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Effect of *Fusarium culmorum* infection on selected physiological and biochemical parameters of barley (*Hordeum vulgare* L.) DH lines

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ABSTRACT

The objective of this study was to facilitate the resistance of barley to *Fusarium culmorum* according to direct (disease rating (DR), fresh weight) and indirect (physiological and biochemical) parameters. Significant correlations were detected between most measured parameters. Hulled lines revealed less root susceptibility to infection of *F. culmorum* expressed in DR and fresh weight. Infection in roots significantly increased phenolics content, especially in most hull-less genotypes, but decreased soluble sugars, pigment content and overall performance index of the PSII photosystem. Significant correlations suggest the possibility of applying the measured indirect parameters in selection of barley DH lines resistant to *F. culmorum* infection.

Introduction

Fusarium seedling blight (FSB) and Fusarium head blight (FHB) caused by the *Fusarium* genera are the most devastating diseases in barely worldwide [1–4]. The seedling and head infection is caused predominantly by *Fusarium culmorum* (W.G.Sm.) Saccm, *Fusarium graminearum* Schwabe and *Fusarium avenaceum* (Fries) Saccardo, depending on climatic conditions [5–8]. The first two species are considered the most important pathogens in Poland and other countries of central Europe [6,9]. The diseases result in the reduction of grain yield and affect the quality of grains by contamination with toxic fungal secondary metabolites (mycotoxins), which may cause several diseases and disorders called mycotoxicoses in humans and domestic animals [4,6,10,11]). Contamination of barley grain with mycotoxins also decreases its use in the malting and brewing industry [12,13]. Barley genotypes vary in their susceptibility to FSB and FHB which is reflected in the various levels of reduction in yield and yield-related traits, e.g., grain weight per ear, 1000-grain weight and percentage of plump grains [14–17]. Infections by the pathogens influence also the reproductivity, as was found by TeKrony and Egli [18]. Because epidemics of Fusarium wilt diseases are a serious problem for crop and sowable material producers, improving the plant resistance is critical.

Plants have developed a number of defense mechanisms to restrain fungal pathogens and the propagation of their toxic metabolites. The natural defensive reaction of a plant organism to infection by a pathogen shows itself, among other things, in the release of hormones, sugars and phenolic compounds. According to Nicholson and Hammerschmidt [19], phenolic compounds exert a toxic action on the pathogen or, by participation in lignification of the cell walls and formation of structural barriers, they prevent penetration of the pathogen into the cells adjacent to the infection site [20–22]. Ferulic acid plays a fundamental role in the resistance of wheat cultivars to *Fusarium*, however, its concentration in mature, well-developed seeds is similar in both susceptible and resistant forms. Resistant cultivars show higher ferulic acid values [23]. Many phenolic compounds occurring in plants also have the properties of signal particles, as such phytoanticipins and phytoalexins, modulators of pathogenesis, and activators of plant disease resistance genes; they play a varied role in resistance processes in plants [20,24–26]. Oxidation of phenolic compounds, as a frequent phenomenon stimulated by infection, leads to the formation of chinones and free radicals, which may block enzymes, the main weapon of pathogens [20,27]. Additionally, oxidized chinones can be directly engaged in stopping the development of pathogens [28].

The phenolic compounds can also act as photoprotectors by limiting the chlorophyll excitation during unfavourable conditions for the photosynthetic apparatus. They can transform high-energy

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radiation into radiation with a lower destructive potential to the photosynthetic apparatus [29]. The levels of photosynthetic pigments (chlorophyll and carotenoids) are directly involved in the photosynthetic apparatus activity and can induce modifications in values of chlorophyll fluorescence parameters [30].

Also soluble sugars have a number of functions associated with defense reactions under stress and photosynthesis [31]. Pathogen infections can lead to sugar accumulation in plant tissues. The total soluble sugar levels are generally decreased due to the inhibition of photosynthesis. The decrease of leaf sugars can be a factor in the promotion of senescence.

Chlorophyll fluorescence measurements may provide a useful measure of the photosynthetic performance of plants; its real strength lies in its ability to give information that is not readily available in other ways. Fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus [32]. There are no literature data providing complex characteristics of phenolics, sugars, assimilation pigment contents and chlorophyll fluorescence parameters in barley after infection with *F. culmorum*, although identifying physiological markers associated with resistance to FSB is an especially compelling consideration for eventual use in selection.

Therefore the objective of this research was to determine the susceptibility of barley DH lines to *F. culmorum* by direct assessment of disease rating (DR) and reduction of fresh weight, and by indirect assessment by using selected physiological and biochemical parameters (phenolic compounds, soluble sugars, chlorophyll *a*, *b* and carotenoids as well as selected chlorophyll fluorescence parameters). To facilitate the most resistant DH lines, disease rating and fresh weight of seedlings were assigned with biochemical and physiological parameters. To test the above associations, correlation coefficients were calculated.

Material and methods

Materials

Material for the study covered 32 spring barley (*Hordeum vulgare* L.) genotypes: 2 parental genotypes (hull-less line 1N86 and hulled line RK63/1), and 30 doubled haploid (DH) lines derived from F_1 hybrids (15 hulled and 15 hull-less). Parental forms differ in susceptibility to *F. culmorum* infection. Line 1N86 was considered as more susceptible and RK63/1 as more resistant. DH lines generated from the crossing of 1N86 and RK63/1 were developed by the *Hordeum bulbosum* technique. Standard procedures were applied for crossing *H. vulgare* with *H. bulbosum* and *in vitro* culture of immature embryos [33,34].

