Genetic approaches to chemotype determination in type B-trichothecene producing *Fusaria*

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Abstract

This review summarises the genetic methods used for chemotype determination of the main *Fusarium* type B-trichothecene producing species. Literature on *Fusarium* chemotype epidemiology over the last 15 years is reviewed in order to describe temporal and spatial chemotype distribution of these fungi worldwide. Genetic approaches used for chemotype determination are also reviewed and discussed, highlighting successes and potential pitfalls of the technique. Results from both genetic and chemical approaches are summarised to compare reliability, advantages and limitations of the two methods. Potential applications of genetic chemotyping to toxigenic *Fusarium* species are evaluated in the light of improving food safety of agricultural products. The use of chemotype determination in population studies, toxin prediction as well as for breeding purpose is described.

Keywords: *TRI* genes, deoxynivalenol, nivalenol, acetylated deoxynivalenol, *Fusarium culmorum*, *Fusarium graminearum* species complex.

Introduction

Among the most studied and harmful toxins produced by *Fusarium* spp. are the sesquiterpene epoxides trichothecenes, secondary metabolites that inhibit eukaryotic protein synthesis and cause severe toxicosis in humans and other animals upon

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ingestion of contaminated grain or their derivatives, affecting intestinal, immune endocrine and neurologic functions (Maresca et al., 2013). Trichothecenes are also highly phytotoxic and play a role in virulence on the host plants (Arunachalam and Doohan 2013; Desmond et al., 2008; Ilgen et al., 2009; Proctor et al 2009; Scherm et al., 2011).

Fusaria may produce different types of toxins depending on differences in the core trichothecene cluster (*TRI* cluster), which includes two regulatory genes (*TRI6* and *TRI10*) and most of the biosynthetic enzymes required for the production of trichothecenes (Alexander et al., 2009, 2011; Kimura et al., 2003; Lee et al., 2001). Depending on the species and chemotype the number of functional core genes in the cluster varies. In *F. graminearum,* for example, the trichothecene gene cluster consists of 10–12 contiguous genes as well as two other genes, *Tri1* and *Tri101*, which are at separate loci outside the main cluster. *Fusarium* trichothecenes can be grouped in two classes based on the presence (B-trichothecenes) *versus* absence (A-trichothecenes) of a keto group at the C-8 position (Ueno et al., 1973). The difference is due to catalytic divergence of the cytochrome P-450 enzymes encoded by *Tri1*. While in *F. graminearum* Tri1p oxygenates both C-7 and C-8 (which results in a hydroxyl at C-7 and a carbonyl at C-8), in *F. sporotrichioides*, only C-8 is hydroxylated by Tri1p (Rep and Kistler, 2010). Among type B-trichothecenes, those having a significant impact on safety issues are: deoxynivalenol (DON), nivalenol (NIV), and their acetylated derivatives 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and 4-acetylnivalenol (4-ANIV, *syn*. fusarenone-X).

Based on the type of trichothecene produced, different chemotypes have been described so far for Fusarium species: chemotype I, producing DON and/or its acetylated derivatives, and chemotype II, producing NIV and/or 4-ANIV (Sydenham et al., 1991). The DON chemotype can be further split into chemotype IA (producing 3-ADON) and IB (producing 15-ADON; Miller et al., 1991). The intact gene cluster in *F. graminearum* results in strain producing NIV where TRI13 cytochrome P450 monoxygenase and TRI7, the associated acetyltransferase, catalyze the C-4 hydroxylation and acetylation. In DON/ADON producers, *TRI13* and *TRI7* show insertions and deletions which determine the lack of functional enzymes able to hydroxylate in C-4 and transacetylate (Lee et al., 2002). The acetylation position determining the 3-ADON or 15-ADON seems to be caused by differential activity of *TRI8* which encodes for a C-3 esterase (Alexander et al., 2011).

Structural differences among toxin chemotypes may have relevant consequences on strain fitness, since the specific pattern of oxygenation and acetylation can modify the bioactivity and hence the (phyto)toxicity of these compounds (Alexander et al., 2009, 2011; Brown et al., 2002, 2004; Ward et al., 2002; Lee et al., 2002).

The discovery of a vast array of secondary metabolites produced by *Fusarium* species has fostered surveys of mycotoxin diversity in many different epidemiological and agricultural conditions. Surveys are routinely conducted in different geographic areas (Barros et al., 2012; Clear et al., 2000a, 2000b; Del Ponte et al., 2012; Desjardin et al., 2000; Edwards, 2009; Giraud et al., 2010; Goertz et al., 2010; Kim et al., 1993; Ok et al., 2011, 2014; Park et al., 2005; Seo et al., 1996; Tanaka et al., 1986; Vanheule et al., 2014; Wagacha et al., 2010; Yoshizawa and Jin, 1995) to identify major toxigenic risks in affected grains. Indeed, trichothecenes are continuously found in cereals and derived food products around the world (Adejumo et al., 2007; Bosch et al., 1992; Gonzales et al., 2008; Nielsen et al., 2014; Poapolathep et al., 2008; Roscoe et al., 2008; Scudamore and Patel, 2009). DON and NIV now represent the two major concerns for safety of wheat and barley products, being the two most abundant toxins detected, as recently reported in a large survey on Canadian grains (Tittlemier et al., 2013).

The purpose of this review is to summarise genetic methods used for chemotype determination of type B-trichothecene producing *Fusarium* spp. Papers published during the last 15 years and reporting on the chemotype identified for sets of isolates were selected, and information on the investigated area as well as on the species and crop have been retained to generate a virtual description of known chemotype distribution worldwide. Moreover, potential applications and limits of genetic chemotyping of *Fusarium* are discussed.

We focus on the *Fusarium graminearum* species complex (FGSC, O'Donnell et al., 2000*)* which presently includes at least 16 species (Aoki et al., 2012), *F. culmorum* and *F. cerealis* (Cooke) Sacc., since these species are considered among the most relevant pathogens on wheat and other cereals (Moss and Thrane, 2004, Osborne and Stein 2007). Production of a type B tricothecene (nivalenol) has been reported also from *F. poae* (Peck) Wollenw. (Jestoi et al., 2008; Thrane et al., 2004; Vogelgsang et al., 2008b) and *F. equiseti* (Corda) Sacc. (Kosiack et al., 2005). However, since they rarely produce significant amounts of other trichothecene B toxins (Kristensen et al., 2005), genetic chemotype determination does not offer additional valuable information and it is therefore not treated in detail here.

Part I.

Why determine the chemotype of an isolate?

Determining the chemotype of an isolate is carried out for two main reasons:1) to obtain epidemiological information on the population colonising a crop in a given area, using chemotype as a proxy in the field;

2) to inform on the toxigenic risk that the presence of a certain chemotype may determine on the food or feed that is produced, with the long term perspective of developing preventive models to decrease the toxigenic risk.

Ward et al. (2002) demonstrated that polymorphism within *TRI* genes is trans-specific and appears to have been maintained by balancing selection acting on chemotype differences. Different trichothecene-type isolates do not just have different trichothecene profiles but can in some instances be regarded as different genetic populations (Mishra et al., 2009), even if they co-occur within the same area and some gene flow may take place between them as shown using VNTR and RFLP markers (Gale et al., 2007; Karugia et al., 2009b; Ward et al., 2008). Gene flow between different populations, yet within a species, seems comparatively limited even if they co-exist, although the factors that inhibit gene flow between populations in the same area are unknown so far (Karugia et al., 2009b). This original observation leads to the idea that monitoring chemotype diversity can be informative for characterising a field population. Indeed, the evolutionary dynamics of the core trichothecene cluster were demonstrated to be essentially uncoupled from the rest of the genome (Ward et al., 2008, Proctor et al., 2009). However, as long as recombination frequency is low, chemotype could be considered as a marker for a genomic background specific to populations or individuals that are distinguished by a variety of phenotypic traits beyond chemotype. Because trichothecene production is associated with the spread of the disease after initial infection in wheat (Mesterházy, 2002), and trichothecene production is a factor affecting not only FHB but also seed diseases (Wang et al., 2006), finding a population with higher toxin production may suggest a stronger impact of the disease. For example, *F. graminearum* populations with 3-ADON chemotype seem to have a higher average toxigenic capacity in wheat and barley (as well as growth rate) in North America compared to NIV and 15-ADON populations (Foroud et al., 2012; Gilbert et al., 2010; von der Ohe, 2010; Ward et al., 2008). Conversely, 3-ADON populations do not differ for pathogenicity and sexual reproduction in different *Fusarium* species from different

137 locations (Alvarez et al., 2010; Gilbert et al., 2010; Purahong et al., 2014; Schmale et al.,
138 2011; Spolti et al., 2014b; von der Ohe, 2010). On another set of isolates, differences in
139 the aggressiveness among chemotypes were reported by Malihipour et al. (2012), who
140 suggested a gradient of aggressiveness from NIV to 15-ADON to 3-ADON chemotypes.
141 Analysing populations carrying the NIV chemotype compared to local DON populations,
142 lower virulence for the NIV populations were observed in *F. asiaticum* from China (Puri et
143 al., 2012; Shen et al., 2012; Zhang et al., 2012), in *F. graminearum* (Foroud et al., 2012;
144 Miedaner et al., 2008), as well as in *F. culmorum* in rye (Miedaner and Reinbrecht,
145 2001). On the contrary, NIV population did not differ in pathogenicity to its DON
146 population counterpart when two different pathogenicity scorings were used (Purahong
147 et al., 2014). Discrepancies between results on aggressiveness among chemotypes can
148 be attributed to the use of chemotype as a proxy of a population. Depending on the gene
149 flow and variability of a certain population in a sampled area, pathogenicity characters
150 may or may not be associated with chemotype data. Aggressiveness is a factor being
151 influenced not only by the characters of the pathogen, but also by its interaction with the
152 host and the environment. Indeed, when large set of resistant cultivars were assayed,
153 the underlying genetic resistance seemed cross-applicable between chemotypes (Clear
154 et al., 2013; Foroud et al., 2012; Horevaij et al., 2011; Perkowski et al., 1997).
155 In order to understand which factors do favour persistence and spread of a chemotype,
156 the hypothesis was formulated that some fitness characters associated with a
157 chemotype can favour its establishment in a given area, as observed in Canada and
158 USA (Puri and Zhong, 2010; Ward et al., 2008), as well as in China for barley (Yang et
159 al., 2008; Zhang et al., 2010a, 2010b) and wheat (Zhang et al., 2012). Indeed, regional
160 difference in chemotype distribution may be influenced by environmental and cultural
161 practices since chemotype variation may confer an adaptive potential to these
162 pathogens and it is likely driven by natural selection: recent migration and introgression
163 were suggested to be the way for new chemotypes with adequate fitness to become
164 established into the resident populations (Desjardins et al., 2008; Gale et al., 2011;
165 Zhang et al., 2012).
166 Of course chemotype diversity cannot *per se* explain all differences that are observed
167 between groups of isolates with different phenotypic characters.. Comparison between
168 population studies and chemotype description is needed (Wang et al., 2011) to clarify the
169 level of variability of a chemotype within a group.

Working on a *F. culmorum* population from an international collection, Miedaner et al. (2013) showed that isolates with 3-ADON and NIV chemotype have a similar genetic background confirming that gene flow occurs also in *F. culmorum*. One hypothesis on the stability of *F. graminearum* 15-ADON chemotype status in New York state is that the original character favouring the spread of the 3-ADON chemotype in North America (Ward et al., 2008) has been transferred to the 15-ADON population, therefore enabling a balance between the two populations (Spolti et al., 2014b). Mechanistic studies are now starting to explore changes by taking into account genetic variability represented by chemotypes at the proteome and transcriptome level (Krishna et al., 2012; Pasquali et al., 2013b) as well as in gene knock-outs (Abou Ammar et al., 2013; Pasquali et al., 2013a). Similarly, the activity of toxin inhibiting compounds has been tested taking into account the different chemotypes (Boutigny et al., 2009, 2010; Kulik et al., 2014). These comparative studies may facilitate the understanding of evolutionary forces acting on the selection of the characters and at the same time may shed light on the mechanisms favouring a certain chemotype in a field, consequently affecting the amount and quality of toxins found in grains.

Among the factors that may have an effect on chemotype selection, fungicides have been suggested by Gale et al. (2007) and their effect was investigated in different papers. With respect to azoles, strobilurins and isopyrazam there seems not to be an effect of chemotype on resistance (Amarasinghe et al., 2013; Beyer et al., 2014; Dubos et al., 2011, 2013; Kulik et al., 2012; Spolti et al., 2014a). On the contrary, carbendazim (MBC) resistance, coupled to higher toxin production (Zhang et al., 2009), seems to be associated with the 3-ADON chemotype in Asia in *F. graminearum* and *F. asiaticum* where MBC sensitivity differed between NIV and DON chemotypes (Zhang et al., 2013a), but this difference was not evident in other studies (Qiu et al., 2014; Wang et al., 2010). Moreover, the 3-ADON chemotype revealed significant advantages over *F. asiaticum* producing NIV, including higher resistance to benzimidazoles (Zhang et al., 2012).

On a small set of isolates, chemotypes differed for fitness characters such as a higher resistance to thermal shock by the 3-ADON chemotype in *F. graminearum* (Vujanovic et al., 2012), or a higher adaptation to oxidative stress by the NIV chemotype (Ponts et al., 2007, 2009). Whether these characters are truly associated with chemotype has to be further investigated on a larger set of isolates.

