Class B-trichothecene profiles of *Fusarium* species as causal agents of head blight

Authors: Emre Yörük[1], Tapani Yli-Mattila[2*]

Affiliations/Addresses:

[1]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Istanbul Yeni Yuzyil University, 34010, Istanbul, Turkey

[2]Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014, Turku, Finland, E-Mail:tymat@utu.fi

[*]**Corresponding author**:

E-Mail: tymat@utu.fi

Tel.: +358-44-0560700

Fax: +358-2-3335549

ABSTRACT

Fusarium head blight is a destructive disease of all small grain cereals worldwide. More than ten *Fusarium* species cause the disease and *F. graminearum* and *F. culmorum* are the major causal agents in Europe, Asia and America continents. The disease leads to loses in crop quality and quantity including contamination with myxotoxins in particular class B-trichothecenes. Class B-trichothecenes include deoxynivalenol (DON), nivalenol (NIV) and their acetylated derivatives (15-acetylated deoxynivalenol (15-ADON), 3-acetylated deoxynivalenol (3-ADON) and 4-acetylated nivalenol (4-ANIV). Distributions of these toxin profiles have been detected in many regions all around the world. 15-ADON, *F. graminearum* and wheat seem to have been major chemotype, causal agent and host plant, respectively. Moreover, more than five host plants including barley, rice and maize, which are economically important, have been associated with *Fusarium* spp. 3-ADON chemotype is predominating northern Europe while NIV chemotype has been reported as locally predominating profile in certain geographic regions. Current modern techniques including PCR and chromatography analysis present reliable, fast and informative output data worldwide. Since the toxicity of mycotoxins, aggressiveness of species and scab resistance of host plants are variable, chemotyping studies could efficiently contribute to disease management strategies.

Keywords: chemotype, detection methods, *Fusarium culmorum*, *Fusarium graminearum*, mycotoxin profiling, trichothecenes

Running title: B-Trichothecene chemotype distribution in *Fusaria*

INTRODUCTION

Plants are interacting with other organisms (bacteria, fungi, nematodes, etc.) and environmental factors (humidity, temperature, light etc.) throughout their life. Under particular/specific conditions, the incidence of diseases could occur as a result of these interactions. Phytopathogenic organisms could lead to devastating diseases on economically important plants when favourable temperature and moisture conditions and susceptible host are present (Dyakov *et al.*, 2007).

Infection of plants by phytopathogenic fungi cause yield loses and reduction in crop quality and quantity. By this way, economic loss occurs and the losses could reach millions of dollar even in local areas (Salas *et al.*, 1999; Windels, 2000). Besides, mycotoxins, produced by fungal pathogen accumulate in crops and contaminated food and live feed, are hazardous for human and animals. Some of these toxic molecules have a stable structure even they are exposed to elevated high temperatures (Gutleb *et al.*, 2002; Lauren and Smith, 2001). Thus, the contamination possibility of crops by these stable molecules and disease epidemics could reach in high levels. Characterization of fungal diseases and mycotoxins are significant in terms of treatment strategies for diseases and development specific diagnostic approaches. Here we describe the class B-trichothecenes profiles in *Fusarium* species associated with head blight disease.

FUSARIUM HEAD BLIGHT

Fusarium head blight (FHB), also known as scab of wheat or ear blight of maize, is one of the most serious fungal diseases worldwide. The disease is at first reported in England in the year 1884 and it is associated with all small grain cereals in particular wheat, barley and maize (Goswami and Kistler, 2004; Parry *et al.*, 1995). In Russian Far East FHB was reported already in 1882 (Yli-Mattila and Gagkaeva, 2015) and even long before this year poisoning of local animals and people has occurred. The disease has devastating effects on wheat and barley especially in humid and semihumid areas throughout the world (Bai and Shaner, 2004). FHB lead to yield loses and reduction in quality and quantity of cereals. The disease becomes widespread because of changes in climatic conditions, crop rotations and other agronomic practices (Bottalico and Perrone, 2002; Miedaner *et al.*, 2008; PARRY *et al.*, 1995; Saharan *et al.*, 2004). As a result of epidemics, billions $ of loses have been recorded in great number of countries (BAI and SHANER, 2004; LORI *et al.*, 2009; Windels, 2000). Especially in the

1990s, loses arising from FHB of wheat were estimated over $3 billion in more than 10 states in America and Canada. An example that, from the year 1993 to 1998 economic loses was reached $300 million in Manitoba. Similar economic loses have also been recorded in European and Asian countries (Bai and Shaner, 2004; Windels, 2000).

During the last years FHB epidemics on maize, wheat, barley and oats were reported by several researchers in Asia (Ji *et al.,* 2007; Li *et al.,* 2009; Yörük and Albayrak, 2012), Europe (Gagkaeva and Yli-Mattila, 2004; Jennings *et al.,* 2004a; Jennings *et al.,* 2004b; Yli-Mattila and Gagkaeva, 2010, 2016; Yli-Mattila, 2012) and America (Scoz *et al.,* 2009; Walker *et al.,* 2000) continents which harbour more than half of human community. Besides causal agents of head blight were isolated from scabby kernels planted in Australia and North Africa (Kammoun *et al.,* 2010; Obanor *et al.,* 2010). The disease has become an important threat for agronomy worldwide and efforts have been currently tried to develop novel strategies for struggling with the disease.

All small grain cereals are the potent host for FHB causal agents. If favourable temperature, moisture and host plant are available, FHB pathogens could infect cereals at different stages in host plant's life. After flowering stage, the anthesis is the most common time to be infected by pathogen microorganisms (Bai and Shaner, 2004; Parry *et al.,* 1995). Disease symptoms are similar in different cereals. Shrivelled kernels and accumulation of mycotoxins, in particular, deoxynivalenol (DON), nivalenol (NIV) and their acetylated derivatives, are two characteristics of the disease (Saharan *et al.,* 2004). At the same time, DNA levels of *F. graminearum* and other *Fusarium* species are increased (Yli-Mattila and Gagkaeva, 2010; Yli-Mattila and Gagkaeva, 2015). Firstly, symptoms are seen as the brown small lesions occurred along rachis and glumes. This is followed salmon-pink to red fungal growth, which could be easily seen on along the spike. Discoloration spreads along spikelet. At the end of the disease, kernels become shrunken (Fig. 1) and even spikes could have no grain (Parry *et al.,* 1995).

The infection causes field loses. The level of yield loses could depend on several factors. Genotypic characteristics of fungal pathogens and Natural resistance level of host plants are of most important factors (Bai and Shaner, 2004; Parry *et al.,* 1995; Saharan *et al.,* 2004). Quellet and Seifert (1993) initiated genotyping studies by using random amplified polymorphic DNA (RAPD) and polymerase chain reaction (PCR) markers in *Fusarium graminearum* isolates associated with head blight in Canada. Among more than two decades, *Fusarium* species were isolated from scabby spikes and genetic variation in local and global isolate collections was investigated by many authors (Chehri *et al.,* 2011; Chung *et al.,* 2008; Gürel *et al.,* 2010; Laday

et al., 2004; Mishra *et al.,* 2006; Yli-Mattila, 2010; Yörük and Albayrak, 2013). Genetic characterization studies are indirectly important in terms of development the novel disease control strategies and the number of genotyping studies is being increased day by day. The second factor is resistance level of the host plant. The number of highly FHB resistant cultivars is limited and *Triticum aestivum* L. cv. Sumai-3 is the most widely used and well-known resistant host (Anand *et al.,* 2003; Bernardo *et al.,* 2007; Wisniewska and Kowalczyk, 2005). Five (I-V) types of resistance to FHB are present in wheat. Type I resistance is associated with the percentage of spikelets with symptoms. Type II resistance is the number of infected spikelets below the inoculation initiation point. Remaining resistance levels are not directly quantified. However, damaged kernels, mycotoxin tolerance and accumulation levels are the markers for these remaining three resistance levels (Bai and Shaner, 2004; Foroud and Eudes, 2009). Since development of plant varieties resistant to head blight with favourable agronomic traits is hard and time-consuming, approaches including genetic characterization of FHB pathogens, mycotoxin measurement, DNA measurements by qPCR and chemotyping, genetic manipulations in pathogen in particular quelling of mycotoxigenic genes seem to be more useful and beneficial.

Fusarium SPECIES ASSOCIATED WITH FHB

Fusarium genus has been linked to head blight and ear blight of all small grain cereals.

Fusarium genus was first described by Link (1809: Mag. Ges. Naturf. Freunde, Berlin 3:10), and sanctioned under Fries1821. *Fusarium* genus is a member of Phylum Ascomycota. The genus whose natural habitat is soil could survive in depth up to 1 meter of soil (Goswami and Kistler, 2004; Parry et al., 1995). Optimum pH and temperature ranges are 5-7 and 24-32 °C, respectively and species can adapt to low temperature ranges and could carry on producing mycotoxins (Doohan *et al.,* 1999; Yörük and Albayrak, 2014). Also, infected host species shows great variation among the genus. While several species has potential to infect wide range of plants which are not closely related including ornamental to forest plants without distinguishing organs, some others have limited host species including cereals (Leslie and Summerell, 2006). The genus has more than 50 species. However, Fusarium head blight disease is associated with 17 *Fusarium* species, *Fusarium graminearum* species complex and *Microdochium nivale* (Bottalico and Perrone, 2002; Davari *et al.,* 2013; Larone, 2011; Saharan *et al.,* 2004). *Fusarium graminearum* and *F. culmorum* are reported as major causal agents of

FHB and ear blight diseases worldwide (Bai and Shaner, 2004; Miedaner *et al.,* 2008; Parry *et al.,* 1995).

Fusarium graminearum (anamorph) is the major causal agent of FHB in many regions worldwide. The telemorph of this fungus is as *Gibberella zeae*. In future, only one name should be used for each fungal species (Hawksworth, 2011). According to Geiser *et al*. (2013) the anamorph name *Fusarium* should be used as the only genus name for all *Fusarium* species instead of different teleomorph names, including *Gibberella*.

Fusarium graminearum is particularly reported as primary FHB pathogen in many countries located in Asia including China (mainly *F. asiaticum*), Iran and Turkey (Haratian *et al.,* 2008; Ji *et al.,* 2007; Tunali *et al.,* 2006), America including USA and Canada (Goswami and Kistler, 2004; MISHRA *et al.,* 2004; SALAS *et al.,* 1999) and most countries in Europe including Germany, Russia and Hungary etc. (Miedaner *et al.,* 2001, 2008; Yli-Mattila, 2010 Yli-Mattila and Gagkaeva, 2016). As it is in many fungal species, haploid phase is predominant in the life cycle of *F. graminearum*. The genome of homothallic species possesses two mating type alleles, *MAT-1* and *MAT-2* (Kerényi *et al.,* 2004). This hemi-biotrophic fungal species is predicted as a model organism and life cycle could be completed in two weeks in laboratory conditions (Bowden and Leslie, 1999; Trail *et al.,* 2002). Genome project of *F. graminearum* was released in the year 2006. Two strains, PH-1 and GZ3639, were used in plasmid, fosmid and bacterial artificial chromosome vectors derived whole genome sequencing process and genome size was noted as 36.1 Mb comprising of 13939 genes, 37575 exon regions and 48.33 %GC content. In regard to genome profiles of closely related fungi whose genome projects have been completed including *Neurosporacrasssa, Magnaporthe grisea, Aspergillus nidulans, F. oxysporum* and *F. verticillioides*, *F. graminearum* genome possess genes associated with transcription factors, hydrolytic enzymes and transmembrane proteins with high number. Single nucleotide polymorphisms (SNP) frequency between two strains is detected as 0-17.5 SNP/kb according to normalization analysis and telomere regions include SNPs with high frequency. Moreover, limited numbers of microsatellite and transposon regions have been linked to repeat-induced point mutation (RIP). Microarray and bioinformatics analysis showed that the species include 382 species-specific genes and 1368 functionally characterized genes, 10000 EST records were deposited in National Center for Biotechnology Information database (Cuomo et al., 2007; Trail, 2009).

