

FcMgv1, *FcStuA* AND *FcVeA* BASED GENETIC CHARACTERIZATION IN *Fusarium culmorum* (W.G. Smith)

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Abstract: *Fusarium culmorum* (W.G. Smith) leads to economic losses in wheat and barley fields in Turkey as well as in many countries worldwide as a result of head blight and crown rot diseases. In this study, *in vitro* growth capacity of 33 *F. culmorum* isolates originating from Turkey and the relationship between phenotypic and genetic characteristics obtained based on similarities of *FcMgv1*, *FcStuA* and *FcVeA* genes were investigated. Linear growth rate values were recorded at 4th and 7th days of incubation. The mean linear growth rate values ranged from 7.58±1.06 to 14.7±1.26mm/day. The isolates F2 and 18F with relatively high linear growth values and the isolates 12F and F19 with relatively low linear growth values, were selected to be used in multiloci based genotyping analysis. *FcMgv1*, *FcStuA* and *FcVeA* genes were amplified in lengths of 1733, 2001 and 1898bp, respectively. The genes were sequenced, aligned and then subjected to BLASTn and to maximum likelihood topology analysis. Nucleotide sequence of each gene showed maximum hit with associated genes deposited in NCBI with 0.0-0.0 E-values and 1188 to 3256 bit scores. Alignment analysis resulted in at least 89% bootstrap support. Moreover, isolates with similar linear growth rates were co-clustered in phylogenetic analysis. The findings obtained in this study showed that the three genes which are essential for fungal survival could be used in genetic characterization analysis and in revealing the associations between their genetic and phenotypic characteristics.

Key words: *Fusarium culmorum*, PCR, genetic characterization, genetic diversity, linear growth rate.

Özet: Dünya çapında pek çok ülkede olduğu gibi Türkiye’de de *Fusarium culmorum* (W.G. Smith) başak yanıklığı ve kök çürüklüğü hastalıkları ile arpa ve buğday tarım alanlarında ekonomik kayıplara yol açar. Bu çalışmada, Türkiye’den köken alan 33 *F. culmorum* izolatının *in vitro* büyüme kapasitesi ile *FcMgv1*, *FcStuA* ve *FcVeA* genetik benzerliği aracılığı ile elde edilen fenotipik ve genetik karakterlerin ilişkisi incelenmiştir. Doğrusal büyüme oranı değerleri inkübasyonun 4. ve 7. günlerinde kaydedilmiştir. Ortalama doğrusal büyüme oranlarının 7,58±1.06 ve 14,7±1.26mm/gün arasında olduğu görülmüştür. Göreceli olarak yüksek LGR değerlerine sahip olduğu belirlenen F2 ile 18F izolatları ile göreceli olarak düşük LGR değerlerine sahip olduğu belirlenen 12F ve F19 izolatları multilokus temelli genotiplendirme analizlerinde kullanılmak üzere seçilmiştir. *FcMgv1*, *FcStuA* ve *FcVeA* genlerine ait sırasıyla 1733, 2001 ve 1898bp ürünler elde edilmiştir. Genler dizilenmiş, bir araya getirilmiş ve BLASTn ile maksimum olasılık topoloji analizi yapılmıştır. Her bir genin nükleotid dizisi NCBI’da 0,0-0,0 E-değeri ve 1188-3256 arası bit skoru vermiştir. Hizalama analizi en az %89 ön yükleme değeri ile sonuçlanmıştır. Ayrıca, benzer büyüme oranına sahip izolatlar filogenetik analizlerde aynı alt kümede yer almıştır. Bu çalışmada elde edilen bulgular, fungal yaşam için gerekli olan bu üç genin, genetik karakterizasyonda ve fenotipik ve genotipik özellikleri arasında ilişki kurulmasında kullanılabileceğini ortaya konmuştur.

