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ORIGINAL PAPER

TRITICUM POLONICUM L. AS PROMISING SOURCE MATERIAL FOR BREEDING NEW WHEAT CULTIVARS

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Abstract

Breeding lines of *Triticum polonicum* (Polish wheat) on average were characterized by significantly the lowest (27.39) number of kernels per spike relative to common wheat (41.04) and durum wheat (32.90). One kernel weight was significantly the highest (57.9 mg) in durum wheat, in particular cv. Malvadur (63 mg). Two-way ANOVA for all analyzed spike and kernel morphological traits revealed no significant differences between the experimental years and no significant year x species interactions, which indicates that the evaluated wheat species responded similarly to weather conditions in both years of the study. The grain of three *Triticum* species differed significantly in protein, ash and fat content. The grain of *T. polonicum* was most abundant in protein (166 g kg^{-1}) relative to durum wheat (131 g kg^{-1}) and common wheat (120 g kg^{-1}). Considerable variations in the protein content of the evaluated lines (from 135 g kg^{-1} to 188 g kg^{-1}) indicate that in Polish wheat effective selection for this trait is possible. *T. polonicum* grain had significantly the highest ash content (22 g kg^{-1}), compared with durum (19 g kg^{-1}) and common wheat (18 g kg^{-1}), and significantly the lowest fat content (18 g kg^{-1}). The grain of the analyzed species did not differ significantly in the fiber content. Similarly to the analyzed spike and kernel properties, the absence of significant year x species interactions suggests that all three wheat species responded similarly to varied weather conditions between the experimental years. In the principal component analysis, the evaluated species were strongly discriminated by yield-related traits and the macronutrient content of grain.

Keywords: macronutrients, Polish wheat, principal component analysis, yield-related traits.

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INTRODUCTION

Allohexaploid (BBAADD) common wheat (*Triticum aestivum* L.) is the most widely cultivated wheat species, which represents 90% of total wheat production in the world (Shewry, Hey 2015). Tetraploid (BBAA) durum wheat (*Triticum durum* Desf.) is the second most economically important wheat species after common wheat, and it accounts for 10% of the wheat grown in the world. Durum wheat is cultivated predominately in the Mediterranean region and in other regions with a similar climate (Shewry, Hey 2015). Several tetraploid wheats could potentially be used as valuable source materials for breeding new varieties (Goncharov 2011). One of them is *Triticum polonicum* L. (Polish wheat) which is classified as a minor cereal. Polish wheat has been poorly investigated, and it has not attracted significant interest from breeders, geneticists or food technology experts. *T. polonicum* is grown on a small scale in southern Spain, southern Italy Algeria, Ethiopia and Asia (Wiwart et al. 2013). The species has awnless or awned spikes and large glumes with a length of up to 4 cm. Kernels are strongly elongated and plump, and 1000-kernel weight can reach 80 g (Wang et al. 2002). Our previous research demonstrated that *T. polonicum* grain is highly abundant in micronutrients, in particular zinc, iron and copper (Bieńkowska et al. 2019), and its flour is suitable for the production of high-quality bread with outstanding sensory attributes (Suchowilska et al. 2019). Despite its name, Polish wheat did not originate in Poland, but in Spain (Linnaeus 1797), although comprehensive research into its nutritional value and agricultural requirements was initiated by Polish scientists several years ago. The present study was undertaken to determine whether *T. polonicum* could become a valuable grain crop and source material for breeding new wheat varieties also with different ploidy levels.

The aim of the study was to analyze variation in the key phenotypic traits and the nutritional value of *T. polonicum* grain as compared with *T. durum* and *T. aestivum* cultivars.

