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Mycotoxin profiles and plumpness of Tritordeum grain after artificial spike inoculation with *Fusarium culmorum* W.G. Smith

Elżbieta Suchowilska [a], Marian Wiwart [a,*], Michael Sulyok [b], Wolfgang Kandler [b], Rudolf Krska [b,c]

[a] Department of Genetics, Plant Breeding and Bioresource Engineering, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-727 Olsztyn, Poland
[b] University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology (IFA-Tulln), Institute of Bioanalytics and Agro-Metabolomics, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria
[c] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, University Road, Belfast BT7 1NN, Northern Ireland, United Kingdom

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ABSTRACT

The responses to artificial spike inoculation with *Fusarium culmorum* were compared in 11 Tritordeum lines, two durum wheat cultivars and one naked barley cultivar. Inoculation of Tritordeum spikes led to a significant decrease in spike weight, kernel weight per spike, and kernel weight (by 18, 28, and 16 %, respectively). Durum wheat responded most strongly to inoculation, particularly with regard to spike weight and kernel weight per spike (decrease of 42 % and 53 %, respectively). Inoculation induced a significant increase in the total concentration of trichothecenes (9902 vs 558 μg/kg in non-inoculated control) and other *Fusarium* toxins (40,207 vs 3250 μg/kg in non-inoculated control) in Tritordeum grain. The content of three *Alternaria* toxins was not significantly modified by inoculation. The principal component analysis (PCA) of all fungal metabolites supported the discrimination of control and inoculated grain, and the results were used to divide the examined Tritordeum lines into two groups with different mycotoxin profiles. The first group (five lines) was more similar to naked barley, whereas the second group (six lines) showed greater similarity to durum wheat. The analyzed Tritordeum lines responded differently to inoculation, which suggests that lines with a low propensity to accumulate *Fusarium* toxins in grain can be selected from the existing gene pool. The study also demonstrated that Tritordeum grain accumulates significantly smaller amounts of mycotoxins than durum wheat grain.

1. Introduction

Fusarium head blight (FHB) is a fungal disease that affects small grain cereals such as wheat, barley, and oat worldwide (Yli-Mattila, 2010). The disease leads to the accumulation of mycotoxins in grain, and it can cause considerable economic losses by decreasing grain yields and grain quality (Khan et al., 2020; Khodaei et al., 2021). Simplified agronomic practices, climate change, and the low resistance of modern cereal cultivars contribute to the spread of fungal epidemics (Vaughan et al., 2016). Fusarium head blight is caused by several Fusarium species. Recent reports of shifts in the populations of FHB pathogens have shown that these populations are dynamic and constantly changing, which is often associated with increased yield losses or changes in the mycotoxins produced in grain (Valverde-Bogantes et al., 2019). In Poland, cereal infections of different severity are also caused by *Fusarium crookwellense* L.W. Burgess, P.E. Nelson & Toussoun, *Fusarium equiseti* (Corda) Sacc., *Fusarium langsethiae* Torp & Nirenberg, *Fusarium sporotrichioides* Sherb., *Fusarium oxysporum* Schl., *Fusarium poae* (Peck) Wollenw., and *Fusarium tricinctum* (Corda) Sacc. (Pszczółkowska et al., 2013).

Abbreviations: 15HCUL, 15-hydroxyculmorin; 3-ADON, 3-acetyldeoxynivalenol; 5-HCUL, 5-hydroxyculmorin; Afs, Aflatoxins; AFUS, Aurofusarin; ALT, Altersetin; APIC, Apicidin; BEA, Beauvericin; BIK, Bikaverin; BUT, Butenolide; Ch-diol, Chlamydospordiol; Ch-ol, Chlamydosporol; CHRY, Chrysogine; CUL, Culmorin; D3G, Deoxynivalenol-3-glucoside; DAS, Diacetoxyscirpenol; DON, Deoxynivalenol; ENA, ENA1, ENB, ENB1, Enniatin A, A1, B, B1, respectively; EQUI, Equisetin; FUMs, Fumonisins; HT-2, HT-2 toxin; INF, Infectopyron; MAS, Monoacetoxyscirpenol; MON, Moniliformin; NIV, Nivalenol; NIV3G, Nivalenol-3-glucoside; OTA, Ochratoxin A; T-2, T-2 toxin; ZEA, Zearalenone; β-ZEA, β-zearalenone.

* Corresponding author.
E-mail address: marian.wiwart@uwm.edu.pl (M. Wiwart).

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Mycotoxins are secondary fungal metabolites that are secreted mainly by toxigenic species of the genera *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* (Khaneghah et al., 2018). Four hundred types of mycotoxins have been identified to date, including zootoxins that pose a threat to humans and animals. This group of metabolites includes aflatoxins (Afs), ochratoxin A (OTA), deoxynivalenol (DON), T-2/HT-2 toxins, fumonisins (FUMs), and zearalenone (ZEA) (Khaneghah et al., 2018). Mycotoxicoses are serious infections that affect mainly the gastrointestinal tract, the nervous system, and skin tissues (Stoyev, 2015; Richard, 2007). These mycotoxins can also act as virulence factors for FHB in cereals, and they generate significant economic losses by reducing yields and rendering grain unsuitable for human or animal consumption (Audenaert et al., 2014). Fusarium head blight poses a particular threat to bread wheat (*Triticum aestivum* L.), the most widely produced species of small grain cereals in the world (Khan et al., 2020). This disease can reduce bread wheat yields by up to 30–50 % in epidemic years (McMullen et al., 1997). However, FHB is especially dangerous for durum wheat which is highly susceptible to infection (Beres et al., 2020).

