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TOOLS FOR *FUSARIUM* MYCOTOXIN REDUCTION IN FOOD AND FEED CHAINS
RESEARCH PAPERS

Fusarium head blight resistance and mycotoxin profiles of four *Triticum* species genotypes

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Summary. Fusarium head blight (FHB) resistance was evaluated for accessions of four *Triticum* species, including bread wheat (modern and old cultivars), spelt, emmer, and einkorn. *Fusarium* head infection, *Fusarium* kernel damage and accumulation of trichothecene toxins (deoxynivalenol, nivalenol) in grains were analysed. Modern bread wheat cultivars were the most susceptible to head infection, and emmer and einkorn accessions were the most resistant. Kernel damage was the least for emmer and spelt and greatest for bread wheat. No significant differences between the four host species were observed for toxin accumulation. However, the greatest amounts of deoxynivalenol were detected in the grains of modern wheat cultivars and the least in old bread wheat cultivars. The greatest amount of nivalenol was detected in einkorn grains and the least in old bread wheat cultivars. Wide variability of resistance of all types in all four species was observed. Accessions resistant to FHB and toxin accumulation in grains were identified.

Key words: *Fusarium culmorum*, *Triticum aestivum*, *Triticum dicoccum*, *Triticum monococcum*, *Triticum spelta*.

Introduction

Fusarium head blight (FHB) is caused by fungi from genus *Fusarium*. All small grain cereal species including species of *Triticum*, can be affected by FHB. Published reports indicate that the host most susceptible to FHB is tetraploid durum wheat (*Triticum turgidum* ssp. *durum*). Less susceptible species are bread wheat (*T. aestivum* ssp. *aestivum*), triticale (x*Triticosecale*), rye (*Secale cereale*), barley (*Hordeum sativum*) and oat (*Avena sativa*) (Langevin *et al.*, 2004). Durum wheat shows low variability of FHB resistance and most cultivars are susceptible to FHB. In contrast, bread wheat exhibits wide variability of resistance to the disease, from susceptible dwarf high-yielding cultivars to highly resistant spring cultivars or landraces e.g. 'Sumai 3', 'Nobeokabozu' and 'Frontana'. These landraces are sources of FHB resistance genes in hexaploid wheat. There are other domesticated *Triticum* species which were cultivated historically and are cultivated presently, to a limited extent (Pagnotta *et al.*, 2005). These are spelt (*T. aestivum* ssp. *spelta*), emmer (*T. turgidum* ssp. *dicoccon*), and einkorn (*T. monococcum* ssp. *monococcum*). All three are hulled, and the first two are closely related to free-threshing bread wheat and durum wheat (Ozkan *et al.*, 2005; Dvorak *et al.*, 2012). FHB resistance of these species was less studied than in major cereals.

Most published reports have been on FHB resistance of emmer wheat. This hulled species is a close relative of durum wheat, lacking FHB resistance genes. Moderately resistant emmer genotypes were identified and could be used as sources of resistance for durum wheat (Terzi *et al.*, 2007). The quantitative trait loci (QTL) controlling FHB resistance in emmer wheat have been identified. For example, Zhang *et al.* (2014) identified QTL on chromosome 5A from emmer wheat, which explained up to 35% of FHB resistance variation. As was summarised by Prat

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et al. (2014), 13 QTL with small to moderate effects have been detected on 11 chromosomes, with alleles improving FHB resistance derived from relative species and durum wheat itself (Buerstmayr *et al*., 2012). The QTL found in tetraploid wheat populations largely overlap with the QTL identified in hexaploid wheat, suggesting a common genetic basis of FHB resistance. Transfer of FHB resistance genes from bread wheat to durum wheat has yet to be achieved. However, there are recent reports of introgression of the *Fhb1* gene in tetraploid backgrounds (Prat *et al*., 2014).

Resistance to FHB was also identified in other tetraploid species such as wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) and Persian wheat (*T. turgidum* ssp. *carthlicum*) (Otto *et al*., 2002; Buerstmayr *et al*., 2003; Oliver *et al*., 2008; Singh *et al*., 2008).

Spelt resistance to FHB has been less investigated, as it is not an important source of resistance for bread wheat including highly resistant genotypes in their gene pool. However, spelt is mostly grown in low input or ecological conditions and belongs to the "healthy food" category (Moudry, 1999; Zieliński *et al*., 2008). Hence, spelt resistance to FHB, and especially to *Fusarium* mycotoxin accumulation, is likely to be important.

This research reported here was part of a project on introduction/re-introduction of old bread wheat cultivars or other *Triticum* species to ecological agriculture in Poland. The substantial goal of the project was evaluation of disease resistance, including resistance to FHB.

Material and methods

Plant material

Resistance to FHB was evaluated in 32 *Triticum* accessions. Most of the accessions (except the modern cultivars) originated from the gene bank of the National Centre for Plant Genetic Resources in the Plant Breeding and Acclimatization Institute, Radzików, Poland. Agronomic performance of these lines was previously evaluated under organic farming conditions (Cyrkler-Degulis and Bulińska-Radomska, 2006). The accessions were: five old bread wheat (*T. aestivum* ssp. *aestivum*) cultivars, six modern bread wheat (*T. aestivum* ssp. *aestivum*) cultivars, seven genotypes/cultivars of spelt (*T. aestivum* ssp. *spelta)*, seven genotypes/cultivars of emmer (*T. turgidum* ssp. *dicoccon*) and seven genotypes/cultivars of einkorn (*T. monococcum* ssp. *monococcum*) (Table 1). All accessions were winter types.

Three *Fusarium culmorum* isolates producing deoxynivalenol (DON) (isolates KF846 and ZFR112) or nivalenol (NIV) (isolate KF350), were used for inoculum production. The two DON chemotype isolates (KF846, ZFR112) originated from Radzików, Poland, and were isolated from wheat heads (Wiśniewska and Kowalczyk, 2005; Ochodzki and Góral 2006). The chemotype isolate KF350 (IPO348) originated from the Netherlands and was also isolated from wheat heads (Snijders and Perkowski, 1990).