MATERIALS AND METHODS

Materials and field experiments

The experimental material consisted of 27 wheat accessions, including 20 breeding lines of *T. polonicum*, 3 cultivars of *T. aestivum* (Torka, Zebra and Kontesa) and 4 cultivars of *T. durum* (Floradur, Duromax, Duroflavus and Malvadur) (Table 1). The experimental material was selected from a collection of more than 100 spring accessions of different origin. All Polish wheat accessions with the most desirable agronomic traits (resistance to lodging, early ripening, and resistance to pathogens) were selected for the study and

Table 1

Experimental material

Line/cultivar	Accession number	Origin
T. polonicum		
POL-1	PL 21801	NCPGR,Poland†
POL-2	PL 21802	
POL-3	PL 22488	
POL-4	PL 22479	
POL-5	PL 23047	
POL-6	PL 20770	
POL-7	PL 22991	
POL-8	PL 22746	
POL-9	PL 22195	
POL-11	PI 56262	NPGS, USA‡
POL-12	Cltr 13919	
POL-12′	Cltr 13919	
POL-12″	Cltr 13919	
POL-14	PI 167622	
POL-14′	PI 167622	
POL-14″	PI 167622	
POL-16	PI 191881	
POL-19	PI 225335	
POL-22	PI 272570	
POL-25	PI 384265	
T. aestivum		
Kontesa (B)		Hodowla Roślin Strzelce, Poland
Torka (A)		Hodowla Roślin Strzelce, Poland
Zebra (E)		DANKO, Hodowla Roślin, Poland
T. durum		
Floradur		Probstdorfer Saatzucht, Austria
Duromax		Saatbau Linz, Austria
Duroflavus		Saatbau Linz, Austria
Malvadur		Probstdorfer Saatzucht, Austria

A – quality cultivar, B – bread-making cultivar, E – elite cultivar (Polish National List of Cultivars, 2013), † National Center for Plant Genetic Resources (NCPGR), Radzików, Poland, ‡ National Plant Germplasm System (NPGS), USA

reproduced as valuable resources for breeding new lines. A two-year (2013 and 2014) field experiment with a randomized complete block design and three replications was performed in Bałcyny, district Ostróda, Warmia and Mazury, Poland (53°36′N latitude, 19°51′E longitude). Plot area was 10 m^2. The preceding crop was winter oilseed rape. N/P/K fertilizer was applied at 40/25/80 kg ha^{-1} once before sowing. At the full ripe stage BBCH 89 (Witzenberger et al. 1989), 30 randomly selected spikes were harvested manually from each plot for morphometric measurements (spike length, spike density, number and weight of grains per spike, one thousand kernel weight) and laboratory analysis (protein, fat, fiber, ash and total phenolic content of grain).

Laboratory analysis

The proximate chemical composition of grain was determined according to the methods described by Wiwart et al. (2013). Grain samples were milled using a Cyclotec 1093 sample Mill (FOSS, Denmark). Crude protein content (N x 5.7) (Hoseney 1994) was determined in two replications in the Büchi system (K-424 Digestion Unit and B-324 Distillation Unit, Switzerland). Crude fat was extracted by the Soxhlet method (Büchi Extraction System B-811, Switzerland), solvent: diethyl ether (POCh Gliwice, Poland), extractor size – 100 ml, 2.5 g analytical samples of air-dried ground grain. Extraction was carried out at a temp. of 60°C for 4 h, in two replications. After ether evaporation, solvent caps containing crude fat were dried for 2 h at 105°C in the exsiccator and weighed. Crude fiber content was determined using the Fibertec 2010 system (FOSS, Denmark) and the Weende method. Ground samples of 2 g were placed in FOSS crucibles with P2 porosity (40–100 μm). The samples were placed in a hot extraction unit, immersed in 1.25% H_2SO_4 (POCh Gliwice, Poland) and boiled for 40 min. Sulfuric acid was removed, the samples were rinsed three times with hot demineralized water, placed in a cold extraction unit and rinsed with acetone (POCh Gliwice, Poland). The samples were dried at 105°C for 3 h, and the amount of fiber was determined in a quantitative analysis. The total phenolic content (TPC) of methanol extracts was determined by a modified Folin-Ciocalteu method (Singleton, Rossi 1956). Briefly, 100 μl of each extract was shaken for 1 min with 500 μl of Folin-Ciocalteu reagent and 6 ml of distilled water. After shaking, 2 ml of 15% Na_2CO_3 was added, and the mixture was shaken again for 0.5 min. Distilled water was added to bring the solution to a final volume of 10 ml. After 2 h, absorbance was read on the UV/visible spectrophotometer Helios γ (Thermo Electron Corporation) at 750 nm (25°C) using glass cuvettes against a blank (100 μl of distilled water instead of the analyzed samples). TPC was assessed by plotting the gallic acid calibration curve (from 1 to 1500 μg ml^{-1}), and it was expressed in milligrams of gallic acid equivalents (GAE) per gram of dried extract (Đordević et al. 2010).

