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Effectiveness of Marker-based Selection for Fusarium Head Blight Resistance in Spring Wheat

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Abstract: The effect of selection for two donor-QTL from Fusarium head blight (FHB) resistant spring wheat variety Sumai 3 on the reduction of deoxynivalenol (DON) content and FHB index was evaluated in field trials over two years (2008, 2009) following artificial inoculation with *Fusarium culmorum*. This study was conducted on populations of recombinant inbred lines derived from two crosses, Sumai 3/Swedget and Sumai 3/SG-S 191-01. DON content and FHB index were significantly reduced in both crosses in the genotype classes with two stacked donor QTL on chromosomes 3B and 5A in comparison to genotype classes with no donor QTL. In the cross Sumai 3/Swedget the selection for QTL alleles from 3B and 5A resulted in a 63.4% reduction in DON content, and a 51.8% reduction in the FHB index. Similarly, there was a 35.9% and 31.9% reduction, respectively, in the cross Sumai 3/SG-S 191-01. The single effect of the donor-QTL allele from 3B was significant only in the cross Sumai 3/Swedjet. The presence or absence of awns affected both DON content and FHB index in both populations, but was only significantly in the cross Sumai 3/SG-S 191-01. In this cross the effect of selection for fully awned genotypes was particularly evident on a reduction of both DON and FHB index in classes with neither donor QTL, or the 3B QTL. However, the data indicate that the "awnedness" effect on FHB resistance may be highly variable and is probably greater on reducing FHB symptoms than on DON content. The results confirmed that marker-based introgression of resistance QTLs on chromosomes 3B and 5A in traditional breeding materials can enrich populations for resistance types, but it was also shown that the effect of marker-based selection need not be large in all crosses and a similar effect can probably be reached by indirect selection for some FHB-related traits.

Keywords: deoxynivalenol; head blight symptoms; FHB-related traits; *Fusarium culmorum*; marker assisted selection; QTL; resistance; *Triticum aestivum*

Fusarium head blight (FHB), also called scab, is a disease of small grain cereals, especially wheat and barley. Mycotoxin contamination of human food and animal feed became a more important feature than the direct yield losses that often occur irregularly. Deoxynivalenol (DON) is also reported to be the most frequent toxin in the conditions of Central Europe, reaching the highest concentration levels. The development and deployment of FHB-resistant cultivars is generally accepted as the most cost effective and environmentally benign way to minimize the infection (Parry *et al.* 1995). Obtaining high FHB resistance in combination with resistance to other diseases, high yielding ability and grain quality, undoubtedly represents a serious problem. As documented by Kosová *et al.* (2009), only moderately resistant varieties are available at present for cultivation in the Central European region, but new opportunities for the utilization of genetically distant sources possessing high FHB resistance may arise by the identification of QTL or genes underlying this resistance.

Selection progress for FHB resistance in wheat is hampered by its quantitative inheritance, the high importance of genotype × environment interactions and the necessity to test at the flowering stage (Miedaner *et al.* 2001, 2006). In genotypes analyzed so far, several FHB resistance loci have been found (Buerstmayr *et al.* 2009). In the Chinese source Sumai 3, a major quantitative trait loci (QTL) on chromosome 3BS explained up to 50% of the phenotypic variation (Anderson *et al.* 2001), and appeared to be primarily associated with type II resistance (Buerstmayr *et al.* 2002). The QTL *Qfhs.ndsu-3BS* was recently re-designated as *Fhb1* (Liu *et al.* 2006). In CM82036 (Sumai 3 × Thornbird) the QTL allele 3BS (*Fhb1*) was verified, and additionally, a QTL on chromosome 5A (*Qfhs.ifa-5A*) was detected, showing not such a large effect as *Fhb1*, and contributing more to resistance to initial infection (type I)(Buerstmayr *et al.* 2002, 2003). It was shown by Miedaner *et al.* (2006) and Wilde *et al.* (2007) that both donor-QTL alleles (3B and 5A) significantly reduced DON content and FHB severity compared to classes with no donor QTL. However, the large range of variation for FHB resistance within the marker-based groups will necessitate an additional phenotypic selection to enhance selection gain (Wilde *et al.* 2007).

