *Avr1 (SIX4)* was detected except for non-pathogenic strains FO-039 and FO-011 (Fig. 1-c). These results were in agreement with previous studies and confirmed the results of older pathogenicity tests on the differential cultivars (Amini, 2009; Fassihiani, 1985; Heidarzadeh, 2006). However, similar to *Avr2 (SIX3),* specific band for *Avr1 (SIX4)* was detected in the non-pathogenic strain FOL012, indicating a probable misclassification of this sample.

Consistence of Race 1 with *Avr2 (SIX3)* allele

Previous studies showed that in the population of Fol, only three sequence polymorphisms were found in *Avr2 (SIX3)* (G121→A, G134→A and G137→ C, which leading to an amino acid change V41→M, R45→H and R46→P respectively). Each of these mutations were found in a single allele that prevents recognition of *Avr2 (SIX3)* by *I-2* and caused race3 arise from race2 (Houterman *et al*., 2009b). Therefore, there are four alleles in the population of Fol three of which (M41, H45 and P46) are exclusively found in Race3 however races 1 and 2 have original allele. As we know that Fol strains investigated in this study belong to race1, the expectation is that they have the allele like race 1. To confirm it, *Avr2 (SIX3)* was amplified in one isolate (FOL022), cloned and sequenced. The result of sequencing showed that variant of *Avr2 (SIX3)* is similar to race1, as expected (Fig. 2).



B

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FOL022     MRFLLLIAMS MTWVCSIAGL PVEDADSSVG QLQGRGNPYC VFPGRRTSST SFTTSFSTEP LGYARMLHRD PPYERAGNSG LNHRIYERSR  [ 90]
AM234063   .......... .......... .......... .......... .......... .......... .......... .......... ..........  [ 90]
GQ268950.1 .......... .......... .......... .......... .......... .......... .......... .......... ..........  [ 90]
GQ268957.1 .......... .......... .......... .......... M......... .......... .......... .......... ..........  [ 90]
GQ268956.1 .......... .......... .......... .......... .....P.... .......... .......... .......... ..........  [ 90]
GQ268955.1 .......... .......... .......... .......... ....H..... .......... .......... .......... ..........  [ 90]

FOL022     VGGLRTVIDV APPDGHQAIA NYEIEVRRIP VATPNAAGDC FHTARLSTGS RGPATISWDA DASYTYYLTI SED*  [164]
AM234063   .......... .......... .......... .......... .......... .......... .......... ....  [164]
GQ268950.1 .......... .......... .......... .......... .......... .......... .......... ....  [164]
GQ268957.1 .......... .......... .......... .......... .......... .......... .......... ....  [164]
GQ268956.1 .......... .......... .......... .......... .......... .......... .......... ....  [164]
GQ268955.1 .......... .......... .......... .......... .......... .......... .......... ....  [164]
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Figure 2 A) Schematic representation of recombinant vector PTG-S3B25C4 and position of amplicon and primers SIX3 F/R and Psh10 F/R. Rep (pMB1): pMB1 origin of replication .Amp R: Ampcillin resistance ORF. F1: f1 origin of ss-DNA replication. **B**) Multiple sequence alignment of *Avr2 (SIX3)* in FOL021 and other FOL isolates in NCBI. Accession numbers of proteins are represented in parentheses. Three SNP in positions V41, R45, R46 are shaded in yellow. Sequences are aligned using Mega 6.

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Characterization of avirulence genotype in Iranian Fol strains

As confirmed above, all Fol strains investigated in this study belonged to race 1. Therefore the expectation is that their avirulence genotype matches with race 1 too. In order to study avirulence genotype of them, since the presence of *Avr1 (SIX4)* and *Avr2 (SIX3)* had been investigated, detection of *Avr3* (*SIX1*) effector gene was performed using PCR specific primers P12-F2B and P12-R1 that anneal outside the ORF. *Avr3 (SIX1)* was amplified in 13 strains (Fig. 1-d). Regarding to non-pathogenic strains, *Avr3* (*SIX1*), as shown in Fig. 1-d, was not amplified in FO-039 and FO-011, while a specific 992 bp band was detected in FOL012.

