Resistance of Winter Wheat Varieties Registered in the Czech Republic to Fusarium Head Blight in Relation to the Presence of Specific *Rht* Alleles

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Abstract: Resistance of 31 winter wheat varieties (bred in 6 European countries) to Fusarium head blight (FHB) was evaluated in field trials lasting for three years (2007, 2008, 2009) after artificial inoculation with *Fusarium culmorum.* The data on deoxynivalenol (DON) content were supplemented by symptom scores and determination of % of Fusarium damaged grains and % reductions of thousand-grain weight and grain weight per spike due to infection. These varieties and also 120 advanced breeding lines from the Úhřetice Breeding Station, SELGEN company were examined for the presence of gibberellic acid (GA) insensitive semi-dwarfing genes to evaluate their effect on FHB. The conditions of experimental years highly influenced the performance of all characters. The highest production of DON occurred in 2008 at a high temperature and high moisture content during the infection period. In all years the moderate resistance to accumulation of DON (at the level of Swiss variety Arina) was detected in the Czech varieties Bakfis, Federer, Baletka, Samanta and Sakura. Among these varieties, Federer showed a low accumulation of DON at a relatively higher symptom expression and greater reduction of grain weight per spike, but the other four varieties and the reference variety Arina expressed resistance in all the examined traits. The varieties Pitbull, Cubus, Kodex and Bagou were found to be highly susceptible to FHB. The presence of the dwarfing allele *Rht-D1b* resulted in a significantly higher mean symptom score and also in a higher affection of the other traits. Nonetheless, the analysis of frequency distributions in symptom scores showed the presence of resistant lines also among the GA insensitive lines, but with a lower frequency than in the group of GA sensitive genotypes. A relatively greater effect on manifestation of the disease had plant height, and therefore it is suggested that the adverse impact of *Rht-D1b* on FHB resistance could be to a high degree excluded by opting for taller *Rht-D1b* genotypes.

Keywords: deoxynivalenol content; disease severity traits; dwarfing allele *Rht-D1b*; *Fusarium culmorum*; head blight; plant height, variety resistance; winter wheat

Fusarium head blight (FHB) belongs to the most damaging diseases, particularly in years with intensive rainfall. In central Europe, the disease is caused by a complex of *Fusarium graminearum*, *F. culmorum* and some species of minor importance. The fungus reduces substantially grain yield and affects grain quality. 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 reported also in the conditions of Central Europe the most frequent toxin reaching the highest concentration levels.

The cultivation of FHB-resistant varieties is thought to be the most viable strategy for controlling disease and reducing mycotoxin contamination (D'Mello *et al.* 1999; Buerstmayr *et al.* 2002). FHB resistance is polygenic and its expression is highly influenced by the environment. It becomes obvious that resistance against FHB in small grain cereals is determined quantitatively by several quantitative trait loci (QTL). It is, however, important that many studies showed common and durable resistance to different *Fusarium* spp. causing FHB (Stack *et al.* 1997; Hollins *et al.* 2003; Mesterházy *et al.* 2005). Though the isolates of different *Fusarium* spp. differed largely in quantitative aggressiveness, they did not show evident qualitative differences in virulence (Mesterházy *et al.* 2005; Akinsamni *et al.* 2006; Šíp *et al.* 2008). Because pathogenic specialization can be classified as low, wheat breeding for general Fusarium resistance may be possible. However, it is also evident that high level of FHB resistance has not yet been obtained in commercially grown European wheat varieties (Kosová *et al.* 2009) and from practical aspects the "double protection" (consisting in growing less susceptible varieties and fungicide treatment) is evidently needed to control the disease more effectively. It was lately demonstrated by Šíp *et al.* (2010) that the joint effect of moderate resistance (varieties Petrus, Simila, Sakura) and application of the fungicide Swing Top was 87% for DON reduction and even 95% for reducing the yield loss.

The mechanisms of plant resistance to FHB are very complex and two major types of host resistance were distinguished: Type I – resistance to initial infection and Type II – resistance to spread of the pathogen within a spike (Schroeder & Christensen 1963). Specific inoculation procedures to evaluate fungal spread in the ear (Type II resistance) involve the introduction of inoculum into central spikelet of an ear at early anthesis (Schroeder & Christensen 1963; Bai & Shaner 1996). Spray inoculation is used for assessment of both Type I and Type II resistance and from breeding aspects it is considered as more suitable because it covers much wider genetic background than the point inoculation does (Mesterházy *et al.* 2008). To minimize year/location effects on results, it appears often necessary to support disease development by irrigation of plots. Low FHB severity due to Type I and Type II resistance usually coincides with low DON because of fewer infected kernels (Bai *et al.* 2001). Other types of resistance described by Mesterházy (1995) are resistance to kernel infection, to toxin (DON) accumulation and tolerance. Wheat resistance to FHB could be described by disease score on head, % of Fusarium damaged grains, kernel weight per head and DON content (Wisniewska *et al.* 2004). Though relationships between these traits are usually significant, their separate evaluation enables to characterize better this complicated disease.

