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The profile of bioactive compounds in the grain of various *x Tritordeum* genotypes

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ABSTRACT

The content of bound phenolic acids, flavonoids and carotenoids was compared in the grain of eleven Tritordeum breeding lines and two cultivars of durum wheat. Ferulic and *t*-cinnamic acids occurred in the highest concentrations and accounted for 89% of total phenolic acids in Tritordeum and 80% in *T. durum*. The total concentration of bound phenolic acids was determined at 2063 mg kg^{-1} on average in Tritordeum, and it was nearly two-fold higher than in durum wheat (1056 mg kg^{-1}). The total concentration of the identified flavonoids in grain was nearly 50% higher in Tritordeum than in durum wheat. The studied lines differed considerably in zeaxanthin content. The lutein/zeaxanthin ratio was closer to 5 in Tritordeum than in durum wheat grain. This ratio of around 5 is generally assumed to deliver health benefits. The grain of the analyzed Tritordeum lines was characterized by significantly higher antioxidant activity than *T. durum* grain.

1. Introduction

Since the beginning of the twentieth century, cereal breeders have been focusing their efforts on developing interspecific amphiploid hybrids to obtain new cereals with improved agronomic performance, increased content of phytochemicals and superior technological quality (Smith, 1942; Loureiro et al., 2007; Wrigley and Bushuk, 2017; Giordano et al., 2019). Breeding efforts gave rise to *x Tritordeum* Ascherson et Graebner, an intergeneric amphiploid of *Triticum durum* Desf. and *Hordeum chilense* Roem. & Schult. This crop constitutes a valuable alternative to other small grain cereals. This novel cereal species has been introduced to European markets in recent years (Martín et al., 1999; Vaquero et al., 2018). Tritordeum is presently available in several European and non-European countries, and it is becoming widely cultivated in Spain, Italy, and Portugal (Visioli et al., 2020). Tritordeum is a rich source of bioactive compounds. According to Atienza et al. (2007), Tritordeum contains 5.2 times more carotenoids than durum wheat, which suggests that it has high functional food potential. Other authors have demonstrated that Tritordeum is characterized not only by a high content of carotenoids (Mellado-Ortega and Hornero-Méndez 2016; Paznocht et al., 2018), but also tocols (Lachman et al., 2018) and phenolic acids (Eliášová and Paznocht, 2017). The health-promoting properties of cereal grain can be attributed to a wide range of phytochemicals with antioxidant activity (Navas-Lopez et al., 2014; Horvat et al., 2020), mainly polyphenols, carotenoids and phytosterols. Phenolics appear to play the most important role in this group of compounds (Călinoiu and Vodnar, 2018; Belobrajdic and Bird, 2013; Luthria et al., 2015). The presence of these metabolites in the human diet considerably decreases the risk of lifestyle diseases, in particular hypercholesterolemia and cancer, and it can reduce the formation of inflammatory metabolites (Rasouli et al., 2017; Krzyżanowska et al., 2010). The grain of modern bread wheat cultivars is becoming less abundant in desirable phenolic compounds (Gotti et al., 2018), and efforts are being made to identify new species of small grain cereals that are rich in these metabolites. Tritordeum appears to be one of such species (Eliášová and Paznocht, 2017; Navas-Lopez et al., 2014; Giordano et al., 2019; Montesano et al., 2021).

The aim of this study was to compare the profile of bound phenolic acids, flavonoids and carotenoids in the grain of eleven Tritordeum breeding lines and two modern cultivars of durum wheat. This is the first

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study to perform such a comprehensive analysis of all three compound groups in Tritordeum grain.

2. Materials and methods

2.1. Materials

The study was performed on eleven spring breeding lines of Tritordeum (HT 440; HT 129; HTC 2083; HTC 2083′ selected from line 2083; HTC 2060; HT 157; HT 352; JB3; HT 444, HT 438; HTC 1324) acquired from Professor Antonio Martín of the Institute for Sustainable Agriculture (CSIC, Spain), courtesy of Petr Martinek of the Agricultural Research Institute Kroměříž, Ltd. (Czech Republic). The reference material comprised two spring cultivars of *T. durum*: Duralis (SAATEN–-UNION, Hannover, Niedersachsen, Germany) and Duranegra (ISTROPOL SOLARY a.s, Horné Mýto, Slovakia).

