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Grain morphology, texture and colour-related compounds of bread wheat cultivars in relation to cultivation regimes and growing location

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Abstract

Digital wheat grain classification systems are primarily based on kernel image and texture features. These traits are highly environment-dependent, so sample-to-sample variation may affect results of grain classification, grading, or discrimination. The main aim of this study was to determine the intra-cultivar variation of wheat kernel size, shape, colour, endosperm hardness and selected chemical compounds (carotenoids, phlobaphenes and proteins) associated with kernel colour. In the study, four winter bread wheat cultivars: two self-pollinated 'Bogatka' and 'Batuta' and two hybrid cultivars 'Hybred' and 'Hymack' were grown in different environments (extensive and intensive cultivation regimes, and three growing locations) to create variation in the kernel image, hardness and chemical compounds to simulate the range of variations encountered in reality. Measurements of kernel colour and dimensions were conducted using digital image analysis, hardness and protein content were analysed using near-infrared reflectance (NIR) spectroscopy, while carotenoids and phlobaphenes were examined using colorimetric assays. The results showed that kernel dimensions and shape were highly intra-cultivar constant, with the highest variation of thickness (up to 7.59%) and volume (up to 10.10%). Surface colour was also relatively constant, with saturation as the most variable feature (up to 11.44%). In contrast, endosperm colour revealing an approx. 2–3-fold higher variation of colour attributes than surface colour, and the highest variation was observed in the case of saturation of the colour (up to 19.67%). Among the tested chemical compounds, carotenoids were the least intra-cultivar variable (up to 14.39%), while phlobaphenes the most intra-cultivar variable (up to 31.87%). Grain hardness was the most intra-cultivar variable feature of the tested grain samples and reached the highest value of 49.02% for cultivar 'Hymack'. The genotype had the greatest effect on kernel colour (up to 50.49% of the source of variation) and carotenoids (52.97%). The environment affected mostly the grain proteins (82.20%) and hardness (72.61%).

Key words: carotenoids, colour, environment, hardness, phlobaphenes, wheat grain.

Introduction

Wheat kernel size, shape and colour are primary physical features that determine the market value of grain and are utilized in grain classification systems. Another important physical trait for grain classification and utilization is grain texture, which differentiates wheat cultivars into two main groups with hard or soft endosperm. This texture is understood as a specific organization of the protein-starch matrix, controlled by the hardness (*Ha*) locus on the short arm of chromosome 5D, and is related to lipid binding proteins known as puroindolines *a* and *b* (Mikulíková, 2007). In contrast, multivariate imaging systems of grain classification often utilize the term texture of kernel image (Panigrahi, Gunasekaran, 2001). This texture is defined as repeating patterns of local variations in image intensity, which are too fine to be distinguished as separate objects (Jain, Karu, 1995). Both endosperm and image texture may be determined using different vision systems; however, near-infrared reflectance (NIR) spectroscopy is proposed as probably the best-known technique able to characterize the global texture of wheat grain (Jaillais et al., 2011). The accuracy of grain sample classification / distinguishing by digital

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systems coupled with advanced statistics, e.g., artificial neural networks (ANN), is very high. For example, the efficiency of texture-based classification of 11 wheat cultivars grown in Poland reached 100% (Zapotoczny, 2011 a; b). Pazoki and Pazoki (2011), using texture, morphology and colour features coupled with ANN classified rain fed wheat grain cultivars with an accuracy of 87%. Shouche et al. (2001) distinguished 15 cultivars of closely-related Indian bread wheat by using 45 shape and size-related parameters. Similarly, discrimination of common wheat cultivars from spelt breeding lines and their single hybrid cultivars was possible based on analysis of grain dimensions, shape and colour features coupled with principle component analysis (Wiwart et al., 2012). According to Choudhary et al. (2008), the classification of wheat grain samples with an accuracy of 89.4% for Canada Western red spring (CWRS) wheat and 99.3% for Canada Western amber durum (CWAD) wheat was possible based on combining the morphological, colour, textural and wavelet features. Dubey et al. (2006), using 45 morphometric features and ANN, correctly identified from 84% to 94% of the analysed wheat cultivars.

The possibility of wheat cultivar discrimination / classification is the result of the inheritance of genes related to dimension and shape (Bergman et al., 2000; Okamoto et al., 2012), colour (Himi, Noda, 2005; Ficco et al., 2014) and endosperm hardness (Morris, Beecher, 2012), which accounts for the inter-cultivar differentiation. However, it is well known that these kernel features are highly affected by the conditions of plant vegetation, with the main impact being climate and fertilization regimes (Li et al., 2012; Lukow et al., 2012). Lukow et al. (2013) stated that the overall variation of the grain colour of commercially-grown Canadian hard white spring wheat ('Snowbird') was mostly attributed to annual fluctuations in climatic conditions (71–79%) and to agro-climates regimens (13–18%). Peterson et al. (2001) concluded that the high variation of colour of 543 hard white wheat grains may contribute to sample misclassification by trained inspectors. The environment-dependent fluctuation of kernel morphology, colour and hardness results from the variation and the interaction of grain carotenoids, phenolic compounds, endosperm texture, moisture and other minor components. All of these features may be affected by the environment, e.g., water stress reduces kernel width and thickness, and these kernels are lighter and redder than those with optimal growth (Konopka et al., 2007). Similarly, heat and light stress and sulphur fertilization affect carotenoid accumulation (Fratianni et al., 2005; 2013; Howitt, Pogson, 2006), while biotic stress can increase the content of grain polyphenols (Moura et al., 2010; Ribera, Zuniga, 2012), which may jointly affect kernel colour (Ficco et al., 2014).

