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Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat

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Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (telomorph = *Gibberella zeae* (Schw.)), is an important wheat disease world-wide. Production of deoxynivalenol (DON) by *F. graminearum* in infected wheat grain is detrimental to livestock and is also a safety concern in human foods. An international collection of 116 wheat lines was evaluated for FHB resistance and concentration of DON in grain. Plants were inoculated with mixed isolates of *F. graminearum* in the greenhouse by injecting conidia into a single spikelet of each spike and in the field by scattering *F. graminearum*-infected wheat kernels on the soil surface. FHB symptoms were evaluated by visual inspection in both the greenhouse and field, and DON was analysed by HPLC. Significant differences in FHB ratings and DON levels were observed among cultivars. In the greenhouse test, visual symptoms varied from no spread of FHB from the inoculated spikelet to spread throughout the spike, and DON levels ranged from trace levels to 283 mg/kg. In the field test, DON ranged from 2.8 to 52 mg/kg. The greenhouse test identified 16 wheat lines from various origins that accumulated less than 2 mg/kg DON. These lines may be useful as sources for breeding wheat cultivars with lower DON levels. Correlation coefficients were significant between FHB symptom ratings, seed quality traits, and DON levels. Thus, the percentage of scabbed spikelets and kernels can be generally used to predict DON levels in harvested wheat grain. In breeding programmes, selection for plants having few scabbed spikelets and scabbed kernels is most likely to result in low DON levels.

Key words: *Triticum aestivum* — *Fusarium graminearum* — DON — mycotoxin — vomitoxin

Fusarium head blight (FHB) or scab of wheat has caused serious epidemics in many wheat growing areas world-wide (Bai and Shaner 1994, McMullen et al. 1997). Although several *Fusarium* species can cause FHB, *Fusarium graminearum* Schwabe (telomorph = *Gibberella zeae* (Schw.) Petch) is primarily responsible for the recent epidemics in the USA and many other countries (Bai and Shaner 1994, McMullen et al. 1997). When warm and wet weather coincides with wheat anthesis and early grain-filling, severe infection can dramatically reduce grain yield and quality.

Infected grain is often contaminated with deoxynivalenol (DON), which is a mycotoxin produced by *F. graminearum*. As epidemics become more frequent and severe in many countries (McMullen et al. 1997), DON contamination of wheat and other small grain crops is becoming a major concern for animal production and human health (Marasas et al. 1984, Luo et al. 1990, Ehling et al. 1997). The maximum acceptable DON levels in wheat grain for human use range from 0.5 to 2 mg/kg in the USA, Canada, and some European countries (Snijders 1990).

DON levels in infected grain vary significantly among wheat cultivars (Miller et al. 1985, Mirocha et al. 1994, Liu et al. 1997). Miller et al. (1985) reported that DON levels in susceptible cultivars were eightfold higher than in resistant cultivars. Mirocha et al. (1994) also observed that resistant cultivars had lower DON levels than did susceptible cultivars. FHB resistant cultivars have been identified from different geographic regions, including the USA, Asia, Europe and South America. However, relatively little information is available on DON levels in many resistant parents currently used in breeding programmes.

Because thousands of lines need to be screened in breeding programmes each year, direct DON measurement may not be feasible for routine breeding applications owing to its high cost and technique requirements. A high correlation between ergosterol and DON levels in wheat kernels inoculated with *Fusarium culmorum* (r = 0.85) indicates that the DON level in wheat kernels is related to fungal biomass (Snijders and Perkowski 1990). Ergosterol level in infected kernels has been proposed as a relatively accurate prediction for DON level. Unfortunately, the analytical procedure to measure ergosterol is no easier than measuring DON, and thus is not a practical alternative to DON analysis for wheat breeders.

In this study, FHB and DON levels were measured in FHB-resistant wheat germplasm from six countries under favourable FHB epidemic conditions. The objectives were to identify germplasm that accumulated low DON levels following inoculation and to evaluate the possibility of using visual FHB ratings to predict DON levels.

