Resistance to Fusarium head blight of winter wheat lines derived from crosses between winter type cultivars and resistant spring wheat 'Sumai 3'

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Introduction

Fusarium head blight is a disease of cereals caused by fungi of the *Fusarium* genus. These fungi produce toxic metabolites - mycotoxins. Head infection by *Fusarium* leads to kernel infection and accumulation of mycotoxins in grain. They pose a threat to human and animal health in the case of consumption of food or feed produced from infected grains. The most important mycotoxins, because of the quantities present in the cereal grain, are deoxynivalenol (DON) and zearalenone (ZEA).

The resistance of wheat to Fusarium head blight (FHB) is a quantitative feature. The presence of numerous loci of quantitative traits (quantitative trait loci – QTL) associated with resistance to this disease was detected. Using molecular markers at least one QTL have been identified on each chromosome of wheat (except the 7D). QTLs explaining the relatively high variability within the FHB resistance were located on chromosomes 3BS, 2DL, 4B, 6B, and 5AS. These QTLs originated from Asian sources, such as. 'Sumai 3', 'Wuhan 1' and 'Nyubai' and from Brazil – 'Frontana'. One of the most effective FHB resistance genes is *Fhb1* (formerly marked as *Qfhs. ndsu-3BS*) originating from 'Sumai 3', which in the different studies explained from 15 to 55% of the variation for the Fusarium spread within the head (resistance type II). This gene also determines the resistance to the toxic effects of deoxynivalenol (DON) through encoding a DON-glucosyl-transferase or regulating the expression of such an enzyme. In accordance with the published results only *Fhb1* shows a stable effect on the resistance of type II and V (resistance to trichothecene accumulation) in different environments and for various genetic backgrounds, while other QTLs give a weak or unstable effect.

Material and methods

Lines of winter wheat were obtained from crosses between Polish winter wheat cultivars ('Begra', 'Korweta' and 'Turnia') and spring wheat cultivar 'Sumai 3' resistant to FHB which was a donor of highly effective FHB resistance gene - *Fhb1*. Lines were selected using pedigree method on the basis of their resistance to FHB (after *Fusarium* inoculation), resistance to other diseases and morphological characters. The best 52 lines of F_{10} generation were tested for FHB resistance in field experiments in two locations in Cerekwica near Poznań and in Radzików near Warsaw.

Infective material was a mixture of three isolates of *Fusarium culmorum.* Inoculation was performed at the stage of full flowering of each line, in both locations with the same isolates. Severity of Fusarium head blight was evaluated about 21 and 28 days post inoculation. The incidence of FHB (percentage of heads infected per plot) and percentage of head infection were determined. From the results obtained, FHB index was calculated (FHBi).

For evaluation of FHB type I resistance wheat heads were sprayed with *F. culmorum* spore suspension at full flowering (http://scabusa.org/pdfs/ptt/cowger_type1-screening_protocol.pdf). Disease was assessed 7 day after inoculation. Number of infected spikelets (infection points = #ip) was recorded in each of 10 heads per line. In addition, 21 days after inoculation FHB index (percentage of infected spikelets per plot) was assessed.

For evaluation of FHB type II resistance wheat heads were point inoculated with spore suspensions of two *F. culmorum* isolates (http://scabusa.org/pdfs/ptt/_Bai_Greenhouse-Screening.pdf). Concentration of suspensions was adjusted to 50,000 spores/ml. A droplet of 50 mcl of suspension was injected into two flowers in the central spikelet of a spike using a self-refilling syringe. After inoculation, high humidity was maintained. Spike infection with *Fusarium* was assessed 21 days after inoculation by calculation of the number of visually infected spikelets (#is) below the infection point.

DNA was isolated from the leaves of five 2-3 week old plants, representing each of the tested accessions i.e.: 55 lines 'S', parental cultivars ('Begra', 'Korweta', 'Sumai 3', 'Turnia'), and two resistant lines: '20828' and 'UNG 136.6.1.1', that had 'Sumai 3' in their pedigree (Table 1). The presence of the gene *Fhb1* was deteced based on the occurrence of the PCR product of 239 bp after amplification of locus of UMN10 marker close linked to *Fhb1* gene (Liu et al., 2008).

