

Behaviour of durum wheat cultivars towards deoxynivalenol content: A multi-year assay in Italy

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Abstract

The deoxynivalenol (DON) occurrence in durum wheat (*Triticum durum* Desf.) kernels is a key food safety issue, hence more and more attempts have been made to increase the availability of resistant cultivars. Within this framework, this work focused on the analysis of the behaviour of several durum wheat cultivars widely grown in Italy in relation to the level of deoxynivalenol contamination in whole milled kernels. A total of 2844 samples were submitted for deoxynivalenol analysis by enzyme-linked immuno-sorbent assay. The statistical analysis by generalised linear models provided a trend ranking of durum wheat cultivars on the basis of the deoxynivalenol contamination level, depending on each cultivation area assayed. This study provided a better understanding of cultivar susceptibility to mycotoxin accumulation in grains. The role of the factor cultivar has clearly emerged. However, other environmental factors, such as growing area, have a great influence on the DON concentration levels in the cultivars tested.

Introduction

Deoxynivalenol (DON) is the most widespread mycotoxin in

small grain cereals; it is mainly produced by toxigenic strains of *Fusarium graminearum* and *Fusarium culmorum*, which are the most important causal agents of fusarium head blight (FHB) disease. DON belongs to the type B group of trichothecenes, which are largely encountered in cereal crops, and the action of this mycotoxin as a virulence factor during fungal pathogenesis is well-known (Arunachalam and Doohan, 2013; Audenaert *et al.*, 2013). Trichothecenes are a large family of chemically related toxic fungal compounds mainly produced by *Fusarium* spp. in cereal grains. These mycotoxins have several toxic effects on eukaryotic cells, including inhibition of protein, DNA and RNA synthesis, increase of the permeability of the intestinal epithelial layer (Yazar and Omurtag, 2008; Gauthier *et al.*, 2013; Antonissen *et al.*, 2014; Pinton and Oswald, 2014). DON may have an emetic effect, but also an immunosuppressive, with the latter also representing a risk for human health (Maresca, 2013; SCF, 2002). Resistance to DON is also considered an important aspect of resistance to FHB (Rocha *et al.*, 2005; Walter *et al.*, 2015).

DON is a powerful tool for *F. graminearum* to grow within the host tissue; the wheat plant can, nevertheless, employ detoxification mechanisms to block the adverse effects of the mycotoxin. Indeed, in addition to the resistance mechanisms that determine the severity of FHB attack on the wheat plant, other mechanisms may influence DON content in kernels, *e.g.*, DON degradation or conjugation and tolerance (Ji *et al.*, 2015). In particular, DON binds covalently to hydrophilic molecules, such as glucose and glutathione (Audenaert *et al.*, 2013; Cirilini *et al.*, 2014).

Several studies are available in literature and report positive correlations between disease incidence and mycotoxin accumulation; however, this issue might be more complex due to a not always positive and significant relationship between wheat varieties, exhibiting high resistance to FHB, and low detected DON levels (Boutigny *et al.*, 2008). Recently, several breeding attempts have been made to combine resistance to pathogen development and resistance to mycotoxin accumulation; however, obtaining new genotypes that are suitable to reduce the level of kernel contamination, and hence limit the risk of human and animal exposition upon food consumption keeps being a major challenge. The complex bases of wheat resistance to FHB are not yet fully understood, however it is known that it is controlled by multiple genes and characterised by large genetic variation. Several studies have been so far successfully carried out to identify traits involved in FHB resistance in soft wheat (*Triticum aestivum* L.); whereas the sources of FHB resistance in durum wheat (*Triticum durum* Desf.) have not been fully identified yet (Buerstmayr *et al.*, 2009, 2012; Jauhar and Peterson, 2009; Lionetti *et al.*, 2015; Ruan *et al.*, 2012). Durum wheat is by far more FHB-susceptible than soft wheat, and its susceptibility to FHB disease and mycotoxin accumulation in kernels is of particular concern in Italy because of its key role in *pasta* production (Bottalico and Perrone, 2002; Boutigny *et al.*, 2008; Foround and Eudes, 2009).

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This work aims at examining the behaviour of several durum wheat cultivars grown in a multi-year period in Italy in relation to deoxynivalenol content detected in kernels. Moreover, it assesses the geographical influence leading to significant genotype-by-area interaction.

Materials and methods

Samples

A total of 2844 durum wheat grain samples, obtained from both varietal trials (Quaranta *et al.*, 2013) and farms, were collected 2006 to 2014.

