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Resistance to *Fusarium* head blight and mycotoxin accumulation among 129 wheat cultivars from different ecological regions in China

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RESEARCH ARTICLE

Abstract

A total of 129 wheat cultivars collected from local breeders in four ecological regions in China was evaluated for *Fusarium* head blight resistance after natural infection under epidemic conditions. The disease index was scored and seven toxins concentrations were determined by UPLC-MS/MS. The disease index ranged from 6.3 to 80.9% and a strong correlation was found between the regions from which the cultivars originate and disease index. The middle and lower reaches of Yangtze River Region showed the highest disease resistance, followed by the upper reaches of the Yangtze River Region. FHB resistance of cultivars from northern and southern Huanghuai Region was lowest and all cultivars in these regions are highly or moderately susceptible. Disease index was significantly correlated with toxin accumulation on nation scale, but no clear correlation was found within most ecological regions. The toxin accumulation was also not well correlated with resistant levels. As the incidence of FHB has increased dramatically over the last decade, improved FHB resistance in cultivars is urgently needed. We recommend that besides scoring for disease index also mycotoxin accumulation in cultivars is incorporated in breeding procedures and the evaluation of cultivars.

Keywords: *Fusarium* head blight, resistance, toxin, disease index, wheat

1. Introduction

Fusarium head blight (FHB), caused mainly by members of the *Fusarium graminearum* species complex, is one of the most damaging diseases of wheat worldwide and is responsible for severe reductions in wheat yield and quality (McMullen *et al.*, 2012; Snijders and Perkowski, 1990). Harvested grains are often contaminated with mycotoxins and these pose a potential threat to public health (Pestka, 2010). Typical toxins are mainly produced by *Fusarium* spp. and include deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), zearalenone (ZEN), T-2 toxin (T-2), HT-2 toxin (HT-2), and nivalenol (NIV) (Miller *et al.*, 1991; Sospedra *et al.*, 2010; Trenholm *et al.*, 1983). In China, the Yangtze river valleys were known as the most severe FHB epidemic areas for a long time, but the disease has been spreading northward since the last decade and now the Huanghuai Region, which is currently the main wheat production area of China has also become one of the most important FHB epidemic regions (He *et al.*, 2019; Zhang *et al.*, 2010). In recent years, FHB epidemics were reported to be more severe and to appear more frequent (Yang *et al.*, 2018). Consequently, the potential hazards of DON contaminated wheat have increased (Qiu *et al.*, 2019). China is the largest wheat producer in the world (Ning, 2018) and yield and quality of wheat directly affects national food security and food safety (Tan *et al.*, 2018).

The use of FHB-resistant wheat cultivars is considered to be the most cost-effective strategy to reduce occurrence and production of mycotoxins in wheat (Buerstmayr *et al.*, 1999; Mesterházy, 1995; Miedaner, 1997). Resistance to FHB is quantitative and can be divided into five types: Type I: resistance to initial infection, type II: resistance to colonisation, type III: limitation of toxin accumulation, type IV: resistance to kernel infection and type V: tolerance to infections (Mesterhazy, 1995; Spanic *et al.*, 2019). Type II resistance has been regarded as the most effective and stably inherited, and its measurement is straightforward with FHB severity scored after point inoculation (Bai and Shaner, 2004). Types I, III, IV and V are generally less investigated compared to type II resistance, and the underlying mechanisms are less clear (Liu *et al.*, 2009). Mycotoxin content, grain yield losses and degree of effects on seed quality by infection are undoubtedly the most important characters from the practical point of view. Breeding for a combination of both FHB resistance and low accumulation of mycotoxins would be greatly facilitated if the resistance to mycotoxin accumulation (Type III) in a genotype could be predicted from indirect, easily determined FHB traits. Besides, the higher level of resistance to FHB will help farmers to lower the risks connected with this devastating disease (to reduce grain yield losses and adverse effects on grain quality). A type III resistance was first reported by Miller *et al.* (1985), where resistant cultivars showed abilities to prevent synthesis and/or to promote degradation of DON, demonstrating the active role of type III resistance and different expression levels between cultivars (Miller and Arnison, 1986). However, the association between FHB severity and DON accumulation in harvested grain is not fully understood. Presently, there are few and conflicting reports on the correlation between FHB severity and mycotoxin accumulation (Chen *et al.*, 2007; Mesterházy, 2002; Tamburic-Ilincic and Miedaner, 2012). Some studies concluded on the highly significant relationship between the level of FHB symptoms and DON contamination, where DON-resistant cultivars were also *Fusarium* resistant (Lemmens *et al.*, 2016), whereas in other studies close to zero or negative correlations were reported (Ji *et al.*, 2015; Paul *et al.*, 2005). Furthermore, most reports are based on artificial inoculation and only target DON.

In an attempt to shed some light on these issues, this study was conducted to compare FHB resistance levels in Chinese wheat cultivars at a national level, to assess the impact of prolonged breeding for reduced infection/FHB (Type II resistance) as well as effects on mycotoxin accumulation (Type III resistance). Specifically, we investigated the regional difference of toxin accumulation and FHB disease resistance and the relationship between them in a nationwide collection of 129 wheat cultivars from four ecological regions. Therefore, natural infected disease severity was scored and seven toxins (DON, 3-ADON, 15-ADON, NIV, ZEN, T-2, HT-2) contamination levels were determined. Finally, the relationship between disease resistance and toxin accumulation of different wheat cultivars is discussed in the light of current breeding programs.

2. Materials and methods

Instruments and reagents

UPLC-MS/MS was performed on a Micromass Quattro Ultima triple-quadrupole mass spectrometer equipped with an ESI source (Waters Xevo TQS, Milford, MA, USA) and Tube Mill control (IKA-Werke GmbH & Co.KG, Staufen, Germany). Multifunctional purification columns (Bond Elut Mycotoxin) were provided by Agilent Technologies (Santa Clara, CA, USA. Acetonitrile and methanol (LC-MS/MS grade) were purchased from Merck KGaA (Darmstadt, Germany). Deionised water (18 MΩ*cm) was produced in the laboratory using a Milli-Q Reagent Water System (Bedford, MA, USA). The standards of DON, 3-ADON, 15-ADON, NIV, ZEN, T-2 and HT-2 were purchased from Pribolab Pte. Ltd. (Singapore, Singapore).