Morphological and molecular investigations resulted in interesting and useful output about the taxonomy of *Fusarium graminearum* species. Aoki and O'Donnell (1999) described a new

Fusarium species, *F. pseudograminearum*, which is formerly known as *F. graminearum* population 1. This novel species recognition was supported by data associated with differences in colony growth rate, conidia width, absence of homothallic perithecia and also *β-tubulin* gene sequence data. Moreover, detailed researches on primary causal agent of FHB, *F. graminearum*, lead to discovering of lineage differentiation and/or recognization of species complex for this fungus. *F. graminearum* species complex is comprised of phylogenetically different species with undistinguishable morphological characteristics, distinct chemotypes and agro-ecological origins (Wang *et al*., 2008, 2011). O'Donnell *et al.* (2000) recognized seven different clades of *F. graminearum* species with distinct genetic structure and geographical distribution by characterization of nucleotide sequence belonging to six nuclear genes. Lineages 2, 3 and 5, lineages 1 and 4 and lineages 6, and 7 were frequently observed in Africa, America and Asia continents, respectively. RAPD-derived SCAR and restriction fragment length polymorphisms markers were adopted in lineage differentiation (Carter *et al.*, 2002). However, in next years the number of species included in *F. graminearum* species complex is increased and recently 15 phylogenetically distinct species have been recognized among *F. graminearum* species complex via multilocus genotyping and molecular marker technologies (O'Donnell *et al.*, 2000; Wang *et al.*, 2008; Yli-Mattila *et al.*, 2009). *F. graminearum sensu stricto* (*F. graminearum* s.s.) is distributed worldwide whereas remaining species are spread among restricted agro-ecological regions. *F. asiaticum, F. vorosii, F. nepalense* and *F. ussurianum* seem to be endemic to Asia while *F. austroamericanum, F. meridionale, F. cortaderiae, F. brasilicum, F. boothii, F. mesoamericanum, F. louisianense* and *F. gerlachii* are specific to America and *F. acacia-mearnsii* and *F. aethiopicum* specific to Africa and Oceania. Prevalence of these species could vary dramatically in association with the geographic region, chemotype and host species. However, *F. asiaticum* has been detected in North America, South America and Europe (Przemieniecki *et al.*, 2014).

In *F. graminearum* and *F. asiaticum* the chemotype profile includes all three chemotypes, and in five species the chemotype profile includes 3-ADON and NIV, while only 3-ADON, 15-ADON or NIV chemotype has been detected in the rest of the species (Astolfi *et al.* 2011; Boutigny *et al.*, 2011; Fernandez-Ortuno *et al.*, 2013; Gale *et al.*, 2011; Karugia *et al.*, 2009; Nielsen *et al.*, 2012; O'DONNELL *et al.*, 2008; Pasquali and Migheli, 2014; Sampietro *et al.*, 2011; Wang *et al.*, 2008, 2011; Zhang *et al.*, 2012). Distribution of chemotypes among *F. graminearum* species complex is variable according to each species. To our knowledge, NIV chemotype is not represented by each species of *F. graminearum* species complex

(Przemieniecki et al., 2014). Isolates of *F. graminearum* s.s. and *F. asiaticum* belonging to three class B-trichothecene chemotype have been recorded whereas 3-ADON and 15-ADON distribution shows great variation for remaining species (Table 1). Similarly, wheat, barley and maize has shown to be a host of *F. graminearum* s.s. and *F. asiaticum*. 3-ADON and 15-ADON chemotypes of *F. graminearum* s.s. have also been found in oats (Yli-Mattila *et al*., 2009; Yli-Mattila and Gagkaeva, 2010). Some of remaining 13 species has not associated with maize or barley. However, this could depend on the restricted and limited number of isolates studied and characterized of these 13 species (Desjardins and Proctor, 2011; Fernandez-Ortuno *et al.,* 2013; Pasquali and Migheli, 2014; Wang *et al.* 2011; Yli-Mattila and Gagkaeva, 2010).

Fusarium culmorum is the secondary predominant pathogen of Fusarium head blight worldwide and it is phylogenetically closely related to *F. graminearum* species complex. Necrotrophic *Fusarium culmorum* has a wide range of host species compared to *F. graminearum* and it also causes several diseases in addition to FHB and root rot (Hornok *et al.,* 2007; Scherm *et al.,* 2011). *Fusarium culmorum* isolates were obtained from scabby kernels planted in many geographically different regions (Bakan *et al.,* 2002; Chung *et al.,* 2008; Jennings *et al.,* 2004b; Kammoun *et al.,* 2010; MIEDANER *et al.,* 2001; Scherm *et al.,* 2011; Scoz *et al.,* 2009; Tunali *et al.,* 2006). In particular *F. culmorum* has been prevalent species in several European countries and the species has been predominating especially regions has cooler and drier climatic conditions compared to *F. graminearum* which has been predominant in warmer and more humid regions. However, generalization on about *F. graminearum/F. culmorum* with warmer/cooler regions could not be always accurate because *F. graminearum* has been replacing *F. culmorum* in cereals in northern Europe (Bai and Shaner, 2004; Bottalico and Perrone, 2002; Parry *et al.,* 1995; Saharan *et al.,* 2004; Yli-Mattila, 2010; Yli-Mattila *et al.,* 2013). No sexual stage has been reported in *F. culmorum* but presence of parasexual stage has been suggested by Miedaner *et al*. (2001, 2008). Only 3-ADON and NIV chemotypes of *F. culmorum* have been found (e.g. Yli-Mattila and Gagkaeva, 2010). Even if there is no released genome project of *F. culmorum,* output related to genome information of *F. culmorum* FcUK99 strain which is 3-acetydeoxynivalenol (3-ADON) producer has been currently accumulated (Baldwin *et al.,* 2010; Scherm *et al.,* 2011). Moreover, nucleotide sequences of specific genes, microsatellite markers, RNA polymerase enzyme genes, genes associated with mycotoxin production and pathogenicity related genes have been released on NCBI database.

In addition to two global head blight causal agents, *F. graminearum* and *F. culmorum*, *F. sporotrichioides, F. poae, F. pseudograminearum* and, *F. cerealis* are reported to be primary

trichothecene-producing pathogens associated with FHB worldwide. These species are generally co-isolated from scabby plants with *F. graminearum* and *F. culmorum.* Predominancy of these species could be correlated with alterations in climatic conditions in local agro-economic regions and changes in crop rotations. Studies on these four species are still limited and present studies have been focused on genotyping and chemotyping (Chandler *et al.,* 2003; Chung *et al.,* 2008; Dinolfo *et al.,* 2010; Isebaert *et al.,* 2009; Mishra *et al.,* 2006; Osborne and Stein, 2007; Tomczak *et al.,* 2002). Moreover, no genome project or genetic manipulation studies revealing functions of specific genes as reported in *F. graminearum* or *F. culmorum* (Brown *et al.,* 2002; Chandler *et al.,* 2003; Kim *et al.,* 2008; Lee *et al.,* 2001) has been published in these four species or in remaining FHB related *Fusarium* species (except for *F. avenaceum* and *F. langsethiae,* Lysoe et al. 2014, 2016). However even if these species have been represented by low infection frequencies in cereals, dramatic and negative effects on economy, mycotoxin contamination profiles and yield lose ranges have been almost similar to that in *F. graminearum* and *F. culmorum* (Bai and Shaner, 2004; Bottalico and Perrone, 2002; Saharan *et al.,* 2004; Windels, 2000). *F. sambucinum* and *F. equiseti* are also class B-trichothecene producer phytopathogens. Studies on their chemotype distribution determination have been currently limited. However, they have been used in general molecular genetic studies including mycoprotein production, species-specific identification, trichothecene production analysis, mating type analysis etc. (Adejumo *et al.,* 2007; Desjardins and Beremand, 1987; Jurado *et al.,* 2005; Kristensen *et al.,* 2007; Miller and Mackenzie, 2000).

MYCOTOXINS PRODUCED BY CAUSAL AGENTS OF FHB

Mycotoxins are secondary metabolites that are produced by fungi. These metabolites have toxigenic effects on both prokaryotic and eukaryotic organisms. *Fusarium* genus is one of the five main mycotoxin producer fungal genera (Gutleb *et al.,* 2002; Niessen, 2007). *Fusaria* produces several types of mycotoxins including; trichothecenes, zearalenone, fumonisin, fusarins, butenolid, enniatins etc. Seven mycotoxin biosynthetic pathways have been reported in *Fusaria,* including, common genes associated with secondary metabolite production such as polyketid synthase, terpene cyclase, cyclic peptide synthetase and cyctochrome P450 (Desjardins and Proctor, 2007). However, it should be mentioned that intraspecific variation in terms of mycotoxins produced and chemotype profiles occur in different *Fusarium* species.

For example, *F. avenaceum* isolates have a potential to produce enniatins and moniliformin, whereas *F. graminearum* produces class B-trichothecenes and zearalenone. The interspecific

variation among *Fusarium* species surely result in different levels of agronomic loses since mycotoxins produced show diversity in toxicity (KIMURA *et al.,* 2007; RYU *et al.,* 1988). Another important note is that *Fusarium* species co-produce mycotoxins meaning that more than one mycotoxin type accumulate on small grain cereals. Despite that, produced mycotoxin profiles for species are generally known and specific. *Fusarium graminearum, F. culmorum* and most other *Fusarium* sp. associated with FHB mainly produce class B-trichothences, zearalenone, fusarin C and butenolide and most commonly accumulated on-cereal myxotoxin is deoxynivalenol, nivalenol and their acetylated derivatives. Class A trichothecenes includes T-2 and HT-2 mycotoxins with more toxigenic effects according to class B trichothecenes (Desjardins and Proctor, 2007; Foroud and Eudes, 2009). The most important class A-trichothecene-producers in cereals are *F. sporotrichioides, F. langsethiae,* which has y been mainly found in Europe and *F. sibiricum*, which has been mainly found in Asia (Yli-Mattila *et al.,* 2011, 2013, 2015)

CLASS B-TRICHOTHECENES AND *tri5* GENE CLUSTER

Trichothecenes are sesquiterpenoid secondary metabolites consisting of incorporated cyclohexane/tetrahydrophane circles. These molecules are classified as four groups as follows: Class A-, B-, C- and D-trichothecenes. Class A- and B-trichothecenes include more than 100 variants (Foroud and Eudes, 2009; Sudakin, 2003). Most frequently isolated types are class A- and B-trichothecenes and class A molecules differ from class B-trichothecenes by possessing an additional carbonyl group at C-8 position. Trichothecenes carry five functionally different groups and generally hydroxyl and acetyl groups are possessed at these positions (Table 2).

T-2 toxin is the major mycotoxin is of class A-trichothecenes whereas deoxynivalenol is the main class B-trichothecene (Foroud and Eudes, 2009; Sudakin, 2003). Farnesyl pyrophosphate is primary metabolite of all kinds of trichothecenes and formation of final product depends on expression of different genes located in trichothecene biosynthetic gene cluster known as *tri5* gene cluster (Hammond-Kosack *et al.,* 2004; Kimura *et al.,* 2007).

Class B-trichothecenes are also known as phytotoxins. Class B-trichothecenes are accumulated on cereals and they are resistant to degradation by high temperature conditions, they protect their stable structure even in food processing conditions. They are harmful to both plants and also for human and animals. They are potential eukaryotic protein synthesis inhibitors by binding ribosomes. Moreover, mortal and chronic toxicoses are caused by feeding with these mycotoxins (Gutleb *et al.,* 2002; Rotter *et al.,* 1996; Sudakin, 2003). These toxins

are produced by several species including *F. graminearum, F. culmorum, F. poae* etc. *Fusarium* isolates and strains producing class B-trichothecenes are divided into two main chemotypes as deoxynivalenol (DON) and nivalenol (NIV) profiles. This phenomenon on about class B-trichothecene classification is also detailed in three chemotypes and/or subchemotypes as (1) 3-acetylated deoxynivalenol (3-ADON) and DON; (2) 15-acetylated deoxynivalenol (15-ADON) and DON and (3) NIV and 4-ANIV (Brown *et al.,* 2002; Chandler *et al.,* 2003; Jennings *et al.,* 2004a, b; Ji *et al.,* 2007; Lee *et al.,* 2002; Miller *et al.,* 1991; Wang *et al.,* 2008; Yörük and Albayrak, 2012). Deoxynivalenol mycotoxin is characteristic mycotoxin in class B-trichothecenes and this molecule deoxy at C-4 position (Fig. 2).

Class B-trichothecenes and remaining trichothecenes are produced by fungus as a result of the expression of genes located in *tri5* gene cluster. Several research groups and laboratories have defined this gene cluster in class B-trichothecene producers *F. graminearum* strains and in class A-trichothecenes producer *F. sporotrichioides* strains (Brown *et al.,* 2004; Hammond-Kosack *et al.,* 2004; Isebaert *et al.,* 2009; Kimura *et al.,* 2003; Lee *et al.,* 2002).

