

Yield reduction and mycotoxin accumulation in barley doubled haploids inoculated with *Fusarium culmorum* (W.G.Sm.) Sacc.

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Abstract. Barley doubled haploids (DH) were examined for their susceptibility to *Fusarium* head blight caused by *Fusarium culmorum*. DH lines were derived from F₁ Maresi (two-rowed) × Pomo (six-rowed) hybrids by the „H. bulbosum“ method. Doubled haploids, parental cultivars and F₁ and F₂ hybrids were inoculated with *Fusarium culmorum* (W.G.Sm.) Sacc., isolate KF350 under field conditions. The kernel infection score, number of kernels per ear, kernel weight per ear, 1000-kernel weight, and kernel fractions were recorded in inoculated and control plants. Samples of kernels were analysed for presence of nivalenol and deoxynivalenol. In the inoculated plants a reduction of kernel number, kernel weight per ear, 1000-kernel weight and percentage of plump kernels was observed. Generally, inoculation caused a significant decrease in the kernel fraction > 2.5 mm, and increase in the fractions 2.5-2.2 and < 2.2 mm. This tendency was more visible in 2-rowed than in 6-rowed lines. The nivalenol content of inoculated doubled haploids ranged from 0.16 to 7.61 mg/kg, whereas their deoxynivalenol content ranged from 0.000 to 0.253 mg/kg. Significant relationships between the kernel infection score and nivalenol content, kernel yield per ear, 1000-kernel weight and kernel fraction > 2.5 mm were observed. Transgression effects were noted in some DH lines, in which the reduction of kernel characters was lower than in parental cultivars. Doubled haploids with a positive and negative transgression for nivalenol and deoxynivalenol content were also recorded.

Key words: barley, doubled haploids, *Fusarium culmorum*, head blight, deoxynivalenol, nivalenol.



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Introduction

Fusarium head blight (=scab) caused by *Fusarium culmorum* is a destructive disease of small grain cereals. Epidemics of *Fusarium* scab of barley in the upper Midwest region of the USA were observed in 1993-1996 (PROM et al. 1997, SALAS et al. 1997). The disease can drastically reduce grain yield and its quality, causing great economic losses (BAI, SHANER 1994, McMULLEN et al. 1997, SCHWARZ et al. 1997).

F. culmorum was found to be able to produce trichothecene mycotoxins, like deoxynivalenol (DON), 3- and 15-acetyldeoxynivalenol (3-AcDON, 15-AcDON), and nivalenol (NIV) (MIROCHA et al. 1994, SNIJDERS, PERKOWSKI 1990, PERKOWSKI et al. 1990, 1995, 1996, 1997a). Grain containing *Fusarium* metabolites can be toxic to animals, particularly to pigs, and to humans (e.g., BAUER et al. 1989, KIM et al. 1993). Research showed that yield reduction and mycotoxin accumulation are dependent on pathogen isolate and plant genotype (MILLER et al. 1985, MASTERHAZY 1989, SNIJDERS, PERKOWSKI 1990, PERKOWSKI et al. 1996, 1997a). Remarkable differences in the susceptibility of barley cultivars and lines were detected by PERKOWSKI et al. (1995, 1996, 1997a). TAKEDA (1990) reported on positive parent-offspring correlation for resistance to *Fusarium* head blight caused by *Gibberella zeae* which occurred between successive generations of five barley crosses. This indicates that the level of susceptibility can be changed by recombination in segregating generations and that selection of more resistant individuals/lines is possible.

During the past two decades extensive research on the use of doubled haploids (DH) in barley breeding was carried out (for review see PICKERING, DEVAUX 1992). DH lines are completely homozygous, and the population of DH lines derived from F₁ hybrids represents a random sample of gametes from F₁ hybrids. For this reason DH lines are also good materials for genetic studies on quantitative and qualitative traits. In the present paper the phenotypic distribution of barley doubled haploids derived from a cross between two- and six-rowed cultivars was studied with respect to grain yield reduction and mycotoxin accumulation after inoculation with *Fusarium culmorum*. The study aims to establish the range of susceptibility of barley DH lines to *Fusarium* head blight and to evaluate the relationships between grain weight reduction, mycotoxin (DON and NIV) content and grain infection score.

Material and methods

Barley doubled haploids (DH) were studied for their susceptibility to *Fusarium* head blight (FHB).

DH lines were derived from F₁ Maresi (two-rowed) × Pomo (six-rowed) hybrids by the „H. bulbosum“ method (KASHA, KAO 1970, ADAMSKI 1979).