Field experiments

Host accessions were sown in field experiments located in Radzików, Central Poland in the years 2007–2009. Field experiments were established as randomized complete block designs. Wheat was sown at 1 m^2 plots in two replicates. Plots were inoculated with a mixture of the *F. culmorum* isolates. No fungicides were applied to the experimental plots.

Fusarium culmorum isolates were separately incubated on autoclaved wheat grain in glass flasks for about 1 week in darkness at 18ºC, and then exposed to UVA light (350nm) with a 16 h photoperiod at 15ºC for about 3 weeks. The culture flasks were shaken thoroughly at 24 h intervals to loosen kernels colonized with mycelium. Grain with visible sporulation on the kernel surfaces was air dried and stored in a refrigerator at 4ºC until used. At the date of inoculation, grain with *Fusarium* mycelium and conidia were suspended in distilled water for 1 h, and then filtered through two layers of cheesecloth to obtain conidium suspensions. The suspensions from each of the three isolates were adjusted to 5 x 10^5 conidia mL^{-1} with haemocytometer enumeration. Equal volumes of conidium suspensions of the three isolates were mixed.

Wheat heads were inoculated at flowering stage with *F. culmorum* conidium suspension at a rate of 100 mL m^{-2}. Each plot was inoculated individually at the beginning of anthesis (ZGS 61), and this was repeated about 3 d later at full anthesis (ZGS 65). Inoculations were carried out in the evenings, when relative air humidity increased.

Fusarium head blight was scored based on the mean percentage of blighted spikelets per infected head (severity) and the percentage of infected heads

Table 1. Accessions of genus *Triticum* evaluated in this study.

No.	Accession name	Species	Country of origin[b]	Accession number[c]
1	Ak-bugda mestnaja	*T. aestivum* ssp. *aestivum*	SUN	14
2	Aldea	″	CSK	19
3	Antonińska S.46	″	POL	39
4	Balta	″	POL	63
5	Biała kaszubska	″	POL	93
6	Clever[a]	″		
7	Kobra[a]	″		
8	Korweta[a]	″		
9	Mewa[a]	″		
10	Tonacja[a]	″		
11	Zyta[a]	″		
12	Hueslers Niederwill 19	*T. aestivum* ssp. *spelta*	DEU	1169
13	Rechbergs Frueher Dinkel	″	DEU	1166
14	Spelt Inz. Droogendijk /39	″	-	1157
15	*T. spelta* 1170	″	DEU	1170
16	*T. spelta* L. *album* 2638	″	-	2638
17	*T. spelta* L. *album* 5044	″	SUN	5044
18	*T. spelta* L. *arduini* 5035	″	SUN	5035
19	E 0718	*T. monococcum* ssp. *monococcum*	SUN	5003
20	E 0719	″	SUN	5004
21	E 0720	″	SUN	5005
22	E 0721	″	SUN	5006
23	E 0722	″	SUN	5007
24	E 0807	″	SUN	5040
25	Winter Einkorn	″	-	1195
26	Bankini	*T. turgidum* ssp. *dicoccon*	SWE	1306
27	Roter Emmer	″	DEU	1183
28	Schwarzer Behaarter	″	DEU	1182
29	*T. dicoccum* 5028	″	SUN	5028
30	*T. dicoccum* 5029	″	SUN	5029
31	*T. dicoccum* 5049	″	SUN	5049
32	*T. dicoccum* 5337	″	SUN	5337

[a] Modern bread wheat cultivars.
[b] SUN, former Soviet Union; CSK, former Czechoslovakia; POL, Poland; DEU, Germany; SWE, Sweden.
[c] Accesion number of gene bank of the National Centre for Plant Genetic Resources in PBAI Radzików.

per plot (incidence). Disease severity was scored on all heads in a plot showing FHB symptoms. Fusarium head blight index (FHBi) was calculated as a combination of disease severity and disease incidence, using the formula:

$$FHBi = \frac{FHB_{severity} \times FHB_{incidence}}{100}$$

The disease was first assessed at approx. 21 d after the last inoculation, and was then assessed twice more at approx. 10 d intervals.

After ripening, 50 heads were harvested manually from each plot and threshed with a laboratory thresher at low wind speed to prevent loss of low weight infected kernels. The percentage of *Fusarium*-damaged kernels (FDK) was visually assessed by dividing each grain sample into two categories: healthy kernels and infected kernels showing different levels of damage (Argyris *et al.*, 2003).

Mycotoxin analyses

Grains were analysed for presence of the *Fusarium* toxins DON and NIV using an HPLC method with UV detection. The extraction of mycotoxins was performed according to the recommendations of the SPE purification column manufacturer (Romer Labs Inc.), with minor modifications, as follows. Five g of representative sample of ground grain from each sample was placed in a 50 mL capacity Falcon tube and extracted with 25 mL of aqueous acetonitrile (84:16 v:v) on shaker for 60 min. The resulting suspension was centrifuged for 5 min at 4000 rpm, and 6 mL of supernatant was purified on Mycosep®227 Trich+ column (Romer Labs Inc.). Four mL of the eluate was evaporated to dryness, redissolved in 500 µL of the mobile phase (acetonitrile in water 10:90, v:v) and filtered through a 0.45 µm pore size nylon filter. Twenty µL of the sample was injected into an HP 1050 HPLC quaternary pump fitted with a LiChrospher 100 RP-C18 250 × 4 mm (5 µm particle size) stainless steel column. DON and NIV were eluted at a flow rate 1.0 mL min^{-1} and detected with a UV detector at wavelength 236 nm (Fazekas *et al.*, 2000). An external standard method was used for quantitative analysis of both toxins. The recovery rate of DON was 82% and NIV 70%, and the detection limit was 0.05 mg kg^{-1}.

Statistical analyses

Statistical analyses were performed using Microsoft® Excel 2010/XLSTAT©-Pro (Version 2013.4.07, Addinsoft, Inc.). Analysis of variance of FHB, FDK, DON and NIV data (incorporating Tukey's pairwise comparison at the 5% probability level) was performed using the 'Mixed models' procedure of XLSTAT. Experimental years constituted a random factor in the model. The relationships between data of FHB, FDK and *Fusarium* toxins were investigated by Pearson correlation analysis using the 'Correlation test' procedure of XLSTAT. Prior to analyses all data were log transformed to stabilize variances. The cereal accessions were grouped according to their resistance (FHBi, FDK, DON, and NIV) using the 'K-means clustering' procedure of XLSTAT, and results were visualised using 'Discriminant analysis' procedure of the same statistical package. Classes obtained from k-means analysis were applied as a qualitative dependent variable in DA analysis.