F. graminearum strains H-11 (15-ADON producer) and F15 (3-ADON producer), *F. graminearum* 88-1 strain (NIV producer) and *F.sporotrichioides* strain NRRL 3299 (T-2 toxin producer) were subjected to *tri5* gene cluster characterization studies (Brown *et al.,* 2001; Kimura *et al.,* 2003; Lee *et al.,* 2001; Lee *et al.,* 2002) (Table 3). Moreover, in following years *tri5* gene cluster in many strains of *F. graminearum* was sequenced totally and interesting results were obtained in particular gene cluster size and number of genes located (see http://www.ncbi.nlm.nih.gov/nuccore/?term=tri5+gene+cluster). Three major differences were reported in *tri5* gene cluster of totally sequenced of reference strains: (1) total size of the gene cluster, (2) full deletion of some genes, and (3) functional activity of definite genes in specific-chemotype-profiles. The complete size of *tri5* gene cluster in three *F. graminearum* strains H-11, 88-1 and GZ3639 is 27022, 30159 and 57840 bp, respectively. This situation is directly associated with specific chemotype profiles and it is due to presence of pseudogenes, insertion/deletions and completely deletion in genes (Brown *et al.,* 2004; Chandler *et al.,* 2003). Besides, number of genes essential in last metabolite production is variable in strains.

However, essentially expressing genes for all kind of different trichothecenes could be accepted as stable. *tri3, tri4, tri6, tri5, tri10, tri9, tri11, tri12*and *tri14* genes (Fig. 3) were reported to critical genes in 3-ADON, 15-ADON and NIV chemotypes (Kimura *et al.,* 2003, 2007). Fig. 3 shows locations and functionality of genes. All genes located in essential 3-

ADON, 15AcDON and NIV production are illustrated as filled boxes, *tri13* and *tri7* which are not essential in DON production are shown as null boxes (Fig. 3).

But Alexander *et al*. (2011) also reported that even if *tri3* is expressed in all three chemotypes, 3-ADON producers do not require *tri3* expression. *tri5* gene cluster is comprised of totally 12 genes including transporter, pathway, and regulatory genes. Properties of genes located in the core gene cluster are mentioned in Table 3.

The information given in Table 2 is generated from data currently deposited in NCBI database and also former literature (Goswami and Kistler, 2004; Kimura *et al.,* 2003, 2007). *tri101* gene and *tri1/tri6* gene cluster are independently located outside of core gene cluster and they are also involved in trichothecene production (Hammond-Kosack *et al.,* 2004; KIMURA *et al.,* 2003). The third important subject is the presence of pseudogenes in different chemotypes. Several researchers confirmed that *tri7* and *tri13* genes are possessed as functional copies on genomes of *Fusarium* isolates of NIV chemotype whereas isolates belonging to DON chemotypes carry non-functional copy (mainly 15-ADON chemotype isolates) or do not contain the genes (mainly 3-ADON chemotype isolates, Chandler *et al.,* 2003; Jennings *et al.,* 2004a; Kimura *et al.,* 2003; Lee *et al.,* 2001; Yli-Mattila, 2010; Yli-Mattila and Gagkaeva, 2010).

REVIEW OF DEVELOPMENTS SO FAR

Class B-trichothecene chemotype distribution in *Fusaria* was more frequently investigated in *F. graminearum* and *F. culmorum* species (Pasquali and Migheli, 2014). Chemotyping studies could be viewed in three approaches. The first approach is the identification of fungus' potential to produce toxin. The second is showing the presence of the toxin type. The third is the determination of presence and quantity of trichothecene molecules. Mycotoxin production potential could be assessed by Polymerase Chain Reaction (PCR) and southern blot (SB) analysis. Precise detection of mycotoxin presence is provided by thin layer chromatography (TLC) analysis. Quantity of mycotoxins is also analysed by semi-quantitative thin layer chromatography, gas chromatography/mass spectrophotometer (GC/MS), high pressure liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) methods (Haratian *et al.,* 2008; Jennings *et al.,* 2004a; Kim *et al.,* 2003; Kimura *et al.,* 2003; Lee *et al.,* 2001, 2002; Leisova *et al.,* 2006; Naef *et al.,* 2006; Tóth *et al.,* 2004). Overall these methods have advantages and disadvantages in comparison by themselves. PCR technique gives fast and reliable result. However, a potential to produce toxin does not always mean that the fungus

produce mycotoxin since toxin production is associated with more than one factor. Similarly, a toxigenic fungus which produces mycotoxin in natural conditions may not secret mycotoxin *in vitro* laboratory conditions (Alexander *et al.*, 2011). Carter *et al*. (2002), Tóth *et al*. (2004) and Llorens *et al*. (2006) reported *Fusarium* sp. with no or very low amount of trichtohecene production. Thus, researchers focused on the combination of two or more methods giving reliable results. In general two chemotype-specific methods, PCR and GC/MS, are commonly used in trichothecene profiling (Haratian *et al.*, 2008; Kim *et al.*, 2003; Lee *et al.*, 2002, 2009). Combined usage of two or more methods is important in terms of presenting of more than one mycotoxin production since in nature *Fusarium* strains/isolates could produce more than one class B-trichothecenes (Pasquali and Migheli, 2014). However, PCR+HPLC+GC/MS (LI *et al.*, 2005), PCR+HPLC+TLC (Kim *et al.*, 2003, 2008) and PCR+GC/MS+southern blot combinations were also used in chemotyping analysis of *Fusaria*. Thus, PCR and chromatographic methods which are fast, reliable and promising are currently used in chemotyping analysis alone or together.

Hue *et al*. (1999) developed PCR assay based on amplification of *tri5* gene enabling distinguishing trichothecene producing *Fusarium* spp. However, polymerase chain reaction based or genetic chemotyping has become popular since the year 2001, which Lee *et al*. (2001) first described genic PCR approach. Great variation in core *tri5* gene cluster is present in *Fusarium* sp. strains with different chemotypes. Ward *et al*. (2002) published detailed study including polymorphism among strains of *F. graminearum* species complex dealing with 15-ADON, 3-ADON and NIV chemotypes. They reported that polymorphism in specific genes directly associated with modification and formation of final trichothecene product is responsible for 3 class-B trichothecene profiles. As an example, Fig. 4 shows CLUSTALW analysis of *tri13* gene which is most commonly targeted in chemotype determination studies.

According to the CLUSTALW analysis of 88-1, H-11 and F15 strains of *F. graminearum* deletion/insertion and single nucleotide polymorphisms, which led to functional or non-functional copies is clearly predicted. The modarate range of average genetic similarity values ranging from 66.62 to 75.23% of *tri13* gene in 88-1, H-11 and F15 strains of *F. graminearum* is an important example of these polymorphisms (Fig. 4). In addition to studies carried out by Lee *et al*. (2001, 2002), Chandler *et al*. (2003) and Kimura *et al*. (2003) reported complete nucleotide sequence information of *tri5* gene cluster. After the release of nucleotide sequence information of genes located in *tri5* gene cluster, several authors developed strategies for distinguishing three chemotypes in *Fusarium* isolates belonging to more than 5 species. Lee *et*

al. (2001, 2002) developed generic PCR assays linked to *tri7* and *tri13* genes for differentiation of DON and NIV chemotypes. Functional copies of *tri7* and *tri13* genes were possessed on genomes of isolates with NIV chemotype. However isolates belonging to 3-ADON and 15-ADON chemotypes have non-functional (disrupted or deleted) copies of *tri7* and *tri13* genes. Thus, generic PCR assay have been efficiently used.

Similarly, Chandler *et al*. (2003) and Waalwijk *et al*. (2003) differentiated two chemotypes since isolates of DON chemotypes have *tri7* and *tri13* genes as disrupted or deleted. Several specific PCR primer sets were developed and two strategies were aimed. First, differences in PCR amplicon sizes yielded two chemotypes, DON or NIV. Second is presence or absence of a PCR band determined two chemotypes. *tri3* have been also targeted generic PCR assays in further analysis of two sub-chemotypes of DON profiles, 3-ADON and 15-ADON (Jennings *et al.,* 2004a, b). Generic PCR with two primer sets result in two amplicons with different sizes which are signs of 3-ADON and 15-ADON chemotypes. Additionally, Wang *et al*. (2008) developed another generic PCR assay including amplification of *tri3* gene resulting with three amplicons different sized. Three PCR profiles differentiated three chemotypes NIV, 3-ADON and 15-ADON. Wang *et al*. (2008) suggested that former studies including presence and absence of a band could give false negative results. Moreover, Lee *et al.* (2002) suggested that in nature some DON-producer isolates could carry functional copy of *tri7* gene even if they did not determine any isolate belonging to DON chemotype with functional *tri7* gene. Also, some *Fusarium* sp. isolates can produce mycotoxins of two chemotypes or two sub-chemotypes (Castanares *et al*., 2014; Gilbert *et al.,* 2001; Salas *et al.,* 1999). Thus, PCR-based approach requires confirmation by secondary methods in chemotyping investigations.

Thin layer chromatography (TLC) is basic and fast method for analysis many kinds of secondary metabolites including mycotoxins produced by fungi. The method is constructed on the movement of a metabolite in the chromatographic system. The system is based on mobile phase including solvent/solvent mixture and stationary phase comprising of solid adsorbent (silica or alumina plate). Trichothecene chemotypes of *Fusarium* species including *F. graminearum, F. culmorum, F. cerealis, F. pseudograminearum* were detected by thin layer chromatography on silica plates with different mixtures of mobile phase (Haratian *et al.,* 2008; Kimura *et al.,* 2003; Rahmani *et al.,* 2009; Tóth *et al.,* 2004; Trigo-Stockli *et al.,* 1994; Völkl *et al.,* 2004). Efficient results on about presence of NIV, 3-ADON and 15-ADON obtained with applied suitable TLC markers has been recorded in these investigations. Further chromatographic analysis or ELISA assays are currently preferred in chemotype determination

analysis. HPLC and GC/MC methods are reliable and suitable chemical approaches for the class B-trichothecene determination as qualitative and quantitative. These methods present precise results and they have been widely used and most frequently preferred in trichothecene profiling studies in *Fusarium graminearum, Fusarium culmorum, Fusarium cerealis, F. pseudograminearum* and *F. poae* species (Carter *et al.,* 2002; Jennings *et al.,* 2004a, b; Kammoun *et al.,* 2010; Kim *et al.,* 2003; Lee *et al.,* 2002, 2009; LI *Et al.,* 2005; Llorens *et al.,* 2006; Salas *et al.,* 1999; Tóth *et al.,* 2004; Walker *et al.,* 2000; Wang *et al.,* 2008). Three strain-specific class B-trichothecene profiles were detected by GC/MS and HPLC analysis in *Fusarium* spp., and precise quantification of mycotoxins produced have been also detected by these investigations. Bily *et al*. (2004) have developed HPLC-based method, which is widely used in chemotyping investigations. However, these high quality methods gave rise to the formation of an extraordinary dilemma about 'a *Fusarium* spp. strain could belong to two chemotypes' (Carter *et al.,* 2002; Gilbert *et al.,* 2001; Pasquali and Migheli, 2014; Salas *et al.,* 1999). In these researches, the presence of both 15-ADON and also 3-ADON mycotoxins had been detected in *Fusarium* spp.. Another theoretical problem is that isolate or strain which is detected as non-trichothecene-producer in laboratory conditions can be indeed a potential producer in nature. Thus, even if more than two methods are used in chemotype determination studies, isolates detected as non-producers could produce trichothecene toxins in natural conditions or, in other words false negative identification could be applied to toxin producer isolates. Nevertheless, this situation is rarely presented.

In general usage of just one of these methods or combination of more than one method gave information about class B-trichothecene profiles of *Fusarium* isolates worldwide. Especially, fungal samples isolated from diseased kernels planted in Asian, European and American countries have been subjected to chemotype identification studies. In Asia, predominantly *Fusarium graminearum* species complex and also *F. culmorum* are widely studied in, Japan, Iran, Turkey, South Korea, Nepal and Syria (Table 4).

According to the investigations surveyed on Asian countries, wheat and *F. graminearum* species complex are the major host and causal agents. Fernandez-Ortuno *et al.* (2013) reported *F. asiaticum, F. ussurianum* and *F. vorosii* are of Asian clade of species complex. These three species have been isolated from wheat and barley. Zhang *et al.* (2012) determined chemotypes of 457 *F. graminearum* species complex -including *F. meridionale, F. graminearum* s.s. and *F. asiaticum*-, 10 *F. cerealis* and 1 *F. culmorum* isolates from China by PCR and HPLC analysis. 3-ADON was the prevalent chemotype in Chinese isolates. Similarly, Karugia *et al.*

(2009) determined *F. asiaticum* as predominant pathogen in wheat samples with chemotypes of NIV (44.7%) and and 3-ADON (53.3%). However, it is clear that chemotype distribution is not clearly associated only with geographic region, species, host and the year survey (sample collection year). In South Korea, both major chemotypes were detected as predominating in different years (Lee *et al*., 2009, 2012). But 15-ADON chemotype seems to be prevalent in *F. graminearum sensu stricto* in eastern Asian countries, except in Russian Far East (Table 4).