Samples coming from experimental trials were collected within the Italian durum wheat network. The trials were conducted according to the best agronomic practices, most in use in the different cultivation areas, and did not include fungicide treatments for controlling FHB. The incidence of wheat as a previous crop was around 15% of total samples, but it was not an experimental factor. Each trial was carried out according to a randomised complete block experimental design (RCBD) with three replications (10 m² plots); the same protocol was used for both year and location. Subsequently, grains from the three-replication plots were bulked and a representative whole grain subsample of about 0.5 kg was taken for milling. Regarding the sample obtained from farms at harvest time, grains were randomly collected from each cultivar trial: after homogenisation, one sample of about 5 kg was taken. A subsequent random subsample of 0.3 kg was taken and submitted to milling operation before analysis. The farms in each Region were chosen by experts from local Institutions and were representative of the local durum wheat cropping system. All sampling operations were substantially based on the criteria of representativeness laid down by the European legislation (EU Commission Regulation No. 401/2006; European Commission, 2006b).

The collection of samples took place in different parts of Italy (8 areas) which are classified as durum wheat growing areas: i) North; ii) Centre-East; iii) Centre-West; iv) Central Apennines; v) Southern Apennines; vi) Southern Adriatic Ionian coast; vii) Sicily; and viii) Sardinia (Figure 1). These areas were identified and selected on the basis of criteria, such as geographical position (North, Centre, South and islands), altitude (plain, coast, Apennines), and distance from the sea. These criteria contribute, in fact, to influencing seasonal temperatures, rainfall amount and distribution. To verify possible differences among wheat cultivars grown in different areas and years, samples were grouped in two sub-datasets, and comparisons were made. Dataset A included 1403 durum wheat samples, collected over the three-year period 2012-2014, and comprised 16 cultivars widely grown in Italy. The second dataset (dataset B) comprised 2080 samples of durum wheat, obtained from 7 out of the 16 cultivars and covering a wider time span than dataset A (2006-2008; 2011-2014), for a total of seven years. The seven cultivars were selected because they were constantly available over the seven years. The choice to split the whole dataset in two sub-datasets (A and B) arose from the need to group the cultivars assayed in more homogeneous clusters on the basis of their representative presence in every year and in any of the growing area considered. In Table 1, the cycle length and the genealogy of the cultivars under analysis are reported. The genealogy shows that some cultivars were derived from common progenitors.

Deoxynivalenol analysis

As regards the determination of DON content, representative grain samples were milled (particle size ≤ 1 mm) by 1093 Cyclotec™ Sample mill (FOSS, Hilleroed, Denmark) and submitted to the mycotoxins analyses by enzyme-linked immuno-sorbent assay (ELISA).

Extraction was performed with aqueous deionised water, according to the Ridascreen® DON method (R-Biopharm AG, Darmstadt, Germany), with limit of detection (LOD) of 18.5 µg/kg. The recovery range declared in the method was 85-110%. Data were acquired as mean of double analysis [coefficient of variation $\leq 10\%$]. The Basic Robotic Immunoassay Operator (BRIO, SEAC, Radim Group, Florence, Italy) was used and the absorbance values were read using Sirio-S Microplate Reader (SEAC, Radim Group). The RIDA® Soft Win software (R-Biopharm AG) was employed for quantitation of DON in samples. Distilled water was obtained from Water Purification System Zeener Power I (Human Corporation, Seoul, Korea). The ELISA method employed for the assessment of DON concentration in durum wheat samples had already been submitted to a validation study by comparison with HPLC analysis, within the research project MICOCER (Brera *et al.*, 2009).

Meteorological data

Data on temperature (°C) and rainfall (mm) on April and May (durum wheat anthesis period) were collected from weather stations located nearby the experimental sites during the 7-year period. The weather conditions during anthesis have a large impact on the *Fusarium* ssp. infection involved in FHB disease in wheat (De

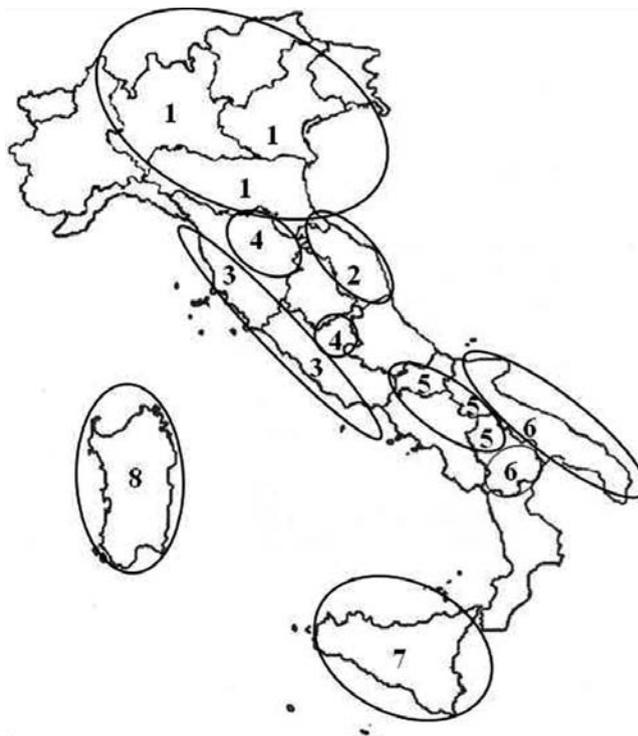


Figure 1. Map of the growing areas of Italy where durum wheat sampling occurred. Numbers are allocated according to the region each area belongs to: 1-North, 2-Centre-East, 3-Centre-West, 4-Central Apennines, 5-Southern Apennines, 6-Southern Adriatic Ionian coast, 7- Sicily, and 8-Sardinia.

