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Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes

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Abstract This review describes the naturally occurring mechanisms in cereals that lead to a reduction of *Fusarium* trichothecene mycotoxin accumulation in grains. A reduction in mycotoxin contamination in grains could also limit fungal infection, as trichothecenes have been reported to act as virulence factors. The mechanisms explaining the low toxin accumulation trait, generally referred to as type V resistance to *Fusarium*, can be subdivided into two classes. Class 1 includes mechanisms by which the plants chemically transform the trichothecenes, leading to their degradation or detoxification. Among the detoxification strategies, glycosylation of trichothecenes is a natural process already reported in wheat. According to the structure and the toxicity of trichothecenes, two other detoxification processes, acetylation and de-epoxidation, can be expressed, at least in transgenic plants. Class 2 comprises mechanisms that lead to reduced mycotoxin accumulation by inhibition of their biosynthesis through the action of plant endogenous compounds. These include both grain constitutive compounds and compounds induced in response to pathogen infection. There are already many compounds with antioxidant properties, like phenolic compounds, peptides or carotenoids, and with prooxidant properties, like hydrogen peroxide or linoleic acid-derived hydroperoxides, that have been described as 'modulators' of mycotoxin biosynthesis. This review addresses for the first time different studies reporting specific *in vitro* effects of such compounds on the biosynthesis of *Fusarium* mycotoxins. A better understanding of the natural processes limiting accumulation of trichothecenes in the plant will open the way to the development of novel breeding varieties with reduced 'mycotoxin risk'.

Keywords FHB resistance · *Fusarium* · Glycosylation · Phenolic compounds · Mycotoxins · Wheat

Abbreviations

3-ADON	3-acetyl-4-deoxynivalenol
15-ADON	15-acetyl-4-deoxynivalenol
4-ABOA	4-acetyl-benzoxazolin-2-one
9S-HPODE	9S-hydroperoxide
13S-HPODE	13S-hydroperoxide
DON	deoxynivalenol
FHB	*Fusarium* head blight
FX	fusarenone X
LC	liquid chromatography
LOX	lipoxygenase

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MS	mass spectrometry
NIV	nivalenol
QTL	quantitative trait loci
TCT B	trichothecene B
UDP glycosyltransferase	uridine diphosphate glycosyltransferase

Introduction

Fusarium head blight (FHB) or 'scab', results from infection of cereal grains by microscopic fungi of the genus *Fusarium* and leads to drastic reductions in crop yield. Furthermore, various species of this genus can produce toxic secondary metabolites, referred to as mycotoxins, which accumulate in the grain. This causes a reduction in grain quality, leading to important economic losses. In addition, given the quantities of cereal-derived food consumed daily, the occurrence of mycotoxins in grains constitutes an important food safety issue.

Among *Fusarium* mycotoxins, trichothecenes are frequently encountered in cereal crops. As these are potent inhibitors of eukaryotic protein synthesis (Rocha et al. 2005), they constitute a toxin family of considerable concern to human and animal health (Bennett and Klich 2003). In Europe, trichothecenes from the type B group (TCT B) produced by *Fusarium graminearum* (teleomorph *Gibberella zeae*) and *Fusarium culmorum* species are predominant (Bottalico and Perrone 2002). They include deoxynivalenol (DON) and its acetylated forms 3-acetyl-4-deoxynivalenol and 15-acetyl-4-deoxynivalenol (3- and 15-ADON) and nivalenol (NIV) and its acetylated form 4-acetylnivalenol or fusarenone X (FX).

In Europe, maximum DON contamination levels acceptable for cereals and maize-based food were established in June 2005 (EC No856/2005) and revised in July 2007 (EC No1126/2007). Grains or derived products exceeding the established limits will not be authorised for human consumption. It is of great concern to reduce DON occurrence in cereals and derived products generated in Europe. Type-B trichothecenes are heat-stable molecules and are not eliminated by current food manufacturing processes (Hazel and Patel 2004). In consequence, the most efficient way to reduce or prevent trichothecene occurrence in food today is to limit their biosynthesis by the fungus before harvest. Since no efficient fungicides are presently available to fully control *Fusarium* development and/or toxin accumulation (Champeil et al. 2004), it is best to prevent the development of the disease in the crop. It has been shown that some cultural practices (ploughing, tillage, preceding crop and choice of cultivar) can contribute to the occurrence of FHB (Champeil et al. 2004). As a consequence, appropriate agronomic practices are generally used to reduce the risk of contamination of wheat with trichothecenes (Champeil et al. 2004; Edwards 2004). Among the different factors that are relevant for that purpose, choice of cultivar can be a determinant.

