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Research Article

Relationship between Wheat Head Blight Levels and Deoxynivalenol, Deoxynivalenol-3-Glucoside Contents

Huijie Zhang[1,2], Li Wu[1,2], Juan Sun[1,2], Yan Zhang[1,2], Xiaoli Dong[1,2], Weixi Li[1,2], Xuexu Hu[1,2], Lijuan Sun[1,2], Bujun Wang[1,2]*

[1]*Institute of Crop Science, Chinese Academy of Agricultural Sciences, China*
[2]*Laboratory of Quality & Safety Risk Assessment for Cereal Products (Beijing), Ministry of Agriculture, China*

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***Corresponding author**

Bujun Wang, Institute of Crop Science, Chinese Academy of Agricultural Sciences, China, Tel: 86-10-82105798; Fax: 86-10-82108742; Email: wangbujun@caas.cn

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Abstract

Field wheat panicles infected with and without wheat head blight were collected in 2012 from Jiangsu province where Fusarium Head Blight (FHB) is prevalent. The collected panicles infected with FHB were assessed in the laboratory for Disease Severity Levels (DSL). A series of wheat samples with varying Rate of Diseased Panicles (RDP) and varying DSL were prepared by adding diseased panicles to healthy ones and analyzed using Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) for their Deoxynivalenol (DON) and Deoxynivalenol-3-Glucoside (D3G) contents. Consistent increases of DON and D3G levels were observed as the RDP and DSL of the wheat samples were enhanced, showing a distinct linear correlation between disease index (DI) of wheat head blight and DON and D3G content. The linear regression models were calculated as Y_{DON}=257.78X_{DI}+2315.61 (r=0.96) and Y_{D3G}=41.81X_{DI}-43.79 (r=0.96). The models were validated with field samples collected in 2013 and showed a good fit for variation trends, but overestimated DON content and underestimated D3G content.

Introduction

FHB (Fusarium head blight) or scab, caused by different species of Fusarium, is a serious worldwide disease of wheat (Triticum aestivum and T. durum), barley and other small grain cereals, and generally leads to reduced grain yield and quality and affects products processed from infected grain [1]. Moreover, grains can be contaminated with mycotoxins, which are secondary metabolites produced by some Fusarium species under certain conditions. Among them, Deoxynivalenol (DON) is the most important toxin in wheat and wheat-derived products due to its worldwide occurrence and potential toxicological impact on animal and human health [2]. Moreover, plants can metabolize DON to a variable extend through enzymatic conjugation to glucose [3-5]. The resulting "masked"mycotoxin Deoxynivalenol-3glucoside (D3G) might be hydrolyzed in the digestive tract of mammals, thus contributing to the total dietary DON exposure of individuals [6]. D3G may also be toxic in its own right, although little is known so far about its potential bioactivity [7].

Approximately 600 million tons of wheat is produced per year worldwide. Much of it is converted to wheat flour for human consumption and processed into various foods, such as breads, pastas, noodles and cakes [8]. Wheat contaminated with mycotoxins may be completely unusable for food or feed purposes.

The relationship between FHB level and DON content in harvested wheat grain has been the subject of discussion among researchers for many years. Results from individual studies have led to conclusions ranging from lack of significant association to strong positive correlations between FHB level and DON content. It was reported that a decreased disease incidence in the field ensures a decreased mycotoxin content of the grain [9-10]. A positive correlation (r=0.84) between Fusarium frequency and mycotoxin concentration among samples from a fungicide trial was found [11]. High correlations between head blight ratings and DON contents, both in segregating materials and in a collection of varieties differing in resistance levels was reported [12-13]. From surveys of barley grain samples, a wide range of correlations between years (r=0.28-0.83) was also reported [14]. Even though most studies reported positive correlations, the amount of mycotoxins per unit of disease index differed considerably between studies. Reviewed findings from 163 individual studies of the association between DON and disease intensity (incidence, diseased head severity) showed that there was an overall significant positive correlation between DON and all commonly used field measures of FHB levels [15]. However, the actual correlation coefficient and amounts of DON differed considerably. It was found that variability between DON content of Fusarium-damaged kernels obtained from wheat grown in northern Germany between 2001 and 2005 was large [16]. In a few instances, low [17-18] or no relationships [19] between FHB and DON were found. Occasionally, disproportionate and negative relationships between DON and disease were reported. For example,

it was reported that elevated levels of DON occurred without visible symptoms of FHB [20]; and disproportionately low levels of DON occurred despite high levels of visual symptoms [20,21]. For D3G there is very limited data available about the relationship between FHB level and D3G in cereals or food.