Introduction

Fusarium culmorum (W.G. Smith) has been reported to be associated with head blight, ear blight and crown rot, diseases of cereals including wheat, barley, and maize (Miedaner *et al.* 2008). This fungus is the second major causal agent of head blight, one of the most destructive diseases affecting cereals worldwide and it is a predominating agent particularly in cooler regions of the world (Yli-Mattila *et al.* 2013, Pasquali & Migheli 2014, Yörük *et al.* 2016). *F. culmorum* is known as a quarantined phytopathogen and it is capable of producing some major and minor mycotoxins such as trichothecenes,

zearalenon, fusarin C, and butanolide. In addition to its hazardous effects on cereals, *F. culmorum* also have adverse effects on human and animal health (e.g. carcinogenic and estrogenic effects) especially through class B trichothecenes and zearalenone (Desjardins & Proctor 2007, Niessen 2007). Therefore, detailed characterization of *F. culmorum* isolates is a requisite in studies considering fight against diseases associated with *F. culmorum*.

The necrotrophic *F. culmorum* is an asexually reproducing fungal plant pathogen, lacking a sexual stage.

However, potential parasexual reproduction is also assumed for *F. culmorum* since two mating types, *MATI-1* and *MATI-2*, were reported to be present approximately in equal proportions (Obanor *et al.* 2010, Albayrak *et al.* 2016). The number of chromosomal nucleotide sequence data of *F. culmorum* has recently increased by registrations of growing sequence data in GenBank and some other databases. However, the number of annotated and well characterized *F. culmorum* genes is still limited. Since the species has a high level of genetic variation worldwide (Miedaner *et al.* 2008), detailed and comprehensive genetic and genomic characterization of isolates are crucial in terms of effective control of the fungus. Several different polymerase chain reaction (PCR) based strategies including single primer based genotyping, sequencing based locus screening, and restriction endonuclease digestion based genotyping have been used in *F. culmorum* genotyping. SCAR-RAPD-PCR (Sequence characterized amplified region-random amplified polymorphic DNA-polymerase chain reaction), inter simple sequence repeat (ISSR), microsatellite PCR, restriction fragment length polymorphisms (RFLP), and single locus genotyping are the most common methods used in *F. culmorum* identification studies. SCAR and AFLP (Amplified fragment length polymorphism) are less frequently used in genotyping studies (Nicholson *et al.* 1998, Miedaner *et al.* 2001, Mishra *et al.* 2003, Chung *et al.* 2008, Albayrak *et al.* 2016). These techniques are generally cost effective, non-laborious, and not time-consuming and they do not need high amount of DNA but minimum prior nucleotide sequence knowledge. On the other hand, non-reproducible bands and inconsistent findings are the two major disadvantages of these techniques. In addition, a majority of these marker techniques do not provide co-dominant markers meaning that there is no possibility to distinguish the heterozygote individuals from the homozygote one (Williams *et al.* 1990, Bornet & Branchard 2001, Llorens *et al.* 2006).

Multilocus genotyping methods are promising in terms of providing a fast, cost effective, reliable, and reproducible strategy for *F. culmorum* genotyping. The most commonly used strategy in *Fusarium* spp. studies is the multiloci genotyping applied for distinguishing of *F. graminearum* (Schwabe) species complex members via 12 different genes of approximately 16kb (O'Donnell *et al.* 2000, Yli-Mattila *et al.* 2009, Sarver *et al.* 2011). In contrast to its efficient use in species complex differentiation of *F. graminearum*, this strategy is not useful for *F. culmorum* which was reported as a monophyletic species (Obanor *et al.* 2010, Przemieniecki *et al.* 2014). The use of multiloci genotyping analysis led so far to precise investigation of even if not all phenotypic characteristics and genotypic similarities and to well recognition of geographic region and genotypic diversity associations. Multiloci genotyping analysis showed that *F. graminearum* could be defined as a species complex with 15 different members (van der Lee *et al.* 2015, Pasquali *et al.* 2016). Despite the relatively long nucleotide sequence regions that have been used in