Moreover, the effect of donor-QTL may be largely dependent on a given genetic background in a breeding population (Anderson *et al.* 2001). The introduction of FHB resistance QTL originating from an exotic source into elite breeding material is often associated with the introgression of several other QTL which may negatively affect agronomically important plant characteristics such as plant height, inflorescense architecture, heading date and final yield (Kosová *et al.* 2009). Parry *et al.* (1995), Miedaner *et al.* (2001) and Klahr *et al.* (2007) reported that morphological characters including plant height, awns and spike compactness are linked to, or have a pleiotropic effect on, FHB resistance in wheat. This may also impact on the magnitude of the effect of the important QTL or genes underlying FHB resistance. Detailed mapping of the FHB resistance QTL as well as the linked QTL determining important agronomical characteristics is necessary in order to make significant progress in FHB resistance.

The objective of this study was to evaluate the effect of selection for the presence of two donor QTL from FHB resistant spring wheat variety Sumai 3, on the reduction of DON content and FHB index, and to justify utilization of this approach in breeding wheat for improved FHB resistance.

MATERIAL AND METHODS

Parents and initial population development

This study was conducted on populations of recombinant inbred lines derived from two crosses: 126 lines came from the cross Sumai 3/Swedget (SuSw) and 162 lines from the cross Sumai 3/SG-S 191-01 (SuSG). The source of high FHB resistance was the spring, fully awned and very early, non-adapted, Chinese variety Sumai 3. This variety showed an average DON content of 9.5 mg/kg, and an average FHB index of 7.5%, in these experiments. The medium-late and tip-awned Swedish spring wheat variety Swedjet, registered in the Czech Republic and possessing high bread-making quality, was characterized on the basis of long experience as moderately susceptible to FHB (average DON content: 45.6 mg/kg; average FHB index: 75.0%). The high yielding advanced breeding line SG-S 191-01 (awnless, medium-early heading winter type) was bred in the Czech Republic by SELGEN com. (pedigree: Samanta/Estica), and registered in 2010 in the Slovak Republic as the variety Sylvie. It is moderately resistant to FHB (average DON content: 27.9 mg/kg; average FHB index: 32.3%). There were no large differences in plant height (not exceeding 10 cm) between these parental varieties.

The lines for both crosses were randomly selected in the $F_{2:3}$. In total, 709 SuSG lines and 568 SuSW lines were examined in the $F_{3:4}$ for the presence or absence of Sumai 3 and recipient QTL alleles on chromosomes 3B and 5A, and only lines not segregating for these alleles were selected. It was also necessary to exclude winter types (SuSG) and many lines of poor vigour and sprouting. It was possible to include, in total, 288 bulked progenies of spring lines at F_5 in a complete analysis of FHB incidence and DON content (126 SuSw lines and 162 SuSG lines).

Identification of specific QTL alleles

Two pieces of leaf, 3 cm long, were collected from individual plants and dried at 40°C.

DNA was extracted from these by the CTAB method (Keb-Llanes *et al.* 2002). DNA was analyzed by standard PCR with the molecular marker *Xbarc133*

(Liu & Anderson 2003), *Xgwm533* and *Xgwm493* (Anderson *et al.* 2001) for the presence of the 3BS QTL, and *Xbarc180* (Buerstmayr *et al.* 2002) for the presence of the 5AS QTL. PCR products were run on 6% polyacrylamide gels on Mega-gel (C.B.S. Scientific) electrophoretic apparatus and visualized by ethidium bromide under UV light.

Out of 162 SuSG lines, 54 lines were classified as lacking donor-QTL alleles on 3B and 5A (class C0), 51 lines as carrying the 3B allele (C1) and 57 lines as carriers of both 3B and 5A alleles (C2). Among the 126 SuSw lines, 49 lines could be classified as C0, 38 lines as C1, and 39 lines as C2.

