Plant Pathol. J. 38(6) : 637-645 (2022)
https://doi.org/10.5423/PPJ.OA.09.2022.0130
eISSN 2093-9280

Research Article Open Access

The Plant Pathology Journal
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Evaluation of Barley and Wheat Germplasm for Resistance to Head Blight and Mycotoxin Production by *Fusarium asiaticum* and *F. graminearum*

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(Received on September 9, 2022; Revised on October 17, 2022; Accepted on October 17, 2022)

Fusarium head blight (FHB) is one of the most serious diseases in barley and wheat, as it is usually accompanied by the production of harmful mycotoxins in the grains. To identify FHB-resistant breeding resources, we evaluated 60 elite germplasm accessions of barley (24) and wheat (36) for FHB and mycotoxin accumulation. Assessments were performed in a greenhouse and five heads per accession were inoculated with both *Fusarium asiaticum* (Fa73, nivalenol producer) and *F. graminearum* (Fg39, deoxynivalenol producer) strains. While the accessions varied in disease severity and mycotoxin production, four wheat and one barley showed <20% FHB severity repeatedly by both strains. Mycotoxin levels in these accessions ranged up to 3.9 mg/kg. FHB severity was generally higher in barley than in wheat, and Fa73 was more aggressive in both crops than Fg39. Fg39 itself, however, was more aggressive toward wheat and produced more mycotoxin in wheat than in barley. FHB severity by Fa73 and Fg39 were moderately correlated in both crops ($r = 0.57/0.60$ in barley and 0.42/0.58 in wheat). FHB severity and toxin production were also correlated in both crops, with a stronger correlation for Fa73 ($r = 0.42/0.82$ in barley, 0.70 in wheat) than for Fg39.

Keywords **:** barley, Fusarium head blight, germplasm, mycotoxin, wheat

Fusarium head blight (FHB) is one of the most critical diseases of small-grain cereals such as wheat and barley. It causes not only damaged grains and yield loss, but also mycotoxin accumulation in infected grains. *Fusarium asiaticum* and *F. graminearum*, which belong to *Fusarium graminearum* species complex (FGSC), are two major contributors to FHB in these crops, particularly in Korea (Del Ponte et al., 2022; Jang et al., 2019; Lee et al., 1985; Shin et al., 2018). To date, five members (*F. graminearum*, *F. asiaticum*, *F. vorosii*, *F. boothii*, and *F. meridionale*) of the FGSC have been reported to be present in Korea with *F. asiaticum* predominant (Lee et al., 2016). These species can produce various mycotoxins including deoxynivalenol (DON) and its acetyl derivatives, nivalenol (NIV) and its acetyl derivative, zearalenone (ZEN), etc. (Desjardins, 2006; Lee et al., 2016). Unlike *F. graminearum*, which is widespread worldwide and mostly produces DON, *F. asiaticum* produces mostly NIV and is distributed with regional limits (van der Lee et al., 2015) and/or in relation to climate and cropping system (Lee et al., 2009; Xu et al., 2021).

In Korea, *F. asiaticum* with NIV chemotype is dominant in barley and wheat fields along with *F. asiaticum* and *F. graminearum* with DON chemotype. Barley and wheat are mostly cultivated consecutively with rice, allowing these crops to share FHB inocula for years and become conducive to infection. FHB severity can be managed by applying host resistance, chemical fungicides, biological control, or cultural practices (Buerstmayr et al., 2020; Chen et al., 2019). FHB resistance is an important agronomic

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Handling Editor : Ki Woo Kim

traits for breeding barley and wheat (Steiner et al., 2017). However, few barley and wheat varieties exhibit strong and stable resistance in the fields (Bai and Shaner, 2004; Chen et al., 2019). Those developed for FHB resistance appeared to lose their resistance quickly over time, or many germplasms identified turned to variable and susceptible to FHB (Ma et al., 2020). In Korea, only a few cultivars are known to be resistant to FHB (Han and Kim, 2005; Kim et al., 2020; Ma et al., 2020; Park et al., 2008), and none of them impart satisfactory FHB or mycotoxin resistance.

In this study, to aid the selection of resources resistant to FHB, we evaluated barley and wheat germplasm accessions by testing FHB severity and mycotoxin accumulation. In the FHB assays, we used both *F. asiaticum* and *F. graminearum* as inocula to investigate whether they could produce similar levels of FHB in barley and wheat because FHB assessment for resistance is usually done with a single species. Here, we report the results of an evaluation of 60 germplasm accessions and a comparison of the FHB severity and mycotoxin production between *F. asiaticum* and *F. graminearum*.

Materials and Methods

Germplasm selection. Sixty germplasm accessions were selected from barley and wheat from the National Agrobiodiversity Center at the Rural Development Administration based on their agronomic traits. The selection was made among those with rapid heading, rapid ripening, and short stems. Additional traits were less cold damage and no lodging for barley, and short awns, high processing suitability, and resistance to FHB, powdery mildew, and leaf rust for wheat.