Materials and Methods

Plant materials: A total of 116 cultivars and breeding lines of wheat, *Triticum aestivum* L., were evaluated for FHB resistance in the greenhouse at the University of Illinois in the autumn of 1997 (Table 1). A subset of 13 cultivars was tested in the greenhouse in the spring of 1997. These cultivars originated from the USA, China, Japan, Austria, Argentina and France. The wheat materials included well-known FHB-resistant Chinese cultivars such as 'Sumai 3', 'Wangshuibai' and 'Ning 7840', some advanced breeding lines from the University of Illinois and some highly susceptible cultivars such as 'Mo94-193' and 'Clark' as susceptible controls (Table 1).

In the field test, the same set of 116 cultivars were planted; only 33 cultivars could be evaluated for disease and DON levels because most of the cultivars were spring wheat and were killed by the cold weather in the winter.

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Table 1: *Fusarium* head blight (FHB) ratings and deoxynivalenol (DON) levels for selected wheat cultivars from different origins based on greenhouse-grown plants in 1997 and field-grown plants in 1998[1]

Cultivar	Origin	Greenhouse DON[1]	PSS	AUDPC	TSW	SG	Field DON	PSS	TSW	SG	PSK
Chinese cultivar											
'Ning 7840'[2]	China	0.00	0.08	1.37	26.5	1.7					
'F 5125'	China	0.38	0.05	0.59	38.4	1.3					
'Sumai 3'[2]	China	0.53	0.08	1.13	27.3	1.7					
'Fumai 3'	China	0.88	0.31	2.92	28.1	2.3					
'Sumai 49'	China	0.99	0.08	1.13	36.5	2.0					
'Ning 8026'	China	1.50	0.28	2.23	28.8	2.0					
'Wangshuibai'[2]	China	1.67	0.09	1.13	34.2	1.7					
'Yangmai 1'	China	1.73	0.32	3.21	22.4	2.3					
'Fu 5114'	China	1.95	0.06	0.89	33.9	1.0					
'Ning 8331'	China	2.19	0.12	1.03	36.8	2.0					
'FSW'	China	2.88	0.17	1.54	24.1	2.3					
'WZHHS'	China	2.29	0.13	1.48	31.0	2.2					
Cultivar and advanced lines from the USA											
'P93D1-10-2'	Indiana	0.01	0.17	1.80	24.6	1.8	23	0.38	17.1	4	39.5
'IL 95-1966'	Illinois	0.7	0.08	0.99	25.8	1.0	22	0.33	20.9	2	20.5
'IL 9634-24851'	Illinois	0.9	0.16	1.31	22.8	1.7					
'Roane'	Virginia	2.0	0.17	1.57	25.5	1.4	15	0.30	19.4	3	21.0
'Bacup'[2]	Minnesota	2.2	0.14	1.03	27.2	2.0					
'IL 93-2283'	Illinois	3.3	0.15	1.22	28.8	3.0					
'IL 94-2426'	Illinois	3.3	0.21	1.97	29.6	2.3					
'OH 552'	Ohio	3.4	0.37	3.34	28.8	2.0	20	0.25	22.0	3.5	20.5
'PA-8769-160'	Pennsylvania	4.0	0.64	4.26	18.9	3.3	44	0.38	12.9	4.5	53.0
'Ernie'[2]	Missouri	6.6	0.57	3.43	17.4	3.0	16	0.21	25.9	2.5	17.5
'Par-55'	Illinois	7.7	0.42	3.26	15.5	4.3	2.8	0.14	16.7	3.3	7.5
'IL 94-1911'	Illinois	8.5	0.59	5.24	21.7	3.7	20	0.20	17.4	3	15.0
'Kaskaskia'	Illinois	8.7	0.71	3.80	18.6	3.0	42	0.39	17	2.7	36.0
'Foster'[2]	Kentucky	8.9	0.71	7.06	10.5	3.9	42	0.50	15.2	4.5	23.5
'PC-2'	Illinois	10.7	0.42	3.26	19.2	3.7	4.7	0.11	26	2.7	1.5
'89-7978'[2]	Illinois	13.7	0.38	2.75	33.6	3.0					
'Freedom'	Ohio	19.3	0.29	2.41	20.0	3.7	31	0.40	15.4	4.5	32.0
'Ag94-1048'[2]	Agripro	21.2	0.34	3.37	16.7	3.8					
'Pontiac'[2]	Agripro	21.6	0.71	5.89	11.6	4.7	14	0.33	16.6	4.3	22.5
'Nel2253'[2]	Illinois	22.9	0.41	3.71	17.1	4.1					
'PB 2571'[2]	Pioneer	35.6	0.53	5.18	17.9	4.0					
'87-2834-1'	Illinois	43.2	0.91	5.99	16.0	4.5	22	0.48	21.6	2.5	18.5
'PB 2555'[2]	Pioneer	73.7	0.74	6.78	14.2	4.5	52	0.79	19.3	4.5	52.5
Cultivars from other countries											
'Shinchunaga'	Japan	0.0	0.12	1.13	19.7	1.7					
'Spartakus'	Austria	1.2	0.07	0.77	26.1	1.7					
'113.92'	Argentina	1.8	0.31	2.69	21.6	3.7					
'111.92'	Argentina	2.2	0.28	2.22	25.7	3.3	43	0.39	19.7	4.5	42.5
'Karat'	Austria	2.6	0.31	1.73	28.6	2.0	42	0.39	10.9	4.5	34.5
'Poncheau'	France	2.8	0.10	0.91	30.6	2.3					
'Perlo'	Austria	3.4	0.09	1.06	22.5	1.5	18	0.24	22.5	4	22.0
'Sanshukomugi'	Japan	3.7	0.34	2.52	17.8	3.7					
Control cultivar											
'Mo 94-193'	Missouri	154.0	1.00	10.94	7.7	4.7					
'Cardinal'	Indiana	216.2	0.76	6.30	12.7	4.3	17	0.38	18.5	4.3	17.5
'Clark'[2]	Indiana	225.0	0.94	8.94	8.1	5.0	17	0.64	21.4	2.8	26.0
'IL 94-6280'	Illinois	283.3	0.94	9.73	4.9	5.0	32	0.75	15.8	4.3	31.5