2.2. Field experiment

In 2020, a field experiment was conducted at the Agricultural Experiment Station in Bałcyny, Poland (53° 360′ N, 19° 510' E) on optimal soils for wheat cultivation. The experiment had random block design with two replicates. Plot size was 9 m^2. NPK fertilizer was applied before sowing at a rate of 120/25/80 kg ha^{-1}. The sowing density of Tritordeum and durum wheat was 400 germinating kernels/m^2. Seeds were not dressed, and fungicides and insecticides were not applied during the growing season. Grain for all analyses was harvested in the over-ripe stage (BBCH 92) (Witzenberger et al., 1989).

2.3. Determination of carotenoids

Carotenoid content was determined according to the method described by Suchowilska et al. (2020). Carotenoids were isolated and quantified in the Acquity UPLC system (Waters, Milford, MA, USA) (Przybylska-Balcerek et al., 2019). Carotenoid extracts in the amount of 0.4 mg were obtained from 10 g specimens of ground kernels that were tittered with a mixture of acetone and petroleum ether (1:1). Plant tissue was separated, and acetone and hydrophilic fractions were removed from the extract by washing with deionized water. The ether extract was obtained with a mixture of carotenoid pigments. The prepared extract was concentrated in a vacuum evaporator at 35 °C until an oily residue was obtained. The residue was dissolved in 2 mL of methanol (Merck, Poland) and subjected to chromatographic analysis. Lutein (LUT), zeaxanthin (ZEA), and β-carotene (β-C) were determined in the Acquity UPLC system (Waters, USA) with a Waters Acquity PDA detector (Waters, Milford, MA, USA). Chromatographic separation was performed on an Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Ireland). Elution was carried out with: A – methanol (MeOH) solvent, B - water, C - tert-butyl methyl ether (TBME) in a 5:1:1 ratio (v/v/v). The elution gradient was applied at a flow rate of 0.4 mL min^{-1}. The column and the samples were thermostatted, where the column temperature was 30 °C, and the test temperature was 10 °C. During the analysis, the solutions were degassed in a Waters device. The injection volume was 10 μL. The separated compounds were registered at a wavelength λ = 445 nm. Compounds were identified based on spectra in the range of 200–600 nm, and retention times were compared with the standards. The LOD was 0.01 mg kg^{-1} for LUT and ZEA, and 0.02 mg kg^{-1} for β-C. The LOQ was 0.1 mg kg^{-1} for LUT and ZEA, and 0.3 mg kg^{-1} for β-C.

2.4. $ABTS^{\bullet+}$ assay

The antioxidant activity of grain was determined in the $ABTS^{\bullet+}$ radical scavenging assay according to the method described by Przybylska-Balcerek et al. (2020) and Suchowilska et al. (2020). The analyses were carried out in a Thermo Multiskan EX Microplate Photometer (Corston, UK). $ABTS^{+}$ radicals were generated from ABTS salt by reacting 3 mM of potassium persulfate with 8 mM ABTS salt in distilled deionized water for 16 h at room temperature in dark. The $ABTS^{+\cdot}$ solution was diluted with a phosphate buffer solution (pH 7.4) containing 150 mM sodium chloride to obtain an initial absorbance of 1.5 at 730 nm. Fresh $ABTS^{+\cdot}$ solution was prepared for each analysis. Reaction kinetics were determined over a 2 h period with readings every 15 min. The reactions were complete in 30 min. The samples and the standards (100 μmol) were reacted with the $ABTS^{+\cdot}$ solution (2900 μmol) for 30 min. Trolox was used as the standard. The results were expressed in terms of $ABTS^{\bullet+}$ radical scavenging activity (μmol TROLOX g^{-1}) per gram of dry matter.