Field trials

A total of 129 wheat cultivars was tested in this study. They were bred by local breeders in four ecological regions in China (Figure 1): (1) the upper reaches of Yangtze River Region (UYR, n=21), including Sichuan, Yunnan and Chongqing, where the climate is wet and warm, a moderate FHB epidemic area; (2) the middle and lower reaches of Yangtze River Region (MLYR, n=25), including Hubei, Zhejiang, the southern part of Anhui and Jiangsu, where the climate is wet and warm, usually rainy during wheat flowering stage, a strong FHB epidemic area; (3) the south of the Huanghuai Region (SH, n=58), including Henan, Shanxi, the northern part of Anhui and Jiangsu, southern part of Shandong, where the climate is warm and dry, irrigation is necessary for wheat growing, a moderate FHB epidemic area; and (4) the north of the Huanghuai Region (NH, n=25), including Hebei and the northern part of Shandong, where the climate is cool and dry, irrigation is necessary for wheat growing, a mild FHB epidemic area. All cultivars were planted at the National Wheat Scab Resistance Evaluation Farm in Nanping (27°43' north latitude, 118°12' east longitude) in Fujian in China in 2014-2015 for resistance evaluation (natural infection). In general, the environmental conditions in the area are very conducive for FHB, as it is rainy and humid from early March to mid-April when wheat is in heading, flowering and milky stages. The average daily maximum temperature is 23 °C degrees and the lowest temperature is 13 °C. In the wheat growing season 2014-2015 weather was favourable for FHB all season. No fungicides were applied on the experimental plot. The cultivars were arranged in a randomised block design in single rows (length 100 cm, line spacing 25-33 cm) which contained approximately 300 plants with three replicates for each cultivar.

Evaluation of resistance

The incidence of FHB was recorded in the late milky stage. Disease severity was scored on 10 random wheat ears per plot, in total 30 wheat ears were investigated per cultivar. Disease severity was estimated visually *in situ* on a 0 to 4 scale: 0, no visible symptoms; 1, <25% affected spikelets; 2, 25-50% affected spikelets; 3, 50-75% affected spikelets; 4, >75% affected spikelets. The disease index was calculated using formula (1), where X_i means the number of infected spikelets, S_i means the scale of disease severity (0-4), X_{max} means the numbers of ears scored (30), and S_{max} stands for the maximum disease severity (4):

$$\text{Disease index} = \sum_{i=0}^{n} \frac{X_i \times S_i}{X_{max} \times S_{max}} \times 100 \qquad (1)$$

The resistant levels are graded by disease index: highly resistant (HR) = disease index below 10; moderately resistant (MR) = disease index between 10 and 25; moderately susceptible (MS) = disease index between 25 and 45; highly susceptible (HS) = disease index higher than 45.

Sample pretreatment

After harvest, all wheat ears were harvest manually for toxin determination from each plot and threshed with a laboratory thresher at low wind speed to prevent loss of low-weight infected kernels. Each sample was further milled to a fine powder with the IKA Tube Mill. Of the sample, 5 g was extracted with 25 ml of 80% (v/v) acetonitrile by shaking for 2 min and centrifuged at room temperature for 3 min at 9,000 rpm. Then, 2 ml of supernatant was sequentially purified by passing through the multifunctional purification column and through an 0.22 mm nylon filter.

UPLC-MS/MS method

Chromatography was performed on a reversed phase UPLC BEH C18 column (100×2.1 mm, 1.7 μm) from Waters Scientific. Mobile phases were methanol (A) and 10 mmol/l of ammonium acetate (B). The gradient program was as follows: 0-3 min, 20-50% A; 3-6 min, 50-90% A; 6-7 min, 90% A; 7-8 min, 90-20% A; 8-10 min, 20% A. The injection volume was 2 μl with a flow rate of 0.3 ml/min and the column temperature was maintained at 40 °C.

The instrument was operated in both positive and negative ion modes. Quantitation was performed using the multiple reaction monitoring (MRM) mode. The source temperature was 150 °C and the desolvation gas temperature was 350 °C. The desolvation gas and nebulizer gas (N_2) were set at 650 and 50 l/h, respectively. The flow rate of the collision gas (Ar) was 0.14 ml/min.

Statistical analysis

The correlation between disease index, toxin content and the regional difference of disease index was processed by generalized linear model (GLM). The comparison of

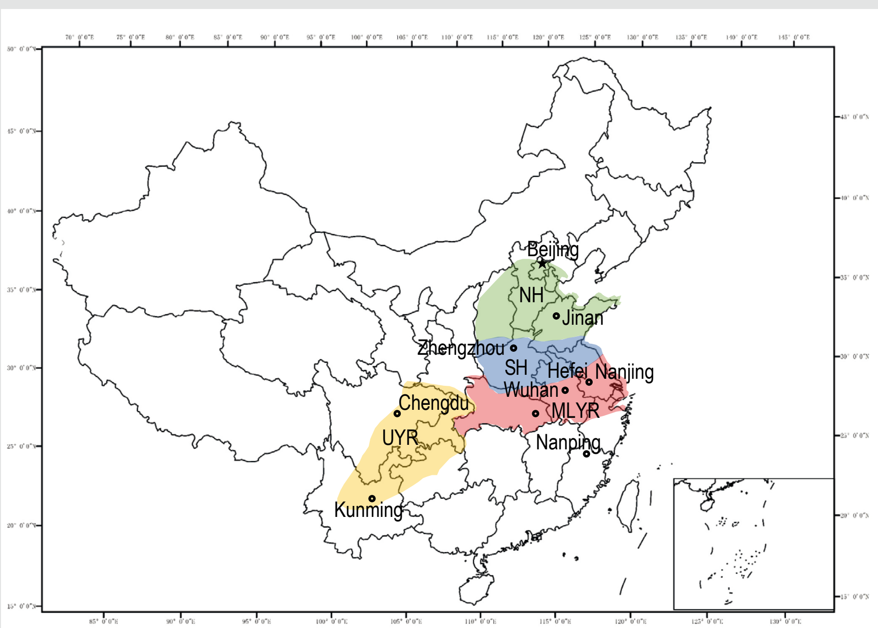

Figure 1. The four ecological regions of wheat production in China. UYR = the upper reaches of Yangtze River Region; MLYR = the middle and lower reaches of Yangtze River Region; SH = the south of thw Huanghuai Region; NH = the north of the Huanghuai Region (NH, n=25). The National Wheat Scab Resistance Evaluation Farm in Nanping.

toxin content among regions and resistant levels was also processed by GLM. All data analysis was performed by R software with glm function.