Slightly similar results were obtained in studies carried out in North and South America countries (Kim *et al*., 2003; Llorens *et al*., 2006; Qu *et al*., 2008; Reynoso *et al*., 2011; Scoz *et al*., 2009; Carter *et al*., 2002; Wang *et al*., 2008). Table 5 shows different *Fusarium* species associated with FHB in North and South America continents. Wheat, *F. graminearum* and DON/15-ADON seem to be predominating host plant, causal agent and chemotype, respectively. However, Salas *et al*. (1999) reported that *F. graminearum, F. aveneceum* and *F. poae* have almost equally account for the disease and NIV chemotype is dominant in *F. poae.* Also, Malihipour *et al*. (2012) proved that NIV chemotype is dominant in *F. cerealis* isolates from Mexico.

In European countries, predominance issue of host plant species, causal agent and chemotype is more complicated. Jennings *et al*. (2004a, b) showed the presence of two major causal agents, *F. graminearum* and *F. culmorum*, with two main class B-trichothecene chemotypes in United Kingdom (Table 6). Especially for these two species, predominancy could be variable according to/associated with agro-ecological regions, climatic conditions, crop rotations etc. Recently, *F. graminearum* has been spreading to the north in Europe and displacing *F. culmorum* (Ward *et al*., 2002; Yli-Mattila, 2010). In addition to wheat, winter rye, maize, rice and barley are also currently infected with *F. graminearum, F. culmorum, F. poae, F. cerealis* and *F. pseudograminearum* (Miedaner *et al*., 2001, 2008; Pasquali and Migheli, 2014; Yli-Mattila *et al*., 2013). In Finland, Norway and many parts of Sweden the main host of *F. graminearum* is oats (Yli-Mattila *et al*., 2013; Yli-Mattila and Gagkaeva, 2016). Limited number of *Fusarium* isolates from France, Italy and Germany have been investigated in chemotyping studies (Carter *et al*., 2002; Kimura *et al*., 2003; Qu *et al*., 2008; Tomczak *et al*., 2002). Nevertheless, Llorens *et al*. (2006) reported NIV was predominating chemotype in *F. graminearum, F. culmorum, F. cerealis, F. poae* and *Gibberella fujikuori* isolates from Spain. On contrary to global 15-ADON distribution of 15-ADON, 3-ADON has been reported as prelavent chemotype in some European countries including northern Europe (Finland, Sweden, Norway and north-western Russia), (Yli-Mattila *et al*., 2009; Yli-Mattila and

Gagkaeva, 2010, 2016). In Denmark 3-ADON of *F. graminearum* is the predominating chemotype in oats, while in other cereals the 15-ADON chemotype of *F. grmainearum* is predominating (Nielsen *et al*., 2011, 2012). Thus, it has been, suggested that there are two main populations of *F. graminearum* in Europe, of which the 3-ADON population is dominant in Central and Southern Europe and has been spreading e.g. to Denmark, while 3-ADON chemotype has been dominant in northern Europe and has recently spread from Finland to north-western Russia (Yli-Mattila et al., 2013). It may be that 3-ADON chemotype is also more specialized to oats, which is more common in northern Europe.

In addition to studies conducted on three major continents, Asia, Europe and America; North Africa and Oceania countries have been also subjected to *Fusarium* spp. studies with limited numbers. Obanor *et al*. (2010, 2013) reported that *F. graminearum* and *F. pseudograminearum* isolates from diseased wheat produced equally DON and NIV in Australia. *F. culmorum* and wheat are reported as major causal agent and host plant in Tunisia (Kammoun *et al*., 2010; Rebib *et al*., 2014). 3-ADON was reported as major mycotoxin in the country among 183 *F. culmorum* isolates. However, number of fungal isolates studied on chemotype determination assays seem to be limited in comparison with Asian, American and European samples.

FUTURE PERSPECTIVES AND CONCLUSIONS

Studies associated with class B-trichothecene producer *Fusarium* species have been increased in recent years. In particular, researchers focused on declaration the novel species in phytopathogenic *Fusarium* spp. Aoki and O'donnell (1999) distinguished former *F. graminearum* group as *F. pseduograminearum*. Moreover, linage number of *F. graminearum* species complex changed few times after discovery of new species (O'Donnell *et al.*, 2000; Przemieniecki *et al.* 2014; Sarver *et al.* 2011; Starkey *et al.* 2007) and the current number is reached to 15. Characterization studies on these novel species is still processed and output on agro-ecological region and chemotypes is increased day by day. Recently, Aoki *et al.* (2015) recognized novel nivalenol producer species *Fusarium dactylidis* sp. nov. which is species sister of *F. pseudograminearum*.

In addition to investigations on the identification of new trichothecene producer species, studies including inhibition or promotion of trichothecene production became popular. Kulik *et al.* (2014) showed that pinoresinol and secoisolariciresinol lignans produced by wheat decreased trichothecene level. Similarly, phenolic acids obtained from *Spirulina* sp. inhibited class B-trichothecene production (Pagnussatt *et al.*, 2014). Lancova et al (2008) detected the

DON-3-Glc in naturally infected barley and as well as in malt and beer samples. Nagl and Schatzmayr (2015) reported that relative portion of these phase II metabolites of DON to free toxin form (DON) is of 20% on average. Analysis of masked forms of deoxynivalenol including deoxynivalenol-3-glucoside (DON-3-Glc), deoxynivalenol-3-glucuronide (DON-3-GlcA) and deoxynivalenol-15-glucuronide (DON-15-GlcA), has become popular in recent years (Michlmayr *et al*., 2015; Generotti *et al*., 2015). Vector-based RNA interference strategy was also used to inhibit trichothecene production in *Fusarium* sp. (Mcdonald *et al.*, 2005; Scherm *et al.* 2011). On contrary, acidic pH and H_2O_2 and amines supplement were recorded as trichothecene production indicators (Kawakami *et al.*, 2014; Merhej *et al.* 2010; Ponts *et al.*, 2009). Species-specific identification and chemotype determination studied have been also studied during last two decades. Zhang *et al.* (2014) and Pallez *et al.* (2014) developed novel efficient PCR-based approaches to detect members of *F. graminearum* species complex at species level and to determine the chemotypes.

Determination of class B-trichothecene profiles of *Fusarium* isolates worldwide could directly and/or indirectly contribute to the development of novel control strategies for FHB disease. First, pre-knowledge about epidemiological distribution of fungal isolates would be gained by chemotyping studies. Since the toxicity of three trichothecene profiles have variation, potential toxigenic risk could be determined by chemotyping in local or global areas where FHB is present. Also, since additional risk factors including crop rotations, climatic changes etc. could directly affect the spread of disease, fast, reliable and precise results become critical and crucial in chemotype determination. For these purposes, researchers have investigated global *Fusarium* spp. samples by modern genetic and chemical techniques in particular PCR and chromatography. As a result of output data originated from global chemotype of *Fusarium* spp., characterization of field populations of fungi, genotypic diversity and aggressiveness of fungus and severity levels of diseased host plants could become more clearly understood.

ACKNOWLEDGEMENTS

The authors express much thanks dealing with the theoretical knowledge share and sharing the experience in *Fusarium* research area to the members of Molecular Biology and Genetics Department of Istanbul University including Dr. Gülruh Albayrak, M.Sc. Aylin Gazdagli, Dr. Berna Tunali a member of Samsun 19 Mayis University Department of Plant Protection, Drs. Kerry O'Donnell and Todd Ward from USDA-ARS in Peoria and Dr. Tatiana Gagkaeva from All-Russian Institute of Plant Protection in St. Petersburg. Our researches on chemotype

distrubution in *Fusarium* species was supported by Research Fundation of Istanbul University, Olvi Foundation, Finnish Cultural Foundation and the Academy of Finland.

CONFLICTS OF INTEREST

'The authors declare no conflict of interest'.

REFERENCES

Adejumo TO, Hettwer U, & Karlovsky P (2007). Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. Int J Food Microbiol 116(3): 350-357.

Alexander NJ, Mccormick SP, Waalwijk C, Lee TVD &Proctor RH (2011). The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. Fungal Genet Biol 48: 485-495.

Alkadri D, Nipoti P, Döll K, Karlovsky P, Prodi A, & Pisi A (2013). Study of fungal colonization of wheat kernels in Syria with a focus on *Fusarium* Species. Int J Mol Sci 14: 5938–5951.

Alvarez CL, Azcarate MP & Pinto VF (2009). Toxigenic potential of *Fusarium graminearum* sensu stricto isolates from wheat in Argentina. Int J Food Microbiol 135: 131–135.

Anand A, Zhou T, Trick HN, Gill BS, Bockus WW &Muthukrishnanl S (2003). Greenhouse and field testing of transgenic wheat plants stably expressing genes for thaumatin-like protein, chitinase and glucanase against *Fusarium graminearum*. J Exp Biol 54(384): 1101-1111.

Aoki T & O'Donnell K (1999). Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. Mycologia 91(4): 597-609.

Aoki T, Vaughan MM, Mccormick SP, Busman M, Ward TJ, Kelly A, O'Donnell K, Johnston PR & Geiser DM (2015). *Fusarium dactylidis* sp. nov., a novel nivalenol toxin-producing species sister to *F. pseudograminearum* isolated from orchard grass (*Dactylis glomerata*) in Oregon and New Zealand. Mycologia 107(2): 409-418.

Astolfi P, Dos Santos J, Schneider L, Gomes LB, Silva CN, Tessmann DJ &Del Ponte EM (2011). Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in Southern Brazil. Int J Food Microbiol 148: 197-201.

Bai G & Shaner G (2004). Management and resistance in wheat and barley to fusarium head blight. Annu Rev Phytopathol 42: 135-161.

Bakan B, Delville CG, Pinson L, Richard-Molard D, Fournier E &Brygoo Y (2002). Identification of *Fusarium culmorum* strains producing large and small amounts of deoxinivalenol. App Environ Microb 68(11): 5472-5479.

Baldwin TK, Urban M, Brown N, & Hammond-Kosack KE (2010). A role for topoisomerase I in *Fusarium graminearum* and *F. culmorum* pathogenesis and sporulation. Mol Plant Microbe In, *23*: 566–577.

Bernardo A, Bai G, Guo P, Xiao K, Guenzi AC & Ayoubi P (2007). *Fusarium graminearum*-induced changes in gene expression between *Fusarium* head blight-resistant and susceptible wheat cultivar. Funct Integr Genomics 7: 69-77.

Bily AC, Reid LM, Savard E, Reddy R, Blackwell BA, Campbell CM, Krantis A, Durst T, Philogene BJR, Arnason JT, & Regnault-Roger C (2004). Analysis of *Fusarium graminearum* mycotoxins in different biological matrices by LC/MS. Mycopathologia 157: 117–126.

Bottalico A, & Perrone G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur J Plant Pathol 108: 611-624.

Bowden RL &Leslie JF (1999). Sexual recombination in *Gibberella zeae*. Phytopathol 89: 182–188.

Boutigny AL, Ward TJ, Van Coller GJ, Flett B, Lamprecht SC, O'Donnell K & Viljoen A (2011). Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genet Biol 48: 914-920.

Boutigny AL, Ward TJ, Ballois N, Iancu G, & Ioos R (2014). Diversity of the *Fusarium graminearum* species complex on French cereals. Eur J Plant Pathol 138: 133–148.

Brown DW, Mccormick SP, Alexander NJ, Proctor R.H. & Desjardins AE (2001). A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. Fungal Genet Biol 32(2): 121-133.

Brown DW, Mccormick SP, Alexander NJ, Proctor RH & Desjardins AE (2002). Inactivation of a cytochrome P-450 is a determinant of trichothecene diversity in Fusarium species. Fungal Genet Biol 36: 224–233.

Brown DW, Dyer RB, McCormick SP, Kendra DF & Plattner RD (2004). Functional demarcation of the *Fusarium* core trichothecene gene cluster. Fungal Genet Biol 41: 454-462.

Carter JP, Rezanoor HN, Holden D, Desjardins AE, Plattner RD & Nicholson P (2002). Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. Eur J Plant Pathol 108: 573-583.