Most *Fusarium* species can produce one or more mycotoxins, and DON, nivalenol (NIV), ZEA and the emerging mycotoxins of the cyclohexadepsipeptide family, mainly enniatins and beauvericin, are most commonly identified in wheat grain. The concentrations of ZEA and DON in the grain of durum wheat and other cereals are controlled and regulated in the European Union, and the maximum permitted levels of DON have been set at 1500 μg/kg in unprocessed durum wheat grain and at 1000 μg/kg in unprocessed barley grain (Commission Regulation (EU) 2024/1022). Enniatins have not yet been considered in the legislation (André et al., 2022).

Tritordeum (×*Tritordeum martinii* A. Pujadas, nothosp. nov.) is a relatively new allopolyploid crop that was obtained by crossing diploid *Hordeum chilense* Roem. & Schult. (HchHch) with tetraploid *Triticum durum* Desf. (AABB) (Martín et al., 1999). The resulting hybrid combines the desirable traits of *H. chilense*, including high carotenoid content of the endosperm and increased resistance to biotic and abiotic stress, with the high technological quality of wheat (Martín et al., 2002). Hexaploid Tritordeum was perceived as an interesting new crop with a similar role to bread wheat in the food industry (Ávila et al., 2021). At present, Tritordeum is cultivated on approximately 600 ha of land in the European Union, 70 % of which is located in Spain, 17 % in Northern Italy, and 12 % in Greece (Landolfi and Blandino, 2023). This cereal crop has been widely researched, and most studies attempted to determine the content of health-promoting phenolic compounds and carotenoids in Tritordeum grain (Suchowilska et al., 2021b; Montesano et al., 2021; Giordano et al., 2019). The optimization of agronomic practices to improve grain yields and the content of basic nutrients in grain has also been studied (Visioli et al., 2020; Suchowilska et al., 2021a). The storage proteins in Tritordeum kernels are less immunogenic than wheat grain proteins, which is why this cereal could be a safer option for consumers with gluten sensitivity (Vaquero et al., 2018).

According to Landolfi and Blandino (2023), Tritordeum can be easily incorporated into the cereal cropping system because it has a similar crop cycle and agronomic requirements to wheat. In comparison with wheat, Tritordeum is characterized by lower grain yields but higher grain protein content. This crop has inherited a good tolerance to abiotic stress and disease from *H. chilense*.

The unquestioned advantages of triticale have inspired breeders to hybridize barely with wheat. The first hybridization attempts were unsuccessful, and the first fertile plants were obtained in 1973 by Kruse who used South American wild barley (*H. chilense*) as the maternal parent (Kruse, 1973). Wild barley can be easily hybridized with the species of the tribe *Triticeae* (Fernández, 1989).

The accumulation of mycotoxins in Tritordeum grain remains insufficiently investigated (Spaggiari et al., 2019; Gozzi et al., 2024). The presence of mycotoxins in the grain of Tritordeum plants inoculated with *Fusarium* pathogens and the responses of different genotypes to infection have not been examined to date. To fill in this knowledge gap, the present study was undertaken to determine and compare the mycotoxin profiles of the grain of 11 Tritordeum breeding lines with two modern cultivars of durum wheat and one spring barley cultivar. This is an especially important consideration in the production of Tritordeum grain which has been recognized for its high nutritional value and health-promoting properties.

2. Materials and methods

2.1. Materials

The experimental material consisted of 11 Tritordeum breeding lines: JB3, HT 129, HT 157, HT 352, HT 438, HT 440, HT 444, HTC 1324, HTC 2060, HTC 2083, and HTC 2083′ (selected from line HTC 2083), which were obtained from Professor Antonio Martín of Instituto de Agricultura Sostenible – CSIC, Spain, with the assistance of Petr Martinek, Eng., of the Agricultural Research Institute Kromeriz, Ltd. in the Czech Republic. Two spring cultivars of *T. durum*, IS Duragold (Istropol Solary a.s, Horné Mýto, Slovakia) and Floradur (Probstdorfer Saatzucht GmbH & CoKG, Austria), and a spring cultivar of naked barley (*Hordeum vulgare* L. var. *nudum*) (cv. Gawrosz, Hodowla Roślin- Strzelce, Poland) were included in the experiment as the reference materials.

2.2. Field experiment

A two-year field experiment was conducted in 2021 and 2022 in the Agricultural Experiment Station in Bałcyny near Ostróda, Poland (DMS: 53° 35′ 49″ N 19° 51′ 15″ E) on optimal soil for wheat cultivation. The experiment had a randomized block design with three replications. Each experimental plot had an area of 12 m^2. The seeding rate was 200 kg/ha for all cereals. Winter rapeseed was the preceding crop in both years. NPK fertilizers (Polifoska 6/20/30, Police, Poland; ammonium nitrate Pulan® 34N, Police, Poland) were applied before sowing. Nitrogen fertilization was also applied in the stem elongation stage. The total fertilization rate reached 80/25/80 kg/ha. Seeds were not dressed, and fungicides and insecticides were not applied during the growing season. Weeds were controlled with the Mustang 306 SE herbicide (Dow AgroSciences, Poland) at 0.6 L/ha in BBCH stage 32 (node 2 at least 2 cm above node 1) (Meier, 2003).