FHB assay. For the FHB assessment, the seeds of each accession were sown in peat soil and vernalized for 4 weeks at 5°C. The seedlings were transplanted into larger pots (25 cm in diameter) in a greenhouse. When the heads started to flower, five heads of each accession were inoculated by spraying (~2 ml/head) with conidia (1×10^5) of *F. asiaticum* #73 (Fa73) and *F. graminearum* Z3639 (Fg39) (McCormick and Alexander, 2002), as previously described (Baek et al., 2020). Fa73 was a strain isolated from Korean rice in 2015 and confirmed for NIV production. These strains were selected based on their ability to produce enough number of conidia and pathogenicity from our laboratory collection. Both strains were maintained at –80°C until use. Sterile water was used as negative control. The inoculated heads were covered with a plastic bag for humidity for three days and, then uncovered. Three weeks after inoculation, treated heads were harvested and scored for FHB severity. The assay was repeated twice during different seasons (March-May 2020 and November 2020-May 2021). FHB severity (%) was calculated as the mean of [(number of grains with FHB symptoms/total number of grains in a head) × 100]. Some heads that turned white after inoculation were excluded from FHB scoring.

Mycotoxin analysis. Harvested grains were completely dried in a hood and ground in a mixer mill (Retsch MM 400, Haan, Germany) for analysis of DON, NIV, 3-acetyl DON (3-ADON), 15-acetyl DON (15-ADON), or ZEN. Toxin analysis was performed as previously described with modifications (Lee et al., 2013). One gram of the ground sample was mixed with 8 ml of a solution of 1% acetic acid in acetonitrile (ACN) and extracted with shaking at 300 rpm for 1 h. After adding 0.25 g NaCl and 1 g $MgSO_4$, the extract was centrifuged at 3,600 rpm for 10 min. The supernatant was mixed with 0.15 g $MgSO_4$, 0.05 g endcapped C18, and 0.05 g primary secondary amine then centrifuged again under the same condition. The supernatant was dried under N_2 gas and the residue was reconstituted with 20% ACN. For liquid chromatography-mass spectrometry (Waters e2695 separation module, Waters 3100 MS detector, Milford, MA, USA) analysis, a Zorbax SB A1 C18 (4.6 × 150 mm, 5 µm, Agilent, Palo Alto, CA, USA) column was used. The flow solvent was a water mixture containing 0.2% formic acid and 5 mM ammonium formate in DW (A) and methanol (B) in a gradient. The mass spectrometer was operated in single ion recording (SIR mode), both in electrospray ionization positive and negative. The mobile phase flow rate was 0.3 ml/min and the injection volume was 10 µl.

Correlation analysis. Pearson's correlation coefficients were calculated using Microsoft Excel for FHB severity and mycotoxin production by *F. asiaticum* and *F. graminearum* in barley and wheat.

Results

FHB severity. The germplasm accessions tested varied in FHB severity by inoculum or assay. However, FHB severity induced by either strain was greater in barley than in wheat. Fig. 1 shows clearly that the blue box (the result of the first assay with Fa73) in barley panel was greater than one in wheat by the values of mean, median, and maximum severity. Other colors show the same pattern. In the first assay, the means of FHB severity by Fa73 were 46.8% and 33.9%, ranging up to 93.6% and 73.6% in barley and