Results

The *Triticum* accessions differed in mean plant heights and spike lengths (Figure 1). On average first parameter was the greatest for spelt (123.7 cm) and the least for bread wheat plants (100.9 cm). Einkorn and emmer had similar mean heights (118.8 cm and 118.2 cm, respectively). The largest variability of mean plant height was observed in bread wheat (78.0 to 133.3 cm). Heights of einkorn, emmer and spelt were less variabile. The ranges of mean plant heights were 109.7 to 129.7 cm for einkorn, 103.3 to 128.3 cm for emmer, and 108.3 to 130.7 cm for spelt.

The spelt and bread wheat accessions had the longest spikes: for spelt, the mean was 10.9 cm (range 9.3 to 13.2 cm), and for bread wheat, 9.1 cm, range 7.2 to 10.8 cm). Old cultivars had slightly longer spikes (mean = 8.7 cm) compared to the modern cultivars (9.6 cm). Diploid and tetraploid *Triticum* species had shorter spikes. For einkorn mean spike length was 6.9 cm (range 5.3 to 8.7 cm) and for emmer, 7.1 cm (range 6.0 to 8.0 cm).

The average FHB index over the three years of experiments was 21.9%. Years 2007 and 2009 were favourable for FHB development, and average FHB indices were similar at 26.0% for 2007 and 26.7% for 2009 (Table 2). In 2008, dry weather prevailed in the period between heading and harvest, so the mean

Figure 1. Mean plant heights and spike lengths of 32 accessions belonging to four *Triticum* species. Boxes represent first quartile, median, mean (+) and third quartile. Circles (o) show minimum and maximum values. Whiskers show lower and upper limits.

Table 2. Monthly average air temperatures and monthly sum of precipitation in June and July of three years in which field evaluations were carried out.

Year	Air temperature [°C]			Precipitation sum [mm]		
	2007	2008	2009	2007	2008	2009
June	19.1	19.0	16.3	72.2	15.0	84.0
July	19.1	19.5	20.0	94.8	21.0	138.6

FHB index was lower at 13.5%. The mean proportion of FDK was greatest in 2007 (19.1%), less in 2009 (13.2%) and least in the dry year 2008 (7.8%). Mean DON concentrations in grain followed this trend and was greatest in 2007 (6.7 mg kg^{-1}), less in 2009 (2.6 mg kg^{-1}) and the least in 2008 (0.8 mg kg^{-1}).

The range of reaction of *Triticum* accessions to *F. culmorum* inoculation was very wide. The mean FHBi ranged from 2.7 to 52.0%, the mean FDK from 2.6 to 35.2%, the mean DON concentration from 0.8 to 8.0 mg kg^{-1}, and the mean concentration of NIV was from 0.3 to 1.9 mg kg^{-1} (Table 3). Differences between the host accessions were statistically significant for all four of these characters.

The most resistant accessions to head infection were emmer 'T. dicoccum 5049' and spelt 'Spelt Inz. Droogendijk /39'. Slight infections also occurred on two genotypes of emmer, two of einkorn, the old bread wheat cultivar 'Biala Kaszubska' and one spelt genotype. The most infected heads were those of the modern cultivar 'Clever'. Four cultivars of bread wheat and two spelt accessions were also heavily infected.

For the FDK assessments, the most resistant accessions were the same as those resistant to FHB. These were 'T. dicoccum 5049' and 'Spelt Inz. Droogendijk /39'. In addition, low amounts of FDK occurred for the two spelt accessions 'T. spelta L. album 2638' and 'T. spelta 1170', the emmer genotype 'T. dicoccum 5337', and einkorn 'E 0721'.

The lowest concentrations of DON were detected in grains of the two spelt genotypes 'Spelt Inz. Droogendijk /39' and 'T. spelta 1170', the emmer genotype 'T. dicoccum 5049' and the old bread wheat cultivar 'Biala Kaszubska'. The highest DON concentration was found in grains of the two modern bread wheat cultivars 'Tonacja' and 'Clever', and in grains of the spelt cultivar 'Rechbergs Frueher Dinkel'. For NIV, the lowest amounts were detected in grains of

Table 3. Mean Fusarium head blight indices (FHBi), proportions of *Fusarium* damaged kernels (FDK%) and concentrations of DON and NIV mycotoxins for different *Triticum* accessions.

No.	Accession	Species	FHBi [%]	FDK [%]	DON [mg kg^{-1}]	NIV [mg kg^{-1}]
1	*T. dicoccum* 5049	Emmer	2.7	2.6	0.964	0.469
2	Spelt Inz. Droogendijk /39	Spelt	2.8	2.8	0.801	0.541
3	Schwarzer Behaarter	Emmer	8.7	13.8	2.074	0.704
4	*T. spelta* 1170	Spelt	8.9	5.0	1.197	0.327
5	E 0720	Einkorn	9.3	13.5	3.398	0.936
6	Biała Kaszubska	Bread wheat	9.5	7.1	0.840	1.020
7	E 0722	Einkorn	9.5	10.8	2.530	0.873
8	Roter Emmer	Emmer	10.8	9.5	2.353	1.280
9	Aldea	Bread wheat	15.3	11.3	2.010	0.583
10	Winter Einkorn	Einkorn	16.7	8.8	3.793	0.977
11	Bankini	Emmer	17.3	15.5	5.425	1.284
12	*T. dicoccum* 5337	Emmer	17.8	4.0	2.189	0.456
13	*T. dicoccum* 5028	Emmer	18.8	11.3	4.728	0.828
14	*T. spelta* L. *album* 2638	Spelt	18.9	3.6	1.654	0.317
15	E 0721	Einkorn	19.3	4.9	1.814	0.494
16	Ak-bugda Mestnaja	Bread wheat	19.6	10.3	2.340	0.556
17	Hueslers Niederwill 19	Spelt	20.3	10.0	3.341	0.529
18	E 0718	Einkorn	21.7	7.8	3.689	1.181
19	*T. dicoccum* 5029	Emmer	25.7	8.8	2.814	0.783
20	E 0719	Einkorn	26.3	10.6	4.659	1.249
21	E 0807	Einkorn	26.7	21.1	5.258	1.903
22	Mewa[a]	Bread wheat	27.9	13.5	4.388	0.838
23	Zyta[a]	Bread wheat	28.4	27.0	2.861	1.223
24	Antonińska S.46	Bread wheat	30.3	15.5	2.063	0.574
25	Rechbergs Frueher Dinkel	Spelt	30.3	22.9	7.957	1.598
26	*T. spelta* L. *arduini* 5035	Spelt	32.7	12.9	3.407	0.863
27	Tonacja[a]	Bread wheat	33.0	30.1	6.080	1.325
28	*T. spelta* L. *album* 5044	Spelt	34.0	17.9	3.763	0.888
29	Korweta[a]	Bread wheat	34.6	26.7	4.972	0.749
30	Kobra[a]	Bread wheat	35.3	21.6	5.673	0.856
31	Balta	Bread wheat	36.6	14.5	3.999	0.748
32	Clever[a]	Bread wheat	52.0	35.8	7.012	1.267
	Mean		21.9	13.5	3.439	0.882
	Tukey's $HSD_{0.05}$		8.5	11.3	2.310	0.757