2.5. Determination of bound phenolic acids

The content of phenolic acids was determined according to the method described by Suchowilska et al. (2020). Phenolic acids were determined in samples of 0.20 g. The samples were placed in sealed 17 mL culture test tubes where alkaline hydrolysis, followed by acid hydrolysis were run. Alkaline hydrolysis was performed by adding 1 mL of distilled water and 4 mL of 2M aqueous sodium hydroxide to the test tubes. Tightly sealed test tubes were heated in a water bath at 95 °C for 30 min. The tubes were cooled (approx. 20 min) and neutralized with 2 mL 6M aqueous hydrochloric acid solution (pH = 2). The samples were then cooled in ice water. Phenolic acids were extracted from the inorganic phase using diethyl ether (2 × 2 mL). The obtained ether extracts were continuously transferred to 8 mL vials. Acid hydrolysis was performed by adding 3 mL of 6M aqueous hydrochloric acid solution to the aqueous phase. Tightly sealed test tubes were heated in a water bath at 95 °C for 30 min. The samples were cooled in ice water and extracted with diethyl ether (2 × 2 mL). The produced ether extracts were continuously transferred to 8 mL vials and evaporated to dryness in a stream of nitrogen. The samples were dissolved in 1 mL MeOH before analysis in the Acquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed on an Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Ireland). The mobile phase had the following composition during gradient elution: (A) acetonitrile with 0.1% formic acid, (B) 0.1% aqueous formic acid solution (pH = 2). The concentration of phenolic acids was determined using an internal standard at λ = 280 nm. Phenolic acids were identified based on comparing the retention time of the analyzed peak with the retention time of the standard. A specific amount of the standard was added to the analyzed samples, and the analysis was repeated twice. The detection threshold was 1 μg g^{-1}. The assayed compounds had the following retention times (min): HBA – 4–hydroxybenzoic acid: 1.94; CA – caffeic acid: 2.80; CGA – chlorogenic acid: 9.56; FA – ferulic acid: 14.20; GA – gallic acid: 7.85; pCA – *p*-coumaric acid: 4.22; PrCA protocatechuic acid: 3.31; SiA – sinapic acid: 12.77; SyA – syringic acid: 15.06; CiA – *t*-cinnamic acid: 11.35; VA – vanillic acid: 6.11; VN – vanillin: 11.79.

2.6. Determination of bound flavonoids

The content of bound flavonoids was determined according to the method described by Suchowilska et al. (2020). Flavonoids were analyzed after alkaline and acidic hydrolysis, as described in subsection 2.5. The samples were dissolved in 1 mL MeOH before analysis in the Acquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed on an Acquity UPLC® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Ireland). The mobile phase had the following composition during gradient elution: (A) acetonitrile with 0.1% formic acid, (B) 0.1% aqueous formic acid solution (pH = 2). The concentration of phenolic compounds was determined using an internal standard at λ = 320 nm. Flavonoids were identified by comparing the retention time of the analyzed peak with the retention time of the standard. A specific amount

of the standard was added to the analyzed samples, and the analysis was repeated twice. The detection threshold was 1 μg g^{-1}. The assayed compounds had the following retention times (min): Ap – apigenin: 23.00; Ka – catechin: 15.88; Km – kaempferol: 24.11; Lu – luteolin: 23.69; Na – naringenin: 14.35; Qu – quercetin: 19.00; Ru – rutin: 21.44; Vi – vitexin: 28.00.

2.7. Statistical analysis

Statistical analyses were performed in Statistica 13 software (TIBCO Software Inc). Data were analyzed by one-way ANOVA, and the significance of differences between means was determined by the Student-Newman-Keuls test. The results were processed by principal component analysis (PCA) and multiple linear regression.

3. Results and discussion

3.1. Antioxidant activity and bound phenolic acids

Thousand kernel weight (TKW) was significantly smaller in the studied Tritordeum lines (39.9 g) than in *T. durum* cultivars (50.4 g). The antioxidant activity of Tritordeum lines was significantly higher (by approx. 26%) than in both *T. durum* cultivars (50.4 g) (Table 1). Eleven bound phenolic acids and vanillin, a vanillic aldehyde, were identified in the grain of the analyzed cereals (Table 1). Ferulic and *t*-cinnamic acids were the dominant compounds, and their concentrations were determined at 1008.9 and 826.5 mg kg^{-1}, respectively, in Tritordeum, and 656.8 and 191.9 mg kg^{-1}, respectively, in both durum wheat cultivars. These metabolites accounted for 89% of all phenolic acids in Tritordeum and 80% in *T. durum*, which implies that the content of the remaining phenolic acids was more balanced in durum wheat than in Tritordeum. The total concentration of all phenolic acids reached 2063 mg kg^{-1} in Tritordeum, and it was nearly two-fold higher than in durum wheat (1056 mg kg^{-1}). It should also be noted that Tritordeum was significantly more abundant in 4–hydroxybenzoic, gallic, *p*-coumaric, protocatechuic, *t*-cinnamic and vanillic acid, but contained less gallic acid than *T. durum*.