Breeding for FHB resistance would be greatly facilitated if the resistance level could be predicted from indirect, easily determined traits. The FHB resistance associated with plant physiological and morphological characteristics is also called passive resistance (Mesterházy 1995). A number of traits including plant height, presence of awns, spike compactness and heading date have been associated with FHB resistance and it is, therefore, important to consider potential pleiotropic effects of such traits on FHB infection. Generally, it has been accepted that taller plant varieties show fewer FHB symptoms compared to shorter ones. It has been postulated that conidia spread more easily to the heads of short varieties because of the reduced distance between leaf layers (Mesterházy 1995). Several QTL studies have found FHB resistance loci associated or coincident with plant height QTL (Gervais *et al.* 2003; Steiner *et al.* 2004; Draeger *et al.* 2007; Klahr *et al.* 2007; Shrinivasary *et al.* 2009). Quite a lot of papers have also been published which examine the effect of allelic constitution at loci determining plant height on chromosomes 4BS and 4DS. Draeger *et al.* (2007) concluded that FHB susceptibility is associated with the dwarfing allele *Rht-D1b* (formerly termed *Rht2*) resulting from linkage or pleiotropy rather than an effect of height per se. Srinivasachary *et al.* (2008) have shown on a mapping population derived from the cross between Spark carrying *Rht-D1a* allele and the FHB susceptible variety Rialto that the enhanced FHB susceptibility associated with *Rht-D1b* was not an effect of plant height per se as other QTL for height segregating in this population have no influence on susceptibility. The following examinations into the effects of semi-dwarfing *Rht-B1* and *Rht-D1* loci (Shrinivasary *et al.* 2009) showed that under high disease pressure both *Rht-B1b* and *Rht-D1b* significantly decreased Type I resistance, however, whilst *Rht-D1b* had no effect on Type II resistance, *Rht-B1b* significantly increased Type II resistance. Population studies of Holzapfel *et al.* (2008) and also some results of practical wheat breeding (Cöster 2010) indicated that the semi-dwarfing allele *Rht-D1b* seems to be the major source for FHB susceptibility in

European winter wheat. Significant genotypic variance for FHB resistance remained also in the *Rht-D1b* subpopulations and, therefore, the selection for moderately FHB resistant genotypes can be feasible also within agronomically beneficial *Rht-D1b* genotypes (Voss *et al.* 2008).

It was shown (Bobková *et al.* 1988; Šíp *et al.* 1988) that the selection environment of the Czech Republic was advantageous for the tall lodging resistant gibberellin acid (GA) insensitive genotypes carrying *Rht-B1b* and *Rht-D1b* genes ("tall dwarfs"), which had high ear productivity through increased grain size and grain number at the level of shorter genotypes. Because many advanced lines characterized by presence or absence of *Rht-D1b* were at disposal in the Breeding Station Úhřetice of the SELGEN company, it was aimed to examine their response to FHB infection. Another objective of this paper was to present three year results of field experiments in which the resistance of modern winter wheat varieties registered in the Czech Republic to accumulation of mycotoxin DON in grain was studied in relation to the other important FHB traits and presence of specific *Rht* alleles following artificial inoculation with *Fusarium culmorum.*

MATERIALS AND METHODS

Plant materials. The response to artificial infection with *Fusarium culmorum* was studied in two separately evaluated sets of winter wheat varieties and advanced breeding lines. The first set contained 30 varieties that could be classified as recommended for cultivation (Akteur, Barryton, Biscay, Bohemia, Cubus, Dromos, Etela, Eurofit, Hedvika, Kerubino, Ludwig, Meritto, Mulan, Rapsodia, Sakura), intended to include into the recommended list (Bakfis, Baletka, Kodex, Megas, Pitbull, Orlando, Sultan), newly registered varieties (Federer, Brilliant, Secese, Seladon, Iridium,Bagou) and varieties tested for a long time as representatives of early variety group (Košútka) and certain baking quality attributes (Samanta). The Swiss variety Arina, generally known as a source of moderate resistance to FHB (Jenny *et al.* 2000), was used in these experiments as a check. The registered varieties were bred in 6 European countries (12 – Czech Republic, 12 – Germany, 2 – France, 2 – Austria, 1 – the Netherlands and 1 – Denmark). Characteristics of the examined varieties that are registered in the Czech Republic are available on the website of the Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic: http://www.ukzuz.cz/Folders/2220-1-Plant+Varieties.aspx. The second set comprised 120 advanced breeding lines (generation F_7–F_9) coming from different crosses between GA responsive and GA insensitive parents (carriers of *Rht-D1b* gene) in which data were obtained on plant height and symptomatic reaction following inoculation with *Fusarium culmorum* in the field conditions.

Description of field experiments, disease evaluation and examined characters. The field tests were conducted in 2007–2009 at the Research Institute of Crop Production in Prague-Ruzyně (a set of registered varieties) and in 2009 at the Breeding Station, Úhřetice, SELGEN comp. (a set of advanced lines). Wheat varieties/lines were planted in hill plots in three replications. Artificial inoculation of spikes with highly pathogenic isolate B of *F. culmorum* (Šíp *et al.* 2002; Chrpová *et al.* 2007) was performed at mid-flowering (GS 64: anthesis half-way) (Zadoks *et al.* 1974). One term spraying of inoculum (conidial suspension 0.8×10^7 ml) onto bunches of 10 flowering spikes randomly selected within hill plots was applied. Inoculated spikes were then kept for 24 h in polythene bags. To minimize year/location effects on results, it appeared necessary in these conditions to support disease development (when needed) by irrigation of plots. Mean pentad temperatures during the period of disease development in individual years are given in Table 1.