[a] Modern bread wheat cultivars.

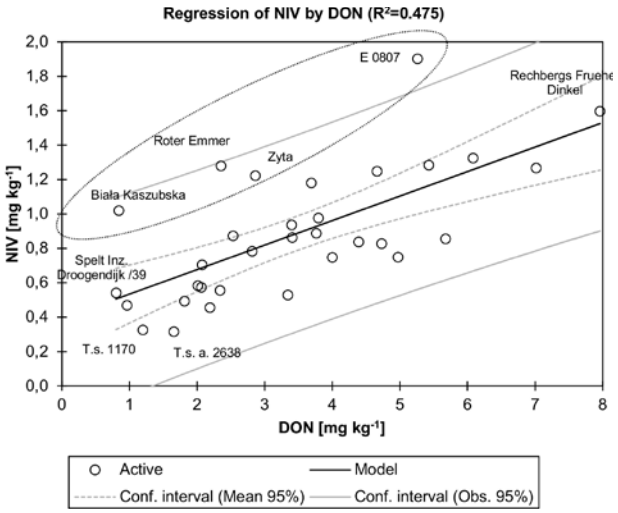

Figure 2. Linear regressions of mean grain NIV and DON concentrations for 32 *Triticum* accessions. Accessions with the highest residuals for NIV are delineated with a dotted ellipse.

the two spelt genotypes 'T. spelta L. album 2638' and 'T. spelta 1170'. Low NIV concentrations also occurred in grain of one accession of einkorn, two accessions of emmer, two cultivars of spelt and three old cultivars of bread wheat. The greatest amounts of NIV were detected in grains of the spelt cultivar 'Rechbergs Frueher Dinkel' and the einkorn genotype 'E 0807'.

Table 4. Mean Fusarium head blight indices (FHBi), proportions of *Fusarium* damaged kernels (FDK%) and concentrations of DON and NIV mycotoxins for different *Triticum* accessions.

Cereal species	FHBi [%]	FDK [%]	DON [mg kg^{-1}]	NIV [mg kg^{-1}]
Emmer	14.5 a	9.4 a	2.935 a	0.829 a
Einkorn	18.5 ab	11.1 a	3.592 a	1.087 a
Spelt	21.1 b	10.7 a	3.160 a	0.723 a
Bread wheat	29.3 c	19.4 b	3.840 a	0.885 a
Old cultivars	22.2	11.7	2.251	0.696
Modern cultivars	35.2	25.8	5.164	1.043

Values followed by the same letter are not statistically significantly different

Bread wheat 'Biała Kaszubska' accumulated more NIV than other low-DON accessions. Similar results were found for spelt 'Roter Emmer', bread wheat 'Zyta' and einkorn 'E 0807' (Figure 2). Comparison of average FHB resistance of the four cereal species showed that emmer had the highest resistance to head infection while bread wheat had the least (Table 4; Figure 3). Differences between species were statistically significant.

The old wheat cultivars showed FHB resistance similar to spelt. The largest variability was for the spelt accessions and for the bread wheat cultivars (Figure 3). For FDK, there were no differences between emmer, einkorn and spelt. Bread wheat kernels were significantly more damaged. However this was due to low resistance in the modern cultivars. The largest variability in FDK occurred in the bread wheat cultivars. No significant differences between species were found for grain concentrations of DON or NIV. The greatest DON amounts were detected in grain of the modern wheat cultivars. In contrast, the greatest concentration of NIV was detected in grain of einkorn, but was similar to that for the modern wheat cultivars. The variabilities of DON and NIV amounts were similar for all species (Figure 3).

Plant height had a significant negative effect on the severity of FHB symptoms on heads and kernels (Table 5). The tall genotypes accumulated less mycotoxins (total content of DON and NIV) than shorter genotypes. Spike length had was not related to FHB index and other resistance parameters. Analysis of correlations for single species showed that the above relationships as regards plant height were due to the presence of dwarf bread wheat cultivars in the studied population. Mean FHB indices and FDK proportions correlated significantly with DON and NIV concentration in grain. The lowest coefficients of correlation were with NIV, because some genotypes showed deviations from linear relationships (Figure 2). For specific species, significant correlations of the the above traits occurred in bread wheat. In the other species, there were mostly no relationships between head infection and kernel damage or toxin accumulation. The exception was for FHBi *vs* FDK in spelt wheat. However, in all three species (einkorn, emmer and spelt) the correlations between FDK and total content of DON and NIV in grains were highly significant.