Castañares E, Albuquerque DR, Dinolfo MI, Pinto VF, Patriarca A & Stenglein SA (2014). Trichothecene genotypes and production profiles of *Fusarium graminearum* isolates obtained from barley cultivated in Argentina. Int J Food Microbiol 179: 57–63.

Chandler E.A., D.R. Simpson, M.A. Thomsett, P. Nicholson (2003): Development of PCR assays to *tri7* and *tri13* trichothecene biosynthetic and characterisation of chemotypes of *Fusarium graminearum, Fusarium culmorum* and *Fusarium cerealis.* Physiol Mol Plant Pathol, *62*: 355-367.

Chehri, K, Salleh B, Yli-Mattila T, Reddy KRN & Abbasi S (2011). Molecular characterization of pathogenic *Fusarium* species in cucurbit plants from Kermanshah province, Iran. Saudi J Biol Sci 18: 341-351.

Chung WH, Ishii H, Nishimura K, Ohshima M, Iwama T & Yoshimatsu H (2008). Genetic analysis and PCR-based identification of major *Fusarium* species causing head blight on wheat in Japan. J Gen Plant Pathol 10: 110-118.

Clear RM, Patrick SK, Gaba D, Roscoe M, Turkington TK, Demeke T, Pouleur S, Couture L, Ward TJ & O'Donnell K. (2006). Trichothecene and zearalenone production, in culture, by isolates of *Fusarium pseudograminearum* from western Canada. Can J Plant Pathol 28: 131–136.

Cornea C, Israel-Roming F, Ciuca M & Voaides C (2013). Natural occurrence of *Fusarium* species and corresponding chemotypes in wheat scab complex from Romania. Rom Biotech Lett 18: 8787–8795.

Cuomo CA, Güldener U, Xu JR, Trail F, Turgeon BG, Di Pietro A, & Adam G (2007). The Fusarium graminearum genome reveals a link between localized polymorphism and pathogen specialization. Science, 317(5843): 1400-1402.

Davari M, Wei SH, Babay-Ahari A, Arzanlou M, Waalwijk C, Van Der Lee TAJ, Zare R, Gerrits Van Den Ende AHG, De Hoog GS & Van Diepeningen AD (2013). Geographic differences in trichothecene chemotypes of *Fusarium graminearum* in the Northwest and North of Iran. World Mycotoxin J 6: 137–150.

Delgado JA, Schwarz PB, Gillespie J, Rivera-Varas V & Secor GA (2010). Trichothecene mycotoxins associated with potato dry rot caused by *Fusarium graminearum*. Phytopathol 100: 290–296.

Desjardins AE & Beremand M (1987). A genetic system for trichothecene toxin production in *Gibberella pulicaris* (*Fusarium sambucinum*). Phytopathol 77: 678-683.

DesjardinsAE & Proctor RH (2007). Molecular biology of *Fusarium* mycotoxins. Int J Food Microbiol 119: 47-50.

Desjardins AE & Proctor RH (2011). Genetic diversity and trichothecene chemotypes of the *Fusarium graminearum* clade isolated from maize in Nepal and identification of a putative new lineage. Fungal Biol 115: 38–48.

Dinolfo MI, Stenglein SA, Moreno MV, Nicholson P, Jennings P & Salerno GL (2010). ISSR markers detect high genetic variation among *Fusarium poae* isolates from Argentina and England. Eur J Plant Pathol 127: 483-491.

Doohan FM, Weston G, Rezanoor HN, Parry DW & Nicholson P (1999). Development and use of a reverse transcription-PCR assay to study expression of *tri5* by *Fusarium* species *in vitro* and in planta. App Environ Microb 65(9): 3850-3854.

Dyakov TY, Dzhavakhlya VG & Korpela T (2007). Comprehensive and Molecular phytopathology. Elsevier, Amsterdam, Netherlands, pp 469.

Fernández-Ortuño D, Waalwijk C, Van Der Lee T, Fan J, Atkins S, West JS & Fraaije BA (2013). Simultaneous real-time PCR detection of *Fusarium asiaticum*, *F. ussurianum* and *F. vorosii*, representing the Asian clade of the *F. graminearum* species complex. Int J Food Microbiol166: 148-154.

Foroud NA & Eudes F (2009). Trichothecenes in cereal grains, Int J Mol Sci, 10: 147-173.

Gagkaeva TY & Mattila TY (2004). Genetic diversity of *Fusarium graminearum*in Europa and Asia, Eur J Plant Pathol 110: 551-562.

Gale LR, Harrison SA, Ward TJ, O'Donnell K, Milus EA, Gale SW & Kistler HC (2011). Nivalenol-Type Populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on Wheat in Southern Louisiana. Phytopathol 101(1): 124-134.

Geiser DM, Aoki T, Bacon CW, Baker SE, Bhattacharyya MK, Brandt ME, ... & Correll JC (2013). One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. Phytopathol, 103(5), 400-408. Generotti S, Cirlini M, Malachova A, Sulyok M, Berthiller F, Dall'asta C & Suman M (2015). Deoxynivalenol and deoxynivalenol-3-glucoside mitigation through bakery production strategies: effective experimental design within industrial rusk-making technology. Toxins 7: 2773-2790.

Gilbert J, Abramson D, Mccallum B & Clear R (2001). Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. Mycopathologia 153: 209–215.

Goswami RS & Kistler HC (2004). Heading for disaster: *Fusarium graminearum* on cereal crop. Mol Plant Pathol 5(6): 515-525.

Gutleb AC, Morrison E & Murk AJ (2002). Cytotoxicity assay for mycotoxins produced by *Fusarium* strains. Environ Toxicol Phar 11: 309-320.

Gürel F, Albayrak G, Diken O, Cepni E & Tunali B (2010). Use of REP-PCR for genetic diversity analyses in *Fusarium culmorum*. J Phytopathol 158: 387-389.

Hammond-Kosack K, Urban M, Baldwin T, Daudi A, Rudd J, Keon J, Lucas J, Maguire K, Kornyukhin D, Jing HC, Bass C & Antoniw J (2004). Plant pathogens: how can molecular genetic information on plant pathogens assist in breeding disease resistant crops. New Directions For a Diverse Planet 44: 1-18.

Haratian M, Sharifnabi B, Alizadeh A & Safaie N (2008). PCR analysis of the tri13 gene to determine the genetic potential of *Fusarium graminearum* isolates from Iran to produce nivalenol and deoxynivalenol. Mycopathologia 166: 109-116.

Hawksworth DLA (2011). New dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. IMA Fungus 2: 155-162.

Hestbjerg H, Felding G & Elmholt S (2002). *Fusarium culmorum* infection of barley seedlings: correlation between aggressiveness and deoxynivalenol content. J Plant Pathol 150: 308–312.

Hornok L, Waalwijk C & Leslie JL (2007). Genetic factors affecting sexual reproduction in toxigenic *Fusarium* species. Int J Food Microbiol 119: 54-58.

Hue FX, Huerre M, Rouffault MA & Bievre CD (1999). Specific detection of *Fusarium* species in blood and tissues by a PCR technique. J Clin Microbiol 37: 2434-2438.

Isebaert S, Saeger SD, Devreese R, Verhoeven R, Maene P, Heremans B & Haesaert G (2009). Mycotoxin-producing *Fusarium* species occurring in winter wheat in Belgium (Flanders) during 2002–2005. J Phytopathol 157: 108-116.

Jennings P, Coates ME, Walsh K, Turner JA & Nicholson P (2004a). Determination of deoxinivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolated from wheat crops in England and Wales. Plant Pathol 53: 643-652.

Jennings P, Coates ME, Turner JA, Chandler EA & Nicholson P (2004b). Determination of deoxinivalenol and nivalenol chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. Plant Pathol 53: 182-190.

Ji L, Cao K, Hu T & Wang S (2007). Determination of deoxinivalenol and nivalenol chemotypes of *Fusarium graminearum* isolates from China by PCR assay. J Phytopathol 155: 505-512.

Jurado M, Va´Zquez C, Patin˜o B & Gonza´Lez-Jae´N MT (2005). PCR detection assays for the trichothecene-producing species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium equiseti* and *Fusarium sporotrichioides*. Syst Appl Microbiol 28: 562-568.

Kammoun LG, Gargouri S, Barreau C, Richard-Forget F & Rabeh HM (2010). Trichothecene chemotypes of *Fusarium culmorum* infecting wheat in Tunisia. Int J Food Microbiol 140: 84–89.

Karugia GW, Suga H, Gale LR, Nakajima T, Tomimura K & Hyakumachi M (2009). Population Structure of the *Fusarium graminearum* Species Complex from a Single Japanese Wheat Field Sampled in Two Consecutive Years. Plant Dis, 93(2): 170-174.

Kawakami A, Nakajima T & Hirayae K (2014). Effects of carbon sources and amines on induction of trichothecene production by *Fusarium asiaticum* in liquid culture. FEMS Microbiol Lett 352: 204–212.

Kerényi ZA, Moretti C, Waalwijk B, Olah L & Hornok L (2004). Mating type sequences in asexually reproducing *Fusarium* species. App Environ Microb 70: 4419-4423.

Kim HS, Lee T, Dawlatana M, Yun SH & Lee YW (2003). Polymorphism of trichothecene biosynthesis genes in deoxynivalenol- and nivalenol-producing *Fusarium graminearum* isolates. Mycol Res 107: 190–197.

Kim JE, Jin J, Kim H, Kim JC, Yun SH & Lee YW (2008). Functional characterization of genes located at the aurofusarin biosynthesis gene cluster in *Gibberella zeae*. Plant Pathol J 24: 8-16.

Kimura M, Tokai T, O'Donnell K, Ward TJ, Fujimura M, Hamamoto H, Shibata T & Yamaguchi I (2003). The trichothecene biosynthesis gene cluster of *Fusarium graminearum* F15 contains a limited number of essential pathway genes and expressed non-essential genes. FEBS Lett 539: 105-110.

Kimura M, TokaiT, Takahashi-Ando N, Ohsato S & Fujimura M (2007). Molecular and genetic studies of *Fusarium* trichothecene biosynthesis: pathways, gene and evolution. Biosci Biotech Bioch 71: 2105-2123.

Kristensen R, Gauthier G, Berdal KG, Hamels S, Remacle J & Holst-Jensen A (2007). DNA microarray to detect and identify trichothecene- and moniliformin-producing *Fusarium* species. J Appl Microbiol 102(4): 1060-1070.

Kulik T, Buśko M, Pszczółkowska A, Perkowski J & Okorski A (2014). Plant lignans inhibit growth and trichothecene biosynthesis in *Fusarium graminearum*. Lett Appl Microbiol 59: 99–107.

Laday M, Juhasz A, Mule G, Moretti A, Szecsi A & Logrieco A (2004). Mitochondrial DNA diversity and lineage determination of Europan isolates of *Fusarium graminearum* (*Gibberella zeae*). Eur J Plant Pathol 110: 545-550.

Lancovaa K, Hajslovaa J, Poustkaa J, Krplovaa A, Zachariasovaa M, Dostalekb P & Sachambula L (2008). Transfer of *Fusarium* mycotoxins and 'masked' deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. Food Addit Contam 25(6): 732-744.

Larone DH (2011). Medically important fungi: a guide to identification. ASM Press, Washington, United States of America, 485 pp.

Lauren DR & Smith WA (2001). Stability of *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearelenone in ground maize under typical cooking conditions. Food Addit Contam 18: 1011-1016.

Lee T, Oh DW, Kim HS, Lee J, Kim YH, Yun SH & Lee YW (2001). Identification of deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae* by using PCR. App Environ Microb 67: 2966–2972.

Lee T, Han YH, Kim KH, Yun SH & Lee YW (2002). *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol- producing chemotypes of *Gibberella zeae*. App Environ Microb 68: 2148–2154.

Lee J, Chang IY, Kim H, Yun SH, Leslie JF & Lee YW (2009). Genetic diversity and fitness of *Fusarium graminearum* populations from rice in Korea. App Environ Microb 75: 3289–3295.

Lee J, Kim H, Jeon JJ, Kim HS, Zeller KA, L.L.A. Carter, J.F. Leslie, Y.W. Lee (2012). Population structure of and mycotoxin production by *Fusarium graminearum* from maize in South Korea. App Environ Microb 78: 2161–2167.

Leisova L, Kucera L, Chrpova J, Sykorova S, Sip V & Ovesna J (2006). Quantification of *Fusarium culmorum* in wheat and barley tissues using Real-Time PCR in comparison with DON content. J Phytopathol 154: 603-611.

