OCCURRENCE OF *FUSARIUM* SPECIES AND DON CONCENTRATION IN KERNELS OF *TRITICUM SPELTA*

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Abstract: *Spelt is an ancient cereal that was cultivated extensively in Europe and is now receiving a renewal interest for its nutritional and agronomic characters. Spelt possesses a unique flavour, higher vitamin content and is more nutritious than wheat. These ancient wheats are attracting the attention of nutritionists who are re-evaluating the healthy and dietary properties attributed to them in the past. The presence of mycotoxins especially deoxynivalenol (DON) is a high concern of grain growers. Contamination in feed and foodstuffs can cause serious health problems and diseases. Six spelt wheat cultivars were cultivated in the Slovak republic under ecological farming conditions on a Haplic luvisol at the location with continental climate. Pre-crop of spelt was peas. The experiment was organized into six randomized blocks in three replicates. During 2007 and 2008 growing seasons, the concentration of DON was detected in Triticum spelta varieties: Bauländer Spelz, Franckenkorn, Holstenkorn, Oberkulmer Rotkorn, Rouquin and Rubiota. The liquid chromatography WATERS BREEZE equipped with binary pump WATERS 1525, RHEODYNE injector and UV detector WATERS 2487 was used with a stainless steel reverse phase 150x3,9 mm, 4 mm spherical particle C18 Nova-Pak column. The effect of varieties on DON concentration was significantly different, with the year x variety interaction also being significant. The lowest DON concentration was determined in Rouquin (165 mg.kg^{-1}), whereas the highest production of mycotoxin was found in Holstenkorn and Oberkulmer Rotkorn (676.4 and 545.4mg.kg^{-1}). In three varieties (Bauländer Spelz, Franckenkorn, Oberkulmer Rotkorn) significant differences of DON concentrations between years were determined. The detected levels of DON did not exceed the maximum levels according to EU regulation 1881/2006. The effect of varietes and years on Fusarium infestation of kernels was significant. The susceptibility of spelt varietes to Fusarium kernel infestation had no influence on DON accumulation in kernels. Correlation coefficient was -0.09. The variety Rouquin showed the lowest FKI and also DON concentration. On the opposite, Bauländer Speltz with the second lowest DON concentration had the highest FKI.*

***Key words**: Triticum spelta, Fusarium, DON, kernels*

INTRODUCTION

Spelt is an ancient cereal that was cultivated extensively in Europe and is now receiving a renewal interest for its nutritional and agronomic characters. Spelt possesses a unique flavour, higher vitamin content and is more nutritious than wheat. These ancient wheats are attracting the attention of nutritionists who are re-evaluating the healthy and dietary properties attributed to them in the past. The fungi from *Fusarium* genera are important pathogens of wheat and other small grain cereals in most parts of the world (CHAKRABORTY *et al*., 2006). The situation may be very complex for diseases like *Fusarium* head blight (FHB), which can be caused by several pathogens (XU et al., 2005). Fungal species of the genera *Fusarium* and *Microdochium* infect developing kernels of cereals causing FHB symptoms. The disease is often associated with the production of mycotoxins and *Fusarium* species are reported to differ greatly in their ability to produce these metabolites (DESJARDINS, 2006). The occurrence of mycotoxins in wheat kernels is a significant threat to its use for food and feed. These fungi survive in the absence of living hosts in infested residues. The mycelium aggressively colonises the stems during senescence so that it has prior occupancy of the tissue at the time before the host plant dies (TRAIL *et al.*, 2005). Crop rotation and farming system are effective tools for management of *Fusarium* diseases in cereals. Incidence and severity of FHB and the composition of *Fusarium* species involved are reported to vary among geographical regions and years due to variation in climatic and weather conditions and cropping practices (KLIX et al., 2008). Conversion to organic management from these conditions usually means considerably drop of yields, during two to tree years of conversion. In the medium and long term, when soil fertility recovers, yields are slightly lower or comparable to the pre-conversion yields (DIERAUER *et al.* 2006). The presence of mycotoxins, especially deoxynivalenol (DON) is a major concern for grain growers using any system (MOSS 1991). Host resistance is the main way to control *Fusarium* head blight (FHB) in wheat. Despite improved levels of resistance to infection and spread in vegetative tissue, the toxin deoxynivalenol (DON) can still accumulate to unacceptable concentration levels. In this study, our objectives were to assess the spelt (*Triticum spelta* L.) varietes grown in ecological farming systems to Fusarium species occurrence in the kernels (RKI) and resistance to toxin accumulation (RTA) and their role in controlling of DON concentration in kernels.