Wolf *et al.*, 2003; Xu, 2003; Fedak *et al.*, 2007; Covarelli *et al.*, 2015; Kelly *et al.*, 2015). Climatic conditions consistently affect composition of *Fusarium* species causing FHB in wheat, especially during wheat anthesis, and together with other factors, such as the lack of crop rotation and the use of mineral fertilisers and pesticides, as it is a practice in conventional systems, heavily influence the FHB occurrence (Bernhoft *et al.*, 2012). The impact of FHB can be limited by adopting measures apt to reduce the inoculum and prevent its dispersal, such as cultural, biological, and chemical control and use of resistant varieties (Magan *et al.*, 2002; Czaban *et al.*, 2015).

Statistical analysis

In order to assess the distribution of data concerning the DON concentration (c) in samples, descriptive statistics [*e.g.*, mean, standard deviation (SD), range (minimum-maximum) DON concentration, median, 75th percentile and 95th percentile] were obtained. The values of DON concentration (c) in samples on wet basis were expressed as mean of duplicate analyses (coefficient of variation $\leq 10\%$). As to results below LOD, half detection limits (9.25 $\mu\text{g}/\text{kg}$) were used in the statistical tests.

EC Regulation No. 1881/2006 and EC Regulation No. 1126/2007 (European Commission, 2006a, 2007) lay out the maximum limit of 1750 $\mu\text{g}/\text{kg}$ for DON in unprocessed durum wheat. The values of DON concentration close to 1750 $\mu\text{g}/\text{kg}$, corrected for recovery, were assessed by a discriminating value of 1340 $\mu\text{g}/\text{kg}$ obtained by applying the Horwitz formula for ELISA methods of analysis for expanded measurement of uncertainty as twice of the relative standard deviation (Brera, 2011). However, the use of the aforesaid discriminant value of 1340 $\mu\text{g}/\text{kg}$ should not be understood as an evaluation of sample compliance because the method of analysis employed is not suitable to this purpose.

The influence of genotype (CV), growing area (GA), year (Y) and the two-way interaction CV \times GA on DON contamination was tested using the powerful statistical technique of generalised linear models (GLZ). The mean response changed as a function of the explanatory variables (CV, GA and Y), and it depended on a linear

predictor through a non-linear canonical link function and a Poisson probability distribution. The statistical model was:

$$\log(\text{DON}) = \beta_0 + \beta_1 \tau_i + \beta_2 \delta_j + \beta_3 \gamma_k + \beta_4 \tau_i \delta_j + e_{ijk} \quad (1)$$

where β_0 =mean effect common to all observations; $\beta_{1,2,3,4}$ =unknown regression parameters; τ_i =cultivar (CV); $i=1, \dots, 16$; δ_j =growing area (GA); $j=1, \dots, 8$; γ_k =year (Y); $k=2012, \dots, 2014$; e_{ijk} =error term.

The goodness of fit for the GLZ model could be based on the deviance statistics, approximated by a chi-square distribution. We used the log-likelihood value to measure the goodness of fit of the regression models.

Results

Meteorological data were quite different among the areas during the 7-year period and the major differences were due to spring rainfalls. Regarding areas, a rainfall increase from Southern to Northern regions, and from coastal to inland areas was observed. The Northern area (area 1) was quite favourable for *Fusarium* spp. infection, as the years under investigation were characterised by rainy springs. No appreciable differences in temperature were observed among areas with the exception of Southern Apennines (area 5), where the lowest mean temperatures were recorded.

Dataset A (1403 durum wheat samples collected in the 3-year period 2012-2014)

The occurrence of DON was observed in 67% of the assayed durum wheat samples reporting low values of contamination. The distribution was highly asymmetric: the average concentration on the dataset was 251 $\mu\text{g}/\text{kg}$; whereas the median value was 37 $\mu\text{g}/\text{kg}$. 95% of samples reported a concentration below 1284 $\mu\text{g}/\text{kg}$. Only 4.8% of total samples exceeded the value of 1750 $\mu\text{g}/\text{kg}$.

The descriptive statistics of DON content in the durum wheat

Table 1. List of analysed cultivars in the 3-year period (dataset A) and in the 7-year period (dataset B).