Leslie JF& Summerell BA (2006). The *Fusarium* laboratuary manual. Blackwell, Manhattan, United States of America, 387 pp.

Li HP, Wu AB, Zhao CS, Scholten O, Löffler H & Liao YC (2005). Development of a generic PCR detection of deoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum*. FEMS Microbiol Lett 243: 505–511.

Li RQ, He R, Zhang YB, Xu YM & Wang JM (2009). Establishment of ISSR reaction system of *Fusarium* and its analysis of genetic diversity. Sci Agricultura Sinica 42(9): 3139-3146.

Llorens A, Hinojo MJ, Mateo R, Medina A, Valle-Algarre FM, Gonzalez-Jaen MT & Jimenez M (2006). Variability and characterization of mycotoxin producing *Fusarium spp.* isolates by PCR-RFLP analysis of the IGS-rDNA region. Anton Leeuw Int J G 89: 465-478. Lori GA, Sisterna MN, Sarando'n SJ, Rizzo I & Chidichimo H (2009). Fusarium head blight in wheat: Impact of tillage and other agronomic practices under natural infection. Crop Prot 28: 495-502.

Lysøe E, Harris LJ, Walkowiak S, Subramaniam R, Divon HH et al. (2016). The Genome of the Generalist Plant Pathogen Fusarium avenaceum Is Enriched with Genes Involved in Redox, Signaling and Secondary Metabolism. PLoS One, 19;9 (11):e112703. doi:10.1371/journal.pone.0112703.

Lysøe E, Frandsen RJN, Divon HH, Terzi V, Orrù L, Lamontanara A, Kolseth AS-K, Nielsen KF,Thrane U. 2016. Draft genome sequence and chemical profiling of *Fusarium langsethiae*, an emerging producer of type A trichothecenes Int J Food Microbiol 221: 29–36.

Malbran I, Mourelos CA, Girotti JR, Balatti PA & Lori GA (2014). Toxigenic capacity and trichothecene production by *Fusarium graminearum* isolates from Argentina and their relationship with aggressiveness and fungal expansion in the wheat spike. Phytopathol 104: 357–364.

Malihipour A, Gilbert J, Piercey-Normore M & Cloutier S (2012). Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of *Fusarium* isolates causing Head Blight in wheat. Plant Dis 96: 1016–1025.

Mcdonald T, Brown D, Keller NP & Hammond TM (2005). RNA silencing of mycotoxin production in *Aspergillus* and *Fusarium* species. Mol Plant-Microbe Interact 18(6): 539-545.

Merhej J, Boutigny AL, Pinson-Gadais L, Richard-Forget F & Barreau C (2010). Acidic pH as a determinant of TRI gene expression and trichothecene B biosynthesis in *Fusarium graminearum*. Food Addit Contam 27: 710–717.

Mert-Türk F & Gencer G (2013). Distribution of the 3-ADON, 15-ADON, and NIV chemotypes of *Fusarium culmorum* in the North-West of Turkey. Plant Prot Sci 49(2): 57-64.

Michlmayr H, Alachová A, Varga E, Kleinová J, Lemmens M, Newmister S, Rayment I, Berthiller F & Adam G (2015). Biochemical characterization of a recombinant UDP-glucosyltransferase from rice and enzymatic production of deoxynivalenol-3-*o*-β-d-glucoside. Toxins 7: 2685-2700.

Miedaner T, Schilling AG & Geiger HH (2001). Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat fields in different countries. J Phytopathol 149: 641-648.

Miedaner T, Cumagun CJR & Chakraborty S (2008). Population genetics of three important head blight pathogens *Fusarium graminearum, F. pseudograminearum* and *F. culmorum.* J Phytopathol 156: 129-139.

Miller JD, Greenhalgh R, Wang YZ & Lu M (1991). Trichothecene chemotypes of three Fusarium species. Mycologia 83: 121–130.

Miller D & Mackenzie S (2000). Secondary metabolites of *Fusarium venenatum* strains with deletions in the Tri5 gene encoding trichodiene synthetase. Mycologia 92:764–771.

Mishra PK, Tewari JP, Clear RM & Turkington TK (2004). Molecular genetic variation and geographical structuring in *Fusarium graminearum*. Ann Appl Biol 145: 299-307.

Mishra PK, Tewari JP, Clear RM & Turkington TK (2006). Genetic diversity and recombination within populations of *Fusarium pseudograminearum* from western Canada. Int Microbiol 9: 65-68.

Naef A, Senatore M & D´Efago GA (2006). Microsatellite based method for quantification of fungi in decomposing plant material elucidates the role of *Fusarium graminearum* DON production in the saprophytic competition with *Trichoderma atroviride* in maize tissue microcosms. FEMS Microbiol Lett 655: 211-220.

Nagl V & Schatzmayr G (2015). Deoxynivalenol and its masked forms in food and feed. Curr Opin Food Sci 5: 43-49.

National Centre for Biotechnology Information (NCBI), 2015. At http://www.ncbi.nlm.nih.gov.

Ndoye M, Zhang JB, Wang JH, Gong AD, Li HP, Qu B, Li SJ & Liao YC (2012). Nivalenol and 15-acetyldeoxynivalenol chemotypes of *Fusarium graminearum* clade species are prevalent on maize throughout China. J Phytopathol 160: 519–524.

Nielsen LK, Jensen JD, Nielsen GC, Jensen JE, Spliid NH, Thomsen IK, Justesen AF, Collinge DB & Jorgensen LN (2011). Fusarium head blight of cereals in Denmark: Species complex and related mycotoxins. Phytopathol 101: 960–969.

Nielsen LK, Jensen JD, Rodríguez A, Jørgensen LN & Justesen AF (2012). TRI12 based quantitative real-time PCR assays reveal the distribution of trichothecene genotypes of *F. graminearum* and *F. culmorum* isolates in Danish small grain cereals. Int J Food Microbiol 157: 384–392.

Niessen, L **(**2007). PCR-based diagnosis and quantification of mycotoxin producing fungi. Int J Food Microbiol 19: 38-46.

Obanor F, Erginbas-Orakci G, Tunali B, Nicol JM & Chakraborty S (2010). *Fusarium culmorum* is a single phylogenetic species based on multilocus sequence analysis. Fungal Biol 114: 753-765.

Obanor F, Neate S, Simpfendorfer S, Sabburg R, Wilson P & Chakraborty S (2013). *Fusarium graminearum* and *Fusarium pseudograminearum* caused the 2010 Head Blight epidemics in Australia. Plant Pathol 62: 79–91.

O'Donnell K, Kistler HC, Tacke BK & Casper HH (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. PNAS 97(14): 7905-7910.

O'Donnell K, Ward TJ, Aberra D, Kistler HC, Aoki T, Orwig N, Kimura M, Bjørnstad A & Klemsdal SS (2008). Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. Fungal Genet Biol 45: 1514-1522.

Ohe CVD, Gauthier V, Tamburic-Ilincic L, Brule-Babel A, Fernando WGD, Clear R, Ward TJ & Miedaner T (2010). A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-

acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur J Plant Pathol 127: 407-417.

Osborne LE & Stein JM (2007). Epidemiology of Fusarium head blight on small-grain cereals. Int J Food Microbiol 119: 103–108.

Pagnussatt FA, Del Ponte EM, Garda-Buffon J & Badiale-Furlong E (2014). Inhibition of *Fusarium graminearum* growth and mycotoxin production by phenolic extract from *Spirulina* sp. Pestic Biochem Phys 108: 21-26.

Pallez M, Pasquali M, Bohn T, Hoffmann L & Beyer M (2014). Validation of a quick PCR method suitable for direct sequencing: identification of *Fusarium* fungal species and chemotypes for preventive approaches in food safety. Food Tech Biotech 52: 351–358.

Pan D, Calero N, Mionetto A & Bettucci L (2013). Trichothecene genotypes of *Fusarium graminearum* from wheat in Uruguay. Int J Food Microbiol 162: 120–123.

Parry DW, Jenkinson P & Mcleod L (1995). *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol 44: 207-238.

Pasquali M & Migheli Q (2014). Genetic approaches to chemotype determination in type B-trichothecene producing *Fusaria*. Int J Food Microbiol 189: 164-182.

Pineiro MS, Scott PM & Kanhere SR (1996). Mycotoxin producing potential of *Fusarium graminearum* isolates from Uruguayan barley. Mycopathologia 132(3): 167-172.

Ponts N, Couedelo L, Pinson-Gadais L, Verdal-Bonnin MN, Barreau C & Richard-Forget F (2009). *Fusarium* response to oxidative stress by H2O2 is trichothecene chemotypedependent. FEMS Microbiol Lett 293: 255–262.

Przemieniecki SW, Kurowski TP & Korzekwa K (2014). Chemotypes and geographic distribution of the *Fusarium graminearum* species complex. Environ Biotech 10(2): 45-54.

Purahong W, Nipoti P, Pisi A, Lemmens M & Prodi A (2013). Aggressiveness of different *Fusarium graminearum* chemotypes within a population from Northern-Central Italy. Mycoscience 55(1): 63-69.

Puri KD & Zhong S (2010). The 3-ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15-ADON population in spring wheat. Phytopathol 100: 1007–1014.

Qu B, Li HP, Zhang JB, Xu YB, Huang T, Wu AB, Zhao CS, Carter J, Nicholson P & Liao YC (2008). Geographic distribution and genetic diversity of *Fusarium graminearum* and *F. asiaticum* on wheat spikes throughout China. Plant Pathol 57: 15-24.

Quellet T & Seifert KA (1993). Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. Phytopathol 83: 1003-1007.

Rahmani A, Jinap S & Soleimany S (2009). Qualitative and quantitative analysis of mycotoxins. Compr Rev Food Sci F 8: 202-251.

Rebib H, Bouraoui H, Rouaissi M, Brygoo Y, Boudabbous A, Hajlaoui MR & Sadfi-Zouaoui N (2014). Genetic diversity assessed by SSR markers and chemotyping of *Fusarium culmorum* causal agent of foot and root rot of wheat collected from two different fields in Tunisia. Eur J Plant Pathol 139: 481–493.

Reynoso MM, Ramirez ML, Torres AM & Chulze SN (2011). Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. Int J Food Microbiol 145: 444–448.

Rotter B, Prelusky DB & Pestka JJ (1996). Toxicology of deoxynivalenol (vomitoxin). J Environ Sci Health 48: 1-34.

Ryu JC, Ohtsubo K, Izumiyama N, Nakamura K, Tanaka T, Yamamura H & Ueno Y (1988): The acute and chronic toxicities of nivalenol in mice. Fundemen Appl Toxicol 11: 38-47.

Saharan MS, Kumar J & Nagarajan S (2004). Fusarium head blight (FHB) or head scab of wheat- a review. PNAS India 3: 255-268.

Salas B, Steffenson BJ, Casper HH, Tacke B, Prom L.K., Jr. Fetch TG & Schwarz PB (1999). *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis 83: 667-674

Sampietro DA, Díaz CG, Gonzalez V, Vattuone MA, Ploper LD, Catalan CAN & Ward TJ (2011). Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. Int J Food Microbiol 145: 359-364.

Sarver BAJ, Ward TJ, Gale LR, Broz K, Kistler HC, Aoki T, Nicholson P, Carter J & O'Donnell K (2011). Novel Fusarium Head Blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet Biol 48: 1096–1107.

Scherm B, Orru M, Balmas V, Spanu F, Azara E, Delogu G, Hammond TM, Keller NP & Migheli Q (2011). Altered trichothecene biosynthesis in *TRI6*-silenced transformants of *Fusarium culmorum* influences the severity of crown and foot rot on durum wheat seedlings. Mol Plant Pathol12(8): 759-771.

Scoz LB, Astolfi P, Reartes DS, Schmale Iii DG, Moraes MG & Del Ponte EM (2009). Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathol 58: 344–351.

Shen CM, Hu TC, Sun HY, Li W, Guo JH & Chen HG (2012). Geographic distribution of trichotecene chemoptypes of *Fusarium graminearum* species complex in major winter wheat production area of China. Plant Dis 96: 1172–1178.

Starkey DE, Ward TJ, Aoki T, Gale LR, Kistler HC, Geiser DM, Suga H, Tóth B, Varga J & O'Donnell K (2007). Globalmolecular surveillance reveals novel fusariumhead blight species and trichothecene toxin diversity. Fungal Genet Biol 44: 1191–1204.

Sudakin, DL (2003). Trichothecenes in the environment: relevance to human health. Toxicol Lett 143: 97-107.