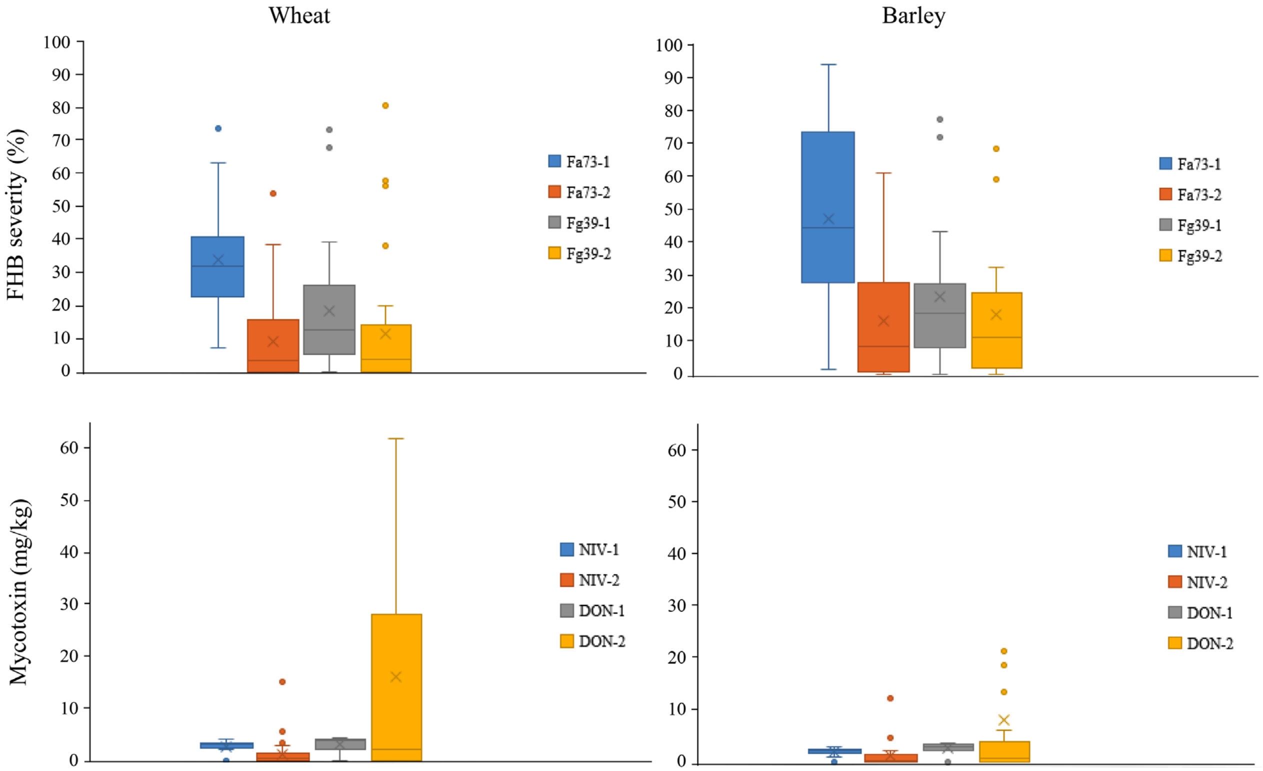

Fig. 1. Comparison of Fusarium head blight (FHB) severity and mycotoxin production by *Fusarium asiaticum* and *F. graminearum* in barley and wheat. Upper plots for FHB severity, lower ones for mycotoxin production, left plots for wheat, and right ones for barley. The numbers after isolate name or mycotoxin indicate assay order. The boxplots were generated with Microsoft Excel.

wheat, respectively (Tables 1 and 2). The means of FHB severity by Fg39 were 22.7% and 18.3%, and up to 77.1% and 73.5% in barley and wheat, respectively. In the second assay, both strains produced much lower means of FHB severity than in the first, and Fg39 produced slightly higher means (18.0% in barley and 11.7% in wheat) than Fa73 (16.2% in barley and 9.3% in wheat). The inoculated heads of some accessions (especially 33% of barley) turned white in the second assay, showing the sensitivity of this assay (Table 1).

The lowest FHB severity was found in one barley (b32) (mean 10.5%, ranging from 8.0% to 15.3%) (Table 1). In wheat, the lowest were two (w49, w51) with means of 11.4% and 4.6%, ranging from 0.9% to 18.2% and from 0 to 18.2%, respectively (Table 2). There were two more wheat accessions with the highest FHB severity of 19.0% (w3) and 19.5% (w12) (Table 2). The rest showed >20% FHB severity. Among the 19 barley accessions with severity values, 18 (95%) showed higher severity by Fa73 than by Fg39 in the first assay, and 14 of them (74%) showed >30% FHB severity by Fa73 (Table 1). Only four of them showed >30% FHB severity by Fg39. In the second assay, six accessions were consistent with higher severity by Fa73 (Table 1), whereas six others turned to the opposite with higher severity by Fg39. Only two and three accessions showed >30% FHB severity by Fa73 and Fg39, respectively. One accession (b60) was consistent with a higher severity of Fg39. In wheat, 28 (85%) of 33 accessions with severity values showed higher severity for Fa73 than for Fg39 in the first assay (Table 2). Nineteen of the 33 accessions (58%) showed >30% FHB severity by Fa73, and four of them had >30% FHB severity by Fg39. In the second assay, 10 accessions maintained higher severity by Fa73, whereas 12 others turned to the opposite with higher severity by Fg39. Only two and four accessions showed >30% FHB severity by Fa73 and Fg39, respectively. None of the wheat accessions were consistently susceptible to Fg39.

Mycotoxin production. Both NIV and DON were detected in most of the accessions analyzed with greater mycotoxin accumulation by Fg39 (Fig. 1). In the first assay, Fa73 produced means of 2.1 and 3.1 mg/kg NIV in barley and wheat, respectively, ranging from 1.1 to 3.0 mg/kg in barley, and from 0.3 to 4.0 mg/kg in wheat (Tables 1 and

Table 1. FHB severity and mycotoxin production in barley[a]

