Deoxynivalenol Distribution in Flour and Bran of Spring Wheat Lines with Different Levels of Fusarium Head Blight Resistance

Zenta Nishio, National Agricultural Research Center for the Hokkaido Region, Shinsei, Memuro, Hokkaido 082-0081, Japan; **Kanenori Takata**, National Agricultural Research Center for Western Region, 6-12-1 Nishi-Fukatsucho, Fukuyama, Hiroshima 721-8514, Japan; **Miwako Ito**, **Masahiko Tanio**, **Tadashi Tabiki**, and **Hiroaki Yamauchi**, National Agricultural Research Center for the Hokkaido Region, Japan; and **Tomohiro Ban**, Kihara Institute for Biological Research, Yokohama City University, 641-12 Maioka-cho, Totsuka-ku, Yokohama, Kanagawa 244-0813, Japan

ABSTRACT

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The level of the fungal toxin deoxynivalenol (DON) in milled flour and its partitioning between flour and bran of 15 spring wheat (*Triticum aestivum*) lines with different levels of Fusarium head blight (FHB; mainly caused by *Fusarium graminearum*) resistance was investigated in Hokkaido, Japan between 2004 and 2007. The DON levels in flour (DON_f) and bran (DON_b) showed positive linear relationships with DON levels in whole kernels (DON_k) ($R^2 = 0.94$, $n = 60$, $P \leq 0.01$ and $R^2 = 0.93$, $n = 60$, $P \leq 0.01$, respectively). Results indicated that the DON partitioning ratio between flour and bran is primarily related to the level of DON_k, and the DON level was approximately doubled in DON_b and halved in DON_f for any level of DON_k. Only one pattern of DON partitioning supported the conventional strategy to select FHB-resistant lines.

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is one of the most widespread and economically important diseases of wheat (*Triticum aestivum* L.) (23). Kernels damaged by FHB are shrunken and discolored, resulting in lower yields and contamination by fungal toxins. Deoxynivalenol (DON) is one of the most common trichothecene toxins produced by *F. graminearum*. This water-soluble toxin, also known as vomitoxin, is responsible for emesis and feed refusal in animals (11,33). DON has been detected in cereal grains grown in Canada (35), the United States (7), the United Kingdom (13), and Japan (34). The recent occurrence of FHB epidemics in Hokkaido, the northernmost island and granary of Japan, has indicated that cultivars developed in Hokkaido are mostly susceptible to FHB compared with southwestern Japanese cultivars, and that levels of DON in flour are of concern (26).

In 1982, the United States Food and Drug Administration (FDA) issued an advisory "level of concern" for DON of 2,000 ppb for wheat grain entering the milling process. In response to the out-

break of FHB in 1993, the FDA revised its previous advisory level to 1,000 ppb in finished wheat products such as flour, germ, and bran intended for human consumption (19). Health Canada (3) issued standards ("Maximum Limits") for DON at 2,000 ppb in uncleaned soft wheat for use in nonstaple foods and at 1,000 ppb for use in baby foods (*under review*). In 2002, on the basis of the report of an FDA/World Health Organization Codex committee (2) on food additives and contaminants, the

Japanese Ministry of Health issued a provisional advisory recommending a "level of concern" of 1,100 ppb DON in wheat grain entering the milling process.

DON contamination can be reduced to some degree by cleaning and removing kernels with low specific gravity (27,31) or through optical seed-sorting technology (9). However, Tanaka et al. (30) and Young et al. (35) reported that about 60% of DON in naturally contaminated wheat remained after milling, and that no appreciable loss of toxins was detected after baking. Levels of DON in food products are affected by the milling processes which separate wheat grains into flour and bran fractions. Previous studies (1,10,12,16,20–22,28,29,32) reported that DON partitioning is generally smaller in flour than in bran, which indicated that the level of DON production is related to the incidence of fungal infection at or near the kernel surface. However, it has been difficult to draw consistent conclusions for DON partitioning between flour and bran because previous studies were carried out using commercially blended wheat samples which included several wheat cultivars with different levels of FHB resistance.