Environmental factors may influence chemotype success. It has been proposed that chemotype specialisation may be driven by a certain host (Yli-Mattila et al., 2013). NIV-producing isolates were found to be more aggressive towards maize compared to DON-producers (Carter et al., 2002) and were associated, in *F. asiaticum*, preferentially to maize in China (Ndoye et al., 2012). Being NIV a pathogenicity factor in maize (Maier et al., 2006), findings that associate an increase in NIV population in areas where preceding crops was maize (Audenaert et al., 2009; Pasquali et al., 2010, Sampietro et al., 2011) are not surprising, despite this association is not consistent in all sampling worldwide. NIV chemotype has been also associated with rice cultivation (Davari et al., 2013; Gale et al., 2011, Lee et al., 2009; Umpierrez et al., 2013). Similarly Nielsen et al. (2012) reported a higher detection of 3-ADON chemotype in oats compared to barley and wheat in northern European conditions. Other reasons for chemotype spread has been postulated by Lee et al. (2012), suggesting that the persistence of the NIV chemotype in *F. asiaticum* (lineage 6) of FGSC is due to its role as a fitness factor towards other microbial communities. By comparing different species within the FGSC in wheat, Goswami and Kistler (2005) found that NIV was accumulated less abundantly by similarly aggressive isolates, hence speculating on its major toxicity accounting for similar pathogenicity results. It is nonetheless evident that aggressiveness is not due to toxin type and production as showed on *F. culmorum* in barley and rye by Miedaner et al. (2004). Gilbert et al. (2011) reported that under controlled conditions, 3-ADON isolates of *F. graminearum* colonise more abundantly the plants when co-inoculated with 15-ADON at 28°C but not at 20°C. This finding was not confirmed in nursery conditions (Clear et al., 2013) and on a different set of isolates (Spolti et al., 2014b). By examining weather variables over the 5-year period on a set of field studies, Gilbert et al. (2014) found no correlation between recovery of a chemotype and temperature or precipitation. It seems therefore difficult to find a clear effect of climatic conditions on chemotype selection.

Within a food safety perspective, understanding the toxigenic potential of the isolates collected from a given area (field, region, county, state, and beyond) may help in guiding risk assessment on toxin contamination at the field/regional scale. Two examples that have been proposed to demonstrate the utility of monitoring chemotype are:

1. the appearance of a chemotype able to produce a toxin with higher toxigenicity in a certain environment [e.g., the toxicity of nivalenol compared to deoxynivalenol (Minervini et al., 2004) as well as reports of a NIV-producing population in Luxembourg (Pasquali et al., 2009), US (Gale et al., 2011), Uruguay (Umpierrez-

Failache et al., 2013; *F. asiaticum*), Brazil (Del Ponte et al., 2012) and China (Lee et al., 2001; Zhang et al., 2012; *F. asiaticum*) suggesting the need to monitor for the increased risk of NIV contamination in the grains].

2. the presence of a population of the pathogen that is able to produce on average a higher amount of toxin on a certain crop [e.g., the case of the 3-ADON population in US and Canada (Foroud et al., 2012; Gilbert et al., 2010; von der Ohe, 2010; Ward et al., 2008)].

It is therefore evident that a continuous monitoring of the chemotype situation may well inform on the risk and the type of population that are present over a certain environment. For this reason, a large set of methods were developed and surveys around the world were carried out. This set of data is considered in the second part of the review.

Part II (methods and surveys)

Molecular genetics methods

Detecting the presence of a certain chemotype requires a chemical method, based on the identification of the product in the substrate or directly in the grain. In some instances chemical analysis can be substituted with antibody-based detection methods, despite reliability of different kits is not fully confirmed and acetylated forms are often cause of cross reactivity phenomena (Tangni et al., 2010). Another complementary method is based on the determination of the genetic structure of the isolate(s) using PCR-derived methods applied to the pure culture or, more recently, also to the whole grain. Chemical analytical methods as well as rapid alternative methods have been revised extensively elsewhere (Cigić and Prosen, 2009; Josephs et al., 2004; Koch, 2004; Köppen et al., 2010; Krska et al., 2001; Maragos and Busman, 2010; Ran et al., 2013) hence they will not be discussed here.

The history of chemotype diversity studies shows that while earlier works were focused on the strain production biochemistry, the appearance of seminal papers on the genetic determinants of the toxin [the genes involved in trichothecene production, mainly studied in *F. sporotrichioides* (Desjardins, 2009)] allowed researcher to postulate and finally to develop tools to differentiate chemotypes based on gene diversity. This information coupled with PCR flexibility and accessibility (Nicholson et al., 2003) led to a significant shift towards the use of genetic chemotyping methods.

The most widely used approach so far aims at detecting strains isolated from plant tissue. It consists in the isolation of single spore colonies from infected spikes and the determination of the chemotype on the isolated strains. Historically this approach led to the identification of trichothecene type B isolates from different crops by measuring the toxin produced by each isolate *in vitro* (Faifer et al., 1990; Gang et al., 1998; Mirocha et al., 1989). More recently, by genetic chemotyping, often combined to chemical confirmation, it has been feasible to obtain information on the distribution of a certain chemotype within a country (Zhang et al., 2012), a field (Suga et al., 2008) or on seed samples (Wang et al., 2012).

The first PCR method developed for discriminating between the DON and the NIV chemotypes (with no distinction between 3- and 15-ADON) was developed by analysing the polymorphism of *TRI* genes, particularly the *TRI7* gene, which has insertions in the non-coding region in DON producers but not in NIV producers. Therefore, a simple sequence length assay allows to distinguish between NIV and DON chemotypes (Lee et al., 2001).

In 2002, the respective function of *TRI7* and *TRI13* was demonstrated to be linked to chemotype diversity (Lee et al., 2002), suggesting that both genes could be used for genetic chemotype distinction. Based on this finding and on additional sequencing work, Chandler et al. (2003) developed a set of primers to amplify *TRI7* and *TRI13* genes and successfully identified DON and NIV chemotypes in *F. graminearum*, *F. culmorum*, and *F. cerealis* by a double assay. Similarly, Waalwijk et al. (2002) developed a *TRI13* primer pair to differentiate between DON and NIV chemotype according to difference in length of the amplified product.

A further optimization of the method developed by Chandler et al. (2003) was proposed by Quarta et al. (2005, 2006). A multiplex PCR assay, based on primer pairs derived from the *TRI3*, *TRI5***,** and *TRI7* genes allowed to identify 3-ADON, 15-ADON and NIV among *F. graminearum, F. culmorum*, and *F. cerealis*. The assay was also validated on plant material (Quarta et al., 2006).

After confirming that the *TRI* set of genes coevolved independently in the three chemotypes, Ward et al. (2002), based on the finding that reciprocally monophyletic groups (corresponding to each of the B trichothecene chemotypes) were strongly supported (bootstrap scores _93%) in *TRI3*, *TRI11*, and *TRI12* gene trees, developed a method based on the polymorphism of two of these genes. *TRI3* and *TRI12* are at the

edges of the cluster and are well conserved within the chemotypes. The method proved to be effective on *F. culmorum*, *F cerealis* as well as on members of the FGSC (Starkey et al., 2007). The analysis of polymorphisms in two genes of the cluster improved the robustness of the assay.

The primers were further optimised for use in a Luminex® assay (Ward et al., 2008). The multiple gene analysis successfully detected discrepancies among *TRI3* and *TRI12* polymorphism in strains obtained from South Africa suggesting the presence of hybrid species (Boutigny et al., 2011). Similarly, new species were detected in Asia and Africa by combining the observation of TRI cluster recombination (O'Donnell et al., 2008; Yli-Mattila et al., 2009) with aspecific signals from species specific identification probes from the Luminex assay**.**The primer couple developed by Li et al. (2005) can discriminate DON and NIV (3-ADON and 15-ADON cannot be distinguished by this assay). The assay is based on the polymorphism found in the intergenic region between *TRI5* and *TRI6*, generating products of two sizes according to the chemotype. It can be coupled with other primers able to amplify a single chemotype as those used in Jennings et al. (2004a, b), that were derived from Chandler et al. (2003) allowing discrimination of the three chemotypes.

Another method based on different sizes of insertions in the *TRI13* gene was developed by Wang et al. (2008). The advantage of this method consists in the use of a single primer pair, being the detection of the three chemotypes linked to the presence of an insertion that is variable according to the chemotype. The method was shown to distinguish Chinese isolates of *F. graminearum* and *F. culmorum* strains.

An alternative method based on *TRI3/TRI6* polymorphism was developed by Suzuki et al. (2010) and aimed to differentiate simultaneously *F. asiaticum* and *F. graminearum* as well as their chemotypes. This approach proved successful for Japanese (Suzuki et al., 2010) and Chinese (Puri et al., 2012) isolates in a multiplexing approach.

More recently, also polymorphism in the *TRI11* gene were used for chemotyping, as reported by Zhang et al. (2010a) on *F. graminearum* isolates obtained from barley, and subsequently used by Talas et al. (2011, 2012a). Similarly, Wang et al. (2012) presented another multiplex assay based on the same gene.

A very promising approach is to develop qPCR multiplex detection and quantification of the chemotypes. This would in principle allow to directly screen grains for the abundance of each chemotype without further isolation of contaminating strains. A qPCR primer and TaqMan® probe set, based on *TRI12* polymorphism, was developed by Kulik (2011) to

allow chemotype quantification *in planta*. A SYBR® green method based on the same gene was used by Nielsen et al. (2012) to quantify chemotypes within a set of grains from Denmark, suggesting also potentialities for studying the evolution of isolate distribution in grain samples collected from historical seed collections. Both methods have been used on isolates sharing similar geographic origin, but potentially they can be used to quantify chemotypes in the field anywhere.

Table S1 summarises the methods for chemotype determination and lists primer set used.

Critical points in genetic chemotyping assays

It is important to underline that without a proper chemical identification of the different trichothecene B types, the genetic methods cannot be considered *per se* sufficient to determine precisely the ability to produce the toxin by any isolate. Indeed, a number of reports have integrated both chemical and genetic determination of the chemotype to overcome this issue.

As many researchers pointed out, the production of a toxin does not always correspond to a certain genetic chemotype (Tan et al., 2012). Sometimes various amounts of different toxins (such as NIV and DON) can be produced by the same isolate (Gilbert et al., 2001; Mugrabi de Kuppler et al., 2011). Moreover, several studies reported the co-production of acetylated forms by single strains, although in different relative amount (Alvarez et al., 2009; Christ et al., 2011; Kawakami et al., 2014; Korn et al., 2011; Mugrabi de Kuppler et al., 2011; Sugiura et al., 1990; Spolti et al., 2014b; Szécsi et al. 2005; Talas et al., 2012b; Ward et al. 2002; Yli-Mattila et al., 2009).

Llorens et al. (2006) reported also co-production by modifying temperature and growth conditions, confirming the role of temperature in influencing toxin production (Walker et al., 2001). Indeed, cultural and laboratory parameters may play a role in the quality of toxin produced (Llorens et al., 2004). At the same time also the crop influences the toxin found as in the case of potato, which transforms DON into NIV, probably by enzymatic activity (Delgado et al., 2011).

Nonetheless, chemical methods may have limitations related to the number of processable samples and the identification of optimal conditions for toxin production. If the methods are applied on single strains and not directly on the grains, similar problems as those described for genetic chemotype may occur, since toxin production *in vitro* is extremely variable and may not represent the real toxigenic potential of a given strain

(Malbrán et al., 2013; Mirocha et al, 1989; Müller and Schwadorf, 1993). Only field inoculation *in planta* can show the real toxigenic capacity of each strain (Gang et al., 1998). In fact, it has been reported that toxin production is strain-dependent as well as substrate-dependent in many *Fusarium* species (Vogelgsang et al., 2008a). Toxin production is often variable among isolates (Spolti et al., 2014b) and some strains do not produce any toxin under laboratory conditions (Tan et al., 2012). Therefore, research on the effects of substrates on the induction/repression of toxin (Gardiner et al., 2009a; Jiao et al., 2008; Kawakami et al., 2014; Pinson-Gadais et al., 2008; Ponts et al., 2006; Sakamoto et al., 2013; Suzuki et al., 2013; Tsuyuki et al., 2011) as well as on chemical and physical parameters (Gardiner et al., 2009b; Hope et al., 2005; Marin et al., 2010; Ryu and Bullerman, 1999; Schmidt-Heydt et al., 2011) are active areas of research.

By considering the history of application of the different genetic chemotyping methods, it is evident that while on average all methods did work quite efficiently, inevitably some failures or contradicting results were obtained by using different methods. For example, the methods developed by Lee et al. (2001) and Chandler et al. (2003), when used by Desjardins et al. (2008) showed a different level of reliability in assigning the chemotype to maize isolates from Nepal. Despite both primer sets were designed on the sequence of the *TRI13* gene (a pseudogene in DON producers) Lee's primers that are located closer to the insertion/deletion sites of degeneration failed to amplify due to the lower degree of stability of the region, thus generating potential false negatives.

Similarly, the *TRI13* length polymorphism method developed by Wang et al. (2008) provided inconsistent results in identifying the three chemotypes. The method was effective when applied to Chinese isolates (Wang et al., 2008) but failed to identify 15-ADON isolates of *F. graminearum sensu stricto* (*s.s.*) outside Asia (Pasquali et al., 2011) and were probably effective only on *F. asiaticum* (Amarasinghe et al., 2011), due to a difference in the size of insertion in the *TRI13* gene.

The method by Quarta et al. (2006) showed contrasting results with some Argentinian isolates giving DON/NIV chemotypes while producing only DON by chemical analysis (Reynoso et al., 2011) as well as with two isolates from barley and wheat from Italy (Quarta et al., 2006), and with some Polish strains (Stephien et al., 2008).

