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Nucleotide polymorphisms and protein structure changes in the *Fg16* gene of *Fusarium graminearum sensu stricto*

Mostafa Abedi-Tizaki, Doustmorad Zafari *

Department of Plant Protection, College of Agriculture, Buali Sina University, Hamedan, Iran

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ABSTRACT

Fusarium graminearum is one of the most important causes of wheat scab in different parts of the world. This fungus is able to produce widespread trichothecene mycotoxins such as nivalenol (NIV) and deoxynivalenol (DON) which are harmful for both human and animals. The *Fg16* target is located in chromosome 1 of the *F. graminearum* genome coding for a hypothetical protein whose function is not yet known. The *Fg16* gene is involved in lipid biosynthesis and leads to sexual development during colonization in wheat stalks. This gene is used to detect *F. graminearum* and determine the lineage of *F. graminearum* complex species. In the present study, polymerase chain reaction–single strand conformational polymorphism (PCR–SSCP) and DNA sequencing methods were employed in screening for genetic variation in 172 *F. graminearum s.s.* isolates. The PCR reaction forced the amplification of 410-bp fragments of *Fg16*. Two single nucleotide polymorphisms (T82C and A352T) and one amino acid exchange (C65S) with three patterns (TA/TA, CT/CT and TA/CT genotypes) were found in the *Fg16* gene fragment. Two haplotypes, 1A and 1B, were identified within *F. graminearum s.s.* populations in northern and western regions of Iran. Two different secondary structures of protein were predicted for CT/CT and TA/CT genotypes of *Fg16* gene. The average diversity levels detected were relatively high (He: 0.3238; He_u: 0.334; Ho: 0.2894; mean PIC: 0.514; mean Shannon's information index: 0.4132; mean number of alleles per locus: 1.473). On the basis of the obtained results, it was revealed that the *Fg16* gene had a high degree of polymorphism that can be considered for future control programming strategies and thus the associations between the SSCP patterns with different traits of *F. graminearum* such as wheat colonization, perithecium formation on stalk tissues and lineage discrimination should be investigated.

1. Introduction

Fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is one of the most important fungal diseases of wheat worldwide that causes serious losses in both yield and quality of grain (Parry et al., 1995). This fungus produces different kinds of mycotoxins, which pose a serious health threat to humans and animals (Arseniuk et al., 1993). It has been estimated that 25% of the world food crops is affected by mycotoxins (Charmley et al., 1995). The most predominant mycotoxins found in small-grain cereals are 8-ketotrichothecenes (type B trichothecenes) such as deoxynivalenol (DON) (also known as vomitoxin) and nivalenol (NIV) and their acetylated derivatives including 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), as well as an estrogenic mycotoxin, zearalenone (Mirocha et al., 1989; Waalwijk et al., 2003).

Both sexual (ascospores) and asexual (macroconidia) propagules are produced by *F. graminearum* but ascospores are generally believed to be the primary inoculum of the disease (Shaner, 2003). Recent studies demonstrated that sexual development in *F. graminearum* is initiated during plant colonization (Guenther and Trail, 2005). The *Fg16* gene target is located at the XM381603 locus of chromosome 1 in the *F. graminearum* genome (http://www-genome.wi.mit.edu/annotation/fungi/fusarium/index.html) coding for a hypothetical protein (FG01427.1) whose function is not yet known.

Recently, it was found that the *Fg16* genes involved in lipid biosynthesis are highly expressed during vegetative growth and early sexual development in culture, and during colonization in wheat stalks (Guenther et al., 2009). Gene expression analysis suggests two phases of cellular growth with respect to lipid metabolism: a biosynthetic phase, where lipids are synthesized and accumulate, and a phase during which stored lipids are utilized to produce fruiting bodies. In *F. graminearum*, the *Fg16* gene, which is involved in mitochondrial lipid oxidation, shows expression typical of lipid oxidation genes (Guenther et al., 2009). Lipid stores are predominantly composed of triacylglycerides, which are characterized by a glycerol backbone and

* Corresponding author.
E-mail address: Zafari_d@yahoo.com (D. Zafari).

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three fatty acyl side chains. The fatty acyl side chains vary (Murphy, 2001) and their composition tends to be species-specific (Weete, 1974).

Gene expression analysis during stages of perithecium development both in planta and in vitro supports the view that lipid biosynthesis occurs during early stages of wheat colonization leading to sexual development and that lipid oxidation occurs as perithecia are developing (Guenther and Trail, 2005). Analysis of gene expression during the stages of wheat stem colonization also revealed sets of genes unique to these stages and lipids accumulate in hyphae colonizing wheat.

Based on the *Fg16* gene, Fg16F/Fg16R primers were designed so that SCAR (sequence characterized amplified regions) types were determined accordingly. Carter et al. (2002) reported six SCAR types, based on PCR product size, among the FGSC isolates examined. These authors suggested that the SCAR product was characteristic for different groups within *F. graminearum sensu lato* (Carter et al., 2002). Polymerase chain reaction analysis specific to *F. graminearum* was performed using primer pair Fg16F/R, which produces polymorphic products with DNA from *F. graminearum* lineages, but no products with DNA from any other fungal species (Nicholson et al., 1998). The sequence of the Fg16F/R product is diagnostic of lineage and may be used simultaneously to detect *F. graminearum* and determine lineage (Carter et al., 2002; Nicholson et al., 1998).

It has recently been reported that *F. graminearum* consists of a number of lineages or groupings (Carter et al., 2000, 2002; O'Donnell et al., 2000). Analysis of a global collection of *F. graminearum* isolates from cereal hosts with primer set (Fg16F/ R) was found to produce one of six different PCR products from each isolate (Carter et al., 2002). Sequence analysis has revealed that the product of the Fg16F/R primer pair is diagnostic of the lineage/group and hence this primer pair may be used to detect *F. graminearum* and simultaneously determine lineage/group. Furthermore, because particular lineages/groups appear to be associated with geographic regions and mycotoxin chemotypes, the Fg16F/R assay can aid the detection of migrants and monitor pathogen movement.

