FUSARIUM SPP. INFECTION OF SPRING BARLEY IN THE CZECH REPUBLIC AND POSSIBILITIES OF INTEGRATED CONTROL OF THIS DISEASE

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Abstract

Three hundred and sixty spring barley genotypes from the world gene collection were tested in a field trial under the artificial infection with conidia suspension of *Fusarium culmorum.* Six barley cultivars registered for growing in the Czech Republic were tested for DON-toxin accumulation after strong scab inoculation. The effect of plant treatment with a fungicide (tebuconazole 250 g/ha) was assessed.

An infection level as well as DON content were significantly reduced by the application of the fungicide. There was significant influence of barley genotype on final reaction under conditions of inoculation and also with or without the fungicide spraying.

The mean level of DON in the trial was 9.2 mg/kg, minimum concentration was 0.36 mg/kg DON and maximum reached 35.4 mg/kg DON. The lowest DON accumulation combined with zero incidence of powdery mildew (*Blumeria graminis* f. sp. *hordei*) and low and medium net blotch (*Drechslera teres*) infection were found in cvs Princesse and Union Firlbecks.

The possibilities of combining genotypically based resistance with the application of effective fungicides are discussed.

Key words: barley, scab, *Fusarium culmorum,* resistance, fungicides, integrated protection systems.

Introduction

In some years, malting barley harvested in Finland, Sweden, Denmark and Scotland has a very heavy infection by *Fusarium*, and thus not all of the harvest will be accepted by the brewing industry (Larsen, 2000). The main cause of the economic loss in malting barley is the presence of deoxynivalenol (DON) or vomitoxin, a mycotoxin produced by the fungus. Studies in Germany revealed that DON has the highest frequency of occurrence in wheat, oats and barley, with a contamination rate of 30—90% (Drochner, Lauber, 2001). In Europe, *Fusarium* infection is responsible for beer gushing, but in North America the presence of mycotoxins discriminates the barley for use in the brewing industry.

Breeding firms develop extensive phytopathological programmes aiming at the detection of donors with higher resistance to FHB. Fruitful international cooperation among gene banks and research centres is one of preconditions of success in this field.

Producers of agrochemicals have developed and registered on the market some fungicidal active ingredients which are effective against FHB (*Fusarium* head blights). One of them is triazole molecule tebuconazole, which has been used as an effective standard in many studies (Jones, 2000; Hudec et al., 2003; Jorgensen, Jensen, 2003).

On the other hand, the FHB epidemic development is controlled by many independent factors including weather conditions and therefore the optimal timing of fungicide application is very difficult to determine (Kaminski, 2003).

Barley growing and breeding have a long-term tradition in the Czech Republic. The collection of genetic resources housed at the Agricultural Research Institute Kromeriz, Ltd., comprises a large number of foreign genotypes but as well as local varieties and landraces. These accessions are assumed to be well adapted to the conditions where they were developed. Therefore, it is useful to recognize their reactions to current biotic stresses that also include infection by the most frequent diseases.

The objective of this programme was to detect cultivars and genotypes within the world barley collection that are less infected by FHB and to assess genotype-dependent reaction of spring barley cultivars to fungicide treatment under the conditions of strong FHB epidemic.

Materials and Methods

1. Reaction of spring barley genotypes to FHB

The trials were conducted in fields of the Agricultural Research Institute Kroměříž, Ltd., in the season of 2003 (average annual temperature 8.7 °C, average annual precipitation 599 mm).

Ninety-eight spring barley genotypes were sown in two replications using a small-plot drill. Each replication was 1 m^2 in size.

The heading date and plant height were assessed in all genotypes.

The trials were artificially inoculated with spores of *Fusarium culmorum*. The concentration of the inoculum was adjusted to 6 million conidia per ml. Inoculation was carried out at full anthesis (DC 65) in five terms depending on genotypic differences. The assessment was carried out in 2—5 weeks after inoculation in the field as the necrotic area of spikes. We assessed twenty spikes for each genotype.