Serious levels of FHB occur every 2 to 3 years in China. The disease is monitored nationwide and forecasts are issued according to the China National Standard (GB/T 15796-2011), 'Rules for Monitoring and Forecast of the Wheat Head Blight'. Three terms are applied: Rate of Diseased Panicles (RDP), Disease Severity Levels (DSL), and Disease Index (DI), to describe FHB prevalence, severity and integration of prevalence and severity, respectively. DSL is termed as the frequency of diseased spikelets on a wheat panicle. RDP is graded into 5 levels, viz. 0.1 %< RDP≤10%, 10 %< RDP≤ 20%, 20% < RDP ≤ 30%, 30% < RDP ≤ 40%, RDP > 40%. DSL is graded into 4 levels, i.e. DSL< 25%, 25% ≤DSL<50%, 50%≤DSL<75%, and DSL≥75%. DI is an integrated indicator of FHB prevalence and severity and is calculated by:

$$I = \frac{\sum(h_i \times i)}{H \times 4} \times 100$$

I - Disease index;

h_i - Diseased panicle number corresponding to severity level;

i - Severity level;

H - Total number of wheat panicles.

The objective of the present research was to investigate the relationship between DON, D3G content and wheat head blight levels and to determine whether a model estimating DON and D3G contamination levels could be developed. The research was carried out by adding Fusarium-infected wheat panicles to non-infected panicles to simulate different RDP, DSL and DI for wheat head blight. Further validation was undertaken, based on uninoculated field samples.

Material and Methods

Wheat panicle collection and sample preparation

Wheat panicles (cultivars Jimai 22, Aikang 58 and Luomai 24) with and without FHB were collected from fields in Xuzhou city, Jiangsu province (E116°22'~118°40', N33°43'~34°58'), at the end of May 2012. Fusarium-infected panicles were separated manually based on color and the degree of grain shriveling in the laboratory, and then added to healthy ones to give samples with varying levels of diseased panicles (5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50% and 70%). For each level of diseased panicles, samples were prepared with 4 severity levels, i.e. the DSL < 25%, 25% ≤ DSL < 50%, 50% ≤ DSL < 75%, and DSL ≥ 75%, respectively. All samples were prepared in triplicate. Samples were then air-dried, threshed and milled in a laboratory mill (Laboratory 3303, Perten, Sweden) in preparation for DON and D3G analysis.

To validate the 2012 results, three fields for each RDP level of wheat head blight (0.1% < RDP ≤ 10%, 10% < RDP ≤ 20%, 20% < RDP ≤ 30%, 30% < RDP ≤ 40%, RDP > 40%) were selected at random and a total of 15 samples of wheat panicles were collected in Huaian city, Jiangsu province (E118°12'~119°36', N31°43'~34°06') at the beginning of June 2013. For each sample, 4 severity levels of FHB-infected wheat were identified and the DI calculated according to the aforementioned formula. All samples were then air-dried, threshed and milled in preparation for DON and D3G analysis.

Chemicals and reagents

Purified water was produced by a Milli-Q system (Millipore Corp., Bedford, MA). DON and D3G standards (both certified purity > 99.9%) were purchased from Supelco Co. (Bellefonte, PA, USA) and LGC (Wesel, Germany), respectively. Stock solutions of DON and D3G were prepared in pure methanol and acetonitrile, respectively, and kept at -20°C in darkness; working solutions were prepared in methanol to 0.1 mg/L and acetonitrile to 0.5 mg/L, respectively, for treatment of wheat samples. Methanol, acetonitrile and formic acid (all High Performance Liquid Chromatography HPLC grade) were purchased from Thermo Fisher Scientific (USA), and ammonium acetate from Sino harm Chemical Reagent Co., Ltd (Beijing).

DON and D3G extraction

25 g representative wheat samples were ground with a Laboratory Mill 3310 with its rotating disc at position 3 (Perten Instruments, Sweden). A 10 g sub-sample was weighed, extracted using 50 mL acetonitrile/ water (80:20 v/v) in an Erlenmeyer flask and were shaken for 1 hour at room temperature (≈20°C). The extract was filtered through filter paper (Whatman n. 4) and a glass microfibre filter (Whatman GF/A). For DON analysis, 8 mL of the filtered extract was cleaned up using multifunctional columns MycoSep 226 (Romer Labs, Inc. Union, MO, and USA). Four mL of the cleaned extract was evaporated to dryness using N-EVAP in a 50°C water bath. The residues were dissolved in 1mL 10 mmol/L ammonium acetate-methanols, then filtered through a 0.22 μm MICRO PES filter membrane (Membrana, Germany), and transferred to a glass vial for analysis. For D3G analysis, the extracts were centrifuged at 8870 rpm for 8 min and 1mL aliquots were filtered through a 0.22 μm filter membrane and transferred to a glass vial for analysis.