3.2. Flavonoids and carotenoids

Eight flavonoids and three carotenoids were identified in the grain of the compared cereals (Table 2). Tritordeum grain was significantly more abundant in flavonoids, excluding quercetin, than durum wheat grain. Significant differences were noted in the concentrations of apigenin, catechin, luteolin and rutin. Rutin content was more than 2.6 higher in Tritordeum grain than in durum wheat grain. This is an interesting result because *T. durum* grain contained 7% more quercetin which forms the glycoside rutin. The above could indicate that quercetin is metabolized differently in the compared cereals. No such relationship was observed in the content of vitexin, an apigenin glycoside. The variations in the concentrations of both metabolites were similar in the compared cereals, and the content of vitexin and apigenin in grain was more than two-fold higher in Tritordeum than in durum wheat. The total concentration of all flavonoids identified in grain and their glycosides was more than 50% higher in Tritordeum than in *T. durum* (223.5 and 143 mg kg^{-1}, respectively).

Interestingly, TKW and the concentrations of the analyzed metabolites were not bound by consistent or logical correlations. Pearson's *r* ranged from −0.859 (for Ru) to 0.740 (for PCA). For 24 variables (phenols, carotenoids and $ABTS^{+}$), the above values were both positive and negative, and they were statistically significant in only 9 cases (Ap, Ru, HBA, CA, CGA, GA, ZEA, total carotenoids and $ABTS^{+}$).

According to the literature, the high lutein content of grain is a feature that distinguishes Tritordeum from other small grain cereals, including *T. durum* (Atienza et al., 2007; Ávila et al., 2021) (Table 2). Lutein and zeaxanthin concentrations were nearly 30% higher in Tritordeum grain than in *T. durum* grain which is generally abundant in both carotenoids. However, significant differences were observed only in the content of lutein and total carotenoids because zeaxanthin concentrations varied considerably across the studied breeding lines (RSD = 32%). Tritordeum was characterized by a narrower lutein/zeaxanthin ratio than durum wheat, which is a positive trait because a lutein/-zeaxanthin ratio should be around 5 (Thurnham, 2007).

The grain of the studied Tritordeum lines was characterized by significantly higher antioxidant activity than both *T. durum* cultivars, which also testifies to its health-promoting properties. The significance of regression coefficients was estimated in a multiple linear regression analysis to predict the value of the dependent variable ($ABTS^{\bullet+}$). In the regression model, the number of independent variables *N* (number of the analyzed metabolites) had to be smaller than the number of objects *n* (number of the analyzed breeding lines); therefore, regression analyses were conducted separately for flavonoids, carotenoids and phenolic acids. Statistically significant ($p < 0.029$) results were noted only in the analysis of phenolic acids, and beta coefficients (standardized values of the regression coefficient) were calculated (Table 3). The results of the above analysis indicate that the antioxidant activity of Tritordeum grain is significantly determined by the concentrations of 4–hydroxybenzoic acid, gallic, *p*-coumaric, protocatechuic, *t*-cinnamic and vanillic acids. The content of the remaining metabolites had no significant effect on $ABTS^{\bullet+}$ values. In a stepwise regression analysis of phenolic acids, the following equation was derived to predict the value of the dependent variable:

$$ABTS^{\bullet+} = 772.602 + 494.551 \times \text{"HBA"} - 10.687 \times \text{"CA"} + 123.236 \times \text{"CGA"} - 0.836 \times \text{"FA"} - 187.607 \times \text{"GA"} - 126.542 \times \text{"PCA"} - 73.097 \times \text{"PrCA"} + 13.075 \times \text{"SiA"} - 36.843 \times \text{"SyA"} + 4.469 \times \text{"CiA"} - 68.262 \times \text{"VA"}$$

Where: HBA– 4–hydroxybenzoic acid, CA– caffeic acid, CGA– chlorogenic acid, FA– ferulic acid, GA– gallic acid, PCA– p-coumaric acid, PrCA protocatechuic acid, SiA– sinapic acid, SyA– syringic acid, CiA– t-cinnamic acid, VA– vanillic acid.

Table 1
Antioxidant activity of grain expressed by $ABTS^{\bullet+}$ (μmol TROLOX kg^{-1}), one thousand kernel weight, and concentrations of bound phenolic acids and vanillin (mg kg^{-1}) in the grain of the studied Tritordeum breeding lines and *T. durum* cultivars.