CV	Cycle length	Genealogy	Dataset A	Dataset B
Anco Marzio	ME	Stot//Altar84/ALD	x	x
Claudio	M	Sel.Cimmyt35/Durango//IS193B/Grazia	x	x
Core	E	Platani/Gianni	x	
Duilio	E	Cappelli//Anhinga/Flamingo	x	x
Dylan	ML	Neodur/Ulisse	x	
Iride	E	Altar84/Ionio	x	x
Kanakis	M	(Unavailable)	x	
Marco Aurelio	M	Orobel//Arcobaleno/Svevo	x	
Massimo Meridio	M	Orobel//Arcobaleno/Svevo	x	
Odisseo	ML	Svevo/Ied24	x	
Ramirez	M	(Unavailable)	x	
Saragolla	E	Iride/Linea PSB0114	x	x
Sculptur	M	(Unavailable)	x	
Simeto	ME	Capeiti8/Valnova	x	x
Svevo	E	Linea Cimmyt/Zenit	x	x
Tirex	ME	Svevo/Nefer	x	

E, early; M, medium; L, late. For each cultivar the length of cycle until anthesis and the genealogy were reported.

samples under investigation are shown in Table 2 and reported according to cultivar; whereas Table 3 shows data according to the cultivation area. In the analysed cultivars, mean values ranged between 162 $\mu\text{g}/\text{kg}$ for Odisseo and 402 $\mu\text{g}/\text{kg}$ for Simeto. The 95th percentile showed values closed to the value of 1750 $\mu\text{g}/\text{kg}$ in the cultivars Massimo Meridio (2730 $\mu\text{g}/\text{kg}$), Marco Aurelio (2214

$\mu\text{g}/\text{kg}$), Duilio (2129 $\mu\text{g}/\text{kg}$), Simeto (1714 $\mu\text{g}/\text{kg}$), Core (1536 $\mu\text{g}/\text{kg}$) and Anco Marzio (1321 $\mu\text{g}/\text{kg}$) corrected for recovery. The cultivars Simeto, Claudio and Duilio showed the highest detected values (14452, 10704 and 8496 $\mu\text{g}/\text{kg}$, respectively).

The incidence rate, intended as percentage of positive samples ($c \geq \text{LOD}$) for DON, out of the total number of assayed samples,

Table 2. Descriptive statistics of deoxynivalenol occurrence contamination (1403 samples concerning 2012-2014 years) according to cultivar: mean, standard deviation, range, median, 75th percentile, 95th percentile.

CV	Whole dataset						Positive samples ($c \geq \text{LOD}$)	
	Mean ($\mu\text{g}/\text{kg}$)	SD ($\mu\text{g}/\text{kg}$)	Range ($\mu\text{g}/\text{kg}$)	Q2 ($\mu\text{g}/\text{kg}$)	Q3 ($\mu\text{g}/\text{kg}$)	P95 ($\mu\text{g}/\text{kg}$)	Samples (%)	Mean ($\mu\text{g}/\text{kg}$)
Anco Marzio	238	561	<LOD-3042	39	173	1321	72	326
Claudio	293	1123	<LOD-10704	37	137	1118	70	413
Core	228	643	<LOD-4560	34	124	1536	61	371
Duilio	355	1057	<LOD-8496	36	227	2129	65	541
Dylan	233	540	<LOD-2900	34	146	1272	67	345
Iride	175	537	<LOD-4713	36	97	769	65	264
Kanakis	249	601	<LOD-3275	28	173	1267	63	392
Marco Aurelio	343	885	<LOD-5394	51	153	2214	72	474
Massimo Meridio	337	890	<LOD-4757	38	138	2730	66	505
Odisseo	162	453	<LOD-3084	30	80	905	63	251
Ramirez	179	394	<LOD-2378	43	129	964	64	276
Saragolla	183	427	<LOD-2428	43	107	1254	66	274
Sculptur	220	579	<LOD-2799	36	109	1299	66	328
Simeto	402	1522	<LOD-14452	44	167	1714	67	598
Svevo	190	454	<LOD-3190	38	95	1215	71	264
Tirex	243	554	<LOD-3817	39	149	1305	76	318

CV, cultivar; SD, standard deviation; Q2, median; Q3, 75th percentile; P95, 95th percentile; LOD, limit of detection (18.5 $\mu\text{g}/\text{kg}$). Results minor than the limit of detection were set to 9.25 for the calculation of means; c=DON concentration ($\mu\text{g}/\text{kg}$). Positive samples: percentage of positive samples and mean.

Table 3. Descriptive statistics of deoxynivalenol occurrence contamination (1403 samples concerning 2012-2014 years) according to cultivation area: mean, standard deviation, range, median, 75th percentile, 95th percentile.