multiloci genotyping, a low level of genetic diversity was detected. However, because presence of high level of intra-specific variation could not be detected by multiloci genotyping via 12 genes, novel strategies can be adapted to *Fusarium* spp. characterization. These novel strategies could be useful in making associations with genetic similarities and some other phenotypic characteristics such as host species, chemotype etc. in *Fusarium* isolates. Yörük & Sefer (2017) used a genotyping strategy based on sequencing of *FgMgv1*, *FgStuA*, and *FgVeA* genes in *F. graminearum* to make association between some characteristics such as 15-acetyldeoxynivalenol producing capacity, radial growth capacity, and genetic similarity. The findings obtained from *FgMgv1*, *FgStuA* and *FgVeA* sequencing and alignment assays showed that genotyping of these genes could be useful in detailed differentiation of fungal species complex members. Since *FgMgv1*, *FgStuA* and *FgVeA* genes whose expressions are essential in sexual/asexual development, cell wall formation and survival of fungal cells possess high level of genetic similarity in terms of nucleotide sequence data, variation in these genes can lead to significant differences in phenotypic characteristics (Hou *et al.* 2002, Jiang *et al.* 2011, Pasquali *et al.* 2013). Macroconidia and/or microconidia production, radial growth rate, host type, and chemotype characteristics can be potential phenotypic traits that can be associated with nucleotide sequence variation. In this study, it was aimed to develop novel multiloci genotyping by amplification and sequencing of *FcMgv1*, *FcVeA*, and *FcStuA* genes and to make association between genetic diversity and phenotypic characteristics.

Materials and Methods

Fungal materials and linear growth rate (LGR) assays

Fusarium culmorum isolates were provided by Dr. Berna Tunali from Ondokuz Mayıs University, Agricultural Faculty, Department of Plant Protection. Each of the 33 isolates (see Table 1) included in the study was given a specific code, purified and identified according to standard morphological and genetic methods (Yörük *et al.* 2016).

Czapek dox agar (CDA) medium was used for *in vitro* growth of the isolates. Fresh *F. culmorum* cultures were initiated by placing the mycelia plug in the middle of the CDA medium. The cultures were incubated at 25°C for 7 days. Linear growth rates (LGR) were calculated as mm/day at 4th and 7th days of incubation (Irzykowska *et al.* 2013). Mean and standard deviation values for LGR data were calculated using column statistics (Graphpad Prism 5.0 software, USA).

Genomic DNA extraction

Genomic DNA (gDNA) of the isolates was isolated from 7-day-old cultures using the CTAB-based DNA isolation kit (BioBasic, Canada). Approximately 100mg of mycelium on the culture surface was collected and after washing with 96% ethanol for 3 minutes, mycelium was homogenized in liquid nitrogen with sterile mortar and

pestle. The homogenate was transferred to a microtube and the binding, washing and elution steps were carried out according to manufacturer's recommendations. The purities and quantities of gDNA was analysed via spectrophotometer (Thermo, USA) and 1% agarose gel electrophoresis. gDNA was visualized under UV light of gel documentation system (MaestroGen, Taiwan) by staining with 0.2µg/mL ethidium bromide. Electrophoresis was carried out under 60V constant power for 60 minutes.

Table 1. Codes, host plants, the region of isolations, the mating types and the isolation years of the *F. culmorum* isolates.