	$ABTS^{\bullet+}$	TKW (g)	HBA	CA	CGA	FA	GA	PCA	PrCA	SiA	SyA	CiA	VA	VN
Tritordeum (n = 11)														
Mean	1896.2**	39.9**	2.67**	64.22**	54.24**	1008.9*	23.17**	17.44**	18.07	16.11	24.03	826.5**	7.31	0.59
Range	231.0	12.1	0.84	17.30	6.40	858.10	11.04	13.24	13.02	14.88	24.90	468.57	5.40	0.73
RSD (%)	4	9	10	9	4	24	14	27	21	31	43	19	26	40
T. durum (n = 2)														
Mean	1501.5	50.4	1.32	87.90	47.65	656.8	11.58	6.15	20.15	11.71	15.70	191.9	4.64	0.56
Range	587.0	7.0	0.18	36.20	9.90	138.30	1.56	1.30	6.50	7.39	9.80	12.64	5.31	0.56
RSD (%)	28	10	10	29	15	15	10	15	23	45	44	5	81	71

TKW- one thousand kernel weight; phenolic acids: HBA – 4–hydroxybenzoic, CA – caffeic, CGA – chlorogenic, FA – ferulic, GA – gallic, PCA – *p*-coumaric, PrCA protocatechuic, SiA – sinapic, SyA – syringic, CiA – *t*-cinnamic, VA – vanillic; VN – vanillin; *, ** differences between Tritordeum and durum wheat are statistically significant at $p < 0.05$ and $p < 0.01$, respectively; RSD-relative standard deviation.

Table 2
Flavonoid and carotenoid concentrations in the grain of 11 Tritordeum breeding lines and two *T. durum* (mg kg^{-1}) cultivars, and the antioxidant activity of grain expressed by $ABTS^{\bullet+}$ activity (μmol TROLOX kg^{-1}).

	Ap	Ka	Km	Lu	Na	Qu	Ru	Vi	LUT	ZEA	β-C	LUT/ZEA	Total carotenoids
Tritordeum (n = 11)													
Mean	17.83**	11.87*	10.70	29.52**	64.05	46.61	35.45**	7.45	12.02**	2.02	2.47**	5.95	17.09**
Range	2.90	8.30	10.27	21.40	58.20	25.80	9.70	11.93	3.19	1.71	1.18	6.70	3.24
RSD (%)	5	24	42	22	38	19	8	72	9	32	15	34	6
T. durum (n = 2)													
Mean	8.64	7.42	9.02	14.55	38.35	50.20	13.60	3.25	9.25	1.58	1.04	6.04	12.17
Range	3.80	1.44	0.10	3.50	1.70	19.00	1.40	0.90	0.21	0.55	0.13	2.22	0.16
RSD (%)	31	14	1	17	3	27	7	20	2	24	9	26	1

Ap – apigenin, Ka – catechin, Km – kaempferol, Lu – luteolin, Na – naringenin, Qu – quercetin, Ru – rutin, Vi – vitexin, LUT – lutein, ZEA – zeaxanthin, β-C – β-carotene; *, ** - differences between Tritordeum and durum wheat are statistically significant at $p < 0.05$ and $p < 0.01$, respectively; RSD-relative standard deviation.

Table 3
Beta coefficients calculated in a stepwise regression analysis for 11 phenolic acids (independent variables) and $ABTS^{\bullet+}$ activity (dependent variable). Adjusted $R^2 = 0.9984$ at $p = 0.029$. Statistically significant results are marked with an asterisk.

HBA* β =	CA β =	CGA β =	FA β =	GA* β =	PCA* β =	PrCA* β =	SiA β =	SyA β =	CIA*β =	VA* β =
1.38	−0.67	2.30	−1.1	-4.9	-3.8	-1.4	0.33	-1.8	6.13	-0.76

Phenolic acids abbreviations see Table 1.

The regression equation was derived for a small number of observations ($n = 11$), but it represents the relationship between the concentrations of phenolic acids and the antioxidant activity of Tritordeum grain under the experimental conditions.