Head blight symptoms were evaluated in three terms (usually 14, 21 and 28 days after inoculation) on a 1–9 scale, where 1 < 5%, 2 = 5–17%, 3 = 18–30%, 4 = 31–43%, 5 = 44–56%, 6 = 57–69%, 7 = 70–82%, 8 = 83–95% and 9 > 95% of the spikelets with FHB symptoms. Visual symptom scores (VSS) are based on average value of three measurements. Determination of other resistance traits was based on seed samples obtained in each plot from inoculated spikes, which were threshed at a low wind not to lose light infected scabby grains. Fusarium damaged (scabby) grains (FDG) were calculated as percentage by total seed number. Tolerance to the infection was expressed as percent reduction (R) from non-inoculated control (C) in the traits thousand grain weight (TGW) and grain weight per spike (GWS). Seeds from infected spikes were analyzed for DON (deoxynivalenol) content.

Table 1. Mean pentad temperature (in °C) during the period of disease development in 2007–2009

		2007	2008	2009
May	12–16	16.0	15.9	12.2
	17–21	15.9	11.5	18.0
	22–26	21.5	14.2	17.9
	27–31	16.0	21.1	11.1
June	1–5	17.3	19.8	12.8
	6–10	21.7	19.3	15.3
	11–15	21.6	14.3	15.6
	16–20	20.9	17.3	16.8
	21–25	19.1	21.7	14.2
	26–30	15.3	20.2	19.3
July	1–5	17.3	20.6	20.9
	6–10	16.8	17.7	17.0
	11–15	20.0	17.8	19.3
Mean temperature		18.4	17.8	16.2
Time of inoculation		14.5.–25.5.	28.5.–5.6.	18.5.–5.6.

Chemical analyses. The content of DON was determined by ELISA with the use of RIDASCREEN® FAST DON kits from R- Biopharm GmbH, Darmstadt, Germany.

DON determination: 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. Samples and standards were applied according to manufacturer instructions. Absorption of final solution was measured at 450 nm, using SUNRISE spectrophotometer. RIDAWIN® software was exploited for the data processing.

Determination of Rht genes presence. Plant responses to exogenous gibberellin (GA) were determined using the method of Gale and Gregory (1977). To distinguish between GA-responsive and GA-insensitive genotypes, the method consisted of a 14 day treatment with 50 ppm GA_3. Measurements of shoot length were taken 10 and 14 days after GA treatment and in control (untreated) plants. A genotype was considered to be GA-sensitive when the increase in shoot length between the GA-treated and control plants was significant.

Genomic DNA was extracted from individual plants using commercial Dneasy Plant Mini Kits (Qiagen) following manufacturers instructions. For detection of both the *Rht-B1* and *Rht-D1* genes (except the *Rht–D1a* allele), located on wheat chromosomes 4B and 4D, respectively, PCR conditions and primers were as published by Ellis *et al.* (2002). To detect the *Rht-D1a* allele, reaction conditions were: initial denaturation at 94°C for 3 min, followed by 45 cycles (94°C for 1 min, 58°C for 1 min and 72°C for 1 min) and finished at 72°C for 10 min. PCR products were separated on agarose gels and visualised using ethidium bromide.

Statistical analysis. The UNISTAT 5.0 package (UNISTAT Ltd., London, UK) was used for statistical analyses and STATISTICA package (StatSoft, Inc., Tulsa, OK) for graphics. Least significant difference (LSD) method based on the *F* distribution was exploited for paired comparisons between the means following analyses of variance, in which the null hypotheses “all population means are equal” were rejected. Pooled variance *t*-test was used to compare the means of two independent samples showing the same variance.

The data obtained from non-inoculated plots were not included in statistical analyses (they were used for determination of reductions of grain yield). Analysis of DON content in control plots (C) showed only traces of grain contamination (average value 0.060 mg/kg). The experiments were not apparently affected by other diseases and pests or abiotic stress factors.

RESULTS AND DISCUSSION

Disease development under different conditions and relations between the examined traits

It follows from evaluation of the response of 31 winter wheat varieties to artificial infection of ears with isolate B of *Fusarium culmorum* in 2007–2009 (Tables 2 and 3) that the conditions of 2008 were the most favourable for the spread of the disease. That year could be characterized by high temperatures in time of inoculation (the end of May and beginning of June – Table 1). The high temperature accompanied by sufficient wetness (when using mist irrigation of plots) favoured early FHB development in the wheat spike and resulted in a high accumulation of DON like in 2006 (Chrpová *et al.* 2007). In 2008, the average DON content was much higher (89.13 mg/kg) than

Table 2. Mean DON content (mg/kg) of 31 winter wheat varieties in three years and inclusion of varieties in homogeneous groups (P = 95%, LSD test)

