Resistance to Fusarium Head Blight in Spring Barley

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Abstract: Fusarium head blight (FHB) is a fungal disease causing substantial yield and quality losses in barley. Genetic variation in deoxynivalenol (DON) content and and important yield traits in response to FHB were studied in 44 spring barley cultivars for two years following artificial inoculation with *Fusarium culmorum* under field conditions. The analysis of variance revealed that the largest effect on DON content and simultaneously on the reduction of thousand grain weight and grain weight per spike were due to the environmental conditions of the year, while the visual disease symptoms depended on the cultivars to a larger extent. All these traits were significantly interrelated. The most resistant cultivars Murasski mochi, Nordic, Krasnodarskij 35, Krasnodarskij 95, Nordus, and Usurijskij 8, together with the resistant check Chevron, showed the lowest DON content, the lowest expression of disease symptoms and the lowest reduction of TGW and GWS. However, most spring barley cultivars registered in the Czech Republic in recent years expressed susceptibility or medium resistance and were considerably affected by the disease. This increases the importance of breeding barley for resistance to FHB.

Keywords: cultivar resistance; *Fusarium culmorum*; head blight; *Hordeum vulgare*; mycotoxin DON

Fusarium head blight (FHB) poses a potential threat to small grain cereals, especially wheat and barley. The major potential risk for both humans and animals caused by the infection is the production of mycotoxins of which deoxynivalenol (DON) and its derivatives appear to be the most important. The deployment of barley cultivars with genetic resistance is the most cost effective and environmentally sound way of controlling FHB. Special attention in breeding for resistance is paid to the detection of FHB resistance sources and their exploitation. A six-rowed, nonmalting barley Chevron, CI 4192 (a landrace from China) and Svanhals (a landrace from Sweden) exhibited low levels of FHB (Steffenson 1999; Šíp *et al*. 2004) and belong to the most widely used FHB resistant cultivars. In the experiments of Buerstmayr *et al*. (2004) the lines with the lowest FHB severity were CIho 4196 and PI 566203. However, the most FHB resistant barley cultivars exhibit poor agronomical characteristics, have poor malting quality and are two-rowed (Zhu *et al.* 1999). Therefore, large effort has been developed to derive more agronomically valuable materials (Rasmusson *et al.* 1999; Steffenson & Smith 2006), as lately reviewed by Kosová *et al*. (2009).

In barley, the assessment of a disease according to symptoms and other measurements is even more complicated than in wheat. Insignificant correlations were found out by Nesvadba *et al.* (2006) between ear infection percentage and DON content and also between the percentage of fusaria in a laboratory test and DON content. By contrast, other authors (Urrea *et al*. 2002; Buerstmayr

Table 1. Mean values of two years, and cultivar and year ranking for DON content, visual symptom score (VSS), reduction of thousand grain weight (TGW-R) and reduction of grain weight per spike (GWS-R)