FHB field tests

Field infection tests including 288 F_5 lines of the two crosses and the parental varieties (in three replicates) were performed at the Crop Research Institute, Prague-Ruzyně in two years (2008 and 2009). The plots were single rows, 1.2 m long, spaced 20 cm apart. Plant spacing within a row was ~ 7.5 cm. Artificial inoculation of spikes with highly pathogenic isolate B of *Fusarium culmorum* (Chrpová *et al.* 2007) was performed with the use of a hand sprayer at the mid-flowering stage (GS 64:anthesis half-way). A conidial suspension with a density of 0.8×10^7 spores/ml was applied. Because of large variation in flowering date between entries, the inoculation times were specific to individual entries over a period of 3 weeks (from 15 June). Disease development was supported (when needed) by mist-irrigation of plots. The evaluation of FHB symptoms was performed at the flowering stage on inoculated spikes, usually 21 days after inoculation (when the symptoms were fully developed and clearly visible). A mean FHB index (Tamburic-Ilincic *et al.* 2007), based on the percentage of infected spikelets, was calculated for each row.

Chemical analyses and data processing

The spikes evaluated for FHB symptoms in individual plots were harvested by hand at full ripening, and dried to minimal water content at a temperature of about 30°C. Samples were threshed, sieved by hand to remove fragments of the rachis and glumes, and carefully cleaned in a machine with adjustable forced air. A representative sample was ground and thoroughly mixed. After that, 5 g of ground sample was shaken (3 min) with 100 ml of distilled water and filtered. 50 µl of the filtrate was used for the test. The content of DON in grains was determined by ELISA with the use of RIDASCREEN® FAST DON kits from R-Biopharm GmbH, Darmstadt, Germany. Samples and standards were applied according to manufacturer instructions. Absorption of final solution was measured at 450 nm, using a SUNRISE spectrophotometer. RIDAWIN® software was used for data processing.

The UNISTAT 5.0 package (UNISTAT Ltd., London, UK) was used for statistical analyses and the STATISTICA package (StatSoft, Inc., Tulsa, OK) for graphics. The least significant difference (LSD) method based on the *F*-distribution was used for paired comparisons between the means.

The experiments were not apparently affected by other diseases and pests or abiotic stress factors.

RESULTS AND DISCUSSION

The effects of donor-QTL alleles on symptom expression and DON content

The DON content and FHB index means for the F_5 lines of the two crosses for the different marker classes (C0, C1 and C2) are given in Table 1. It is clear that genotypes within QTL classes reacted quite similarly for DON content and FHB rating. When compared to the class with no donor QTL (C0), both DON content and FHB index, in both crosses, were significantly reduced in genotypes with two stacked donor 3B and 5A QTL (C2). However, the single effect of the donor-QTL 3B allele (C1) was significant only in the cross Sumai 3/Swedjet (SuSw). In this cross, the selection for 3B and 5A QTL alleles resulted in a 63.4% reduction of DON content and a 51.8% reduction of FHB index, while it was respectively, 35.9% and 31.9%, in the cross Sumai 3/SG-S 191-01 (SuSG). Similarly Miedaner *et al.* (2006) found the highest effect for the stacked donor-QTL 3B and 5A alleles jointly reduced DON content by 78% and FHB rating by 55% compared to the susceptible QTL class.

A large effect of marker-based selection in the cross SuSw is also evident from Figure 1 in which 97% of C2 genotypes could be included in the DON class interval 0–20 mg/kg. A low DON content (< 10 mg/kg) was detected in 27/39 C2 genotypes in SuSw (69%), while it was only 16/57 in SuSG (28%).