SCAR analysis using Fg16F/Fg16R was previously used to distinguish *F. graminearum* and Fusarium asiaticum isolates from China (Qu et al., 2008). However, differentiation of *F. asiaticum* from Fusarium meridionale based on SCAR fragment size was not reliable. The use of SSCP analysis (single strand conformation polymorphism) enabled unequivocal resolution of *F. meriodionale* from *F. asiaticum*.

The objectives of the present study were the identification of *Fg16* gene mutations by PCR–SSCP and DNA sequencing methods and evaluation of the association between these mutations and protein structure changes in *F. graminearum sensu stricto* (*s.s.*) isolates.

2. Materials and methods

2.1. Sampling and Fusarium isolates

Diseased wheat spikes were taken during 2013–2014 from 245 fields in the northern and western regions of Iran that were separated by at least 10 km, and the numbers of the collected wheat spikes with clear FHB symptoms were proportional to the acreage of wheat infected by the disease. Each field was arbitrarily divided into 5 circular plots approximately 100 m in diameter, and 3–5 samples (spikes with visible infection symptoms) were randomly taken from each plot and then samples were pooled in each field. These collections represent samples from all the areas in the northern and western regions of Iran with a known history of FHB occurrences in wheat, covering 6 provinces including Golestan and Mazandaran (Northern regions), Hamedan, Kurdistan, Kermanshah and Lorestan (western regions).

After that the diseased wheat spikes were surface-sterilized and cultured in potato dextrose agar (PDA) and carnation leaf agar (CLA) media, *Fusarium* isolates were obtained by single spore isolation and identified by the methods described previously via Nelson et al. (1983). In total 172 isolates of *F. graminearum* were used in this study.

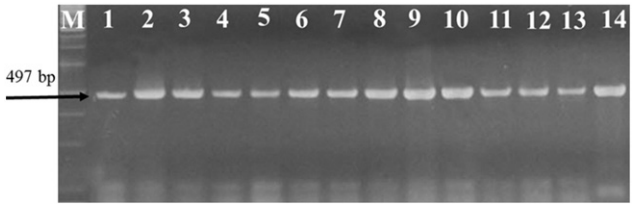


Fig. 1. PCR amplification products with Fg16F/Fg16R primer set. 1 to 14 isolates produce a 497 bp fragment. Product sizes for all isolates corresponded to SCAR type 1. M: marker(1 kb).

2.2. DNA extraction from fungal cultures

All isolated were inoculated with mycelia disks excised from the margin of 10-day-old PDA cultures in 100 ml Erlenmeyer flasks containing 20 ml of PDB liquid medium (Merck, Germany). Submerged fungi cultures were incubated on a rotary shaker at 120 rpm for 8 days at 25 °C. Mycelia were harvested by filtration through Whatman paper 1, ground to fine powder with liquid nitrogen and keep at −80 °C for further DNA extraction. Total genomic DNA was extracted from dried mycelium using the CTAB method as described by Nicholson et al. (1997).

2.3. SCAR and phylogenetic species analysis

SCAR analysis was used to identify the *Fusarium* isolates. It was previously demonstrated that SCAR typing, based on the size of the PCR product obtained with primer set Fg16F/Fg16R, resolved isolates of *F. graminearum* (type 1) and *F. asiaticum* (type 5) (Qu et al., 2008). A 410 bp DNA fragment specific for SCAR group I and a 497 bp fragment for SCAR group V were generated, respectively. Primer set Fg16F/Fg16R was used for SCAR analysis as described previously (Nicholson et al., 1998). Amplifications were carried out using 50 ng of DNA template with thermal cycling consisting of 30 cycles of denaturation (94 °C, 1.5 min), annealing (60 °C, 1 min) and extension (72 °C, 2 min). PCR products were separated on 2% agarose gels.

Portions of three phylogenetically informative genes (3-*O*-acetyltransferase (*Tri101*), translation elongation factor 1α (*EF-1α*) and reductase (*RED*)) from 10 isolates of *F. graminearum* were amplified and sequenced as described previously (O'Donnell et al., 2000, 2004). Lineage and phylogenetic species of *F. graminearum* were analysed and classified according to the methods of O'Donnell et al. (2000, 2004).

2.4. Single-strand conformation polymorphism (SSCP) analysis

The SSCP analysis of *Fg16* gene was carried out as follows. A 5 μL PCR product sample of BMPR-1B gene exon 8 was added to 10 μL of SSCP gel loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, 95% formamide, 20 mM EDTA) and mixed. After heat denaturation at 97 °C for 8 min, the samples were immediately chilled on ice to prevent heteroduplex formation and then run (18 h, 300 V, 5 °C) on 15% acrylamide:bis-acrylamide gels (29:1 acrylamide to bisacrylamide), without glycerol in 1× TBE buffer on a 21 × 22 cm gel casting vertical electrophoresis (Payapajoohesh Pars, Iran). For SSCP analysis of Fg16, 4 μL of PCR sample was aliquoted into separate tubes, then 7 μL of SSCP gel loading dye was added and mixed. After heat denaturation at 98 °C for 10 min, the samples were immediately chilled on ice and then run (22 h, 300 V, 5 °C) on 8% acrylamide:bis-acrylamide gels (29:1 acrylamide to bisacrylamide), in 1× TBE buffer. DNA visualization in the polyacrylamide gel after electrophoresis was done by silver staining (Sanguinetti et al., 1994).