Plate assay for resistance

Inoculation of semi-germinated seeds was performed with an isolate of *F. culmorum* (IPO348-01, ITEM6249 – nivalenol chemotype, Plant Breeding Institute, Wageningen) cultured in Petri dishes on PDA medium (Potatoe Dextrose Agar – Sigma) at a temperature of 22 °C without access to light, in a microbiological incubator (B 6060 – Heraeus, USA) over a period of seven days. The kernels were surface disinfected for 15 min with 20% Domestos solution (commercial bleach, with sodium hypochlorite as the active ingredient) and substantially washed three times in sterile water and placed on blotting paper for 24 h for germination. Then the semi-germinated seeds were transferred onto PDA medium discs (ø 4 mm) overgrown with *F. culmorum* mycelium. Barley kernels placed on sterile medium discs were the control. The assay for resistance was carried out in an air-conditioned chamber over a period of 7 days at 22 °C/20 °C, with 130 μE m^{-2} s^{-1} lighting, 12/12 h photoperiod and 100% relative humidity (RH). To determine the effect of infection on seedling development, direct assessment using a disease rating (DR) [35] was calculated according to the formula:

$$DR\% = 100^{*}(n_i D_i)/ND_{max}$$

where n_i – the number of plants of the *i*th category, D_i – numerical value of the *i*th category, N- the total number of plants in the sample and D_{max} – is the maximum scale value [36].

The leaves and roots fresh weight (FW, mg) of inoculated and control seedlings were also determined in three replications.

Plant tissue preparation

For all biochemical tests and pigments determination infected and free of pathogen, leaves and roots were lyophilized separately in high vacuum at 40 μbar, coil temperature −52 °C (lyophiliser: FreeZone 6L, Labconco, USA). Then tissue was homogenized using a ball mill (MM400, Retsch, Germany) for 5 min at frequency 25 Hz.

All the determinations were done in three biological and analytical replications, using plant tissue obtained from seedlings after a period of 7 days when the plate assay for resistance was completed.

The total phenolic compounds content

The content of phenolic compounds was determined by the Folin and Ciocalteu reagent [38]. Twenty mg of plant tissue (roots, leaves of both combinations; inoculated and control) was extracted in 1 ml of 80% ethanol, then centrifuged at 2,000 *g* (Universal 32R, Hettich, Germany) for 10 min. An aliquot of extract (20 μl) was added to the reaction mixture, which consisted of 0.5 ml of 25% Na_2CO_3 and 0.125 ml of Folin and Ciocalteu reagent (diluted with distilled water in use within one day in a ratio of 1:1). After 30 min when the reaction was completed, absorbance of the reaction mixture was measured spectrophotometrically with a microplate reader (Synergy II, Bio-Tek, USA) at the wavelength 760 nm. The content of phenolics was defined as μg of chlorogenic acid per 1 g dry weight (DW) of plant tissue.

The soluble sugar content

The total amount of soluble sugar was determined by Dubois et al. [39]. Twenty mg of plant tissue (roots, leaves of both combinations; inoculated and control) was extracted in 1 ml of 80% ethanol, then centrifuged at 2,000 *g* (Universal 32R, Hettich, Germany) for 10 min. An aliquot of extract (20 μl) was added to the reaction mixture, which consisted of 0.4 ml H_2O, 0.4 ml of 5% phenol and 2 ml H_2SO_4. After 20 min when the reaction was completed, absorbance of the reaction mixture was measured spectrophotometrically with a microplate reader (Synergy II, Bio-Tek, USA) at 490 nm. The content of soluble sugar was expressed in μg per 1 g dry weight (DW) of plant tissue.

Chlorophyll and carotenoid contents

Twenty mg of dry weight were then extracted in 1 ml of 80% ethanol and left overnight in 4 °C with no light, then centrifuged at 2,000 *g* (Universal 32R, Hettich, Germany) for 10 min. An aliquot of extract (200 μl) was added to microplate wells and absorbance at 470, 648, 664 nm was measured spectrophotometrically with a microplate reader (Synergy II, Bio-Tek, USA). The concentrations of chlorophyll *a*, *b* and carotenoids were determined according to the following equations [37]:

Table 1
Mean disease rating (DR, %) and fresh weight (mg) of root and leaf of the control (C) and inoculated (I) with *Fusarium culmorum* hulled and hull-less barley DH lines.