3. Results

Fusarium head blight severity in wheat cultivars

FHB resistance evaluation identified 3 cultivars that were highly resistant (HR), 20 cultivars were moderate resistant (MR), 53 cultivars were moderately susceptible (MS), and 53 cultivars were highly susceptible (HS), accounting for 2.3, 15.5, 41.1 and 41.1% of the total number of samples, respectively. This indicated most of wheat cultivars (82.2%) were susceptible to FHB. The three highly resistant cultivars were all from MLYR, accounting for 12% of the samples in this region. Fifteen moderately resistant cultivars and seven moderately susceptible cultivars were also found, accounting for 60 and 28%, respectively, while no highly susceptible cultivars were identified in this region (Figure 2A).

The resistant level composition of cultivars from UYR proved significantly different (P=0.003, Fisher's exact test) from MLYR. Cultivars from UYR consisted of five moderately resistant cultivars, 14 moderately susceptible cultivars and two highly susceptible cultivars – accounting for 23.8, 66.7 and 9.5% of the total samples in this region, respectively. No resistant cultivars were found in the Huanghuai Region. Most cultivars from both SH and NH were highly susceptible (62.1 and 60.0%, respectively) (Figure 2A). No significant difference (P=1.00, Fisher's exact test) of resistant levels composition was observed between these two regions.

Regression analysis by GLM revealed significant differences (P<0.001) on disease index among regions: disease index of cultivars from MLYR (20.0±10.9) was significantly lower than other regions, indicating significant higher FHB resistance of cultivars from this region. Disease index of UYR (32.2±9.9) was higher than MLYR, but significantly lower than the SH (50.8±11.9) and NH (47.0±9.0) groups. There is no significant difference on disease index between SH and NH group (Figure 2B).

UPLC-MS/MS method validation

The analytical performance of the method was assessed by evaluating key parameters, such as signal linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy and precision. The correlation coefficients and linear ranges of the calibration curves for seven toxins in wheat were listed in Supplementary Table S1. A good linear relationship was found (R^2>0.99) between the peak area ratios and the concentrations of the standard solutions of the analytes. The LODs and LOQs of seven toxins were calculated on the basis of signal-to-noise ratios (S/N) of 3 and 10, respectively. The LODs and LOQs were 2.1 and 6.9 mg/kg for DON, 0.8 and 2.6 mg/kg for 3-ADON, 1.1 and 3.7 mg/kg for 15-ADON, 50 and 185 mg/kg for NIV, 2.1 and 7.1 mg/kg for ZEN, 0.2 and 0.6 mg/kg for T-2, 1.2 and 4.1 mg/kg for HT-2, respectively.

The accuracy and precision of this method are presented in Supplementary Table S2. All compounds gave acceptable recoveries and relative standard deviations at three spiked levels, in the range of 78.0-109.4% and 3.7-16.5%, respectively. The performance of the method could satisfy the requirements of the European Union (No 401/2006) (EC, 2006).

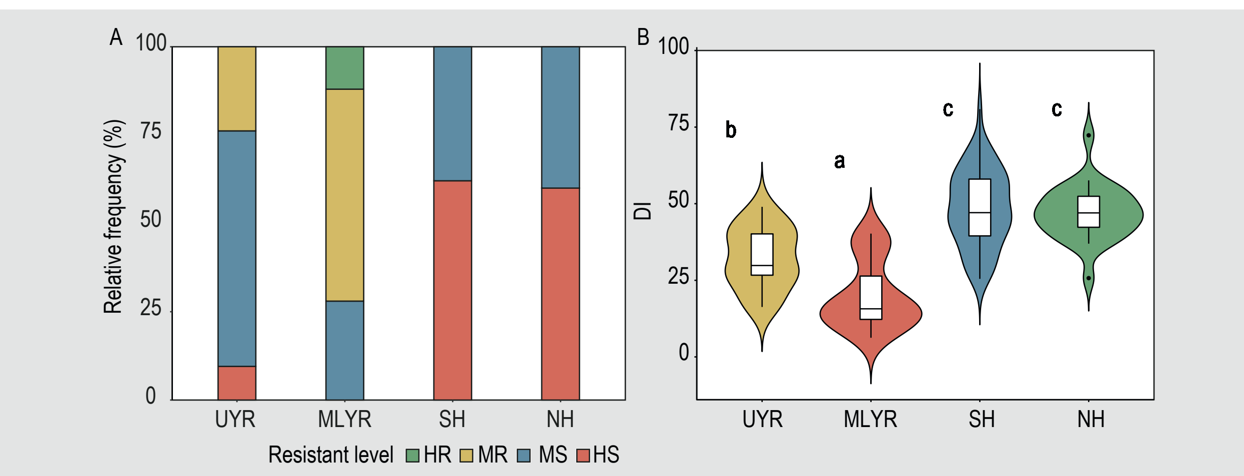

Figure 2. Disease resistance of cultivars in different ecological regions. (A) Relative frequency of *Fusarium* head blight resistant levels in the four regions. HR = high resistant; MR = moderate resistant; MS = moderate susceptible; HS = high susceptible. (B) Disease index (DI) of cultivars in the four regions. Width of the coloured part indicate the abundance of cultivars at this disease severity. The different letters for each region indicate significant difference at P=0.01. UYR = upper Yangtze river; MLYR = middle and lower Yangtze river; SH = south of Huanghuai region; NH = north of Huanghuai region.