DON and D3G analysis

DON and D3G analyses were carried out using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS, XEVO-TQ, Waters, USA) equipped with an Electrospray Ionization Source (ESI). In order to separate the analyte a reversed-phase C18 stainless column (50 ×2.1 mm, particle size 1.7 um, Waters, USA) was used. The multiple reaction monitoring (MRM) modes was used, with capillary voltage maintained at 2.5 KV, cone voltage 20 V, and column temperature set at 26°C. For DON analysis, the ESI interface was operated in positive-ionization (ESI^+) mode and desolvation temperature was 450°C. Nitrogen was used as the desolvation gas with a flow rate of 800 L/h. The solvent system consisted of methanol (solvent A) and 10 mmol/L ammonium acetate (solvent B). The following gradient was applied with a flow rate of 0.3 mL/min: 0 - 5.5 min, a linear increase from 20 to 85%A; followed by a linear increase from 85% to 100% A for 5.5 - 5.8 min; a linear decrease from 100% to 20% A for 5.8 - 6.0 min, followed by an isocratic washout step of 20% A for 2 min. For D3G analysis, the ESI interface was operated in negative-ionization (ESI-) mode, desolvation temperature was 500°C, and the nitrogen flow rate was 1000 L/h. The solvent system consisted of acetonitrile (solvent A) and 0.1% formic acid-water (solvent B), and the following gradient was applied with

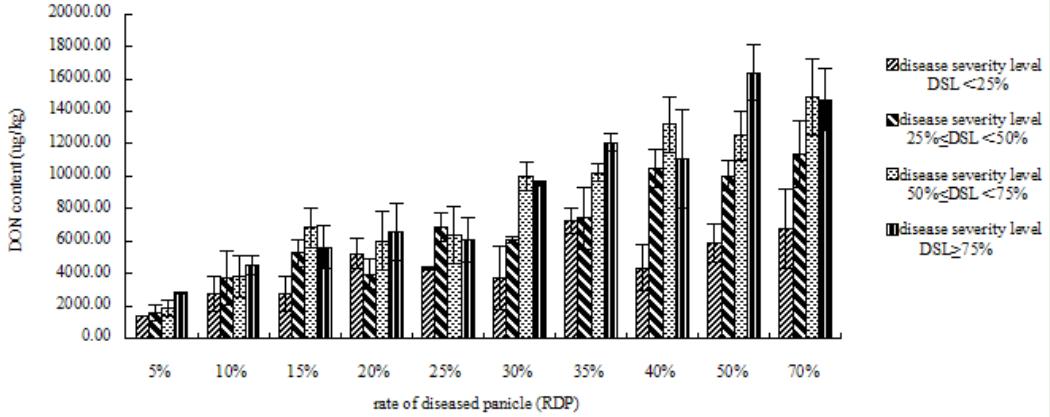

Figure 1: Deoxynivalenol (DON) content corresponding to Rate of Diseased Panicles (RDP) and Disease Severity Levels (DSL) of wheat head blight.

a flow rate of 0.25 mL/min: 0 - 4.0 min, a linear increase from 20 to 40% B; followed by a linear decrease from 40% to 20% B for 4.0 - 5.0 min. Target analytes were identified according to their retention time (DON, 1.09 min; D3G, 0.5 min) and accurate mass (DON, m/z 203.1; D3G, m/z 247.2) and quantification were performed using external standards and peak area measurements.

Method validation

Validation of the methods developed herein consisted of the determination of the recovery rate, linearity, and the limits of detection and quantification of DON and D3G in wheat. To determine the recovery rate, blank wheat samples were experimentally spiked with three different concentrations for DON (25, 50 and 100 μg/kg) and D3G (50, 100, and 500 μg/kg). Triplicates of each concentration were analyzed. Recoveries of DON and D3G ranged from 83.5 to 101.1% and 71.5 to 98.6%, with an overall average of 90.9% and 82.0%, and an average relative standard deviation (RSD) of 5.1% and 13.0%, respectively. It was linear in the range of 0.5-250 μg/kg for DON and 1-1000 μg/kg for D3G, with a coefficient of correlation of 0.998 and 0.999, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were defined as the smallest analyte amount detected with at least a 3:1 and 10:1 signal-to-noise ratio obtained from a disease free-sample, and LOD and LOQ for DON and D3G were 0.5 and 1.5 μg/kg, and 3.0 and 5.0 μg/kg, respectively.