IT No.[b]	Serial No.	FHB severity (%)				Mycotoxin (mg/kg)[c]							
		1st assay		2nd assay		1st assay		2nd assay					
		Fa73-1	Fg39-1	Fa73-2	Fg39-2	NIV-1	DON-1	NIV-2	DON-2	3-ADON-2	15-ADON-2	DON total-2	ZEN-2
291150	b24	44.6 ± 21.6	18.4 ± 9.6	26.8 ± 13.1	21.8 ± 20.1	1.8 ± 0.1	3.1 ± 0.1	0.0 ± 0.0	0.9 ± 0.0	0.0 ± 0.0	0.5 ± 0.0	1.4	0.0 ± 0.0
291515	b25	60.1 ± 20.4	11.1 ± 7.5	6.9 ± 7.4	1.8 ± 4.1	2.0 ± 0.0	3.2 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.3	0.0 ± 0.0
291563	b26	wh	wh	6.8 ± 7.2	13.5 ± 13.1	2.3 ± 0.0	3.0 ± 0.1	0.9 ± 0.1	13.6 ± 11.8	0.1 ± 0.1	3.7 ± 3.2	17.4	0.0 ± 0.0
291699	b27	14.7 ± 6.3	3.8 ± 5.6	wh	wh	1.8 ± 0.1	2.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
291746	b28	wh	wh	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.1	2.9 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.3	0.0 ± 0.0
291749	b29	wh	wh	na	na	2.4 ± 0.3	2.7 ± 0.9	-	-	-	-	-	-
306775	b31	39.5 ± 22.7	21.4 ± 16.8	na	na	1.6 ± 0.1	2.3 ± 0.2	-	-	-	-	-	-
315405	b32	15.3 ± 13.0	8.0 ± 12.9	8.3 ± 6.9	10.3 ± 14.0	1.2 ± 0.0	2.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
319783	b33	31.1 ± 14.8	14.1 ± 14.7	wh	wh	1.8 ± 0.0	2.6 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.9 ± 0.8	1.1	0.0 ± 0.0
319785	b34	25.7 ± 24.5	16.7 ± 26.0	wh	wh	1.1 ± 0.0	2.3 ± 0.3	0.2 ± 0.2	0.2 ± 0.0	0.0 ± 0.0	0.8 ± 0.1	1.0	0.0 ± 0.0
320146	b35	91.5 ± 2.4	77.1 ± 7.1	11.4 ± 14.1	11.3 ± 5.4	2.2 ± 0.2	3.0 ± 0.1	0.3 ± 0.0	2.7 ± 4.7	0.0 ± 0.0	0.3 ± 0.4	3.0	0.0 ± 0.0
320183	b36	93.6 ± 3.2	38.3 ± 16.9	wh	wh	2.3 ± 0.0	3.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.3	0.0 ± 0.0
320301	b37	78.7 ± 30.8	4.8 ± 6.7	28.4 ± 23.8	8.7 ± 8.9	2.1 ± 0.1	3.3 ± 0.1	0.1 ± 0.2	1.4 ± 1.2	0.0 ± 0.0	0.4 ± 0.4	1.8	0.1 ± 0.2
320302	b38	56.9 ± 32.7	24.1 ± 27.6	39.3 ± 15.2	32.6 ± 32.6	2.2 ± 0.1	3.3 ± 0.1	4.6 ± 0.4	1.9 ± 3.2	0.0 ± 0.1	0.3 ± 0.5	2.2	0.0 ± 0.0
320541	b39	31.2 ± 14.5	15.5 ± 17.8	0.0 ± 0.0	1.3 ± 2.3	2.6 ± 0.2	3.7 ± 0.1	0.0 ± 0.0	0.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.4	0.0 ± 0.0
320564	b40	wh	wh	1.1 ± 2.5	12.3 ± 13.1	1.5 ± 0.1	0.3 ± 0.1	1.6 ± 0.1	2.5 ± 4.4	0.1 ± 0.1	0.3 ± 0.5	2.9	0.0 ± 0.0
320582	b41	35.3 ± 30.4	5.6 ± 5.2	61.0 ± 9.8	68.3 ± 7.5	2.8 ± 0.1	3.6 ± 0.2	12.4 ± 1.4	120.5 ± 3.2	1.9 ± 0.0	11.0 ± 0.8	133.5	0.0 ± 0.0
320584	b42	53.7 ± 8.6	27.6 ± 17.3	wh	wh	-	-	1.9 ± 0.5	6.1 ± 0.2	0.2 ± 0.0	0.5 ± 0.0	6.8	0.0 ± 0.0
320833	b43	wh	wh	wh	wh	3.0 ± 0.0	3.3 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
320840	b44	77.6 ± 12.4	26.7 ± 12.1	wh	0.0 ± 0.0	2.2 ± 0.1	3.6 ± 0.2	0.0 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	0.7	0.0 ± 0.0
320859	b45	1.7 ± 3.3	0.0 ± 0.0	wh	wh	-	-	2.2 ± 0.3	4.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.0	4.9	0.0 ± 0.0
327830	b58	73.4 ± 13.3	43.3 ± 16.2	27.7 ± 24.0	24.7 ± 11.7	2.7 ± 0.1	3.6 ± 0.1	2.1 ± 0.2	21.4 ± 2.1	0.5 ± 0.1	0.8 ± 0.0	22.7	0.1 ± 0.1
331194	b59	27.7 ± 33.1	21.1 ± 27.8	8.8 ± 7.7	4.4 ± 3.9	2.3 ± 0.0	3.4 ± 0.2	1.1 ± 0.1	1.8 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	2.2	0.0 ± 0.0
331562	b60	44.6 ± 49.3	71.8 ± 41.0	0.0 ± 0.0	59.2 ± 22.3	2.4 ± 0.2	3.6 ± 0.1	0.0 ± 0.0	18.6 ± 3.4	0.4 ± 0.0	1.8 ± 0.3	20.9	0.0 ± 0.0
Dahyang		38.9 ± 18.1	3.8 ± 5.2	69.1 ± 23.5	na	1.1 ± 0.1	3.5 ± 0.2	-	-	-	-	-	-
Mean[d]		46.8	22.7	16.2	18.0	2.1	3.0	1.3	9.0	0.2	1.1	10.2	0.0
Median[d]		44.6	18.4	8.5	11.3	2.2	3.2	0.2	1.2	0.0	0.4	1.8	0.0
Max[d]		93.6	77.1	61.0	68.3	3.0	3.7	12.4	120.5	1.9	11.0	133.5	0.1
Min[d]		1.7	0.0	0.0	0.0	1.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0

