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The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain

Monika ZRCKOVÁ[1,*], Ivana CAPOUCHOVÁ[1], Marie ELIÁŠOVÁ[2], Luboš PAZNOCHT[2], Kateřina PAZDERŮ[1], Petr DVOŘÁK[1], Petr KONVALINA[3], Matyáš ORSÁK[2], Zdeněk ŠTĚRBA[3]

[1]*Department of Plant Production, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Department of Chemistry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[3]*Department of Agroecosystems, Faculty of Agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic*

**Corresponding author: zrckova@af.czu.cz*

ABSTRACT

Zrcková M., Capouchová I., Eliášová M., Paznocht L., Pazderů K., Dvořák P., Konvalina P., Orsák M., Štěrba Z. (2018): The effect of genotype, weather conditions and cropping system on antioxidant activity and content of selected antioxidant compounds in wheat with coloured grain. Plant Soil Environ., 64: 530–538.

The aim of the study was to evaluate total antioxidant activity (TAA) and total content of carotenoids (TCC), anthocyanins (TAC), phenolics (TPC) and phenolic acids (PAs) in grain of selected pigmented wheat genotypes and traditional control cultivar cultivated under organic and conventional cropping systems in two-year trials. All of the evaluated parameters were significantly affected both by genotype and evaluated environmental factors. While in TPC, PAs and TCC the effect of years prevailed, TAC was affected mainly by genotype. The effect of genotype and year in TAA was comparable. TPC ranged from 581.71 mg/kg (control cv. Annie) to 723.60 mg/kg (cultivar with purple pericarp PS Karkulka), total PAs content from 711.77 mg/kg (cv. PS Karkulka) to 849.47 mg/kg (cv. Skorpion with blue aleurone). TCC varied from 1.56 mg/kg (cv. PS Karkulka) to 5.32 mg/kg (cv. Citrus with yellow endosperm). The highest TAC (63.23 mg/kg) was found in cv. Skorpion, the lowest (12.70 mg/kg) in cv. AF Jumiko with purple pericarp. Anthocyanins were not detected in cvs. Annie and Citrus. TAA varied from 162.68 mg/kg in cv. Annie to 226.71 mg/kg in breeding line KM 53-14 with blue aleurone. Higher TAA and antioxidants contents and lower grain yields were observed in organic cropping system and in drier year 2016.

Keywords: *Triticum aestivum* L.; cereals; phytochemical; abiotic stress; water deficit

Wheat is the most widely grown cereal crop in the world and bread wheat represents a staple food for human nutrition. The consumption of grain and especially whole grain products is associated with a number of health benefits which may be related in part to the contents of different phytochemicals (Shewry and Hey 2015). They can act as antioxidants and help in prevention of cardiovascular diseases, diabetes, inflammation, cancer, obesity and aging (Garg et al. 2016). Some of these

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phytochemicals significantly influence the grain colour. The purple colour is due to anthocyanins in the pericarp layer, while blue colour is due to anthocyanins in the aleurone layer (Lachman et al. 2017). Carotenoids are responsible for the yellow endosperm colour in cereal grains (Paznocht et al. 2018). Phenolic acids (PAs) represent the most common phenolic compounds in cereal grains (Martini et al. 2015). PAs are secondary metabolites which, as many other antioxidants, are synthesized as a part of multifunctional defence system against biotic and abiotic stresses in plants (Lattanzio et al. 2006). They are, similarly as other phenolic compounds, mainly present in the outer layers of grains (Žilić et al. 2011) and apparently act as a defence against herbivores, microbes, viruses or competing plants as well as they protect the plant from oxidation (Lattanzio et al. 2006).

In recent years, many studies have dealt with antioxidant compounds in various cereal species such as common wheat (Stracke et al. 2009a, Žilić et al. 2011, Zuchowski et al. 2011), durum wheat (Žilić et al. 2011, Ficco et al. 2014, Martini et al. 2015), einkorn wheat and emmer wheat (Lachman et al. 2013). The most of them have also shown a significant impact of genotype on the occurrence of antioxidants and, therefore, on the antioxidant activity of cereals. However, only a few papers have specifically dealt with these phytochemicals in wheat with coloured grain (Abdel-Aal et al. 2016, Garg et al. 2016, Lachman et al. 2017, Paznocht et al. 2018).

