

## Evaluation of Resistance to Fusarium Head Blight in Spring Wheat Genotypes Belonging to Various *Triticum* Species

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### Abstract

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Response of 35 spring wheat varieties and lines (of four *Triticum* species) to spray inoculation with *Fusarium culmorum* was evaluated in field experiments over three years (2010–2012). Data on mycotoxin deoxynivalenol (DON) content were complemented by symptom scores and determination of the percentage of Fusarium damaged kernels and percent reduction of thousand grain weight and of grain weight per spike due to infection. Resistance to Fusarium head blight (FHB) determined on the basis of the five mentioned traits was variable in all the examined genotype groups and very high only in the non-adapted check variety Sumai 3. The common wheat landrace Červená perla, four *T. dicoccum* genotypes (May Emmer, Weisser Sommer, Tábor, and Rudico), *T. spelta* (Ruzyně), and the commercially grown bread wheat variety Vánek can be considered as moderately resistant to FHB. DON accumulation was significantly higher in the modern common wheat varieties than in the other *Triticum* species and common wheat landraces. The latter nonetheless showed similar average reductions in grain weight per spike due to infection as did current spring wheat varieties. It is particularly important that DON content was found to be low and least variable not only in Sumai 3 but also in some *T. dicoccum* (Rudico and Tábor) and *T. spelta* (Ruzyně) genotypes. It was documented that FHB-resistant emmer and spelt wheat materials have some outstanding grain-quality parameters (e.g. very high protein content) and can be progressively utilized particularly in breeding wheat for alternative use and growing in organic farming systems. It is important to make substantial progress towards developing resistance in common spring wheat, because most current varieties other than Vánek and Trappe were found to be moderately susceptible or susceptible to FHB.

**Keywords:** common wheat; DON content; einkorn; emmer; *Fusarium culmorum*; head blight resistance; spelt

Fusarium head blight (FHB) poses a serious threat to small grain cereals, especially wheat and barley. In addition to causing losses in yield and grain quality, FHB presents a major potential risk to both humans and animals because the infection produces mycotoxins. These are either oestrogenic zearalenone (ZEA or ZON) or non-macrocyclic trichothecenes, of which deoxynivalenol (DON)

appears to be the most important. PLACINTA *et al.* (1999) concluded that, on a global scale, cereal grains and animal feed may be subjected to multiple contaminations with trichothecenes, zearalenone, and fumonisins, the major mycotoxins of *Fusarium* fungi.

While the best way to prevent or reduce Fusarium infection is to grow cultivars with high levels of

disease resistance, it is also evident that a high degree of FHB resistance has not yet been obtained in commercially grown European wheat varieties.

Resistance to FHB is a quantitative trait that is governed by polygenes, and quantitative trait loci have been detected on all wheat chromosomes (BUERSTMAYR *et al.* 2009; LIU *et al.* 2009; LÖFFLER *et al.* 2009). Great genetic variation for FHB resistance is available in the wheat gene pool, but often the regionally best-adapted and most highly productive varieties are susceptible to FHB (BUERSTMAYR *et al.* 2009).

Evolution of crop plants from their wild progenitors through domestication involved massive erosion of the original genetic resources, especially as a result of modern agriculture, thus leaving current genotypes vulnerable and susceptible to abiotic and biotic environmental stresses (XIE & NEVO 2008). Ancient wheat (the original genetic resources) offer the best hope for crop improvement, because they may carry adaptive complexes to abiotic and biotic stresses (FELDMAN & SEARS 1981; NEVO 2004). Knowledge as to the response of ancient wheat to *Fusarium* spp. infection is especially important both for breeding resistance into cereals and the application of suitable plant protection practices in conventional and organic farming systems. *Triticum dicoccum* (Schrank) Schuebl, *Triticum monococcum* L., and *Triticum spelta* L., also known as emmer, einkorn, and spelt, respectively, were among the earliest Triticeae domesticated by man. Current trends towards low-impact and sustainable agriculture, as well as an increase in the utilization of organic and so-called “functional food” products, suggest that these ancient wheat species still can play a certain role in human nutrition (BRANDOLINI *et al.* 2008). Information on the response of hulled wheat to *Fusarium* infection is still scarce. WIWART *et al.* (2004) reported that the response of spelt to spike infection by *F. culmorum* is slightly stronger than that of common wheat, which may be related to the fact that husked spelt grains create more favourable conditions for pathogen growth. Spelt husks in particular were found to contain considerable concentrations of trichothecenes (WIWART *et al.* 2009). Although susceptibility to FHB appears very common in wild emmer wheat, a few accessions can be utilized as potential sources of FHB resistance. BUERSTMAYR *et al.* (2003) tested 150 accessions of wild emmer coming mainly from different habitats in Israel for resistance to fungal