The content of the examined metabolites in Tritordeum grain was processed by principal component analysis (PCA) (Fig. 1; Table 4). The identified compounds were analyzed jointly, without a division into flavonoids, phenolics and carotenoids for the maximum discrimination between breeding lines. The first two principal components (PC1 and PC2) explained most (68.5%) of the total variance, which indicates that durum wheat cultivars and Tritordeum lines were strongly discriminated, mainly by PC1. All significant correlations between the variables and PC1 (excluding gallic acid) were negative, which can be directly attributed to the lower content of the analyzed metabolites in *T. durum* than in Tritordeum grain. The analyzed breeding lines formed two distinct clusters. The first cluster was composed of lines JB 3, HTC 2083, HT 438, HTC 1324 and HT 444, and the second cluster contained the remaining lines, excluding HTC 2083′ which was located at mid-distance between the clusters. The location of breeding line HTC 2083' in the PCA plot can be attributed to its "intermediate" metabolite profile. This line was derived from two previously selected HTC 2083 plants that differed considerably from the remaining plants in spike morphology. It should be noted that Tritordeum lines were discriminated by both PC1 and PC2. Syringic acid and kaempferol were characterized by the highest discriminatory power (the sum of variable contributions to PC1 and PC2 were equal to 0.161 and 0.130, respectively), whereas vanillic acid, vanillin and zeaxanthin were characterized by the weakest discriminatory power (the sum of variable contributions to PC1 and PC2 were determined at 0.029, 0.024 and 0.015, respectively). Vanillic acid and zeaxanthin were not significantly

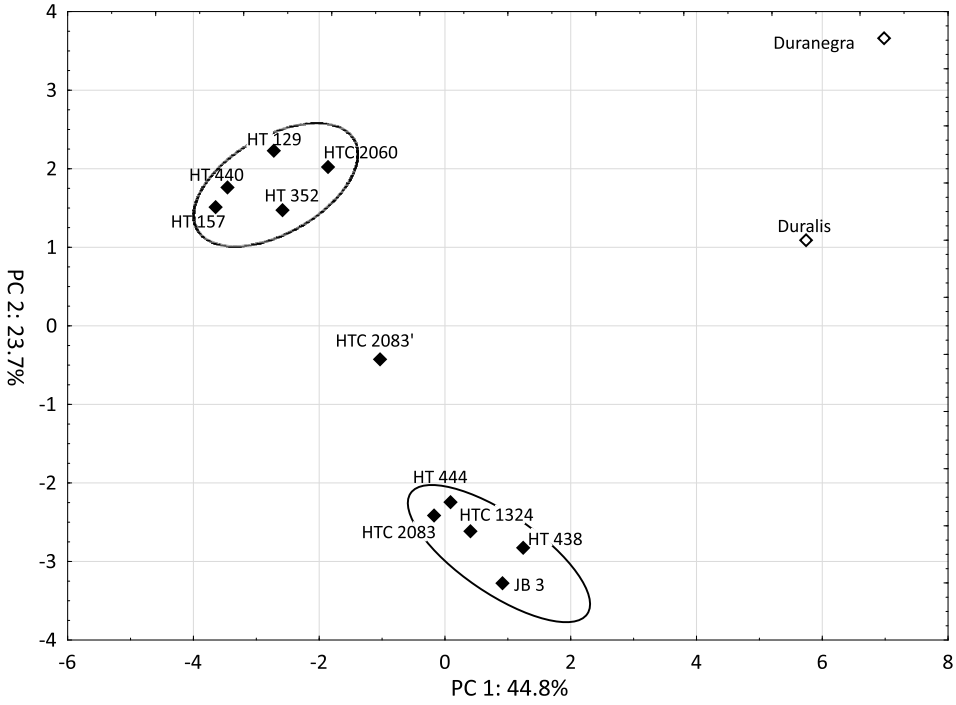

Fig. 1. PCA results for 11 Tritordeum breeding lines, two *T. durum* cultivars and 23 metabolites and $ABTS^{\bullet+}$ (see Table 4).

Table 4
Correlations between variables and PCs, and variable contributions to PC1 and PC2 for the analyzed Tritordeum lines, *T. durum* cultivars and 23 metabolites and ABTS$^{\bullet+}$