FHB, Fusarium head blight; NIV, nivalenol; DON, deoxynivalenol; 3-ADON, 3-acetyl DON; 15-ADON, 15-acetyl DON; ZEN, zearalenone; wh, white head; na, not assessed; -, not analyzed.

[a] Values are presented as mean ± standard deviation.

[b] IT No., accession number designated by National Agrobiodiversity Center.

[c] DON total = sum level of DON, 3-ADON, and 15-ADON. Limit of detection of mycotoxins (mg/kg): 0.02, 0.015, 0.03, 0.04, 0.003 for NIV, DON, 3-ADON, 15-ADON, ZEN, respectively.

[d] Calculation was done with germplasm data only.

Table 2. FHB severity and mycotoxin production in wheat[a]

IT No.[b]	Serial No.	FHB severity (%)				Mycotoxin (mg/kg)[c]						
		1st assay		2nd assay		1st assay		2nd assay				
		Fa73-1	Fg39-1	Fa73-2	Fg39-2	NIV-1	DON-1	NIV-2	DON-2	3-ADON-2	15-ADON-2	DON total-2
15872	w1	73.6 ± 12.4	68.1 ± 18.2	23.2 ± 1.6	0.0 ± 0.0	3.1 ± 0.1	3.7 ± 0.3	2.9 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
15996	w2	63.4 ± 11.2	29.1 ± 28.7	14.0 ± 20.5	1.4 ± 1.9	2.1 ± 1.2	3.8 ± 0.1	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
16036	w3	19.0 ± 21.9	11.7 ± 10.2	2.3 ± 3.2	7.4 ± 7.1	3.0 ± 0.4	3.9 ± 0.0	0.2 ± 0.0	1.5 ± 0.2	0.0 ± 0.0	0.5 ± 0.0	1.9
16069	w4	23.3 ± 14.8	8.0 ± 7.4	17.8 ± 5.0	0.8 ± 1.4	2.9 ± 0.3	4.1 ± 0.3	2.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
16077	w5	32.1 ± 5.8	28.2 ± 11.0	6.7 ± 2.7	5.1 ± 8.7	2.9 ± 0.5	4.0 ± 0.1	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
16251	w6	9.6 ± 19.6	22.3 ± 16.1	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 0.2	4.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
16252	w7	13.3 ± 6.4	3.4 ± 4.8	38.5 ± 26.4	20.2 ± 11.3	3.3 ± 0.4	1.8 ± 0.1	15.1 ± 0.6	19.4 ± 1.1	0.3 ± 0.0	5.9 ± 0.6	25.6
16253	w8	58.7 ± 6.9	38.6 ± 22.4	24.2 ± 14.4	1.9 ± 3.1	3.4 ± 0.0	4.0 ± 0.2	3.4 ± 0.3	2.0 ± 0.1	0.0 ± 0.0	0.6 ± 0.0	2.7
16254	w9	41.5 ± 8.4	12.7 ± 9.2	24.4 ± 7.9	10.9 ± 7.7	2.8 ± 0.3	3.8 ± 0.2	5.4 ± 0.8	24.4 ± 0.9	0.3 ± 0.0	7.1 ± 0.7	31.8
16261	w10	57.9 ± 25.9	26.9 ± 9.9	18.8 ± 21.4	5.2 ± 5.1	2.8 ± 0.2	3.8 ± 0.2	1.3 ± 0.1	7.7 ± 1.6	0.1 ± 0.1	3.2 ± 1.0	11.0
16459	w11	32.3 ± 8.6	39.2 ± 19.1	7.8 ± 2.7	3.6 ± 5.4	3.0 ± 0.2	3.6 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
16552	w12	7.4 ± 8.3	19.5 ± 9.0	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 1.3	3.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
16575	w13	47.0 ± 22.9	21.7 ± 5.3	15.1 ± 16.4	3.3 ± 3.3	3.1 ± 0.2	3.8 ± 0.2	1.0 ± 0.0	10.3 ± 0.3	0.1 ± 0.0	2.3 ± 0.1	12.7