Table 1. Origin of 'S' lines and check cultivars and lines

No.	Lines/ cultivars	Pedigree	FHB resistance
1	S1 – S7	Begra (= Grana x Bezostaja 1) x Sumai 3 (= Funo x Taiwanmai)	
2	S8 – S29	Korweta (=CHD-3672-72-77 x Gama) x Sumai 3	
3	S30 – S59	Turnia [= (Polanka x DED-739-75) x (Polanka x TAW-6505-74)] x Sumai 3	
4	20828[1, 3]	Capo x Sumai 3	VR
5	A40-19-1-2[1, 3]	Capo x SVP 72017-17-5-10-1	VR
6	Arina[2, 3]	Moisson x Zenith	R
7	Fregata	Kobra × Astron	R
8	UNG 136.6.1.1[2, 3]	(Sagvari x Nobeokabozu) x (Mini-Mano x Sumai 3)	VR
9	SMH 8694[3]	SMH 7297 x Rapsodia	S
10	SMH 8816[3]	SMH 7293 x Rapsodia	S
11	Tonacja[3]	Jubilatka × SMH 8134	MS

[1] Buerstmayr et al. 2008; [2] Buerstmayr et al. 1999; [3] Góral et al. 2015

Results

Table 2. Presence of the resistance allele at the locus of UMN10 marker linked to *Fhb1* gene in 61 'S' lines, parental cultivars and resistant control checks

Allele 239 bp[1]	Allele 236 bp[2]	Allele 239 bp/ 236 bp[3]
Sumai 3; S 01; S 02; S 03; S 04; S 05; S 10; S 11; S 12; S 13; S 26; S 27; S 29; S 30; S 32; S 33; S 39; S 42; S 43; S 44; S 45; S 46; S 48; S 50; S 51; S 52; S 55; S 56; S 57; S 59; **UNG 136.6.1.1**	**Begra**; **Korweta**; **Turnia**; S 07; S 08; S 09; S 14; S 15; S 16; S 18; S 19; S 20; S 21; S 22; S 24; S 25; S 31; S 34; S 35; S 37; S 38; S 40; S 41; **20828**	S 06; S 23; S 28

[1] amplification of allel 239 bp in locus of marker UMN10 indicates the presence of resistance gene *Fhb1*; [1] amplification of allel 236 bp in locus of marker UMN10 indicates the absence of resistance gene *Fhb1*; [3] heterogenic lines

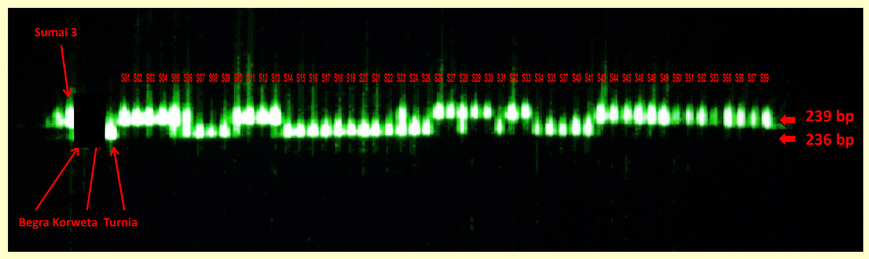


Figure 1. Amplification products of marker UMN10 associated with *Fhb1* gene on winter wheat lines generated from crosses between winter type cultivars and resistant spring wheat 'Sumai 3'