Twenty-four DH lines, 11 two-rowed and 13 six-rowed, along with initial cultivars and F₁ and F₂ hybrids, were examined in a field experiment carried out in the complete randomized block design with three replications. The materials were planted in 2 m² plots with 10 × 3 cm spacing between plants. At the stage of full anthesis 180 heads of each line (60 heads per plot) were inoculated by brushing single heads with 2 mL of conidial suspension (5 × 10⁶ in 1 mL) of *Fusarium culmorum* (W.G.Sm.) Sacc., isolate KF350. Non-inoculated plots of the same size were used as controls. Inoculated heads were covered for 48 h with plastic bags. At the stage of full maturity heads from inoculated and control plants were collected and threshed manually.

The infection score of kernels was estimated in 0-5 scale, where 0 means no infection and 5 denotes the highest infection. The number of kernels per ear, kernel weight per ear, 1000-kernel weight, and kernel fractions (the proportion of kernels that passed through a 2.8 mm sieve, a 2.5 mm sieve and a 2.2 mm sieve) were recorded in inoculated and control plants. Samples of kernels originating from inoculated and control plants were analysed for presence of trichothecene mycotoxins (nivalenol, NIV, and deoxynivalenol, DON). Samples were extracted with aqueous acetonitrile and purified using charcoal columns (SNIJDERS, PERKOWSKI 1990). The extract was dissolved by sonification for 2 min in chloroform/acetonitrile (4 : 1). Analyses were performed on a HP 6890 gas chromatograph coupled with a mass spectrometer. Trichothecenes were detected and quantified in a Selected Ion Monitoring mode. DON and NIV were analysed as trimethylsilyl derivatives. The quantitation limit of the complete method was 5-10 µg/kg, and average recoveries of the toxin were 78-91% (PERKOWSKI et al. 1997b).

Two-way analysis of variance was carried out to study the influence of genotypes and inoculation on phenotypic variability of DH lines with respect to yield structure characters, and one-way analysis of variance with respect to mycotoxin accumulation. Correlation coefficients were calculated to examine the relationships between characters under study.

Results

Phenotypic values of yield structure characters (averages over replications) and kernel infection score observed in inoculated and control plants are presented in Tables 1 and 2. Analysis of variance showed the significant effect of genotypes and inoculation on variability of the studied characters in DH lines (Table 3). It may be noticed that the influence of inoculation was greater than that of genotypes, which is reflected in higher values of F-statistic for inoculation than for genotypes.

The infection score in DH lines ranged from 1 to 5, whereas in parental cultivars Maresi and Pomo it was 2 and 3, respectively. In the inoculated plants

Table 1. Kernel infection score and yield structure characters in barley DH lines inoculated (I) and non-inoculated (C) with *F. culmorum*

Genotype	Infection score*		No. of kernels per ear		Kernel weight per ear (g)		1000-kernel weight (g)	
	I	C	I	C	I	C	I	C
2-rowed DH lines								
MP 7	2.0	0	23.1	23.4	1.06	1.24	45.5	53.6
MP 33	3.7	0	20.4	22.6	0.78	1.01	34.7	49.4
MP 39	4.0	0	18.9	20.1	0.79	1.11	41.6	55.4
MP 56	2.7	1	22.6	24.2	1.07	1.16	44.3	51.6
MP 98	3.5	0	24.0	25.5	1.10	1.29	43.2	53.6
MP 104	3.0	0	22.9	24.0	0.87	0.91	36.3	39.6
MP 105	5.0	0	22.1	23.6	0.83	1.15	37.4	48.5
MP 112	3.7	0	23.8	24.6	0.98	1.28	41.3	52.2
MP 115	3.0	1	25.9	27.0	1.27	1.32	46.8	51.0
MP 118	3.7	0	22.7	23.5	0.94	1.15	39.9	50.3
MP 144	1.0	0	22.0	22.3	1.04	1.16	46.6	52.8
6-rowed DH lines								
MP 2	3.3	0	33.6	37.6	1.13	1.57	33.6	41.7
MP 22	2.7	0	43.8	50.8	1.13	1.58	25.9	31.2
MP 32	4.0	0	32.7	35.4	1.25	1.42	38.4	40.1
MP 50	4.0	0	53.2	54.2	1.26	1.63	23.3	30.6
MP 51	3.7	0	44.6	49.0	1.07	1.55	24.0	31.6
MP 62	4.0	0	35.3	38.0	0.92	1.07	24.1	30.4
MP 79	4.0	0	39.4	50.4	0.97	1.58	24.6	31.3
MP 80	2.7	2	38.6	39.4	1.33	1.44	33.7	37.2
MP 85	1.7	0	49.4	62.3	1.69	2.21	34.1	35.6
MP 113	5.0	0	40.0	47.4	0.69	1.08	17.5	22.7
MP 127	1.3	0	55.4	58.7	1.61	1.87	28.8	31.9
MP 135	4.5	0	29.2	40.2	0.79	1.18	26.9	29.2
MP 136	5.0	0	34.2	42.7	0.97	1.13	23.0	29.2
Maresi	2.0	0	22.3	23.0	0.92	1.08	39.8	48.4
Pomo	3.0	0	55.4	58.8	1.61	2.19	29.2	37.2
F ₁	3.0	0	28.6	29.9	1.08	1.27	39.4	44.5
F ₂	3.0	0	31.5	33.0	1.01	1.23	32.0	36.9