[1] DON is the average value in mg/kg; PSS is the proportion of scabbed spikelets in a spike; AUDPC is area under FHB progress curve; TSW is 1000-seed weight in g; SG is the seed grade on a 1–5 scale; PSK is the percentage of scabbed kernels.
[2] Cultivars were selected for repeated experiment in the greenhouse.

FHB evaluation: In the greenhouse test, a droplet of conidia (approximately 1000 spores) was injected with a hypodermic syringe into a central floret of selected spikes at anthesis. The inoculum of *F. graminearum* was a mixture of field isolates that originated from five scabbed seeds collected in 1996 at the Crop Sciences Research and Education Centre of the University of Illinois. Mung bean liquid medium was used to produce the conidial inoculum (Bai and Shaner 1994). The inoculated plants were misted with tap water from overhead misting nozzles installed inside an inoculation chamber that consisted of a frame covered with a polythene sheet on a greenhouse bench. The misting schedule was 10 s every 30 min for 3 days. Temperatures within the moist chamber were 23–25°C and relative humidity was 100%. On the fourth day after inoculation, plants were returned to their original positions on the greenhouse benches. Greenhouse temperatures averaged 25°C during the day with a range of 19–30°C and 19°C at night with a range of 17–21°C.

In the greenhouse, scabbed spikelets were counted 3, 9, 15, and 21 days after inoculation. The number of spikelets on each inoculated spike was counted on day 21. Disease severity was calculated as the proportion of scabbed spikelets (PSS) per infected spike at 21 days after inoculation. From these data the area under the disease progress curve (AUDPC) was calculated for each plant according to Shaner and

Finney (1977). Both inoculated and uninoculated spikes from each pot were harvested and hand-threshed separately. Seeds were counted and weighed, and 1000-seed weight (TSW) was calculated. Seed grade (SG) was visually scored using a 1–5 rating, where 1 indicated the lowest and 5 the highest incidence of scabbed seeds.

The greenhouse test was conducted in a completely randomized block design with three replications (pots). Each replication had three inoculated plants. Visual FHB ratings were analysed on a single-plant basis. SG, TSW and DON level were based on bulked seeds from the three inoculated plants in each pot.