spread caused by *Fusarium graminearum*. While most lines were highly susceptible, a few accessions exhibited moderate levels of resistance. XIE and NEVO (2008) showed that wild emmer (*Triticum dicoccoides*), the progenitor of cultivated wheat, harbours rich genetic resources in many traits useful for wheat improvement, including resistance to FHB. KONVALINA *et al.* (2011) found a rather low contamination of grain with the mycotoxin DON in the examined hulled wheat varieties (einkorn, emmer wheat, spelt wheat) grown in organic farming systems, which may be a consequence of eliminating hulls before the processing of grains.

The present study aimed to (1) evaluate resistance to FHB infection and contamination of grain with DON in selected genetically and evolutionarily very distant spring wheat germplasm that may be variously utilized in human and animal nutrition and wheat-growing systems, and (2) find new resistance sources usable in breeding processes.

## MATERIAL AND METHODS

**Plant materials.** The examined spring wheat accessions (Table 1) came from the Gene Bank of the Crop Research Institute in Prague-Ruzyně. Genetic resources of einkorn (*Triticum monococcum* L.), emmer wheat (*Triticum dicoccum* [Schrank] Schuebl), spelt wheat (*Triticum spelta* L.), and landraces of bread wheat (intermediate form – LI; *Triticum aestivum* L.) were chosen after evaluating an entire set containing 173 accessions during 2008 using both standard criteria as well as certain criteria valuable for growing under organic farming conditions (turf shape in early stage of development, length of upper internodium, resistance to lodging, special grain quality). The experimental set also included 11 common wheat varieties (CV; *Triticum aestivum* L.) commercially used in the Czech Republic. The non-adapted Chinese common wheat variety Sumai 3, which is highly resistant to FHB, was used as a check. Characteristics of the original genetic resources belonging to different *Triticum* species are available at [http://genbank.vurv.cz/genetic/resources/asp2/default\\_a.htm](http://genbank.vurv.cz/genetic/resources/asp2/default_a.htm) and characteristics of the registered bread wheat varieties are on the website of the Central Institute for Supervising and Testing in Agriculture (<http://www.ukzuz.cz/ChangeLang.aspx?Lang=EN>).

**Field experiments, disease evaluation, and examined characteristics.** The field tests were

Table 1. Characteristics of wheat varieties and variety and year mean values for deoxynivalenol (DON) content, visual symptom scores (VSS) on 1–9 scale (1 – without symptoms), % of Fusarium damaged kernels (FDK), reduction of thousand grain weight (TGW-R) and reduction of grain weight per spike (GWS-R)