Variety	Year of registration	Country of origin*	Classification**	Average	2007	2008	2009
Bakfis	2008	CZ	MR	15.1^{a}	11.6^{a}	17.7^{a}	16.0^{a}
Arina		CH	MR	20.3^{a}	9.9^{a}	32.4^{abc}	18.8^{ab}
Federer	2009	CZ	MR	30.9^{ab}	16.0^{ab}	38.1^{abc}	38.7^{abcde}
Baletka	2008	CZ	MR	31.4^{ab}	21.2^{ab}	39.4^{abc}	33.7^{abcd}
Samanta	1993	CZ	MR	33.4^{ab}	27.7^{ab}	29.3^{ab}	43.3^{abcdef}
Sakura	2007	CZ	MR	36.6^{abc}	29.8^{abc}	41.5^{abc}	36.2^{abcde}
Brilliant	2009	DE	M	40.8^{abcd}	12.4^{ab}	39.8^{abc}	51.3^{cdefgh}
Dromos	2006	DE	M	45.1^{abcd}	22.3^{ab}	75.1^{abcde}	37.8^{abcde}
Košútka	1981	CZ	M	45.3^{abcd}	44.9^{abcd}	67.9^{abcde}	23.2^{abc}
Sultan	2008	CZ	M	48.1^{abcd}	45.0^{abcd}	56.8^{abcd}	41.4^{abcde}
Hedvika	2004	NL	M	48.6^{abcd}	18.0^{ab}	74.8^{abcde}	42.2^{abcdef}
Orlando	2008	DK	M	58.4^{bcde}	38.8^{abcd}	95.9^{bcdef}	40.6^{abcde}
Akteur	2004	DE	M	59.2^{bcde}	29.5^{ab}	100.4^{cdef}	47.7^{bcdefg}
Secese	2009	CZ	M	62.1^{bcde}	74.9^{def}	47.0^{abcd}	59.4^{defgh}
Mulan	2007	DE	M	62.3^{bcde}	42.7^{abcd}	89.4^{bcdef}	54.8^{defgh}
Ludwig	2000	AT	M	65.1^{bcde}	59.7^{bcd}	78.0^{abcdef}	61.8^{defghi}
Megas	2008	DE	M	65.4^{bcde}	38.2^{abcd}	112.8^{defg}	36.2^{abcde}
Meritto	2003	CZ	M	65.7^{bcde}	71.8^{cdef}	92.7^{bcdef}	32.5^{abcd}
Biscay	2005	DE	MS	72.0^{def}	29.9^{abc}	114.9^{defg}	71.2f^{ghijk}
Seladon	2009	CZ	MS	75.0^{def}	54.1^{bcd}	74.5^{abcde}	96.5^{kl}
Etela	2006	CZ	MS	76.9^{def}	62.8^{bcd}	89.9^{bcdef}	77.9^{hijkl}
Iridium	2009	FR	MS	78.8^{defg}	77.8^{def}	100.6^{cdef}	58.1^{defgh}
Kerubino	2007	DE	MS	88.9^{efgh}	109.3^{fgh}	93.1^{bcdef}	64.3^{efghij}
Eurofit	2006	AT	MS	89.3^{efgh}	126.0^{gh}	63.4^{abcde}	78.4^{hijkl}
Rapsodia	2003	DE	MS	90.2^{efgh}	43.2^{abcd}	152.2^{fg}	75.2^{ghijkl}
Bohemia	2007	CZ	MS	90.5^{efgh}	73.7^{def}	152.9^{fg}	44.9^{abcdef}
Barryton	2007	DE	MS	91.5^{efgh}	65.6^{bcd}	154.4^{fg}	54.6^{defgh}
Pitbull	2008	DE	S	102.7^{fgh}	59.1^{bcd}	156.1^{fg}	93.0^{jkl}
Cubus	2004	DE	S	113.5^{gh}	103.4^{efg}	172.9^{g}	64.1^{efghij}
Kodex	2008	DE	S	117.4^{h}	70.7^{cde}	179.1^{g}	102.2^{l}
Bagou	2009	FR	S	122.1^{h}	145.0^{h}	130.1^{efg}	91.2^{ijkl}
Average				65.9	52.8	89.1	54.4

*Country where the variety was bred; **MR = moderate resistance; M = medium response; MS = moderate susceptibility; S = susceptibility

Means in columns followed by the same letter are not significantly different from each other ($P < 0.05$)

Table 3. Mean values (2007–2009) and variety and year ranking for DON content, visual symptom score (VSS), % of Fusarium damaged grains (FDG), reduction of thousand grain weight (TGW-R), and reduction of grain weight per spike (GWS-R); results of traits evaluation with respect to the presence of Norin 10 *Rht* genes in the examined varieties

Variety/year/response to GA (*Rht* gene)		DON content		VSS		FDG		TGW-R		GWS-R	
		mg/kg	rank	1–9	rank	%	rank	%	rank	%	rank
Bakfis	GA-S	15.1	1	2.87	2	29.9	1	19.8	1	35.0	1
Arina	GA-S	20.3	2	2.11	1	34.6	2	22.1	3	39.3	3
Baletka	*Rht-B1b*	31.4	4	3.26	6	44.2	3	21.9	2	42.9	4
Samanta	GA-S	33.4	5	3.36	7	51.0	5	26.1	6	38.9	2
Dromos	GA-S	45.1	8	3.25	5	49.7	4	26.1	5	48.7	5
Sakura	GA-S	36.6	6	3.19	4	62.6	8	28.0	8	50.3	6
Košútka	GA-S	45.3	9	3.83	16	51.6	6	25.6	4	50.4	7
Federer	GA-S	30.9	3	3.66	11	55.5	7	34.7	17	55.6	14
Brilliant	*Rht- D1b*	40.8	7	3.17	3	63.9	9	39.3	24	52.2	10
Mulan	GA-S	62.3	15	3.50	8	68.4	15	30.5	9	53.2	13
Hedvika	GA-S	48.3	11	3.77	12	70.3	18	33.0	12	51.9	8
Meritto	GA-S	65.7	18	3.83	15	66.2	12	31.7	10	52.9	11
Ludwig	GA-S	65.1	16	3.56	9	68.9	16	33.5	16	53.0	12
Megas	GA-S	65.4	17	3.58	10	64.0	10	38.4	21	57.9	17
Orlando	GA-S	58.4	12	3.77	13	71.0	19	33.3	14	59.2	19
Sultan	GA-S	48.1	10	4.05	18	67.2	13	33.4	15	64.4	23
Akteur	GA-S	59.2	13	4.13	19	65.8	11	38.5	22	59.3	20
Barryton	GA-S	91.5	27	3.98	17	74.0	24	27.2	7	56.8	15
Bohemia	GA-S	90.5	26	3.81	14	68.1	14	40.9	29	52.0	9
Secese	GA-S	62.1	14	4.97	31	71.6	21	32.4	11	57.1	16
Etela	GA-S	76.9	21	4.56	26	69.7	17	36.8	20	66.1	25
Cubus	*Rht- D1b*	113.5	29	4.31	22	73.7	22	36.7	19	58.3	18
Eurofit	GA-S	89.3	24	4.21	20	71.1	20	40.8	27	61.4	21
Rapsodia	*Rht- D1b*	90.2	25	4.35	23	80.8	30	33.2	13	62.9	22
Iridium	*Rht- D1b*	78.8	22	4.23	21	73.9	23	35.9	18	69.4	29
Kerubino	GA-S	88.9	23	4.44	25	74.2	25	40.8	26	65.1	24
Biscay	*Rht- D1b*	72.0	19	4.41	24	78.1	28	39.4	25	67.0	27
Seladon	GA-S	75.0	20	4.56	27	75.9	27	40.9	28	66.7	26
Bagou	*Rht- D1b*	122.1	31	4.76	30	74.4	26	38.5	23	71.8	30
Pitbull	*Rht- D1b*	102.7	28	4.67	29	80.5	29	48.9	31	67.0	28
Kodex	*Rht- D1b*	117.3	30	4.63	28	86.9	31	47.7	30	72.1	31
2007		52.8	1	3.59	2	58.1	1	30.3	1	48.3	1
2009		54.4	2	3.32	1	78.0	3	33.1	2	54.1	2
2008		89.1	3	4.92	3	64.1	2	40.2	3	70.4	3
GA sensitive		59.7		3.82		64.1		33.0		55.1	
GA insensitive: *Rht-D1b*		92.2		4.32		76.5		40.0		65.1	
$\|t\|$-statistic		3.391**		2.549*		2.961**		2.867**		3.107**	
Total average		65.9		3.90		65.7		34.1		56.8	