Mycotoxins accumulation in different wheat cultivars and regions

The contents of seven different toxins (DON, 3-ADON, 15-ADON, NIV, ZEN, T-2 and HT-2) in the 129 wheat samples were determined. HT-2 was not found in any of the samples and the contamination of the other six toxins are summarised in Table 1. ZEN and type B trichothecenes (DON, 3-ADON, 15-ADON and NIV) were detected in a high fraction (>90.0%) of the samples. The type A trichothecene – T-2 was detected at significantly lower frequency (58.9%). Among the toxins, the DON content was significant higher (P<0.01) than other toxins, which ranged from 0.09-9.87 mg/kg with a mean of 2.35 mg/kg, accounting for 68.3% of the total toxins accumulation, followed by ZEN ranging from 0.00-1.91 mg/kg with a mean of 0.41 mg/kg, T-2 ranging from 0.00-0.57 mg/kg with a mean of 0.28 mg/kg, NIV ranging from 0.09-0.56 mg/kg with a mean of 0.21 mg/kg, and 15-ADON ranging from 0.00-0.54 mg/kg with a mean of 0.11 mg/kg, respectively. The 3-ADON content was the lowest and ranged from 0.00-0.48 mg/kg with a mean of 0.09 mg/kg. ANOVA analysis also showed a significant difference (P<0.01) in mycotoxin accumulation among these cultivars, indicating their significant different mycotoxin accumulation resistance.

To investigate the relation between cultivar source and resistance to toxin accumulation, all cultivars were divided into four groups according to the ecological regions. The results are illustrated in Figure 3 and Supplementary Table S3. Like FHB resistance, toxin content varied among the cultivars within each group, but significant regional differences (P<0.01) among the groups were observed, indicating significantly different resistance levels to the accumulation of toxins of wheat cultivars from different ecological regions. We found a similar regional toxin accumulation profile of DON, 3-ADON, 15-ADON, ZEN and T-2, cultivars from the two groups along the Yangtze River (UYR and MLYR) and showed a significant lower (P<0.01) content of these toxins than in the Huanghuai Region (NH and SH). The NIV accumulation profile is a little different, as NIV content of the NH group is significant higher (P<0.01) than in the other groups, while MLYR showed a significantly higher (P<0.01) NIV accumulation than UYR. The NIV content of wheat cultivars in the SH group were more variable, so no significant difference was observed with cultivars from either MLYR or UYR. We also found a very low positive rate of T-2 in MLYR (4%, 1/25) and UYR (0%, 0/21) groups, whereas it proved much higher in SH (88%, 51/58) and NH (100%, 25/25). The toxin and disease information are summarised in Supplementary Table S4.

Relationship between *Fusarium* head blight and toxin accumulation resistance

All wheat cultivars were grouped into four resistant levels and the toxins contents among these groups were compared. As shown in Figure 4, the toxin profiles indicate a clear association with resistant level groups except for NIV, as four resistance level groups showed similar NIV content. However, ANOVA analysis showed that no significant difference was observed between some resistant level groups. For example, DON accumulation of HR group was not significantly different from MR (P=0.99) and MS (P=0.17) group. Besides, cultivars in HR, MR and MS groups showed similar level of ZEN, 3-ADON and 15-ADON. This indicates toxin accumulation resistance is not straightforward positively related with FHB resistant levels.

Regression analysis between disease index and toxin accumulation was also performed by GLM. Different from resistant levels, disease index and toxin content of all 129 cultivars showed a significant positive relation except for NIV. However, the relationship was not significant within most ecological regions (19/23) (Table 2). The disease index of cultivars in UYR significantly correlated with ZEN and disease index of SH showed significant relation with DON and its acetylated derivatives. However, the disease index of Huamai 1028 (13.64), Ruiquanmai 24 (25.48), Han 115276 (25.71), Zhumai 328 (34.62) and Xinnong 518 (39.02) were lower, while they have a high total toxin content of 3.13 mg/kg, 4.32 mg/kg, 4.33 mg/kg, 6.14 mg/kg and 8.21 mg/kg respectively. In contrast, the disease index of R802 (41.07),

Table 1. Mycotoxin accumulation in 129 wheat cultivars.

Toxins	Minimum (mg/kg)	Maximum (mg/kg)	Average (mg/kg)[1]	Coefficients variation (%)	Contamination rate (%)
DON	0.09	9.87	2.35A	75.3	100
3-ADON	0	0.48	0.09C	90.7	99.2
15-ADON	0	0.54	0.11C	88.8	93.8
NIV	0.09	0.56	0.21BC	32.5	100
ZEN	0	1.90	0.41B	93.3	94.6
T-2	0	0.57	0.28BC	85.9	58.9

[1] Values followed by different letters are significantly different at P=0.01.

