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 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 f16 gene. The average diversity levels detected were relatively high (He: 0.3238; Heu: 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.

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 (Guenter 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 (Guenter 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 (Guenter et al., 2009). Lipid stores are predominantly composed of triacylglycerides, which are characterized by a glycerol backbone and

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**A B S T R A C T**

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 f16 gene. The average diversity levels detected were relatively high (He: 0.3238; Heu: 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.

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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 the lineage/group 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 product obtained with primer set Fg16F/Fg16R, resolved isolates of 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, 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.

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 fungus 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 kept 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 1a (EF-1a) 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 gel 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 1a (EF-1a) 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).
2.5. 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 (Heu) 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 ($\chi^2$).

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 (EF1α) 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 sensu stricto (s.s.) isolates were 54.16%, 60% and 56%, in Kurdistan, Lorestan and Golestan.

| 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         |

Table 1 Frequencies of Fg16 genotypes in randomly selected F. graminearum s.s. isolates in six populations.
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. 3.

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→CT); N: nucleotide sequence is the heterozygote genotype.
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).

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; Heu = 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 isolates.
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 current 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 TFIIA), 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 ω-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 1 (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.

### 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% |
| IA (280)              | 13.50 mg/ml                | 11.47 mg/ml | 11.59 mg/ml |
| Isoelectric point     | 9.83                       | 9.87 | 9.87 |
| 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 |

**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.

**Fig. 7.** Tertiary structure of *Fg16* protein before mutation (a) and after mutation (change cysteine to serine amino acid (C65S) (b).
Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32, 314–331.

Carter, J.P., Rezanoor, H.N., Desjardins, A.E., Nicholson, P., 2000. Variation in Fusarium graminearum isolates from Nepal associated with their host of origin. Plant Pathol. 49, 1–10.

Carter, J.P., Rezanoor, H.N., Holden, D., Desjardins, A.E., Plattner, R.D., Nicholson, P., 2002. Variation in pathogenicity associated with the genetic diversity of Fusarium graminearum. Eur. J. Plant Pathol. 108, 573–583.

Charmley, L.L., Trenholm, H.L., Prelusky, D.A., Rosenberg, A., 1995. Economic losses and decontamination. Nat. Toxins 3, 199–203.

Cuomo, C.A., Güldener, U., Pietro, A.D., Walton, J.D., Ma, L.J., Bücking, H., Shachar-Hill, Y., Trail, F., 2007. The Fusarium graminearum genome reveals a link between localized polymorphism and pathogen specialization. Science 317, 1400–1402.

Edwards, A.W.F., 2008. G. H. Hardy (1908) and Hardy–Weinberg Equilibrium. Genetics 179 (3), 1143–1150.

Guenther, J.C., Hallen-Adams, H.E., Bücking, H., Shachar-Hill, Y., Trail, F., 2001. Tricyclicgycolic acid metabolism by Fusarium graminearum during colonization and sexual development on wheat. Mol. Plant-Microbe Interact. 12 (2), 1492–1503.

Guenther, J.C., Trail, F., 2005. The development and differentiation of Gibberella zeae (anamorph: Fusarium graminearum) during colonization of wheat. Mycologia 97, 229–237.

Laita, J.H., Lee, B.M., Wright, P.E., 2001. Zinc finger proteins: new insights into structural and functional diversity. Curr. Opin. Struct. Biol. 11, 39–46.

Mac Pherson, S., Larochelle, M., Turcotte, B., 2006. A fungal family of regulatory factors: the zinc cluster proteins. Microbiol. Mol. Biol. Rev. 60, 563–604.

Maggio-Hall, L.A., Keller, N.P., 2004. Mitochondrial beta-oxidation in Aspergillus nidulans. Mol. Microbiol. 54, 1173–1185.

Mirocha, C.J., Abbas, H.K., Windels, C.C., Xie, W., 1989. Variation in deoxynivalenol, 15-acetoxyscirpenol, 3-acetoxydeoxynivalenol, and zearalenone production by Fusarium graminearum isolates. Appl. Environ. Microbiol. 55, 1313–1316.