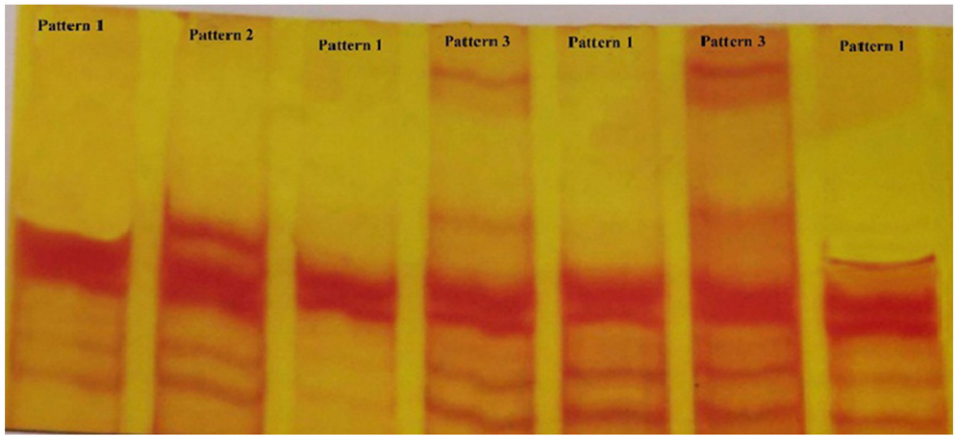


Fig. 2. Different SSCP patterns (TA/TA, CT/CT and TA/CT genotypes) of 479 bp fragment of *Fg16* gene.

2.5. DNA sequencing

Two samples of each SSCP pattern were randomly selected for DNA sequencing. The primers used for sequencing were the same as those for the PCR reaction. The PCR products were sequenced by Bioneer Co., Korea. Sequence alignments, translations and comparisons were carried out using ExPASy translate tools website (http://us.expasy.org/translatetool/) and MegAlign module of DNASTAR software (DNASTAR Inc., Madison, WI. USA) by Clustal W method.

2.6. Protein structure

To predict the secondary and tertiary structures of protein referring to the sequences and different genotypes *Fg16* genes, the sequences of amplified fragments of the studied gene (*Fg16*) were retrieved from NCBI GenBank databases and DNASTAR, EditSeq software (DNASTAR Inc., Madison, WI. USA) was used to enter the mutations. Then, ExPASy translate tools website (http://us.expasy.org/translatetool/) and MegAlign module of DNAstar, software (DNASTAR Inc., Madison, WI, USA) were used to translate the nucleotide sequences to amino acid chains. Secondary and tertiary structures of proteins were predicted using Psipred section of SWISS-MODEL available in the ExPASy website (http://swissmodel.expasy.org) and Protean section of DNASTAR software (DNASTAR Inc., Madison, WI, USA).

2.7. Statistical analysis

Population genetic indices, namely, gene heterozygosity (expected heterozygosity (He), expected unbiased heterozygosity (He_u) and observed heterozygosity), effective allele number (Ne) and Shannon's information index were calculated by POPGENE 32 software version 1.32 (version 1.32; Molecular Biology and Biotechnology Center, University of Alberta, Edmonton, Canada) (Nei, 1973). Polymorphisms were tested for deviations from Hardy–Weinberg equilibrium by chi-square test (χ^2).

3. Results

3.1. SCAR analysis and identification of F. graminearum sensu stricto (s.s.) lineage

Based on morphological identification, all 172 isolates from across six populations of Iran were identified as *F. graminearum*. All isolates were subjected to SCAR analysis using the Fg16F/R primer set. One-product sizes were observed, corresponding to SCAR types 1 by Carter et al. (2002). PCR assays with the primer pair Fg16F/Fg16R revealed that 172 *F. graminearum* isolates produced a 410 bp fragment (Fig. 1). Sequencing analyses of the three phylogenetically informative genes (3-*O*-acetyltransferase (*Tri101*), translation elongation factor 1α (*EF-1α*) and reductase (*RED*)) from 10 isolates of *F. graminearum* showed that they were fully congruent with sequence-characterized amplified region (SCAR) grouping I and is typical of lineage 7, *F. graminearum sensu stricto* (*s.s.*).

3.2. SSCP analysis of Fg16

SSCP analysis of *Fg16* in *F. graminearum* (*s.s.*) isolates revealed three distinct patterns (TA/TA, CT/CT and TA/CT genotypes) on polyacrylamide gels (Fig. 2). Pattern 3 (TA/CT genotype) showed 6 major bands on the gel, but Pattern 1 (TA/TA genotype) and Pattern 2 (CT/CT genotype) showed 4 and 5 bands on the gel, respectively (Fig. 2) (Table 1). The high frequency percentage of TA/TA, CT/CT and TA/CT genotypes of *Fg16* gene in 172 randomly selected *F. graminearum* (*s.s.*) isolates were 54.16%, 60% and 56%, in Kurdistan, Lorestan and Golestan

Table 1
Frequencies of Fg16 genotypes in randomly selected *F. graminearum s.s.* isolates in six populations

Frequency						
Northern populations		Western populations				
	Golestan	Mazandaran	Hamadan	Kurdistan	Lorestan	Kermanshah
Genotype						
TA/TA	7 (28)	5 (45.46)	12 (31.58)	13 (54.16)	14 (28)	10 (41.67)
CT/CT	4 (16)	–	21 (55.26)	11 (45.84)	30 (60)	14 (58.33)
TA/CT	14 (56)	6 (54.54)	5 (13.16)	–	6 (12)	–
Total	25 (100)	11 (100)	38 (100)	24 (100)	50 (100)	24 (100)
Haplotype						
1A	56	73	38	54	34	42
1B	44	27	62	46	66	58