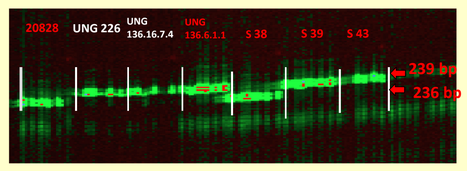
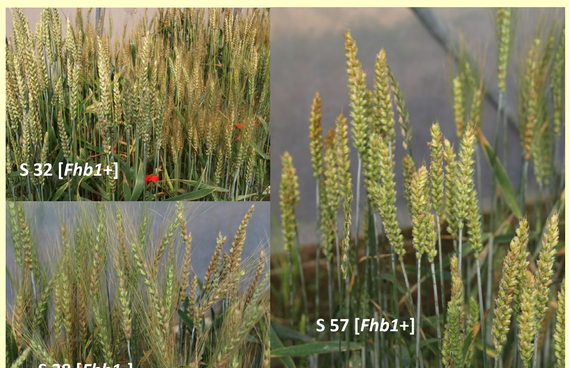


Figure 2. Amplification products of marker UMN10 associated with *Fhb1* gene on three winter wheat lines generated from crosses between winter type cultivars and resistant spring wheat 'Sumai 3' and resistant check lines.

Analysis of the presence of allele 239 bp of molecular marker UMN10 revealed its lack in maternal cultivars i.e. 'Begra', 'Korweta', and 'Turnia' (Tab. 2, Fig. 1). The presence of this allele was confirmed in the paternal cultivar 'Sumai 3'. Resistant allele of UMN10 was detected in 56% (29) of lines 'S' which indicates that they possess *Fhb1* gene. Three lines were heterogenic as both resistant and susceptible alleles were found. *Fhb1* was present in one highly resistant check line 'UNG 136.6.1.1' but not in the other '20828' (Fig. 2).

'S' lines showed high resistance to FHB under field conditions. Average FHB index was 3.4%. The range of variation of FHB index was 1.0 – 6.0%. The most resistant were 5 lines derived from a cross 'Turnia' x 'Sumai 3' ('S 55', 'S 45', 'S 46', 'S 48' and 'S 44'). These lines included *Fhb1* gene. Highly FHB resistant lines were also found in the group without presence of the gene *Fhb1*, for example 'S 31', 'S 38', 'S 08'. The highest susceptibility (FHBi >5.0%) showed five lines ('S 40', 'S 26', 'S 41', 'S 22', 'S 34'). One of them 'S 26' – had *Fhb1* gene, while in the others the presence of the gene was not detected. Resistance of 30 lines was higher than the resistance of 'UNG 136.6.1.1' line containing the *Fhb1*. However, higher resistance under field conditions was shown by line '20828', which did not possess the gene *Fhb1.* FHB index for 23 lines was lower than observed for this standard line. All 'S' lines were more resistant than 'Arina', 'Tonacja', 'SMH 8694', and 'SMH 8816' check cultivars.



FHB symptoms on heads of highly resistant lines 'S' 21 days after spray inoculation with *F. culmorum* in mist irrigated poly tent.