Murphy, D.J., 2001. The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Prog. Lipid Res. 40, 325–438.

Nietl, W.J., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. 70, 3321–3323.

Nelson, P.E., Tousson, T.A., Marasas, W.F.O., 1983. Fusarium Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park (PA).

Nicholson, P., Rezanoor, H.N., Simpson, D.R., Joyce, D., 1997. Detection and quantification of the cereal eye spot fungi Tapesia yallundae and Tapesia aecium using a PCR assay. Plant Pathol. 46, 842–856.

Nicholson, P., Simpson, D.R., Weston, C., Rezanoor, H.N., Lee, A.K., Parry, D.W., Joyce, D., 1998. Detection and quantification of Fusarium culmorum and Fusarium graminearum cereals using PCR assays. Physiol. Mol. Plant Pathol. 53, 17–37.

O’Donnell, K., Kistler, H.C., Tacke, B.K., Casper, H.H., 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of Fusarium graminearum, the fungus causing wheat scab. Proc. Natl. Acad. Sci. U. S. A. 97, 7905–7910.

O’Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C., Aoki, T., 2004. Genealogical concordance between the mating type loci and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the Fusarium graminearum clade. Fungal Genet. Biol. 41, 600–623.

Pace, N.J., Weerapan, E., 2014. Zinc-binding cytochrome: diverse functions and structural motifs. Biomolecules 4, 419–434.

Parry, D.W., Jenkinson, P., MacLeod, L., 1995. Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol. 44, 207–238.

Qu, B., 2002. Genetic Diversity of Fusarium graminearum in China and its Comparison with the Isolates from of Nepal, Europe, and USA. Hauzhong Agricultural University, Wuhan, China PhD thesis.

Qu, R., Li, H.P., Zhang, J.B., Huang, T., Carter, J., Liao, Y.C., Nicholson, P., 2008. Comparison of genetic diversity and pathogenicity of Fusarium head blight pathogens from China and Europe by SSCP and seedling assays on wheat. Plant Pathol. 57, 642–651.

Sanguinetti, C.J., Dias, Neto E., Simpson, A.J.G., 1994. Rapid silver staining and recovery of Aspergillus nidulans genome reveals a link between localized polymorphism and pathogen specialization. Science 317, 1400–1402.

Shaner, G.E., 2003. Epidemiology of Fusarium Head Blight of Small Grain Cereals in North America. In: Leonard, J., Bushnell, W.R.K. (Eds.), Fusarium Head Blight of Wheat and Barley. American Phytopathological Society Press, St. Paul, MN, USA.

Waalwijk, C., Kastelein, P., Vries, I., Kerényi, Z., van der Lee, T., Hesselink, T., Köhl, J., Kema, H., 1994. Rapid silver staining and recovery of PCR products separated on polycrylamide gels. Biotechn. Tech. 17, 914–921.

Whelan, J., Landegren, U., Khatib, Z., Van der Lee, T., Hesselink, T., Köhl, J., Kema, H., Shachar-Hill, Y., Trail, F., 2001. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of Fusarium graminearum, the fungus causing wheat scab. Proc. Natl. Acad. Sci. U. S. A. 97, 7905–7910.

Wee, J.D., 1974. Fungal Lipid Biochemistry: Distribution and Metabolism. Plenum Press, New York.

Yang, L., van der Lee, T., Yang, X., Yu, D., Waalwijk, C., 2008. Fusarium populations on Chinese barley show a dramatic gradient in mycotoxin profiles. Physiopathology 98, 719–727.

Zhang, J.B., Li, H.P., Dan, J.D., Qu, B., Xu, Y.B., Zhao, C.S., Liao, Y.C., 2007. Determination of the deoxynivalenol mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the Fusarium graminearum clade from China. Mycol Res. 111, 967–975.

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 the mutations could disrupt the Hardy–Weinberg equilibrium. In all populations, two mutations at 82 and 352 nucleotides was observed, in which the mutations could disrupt the Hardy–Weinberg equilibrium. In all populations, two mutations at 82 and 352 nucleotides was observed, in which the mutations could disrupt the Hardy–Weinberg equilibrium.