K-means analysis grouped the accessions of four *Triticum* species into three groups (Figure 4). One group included 12 accessions of three old bread

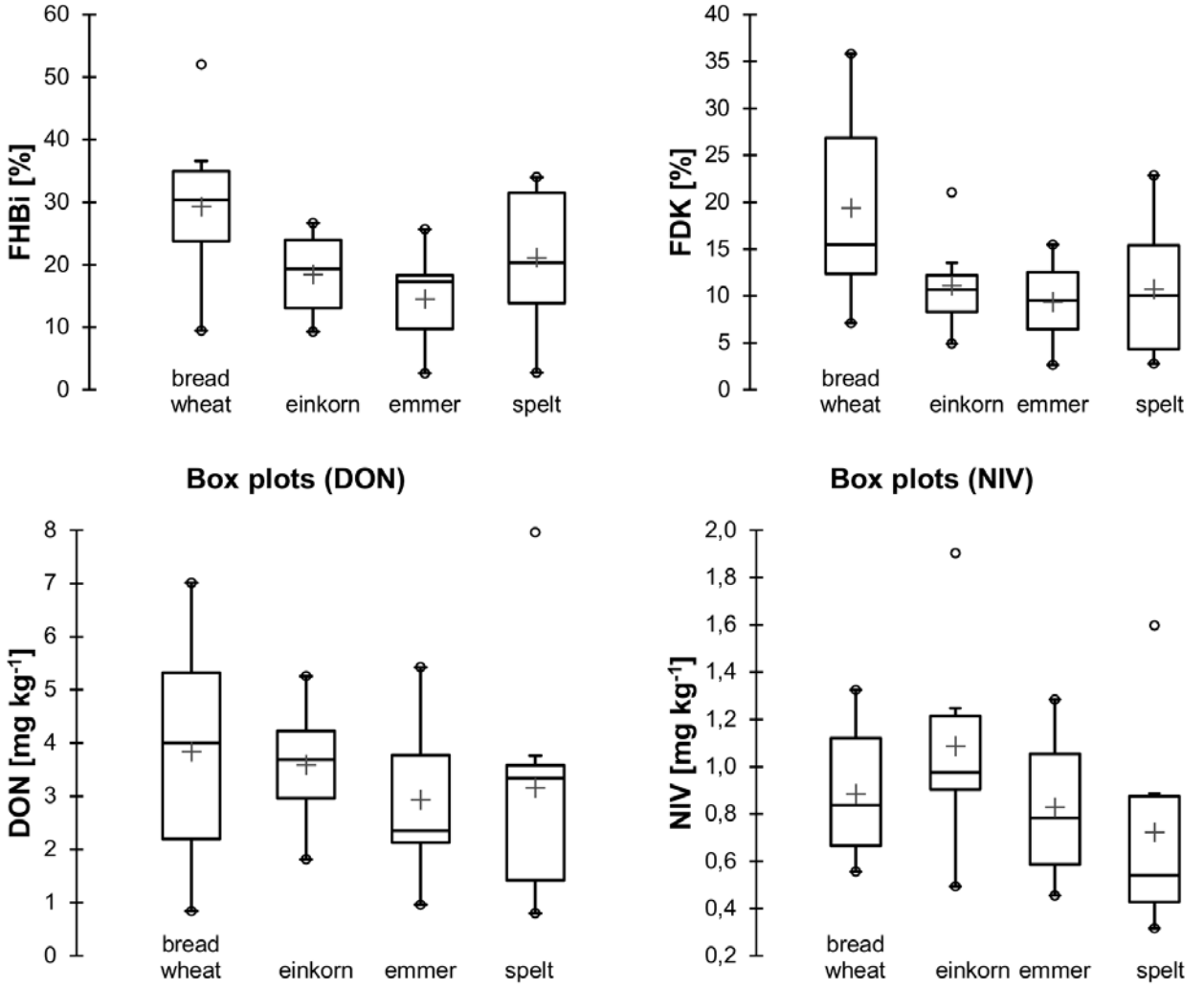

Figure 3. Mean Fusarium head blight indices (FHBi), *Fusarium* damaged kernels (FDK) and grain concentrations of DON and NIV mycotoxins for four *Triticum* species. Box plots represent first quartile, median, mean (+) and third quartile. Circles show minimum and maximum values. Whiskers show lower and upper limits.

wheat cultivars, four spelt cultivars, three emmer cultivars and two einkorn genotypes ('E 0721' and 'E 0722'). The highest resistance to FHB and toxin accumulation also characterized this group (Table. 6). Einkorn accession 'E 0721' accumulated the least amount of toxins among all the tested accessions. A second group comprised 13 accessions including two old and one modern ('Mewa') bread wheat cultivars, two cultivars of spelt, four of emmer and three of einkorn. Medium resistance to FHB and medium trichothecene accumulation characterized this group. A third group comprised five modern bread wheat cultivars, one spelt cultivar ('Rechbergs Frueher Dinkel') and one einkorn accession ('E 0807'). The accessions in this group were the most susceptible to FHB, had the greatest proportions of FDK and accumulated the greatest amounts of DON and NIV toxins.

Discussion

In all studied species, diversity occurred in morphology and resistance to FHB. Plant height was the most diverse in common wheat. This was due to the presence of modern cultivars with a strongly short

Table 5. Correlation matrix for mean plant parameters, Fusarium head blight indices and mycotoxin concentrations in grain, for 32 *Triticum* accessions.

Variables	Plant height [cm]	Spike length [cm]	FHBi [%]	FDK [%]	DON [mg kg^{-1}]	NIV [mg kg^{-1}]
Spike length [cm]	**0.208**					
FHBi [%]	**-0.592**	0.049				
FDK [%]	**-0.481**	0.002	**0,762**			
DON [mg kg^{-1}]	-0.332	-0.194	**0,714**	**0,765**		
NIV [mg kg^{-1}]	0.011	-0.342	**0,357**	**0,592**	0,689	
DON+NIV [mg kg^{-1}]	-0.286	-0.230	**0.684**	**0.770**	**0.992**	**0.777**

All coefficients in bold are different from 0 with a significance level $P \leq 0.05$.