The inoculum was prepared according to the procedure described by Suchowilska et al. (2010). Two strains of *F. culmorum* from the authors' collection (previously identified as the 15-ADON chemotype) had been isolated from bread wheat grain grown in north-eastern Poland and displaying symptoms of FHB. Species identity and chemotype were confirmed by PCR using primers specific for *Tri3* and *Tri13* genes, according to the procedures described by Jennings et al. (2004). The isolates were grown on potato dextrose agar (PDA) (Merck, Darmstadt, Germany) at 22 °C for 14 days. The photoperiod consisted of 12 h of darkness and 12 h of near-UV irradiation (36 W TLD; Philips, Amsterdam, the Netherlands) to promote sporodochial growth. Under these conditions, the fungus rapidly produced large amounts of uniform conidial spores that were used to prepare the inoculum. Inoculation was carried out by spraying an aqueous suspension of 5×10^5 conidial spores per mL onto the spikes with a backpack sprayer (Marolex Titan 12, Poland) in the full flowering stage (BBCH 65). The spikes were inoculated in the evening (at sunset) with 100 mL of the inoculum per 1 m^2 of plot area. The inoculation was repeated after 48 h. Control group lines and cultivars were grown under the same conditions, but without inoculation. Mature spikes were harvested manually from control and inoculated plants. Spike weight, kernel weight per spike, kernel number per spike, and kernel weight were determined.

2.3. Identification of Fusarium mycotoxins by LC-MS/MS

To identify *Fusarium* mycotoxins in grain, subsamples of 150 g were

obtained with a riffle divider and ground in a mill (Cyclotec™ 1093, Foss Tecator, Sweden, 1 mm mesh size). *Fusarium* mycotoxins were extracted in a rotary shaker with a dilution solvent composed of acetonitrile/water/acetic acid (79:20:1 v/v/v), applied at 20 mL per 5 g of grain for 90 min. The extracts were transferred to glass vials using Pasteur pipettes, and 350 µL aliquots were diluted with the same volume of the dilution solvent (acetonitrile/water/acetic acid 20:79:1, v/v/v). The extracts were stirred, and 5 µL of the diluted extract was injected into the LC–MS/MS system without further pre-treatment. The entire procedure was simplified for validation purposes to decrease the amounts of standards needed for spiking. *Fusarium* mycotoxins were identified and quantified according to the procedure described by Sulyok et al. (2020) with the use of a QTrap5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIon spray electrospray ionization (ESI) source and a 1290 Series UHPLC System (Agilent Technologies, Waldbronn, Germany). The analytes were separated on a Gemini C18 column (150 × 4.6 mm i.d., 5 µm particle size) with a 4 × 3 mm precolumn with the same characteristics (Phenomenex, Torrance, CA, USA). The analysis was performed with the use of a fully validated method for identifying >500 mycotoxins and other secondary metabolites, as described by Sulyok et al. (2020).

2.4. Statistical analysis

The results of spike biometric measurements were processed by analysis of variance (ANOVA). The distribution of mycotoxin concentrations was assessed for normality with the Shapiro-Wilk test. Significance of differences between control and inoculated treatments were determined in the Mann-Whitney *U* test. The results were subjected to principal component analysis (PCA). Data were processed statistically in Statistica 13 (TIBCO, 2017). Figure drawings were generated in CorelDraw® X8 (Corel Corporation, 2016).

3. Results

3.1. Weather conditions

Weather conditions during the growing season in both experimental years are presented in Fig. 1. In 2021, the lowest precipitation was noted in July (during flowering), and relatively high temperatures persisted until plant harvest in August. Harvest was delayed due to high precipitation in August. Abundant rainfall promotes the growth of fungal pathogens of the genera *Cladosporium* and *Alternaria* which cause sooty mold in cereals. In 2022, weather conditions were generally unfavorable for cereal growth, and low monthly precipitation delayed successive phenological stages. In 2022, July was a dry month with temperatures lower than those noted in 2021, which limited the spread of FHB.

3.2. The effect of inoculation on spike and kernel weight

Spike weight, kernel weight per spike, kernel number per spike, and kernel weight in the evaluated cereals are presented in Table 1. *Triticum durum* responded to *Fusarium* inoculation with the greatest reduction in spike weight, kernel weight per spike, and kernel weight (by 42, 53, and 25 % on average, respectively). In the analyzed Tritordeum lines, inoculation decreased the above parameters by 18 %, 28 %, and 16 %, respectively. Naked barley was least sensitive to inoculation; the evaluated parameters did not decrease by >8 %, and no significant differences were observed between control and inoculated plants.

3.3. Mycotoxin concentrations in grain

This is the first study to compare the content of fungal metabolites in Tritordeum grain after artificial inoculation with *F. culmorum* with the grain of non-inoculated plants grown in a field experiment. A total of 31