| Code | Host | Region/Province | Mating | Year |
|--------|--------|-----------------|-----------------|------|
| F1 | Wheat | Marmara | <i>MAT1-1</i> | 2006 |
| F2 | Wheat | Marmara | <i>MAT1-2</i> | 2006 |
| F3 | Wheat | Konya | <i>MAT1-2</i> | 2006 |
| F4 | Wheat | Marmara | <i>MAT1-2</i> | 2006 |
| F10 | Wheat | Bilecik | <i>MAT1-1</i> | 2006 |
| F12 | Wheat | Balıkesir | <i>MAT1-2</i> | 2006 |
| F14 | Wheat | Bilecik | <i>MAT1-2</i> | 2006 |
| F15 | Wheat | Sinop | <i>MAT1-1</i> | 2006 |
| F16 | Wheat | Konya | <i>MAT1-1</i> | 2006 |
| F17 | Wheat | Konya | <i>MAT1-1</i> | 2006 |
| F19 | Wheat | Konya | <i>MAT1-1</i> | 2006 |
| F20 | Wheat | Bilecik | <i>MAT1-2</i> | 2006 |
| F21 | Wheat | Uşak | <i>MAT1-1</i> | 2006 |
| F24 | Wheat | Konya | <i>MAT1-2</i> | 2006 |
| 8F | Wheat | Ankara | <i>MAT1-2</i> | 2009 |
| 9F | Wheat | Isparta | <i>MAT1-1</i> | 2008 |
| 10F | Wheat | Samsun | <i>MAT1-2</i> | 2007 |
| 11F | Wheat | Corum | <i>MAT1-2</i> | 2009 |
| 12F | Wheat | Amasya | <i>MAT1-2</i> | 2009 |
| 13F | Wheat | Konya | <i>MAT1-1</i> | 2008 |
| 17F | Wheat | Ankara | <i>MAT1-1/2</i> | 2009 |
| 18F | Wheat | Eskişehir | <i>MAT1-1/2</i> | 2010 |
| 19F | Wheat | Eskişehir | <i>MAT1-1/2</i> | 2010 |
| 20F | Barley | Afyon | <i>MAT1-1</i> | 2011 |
| 14-1TR | Barley | Sivas | <i>MAT1-2</i> | 2014 |
| 14-2TR | Wheat | Samsun | <i>MAT1-1</i> | 2014 |
| 15-1TR | Wheat | Tekirdağ | <i>MAT1-1/2</i> | 2015 |
| 14-3TR | Wheat | Yozgat | <i>MAT1-1/2</i> | 2014 |
| 09-1TR | Wheat | Kastamonu | <i>MAT1-1/2</i> | 2009 |
| 15-2TR | Wheat | Edirne | <i>MAT1-2</i> | 2015 |
| 15-3TR | Barley | Tekirdağ | <i>MAT1-2</i> | 2015 |
| 15-4TR | Wheat | Edirne | <i>MAT1-1/2</i> | 2015 |
| 14-8TR | Wheat | Amasya | <i>MAT1-2</i> | 2014 |

FcMgv1, FcStuA and FcVeA amplification and cloning

Allele specific or chromosomal nucleotide sequence data of *FcMgv1*, *FcStuA* and *FcVeA* genes were obtained from the GenBank with accession numbers AF492766.1, HG970332.2 and HQ436464.1, respectively. The primers of 20 nucleotides in length were designed using the “Primer 3” software (Rozen & Skaletsky 2000) for the polymerase chain reaction (PCR). PCRs were carried out in a reaction volume of 50µL including 1X PCR mix (Takara, Japan), 10pmol of each primer (Table 2), and 50ng of DNA. PCR amplification was performed by pre-denaturation at 98°C for 2min, followed by 34 cycles of amplification at 98°C for 30s, 55°C for 30s, 72°C for 3min and final extension at 72°C for 5min. PCR bands were separated on 1.5% agarose gels. Qualitative analysis of bands was carried out via gel documentation system.

Table 2. Primers used in this study.

| Primer name | Sequence 5'-3' | Amplicon Size |
|-------------|---------------------------|---------------|
| MGVSPANF | ATGGGCGACCT ACAAGGACG | 1733bp |
| MGVSPANR | TTATCTTCGAG AAGCATCCA | |
| STUASPANF | ATGAACCAAA GTCATCACCA | 2001bp |
| STUASPANR | CTATCCAAAGG ACTGTTGCC | |
| VELVETSPANF | CTGGGTTTCCTC TCTGCCTTA | 1898bp |
| VELVETSPANR | TGTCGCTCATG TATCTTCCAT | |

PCR bands were purified from agarose gels by using a gel extraction kit (BioBasic, Canada). Pieces (100-200mg) including amplicons were cut from the gels and the purification was carried out according to the protocol provided by the manufacturer.