Despite relatively high genetic weighing, some environmental factors influence the antioxidant compounds development, too (Ficco et al. 2014). Many of the antioxidants are known to be produced by plants in response to abiotic (e.g. wounding and heat, water and nutrient) and biotic (pest attacks and disease) stress (Barański et al. 2014). Regarding to weather conditions, some authors observed an increased antioxidants synthesis in different cereals grown under water deficit and higher average temperatures during the grain filling (Fratianni et al. 2013, Paznocht et al. 2018), others registered a negative correlation between high temperatures and antioxidants contents (Mattera et al. 2017). Paznocht et al. (2018) suppose that cultivars originating in climate that can be found in the Czech Republic might react differently to increased temperature and decreased precipitation compared to cultivars of different origin. These findings might be useful for selecting wheat genotypes naturally rich in antioxidant compounds, also in relation to the choice of the more suitable growing areas (Martini et al. 2015).

Cultivation system may also affect antioxidants contents in crops. There is evidence that differences in fertilization regimes between organic and conventional production systems (and, in particular, the non-use of high mineral N fertilizer inputs) are significant drivers for higher antioxidant concentrations in organic crops. Many studies dealt with the effect of organic and conventional cropping systems on the contents of antioxidants in different crops, including cereals (Zuchowski et al. 2011, Almuayrifi 2013, Barański et al. 2014). However, data related to the effect of organic and conventional cropping systems on concentrations of different antioxidant compounds in grain of less traditional, pigmented wheat are still scarce.

Wheat breeders are currently attempting to develop new types of colour-grained wheat cultivars with improved properties including quality and yield (Martinek et al. 2013). Nevertheless, more data are needed regarding the antioxidant compounds in this wheat, as this could lead to new opportunities for breeding and commercial production of value-added colour-grained cultivars rich in health-beneficial components. Therefore, the objective of this study was to compare the less traditional, pigmented wheat genotypes with traditional common wheat and to assess the impact of weather conditions and cropping system on evaluated antioxidant compounds contents.

MATERIAL AND METHODS

Plant material. The exact field plot trials with collection of 6 winter wheat genotypes (Table 1) were carried out during the 2015/2016 and 2016/2017 growing seasons at the experimental station of the Czech University of Life Sciences in Prague-Uhříněves (central part of Bohemia, 295 m a.s.l., average annual temperature 8.4°C, average sum of precipitation 575 mm). The field trials were established using the random blocks, in 3 replicates, with experimental plot average area of 10 m^2. The trials were carried out under organic and conventional cropping systems. Red clover was used as a preceding crop of wheat in both cropping systems. Treatment of the wheat

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Table 1. Basic characteristics of evaluated wheat genotypes

Genotype	Country of origin	Genotype character	Grain colour
Annie	CZE	cultivar	standard commercial cultivar (check cultivar)
KM 53-14	CZE	breeding line	blue aleurone
Skorpion	AUT	cultivar	blue aleurone
AF Jumiko	CZE	cultivar	purple pericarp
PS Karkulka	SVK	cultivar	purple pericarp
Citrus	DEU	cultivar	yellow endosperm

stands by weeding harrows was used during the vegetation; no fertilizers and pesticides were applied to wheat cultivated under organic cropping system. Nitrogen fertilization in the total dose of 120 kg N/ha and treatment by herbicide (Agritox 50SL; 1.0 L/ha), fungicide (Amistar XTRA; 2.5 L/ha) and insecticide (Nurelle D; 0.6 L/ha) were applied to wheat cultivated conventionally.

As for the weather conditions (Table 2), the period of grain formation and maturing both in 2016 and 2017 was similar in the average temperatures. With regard to precipitation, year 2016 was drier and reached only 60% of precipitation in the evaluated period compared to 2017.

Grain samples. Grain samples obtained after the field plot trials harvest were ground using the IKA analytical mill (Janke & Kunkel Co., Stanfen, Germany) to pass through 0.5 mm screen (35 mesh) and were homogenised well. Dry matter (DM) was determined by drying of meal at 105°C for 24 h. Three replicates were made in all of the following analyses.