GA = gibberellic acid; GA-S = GA sensitive; $^{*}P < 0.05$; $^{**}P < 0.01$

in 2007 (52.75 mg/kg) and 2009 (54.43 mg/kg). Also the other traits, except for percentage of Fusarium damaged grains, were in 2008 more affected by the disease than in the other years. In 2009, the disease developed at a relatively lower daily temperature and in 2007 at a higher fluctuation of temperature values, but the average DON content was in these years rather similar.

Besides DON content, the disease severity was also characterized by symptom expression (VSS), percentage of Fusarium damaged grains (FDG) and reductions of thousand-grain weight (TGWR) and grain weight per spike (GWSR) due to infection. As shown in Table 4, all these traits were significantly interrelated, but it is evident that particularly the relations of DON with VSS, FDG and GWSR were in 2007 not so tight as in the other years, which could probably be ascribed to the above mentioned temperature fluctuations during the period of disease development causing different impacts on manifestation of different traits. The relations of DON with VSS, FDG, TGWR and GWSR were close in 2009, when correlation coefficients ranged between 0.78 and 0.84 (DON vs GWSR – yield loss), while it was 0.54–0.71 in 2008 with the most severe infection and only 0.41–0.56 in 2007. According to these results, it can be expected that the conditions of 2009, when the inspection into the effects of *Rht* genes on FHB resistance was performed in advanced breeding lines, were favourable to make implications for DON content from VSS.

Evaluation of the variety resistance to accumulation of DON mycotoxin and performance in the other FHB traits examined

It can be deduced from the correlation studies above that the classification of variety resistance (tolerance) to FHB and accumulation of DON in grain is undoubtedly connected with the inclusion of suitable characters and the accuracy of their determination. In this study, great attention was paid to DON content, which can be reckoned as the character of crucial importance. Differences between varieties in the accumulation of DON mycotoxin following FHB infection in individual years are clear from Table 2. Using multiple comparisons from ANOVA, the varieties could be arranged in each year and over examined years into homogeneous groups of a similar resistance level. These groups were often large (particularly when examined over years), which may indicate a high affection of variety DON content by environmental conditions. Therefore, a multiple testing is evidently necessary. On the basis of three-year results, the varieties could be classified from these aspects as showing moderate resistance (MR), medium response (M), moderate susceptibility (MS) and susceptibility (S). When compared with the response of moderately resistant reference variety Arina, a great majority of included varieties could be reckoned to be medium responsive or quite susceptible to accumulation of DON. The highest resistance level to accumulation of DON was found

Table 4. Coefficients of correlation between the examined traits in three years (31 varieties)

Combination of traits*	2007		2008		2009	
	r	*P*	*r*	*P*	*r*	*P*
DON vs VSS	0.563	0.0006	0.711	0.0000	0.799	0.0000
DON vs FDG	0.466	0.0047	0.829	0.0000	0.794	0.0000
DON vs TGWR	0.685	0.0000	0.536	0.0011	0.783	0.0000
DON vs GWSR	0.414	0.0114	0.665	0.0000	0.839	0.0000
VSS vs FDG	0.585	0.0003	0.820	0.0000	0.619	0.0001
VSS vs TGWR	0.585	0.0003	0.570	0.0005	0.501	0.0021
VSS vs GWSR	0.733	0.0000	0.876	0.0000	0.744	0.0000
FDG vs TGWR	0.622	0.0001	0.679	0.0000	0.728	0.0000
FDG vs GWSR	0.650	0.0001	0.845	0.0000	0.794	0.0000
TGWR vs GWSR	0.760	0.0000	0.651	0.0000	0.781	0.0000