The field test was conducted in 1998 at the Crop Sciences Research and Education Centre of the University of Illinois, Urbana, IL. Wheat cultivars were planted in 0.9-m long rows in the autumn of 1997 and arranged in a completely randomized block design with two replications. When the wheat reached the boot stage in the spring of 1998, scabbed wheat kernels were scattered on the soil surface in the plot areas at a density of 60 kg/ha. To make inoculum, autoclaved wet wheat kernels in a 1-l flask were inoculated with conidia derived from the same isolate mixture used in greenhouse experiments and incubated at room temperature for about 3 weeks. The flask containing infected kernels was shaken daily. From flowering to early dough stages, plots were misted daily for two 30-min periods starting at 09.00 h and 15.00 h, respectively, with an overhead misting system. PSS in a spike was recorded at late dough stage by selecting 10 spikes per row. All wheat spikes were harvested and threshed. Percentage of scabbed kernels (PSK) was calculated based on the number of kernels with discoloration in the randomly selected 200 kernels. SG was scored as described in the greenhouse experiments. All the data were analysed by using STATVIEW (SAS Institute Inc. Cary, NC, USA) according to Steel and Torrie (1980).

DON analysis: In the greenhouse tests, seeds from the three inoculated plants of each pot were pooled and ground for DON analysis. Seeds from uninoculated spikes of selected cultivars were also analysed for DON content. The total ground sample was extracted by shaking with 5 ml acetonitrile–water (86 : 14, v/v)/g of sample for 3 h. The extract was filtered into a vial using Whatman filter paper (Whatman International Ltd, Maidstone, England). DON in the extract was measured by high-pressure liquid chromatography (HPLC) with detection by electrospray ionization mass spectroscopy (ESI-MS) (Finnigan LCQ, San Jose CA, USA). The small sample size precluded sample cleanup, so crude extracts were diluted appropriately, and 10 μl aliquots were injected directly. Quantification was by comparison of signal for DON in the sample with the signal from a standard curve obtained from injection of DON standards. The detection limit of the method was approximately 0.5 mg/kg. The same method was used for DON analysis from field samples except that the sample size was standardized to 10 g. For selected samples, the DON levels were confirmed by gas chromatography (GC)/MS (Finnigan TSQ-700, San Jose CA) of the trimethyl-silyl derivative with detection in the negative CI mode. Since mean DON contents for individual cultivars from the greenhouse test were highly correlated with their variances ($r = 0.91$), $\mathrm{Log}(X + 1)$ was used for transformation of DON content data before variance analysis.

Results

FHB ratings in wheat cultivars

In the greenhouse tests, all inoculated wheat cultivars showed FHB symptoms following single floret inoculation, but wheat cultivars differed significantly in PSS and in AUDPC (Table 1). Resistant cultivars had as few as 5% infected spikelets ('Fu 5125'), whereas susceptible cultivars had up to 100% of infected spikelets ('Mo94–193', Table 1). In this study, the majority of resistant cultivars (64%) with fewer than 10% infected spikelets were from Chinese sources. However, 10 cultivars or advanced breeding lines from USA breeding programmes had fewer than 20% infected spikelets, indicating significant progress in improving levels of resistance to FHB in wheat in recent years.

Table 2: Correlation coefficients[1] between visual disease ratings and deoxynivalenol (DON) levels calculated from 1997 greenhouse and 1998 field data

	PSS[2]	TSW	SG	DON	AUDPC[3]
PSS	0.55*	−0.74*	0.84*	0.65*	0.94*
TSW	0.34	0.13	−0.76*	−0.52*	−0.72*
SG	0.34	−0.66*	0.03	0.56*	0.80*
DON	0.50*	−0.52*	0.52*	0.02	0.75*
PSK[3]	0.54*	−0.55*	−0.67*	0.82*	—

* Correlation coefficients are significant at $P < 0.01$.
[1] Above diagonal are correlation coefficients from the greenhouse data; on diagonal are correlation coefficients between the greenhouse and field data; below diagonal are correlation coefficients from the field data.
[2] PSS is proportion of scabbed spikelets in a spike; TSW is 1000-seed weight from infected plants for a cultivar; DON is DON level in mg/kg for a cultivar; SG is the seed grade for a cultivar on a 1–5 scale; PSK is the percentage of scabbed kernels; and AUDPC is area under disease progress curve.
[3] AUDPC was not measured in the field experiment and PSK were not measured in the greenhouse experiment.