Total phenolics content (TPC). The TPC was evaluated according to Eliášová and Paznocht (2017). Briefly, 2.5 g of meal was extracted with 25 mL of 0.1% HCl in methanol. 2 mL of extract were reacted with 2.5 mL of the Folin-Ciocalteu reagent with addition of 7.5 mL of 20% sodium carbonate and filled up with pure water to 50 mL. After 2 h the solution was measured spectrophotometrically at 765 nm. The results were quantified using external calibration and expressed as mg of gallic acid per kg of DM.

Total antioxidant activity using DPPH (TAA). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical cation scavenging activity of methanolic extracts was evaluated according to Eliášová and Paznocht (2017). Briefly, 2.5 g of meal was extracted with methanol. The extracts were filled up with methanol to 25 mL and stored in darkness at the room temperature for one week. 100 µL of extract was mixed with 1 mL of DPPH methanolic solution, incubated for 20 min and measured at wavelength 515 nm. The results were quantified using external calibration and expressed as mg of Trolox equivalent antioxidant activity (TEAC) per kg of DM.

Total phenolic acids content (PAs). For extraction and chromatographic separation, a method published by Martini et al. (2015) with some modi-

Table 2. Average temperature and sum of precipitation from anthesis to grain maturity

Decade	Month	Average temperature (°C)		Σ of precipitation (mm)	
		2016	2017	2016	2017
1st	June	19.97	18.00	17.00	23.60
2nd		19.95	18.95	10.40	25.20
3rd		20.52	20.91	18.00	51.80
1st	July	20.55	19.90	3.60	9.40
2nd		19.14	19.40	19.00	15.40
3rd		20.24	21.56	32.30	41.80
Average temperature		20.06	19.79		
Σ of precipitation				100.30	167.20
Average temperature (long-term standard)		17.25			
Σ of precipitation (long-term standard)				148.00	

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fications was used. Briefly, 0.25 g of meal was hydrolysed with 14 mL of 2 mol/L aqueous sodium hydroxide for 1 h at the room temperature. 7 mL of 4 mol/L HCl was added to adjust acidic pH (1–2). Two mL of hydrolysate were transferred into 8 mL glass vial and twice extracted with 2 mL of ethylacetate. Combined supernatants of upper organic phase were removed to another glass vial, evaporated to dryness under the nitrogen stream and reconstituted with 1 mL of 70% aqueous methanol, filtered through a syringe filter into an amber HPLC vial and analysed by HPLC-DAD. The analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) with diode array detector. The analytes were separated by an Omnispher C18 HPLC column (250 × 4.6 mm; particle size 5 µm; Agilent, Inc., Santa Clara, USA) and detected at two different wavelengths, 280 nm and 325 nm. The results were expressed in mg per kg of DM.

Total anthocyanin content (TAC). The TAC was determined using a method described by Syed Jaafar et al. (2013) with minor modifications. Briefly, 2.5 mL of 1 mol/L HCl/MeOH (15:85, v/v) was added to 400 mg of meal and was shaken for 30 min. Then the sample was centrifuged and the supernatant was removed. The extraction was repeated three more times and all the four supernatants were collected. The extract volume was adjusted to 10 mL with extraction solvent. The TAC was determined spectrophotometrically at 529 nm. The total anthocyanin content was quantified using external calibration and expressed as mg of cyanidin-3-glucoside equivalent per kg of DM.

Total carotenoids content (TCC). The TCC was determined according to Paznocht et al. (2018). Briefly, 2 g of meal were twice extracted with 12 mL of ethanol/acetone/hexane mixture (1:1:2, v/v/v), centrifuged and combined supernatants were evaporated to dryness. The dry residue was reconstituted with 2 mL ethanol/acetone (3:2, v/v) containing 0.2% BHT and filtered through a syringe filter into an amber HPLC vial. The HPLC-DAD analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) with diode array detector. The analytes were separated on an YMC C30 Carotenoid Column (150 × 3.0 mm, S-3 µm, YMC Co., Kyoto, Japan) and detected at wavelength 445 nm. The TAC was expressed in mg per kg of DM.