* in 0-5 scale, where 0 means no infection and 5 denotes the highest infection.

Table 2. Kernel fractions (%) in barley DH lines inoculated (I) and non-inoculated (C) with *F. culmorum*

Genotype	Kernel fraction					
	> 2.5 mm		2.5-2.2 mm		< 2.2 mm	
	I	C	I	C	I	C
2-rowed DH lines						
MP 7	77.6	91.1	18.5	2.7	3.9	6.2
MP 33	53.5	88.0	29.9	6.8	16.6	5.2
MP 39	85.6	95.0	11.7	3.6	2.8	1.4
MP 56	75.5	80.7	14.8	12.9	9.7	6.4
MP 98	74.0	85.5	23.4	5.2	2.6	9.3
MP 104	59.2	69.5	26.8	18.2	14.0	12.3
MP 105	64.7	85.0	22.3	9.5	13.0	5.5
MP 112	52.8	81.0	28.2	15.4	19.0	3.6
MP 115	85.1	92.3	11.7	3.4	3.2	4.3
MP 118	89.8	93.3	7.6	3.0	2.6	3.7
MP 144	86.6	86.1	10.6	12.6	2.8	1.3
6-rowed DH lines						
MP 2	73.0	75.4	14.3	11.4	12.7	13.2
MP 22	62.3	57.3	9.0	8.0	28.7	34.7
MP 32	76.5	79.6	12.1	13.6	11.4	6.9
MP 50	28.4	32.0	26.3	32.8	45.3	35.2
MP 51	43.8	54.5	18.7	9.0	37.5	36.5
MP 62	49.7	55.5	14.7	11.7	35.6	32.8
MP 79	35.1	48.4	23.6	26.1	41.3	25.5
MP 80	72.5	73.4	9.9	11.9	17.6	14.7
MP 85	61.5	65.3	20.6	21.6	17.9	13.1
MP 113	13.8	20.9	17.2	20.1	69.0	59.0
MP 127	42.7	44.4	24.7	23.3	32.6	32.3
MP 135	64.0	69.6	14.3	13.4	21.7	17.0
MP 136	51.8	67.5	13.1	6.1	35.1	26.3
Maresi	72.4	78.6	17.3	14.5	10.4	6.9
Pomo	49.4	66.9	28.4	23.5	22.2	9.6
F ₁	79.2	78.2	10.3	12.7	10.4	9.1
F ₂	72.5	85.4	13.4	7.1	14.0	7.5

Table 3. Analysis of variance for yield structure characters of barley DH lines

Source of variation	D.F.	F-statistic						F _{0.05}	F _{0.01}
		no. of kernels per ear	kernel weight per ear	1000-kernel weight	kernel fraction > 2.5 mm	kernel fraction 2.5-2.2 mm	kernel fraction < 2.2 mm		
Inoculation (I)	1	14.0	184.5	343.6	100.6	94.1	60.6	3.92	6.85
Genotype (G)	27	87.6	29.9	84.9	71.4	22.7	111.9	1.61	1.95
Interaction I×G	27	2.8	2.8	3.3	4.6	7.2	4.3	1.61	1.95
Error	110								