Data analysis

The results of all analyses were processed statistically using Statistica 12 software (StatSoft, 2014).The significance of differences between means was estimated by analysis of variance (ANOVA), and mean values were compared by the multiple Student-Newman-Keuls (SNK) test. All analyzed traits were subjected to principal component analysis (PCA). Principal component analysis is a classical multivariate technique used for data analysis, compression and visualization of data sets (Bishop 2006).The analysis reflects the importance of the major contributor to the total variation at each axis of differentiation (Hussain et al. 2014). PCA biplots support discrimination and the determination of correlations between variables and the two principal components (PC1 and PC2). The biplots illustrate the strength and direction of the correlations between a variable and a principal component (PC).

RESULTS AND DISCUSSIONS

Weather conditions

Polish wheat is a thermophilic crop, and weather conditions during the growing season of 2013 did not support its growth and development (Figure 1).

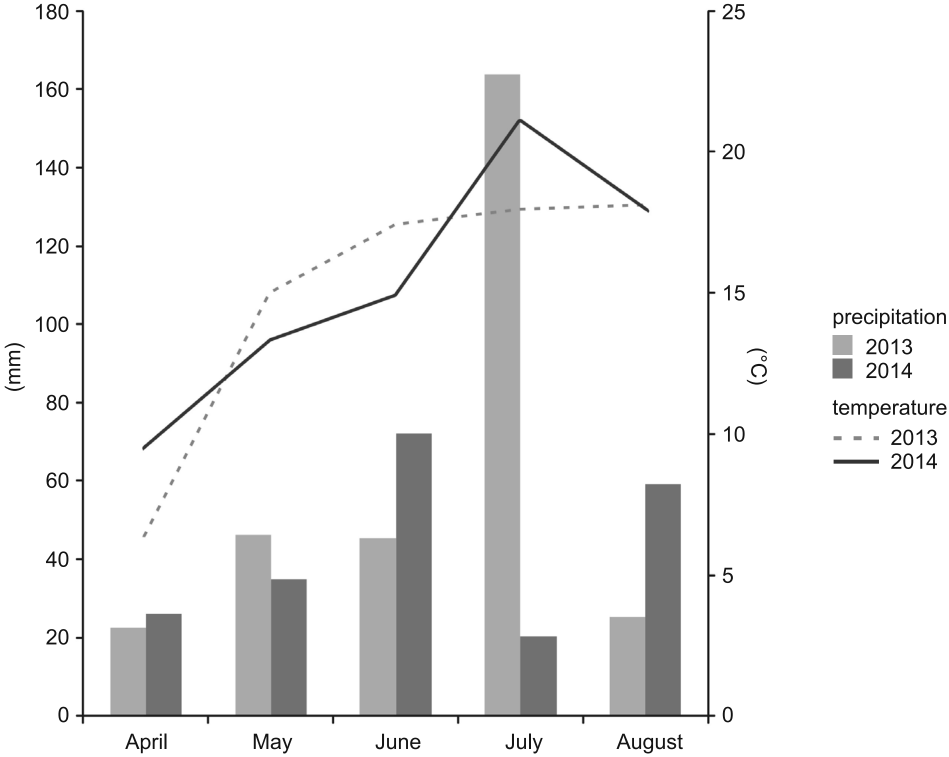

Fig. 1. Precipitation and average temperature in the growing seasons of 2013 and 2014 at the Agricultural Experiment Station in Bałcyny, Poland