Table 1. Mean DON content in 2009 and FHB index from two years (2008 and 2009) for different marker classes and morphotypes in spring F_5 lines of two crosses

QTL class/morphotype	Sumai 3/Swedget (SuSw)		Sumai 3/SG-S 191-01 (SuSG)	
	DON content (mg/kg)	FHB index (%)	DON content (mg/kg)	FHB index (%)
3B + 5A (C2)	8.53^{a}	25.63^{a}	18.92^{a}	24.20^{a}
3B (C1)	14.76^{b}	40.08^{b}	23.67^{ab}	36.59^{b}
None (C0)	23.31^{c}	53.22^{c}	29.50^{b}	35.54^{b}
Fully awned spike	14.36^{a}	38.72^{a}	19.21^{a}	22.06^{a}
Awnless (tip-awned) spike	18.06^{a}	43.37^{a}	31.66^{b}	47.70^{b}
C2/awned spike	9.21^{a}	20.11^{a}	18.57^{a}	19.12^{a}
C2/awnless spike	7.95^{a}	31.16^{ab}	19.75^{ab}	35.22^{bc}
C1/awned spike	17.72^{bc}	41.57^{bc}	17.74^{a}	21.81^{ab}
C1/awnless spike	11.23^{ab}	38.24^{bc}	32.56^{bc}	59.50^{d}
C0/awned spike	23.66^{c}	49.21^{cd}	21.64^{ab}	26.28^{ab}
C0/awnless spike	22.84^{c}	58.57^{d}	39.51^{c}	47.22^{cd}
Heading: early	20.86^{a}	56.15^{b}	24.71^{ab}	25.15^{a}
Heading: medium	15.66^{a}	40.65^{a}	28.31^{b}	41.42^{b}
Heading: late	15.14^{a}	34.69^{a}	20.31^{a}	28.63^{a}
Mean of selected plants*	31.66	36.11	29.79	26.80
Total population mean	16.32	40.84	23.93	31.83

*Plants perspective for utilization in wheat breeding for Central European conditions
Means in columns followed by the same letter are not significantly different from each other ($P < 0.05$)

For FHB index, the frequencies of different marker classes were similar in both crosses, and the frequency of susceptible genotypes was higher than with DON. The lower effectiveness of marker-based selection in SuSG was evidently due to the relatively smaller effect of the 3B QTL allele (not significant for FHB index and only a 20% effect on reduction of DON content) (Table 1). Large differences in % variation explained by markers in the 3BS QTL region (25/42%) have been detected by Anderson *et al.* (2001), and they are also clear from the review study of Buerstmayr *et al.* (2009). These differences may result from a coincidence of QTL for FHB and other FHB-related traits as described by Srinivasachary *et al.* (2008a, b). Additionally, the winter habit of parental line SG-S 191-01 in the SuSG cross, and probably the greater genetic divergence could contribute to the lowering of the 3B and 5A allele effects, because numerous studies of winter wheat germplasm indicate that, in most cases, the FHB resistance is due to numerous QTL of moderate to small effect (Gervais *et al.* 2003; Schmolke *et al.* 2005; Srinivasachary *et al.* 2008a).

The impact of FHB related traits on the effectiveness of marker-based selection

It is evident that some morphological characters including plant height, awns, spike compactness or heading time have an influence on FHB index and DON accumulation (Mesterházy 1995; Buerstmayr *et al.* 2000; Steiner *et al.* 2004), and indirect selection for desirable performance in these traits may help breeders to identify and discard susceptible early generation lines (Tamburic-Ilincic *et al.* 2007). In this study, special attention was paid to traits showing large variation in the crosses examined, mainly the presence of awns and heading date. Differences in plant height, not large in these experiments, were not examined, but lately it was shown by Chrpová *et al.* (2010) that in the conditions of Central Europe, selection for