One obvious approach against the mycotoxin threat consists of breeding varieties for resistance to FHB disease. This strategy could limit mycotoxin contamination in grains. However, a direct relationship between resistance to FHB and resistance to toxin contamination of the infected grain remains a subject of controversy. In fact, some authors claim a correlation between FHB symptoms and trichothecene level in wheat in contrast to other literature data that show that infection severity alone is not enough to predict a potential level of toxin concentration in wheat grain (Mesterházy et al. 1999; Snijders 2004). Although there is a general trend showing that the higher the level of resistance of a genotype the lower the toxin accumulation, varieties with low apparent FHB symptom severity and high DON content, and *vice versa*, have also been described (Mesterházy et al. 1999). Because FHB symptoms and *Fusarium* trichothecene levels are not always correlated, breeding for FHB-resistant wheat varieties with lower mycotoxin levels in the grain is not straightforward and presents some difficulties.

Besides the resistance mechanisms that modulate the severity of FHB, there may be additional mechanisms that lead to a decrease in accumulation of TCT B in kernels. In addition to quantitative trait loci (QTL) controlling resistance to FHB, a QTL linked to the control of trichothecene accumulation has recently been characterised in wheat (Lemmens et al. 2005). As trichothecenes have been shown to act as virulence factors for fungal infection (Proctor et al. 1995), limiting mycotoxin production in the kernel would consequently reduce fungal infection.

No FHB resistant varieties are yet commercially available for durum wheat. In a recent study, the occurrence of different sensitivities to TCT B accumulation was demonstrated for a collection of *Triticum turgidum*

subsp. *durum* lines inoculated with a nivalenol-producing strain of *F. culmorum* (Favre et al. 2004). While only slight differences in the levels of fungal biomass were observed for the different lines, four of the lines exhibited lower TCT B contamination. The current hypothesis is that those wheat lines may either contain endogenous compounds able to inhibit *Fusarium* trichothecene biosynthesis or possess biological activities able to degrade or modify the toxins. Such a variation in TCT B production in different lines of the same crop suggests that, even for cereal species in which no resistance to pathogen development can be selected, varieties accumulating lower toxin can be produced. The identification of the precise chemical or biological nature of the factors responsible for the differences in susceptibility to toxin accumulation among varieties will help plant geneticists to breed for varieties with such desirable characteristics. A breeding strategy to combine both resistance to pathogen development and to toxin accumulation would probably lead to new genotypes able to efficiently limit the 'mycotoxin risk'.

Schroeder and Christensen (1963) distinguished between two types of resistance to FHB in wheat: type I resistance that operates against initial infection, and type II resistance that operates against the spread of the pathogen within the host. Later, other types of resistance were defined from characteristics observed in FHB-resistant wheat: ability to resist kernel infection (type III), tolerance to infection (type IV), and resistance to DON accumulation (type V; Miller et al. 1985; Mesterházy 2002).

The aim of this review is to focus on the natural processes limiting *Fusarium* trichothecene accumulation in cereals (type V resistance), especially in wheat. These processes include both chemical transformation of the toxins by plant metabolic activities and inhibition of their biosynthesis by natural plant compounds. We propose to divide the type V resistance into two components of resistance: (V-1) resistance to trichothecene accumulation by metabolic transformation of the toxin and (V-2) resistance *via* inhibition of trichothecene biosynthesis.

Metabolic transformation of *Fusarium* trichothecenes

The occurrence of chemical transformation of *Fusarium* trichothecenes *in planta* has long been suspected from studies devoted to DON accumulation in kernels. Miller et al. (1983) observed a decrease of DON and 15-ADON levels during the growing season in field maize inoculated with *F. graminearum* and hypothesised that this decrease could be explained by a chemical transformation of the mycotoxins by plant enzymes. This reduction in toxin levels was also noticed in wheat either artificially or naturally infected with *F. graminearum* (Scott et al. 1984; Miller and Young 1985). Later, different studies described interactions between plant components and trichothecenes. For example, during germination of barley seeds spiked with deoxynivalenol, a 77% degradation of DON was observed within 5 days (El-Banna 1987). In sweet potato root tissues, ^{14}C-labelled deoxynivalenol was rapidly metabolised and converted into several unknown metabolites (Fujita and Yoshizawa 1990). Miller et al. (1985) experimentally infected a set of spring cereals (wheat, rye and triticale; 20 cultivars) with *F. graminearum*, and observed that there was proportionally less DON in the resistant cultivars than in the susceptible ones when compared with the expected values based on fungal biomass measurements. Yao et al. (1996) showed that extracts of leaves from two varieties of wheat resistant to FHB (Sumaï 3 and Fan 9) transformed DON into an unknown compound while no transformation was noticed in varieties susceptible to FHB. Miller et al. (1985) suggested that resistance in some cultivars might result in part from degradation or transformation of the toxins.