High precipitation and relatively low temperature contributed to lodging and inhibited grain formation in spikes. In 2014, total precipitation was lower than in 2013 (213 mm) and was below the long-term average (316 mm), which improved stand uniformity, increased stand density and 1000-kernel weight after harvest. High precipitation in the first ten days of August (37.3 mm) prolonged grain harvest. However, *T. polonicum* is not susceptible to grain shattering.

Biometric parameters of spikes and yield components

On average, *T. polonicum* plants were significantly taller (110.48 cm) than *T. aestivum* (84.42 cm) and *T. durum* (76.57 cm) plants (Table 2). Lines Pol-1 (126.37 cm) and Pol-16 (119.98cm) were the tallest, and lines Pol-11

Table 2

The mean values of biometric parameters and yield components of *T. polonicum* breeding lines and *T. durum* and *T. aestivum* cultivars in a two-year field experiment

Specification	PH (cm)	SL (cm)	SW (g)	SD	NKS (kernels)	KWS	OKW (mg)
T. polonicum (n=17)	110.48[a]	8.38[a]	1.95[b]	13.96[b]	27.39[c]	1.34[b] (68.7%)	49.78[b]
Mean 2013-2014	112.96 - 108.54	8.66 - 8.14	1.99 - 1.91	14.91 - 13.16	29.56 - 25.56	1.35 - 1.32	46.91 - 52.22
RSD (%) 2013-2014	12.90 - 10.71	18.21 - 16.66	17.59 - 19.60	14.32 - 8.42	23.02 - 21.62	22.60 - 22.15	13.84 - 16.99
T. durum (n=4)	76.57[b]	6.11[b]	2.50[a]	13.28[b]	32.90[b]	1.88[a] (75.2%)	57.92[a]
Mean 2013-2014	78.18 - 74.97	6.15 - 6.08	2.50 - 2.50	13.35 - 13.20	34.32 - 31.49	1.93 -1.84	56.11 -59.73
RSD (%) 2013-2014	6.10 - 7.41	12.91 - 10.53	14.24 - 9.80	4.68 - 4.67	11.17 - 9.06	13.07 - 8.08	6.60 - 5.81
T. aestivum (n=3)	84.42[b]	7.98[a]	2.22[ab]	16.63[a]	41.04[a]	1.81[a] (81.5%)	44.16[b]
Mean 2013-2014	89.37 - 79.47	8.10 - 7.87	2.10 - 2.33	16.23 - 17.03	38.93 - 43.16	1.71 - 1.92	43.82 - 44.49
RSD (%) 2013-2014	6.18 - 7.30	4.45 - 4.09	0.00 - 4.95	8.93 - 6.55	3.65 - 5.66	1.22 - 3.42	2.82 - 2.28
Mean Means 2013-2014	101.57 104.22 - 99.32	7.95 8.18 - 7.77	2.08 2.09 - 2.07	14.18 14.82 - 13.66	30.01 31.53 - 28.75	1.49 1.50 - 1.49	50.43 48.06 - 52.51

RSD (%) – relative standard deviation, PH – plant height, SL – spike length, SW – spike weight, SD – spike density (average number of spikelets per 10 cm length of spike), NKS – number of kernels per spike, KWS – kernel weight per spike, the average proportion of kernel weight in spike weight is given in parentheses in the KWS column. OKW – one kernel weight. Mean values followed by the same letter do not differ significantly at $p<0.05$ according to multiple Student-Newman-Keuls test. *F*-values for: plant height $F_{(2.42)}$ =76.201, $p<0.001$, spike length $F_{(2.42)}$ = 9.419, $p<0.001$, spike weight $F_{(2.42)}$ = 9.342, $p<0.001$, spike density $F_{(2.42)}$ = 8.694, $p<0.001$, number of kernels per spike $F_{(2.42)}$ = 14.955, $p<0.001$, kernel weight per spike $F_{(2.42)}$ = 17.118, $p<0.001$, 1000-kernel weight $F_{(2.42)}$ =7.254, p = 0.002.