The DON-toxin content in harvested grains of the barley genotypes was detected using an ELISA method. Data on *Fusarium* infection were completed with field reactions to other leaf diseases. Powdery mildew (*Blumeria graminis*

f. sp. *hordei*), net blotch (*Pyrenophora teres*) and leaf stripe disease (*Helminthosporium gramineum*) were assessed during the whole growing season. The results were statistically compared.

2. Reaction of spring barley genotypes to *tebuconazole* treatment under the conditions of strong FHB infection

Six spring barley genotypes registered for growing in the Czech Republic were sown in twelve replications using a micro-plot seeder in years 2002 and 2003. Each replication was 1 m^2 in size.

The trials were artificially inoculated with spores of *Fusarium culmorum*. The concentration of the inoculum was adjusted to 6 million conidia per ml. Six replications were sprayed with the fungicide Horizon 250 EC (a.i. tebuconazole 250 g/l) and three of them 24 hours later inoculated with *F. culmorum*. Four variants were established:

- A: no FHB infection,
- B: FHB infection,
- C: no FHB infection +treatment with tebuconazole 250 g/ha,
- D: FHB infection + treatment with tebuconazole 250 g/ha.

The content of DON mycotoxin in harvested grains was detected using an ELISA method in all variants.

Results and Discussion

Experiment 1:

ANOVA confirmed highly significant differences in grain contamination by DON mycotoxin (Table 1). The mean value of DON was 9.24 mg/kg. The highest level was found in the cultivar Philadelphia (25.4 mg/kg). Another cultivars exhibiting significantly high DON concentrations were: Early Chevalier, Ceres, Morgenrot, Ymer, Isaria Nova and Provost.

Table 1

ANOVA of DON content (mg/kg) in infected grains of the genotypes assessed

Source of variation	d.f.	Mean square	Significance
genotype	97	113.973	hs
residual	196	0.0186	

Note: hs = significant at α 0.01.

Among 11 cultivars with the lowest DON content, there were seven cultivars whose percentage of visual infection by FHB assessed in the field was low, too. Two genotypes displayed medium and two genotypes — high infection. The important traits of genotypes with low DON in grains are summarized in Table 2.

However, the non-significant correlation was found between grain infection and DON contamination after harvest for all tested cultivars (Table 3). Another analyses indicate that the heading date did not affect final fusarium infection, but plant height at the heading stage was in highly significant negative correlation with an infection level. Higher susceptibility to FHB was accompanied by increased susceptibility to net blotch at high significance. No relationship was found between the susceptibility to FHB and that to powdery mildew and leaf stripe disease.

Table 2

Agronomically important traits of barley genotypes with low DON accumulation in grains

Genotype	DON (mg/kg)	Fusarium head blight	Heading date	Height (cm)	*B. graminis*	*P. teres*	*H. gramineum*
PRINCESSE	0.357	LS	06.06.	80	R	LS	HS
SELECTA HANAK. 1	0.766	MS	10.06.	105	LS	LS	LS
UNION FIRLBECKS	1.058	LS	10.06.	80	R	MS	R
SPARTAN	1.226	LS	10.06.	85	MS	LS	R
JERSEY	1.300	HS	06.06.	80	R	HS	R
KM 1192	1.326	LS	10.06.	75	R	LS	R
DIGGER	1.385	LS	10.06.	75	LS	LS	LS
DIPLOM	1.500	HS	06.06.	87	LS	HS	HS
DOMEN	1.538	LS	10.06.	90	LS	LS	LS
HODONÍNSKÝ KVAS	1.771	MS	06.06.	105	MS	LS	LS
OPÁL	1.959	LS	06.06.	95	R	LS	LS

Note: field disease assessment key: R — resistant, LS — low susceptible, MS — medium susceptible, HS — highly susceptible.

Table 3

Correlation coefficients between *Fusarium culmorum* infection traits (spike infection and DON content) and other characteristics

	Spike infection	Significance	DON (mg/kg)	Significance
Spike infection		ns	0.07	ns
DON (mg/kg)	0.07	ns		ns
Heading date	0.00	ns	–0.18	ns
Plant height (cm)	0.02	ns	–0.27	ns
B. graminis	0.00	ns	–0.17	ns
P. teres	–0.13	ns	0.39	hs
H. graminearum	0.08	ns	–0.07	ns

Note: ns = non-significant, hs = significant at α 0.01.