Such mechanisms would be supported by the identification of either the bypass products or degradation metabolites, or by the identification of plant chemical transformation activities on trichothecenes. Some recent works, described in the following paragraph, clearly demonstrate that such mechanisms do exist.

Glycosylation: a natural TCT B detoxification process for cereals

A detoxification process for plants consists of reducing the toxicity of pollutants by chemical modification and sequestering into the vacuole (Coleman et al. 1997). Chemical transformation may involve conjugation of the toxic compound to polar substances such as sugars, amino acids, or sulphates (Berthiller et al. 2005). So far, conjugation of TCT B to sugars is

the only modification reported to occur *in planta* (Berthiller et al. 2005). In general, glycosylation transforms toxic metabolites into stable and non-reactive storage forms with increased water solubility and the reactive site of the toxin is blocked by the addition of the sugar residue, leading to a reduced toxicity for the plant (Jones and Vogt 2001).

Miller and Arnison (1986) observed that the FHB resistant wheat cv. Frontana was able to metabolise 18% of ^{14}C deoxynivalenol added to suspension cultures while the FHB susceptible cv. Casavant converted only a small amount (<5%) of ^{14}C DON. In the Frontana cultures, three derived products were formed and one of them was identified as a possible glycoside of deoxynivalenol based on its molecular weight. However, this product was not fully characterised at the time of the study. Savard (1991) was the first to chemically synthesise a glycoside derivative of deoxynivalenol. Thus, with standards available, analytical methods could be developed and the presence of conjugated mycotoxins could be investigated in plant extracts. First, a glycoside of deoxynivalenol was isolated from maize cell suspension cultures incubated with DON (Sewald et al. 1992). Poppenberger et al. (2003) identified an UDP glycosyltransferase in *Arabidopsis thaliana* able to catalyse glycosylation of DON and showed that the glycosylated form had a strongly reduced capacity to inhibit protein synthesis. Later, the natural occurrence of glycoside derivatives of deoxynivalenol was reported in artificially (Berthiller et al. 2005; Dall'Asta et al. 2005) and naturally (Berthiller et al. 2005) infected cereals. In their study, Lemmens et al. (2005) even established a close relationship between the ratio of DON glycoside/DON and DON resistance in DON-treated wheat. Moreover, in their report, QTL analysis linked DON resistance and *Qfhs.ndsu-3BS*, QTL hypothesised to encode a DON-glycosyltransferase or to regulate the expression of such an enzyme. In such DON-resistant cultivars, trichothecenes seem to accumulate mainly as DON glycoside and in addition, the total content of DON+DON glycoside appears one order of magnitude lower in the resistant lines compared with the sensible ones (Lemmens et al. 2005). New wheat cultivars with increased glycosyltransferase activity can now be selected by introgression of the *Qfhs.ndsu-3BS* QTL into sensible cultivars.

Detoxification of trichothecenes by glycosylation leading to so-called masked mycotoxins could be an important natural mechanism used by plants for resistance to mycotoxin accumulation. A mechanism leading to zearalenone glycosylation has also been described (Schneweis et al. 2002) showing that it is not limited to trichothecenes. However, although glycosylated compounds appear to be less toxic (Poppenberger et al. 2003; Wu et al. 2007), the possibility that they can be re-converted into the original toxic form by hydrolysis during food/feed processing or in the digestive tract of humans and animals cannot be excluded (Gareis et al. 1990). The masked mycotoxins are often not detected when using the routine methods of mycotoxin analysis (Berthiller et al. 2005). Because of the increased water solubility of glycosylated compounds, the solvent generally used in the extraction process is not adapted for an efficient extraction of the conjugated mycotoxins, and in consequence, they can get lost during the clean-up process (Berthiller et al. 2005). Since very sensitive LC-MS/MS methods are now available for structural characterisation and quantification of trichothecene glycosides (Berthiller et al. 2005), an emphasis should be placed on tracking down these masked mycotoxins in field sample analysis.

