Deoxynivalenol contamination in Tunisian barley in the 2009 harvest

F. Bensassiab, I. Rjibac, A. Zarrouka, A. Rhoumad, M.R. Hajlaouib and H. Bachaa*

aLaboratory for Research on Biologically Compatible Compounds, Faculty of Dentistry, Rue Avicenne, 5019 Monastir, Tunisia; bLaboratory of Plant Protection, National Institute for Agricultural Research, INRA Tunisia, Rue Hedi Karray, 2049 Ariana, Tunisia; cLaboratory of Biochemistry, UR 'Human Nutrition and Metabolic Disorders' Faculty of Medicine, 5019 Monastir, Tunisia; dResearch Unit of Plant Protection and Environment, Olive Tree Institute, BP 208, Mahrajene City, Tunis 1082, Tunisia

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In Tunisia, barley is commonly used in human consumption in a variety of food forms. In this regard, a high quality of this agricultural product is always demanded by consumers. A survey of the natural occurrence of deoxynivalenol (DON), the most common Fusarium mycotoxin in small grain cereals, in barley harvested in the main cropping regions in Northern Tunisia in the 2009 harvest was conducted. A total of 72 samples were analysed for DON using high-performance liquid chromatography (HPLC) with a UV visible detector set at 220 nm. Between 36% and 100% of the samples were positive for DON with averages ranging from 1.2 to 2.4 mg kg\(^{-1}\). A positive correlation between DON levels and temperature was seen; on the other side no correlation between DON contents and rainfall was observed. In this study we notably showed the effect of regions on DON contamination.

Keywords: cereals; mycotoxins; trichothecenes

Introduction

The agricultural system is much diversified in Tunisia, including mostly cereals, mainly located in the Northern part of the country. Among cereals, barley is one of the most important, covering about 34% of the whole cultivated cereal surface in 2009. It is commonly used in human consumption in a variety of food forms after being transformed (Slavin et al. 2000). In Tunisia, there is a traditional alimentary habit for barley consumption. Indeed, people during the fasting month (Ramadhan) consume large amounts of barley grains in a local food called ‘El Frik’, which is taken as a soup. Moreover, barley is used by Tunisian manufacturers in the brewing industry.

Barley grains are clinically proven to be efficient sources of soluble and insoluble dietary fibre which have several health benefits such as reducing: plasma cholesterol, the glycaemic index and the risk of colon cancer (Brennan and Cleary 2005; Izydorczyk et al. 2008). Unfortunately, this cereal grain is most of the time a target for fungal infection in the field. The most important moulds associated with barley are Fusarium species which cause diseases across the world (Bottalico and Perrone 2002). The most significant is Fusarium head blight (FHB) (McMullen et al. 1997; Spunarova et al. 2002), which is predominantly caused by \(F.\) graminearum, \(F.\) culmorum, \(F.\) avenaceum and Microdochium nivale (Edwards 2004). Fusarium infections can render barley commercially unusable for feed, food and malt production (Hysek et al. 2002) because many of these Fusarium pathogens may produce mycotoxins (Desjardins 2006) which are unavoidably ingested by humans or animals.

Deoxynivalenol (DON), a representative mycotoxin of the trichothecene B group, is one of the most widespread cereals contaminants worldwide (Larsen et al. 2004; Krysinska-Traczyk et al. 2007). Several studies have showed mainly the occurrence of DON in barley and its derivative commodities such as malt and beer (Schwarz et al. 1995; Maenetje and Dutton 2007). In fact, DON is efficiently transferred to barley products owing to its relatively good thermal stability (Schwarz et al. 1995). Nowadays, no regulations are available concerning DON levels in cereal-based foodstuff such as beer; maximum limits are fixed only for raw barley. Indeed, the European Community (Regulation 1881/2006) has set the DON limit to 1250 \(\mu\)g kg\(^{-1}\) for unprocessed cereals (other than maize, durum wheat and oats) (European Commission 2006). Grains highly contaminated with DON constitute a serious threat to consumers (Pestka and Smolinski 2005).