We assessed the field reaction to FHB in a large collection of genebank accessions which are variable in many agronomic and growth parameters. The main attention was given to resistance to fungal diseases.

There are two cultivars with low DON accumulation which were registered in former Czechoslovakia before the Second World War. 'Selecta Hanak 1', registered in 1926, is a midlate cultivar (111 days), susceptible to powdery mildew, medium susceptible to net blotch and rust. The spike has a high grain number (26) with relatively high TKW. 'Hodonínský Kvas', registered in 1937, is also a midlate cultivar (105—115 days), susceptible to powdery mildew, medium susceptible to rust, but highly resistant to net blotch.

There are two cultivars with a low DON grain content which have been registered for growing in the Czech Republic after 2000 and which are now planted on a large area of malting barley fields. The first of them is 'Jersey' (Cebeco Zaden, B.V. Vlijmen, The Netherlands). The midlate genotype with medium height of stem (76 cm), high resistance to powdery mildew (Mlo gene), medium susceptibility to scald and high susceptibility to net blotch, and a high level of malting quality. The second one is 'Diplom' (Nordsaat Saatzucht, Germany). Medium resistance to powdery mildew, susceptibility to net blotch and rust is combined with high malting quality.

The genotypes with low DON accumulation include 'KM 1162' which was bred by the former Cereal Research Institute Kromeriz. The midlate cultivar has medium plant height (71—80 cm), medium resistance to resistance to powdery mildew, and it could be used as a resistance source to net blotch and rust.

This short review of some accessions from our experiment with low DON accumulation evidences that the higher resistance to FHB can be found on variable genetic background and can be influenced by different factors. We did not find any significant correlation between traits of FHB resistance and agronomic parameters: plant height or heading date. The influence of both these factors, which can significantly change the disease rate under natural epidemic conditions, is completely eliminated under conditions of artificial infection with conidia suspension, which was made regularly when particular genotypes coming to the flowering stage. On the other hand, the significant correlation was found between DON content and net blotch resistance. This finding could probably stand for the set of genotypes tested in this project only.

Experiment 2:

ANOVA showed a highly significant effect of the genotype and fungicide treatment on final DON content (Table 4). The highest level of mycotoxin content was found in cv. TOLAR under artificial inoculation with FHB (Table 5). *Tebuconazole* reduced grain contamination with DON to 60% compared with the non-treated control. High infection by FHB resulting in a high DON level was also found in cv. AKCENT. On the contrary, the lowest DON content was found in both treated and non-treated variants of cv. JERSEY.

Table 4

ANOVA of DON (mg/kg) grain content

Source of variation	d.f.	f-Ratio	Significance
Treatment	3	54.96	hs
Year	1	0.08	ns
Genotype	5	13.34	hs

Note: hs — significant at 0.01, ns — nonsignificant.

Table 5

Two years mean DON level (mg/kg) in selected spring barley cultivars

	A	B	C	D
OLBRAM	0.14	12.20	0.12	6.78
TOLAR	0.59	27.15	0.21	15.80
KOMPAKT	0.48	15.45	0.17	11.64
SCARLETT	0.46	16.60	0.18	12.68
AKCENT	2.37	19.55	0.74	18.37
JERSEY	0.16	1.35	0.30	1.14
Mean	0.70	17.41	0.29	11.56

Note: - A: no FHB infection,
- B: FHB infection,
- C: no FHB infection + treatment with tebuconazole, 250 g/ha,
- D: FHB infection + treatment with tebuconazole, 250 g/ha.

The possibility to reduce the risk of high DON occurrence in harvested grain is strongly influenced by genetic backround of grown cultivars. The efficacy of fungicide treatment against FHB can reduce DON content under the level acceptable for human consumption in moderately or highly resistant genotypes only. The combination of both approaches is therefore the strategy of protection against FHB in spring barley growing.

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