MATERIAL AND METHODS

Field experiments with six varietes of *T. spelta* was carried out at Dolná Malanta, Western Slovakia (18°07´E, 48°19´N) from 2007 to 2008 on a Haplic Luvisol developed at proluvial sediments mixed with loess. The altitude of the experimental plots is 178 m. The location has a continental climate with an average temperatures 19,7°C in July and - 1,7°C in January, an average annual precipitation is 561 mm.

Table 1

Crop management data for *T. spelta*, 2007-2008; climatic data during vegetative period

Year	Sowing date	Harvest date	Rainfall (mm)	Average temperature (°C)
2007	2.10. 2006	2.07. 2007	348,4	11,3
2008	9.10. 2007	28.07. 2008	493,5	8.9

For estimating of *Fusarium* species occurrence in the kernels, the samples (symptom free grains) were separated from yield in each cultivar at the time of harvest. In the laboratory, the kernels (220 from each variant) were surface sterilized with NaOCl (3% for 3 min), rinsed twice with sterile water, dried on filter paper, transferred onto PDA agar and incubated for 7 days at 21 ± 2 °C, 12/12 photoperiod to record the percentage of kernels infected by *Fusarium* spp. Fungal cultures were separated and transferred to PDA to obtain pure cultures for the morphological identification of species according to NELSON et al. (1983). The occurrence of *Fusarium* species in each cultivar were expressed as isolation frequency (IF) according to GONZÁLES et al. (1999).

The concentration of deoxynivalend (DON) was detected in the kernel samples by using of HPLC method. The liquid chromatography WATERS BREEZE (Lambda Life, Slovakia) equipped with binary pump WATERS 1525, RHEODYNE injector and UV detector WATERS 2487 was used with a stainless steel reverse phase 150x3,9 mm, 4 μm spherical particle C18Nova-Pak column (Waters, Milford, MA, USA). Rotary vacuum evaporator RVO 400 (INGOS, Prague, Czech republic) for evaporating samples was used. 50 μL of the samples were injected into the HPLC column heated on 35°C and determined by UV detector set to 218 nm for DON. Mobile phase were mix of A: water, B: acetonitrile with using gradient: 0-5 min 10 % B, 6-16 min 10-25 % B, 1735 min 25-60 % B, 36-40 min 60 % B, 41 min 10 % B. Flow rate was 0,6 mL/min mobile phase. Retention time was for DON 13,4 min.

RESULTS AND DISCUSSION

All of the six tested varietes in 2007 and 2008 were DON positive. The overall levels of DON from spelt wheat samples are given in Table 1. The obtained results indicate that spelt grain contain considerable concentrations of trichothecenes. The effect of varieties on DON concentration was significantly different, with the year x variety interaction also being significant. The lowest DON concentration was determined in Rouquin (165 μg.kg^{-1}), whereas the highest production of mycotoxin was found in Holstenkorn and Oberkulmer Rotkorn (676,4 and 545,4 μg.kg^{-1}). In three varieties (Bauländer Spelz, Franckenkorn, Oberkulmer Rotkorn) significant differences of DON concentrations between years were determined. Average DON contamination of samples from 2007 was higher than the contamination of samples from 2008, but difference was not significant. In two consecutive years, not significantly different concentration of DON was determined in kernels of Holstenkorn, Rouquin and Rubiota. In other three varietes, the effect of year on DON concentration was significant. In Bauländer Speltz and Oberkulmer Rotkorn higher DON concentration was found in 2007, in Franckenkorn better conditions for grain contamination were in 2008. In any case the levels of DON did not exceed the maximum levels according to EU regulation 1881/2006. All of the six tested varietes in 2007 and 2008 were infested by *Fusarium* species. The overall levels of *Fusarium* infestation of grains are given in Table 1. The effect of varietes and years on *Fusarium* infestation of kernels (FKI) were significantly different, interaction variety x year was also significant. In 2008, with higher level of precipitations during vegetative period of spelt wheat, the average FKI was significantly higher. The highest average infestation was recorded in Bauländer Speltz and Franckenkorn, the varietes Rouquin and Rubiota showed the lowest level of FKI. The effect of year on *Fusarium* infestation of kernels was significant for Bauländer Spelz, Franckenkorn and Holstenkorn, in other three varietes not significant differences in years and FKI was found. The pattern of weather influence on FKI and DON concentration was different.