Bioinformatics assays

The sequencing process was maintained based on Sanger dideoxy termination method using “DYEnamic ET Terminator Cycle Sequencing” kit (Amersham, USA) on ABI PRISM 310 system. The nucleotide sequence was displayed in chromatograms using the “Chromas Pro” software. The sequences translated to FASTA format were subjected to BLASTN analysis. The nucleotide sequences generated via forward and reverse primers were aligned via DNA Dragon software. Assembled DNA sequences were used in multiple alignment analysis via ClustalW 1.8. software and in neighbor joining (NJ) topology analysis via Mega 6.0 software (Tamura *et al.* 2013). Pairwise distance and bootstrap support values were recorded for each assay. Accession numbers for *FgMgv1*, *FgStuA* and *FcVeA* genes (see above) were used as reference control in alignment analysis. The potential amino acids changes were characterized in “HOPE amino acid mutation” analysis.

Results

LGR analysis and fungal sample selection

Single spore *F. culmorum* isolates were grown successfully on CDA media with a main pigmentation of red to tan colour (data not shown). Each isolate was used

in LGR assays. The minimum and maximum mean LGR values of the isolates were 7.58 ± 1.06 and 14.7 ± 1.26 mm/day, respectively (Fig. 1). Isolates with relatively high (F2 and 18F) and low (12F and F19) LGR values were used in multilocus sequencing based genotyping analysis.

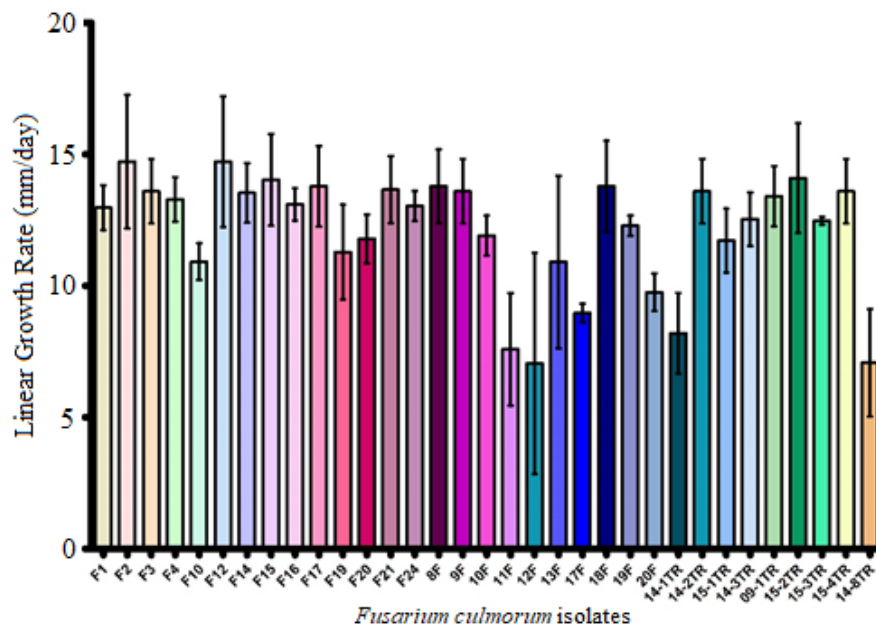


Fig. 1. LGR data of the *F. culmorum* isolates.