and Pol-6 (94.93 and 96.30 cm, respectively) were the shortest. The average spike length in the evaluated Polish wheat lines (8.38 cm) was comparable to *T. aestivum* (7.98 cm) and was significantly higher than in *T. durum* (6.11 cm). Considerable plant height can contribute to lodging, in particular under adverse weather conditions and excessive nitrogen fertilization. Low spike density could be regarded as a positive trait because loose spikes are less susceptible to infections caused by pathogenic fungi, in particular species of the genus *Fusarium* (Jones et al. 2018). In cereals, the key yield components are density of spikes, kernel weight and the number of kernels per spike (Martino et al. 2015). On average, the kernels of the evaluated lines of Polish wheat were characterized by significantly lowest weight (1.34 g) relative to *T. durum* (2.50 g) and *T. aestivum* (2.22 g). The number of kernels per spike was significantly the highest in common wheat (41.04), followed by durum wheat (32.90), and it was significantly the lowest in *T. polonicum,* but the selected Polish wheat lines were characterized by a very high number of kernels per spike that reached 36 (Pol-12′) and even 39 (Pol-1). Selective breeding for this trait could be very important in high-yielding cultivars. In both years of the study, one kernel weight (OKW) was significantly the highest in *T. durum* (57.9 mg). The value of this trait was the highest in cv. Malvadur (63 mg), and it was similar in the remaining cultivars (approx. 56 mg). The average OKW in Polish wheat (49.78 mg) exceeded that of common wheat (44.2 mg), but the o bserved difference was not statistically significant. The highest OKW was determined in lines Pol-4 (64.4 mg) and Pol-19 (62.8 mg) of Polish wheat, and in common wheat cv. Torka (44.9 mg). Two-way ANOVA for all seven spike and kernel parameters revealed no significant differences between years and no significant year x species interactions, which indicates that the evaluated species responded similarly to weather conditions in both experimental years.

PCA analysis showed that the variables (grain quality traits) positioned close to the correlation circle of radius 1, which corresponds to the maximum absolute value of the correlation coefficient, had greater discriminatory power (Figure 2). Both principal components explained 81.6% of total variance. The number of kernels per spike, kernel weight per spike and the number of spikelets per spike had the greatest discriminatory power, whereas plant height and spike length had the smallest discriminatory power. Strong discrimination can be attributed to the different values of yield components in most *T. polonicum* lines relative to common wheat and durum wheat.

Concentrations of macronutrients and phenolics in grain

Wheat grain is an essential part of human diet around the world, and its quality is a very important consideration (Ahmed, Hassan 2015). The chemical composition of grain is influenced mainly by genetic and environmental factors, and it is the main determinant of cereals' nutritional value (Biel et al. 2016). The protein content of grain is one of the most important traits