Genotype	DR (%)				Fresh weight (mg)			
	Leaf		Root		Leaf		Root	
	C	I	C	I	C	I	C	I
Hulled								
R63N/1	0	9.7	0	51.5	108.2	85.6	95.8	36.4
R63N/3	0	12.0	4.4	57.3	70.7	85.5	54.0	38.5
R63N/4	0	6.2	2.9	49.2	79.3	85.2	85.2	38.3
R63N/9	0	4.9	6.2	43.7	99.7	85.7	90.3	42.8
R63N/18	0	7.3	0	54.3	103.7	88.6	107.4	41.9
R63N/21	0	5.2	1.3	49.5	86.9	81.9	90.5	40.1
R63N/22	0.7	8.0	7.3	54.7	114.5	91.1	100.3	37.9
R63N/27	0	5.4	2.3	65.2	77.9	70.8	98.7	27.3
R63N/28	0	2.0	1.3	68.0	101.9	81.9	81.3	27.5
R63N/34	0	5.9	3.8	63.1	87.0	66.2	88.2	26.1
R63N/35	0	4.9	2.2	72.9	62.3	50.2	66.8	17.9
R63N/61	0	10.3	5.4	57.9	80.7	69.4	93.7	29.2
R63N/63	0	11.6	9.3	60.0	89.6	81.5	102.0	34.1
R63N/67	0	14.8	13.8	58.7	80.5	68.5	95.1	29.9
R63N/74	0	8.4	8.1	65.3	92.4	66.7	101.8	22.0
RK63/1 (parental line)	0	7.0	6.3	58.7	78.0	72.8	88.4	45.8
Mean	**0.04**	**7.7**	**4.7**	**58.1**	**88.3**	**77.0**	**90.0**	**33.5**
Hull-less								
R63N/19	0	72.3	0	83.0	48.9	27.9	21.5	10.3
R63N/46	0	48.6	0	77.1	80.6	52.1	47.6	17.4
R63N/47	0	51.4	5.2	84.3	102.4	47.3	79.4	19.7
R63N/14	0	61.3	0	82.3	68.7	32.7	40.1	12.4
R63N/20	0	32.2	0	70.6	88.6	55.8	42.0	13.2
R63N/52	0	46.2	0	81.6	66.3	34.8	43.7	10.3
R63N/65	0	31.9	3.1	72.5	80.5	54.3	64.8	24.7
R63N/24	0	14.1	1.1	60.7	85.9	67.6	67.0	24.1
R63N/31	0	37.4	2.5	72.4	82.7	61.5	51.8	20.1
R63N/42	0	33.3	1.1	73.9	114.9	63.1	30.8	20.2
R63N/43	0	26.7	0	75.6	58.8	44.6	27.3	9.2
R63N/55	0	37.0	0	77.0	96.7	65.6	28.3	12.0
R63N/70	0	43.7	7.5	70.6	96.1	62.8	44.2	18.9
R63N/71	0	29.9	0	70.8	90.8	69.6	61.2	18.4
R63N/75	0	19.1	2.5	72.9	95.2	65.1	60.2	16.8
1N/86 (parental line)	0	58.4	8.6	89.8	58.9	26.5	24.4	6.8
Mean	**0**	**40.2**	**2.0**	**75.9**	**82.3**	**52.0**	**45.9**	**15.9**
LSD	**7.99**		**8.44**		**23.29**		**22.54**	

$$Chl_a(\mu g/ml) = 12.7*A664 - 2.7*A648$$

$$Chl_b(\mu g/ml) = 22.9*A648 - 4.7*A664$$

$$Car(\mu g/ml) = (1000*A470 - 2.13*Chla - 97.64*Chlb)/209$$

where Chl $_a$ = *chlorophyll a*, Chl $_b$ = *chlorophyll b*, Car = carotenoids, A470 = absorbance at 470 nm, A648 = absorbance at 648 nm, A664 = absorbance at 664 nm.

Concentrations of chlorophyll and carotenoids are expressed in pigment contents per gram dry weight (μg/g DW).

Chlorophyll a fluorescence parameters

Chlorophyll fluorescence was measured on the fully developed 7-day seedling leaf using a portable fluorometer (Handy PEA; Hansatech Instruments, King's Lynn, UK) at 24 °C after 20 min for the leaves to adapt to the dark conditions on the day of sampling. Fluorescence intensity was measured with a PINphotodiode after being passed through a long-pass filter. Changes in fluorescence were registered during irradiation of 10 μs to 1 s. During the initial 2 ms, data were collected every 10 μs with 12-bit resolution. After this period, the frequency of measurements was reduced automatically. The measurements were done for each line with three plant replicates. The parameters: Fv/Fm (the maximum photochemical efficiency) and PI (overall performance index of PSII photochemistry) were calculated per excited leaf cross-section.

Statistical analysis

For the examined parameters, two-factor variance analyses using the independent system were done. The distinguished sources of variability were tested using the fixed model. The evaluation of the correlations between characteristics was performed on the basis of the Pearson linear correlation coefficient. Statistical analysis was performed with the application of Statistica StatSoft, Inc. [40].

Results

Spring barley (*Hordeum vulgare* L.) DH lines (15 hulled and 15 hull-less) and parental genotypes (breeding lines 1N86 and R63/1) were exposed to the *F. culmorum* infection. DH lines revealed significant differences in susceptibility to infection (Table 4). The results of the DH lines evaluation using the rating scale expressed in disease rating (DR) showed a varied intensity of disease symptoms, both in hulled and hull-less genotypes (Table 1). It is worth noting

Table 2
Mean phenolics (μg/g DW) and soluble sugars (μg/g DW) in root and leaf of the control (C) and inoculated (I) with *Fusarium culmorum* hulled and hull-less barley DH lines.