Table 1. Origin, quality class, and reported Fusarium head blight (FHB) resistance of 15 spring wheat lines naturally infected in Hokkaido, Japan from 2004 to 2007^a

Lines ^b	Origin	Class	Reported FHB resistance ^c	Reference ^d
Sumai 3 (Kyushu)	Jiangsu, China via Kyushu, Jpn.	Soft red	R	4, 15
Sumai 3 (Austria)	Jiangsu, China via Austria	Soft red	R	4, 26
Sumai 3 (CIMMYT)	Jiangsu, China via CIMMYT	Soft red	R	4
Norin 61	Kyushu, Japan	Soft red	MR	5
Saikai 165	Kyushu, Japan	Soft red	R	5
Fujian5114	Fujian, China	Soft red	R	4
Nobeokabo	Kyushu, Japan	Soft red	R	5
NING7840	Jiangsu, China	Soft red	R	4
Alsen	North Dakota, United States	Hard red	MR	24
Shinchunaga	Kyushu, Japan	Soft red	MR	5
Bacup	Minnesota, United States	Hard red	MR	8
Haruyokoi	Hokkaido, Japan	Hard red	M	18
Harunoakebono	Hokkaido, Japan	Hard red	M	18
Haruyutaka	Hokkaido, Japan	Hard red	S	18
Gabo	Sydney, Australia	Soft white	VS	5

^a The DON partitioning ratio in flour was calculated by ((DON level in flour (ppb))/(DON level in kernels (ppb))) × flour yield (%).

^b CIMMYT = International Maize and Wheat Improvement Center.

^c R = resistant, MR = moderate resistant, M = moderate, S = susceptible, and VS = very susceptible.

^d Related references for the levels of FHB resistance.

Corresponding author: Z. Nishio
E-mail: zenta@affrc.go.jp

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From a practical perspective, the knowledge of DON partitioning between flour and bran of wheat lines would be beneficial for obtaining flour with low DON levels. Although the partitioning of DON in mill streams has been reported for a

number of commercial wheat lines (1,10, 12,16,20–22,28,29,32), the risk of DON accumulation in flour and of its partitioning between grain components has not been investigated in depth. The objective of this study was to determine how DON

in wheat kernels is partitioned between flour and bran and whether the partitioning is different for resistant and susceptible wheat cultivars.

MATERIALS AND METHODS

Plant materials. In total, 15 spring wheat lines (Table 1), including 3 ‘Sumai 3’ derivatives (4,26) from the International Maize and Wheat Improvement Center, Kyushu, Japan, as well as some lines from Austria, were planted at the National Agricultural Research Center for the Hokkaido Region (Memuro) (42.9°N, 143.1°E). Soft white ‘Gabo’ is a commercial Australian cultivar which is imported to Japan for white salted noodle (Udon) production, and it was employed as an FHB-susceptible line which has similar susceptibility to its descendant cv. Gamenya (5). Each experimental unit consisted of a single 2-m-long row, laterally 0.72 m distant from the adjoining row. All lines were planted for 4 years, and received nitrogen fertilizer at approximately 60 kg ha⁻¹ at seeding in April of each year. Flowering occurred at the end of June, and all lines flowered and matured within about 10 days each year. The mean air temperature between June 20 and 30 was 15.4°C in 2004, 18.1°C in 2005, 19.1°C in 2006, and 17.2°C in 2007. The plots were arranged using a complete block design, and the order of lines was inverted in each block next to each other. All tests were conducted with three replicates of each line for each year.

DON analysis. Grain from each plot was threshed, conditioned to 14% moisture, and milled in a Quadrumat Junior test mill (Brabender GmbH & Co. KG, Duisburg, Germany). Flour and bran samples were obtained from the milling, and flour yield (%) was calculated as:

$$100 \times [\text{flour weight (g)}] / [\text{flour weight (g)} + \text{bran weight (g)}] \quad (1)$$

The DON level was determined using a ‘Fast DON’ enzyme-linked immunosorbent assay (ELISA) kit (Azmax Corp., Chiba, Japan) that contained standards of 3.7, 11.1, 33.3, and 100.0 ppb. Flour or bran samples (2 g) were ground for 1 min in 50 ml of distilled water, using a high-speed ‘Hiscoteron’ homogenizer (Microtecnic Corp., Chiba, Japan). The sample was centrifuged (60 s at 12,000 × g) and the supernatant appropriately diluted for the test. The ELISA was conducted twice for each sample, and mean values reported in ppb. The DON level in the kernel was calculated as:

$$\text{DON}_k = [(\text{DON}_f \times \% \text{ flour yield}) / 100] + [\text{DON}_b \times (100 - \% \text{ flour yield}) / 100] \quad (2)$$

where DON_k, DON_f, and DON_b = flour levels in whole kernels, flour, and bran, respectively.