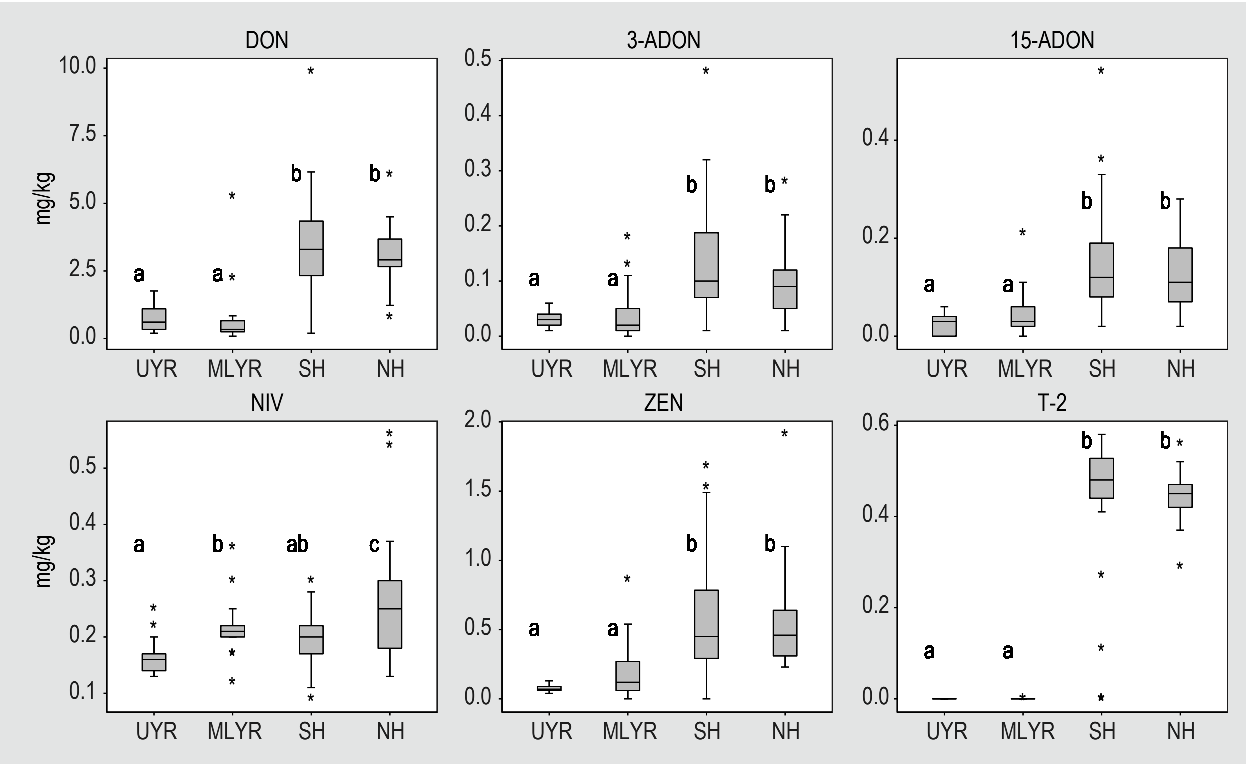

Figure 3. Content of six toxins in 129 wheat cultivars. The different letters for each toxin indicate significant difference at *P*=0.01. DON = deoxynivalenol; ADON = acetyl-deoxynivalenol; NIV = nivalenol; ZEN = zearalenone; T-2 = T-2 toxin; UYR = upper Yangtze river; MLYR = middle and lower Yangtze river; SH = south of Huanghuai region; NH = north of Huanghuai region. Asterisks represent outliers.

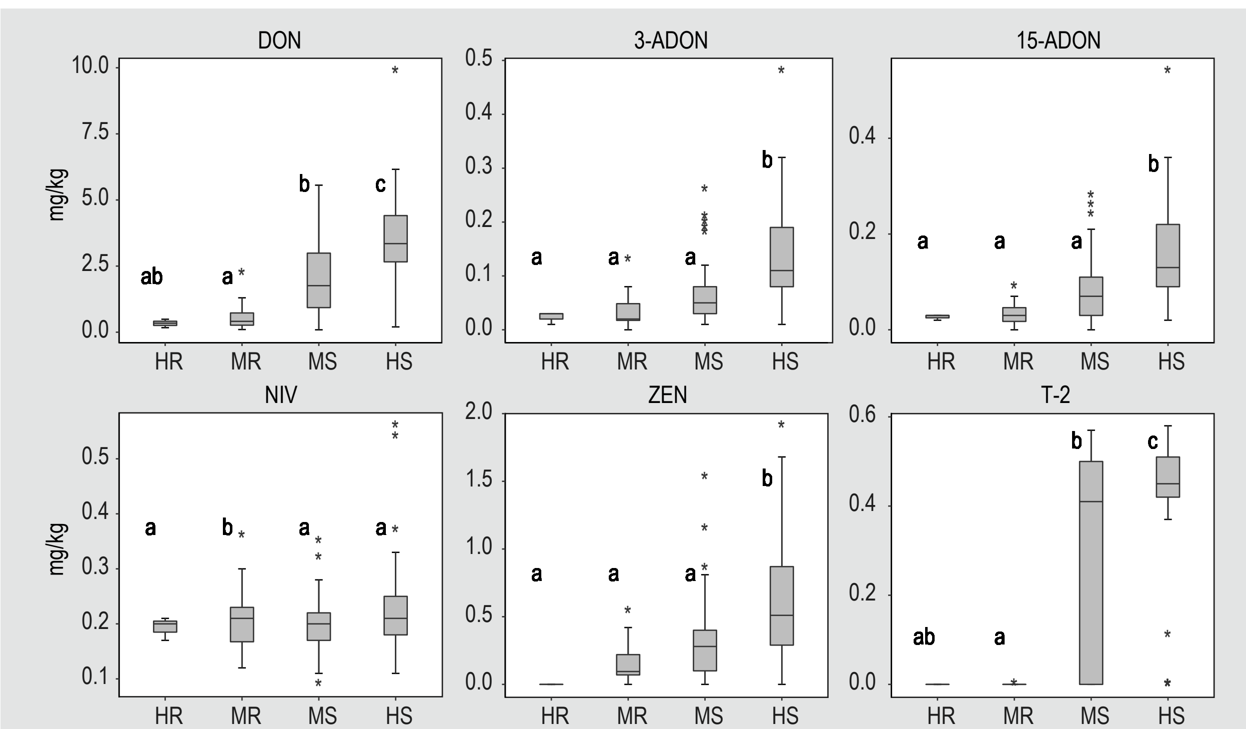

Figure 4. Contamination of six toxins in cultivars with different resistant levels. The different letters for each toxin indicate significant difference at *P*=0.01. DON = deoxynivalenol; ADON = acetyl-deoxynivalenol; NIV = nivalenol; ZEN = zearalenone; T-2 = T-2 toxin; HR = high resistant; MR = moderate resistant; MS = moderate susceptible; HS = high susceptible. Asterisks represent outliers.