Statistical analysis. The results were statistically analysed by the analysis of variance (ANOVA) method, with expression of the Fisher's *F*-value. The differences between mean values were evaluated by the Tukey's *HSD* (honestly significant difference) test in the SAS program (version 9.4, SAS Institute, Carry, USA) at the level of significance $P = 0.05$.

RESULTS AND DISCUSSION

The effect of genotype on the content of evaluated antioxidant compounds. The results of ANOVA related to six wheat genotypes, grown over two years in two different cropping systems (organic and conventional) are given in Table 3. The analysis shows that genotype (G) and its interactions with crop year (Y) and cropping system (S) significantly affect all the parameters under study. Nevertheless, only the TAC was mostly affected by G factor and impact of Y and S factors was lesser. In case of TAA, similar impacts of G

Table 3. The effect of genotype, cropping system and year on the content of evaluated antioxidants in the wheat grain and yield of grain (ANOVA, Fisher's *F* values)

	Total phenolics content	Phenolic acids	Total carotenoids content	Total anthocyanin content	Total antioxidant activity	Grain yield
Genotype (G)	34.19***	22.64***	7698.97***	1826.75***	38.82***	55.57***
Year (Y)	178.66***	30.74***	9540.05***	1379.07***	36.71***	207.43***
Cropping system (S)	23.39***	6.00**	27.55***	490.99***	18.91***	834.23***
G × Y	3.40*	11.74***	1040.32***	29.79***	5.07**	13.07***
G × S	5.47**	8.09***	8.28***	8.62**	0.96	3.59**
Y × S	30.97***	12.15**	3.52	8.57**	2.59	5.01**
G × Y × S	1.88	7.53**	6.64***	4.35*	1.38	1.14

$P < 0.05$*; $P < 0.01$**; $P < 0.001$***

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and Y were observed. The impact of S was lesser but still statistically significant. The TPC, PAs and TCC appear mostly influenced by the Y factor and less by G, S and their interactions.

According to Martini et al. (2015), yellow-coloured pigments and TAA in durum wheat were mainly affected by genotype, differently from the content of PAs and TPC, which appears to be mostly affected by environmental factors. Our results are in accordance with these findings, with exception of TCC, where the prevailing effect of year was observed. Lachman et al. (2017) reported that anthocyanin levels are highly significantly affected by genotype, but the effects of environment are significant, too. Our results confirm the high heritability for anthocyanins as well as significant effects of year and cropping system.

The results of Tukey's test describing significance between the mean values of genotypes, cropping systems and years are shown in Table 4, a more detailed view on individual genotypes is given in Table 5.

Control cv. Annie reached the lowest TPC of all the evaluated genotypes. However, the difference in TPC between cv. Annie and cv. Skorpion with blue aleurone was statistically insignificant. These results indicate that TPC in traditional wheat cultivars may be on the same or similar level as in wheat with coloured grain. Our results of TPC in wheat correspond with the results of Eliášová and Paznocht (2017), but are significantly lower compared to the results presented by Abozed et al. (2014). The PAs content was higher in total compared to TPC. Cv. Annie, belonging to the genotypes with lower total PAs content, did not differ statistically from genotypes with purple pericarp. Cv. Annie also reached comparable values of TCC as genotypes with blue aleurone and purple pericarp, with the exception of cv. AF Jumiko with purple pericarp and especially, in accordance with Paznocht et al. (2018), cultivar with yellow endosperm where TCC was significantly higher. According to Garg et al. (2016) and Syed Jaafar et al. (2013), the highest contents of anthocyanin are present in black grained wheats, followed by blue-grained wheats and wheats with purple pericarp. However, some purple wheat may contain higher anthocyanin levels than blue wheats (Abdel-Aal et al. 2016). Our results show that the TAC was substantially higher in genotypes with blue aleurone compared to the purple pericarp ones. In cvs. Annie and Citrus, anthocyanins were not detected. The highest TAA was determined in genotypes with purple pericarp and blue aleurone,

Table 4. The content of evaluated antioxidant compounds, antioxidant activity and yield of grain in the wheat genotypes, years and cropping systems (Tukey's *HSD* (honestly significant difference) test)