*for explanation of symbols see Table 3

in the modern variety Bakfis, which appeared to be more stable over years than Arina. Another 9 varieties (Federer, Baletka, Samanta, Sakura Brilliant, Dromos, Košútka, Sultan and Hedvika) were included in the same "a" group, however, as seen in Table 2, some of them expressed high instability over years (5 lastly mentioned varieties), and therefore only six varieties (Bakfis, Arina, Federer, Baletka, Samanta and Sakura), significantly differing from the MS and S groups, could be considered as moderately resistant to accumulation of DON. The information about the moderate resistance of varieties Bakfis and Federer, together with the description of other important properties of these varieties, was lately provided by Laml and Pánek (2008, 2010) and about high resistance of Sakura by Horčička *et al.* (2007) and Šíp *et al.* (2010). Concerning the examined set of registered winter wheat varieties, Bagou, Kodex, Cubus and Pitbull were found to be the most susceptible to accumulation of DON mycotoxin.

In Table 3, variety and year ranking (in an ascending order) is available for all examined traits. It is clear from this table that most varieties showed a similar resistance level in all traits, however, it was also possible to detect different rank orders in a variety resistance to e.g. accumulation of DON, tolerance to the disease or symptom expression, which may indicate the presence of different resistance types. The comparison of variety ranking for DON with the ranking obtained on the basis of all examined traits showed the highest differences in the varieties Barryton, Biscay, Seladon, Bohemia, Sultan and Meritto. The varieties Biscay and Seladon that were classified as moderately susceptible to accumulation of DON showed a very high susceptibility in all the other traits measuring the disease incidence. In previous experiments (Chrpová *et al.* 2007), Biscay expressed a very high DON content, as well. The varieties Bohemia and Barryton showed in these experiments a high susceptibility to accumulation of DON, but a relatively lower expression of FHB symptoms and lower grain yield losses. Šíp *et al.* (2010) reported from spray inoculated 10m^2 plots about medium responsiveness of Bohemia from different aspects. A low disease incidence is stated for this variety in systematic surveys of grain samples collected in the Czech Republic by the State Phytosanitary Administration (data not presented here), which indicated a relatively high resistance to initial infection (Type I) in this variety. In the conditions of natural infection (in agricultural practice), the components of its passive resistance (such as greater plant height and loose spike) could be better expressed than in infection tests. A lower disease incidence and a high susceptibility to accumulation of DON were characteristic of the variety Ebi in the previous experiments (Chrpová *et al.* 2007; Šíp *et al.* 2007). On the other hand, Sultan and apparently also Federer (at a higher resistance level) can be classified according to these experiments as varieties producing less DON at a higher symptom expression and greater grain weight reduction. Bai *et al.* (2001) reported that severe visual symptoms may not always be associated with high DON levels, especially in the varieties possessing a moderate Type II resistance.

Analysis of relations between the variety response to FHB and *Rht* genes presence

As shown in Table 3, the presence of the semi-dwarfing gene *Rht-D1b* resulted in a significantly higher mean DON content and a higher performance in all other FHB traits. All the varieties that were the most susceptible to accumulation of DON (Bagou, Kodex, Cubus and Pitbull) carry this gene, which was also detected in the susceptible varieties Iridium, Biscay and Rapsodia, and in the variety Brilliant with a higher but less stable resistance. The effect of *Rht-B1b* on FHB resistance could not be evaluated in this variety set, because only one variety (a moderately resistant variety Baletka) was found to carry this gene. The association of FHB susceptibility with the dwarfing allele *Rht-D1b* was recently documented in many publications (Draeger *et al.* 2007; Holzapfel *et al.* 2008; Srinivasary *et al.* 2008; Voss *et al.* 2008) and this association is also supported by the finding that e.g. UK winter wheat varieties, the great majority of which carry the *Rht-D1b* allele, are generally highly susceptible to FHB (Draeger *et al.* 2007; Gosman *et al.* 2007). In addition, several studies revealed that FHB resistance QTL is often co-localized with QTL for other traits such as plant height, heading date or DON content (Schmolke *et al.* 2005; Gervais *et al.* 2007; Klahr *et al.* 2007) confirming the general complexity of FHB response.

To elucidate the effect of *Rht-D1b* on FHB resistance, the relation of FHB resistance to plant