Table 2. *P*-value of generalised linear regression between mycotoxin content and disease index in different ecological regions.[1,2]

Mycotoxins	Regions				
	Total	UYR	MLYR	SH	NH
DON	3.28×10^{-13}**	0.818	0.111	0.017*	0.401
ZEN	2.08×10^{-8}**	0.016*	0.131	0.093	0.124
3-ADON	2.86×10^{-10}**	0.801	0.061	0.003**	0.154
15-ADON	2.92×10^{-11}**	0.392	0.074	0.002**	0.262
NIV	0.258	0.366	0.913	0.546	0.799
T-2	1.84×10^{-11}**	NA[3]	0.406	0.834	0.434

[1] DON = deoxynivalenol; ADON = acetyl-deoxynivalenol; NIV = nivalenol; ZEN = zearalenone; T-2 = T-2 toxin; UYR = upper Yangtze river; MLYR = middle and lower Yangtze river; SH = south of Huanghuai region; NH = north of Huanghuai region.

[2] Significant related at * P=0.05; ** P=0.01.

[3] No T-2 was detected in this region.

Chuan 12147 (45.45), Nan 12 pin B991 (48.98), Qimai 2 (53.79), Xumai 0054 (67.14) were higher, however they have a low total toxin content of 0.76 mg/kg, 0.94 mg/kg, 0.67 mg/kg, 1.98 mg/kg, and 0.75 mg/kg respectively. PCA analysis of disease index and multi-toxins revealed that cultivars from Yangtze River regions (UYR and MLYR) and Huanghuai regions (NH and SH) are clearly separated (Figure 5), indicating significant different resistant levels to FHB and toxin accumulation between the two regions. Further analysis was also made on the two main toxins DON and ZEN: the dots distributed dispersedly in the dot plot (Figure 6A) indicate a weak, but significant, correlation between toxin content and disease index.

We used the overlap in the disease index (25<disease index<47) between Yangtze River regions (UYR and

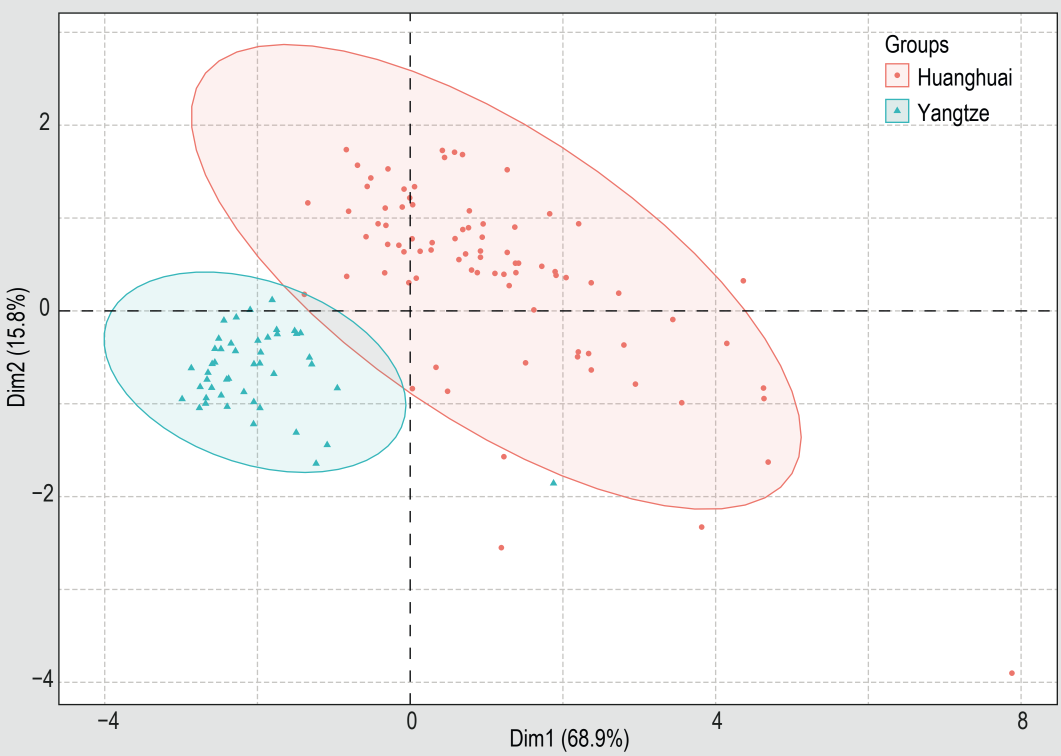

Figure 5. Principle component analysis of disease severity and six toxins content of cultivars from different regions.