Variety or accession name/year	Botanical classification	Plant height (cm)	Year of registration/ accession No.	DON (mg/kg)	VSS (1–9)	FDK	TGW-R		Rank (5 traits)
							%	GWS-R	
Sumai 3	<i>T. aestivum</i> – check	105		5.2	1.9	14.2	11.1	17.6	1.4
Červená perla	<i>T. aestivum</i> – LI	124	01C0100124	8.1	3.0	15.3	12.5	25.8	4.2
May-Emmer	<i>T. dicoccum</i>	138	01C0203990	8.2	2.5	16.5	7.3	34.1	4.6
Weisser Sommer	<i>T. dicoccum</i>	137	01C0203993	11.1	2.6	18.4	12.0	28.6	5.4
<i>T. dicoccum</i> (Tábor)	<i>T. dicoccum</i>	127	01C0204318	7.3	3.4	18.4	10.4	27.8	5.6
<i>T. spelta</i> (Ruzyně)	<i>T. spelta</i>	129	01C0201257	6.5	2.5	19.3	14.9	34.5	6.0
Vánek	<i>T. aestivum</i> – CV	98	2004	14.5	3.1	24.6	14.9	25.7	8.0
Rudico	<i>T. dicoccum</i>	126	01C0200948	7.1	3.5	22.7	16.1	32.2	8.8
<i>T. monococcum</i> (Georgia)	<i>T. monococcum</i>	123	01C0204038	23.8	2.5	18.3	13.3	37.5	10.4
Schwedisches Einkorn	<i>T. monococcum</i>	102	01C0204053	13.5	3.4	35.0	15.4	28.8	10.8
<i>T. spelta</i> (Tábor 2)	<i>T. spelta</i>	122	01C0204323	9.1	3.6	22.5	18.6	33.9	10.8
Kaštická přesívka 203	<i>T. aestivum</i> – LI	113	01C0200031	14.0	3.3	26.8	16.5	35.6	11.6
<i>T. monococcum</i> No.8910	<i>T. monococcum</i>	117	01C0204542	14.5	3.4	26.3	22.5	41.9	14.0
<i>T. spelta</i> (Tábor 1)	<i>T. spelta</i>	121	01C0204322	11.4	3.6	23.5	22.7	44.1	14.6
Rosamova česká červená přesívka	<i>T. aestivum</i> – LI	124	01C0200051	13.8	4.3	27.2	20.9	34.6	15.4
Trappe	<i>T. aestivum</i> – CV	95	2007	22.7	3.4	28.4	25.7	34.3	16.2
<i>T. dicoccum</i> (Tapioszele)	<i>T. dicoccum</i>	140	01C0201280	16.5	3.3	29.0	24.1	45.0	16.4
<i>T. spelta</i> (Kew)	<i>T. spelta</i>	128	01C0200984	19.6	3.4	34.6	24.6	42.3	17.5
Postoloprtská přesívka 6	<i>T. aestivum</i> – LI	118	01C0200043	22.9	3.6	35.4	25.0	41.9	20.2
<i>T. dicoccum</i> (Palestine)	<i>T. dicoccum</i>	128	01C0201261	16.6	3.9	40.5	28.4	39.2	21.0
KWS Scirocco	<i>T. aestivum</i> – CV	97	2011	22.7	3.7	34.8	25.1	49.3	21.6
Izzy	<i>T. aestivum</i> – CV	100	2011	29.0	4.4	40.8	22.9	37.7	23.0
Dafne	<i>T. aestivum</i> – CV	98	2011	31.0	4.0	38.5	30.9	41.3	24.2
<i>T. spelta</i> (VIR St.Petersburg)	<i>T. spelta</i>	121	01C0204865	21.2	4.2	35.8	34.7	47.5	25.0
KWS Chamsin	<i>T. aestivum</i> – CV	92	2012	34.4	4.2	38.7	28.6	46.5	25.6
<i>T. monococcum</i> (Albania)	<i>T. monococcum</i>	119	01C0204044	27.2	3.6	50.1	30.4	51.0	26.6
Špaldá bílá jarní	<i>T. spelta</i>	124	01C0200982	25.7	4.2	38.3	37.4	53.0	27.6
<i>T. spelta</i> No 8930	<i>T. spelta</i>	127	01C0204506	22.9	4.7	39.2	33.5	50.7	28.2