Cultivar/year	Country of origin	Year of registration/ accession No.	Quality*	DON content				VSS		TGW-R		GWS-R		Classification***
				Ruzyně		6 experiments**				Ruzyně				
				(mg/kg)	rank	(mg/kg)	rank	(1–9)	rank	%	rank	%	rank	
Murasski mochi	US	03C0602047		5.0^{a}	2	5.1	2	3.50	3	0.7	2	6.4	2	R-MR
Nordic	US	03C0601593		4.4^{a}	1	5.8	3	3.32	2	0.8	3	14.0	6	R-MR
Krasnodarskij 35	SU	03C0600328		9.4^{abc}	4	5.9	4	4.35	8	8.0	5	4.0	1	R-MR
Krasnodarskij 95	SU	03C0601541		8.3^{ab}	3	4.8	1	3.92	5	9.3	6	15.8	9	R-MR
Chevron	CH	03C0601152		9.8^{abcd}	5	9.5	6	3.10	1	0.1	1	26.7	15	R-MR
Nordus	DE	1998	M	12.4^{abcd}	6	8.0	5	4.00	6	8.0	4	14.7	7	R-MR
Ussurijskij 8	SU	03C0600255		16.2^{abcd}	7	9.7	7	4.55	9	10.1	7	11.1	4	R-MR
Maris Mink	UK	03C0601412		23.4^{abcdef}	9	11.6	9	3.82	4	10.1	8	11.6	5	MR
Madeira	DE	1999	M	20.0^{absde}	8	11.3	8	5.23	17	14.0	10	10.8	3	MR
Primus	SW	1995	F	25.9^{abcdef}	12	13.2	10	4.65	10	14.3	11	15.7	8	MR
Morrison	CA	03C0602303		29.1^{abcdef}	15	16.6	12	4.75	12	13.8	9	18.3	10	MR
Nepolegajuscij	SU	03C0600387		32.3^{abcdefgh}	18	16.4	11	4.32	7	16.9	12	24.9	11	M
Prosa	AT	1998	F	24.3^{abcdef}	10	19.9	19	4.65	11	23.2	14	26.1	13	M
Tocada	DE	2006	F	25.0^{abcdef}	11	16.7	13	5.15	15	24.5	15	27.6	18	M
Prudencia	US	03C0602728		43.8^{defghijkl}	32	18.4	14	4.98	13	22.5	13	25.9	12	M
Madonna	DE	1998	M	27.3^{abcdef}	14	19.2	18	5.24	18	25.6	17	28.9	20	M
Saloon	UK	2002	M	26.2^{abcdef}	13	18.6	17	5.83	23	25.9	18	29.7	21	M
Calgary	FR	2003	M	30.7^{abcdefg}	16	25.8	29	5.17	16	27.7	24	28.4	19	M
Orthega	DE	1999	F	31.2^{abcdefg}	17	18.4	15	5.07	14	29.9	30	35.1	32	M
Pribina	SK	2005	F	34.0^{bcdefgh}	20	21.9	21	5.85	25	27.6	22	32.1	27	M
Philadelphia	DE	2002	M	34.6^{bcdefgh}	21	21.0	20	6.15	34	28.1	25	29.8	22	M
Sebastian	DK	2005	M	37.1^{cdefgh}	22	28.4	35	6.17	36	26.6	20	26.2	14	M
Heris	CZ	1998	F	38.3^{defghi}	24	26.5	32	5.40	19	28.3	28	31.2	24	M
Bojos	CZ	2005	M	39.3^{defghij}	25	22.8	22	5.68	22	28.1	27	35.8	35	M
Xanadu	DE	2006	M	41.5^{defghijk}	29	28.7	37	5.85	26	24.7	16	32.2	29	M