Statistical analysis. Analysis of variance (ANOVA) was performed on DON partitioning ratio in flour, using a software

Fig. 1. Relationship of deoxynivalenol (DON) levels between flour and kernels across 15 wheat lines, naturally infected by *Fusarium* head blight in Hokkaido, Japan from 2004 to 2007. Linear regression analysis was carried out on pairwise comparisons **A**, between DON levels in flour and kernels and **B**, between bran and kernel. DON levels in flour and kernels are presented on double logarithmic scales.

R version 2.8.0 (R Foundation for Statistical Computing, Vienna). Cultivars were considered as a fixed effect for this analysis, while years and replications were considered random. DON_k , DON_f , and DON_b among lines during the tested years showed wide ranges (19.9 to 13,411.8, 12.6 to 12678.8, and 26.3 to 15,059.2 ppb, respectively), and the data points were shown in double logarithmic plots. Untransformed DON levels of each line across 4 years were analyzed to compare the relationships between DON_k and DON_f , and between DON_k and DON_b , by linear regression analysis.

RESULTS AND DISCUSSION

ANOVA for the DON partitioning ratio in flour indicated no significant difference among cultivars ($P = 0.07$). Regression analysis exploring relationships between DON_f , DON_b , and DON_k levels showed there to be positive linear relationships between DON_k and DON_f levels ($R^2 = 0.94$, $n = 60$, $P < 0.01$; Fig. 1A) and between DON_k and DON_b levels ($R^2 = 0.93$, $n = 60$, $P < 0.01$; Fig. 1B). Results indicated that the DON partitioning ratio in flour and bran is primarily related to the level of DON_k (Figs. 1A and B), and the DON level was approximately doubled in DON_b and halved in DON_f for any level of DON_k .

Previous reports indicated that levels of DON or zearalenone were generally higher in bran than in flour. Abbas et al. (1) tested five U.S. wheat samples with DON ranging from 7,900 to 9,500 ppb, and found less concentration of DON in the flour (1,100 to 3,100 ppb) than in the bran (11,300 to 21,300 ppb). Trigo-Stockli et al. (32) tested 27 wheat samples ranging from 600 to 3,800 ppb, and only 3 flour samples contained DON that exceeded 1,000 ppb, whereas 10 bran samples exceeded 1,000 ppb. Gärtner (12) tested eight spring wheat cultivars and found less concentration of DON in the reduction flour (2,500 to 16,600 ppb) than in the bran (8,300 to 122,000 ppb). Tanaka (29) tested two spring wheat cultivars, and DON content in bran was 2.3 to 2.4 times that of the original wheat. The high level of toxin in the bran may be attributed to the prevalence of the *F. graminearum* fungus in the aleurone and pericarp tissues (6). Young et al. (35) also reported a strong positive correlation between ergosterol and DON levels in grain, and suggested that fungal infections were greater at or near the surface of the kernels.

In this study, we showed the positive linear relationship between DON_k and DON_f levels and its relation to DON partitioning ratio in flour using various wheat lines with different levels of FHB resistance. Bechtel et al. (6) found hyphae throughout the starchy endosperm in *F. graminearum*-infected kernels, and the alteration in fracturing characteristics of heavily infected kernels in lines such as

Gabo is considered to be one of the causes of the greater DON_f obtained from such kernels (Fig. 1A). The relationship between locality of fungal biomass and DON levels remains to be elucidated. Further investigation should be carried out to elucidate the relationship between sites of DON accumulation and of fungal biomass in the grain, in order to understand the mechanism of DON localization and distribution.

Generally, a greater flour yield is preferred in the milling industry, and hard wheat has a greater flour yield than soft wheat (14). Mean flour yield ranged from 57.4% ('Shinchunaga', soft red) to 73.9% ('Haruyutaka', hard red). The DON level in kernels was not significantly correlated with flour yield ($R^2 = 0.03$, $n = 60$, $P > 0.05$). Dexter et al. (10) reported that flour yield was not influenced by an increase in FHB-damaged kernels. Similarly, Hatcher et al. (17) found no significant influence of DON levels on flour yield. Because the flour yield from the Brabender Quadramat Jr. was sufficiently similar to that from the experimental mill for a large-scale testing (25), the DON partitioning pattern in this study could be applicable to DON levels in large-scale milling.

The most effective way to reduce the risk of DON contamination is to cultivate FHB-resistant wheat cultivars. Our study indicated that the DON partitioning between flour and bran is related to the level of DON in whole kernels. The results support the conventional strategy to select FHB-resistant lines based on only one pattern of DON partitioning.

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