Metabolite		Correlations between variables and PCs*		Variable contributions		
		PC 1	PC 2	PC 1	PC 2	Σ for PC1 and PC2
Flavonoids	Apigenin	−0.828		0.065	0.044	0.109
	Catechin	−0.772		0.057	0.021	0.077
	Kaempferol		0.846	0.007	0.123	0.130
	Luteolin	−0.904		0.078	0.009	0.087
	Naringenin	−0.757	−0.601	0.054	0.062	0.116
	Quercetin		0.592	0.013	0.060	0.073
	Rutin	−0.837		0.067	0.033	0.099
	Vitexin	−0.672	−0.665	0.043	0.076	0.119
Phenolic acids	4-hydroxybenzoic	−0.889		0.075	0.014	0.089
	Caffeic	0.564	−0.702	0.030	0.085	0.115
	Chlorogenic	−0.598	0.612	0.034	0.064	0.098
	Ferulic	−0.768		0.056	0.022	0.078
	Gallic	−0.827		0.065	0.008	0.073
	p-coumaric	−0.815		0.063	0.003	0.066
	Protocatechuic		0.626	0.017	0.067	0.085
	Sinapic		0.695	0.002	0.083	0.085
	Syringic		0.962	0.002	0.159	0.161
	t-Cinnamic	−0.860		0.070	0.003	0.073
	Vanillic	−0.543		0.028	0.001	0.029
Carotenoids	Lutein	−0.631		0.038	0.008	0.046
	Zeaxanthin			0.015	0.000	0.015
	Beta-carotene	−0.779		0.058	0.010	0.067
	Vanillin			0.013	0.011	0.024
	ABTS$^{\bullet+}$	−0.729		0.051	0.034	0.085

* only values that are significant at $p < 0.01$ are presented.

correlated with PC1 or PC2, and they made a very small contribution to both PCs, which indicates that their discriminatory power was negligent.

The present findings cannot be broadly discussed due to the general scarcity of published studies that would comprehensively investigate the concentrations of different phenolics and carotenoids in Tritordeum grain. In this study, Tritordeum lines were compared with two *T. durum* cultivars despite the fact that Tritordeum grain is more similar to bread wheat grain in terms of technological quality and applicability. Durum wheat is one of the two parent components of the studied amphiploid (Martín et al., 1999); therefore, it was assumed *a priori* that the profile of the analyzed metabolites in Tritordeum grain would be more similar to that of *T. durum* than *T. aestivum* grain. The two selected cultivars differ in processing parameters, they were derived from two programs conducted by different breeding companies, and they were included in the study as reference materials. The aim of this study was to determine the range of variation in the analyzed Tritordeum lines, as well as to identify differences in the metabolic profile of Tritordeum grain and the grain of modern durum wheat cultivars. Montesano et al. (2021) observed a relatively high content of free phenolic acids in Tritordeum grain and reported the highest concentrations of ferulic and sinapic acids in line HT621. Cinnamic acid and gentisic acid were identified for the first time in Tritordeum grain in the cited study. In the current experiment, Tritordeum grain also contained *t*-cinnamic acid, whereas gentisic acid was not found. Interestingly, the differences in the content of bound ferulic acid in grain between Tritordeum and durum wheat, reported by the cited authors, were far less pronounced than those noted in the present study. In the cited experiment, durum wheat was significantly more abundant in *t*-cinnamic acid than Tritordeum grain, which was not corroborated by this study. The grain of the examined breeding lines was characterized by high concentrations of carotenoids due to very high lutein content, and similar observations were previously made by Atienza et al. (2007) and Giordano et al. (2019). This is the first study to investigate flavonoid concentrations in Tritordeum grain, and our results cannot be confronted with other authors' findings. The total concentration of the eight analyzed flavonoids was 54% higher in Tritordeum than in *T. durum* grain. This result indicates that Tritordeum can be a promising candidate for the production of functional foods with health-promoting properties.

4. Conclusions

The results of a comprehensive analysis of the main bioactive components, including carotenoids, phenolic acids and flavonoids, and the antioxidant activity of Tritordeum grain clearly indicate that Tritordeum grain is characterized by a highly desirable metabolite profile in terms of nutritional value and potential health benefits. Considerable variations in the content of the analyzed compounds in grain suggest that Tritordeum lines can be selectively bred to obtain genotypes with higher concentrations of these metabolites. Tritordeum lines were strongly discriminated in PCA. Phenolic acids and flavonoids were characterized by the highest discriminating power, and the discriminating power of carotenoids was relatively lowest, which indicates that selective breeding of Tritordeum lines for increased concentrations of phenolic acids and flavonoids can be more successful than selective breeding for carotenoids. However, further genetic research and breeding efforts are needed to validate these assumptions.

Author statement

Elżbieta Suchowilska: Conceptualization, Investigation, Writing - original draft.

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Anna Przybylska-Balcerek: chemical analysis.

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Declaration of competing interest

The authors do not have conflict of interest to declare for this paper.

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Appendix A. Supplementary data

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