There are many factors involved in mycotoxin contamination, but the most important is the climate.
Numerous studies have showed that barley infestation with *Fusarium* spp. and consequently DON accumulation are chiefly dependent on climatic conditions (Salas et al. 1999; Pan et al. 2007). Meteorological conditions in Tunisia favour such a situation: mould infestation and mycotoxin synthesis (Hadidane et al. 1985; Bacha et al. 1988). Therefore, the main aim of this study was to survey the natural occurrence of DON in barley grains using an efficient HPLC/UV method and to relate this with climatic conditions in Tunisia, especially temperature and the amount of rainfall during the spring of the crop year 2009, which is revealed to be the most successful crop year this decade in terms of productivity and quality.

**Materials and methods**

**Materials**

All reagents used in the analysis were of analytical grade purity. The used solvents were obtained from Fisher Scientific (Fisher Chemicals HPLC, France). For the clean-up step, DONPREP HPLC immunoaffinity columns (IAC) were supplied by R-Biopharm Rhone Ltd (R-Biopharm, Germany).

DON standard was purchased from Sigma Chemicals (St. Louis, MO, USA). It was dissolved in acetonitrile at concentrations of 5 mg ml\(^{-1}\) and stored at \(-20^\circ\text{C}\). Composite working standard solutions were prepared by diluting DON standard stock solution (5 mg ml\(^{-1}\)) in the mobile phase (acetonitrile:water, 5:95, v/v) and kept at 4 °C.

**Sampling**

Barley grains for human consumption were collected during the crop year 2009 from the major cropping areas in Tunisia including Jendouba, Beja, Bizerte, Zaghouan and Ben Arous. A total of 72 samples of barley were taken directly from farmers’ fields immediately after the harvest and before being delivered to the silos. All barley varieties used in this survey were planted in September and harvested in July. The total weight of the composite sample was 3 kg. After homogenisation, a subsample of approximately 1 kg kernels was used before the determination of DON. Samples were packed in plastic bags and stored at 4°C until laboratory use.

Meteorological data (temperature and rainfall) were provided by the National Institute of Meteorology (Tunisia), and due to minimum differences the means are showed in Figure 1.

**Sample preparation**

The method used for DON extraction from the barley samples was adapted according to both the IACs manufacturer’s instructions and Kotal and Radova (2002), with some specific modifications. A total of 25 g of the finely ground sample were placed in a blender jar with 200 ml of HPLC-grade water. The mixtures were shaken for 30 min. After shaking, samples were centrifuged for 15 min at 4000 rpm. A total of 2 ml of the supernatant were passed through the immunoaffinity column (IAC DONPREP) at a flow rate of 1 drop/s\(^{-1}\). After washing the column twice with 5 ml of distilled water, the toxin was slowly eluted with 5 ml of 100% methanol. The eluate was evaporated to dryness. The dried residue was dissolved in 1 ml of mobile phase (acetonitrile:water, 5:95, v/v) then stored at 4°C until HPLC analysis.

**Procedure for HPLC**

DON analysis was performed using the HPLC Agilent 1100 model equipped with a quaternary isocratic pump (Quart-Pump G1311A) and UV visible detector (UV-LAMBDA 1010 ICS) set at 220 nm packed with a C\(_{18}\) stainless steel reverse phase column (Spherisorb ODII, Leonberg) column. A total of 70 µl of the extract were injected onto the UV-HPLC System set at a wavelength of 220 nm. The mobile phase was delivered at a constant flow rate of 0.7 ml min\(^{-1}\). DON levels were obtained by measurement of peak area at DON retention time compared with the standard solutions used for the calibration curve (\(r = 0.999\)). The Data Station was Agilent ChemStation.
Statistical analysis

Data were subjected to analysis of variance (ANOVA) with SPSS 13 Microsoft version. The significance of mean differences between regions was determined using Duncan’s multiple range test, and responses were judged significant at $p < 0.05$. Due to differences in the number of samples between regions, the Type III sum of squares test was used.