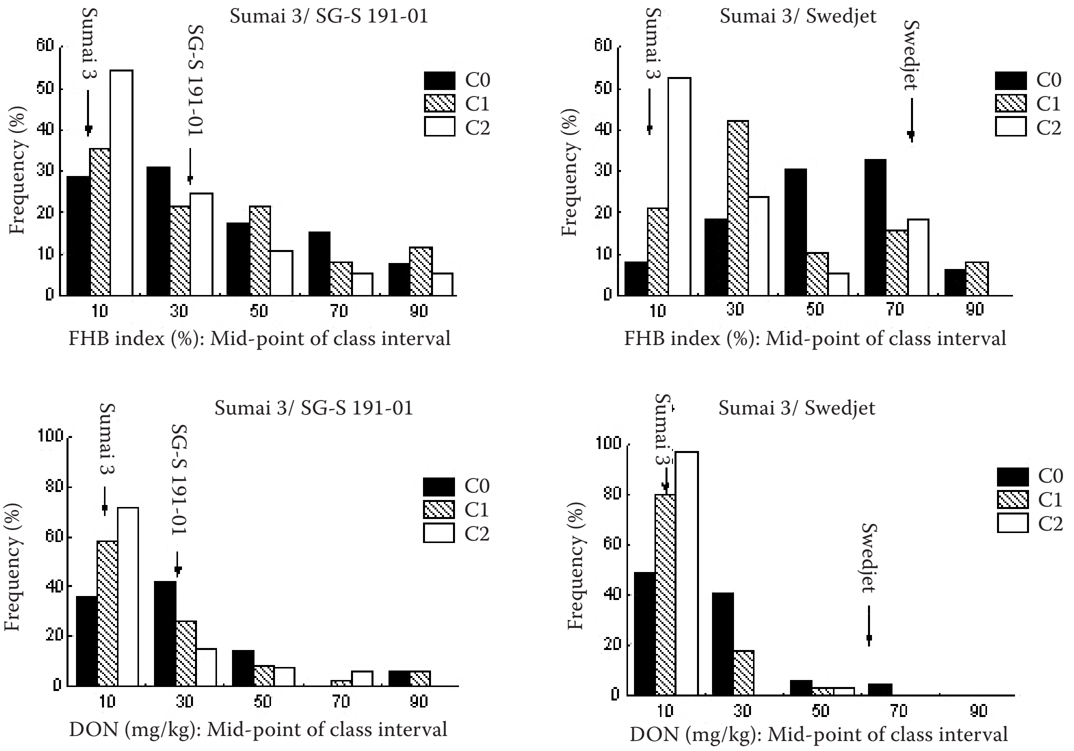

Figure 1. Frequency distribution (%) of 162 Sumai 3/SG-S 191-01 and 126 Sumai 3/Swedjet F5 lines for mean FHB index and DON content and three QTL classes (C0, C1 and C2)

greater plant height may contribute significantly to increased FHB resistance.

As documented in Table 1, the presence or absence of awns affected both DON content and FHB index, significantly in the cross SuSG. Thus, it was examined whether simultaneous selection for long awns and the 3B and 5A alleles derived from Sumai 3 could increase the effectiveness of marker based selection. In the cross SuSG, the effect of selection for fully awned genotypes was particularly evident on a reduction of both DON and FHB index in C0 (with no donor QTL) and C1 (3B) genotype classes. In the C2 (3B + 5A) class, the effect of selection for the presence of awns was small on a reduction of DON (6%), but relatively high on a reduction of FHB index (48%). However, the data obtained in the SuSw cross leads to the conclusion that the "awnedness" effect on FHB resistance may be highly variable, and probably greater on reducing FHB symptoms than on DON content. Snijders (1990) suggested that awns could be used as a marker for selecting FHB resistant lines among progenies from crosses in which the resistant parent carries awns. Tamburic- Ilincic *et al.* (2007) found that awned genotypes had a lower FHB index than awnless ones, but they argue that with the use of the spray-inoculation method, awned spikes could receive less inoculum than awnless spikes. Mesterházy (1995) reported that awned wheat cultivars showed more disease symptoms under natural infection, suggesting that they might accumulate more air-borne spores due to a larger spike surface and they could retain humidity over longer periods than awnless ones. Srinivasachary *et al.* (2008b) suggest that QTL for both FHB resistance/susceptibility and awns/no awns are located on 2B, and that they are sufficiently closely linked to provide a means to enhance FHB resistance in breeding programmes by selecting for either the presence or absence of awns.