Acetylation and de-epoxidation: additional chemical modifications that reduce toxicity of TCT B

Based on the relationship between structure and toxicity of trichothecenes, acetylation and de-epoxidation are two possible mechanisms that could reduce the toxicity of trichothecenes (Eriksen 2003; Kimura et al. 2006). Can these strategies be used in plants to protect themselves against trichothecene accumulation?

Acetylation has been described as a trichothecene detoxification process used by *Fusarium* species to protect themselves from their own toxins. First, Kimura et al. (1998) isolated the *Tri101* gene from *F. graminearum*, which encodes a trichothecene 3-*O*-acetyltransferase catalysing the transfer of an acetyl group to the C-3 hydroxyl group of trichothecenes. The authors showed that the 3-*O*-acetylation of trichothecenes led to a reduced toxicity on *in vitro* protein synthesis in the rabbit reticulocyte lysate translation system. The acetylated forms 15-ADON and 3-ADON have generally been reported as being less toxic for animals and humans (Atkinson and Miller 1984; Eriksen et al. 2004). McCormick et al. (1999) observed that expression of *Tri101* in the yeast species *Saccharomyces cerevisiae* and *Schizosacchar-*

omyces pombe increased their resistance to trichothecenes. Since trichothecene production has been suggested as a virulence factor, Kimura et al. (1998) hypothesised that transgenic plants expressing *Tri101* could contribute to the control of FHB by decreasing *Fusarium* aggressiveness. Various studies with wheat (Okubara et al. 2002), barley (Manoharan et al. 2006), and rice (Kimura et al. 2006; Ohsato et al. 2007) have shown that the expression of a transgenic *Tri101* gene can reduce DON accumulation or FHB symptoms in greenhouse tests but this mechanism was not effective in field experiments (Okubara et al. 2002; Manoharan et al. 2006). To date, no reports on the natural occurrence of trichothecene acetylation as a detoxification process in plants have been published.

There are conflicting observations concerning the phytotoxicity of acetylated DON to cereal plants. In a study on rice seedlings, Ohsato et al. (2007) reported that DON was not phytotoxic to transgenic lines expressing *Tri101*. In contrast, other data reported that acetylated DON was nearly as toxic as DON for cereal plants (Wang and Miller 1988; Wakulinski 1989; Bruins et al. 1993; Eudes et al. 2000). According to Ohsato et al. (2007), the apparent toxicity of 3-ADON observed in studies mentioned earlier with non-transgenic plants may be attributed to C-3 de-acetylation inside the cells. According to Mitterbauer and Adam (2002), the efficiency of overexpression of an acetyl transferase in plants could be severely limited by the reconversion of the acetylated precursors to DON by plant de-acetylases. This could be experimentally demonstrated by tracing the re-conversion of 3-ADON applied to plants to DON. In contrast, Kimura et al. (2006) supported the idea that transgenic expression of *Tri101* could continuously eliminate the C-3 deacetylated trichothecenes from plant cells. More studies are needed to resolve the ambiguities concerning the phytotoxicity and stability of acetylated derivatives of DON in cereals and the problems concerning the stability of expression of *Tri101* in transgenic plants before *Tri101* can be part of a biotechnological application. Recent work reported a significant difference in the catalytic properties of *Tri101* orthologs from *F. sporotrichioides* and *F. graminearum* to inactivate DON (Garvey et al. 2007). New forms of acetyl transferase with an increased efficiency to acetylate the DON and which can be stably expressed *in planta* have to be investigated in order to generate transgenic plants that could be substantially protected against FHB and DON accumulation under field conditions.

Trichothecenes constitute a family of sesquiterpenoids with a 12,13 epoxide ring, which confers the toxicity to these molecules (Eriksen 2003). The epoxide reduction corresponds to the removal of the oxygen from the epoxide group to yield a carbon–carbon double bond. Eriksen et al. (2004) used a cell toxicity test to show that the IC50 values for the DON and NIV de-epoxides were respectively 55 and 54 times higher than for the corresponding toxins. Other studies showed that de-epoxide metabolites of the type A trichothecenes T-2 toxin and diacetoxyscirpenol were less toxic than the corresponding toxins with an intact epoxide ring (Swanson et al. 1987, 1988). However, no de-epoxide forms of trichothecenes have been reported yet *in planta*. Although phytotoxicity tests of de-epoxide trichothecenes on cereals have not been performed, transgenic strategies using expression of a gene encoding an enzyme able to de-epoxidise the trichothecene molecule could be of great interest for reducing toxin toxicity. As recently reported by Binder (2007), several authors previously described a de-epoxydation reaction of ruminal or intestinal flora. Binder et al. (2000) were able for the first time to isolate out of bovine rumen fluid a pure bacterial strain of a new species of the genus *Eubacterium* which was able to detoxify trichothecenes. An epoxidase from this strain can enzymatically reduce different type A trichothecenes and deoxynivalenol to non-toxic de-epoxide metabolites (Fuchs et al. 2002; Schatzmayr et al. 2006). However, the genes involved in the detoxification pathway remain to be identified.