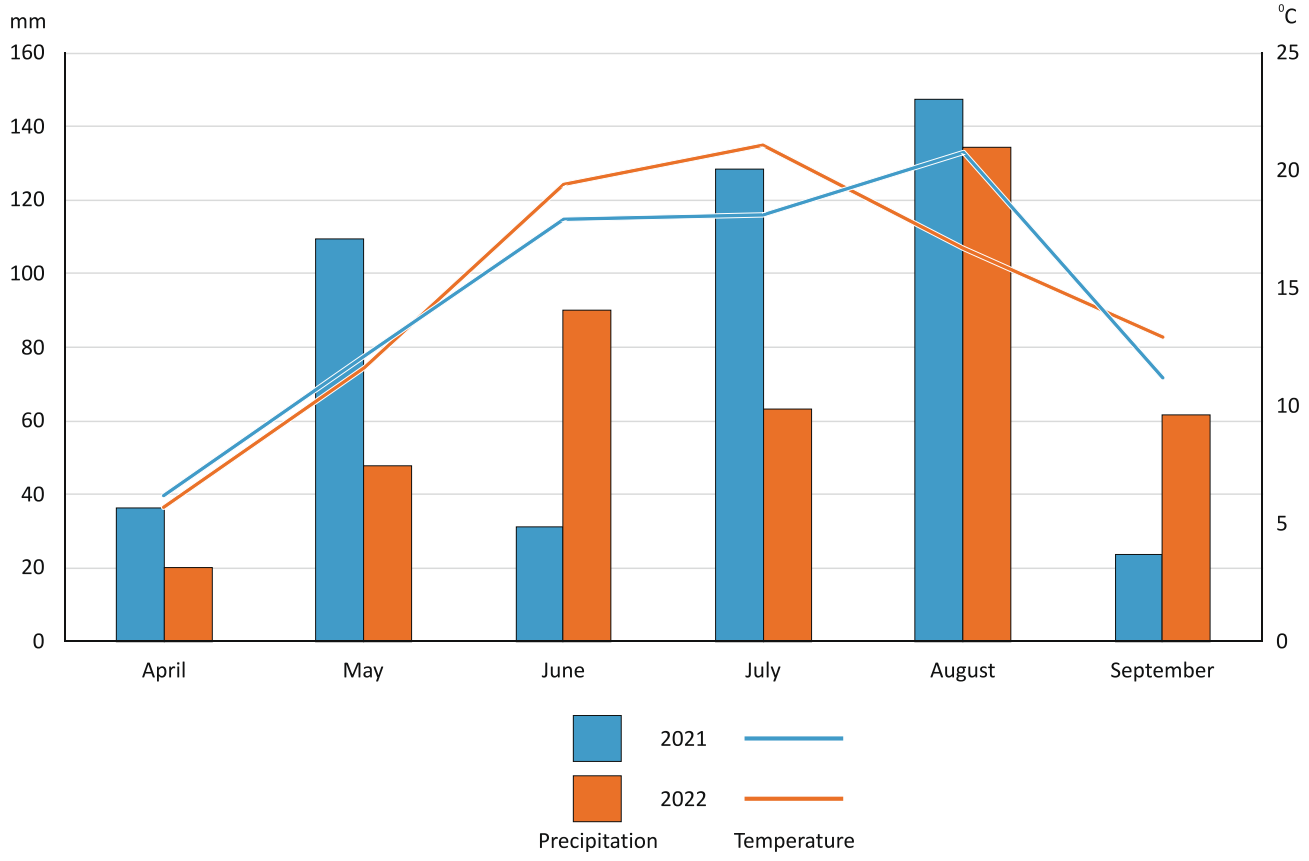

Fig. 1. Weather conditions in the Agricultural Experiment Station in Bałcyny during the growing seasons of 2021 and 2022.

Table 1
Spike weight (SW) (g), kernel weight per spike (KWS) (g), kernel number per spike (KNS) and kernel weight (KW) (mg) in the analyzed Tritordeum lines and *T. durum* and *H. sativum* cultivars inoculated with *F. culmorum* (mean values for the two-year experiment).

Trait	Tritordeum ($n = 11$)		*T. durum* ($n = 2$)		*H. sativum* var. *nudum* (n = 1)		Total ($n = 14$)	
	C	I	C	I	C	I	C	I
SW	1.67	1.37*	2.29	1.32*	1.25	1.15	1.77	1.35**
Decrease (%)	18 %		42 %		8 %		23 %	
KWS	1.29	0.93**	1.60	0.75*	0.97	0.89	1.34	0.90**
Decrease (%)	28 %		53 %		8 %		33 %	
KNS	37.37	31.93	38.28	23.87*	22.15	21.73	37.51	30.05*
Decrease (%)	15 %		38 %		2 %		18 %	
KW	34.14	28.80**	41.91	31.37*	43.9	41.07	35.34	30.04**
Decrease (%)	16 %		25 %		6 %		17 %	

*, ** - differences between control (C) and inoculated (I) treatments are significant at $p < 0.05$ and $p < 0.01$, respectively. Decrease (%) – decrease in the value of the examined trait after inoculation relative to control.

mycotoxins, including 30 metabolites produced by fungi of the genus *Fusarium* and three metabolites produced by fungi of the genus *Alternaria*, were identified in the analyzed grain in both years of the study (Tables S1, 2). Deoxynivalenol was the most prevalent mycotoxin that was determined in all samples of control and inoculated grain. After inoculation, the mean concentration of DON increased >19 times in Tritordeum grain (6425 vs 333.5 μg/kg) and nearly 72 times in the grain of both durum wheat cultivars (15,204.3 vs 211.8 μg/kg) (Table 2). Inoculation led to a highly significant increase in the content of all group B trichothecenes, in particular 3-ADON whose concentration increased >73 times in Tritordeum grain and >173 times in durum wheat grain on average. Significant differences were also noted in the content of NIV-3G which increased >25 times in Tritordeum grain and >1300 times in durum wheat grain after inoculation relative to control grain. After inoculation, total trichothecene content was highest in *T. durum* (22,266.5 μg/kg) and lowest in *H. sativum var nudum* (3041.8 μg/kg). Trichothecene levels were generally low in control grain, and they were determined at 558 μg/kg in Tritordeum on average. In both control and inoculated grain, the total content of the remaining *Fusarium* toxins was highest in *T. durum* (11,629 μg/kg in control and 100,725.8 μg/kg in inoculated grain) and lowest in barley. Grain was also analyzed for the presence of three toxins produced by *Alternaria* spp. The most prevalent *Alternaria* mycotoxins were infectopyron (INF) and altersetin (ALT), and their concentrations were 1.57 and 9.64 times higher in control than in inoculated Tritordeum grain. The total content of all analyzed mycotoxins was lower in Tritordeum grain than in *T. durum* grain. A comparison of control and inoculated grain of both species revealed that toxin concentrations were more than twice lower in Tritordeum grain than in durum wheat grain.