16666	w14	26.5 ± 11.1	9.2 ± 13.4	23.9 ± 15.4	56.3 ± 24.0	3.4 ± 0.2	3.9 ± 0.1	2.8 ± 0.4	40.1 ± 3.9	0.9 ± 0.1	13.2 ± 1.3	54.1
198184	w15	wh	wh	2.9 ± 2.6	7.4 ± 2.5	3.2 ± 0.1	3.5 ± 0.1	0.2 ± 0.0	25.4 ± 3.0	0.3 ± 0.0	6.2 ± 0.5	31.8
198249	w16	wh	wh	wh	wh	-	-	0.0 ± 0.0	0.7 ± 0.1	0.0 ± 0.0	0.5 ± 0.0	1.3
205581	w17	40.6 ± 32.3	4.8 ± 8.2	12.5 ± 14.6	15.3 ± 9.8	-	-	0.4 ± 0.0	29.0 ± 2.9	0.2 ± 0.0	6.2 ± 0.8	35.5
210438	w18	wh	wh	wh	wh	0.3 ± 0.0	3.8 ± 0.0	1.6 ± 0.2	2.3 ± 2.0	1.0 ± 0.1	4.3 ± 0.2	7.6
210451	w19	35.6 ± 16.0	15.1 ± 10.9	0.0 ± 0.0	0.0 ± 0.0	3.1 ± 0.1	3.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
210452	w20	33.1 ± 4.4	19.1 ± 12.0	0.0 ± 0.0	4.4 ± 8.8	2.8 ± 0.3	3.7 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.2
210484	w21	27.4 ± 12.0	4.2 ± 9.4	2.6 ± 1.8	16.8 ± 12.0	3.2 ± 0.1	4.0 ± 0.1	0.3 ± 0.0	51.2 ± 0.8	0.2 ± 0.0	4.1 ± 0.3	55.5
214604	w22	31.3 ± 19.3	26.0 ± 10.7	12.3 ± 8.3	57.8 ± 34.0	3.5 ± 0.3	4.0 ± 0.1	1.3 ± 0.1	61.8 ± 3.2	0.4 ± 0.1	17.5 ± 0.6	79.7
269367	w23	32.4 ± 17.5	6.1 ± 4.2	3.9 ± 3.3	13.7 ± 10.5	3.6 ± 0.3	4.1 ± 0.1	0.5 ± 0.1	8.7 ± 0.8	0.0 ± 0.0	1.6 ± 0.4	10.2
301705	w30	54.2 ± 14.0	18.0 ± 34.6	54.1 ± 28.2	80.5 ± 15.6	-	-	2.1 ± 0.0	40.3 ± 1.2	0.3 ± 0.0	13.6 ± 1.0	54.2
323562	w46	26.5 ± 6.8	9.5 ± 7.2	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 0.4	3.8 ± 0.2	0.0 ± 0.0	6.1 ± 0.1	0.0 ± 0.0	3.9 ± 0.6	10.0
323778	w47	20.4 ± 19.8	19.8 ± 28.4	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 0.2	3.9 ± 0.6	0.0 ± 0.0	54.9 ± 9.2	0.4 ± 0.1	10.3 ± 2.3	65.6
324305	w48	27.3 ± 16.8	4.8 ± 8.7	2.6 ± 3.6	0.0 ± 0.0	3.4 ± 0.3	3.3 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
324467	w49	18.2 ± 11.9	12.7 ± 12.3	0.9 ± 1.9	13.9 ± 27.8	3.8 ± 0.0	-	0.0 ± 0.0	41.1 ± 0.6	0.3 ± 0.0	10.7 ± 1.0	52.1
325290	w50	33.8 ± 24.2	73.5 ± 10.9	0.0 ± 0.0	0.0 ± 0.0	-	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
325587	w51	18.2 ± 8.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
325689	w52	35.9 ± 31.0	27.4 ± 30.9	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
325968	w53	39.2 ± 25.0	3.9 ± 6.7	4.4 ± 9.8	13.7 ± 15.0	4.0 ± 0.0	4.4 ± 0.1	0.4 ± 0.4	35.0 ± 1.5	0.4 ± 0.0	13.5 ± 0.4	48.8
326055	w54	62.3 ± 13.4	2.6 ± 5.8	4.4 ± 3.7	38.3 ± 22.3	4.0 ± 0.3	4.1 ± 0.0	0.5 ± 0.1	97.4 ± 1.6	1.1 ± 0.1	33.8 ± 0.1	132.3
326070	w55	26.6 ± 6.5	11.0 ± 8.5	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.9	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0