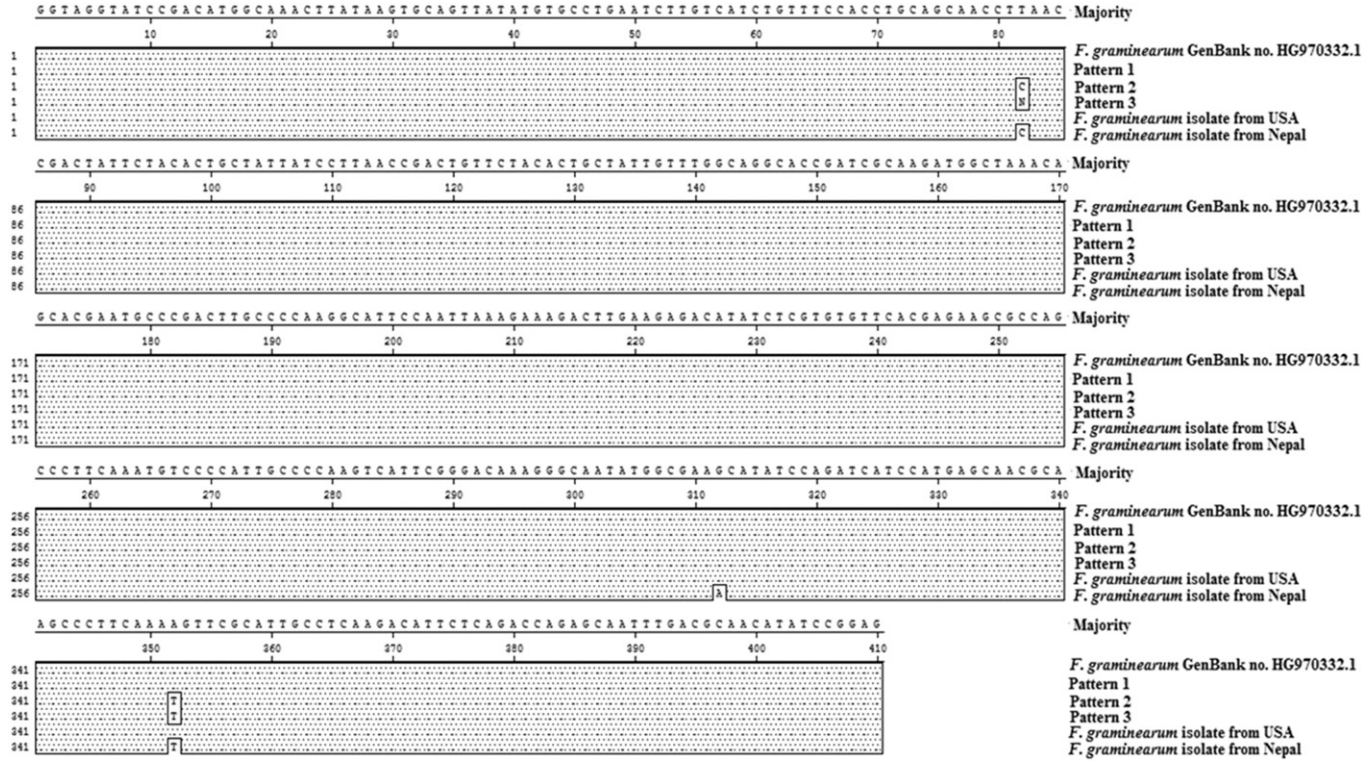


Fig. 3. The comparison between sequences of 1, 2 and 3 patterns and GenBank accession number HG970332.1, with other sequences in NCBI for *F. graminearum s.s.*

populations, respectively (Table 1). Results indicated that SCAR type 1 consisted of two haplotypes, 1A and 1B (Table 1). Analysis of the SSCP dataset by UPGMA resolved three main clusters (data not shown). Clusters A, B and C contained isolates with Patterns 1, 2 and 3, respectively. Clusters B and C contained haplotype 1B while cluster A only contained isolates with haplotype 1A (data not shown). DNA sequencing of the Fg16F/R product confirmed that haplotype 1B differed from that of 1A at nucleotide 82 (T–C) and 352 (A–T) (Table 1). High-frequency percentages of 1A and 1B haplotypes were 73% and 66% in Mazandaran and Lorestan populations (Table 1).

3.3. Sequence analysis and single nucleotide polymorphism (SNP) loci screening

Two single nucleotide polymorphisms (T82C and A352T) and one amino acid exchange with three patterns (TA/TA, CT/CT and TA/CT genotypes) were found in the *Fg16* gene fragment (Fig. 3). The results obtained by DNA sequencing showed a transition of T→C at position 82 and A→T at position 352 of the amplified fragment of *Fg16* gene in pattern 2 (CT/CT genotype)(Figs. 3 and 4). Moreover, pattern 3 (TA/CT genotype), showed a transition T→C, T (heterozygote) at position 82 and A→T at position 352 and, in pattern 1 (TA/TA genotype), no change was observed at positions 82 and 352 in comparison to the referring sequence (GenBank accession number HG970332.1) (Figs. 3 and 4). However, mutations at position 82 did not show any exchanged amino acid (L16L) but one amino acid exchange (C65S) was observed at position 352 (Fig. 5).

3.4. Nucleotide sequence comparison

The results of alignment analysis as sequence similarity between the studied fragment and some reported sequences are presented in

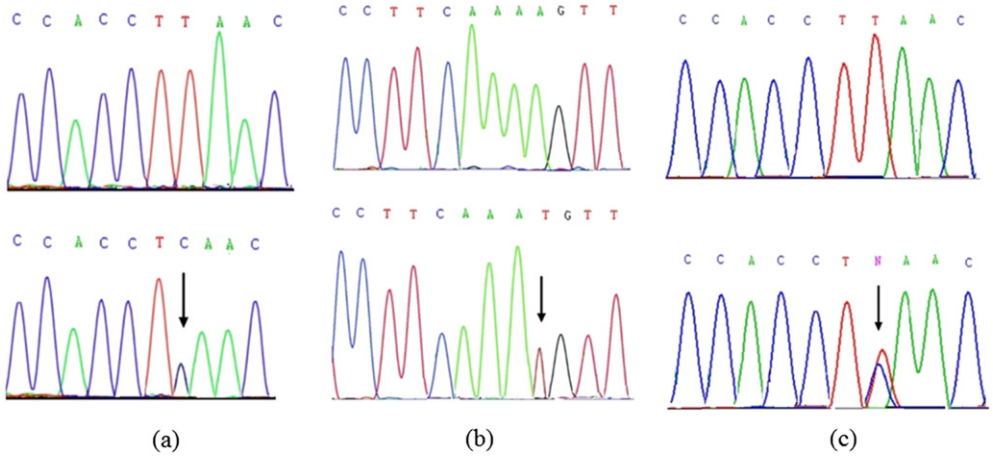


Fig. 4. Sequencing results and three genotypes of *Fg16* gene. (a) Mutation at position 82 (T→C); (b) mutation at position 352 (A→T); (c) mutation at position 82 (T→C,T); N: nucleotide sequence is the heterozygote genotype.