Area	Whole dataset						Positive samples ($c \geq \text{LOD}$)	
	Mean ($\mu\text{g}/\text{kg}$)	SD ($\mu\text{g}/\text{kg}$)	Range ($\mu\text{g}/\text{kg}$)	Q2 ($\mu\text{g}/\text{kg}$)	Q3 ($\mu\text{g}/\text{kg}$)	P95 ($\mu\text{g}/\text{kg}$)	Samples (%)	Mean ($\mu\text{g}/\text{kg}$)
North	1016	1633	<LOD-14452	401	1263	3528	96	1057
Centre-East	177	359	<LOD-3223	53	145	872	78	224
Centre-West	153	314	<LOD-2479	71	167	443	84	181
Central Apennines	215	466	<LOD-2770	53	161	1118	74	286
Southern Apennines	21	29	<LOD-289	<LOD	21	76	32	47
Southern Adriatic Ionian coast	48	58	<LOD-375	27	56	137	73	62
Sicily	24	26	<LOD-196	<LOD	27	76	45	43
Sardinia	37	35	<LOD-163	28	52	112	58	57

SD, standard deviation; Q2, median; Q3, 75th percentile; P95, 95th percentile; LOD, limit of detection (18.5 $\mu\text{g}/\text{kg}$). Results minor than the limit of detection were set to 9.25 for the calculation of means; c=DON concentration ($\mu\text{g}/\text{kg}$). Positive samples: percentage of positive samples and mean.

Table 4. Descriptive statistics of deoxynivalenol occurrence contamination (1403 samples concerning 2012-2014 years) according to growing year: mean, standard deviation, range, median, 75th percentile, 95th percentile.

Year	Whole dataset						Positive samples ($c \geq \text{LOD}$)	
	Mean ($\mu\text{g}/\text{kg}$)	SD ($\mu\text{g}/\text{kg}$)	Range ($\mu\text{g}/\text{kg}$)	Q2 ($\mu\text{g}/\text{kg}$)	Q3 ($\mu\text{g}/\text{kg}$)	P95 ($\mu\text{g}/\text{kg}$)	Samples (%)	Mean ($\mu\text{g}/\text{kg}$)
2012	150	374	<LOD-2799	29	91	837	64	230
2013	402	1201	<LOD-14452	45	181	2484	70	567
2014	204	426	<LOD-2770	40	138	1181	68	296

SD, standard deviation; Q2, median; Q3, 75th percentile; P95, 95th percentile; LOD, limit of detection (18.5 $\mu\text{g}/\text{kg}$). Results less than the limit of detection were set to 9.25 for the calculation of means; c=DON concentration ($\mu\text{g}/\text{kg}$). Positive samples: percentage of positive samples and mean.

was 67% in the 1403 durum wheat samples with an average concentration of DON equal to 369 $\mu\text{g}/\text{kg}$. The incidence of DON varied depending on the cultivar, area and year. Within each cultivar, more than 60% of samples resulted positive. In particular, samples belonging to the cultivar Tirez, Marco Aurelio, Anco Marzio, Svevo and Claudio resulted the most contaminated, although the highest mean values ($c > 500 \mu\text{g}/\text{kg}$) were recorded by Simeto, Duilio and Massimo Meridio (Table 2). As shown in Table 3, DON levels were heavily influenced by the geographical growing area: Northern Italy showed, for instance, considerable amount of DON with a mean value (1016 $\mu\text{g}/\text{kg}$) far higher than those detected in the other areas. Furthermore, the negligible concentrations of this mycotoxin were also confirmed both in Southern Italy, Sicily and Sardinia. The lowest incidence and means of DON were recorded in Southern Apennines, Sicily and Sardinia, where means of positive samples were lower than 60 $\mu\text{g}/\text{kg}$. In particular, in Southern Apennines only 32% of samples exceeded the detection limit with a very low mean value of DON (47 $\mu\text{g}/\text{kg}$). On the other hand, the highest values were obtained for samples of grains grown in Northern Italy, where DON contamination affected 96% of samples, with an average concentration of 1057 $\mu\text{g}/\text{kg}$. As regards the three years assayed, namely 2012-2014 (Table 4), the highest DON levels were observed in 2013: the mean value was 402 $\mu\text{g}/\text{kg}$, the 95th percentile far exceeded the value of 1750 $\mu\text{g}/\text{kg}$, and the maximum reached 14452 $\mu\text{g}/\text{kg}$. Over that year, 70% of samples resulted positive to DON. In 2012, DON was present in similar percentages of positivity (64% of samples), but with an average level of only 150 $\mu\text{g}/\text{kg}$. DON values during 2014 were comparable with those of 2012.

The GLZ analysis showed that all single factors and the interaction between cultivar and area were highly significant ($P < 0.0001$), although a marked difference in χ^2 values among factors was highlighted (Table 5). The growing area was the main contributing factor to deoxynivalenol content ($\chi^2 = 114540$), followed by year ($\chi^2 = 5504$), cultivar ($\chi^2 = 1239$) and the two-way ($\text{CV} \times \text{GA}$) interaction ($\chi^2 = 281$).