To examine environmental variation in FHB severity between experiments under greenhouse conditions, a subset of 13 cultivars representing various levels of FHB resistance was re-evaluated (Table 1). The differences in PSS and AUDPC were significant among cultivars within a test, but not between the replicated tests (data not shown). The correlation coefficient for PSS was highly significant between the two tests ($r = 0.91$), indicating the high reproducibility of the visual FHB ratings in the greenhouse experiments.

FHB severity ranged from 11% ('PC-2') to 79% ('PB 2555') in the field test (Table 1). Resistant and moderately resistant cultivars had higher disease levels in the field than in the greenhouse, but more susceptible cultivars showed the opposite. Despite large effects of environmental variation on disease severity, the correlation coefficient between field and greenhouse data was still significant for PSS (Table 2).

Variation in DON levels among the wheat germplasm

Seventeen samples selected from the greenhouse test, representing various levels of FHB ratings, were analysed by HPLC/MS and GC/MS methods. DON was determined as an average of three measurements for each sample. The correlation coefficient between sample means of the two measuring methods was highly significant ($r = 0.91$), demonstrating consistency between these two measuring methods. Because the HPLC method provides lower variability than the GC/MS method and does not require derivatization, it was selected to measure DON in this study.

In the greenhouse test, there was no detectable DON contamination in kernels of uninoculated wheat spikes. Almost all wheat cultivars had measurable DON in inoculated spikes. In the two replicated experiments, the difference in DON levels was noted but was not significant in a variance analysis (data not shown). Variation in DON levels between replicated samples within the same experiment was similar to, or larger than variation in DON levels between experiments. In the greenhouse study, DON for each cultivar was assessed in three replications with three measurements per replication: thus, the results provided a relatively reliable estimate of DON levels in wheat cultivars.

In the greenhouse test, DON in infected grain ranged from trace to 283 mg/kg, depending on the cultivars tested (Table 1). DON levels were relatively low in more than half of the cultivars tested (less than 10 mg/kg) under favourable FHB epidemic conditions. About 10% of the cultivars had over 50 mg/kg DON, but only four susceptible controls had more than 150 mg/kg DON (Table 1). Sixteen cultivars, including 'Shinchunaga', 'Sumai 3', 'Ning 7840', 'Fu 5125', 'P93D1-10-2', and 'IL 95-1966', had very low DON levels (< 2 mg/kg) and originated in all six countries where FHB epidemics are frequent (Table 1). Nine of the 16 cultivars with low DON levels were from China. Some of the advanced breeding lines from the University of Illinois also contained low levels of DON. Among them, eight lines had less than 5 mg/kg DON and two had less than 1 mg/kg DON. These results indicate significant progress has been made to reduce DON levels in wheat through breeding.

DON levels from field infected seeds ranged from 2.8 to 52 mg/kg (Table 1). The maximum DON level was much lower (52 mg/kg) than that from the greenhouse experiment (283 mg/kg).

Table 3: Minimum, maximum and mean deoxynivalenol (DON) levels in mg/kg for wheat cultivars in four different FHB resistance categories based on proportion of scabbed spikelets (PSS)

PSS	0–0.2	0.21–0.5	0.51–0.8	0.81–1
Minimum	0	0.9	4	43.2
Maximum	5.8	44.9	216.3	283.3
Mean	2.3	11.3	34.0	127.9
Number of cultivars	25	57	27	7

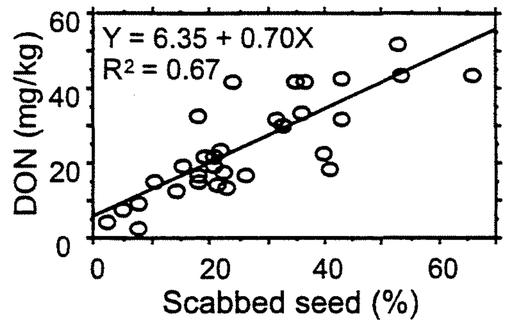

Fig. 2: Regression of DON on percentage of scabbed seeds from the field test of 1998

Association between DON levels and FHB ratings

In the greenhouse test, PSS, AUDPC, TSW and SG were significantly correlated with each other and they were all significantly correlated with DON levels (Table 2, Fig. 1). Among them, the correlation coefficient (r) between DON and AUDPC was the highest, and between DON and PSS was second highest. Cultivars with low DON usually had low FHB severity (Tables 1 and 3). Most cultivars accumulating less than 2 mg/kg DON had less than 20% of scabbed spikelets. Twenty-five cultivars with less than 20% of scabbed spikelets had an average of 2.3 mg/kg DON (Table 3), with little variation among them. In the field test, significant associations were detected between DON and PSS, TSW, SG and PSK (Table 2, Fig. 2). The results suggest that selecting highly resistant cultivars based on visual FHB symptoms on infected spikes and grains can be expected to lead to new cultivars with low DON levels.