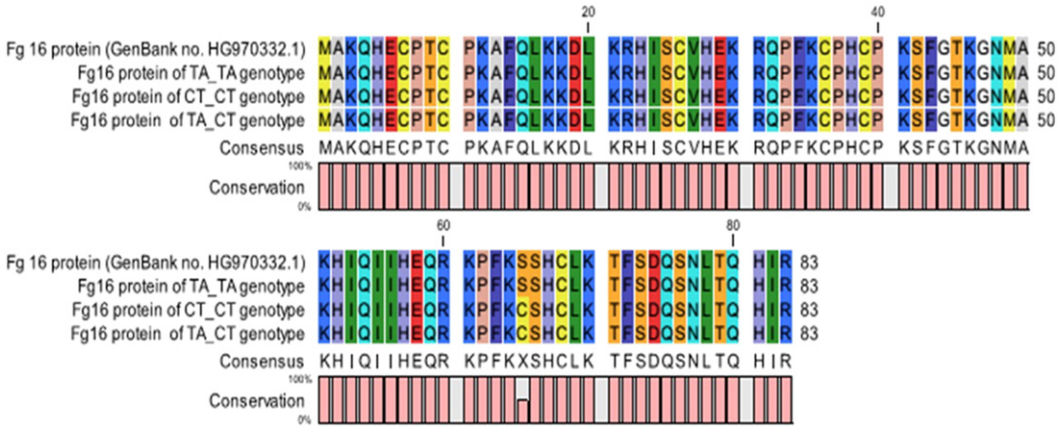


Fig. 5. Comparative alignment of conceptualized full protein sequences of *Fg16* gene, based on genotype sequences in *F. graminearum s.s.* with NCBI reference sequence.

Table 2. The sequence similarity was found to be higher than 99.2% between the observed genotypes and other reported sequences, including GenBank no. HG970332.1, *F. graminearum* isolate from the USA and *F. graminearum* isolate from Nepal (Carter et al., 2000) (Table 2). The designated TA/TA genotype had a 100% homology with sequences of GenBank no. HG970332.1 *F. graminearum* isolate from the USA (Carter et al., 2002) and 99.2% homology with CT/CT genotype, TA/CT genotype and *F. graminearum* isolate from Nepal (Carter et al., 2002) (Table 2). Similarity of the CT/CT with TA/CT genotype and *F. graminearum* isolate from Nepal was 99.5% (Table 2).

3.5. Secondary and tertiary protein structure changes of Fg16 gene

CT/CT and TA/CT genotypes of *Fg16* gene showed various predictions for secondary protein structure in comparison to the GenBank sequence. The sequence of the TA/TA genotype was similar to the referring sequence (GenBank accession number HG970332.1) (Fig. 6). The most changes in the CT/CT genotype included alpha amphipathic regions, antigenic index and surface probability plot in the secondary structure of protein (Fig. 6). Moreover, the TA/CT genotype showed a difference in antigenic index and surface probability plot in the secondary structure of protein in comparison to the referring sequence (Fig. 6).

Predictions of the tertiary structure showed that this protein belongs to a class of zinc finger proteins. By investigation of the tertiary structure of proteins, we found that mutations in the *Fg16* gene and changes in cysteine to serine amino acid had no effect on the tertiary structure of the protein (Fig. 7), because the SNPs had no effect on the folding polypeptide chain and the tertiary structure skeleton remained intact (Fig. 7). According to some properties of Fg16 protein, it was found that mutations (CT/CT and TA/CT genotypes) leading to changes in molecular weight, 1 μg, isoelectric point, absorption at a wavelength of 280 nm (1A), charge at pH 7, instability index but had no effect on polypeptide chain length, molar extinction coefficient and aliphatic index (Table 3).

Table 2
Similarity and divergence percentages of the observed genotypes and other reported sequences for *F. graminearum s.s.* isolates.

Percent identity

Divergence	1	2	3	4	5	6		
1		100	99.2	99.2	100	99.0	1	GenBank no. HG970332.1
2	0.0		99.2	99.2	100	99.0	2	TA/TA genotype
3	0.8	0.8		99.7	99.2	99.5	3	CT/CT genotype
4	0.8	0.8	0.3		99.0	99.5	4	TA/CT genotype
5	0.0	0.0	0.8	1		99.0	5	F. graminearum isolate from USA
6	1	1	0.5	0.5	1		6	F. graminearum isolate from Nepal
	1	2	3	4	5	6		