Results

Relationship between DON, D3G contents and wheat head blight levels

DON and D3G contents in wheat samples corresponding to different RDP and DSL are shown in figure 1 and figure 2. On average, DON and D3G contents ranged from 1,897.99 to 11,933.32 μg/kg and from 211.14 to 1,956.34 μg/kg, respectively, as RDP increased from 5 to70%. DON contents rose proportionally with increasing DSL when RDP were set at 5%, 10%, 35%, and 50%. However, disproportionately variations were observed when RDP were set at 15%, 20%, 25%, 30%, 40%, and 70%. For D3G, contamination levels varied only slightly when the RDP levels were less than 25%, but increased significantly when RDP levels were greater than 30%. At different RDP levels, D3G contents were highly varied and did not show a corresponding increase with the increase of DSL.

The DI of wheat head blight was calculated according to the aforementioned formula. DON and D3G concentrations of wheat samples corresponding to elevated DI are presented in figure 3. There were distinct linear correlations between DI of FHB and DON, D3G contents; expressed as $Y_{DON}=257.78X_{DI} + 2315.61$ (r=0.96) and $Y_{D3G}=41.81X_{DI}-43.79$ (r=0.96) (Figures 1-3).

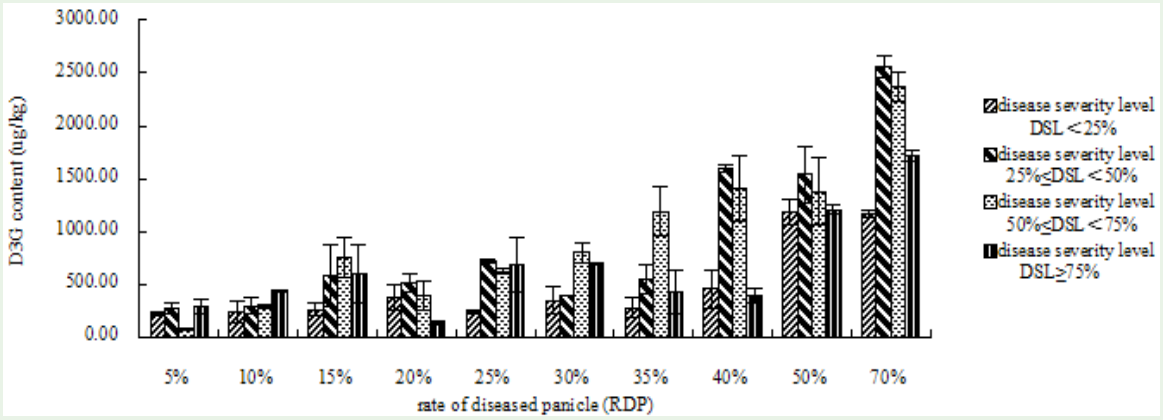

Figure 2: Deoxynivalenol-3-Glucoside (D3G) content corresponding to Rate of Diseased Panicles (RDP) and Disease Severity Levels (DSL) of wheat head blight.

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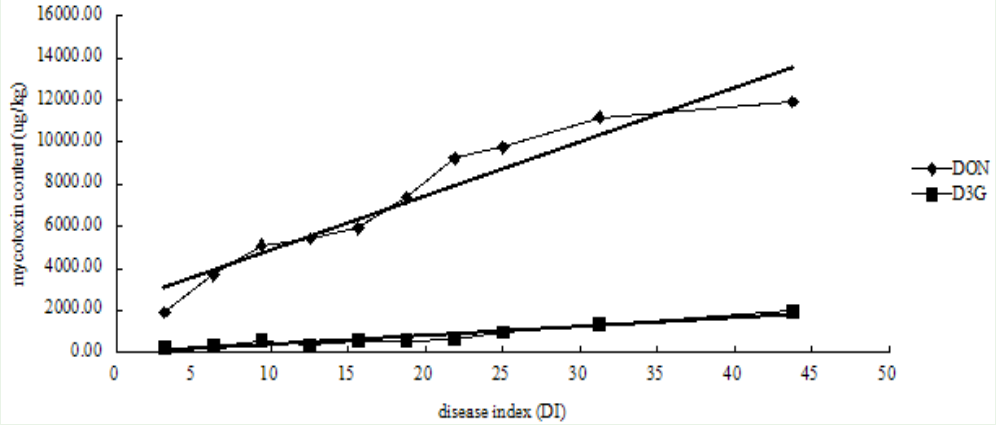