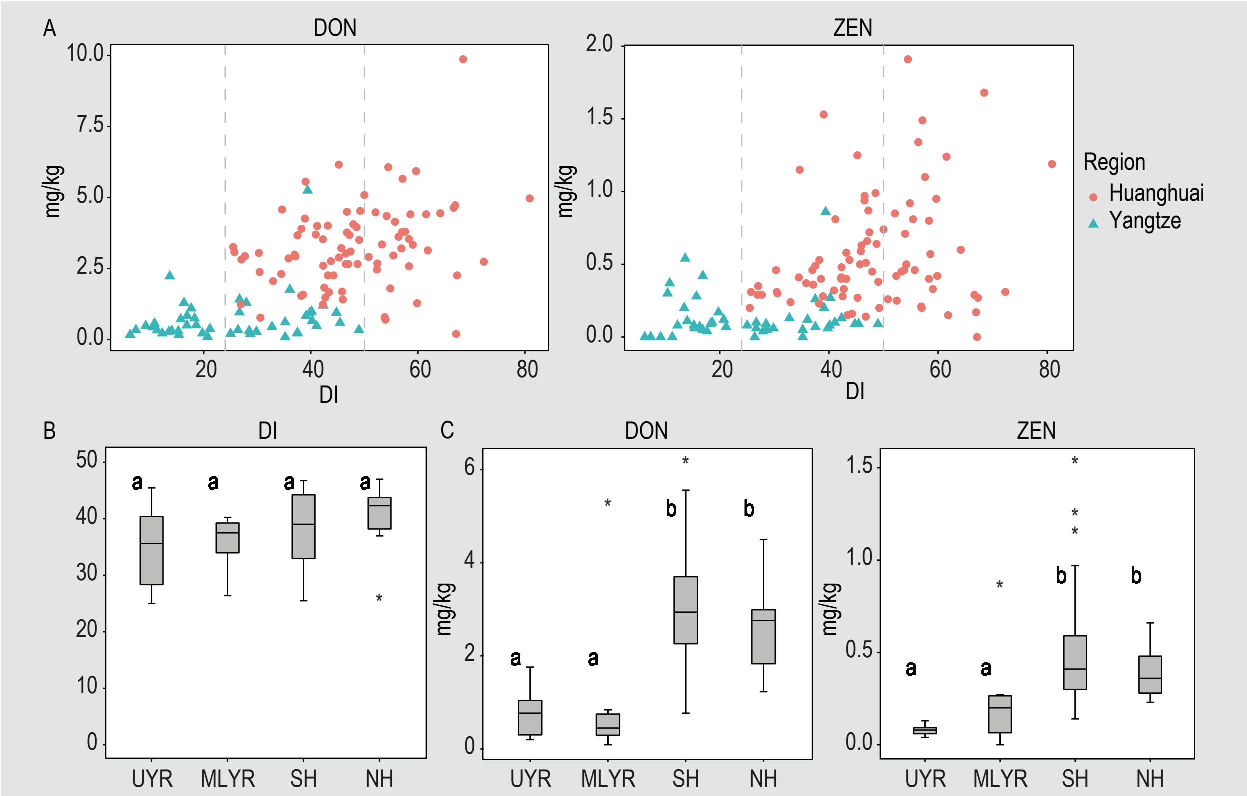

Figure 6. Correlation between disease index (DI) and toxin content (deoxynivalenol (DON) and zearalenone (ZEN)). (A) Dot plot of DI and mycotoxin content, region between the dotted lines indicated overlapped part of Yangtze River and Huanghuai Region on disease index (25<DI<47). (B) DI of cultivars in the subpopulation (25<DI<47) from different ecological regions. (C) DON and ZEN accumulation of cultivars in the subpopulation (25<DI<47) from different ecological regions. The different letters indicate significant difference at *P*=0.01. UYR = upper Yangtze river; MLYR = middle and lower Yangtze river; SH = south of Huanghuai region; NH = north of Huanghuai region. Asterisks represent outliers.

MLYR) and Huanghuai regions (NH and SH) (Figure 6A) to make a direct comparison. The cultivars in this interval were extracted as a subpopulation. In this subpopulation, cultivars in the four ecological regions showed a similar level (P=0.077) of FHB resistance (disease index) (Figure 6B), however, DON and ZEN accumulation was significant different. The toxin contents of cultivars from Yangtze River regions (UYR and MLYR) were significant lower (P<0.01) than in the Huanghuai regions (NH and SH) (Figure 6C). This reveals that under the same FHB resistance level, cultivars from the Yangtze River regions have a higher level of resistance to toxin accumulation than those from the Huanghuai regions.

4. Discussion

Breeding for disease resistance is one of the most important and cost-effective strategies for plant disease control. Chinese wheat breeders began to select plants or spikes with resistance to FHB in the disease epidemic areas since the late 1950s (Yao and Lu, 2000). Dozens of moderate resistant cultivars have been released and adopted for wheat production in the middle and lower reaches of Yangtze River Region during recent decades (Cheng *et al.*, 2012). However, 96% of the cultivars released in China are moderately or highly susceptible to FHB, only 4% (12/302) of the cultivars released from the national cultivar trials from 2005 to 2016 had moderate resistance to FHB (Ma *et al.*, 2019). All moderate resistance cultivars were bred in Jiangsu Province (MLYR) and the cultivars in other regions are all susceptible including the cultivars grown in the largest wheat producing area – the Huanghuai Region – where approximately 60% of China's wheat is produced.

We evaluated FHB resistance of 129 cultivars from local breeders in different ecological regions. Both the disease index and resistant level composition demonstrate that the resistance level of cultivars from MLYR was significantly higher than those in other regions. The climate in the middle and lower reaches of Yangtze River in China is humid and rainy, and the area has traditionally been a region of severe FHB epidemics and outbreaks. In this region, farmers are more likely to select FHB resistant cultivars. The strong selection pressures may also have provided conditions for the natural mutations of scab resistance to be preserved in wheat. In addition, the long history of targeted breeding may have fixed the resistance in local population and improved the overall FHB resistant level. A final, and perhaps decisive reason is the strict regulation of national and provincial cultivar trials, determining that highly susceptible cultivars cannot be released and marketed in this region. Our results indicate that this regulations significantly enhanced FHB resistance of cultivars grown in this area. No highly susceptible cultivars were found in MLYR Region. UYR is a mountain area, relative humidity is high with a lot of fog and less sunshine, the average temperature is a little lower than MLYR region. NIV producing *Fusarium asiaticum* is the predominant pathogen for FHB in this area (Yang *et al.*, 2018; Zhang *et al.*, 2012). Several previous reports have shown that this population was significant less virulent than *F. asiaticum* with 3-ADON chemotype, which dominates the MLYR region (Shen *et al.*, 2012; Zhang *et al.*, 2012). Lower biological and environmental selection pressures lead to the lower FHB resistance than in the MLYR region. Climate change as well as changes in farming systems such as straw incorporation and no-till farming in recent years, allowed FHB to gradually spread to northern parts (NH and SH) of China, where it is now a threat to the main wheat production area in China (Qiu *et al.*, 2014; Zhang *et al.*, 2007). In these regions, the frequency of FHB outbreaks increased only recently, and FHB resistance had low priority in breeding programs. Consequently, disease resistant cultivars in the Huanghuai wheat area are currently lacking (Zhou *et al.*, 2018). The popular wheat cultivars in the south of Huanghuai wheat growing area were found to be susceptible to FHB (Zhang *et al.*, 2018). In this study, cultivars from SH and NH region showed the same susceptibility to FHB and are significantly more susceptible that cultivars originating from the MLYR and UYR regions. No (moderate) resistant cultivar was identified and more than 60% of the cultivars were highly susceptible cultivars in the SH and NH regions. Due to different vernalization requirements, almost all moderately resistant cultivars, bred in the Yangtze River region, cannot be grown in northern regions, such as SH and NH. Therefore, due to lacking local resistance germplasms, involvement of suitable FHB resistant cultivars from MLYR in the breeding program in the Huanghuai Region (SH and NH) could be an effective strategy for rapid improving of FHB resistance level of local cultivars.