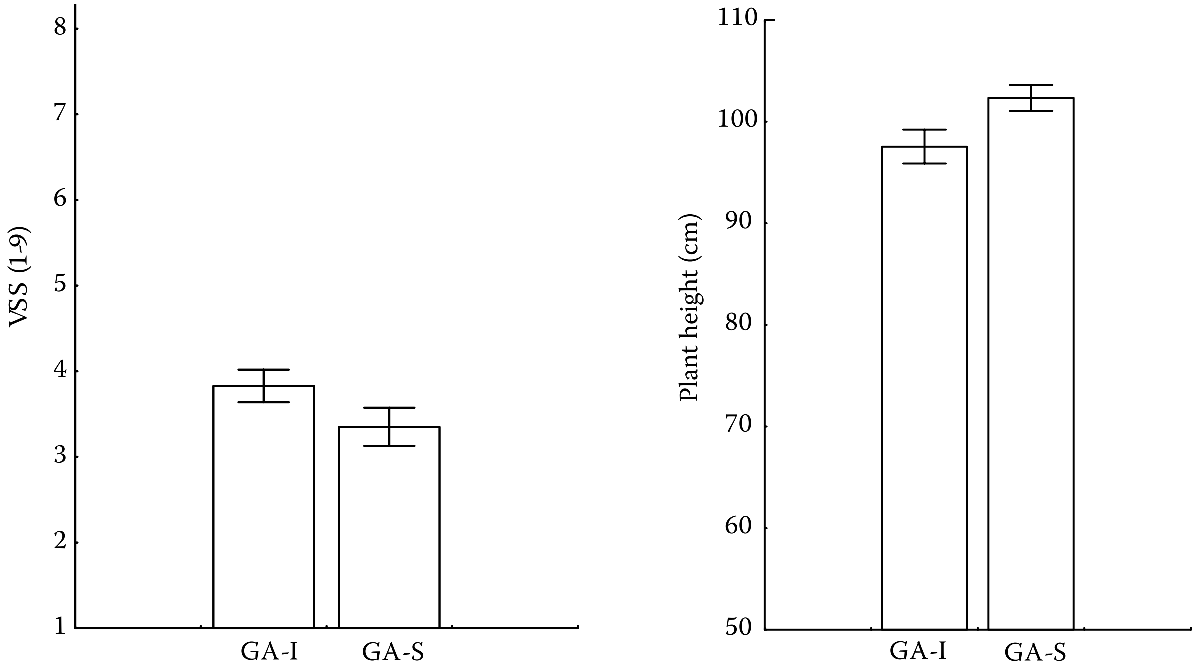

Figure 1. Mean VSS (visual symptom score on 1–9 scale; 1 = low incidence) and plant height of gibbrellic acid (GA) insensitive (GA-I) (*Rht-D1b*) and sensitive (GA-S) wheat lines; the bars are 95% confidence interval

height was studied in a set of 120 adapted advanced breeding lines, of which 64 were GA insensitive (*Rht-D1b*) and 56 GA sensitive genotypes. This set of lines was developed within a so called "gibberellin" programme aimed at the obtaining of high yielding and lodging resistant *Rht-D1b* genotypes (Bobková *et al.* 1988), from which widely grown Czech varieties, such as Šárka, Vlasta, Mladka and Rheia, were derived. The results of this analysis are shown in Figures 1 and 2, and Table 5. In the recommended system of selecting "tall GA insensitive" genotypes (Šíp *et al.* 1988), plant height was at the presence of *Rht-D1b* reduced by appr. 5 cm (4.7%) when compared to the GA sensitive genotypes and s^2 was significantly higher in the group of GA insensitive lines (44.22/22.65; $F = 1.95 > F_{0.05} = 1.56$). The presence of *Rht-D1b* resulted in a significantly higher mean FHB symptom score (Figure 1) of 14% if compared to the mean score of *Rht-D1a* (GA sensitive) group of lines, which showed quite a similar variation (s^2) in FHB scores as the *Rht-D1b* genotypes (0.692/0.578 = 1.19). A shift to a greater susceptibility for GA insensitive (GA-I) genotypes is clearly visible in the histograms representing frequency distributions (Figure 2). VSS corresponding to the greatest frequency (mode) was 4.5 for the GA-I group, while it was 3.5 for the GA-S group, however, the variation was almost the same. Therefore, it is possible to conclude from these results that we can obtain the FHB resistant genotypes also among the *Rht-D1b* genotypes, but with a relatively lower frequency than among the *Rht-D1a* genotypes.

In the breeding programme at Úhřetice, which is generally oriented towards the development of relatively taller varieties, it was particularly important to recognize whether a greater plant height could moderate an adverse effect of the *Rht-D1b* gene. To answer this question, a set of 120 lines was subdivided into three plant height (PH) classes (I: 75–95 cm, II: 96–100 cm and III: 101–111 cm) in which the effect of the presence and absence of the *Rht-D1b* gene was examined. It is clear from Table 5 that, unlike in all lines, in each PH category the differences between GA-insensitive and GA-sensitive groups of lines were not statistically significant, and irrespective of GA response, the VSS means decreased in dependence on plant height from 3.99 (I) to 3.36 (III), which indicates a relatively higher effect of increased plant on reduction of VSS (19%) than in the case of the absence of *Rht-D1b* gene (14%). These findings indicate that the negative effect of *Rht-D1b* on FHB resistance can be excluded, to a high degree, through selecting for taller *Rht-D1b* genotypes. In the most desirable plant height class of 96–100 cm