So far, the method developed by Ward et al. (2002) and further improved and implemented in a Luminex® system (Ward et al., 2008) proved very reliable in discriminating polymorphisms linked to chemotype and it is probably the most used worldwide on international collections of B-clade isolates. Alexander et al. (2011)

406 reported a misamplification for three *F. meridionale* isolates, suggesting that methods
407 targeting the functional domain generating the toxin may further improve the precision of
408 a PCR test linking effectively a certain mutation / polymorphism in a gene with its
409 function within the process of toxin biosynthesis. The recent identification of the TRI8
410 catalytic region as determinant of the 3-ADON generation (Alexander et al., 2011) may
411 be promising, since a genetic method based on functional domains would further
412 increase the reliability of the analysis. For a PCR assay to have broad utility, it must be
413 able to detect not only an allele that results in an inactivated protein, but all the alleles
414 resulting in an inactivated protein - an incredibly difficult task indeed (Reynoso et al.,
415 2011). Despite that, knowledge on the linkage relationships and evolutionary dynamics
416 involving selection on sets of genes at either end of the core trichothecence cluster
417 allows to confidently use the most used method so far which includes *TRI3-TRI12*
418 polymorphism and well as those based on *TRI3* and *TRI7* polymorphisms.It is evident
419 that a continuous monitoring using complementary methods is still needed (Nicholson et
420 al., 2004; Desjardin et al., 2008), therefore PCR validation of the method should rely on
421 chemical measures of toxin produced by each tested isolate.
422
423 **Surveys**
424 Chemotype studies worldwide have increased 20 times over the last 10 years. At
425 present, data on chemotype distribution of FGSC are available from all continents (see
426 Table 1 for details), being *F. graminearum s.s.* the most studied species. Given the lower
427 general importance of *F. culmorum* as primary cause of FHB, less work has been
428 devoted to chemotype determination in this species. However, since it was shown that
429 toxin translocation may occur from roots (Covarelli et al., 2012; Winter et al., 2013)
430 further attention on this species should be expected where environmental conditions
431 favour foot and root (crown) rot disease (Scherm et al., 2013). Chemotype determination
432 and population studies can therefore help in managing also crown rot diseases and their
433 associated toxins (Rebib et al., 2014).
434 Historically, since the pioneering studies by Mirocha et al. (1989) and by Miller et al.
435 (1991), it became apparent that a regional relationship could be sometimes postulated
436 between the geographic origin and the production of of NIV or 15-ADON or 3-ADON as
437 the major isomer (Bottalico and Perrone, 2002). This phenomenon can be due to
438 different species colonising the region as not all species are able to produce all
439 chemotypes (see Aoki et al., 2012 for a review in FGSG). For this reason a shift in

species could also be associated to a shift in toxin type (Astolfi et al., 2011; Audenaert et al., 2009; Beyer et al., 2014; Bottalico and Perrone, 2002; Malihipour et al., 2012; Pasquali et al., 2010; Yang et al., 2008; Yli-Mattila, 2010).

From an analysis of the chemotyping studies carried out since 2000, it is evident that the majority of reports were carried out on wheat while investigation on other cereals are more limited, although scattered information is available also for crops that are not notoriously target of trichothecene B contamination, such as asparagus, banana, etc..

Information from all continents are available but not all reports include complete information on the isolates analysed nor precise characterisation of the species that sometimes is based only on morphological observations or on the use of putatively species-specific primers, hence making it impossible to further using the dataset for comparison. It is desirable that a more coordinated effort, leading to common protocols for sampling, chemotype determining and data reporting in a more accessible way could facilitate the effort of understanding which factor do favour establishment and persistence of a certain chemotype.

The usefulness of genetic chemotyping studies was proven by the identification of novel groups and species. For instance, the NIV population reported in Louisiana by Gale et al. (2011) was then identified as a new species using multiple genotyping techniques (Sarver et al., 2011). Similarly, studies pinpointing inconsistencies among results of chemotyping on two *TRI* genes coupled with species-specific detection probes led to the identification of a new species in Ethiopia (O'Donnell et al., 2008).

Shift in species population has been reported in many surveys (Fredlund et al., 2013; Nielsen et al., 2011; Xu et al., 2005), but chemotype shift in certain areas is somewhat a novel report that would probably become more popular in the future (Beyer et al., 2014; Guo et al., 2008; Nielsen et al., 2012; Waalwijk et al., 2002; Ward et al., 2008). Analysing historical samples, Nielsen et al. (2012) showed that the presence of a certain chemotype is associated to historical periods. It is advisable that the availability of faster and more effective tools for chemotype determination would facilitate the identification of factors driving such shift.

For practical purposes, studying chemotype diversity for breeding against FHB susceptibility has been acknowledged (Gilbert et al., 2010; Gosman et al., 2010; He et al., 2013; Spolti et al., 2012; van der Ohe et al., 2010). In particular, testing local genetic diversity including toxin abilities in breeding programmes is considered essential to develop locally adapted varieties (Horevaj et al., 2011). Indeed, differences in the

474 characteristics of the pathogen (species/isolate) used in breeding programs (Warzecha
475 et al., 2010) may affect reaction of host genotypes, leading to erroneous results, and
476 therefore explaining different reactions to FHB in different geographical zones
477 (Malihipour et al., 2012).
478 Whether chemotype diversity needs to be assessed constantly is a matter of debate.
479 Studying the prevalence of 15-ADON and 3-ADON chemotype on barley, Clear et al.
480 (2013) found no strong effect on the chemotype prevalence as being determined by
481 resistance factors from the plant, suggesting on the contrary a potential role of micro-
482 environmental factors. While it is evident that highly resistant cultivars do not recognise
483 chemotype diversity as the pathogenic process is hindered (Foround et al., 2012),
484 breeding activities that will likely explore the susceptibility to toxin accumulation need to
485 consider also chemotype diversity as well as species interaction (Xu et al., 2007). Given
486 the challenge to toxin contamination posed by new evolving populations of *F.*
487 *graminearum* (Foroud et al., 2012), breeding programs should include a panel of isolates
488 and chemotypes to better cover natural variability of populations causing FHB.
489
490 **Future challenges**
491 By acknowledging the potential informativeness of chemotype diversity, the table
492 presented here should be considered with caution as it includes experiments carried out
493 with different rigour, different methods and using different techniques. It would be
494 therefore extremely valuable to generate a technically homogeneus and accessible map
495 of chemotypes where homogeneous data and methods could allow a true comparison of
496 the situation during years and among countries.
497 A small step towards improving research on chemotype diversity would be to include
498 these data in any fungal collection. Instruction on how the sampling was performed as
499 well as detailed information on cultural practices and location is indeed essential in order
500 to address questions such as which conditions are more favourable to a given
501 chemotype. Indeed, understanding which factor do play a role in chemotype prevalence
502 may help limiting the toxigenic risk associated to the spread of a certain chemotype.
503 Further research on the tools for chemotype determination is needed. The different
504 methods developed so far provide information that are at least partially discordant and
505 may fail to predict real toxin production by the strains. At the same time chemical
506 conditions simulating the behaviour of a strain in the field are not defined yet and do not
507 allow any preventive approach. Considering the need for preventive tools to limit

mycotoxin contamination in food and feed, it appears essential developing molecular tools that are able to predict toxin contamination at an early stage of infection in the field based on the chemotype spread over a certain area. At the same time, it would be important to identify conditions regulating toxin production in the field, by taking into account the environment/plant/pathogen interactions.

Paramount in a food safety perspective is the role that the host plant can play in transforming a toxin, hence determining a different level of toxin contamination. The ability of potato to transform DON into NIV (Delgado et al., 2010) due to enzymatic activity requires further attention. Similarly, the general issue of masked mycotoxins (Berthiller et al., 2013), which can be partially prevented by monitoring the producing microrganism and not the product itself or all its closely related metabolites in any sample, requires further understanding at both the toxicological and epidemiological viewpoints.

A larger coverage of crops and environments where *Fusarium* species may produce type B-trichothecenes is also needed in order to better monitor all potential toxigenic risks in food and feed. It has been emphasised that understanding host-specific differences in pathogen composition is crucial in the development of pathogen and mycotoxin control strategies, and could lead to novel approaches to achieve improved resistance in commercial cultivars (Boutigny et al., 2011).

Exploitation of molecular approaches to chemotype quantification such as those based on qPCR in grains may help guiding epidemiological studies and may lead to a better understanding of correlations between fungal populations and toxin production (Yli Mattila et al., 2008, 2009b). There is a need for rapid and cheap tools able to predict effectively toxin productivity in the field. Bakan et al. (2002) attempted to build a marker for isolates based on strain toxin productivity, linking polymorphism of a regulatory region to toxin production but no further validation of the method was carried out. As toxin production is subject to a series of regulation layers (Audenaert et al., 2013; Gardiner et al., 2009a, 2009b; Hope et al., 2005; Mereji et al., 2010; Reverberi et al., 2010; Seong et al., 2009) actual diagnostic methods based exclusively on DNA polymorphism can probably be used as a simple warning method for toxin risk. Despite mapping a population by genetic chemotyping would evidently be only an approximation of the field situation, it can result anyway in an effective monitoring of potential food threats.

Finally, the discovery of novel metabolites belonging to type B-trichothecene (Fruhmann et al., 2014) is also suggesting that genetic chemotyping determination requires

continuous monitoring of the markers used that need to be coupled with genetic research on diversity in order to develop novel and more precise markers for toxin prevention.

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References

1. Abedi-Tizaki, M., Sabbagh S., K., 2013. Detection of 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol and nivalenol-chemotypes of *Fusarium graminearum* from Iran using specific PCR assays. Plant Knowledge Journal 2, 38.
2. Abedi-Tizaki, M., Sabbagh S.K., Naeini, M.M., Sepehrikia, S., 2013b Chemotyping of *Fusarium graminearum* using *Tri13* trichothecene biosynthetic gene. Journal of Crop Protection 2, 487-500.
3. Abou Ammar, G., Tryono, R., Döll, K., Karlovsky, P., Deising, H.B., Wirsel, S.G.R., 2013. Identification of ABC transporter genes of *Fusarium graminearum* with roles in azole tolerance and/or virulence. PLoS ONE 8, e79042.
4. Adler, A., Lew, H., Brunner, S., Oberforster, J., Hinterholzer, J., Kullnig Gradinger, C.M., Mach, R.L., Kubicek, C.P., 2002. *Fusaria* in Austrian cereals – change in species and toxins spectrum. Journal of Applied Genetics 43 A, 11-16.
5. Adejumo, T.O., Hettwer, U., Karlovsky, P., 2007. Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. International Journal of Food Microbiology 116, 350–357.

574 6. Alexander, N.J., McCormick, S.P., Waalwijk, C., van der Lee, T., Proctor, R.H., 2011. The
575 genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. Fungal
576 Genetics and Biology 48, 485-495.

577 7. Alexander N.J., Proctor, R.H., McCormick, S.P., 2009. Genes, gene clusters, and
578 biosynthesis of trichothecenes and fumonisins in *Fusarium*. Toxins Reviews 28, 198-215.

579 8. Alkadri, D., Nipoti, P., Döll, K., Karlovsky, P., Prodi, A., Pisi, A., 2013. Study of fungal
580 colonization of wheat kernels in Syria with a focus on *Fusarium* Species. International
581 Journal of Molecular Sciences 14, 5938-5951.

582 9. Alvarez, C.L., Azcarate, M.P., Pinto, V.F., 2009. Toxigenic potential of *Fusarium*
583 *graminearum* sensu stricto isolates from wheat in Argentina. International Journal of
584 Food Microbiology 135, 131-135.

585 10. Alvarez, C.L., Somma, S., Moretti, A., Fernández Pinto, V., 2010. Aggressiveness of
586 *Fusarium graminearum sensu stricto* isolates in wheat kernels in Argentina. Journal of
587 Phytopathology 158, 173-181.

588 11. Amarasinghe, C.C., Tamburic-Ilincic, L., Gilbert, J., Brûlé-Babel, A.L., Dilantha Fernando,
589 W.G., 2013. Evaluation of different fungicides for control of fusarium head blight in wheat
590 inoculated with 3-ADON and 15-ADON chemotypes of Fusarium graminearum in
591 Canada. Canadian Journal of Plant Pathology 35, 200–208.

592 12. Amarasinghe, C., Wang, J.-H., Liao, Y.-C., Fernando, W.G.D., 2011. Difference in TRI13
593 Gene Sequences between the 3-Acetyldeoxynivalenol Producing *Fusarium*
594 *graminearum* Chemotypes from Canada and China. International Journal of Molecular
595 Sciences 12, 6164–6175.

596 13. Aoki, T., Ward, T.J., Kistler, H.C., O'Donnell, K., 2012. Systematics, phylogeny, and
597 trichothecene mycotoxin potential of Fusarium head blight cereal pathogen. Mycotoxins
598 62, 91-102.

599 14. Arunachalam, C., Doohan, F.M., 2013. Trichothecene toxicity in Eukaryotes: cellular and
600 molecular mechanisms in plants and animals. Toxicology Letters 217, 149-158.

601 15. Astolfi, P., dos Santos, J., Schneider, L., Gomes, L.B., Silva, C.N., Tessmann, D.J., Del
602 Ponte, E.M., 2011. Molecular survey of trichothecene genotypes of *Fusarium*
603 *graminearum* species complex from barley in southern Brazil. International Journal Food
604 Microbiology 148, 197–201.

605 16. Astolfi, P., Reynoso, M.M., Ramirez, M.L., Chulze, S.N., Alves, T.C.A., Tessmann, D.J.,
606 Del Ponte, E.M., 2012. Genetic population structure and trichothecene genotypes of

607 *Fusarium graminearum* isolated from wheat in southern Brazil. Plant Pathology 61, 289–
608 295.

609 17. Audenaert, K., Van Broeck, R., Bekaert, B., De Witte, F., Heremans, B., Messens, K.,
610 Höfte, M., Haesaert, G., 2009. Fusarium head blight (FHB) in Flanders: population
611 diversity, inter-species associations and DON contamination in commercial winter wheat
612 varieties. European Journal of Plant Pathology 125, 445–458.

613 18. Audenaert, K., Vanheule, A., Höfte, M., Haesaert, G., 2013. Deoxynivalenol: a major
614 player in the multifaceted response of *Fusarium* to its environment. Toxins 6, 1–19.

615 19. Bakan, B., Pinson, L., Cahagnier, B., Melcion, D., Sémon, E., Richard-Molard, D., 2001.
616 Toxigenic potential of *Fusarium culmorum* strains isolated from French wheat. Food
617 Additives and Contaminants 18, 998–1003.

618 20. Bakan, B., Giraud-Delville, C., Pinson, L., Richard-Molard, D., Fournier, E., Brygoo, Y.,
619 2002. Identification by PCR of *Fusarium culmorum* strains producing large and small
620 amounts of deoxynivalenol. Applied and Environmental Microbiology 68, 5472-5479.