The susceptibility of wheat cultivars to *Fusarium* kernel infection had no significant influence on DON accumulation in kernels. Some genotypes consistently had low *Fusarium* infestation in their grain despite of increased DON contamination. Additionally, some genotypes consistently had low DON concentration in their grain and increasing kernel infestation suggests a higher resistance to toxin accumulation (RTA) in these genotypes. Variety Rouquin showed the lowest *Fusarium* infestation and also DON contamination. It seems to be the most resistant genotype to *Fusarium* infection (FKI) and DON contamination (RTA) of the kernels among the tested cultivars of *Tr. spelta*. Opposite, the Holstenkorn showed the highest susceptibility to DON contamination, with medium *Fusarium* infestation. The correlation between *Fusarium* infestation and DON contamination was very low, correlation coefficient is -0,09. The good example of different susceptibility to kernels infestation and resistance to DON forming is demonstrated by cv. Bauländer Speltz. It was infested the most, but the DON content is in medium level in both the years. The variation for FKI and RTA explained a significant fraction of the variation for DON among genotypes with moderate kernel infestation using independent grain samples. Thus, variation for RTA was important in explaining variation for DON among genotypes with acceptable levels of resistance to fungal infection and spread. This work indicates that there is a need for better understanding of relationship between RTA and FKI.

Table 1

DON concentration and *Fusarium* infestation of *Tr. spelta* kernels, in 2007 and 2008.

Variety	DON (µg.kg^{-1})			*FKI (%)		
	2007	2008	Average	2007	2008	Average
Bauländer Spelz	407,5 b	164,5 a	286,0 AB	15,75 a	40,67 b	28,21 D
Franckenkorn	230,7 a	570,2 b	400,5 B	6,37 a	35,32 b	20,85 C
Holstenkorn	617,2 n.s.	735,5 n.s.	676,4 D	17,77 b	5,55 a	11,66 B
Oberkulmer Rotkorn	722,7 b	368,0 a	545,4 C	14,61 n.s.	11,80 n.s.	13,21 B
Rouquin	154,2 n.s.	175,7 n.s.	165,0 A	7,51 n.s.	3,67 n.s.	5,59 A
Rubiota	530,0 n.s.	455,2 n.s.	492,6 BC	0,95 n.s.	0,93 n.s.	0,94 A
Average	443,7	411,5		10,49 a	16,32 b	

*FKI – Infestation of kernels by *Fusarium* species

Values within rows followed by different small letters are significantly different (LSD test, α = 0,01)

Values within rows followed by different capital letters are significantly different (LSD test, α = 0,01)

CONCLUSIONS

Concentrations of DON in kernels of six *Tr. spelta* varietes were significantly influenced by variety and interaction of year and variety. The lowest DON concentration was determined in Rouquin, the highest production of mycotoxin was found in Holstenkorn and Oberkulmer Rotkorn. The detected levels of DON did not exceed the maximum levels according to EU regulation 1881/2006. The effect of varietes and years on *Fusarium* infestation of kernels was significant. The susceptibility of spelt varietes to *Fusarium* kernel infestation had no influence on DON accumulation in kernels. Correlation coefficient was -0,09. The variety Rouquin showed the lowest FKI and also DON concentration. On the opposite, Bauländer Speltz with the second lowest DON concentration had the highest FKI. There is a need for better understanding of the relationship between resistance to toxin accumulation in grain and *Fusarium* infection of kernels.

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