As the other interactions in the complete model were not significant, a reduced model was used. The model took into consideration the only interaction which was significant ($\text{CV} \times \text{GA}$). Interactions between the remaining factors are not shown because not significant. Moreover, the behaviour of the 16 cultivars assayed in the three-year period was highlighted by applying the GLZ analysis of data concerning the DON content.

The GLZ analysis allows to distinguish and order the varieties under analysis on the basis of each factor (*i.e.*, area, year and cultivar), as well as of the interaction $\text{CV} \times \text{GA}$. Hence, the variety classification obtained upon ranking does not straight match the classification deriving from the means (Table 6). In order to assess any change of cultivar ranking due to the different geographical position of the cropping areas, the data regarding the three areas with a major presence of DON (*i.e.*, North, Centre-East and Central-Apennines) were selected (Table 7). Despite some differences among the growing areas, the results confirmed the general trend. In fact, the cultivars Simeto and Duilio resulted highly susceptible to DON accumulation in kernels in all areas assayed, whereas Odisseo appeared the most resistant. The Iride CV behaviour was greatly influenced by the growing areas: high values of DON content were observed in East-Central Italy, Central Apennines and Northern Italy (mean = 303 $\mu\text{g}/\text{kg}$, 95th percentile = 1268 $\mu\text{g}/\text{kg}$), whereas lower values were obtained in the remaining areas of Italy (mean = 43 $\mu\text{g}/\text{kg}$, 95th percentile = 132 $\mu\text{g}/\text{kg}$). It could explain the different position in cultivar ranking related to the measured DON content.

Dataset B (2080 durum wheat samples collected in two periods, 2006-2008 and 2011-2014)

The GLZ analysis of the second dataset, comprising seven durum wheat cultivars over a 7-year period, was useful to confirm both the general trend of cultivar behaviour towards the DON contamination and the influence of several environmental factors. DON content in kernels was mainly influenced by the cropping area ($\chi^2 = 526328$; $P < 0.0001$), but also by the year ($\chi^2 = 21966$; $P < 0.0001$), cultivar ($\chi^2 = 11545$; $P < 0.0001$) and the two-way interaction $\text{CV} \times \text{area}$ ($\chi^2 = 5168$; $P < 0.0001$). An increased susceptibility to higher concentration of DON was highlighted for the cultivars Simeto and Duilio, whereas Saragolla and Svevo showed a trend of lower contamination levels (Table 7).

Discussion

Genotype-by-environment interactions and the complex nature of FHB are among the most important aspects to be considered for breeding challenges (Bai and Shaner, 1994; Buerstmayr *et al.*, 2013). However, nowadays no full indication exists about the wheat cultivars with a high level of resistance to *Fusarium* spp. that might limit yield loss and mycotoxin accumulation. As the utilisation of FHB-resistant cultivars could be an effective approach for the protection of wheat against FHB disease, new sources of FHB resistance have increasingly been in high demand among wheat breeders.

Resistant cultivars can develop active defense reactions during infection and thus determine a lower accumulation of DON in the tissues of infected spikes of resistant wheat cultivars (Kang and Buchenauer, 2000). Wheat resistance to FHB is affected by the relationship between symptom intensity and toxin production, and this inheritance has a quantitative and polygenic nature.

It is known that DON plays a key role as a powerful virulence factor during fungal pathogenesis; resistance to this mycotoxin is therefore an important component of resistance against FHB. Resistance aspects determine the severity of FHB (I-IV resistance), however, other mechanisms may influence DON content in kernels, which limit the trichothecene accumulation in cereals, including the transformation of the mycotoxin that is named type V-1 (Boutigny *et al.*, 2008). Several studies are available in literature about the mechanisms of resistance to DON which comprise the conversion of DON into less toxic metabolites, such as deoxynivalenol-3-*O*-glucoside (D3G), with a good correlation between FHB resistance and DON conversion rate, expressed as $[\text{D3G}]/[\text{DON}]$ ratio in durum wheat (Audenaert *et al.*, 2013; Berthiller *et al.*, 2005; Cirlini *et al.*, 2014; Kluger *et al.*, 2015; Lemmens *et al.*, 2005) and DON-glutathione (DON-GSH) (Krska

Table 5. χ^2 values and significance levels from generalised linear models regression for deoxynivalenol occurrence (1403 samples concerning 2012-2014 years).

Factor	χ^2	$\text{Pr} > \chi^2$
CV	1239	<0.0001
GA	114540	<0.0001
Year	5504	<0.0001
CV*GA	281	<0.0001

CV, cultivar; GA, growing area.

et al., 2014; Stanic *et al.*, 2014). D3G is also defined as *masked* form of DON. This compound has a lower acute toxicity compared to his parent mycotoxin particularly in the binding to the ribosome and its intestinal toxicity (Pierron *et al.*, 2016). However, it can be hydrolysed by several intestinal bacteria releasing free DON and this fact is of great toxicological concern (Berthiller *et al.*, 2011).