Table 1 to be continued

Cultivar/year	Country of origin	Year of registration/ accession No.	Quality*	DON content				VSS		TGW-R		GWS-R		Classification***
				Ruzyně		6 experiments**				Ruzyně				
				(mg/kg)	rank	(mg/kg)	rank	(1–9)	rank	%	rank	%	rank	
Ebson	CZ	2002	M	37.6^{defghi}	23	23.5	25	6.27	40	26.9	21	33.4	30	M
Jersey	NL	2000	M	$40.0^{defghij}$	26	23.3	23	6.02	30	31.5	35	32.0	26	M
Scarlett	DE	1997	M	$50.1^{fghijkl}$	37	26.5	30	6.08	31	28.5	29	27.3	16	MS
Cristalia	FR	03C0602736		$33.3^{abcdefgh}$	19	18.6	16	6.27	39	30.4	32	41.2	42	MS
Tolar	CZ	1997	M	$43.7^{defghijk}$	31	25.8	28	6.10	33	30.3	31	32.1	28	MS
Faustina	DE	2003	M	59.8^{hijkl}	40	36.5	41	6.17	35	26.1	19	27.6	17	MS
Sabel	UK	2001	M	$46.4^{efghijkl}$	35	28.4	36	6.10	32	27.7	23	34.0	31	MS
Kompakt	SK	1995	M	$41.5^{defghijk}$	28	27.5	33	5.67	21	35.9	42	39.2	39	MS
Nitran	SK	2004	M	64.2^{ijkl}	41	39.3	42	5.85	24	30.6	33	31.3	25	MS
Diplom	DE	2002	M	$42.2^{defghijk}$	30	25.4	26	5.93	27	36.9	43	40.3	40	MS
Biatlon	UK	2003	M	$51.3^{fghijkl}$	38	25.6	27	5.98	28	32.4	36	37.9	37	MS
Malz	CZ	2002	M	$48.6^{fghijkl}$	36	29.8	38	6.18	37	28.1	26	35.4	33	MS
Respekt	CZ	2003	M	$41.0^{defghijk}$	27	26.5	31	5.98	29	38.1	44	43.4	43	MS
Prestige	FR	2002	M	$45.1^{efghijkl}$	33	23.4	24	6.23	38	33.9	39	41.1	41	MS
Radegast	CZ	2005	M	57.1^{ghijkl}	39	31.3	39	5.65	20	34.0	40	38.9	38	S
Pedant	CZ	2003	M	73.1^{l}	44	57.1	44	6.50	43	33.6	37	30.3	23	S
Class	UK	2005	M	$46.3^{efghijkl}$	34	27.9	34	6.27	41	34.8	41	43.8	44	S
Braemar	UK	2006	M	65.7^{jkl}	42	42.1	43	6.42	42	30.7	34	35.6	34	S
Bolina	DE	2004	F	68.4^{kl}	43	31.6	40	6.67	44	33.6	38	36.9	36	S
2008				15.1^{a}		15.5	a	4.63		12.7	a	18.1	a	
2009				57.0^{b}		27.5	b	6.14		34.9	b	38.5	b	
Total average				36.0		22.2		5.39		23.9		28.4		

*M – malting quality; F – feeding quality; **means of experiments performed in Ruzyně, Stupice and Kroměříž in 2008 and 2009
***R – resistance; 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 ($P < 0.05$)

et al. 2004; Špunarová *et al.* 2005) reported significant correlations between FHB severity and DON accumulation.

The objective of this paper was to present results of artificially inoculated field experiments in which 44 spring barley cultivars were examined over two years for the accumulation of the mycotoxin DON in grain and the important FHB severity traits to investigate genetic variation in these traits and to identify possible new sources for resistance breeding.

MATERIAL AND METHODS

Material for this study comprised 33 spring barley cultivars that were registered in the Czech Republic during 1995–2006, together with 11 potential resistance sources available at http://genbank.vurv.cz/genetic/resources/asp2/default_a.htm (Accession number is given in Table 1). A six-rowed cultivar Chevron (landrace from Switzerland) was used as a resistant check. The detailed study is based on experiments performed for two years (2008, 2009) at the Prague-Ruzyně location. The cultivars were planted in hill plots in three replications. Artificial inoculation of spikes with highly pathogenic isolate B of *Fusarium culmorum* (Šíp *et al.* 2002; Chrpová *et al.* 2007) was performed at the phase of full flowering. The spraying of the inoculum (conidial suspension 0.8×10^7/ml) onto bunches of 10 flowering spikes randomly selected within hill plots was applied on one date. Inoculated spikes were then kept in polythene bags for 24 hours. To minimize year/location effects on results, it appeared necessary in these conditions to support the disease development (when needed) by irrigation of plots. Head blight symptoms were evaluated on three dates (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 the 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 mildly infected scabby grains. Tolerance to the infection was expressed as percent reduction (R) in the traits of thousand grain weight (TGW) and grain weight per spike (GWS) compared to the non-inoculated control (C). Seeds from infected spikes were analysed for DON (deoxynivalenol) content determined by ELISA with the use of RIDASCREEN® FAST DON kits from R-Biopharm GmbH, Darmstadt, Germany. The detailed description of DON content determination was provided by Chrpová *et al.* 2007. The UNISTAT 5.0 package (UNISTAT Ltd., London, UK) was used for statistical analyses and Statistica package (StatSoft, Inc., Tulsa, Oklahoma, USA) for graphics. To get broader evidence, data obtained with this material set in the locations Stupice and Kroměříž were also used. However, these data could not be included in detailed examination due to slightly modified methodology.