Table 2. Continued

IT No.^{b}	Serial No.	FHB severity (%)				Mycotoxin (mg/kg)^c						
		1st assay		2nd assay		1st assay		2nd assay				
		Fa73-1	Fg39-1	Fa73-2	Fg39-2	NIV-1	DON-1	NIV-2	DON-2	3-ADON-2	15-ADON-2	DON total-2
326236	w56	22.4 ± 7.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 0.5	3.8 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
326672	w57	30.7 ± 6.2	12.2 ± 12.3	0.0 ± 0.0	19.2 ± 34.7	3.9 ± 0.3	4.3 ± 0.0	0.0 ± 0.0	22.0 ± 1.5	0.1 ± 0.0	5.1 ± 0.5	27.2
Geumgang		32.2 ± 9.0	12.3 ± 10.1	30.5 ± 13.5	na	3.9 ± 0.2	4.3 ± 0.1	-	-	-	-	-
Mean^d		33.9	18.3	9.3	11.7	3.1	3.7	1.2	16.2	0.2	4.6	20.9
Median^d		32.1	12.7	3.4	4.0	3.2	3.8	0.3	2.2	0.0	1.1	5.1
Max^d		73.6	73.5	54.1	80.5	4.0	4.4	15.1	97.4	1.1	33.8	132.3
Min^d		7.4	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0

FHB, Fusarium head blight; NIV, nivalenol; DON, deoxynivalenol; 3-ADON, 3-acetyl DON; 15-ADON, 15-acetyl DON; wh, white head; na, not assessed; -, not analyzed.

[a] Values are presented as mean ± standard deviation.

[b] IT No., accession number designated by National Agrobiodiversity Center.

[c] DON total = sum level of DON, 3-ADON, and 15-ADON. Limit of detection of mycotoxins (mg/kg): 0.02, 0.015, 0.03, 0.04 for NIV, DON, 3-ADON, 15-ADON, respectively.

[d] Calculation was done with germplasm data only.

2). Fg39 produced means of 3.0 and 3.7 mg/kg DON in barley and wheat, respectively, ranging from 0.3 to 3.7 mg/kg in barley, and up to 4.4 mg/kg in wheat. In the second assay, Fa73 produced decreased level of NIV than the first but with increase in some accessions up to 12.4 mg/kg (barley) and 15.1 mg/kg NIV (wheat) (means of 1.3 mg/kg and 1.2 mg/kg in barley and wheat). In contrast, Fg39 produced increased level of DON up to 120.5 mg/kg and 97.4 mg/kg in barley and wheat, respectively (means of 9.0 mg/kg and 16.2 mg/kg in barley and wheat). However, all medians were much lower than the first. It is notable that the highest DON and NIV levels were detected from the very same accession with >60% FHB severity (b41) (Table 1), indicating the susceptibility of this accession especially to mycotoxin accumulation. Regardless of the FHB severity of the accessions, the level of DON detected was higher than that of NIV in most accessions. Additional analysis of the acetyl derivatives of DON in the second assay showed that both 3-ADON and 15-ADON were produced in 29% and 75% of barley, and in 42% and 61% of wheat, respectively. Between 3-ADON and 15-ADON, the levels of 15-ADON were higher than 3-ADON levels in all accessions with one exception. The accessions with >10 mg/kg DON also accumulated 15-ADON up to 33.8 mg/kg, ranging in sum production of DON and acetyl DONs from 12.7 to 132.3 mg/kg. The five accessions with <20% FHB severity accumulated either NIV or DON up to 3.9 mg/kg at maximum, except one (w49) with accumulation of 41.1 mg/kg of DON and 10.7 mg/kg of 15-ADON. ZEN was detected in only 8% of barley (2/22), with mean of 0.1 mg/kg (Table 1).

Correlation between FHB severity and mycotoxin levels in barley and wheat. To investigate the associations between FHB severity and toxin level by *F. asiaticum* and *F. graminearum*, correlation was analyzed between disease severities by both strains in each crop, toxin levels by both strains in each crop, and between disease severity and toxin level by each strain in each crop. Firstly, FHB severities between two strains (Fa73 and Fg39) were moderately correlated in both crops (r = 0.57 and 0.60 in barley, 0.42 and 0.58 in wheat) (P < 0.05) (Table 3). Second, mycotoxins between the two strains (NIV by Fa73 and DON by Fg39) were highly correlated in barley (r = 0.69 and 0.91), but not in wheat (r = –0.08 and 0.06). Third, FHB severity by either strain was correlated with mycotoxin levels, especially in barley. In barley, the correlation between FHB and NIV was moderately high (r = 0.42 and 0.82), whereas that between FHB and DON was less consistent (r = 0.22 and 0.76). In wheat, however, the correlations were high only

Table 3. Correlation coefficients calculated among FHB severity and mycotoxin production by *Fusarium asiaticum* and *F. graminearum* in barley and wheat

Crop	Barley			Wheat		
	FHB by Fg39	NIV by Fa73	DON by Fg39	FHB by Fg39	NIV by Fa73	DON by Fg39
FHB by Fa73	0.57*/0.60*	0.42*/0.82*	0.48*/0.70*	0.42*/0.58*	0.049/0.70*	0.086/0.14
FHB by Fg39		0.31/0.70*	0.22/0.76*		–0.33/0.20	–0.077/0.66*
NIV by Fa73			0.69*/0.91*			–0.080/0.060

FHB, Fusarium head blight; NIV, nivalenol; DON, deoxynivalenol.
*$P < 0.05$.

in the second assay ($r = 0.76$ by Fa73, 0.66 by Fg39). Due to the different numbers of datasets, disease severity or mycotoxin levels between wheat and barley were not analyzed.