Statistical analyses of mycotoxin concentrations pose a challenge, mainly because the results are not normally distributed, which prevents the application of popular parametric tests, such as ANOVA and multiple significance tests. As a result, statistical significance can be determined only with the use of non-parametric methods. Mycotoxin concentrations in the analyzed cereals were ranked and compared by the non-parametric Mann-Whitney *U* test, and the results are presented in Table S2. The test revealed highly significant differences in DON, D3G, ADON, NIV, and NIV3G levels between control and inoculated grain of Tritordeum and *T. durum*. In durum wheat, control and inoculated grain differed highly significantly also in the concentrations of β-ZEA, CUL, 15HCUL, 5HCUL, and CHRY. In both Tritordeum and *T. durum*, the content of *Alternaria* mycotoxins was significantly higher in control than in inoculated grain (Table S2).

The mean concentrations of all mycotoxins identified in the analyzed cereal grain in both years of the study were subjected to PCA, and the results are presented in Fig. 2. The first two principal components (PC1 and PC2) explained 70.6 % of total variance, and they strongly discriminated between control and inoculated grain (Fig. 2). In addition, Tritordeum lines were divided into two groups that were characterized by similar mycotoxin profiles of grain in both control treatments and treatments inoculated with *F. culmorum*. The first group comprised the grain of Tritordeum lines HT 157, HT 438, HT 444, HTC 1324, and HTC 2083 which was characterized by high intergroup similarity, as well as similarity to the grain of barley cv. Gawrosz. In the second group, the mycotoxin profiles of grain were similar to the profiles noted in both durum wheat cultivars (Fig. 2). This discrimination can be attributed to the fact that group 1 grain accumulated smaller amounts of mycotoxins, in particular trichothecenes (S1, S3, Fig. 2). The above observation was made in both control and inoculated grain. Interestingly, spike weight, kernel weight per spike, kernel number per spike, and kernel weight were significantly higher in group 1 than in group 2 lines (Table S3). Highly significant differences in the mean concentration of 14 trichothecenes were noted between groups. In group 2, the total content of these fungal metabolites was more than six times higher in control grain and more than twice higher in inoculated grain relative to group 1 (Table S3). Deoxynivalenol levels in Tritordeum grain were bound by negative correlations with the analyzed spike and grain parameters (spike weight, kernel weight per spike, kernel number of spike, and kernel weight). The values of Pearson's correlation coefficient ranged from −0.16 (DON x KW in control grain) to −0.61 and −0.70 (DON x KNS in control grain and DON x KWS in inoculated grain, respectively). However, only the two latter values were statistically significant.

The contribution of the analyzed variables (mycotoxins) to PC1 and PC2 and the strength of the correlations between variables and PCs (*r*) are presented in Table S4. The contribution of variables to both PCs is presented graphically in Fig. 3. These results clearly indicate that *F. culmorum* mycotoxins, which were detected in large quantities in inoculated grain, made a significant contribution to PC1, whereas the remaining *Fusarium* and *Alternaria* mycotoxins made a contribution to PC2.

4. Discussion

The responses of Tritordeum breeding lines to artificial spike inoculation with *F. culmorum* were examined in a two-year field experiment, with special emphasis on mycotoxin concentrations in grain. Tritordeum grain has high nutraceutical value and a high content of protein and micronutrients, but the presence of toxic fungal metabolites can undermine its suitability for the production of health-promoting and functional foods (De Caro et al., 2024). In this study, two durum wheat cultivars and one naked barley cultivar were used as the reference materials. Durum wheat is generally more prone to FHB than bread wheat due to genetic and morphological traits, although susceptibility to infection differs considerably across varieties, in particular in common wheat genotypes (Giancaspro et al., 2018). Naked barley was the second reference cereal. Despite the fact that Tritordeum is a hybrid species obtained by crossing *T. durum* and *H. chilense*, *H. sativum* was selected for the current experiment for two reasons. Firstly, no information was