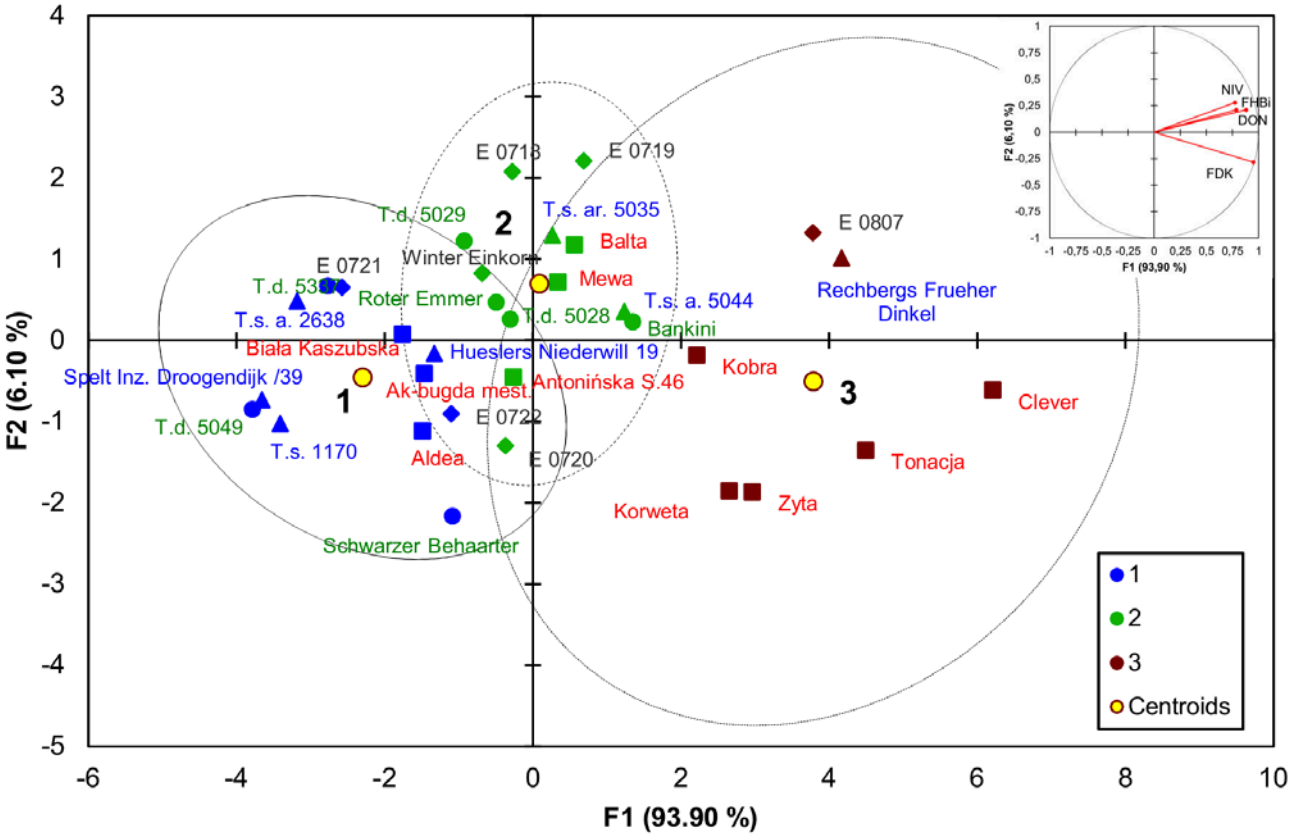

Figure 4. Discriminant analysis plot of three groups created with k-means analysis (Table 6). The first and second discriminant axes account for, respectively, 93.9% and 6.1% discriminant power. Ellipses indicate 95% probability for each group. Squares, red label – bread wheat; triangles, blue label – spelt; circles, green label – emmer; diamonds, black label – einkorn. The correlation circle (upper right) shows the correlations of the initial variables with the two factors.

Table 6. Mean Fusarium head blight indices (see Table 3) and grain mycotoxin concentrations for three *Triticum* accession groups, created with k-means analysis.

Group	Number of accessions	FHBi [%]	FDK [%]	DON [mg kg^{-1}]	NIV [mg kg^{-1}]
1	12	12.8	7.2	1.813	0.572
2	13	23.7	12.3	3.729	0.956
3	7	34.3	26.4	5.688	1.274
Mean		21.9	13.5	3.439	0.882

ened straw, including 'Clever' and 'Kobra'. Cultivar 'Clever' possesses the dwarfing gene *Rht2* (Chrpová *et al.*, 2003). The head length was clearly larger in the hexaploid (sub)species (bread wheat and spelt). The diploid einkorn species, and tetraploid emmer had shorter heads, similar in average length to each other.

Resistance to head infection by *Fusarium* (measured with FHB index) was the least in the bread wheats. However, accessions of this species can be separated into two groups with different characteristics. One comprised 'modern' cultivars, which have been present in the Poland National Register for the last 10 years. Heads of these cultivars were approx. 50% more infected than heads of the second group of 'old' wheat cultivars. These reacted similarly to the spelt cultivars. Tetraploid and diploid species had on average the most resistant heads and emmer was the most resistant host type.

We found a similar pattern for *Fusarium* damaged kernels. The modern bread wheat cultivars had the most damaged kernels and emmer accessions had the lowest amounts of kernel damage. However, there were no significant differences between emmer, einkorn, spelt and old bread wheat for *Fusarium* kernel damage.

Packa *et al.* (2013) found that spring spelt accessions were the most resistant to FHB after inoculation with *F. culmorum*. Emmer and einkorn were more susceptible and their reactions were similar. Spring bread wheat accessions were less resistant than spelt but more than emmer and einkorn. However, two of three studied wheat cultivars (Sumai 3 and Frontana) were highly resistant checks. On the other hand, Suchowilska *et al.* (2007) observed that after inoculation of heads with *F. culmorum*, spelt produced more kernels characterized as as *Fusarium* damaged kernels than bread wheat. This species also showed severe reduction of the yield components which corresponded to the earlier results obtained by Wiwart *et al.* (2004). Those authors considered spelt to be more susceptible to head infection than bread wheat. The results published by Packa *et al.* (2013) and Suchowilska (2007) are inconsistent, although it should be noted that different spelt populations were examined in their studies. Packa *et al.* (2013) accessions originating from gene banks, while Suchowilska (2007) examined cultivars of spelt. In the present research we found large variability in spelt from resistant 'Spelt Inz. Droogendijk /39' to susceptible 'Rechbergs Frueher Dinkel'. Moreover, some accessions with severely infected heads (e.g. 'T. spelta L. arduini 5035') had low proportions of damaged kernels.