621 21. Barros, G., Zanon, M.S.A., Abod, A., Oviedo, M.S., Ramirez, M.L., Reynoso, M.M.,
622 Torres, A., Chulze, S., 2012. Natural deoxynivalenol occurrence and genotype and
623 chemotype determination of a field population of the *Fusarium graminearum* complex
624 associated with soybean in Argentina. Food Additives & Contaminants Part A 29, 293–
625 303.

626 22. Baturo-Ciesniewska, A., Suchorzynska, M., 2011. Verification of the effectiveness of
627 SCAR (sequence characterized amplified region) primers for the identification of Polish
628 strains of *Fusarium culmorum* and their potential ability to produce B-trichothecenes and
629 zearalenone. International Journal of Food Microbiology 148, 168–176.

630 23. Ben Amar, A., Oueslati, S., Ghorbel, A., Mliki, A., 2012. Prediction and early detection of
631 mycotoxigenic *Fusarium culmorum* in wheat by direct PCR-based procedure. Food
632 Control 23, 506–510.

633 24. Beyer, M., Pogoda, F., Pallez, M., Lazic, J., Hoffmann, L., Pasquali, M., 2014. Evidence
634 for a reversible drought induced shift in the species composition of mycotoxin producing
635 Fusarium head blight pathogens isolated from symptomatic wheat heads. International
636 Journal of Food Microbiology, 182-183, 51-56.

637 25. Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S.D., Haesaert, G., Karlovsky, P., Oswald,
638 I.P., Seefelder, W., Speijers, G., Stroka, J., 2013. Masked mycotoxins: a review.
639 Molecular Nutrition and Food Research 57, 165–186.

640 26. Blaney, B.J., Dodman, R.L. 2002. Production of zearalenone, deoxynivalenol, nivalenol,
641 and acetylated derivatives by Australian isolates of *Fusarium graminearum* and *F.*
642 *pseudograminearum* in relation to source and culturing conditions. Australian Journal of
643 Agricultural Research 53, 1317-1326.

644 27. Bosch, U., Mirocha, C.J., 1992. Toxin production by Fusarium species from sugar beets
645 and natural occurrence of zearalenone in beets and beet fibers. Applied and
646 Environmental Microbiology 58, 3233–3239.

647 28. Bottalico, A., Perrone, G., 2002. Toxigenic *Fusarium* species and mycotoxins associated
648 with head blight in small-grain cereals in Europe. European Journal of Plant Pathology
649 108, 611-624.

650 29. Boutigny, A.L., Atanasova-Pénichon, V., Benet, M., Barreau, C., Richard-Forget, F.,
651 2010. Natural phenolic acids from wheat bran inhibit *Fusarium culmorum* trichothecene
652 biosynthesis *in vitro* by repressing *Tri* gene expression. European Journal of Plant
653 Pathology 127, 275–286.

654 30. Boutigny, A.L., Barreau, C., Atanasova-Penichon, V., Verdal-Bonnin, M.-N., Pinson-
655 Gadais, L., Richard-Forget, F., 2009. Ferulic acid, an efficient inhibitor of type B
656 trichothecene biosynthesis and *Tri* gene expression in *Fusarium* liquid cultures.
657 Mycological Research 113, 746-753.

658 31. Boutigny, A.L., Ward, T.J., Ballois, N., Iancu, G., Ioos, R., 2014. Diversity of the *Fusarium*
659 *graminearum* species complex on French cereals. European Journal of Plant Pathology
660 138, 133–148.

661 32. Boutigny, A.L., Ward, T.J., Van Coller, G.J., Flett, B., Lamprecht, S.C., O'Donnell, K.,
662 Viljoen, A., 2011. Analysis of the *Fusarium graminearum* species complex from wheat,
663 barley, and maize in South Africa provides evidence of species-specific differences in
664 host preference. Fungal Genetics and Biology 48, 914–920.

665 33. Brown, D.W., Dyer, R.B., McCormick, S.P., Kendra, D.F., Plattner, R.D., 2004. Functional
666 demarcation of the *Fusarium* core trichothecene gene cluster. Fungal Genetics and
667 Biology 41, 454-462.

668 34. Brown, D.W., McCormick, S.P., Alexander, N.J., Proctor, R.H., Desjardins, A.E., 2002.
669 Inactivation of a cytochrome P-450 is a determinant of trichothecene diversity in
670 *Fusarium* species. Fungal Genetics and Biology 36, 224–233.

671 35. Burlakoti, R.R., Ali S., Secor, G.A., Neate, S.M., McMullen, M.P., Adhikari, T.B., 2008a.
672 Comparative mycotoxin profiles of *Gibberella zeae* populations from barley, wheat,
673 potatoes, and sugar beets. Applied and Environmental Microbiology 74, 6513-6520.

674 36. Burlakoti, R.R., Ali S., Secor, G.A., Neate, S.M., McMullen, M.P., Adhikari, T.B., 2008b.
675 Genetic relationships among populations of *Gibberella zeae* from barley, wheat, potato,
676 and sugar beet in the upper Midwest of the United States. Phytopathology 98, 969-976.

677 37. Burlakoti, R.R., Neate, S.M., Adhikari, T.B., Gyawali, S., Salas, B., Steffenson, B.J.,
678 Schwarz, P.B., 2011. Trichothecene profiling and population genetic analysis of
679 *Gibberella zeae* from barley in North Dakota and Minnesota. Phytopathology 101, 687–
680 695.

681 38. Burlakoti, R.R., Rolando Estrada, V., Rivera-Varas, V., Boddeda, A., Secor, G.A. Adhikari,
682 T.B., 2007. Real-time PCR quantification and mycotoxin production of *Fusarium*
683 *graminearum* in wheat inoculated with isolates collected from potato, sugar beet, and
684 wheat. Phytopathology 97, 835-841.

685 39. Carter, J.P., Rezanoor, H.N., Holden, D., Desjardins, A.E., Plattner, R.D., Nicholson, P.,
686 2002. Variation in pathogenicity associated with the genetic diversity of *Fusarium*
687 *graminearum*. European Journal of Plant Pathology 108, 573-583.

688 40. Castañares, E., Dinolfo, M.I., Moreno, M.V., Berón, C., Stenglein, S.A., 2013. *Fusarium*
689 *cerealis* associated with barley seeds in Argentina. Journal of Phytopathology 161, 586–
690 589.

691 41. Castañares, E., Albuquerque, D.R., Dinolfo, M.I., Pinto, V.F., Patriarca, A., Stenglein,
692 S.A., 2014. Trichothecene genotypes and production profiles of *Fusarium graminearum*
693 isolates obtained from barley cultivated in Argentina. International Journal of Food
694 Microbiology,179, 57-63.

695 42. Chandler, E.A., Simpson, D.R., Thomsett, M.A., Nicholson, P., 2003. Development of
696 PCR assays to *Tri7* and *Tri13* trichothecene biosynthetic genes, and characterisation of
697 chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*.
698 Physiological and Molecular Plant Pathology 62, 355-367.

699 43. Chelkowski, J., Gromadzka, K., Stepien, L., Lenc, L., Kostecki, M., Berthiller, F., 2012.
700 Fusarium species, zearalenone and deoxynivalenol content in preharvest scabby wheat
701 heads from Poland. World Mycotoxin Journal 5, 133–141.

702 44. Christ, D.S., Märländer, B., Varrelmann, M., 2011. Characterization and mycotoxigenic
703 potential of *Fusarium* species in freshly harvested and stored sugar beet in Europe.
704 Phytopathology 101, 1330–1337.

705 45. Cigić, I.K., Prosen, H., 2009. An overview of conventional and emerging analytical
706 methods for the determination of mycotoxins. International Journal of Molecular
707 Sciences 10, 62-115.

708 46. Clear, R.M., Tucker, J.R., Gaba, D., Patrick, S.K., Lee, S.J., Demeke, T., Tittlemier, S.A.,
709 Legge, W.G., Gräfenhan, T., 2013. Deoxynivalenol levels and chemotype frequency in
710 barley cultivars inoculated with two chemotypes of *Fusarium graminearum*. Canadian
711 Journal of Plant Pathology 35, 37–45.

712 47. Clear, R.M., Patrick, S.K., Gaba, D., 2000a. Prevalence of fungi and fusariotoxins on
713 barley seed from western Canada, 1995 to 1997. Canadian Journal of Plant Pathology
714 22, 44-50.

715 48. Clear, R.M., Patrick, S.K., Gaba, D., 2000b. Prevalence of fungi and fusariotoxins on oat
716 seed from western Canada, 1995 to 1997. Canadian Journal of Plant Pathology 22,
717 310-314.

718 49. Clear, R.M., Patrick, S.K., Gaba, D., Roscoe, M., Turkington, T.K., Demeke, T., Pouleur,
719 S., Couture, L., Ward, T.J., O'Donnell, K., 2006. Trichothecene and zearalenone
720 production, in culture, by isolates of *Fusarium pseudograminearum* from western
721 Canada. Canadian Journal of Plant Pathology 28, 131–136.

722 50. Cornea, C., Israel-Roming, F., Ciuca, M., Voaides, C., 2013. Natural occurrence of
723 *Fusarium* species and corresponding chemotypes in wheat scab complex from Romania.
724 Romanian Biotechnological Letters 18, 8787-8795.

725 51. Covarelli, L., Beccari, G., Steed, A., Nicholson, P., 2012. Colonization of soft wheat
726 following infection of the stem base by *Fusarium culmorum* and translocation of
727 deoxynivalenol to the head. Plant Pathology 61, 1121-1129.

728 52. Davari, M., Safaie, N., Darvishnia, M., Taleshmikaeel, R.D., 2014. Occurrence of
729 deoxynivalenol producing isolates of *Fusarium graminearum* species complex
730 associated with Head Blight of wheat in Moghan area. Journal of Crop Protection 3, 113-
731 123.

732 53. Davari, M., Wei, S.H., Babay-Ahari, A., Arzanlou, M., Waalwijk, C., van der Lee, T.A.J.,
733 Zare, R., Gerrits van den Ende, A.H.G., de Hoog, G.S., van Diepeningen, A.D., 2013.
734 Geographic differences in trichothecene chemotypes of *Fusarium graminearum* in the
735 Northwest and North of Iran. World Mycotoxin Journal 6, 137-150.

736 54. Delgado, J.A., Schwarz, P.B., Gillespie, J., Rivera-Varas, V., Secor. G.A., 2010.
737 Trichothecene mycotoxins associated with potato dry rot caused by *Fusarium*
738 *graminearum*. Phytopathology 100, 290-296.

739 55. Del Ponte, E.M., Garda-Buffon, J., Badiale-Furlong, E., 2012. Deoxynivalenol and
740 nivalenol in commercial wheat grain related to Fusarium head blight epidemics in
741 southern Brazil. Food Chemistry 132, 1087–1091.

742 56. Desjardins, A.E. 2008. Natural product chemistry meets genetics: when is a genotype a
743 chemotype? Journal of Agricultural and Food Chemistry 56, 7587-7592.

744 57. Desjardins, A.E., 2009. From yellow rain to green wheat: 25 years of trichothecene
745 biosynthesis research. Journal of Agricultural and Food Chemistry 57, 4478–4484.

746 58. Desjardins, A.E., Busman, M., Manandhar, G., Jarosz, A.M., Manandhar, H.K., Proctor,
747 R.H., 2008. *Gibberella* Ear Rot of maize (*Zea mays*) in Nepal: distribution of the
748 mycotoxins nivalenol and deoxynivalenol in naturally and experimentally infected maize.
749 Journal of Agricultural and Food Chemistry 56, 5428-5436.

750 59. Desjardins, A.E., Jarosz, A.M., Plattner, R.D., Alexander, N.J., Brown, D.W., Jurgenson,
751 J.E., 2004. Patterns of trichothecene production, genetic variability, and virulence to
752 wheat of *Fusarium graminearum* from smallholder farms in Nepal. Journal of Agricultural
753 and Food Chemistry 52, 6341-6346.

754 60. Desjardins, A.E., Manandhar, G., Plattner, R.D., Maragos, C.M., Shrestha, K.,
755 McCormick, S.P., 2000. Occurrence of *Fusarium* species and mycotoxins in Nepalese
756 maize and wheat and the effect of traditional processing methods on mycotoxin levels.
757 Journal of Agricultural and Food Chemistry 48, 1377-1383.

758 61. Desjardins, A.E., Proctor, R.H., 2011. Genetic diversity and trichothecene chemotypes of
759 the *Fusarium graminearum* clade isolated from maize in Nepal and identification of a
760 putative new lineage. Fungal Biology 115, 38–48.

761 62. Desmond, O.J., Manners, J.M., Stephens, A.E., Maclean, D.J., Schenk, P.M., Gardiner,
762 D.M., Munn, A.L., Kazan, K., 2008. The *Fusarium* mycotoxin deoxynivalenol elicits
763 hydrogen peroxide production, programmed cell death and defence responses in wheat.
764 Molecular Plant Pathology 9, 435-445.

765 63. Dubos, T., Pasquali, M., Pogoda, F., Casanova, A., Hoffmann, L., Beyer, M., 2013.
766 Differences between the succinate dehydrogenase sequences of isopyrazam sensitive
767 *Zymoseptoria tritici* and insensitive *Fusarium graminearum* strains. Pesticide
768 Biochemistry and Physiology 105, 28–35.

769 64. Dubos, T., Pasquali, M., Pogoda, F., Hoffmann, L., Beyer, M., 2011. Evidence for natural
770 resistance towards trifloxystrobin in *Fusarium graminearum*. European Journal of Plant
771 Pathology 130, 239–248.

772 65. Edwards, S.G., 2009. Fusarium mycotoxin content of UK organic and conventional
773 barley. Food Additives & Contaminants Part A 26 8, 1185-1190.

774 66. Ellis, M.L., Munkvold, G.P., 2014. Trichothecene genotype of *Fusarium graminearum*
775 isolates from soybean (*Glycine max*) seedling and root diseases in the United States.
776 Plant Disease 98, 1012.