Moderately resistant and moderately susceptible cultivars usually had higher DON levels than resistant cultivars, but there were exceptions (Table 1, Fig. 1). Cultivars 'Ernie', 'Par-55', 'IL 84-3206', 'IL 90-6364' and 'IL-94-1911' had diseased spikelets from 40% to 60% in the greenhouse, but their DON levels were low in the greenhouse (less than 9 mg/kg) and in the field (DON from 2.8 to 20 mg/kg). 'Par-55' was moderately resistant with 7.7 mg/kg DON in the greenhouse test, but showed very good resistance (14% diseased spikelets) with the lowest DON (2.8 mg/kg) in the field test. These results suggest that severe visual symptoms may not always be associated with high DON levels, especially for those cultivars with a moderate Type II resistance.

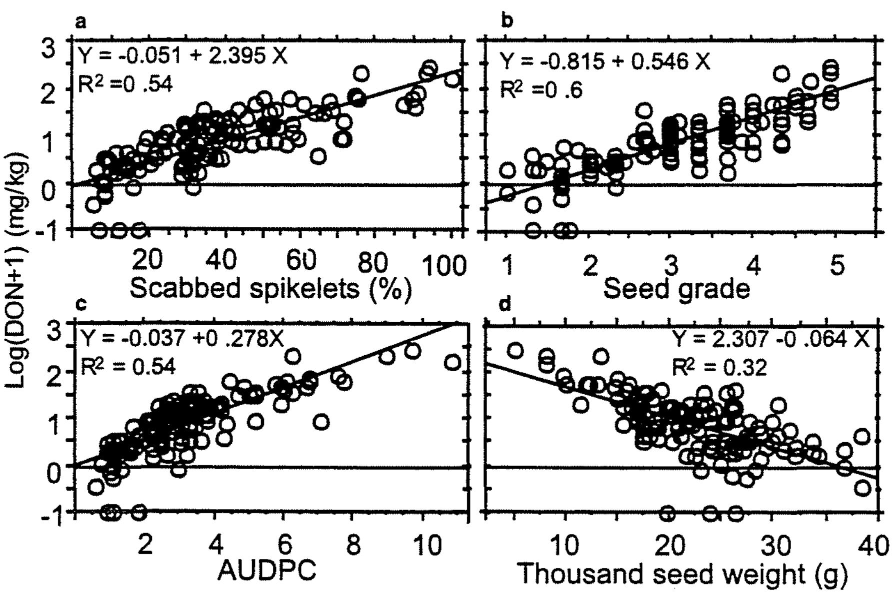

Fig. 1: Regression of Log(DON + 1) on percentage of scabbed spikelets (a), seed grade (b), AUDPC (c), 1000-seed weight (d) from the greenhouse test of 1997

Discussion

Genetic variation among wheat cultivars in levels of DON produced following infection by *Fusarium* pathogens has been evaluated under various conditions (Mirocha et al. 1994, Lemmens et al. 1997, Mesterhazy 1997). In the present study, 116 cultivars were evaluated in the greenhouse. DON levels ranged from trace to 283 mg/kg, depending on cultivars. Many Chinese cultivars not only have Type II resistance, but also accumulate low DON levels in the kernels of infected spikes. Seven of the nine low-DON Chinese cultivars included 'Sumai 3' in their pedigree, suggesting that scab resistance and low DON in these cultivars were inherited from 'Sumai 3'. Low DON levels in the cultivars 'Sumai 3' and 'Ning 7840' observed in this study were also reported by others (Mirocha et al. 1994, Mesterhazy 1997). Therefore, 'Sumai 3' and its derivatives should be good parents for breeding resistant cultivars that would be expected to produce seeds with low levels of DON under heavy disease pressure. That the resistant germplasm has DON levels as low as 1 mg/kg under favourable disease conditions indicates that DON contamination in harvested grain can be reduced to an acceptable level under natural epidemic conditions through breeding. About 10 cultivars or advanced breeding lines from USA breeding programmes demonstrated low levels of disease and DON, indicating significant progress in recent years toward improving wheat resistance to FHB and DON.