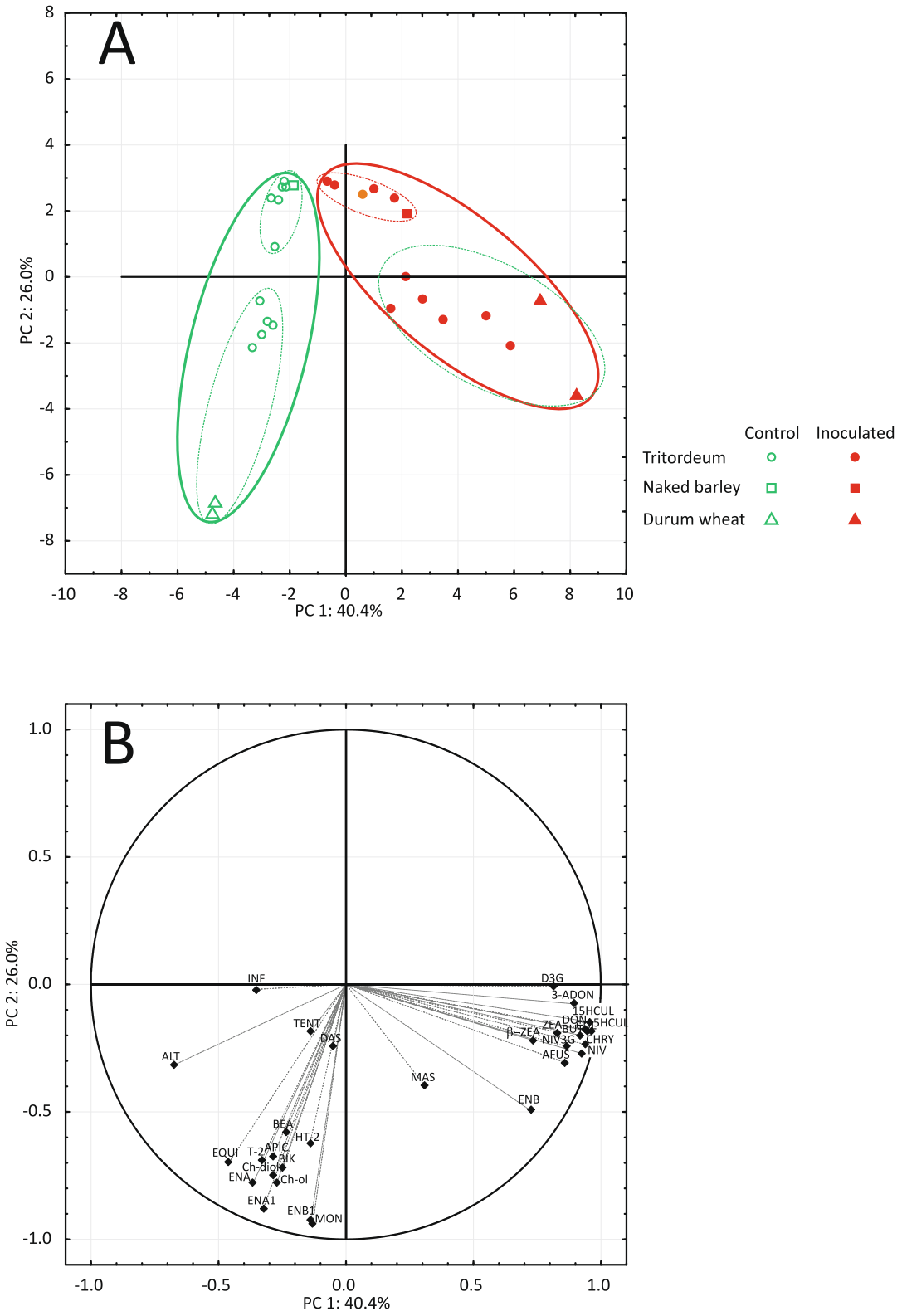

Fig. 2. PCA results for 31 mycotoxins and all cereals analyzed in the two-year field experiment. A. Score plot, B. Variable loading plot. The ellipses drawn with dashed lines surround two groups of breeding lines with different mycotoxin concentrations and profiles.

available about the genotype of *H. chilense* used for developing the analyzed Tritordeum lines. Secondly, the only *H. chilense* accession in the authors' possession is characterized by irregular onset of heading, which significantly complicates spike inoculation.

Most research conducted on Tritordeum focused on the quality and health-promoting properties of grain, whereas the risk of FHB in this cereal species has been rarely investigated. According to Landolfi and Blandino (2023), recent research suggests that the first commercial cultivars of Tritordeum are highly susceptible to FHB and that the phytosanitary risk during production is similar to that noted in durum