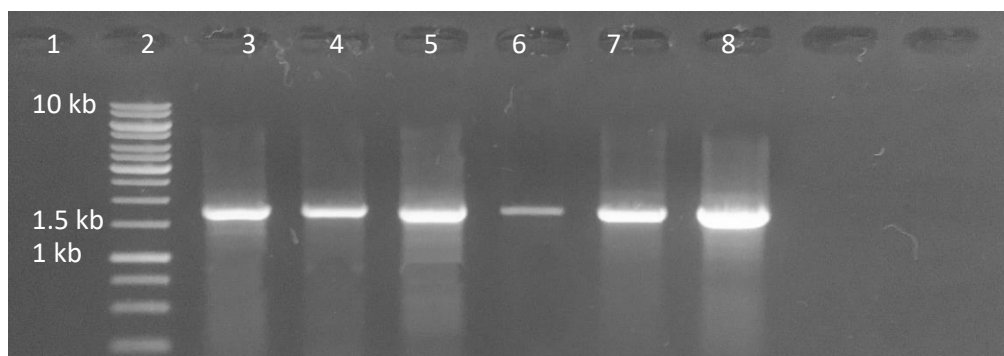


Fig. 2. *FcMgv1*, *FcStuA*, and *FcVeA* genes amplified from F2, F19 isolates. 1: negative control, 2: 1kb size marker (Thermo, ABD), 3, 5, 7: from F2 isolate, 4, 6 and 8: from F19 isolate, 3, 4; 5, 6 and 7, 8 *FcMgv1*, *FcStuA* and *FcVeA* PCR product, respectively.

gDNA Isolation and PCR analysis

gDNAs were extracted from 7-day-old cultures of the isolates F2, 18F, 12F and F19. High quality ($A_{260/280}=1.7-1.9$) and quantity ($1-2.5 \mu\text{g}/\mu\text{L}$) of gDNAs were obtained from 100mg fresh mycelia.

FcMgv1, *FcStuA* and *FcVeA* genes were used in genetic characterizations. 1733, 2001 and 1898bp amplicons were obtained from each isolate (Fig. 2). PCR amplicons purified from agarose gels were checked on agarose gel again. The PCR bands visible on gels (approximately $20-100 \text{ng}/\mu\text{L}$) were subjected to sequencing analysis.

Alignment analysis

Nucleotide sequence data of *FcMgv1*, *FcStuA* and *FcVeA* genes were screened and subjected to BLASTn

analysis. The similarity of the sequence data with target associated genes recorded on GenBank (Table 3) was found to be significant. The E-values and maximum bit scores ranged from 0.0 to 0.0 and from 1188 to 3256, respectively.

The assembled *FcMgv1*, *FcStuA* and *FcVeA* sequences were subjected to ClustalW and NJ topology analysis. The nucleotide sequence data obtained from the three genes were merged and used as a single locus in homology analysis. According to ClustalW analysis minimum bootstrap support value was 89% (Fig. 3). The minimum and maximum genetic similarity values were ranged from 89 to 99%. According to topology analysis, isolates with the similar LGR values were included in the same sub-cluster and they were genetically closer to each other (Fig. 3).

Alignment analysis showed that isolates with similar LGR values had the same type of amino acid changes in 430 and 693 points (Fig. 4). HOPE amino acid mutation analysis showed that these mutations occurred as conversion of proline to leucin and glycine to serine could directly affect ligand and substrate binding interactions with proteins coded by the genes used.

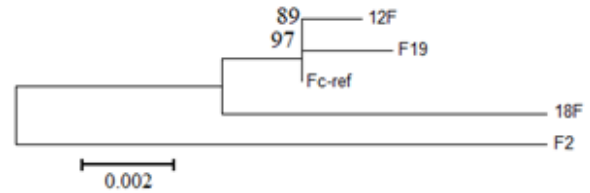


Fig. 3. Maximum Likelihood phylogram of the isolates. Bootstrap support values above 70% are shown above the branches.