Genotype	Phenolics (μg/g DW)				Soluble sugars (μg/g DW)			
	Leaf		Root		Leaf		Root	
	C	I	C	I	C	I	C	I
Hulled								
R63N/1	65.0	68.6	150.0	111.0	199.1	210.6	92.0	108.6
R63N/3	71.6	105.4	176.8	152.8	192.1	197.2	157.1	104.2
R63N/4	68.3	81.7	205.6	125.9	206.4	196.6	118.1	136.2
R63N/9	66.8	69.7	203.8	169.9	194.4	221.5	85.4	122.6
R63N/18	98.2	90.1	193.8	193.1	209.8	208.2	164.8	112.2
R63N/21	95.1	90.5	165.0	135.4	203.3	185.6	144.2	95.8
R63N/22	67.2	80.8	144.4	149.4	221.2	215.5	136.2	107.6
R63N/27	83.1	68.0	138.1	148.8	219.9	236.2	93.4	136.1
R63N/28	86.8	85.7	120.2	138.7	259.8	252.4	144.2	117.7
R63N/34	64.9	59.0	148.8	153.7	230.3	214.3	87.1	100.9
R63N/35	92.4	93.4	137.1	154.9	202.3	235.0	156.5	125.4
R63N/61	87.5	85.8	122.7	155.1	171.1	203.7	112.9	111.5
R63N/63	92.3	93.5	93.9	111.2	205.2	185.0	139.3	107.4
R63N/67	91.7	80.5	149.3	138.1	221.4	208.6	102.1	105.0
R63N/74	70.4	69.4	107.3	112.4	242.8	231.3	85.8	120.9
RK63/1 (parental line)	106.0	103.6	135.8	123.3	165.1	184.7	76.7	67.2
Mean	**81.7**	**82.9**	**149.5**	**142.1**	**209.0**	**211.6**	**118.5**	**111.2**
Hull-less								
R63N/19	90.0	99.1	104.7	108.5	190.7	154.2	112.9	162.6
R63N/46	93.8	116.1	138.0	123.2	226.3	206.0	260.1	110.0
R63N/47	86.8	104.9	116.7	139.1	181.7	192.3	147.4	143.5
R63N/14	117.2	97.4	107.7	134.9	196.4	165.6	143.9	122.9
R63N/20	79.4	84.5	156.3	146.8	206.7	182.5	147.1	131.2
R63N/52	99.9	88.8	123.0	172.0	198.0	150.8	181.2	130.0
R63N/65	92.0	88.9	144.2	141.0	196.0	213.6	171.8	146.8
R63N/24	99.0	92.6	147.7	189.0	171.0	212.6	135.6	131.8
R63N/31	100.5	86.8	133.9	146.3	229.8	184.4	133.2	109.2
R63N/42	93.7	100.2	85.6	178.4	231.0	207.8	79.2	154.5
R63N/43	115.1	94.9	184.7	179.3	184.0	222.0	155.6	103.6
R63N/55	112.1	113.4	143.1	253.9	175.5	155.5	100.0	124.0
R63N/70	111.7	99.8	154.1	150.3	184.3	173.6	121.4	111.9
R63N/71	143.8	119.0	95.9	147.1	208.5	192.6	132.0	119.6
R63N/75	97.0	83.3	132.4	195.7	182.7	165.9	107.3	100.1
1N/86 (parental line)	96.2	107.2	75.7	223.3	222.6	178.8	138.4	252.7
Mean	**101.8**	**98.6**	**127.7**	**164.3**	**199.1**	**184.9**	**141.7**	**134.6**
LSD	**21.26**		**39.15**		**19.49**		**28.75**	

Table 3
Mean chlorophyll *a*, *b*, carotenoids (μg/g DW) and selected chlorophyll fluorescence parameters (Fv/Fm, PI) in control (C) and inoculated (I) with *Fusarium culmorum* hulled and hull-less barley DH lines.