777 67. Faifer, G.C., Sala De Miguel, M., Godoy, H.M., 1990. Patterns of mycotoxin production
778 by *Fusarium graminearum* isolated from argentine wheat. Mycopathologia 109, 165-170.

779 68. Fernandez Pinto, V. E., Terminiello, L.A., Basilico, J.C., Ritieni, A. 2008. Natural
780 occurrence of nivalenol and mycotoxigenic potential of *Fusarium graminearum* strains in
781 wheat affected by head blight in Argentina. Brazilian Journal of Microbiology 39, 157-
782 162.

783 69. Foroud, N.A., McCormick, S.P., MacMillan, T., Badea, A., Kendra, D.F., Ellis, B.E.,
784 Eudes, F., 2012. Greenhouse studies reveal increased aggressiveness of emergent
785 Canadian *Fusarium graminearum* chemotypes in wheat. Plant Disease 96, 1271-1279.

786 70. Fredlund, E., Gidlund, A., Sulyok, M., Börjesson, T., Krska, R., Olsen, M., Lindblad, M.,
787 2013. Deoxynivalenol and other selected *Fusarium* toxins in Swedish oats - occurrence
788 and correlation to specific *Fusarium* species. International Journal of Food Microbiology
789 167, 276–283.

790 71. Fruhmann, P., Mikula, H., Wiesenberger, G., Varga, E., Lumpi, D., Stöger, B., Häubl, G.,
791 Lemmens, M., Berthiller, F., Krska, R., Adam, G., Hametner, C., Fröhlich, J., 2014.
792 Isolation and structure elucidation of pentahydroxyscirpene, a trichothecene *Fusarium*
793 mycotoxin. Journal of Natural Products 77, 188–192.

794 72. Gale, L.R., Harrison, S.A., Ward, T.J., O'Donnell, K., Milus, E.A., Gale, S.W., Kistler,
795 H.C., 2011. Nivalenol-type populations of *Fusarium graminearum* and *F. asiaticum* are
796 prevalent on wheat in southern Louisiana. Phytopathology 101, 124–134.

797 73. Gale, L.R., Ward, T.J., Balmas, V., Kistler, H.C., 2007. Population subdivision of
798 *Fusarium graminearum* sensu stricto in the Upper Midwestern United States.
799 Phytopathology 97, 1434-1439.

800 74. Gang, G., Miedaner, T., Schuhmacher, U., Schollenberger, M., Geiger, H.H., 1998.
801 Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in
802 aggressiveness toward winter rye. Phytopathology 88, 879–884.

803 75. Gardiner, D.M., Kazan, K., Manners, J.M., 2009a. Nutrient profiling reveals potent
804 inducers of trichothecene biosynthesis in *Fusarium graminearum*. Fungal Genetics and
805 Biology 8, 604-613.

76. Gardiner, D.M., Osborne, S., Kazan, K., Manners, J.M., 2009b. Low pH regulates the production of deoxynivalenol by *Fusarium graminearum*. Microbiology 155, 3149-3156.

77. 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.

78. Gilbert, J., Brûlé-Babel, A., Guerrieri, A.T., Clear, R.M., Patrick, S., Slusarenko, K., Wolfe, C., 2014. Ratio of 3-ADON and 15-ADON isolates of *Fusarium graminearum* recovered from wheat kernels in Manitoba from 2008 to 2012. Canadian Journal of Plant Pathology, 36, 54-63.

79. Gilbert, J., Clear, R.M., Patrick, S., Slusarenko, K., Wolfe, C., (2011). Ratio of 3-ADON and 15-ADON isolates of *Fusarium graminearum* recovered from wheat plants inoculated and incubated at various temperatures. Proceedings of the Plant Canada Meeting (p. 172). Halifax, Nova Scotia, July 17–21, 2011. Saint Mary's University, Halifax, Nova Scotia.

80. Gilbert, J., Clear, R.M., Ward, T.J., Gaba, D., Tekauz, A., Turkington, T.K., Woods, S.M., Nowicki, T., O Donnell, K., 2010. Relative aggressiveness and production of 3- or 15-acetyl deoxynivalenol and deoxynivalenol by *Fusarium graminearum* in spring wheat. Canadian Journal of Plant Pathology 32, 146-152.

81. Giraud, F., Pasquali, M., El Jarroudi, M., Vrancken, C., Brochot, C., Cocco, E., Hoffmann, L., Delfosse, P., Bohn, T., 2010. Fusarium head blight and associated mycotoxin occurrence on winter wheat in Luxembourg in 2007/2008. Food Additives & Contaminants Part A 27, 825-835.

82. Goertz, A., Zuehlke, S., Spiteller, M., Steiner, U., Dehne, H.W., Waalwijk, C., Vries, I., Oerke, E.C., 2010. *Fusarium* species and mycotoxin profiles on commercial maize hybrids in Germany. European Journal of Plant Pathology 128, 101-111.

83. González, H.H.L., Moltó, G.A., Pacin, A., Resnik, S.L., Zelaya, M.J., Masana, M., Martínez, E.J., 2008. Trichothecenes and mycoflora in wheat harvested in nine locations in Buenos Aires province, Argentina. Mycopathologia 165, 105-114.

84. Gosman, N., Srinivasachary, Steed, A., Chandler, E., Thomsett, M. and Nicholson, P., 2010. Evaluation of type I fusarium head blight resistance of wheat using non-deoxynivalenol-producing fungi. Plant Pathology 59, 147–157.

85. Goswami, R.S., Kistler, H.C., 2005. Pathogenicity and *in planta* mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology 95, 1397-1404.

840 86. Guo, X.W., Fernando, W.G.D., Seow-Brock, H.Y., 2008. Population structure, chemotype
841 diversity, and potential chemotype shifting of *Fusarium graminearum* in wheat fields of
842 Manitoba. Plant Disease 92, 756-762.

843 87. Haratian, M., Sharifnabi, B., Alizadeh, A., Safaie, N., 2008. PCR analysis of the *Tri13*
844 gene to determine the genetic potential of *Fusarium graminearum* isolates from Iran to
845 produce nivalenol and deoxynivalenol. Mycopathologia 166, 109-116.

846 88. He, X., Singh, P.K., Duveiller, E., Schlang, N., Dreisigacker, S., Singh, R.P., 2013.
847 Identification and characterization of international Fusarium Head Blight screening
848 nurseries of wheat at CIMMYT, Mexico. European Journal of Plant Pathology 136, 123-
849 134.

850 89. Hestbjerg, H., Felding, G., Elmholt, S., 2002. *Fusarium culmorum* infection of barley
851 seedlings: correlation between aggressiveness and deoxynivalenol content. Journal of
852 Phytopathology 150, 308-312.

853 90. Hope, R., Aldred, D., Magan, N., 2005. Comparison of environmental profiles for growth
854 and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat
855 grain. Letters in Applied Microbiology 40, 295–300.

856 91. Horevaj, P., Gale, L.R., Milus, E.A., 2011. Resistance in winter wheat lines to initial
857 infection and spread within spikes by deoxynivalenol and nivalenol chemotypes of
858 *Fusarium graminearum*. Plant Disease 95, 31–37.

859 92. Ilgen, P., Hadeler, B., Maier, F.J.,Schäfer, W., 2009. Developing kernel and rachis node
860 induce the trichothecene pathway of *Fusarium graminearum* during wheat head
861 infection. Molecular Plant-Microbe Interactions 22, 899-908.

862 93. Jennings, P., Coates, M.E., Turner, J.A., Chandler, E.A., Nicholson, P., 2004.
863 Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium culmorum*
864 isolates from England and Wales by PCR assay. Plant Pathology 53, 182-190.

865 94. Jennings, P., Coates, M.E., Walsh, K.,Turner, J.A., Nicholson, P., 2004. Determination of
866 deoxynivalenol- and nivalenol-producing chemotypes of *Fusarium graminearum* isolated
867 from wheat crops in England and Wales. Plant Pathology 53, 643-652.

868 95. Jestoi, M.N., Paavanen-Huhtala, S., Parikka, P., Yli-Mattila, T., 2008. *In vitro* and *in vivo*
869 mycotoxin production of *Fusarium* species isolated from Finnish grains. Archives of
870 Phytopathology and Plant Protection 41, 545-558.

871 96. Ji, L., Cao, K., Hu, T., Wang, S., 2007. Determination of deoxynivalenol and nivalenol
872 chemotypes of *Fusarium graminearum* isolates from china by PCR assay. Journal of
873 Phytopathology 155, 505-512.

874 97. Jiao, F., Kawakami, A., Nakajima, T., 2008. Effects of different carbon sources on
875 trichothecene production and *Tri* gene expression by *Fusarium graminearum* in liquid
876 culture. FEMS Microbiology Letters 285, 212-219.

877 98. Josephs, R.D, Derbyshire, M., Stroka, J., Emons, H., Anklam, E., 2004. Trichothecenes:
878 reference materials and method validation. Toxicology Letters 153, 123-132.

879 99. Kammoun, L.G., Gargouri, S., Barreau, C., Richard-Forget, F., Rabeh Hajlaoui, M., 2010.
880 Trichothecene chemotypes of *Fusarium culmorum* infecting wheat in Tunisia.
881 International Journal of Food Microbiology 140, 84-89.

882 100. Karugia, G.W., Suga, H., Gale, L.R., Nakajima, T., Tomimura, K., Hyakumachi,
883 M., 2009a. Population structure of the *Fusarium graminearum* species complex from a
884 single Japanese wheat field sampled in two consecutive years. Plant Disease 93, 170-
885 174.

886 101. Karugia, G.W., Suga, H., Gale, L.R., Nakajima, T., Ueda, A., Hyakumachi, M.,
887 2009b. Population structure of *Fusarium asiaticum* from two Japanese regions and
888 eastern China. Journal of General Plant Pathology 75, 110–118.

889 102. Kawakami, A., Nakajima, T., Hirayae, K., 2014. Effects of carbon sources and
890 amines on induction of trichothecene production by *Fusarium asiaticum* in liquid culture.
891 FEMS Microbiology Letters, 352, 204-212.

892 103. Kim, J.C., Kang, H.J., Lee, D.H., Lee, Y.W., Yoshizawa, T., 1993. Natural
893 occurrence of *Fusarium* mycotoxins (trichothecenes and zearalenone) in barley and corn
894 in Korea. Applied and Environmental Microbiology 59, 3798-3802.

895 104. Kim, H.S, Lee, T., Dawlatana, M., Yun, S.H., Lee, Y.W., 2003. Polymorphism of
896 trichothecene biosynthesis genes in deoxynivalenol- and nivalenol-producing *Fusarium*
897 *graminearum* isolates. Mycological Research 107, 190-197.

898 105. Kimura, M., Tokai, T., O'Donnell, K., Ward, T.J., Fujimura, M., Hamamoto, H.,
899 Shibata, T., Yamaguchi, I., 2003. The trichothecene biosynthesis gene cluster of
900 *Fusarium graminearum* F15 contains a limited number of essential pathway genes and
901 expressed non-essential genes. FEBS Letters 539, 105-110.

902 106. Koch, P., 2004. State of the art of trichothecenes analysis. Toxicology Letters
903 153, 109-112.

904 107. Konkz, Z., Naár, Z., Kiss, A., Szécsi, Á., 2008. Determination of the trichothecene
905 mycotoxin chemotypes of *Fusarium graminearum* isolates from Hungary by PCR assay.
906 Cereal research Communications 36S6, 637-639.

907 108. Köppen, R., Koch, M., Siegel, D., Merkel, S., Maul, R., Nehls, I., 2010.
908 Determination of mycotoxins in foods: current state of analytical methods and limitations.
909 Applied Microbiology and Biotechnology 86, 1595-1612.

910 109. Korn, U., Müller, T., Ulrich, A., Müller, M.E.H., 2011. Impact of aggressiveness of
911 *Fusarium graminearum* and *F. culmorum* isolates on yield parameters and mycotoxin
912 production in wheat. Mycotoxin Research 27, 195–206.

913 110. Kosiak, E.B., Holst-Jensen, A., Rundberget, T., Gonzalez Jaen, M.T., Torp, M.,
914 2005. Morphological, chemical and molecular differentiation of *Fusarium equiseti*
915 isolated from Norwegian cereals. International Journal of Food Microbiology 99, 195–
916 206.

917 111. Krishna K.D., Yan C., Zhong S., 2012. RNA-seq revealed gene expression
918 differences between 3ADON and 15ADON populations of *F. graminearum in vitro* and *in*
919 *planta*. National Fusarium Head Blight Forum, p. 121-122.

920 112. Kristensen, R., Torp, M., Kosiak, B., Holst-Jensen, A., 2005. Phylogeny and
921 toxigenic potential is correlated in Fusarium species as revealed by partial translation
922 elongation factor 1 alpha gene sequences. Mycological Research 109, 173–186.

923 113. Krska, R., Baumgartner, S., Josephs, R., 2001. The state-of-the-art in the
924 analysis of type-A and -B trichothecene mycotoxins in cereals. Fresenius' Journal of
925 Analytical Chemistry 371, 285-299.

926 114. Kulik, T., 2011. Development of TaqMan assays for 3ADON, 15-ADON and NIV
927 Fusarium genotypes based on *Tri12* gene. Cereal Research Communications 39, 200-
928 214.

929 115. Kulik, T., Buśko, M., Pszczółkowska, A., Perkowski, J., Okorski, A., 2014. Plant
930 lignans inhibit growth and trichothecene biosynthesis in *Fusarium graminearum*. Letters
931 in Applied Microbiology 59, 99-107.

932 116. Kulik, T., Lojko, M., Jestoi, M., Perkowski, J., 2012. Sublethal concentrations of
933 azoles induce *Tri* transcript levels and trichothecene production in *Fusarium*
934 *graminearum*. FEMS Microbiology Letters 335, 58-67.

935 117. Irzykowska, L., Bocianowski, J., Baturo-Cieśniewska, A., 2013. Association of
936 mating-type with mycelium growth rate and genetic variability of *Fusarium culmorum*.
937 Central European Journal of Biology 8, 701-711.