Avirulence genotypes of the strains we studied are summarized in table 1. Of the 21 strains in the collection, 14 strains had the avirulence genotype similar to FOL race 1 that, remarkably, among them FOL012 had been previously reported as non-pathogenic strain. Furthermore, the avirulence genotype similar to non-pathogenic strains was identified in 2 strains in our collection. Interestingly, we identified a new avirulence genotype in 5 strains that had been previously reported as race 1 (Amini, 2009; Heidarzadeh, 2006). They had only two *Avr* genes (*Avr1 (SIX4)* and *Avr2 (SIX3)*) whereas *Avr3 (SIX1)* was not detected in them. Further studies are being conducted on these strains.

We were interested to assess the virulence of FOL012 strain on tomato plant. The pathogenicity assay was performed on 10-day old seedlings without any identified resistance genes against Fol (Bonny Best). Results revealed that FOL021 (as positive control) was able to cause high degree of virulence (disease index 5) in tomato, while no symptom of wilting was observed in control plants. FOL012, non-pathogenic strain which had avirulence genotype like race1, was able to colonize the xylem of stem and caused yellowing and very slight wilt symptom on tomato plant therefore we reasoned that this strain originally had been misidentified as non-pathogenic strain (Fig. 1-e).

Degree of virulence in Iranian Fol strains

Previously, Rep *et al*. (2005) founded a DNA polymorphism in *Avr3 (SIX1)* (G490 →A) leads to an amino acid change (E164 → K) which confers a higher virulence to Fol than the E164 variant. To determine the relation between *Avr3(SIX1)* alleles and power of pathogenicity of Iranian Fol strains, *Avr3 (SIX1)* sequence was analyzed in Fol strains with high (disease indices 4 and 5) and low (disease indices 2 and 3) degrees of virulence in susceptible tomato cultivar (Bonny Best). In this regard, a pair of external primers (SIX1-F/R) that located 84 bp upstream of the start codon and 83 bp downstream of the stop codon was designed. Results of sequencing demonstrated that all *Avr3 (SIX1)* sequences were identical and belonged to E164 variant. In addition, in Iranian Fol strains, there was no correlation between degree of virulence and amino acid variation of *Avr3 (SIX1)* at position 164 (Fig. 3).

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[              444 444 444 444 444 444 444 444 444 444 455 555 555 555 555 555 555 555 555 555 555 555 555 555 555 555 555
[              677 777 777 778 888 888 888 999 999 999 900 000 000 001 111 111 111 222 222 222 233 333 333 334 444 444
[              901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456
#AJ608702.1    TCA GTG ACC AGA GCT TGC CCA GAG GGC CAG GAG TGC ACC ACT TTC AAT GCG TAC AAC TTT CGT AAC CGT CAC CAT CAG
#GQ268948.1    ... ... ... ... ... ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL013         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL012         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL030         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL021         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL022         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL023         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

[              555 555 555 555 555 555 555 555 555 555 555 555 555 555 555 555 555 555 556 666 666 666 666 666 666 666 666
[              444 555 555 555 566 666 666 667 777 777 777 888 888 888 899 999 999 990 000 000 000 111 111 111 122 222
[              789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234
#AJ608702.1    GTT ACT TTT CCT GTC TGT GGG CCC CGA ATT GAG GTG AAG GAC AGA CAT GAT ATA GGG ATA CAC ACG GAG TGG CAG GGA
#GQ268948.1    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL013         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL012         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FOL030         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
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Figure 3 Multiple sequence alignment of *Avr3 (SIX1)* in Iranian FOL strains. All *Avr3 (SIX1)* sequences were identical and belong to E164 variant.

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Discussion

The current study showed that results of molecular identification in most of the Iranian strains we examined, were consistent with earlier findings (Houterman *et al*., 2009b; Lievens *et al*., 2009), and avirulence genotype of them was consistent with result of older pathogenicity tests on the differential cultivars (Amini, 2009; Fassihiani, 1985; Heidarzadeh, 2006). However few contrasts were observed in our collection and further studies are being conducted on them. One of the unanticipated finding was that all three *Avr* genes were present in strain FOL012, which had been previously classified as non-pathogenic *F. oxysporum*. In planta assay confirmed the result of molecular identification, and demonstrated that this PCR assay is very efficient in distinguishing between a non-pathogenic strain and a virulent strain with low pathogenic power. Such misclassifications had previously been observed (Lievens *et al*., 2009; Van Der Does *et al*., 2008) and were attributed to the fact that greenhouse virulence test is based on disease symptoms, therefore inherent problems such as environmental conditions and methods of inoculation can influence symptom expression of disease in a pathogenicity test (Correll *et al*., 1986); while this PCR assay is based on the direct detection of pathogen in plant tissue.