Table 1 to be continued

Variety or accession name/year	Botanical classification	Plant height (cm)	Year of registration/ accession No.	DON (mg/kg)	VSS (1–9)	FDK	%		Rank (5 traits)
							TGW-R	GWS-R	
Seance	<i>T. aestivum</i> – CV	88	2008	41.4	4.5	47.6	30.4	43.6	28.4
Astrid	<i>T. aestivum</i> – CV	96	2012	38.9	4.6	42.4	32.2	46.8	29.0
Septima	<i>T. aestivum</i> – CV	82	2008	40.9	4.9	50.3	37.2	46.9	31.6
Tercie	<i>T. aestivum</i> – CV	81	2008	45.8	4.6	54.9	38.4	49.7	32.8
SW Kadrij	<i>T. aestivum</i> – CV	95	2006	51.3	4.6	44.0	40.1	54.1	33.2
<i>T. dicoccon</i> (Dagestan ASSR)	<i>T. dicoccam</i>	114	01C0204016	31.0	5.1	53.3	44.6	60.0	33.6
<i>T. dicoccon</i> (Brno)	<i>T. dicoccam</i>	112	01C0204022	37.9	5.6	61.1	41.2	65.6	34.8
2012				24.4	3.6	32.3	23.5	36.9	1.6
2010				19.4	4.0	29.6	24.4	40.6	1.8
2011				22.3	3.8	39.7	27.2	45.4	2.6
Total average				22.4	3.8	33.9	25.1	41.5	

LI – landraces – intermediate form; CV – commercial varieties

conducted during 2010–2012 at the Crop Research Institute in Prague-Ruzyně. Wheat varieties and lines were planted in hill plots in three replications. Spikes were inoculated artificially with highly pathogenic isolate B of *F. culmorum* (ŠÍP *et al.* 2002) at mid-flowering (GS 64: anthesis half-way) (ZADOKS *et al.* 1974). Inoculum (conidial suspension  $0.8 \times 10^7$ /ml) was sprayed one time onto bunches of 10 flowering spikes randomly selected within the hill plots. Inoculated spikes were then kept covered for 24 h using polythene bags. To minimize year and location effects on results, it appeared necessary in these conditions to support disease development (as needed) by irrigating the plots. In general, there were applied inoculation techniques described (developed and described) by MESTERHÁZY (1978, 1997).

Head blight symptoms were evaluated at three time points (usually 14, 21, and 28 days after inoculation) on a scale of 1 to 9, 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 showing FHB symptoms. Visual symptom score (VSS) is 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 flow in order not to lose light infected, scabby grains. Fusarium damaged (scabby) kernels (FDK) were calculated as a percentage of total seed number. Tolerance to the infection was expressed as percentage reduction (R) versus the non-inoculated control (C) according to the traits thousand grain weight (TGW) and grain weight per spike (GWS). Seeds from infected spikes were analysed for DON (deoxynivalenol) content, which was determined by ELISA using RIDASCREEN® FAST DON kits from R-Biopharm GmbH, Darmstadt, Germany (CHRPOVÁ *et al.* 2007).

Complementary data on important plant growth type and grain-quality characteristics in the selected promising materials were obtained from the 2010–2012 experiments. Crude grain protein content was evaluated by the Kjeldahl method (ISO 1871). Also performed were the Zeleny sedimentation (ISO 5529) and Hagberg falling number (falling number) (ISO 3093) tests.

The UNISTAT 5.0 package (UNISTAT, London, UK) was used for statistical analyses and the STATISTICA package (StatSoft, Tulsa, OK, USA) for graphics.

## RESULTS AND DISCUSSION

Response to artificial infection of ears with isolate B of *Fusarium culmorum* was evaluated in 35 spring wheat varieties and lines during 2010–2012. In addition to mycotoxin DON content, also examined as indicators of disease severity were symptom expression (VSS), percentage of Fusarium damaged kernels (FDK), and reductions of thousand grain weight (TGW-R) and grain weight per spike (GWS-R) due to infection (Table 1). All these traits were significantly and positively interrelated ( $r = 0.72–0.90$ ;  $P < 0.001$ ), and the development of Fusarium head blight was similar in all three years of testing. DON content correlated more closely with FDK ( $r = 0.84$ ) and TGW-R ( $r = 0.82$ ) than with GWS-R ( $r = 0.72$ ) and VSS ( $r = 0.75$ ). Tested materials are arranged in Table 1 according to average rank for the five traits and it can be implied from this table that genotype reactions to different traits were not similar (e.g. *T. monococcum*. Georgia yielded almost three times more DON than May Emmer at the same level of visual symptoms). It appeared again that not only FHB visual symptoms, but DON, FDK and yield loss traits should be examined in order to demonstrate better the complex nature of FHB resistance (MESTERHÁZY *et al.* 1999). The check variety Sumai 3 demonstrated the highest resistance as determined on