938 118. Langseth, W., Ghebremeskel, M., Kosiak, B., Kolsaker, P., Miller, D., 2000.
939 Production of culmorin compounds and other secondary metabolites by *Fusarium*

940 *culmorum* and *F. graminearum* strains isolated from norwegian cereals. Mycopathologia
941 152, 23-34.

942 119. Lee, J., Chang, I.Y., Kim, H., Yun, S.H., Leslie, J.F., Lee, Y.W., 2009. Genetic
943 diversity and fitness of *Fusarium graminearum* populations from rice in Korea. Applied
944 and Environmental Microbiology 75, 3289-3295.

945 120. Lee, J., Kim, H., Jeon, J.J., Kim, H.-S., Zeller, K.A., Carter, L.L.A., Leslie, J.F.,
946 Lee, Y.W., 2012. Population structure of and mycotoxin production by *Fusarium*
947 *graminearum* from maize in South Korea. Applied and Environmental Microbiology 78,
948 2161–2167.

949 121. Lee, T., Han, Y.K, Kim, K.H., Yun, S.-H., Lee, Y.W., 2002. *Tri13* and *tri7* determine
950 deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Applied and
951 Environmental Microbiology 68, 2148-2154.

952 122. Lee, T., Oh, D.W., Kim, H.S., Lee, J., Kim,Y.H., Yun, S.H., Lee, Y.W., 2001.
953 Identification of deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*
954 by using PCR. Applied and Environmental Microbiology 67, 2966-2972.

955 123. Lenart, A.M., Klimek-Kopyra, A., Boroń, P.M., 2013. Morphological and molecular
956 identification and PCR amplification to determine the toxigenic potential of *Fusarium spp.*
957 isolated from maize ears in southern Poland. Phytoparasitica 41, 241–248.

958 124. Li, H.P., Wu, A.-B., Zhao, C.-S., Scholten, O., Löffler, H., Liao, Y.C., 2005.
959 Development of a generic PCR detection of deoxynivalenol- and nivalenol-chemotypes
960 of *Fusarium graminearum*. FEMS Microbiology Letters 243, 505-511.

961 125. Llorens, A., Hinojo, M.J., Mateo, R., Gonzalez-Jaen, M.T., Valle-Algarra, F.M.,
962 Logrieco, A., Jimenez, M., 2006. Characterization of *Fusarium* spp. isolates by PCR-
963 RFLP analysis of the intergenic spacer region of the rRNA gene (rDNA). International
964 Journal of Food Microbiology 106, 297–306.

965 126. Llorens, A., Mateo, R., Hinojo, M.J., Valle-Algarra, F.M., Jiménez, M., 2004.
966 Influence of environmental factors on the biosynthesis of type B trichothecenes by
967 isolates of *Fusarium* spp. from spanish crops. International Journal of Food Microbiology
968 94, 43-54.

969 127. Ludewig, A., Kabsch, U., Verreet, J.A., 2005. Mycotoxin production of 31
970 *Fusarium graminearum* isolates and aggressiveness against wheat seedlings. Zeitschrift
971 für Pflanzenkrankheiten und Pflanzenschutz 112, 124-133.

972 128. Maier, F.J., Miedaner, T., Hadeler, B., Felk, A., Salomon, S., Lemmens, M.,
973 Kassner, H., Schäfer, W., 2006. Involvement of trichothecenes in fusarioses of wheat,

barley and maize evaluated by gene disruption of the trichodiene synthase (*Tri5*) gene in three field isolates of different chemotype and virulence. Molecular Plant Pathology 7, 449-461.

129. Malbrán, I., Mourelos, C.A., Girotti, J.R., Balatti, P.A., Lori, G.A., 2014. Toxigenic capacity and trichothecene production by *Fusarium graminearum* isolates from Argentina and their relationship with aggressiveness and fungal expansion in the wheat spike. Phytopathology 104, 357-364.

130. 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 Disease 96, 1016-1025.

131. Maragos, C.M., Busman, M., 2010. Rapid and advanced tools for mycotoxin analysis: a review. Food Additives & Contaminants: Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 27, 688-700.

132. Maresca, M., 2013. From the gut to the brain: journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. Toxins 5, 784-820.

133. Marin, P., Jurado, M., Magan, N., Vazquez, C., Gonzalez-Jaen, M.T., (2010) Effect of solute stress and temperature on growth rate and *TRI5* gene expression using real time RT-PCR in *Fusarium graminearum* from Spanish wheat. International Journal of Food Microbiology 140, 169-174.

134. Medina, A., Valle-Algarra, F.M., Mateo, R., Gimeno-Adelantado, J.V., Mateo, F., Jiménez, M., 2006. Survey of the mycobiota of Spanish malting barley and evaluation of the mycotoxin producing potential of species of *Alternaria*, *Aspergillus* and *Fusarium*. International Journal of Food Microbiology 108, 196-203.

135. Merhej, J., Boutigny, A.L., 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 Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 27, 710-717.

136. Mert-Türk, F., Gencer, R., 2013. Distribution of the 3-AcDON, 15-AcDON, and NIV chemotypes of *Fusarium culmorum* in the North-West of Turkey. Plant Protection Science 49, 57–64.

137. Mesterházy, A., 2002. Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to Fusarium head blight. European Journal of Plant Pathology 108, 675-684.

138. Miedaner T., Caixeta, F., Talas, F., 2013. Head-blighting populations of *Fusarium culmorum* from Germany, Russia, and Syria analyzed by microsatellite markers show a recombining structure. European Journal of Plant Pathology 137, 743-752.

139. Miedaner, T., Cumagun, C.J.R., Chakraborty, S., 2008. Population genetics of three important head blight pathogens *Fusarium graminearum*, *F. pseudograminearum* and *F. culmorum*. Journal of Phytopathology 156, 129-139.

140. Miedaner, T., Reinbrecht, C., 2001. Trichothecene content of rye and wheat genotypes inoculated with a deoxynivalenol- and a nivalenol-producing isolate of *Fusarium culmorum*. Journal of Phytopathology 149, 245–251.

141. Miedaner, T., Reinbrecht, C., Schilling, A.G., 2000. Association among aggressiveness, fungal colonization, and mycotoxin production of 26 isolates of *Fusarium graminearum* in winter rye head blight. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 107, 124–134.

142. Miedaner, T., Schilling, A.G., Geiger, H.H., 2004. Competition effects among isolates of *Fusarium culmorum* differing in aggressiveness and mycotoxin production on heads of winter rye. European Journal of Plant Pathology 110, 63–70.

143. Miller, J.D., Greenhalgh, R., Wang, Y.Z., Lu, M., 1991. Trichothecene chemotypes of three Fusarium species. Mycologia 83, 121-130.

144. Minervini, F., Fornelli, F., Flynn, K.M., 2004. Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. Toxicology in Vitro 18, 21-28.

145. Mirocha, C.J., Abbas, H.K., Windels, C.E., Xie, W., 1989. Variation in deoxynivalenol, 15-acetyldeoxynivalenol, 3- acetyldeoxynivalenol, and zearalenone production by *Fusarium graminearum* isolates. Applied and Environmental Microbiology 55, 1315-1316.

146. Mishra, P.K., Tewari, J.P., Turkington, T.K., Clear, R.M., 2009. Genetic evidence for a recent geographic expansion of 15-acetyldeoxynivalenol chemotypes of *Fusarium graminearum* in Canada. Canadian Journal of Plant Pathology/Revue Canadienne de Phytopathologie 31, 468-474.

147. Molto, G.A., Gonzalez, H.H.L., Resnik, S.L., Pereyra Gonzalez, A., 1997. Production of trichothecenes and zearalenone by isolates of *Fusarium* spp. from argentinian maize. Food Additives and Contaminants 14, 263-268.

148. Monds, R.D., Cromey, M.G., Lauren, D.R., Di Menna, M., Marshall, J., 2005. *Fusarium graminearum*, *F. cortaderiae* and *F. pseudograminearum* in New Zealand: molecular phylogenetic analysis, mycotoxin chemotypes and co-existence of species. Mycological Research 109, 410-420.

149. Moss, M.O., Thrane, U., 2004. Fusarium taxonomy with relation to trichothecene formation. Toxicolology Letters 153, 23–28.

150. Mudili, V., Siddaih, C.N., Nagesh, M., Garapati, P., Naveen Kumar, K., Murali, H.S., Yli-Mattila, T., Batra, H.V., 2014. Mould incidence and mycotoxin contamination in freshly harvested maize kernels originated from India. Journal of Science Food and Agriculture. In press (DOI: 10.1002/jsfa.6608).

151. Mugrabi de Kuppler, A.L., Steiner, U., Sulyok, M., Krska, R., Oerke, E.-C., 2011. Genotyping and phenotyping of *Fusarium graminearum* isolates from Germany related to their mycotoxin biosynthesis. International Journal of Food Microbiology 151, 78-86.

152. Müller, H.-M., and Schwadorf, K., 1993. A survey of the natural occurrence of *Fusarium* toxins in wheat grown in a southwestern area of Germany. Mycopathologia 121, 115-121

153. Muthomi, J. W., Schütze, A., Dehne, H.W., Mutitu, E.W., Oerke, E.C., 2000. Characterization of *Fusarium culmorum* isolates by mycotoxin production and aggressiveness to winter wheat. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 107, 113– 123.

154. Ndoye, M., Zhang, J.B., Wang, J.H., Gong, A.D., Li, H.P., Qu, B., Li, S.J., Liao, Y.C., 2012. Nivalenol and 15-acetyldeoxynivalenol chemotypes of *Fusarium graminearum* clade species are prevalent on maize throughout China. Journal of Phytopathology 160, 519-524.

155. Nicholson, P., Chandler, E., Draeger, R.C., Gosman, N.E., Simpson, D.R., Thomsett, M., Wilson, A.H., 2003. Molecular tools to study epidemiology and toxicology of Fusarium head blight of cereals. European Journal of Plant Pathology 109, 691-703.

156. Nicholson, P., Simpson, D.R., Wilson, A.H., Chandler, E., Thomsett, M., 2004. Detection and differentiation of trichothecene and enniatin-producing Fusarium species on small-grain cereals. European Journal of Plant Pathology 110, 503-514.

157. Nielsen, L.K., Cook, D.J., Edwards, S.G., Ray, R.V., 2014. The prevalence and impact of Fusarium head blight pathogens and mycotoxins on malting barley quality in UK. International Journal of Food Microbiology 179, 38–49.

158. Nielsen, L.K., Jensen, J.D., Nielsen, G.C., Jensen, J.E., Spliid, N.H., Thomsen, I.K., Justesen, A.F., Collinge, D.B., Jørgensen, L.N., 2011. Fusarium head blight of cereals in Denmark: species complex and related mycotoxins. Phytopathology 101, 960–969.

159. Nielsen, L.K., Jensen, J.D., Rodríguez, A., Jørgensen, L.N., Justesen, A.F., 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. International Journal of Food Microbiology 157, 384-392.

160. 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 Pathology 62, 79-91.

161. O'Donnell, K., Kistler, H.C., Tacke, B.K., Casper, H.H., 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proceedings of the National Academy of Sciences of the United States of America 97, 7905-7910.

162. O'Donnell, K., Ward, T.J., Aberra, D., Kistler, H.C., Aoki, T., Orwig, N., Kimura, M., Bjørnstad, S., Klemsdal, S.S., 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. Fungal Genetics and Biology 45, 1514-1522.

163. Ok, H.E., Choi, S.W., Chang, H.J., Chung, M.S., Chun, H.S., 2011. Occurrence of five 8-ketotrichothecene mycotoxins in organically and conventionally produced cereals collected in Korea. Food Control 22, 1647–1652.

164. Ok, H.E., Kim, D.M., Kim, D., Chung, S.H., Chung, M.S., Park, K.H., Chun, H.S., 2014. Mycobiota and natural occurrence of aflatoxin, deoxynivalenol, nivalenol and zearalenone in rice freshly harvested in South Korea. Food Control 37, 284–291.

165. Osborne, L.E., Stein, J.M., 2007. Epidemiology of Fusarium head blight on small-grain cereals. International Journal of Food Microbiology 119, 103-108.

166. 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 Technology and Biotechnology 52, 351-358.

1105 167. Pan, D., Calero, N., Mionetto, A., Bettucci, L., 2013. Trichothecene genotypes of
1106 *Fusarium graminearum* from wheat in Uruguay. International Journal of Food
1107 Microbiology 162, 120-123.

1108 168. Park, J.W., Choi, S.Y., Hwang, H.J., Kim, Y.B., 2005. Fungal mycoflora and
1109 mycotoxins in Korean polished rice destined for humans. International Journal of Food
1110 Microbiology 103, 305–314.

1111 169. Pasquali, M., Beyer, M., Bohn, T., Hoffmann, L., 2011. Comparative analysis of
1112 genetic chemotyping methods for *Fusarium*: Tri13 polymorphism does not discriminate
1113 between 3- and 15-acetylated deoxynivalenol chemotypes in *Fusarium graminearum*.
1114 Journal of Phytopathology 159, 700–704.

1115 170. Pasquali, M., Giraud, F., Brochot, C., Cocco, E., Hoffmann, L., Bohn, T., 2010.
1116 Genetic Fusarium chemotyping as a useful tool for predicting nivalenol contamination in
1117 winter wheat. International Journal of Food Microbiology 137, 246-253.

1118 171. Pasquali, M., Giraud, F., Brochot, C., Hoffmann, L., Bohn, T., 2009. First report of
1119 the nivalenol chemotype of *Fusarium graminearum* causing Head Blight of wheat in the
1120 Grand Duchy of Luxembourg. Plant Disease 93, 1217.

1121 172. Pasquali, M., Serchi, T., Renaut, J., Hoffmann, L., Bohn, T., 2013a. 2D difference
1122 gel electrophoresis reference map of a *Fusarium graminearum* nivalenol producing
1123 strain. Electrophoresis 34, 505–509.

1124 173. Pasquali, M., Spanu, F., Scherm, B., Balmas, V., Hoffmann, L., Hammond-
1125 Kosack, K.E., Beyer, M., Migheli, Q., 2013b. *FcStuA* from *Fusarium culmorum* controls
1126 wheat foot and root rot in a toxin dispensable manner. PLoS ONE 8, e57429.