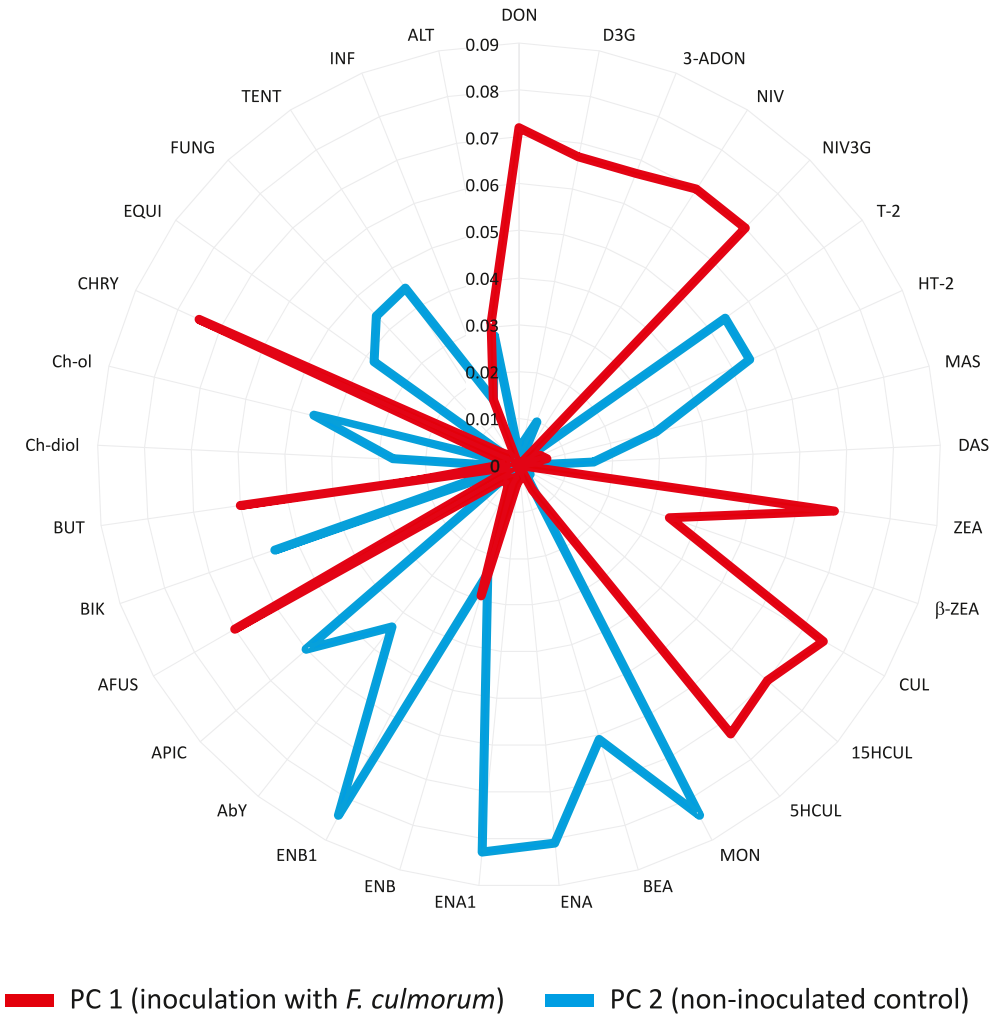

Fig. 3. The contribution of the analyzed variables (mycotoxins) to the first two principal components (PC1 and PC2).

wheat processing. Spaggiari et al. (2019) analyzed the mycotoxin profiles and the distribution of native and modified *Fusarium* mycotoxins in whole grain and pearled grain fractions of Tritordeum, wheat, and barley, and they found that the concentration of *Fusarium* toxins was highest in the outer layer and lower in the endosperm. These authors reported that the content of DON in whole kernels of two Tritordeum cultivars (Aucan and Bulel) ranged from 3209 to 6354 μg/kg, and it was only somewhat lower than that determined in a durum wheat cultivar. The analyzed cereal species were not inoculated, but the reported values were significantly higher than those noted in this study. In turn, Gozzi et al. (2024) examined mycotoxin levels in the grain and straw of Tritordeum, barley, bread wheat, and durum wheat. The content of *Fusarium* toxins in grain differed significantly across the experimental years, whereas DON and D3G levels were only somewhat higher in Tritordeum than in durum wheat grain. The cited study involved Tritordeum cv. Coique as well as two Tritordeum cultivars that had been previously examined by Spaggiari et al. (2019).

This is the first study to examine the responses of Tritordeum plants to artificial spike inoculation with *Fusarium* pathogens, and the results cannot be compared with other authors' findings. The response to spike inoculation was weaker in 11 Tritordeum lines than in durum wheat cultivars, including the four evaluated yield-related traits and mycotoxin concentrations. The fact that in both reference cultivars of durum wheat, inoculation induced the greatest decrease in grain plumpness and led to a substantial increase in mycotoxin levels is not surprising because *T. durum* is particularly susceptible to infections by FHB pathogens (Miedaner et al., 2017; Haile et al., 2019). In turn, mycotoxin levels were low in the reference naked barley cultivar, both in control and inoculated grain, and barley responded weakly to pathogens causing FHB. In the PCA, Tritordeum lines were divided into two groups based on mycotoxin concentrations in grain, which was quite unexpected. Five Tritordeum lines whose grain was similar to the grain of naked barley in terms of mycotoxin levels and profiles were characterized by significantly higher values of yield-related traits. In both groups, artificial inoculation with *F. culmorum* led to a significant decrease in spike weight and kernel weight per spike, but no significant differences in kernel weight were observed between groups. This observation suggests that in inoculated plants, the decrease in kernel weight per spike resulted mainly from a reduction in the number of kernels per spike and, to a lesser degree, from a decrease in kernel weight. Deoxynivalenol levels were bound by negative and mostly non-significant correlations with the four examined phenotypic traits, which points to a certain relationship between the pathogenicity and toxin-producing ability of *F. culmorum*. The relationship between these traits has been analyzed extensively in this *Fusarium* pathogen. According to a review article by Wagacha and Muthomi (2007), trichothecenes produced by *F. culmorum* are a major determinant of fungal spread and disease development in Triticeae. Resistance to DON accumulation is one of the five identified mechanisms of resistance to FHB (type 4). The remaining types of resistance include resistance to primary infection (type 1), resistance to the spread of infection (type 2), kernel resistance (type 3), and tolerance (type 5) (Mesterhazy, 2024). The significance and prevalence of these

Table 2
Mean concentrations (μg/kg) of the analyzed mycotoxins in the grain of the examined in two-year experiment Tritordeum lines and *T. durum* and *H. sativum* cultivars.