Discussion

Germplasm accessions showed diversity in FHB resistance and mycotoxin accumulation. Most accessions (>91%) showed >20% FHB severity caused by either *F. asiaticum* or *F. graminearum*, and none of the accessions were free from mycotoxin accumulation. Among the five accessions with <20% FHB severity, barley (b32) was associated with the lowest level of mycotoxin (up to 2.2 mg/kg), suggesting the possibility of double FHB resistance (type II-resistance to spread and type III-resistance to toxin accumulation) (Chen et al., 2019). These results demonstrate that such FHB resistance can be secured and that FHB resistance is better regarded not as a single disease resistance but as a double resistance to FHB and mycotoxins. The resistant accessions revealed in this study can be useful resources for breeders. Both FHB and mycotoxin production are significantly affected by environmental and experimental conditions. Such effects of plant conditions and micro-environments can cause inconsistent evaluations. Although we tried to keep the assay conditions such as inoculum concentration, pathogen viability and plant conditions consistent, there could be variations in the temperature and humidity of the greenhouse between experiments as the accessions were tested in several months during a season. Thus, higher FHB severity should be counted, even with discrepancies between assays, because they reflect the susceptibility of a tested variety or accession under given environments.

Comparison of FHB severity by both *F. asiaticum* and *F. graminearum* was conducted in the germplasm for the first time in this study. The barley and wheat accessions showed different responses to *F. asiaticum* and *F. graminearum* under the test conditions. Our results suggest to use both species for FHB assessment in barley and wheat in Korea. FHB severity was generally higher in barley than in wheat in both strains, showing that barley was more susceptible to FHB, confirming our previous results (Baek et al., 2020). Of these two species, NIV-producing *F. asiaticum* was more aggressive than DON-producing *F. graminearum* in both crops. However, the latter had a tendency to be more aggressive toward wheat. Although the pathogenicity of Fa73 appeared to be higher than that of Fg39, the absolute amount of mycotoxins produced was greater by Fg39. The mycotoxin levels were higher in wheat for both DON and NIV, indicating that wheat was more prone to mycotoxin accumulation. These results differ from those of a previous study where DON levels were not significantly different between barley and wheat, and DON-producing *F. graminearum* was less aggressive than NIV-producing *F. asiaticum* in wheat (Baek et al., 2020). This work, however, was based on a lower number (14 in total) of cultivars, making our results more representative. Other studies reported that NIV-producing *F. asiaticum* and DON-producing *F. graminearum* were not significantly different on virulence in wheat cultivars (Jang et al., 2019; Shin et al., 2018). However, these results were based on a single cultivar or on a single pathogenicity test, respectively. Notably, DON production is accompanied by acetyl derivatives such as 3-ADON and 15-ADON. The toxicity of these acetyl DON derivatives is less than that of DON, but they still contribute to total toxicity (Knutsen et al., 2017). However, little information is available on the occurrence of acetyl DON derivatives in Korean cereals. Although Korean cereals are prevalent in NIV-producing *F. asiaticum*, they remain contaminated with DON, indicating that acetyl DON derivatives co-occur, as shown in this study. For food safety purposes, it is necessary to monitor DON derivatives in cereals and cereal-based products. Differences in FHB severity between *F. asiaticum* and *F. graminearum* and the superiority of *F. graminearum* in mycotoxin production (Gale et al., 2011; Jang et al., 2019) should be considered, especially for wheat breeding, as this crop is challenged more by *F. graminearum*. As wheat cultivation is currently encouraged in Korea to increase wheat self-sufficiency (Kim et al., 2020), it is important to secure wheat breeding resources that are resistant to both FHB and mycotoxins.

Our analyses confirmed previous results that FHB severity and toxin production are correlated in wheat and barley (Choo et al., 2004; Geddes et al., 2008; He et al., 2015; Yan et al., 2022). Unlike the previous studies, we used both *F. asiaticum* and *F. graminearum* at the same time and were able to compare these species. The highest levels of DON and NIV were associated with high FHB severity in both crops, especially *F. graminearum* (accessions b41 and w54). However, it is controversial to conclude because some studies found no correlation between FHB and DON concentration in these crops (Gilbert et al., 2002; Khanal et al., 2021) and all test conditions were different. The strong correlation between DON and NIV levels only in barley, and higher correlation between FHB severity and toxin production with *F. asiaticum* in barley accessions than wheat or with *F. graminearum* might be another proof of the co-evolution of *F. asiaticum* and barley for a longer period than wheat, as speculated previously (Baek et al., 2020). Further studies are necessary to elucidate the basis of this association between barley and *F. asiaticum*.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This study was carried out with the support of "Research Program for Agricultural Science & Technology Development (Project No. PJ014895)", National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

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