1127 174. Perkowski, J., Kiecana, I., Schumacher, U., Mueller, H.-M., Chełkowski, J.,
1128 Goliński, P., 1997. Head infection and accumulation of fusarium toxins in kernels of 12
1129 barley genotypes inoculated with *Fusarium graminearum* isolates of two chemotypes.
1130 European Journal of Plant Pathology 103, 85-90.

1131 175. Pinson-Gadais, L., Richard-Forget, F., Frasse, P., Barreau, C., Cahagnier, B.,
1132 Richard-Molard, D., Bakan, B., 2008. Magnesium represses trichothecene biosynthesis
1133 and modulates *Tri5*, *Tri6*, and *Tri12* genes expression in *Fusarium graminearum*.
1134 Mycopathologia 165, 51–59.

1135 176. Poapolathep, A., Poapolathep, S., Klangkaew, N., Sugita-Konishi, Y., Kumagai,
1136 S., 2008. Detection of deoxynivalenol contamination in wheat products in Thailand.
1137 Journal of Food Protection 71, 1931-1933.

177. Ponts, N., Couedelo, L., Pinson-Gadais, L., Verdal-Bonnin, M.N., Barreau, C., Richard-Forget, F., 2009. Fusarium response to oxidative stress by H_2O_2 is trichothecene chemotype-dependent. FEMS Microbiology Letters 293, 255-262.

178. Ponts, N., Pinson-Gadais, L., Barreau, C., Richard-Forget, F., Ouellet, T., 2007. Exogenous H_2O_2 and catalase treatments interfere with *Tri* genes expression in liquid cultures of *Fusarium graminearum*. FEBS Letters 581, 443-447.

179. Ponts, N., Pinson-Gadais, L., Verdal-Bonnin, M., Barreau, C., Richard-Forget, F., 2006. Accumulation of deoxynivalenol and its 15-acetylated form is significantly modulated by oxidative stress in liquid cultures of *Fusarium graminearum*. FEMS Microbiology Letters 258, 102–107.

180. Proctor, R.H., McCormick, S.P., Alexander, N.J., Desjardins, A.E., 2009. Evidence that a secondary metabolic biosynthetic gene cluster has grown by gene relocation during evolution of the filamentous fungus *Fusarium*. Molecular Microbiology 74, 1128-1142.

181. Prodi, A., Purahong, W., Tonti, S., Salomoni, D., Nipoti, P., Covarelli, L., Pisi, A., 2011. Difference in chemotype composition of *Fusarium graminearum* populations isolated from durum wheat in adjacent areas separated by the Apennines in Northern-Central Italy. The Plant Pathology Journal 27, 354–359.

182. Prodi, A., Salomoni, D., Bertacchini, E., Alkadri, D., Pisi, A., Tonti, S., Alberti, I., Prà, M., Pancaldi, D., Covarelli, L., Nipoti, P., 2012. Determination of deoxynivalenol and nivalenol producing chemotypes of *Fusarium graminearum* isolated from durum wheat in different Italian regions. Plant Breeding and Seed Science 64, 75–80.

183. Prodi, A., Tonti, S., Nipoti, P., Pancaldi, D., Pisi, A., 2009. Identification of deoxynivalenol and nivalenol producing chemotypes of *Fusarium graminearum* isolates from durum wheat in a restricted area of northern Italy. Journal of Plant Pathology 91, 727-731.

184. Purahong W, P.N., 2014. Aggressiveness of different *Fusarium graminearum* chemotypes within a population from Northern-Central Italy. Mycoscience 55, 63-69.

185. Puri, K.D., Saucedo, E.S., Zhong, S., 2012. Molecular characterization of Fusarium Head Blight pathogens sampled from a naturally infected disease nursery used for wheat breeding programs in China. Plant Disease 96, 1280–1285.

186. Puri, K.D., 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. Phytopathology 100, 1007–1014.

187. Qiu, J., Xu, J., Shi, J., 2014. Molecular characterization of the *Fusarium graminearum* species complex in Eastern China. European Journal of Plant Pathology 139, 811-823.

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

189. Quarta, A., Mita, G., Haidukowski, M., Logrieco, A., Mulè, G., Visconti, A., 2006. Multiplex PCR assay for the identification of nivalenol, 3- and 15-acetyl-deoxynivalenol chemotypes in *Fusarium*. FEMS Microbiology Letters 259, 7-13.

190. Quarta, A., Mita, G., Haidukowski, M., Santino, A., Mulè, G., Visconti, A., 2005. Assessment of trichothecene chemotypes of *Fusarium culmorum* occurring in Europe. Food Additives and Contaminants 22, 309-315.

191. Ramirez, M.L., Reynoso, M.M., Farnochi, M.C., Chulze, S., 2006. Vegetative compatibility and mycotoxin chemotypes among *Fusarium graminearum* (*Gibberella zeae*) isolates from wheat in Argentina. European Journal of Plant Pathology 115, 139-148.

192. Ran, R., Wang, C., Han, Z., Wu, A., Zhang, D., Shi, J., 2013. Determination of deoxynivalenol (DON) and its derivatives: current status of analytical methods. Food Control 34, 138-148.

193. Rebib, H., Bouraoui, H., Rouaissi, M., Brygoo, Y., Boudabbous, A., Hajlaoui, M.R., 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. European Journal of Plant Pathology 139, 481-493.

194. Rep, M., Kistler, H.C., 2010. The genomic organization of plant pathogenicity in Fusarium species. Current. Opinion in Plant Biology 13, 420–426.

195. Reverberi, M., Ricelli, A., Zjalic, S., Fabbri, A.A., Fanelli, C., 2010. Natural functions of mycotoxins and control of their biosynthesis in fungi. Applied Microbiology and Biotechnology 87, 899-911.

196. Reynoso, M.M., Ramirez, M.L., Torres, A.M., Chulze, S.N., 2011. Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. International Journal of Food Microbiology 145, 444–448.

197. Roscoe, V., Lombaert, G.A., Huzel, V., Neumann, G., Melietio, J., Kitchen, D., Kotello, S., Krakalovich, T., Trelka, R., Scott, P.M., 2008. Mycotoxins in breakfast cereals

from the Canadian retail market: A 3-year survey. Food Additives & Contaminants 25, 347-355.

198. Ryu, D., Bullerman, L.B., 1999. Effect of cycling temperatures on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883. Journal of Food Protection 62, 1451-1455.

199. Sakamoto, N., Tsuyuki, R., Yoshinari, T., Usuma, J., Furukawa, T., Nagasawa, H., & Sakuda, S., 2013. Correlation of ATP citrate lyase and acetyl CoA levels with trichothecene production in *Fusarium graminearum*. Toxins 5, 2258-2269.

200. Sampietro, D. A., Apud, G. R., Belizán, M. M. E., Vattuone, M. A., Catalán, C.A.N., 2013. Toxigenic potential of *Fusarium graminearum* isolated from maize of Northwest Argentina. Brazilian Journal of Microbiology 44, 417–422.

201. Sampietro, D.A., Ficoseco, M.E.A., Jimenez, C.M., Vattuone, M.A., Catalán, C.A., 2012. Trichothecene genotypes and chemotypes in *Fusarium graminearum* complex strains isolated from maize fields of northwest Argentina. International Journal of Food Microbiology 153, 229–233.

202. Sampietro, D.A., Díaz, C.G., Gonzalez, V., Vattuone, M.A., Ploper, L.D., Catalán, C.A.N., Ward, T.J., 2011. Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. International Journal of Food Microbiology 145, 359–364.

203. Sarver, B.A.J., Ward, T.J., Gale, L.R., Broz, K. Kistler, H.C., 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 Genetics and Biology 48, 1096-1107.

204. Shen, C., Hu, Y., Sun, H., Li, W., Guo, J., Chen, H., 2012. Geographic Distribution of trichothecene chemotypes of the *Fusarium graminearum* species complex in major winter wheat production areas of China. Plant Disease 96, 1172–1178.

205. Scherm, B., Balmas, V., Spanu, F., Pani, G., Delogu, G., Pasquali, M., Migheli, Q., 2013. *Fusarium culmorum*: causal agent of foot and root rot and head blight on wheat. Molecular Plant Pathology 14, 323–341.

206. Scherm, B., Orrù, M., Balmas, V., Spanu, F., Azara, E., Delogu, G., Hammond, T.M., Keller, N.P., 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. Molecular Plant Pathology 12, 759-771.

207. Schmale, D.G., Ross, S.D., Fetters, T.L., Tallapragada, P., Wood-Jones, A.K., Dingus, B., 2012. Isolates of *Fusarium graminearum* collected 40–320 meters above ground level cause *Fusarium* head blight in wheat and produce trichothecene mycotoxins. Aerobiologia 28, 1-11.

208. Schmale, D.G., Wood-Jones, A.K., Cowger, C., Bergstrom, G.C., Arellano, C., 2011. Trichothecene genotypes of *Gibberella zeae* from winter wheat fields in the eastern USA. Plant Pathology 60, 909–917.

209. Schmidt-Heydt, M., Parra, R., Geisen, R., Magan, N., 2011. Modelling the relationship between environmental factors, transcriptional genes and deoxynivalenol mycotoxin production by strains of two *Fusarium* species. Journal of the Royal Society Interface 54, 117-126.

210. Scoz, L.B., Astolfi, P., Reartes, D.S., Schmale III, D.G., Moraes, M.G., Del Ponte, E.M., 2009. Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. Plant Pathology 58, 344-351.

211. Scudamore, K.A., Patel, S., 2009. Occurrence of *Fusarium* mycotoxins in maize imported into the UK, 2004-2007. Food Additives & Contaminants: Part A 26 3, 363-371.

212. Seo, J.A., Kim, J.C. Lee, D.H., Lee, Y.W., 1996. Variation in 8-ketotrichothecenes and zearalenone production by *Fusarium graminearum* isolates from corn and barley in Korea. Mycopathologia 134, 31-37.

213. Seong, K.-Y., Pasquali, M., Zhou, X., Song, J., Hilburn, K, McCormick, S., Dong, Y., Xu, J.-R., Kistler, H.C., 2009. Global gene regulation by *Fusarium* transcription factors *Tri6* and *Tri10* reveals adaptations for toxin biosynthesis. Molecular Microbiology 72, 354-367.

214. Shen, C.M., Hu, T.C., Sun, H.Y., Li, W., Guo, J.H., Chen, H.G., 2012. Geographic distribution of trichotecene chemoptypes of *Fusarium graminearum* species complex in major winter wheat production area of China Plant Disease 96, 1172-1178.

215. Somma, S., Petruzzella, A.L., Logrieco, A.F., Meca, G., Cacciola, O.S., Moretti, A., 2014. Phylogenetic analyses of *Fusarium graminearum* strains from cereals in Italy, and characterisation of their molecular and chemical chemotypes. Crop and Pasture Science 65, 52–60.

216. Spolti, P., Barros, N.C., Gomes, L.B., dos Santos, J., Ponte, E.M.D., 2012. Phenotypic and pathogenic traits of two species of the *Fusarium graminearum* complex possessing either 15-ADON or NIV genotype. European Journal of Plant Pathology 133, 621-629.

217. Spolti, P., Del Ponte, E., Cummings, J.A., Dong, Y., Bergstrom, G.C., 2014b. Fitness attributes of *Fusarium graminearum* isolates from wheat in New York possessing a 3-ADON or 15-ADON trichothecene genotype. Phytopathology 104, 515-519.

218. Spolti, P., Del Ponte, E., Dong, Y., Cummings, J.A., Bergstrom, G.C., 2014a. Assessment of triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Disease 98, 607-613.

219. Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Tóth, B., Varga, J., O'Donnell, K., 2007. Global molecular surveillance reveals novel fusarium head blight species and trichothecene toxin diversity. Fungal Genetics and Biology 44, 1191-1204.

220. Stepień, Ł., Popiel, D., Koczyk, G., Chełkowski, J., 2008. Wheat-infecting *Fusarium* species in Poland - their chemotypes and frequencies revealed by PCR assay. Journal of Applied Genetics 49, 433-441.

221. Suga, H., Karugia, G.W., Ward, T., Gale, L.R., Tomimura, K., Nakajima, T., Miyasaka, A., Koizumi, S., Kageyama, K., Hyakumachi, M., 2008. Molecular characterization of the *Fusarium graminearum* species complex in Japan. Phytopathology 98, 159-166.

222. Sugiura, Y., Watanabe, Y., Tanaka, T., Yamamoto, S., Ueno, Y., 1990. Occurrence of *Gibberella zeae* strains that produce both nivalenol and deoxynivalenol. Applied and Environmental Microbiology 56, 3047-3051.

223. Sumíková, T., Gabrielová, L., Kučera, L., Žabka, M., Chrpová, J., 2013. Mycotoxin production, chemotypes and diversity of Czech *Fusarium graminearum* isolates on wheat. Czech Journal of Food Science 31, 407-412.

224. Suzuki, T., Kim, Y.-K., Yoshioka, H., Iwahashi, Y., 2013. Regulation of metabolic products and gene expression in *Fusarium asiaticum* by agmatine addition. Mycotoxin Research 29, 103–111.

225. Suzuki, F., Koba, A., Nakajima, T., 2010. Simultaneous identification of species and trichothecene chemotypes of *Fusarium asiaticum* and *F. graminearum* sensu stricto by multiplex PCR. Journal of General Plant Pathology 76, 31-36.

226. Sydenham, E.W., Marasas, W.F.O., Thiel, P.G., Shephard, G.S., Nieuwenhuis, J.J., 1991. Production of mycotoxins by selected *Fusarium graminearum* and *F. crookwellense* isolates. Food Additives & Contaminants Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment 8, 31.

227. Szécsi, Á., Bartók, T., 1995. Trichothecene chemotypes of *Fusarium graminearum* isolated from corn in Hungary. Mycotoxin Research 11, 85–92.