Inhibition of *Fusarium* trichothecenes biosynthesis by kernel compounds

Another alternative strategy to limit mycotoxin content in grains consists of reducing their biosynthesis during the growth of the plant. We previously raised the hypothesis that resistance to *Fusarium* trichothecene accumulation in some durum wheat cultivars could be explained by a particular biochemical composition of the kernel, rich in specific endogenous compounds able to reduce trichothecene biosynthesis (Pinson-Gadais et al. 2007). Several studies indicated an inhibitory effect of plant secondary metabolites on mycotoxin production. These include both constitu-

tive compounds of kernels and compounds induced in response to pathogen infection. Trichothecenes are synthesised from trichodiene by a series of oxygenations known to require molecular oxygen (Desjardins et al. 1993). Therefore, changes in the oxidative parameters of the nutrient source, the kernels, can interfere with the secondary metabolism of the fungus and modulate the levels of trichothecene production (Ponts 2005).

Different compounds with antioxidant properties, like phenolic compounds, peptides or carotenoids, and with pro-oxidant properties, like hydrogen peroxide or linoleic acid-derived hydroperoxides, have been presumed to modulate biosynthesis of mycotoxins (Burow et al. 1997; Huang et al. 1997; Norton 1997; Hua et al. 1999; Ponts et al. 2006). Here, we review different studies reporting *in vitro* effects of such compounds on biosynthesis of trichothecenes.

Secondary metabolites with pro-oxidant properties

In planta, substrate composition may be greatly modified upon invasion by pathogens, triggering several defence mechanisms in the host. Among the broad range of defence responses, the generation of reactive oxygen species, such as hydrogen peroxide (H_2O_2) is one of the earliest events (Repka 1999; Kachroo et al. 2003). This molecule orchestrates the plant hypersensitive disease resistance response (Levine et al. 1994). In the interaction between *F. graminearum* and wheat, proteins with antioxidant function are observed 5 days after inoculation suggesting an oxidative burst of H_2O_2 inside the infected tissues (Zhou et al. 2005). Because many oxidation steps are involved in trichothecene biosynthesis, the very strong oxidant characteristics of H_2O_2 may interfere with fungal metabolism and modulate toxin yield (Ponts 2005). Previous data indicate that DON production by *Fusarium* requires weak oxidant conditions (Miller and Blackwell 1986). However, our recent *in vitro* experiments showed that exogenous H_2O_2 leads to enhanced DON/ADON production by *F. graminearum* (Ponts et al. 2003, 2006) and up-regulates expression of various *Tri* genes involved in trichothecene biosynthesis (Ponts et al. 2007). Relieving the fungus from H_2O_2 stress by addition of catalase in the medium leads to a significant down-regulation of these genes and a strong decrease in trichothecene biosynthesis (Ponts et al. 2007). This result reinforces the hypothesis that oxidative conditions encountered by *F. graminearum* during its development on the spike can be a determinant factor in the level of induction of toxin biosynthesis. Thus, the efficiency of the oxidative burst will not only act on the development of the fungus in the infected kernels but also have an effect on the level of the virulence factors, the trichothecenes produced by the fungus. This may explain in part the difference observed between various wheat cultivars in levels of resistance to FHB and of mycotoxin accumulation. In addition, Ponts et al. (2006) observed that the regulation of TCT B accumulation induced by an oxidative stress may be compound dependent, as paraquat, another pro-oxidant molecule, inhibits their production. The results also suggested that H_2O_2 effect on TCT B production could be chemotype dependent, as mycotoxin accumulation in liquid culture of a nivalenol and fusarenone X-producing *F. graminearum* strain was not affected by the H_2O_2 treatment. From this study on the effects of pro-oxidants on trichothecene synthesis, the authors concluded that the difference in *F. graminearum* virulence levels could partially be explained by an adaptation of *Fusarium* to H_2O_2 stress.