Genotype	Chlorophyll (μg/g DW)				Carotenoids (μg/g DW)		Fluorescence parameters			
	a		*b*				Fv/Fm		PI	
	C	I	C	I	C	I	C	I	C	I
Hulled										
R63N/1	19.2	17.7	11.3	10.7	1.8	1.6	0.804	0.806	1.265	1.185
R63N/3	17.2	17.0	10.7	10.9	1.6	2.1	0.822	0.818	1.378	1.361
R63N/4	11.2	15.2	8.6	9.9	0.8	1.8	0.811	0.816	1.290	1.250
R63N/9	18.6	14.1	11.9	9.8	2.3	1.7	0.817	0.810	1.557	1.273
R63N/18	20.0	10.8	12.6	9.0	2.3	1.6	0.790	0.802	1.094	0.994
R63N/21	17.5	10.1	11.6	7.5	2.4	0.5	0.813	0.814	1.358	1.355
R63N/22	18.0	13.0	12.2	8.9	1.5	1.8	0.824	0.821	1.430	1.239
R63N/27	7.7	8.4	5.8	6.9	0.4	0.1	0.824	0.817	1.404	1.475
R63N/28	20.6	11.8	11.1	7.9	2.3	1.3	0.824	0.813	1.598	1.157
R63N/34	8.8	7.2	6.8	6.3	0.5	0.2	0.821	0.823	1.345	1.497
R63N/35	14.9	5.8	9.4	4.9	1.2	0.2	0.816	0.816	1.449	1.458
R63N/61	17.4	8.3	10.8	6.6	1.9	0.3	0.812	0.813	1.205	1.472
R63N/63	19.7	9.9	11.8	7.4	2.2	0.5	0.817	0.804	1.717	1.373
R63N/67	16.2	9.2	9.9	6.8	2.0	1.2	0.813	0.815	1.648	1.576
R63N/74	17.1	11.1	10.6	7.1	1.7	1.1	0.803	0.816	1.067	1.302
RK63/1 (parental line)	24.3	11.9	15.2	8.3	2.3	1.0	0.819	0.825	1.478	1.865
Mean	**16.8**	**11.3**	**10.6**	**8.1**	**1.7**	**1.0**	**0.814**	**0.814**	**1.393**	**1.365**
Hull-less										
R63N/19	7.3	4.6	6.4	4.5	0.3	0.2	0.812	0.809	1.284	1.247
R63N/46	7.0	5.9	5.3	4.9	0.4	0.4	0.798	0.797	1.180	1.016
R63N/47	12.9	7.5	8.7	6.4	0.9	0.9	0.801	0.777	1.030	0.571
R63N/14	15.8	5.9	9.3	5.1	1.7	0.4	0.805	0.812	1.237	1.167
R63N/20	20.2	7.1	12.0	6.0	2.5	0.4	0.825	0.818	1.843	1.430
R63N/52	15.1	5.6	9.8	5.1	1.9	0.3	0.808	0.819	1.560	1.463
R63N/65	11.4	5.4	8.1	4.9	1.8	0.3	0.806	0.811	1.117	1.162
R63N/24	22.8	16.8	12.5	10.4	2.7	2.0	0.823	0.816	1.938	1.675
R63N/31	15.5	10.1	9.4	8.2	1.7	1.2	0.793	0.806	1.296	0.972
R63N/42	16.6	10.3	10.5	7.8	1.8	1.5	0.824	0.795	1.773	1.528
R63N/43	15.0	5.0	9.1	4.3	1.4	0.3	0.820	0.809	1.588	1.061
R63N/55	10.3	9.9	7.4	8.4	0.5	0.4	0.817	0.818	1.583	1.326
R63N/70	11.4	10.3	7.6	6.4	1.4	1.3	0.815	0.808	1.589	1.086
R63N/71	20.2	9.8	10.8	7.1	2.8	0.8	0.815	0.800	1.435	0.958
R63N/75	8.6	6.4	6.6	5.4	0.4	0.4	0.817	0.815	1.168	1.250
1N/86 (parental line)	8.8	6.1	6.8	5.4	0.5	0.3	0.820	0.811	1.592	1.310
Mean	**13.7**	**7.9**	**8.8**	**6.3**	**1.4**	**0.7**	**0.812**	**0.808**	**1.451**	**1.201**
LSD	**2.82**		**1.65**		**0.44**		**0.0151**		**0.3062**	

that hulled lines revealed less root susceptibility to infection of *F. culmorum* expressed in DR (hulled - 58.1%, hull-less - 75.9%) and in fresh weight (hulled − 33.5 mg, hull-less − 15.9 mg). The most susceptible hulled line was R63N/35 (72.9%) and hull-less line was 1N86 (89.8%). The least susceptible hulled line was R63N/9 (43.7%) and hull-less was R63N/24 (60.7%). Considering the leaves infection score, the most susceptible hulled line was R63N/67 (14.8%) and hull-less line was R63N/19 (72.3%), the least susceptible was hulled line R63N/28 (2.0%) and hull-less was R63N/24 (14.1%). The susceptibility of hull-less line R63N/24 was comparable to the mean DR value for hulled lines, which generally showed fewer infection symptoms. This line was also one of the best considering the fresh weight of roots and leaves.

It should be noted that some of the lines revealed higher resistance when we consider leaf infection score presented in DR. In the group of hulled lines, only RK63N/28 had a lower score (2.0) but it was statistically insignificant; the rest of the lines in this group were infected at the same level. In the group of hull-less lines, one line (R63N/47) was infected at the same level as the parental hull-less form and one (R63N/19) was infected more severely. The rest of the lines were less damaged compared to parental form 1N86. Considering the root infection score expressed in DR, which might be more reliable because FSB first attacks roots, only one line (R63N/9) in the group of hulled lines was statistically less infected than the parental form RK63/1. In the group of hull-less lines, four lines (R63N/19, R63N/47, R63N/14, R63N/52) were infected

Table 4
Significance of F values for disease rating (DR, %), fresh weight (mg), phenolics (μg/g DW), soluble sugars (μg/g DW) of root and leaf, chlorophyll *a*, *b* (μg/g DW), carotenoids (μg/g DW), and selected chlorophyll fluorescence parameters (Fv/Fm, PI) of leaves in the control and inoculated with *Fusarium culmorum* hulled and hull-less barley DH lines.

Source of variability	DF	DR (%)		Fresh weight (mg)		Phenolics (μg/g DW)		Soluble sugars (μg/g DW)		Chlorophyll (μg/g DW)		Carotenoids (μg/g DW)	Chlorophyll fluorescence parameters	
		Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	*a*	*b*		Fv/Fm	PI
Inoculation A	1	1128.25**	7147.22**	100.24**	461.58**	1.02 ns	17.39**	0.96 ns	7.79**	494.36**	299.17**	329.53**	3.00 ns	25.75**
Genotype B	31	24.29**	7.02**	7.00**	10.33**	7.96**	5.70**	24.24**	11.57**	27.03**	20.11**	29.35**	4.00**	7.22**
A x B	31	24.38**	9.11**	1.50ns	2.92**	1.56*	5.06**	0.22 ns	10.24**	8.64**	6.06**	11.67**	2.00**	2.31**
Error	128	24.41	27.30	0.0002	0.0002	173.00	587.00	145.00	316.00	3.04	1.04	0.0751	0.0003	0.0359

* − significant at $p < 0.05$; ** − significant at $p < 0.01$; ns − not significant.

similarly to the parental hull-less line, and the rest were less infected. When we consider leaf weight in the group of hulled lines, we found 9 lines with higher mass but the differences were not statistically significant. In the group of hull-less lines, there were ten lines with higher leaf weight compared to the hull-less parent. It is noticeable that FSB affects the root and its development severely. It reduced the mean value by over 63%, and all of the hulled DH lines roots were less developed and had lower weight compared to the parental form RK63/1. In the group of hull-less lines, FSB caused an even higher reduction of root mean weight, over 65%. All hull-less progenies revealed higher root mass, but the differences were not statistically significant when compared to parental hull-less lines 1N86 (Table 1).