		TPC	PAs	TCC	TAC	TAA	Yield (t/ha)
		(mg/kg dry matter)					
Genotype	Annie/control	581.71^{d}	714.61^{d}	1.61^{cd}	nd	162.68^{d}	8.20^{a}
	Citrus	659.81^{c}	778.85^{bc}	5.32^{a}	nd	195.12^{c}	7.78^{b}
	AF Jumiko	695.50^{b}	751.72^{cd}	2.18^{b}	12.70^{d}	212.70^{b}	8.21^{a}
	PS Karkulka	723.60^{a}	711.77^{d}	1.56^{d}	20.47^{c}	201.17^{bc}	7.40^{c}
	KM 53-14	645.09^{c}	818.49^{ab}	1.62^{cd}	57.25^{b}	226.71^{a}	7.75^{b}
	Skorpion	596.83^{d}	849.47^{a}	1.64^{c}	63.23^{a}	206.31^{c}	7.06^{d}
	$HSD_{0.05}$	39.44	49.17	0.07	2.87	12.96	0.25
Year	2016	701.70^{a}	810.65^{a}	3.00^{a}	42.41^{a}	210.09^{a}	7.37^{b}
	2017	599.15^{b}	730.99^{b}	1.64^{b}	34.41^{b}	191.48^{b}	8.09^{a}
	$HSD_{0.05}$	15.43	19.23	0.03	1.52	5.07	0.10
Cropping system	ECO	670.51^{a}	796.96^{a}	2.36^{a}	39.59^{a}	205.45^{a}	7.01^{b}
	CONV	630.34^{b}	744.67^{b}	2.28^{b}	37.22^{b}	196.12^{b}	8.45^{a}
	$HSD_{0.05}$	15.43	19.23	0.03	1.52	5.07	0.10

nd – non detected; TPC – total content of phenolics; PAs – total content of phenolic acids; TCC – total content of carotenoids; TAC – total content of anthocyanins; TAA – total antioxidant activity; ECO – organic; CONV – conventional cropping system

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Table 5. The content of evaluated antioxidant compounds, antioxidant activity and yield of grain in individual wheat genotypes (Tukey's *HSD* (honestly significant difference) test)