The plant lipoxygenase (LOX) pathway produces metabolites implicated in pathogen resistance (Gardner 1991). It has been shown with *Aspergillus* species that oxidation products of lipids, induced by stress conditions, can also affect aflatoxin biosynthesis (Fabbri et al. 1983; Fanelli and Fabbri 1989). Several *in vitro* studies demonstrated an effect of LOX products on aflatoxin production, including inhibitory effects (Goodrich-Tanrikulu et al. 1995; Burow et al. 1997) but also activating effects (Fabbri et al. 1983; Passi et al. 1984; Fanelli et al. 1989). Burow et al. (1997) showed that 13S-hydroperoxide (13S-HPODE), one of the LOX-derived metabolites from linoleic acid, can decrease aflatoxin biosynthesis *in vitro* while 9S-HPODE did not. Derivatives of 13S-HPODE like methyl jasmonate (Goodrich-Tanrikulu et al. 1995; Vergopoulou et al. 2001) or aldehyde products of 13S-HPODE (Castoria et al. 1989; Zeringue et al. 1996) can also modulate aflatoxin production. *In planta*, past studies showed a relationship between the percentage of polyunsaturated fatty acids of lipid extracts from seeds and aflatoxin production (Fabbri et al. 1983; Passi et al. 1984). The authors attributed this result to the fact that

polyunsaturated fatty acids are more easily peroxidisable than the mono-unsaturated fatty acids. These results suggest that the activity of the plant lipoxygenase system may influence mycotoxin production. Regarding trichothecenes, there are few studies on the effect of the plant lipoxygenase pathway on their production. A recent *in vitro* experiment performed in our laboratory showed that 13S-HPODE can activate TCT B production by *F. graminearum* (Ponts 2005). However, this is a preliminary result and further studies are necessary to clarify the effect of LOX products on trichothecene production.

Secondary metabolites with antioxidant properties

Since plant metabolites with pro-oxidant properties have a strong effect on trichothecene biosynthesis, we could reasonably suppose that plant secondary metabolites with antioxidant properties may also have a modulator effect on trichothecene biosynthesis. Among the secondary metabolites of cereal kernels with antioxidant properties, carotenoids, peptides, and especially phenolic compounds have been studied for their efficiency to reduce mycotoxin biosynthesis (Norton 1997; Bily 2003; Chen et al. 2006).

Literature data concerning the effect of carotenoids on mycotoxin biosynthesis are scarce. Some studies report an inhibitory effect of carotenoids on aflatoxin biosynthesis by *Aspergillus* (Norton 1997; Wicklow et al. 1998). As the amount and type of carotenoids present in cereal grains are subject to variations between species, cultivars and environmental growing conditions (Hentschel et al. 2002; Konopka et al. 2006; Abdel-Aal et al. 2007), more studies evaluating the effect of these antioxidant molecules on TCT B production by *Fusarium* species are needed. Preliminary *in vitro* work from our laboratory suggests an inhibitory effect of lutein extracted from durum wheat bran on TCT B production by *F. culmorum,* and also of a carotenoid extract from maize kernels on fumonisin production by *F. verticillioides* (unpublished data).

Plant seeds contain high levels of many antimicrobial and antifungal proteins including pathogenesis-related proteins, enzyme inhibitors, hydrolytic enzymes like chitinases and glucanases, ribosome-inactivating proteins, lipid transfer proteins, and small peptides such as defensins, lectins, and thionins (Apel et al. 1990; Vigers et al. 1991; Duvick et al. 1992; Van Loon and Van Strien 1999; Muthukrishnan et al. 2001). Such antifungal defence compounds may be expressed constitutively by the host plants or induced upon pathogen infection (Fritig et al. 1998). Small peptides and proteins have been characterised in maize and wheat kernels that have antifungal activity against *Fusarium* (Duvick et al. 1992; Huynh et al. 1992a, b; Egorov et al. 2005) and *Aspergillus* species (Neucere and Godshall 1991; Chen et al. 1998). Some authors have suggested a relationship between maize kernel resistance to *Aspergillus* and expression of antifungal proteins (Huang et al. 1997; Chen et al. 1998, 2006). Doohan et al. (2000) identified antifungal proteins from seed extracts of the FHB-resistant wheat cv. Arina. According to the molecular mass of this antifungal protein, they suggested chitinases, ribosome-inactivating proteins, permatins or glucanases as possible candidates. Strikingly, few studies attempting to understand the effect of these antifungal proteins or peptides from kernels on mycotoxin production have been reported. The inhibition of *Aspergillus* aflatoxin production by maize seed proteins (Nagarajan and Bhat 1972; Huang et al. 1997; Chen et al. 2006) or cotton seed proteins (McCormick et al. 1988) has been examined. Thus, as with carotenoids, studies evaluating the effect of antifungal seed proteins and peptides on TCT B production by *Fusarium* species are required.