3.6. Genetic diversity of F. graminearum s.s. populations

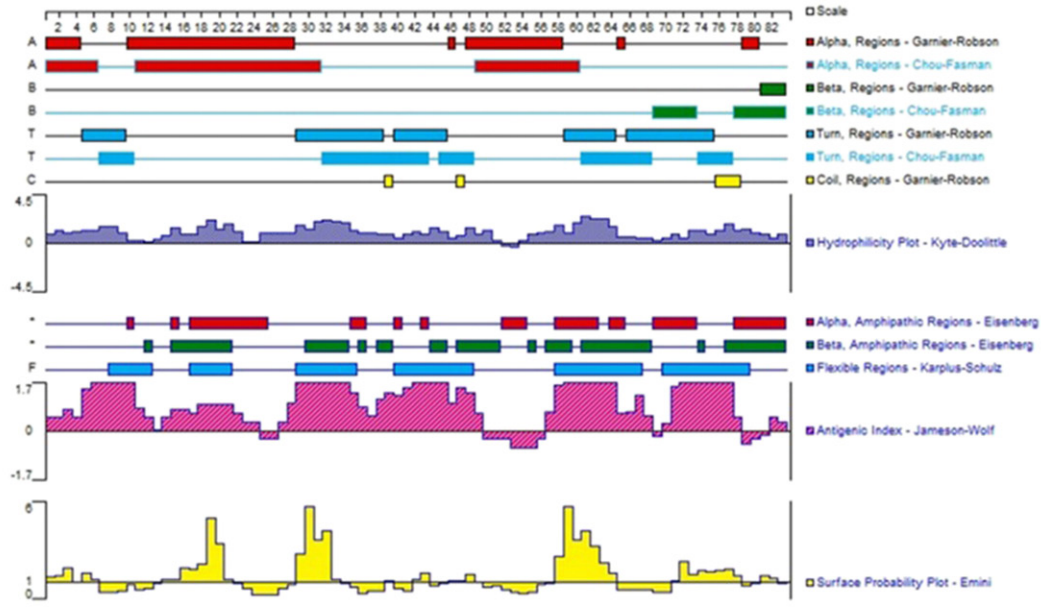
Regarding measures of genetic diversity (Table 4), the population with the highest unbiased heterozygosity was that of Lorestan followed by Hamedan while the population with the lowest expected unbiased heterozygosity was that of Kurdistan and Kermanshah, followed by Golestan and Mazandaran. With the exception of these last four populations, the Lorestan and Hamedan populations showed levels of expected heterozygosity higher than 0.4600 (Table 4). Table 4 also shows the mean heterozygosity for the total sample, the fact that there is a difference between the expected (He) and observed (Ho) heterozygosity suggests a tendency towards heterozygote deficiency. The PIC values were between 0.290 for the Mazandaran population and 0.772 for the Lorestan population (Table 4). A high PIC value depends on the number and frequency distribution of the alleles measured; populations with PIC values exceeding 0.500 being considered more informative (Botstein et al., 1980).

The estimates of Shannon's information index (I) for all loci in each of the populations ranged from 0.2093 to 0.6038 (Table 4). In the present study, the highest PIC values were obtained for those populations with a high number of alleles (e.g. Lorestan, Hamedan and Kermanshah populations) or which showed a more homogeneous allele frequency distribution even when the number of alleles detected were low (e.g. Kurdistan and Mazandaran populations) and for those alleles presenting both of these characteristics (e.g. Golestan population). Our study shows that the Fg16 marker with the highest heterozygosity and PIC values could be included in future genetic diversity studies of *F. graminearum s.s.* populations. The average diversity levels detected were relatively high ($He = 0.3238$; $He_u = 0.334$; $Ho = 0.2894$; mean $PIC = 0.514$; mean number of alleles per locus: 1.473). We compared genotype frequencies with Hardy–Weinberg expectations for each population. The genotypes at these SNP positions in all populations in the *Fg16* gene exhibited significant deviations ($P < .0001$) from Hardy–Weinberg equilibrium.

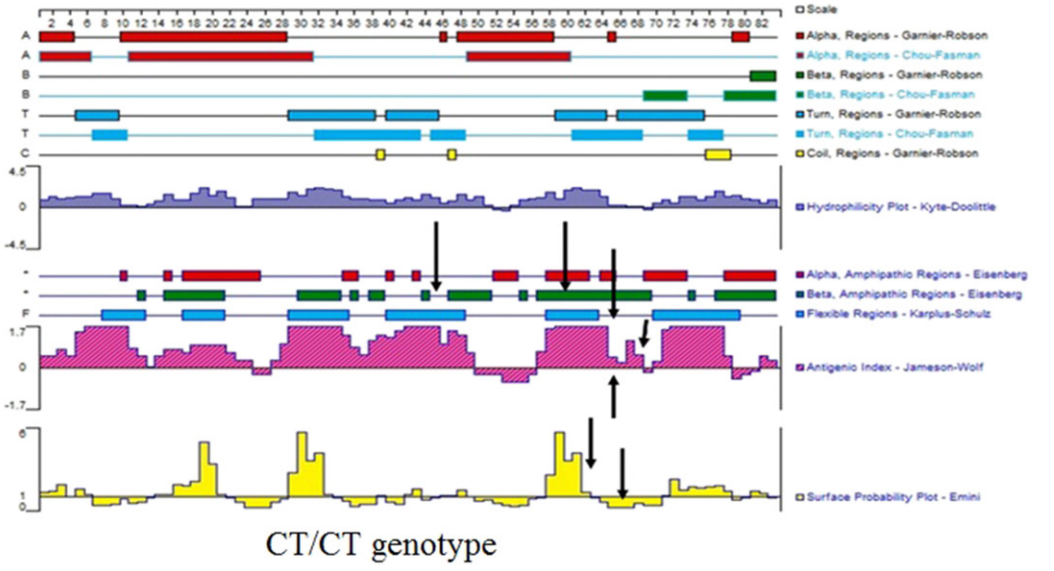
However, the heterozygosity found in our sample of Iranian *F. graminearum s.s.* isolates is considerably higher. Our present analysis contributes to the genetic characterization and population dynamic of the Iranian *F. graminearum s.s.* population.