Suga H, Karugia GW, Ward T, Gale LR, Tomimura K, Nakajima T, Miyasaka A, Koizumi S, Kageyama K & Hyakumachi M (2008). Molecular characterization of the *Fusarium graminearum* species complex in Japan. Phytopathol 98: 159–166.

Talas F, Parzies HK & Miedaner T (2011). Diversity in genetic structure and chemotype composition of *Fusarium graminearum* sensu stricto populations causing wheat head blight in individual fields in Germany. Eur J Plant Pathol 131: 39–48.

Tomczak M, Wisniewska H, Stepien L, Kostecki M, Chelkowski J & Golinski P (2002). Deoxynivalenol, nivalenol and moniliformin in wheat samples with head blight (scab) symptoms in Poland (1998–2000). Eur J Plant Pathol 108: 625-630.

Tóth B, Mesterhazy A, Nicholson P, Teren J & Varga J (2004). Mycotoxin production and molecular variability of European and American isolates of *Fusarium culmorum.* Eur J Plant Pathol 110: 587-599.

Tóth B, Merterhazy A, Horvath Z, Bartok T, Varga M & Varga J (2005). Genetic variability of central European isolates of the *Fusarium graminearum* species complex. Eur J Plant Pathol 113: 35-45.

Trail F, Xu H, Loranger R & Gadoury D (2002). Physiological and environmental aspects of ascospore discharge in *Gibberella zeae*. Mycologia 94: 181–189.

Trail, F (2009). For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. Plant Physiol 149: 103-110.

Trigo-Stockli DM, Deyoe CW, Satumbaga RF & Pedersen JR (1994). Distribution of deoxynivalenol and zearalenone in milled fractions of wheat. Cereal Chem 73(3): 388-391.

Tunali B, Özseven İ, Büyük O, Erdurmuş D & Demirci A (2006). Fusarium head blight and deoxynivalenol accumulation of wheat in Marmara region and reactions of wheat cultivars and lines to *F. graminearum* and*Fusarium culmorum*. Plant Pathol J 5(2): 150-156.

Venkataramana M, Shilpa P, Balakrishna K, Murali HS & Batra HV (2013). Incidence and multiplex PCR based detection of trichothecene chemotypes of *Fusarium culmorum* isolates collected from freshly harvested maize kernels in Southern India. Braz J Microbiol 44: 401–406.

Völkl A, Vogler B, Schollenberger M & Karlovsky P (2004). Microbial detoxification of mycotoxin deoxynivalenol. J Basic Microbiol 44(2): 147–56.

Waalwijk C, Kastelein P, De Vries I, Kerenyi Z, Van Der Lee T & Hesselink T (2003). Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur J Plant Pathol 109: 743–754.

Walker SL, Leath S, Jr. Hagler WM & Murphy JP (2000). Variation among isolates of *Fusarium graminearum* associated with fusarium head blight in North Carolina. Plant Dis 85: 404-410.

Wang JH, Li HP, Qu B, Zhang JB, Huang T, Chen FF & Liao YC (2008). Development of a generic PCR detection of 3-acetyldeoxy-nivalenol-, 15-acetyldeoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum* clade. Int J Mol Sci 9: 2495–2504.

Wang JH, Ndoye M, Zhang JB, Li HP & Liao YC (2011). Population structure and genetic diversity of the *Fusarium graminearum* species complex. Toxins 3(8):1020-1037.

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K (2002). Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. PNAS 99: 9278-9283.

Windels, CR (2000). Economic and Social Impacts of Fusarium Head Blight: Changing Farms and Rural Communities in the Northern Great Plains. Phytopathol, 90(1): 17-21.

Wisniewska H & Kowalczyk K (2005). Resistance of cultivars and breeding lines of spring wheat to *Fusarium culmorum* and powdery mildew. J Appl Genet 46(1): 35-40.

Yli-Mattila T, Gagkaeva T, Ward TJ, Aoki T, Kistler HC & O'Donnell K (2009). A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian far east. Mycologia 101: 841–852.

Yli-Mattila, T (2010). Ecology and evolution of toxigenic *Fusarium* species in cereals in Northern Europe and Asia. J Plant Pathol 92: 7–18.

Yli-Mattila, T. and Gagkaeva, T. 2010. Molecular chemotyping of *Fusarium graminearum, F. culmorum* and *F. cerealis* isolates from Finland and Russia. In Y. Gherbawy, K. Voigt (eds.) Molecular identification of fungi. Springer Verlag, Berlin, Heidelberg, New York: 159-177. 507 pp.

Yli-Mattila T, Ward TJ, O'Donnell K, Proctor RH, Burkin AA, Kononenko GP, Gavrilova OP, Aoki T, Mccormick SP & Gagkaeva, T (2011). *Fusarium sibiricum* sp. nov, a novel type A trichothecene-producing *Fusarium* from northern Asia closely related to *F. sporotrichioides* and *F. langsethiae*. Int J Food Microbiol 147(1): 58-68.

Yli-Mattila, T. 2012 Morphological and Molecular Taxonomy of Highly Toxigenic Fusarium Species from Small Cereal Grains in Northern Europe and Asia. Systematics and Evolution of Fungi. *Editors* J.K. Misra, J.P. Tewari, K. Deshmukh, Science publishers, New Hampshire, USA: 277-303.

Yli-Mattila, T, Rämö S, Hietaniemi V, Hussien T, Carlobos-Lopez AL & Cumagun CJR (2013). Molecular quantification and genetic diversity of toxigenic *Fusarium* species in Northern Europe as compared to those in Southern Europe. Microorganisms 1: 162–174.

Yli-Mattila, T., Garvrilova, O., Hussien, T. and Gagkaeva, T. 2015. Identification of the first *Fusarium sibiricum* isolate in Iran and *Fusarium langsethiae* isolate in Siberia by morphology and species-specific primers. J. Plant Pathol. 97: 183-187.

Yli-Mattila, T. and Gagkaeva, T. 2016. *Fusarium* toxins in cereals in northern Europe and Asia. In Applications of Fungi and their Management Strategies (Edited by Drs. Deshmukh, Misra, Tewari & Papp): 293-317, CRC Press.

Yörük E & Albayrak G (2012). Chemotyping of *Fusarium graminearum* and *F. culmorum* isolates from Turkey by PCR assay. Mycopathologia 173: 53-61.

Yörük E & Albayrak G (2013). Genetic characterization of *Fusarium graminearum* and *F. culmorum* isolates from Turkey by using random-amplified polymorphic DNA, Genet Mol Res 12(2). 1360-72.

Yörük E & Albayrak G (2014). *Tri4* and *tri5* gene expression analysis in *Fusarium graminearum* and *F. culmorum* isolates by qPCR. Plant Pathol J *13(2)*: 133-138.

Yörük E, Gazdağli A & Albayrak G (2014). Class B trichothecene chemotyping in *Fusarium* species by PCR assay. Genetika 46(3): 661-669.

Zhang JB, Li HP, Dang FJ, Qu B, Xu JB, Zhao CS & Liao YC (2007). Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the *Fusarium graminearum* clade from China. Mycol Res 111: 967–975.

Zhang H, Theo VDL, Waalwijk C, Chen W, Xu J, Xu J, Zhang Y & Feng H (2012). Population Analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. PlosOne 7(2): 1-13.

Zhang X, Ma HX, Zhou YJ, Xing JC, Chen JH, Yu GH, Sun XB & Wang L (2014). Identification and Genetic Division of *Fusarium graminearum* and *Fusarium asiaticum* by Species-Specific SCAR Markers. J Phytopathol 162: 81-88.

Table 1 *Generalized chemotype profile and suggested origin for the species of F. graminearum species complex*

Species	**Origin**	**Host**			**Chemotype**		
		Wheat	**Maize**	**Barley**	**3-ADON**	**15-ADON**	**NIV**
F. graminearum s.s.	Cosmopolitan	+	+	+	+	+	+
F. asiaticum	Asia	+	+	+	+	+	+
F. vorosii	Asia	+	nr[1]	+	nr	+	nr
F. ussurianum	Asia	+	nr	+	+	nr	nr
F. nepalense	Asia	+	+	nr	nr	+	nr
F. austroamericanum	South America	+	+	+	+	nr	+
F. meridionale	South America	+	+	+	nr	nr	+
F. cortaderiae	South America	+	+	+	+	nr	+
F. brasilicum	South America	+	nr	+	+	nr	+
F. boothii	Central America	+	+	+	nr	+	nr
F. mesoamericanum	Central America	nr	nr	nr	+	nr	+
F. gerlachii	North America	+	nr	nr	nr	nr	+
F. louisianense	Central America	nr	nr	nr	nr	nr	+
F. acacia-mearnsii	Africa/Oceania	+	nr	nr	+	nr	+
F. aethiopicum	Africa	+	nr	nr	nr	+	nr

[1]nr means there is no present report about specific host or chemotype

Table 2. *Five side chains of class B-trichothecenes*

Class B-trichothencenes	**C position**				
	C-3	**C-4**	**C-15**	**C-7**	**C-8**
DON	-OH	-H	-OH	-OH	=O
3-ADON	-OA	-H	-OH	-OH	=O
15-ADON	-OH	-H	-OA	-OH	=O
NIV	-OH	-OH	-OH	-OH	=O

Table 3. *Genes located in core tri5 gene cluster and their activities in F. graminearum strains H-11 (LEE et al., 2001), 88-1 (LEE et al., 2002) and F15 (KIMURA et al., 2003)*

Gene	**Product**	**Size in bp**	**Exon/intron**	**Activity in three *F. graminearum* strains**		
				H-11	**88-1**	**F15**
tri8	Trichothecene C-3 deacetylase	1334	1/0	+[2]	+[2]	+[2]
tri7	3-acetyltrichothecene 4-*O*-acetyltransferase	1376	2/1	X[3]	-[4]	+[2]
tri3	3-acetyltrichothecene 15-*O*-acetyltransferase	1762	4/3	+[2]	+[2]	![5]
tri4	Multifunctional (in four steps) oxygenase	1741	4/3	+[2]	+[2]	+[2]
tri6	Transcription factor	656	1/0	+[2]	+[2]	+[2]
tri5	Trichodiene synthase	1179	2/1	+[2]	+[2]	+[2]
tri10	Regularotry protein	1352[1]	2/1	+[2]	+[2]	+[2]
tri9	*TRI9* with unknown function	131	1/0	![5]	![5]	![5]
tri11	C-15 hydroxylase	1740	5/4	+[2]	+[2]	+[2]
tri12	Final product transporter/efflux pump	1904	3/2	+[2]	+[2]	+[2]
tri13	3-acetyltrichothecene C-4 hydroxylase	1801	2/1	X[3]	X[3]	+[2]
tri14	Hypothetical protein	1176	2/1	![5]	![5]	![5]

[1] means the information obtained from AF326571.1 and AY102604.1,

[2]+ means expressed gene,

[3]x means pseudogene,

[4]- means deleted gene,

[5]! means gene with no crucial expression

Table 4. *Class B-trichothecene profile distribution of Fusarium spp. in Asian countries. Fg: F. graminearum, Fc: F. culmorum, Fgss: F. graminearum sensu stricto, Fmer: F. meridionale, Fps: F. pseudograminearum, Fce: F. cerealis, Fa: F. asiaticum, Fu: F. ussurianum, Fv: F. vorosii and Fgsc : F. graminearum species complex*

Author(s)	**Country(ies)**	**Species**	**Host(s)**	**Method(s)**	**No of isolates**		
					3-ADON	15-ADON	NIV
Lee *et al*. (2001)	South Korea	*Fg*	Rice	PCR, GC/MS	31[1]		321
Carter *et al*. (2002)	Nepal	*Fg*	Maize, Rice, Wheat	GC/MS	26[1]		10
Kim *et al*. (2003)	South Korea	*Fg*	Wheat, Barley, Corn	PCR, SB GC/MS	34[1]		76
Li *et al*. (2005)	China	*Fg*	Wheat	PCR, HPLC GC/MS	310[1]		54
Ji *et al*. (2007)	China	*Fg*	Wheat, Maize, Barley	PCR	48	203	3
Zhang *et al*. (2007)	China	*Fg*, *Fa*	Wheat	PCR	155	91	53
Haratian *et al*. (2008)	Iran	*Fg*	Wheat	PCR, TLC	11[1]		46
Qu *et al*. (2008)	China, Nepal Japan	*Fgsc*	Wheat, Maize, Rice	PCR	26	3	10