Among the secondary metabolites with antioxidant properties, phenolic compounds have often been described as inhibitors of both fungal growth and mycotoxin production (Guiraud et al. 1995; Hua et al. 1999). Phenolic compounds are present in all plants, and they or their oxidation products have been shown to have a role in disease resistance (Friend 1981; Matern and Kneusel 1988, Nicholson and Hammerschmidt 1992). Some phenolic compounds are linked to various cell wall components (Wallace and Fry 1994). In response to pathogen infection, they can be either released from the cell wall or massively synthesised by the plant, accumulating rapidly at the infection site (Nicholson and Hammerschmidt 1992). They can thus operate in defence response through inactivation of fungal enzymes or reinforcement of plant structural components (Bell 1981), such as the host cell wall which acts as a mechanical barrier against the pathogen, limiting the diffusion of toxins released by the pathogens into the host cell and the flux of nutrients from the host cell to the pathogen (Siranidou et al. 2002).

During the infection process, the fungal mycelium progresses from the surface of the kernel to the endosperm (McKeehen et al. 1999). Hence, the biochemical organisation of the outer layers can interact with the penetration of *Fusarium* species (McKeehen et al. 1999). The cell walls of kernels are rich in phenolic compounds (Naczk and Shahidi 2004). Phenolic compounds have been shown to inhibit the *in vitro* growth and reproduction of a wide array of fungal genera (Guiraud et al. 1995; Aziz et al. 1998). Several reports suggest that resistance to *Fusarium* is correlated with kernel phenolic content in maize at maturity (Reid et al. 1992; Assabgui et al. 1993; Bily et al. 2003) and wheat (McKeehen et al. 1999; Siranidou et al. 2002). In these studies, the phenolic compounds associated with FHB resistance are flavonoids (Reid et al. 1992) and phenolic acids, especially ferulic and *p*-coumaric acid (Assabgui et al. 1993; McKeehen et al. 1999; Siranidou et al. 2002; Bily et al. 2003). In response to inoculation with *F. graminearum*, Reid et al. (1992) observed that the concentration of phenolic compounds in the silk tissue increased in the resistant maize cultivars and decreased in the susceptible ones. In the latter case, the authors explained this decrease in phenolic compounds either by their degradation through the pathogen or by an inhibition of their biosynthesis through fungal trichothecenes, which are known as potent inhibitors of protein biosynthesis. In the former case, Reid et al. (1992) suggested that either the cultivar is resistant to the toxin or may be able to detoxify it. Thus, in the resistant cultivar, the metabolism is not affected by the toxin and the plant reacts by synthesising more phenolic compounds that are targeted to the infection site. This led the authors to hypothesise that this increase in phenolic compounds that seems to be induced in resistant genotypes, may play a crucial role in resistance to trichothecene accumulation.

Several *in vitro* experiments have described an inhibitory effect of various phenolic compounds on mycotoxin production. The inhibitory effect of different phenolic compounds on aflatoxin production by *Aspergillus* species has been widely reported in the literature (Chipley and Uraih 1980; Mallozzi et al. 1996; Hua et al. 1999; Norton 1999; Nesci and Etcheverry 2006). Interestingly, several natural phenolic compounds (quinones, coumarins, flavonoids, phenolic acids) isolated from various plants were shown to be potent inhibitors of aflatoxin formation (Lee et al. 2001; Mahoney and Molyneux 2004). Similarly, phenolic acids were found to be effective inhibitors of fumonisin B1 production by *F. verticillioides* (Beekrum et al. 2003). Miller et al. (1996) and Desjardins et al. (1988) reported an inhibitory effect of respectively 4-acetylbenzoxazolin-2-one (4-ABOA) and naturally occurring flavonoids and furanocoumarins on TCT B biosynthesis. A more recent study suggested that soluble phenolic compounds of maize germ could reduce trichothecene accumulation (Bakan et al. 2003). The same year, Bily (2003) reported that caffeic acid, ferulic acid, *p*-coumaric acid, vanillic acid and 3-hydroxybenzoic acid can reduce TCT B production by *F. graminearum*. Interestingly, in the last two studies, toxin inhibition by phenolic compounds occurred at concentrations where fungal growth was not affected, suggesting that the effect of phenolic compounds on mycotoxin accumulation was not the result of a fungitoxic effect but a specific effect on toxin biosynthesis. Bily (2003) showed that ferulic acid acted on the TCT B biosynthesis pathway but not on the steps involved in the initiation of production. This is in agreement with a previous report from Desjardins et al. (1988) showing that in cultures of *F. sporotrichioides* treated with flavones or furanocoumarins, accumulation of the final toxin decreased while there was an increased accumulation of trichodiene (the first intermediate in trichothecene biosynthesis). Their interpretation was that conversion of trichodiene to oxygenated trichothecene was blocked by the phenolic compounds tested, therefore impeding the production of any toxic trichothecenes with a 12, 13-epoxide group.