a reduction of kernel number and kernel weight per ear, as well as 1000-kernel weight and percentage of plump kernels was observed (Table 4). Generally, inoculation caused a significant decrease in kernel fraction > 2.5 mm, and increase in the fractions 2.5-2.2 and < 2.2 mm. This tendency was more visible in 2-rowed than in 6-rowed lines, probably because 6-rowed lines have a relatively low percentage of kernels > 2.5 mm; the average share of the fraction > 2.5 mm in the non-inoculated 2-rowed lines was 86.1%, and in the non-inoculated 6-rowed lines it amounted to 57.2% (Table 2). The studied doubled haploids differed in the reduction of yield characters after inoculation with *F. culmorum*. A wide range of reduction was observed in respect of kernel weight per ear (from 96.2 to 61.4% of the control) and 1000-kernel weight (from 95.8 to 70.2% of the control). In the case of the kernel fraction > 2.5 mm, no reduction was noticed in two lines (MP144, MP22), and a maximum reduction (about 60% of the control) in two lines (MP33 and MP113).

The isolate KF350 of *F. culmorum*, that was used for inoculation, was the nivalenol type which produced under laboratory conditions mostly nivalenol and a small amount of deoxynivalenol. The nivalenol and deoxynivalenol content of infected kernels is presented in Table 5. It should be noted that accumulation of NIV and DON in kernels of non-inoculated plants was very low and amounted to (average over the lines) 0.01 and 0.00 mg/kg, respectively. Analysis of variance showed that variation of the lines in mycotoxin accumulation was significant (Table 6). The nivalenol content of parental cultivars was similar: 1.00 mg/kg for Pomo and 0.65 mg/kg for Maresi. The nivalenol content of inoculated doubled haploids ranged from 0.16 (MP32) to 7.61 (MP113) mg/kg, whereas their deoxynivalenol content ranged from 0.000 (MP7, MP32) to 0.253 (MP113) mg/kg.

Correlation coefficients between the studied characters in inoculated DH lines are presented in Table 7. Significant relationships between the kernel infection

Table 4. Reduction of kernel weight and kernel size (expressed as a percentage of the control) in barley DH lines inoculated with *F. culmorum*

Genotype	No. of kernels per ear	Kernel weight per ear	1000-kernel weight	Kernel fraction (mm)		
				> 2.5	2.5-2.2	< 2.2
2-rowed DH lines						
MP 7	98.7	85.4	84.8	85.2	685.2	62.9
MP 33	90.3	77.2	70.2	60.8	439.7	319.2
MP 39	93.9	71.2	75.1	90.0	325.0	200.0
MP 56	93.4	92.2	85.8	93.6	114.7	151.6
MP 98	94.1	85.2	80.6	86.5	450.0	27.9
MP 104	95.4	95.6	91.7	85.2	147.2	113.8
MP 105	93.6	72.1	77.1	76.1	234.7	236.4
MP 112	97.1	76.6	79.1	65.1	183.1	527.7
MP 115	95.9	96.2	91.8	92.2	344.1	74.4
MP 118	96.6	81.7	79.3	96.2	253.3	70.3
MP 144	98.6	89.6	88.2	100.6	84.1	215.4
6-rowed DH lines						
MP 2	89.3	71.9	80.5	96.8	125.4	96.2
MP 22	86.2	71.5	83.0	108.7	112.5	82.7
MP 32	92.4	88.0	95.7	96.2	89.0	165.2
MP 50	98.1	77.3	76.1	88.8	80.2	128.7
MP 51	91.0	69.0	75.9	80.4	207.8	102.7
MP 62	92.9	85.9	79.3	89.5	125.6	108.5
MP 79	78.2	61.4	78.6	72.5	90.4	162.0
MP 80	97.9	92.3	90.6	98.8	93.2	119.7
MP 85	79.3	76.5	95.8	94.2	95.4	136.6
MP 113	84.4	63.9	77.1	66.0	85.5	117.0
MP 127	94.3	86.1	90.3	96.1	106.0	100.9
MP 135	72.6	66.9	92.1	92.0	106.7	127.6
MP 136	77.5	85.8	78.8	76.7	211.3	133.4
Maresi	96.9	85.2	82.2	92.1	119.3	150.9
Pomo	94.2	73.5	78.5	73.9	120.9	231.1
F ₁	95.6	85.0	88.5	101.2	81.1	114.3
F ₂	95.4	82.1	86.7	84.9	188.7	186.7

Table 5. Nivalenol and deoxynivalenol content (mg/kg) in kernels of barley DH lines inoculated with *F. culmorum*