Wan *et al.* (1997) observed that diploid and tetraploid wheats were severely susceptible to FHB. Two accessions of *T. monococcum* were highly susceptible, having the greatest disease ratings. However, in tetraploid wheats some showed moderate resistance (8%), including two studied accessions of emmer wheat and 24% of accessions of a wild relative of emmer, *T. dicoccoides*. Six years earlier, Saur (1991) evaluated FHB resistance of 64 accessions of *T. monococcum* together with many species of *Aegilops*. Most were susceptible to FHB. Only the frequency of resistant genotypes was greater in *T. monococcum* (20%) and *Ae. speltoides* (27%). In our study, tetraploid winter emmer was the most resistant to FHB, especially in terms of kernel damage. For diploid einkorn, winter accessions were of medium resistance, comparable to the old bread wheats. In our paper on spring *Triticum* species (Góral *et al.*, 2012), we found, similar to winter species, that emmer was the most resistant to head and kernel *Fusarium* infection. We conclude that results obtained in different studies greatly depend on selection of plant material – whether cultivars, landraces or gene bank accessions.

As mentioned above, we found significant differences in head and kernel *Fusarium* infection/damage. However, for accumulation of two trichothecene toxins, we did not observe significant differences, with only a tendency for average DON concentration. Species could be ranked from the least contamination to the greatest, as follows: emmer, spelt, einkorn, and bread wheat. The lowest concentration of DON was

found in old bread wheat cultivars and the greatest was in modern cultivars of this species. Similar results were obtained for NIV. No significant differences between species were found. The lowest amount of NIV was detected in spelt grain, next in emmer and bread wheat grain. The greatest amount of this toxin was found in einkorn grain. Bread wheat groups differed substantially in NIV contents. Modern cultivars accumulated approx. 50% more NIV that old ones. NIV levels were similar to that for einkorn. On the other hand NIV concentrations level in grains of old cultivars were less than those for spelt. Wiwart *et al.* (2009) compared concentrations of *Fusarium* metabolites in grains of spelt and common wheat and found that the total concentration of mycotoxins was slightly less in T. *spelta*, whereas bread wheat grains contained lower concentrations of ergosterol (a measure of fungal biomass). These authors also observed that spelt husks contained considerable concentrations of trichothecenes. Similar observations were made in einkorn for B trichothecenes with greater concentrations recorded in glumes than in grains (Perkowski *et al*., 2011). Wiwart et al. (2011) compared three cultivars of winter type cereals – durum wheat, bread wheat and spelt. They found significantly lower accumulation of trichothecenes and ergosterol in grain of spelt cultivar as compared to bread wheat. Suchowilska *et al.* (2010) compared the lines of three hulled *Triticum* species (einkorn, emmer, spelt) with mycotoxin concentrations in the grain of 'Sumai 3', which is a highly FHB resistant spring wheat. They found many host forms had lower toxin levels (in particular trichothecenes) than this cultivar. On the other hand, DON concentrations in naturally infected grain samples of four *T. monococcum* lines, eight *T. dicoccum* lines and all five *T. spelta* lines were at the level of 'Sumai 3'. After inoculation, DON concentrations increased drastically, but the amounts of this toxin were equal to or lower than in 'Sumai3' in one line of *T. monococcum*, three lines of *T. Spelta* and three lines of *T. dicoccum* (Konvalina *et al*., 2011). Russian spring spelt cultivars ('Spalda bila jarni' and 'VIR St. Petersburg') were the least infected, and accumulated the lowest amount of DON. On the other hand, landraces of bread wheat and modern bread wheat cultivars accumulated the greatest amounts of DON in grain. Emmer and einkorn cultivars contained the lowest DON concentrations.

In summary, this study has demonstrated the wide variability in FHB resistance in winter type accessions of four *Triticum* species. This variability was also found for concentrations of two trichothecene toxins in grains. The variability is well known for *T. aestivum* genotypes, but less is known about FHB resistance in the three other species studied here, particularly in winter forms. Resistant or moderately resistant genotypes were identified, and these can be used in resistance breeding for organic farming or as sources of resistance in durum wheat. The potential for selection of FHB and toxin resistance in the three old *Triticum* forms (einkorn, emmer, spelt) has also been demonstrated, which are predominantly grown for "healthy food" production.

Literature cited

Argyris J., D. Van Sanford and D. TeKrony, 2003. *Fusarium graminearum* infection during wheat seed development and its effect on seed quality. *Crop Science* 43, 1782–1788.

Buerstmayr H., M. Stierschneider, B. Steiner, M. Lemmens, M. Griesser, E. Nevo and T. Fahima, 2003. Variation for resistance to head blight caused by *Fusarium graminearum* in wild emmer (*Triticum dicoccoides*) originating from Israel. *Euphytica* 130, 17–23.

Chrpová J., M. Škorpík, P. Prášilová and V. Sip, 2003. Detection of *Norin 10* dwarfing genes in winter wheat varieties registered in the Czech Republic. *Czech Journal of Genetics and Plant Breeding* 39, 89–92.

Buerstmayr M., K. Huber, J. Heckmann, B. Steiner, J.C. Nelson and H. Buerstmayr, 2012. Mapping of QTL for Fusarium head blight resistance and morphological and developmental traits in three backcross populations derived from *Triticum dicoccum* × *Triticum durum*. *Theoretical and Applied Genetics* 125, 1751–1765.

Cyrkler-Degulis M. and Z. Bulińska-Radomska, 2006. Yielding and healthiness of cultivars and populations of four winter wheat species under organic agriculture conditions. *Journal of Research and Applications in Agricultural Engineering* 51, 17–21. (in Polish)

Dvorak J., K.R. Deal, M.C. Luo, F.M. You, K. Von Borstel and H. Dehghani, 2012. The origin of spelt and free-threshing hexaploid wheat. *Journal of Heredity* 103, 426–441.