For breeding of wheat with resistance to FHB, not only disease management but also mitigating mycotoxin contamination is important. Evaluation of FHB resistance has been used as an indirect way to predict mycotoxin accumulation resistance of wheat cultivars. In China, the evaluation for cultivar registration and marketing in some FHB epidemic provinces critically depends on the FHB resistance evaluation. There are some contradictory reports on the relationship between FHB and mycotoxin accumulation resistance. Miedaner *et al.* (2003) reported that correlations between FHB ratings and DON content were high in segregating materials. Bai *et al.* (2001) investigated an international collection of 116 wheat cultivars and found a significant correlation between FHB symptom ratings and DON levels and concluded the disease evaluation can be generally used to predict DON levels in harvested wheat grain. However, Chen *et al.* (1995) inoculated five wheat varieties with a strain of *F. graminearum* and found no correlation between DON concentration and FHB severity. Liu *et al.* (1997) also found no correlation between DON concentration and FHB severity in wheat, barley and oats after inoculation with a mixture of *Fusarium culmorum* strains. Ji *et al.* (2015) evaluated the severity of FHB and DON content of 122 wheat varieties obtained from the Nordic Genetic Resource Center and the DON contamination levels did not increase consistently with increased FHB incidence.

Almost all the previous studies were based on artificial inoculation, so disease index mainly represents type II resistance – e.g. resistance to colonisation. In this study, we made an evaluation of FHB severity of 129 cultivars by natural infection, which we propose reflects more the field situation and also represents the overall resistance including all types of resistance. We found a significant correlation between disease index and most toxins of the whole population, but no clear correlation within most ecological regions. This indicated the correlation only exist on a global level with a wide geographical distribution. Nevertheless, also in our study we observed that although significant, the correlation was not strong. For a large part, the significant correlation is due to low levels of FHB correlating with low levels of toxin, but the toxin accumulation (DON, ZEN) varied much more with the increase of disease index (Figure 6A and 6B). These results demonstrate that selection of cultivars with low toxin accumulation by disease evaluation could result in overlooking cultivars that show type III resistance with low toxin accumulation. Toxin contamination of cultivars from the Huanghuai Region is significantly higher than in cultivars from Yangtze River Region with a similar resistance level, indicating cultivars bred in Yangtze River Region have a higher level of resistance to toxin accumulation and the toxin resistance is partly independent from disease severity. There are five types of FHB resistance in wheat, of which type II resistance has been regarded as the most effective and well-studied (Bai and Shaner, 2004), while others are less investigated. Type III (toxin accumulation) resistance is generally supposed to be independent resistance mechanism, but it has shown association with type I and/or type II resistance. For example, Liu *et al.* (2009) summarized the quantitative trait loci (QTL) for different FHB resistance components and found that only 25 out of 209 QTL were for types III resistance, and none of them was independent from type I and/or type II resistance. Recently, He *et al.* (2019) reported a QTL on wheat 3DL chromosome showing major effects only on

DON content, and a QTL on 3BL chromosome exhibiting major effects on DON in *Fusarium* damaged kernels and minor effects on field FHB resistance. This may explain the contradictory reports above, and the results obtained in this study. High frequency of association between types III and type I and/or type II resistance results in significant correlation between disease index and toxin content on a large-scale analysis. However, the independent type III resistant genes (QTL) will contribute to additional toxin accumulation resistance and decrease the correlation with disease severity. Therefore, harbouring QTLs specific to toxin content reduction may be the reason of high toxin accumulation resistance of cultivars in Yangtze River Region, whereas due to lack of these QTLs, the cultivars in Huanghuai Region with the same disease resistant level showed significant higher toxin contamination.

Along with the climate change and popularisation of minimal and no tillage, FHB has spread northward in China since the last decade and the Huanghuai region has become a high-risk area for FHB epidemics and mycotoxin contamination, especially the southern Huanghuai region where the disease severity was almost comparable to Yangtze River region in the last few years. In this study, based on a nationwide investigation, we found both FHB disease and mycotoxin accumulation resistance of wheat cultivars are low in the Huanghuai region. We propose that the strategy that was previously applied in the MLYR area will also be used in the Huanghuai region. Improvement of FHB resistance of cultivars by breeding programs in this region is urgently needed to reduce FHB and the resulting yield loss, quality loss and mycotoxin accumulation. Also setting minimal requirements for the list of selected cultivars could help to mitigate FHB and mycotoxin problems. Furthermore, our research demonstrates that understanding the relationship between FHB severity and mycotoxin contamination is important. Due to the high cost of mycotoxin measurement, visual disease rating is widely used as an indirect selection criterion to select wheat genotypes with low mycotoxin accumulation and, it is also the standard procedure for cultivar registration in many countries including China. However, we found this procedure on its own is not robust enough because of the poor correlation between disease severity and toxin content. Therefore, we suggest that mycotoxin determination is to be included in breeding procedures and used as a reference in evaluation of national cultivar trials.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/WMJ2019.2501.

Table S1. Linear relationships and sensitivity of seven toxins.

Table S2. Recoveries and RSDs of seven toxins in wheat.

Table S3. Toxin accumulation of cultivars from four ecological regions.

Table S4. The disease index, resistant levels and toxin content of 129 cultivars.

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Conflict of interest

The authors declare no conflict of interest.

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