RESULTS AND DISCUSSION

Analyses of variance of data obtained in the Ruzyně experiments showed a highly significant effect of cultivar on all examined traits (Table 2); also the interaction between cultivar and year sig-

Table 2. *F* values from analyses of variance and % variation (%var) for DON content, visual symptom scores (VSS) and reductions of thousand grain weight (TGW-R) and grain weight per spike (GWS-R) in two-year experiments at the Ruzyně location

Source of variation	df	DON content (mg/kg)		VSS (1–9)		TGW-R (%)		GWS-R (%)	
		F value	%var	*F* value	%var	*F* value	%var	*F* value	%var
Cultivar	43	11.4**	29.1	9.8**	47.3	7.1**	33.5	3.2**	24.3
Year	1	812.4**	48.1	237.2**	26.6	330.4**	36.4	138.2**	25.1
Cultivar × year	43	5.3**	13.6	1.5*	7.1	2.7**	12.7	2.5**	19.8
Explained variation	87		90.9		80.4		80.9		68.5
Error	176		9.1		19.6		19.1		31.5

df – degree of freedom; $^{**}P < 0.01$; $^{*}P < 0.05$

Table 3. Characterization of resistance sources in comparison with commercially grown cultivars (means of 2008 and 2009)

Source of resistance	FD	SM	TGW (g)	GWS (g)
Murasski mochi	8	6	27.1	1.03
Nordic	9	6	34.9	1.96
Krasnodarskij 35	16	2	43.6	1.09
Krasnodarskij 95	15	2	46.4	1.29
Chevron	20	6	32.2	1.73
Nordus	15	2	43.2	1.86
Ussurijskij 8	15	2	48.3	1.18
Mean of commercially grown cultivars	14.2		49.5	1.39

FD – flowering date in days after 1 June; SM – spike morphology; 2-rowed; 6-rowed; TGW – thousand grain weight; GWS – grain weight per spike

nificantly affected particularly the content of DON and the examined yield characters, which implies a necessity of multi-year testing. The interaction with year was relatively lowest for the evaluation of disease symptoms. Explained variation was high for DON content and low for the reduction of grain weight per spike (GWS-R).

In this study, great attention was paid to DON content which can be taken as the character of crucial importance. As shown in Table 1, high resistance (R-MR) to DON accumulation was detected in the cultivars Murasski mochi, Nordic, Krasnodarskij 35, Krasnodarskij 95, Chevron, Nordus, and Ussurijskij 8. High resistance in the cultivars Nepolegajuscij, Nordic, Murasski mochi, Ussurijskij 8 and Morrison was detected by McCallum *et al.* (2004). Basic characteristics of the detected resistance sources are given in Table 3. When compared with the response of the moderately resistant reference cultivar Chevron, a great majority of the included cultivars could be considered as medium responsive or quite susceptible to DON accumulation. It is encouraging that we could find a resistant or moderately resistant response to FHB in some adapted (registered) older varieties, particularly in the German cultivars Nordus and Madeira or Swedish Primus. Similar genotypic classification like for DON was obtained in this set of cultivars for visual symptom score (VSS) and often also for TGW-R and GWS-R. A highly significant positive correlation was detected between VSS and DON content ($r = 0.76$; $P < 0.01$), as well as between DON content and GWS-R ($r = 0.62$; $P < 0.01$) and between DON content and TGW-R ($r = 0.71$; $P < 0.01$). However, it is clear from Table 2 that low DON content was not always connected with low yield reduction. Especially Chevron, producing low amounts of DON, showed a relatively high yield reduction. Obviously, observations of yield traits or visual assessment of the disease cannot replace direct determination of mycotoxin content in barley (Šíp *et al.* 2004). These results are supported by the findings of Jones and Mirocha (1999), who found out that the DON concentration in barley could not be effectively estimated by yield trials. An example is the cultivar Cristalia with relatively lower DON content and high reduction of GWS. On the contrary, Faustina showed susceptibility to DON accumulation and medium reductions of yield components. The susceptible cultivars Radegast, Pedant, Class, Braemar and Bolina expressed above-average performance in all traits measuring the FHB severity.