4. Discussion

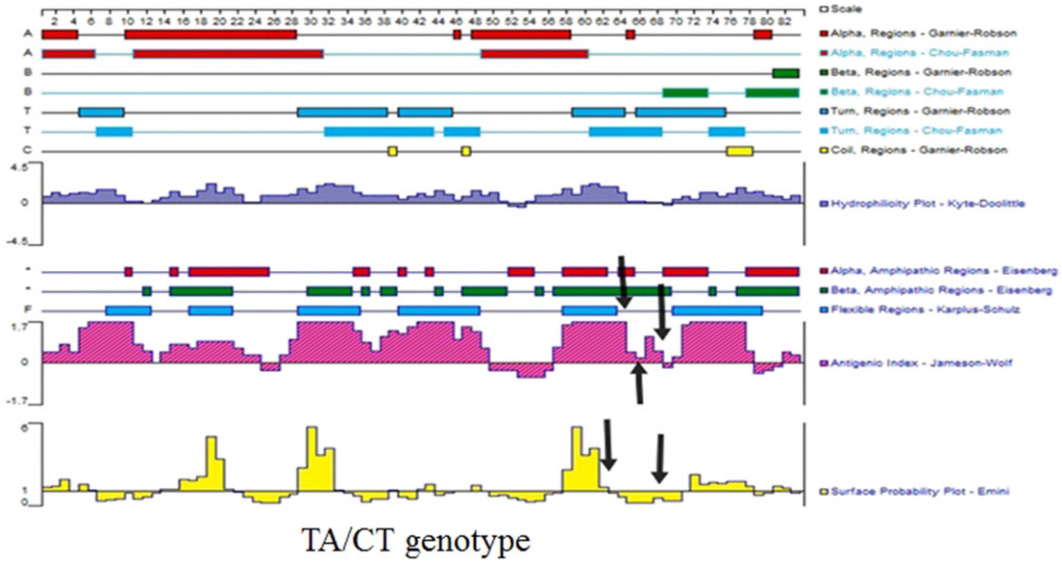
An SNP is a single base pair mutation at a specific locus, usually consisting of two alleles that can be amplified by two pairs of primers in one PCR reaction. Cuomo et al. (2007) identified more than 10,000



GenBank Accession number HG970332.1 and TA/TA genotype



CT/CT genotype



TA/CT genotype

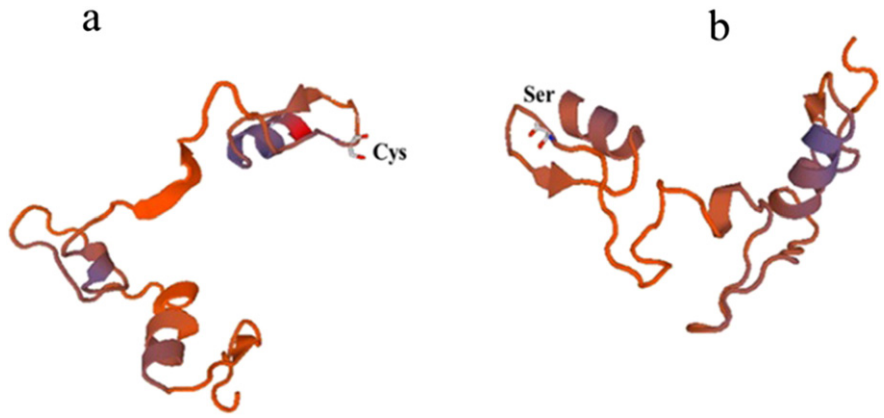


Fig. 7. Tertiary structure of Fg16 protein before mutation (a) and after mutation (change cysteine to serine amino acid (C65S) (b).

Table 3
Comparisons of statistics between Fg16 protein before mutation and after mutation.

Fg16 characteristics	TA/TA + GenBank HG970332.1	CT/CT	TA/CT
Molecular weight	9719.40 kDa	9834.38 kDa	9735.46 kDa
Length	83 aa	83 aa	83 aa
1 μg	102.887 pmol	103.795 pmol	102.217 pmol
Molar Extinction coefficient	720 ± 5%	840 ± 5%	840 ± 5%
1A (280)	13.50 mg/ml	11.47 mg/ml	11.59 mg/ml
Isoelectric point	9.83	9.71	9.71
Aliphatic index	49.40	49.40	49.40
Charge at pH 7	13.05	13.02	13.02
Instability index	72.29	69.97	69.97

SNPs from a comparison of two *F. graminearum s.s.* strains (strains PH-1 and GZ3639), which were frequently located near telomeres and within other discrete chromosomal segments. *F. graminearum* genes specifically expressed during plant infection (including predicted secreted proteins, major facilitator transporters, amino acid transporters, and cytochrome P450s) are all overrepresented in high SNP density regions, which may allow the fungus to adapt rapidly to changing environments or hosts (Cuomo et al., 2007). Perhaps the SNP have the greatest potential in elucidating the dynamics of host–pathogen interactions (Wang et al., 2011).

In the present study, two SNP (T82C and A352T) and one amino acid exchange (C65S) were found in the *Fg16* gene fragment. Prediction of the tertiary structure of proteins showed that this protein is a class of zinc finger proteins. Zinc-binding proteins form one of the largest families of transcriptional regulators in eukaryotes, displaying variable secondary structures and enormous functional diversity (Pace and Weerapana, 2014).

The majority of zinc finger proteins bind to DNA (and to RNA in the case of TFIIIA), thereby playing important roles in transcriptional and translational processes (Mac Pherson et al., 2006). Newly identified zinc finger proteins are also involved in many other physiological roles, including mediating protein–protein interactions, chromatin remodeling, protein chaperoning, lipid binding and zinc sensing (Laity et al., 2001).

Suggesting that lipid accumulation precedes sexual reproduction and that the oxidation of lipids accompanies fruiting body development, Guenther et al. (2009) reported that genes involved in lipid biosynthesis are highly expressed during vegetative growth and early sexual development, and during colonization in wheat stalks. Genes involved in β-oxidation showed higher transcript levels during sexual development in wheat stalks, when lipids would be used for energetics and building cellular structures. Gene expression analysis suggests two phases of cellular growth with respect to lipid metabolism: a biosynthetic phase, where lipids are synthesized and accumulate, and a phase during which stored lipids are utilized to produce a fruiting body (Guenther et al., 2009). Maggio-Hall and Keller (2004) have shown that β-oxidation of fatty acids takes place both in peroxisomes (branched and very long-chain fatty acids) and in mitochondria (short-chain and long-chain).