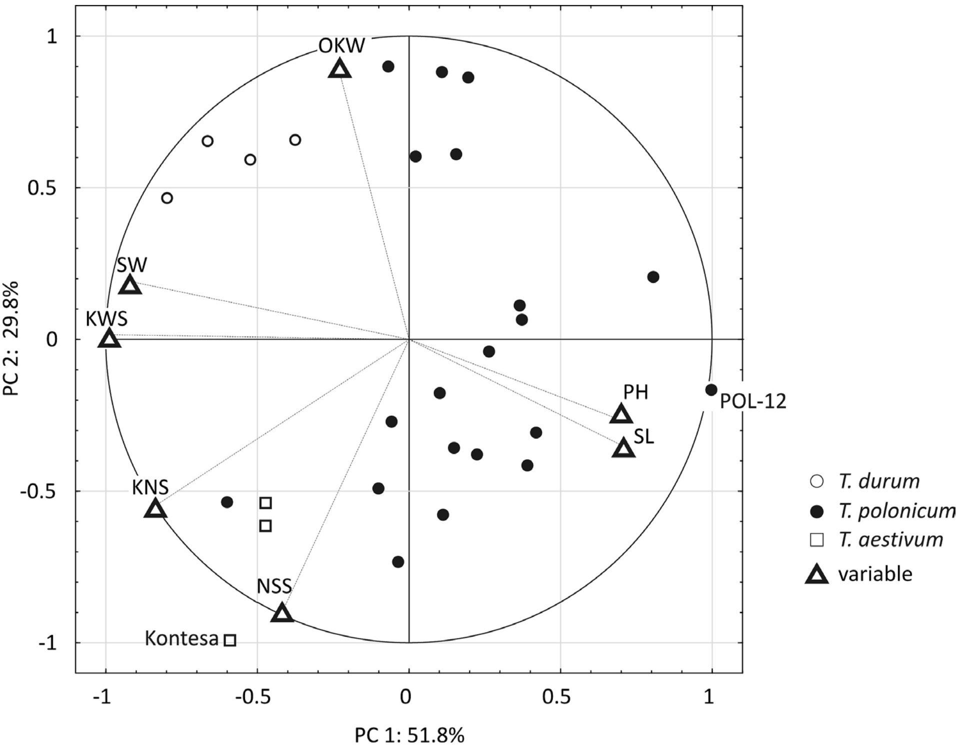

Fig. 2. PCA biplot for all evaluated lines and cultivars: PH – plant height, SL – spike length, NSS – number of spikelets per spike, NKS –number of kernels per spike, KWS – kernel weight per spike, SW – spike weight, OKW – one kernel weight. POL-12 (*T. polonicum*) and Kontesa (*T. aestivum*) differed most significantly from the remaining lines and cultivars

that imparts nutritional value to grain and influences the quality of flour-based products (Balyan et al. 2013). Grain size and weight are important qualitative traits. Large and heavy kernels have a larger endosperm and a smaller proportion of aleuronic layers and the external pericarp (Arzani, Ashraf 2017).

The grain of *T. polonicum* was significantly most abundant in protein (166 g kg^{-1} DM) compared with durum wheat (131 g kg^{-1} DM) and common wheat (120 g kg^{-1} DM) – Table 3. Considerable differences in the protein content among the evaluated lines (from 134 g kg^{-1} DM in Pol-12′ to 188 g kg^{-1} DM in Pol-12 on average) indicate that wheat can be bred selectively for this trait. In *T. durum,* the protein content was the highest in cv. Malvadur 138 g kg^{-1} DM), whereas the lowest value of this parameter was noted in three common wheat cultivars (max of 124 g kg^{-1} DM in cv. Zebra). The grain of *T. polonicum* had significantly the lowest fat content (18 g kg^{-1} DM), although high values of this parameter were observed in lines Pol-12″ and Pol-12′ (22 g kg^{-1} DM and 21 g kg^{-1} DM, respectively).

The crude ash content of grain is an important determinant of milling

Table 3

Mean values of major components of three *Triticum* sp. species in a two-year experiment