Infection did not have a statistically significant influence on total phenolics content in leaves of both hulled and hull-less lines, whereas the content of phenolics varied significantly among genotypes (Table 4). Also a significant interaction between inoculation and genotypes was noted. The highest content of phenolics in leaves was in hulled line R63N/3 (105.4 μg/g DW) and hull-less R63N/46 (116.1 μg/g DW). Among all lines tested, only these synthesized 30% and 20% more phenolics, respectively, compared to the control plants (Table 2). In contrast, root infection caused a significant increase of phenolics content, especially in most hull-less genotypes. The mean concentration of phenolics in hulled lines was 142.1 μg/g DW, whereas in hull-less lines it was 164.3 μg/g DW. The highest phenolics content in roots was observed in hulled line R63N/61, and hull-less line R63N/55.

Considering the total phenolics content in the group of hulled lines, 5 lines possessed a statistically significant lower amount (the lowest value posses line R63N/34) compared to the hulled parent, and in the group of hull-less DH lines, two lines (R63N/20 and R63N/75) possessed a lower amount of the compound.

The content of soluble carbohydrates in leaves was not significant considering inoculation, but depended only on the genotype (Table 4). However, in roots, inoculation caused a significant decrease of soluble sugar content compared to uninfected plants. The highest decrease of sugars (63%) was observed in hull-less line R63N/46 and over 30% in hulled lines R63N/3 and R63N/21 (Table 2).

Regarding soluble sugars, ten hulled DH lines had a higher amount of this compound compared to the hulled parent (the highest amount line R63N/28), and only four DH hull-less lines had a higher amount (the highest amount line R63N/43) compared to the hull-less parent. Generally in roots there were less soluble sugars then in leaves, but in most hulled lines, except line R63N/21, there were more soluble sugars then in the parental hulled line, in all hull-less lines there were significantly less soluble sugars compared to the hull-less parental form (Table 2).

A markedly lower level of pigments was detected after inoculation (Table 4). The largest decrease was observed for carotenoids (40% for hulled lines, 50% for hull-less lines), then for chlorophyll *a* (33% for hulled lines, 42% for hull-less lines), and chlorophyll *b* (20% for hulled lines, 28% for hull-less lines) (Table 3). The level of pigments was not reduced after inoculation in only two hulled lines (R63N/3, R63N/4).

Three hulled lines (R63N/1, R63N/3, R63N/4) had a significantly higher amount of chlorophyll *a* when compared to the hulled parent. In the group of hull-less lines, four lines (R63N/24, R63N/31, R63N/42, R63N/55, R63N/70) revealed the same tendency. None of the hulled lines had a significantly higher amount of chlorophyll *b* compared to the hulled parent. In contrast, there were four lines (R63N24, R63N31, R63N/42, R63N/71) with a significantly higher amount of chlorophyll *b* when compared to the hull-less parent. The most affected pigment component was carotenoids, but in the group of hulled lines, there were some lines (R63N/1, R63N/3, R63N/4, R63N/9, R63N/18, R63N/22) with a higher amount compared to the hulled parent. Comparing the hull-less parent to the group of hull-less lines, the same tendency was observed in the following lines: R63N/47, R63N/24, R63N/31, R63N/42, R63N/71.

Analysis of variance showed highly significant differences between the DH lines and the interaction of chlorophyll fluorescence parameters: the maximum photochemical efficiency (Fv/Fm), and the overall performance index of PSII photochemistry (PI). Inoculation significantly affected PI but not Fv/Fm (Table 4). Lower mean PI values for inoculated plants compared to the control was observed for hull-less lines more than hulled ones, 17% and 2% respectively (Table 3).

When taking into consideration the Fv/Fm (the maximum photochemical efficiency) parameter, there were no significant differences between the parental hulled form and the DH hulled progenies; two lines had a similar value for this parameter (R63N/22 and R63N/34). The same was noted in the group of hull-less lines. Although there were five lines with a higher Fv/Fm value compared to hull-less parent, the differences were also not statistically significant. Only one line of all hulled lines had a similar value of PI (overall performance index of PSII photochemistry) to the parental hulled form (R63N/1), whereas the rest of the lines possessed lower values. In the group of hull-less lines, one line (R63N/24) had a significantly higher PI value when compared to the hull-less parental form. It is worth noting that this line also had a higher amount of all studied pigments (Table 3).