Fazekas B., Hajdu, E.T., Tar, A.K. and J. Tanyi, 2000. Natural deoxynivalenol (DON) contamination of wheat samples grown in 1998 as determined by high-performance liquid chromatography. *Acta Veterinaria Hungarica* 48, 151-160

Góral T., P. Ochodzki and Z. Bulińska-Radomska, 2012. Resistance to Fusarium head blight caused by *Fusarium culmorum* and *Fusarium* mycotoxins content in grain of species of spring cereals intended for organic farming. *Biuletyn IHAR 263,* 43–54. (in Polish)

Konvalina P., I. Capouchová, Z. Stehno, J. Moudrỳ Jr and J. Moudrỳ, 2011. *Fusarium* identification by PCR and DON content in grain of ancient wheat. *Journal of Food, Agriculture & Environment* 9, 321–325.

Langevin F., F. Eudes and A. Comeau, 2004. Effect of trichothecenes produced by *Fusarium graminearum* during

Fusarium head blight development in six cereal species. *European Journal of Plant Pathology* 110, 735–746.
Moudry J., 1999. Productivity of spelt wheat (*Triticum spelta* L.) spike. *Scientia Agriculturae Bohemica* 30, 15–26.
Ochodzki P. and T. Góral, 2006. Production of mycotoxins by selected *Fusarium graminearum* and *F. culmorum* isolates cultured on rice and wheat. Conference Papers of 28. Mykotoxin-Workshop, Bydgoszcz, Poland, 29–31 May, 2006. p. 73.
Oliver R.E., X. Cai, T.L. Friesen, S. Halley, R.W. Stack and S.S. Xu, 2008. Evaluation of Fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). *Crop Science* 48, 213.
Otto C.D., S.F. Kianian, E.M. Elias, R.W. Stack and L.R. Joppa, 2002. Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. *Plant Molecular Biology* 48, 625–632.
Ozkan H., A. Brandolini, C. Pozzi, S. Effgen, J. Wunder and F. Salamini, 2005. A reconsideration of the domestication geography of tetraploid wheats. *Theoretical and Applied Genetics* 110, 1052–1060.
Packa D., D. Załuski, Ł. Graban, W. Lajszner and M. Hościk, 2013. The response of diploid, tetraploidand and hexaploid wheats to *Fusarium culmorum* (W.G.Smith) Sacc. inoculation. *Polish Journal of Agronomy*12, 38–48. (in Polish)
Pagnotta M.A., L. Mondini and M.F. Atallah, 2005. Morphological and molecular characterization of Italian emmer wheat accessions. *Euphytica* 146, 29–37.
Perkowski J., M. Wiwart, K. Stuper, M. Buśko, A. Matysiak and E. Suchowilska, 2011. Concentration of fungal biomass and trichothecenes in different parts of einkorn. *Annals of Agricultural and Environmental Medicine* 18, 89–98.
Prat N., M. Buerstmayr, B. Steiner, O. Robert and H. Buerstmayr, 2014. Current knowledge on resistance to Fusarium head blight in tetraploid wheat. *Molecular Breeding* 34, 1689–1699.
Saur L., 1991. Sources of resistance to head blight caused by *Fusarium culmorum* in bread wheat and related species. *Agronomie* 11, 535–541. (in French)
Singh A.K., R.E. Knox, F.R. Clarke, J.M. Clarke, G. Fedak, A. Singh and R. DePauw, 2008. Fusarium head blight QTL mapping in durum wheat and *Triticum carthlicum* sources of resistance. In: Proceedings of the 11th International Wheat Genetics Symposium, Brisbane, Australia, 845–847.
Snijders C. and J. Perkowski, 1990. Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. *Phytopathology* 80, 566–570.
Suchowilska E., W. Kandler, M. Sulyok, M. Wiwart and R. Krska, 2010. Mycotoxin profiles in the grain of *Triticum monococcum*, *Triticum dicoccum* and *Triticum spelta* after head infection with *Fusarium culmorum*. *Journal of the Science of Food and Agriculture* 90, 556–565.
Suchowilska E., M. Wiwart and A. Borusiewicz, 2007. The reaction of the selected *Triticum aestivum*, *Triticum spelta* and *Triticum dicoccum* genotypes to spike infection by *Fusarium culmorum*. In: Proceedings of the Conference Organic farming 2007, Praha Suchdol, 6–7 February 2007, 172–174.
Terzi V., C. Morcia, A. M. Stanca, L. Kucera, C. Fares, P. Codianni, N. Di Fonzo and P. Faccioli, 2007. Assessment of genetic diversity in emmer (*Triticum dicoccon* Schrank) x durum wheat (*Triticum durum* Desf.) derived lines and their parents using mapped and unmapped molecular markers. *Genetic Resources and Crop Evolution* 54, 1613–1621.
Wan Y.-F., C. Yen and J.-L. Yang, 1997. Sources of resistance to head scab in *Triticum*. *Euphytica* 94, 31–36.
Wiśniewska H. and K. Kowalczyk, 2005. Resistance of cultivars and breeding lines of spring wheat to *Fusarium culmorum* and powdery mildew. *Journal of Applied Genetics* 46, 35–40.
Wiwart M., J. Perkowski, H. Jackowiak, D. Packa, A. Borusiewicz and M. Buśko, 2004. Response of some cultivars of spring spelt (*Triticum spelta*) to *Fusarium culmorum* infection. *Bodenkultur* 55, 103–111.
Wiwart M., W. Kandler, J. Perkowski, F. Berthiller, B. Preinerstorfer, E. Suchowilska, M. Buśko, M. Laskowska and R. Krska, 2009. Concentrations of some metabolites produced by fungi of the genus *Fusarium* and selected elements in spring spelt grain. *Cereal Chemistry* 86, 52–60.
Wiwart M., J. Perkowski, M. Budzyński, E. Suchowilska, M. Buśko and A. Matysiak, 2011. Concentrations of ergosterol and trichothecenes in the grains of three *Triticum* species. *Czech Journal of Food Sciences* 29, 430–440.
Zhang Q., J.E. Axtman, J.D. Faris, S. Chao, Z. Zhang, T.L. Friesen, S. Zhong, X. Cai, E.M. Elias, S.S. Xu, 2014. Identification and molecular mapping of quantitative trait loci for Fusarium head blight resistance in emmer and durum wheat using a single nucleotide polymorphism-based linkage map. *Molecular Breeding* 34, 1677–1687.
Zieliński H., A. Ceglińska and A. Michalska, 2008. Bioactive compounds in spelt bread. *European Food Research and Technology* 226, 537–544.

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