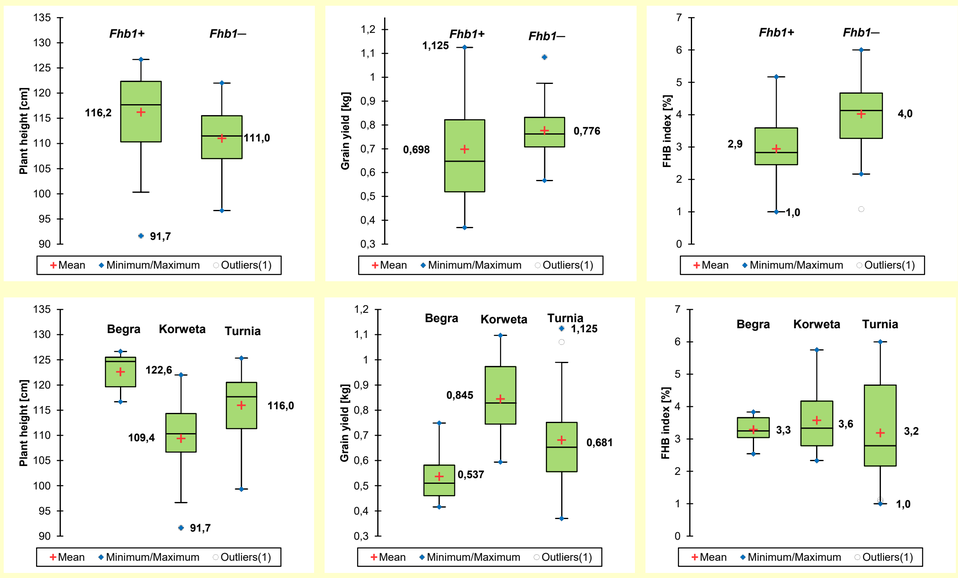


Figure 3. Comparison of plant height, grain yield per 1 m^2 and Fusarium head blight (FHB) index in 52 lines 'S' depending on presence of *Fhb1* gene and parental cultivar.

FHB symptoms on heads of highly resistant check lines 21 days after spray inoculation with *F. culmorum* in mist irrigated poly tent. Lines differing in type I resistance.

The average plant height of the lines with the *Fhb1* gene was 116.3 cm, whereas without the gene – 111.0 cm (Fig. 3). The difference between the groups was statistically significant. The range of variation in the first group was 91.7 – 126.7 cm, and in the second it was 96.7 – 122.0 cm. The shortest was line 'S 13' possessing *Fhb1* gene. Three groups of lines depending on the parental cultivar varied in average plant height. For 'Begra' cultivar it was 122.6 cm, for 'Korweta' cultivar – 109.4 cm and for 'Turnia' cultivar – 116.0 cm. Differences between the groups were statistically significant.

The average grain yield per 1m^2 for lines with *Fhb1* gene was 0.682 kg, and without the gene – 0.776 kg. The difference between the groups was statistically significant. The range of variation in the first group was 0.370 – 1.125 kg, in the second it was 0.567 – 1.084 kg. The highest grain yield was found for line 'S 30' possessing *Fhb1* gene.

Three groups of lines depending on the parental cultivar varied in grain yield. For 'Begra' progenies it was 0.531 kg, for 'Korweta' cultivar – 0.845 kg, and for 'Turnia' cultivar – 0.681 kg. The difference in yield between the lines obtained from 'Korweta' and the others was statistically significant.

FHB index for lines with gene *Fhb1* was 2.9% (1.0 – 5.2%), while for the lines without this gene – 4.0% (2.2 – 6.0%). The difference between the groups was statistically significant. Three groups of lines depending on the parental cultivars were characterized by a similar average FHB index. Differences between groups were not significant.

Symptoms of Fusarium head blight on wheat heads point inoculated with *Fusarium culmorum* (21 dpi)

Susceptibility to yellow rust was observed in 19 lines, including all lines obtained from crosses with 'Begra' cultivar. In lines obtained from the cross with 'Korweta' yellow rust was observed in one line. The highest susceptibility showed 4 lines – 'S 05', 'S 40', 'S 43', and 'S 59. Occurrence of yellow rust on the leaves had a significant negative effect on grain yield. The correlation coefficient was r =–0.624.

The average flowering time for lines with the *Fhb1* gene was 157.3 days, and without the gene – 156.6 days. The difference between the groups was statistically significant. Later flowering time negatively affected grain yield. Correlation coefficient was r = – 0.565.

The average resistance type I was to 1.5 infection points (#ip), at the range of 1.0 to 2.7 #ip. For the 'S' lines it was 1.4 pi and 1.0 – 2.0 #ip. The maximum number of points of infection on individual spikes was 3.0 #ip. The highest resistance type I showed lines with *Fhb1* gene: 'S 48', 'S 49', 'S 57 and check cultivars: '20828 and 'Slade'. The lowest resistance was found in lines: 'S 40', 'S 39', 'S 15', and 'S 23' and check cultivars – durum 'Komnata, resistant 'UNG 136.6.1.1' and susceptible: 'SMH 8816', 'SMH 8694', and 'Belenus'.