Table 3. BLASTn analysis of three genes aligned in this study.

| Sample | Gene | Corresponding organism | Accession no | E value | Bit score |
|--------|---------------|------------------------|--------------|---------|-----------|
| F2 | <i>FcMgv1</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 1653 |
| F2 | <i>FcStuA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 3256 |
| F2 | <i>FcVeA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 2761 |
| 18F | <i>FcMgv1</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 1687 |
| 18F | <i>FcStuA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 3201 |
| 18F | <i>FcVeA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 2854 |
| 12F | <i>FcMgv1</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 2843 |
| 12F | <i>FcStuA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 3201 |
| 12F | <i>FcVeA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 1188 |
| F19 | <i>FcMgv1</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 1698 |
| F19 | <i>FcStuA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 3253 |
| F19 | <i>FcVeA</i> | <i>F. culmorum</i> | LT598659.1 | 0.0 | 1995 |

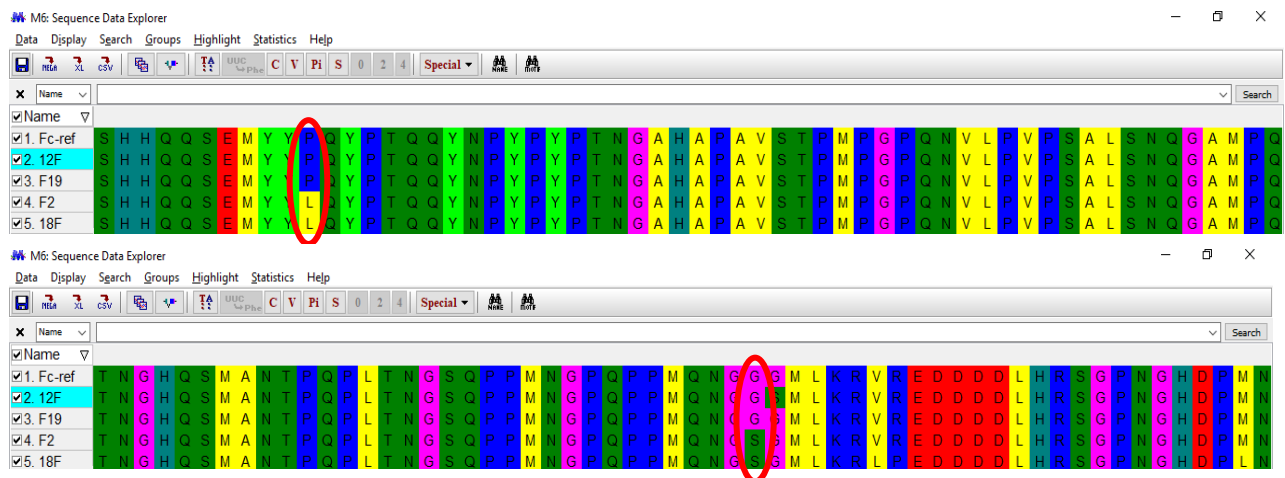


Fig. 4. Phylogeny analysis obtained with MEGA 6.0 software. The boxes within the red circle show the mutations. Fc-ref means the merged nucleotide sequences of *FcMgv1*, *FcStuA* and *FcVeA*, respectively.

Discussion

Fusarium head blight and crown rot of cereals are among the fungi-based destructive diseases worldwide (Miedaner *et al.* 2008, Yli-Mattila *et al.* 2013, Pasquali & Migheli 2014). Diagnosis and genetic characterization of causal agents of these diseases form the crucial step in disease management. The comprehensive genotyping strategies would provide useful data and knowledge in order to fight with *F. culmorum* which is major causal agent of head blight and crown rot in many countries. The majority of studies on population structure of *F.*

culmorum mainly include the use of molecular marker techniques. These techniques are generally associated with targeting and amplifying unknown or random sites on genomes. These marker strategies have some disadvantageous in usage because they do not allow obtainment of co-dominant markers, the data obtained through these strategies are non-reproducible and they are likely to yield false positive results (Miedaner *et al.* 2008, Ward *et al.* 2008). However, multilocus genealogical concordance analysis provides more allele specific and reliable findings about genetic characteristics of fungal