DON measurement is expensive and not feasible for routine breeding practice. Significant correlation between FHB ratings and DON levels indicates the possibility of using visual disease ratings as an indirect selection criterion to select wheat genotypes with low DON: the cultivars with low DON levels in this study were all selected based on evaluation of FHB symptoms and were not selected directly on DON levels. Results from this study clearly demonstrate that cultivars with high resistance all had low DON levels. Therefore, selecting cultivars with low levels of FHB damage in a breeding programme is likely to result in selecting genotypes that produce low DON levels.

Several parameters can reflect the degree of FHB damage: PSS, AUDPC, PSK, TSW and SG. PSS is easy to measure and highly correlated with DON and other parameters in both greenhouse and field conditions. In addition, significant association was detected for PSS between greenhouse and field data. Therefore, it can be used as a criterion for indirect selection of low DON in breeding programmes. Seed grading can be another useful criterion for DON prediction. Unlike rating visual symptoms in the field, seed grading does not have to be done in a specific time and large numbers of seed samples can be visually scored quickly after harvest. PSK is more reliable parameter than SG because a higher correlation coefficient was observed between it and DON ($r = 0.82$) than between DON and SG ($r = 0.52$) in the field experiment. TSW is easy to measure and can roughly estimate the weight reduction from *F. graminearum* infection in a severe epidemic. However, using kernel weight reduction due to infection instead of TSW may be more appropriate to predict DON levels (Snijders and Perkowski 1990). Unfortunately, the lack of effective fungicides makes it difficult to conduct experiments that include disease-free control treatments in field. Thus, PSK and SG appear to be useful parameters for DON prediction. It is recommended that selecting for fewer scabbed spikelets and fewer scabbed kernels in a breeding programme is likely to result in the selection of genotypes that produce low DON levels. Considering the selection cost and efficiency, selection in early generations should focus on field symptoms of FHB. Evaluation of kernel infection can be done by visual inspection. For selection in advanced generations, both PSS and PSK should be evaluated in both greenhouse and field.

Correlations between field and greenhouse were not significant for DON levels. Several factors may have contributed to a lower correlation between the two test conditions. In the greenhouse, the environment was optimized to favour early and fast FHB development in a wheat spike, which may not only cause early DON accumulation but also hinder seed development. Early infection and DON accumulation both increases the total amount of DON in an infected kernel and decreases kernel weight, which consequently increases the DON concentration. In addition, in the greenhouse only kernels from inoculated spikes were hand-threshed for DON analysis, therefore, badly shrivelled and infected kernels were included in the analysis. In contrast, in the field test, the plants were infected more naturally, and disease levels could have been affected by temperature, moisture and production of ascospore inoculum at the flowering stage. Those factors that affect disease levels in field conditions will also affect DON accumulation. Also, both infected and uninfected spikes from the field plots were harvested and threshed. The seeds from uninfected spikes may dilute DON levels, and the threshing machine may also blow away some severely infected light seeds that could be expected to have high DON levels. Therefore, DON levels measured from greenhouse tests can be considered as potential upper limits for DON levels. It is informative to measure differences in DON levels among cultivars under greenhouse inoculation conditions by spray inoculation, which may predict more reliably the cultivar performance under the worst case conditions of natural disease pressure in the field.

In general, moderately resistant and moderately susceptible cultivars had higher DON levels than resistant cultivars. But, relatively low DON levels have been detected in some moderately resistant or moderately susceptible cultivars in this study. Possible explanations are that the plant blocks synthesis or promotes degradation of DON in these cultivars (Miller et al. 1985, Snijders and Perkowski 1990). Owing to the quantitative nature of FHB resistance, moderate resistance may be more easily integrated into elite breeding lines. Therefore, in addition to greenhouse and field disease evaluation, direct DON analysis should be conducted for promising breeding lines with moderate resistance to FHB. Nonetheless, wheat breeders should aim at high levels of FHB resistance, which will not only minimize yield loss under severe epidemics, but also reduce DON to a safe level for human and animal consumption.

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