Specification	Ash (g kg^{-1} DM)	Fiber (g kg^{-1} DM)	Protein (g kg^{-1} DM)	Fat (g kg^{-1} DM)	TPC (μg kg^{-1}) samples
T. polonicum (n=17)	22^{a}	26	166^{a}	18^{b}	2837
Mean 2013-2014	23 - 22	28 - 24	174 - 160	19 - 17	3271 - 2403
RSD (%) 2013-2014	11.16 - 5.32	9.03 - 12.53	8.91 - 11.54	11.73 - 12.05	5.32 - 4.98
T. durum (n=4)	19^{b}	24	131^{b}	20^{a}	2910
Mean 2013-2014	19 - 20	23 - 24	136 - 126	19 - 21	3307 - 2514
RSD (%) 2013-2014	3.74 - 1.26	11.76 - 8.21	3.41 - 6.96	12.50 - 11.44	5.71 - 6.10
T. aestivum (n=3)	18^{b}	26	120^{b}	21^{a}	2716
Mean 2013-2014	18 - 18	25 - 27	131 - 190	20 - 23	3127 - 2306
RSD (%) 2013-2014	4.62 - 4.16	2.95 - 10.10	2.58 - 5.84	8.31 - 4.84	6.33 - 7.15
Mean Means 2013-2014	21 (20 - 21)	25 (27 - 24)	155** (162 - 147)	0.19 (19 - 19)	2834** (3259 - 2409)

RSD – relative standard deviation (%), DM – dry matter. Mean values followed by the same letter do not differ significantly at p<0.01 according to multiple Student-Newman-Keuls test. F-values for: ash $F_{(2.42)}$ = 19.548, p<0.001, protein $F_{(2.42)}$ = 35.653, p<0.001, fat $F_{(2.42)}$ = 7.257, p = 0.019. ** difference between years, significant at p<0.01; F-values for protein $F_{(1,42)}$ = 7.547, p = 0.009, and TPC $F_{(1,42)}$ = 196.810, p<0.001.

quality (Biel et al. 2016). The studied lines of *T.polonicum* were characterized by significantly the highest ash content (22 g kg^{-1} DM) relative to *T. durum* (19 g kg^{-1} DM) and *T. aestivum* (18 g kg^{-1} DM) – Table 3. Crude ash content exceeded 2 g kg^{-1} DM in the grain of all *T. polonicum* lines, excluding Pol-12″. The grain of the tested species did not differ significantly in the fiber content, which was the highest in common wheat (26 g kg^{-1} DM) and the lowest in durum wheat (240 g kg^{-1} DM). It should be noted that the analyzed macronutrients were not bound by strong correlations, and the Pearson's correlation coefficient was the highest for fat and fiber content, which was determined at 0.575 (p<0.05) for both experimental years (0.679 in the first year (p<0.05) and only 0.160 in the second year). These results indicate that selection can be effective for each of these traits separately.

The results of two-way ANOVA revealed significant differences in the protein, ash and fat content of grain between the three evaluated *Triticum* species. The presence of year x genotype interactions was not observed for any of the above traits, which implies that all of the analyzed species responded similarly to varied weather conditions in the two-year study. The protein and total phenolic content of grain differed significantly between experimental years (Table 3). Similarly to the morphological parameters