These results document the possibility of evaluating barley resistance to FHB in field conditions on the basis of DON content determination, visual scoring and with respect to yield reduction. When the mist irrigation of plots was used, it was possible to detect large differences in the cultivar response and select cultivars that could be exploited for a desirable improvement of FHB resistance in barley.

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References

Buerstmayr H., Legzdina L., Steiner B., Lemmens M. (2004): Variation for resistance to FHB in spring barley. In: Proc. 9th Int. Barley Genet. Symposium Brno, 308–315.

Chrpová J., Šíp V., Matějová E., Sýkorová S. (2007): Resistance of winter wheat varieties registered in the Czech Republic to mycotoxin accumulation in grain following inoculation with *Fusarium culmorum*. Czech Journal of Genetics and Plant Breeding, **43**: 44–52.

Jones R.K., Mirocha C.J. (1999): Quality parameters in small grains from Minnesota affected by Fusarium head blight. Plant Disease, **83**: 506–511.

Kosová K., Chrpová J., Šíp V. (2009): Cereal resistance to Fusarium head blight and possibilities of its improvement through breeding. Czech Journal of Genetics and Plant Breeding, **45**: 87–105.

McCallum B.D. Tekauz A. Gilbert J. (2004): Reaction of a diverse collection of barley lines to Fusarium head blight. Plant Disease, **88**: 167–174.

Nesvadba Z., Vyhnánek T., Ježíšková I., Tvarůžek L., Špunarová M., Špunar J. (2006): Evaluation of spring barley genotypes with different susceptibility to Fusarium head blight using molecular markers. Plant, Soil and Environment, **52**: 485–491.

Rasmusson D.C., Wilcoxson R.D., Dill-Macky R., Schiefelbein E.L., Wiersma J.V. (1999): Registration of MNBrite barley. Crop Science, **39**: 290.

Steffenson B.J. (1999): Combating *Fusarium* head blight of barley: an emerging threat to malting barley quality throughout the world. In: EBC Congress, 531–538.

Steffenson B.J., Smith K.P. (2006): Breeding barley for multiple disease resistance in the Upper Midwest region of the USA. Czech Journal of Genetics and Plant Breeding, **42**: 79–85.

Šíp V., Sýkorová S., Stuchlíková E., Chrpová J. (2002): The effect of infection with *Fusarium culmorum* L. on deoxynivalenol content in grain of selected winter wheat varieties. Journal of Applied Genetics, **43A**: 319–332.

Šíp V., Tvarůžek L., Chrpová J., Sýkorová S., Leišová L., Kučera L., Ovesná J. (2004): Effect of Fusarium head blight on mycotoxin content in grain of spring barley cultivars. Czech Journal of Genetics and Plant Breeding, **40**: 91–101.

Špunarová M., Ovesná J., Tvarůžek L., Kučera L., Špunar J., Hollerová I. (2005): The use of molecular markers for characterisation of spring barley for breeding to Fusarium head blight. Plant Soil and Environment, **51**: 483–490.

Urrea C.A., Horsley R.D., Steffenson B.J., Schwarz P.B. (2002): Heritability of Fusarium head blight resistance and deoxynivalenol accumulation from barley accession CIho 4196. Crop Science, **42**: 1404–1408.

Zhu H., Gilchrist L., Hayes P., Kleinhofs A., Kudrna D., Liu Z., Prom L., Steffenson B., Toojinda T., Vivar H. (1999): Does function follow form? Principal QTLs for *Fusarium* head blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a doubled-haploid population of barley. Theoretical and Applied Genetics, **99**: 1221–1232.

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