As phenolic acid profiles vary between wheat varieties (Lempereur et al. 1997; Moore et al. 2006; Mpofu et al. 2006), they could be good candidate compounds for the reduced TCT B biosynthesis trait in kernels. Recent work in our laboratory showed that a phenolic fraction extracted from wheat bran exhibited a strong inhibitory effect on *in vitro* DON/ADON biosynthesis by *F. culmorum* and suggested a possible role of phenolic acids in resistance to trichothecene accumulation in durum wheat.

Conclusion

Up to now, most cereal breeding approaches to develop varieties with high levels of FHB resistance

have focused on selection for reduced FHB symptoms. As trichothecenes have been shown to act as virulence factors, an alternative strategy to limit FHB and its effects could consist of reducing the mycotoxin contamination of kernels. This would limit fungal infection by decreasing or removing the fungal virulence factors. An improved breeding strategy would be to select for varieties exhibiting natural mechanisms of plant resistance to both fungal spread and mycotoxin accumulation.

Cereals can deploy various natural mechanisms to reduce mycotoxin accumulation. Among these natural mechanisms, we reported here that cereal plants can promote degradation or detoxification of mycotoxins (type V-1 resistance) or prevent their biosynthesis (type V-2 resistance).

Concerning the detoxification mechanisms, glycosylation of TCT B is a recently described natural process encountered in wheat. Based on the structure and toxicity of trichothecenes, two other mechanisms of chemical modification can reduce the toxicity of TCT B: acetylation and de-epoxidation. To our knowledge, the occurrence of these two mechanisms has not yet been reported to occur naturally in cereals. Studies with transgenic plants containing a *Fusarium* gene that acetylates TCT B as a detoxification process have been promising. However, before using heterologous detoxification genes in cereals, it may be worthwhile to investigate first the phytotoxicity and the stability of acetylated and de-epoxyde derivatives of TCT B. If proven relevant, the genes conferring the ability to detoxify trichothecenes could be introduced in susceptible genotypes, using either marker-assisted selection or transgenic approaches.

Plants can also prevent mycotoxin biosynthesis by the fungus. In fact, some compounds, constitutive in the kernels or induced as a response to pathogen attack, can effectively reduce mycotoxin production. Literature data describing kernel phenolic compounds that inhibit biosynthesis of various mycotoxins, including trichothecenes, are numerous. It is possible that many other kernel compounds can interfere with mycotoxin metabolism. These include carotenoids, antifungal peptides, hydrogen peroxide and oxidation products of lipids. Unfortunately, many studies that showed an inhibitory effect of some of these compounds on fungal growth have not looked at their effect on mycotoxin biosynthesis. Recent efforts in this direction from different groups, including our laboratory, appear to be very promising and suggest that, at least *in vitro*, various kernel compounds can greatly modulate accumulation of trichothecenes. Such studies have to be extended to confirm these results and to identify new kernel compounds that might inhibit mycotoxin biosynthesis. In this review, we reported different compounds that modulated *in vitro* TCT B biosynthesis by *Fusarium*. However, the question remains whether these compounds are effective *in planta*. An exciting challenge for the near future is to answer this question as this could open the way for selecting varieties exhibiting appropriate kernel composition, rich in such endogenous compounds.

Thus, a strategy of 'pyramiding' resistance QTL, genes for detoxification, and improved composition in kernel compounds limiting trichothecene accumulation could lead to a palette of new varieties, increasing the choices for cultural practices aiming at reducing the mycotoxin risk in cereals.

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