The Spearman test of association was applied in order to evaluate the correlation between DON levels and climatic factors (rainfall and temperature).

Results and discussion

Method validation

Validation consists of the determination of the recovery rate, linearity, reproducibility, repeatability, and limits of detection and quantification of the whole method of DON analysis in barley. To determine the recovery rate, blank grains were experimentally spiked with two different concentrations of DON: 15 and 3.5 mg kg$^{-1}$. A triplicate of each concentration was analysed. Recoveries were 65% and 83% for 15 mg kg$^{-1}$ and 3.5 mg kg$^{-1}$ DON, respectively. The average relative standard deviation (RSD) of the whole method was 9%.

Chromatograms obtained showed that DON has a retention time ($R_t$) of nearly 7.8 ± 0.3 min. Linearity was confirmed using the calibration curve for each DON concentration. It was linear in the range 0.5–20 µg ml$^{-1}$ (20, 10, 5, 2, 1 and 0.5 µg ml$^{-1}$) with a coefficient of correlation $r = 0.999$.

The limit of detection (LOD) was defined as the smallest DON amount detected with at least a 3:1 signal-to-noise ratio for which the method was validated; it was 0.03 mg kg$^{-1}$. Based on the LOD and LOQ values, the analytical performance of the method seems to be high comparatively with other studies (Cahill et al. 1999; Kotal and Radova 2002; Hafner et al. 2007).

Analysis of barley samples

The occurrence of DON in the investigated samples is summarised in Table 1. From 72 samples, about 57% were positive for DON with frequencies between 36% and 100% according to the regions with levels ranging between 0.5 and 3.6 mg kg$^{-1}$ (Figure 2). In some samples, DON levels exceeded the permissible maximum level fixed by European Commission Regulation 1881/2006 to 1.25 mg kg$^{-1}$ for barley intended for human and animal consumption (European Commission 2006). The natural occurrence of DON in barley has been reported in several countries (Molto et al. 2000; Tutelyan 2004). Similar frequency and DON levels as in this study were reported in the southwest of Uruguay in 1996, 1998 and 1999 (Pan et al. 2007). Several other investigations noticed high DON contamination in freshly harvested barley samples such as from Germany and Great Britain (Tanaka et al. 1988; Müller et al. 1997; Meister 2009; Turner et al. 2009).

In general, the contamination levels were low in all barley samples when compared with levels obtained in durum wheat in Northern Tunisia during the crop year 2009 (unpublished data). This difference depends mainly on the category of the gramineous host infected by the FHB. FHB is known to cause significant yield losses and quality reduction, mainly due to the ability of *Fusarium* species to produce mycotoxins (Schwarz et al. 2001). Indeed, FHB pathogens are less aggressive
on barley than on wheat. These *Fusarium* species may grow in a saprophytic manner or produce low levels of mycotoxins on barley contrarily to other cereal grains (Salas et al. 1999; Brennan et al. 2003; Osborne and Stein 2007). In such a case, the role of the host cannot be neglected. Previous investigations conducted in many countries such as Norway, the UK and Hungary are in agreement with our observation that barley was most of the time less contaminated with the trichothecenes such as DON than other cereals (Langseth and Rundberget 1999; Prickett et al. 2000; Rafai et al. 2000). In the literature the majority of the research on FHB was concerned with wheat, which is known to be the most susceptible to FHB and mycotoxin contamination throughout the world (Bottalico 1998; Windels 2000; Birzele et al. 2002; Edwards 2007). In fact, barley was known as a non-susceptible host to FHB infection, but further research has shown that it was as susceptible as wheat to FHB and consequently mycotoxin biosynthesis (Tekauz et al. 2000). According to Edwards (2007), this susceptibility may be due to a fundamental shift in the pathogen population or particularly changes in the varieties grown.