In this work, the evaluation of DON content in durum wheat samples was carried out by Ridascreen®DON which employs an antibody showing a declared cross-reactions with other DON-conjugated compounds (*e.g.*, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol). Moreover, besides these interfering compounds, a marked cross-reaction between antibody and D3G

Table 6. Descriptive statistics of the cultivars assayed in 3-year period (2012-2014, dataset A) and in 7-year period (2006-2008 and 2011-2014, dataset B) of deoxynivalenol occurrence mean concentration ($\mu\text{g}/\text{kg}$) in each cultivation area.

		North	Centre East	Centre West	Central Apennines	Southern Apennines	Southern Adriatic	Southern Ionian coast	Sicily	Sardinia
Dataset A	Anco Marzio	912	164	144	195	30	29	23	49	
	Claudio	1618	152	61	173	<LOD	42	28	55	
	Core	806	236	72	157	22	42	19	27	
	Duilio	1490	290	171	377	29	38	<LOD	42	
	Dylan	618	143	577	129	<LOD	56	23	22	
	Iride	781	159	108	251	24	37	<LOD	55	
	Kanakis	904	228	67	207	<LOD	25	25	34	
	Marco Aurelio	1281	202	104	335	<LOD	39	29	50	
	Massimo Meridio	1226	250	102	353	<LOD	30	22	22	
	Odisseo	584	89	71	150	<LOD	38	21	22	
	Ramirez	591	203	122	162	<LOD	25	30	36	
	Saragolla	828	155	76	165	23	59	28	33	
	Sculptur	863	124	89	123	<LOD	28	22	34	
	Simeto	2198	412	192	452	36	59	25	48	
	Svevo	777	115	96	150	<LOD	47	30	33	
Tirex	804	163	310	131	26	48	40	24		
Dataset B	Anco Marzio	619	188	130	152	35	30	28	81	
	Claudio	846	338	102	106	23	26	24	38	
	Duilio	992	341	178	134	25	41	20	44	
	Iride	658	239	135	126	22	33	21	44	
	Saragolla	564	167	92	113	30	53	40	47	
	Simeto	1464	428	211	222	19	34	27	50	
	Svevo	583	70	78	111	<LOD	26	30	35	

LOD, limit of detection (18.5 $\mu\text{g}/\text{kg}$). Results less than the limit of detection were set to 9.25 for the calculation of means. Deoxynivalenol mean values ($\mu\text{g}/\text{kg}$) in the cultivation areas.

Table 7. Generalised linear models analysis: detailed ranking of the cultivars assayed in 3-year period (2012-2014, dataset A) and in 7-year period (2006-2008 and 2011-2014, dataset B) in three cultivation areas from the minimum (up) to the maximum (bottom) deoxynivalenol occurrence mean content ($\mu\text{g}/\text{kg}$).

	Total areas	Cultivation areas		
		North	Centre-East	Central Apennines
Dataset A	Iride ^a	Odisseo ^a	Odisseo ^a	Sculptur ^a
	Odisseo ^{a,b}	Ramirez ^a	Sculptur ^b	Tirex ^a
	Dylan ^{b,c}	Dylan ^b	Svevo ^c	Dylan ^a
	Ramirez ^c	Svevo ^c	Dylan ^d	Svevo ^b
	Saragolla ^d	Iride ^d	Anco Marzio ^e	Odisseo ^b
	Svevo ^e	Tirex ^e	Tirex ^e	Core ^{b,c}
	Tirex ^e	Core ^f	Claudio ^f	Ramirez ^c
	Core ^f	Saragolla ^g	Marco Aurelio ^g	Saragolla ^c
	Anco Marzio ^g	Sculptur ^h	Ramirez ^g	Anco Marzio ^d
	Sculptur ^h	Kanakis ⁱ	Saragolla ^h	Kanakis ^d
	Kanakis ^h	Anco Marzio ⁱ	Kanakis ⁱ	Claudio ^e
	Marco Aurelio ⁱ	Massimo Meridio ^j	Iride ^f	Iride ^f
	Massimo Meridio ^j	Marco Aurelio ^k	Core ^k	Marco Aurelio ^g
	Duilio ^j	Duilio ^l	Massimo Meridio ^k	Massimo Meridio ^h
	Claudio ^k	Claudio ^m	Duilio ^l	Duilio ^l
Simeto ^l	Simeto ⁿ	Simeto ^m	Simeto ^j	
Dataset B	Saragolla ^a	Svevo ^a	Svevo ^a	Svevo ^a
	Svevo ^a	Saragolla ^a	Saragolla ^b	Saragolla ^b
	Anco Marzio ^b	Anco Marzio ^b	Anco Marzio ^c	Claudio ^c
	Iride ^c	Iride ^c	Duilio ^d	Anco Marzio ^d
	Claudio ^d	Claudio ^d	Claudio ^d	Iride ^e
	Duilio ^e	Duilio ^e	Simeto ^e	Duilio ^f
	Simeto ^f	Simeto ^f	Iride ^f	Simeto ^g

^{a-g}Cultivars within columns of each dataset followed by different letters are significantly different at $P < 0.05$.

was relieved (Berthiller *et al.*, 2013; Zachariasova *et al.*, 2008).