the basis of all examined traits (average ranking 1.4), followed by Červená Perla (*T. aestivum* – LI), May Emmer (*T. dicoccum*), Weisser Sommer (*T. dicoccum*), *T. dicoccum* (Tábor), *T. spelta* (Ruzyně), Vánek (*T. aestivum* – CV), and Rudico (*T. dicoccum*) (average rankings 4.2–8.8). Above average or medium performance in a majority of traits was characteristic of 10 varieties and lines with rankings 10.4–17.5 (ancient wheat materials and contemporary common wheat variety Trappe). The other tested materials (rankings 20.2–34.8) can be considered as moderately susceptible or susceptible to FHB.

DON content was given particular attention in this study, as it can be reckoned the crucially most important characteristic. The differences in DON accumulation within the evaluated *Triticum* species can be seen in Figure 1. The highly resistant check variety Sumai 3 showed the lowest and least variable DON accumulation. Accumulation of DON was also relatively low and less variable in *T. spelta* (Ruzyně), *T. dicoccum* variety Rudico, and *T. dicoccum* (Tábor). Low but rather variable accumulation of DON was detected in Červená perla, May Emmer, and *T. spelta* (Tábor 2). While it is also evident from Figure 1 that variation in DON content was high in all examined genotypic groups, the average DON content of varieties currently in commercial use (33.9 mg/kg) was significantly

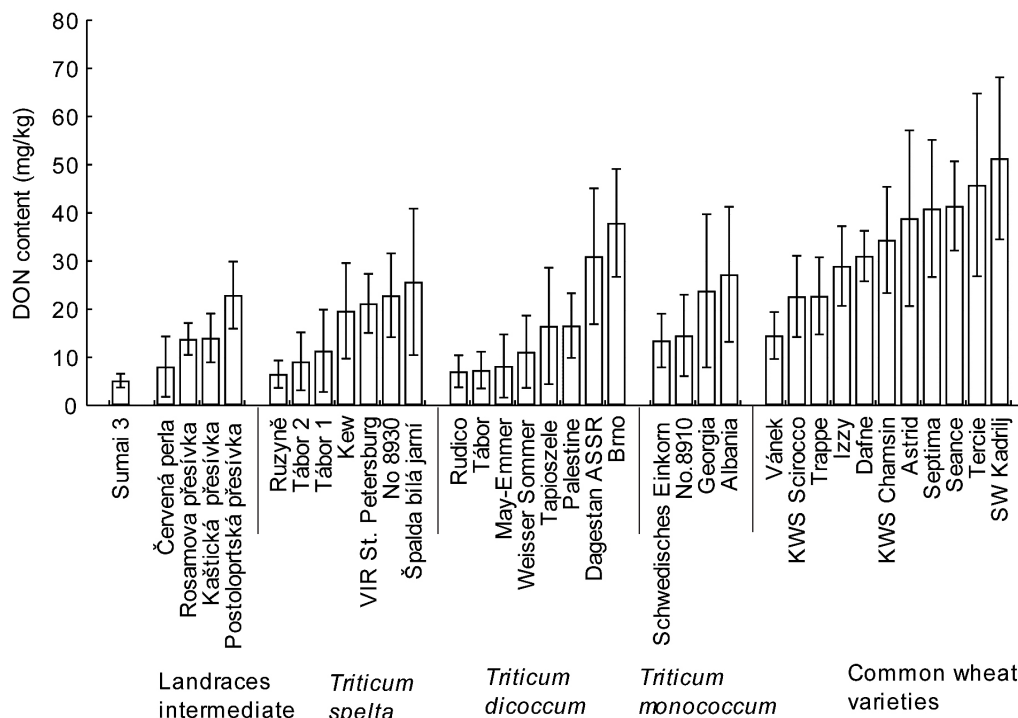


Figure 1. Content of mycotoxin deoxynivalenol in ancient and current spring wheat genotypes after artificial infection