Toxin		Tritordeum (n = 11)		*T. durum* (n = 2)		*H. sativum* var. *nudum* (n = 1)		Total (n = 14)	
		C	I	C	I	C	I	C	I
Trichothecenes	DON	333.5	6425.0**	211.8	15,204.3	0.8	4200.0	292.3	7520.3**
	D3G	63.6	850.6**	63.8	827.4	0.8	1713.7	59.1	908.9**
	3-ADON	4.6	186.2**	2.4	415.3	2.4	436.2	4.1	236.7**
	NIV	116.4	1949.0*	74.6	5138.6	7.9	648.9	102.6	2311.8**
	NIV3G	18.2	464.4**	0.5	672.6	0.5	199.7	14.4	475.2**
	T-2	2.1	1.9	10.8	0.4	0.4	0.4	3.2	1.6
	HT-2	12.1	15.7	47.1	3.4	0.7	20.0	16.3	14.2
	MAS	4.6	5.7	2.0	3.9	3.3	2.4	4.1	5.2
	DAS	3.1	3.5	8.4	0.7	7.8	20.4	4.2	4.3
	Σ	558.1	9902.0**	421.4	22,266.5**	23.9	3041.8	500.5	11,478.3**
Other Fusarium toxins	ZEA	6.2	224.3**	18.8	1224.8	0.1	266.0	7.6	370.2**
	β-ZEA	1.46	12.05	1.45	26.5	1.5	1.5	1.5	13.4*
	CUL	596.3	9126.3**	307.0	17,741.8	8.8	8550.9	513.0	10,316.0**
	15HCUL	305.2	12,291.4**	164.5	24,250.7	3.1	11,642.7	263.5	13,953.5**
	5HCUL	170.7	4754.6**	80.9	10,541.8	5.0	3175.4	146.0	5468.5**
	MON	534.8	339.5	1940.0	977.2	41.3	17.6	700.3	407.6
	BEA	10.1	3.4	18.8	8.3	1.3	0.2	10.7	3.9**
	ENA	7.2	3.1	15.2	3.1	1.0	0.4	7.9	2.9*
	ENA1	76.1	37.7	210.8	56.2	14.1	6.5	90.9	38.1
	ENB	327.1	862.8	828.4	1852.9	300.7	196.4	396.8	956.6
	ENB1	420.4	279.4	1100.3	535.3	107.2	53.0	495.1	299.8
	APIC	14.2	4.9	67.4	14.5	7.3	0.1	21.3	5.9
	AFUS	532.8	10,579.9**	4215.6	39,239.6	38.2	4194.8	1023.6	14,218.0**
	BIK	6.4	3.1	50.2	13.6	1.8	0.2	12.4	4.4*
	BUT	130.1	1128.6	176.6	2395.8	11.0	1444.4	128.2	1332.2**
	Ch-diol	2.3	0.3	59.4	10.2	0.0	0.0	10.3	1.7
	Ch-ol	24.6	2.47	466.8	106.0	0.2	0.2	86.0	17.1
	CHRY	36.9	530.49**	87.8	1565.3	17.6	682.7	42.8	689.2**
	EQUI	46.9	22.4	203.0	7.2	3.0	2.4	66.0	18.8*
	Σ	3249.8	40,206.7**	10,013.0	100,570.8**	563.2	30,235.4**	4023.9	48,117.8**
Alternaria toxins	TENT	4.8	2.9	5.4	3.1	1.4	0.1	4.6	2.8*
	INF	758.5	481.4	465.6	191.7	244.4	121.0	679.9	414.3**
	ALT	218.8	22.7	447.7	17.3	32.8	0.5	238.2	20.4**
	Σ	982.1	507.1	918.6	212.1	278.7	121.6	922.8	437.4
Total		4790.1	50,615.8	11,353.0	123,049.4	865.8	3398.8	5801.2	60,033.5

*, ** - differences between control (C) and inoculated (I) treatments are significant at $p < 0.05$ and $p < 0.01$, respectively (in the non-parametric Mann-Whitney U test; see Table S2). DON – Deoxynivalenol; D3G-Deoxynivalenol-3-glucoside; NIV – Nivalenol; 3-ADON - 3-acetyldeoxynivalenol; NIV3G - Nivalenol-3-glucoside; T-2 – T-2 toxin; HT-2 – HT-2 toxin; MAS – Monoacetoxyscirpenol; DAS – Diacetoxyscirpenol; ZEA – Zearalenone; β-ZEA - β-zearalenone; CUL – Culmorin; 15HCUL – 15-hydroxyculmorin; 5-HCUL – 5-hydroxyculmorin; MON -Moniliformin; BEA – Beauvericin; ENA, ENA1, ENB, ENB1 – Enniatin A, A1, B, B1, respectively; APIC – Apicidin; AFUS – Aurofusarin; BIK - Bikaverin; BUT - Butenolide; Ch-diol – Chlamydospordiol; Ch-ol- Chlamydosporol; CHRY - Chrysogine; EQUI - Equisetin; TENT - Tentoxin; INF - Infectopyron; ALT - Altersetin.

resistance mechanisms in Tritordeum is difficult to determine due to a limited number of research studies and a very small number of analyzed Tritordeum cultivars.

Tritordeum grain is highly suitable for the production of functional foods with a high nutritional value. The present study was undertaken to determine the extent to which mycotoxin contamination can compromise the health-promoting properties of Tritordeum grain. The analyzed breeding lines responded differently to artificial spike inoculation with *F. culmorum*, which suggests that lines (or cultivars) with a low propensity to accumulate toxic metabolites produced by FHB pathogens can be selected from the existing gene pool. The study also demonstrated that Tritordeum grain accumulates significantly smaller amounts of mycotoxins than durum wheat grain.

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CRediT authorship contribution statement

Elżbieta Suchowilska: Writing – original draft, Methodology, Investigation, Conceptualization. **Marian Wiwart:** Writing – review & editing, Supervision. **Michael Sulyok:** Methodology, Investigation. **Wolfgang Kandler:** Investigation. **Rudolf Krska:** Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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