Attention was also paid to the differences in heading time (in the 18 days range). It is apparent (Table 1) that the effects of lateness or earliness on the traits and crosses were not consistent, and differences between three heading date classes were often not significant. On average, earliness appeared to contribute significantly to an increase of the FHB index in SuSw, but in SuSG the highest symptom expression was found in the intermediate heading date class, similar to DON content. It is obvious that FHB development, and accumulation of DON, in early and late flowering genotypes are highly affected by the conditions of individual years, and any correlation between heading date and FHB traits may be of opposite direction in individual years (Tamburic-Ilincic *et al.* 2007). In these experiments both crosses did not differ in average heading date and the correlation between heading date and FHB index was insignificant in both years for SuSG and in 2008 for SuSw ($r = –0.06 – 0.04$). The correlation between heading date and FHB index was significantly negative in SuSw in 2009 ($r = –0.34$; $P < 0.001$), and for DON content vs. heading date in both crosses ($r_{\text{SuSG}} = –0.14$; $r_{\text{SuSw}} = –0.20$; $P < 0.05$). This suggests that earliness could contribute to increased DON content in 2009. The simultaneous effect of selecting for lateness and the 3B and 5A QTL (C2) on a reduction of DON content was 77% in SuSw in comparison with the C0 class (without respect to heading date C2/C0 = 63%), and 44% in SuSG (C2/C0 = 36%). However, this increase of selection effectiveness (by about 10%) can hardly be justifiable under different weather conditions and in different hybrid populations. McCartney *et al.* (2007) concluded that improvements in FHB resistance can be made without adverse changes in days to anthesis.

From a breeding perspective, in both crosses, there were selected lines showing suitable plant height, heading date, plant growth vigour and spike productivity. It was possible to find 22 lines in SuSG and 9 lines in SuSw with a desirable plant type. Data on their DON content and FHB index are presented in Table 1. Out of 31 lines, 18 lines were carriers of 3B (58%) and eight of both the 3B and 5A QTL alleles (26%). Large variation in both DON content and FHB index was characteristic also for these lines, but it was possible to detect 6 lines (19%) with a DON content lower than 10 mg/kg and 9 lines (29%) with a FHB index lower than 10%. Selection within large populations and/or an application of recurrent backcrossing will undoubtedly be needed to meet demands for high and stable yield and quality performance in combination with resistance to the relevant diseases and pests, including resistance to FHB. Further examination of the effects of FHB resistance alleles on different agronomically important traits is very important from a practical breeding perspective. In the experiments of McCartney *et al.* (2007) the Sumai 3 5AS allele was significantly associated with reduced grain protein content.

In conclusion, these results confirmed that marker-based introgression of resistance QTLs on chromosomes 3B and 5A in traditional breeding materials may result in a substantial increase in resistance within a short time, but it was also evident that the effect of marker-based selection need not be large in all crosses, and similar effects can probably be reached by indirect selection for some FHB-related traits. In the cross SuSG, a similarly low average DON content and FHB index, as after selecting for QTLs 3B and 5A, could be reached after selection for awned spikes. The presence of these QTL did not also exclude the occurrence of susceptible genotypes. Therefore, it could be advantageous to integrate the marker-based approach with indirect selection for FHB-related morphological traits and additional phenotypic selection (Wilde *et al.* 2007). More attention should be paid to specific properties of recipient genotypes – particularly to take into account the expected contribution of certain traits to FHB resistance, and it may be also feasible to perform selection for other specific QTLs that were found to be associated with FHB (Srinivasachary *et al.* 2008b). To apply the marker-based selection effectively in different hybrid populations, more diagnostic markers should be developed for the most repeatable QTL reported (Buerstmayr *et al.* 2009).

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