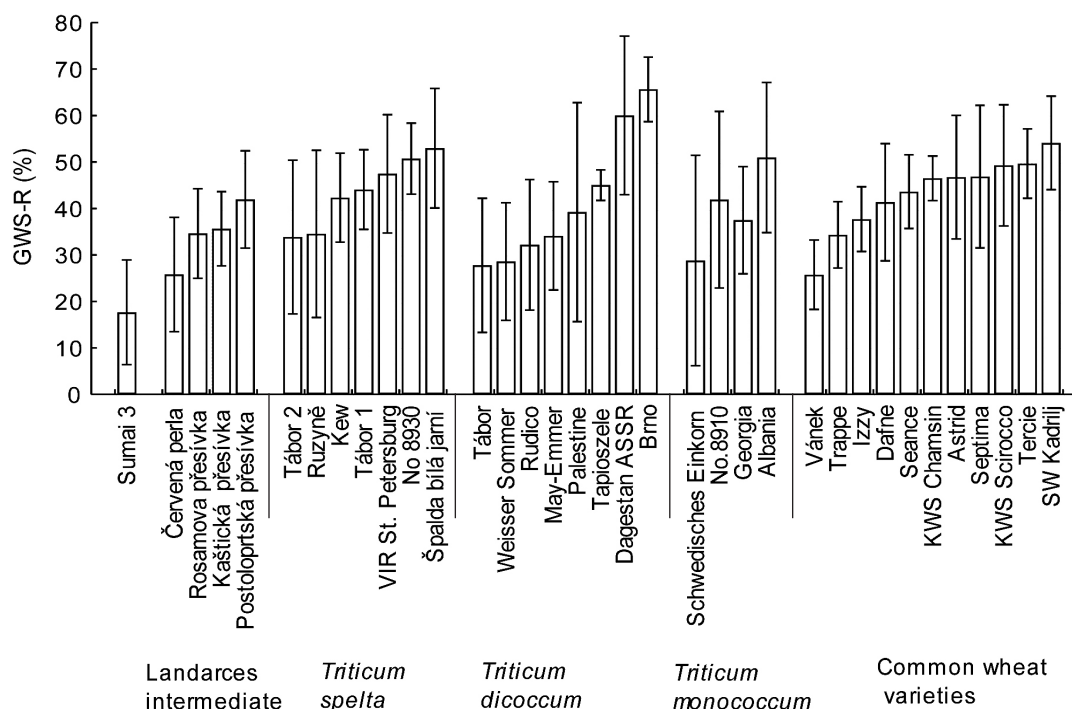


Figure 2. Reduction of grain weight per spike in ancient and current spring wheat genotypes after artificial infection

higher (LSD test;  $P < 0.05$ ) than in ancient materials belonging to *T. aestivum* – LI, *T. spelta*, *T. dicoccum*, and *T. monococcum* groups (17.3 mg/kg) and in the check variety Sumai 3 (5.2 mg/kg). The highest DON content was detected in some modern bread wheat varieties (especially in SW Kadrij), but the elite bread wheat variety Vánek showed relatively low DON content.

Examination of the second most important characteristic, effect on grain yield, however, showed significantly lower reduction of grain weight per spike (GWS-R) only in the check variety Sumai 3 (17.6%), while average GWS-R values for the modern, commercially grown varieties, hulled wheat species (einkorn, emmer, and spelt wheat), and landraces of intermediate type, respectively, were 43.3%, 42.6%, and 33.8%. As shown in Figure 2, variation in GWS-R was especially high in the *T. monococcum* and *T. dicoccum* genotypic groups and relatively lower in some commercial varieties (moderately resistant Vánek and medium resistant Trappe).