The DON amounts in the different prospected regions decreased as follows: Bizerte > Ben Arous > Zaghouan > Beja > Jendouba, with means of 2.2, 2.0, 1.8, 1.2 and 0.4 mg kg$^{-1}$, respectively. These differences may be attributed principally to the impact of location, weather conditions, fungal sporulation, cultural practices and cultivar properties on DON accumulation (Glenn 2007).

According to Duncan’s test, the prospected regions were classified in three groups as shown in Table 1. Indeed, we noted that only the region of Jendouba was lowly contaminated with DON; Beja was moderately contaminated; whereas Bizerte was the most contaminated and also included the highest DON content,
about three-fold higher than the European limit. The results clearly showed the effect of regions on DON accumulation in barley \( (p < 0.05) \). The impact of location was also observed in Northern America, where DON contents varied between regions (Trucksess et al. 1995; Salas et al. 1999).

According to the Spearman test, when comparing mean temperature with the average DON levels either in positive samples or in the whole region, a high positive correlation existed for the March–May period when flowering occurs with \( r = 0.984 \) and 0.981, respectively \( (p < 0.01) \). In contrast to temperature, no correlation was shown between total rainfall and DON levels, even if in Uruguay positive correlation was seen between DON contents and both temperature and rainfall (Pan et al. 2007). The absence of correlation between DON and rainfall can be explained by the irregularity of precipitation during spring 2009 (Figure 1).

The production of mycotoxins on crops is highly susceptible to weather factors. When climate change occurs, mycotoxin production is affected (Sanchis and Magan 2004; Miraglia et al. 2009). According to Magan et al. (2003), the climate represents the key agro-ecosystem triggering fungal colonisation and mycotoxin production. During the spring, warm and moist conditions represent the critical period for FHB infection which coincides with the flowering period of barley (Dalcero et al. 1997; Birzele et al. 2002; Bushnell et al. 2003; Shaner 2003). Indeed, the weather conditions around flowering are very important and responsible for the variation in DON amounts during this period (Hooker et al. 2002). Our results are in agreement with previous works showing that temperature, in particular, played an important role in FHB incidence and mycotoxin biosynthesis (Jennings et al. 2004; Isebaert et al. 2009; Paterson and Lima 2010).

It is known that there are both toxigenic and non-toxigenic strains of *Fusarium* which influence the level of mycotoxin in small grains within a single country (Langseth and Elen 1997; Beyer et al. 2005) because many of FHB pathogens do not produce DON (Desjardins and Proctor 2001; Desjardins 2006). For example, the region of Jendouba was the least contaminated; this may be attributed to the dominance of non-DON producer species versus DON producers.

In addition to spore type, the differences between cultivating regions may be explained by different farming practices. Soil cultivation including ploughing, minimum tillage and no till may reduce the *Fusarium* inoculum and subsequently mycotoxin accumulation for the following crop (Obst et al. 1997; Edwards 2004). Besides, the use of a moderate or highly susceptible barley variety may reflect differences in FHB severity and so mycotoxin contents in grains. Indeed, the barley response to infection development may vary hugely according to the genetic resistance to the pathogen and physiological condition of the used variety (Mesterhazy 2003; Osborne and Stein 2007).

To the best of our knowledge, this is the first study showing the natural occurrence of DON in barley produced in Tunisia. In light of the obtained results, the use of contaminated barley suggests a possible contamination of some end products such as beer with DON.

### Conclusion

The results collected in this survey revealed the contamination of unprocessed barley with the *Fusarium* toxin DON at different averages between regions in Northern Tunisia. Although no correlation between DON levels and rainfall was seen in the 2009 harvest, monitoring for DON in barley crops must not be limited to this survey but must be carried out regularly, particularly in crops with heavy rainfall during flowering. The regular screening for mycotoxins is urgent in Tunisia; the situation may reveal a tendency for increasing contamination levels which constitutes a serious economic loss in the industry and a threat to human health.

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