		Annie	Citrus	AF Jumiko	PS Karkulka	KM 53-14	Skorpion
Total content of phenolics (mg/kg DM)	2016	642.40^{a}	699.67^{a}	776.85^{a}	757.75^{a}	694.45^{a}	639.08^{a}
	2017	521.02^{b}	619.95^{b}	614.15^{b}	689.45^{b}	595.73^{b}	554.58^{b}
	$HSD_{0.05}$	16.39	49.53	26.72	52.22	23.05	23.28
	ECO	593.68^{a}	663.43^{a}	700.10^{a}	780.75^{a}	659.40^{a}	625.70^{a}
	CONV	569.73^{b}	656.18^{a}	690.90^{a}	666.45^{b}	630.78^{b}	567.97^{b}
	$HSD_{0.05}$	16.39	49.53	26.72	52.22	23.05	23.28
Total content of phenolic acids (mg/kg DM)	2016	767.60^{a}	793.49^{a}	769.16^{a}	780.58^{a}	843.78^{a}	909.26^{a}
	2017	661.61^{b}	764.21^{a}	734.29^{a}	642.95^{b}	793.19^{a}	789.69^{a}
	$HSD_{0.05}$	27.28	45.08	51.03	31.08	51.18	40.76
	ECO	736.39^{a}	795.07^{a}	751.86^{a}	775.32^{a}	839.99^{a}	883.15^{a}
	CONV	692.82^{b}	762.63^{a}	751.59^{a}	648.21^{b}	796.99^{a}	815.80^{b}
	$HSD_{0.05}$	27.28	45.08	51.03	31.08	61.18	40.76
Total content of carotenoids (mg/kg DM)	2016	2.16^{a}	7.10^{a}	2.72^{a}	1.96^{a}	2.07^{a}	1.97^{a}
	2017	1.07^{b}	3.54^{b}	1.64^{b}	1.15^{b}	1.16^{b}	1.31^{b}
	$HSD_{0.05}$	0.07	0.16	0.03	0.05	0.05	0.04
	ECO	1.62^{a}	5.29^{a}	2.29^{a}	1.63^{a}	1.64^{a}	1.66^{a}
	CONV	1.60^{a}	5.34^{a}	2.06^{b}	1.48^{b}	1.59^{b}	1.62^{b}
	$HSD_{0.05}$	0.07	0.16	0.03	0.05	0.05	0.03
Total content of anthocyanins (mg/kg DM)	2016	nd	nd	14.02^{a}	22.60^{a}	63.21^{a}	69.81^{a}
	2017	nd	nd	11.38^{b}	18.34^{b}	51.29^{b}	56.65^{b}
	$HSD_{0.05}$	nd	nd	1.79	2.16	3.49	3.87
	ECO	nd	nd	13.09^{a}	21.09^{a}	59.00^{a}	65.17^{a}
	CONV	nd	nd	12.31^{a}	19.84^{a}	55.48^{b}	61.27^{b}
	$HSD_{0.05}$	nd	nd	1.79	2.16	3.49	3.87
Total antioxidant activity (mg/kg DM)	2016	169.40^{a}	202.92^{a}	217.72^{a}	211.42^{a}	236.48^{a}	222.57^{a}
	2017	155.97^{b}	187.32^{b}	207.68^{b}	190.92^{b}	216.93^{b}	190.05^{b}
	$HSD_{0.05}$	5.79	9.62	8.38	5.69	6.60	20.74
	ECO	166.35^{a}	196.83^{a}	212.82^{a}	207.32^{a}	232.32^{a}	217.05^{a}
	CONV	159.02^{b}	193.40^{a}	212.58^{a}	195.02^{b}	221.10^{b}	195.57^{b}
	$HSD_{0.05}$	5.79	9.62	8.38	5.69	6.60	20.74
Grain yield (t/ha)	2016	7.83^{b}	7.56^{b}	8.07^{b}	7.23^{b}	7.12^{b}	6.44^{b}
	2017	8.57^{a}	8.00^{a}	8.36^{a}	7.57^{a}	8.38^{a}	7.67^{a}
	$HSD_{0.05}$	0.37	0.22	0.37	0.16	0.23	0.27
	ECO	7.53^{b}	7.06	7.57^{b}	6.82^{b}	6.86^{b}	6.24^{b}
	CONV	8.87^{a}	8.49	8.86^{a}	7.97^{a}	8.63^{a}	7.87^{a}
	$HSD_{0.05}$	0.37	0.22	0.37	0.16	0.23	0.27

DM – dry matter; nd – non detected; ECO – organic; CONV – conventional cropping system

but differences between them and cultivar with yellow endosperm were insignificant in some of cases. The TAA in the control cv. Annie was the lowest and significantly different from the others.

The effect of weather conditions. Besides the genotype, a significant impact of weather conditions on all evaluated antioxidant compounds contents was determined and it prevailed in TPC,

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PAs and TCC. The 2016 season was marked by slightly higher average temperatures and substantially lower precipitation during the time from anthesis to wheat maturity compared to 2017. Therefore, it could be possible to suppose that in 2016, when the contents of all evaluated antioxidant compounds were higher, evaluated wheat genotypes were exposed to higher weather stress. The fact that many antioxidants are produced by plants in response to abiotic stress, like water stress and/or heat stress has been known (Barański et al. 2014). Our results are in agreement with findings of Paznocht et al. (2018) and Fratianni et al. (2013) who observed an increased carotenoid synthesis in wheat with coloured grain grown under water deficit or Alexieva et al. (2001) who registered an increase in soluble phenols and anthocyanins in wheat subjected to drought.

The effect of the cropping system. Our results (Table 3) show that cropping system affects significantly the content of all evaluated antioxidant compounds, although at lower level compared to year and genotype. It is evident from the results (Table 4) that there were statistically significant differences among the cropping systems in the content of all evaluated antioxidant compounds – higher contents of antioxidants in organic cropping system were determined. Organic and conventional wheat usually differed significantly in concentrations of the determined compounds even in individual genotypes, although not in all the cases (Table 5).