The DON concentration detected in samples is to be intended as the whole form of DON recognised by the antibody employed. The quite similar trend of percentage of samples positive for DON (from 61 to 76%) within the 16 cultivars against different values of 95th percentile (from 769 to 2730 µg/kg) suggested a different response to accumulate the mycotoxin in the host kernels. Indeed, the different behaviour towards DON accumulation in kernels allowed ranking cultivars on the basis of significant differences about the mycotoxin content. To this aim, the choice to select the cultivation areas with greater levels of contamination allowed to better identify the response of each cultivar to the fungal aggressiveness in terms of DON production. The classification of cultivars within the *total areas* group did not differ a lot from each single area of cultivation with a major risk of contamination. The susceptibility of cultivars, emerged within the tested area, has substantially a similar trend than in the areas reported in Table 7. The Odisseo CV showed a minor tendency to the DON accumulation in kernels all over the cultivation areas assayed. Moreover, in case of higher exposition of plants to DON contamination, such as in the North, or more in general in the case of the assayed *total areas*, the cultivars Iride, Dylan, Ramirez and Svevo showed a fairly good resistance to the mycotoxin occurrence. Our results also hinted that the length of the wheat crop cycle does not affect the levels of DON contamination detected in kernels. This outcome further highlights the importance of the cultivar own capability to actively counteract the fungal aggressiveness. The slight differences in the position of each of the 16 cultivars within the area also highlighted the importance of environmental conditions on their behaviour with regard to DON content in kernels.

In case of FHB outbreak on wheat plants, due to the landing of fungi, such as *F. graminearum* and *F. culmorum*, the growth of mycelia and the ability to produce mycotoxins are greatly influenced by many factors and interactions thereof, such as the aggressiveness of the fungal species, the host susceptibility and the cultivar resistance level. The latter are also strongly related to plant exposure to FHB risk and mycotoxin accumulation due to a several environmental factors involving key parameters (*e.g.*, temperature, relative humidity, rainfall, previous crop and crop residue management) (Cromery *et al.*, 2002; Lori *et al.*, 2009; Wegulo, 2012). The area was the main contributing factor to the DON occurrence, as it takes into account both the high climate and geographic variability of Italy.

These data also match results obtained in a previous study on the occurrence of trichothecenes in organic and conventional durum wheat in Italy, where the major influence of the area as a single factor and its interaction with the year was showed (Quaranta *et al.*, 2010). It also emerged that the importance of the effect of the growing year should not be overlooked as shown in previous works (Aureli *et al.*, 2015; Camerini *et al.*, 2015). As to the importance of the environment, the higher mean and maximum values of DON content detected in 2013 should be reasonably connected with the high amount of rainfall occurring in May in Northern and Central Italy.

This work provides information about the behaviour of several durum wheat cultivars, widely grown in Italy, in function of the level of deoxynivalenol contamination in whole milled kernels. It hence contributes to bringing new knowledge to a field where there is still a limited availability of information, that is, resistance sources for durum wheat against Fusarium Head Blight, with emphasis on plant capability to contrast the production and/or accumulation of mycotoxins in kernels. The results obtained by the statistical analysis (GLZ), especially for areas with a major DON

contamination, highlight a general trend ranking of cultivars based on their sensitivity to DON accumulation in raw matter.

Among the cultivars investigated in this work, Odisseo CV generally showed a minor sensitivity, whereas a higher susceptibility to DON contamination was revealed for Simeto and Duilio. The analysis of all cultivars allowed highlighting that the length of wheat crop cycle does not affect the levels of DON contamination detected in kernels.

The major influence of the area factor and its two-way interaction with the cultivar factor was shown. Because of the role played by the area factor, slight differences along the cultivar ranking for each area assayed were also outlined. This result confirms that it represents one of the most important factors together with the year factor able to heavily influence the DON accumulation occurring in durum wheat grain in Italy (Quaranta *et al.*, 2010; Aureli *et al.*, 2015). Therefore, in spite of the expanded sowing of this species towards the Northern region of Italy, both the Centre-South and islands areas remain the more suitable regions for growing this type of culture.

Conclusions

This study is a first attempt of forward-looking approaches to a better understanding of cultivar susceptibility to mycotoxin accumulation in grains. It has clearly emerged the pivotal role of the factor cultivar. However, other factors, such as environment and agronomic practices, have not to be neglected. It is worth highlighting that the obtained information is likely of use for farmers when deciding for the most suitable cultivar, and of interest for breeding programmes in durum wheat.

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