Correlation coefficients between traits are given in Table 5. Disease ratings (DR) for leaves and roots have a highly significant negative correlation with plant fresh weight. A highly significant positive correlation was observed between phenolics in leaves, and leaves and roots DR. Phenolics in leaves were negatively correlated with soluble sugars in leaves, in contrast to a positive correlation in roots. The amount of phenolics in leaves was negatively correlated with the Fv/Fm parameter and leaf weight. The content of soluble sugars in leaves was positively correlated with pigments and fresh weight of plants, but negatively with leaf and root DR. Opposite of leaves, soluble sugars accumulated in roots were negatively correlated with pigments and positively with DR values. The content of pigments had a highly significant negative correlation with DR of leaves and roots, and a positive correlation with the fresh weight of leaves and roots. Other significantly negative correlations were observed between chlorophyll fluorescence parameters and the DR of leaves and roots.

Discussion

F. culmorum is a fungal pathogen causing seedling blight and root rot; although the diseases have less impact than Fusarium head blight in barley and wheat in general, both can significantly reduce the seedling emergence and establishment as a result of infection and can result in notable yield reduction, especially under high soil moisture favourable for fungi development [41].

The results expressed in disease rating (DR) and in fresh weight of seedlings showed a varied intensity of disease symptoms, both in hulled and hull-less genotypes. Notably, hulled lines revealed less root susceptibility to infection of *F. culmorum* expressed in DR (hulled – 58.1%, hull-less – 75.9%) and in fresh weight (hulled – 33.5 mg, hull-less – 15.9 mg). Our results correspond with results of Warzecha et al. [42], in which less intense symptoms of the disease, both on leaves and on roots, were found in husked oat cultivars Stoper and Cwał when compared to naked cultivars. In naked cultivars, the weight of leaves was decreased by 20%, as compared with husked cultivars. Also in the case of the husked form, the rating scale evaluation (no visual symptoms of the disease on seedling

Table 5
Matrix of the correlation coefficients between direct parameters: disease rating (DR, %), fresh weight (mg) and indirect parameters: phenolics (μg/g DW), soluble sugars (μg/g DW), chlorophyll *a*, *b* (μg/g DW), carotenoids (μg/g DW), chlorophyll fluorescence parameters (Fv/Fm, PI) of hulled and hull-less barley seedlings after inoculation with *Fusarium culmorum*.

Parameters	DR (%)		Fresh weight (mg)		Phenolics (μg/g DW)		Soluble sugars (μg/g DW)		Chlorophyll (μg/g DW)		Carotenoids (μg/g DW)	Chlorophyll fluorescence parameters	
												Fv/Fm	PI
	Leaf (1)	Root (2)	Leaf (3)	Root (4)	Leaf (5)	Root (6)	Leaf (7)	Root (8)	*a* (9)	*b* (10)	(11)	(12)	(13)
1	1.00												
2	0.77**	1.00											
3	−0.36**	−0.28**	1.00										
4	−0.32**	−0.27**	0.82**	1.00									
5	0.47**	0.40**	−0.25*	−0.11	1.00								
6	0.09	0.14	−0.13	−0.14	0.15	1.00							
7	−0.58**	−0.37**	0.27**	0.25*	−0.32**	−0.14	1.00						
8	0.47**	0.43**	−0.09	−0.12	0.17	0.24*	−0.12	1.00					
9	−0.52**	−0.67**	0.36**	0.37**	−0.13	−0.07	0.29**	−0.24*	1.00				
10	−0.51**	−0.64**	0.31**	0.31**	−0.12	0.02	0.24*	−0.24*	0.95**	1.00			
11	−0.35**	−0.54**	0.28**	0.29**	−0.08	−0.05	0.28**	−0.17	0.84**	0.77**	1.00		
12	−0.31**	−0.25*	0.17	0.06	−0.27**	0.17	−0.01	−0.18	0.11	0.09	0.00	1.00	
13	−0.26*	−0.16	0.28**	0.26*	−0.13	0.06	0.02	−0.05	0.12	0.09	−0.01	0.61**	1.00

*– significant at $p < 0.05$; ** – significant at $p < 0.01$.

leaves) as well as seedling weight values may suggest their greater resistance to *F. culmorum* [42].

For the whole set of DH lines, the mean values of the seedling root weight decreased more than twice (2.6 times) as much as the leaf weight, confirming the results of evaluation in DR where the disease symptoms were three times more severe in roots, both in hulled and in hull-less lines. Other authors [37,42,43] show the destruction caused by Fusarium seedling blight is much greater in the root system, and that is why the root infection score is considered more reliable than the leaf infection score. As a result of root system damage, physiological processes – those connected with uptake and transport of water and mineral salts, as well as distribution of assimilates – become disrupted, which later may have a negative effect on the development of plants.

The analysis of variance revealed that inoculation, genotype, and the interaction of both factors were significant for almost all measured biochemical parameters (apart from phenolics and soluble sugars in leaves for the first factor – inoculation, as well as the interaction of inoculation and genotype). It means that in DH population obtained from F_1 generation of two parents differ in their resistance/susceptibility to *F. culmorum* infection, the progeny react in different way.