The results generally confirm the conclusions of Barański et al. (2014) that organic crops are usually richer in antioxidant compounds and are in accordance with the findings of some other authors, too. Zuchowski et al. (2011) evaluated the content of selected phenolic acids in wheat from organic and conventional cropping systems. Their study demonstrated statistically significant influence of cropping system on the level of total phenolic acid content in favour of organic cultivation, although no differences in some of phenolic acids were observed. Levels of phenolics in organic crops were reported to be significantly higher in some publications concerning fruits and vegetables (Mitchell et al. 2007, Stracke et al. 2009b). On the other hand, the study of Stracke et al. (2009a) was performed to evaluate the concentrations of carotenoids and phenolic acids in wheat cultivars grown under organic and conventional conditions. The results indicate that climate factors have a greater impact on the phytochemical concentrations in the wheat grain than the production method (organic/conventional).

Elevated concentrations of antioxidant compounds in organic products can be explained by changes in plant metabolism caused by differences in the soil nitrogen availability in organic and conventional management methods (carbon/nutrient balance hypothesis) (Bloksma et al. 2007, Massad et al. 2012). In organic production, in which no synthetic fertilizers are allowed, nitrogen availability is usually expected to be lower. This leads to intensification of biosynthesis of carbon-containing compounds, including non-nitrogen secondary metabolites. When nitrogen is more readily available, plants will more intensively synthesise proteins and other nitrogen-containing compounds (Zuchowski et al. 2011). However, it should be also taken into account that the probable N deficiency of organically cultivated fields can lead to organic cereal grains with lower values of TKW. As smaller wheat kernels have a higher surface/volume ratio, they also have a higher percentage of pericarp and aleurone layer, parts containing the majority of antioxidant compounds (Zuchowski et al. 2011).

In our experiments, the same preceding crop (red clover) was used in both cropping systems. In the conventional system, the total dose of nitrogen of 120 kg N/ha applied in mineral fertilizer (nitrate form) in two partial doses of 60 kg N/ha was used. Despite the fact there are not sufficient data to discuss the potential effect of nitrogen availability on evaluated antioxidant compounds in wheat grain on the basis of soil parameters, it is possible to presume that the availability of nitrogen in the conventional cropping system was higher. This indicates the fact that the yields of grain in the conventional cropping system were by 17% in genotypes cvs. AF Jumiko and PS Karkulka, 18% in cv. Annie, 20% in cv. Citrus, 26% in cvs. Skorpion and KM 53-14 higher compared to the genotypes cultivated organically.

Moreover, it is well known that many of antioxidants found in higher concentrations in organic crops are produced by plants in response to biotic (pest attacks and disease) stress and form a part of plant's constitutive and inducible resistance mechanisms to pest and diseases (Nicholson and Hammerschmidt 1992). In our field trials in comparison with conventional cultivation, wheat genotypes grown under organic cropping system were

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much more damaged by the pests of *Oulema* spp. and fungal diseases caused by *Puccinia* spp. and *Phaeospheria nodorum*. Therefore, it is possible to assume they were exposed to higher biotic stress, compared to genotypes cultivated conventionally, with fungicide and insecticide protection. However, Almuayrifi (2013) demonstrated that non-use of synthetic pesticides and fungicides had no effect on phenolic acid and flavonoid concentrations. According to Barański et al. (2014) there are no sound published data for a causal link between higher pest/disease incidence and antioxidant concentrations in organic crops.

Grain yield. The successful introduction of cultivars with coloured grain into practice will depend on the level of yield and agronomic properties comparable to commercially used cultivars. At present, yields of coloured wheat genotypes are usually lower compared to traditional commercial cultivars. However, selected pigmented lines with commercial potential are able to give yield equivalent to the high yielding cultivars (Garg et al. 2016). In Austria, a commercial cv. Skorpion with blue grain was released with about 25% lower yield in comparison with the control cultivars (Martinek et al. 2014). Cv. Skorpion, included even to our wheat set, reached the yield only about 14% lower in comparison with the control cultivar. Moreover, cv. AF Jumiko with purple pericarp reached the same yield as the control cv. Annie.

In conclusion, variation of antioxidant compounds in the wheat grain depends on genotype, weather conditions and cultivation system. Organic cultivation may help increase the level of antioxidants in wheat grain. Coloured wheat cultivars are the important source of colour components and therefore they can be used for specific production of baking products, mainly made from the whole grain. Moreover, some of them are able to give the same or almost the same grain yields as traditional wheat cultivars.

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