Grain hulling could be one of the factors that condition passive resistance to infection, because hard husks that cling tightly to the kernel may put up an effective barrier to mycelial filaments (BURSTMAYER *et al.* 2003; SUCHOWILSKA *et al.* 2010). More frequent occurrence of lower DON content in grain of the hulled wheat species can

also be connected with peeling off the hulls before grain processing (KONVALINA *et al.* 2011). Greater plant height (in these experiments on average by 30 cm compared to modern common wheat varieties) may be another advantage of older varieties and wild wheat relatives under natural infection conditions, along with the presence of awns and advantageous spike morphology (MESTERHÁZY 1995). According to BURSTMAYER *et al.* (2003), especially smaller and looser spikes that possess harder glumes and brittle rachises may contribute significantly to passive resistance in *T. dicoccoides*. SUCHOWILSKA *et al.* (2010) found that einkorn, emmer, and spelt differ significantly with regard to the mycotoxin profiles of their grains.

Differences between old and modern wheat varieties detected in our experiments were similar to those in the experiments of GORAL *et al.* (2008). Those authors had suggested that the differences may stem from loss of resistance during the breeding process. Detection of great variation for FHB resistance in the genus *Triticum* is undoubtedly common to many investigations, and it is obvious that the occurrence of high resistance (at the level of Sumai 3) is likely to be very rare also among wild wheat relatives. These experiments indicate that the choice of resistant genotypes in the hulled wheat species should take into consideration not only resistance to mycotoxin accumulation, but also

Table 2. Characterization of resistance sources in comparison with commercially grown varieties SW Kadrilj and Vánek (means of 2010–2012)

Variety	FD	TGW (g)	GWS (g)	Other important characters		
				protein content (%)	Zeleny sedimentation volume (ml)	falling number (s)
Červená perla	19	33.3	1.57	12.1	33	311
May-Emmer	20	32.1	1.44	16.8	22	346
Weisser Sommer	20	33.4	1.47	16.2	18	326
<i>T. dicoccum</i> Tábor	22	33.3	1.42	15.8	12	317
<i>T. spelta</i> Ruzyně	12	44.9	1.39	14.6	26	334
Rudico	20	32.7	1.38	16.1	14	340
SW Kadrilj	8	43.5	1.67	14.0	56	225
Vánek	7	47.0	1.95	14.0	62	220

FD – flowering date in days after 1 June; TGW – thousand grain weight; GWS – grain weight per spike

other important components of resistance, mainly effect on grain yield. Relatively lower variation in GWS-R detected among modern, common wheat



Figure 3. Spike morphotype of spring wheat varieties and lines that showed resistance to FHB. From left: Červená perla (*T. aestivum* – LI), May Emmer (*T. dicoccum*), *T. dicoccum* (Tábor), Rudico (*T. dicoccum*), *T. spelta* (Ruzyně) and Vánek (*T. aestivum* – CV)

varieties (Figure 2) may indicate their better ability to cope with these stress conditions in comparison with other *Triticum* species.

Table 2 presents performance data for some important plant-type and grain-quality characteristics of ancient wheat materials possessing resistance to FHB and of the widely grown common wheat varieties SW Kadrilj and Vánek. Divergence in spike morphology of selected FHB resistant materials is illustrated in Figure 3. It is evident that especially resistant emmer wheat varieties and lines may be progressively used for the production of protein rich and healthy bio-foods. The emmer wheat variety Rudico, for which a certificate of legal protection was obtained, has demonstrated, in addition to very high protein content, multiple resistance to fungal diseases, and it can be recommended for growing in organic farming systems (STEHNO 2007). These experiments also confirmed this variety's resistance to FHB. The detection of moderate FHB resistance in the current common spring wheat variety Vánek adapted to these environmental conditions can be considered particularly important, because this elite (E) bread wheat variety possesses many other desirable characteristics. By finding new and genetically distant sources of FHB resistance, this study has enabled the creation of new opportunities for wheat breeding.

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