228. Szécsi, Á., Bartók, T., Varga, M., Magyar, D., Mesterházy, Á., 2005. Determination of trichothecene chemotypes of *Fusarium graminearum* strains isolated in Hungary. Journal of Phytopathology 153, 445-448.

229. Talas, F., Kalih, R., Miedaner, T., 2012a. Within-field variation of *Fusarium graminearum* isolates for aggressiveness and deoxynivalenol production in wheat head blight. Phytopathology 102, 128–134.

230. Talas, F., Parzies, H.K., 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. European Journal of Plant Pathology 131, 39-48.

231. Talas, F., Würschum, T., Reif, J.C., Parzies, H.K., Miedaner, T., 2012b. Association of single nucleotide polymorphic sites in candidate genes with aggressiveness and deoxynivalenol production in *Fusarium graminearum* causing wheat head blight. BMC Genetics 13, 14.

232. Tan, D.C., Flematti, G.R., Ghisalberti, E.L., Sivasithamparam, K., Chakraborty, S., Obanor, F., Jayasena, K., Barbetti, M.J., 2012. Mycotoxins produced by *Fusarium spp*. associated with Fusarium head blight of wheat in Western Australia. Mycotoxin Research 28, 89–96.

233. Tan, M.K., Simpfendorfer, S., Backhouse, D., Murray, G.M., 2004. Occurrence of fusarium head blight (FHB) in southern NSW in 2000: identification of causal fungi and determination of putative chemotype of *Fusarium graminearum* isolates by PCR. Australasian Plant Pathology 33, 385-392.

234. Tanaka, T., Hasegawa, A., Matsuki, Y., Lee, U.S., Ueno, Y., 1986. A limited survey of *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone in 1984 UK harvested wheat and barley. Food Additives and Contaminants 3, 247-252.

235. Tangni, E.K., Motte, J.C., Callebaut, A., Pussemier, L., 2010. Cross-reactivity of antibodies in some commercial deoxynivalenol test kits against some fusariotoxins. Journal of Agricultural and Food Chemistry 58, 12625–12633

236. Tateishi, H., Miyake, T., Mori, M., Kimura, R., Sakuma, Y., Saishoji, T., 2010. Sensitivity of Japanese *Fusarium graminearum* species complex isolates to metconazole. Journal of Pesticide Science 35, 419–430.

237. Thrane, U., Adler, A., Clasen, P.-E., Galvano, F., Langseth, W., Lew, H., Logrieco, A., Nielsen, K.F., Ritieni, A., 2004. Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae*, and *Fusarium sporotrichioides*. International Journal of Food Microbiology 95, 257–266.

238. Tittlemier, S.A., Gaba, D., Chan, J.M., 2013. Monitoring of *Fusarium* trichothecenes in Canadian cereal grain shipments from 2010 to 2012. Journal of Agricultural and Food Chemistry 61, 7412-7418.

239. Tóth, B., Mesterházy, Á., Horváth, Z., Bartók, T., Varga, M., Varga, J., 2005. Genetic variability of central european isolates of the *Fusarium graminearum* species complex. European Journal of Plant Pathology 113: 35-45.

240. Tóth, B., Mesterházy, Á., Nicholson, P., Téren, J., Varga, J., 2004. Mycotoxin production and molecular variability of european and american isolates of *Fusarium culmorum*. European Journal of Plant Pathology 110, 587-599.

241. Tsuyuki, R., Yoshinari, T., Sakamoto, N., Nagasawa, H., Sakuda, S., 2011. Enhancement of trichothecene production in *Fusarium graminearum* by cobalt chloride. Journal of Agricultural and Food Chemistry 59, 1760–1766.

242. Ueno Y, Nakajima M, Sakai K, Ishii K, Sato N, Shimada N., 1973. Comparative toxicology of trichothecene mycotoxins: Inhibition of protein synthesis in animal cells. Journal of Biochemistry (Tokyo) 74, 285–296.

243. Umpierrez-Failache, M., Garmendia, G., Pereyra, S., Rodriquez-Haralambides, A., Ward, T.J., Vero, S., 2013. Regional differences in species composition and toxigenic potential among Fusarium head blight isolates from Uruguay indicate a risk of nivalenol contamination in new wheat production areas. International Journal of Food Microbiology 166, 135-140.

244. Vanheule, A., Audenaert, K., De Boevre, M., Landschoot, S., Bekaert, B., Munaut, F., Eeckhout, M., Höfte, M., De Saeger, S., Haesaert, G., 2014. The compositional mosaic of *Fusarium* species and their mycotoxins in unprocessed cereals, food and feed products in Belgium. International Journal of Food Microbiology 181C, 28–36.

245. Venkataramana, M., Shilpa, P., Balakrishna, K., Murali, H.S., Batra, H.V., 2013. Incidence and multiplex PCR based detection of trichothecene chemotypes of *Fusarium culmorum* isolates collected from freshly harvested maize kernels in Southern India. Brazilian Journal of Microbiology 44, 401–406.

246. Vogelgsang, S., Sulyok, M., Bänziger, I., Krska, R., Schuhmacher, R., Forrer, H.-R., 2008a. Effect of fungal strain and cereal substrate on *in vitro* mycotoxin production by *Fusarium poae* and *Fusarium avenaceum*. Food Additives & Contaminants Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment 25, 745-757.

247. Vogelgsang, S., Sulyok, M., Hecker, A., Jenny, E., Krska, R., Schuhmacher, R., Forrer, H.-R., 2008b. Toxigenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on wheat. European Journal of Plant Pathology 122, 265–276.

248. von der Ohe, C., Gauthier, V., Tamburic-Ilincic, L., Brule-Babel, A., Dilantha Fernando, W.G., Clear, R., Ward, T.J., 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. European Journal of Plant Pathology 127, 407-417.

249. Vujanovic, V., Goh, Y.K., Daida, P., 2012. Heat- and cold-shock responses in *Fusarium graminearum* 3 acetyl- and 15 acetyl-deoxynivalenol chemotypes. Journal of Microbiology 50, 97–102.

250. 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. European Journal of Plant Pathology 109, 743–754.

251. Wagacha, J.M., Steiner, U., Dehne, H.W., Zuehlke, S., Spiteller, M., Muthomi, J.W., Oerke, E.-C., 2010. Diversity in mycotoxins and fungal species infecting wheat in Nakuru District, Kenya. Journal of Phytopathology 158, 527-535.

252. Walker, S.L., Leath, S., Hagler, W.M., Murphy, J.P., 2001. Variation Among Isolates of *Fusarium graminearum* associated with Fusarium Head Blight in North Carolina. Plant Disease 85, 404–410.

253. Wang, J.H., Li, H.P. Qu, B., Zhang, J.B., Huang, T., Chen, F.F., Liao, Y.C. 2008. Development of a generic PCR detection of 3-acetyldeoxy-nivalenol-, 15-acetyldeoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum* clade. International Journal of Molecular Sciences 9, 2495-2504.

254. Wang, J.H., Ndoye, M., Zhang, J.B., Li, H.P., Liao, Y.C. 2011. Population structure and genetic diversity of the *Fusarium graminearum* Species Complex. Toxins 3, 1020-1037.

255. Wang, J.H., Zhang, J.B., Chen, F.F., Li, H.P., Ndoye, M., Liao, Y.C., 2012. A multiplex PCR assay for genetic chemotyping of toxigenic *Fusarium graminearum* and

wheat grains for 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol and nivalenol mycotoxin. Food, Agriculture and Environment 10, 505-511.

256. Wang, H., Hwang, S.F., Eudes, F., Chang, K.F., Howard, R.J., Turnbull, G.D., 2006. Trichothecenes and aggressiveness of *Fusarium graminearum* causing seedling blight and root rot in cereals. Plant Pathology 55, 224–230.

257. Wang, X., Cui, Y., Fan, F., Song, Y., Ren, J., Meng, Q., Xu, W., Jiang, L., 2010. Phylogenetic, carbendazim sensitivity and mycotoxin genotype analyses of *Fusarium graminearum* Complex species isolated from wheat Fusarium Head Blight in China. Journal of Phytopathology 158, 576-578.

258. Wanyoike, M.V., Walker, F., Buchenauer, F., 2002. Relationship between virulence, fungal biomass and mycotoxin production by *Fusarium graminearum* in winter wheat head blight. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 109, 589-600.

259. Ward, T.J., Bielawski, J.P., Kistler, H.C., Sullivan, E., O'Donnell, K., 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proceedings of the National Academy of Sciences of the United States of America 99, 9278-9283.

260. Ward, T.J., Clear, R.M., Rooney, A.P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser, D.M., Nowicki, T.W., 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genetics and Biology 45, 473-484.

261. Warzecha, T., Adamski, T., Kaczmarek, Z., Surma, M., Goliński, P., Perkowski, J., Chełkowski, J., Wiśniewska, H., Krystkowiak, K., Kuczyńska, A., 2010. Susceptibility of hulled and hulless barley doubled haploids to *Fusarium culmorum* head blight. Cereal Research Communications 38, 220-232.

262. Winter, M., Koopmann, B., Döll, K., Karlovsky, P., Kropf, U., Schlüter, K., von Tiedemann, A., 2013. Mechanisms regulating grain contamination with trichothecenes translocated from the stem base of wheat (*Triticum aestivum*) infected with *Fusarium culmorum*. Phytopathology 103, 682–689.

263. Xu, X., Nicholson, P., Ritieni, A., 2007. Effects of fungal interactions among Fusarium head blight pathogens on disease development and mycotoxin accumulation. International Journal of Food Microbiology 119, 67-71.

264. Xu, X.M., Parry, D.W., Nicholson, P., Thomsett, M.A., Simpson, D., Edwards, S.G., Cooke, B.M., Doohan, F.M., Brennan, J.M., Moretti, A., Tocco, G., Mulé G., Hornok, L., Giczey, G., Tatnell, J., 2005. Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. European Journal of Plant Pathology 112, 143-154.

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

266. Yli-Mattila, T., 2010. Ecology and evolution of toxigenic *Fusarium* species in cereals in Northern Europe and Asia. Journal of Plant Pathology 92, 7-18.

267. Yli-Mattila, T., Gagkaeva, T., 2010. Molecular chemotyping of *Fusarium graminearum, F. culmorum*, and *F. cerealis* isolates from Finland and Russia. In: Gherbawy, Y., Voigt, K. (Eds.), Molecular Identification of Fungi, Part 1, Springer, Berlin, pp. 159-177.

268. Yli-Mattila, T., Gagkaeva, T., Ward, T.J., Aoki, T., Kistler, H.C., O'Donnell, K., 2009a. 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.

269. Yli-Mattila, T., Paavanen-Huhtala S., Parikka P., Hietaniemi V., Jestoi M., Gagkaeva T., Sarlin T., Haikara A., Laaksonen S., Rizzo A., 2008. Real-time PCR detection and quantification of *Fusarium poae*, *F. graminearum*, *F. sporotrichioides* and *F. langsethiae* as compared to mycotoxin production in grains in Finland and Russia. Archives of Phytopathology and Plant Protection 41, 243-260.

270. Yli-Mattila, T., Parikka, P, Lahtinen, T., Ramo, S., Kokkonen, M., Rizzo, A., Jestoi, M., Hietaniemi, V., 2009b. *Fusarium* DNA levels in Finnish cereal grains .In: Gherbawy, Y., Mach, L., Rai, M., (Eds.) Current Advances in Molecular Mycology, , Nova Science Publishers Inc., New York, pp. 107-138.

271. Yörük, E., Albayrak, G., 2012. Chemotyping of *Fusarium graminearum* and *F. culmorum* isolates from Turkey by PCR assay. Mycopathologia 173, 53–61.

272. Yoshizawa, T., Jin, J.Z., 1995. Natural occurrence of acetylated derivatives of deoxynivalenol and nivalenol in wheat and barley in Japan. Food Additives and Contaminants 12, 689-694.

273. Zhang, H., Van der Lee, T., Waalwijk, C., Chen, W., Xu, J., Zhang, Y., Feng, J., 2012. Population analysis of the *Fusarium graminearum* Species Complex from wheat in China show a shift to more aggressive isolates. PLoS ONE 7, e31722.

274. Zhang, H., Zhang, Z., van der Lee, T., Chen, W.Q., Xu, J., Xu, J.-S., Yang, L.-J., Yu, D., Waalwijk, C., Feng, J., 2010a. Population genetic analyses of *Fusarium asiaticum* populations from barley suggest a recent shift favoring 3-ADON producers in Southern China. Phytopathology 100, 328-336.

275. Zhang, J.B., Li, H.P., Dang, F.J. Qu, B., Xu, J.B., Zhao, C.S., Liao, Y.C., 2007. Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the *Fusarium graminearum* clade from China. Mycological Research 111, 967-975.

276. Zhang, J.B., Wang, J.H., Gong, A.D., Chen, F.F., Song, B., Li, X., Li, H.P., Peng, C.H., Liao, Y.C., 2013b. Natural occurrence of Fusarium Head Blight, mycotoxins and mycotoxin-producing isolates of *Fusarium* in commercial fields of wheat in Hubei. Plant Pathology 62, 92-102.

277. Zhang, L., Jia, X., Chen, C., Zhou, M., 2013a. Characterization of carbendazim sensitivity and trichothecene chemotypes of *Fusarium graminearum* in Jiangsu Province of China. Physiological and Molecular Plant Pathology 84, 53-60.

278. Zhang, Y.J., Yu, J.J., Zhang, Y.N., Zhang, X., Chen, C.J., Wang, J.X., Hollomon, D.W., Fan, P.S., Zhou, M.G., 2009. Effect of carbendazim resistance on trichothecene production and aggressiveness of *Fusarium graminearum.* Molecular Plant-Microbe Interaction 22, 1143-1150.

279. Zhang, Z., Zhang, H., van der Lee, T., Chen, W.Q., Arens, P., Xu, J., Xu, J.-S., Yang, L.-J., Yu, D.-Z., Waalwijk, C., Feng, J., 2010b. Geographic substructure of *Fusarium asiaticum* isolates collected from barley in China. European Journal of Plant Pathology 127, 239-248.