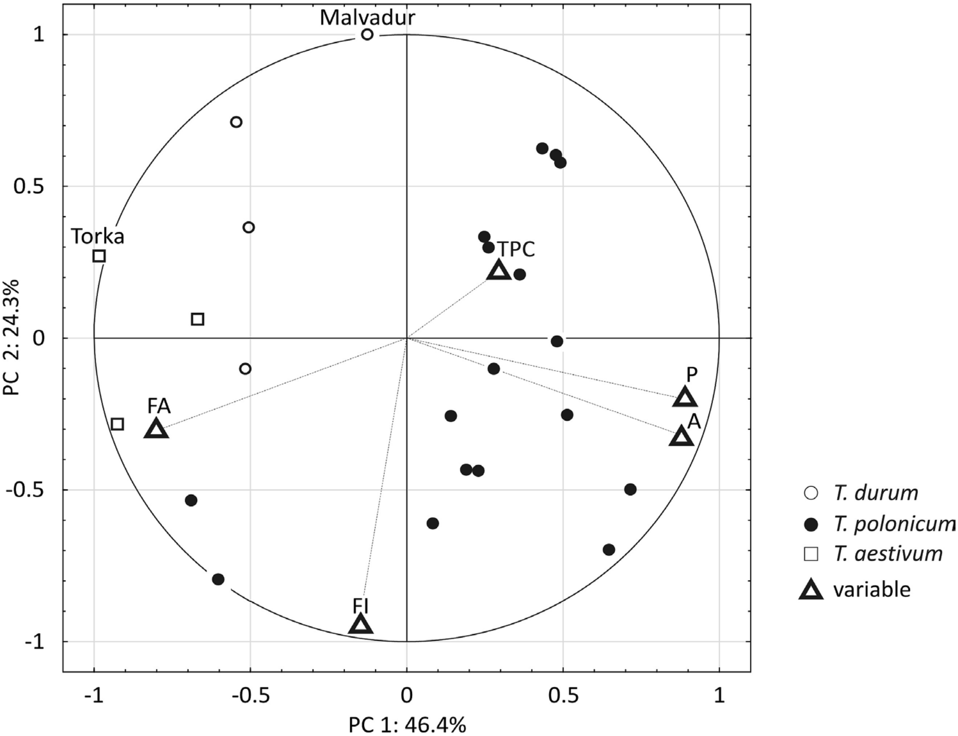

Fig. 3. PCA biplot for the concentrations of major nutrients and phenolics in the grain of all evaluated lines and cultivars. P – crude protein, A – ash, FI – crude fiber, FA – crude fat, TPC – total phenolic content. Cultivars Torka (*T. aestivum*) and Malvadur (*T. durum*) differed most significantly from the remaining lines and cultivars

of spikes and kernels and plant height, the above traits strongly discriminated the three evaluated wheat species in PCA (Figure 3). The first two principal components, PC1 and PC2, explained 70.7% of total variance. Fiber, protein and ash content of grain had the greatest discriminatory power. The fat content of grain was a parameter with lower discriminatory power, and the analyzed species were least effectively discriminated based on the TPC of grain. The PCA biplot corresponds to the results presented in Table 3, and it indicates that the grain of common wheat cultivars was had the lowest content of ash and protein. Only minor differences in the TPC of grain were observed in the analyzed wheat species. The average value of this parameter was lowest in *T. aestivum* (2716 μg kg^{-1}) and highest in *T. durum* (2910 μg kg^{-1}). None of the studied *T. polonicum* lines were more abundant in phenolic compounds than common wheat or durum wheat cultivars. Interestingly, the average TPC of grain was around 35% lower (2409 μg kg^{-1}) in the second year than in the first year of the study (3259 μg kg^{-1}). This difference could be attributed to varied weather conditions between the experimental years.

CONCLUSION

Triticum polonicum could be promising source material for breeding new wheat varieties. Polish wheat was first used as breeding material in the 1990s in the International Maize and Wheat Improvement Center in Mexico. Due to its high processing suitability and valuable functional properties, attempts were made to cross *T. polonicum* with selected species of the genus *Aegilops,* related to common wheat. The research gave rise to wheat with record-breaking yield of up to 18 t ha^{-1} (Holden 1998). However, the produced hybrid had very high nutritional requirements, and its production not only was very expensive, but also exerted adverse effects on the soil environment. Similarly to other tetraploid *Triticum* species, Polish wheat is quite tolerant to water deficit. In view of global climate change, new research programs should be initiated to cross *T. polonicum* with other wheat species and to promote the cultivation of Polish wheat as a new grain crop in regions with a warmer climate as well as in Europe. The yield potential of the analyzed breeding lines of *T. polonicum* is difficult to estimate because such an evaluation would require large-area field experiments with varied weather and soil conditions. However, our findings suggest that Polish wheat yields can reach 4 t ha^{-1} under conducive agronomic conditions. The analyzed crop has considerable potential for breeding and food technology. Polish wheat grain and flour can be aviable alternative for consumers who have an interest in new cereal products with high nutritional value.

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