In *F. graminearum*, the presence of homologues of the *Aspergillus nidulans* mitochondrial pathway genes *echA* (enoyl-CoA hydratase, FGSG_00704; gene 16) and *scdA* (short chain acyl-CoA dehydrogenase, FGSG_09661; gene 23) indicates the presence of a lipid oxidation pathway (Guenther et al., 2009). These genes, involved in mitochondrial lipid oxidation, show expression typical of lipid oxidation genes. In the current study, SNPs in the *Fg16* gene lead to an exchange in the cysteine to serine amino acid (C65S) and this protein predicted is a class of zinc finger proteins, perhaps the SNPs have the greatest potential in elucidating the dynamics of host–pathogen interactions (Wang et al., 2011).Yang et al. (2008) characterized *Fusarium* isolates at the species level by a robust set of diagnostic primers based on SNPs among members of the *Fg* complex. Based on the SNPs in ammonia ligase 2 (CTPS2) and translation elongation factor 1α (*TEF-1α*) genes between different species within the *F. graminearum* clade, species-specific primers were designed. According to differences in the length of the ammonia ligase 2 (CTPS2) gene-derived amplicons, *F. asiaticum*, *F. meridionale*, and other members of the *F. graminearum* clade can be distinguished (Yang et al., 2008). *F. meridionale* isolates have a thymidine at nucleotide 306 in the partial sequence of the ammonia ligase 2 gene, while there is a cytosine in the other species of the *F. graminearum* clade. In the partial sequence of the *TEF-1α* gene, there is a cytosine at nucleotide 364 in *F. graminearum s.s.* isolates while other species have a thymidine at this position (Yang et al., 2008).

In addition, numerous SNP-based trichothecene chemotype assays were developed from the trichothecene biosynthetic pathway genes.

Fig. 6. Changes in the secondary structure of protein and the comparison between the referring sequence (GenBank accession number HG970332.1) and different genotypes TA/TA, CT/CT and TA/CT genotypes of *Fg16* gene. Differentiations are presented by the ↓ symbol.

Table 4
Population size (n), effective number of alleles (ne), Shannon's information index (I), expected heterozygosity (He), expected unbiased heterozygosity (He_u) and observed heterozygosity (Ho) in six populations *F. graminearum s.s.*

Population	*n*	*ne*	I	PIC	Heterozygosity		
					He	He_u	Ho
Golestan	25	1.310	0.3227	0.399	0.2305	0.2365	0.1941
Mazandaran	11	1.145	0.2093	0.290	0.1527	0.1536	0.1065
Hamedan	38	1.759	0.5385	0.699	0.4634	0.4726	0.4152
Kurdistan	24	1.331	0.3757	0.414	0.2411	0.2687	0.2176
Kermanshah	24	1.423	0.4292	0.511	0.3345	0.3512	0.3080
Lorestan	50	1.874	0.6038	0.772	0.5208	0.5214	0.4954
Mean		1.473	0.4132	0.514	0.3238	0.334	0.2894

Two sets of multiplex primers specific to individual chemotypes were designed from the *Tri3* and *Tri12* genes (Ward et al., 2008). Zhang et al. (2007) also developed an effective set of diagnostic primers based on SNPs among three different chemotype isolates and determined that there was a recent shift to the 3-AcDON chemotype. The high genetic diversity of this group of genes suggests that the fungus has great capacity for adaptability and genetic change during its interaction with a host species. The richness in SNPs in the fungal genome combined with real-time PCR assays would provide a powerful tool for the development of markers for various identifications within the *Fg* complex (Wang et al., 2011).

The low haplotype diversity detected by the SSCP markers indicates that it is too conserved for population genetic studies of the *Fg* complex. SSCP analysis of the Fg16F/R PCR amplicon differentiated *F. graminearum s.s.*, *F. asiaticum* and *F. meridionale* from China and revealed three haplotypes among sequence-characterized amplified region (SCAR) type 1 *F. graminearum s.s.* isolates: 1A, 1B and 1C, while the remaining SCAR types displayed the same SSCP patterns with no polymorphism (Qu et al., 2008). All *F. graminearum s.s.* isolates from China and Europe, and 5 of the seven isolates from the USA were haplotype 1A. The remaining two American isolates (A2 and A5) were haplotype 1B (Qu et al., 2008). DNA sequencing of the Fg16F/R product confirmed that haplotype 1B differed from that of 1A at nucleotide 348 (C–T) and 412 (T–A)(Qu, 2002). In the present study, SSCP assays of 172 isolates from Iran indicated that all isolates were *F. graminearum* (1A and 1B haplotypes) and haplotypes differed at nucleotide 82 (T→C) and 352 (A→T) (Table 1). Three haplotypes (haplotypes 3, 4 and 5) were observed among *F. asiaticum* isolates from Nepal while two haplotypes were observed among the limited number of isolates from Europe (haplotypes 1A and 6) and the USA (haplotypes 1A and 1B) (Carter et al., 2002).

In this study, none of the populations was in Hardy–Weinberg õequilibrium. Many factors affect the Hardy–Weinberg equilibrium such as selection, mutation, small population size and migration (Edwards, 2008). In all populations, two mutations at 82 and 352 nucleotides was observed, in which the mutations could disrupt the Hardy–Weinberg equilibrium.

Thus, according to SNP mutations, SSCP analysis using Fg16F/Fg16R can be used to distinguish the *F. graminearum* clade. However, there is little knowledge about the *Fg*16 gene and its function, thus, identification polymorphisms of *Fg16* gene could display the genetic diversity and help to predict population dynamics, genetic diversity and associate these changes with different traits of the *F. graminearum* clade. The results showed that the *Fg16* gene had a high degree of polymorphism, which can be considered for future control programming strategies and thus the associations between the SSCP patterns (genotypes) with different traits of *F. graminearum* such as wheat colonization, perithecium formation on stalk tissues and lineage discrimination should be investigated.

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