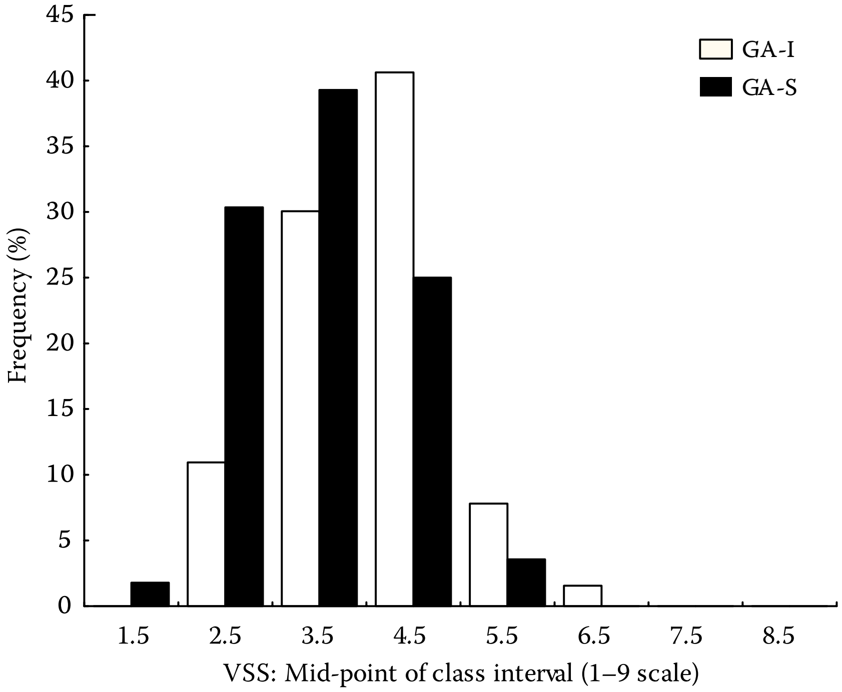

Figure 2. Frequency distribution (%) of 64 VSS (visual symptom score) values obtained in the gibbrellic acid (GA) insensitive (GA-I) group of lines and 56 VSS values obtained in the GA sensitive (GA-S) group of lines

the means of VSS for GA-I and GA-S genotypes are very similar. The respective VSS means 3.78 and 3.60 indicate only a 5% increase in symptom scores, which corresponds with the reduction of PH caused by the presence of *Rht-D1b.* Only in *Rht-D1b* genotypes, that were more variable in PH, the correlation between PH and VSS was significantly negative ($r = -0.311$; $P < 0.01$). In GA sensitive genotypes the respective correlation coefficient was –0.193 ($P > 0.05$).

The obtained results indicate that selection for tall GA insensitive genotypes may compensate, to a high degree, for injury caused by the presence of the Norin 10 semi-dwarfing gene *Rht-D1b*. With a high prevalence of relatively shorter *Rht-D1b* genotypes occurring in the randomly selected populations, Voss *et al.* (2008) reported much higher mean FHB ratings (22–53%) compared to mean FHB ratings of the *Rht-D1a* subpopulations. Selecting taller GA insensitive genotypes is an approach that was found advantageous for the examined Central European conditions (Šíp *et al.* 1988), and it can be feasible also for the high yielding environments of Northwest Europe (Mathews *et al.* 2006), where not using the *Rht-D1b* allele would lead to a considerable yield penalty. The obtained results indicate that it is possible to achieve improved FHB resistance also in genotypes carrying *Rht-D1b*, however, in accordance with the findings of Voss *et al.* (2008)

Table 5. Results of testing the differences between mean VSS (visual symptom score on 1–9 scale; 1 = low incidence) of GA insensitive (*Rht-D1b*) and GA sensitive lines in relation to different plant height categories

	Class interval of plant height (cm)			
	all lines (n = 120)	75–95 (n = 22)	96–100 (n = 41)	101–111 (n = 57)
Total mean	3.59	3.99	3.69	3.36
Mean of GA insensitive lines	3.83	4.21	3.78	3.51
Mean of GA sensitive lines	3.35	3.78	3.60	3.22
t-statistic	3.2750	0.8564	0.8524	1.2444
Probability	0.0014	0.4019	0.3992	0.2895

it becomes increasingly difficult (but not impossible) with large height reductions. To achieve a greater success, the marker selection of *Rht-D1b* genotypes prior to selection for FHB response can be recommended. Nevertheless, it could be more convenient to exploit, in wheat breeding for short FHB resistant genotypes as parents, the short GA sensitive genotypes, such as FHB resistant variety Apache that appeared to possess highly effective height-reducing genes or QTL with less impact on FHB susceptibility (Voss *et al.* 2008).

CONCLUSIONS

The mycotoxin content, grain yield losses and degree of effects on seed quality by infection are undoubtedly the most important characters from a practical point of view. A higher level of resistance to FHB will help farmers to lower the risks accompanied with this devastating disease (reduced grain yield losses and adverse effects on grain quality). The obtained results could help to improve classification of varieties from these aspects, which is desirable for recommendation on their use in practice and breeding.

It was found, that only a few varieties among the examined 30 Czech and foreign winter wheat varieties possess high and stable resistance to FHB, but it is delightful that besides the old reference Swiss variety Arina, all the five varieties that could be included in the first group of moderately resistant varieties originate from the Czech Republic.

Breeding for FHB resistance and low accumulation of mycotoxins would be greatly facilitated if the resistance to mycotoxin accumulation in a genotype could be predicted from indirect, easily determined FHB traits. However, the correlation analyses indicate that due to the highly variable weather conditions in different years and also due to the multi-component nature of FHB resistance, the inspection into the five FHB traits was beneficial for recognizing the specificity of variety response to FHB (resistance to individual components), even though all the examined traits were significantly interrelated. It is also obvious that symptom scores or the traits measuring damage to wheat seed due to infection are more easily to exploit particularly in early hybrid generations, in order to predict the resistance level. However, in accordance with the experiments of Mesterházy *et al.* (2005), this research demonstrated that in the later stages of the breeding process, the determination of DON content, besides other important characters, was evidently necessary so that it could be possible to fully describe the state.

The obtained results also brought evidence of the threat connected with the selection of *Rht-D1b* genotypes. The presence of this gene caused a significant decrease in resistance level, but drastically only in the short *Rht-D1b* genotypes. Since it was found out that the level of FHB resistance among these genotypes is significantly related to plant height, it appeared desirable to minimize the risk through selecting taller GA insensitive (*Rht-D1b*) genotypes. However, in the breeding programmes focused on the development of short genotypes it can be recommended to avoid introducing *Rht-D1b* and exploit “an additional shortness” (characterized by sensitivity to gibberellic acid) with a lower impact on FHB susceptibility.

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