Previous studies showed that a point mutation in *Avr3 (SIX1)* that caused a change of glutamate to lysine at its protein can lead to enhanced virulence of Fol on a susceptible tomato cultivar (Rep, 2005). The final section of present study was designed to determine if degree of virulence of Iranian Fol strains is correlated with allelic variation of *Avr3 (SIX1)*. However we had hoped to find a molecular marker that correlated with degree of virulence, our finding showed that all Iranian strains belong to E-variant and degree of virulence of them did not correlate with the allelic variation of *Avr3 (SIX1)* in them. Previous studies showed that Fol isolates belong to four VCG groups (0030, 0031, 0032 and 0034) (Cai *et al*., 2003; Katan and Primo, 1999). Van der Does *et al*. (2008) showed VCG0030 had both alleles (E and K) but VCG0031 and VCG0032 had exclusively E-allele and VCG0033 had K-allele. It is therefore likely that such connections exist between allelic variations of *Avr3 (SIX1)* and VCG groups of Iranian FOL strains. Unfortunately, VCG group of Iranian FOL stains is unknown and so far, no investigation has been performed on them; therefore further research in this field is recommended.

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شناسایی ملکولی فرم‌تخصصی و هویت‌نژادی در جدایه‌های ایرانی *Fusarium oxysporum* f. sp. *Lycopersici*: تشخیص ژن‌های غیربیماری‌زایی

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چکیده: قارچ *Fusarium oxysporum* f. sp. *lycopersici* (Fol) عامل پژمردگی آوندی و یکی از مهم‌ترین عوامل بیماری‌زای گیاهی در ایران است. تاکنون چهار ژن مقاومت تک‌ژنی در گوجه‌فرنگی شناسایی شده‌اند که در تعیین نژاد این بیمارگر به‌کارگرفته می‌شوند. در مقابل ژن‌های غیربیماری‌زایی *Avr1*،*Avr2* و *Avr3* در نژادهای FOL شناسایی‌شده‌اند که در این بین *Avr2* فقط در فرم اختصاصی FOL حضور دارد. از این‌رو سه ژن فوق می‌توانند نشانگرهای مناسبی جهت تشخیص فرم تخصصی و هویت نژادی در این عامل بیماری‌زا باشند. این فرضیه در جدایه‌هایی از کشورهای مختلف مورد بررسی قرارگرفته، ولیکن تاکنون در جدایه‌های ایرانی بررسی نشده است. هم‌چنین مطالعات قبلی نشان‌داده‌اند که یک جهش نقطه‌ای در *Avr3* می‌تواند سبب افزایش توان بیماری‌زایی FOL در ارقام حساس گوجه‌فرنگی گردد. در این تحقیق به‌منظور شناسایی فرم‌تخصصی و هویت نژادی، ژن‌های *Avr* در مجموعه‌ای از جدایه‌های ایرانی مورد مطالعه قرارگرفت. نتایج حاصل نشان‌داد که این روش سنجش ملکولی در تفکیک جدایه‌های غیربیماری‌زا از جدایه‌های بیماری‌زا با توان بیماری‌زایی پایین بسیار مؤثر است. هم‌چنین براساس نتایج حاصل اکثر جدایه‌های مورد بررسی دارای ژنوتیپ غیربیماری‌زایی مشابه با نژاد یک بودند. علاوه براین به‌منظور بررسی این نکته که آیا تنوع آللی در *Avr3* می‌تواند جدایه‌هایی با توان بیماری‌زایی مختلف را از یک‌دیگر تفکیک نماید، ژن فوق در جدایه‌هایی با توان بیماری‌زایی بالا و پایین تعیین‌توالی گردید. نتایج حاصل نشان‌داد که در جدایه‌های ایرانی ارتباطی میان توان بیماری‌زایی آن‌ها و تنوع آللی در *Avr3* وجود ندارد.

واژگان کلیدی: ژنوتیپ غیربیماری‌زایی، ژن اثرگزار، پژمردگی آوندی، تشخیص ملکولی

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