The average type II resistance was 1.4 infected spikelets (#is), at the range of 0.6 to 5.3 #is. For the 'S' lines it was respectively 0.9 and 0.7 – 1.3 #is. The highest resistance of type II showed lines: 'S 38' (w/o *Fhb1*) and 'S 45' (*Fhb1*) and two resistant checks: 'A40-19-1-2' and 'UNG 136.6.1.1'. For the 15 lines 'S' head infection was below 1.0 #is what means that symptoms were limited to single floret in the most of heads (scored as 0.5 #is). Lowest resistance has been detected in susceptible checks – 'SMH 8816' and 'SMH 8694' and cultivars: 'Belenus', 'Slade, ''Komnata' (durum) and 'Meister'. Among the 'S' lines low resistance type II showed – 'S 05', 'S 30', 'S 52' (with *Fhb1*) and 'S 15' (w/o *Fhb1*).

Using the principal components analysis (PCA), it was possible to detect lines 'S' combining desirable features such as resistance to Fusarium head blight, yellow rust resistance, high grain yield, earliness and plant height at the level of 'Tonacja' cultivar (Fig. 4). These were the lines: without *Fhb1 gene* – 'S 08', 'S 16', 'S 19', 'S 24, and 'S 25'; possessing *Fhb1* gene – 'S 10', 'S 11', 'S 12', 'S 13', 'S 27', 'S 29', 'S 30', and 'S 32'.

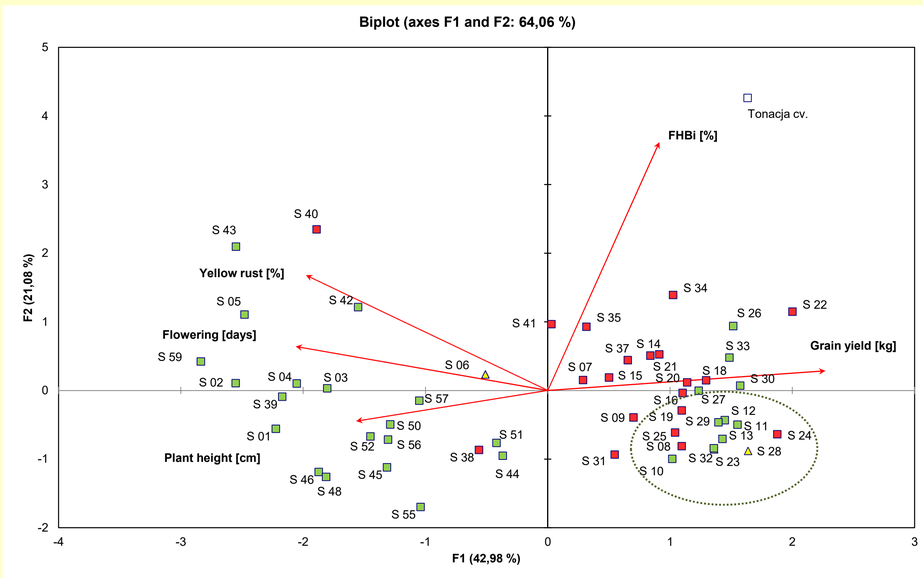


Figure 4. Biplot of the principal component analysis for 52 winter wheat lines 'S' and cultivar 'Tonacja'. Two first components explained 64.06% of variability of flowering time, plant height, grain yield, FHB index (FHBi) and yellow rust resistance. Lines combining all positive characters marked with circle.

■ – lines containing *Fhb1* gene, ■ – lines without *Fhb1* gene, ▲ – heterogenic lines

Conlclusions

1. The presence of the gene *Fhb1* was detected in 56% of lines 'S' using molecular marker UMN10 close linked to this gene.
2. Lines 'S' had on average high resistance of type II (no pathogen spread), while resistance type I was variable (from 1 to 3 infection points).
3. Lines with *Fhb1* resistance gene had on average higher resistance to FHB, taller plants and lower grain yield compared to lines without the gene.
4. In both groups of lines (with or without *Fhb1*) lines with a very high resistance, short plants and high yields of grain were identified.

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