Suga *et al*. (2008)	Japan	*Fg, Fa*	Wheat, Barley	PCR, GC-MS	100	13	170
Wang *et al*. (2008)	Nepal, China	*Fgsc*	Rice, Maize, Wheat	PCR, HPLC GC/MS	6	13	8
Karugia *et al.* (2009)	Japan	*Fa, Fgss*	Wheat	PCR, HPLC	99	0	84
Yli-Mattila and Gagkaeva (2010) Yli-Mattila *et al.* (2009)	Russia	*Fg, Fc, Fu, Fv, Fce*	Wheat, Barley, Oats	PCR	20/55	26/45	6/0
Yli-Mattila and Gagkaeva (2010)	China	*Fg, Fce*	Wheat	PCR	2	9	4
Desjardins and Proctor (2011)	Nepal	*Fgsc*	Maize	PCR, HPLC	48[1]		148
Lee *et al*. (2012)	South Korea	*Fgsc*	Maize	PCR, TLC	438[1]		54
Ndoye *et al*. (2012)	China	*Fg, Fa*	Maize		1	269	350
Shen *et al*. (2012)	China	*Fg, Fa*	Wheat	PCR	300	182	48
Yörük and Albayrak (2012)	Turkey	*Fg, Fc*	Wheat, Maize, Barley	PCR	20	11	1
Zhang *et al.* (2012)	China	*Fmer, Fgss, Fa, Fc, Fce*	Wheat	PCR, HPLC	172	176	109
Alkadri *et al*. (2013)	Syria	*Fg, Fc*	Wheat	PCR, HPLC-MS/MS	4	0	10
Davari *et al*. (2013)	Iran	*Fg*	Wheat	PCR	3	121	11
Mert-Türk and Gencer (2013)	Turkey	*Fc*	Wheat	PCR	16	39	2

Venkataramana *et al*. (2013)	India	*Fc*	Maize	PCR, HPLC	54[1]		34
Yörük *et al.* (2014)	Turkey, Iran	*Fg, Fc, Fps, Fpo*	Wheat, Barley	PCR	11	6	0

[1] means no further differentiation of DON chemotype

Table 5. *Class B-trichothecene profile distribution of Fusarium spp. in North and South America countries. Fg: F. graminearum, Fgss: F. graminearum sensu stricto, Faust: F. austroamericanum, Fb: F. boothii, Fmer: F. meridionale, Fa: F. asiaticum, Fave: F. aveneceum, Fps: F. pseudograminearum, Fsp: F. sporotrichioides and Fgsc: F. graminearum species complex*

Author(s)	**Country(ies)**	**Species**	**Host(s)**	**Method(s)**	**No of isolates**		
					3-ADON	15-ADON	NIV
Pineiro *et al* . (1996)	Uruguay	*Fg*	Barley	GC/MS	0	11	0
Salas *et al.* (1999)	USA	*Fg, Fpo, Fave, Fsp*	Wheat	GC/MS	11[1]		9
Walker *et al.* (2000)	USA	*Fg*	Wheat	GC	66[1]		0
Gilbert *et al.* (2001)	Canada	*Fg*	Wheat, Barley, Corn, Weeds	GC	3	14[2]	2
Clear *et al.* (2006)	Canada	*Fps*	Wheat	GC	122	2	1
Qu *et al.* (2008)	USA	*Fgsc*	Wheat	PCR	0	7	0
Wang *et al*. (2008)	USA	*Fgsc*	Wheat	PCR, HPLC GC/MS	0	5	0
Alvarez *et al.* (2009)	Argentina	*Fgss*	Wheat	GC	75	115	34
Scoz *et al.* (2009)	Brazil	*Fgss, Fmer*	Wheat	PCR	0	76	6
Delgado *et al.* (2010)	USA	*Fg*	Potato, Wheat, Sugar beet	PCR, TLC, GC/MC	12[1]		2
Ohe *et al.* (2010)	Canada	*Fg*	Wheat	ELISA	12	12	0
Puri and Zhong (2010)	USA	*Fgss*	Wheat	PCR, GC/MS	4	111	5
Astolfi *et al.* (2011)	Brazil	*Fgss,Fmer, Faust*	Barley	PCR	4	61	27
Gale *et al.* (2011)	Louisiana	*Fa, Fgss*	Wheat	PCR, GC/MS	6	253	48
Sampietro *et al.* (2011)	Argentina	*Fmer, Fb*	Maize	PCR	0	10	56
Pan *et al.* (2013)	Uruguay	*Fgss*	Wheat	PCR	0	110	1

Castanares *et al.* (2014)	Argentina	*Fgss*	Barley	PCR, GC/MS	16[2]	110	1[2]
Malbran *et al.* (2014)	Argentina	*Fg*	Wheat	PCR, ELISA	0	112	0

[1] means no further differentiation of DON chemotype

[2] means co-production of two or three mycotoxins

Table 6. *Class B-trichothecene profile distribution of Fusarium spp. in European countries. Fg: F. graminearum, Fcor: F. cortaderiae, Fc: F. culmorum, Fgss: F. graminearum sensu stricto, Fb: F. boothii, Fgsc: F. graminearum species complex*

Author(s)	**Country(ies)**	**Species**	**Host(s)**	**Method(s)**	**No of isolates**		
					3-ADON	15-ADON	NIV
Bakan *et al.* (2002)	France	*Fc*	Wheat	GC/MS	10	3[2]	0
Carter *et al.* (2002)	France, Germany, UK,	*Fg*	Wheat	GC/MS	13[1]		1
Hestbjerg et al. (2002)	Austria, Denmark, Norway	*Fc*	Wheat	HPLC	77	0	9
Jennings *et al.* (2004a)	England, Wales	*Fg*	Wheat	PCR, HPLC	4	76	21
Jennings *et al.* (2004b)	England, Wales	*Fc*	Wheat	PCR	88	0	65
Tóth *et al.* (2005)	Hungary, Austria	*Fgsc*	Wheat	PCR, HPLC	2[2]	21	0
Qu *et al.* (2008)	France, Germany, Italy, UK, Sweden	*Fgsc*	Wheat	PCR	5	17	2
Yli-Mattila *et al.* (2009)	Russia, Finland	*Fg*	Wheat, Barley, Oat	PCR	43	43	0
Yli-Mattila and Gagkaeva (2010)	Finland	*Fg*	Wheat, Barley, Oat	PCR	12	0	0
		Fc	Wheat, Barley	PCR	13	0	0
	Russia	*Fc, Fce*	Potato, Cirsium, Wheat, Barley	PCR	10	0	1
	Germany	*Fg*	Oat	PCR	0	4	1

Wang *et al.* (2008)	France, Germany, Italy, UK	*Fgsc*	Wheat, Barley	PCR, HPLC GC/MS	0	18	3
Talas *et al.* (2011)	Germany	*Fgss*	Wheat	PCR	23	311	4
Nielsen *et al.* (2012)	Denmark	22 *Fusarium* sp. with 6 members of Fgsc	Wheat, Barley, Triticale, Rye, Oats	PCR	36	7	11[2]
Cornea *et al.* (2013)	Romania	*Fg*, *Fc*	Wheat	PCR, HPLC	8	71	0
Purahong *et al.* (2013)	Italy	*Fg*	Wheat	PCR	5	21	6
Boutigny *et al.* (2014)	France	*Fgss*, *Fcor*, *Fb*	Wheat, Maize, Barley	PCR	1	255	38

[1] means no further differentiation of DON chemotype

[2] means co-production of two or three mycotoxins

Figures

Fig.1

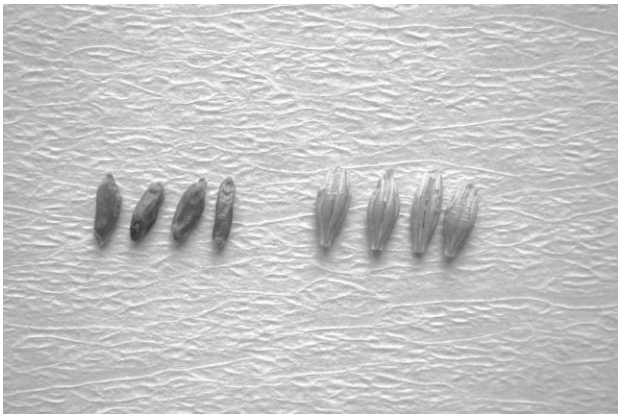

Fig.2

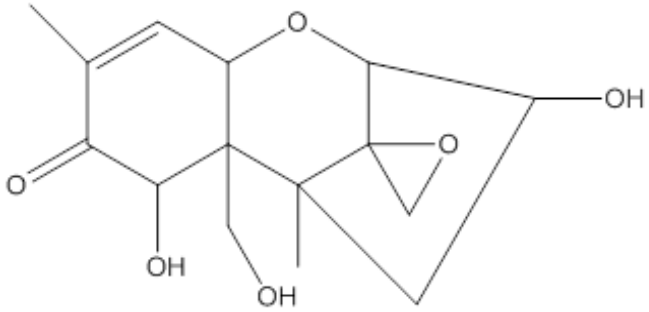

Fig. 3

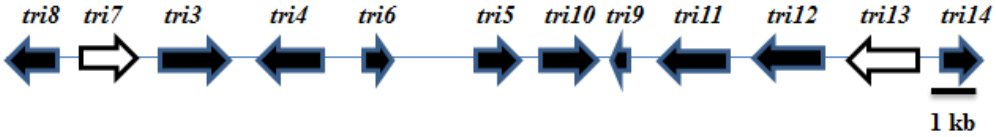
tri8
tri7
tri3
tri4
tri6
tri5
tri10
tri9
tri11
tri12
tri13
tri14
1 kb

Fig.4


H-11 TGCGTGTCAATCTTGGACAAGTGCTGGAGTGAGGATGTAGCTCCACCGCATCGAAGAGTC 531
F15 TGCGTGTCGCCCTTGGACAAGTGTTGAAGTGCGGGTGTAGCTCGACCGCATCGAAGAGTC 540
88-1 TGCGTGTCAACCATGGCCAAGCGTTGAAGTGCCGATGTAGCTCCACCGCATCGAAGACTC 529
H-11 TCTTCAAAGACTGCTTCAAGGTGGAGGATTCGCGCAAAG--TAGCTCCAGGAAAGT---- 585
F15 TCTTCAATGGCTGTTTCAAGGTAGGGGATTCGCGCAAGG--TAGCTCTAGGAAAGT---- 594
88-1 TCTTCAATGACTGCTTCAAGGTAGGGGATTCGAGCAGCTGCTAGCTCTTGGAAAGTGGGA 589
H-11 ----------
F15 ----------
88-1 CTGCGGTTTTCGGATTTGGCCTTGGGGAACGCTGACTGCAAAGCCTGTCGGAGTCGCTTC 649
H-11 ------CCACTATTGCG---------- 596
F15 ------CCACTGTTGCA---------- 605
88-1 TGGGCGCCAGCATTGCCTGTAAGGAACTTCAAGCCCCACAGAGTGGTGGTGCTCGTTGTG 709
H-11 ----------
F15 ----------
88-1 TCATAACCTCCGAGGATCAGTCCGAATAGCTAGAATAACCCAACGTATCAGCTTCCATCA 769
H-11 -----ATCTGTGATTGAGTCTTTGTGCTTACCTCTCCTTGTATCATCGTTGACAATTAAT 651
F15 -----ATCTGTGATTGAGTCTTTGTGCTTACCTCTTCTTGCATCATCGTTGATACATAAT 660
88-1 TCTTAATATGTCATTGAGTCGTTGTGCTTACCTCTCCTTGCATCATTGTTGACAAATAGT 829
H-11 TTCGGCTCCGAATTTTCGCTTCAGCCAACAATGTTTCACGTAGCATCATCTGTTCCAACG 711
F15 TCAAGCTCCGATTTTTCGTTTCAGCCAGTAATGTTTCACGTAGCACCATCTGTTCCAACG 720
88-1 TTGGACTCCGATTTTCCGCTTTGGCCAACAATGTTTCACGTAGCACCATCTGTTCCAAAG 889

Figure Legends

Fig. 1 Shrivelled kernels obtained from scabby barley spikes in Çorum region of Turkey (four grains left side) and healthy kernels of *Hordeum vulgare* L. cv. Tokak157/37

Fig. 2 Schematization of DON molecule generated by ChemOffice software V14 (Cambridge, England)

Fig.3 Genes located in *tri5* gene cluster

Fig.4 CLUSTALW analysis of *tri13* gene of *F. graminearum* 88-1 (NIV chemotype), H-11 (15-ADON chemotype) and F15 strains (3-ADON chemotype). '-' means deletions, '*' means conserved nucleotide sequences.