Genotype	Nivalenol	Deoxynivalenol
2-rowed DH lines		
MP7	0.18	0.000
MP 33	2.98	0.016
MP 39	2.53	0.033
MP 56	1.03	0.020
MP 98	3.59	0.033
MP 104	2.37	0.053
MP 105	3.99	0.053
MP 112	0.76	0.066
MP 115	0.56	0.006
MP 118	2.30	0.043
MP 144	0.51	0.020
6-rowed DH lines		
MP 2	0.53	0.030
MP 22	2.30	0.076
MP 32	0.16	0.000
MP 50	1.24	0.026
MP 51	1.38	0.013
MP 62	2.42	0.030
MP 79	2.26	0.060
MP 80	1.21	0.020
MP 85	0.81	0.023
MP 113	7.61	0.253
MP 127	0.60	0.036
MP 135	5.15	0.090
MP 136	0.43	0.003
Pomo	1.00	0.020
Maresi	0.65	0.013
F ₁	1.05	0.032
F ₂	1.02	0.020
6-rowed DH : 2-rowed DH	1.06	1.640

Table 6. Analysis of variance for nivalenol and deoxynivalenol content in kernels of barley DH lines inoculated with *F. culmorum*

Source of variation	D.F.	F-statistic		F _{0.05}	F _{0.01}
		nivalenol	deoxynivalenol		
Genotypes	27	2.156	2.662	1.67	2.07
Error	84				

Table 7. Correlation coefficients between infection score, mycotoxin content and yield structure characters in barley DH lines inoculated with *F. culmorum*

Character	Infection score	NIV	DON
NIV	0.56**		
DON	0.38	0.83**	
No. of kernels per ear	-0.38	-0.37	-0.33
Kernel weight per ear	-0.44*	-0.51**	-0.54**
1000-kernel weight	-0.49**	-0.29	-0.17
Kernel fraction			
> 2.5 mm	-0.51**	-0.37	-0.32
2.5-2.2 mm	0.01	-0.03	-0.27
< 2.2 mm	0.17	-0.06	0.03

* P ≤ 0.05

** P ≤ 0.01

score and nivalenol content, kernel yield per ear, 1000-kernel weight and kernel fraction >2.5 mm were observed. Accumulation of mycotoxins (both nivalenol and deoxynivalenol) appeared significantly higher in the lines with a higher reduction of kernel weight per ear. A high and positive correlation occurred between nivalenol and deoxynivalenol content ($r = 0.83$, $P < 0.01$).

Discussion

In the present paper the results of studies on the phenotypic distribution of susceptibility of two- and six-rowed barley DH lines to *Fusarium* head blight caused by *F. culmorum* are outlined. The susceptibility was measured by the reduction of kernel yield and fraction of plump kernels. It was observed that two-rowed lines were more resistant than six-rowed. Similar results were reported by

TAKEDA (1990) who studied the resistance of barley hybrids to *Gibberella zeae*. This may be connected with the morphological structure of 6-rowed spikes which is more suitable to keep a higher moisture and, consequently, to spread infection within a spike than that of 2-rowed spikes.

A significant correlation was found between the level of kernel infection score and reduction of kernel weight per ear, 1000-kernel weight and kernel fraction >2.5 mm. A negative correlation between mycotoxin content and kernel weight per ear was also observed. A similar relationship between toxin content and kernel weight was found by SNIJDERS and PERKOWSKI (1990) in wheat genotypes inoculated with *F. culmorum*.

In the present study susceptibility of parental cultivars Maresi and Pomo to *F. culmorum* was similar. DH lines derived from F₁ Maresi × Pomo hybrids were observed to vary in their susceptibility to *F. culmorum*. This was expressed in the level of reduction of kernel weight and the proportion of plump kernels. This suggests that the genetic background for susceptibility to *F. culmorum* in the parents was not the same. Transgression effects were observed in some DH lines, for example in MP115 (two-rowed) and MP80 (six-rowed), in which the reduction of kernel characters was lower than in parental cultivars. Doubled haploids with a positive and negative transgression were also recorded for nivalenol and deoxynivalenol content. The lines MP7 and MP32 were distinguished as genotypes with a very low accumulation of mycotoxins, whereas MP105 and MP113 as genotypes with a relatively high mycotoxin content. It should be noted that no significant differences in mycotoxin content were found between parents. This fact suggests that transgression effects observed in the DH population may be caused by dispersion of (+) and (–) alleles conditioning susceptibility to *F. culmorum* and mycotoxin accumulation in parental cultivars Maresi and Pomo, and association of these alleles in extreme DH lines (POWELL, THOMAS 1992, SURMA et al. 1998).

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