Figure 3: Deoxynivalenol (DON) and Deoxynivalenol-3-Glucoside (D3G) content corresponding to Disease Index (DI) of wheat head blight in simulation experiment.

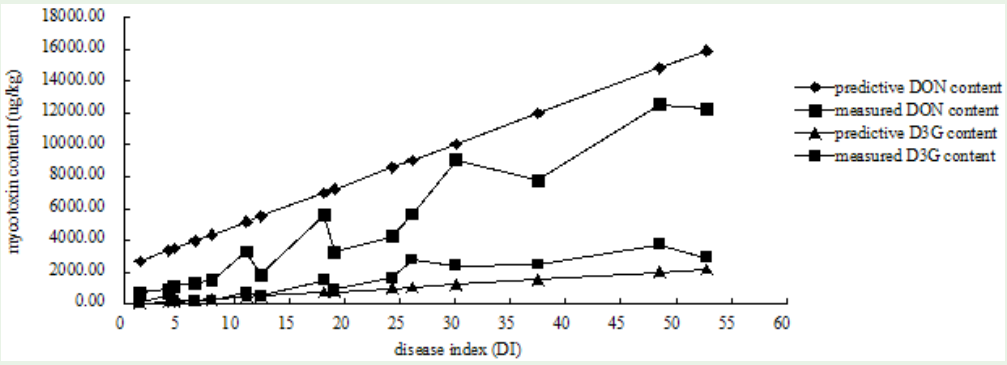

Figure 4: Predictive and measured contents of Deoxynivalenol (DON) and Deoxynivalenol-3-Glucoside (D3G) in wheat infected with wheat head blight.

Validation of the regression model

Predictive contents of DON and D3G of 15 wheat samples with different levels of FHB collected from Jiangsu fields in 2013 were calculated according to the regression equation generated in 3.1 and were presented together with the measured contents in figure 4. The R-squared values of 0.96 suggest that the linear regressions are useful when estimating the variation trends of DON and D3G, but predictive contents of DON when compared with the measured contents were overestimated by 59% whereas the predictive contents of D3G when compared with the measured contents were underestimated by 42%. There was strong correlation between the predictive contents of D3G and the measured contents of D3G when D3G values were lower than 1,000 μg/kg (Figure 4).

Discussion

Considerable effort has been undertaken to relate variation in FHB levels to mycotoxin concentrations, and most of the studies reported positive correlations. Our research confirmed there is highly positive correlation between DON content and FHB levels of wheat, but the correlation coefficient and amounts of DON differed considerably with previous studies. Also, our results indicated that there is a significantly positive relationship between D3G contamination levels and FHB levels.

Our research showed the regression models fitted well with the variation trend of DON and D3G in wheat contaminated with FHB, but it is difficult to accurately forecast mycotoxin contamination levels. The reasons might relate to wheat varieties, Fusarium species, or epidemiological conditions. It was suggested that resistance to FHB and mycotoxin accumulation may be controlled by different genes [22]. In the present research we collected panicle samples from different cultivars and mixed them in order to avoid any effect of cultivars on mycotoxin content. Infection with all FHB pathogen species causes similar degrees of shrivelling and discoloration of the grain, but spore type influences the level of DON in wheat grain [23], and different FHB pathogen species have different abilities of producing DON [14,24]; for example, some strains do not produce DON [25]. The Fusarium species effect on DON content was not considered in this research as the Fusarium species in Chinese wheat production areas do not change year by year. We suggest that climatic conditions during 2012 were a major factor that influenced the results because FHB levels are heavily dependent on favourable epidemiological conditions [26,27]. In 2012, Jiangsu province experienced prolonged periods of rain during the wheat flowering stage which created favourable conditions for the occurrence and spread of wheat head blight. However, such weather conditions were not repeated in 2013 and so disease was restricted to only a small part of Jiangsu province. Moreover, climate affects not only the epidemic

species, but also the level of mycotoxin production. Further efforts may be made to improve the forecasting model.

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