Since infection is a state of stress for plant tissue and many biochemical changes might be reported during pathogenesis, such translocation of water and nutrients decreases, cell membrane permeability changes. Also an increased respiration can be observed, which are connected with synthesis of proteins, enzyme activation, division and growth of the cells, gathering of compounds such as ion fluxes, phenolics, reactive oxygen species (ROS), nitric oxide [44,45]. All the changes during the infection process might have a huge impact on pigment production and its activity, resulting in chlorophyll fluorescence changes and affecting effectiveness of PSII. The natural defensive reaction of a plant organism to infection by a pathogen shows itself, among other things, in an increased release of phenolic compounds, the components of the cell walls, and their intensive collection and synthesis at the infection site [19]. We observed that phenolic compounds in roots increased in most genotypes after inoculation, as described by other authors [46]. The function of phenolic compounds may be varied e.g. toxic action on the pathogen, participation in lignification of the cell walls, of structural barriers, blocking the spread of the pathogen to non-infected tissue [20–22]. Phenolics could also be considered as signal particles and activators of plant disease resistance genes [20,24–26]. Phenolic compounds could be oxidized as a result of infection, produce chinones and free radicals and block pathogens [20,27].

The sugars are a source of carbon for the mycelium of pathogens and are the signal for initiating defense reactions in plants and they originated from decomposed polysaccharides in cell walls by pathogen enzymes. In our studies inoculation caused a significant decrease of soluble sugar content in roots compared to uninfected plants. The largest decrease of sugar among hull-less lines was two times larger (line R63N/46 – 63%) than hulled lines (R63N/3 and R63N/21 – over 30%). The results correspond with the direct test, proving that hull-less lines were more susceptible to infection then hulled lines.

Sugars could limit the spread of the pathogen by isolating the infected cells, and protect the tissues against water loss [47,48]. Soluble sugars directly and indirectly play a significant role in resistance processes. A high concentration of soluble sugars may directly limit the pathogen colonization of the cells as a result of increased osmotic potential [49]. Fungal pathogens of plants have a definite range of water potential necessary for optimal growth and development, and accumulation of sugars and other osmotically active substances decreases water potential in the host cells and may limit the pathogen development [50]. This is how the resistance of grasses to a complex of pathogens causing pink snow mold was explained; additionally, the cultivars resistant to that complex of pathogens were characterized by low water content [51,52]. Indirectly, sugars may affect plant disease resistance genes; hexoses induce the expression of many genes by hexokinase signal transduction, e.g., by activating the genes responsible for the production of peroxidase and pathogenesis-related proteins. Hexoses can also be a source of defensive compound precursors [47,48].

It is worth noting that phenolics in leaves positively correlated with soluble sugars in roots. Some authors found correlation between carbohydrate content during pathogenesis and increased level of phenolics, which play a crucial role in the pathogenesis-related defense mechanism [46,53].

Increased yield means increased harvest index or total plant above-ground biomass. Thus biomass is a main target for breeders, and photosynthetic efficiency is a critical physiological determinant

of net carbon gain. Therefore, a look into plant productivity can be obtained by studying the activity components of the photosynthetic apparatus, for example chlorophyll fluorescence kinetics and assimilation pigment contents [54,55]. Those kinds of studies supply key information about photochemical efficiency of photosystem II (PSII) and the amount of energy trapped during photosynthesis. Chlorophyll fluorescence and pigment contents have become the most recognizable and useful traits in photosynthesis research available to plant physiologists [32,56]. All the changes during the infection process might have a huge impact on pigment production and its activity, resulting in chlorophyll fluorescence changes and affecting effectiveness of PSII. In our study we observed a significantly lower level of pigments after inoculation. Similar results were presented by other authors who studied pigment content in oat after inoculation [42]. The largest decrease was observed for carotenoids, then for chlorophyll *a*, and chlorophyll *b*. Analysis of variance showed highly significant differences between the DH lines and the interaction for chlorophyll fluorescence parameters: the maximum photochemical efficiency (Fv/Fm), and the overall performance index of PSII photochemistry (PI). Inoculation significantly affected PI but not Fv/Fm, although a significant interaction for this parameter was observed between genotype and inoculation as well as for a single factor – genotype. Lower mean PI values for inoculated plants compared to the control were observed for hull-less lines than hulled ones. Higher activity of the photosynthetic apparatus may result in a more efficient primary carbohydrate metabolism, constituting the basis of the secondary metabolism related to e.g. synthesis of phenolics important for the defense mechanisms during pathogenesis [46]. Therefore, further studies could explore two hulled lines (R63N/3, R63N/4) where the level of pigments was not reduced after inoculation.

Conclusions

Physiological parameters could be utilized as markers associated with resistance to FSB, which is an especially compelling consideration for eventual use in selection. The limitation is significant correlation with direct assessment tests. To facilitate the most resistant DH lines, calculation of correlation coefficients of disease rating and fresh weight of seedlings assigned with biochemical and physiological parameters were performed. Results suggested a strong relation (significant positive correlation) between the DR of roots and the following physiological parameters:phenolics in leaves, soluble sugars in roots, and between fresh weight of roots and the following physiological parameters: chlorophyll *a*, *b*, carotenoids and PI.

A significant correlation between chlorophyll *a*, *b*, carotenoids and PI clearly reveals that higher content of chlorophyll *a*, *b*, carotenoids, and PI are related to less root fresh weight reduction after infection, which means that the examined genotypes are more resistant. Negative correlation coefficients were found between DR of roots and the following physiological parameters:soluble sugars in leaves, chlorophyll *a*, *b*, carotenoids, and maximum photochemical efficiency (Fv/Fm). Therefore low values of soluble sugars in leaves, chlorophyll *a*, *b*, and carotenoids, and maximum photochemical efficiency (Fv/Fm) suggest that genotypes are less resistant.

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