samples. Moreover, co-dominant markers could be provided via multiloci genotyping. *F. graminearum* species complex members have been successfully identified via genealogical concordance analysis of genes with highly conserved nucleotide sequence data (O'Donnell *et al.* 2000, Yli-Mattila *et al.* 2009, Sarver *et al.* 2011). However, there is no additional usage of these conserved markers for making correlation between genetic diversity and some phenotypic characteristics including radial growth capacity, host type, macroconidium reproduction, chemotype differences or mating type. Yörük & Sefer (2017) used *FgMgv1*, *FgStuA* and *FgVeA* genes in order to make association with genetic similarity and chemotype and radial growth rate in *F. graminearum*. The results obtained from *FgMgv1*, *FgStuA* and *FgVeA* sequencing in *F. graminearum* showed that nucleotide sequence of these genes could be used as a supportive tool for detailed characterization of hemi-biotrophic species. However, there exist no data about detailed analysis of these genes for monophyletic species such as *F. culmorum*, *F. semitectum*, *F. poae* etc. and about amino acids alignments. Also, genotypic diversity and bootstrap support values obtained from this study were relatively close to the results obtained from individuals of *F. graminearum* species complex (O'Donnell *et al.* 2000, Yli-Mattila *et al.* 2009, Sarver *et al.* 2011). It was shown that *Mgv1*, *StuA* and *VeA* genotyping could be useful in differentiation of highly divergent individuals of fungal populations. In this study, we used *FcMgv1*, *FcStuA* and *FcVeA* genes for the first time as a simple gene set for making association between genetic and phenotypic characteristics in LGR and associated amino acid sequence alterations of *FcMgv1*, *FcStuA* and *FcVeA* were evaluated for necrotrophic fungus *F. culmorum* for the first time. These three genes could also be used in detailed genetic characterization of phytopathogenic fungi which could lead to devastating diseases worldwide such as *F. fujikori* (Niren-berg) species complex and *F. oxysporum* (Sacc.) species complex. Aggressiveness, host range and genotypic diversity in members of these species complexes are issues which are not well known. Thus, in addition to

genotyping of *F. culmorum* which is monophyletic phytopathogen, these three genes could be used in genotyping of fungal species complexes. Moreover, these genes could provide additional data for some important characteristics such as chemotype profiles which is still problematic worldwide (Pasquali & Migheli 2014, Pasquali *et al.* 2016). These kinds of novel approaches could contribute to disease management of monophyletic and polyphyletic fungi with different chemotypes.

We used, considering the data of LGR analysis, the isolates with relatively low and high *in vitro* growth capacity in genotyping analysis. Almost all Turkish *F. culmorum* isolates showed a high level of *in vitro* growth capacity compared to *Fusarium* spp. isolates subjected to LGR analysis in previous studies. (Chung *et al.* 2008, Irzykowska *et al.* 2013). Nucleotide sequence data of *FcMgv1*, *FcStuA* and *FcVeA* genes were firstly subjected to detailed BLASTn and multiple nucleotide alignment analysis. Predictably, each nucleotide sequence data showed maximum E-value with corresponding gene located at *F. culmorum* chromosomes deposited on GenBank. Assembled sequences were used in multiple alignment and phylogeny assays. Multiple alignment analysis provided effective results with bootstrap support more than 89%. Maximum likelihood topology analysis showed that isolates with the similar LGR values were co-clustered in the same sub-clusters. Moreover, isolates with the similar characteristics showed the same amino acid changes in comparison to isolates with distinct characteristics. Protein modelling analysis showed that the mutations detected in the genes subjected to the multilocus genotyping analysis could lead to alterations in structure and affinity and changes in 3D configurations in the proteins coded by these genes. Our present findings showed that the genes used in this study could be adapted to novel genotyping studies which could be useful in making association of genetic and phenotypic characteristics in *Fusarium* spp. Different isolates originating from different regions should be used in further studies in order to support the techniques used in the present study.

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