Knowledge on the impact of genotype and growing conditions on wheat kernel morphology, colour and texture is still incomplete, especially in terms of intra-cultivar variation under the impact of the environment. The main aim of this study was to determine the intra-cultivar variation of image features, hardness and some chemical compounds (carotenoids, phlobaphenes and proteins) associated with kernel colour caused by two variables (cultivation regime and growing location).

Materials and methods

Sample description. The material included 24 samples of grain of four common white winter wheat (*Triticum aestivum* L.) cultivars: two self-pollinated 'Bogatka' and 'Batuta', breeder "Danko" (Poland), and two hybrid cultivars 'Hybred' and 'Hymack', breeder "Saaten-Union" (France). 'Batuta' and 'Bogatka' are bread cultivars with semi-early time of ear formation and maturing. They are characterized by good grain uniformity with good baking quality. 'Hybred' and 'Hymack' are high-yielding bread cultivars, with good tolerance to ear and root base diseases. The well-developed root system allows for their cultivation in less fertile soils. Wheats were cultivated in 2013–2014 in three locations in south-eastern Poland: Przecław (50°19′ N, 21°48′ E), Nowy Lubliniec (50°29′ N, 23°09′ E) and Dukla (49°55′ N, 21°68′ E), and in two extreme fertilization and plant control regimes: 1) extensive – cultivation without the use of nitrogen (N) fertilization and pesticides, and 2) intensive – cultivation with the use of 120 kg ha^{-1} N, as well as herbicide (a.i. iodosulfuron methyl sodium, amidosulfuron and fenoxaprop-P-ethyl) + insecticide (a.i. lambda-cyhalothrin) + fungicide (a.i. epoxiconazole, kresoxim-methyl, fenpropimorph and dimoxystrobin, epoxiconazole) + growth regulator (a.i. trinexapac-ethyl) treatments.

The experiments were located in Przecław – *Gleic Fluvisol* (*FLgl*), Dukla – *Haplic Cambisol* (*CMha*) and Nowy Lubliniec – *Haplic Luvisol* (*LVha*), according to WRB (2014). The typical physical and chemical properties of the soil assessed before the experiment are given in Table 1.

Table 1. Basic soil characteristics in south-eastern Poland

Location	pH in KCl	Total organic C g kg^{-1}	P mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	STC
Przecław	6.33	13.1	81	172	146	CL
Dukla	6.26	11.9	69	195	91	SC
Nowy Lubliniec	6.07	10.1	110	186	93	LS

STC – soil textural class; CL – clay loam, SC – silty clay, LS – loamy sand

Weather conditions. The data of the weather conditions were obtained from local observation measurement units located at three Experimental Stations in Przecław (50°19′ N, 21°48′ E), Dukla (49°55′ N, 21°68′ E) and Nowy Lubliniec (50°29′ N, 23°09′ E) (Table 2).

After harvesting, grain samples of approx. 300 g were manually cleaned from all foreign materials and broken kernels and stored at 8 ± 2°C. Before further analyses, the required amount of grain was removed from the refrigerator and equilibrated at a temperature of

Table 2. Weather conditions in 2013–2014 and the multi-annual average of 1956–2010 in south-eastern Poland

Years	Location								
	Przecław			Dukla			Nowy Lubliniec		
	T	R	D	T	R	D	T	R	D
2013–2014	9.0	776	+0.5 +163	9.2	919	+1.3 +47	9.2	805	+1.0 +162
1956–2010	8.5	613	–	7.9	872	–	8.2	643	–

T – mean air temperature (°C) from sowing to harvest, R – sum of rainfall (mm) from sowing to harvest, D – deviations from multi-annual average temperature (°C) / sum rainfall (mm)

21 ± 1°C and moisture of 40 ± 8% relative humidity for at least 48 hours. Prior to chemical analyses, the grain was ground to obtain particles smaller than 300 μm.

Grain morphology and colour analyses. The images of 100 single kernels for each of the 24 samples were acquired by a high resolution, low-noise colour camera CCD Nikon DXM 1200 and analysed by software *LUCIA G*, v. 4.8. The frame grabber was at a resolution of 1 280 × 1 024 pixels. The kernels were examined from a distance (lens-to-object) of 13 cm. The light source was a Kaiser RB 5004 HF – high frequency daylight copy light set with 4 × 36 W fluorescent light tubes, colour temperature about 5 400 K (Kaiser Fototechnik GmbH & Co.KG, Germany). Kernel length, width and surface colour were derived from the projected area of a single kernel arranged with the crease-side down. Kernel thickness and endosperm colour were measured after hand preparation of the kernel barrel (one cut in half and the second cut above the germ), which was carefully polished with fine-grained abrasive paper. The linear dimensions of each kernel were determined with a precision of 0.05 mm. Kernel elongation, volume and circularity were calculated from the formulas (Lucia User's Guide 2001. System for image processing and analysis. Laboratory imaging): elongation = length / width; volume = $(\pi \times \text{width}^2) \times (\text{length} - \text{width}) / 4 + \pi \times \text{width}^3 / 6$; circularity = $4 \times \pi \times \text{area} / \text{perimeter}^2$.

The colour features of kernels were measured in the HSI colour model space, in which H (hue) represents the hue of colour (in a range of 0–360°), S (saturation) – the degree to which the colour expresses its hue (in a range of 0–100%) and I (intensity) – the visual sensation of brightness / intensity of colour (in a range of 0–100%). The H value of each kernel was determined with a precision of 1°, while S and I values of the kernel with a precision of 0.05%. Before the analysis, calibration to a standard white reflective plate was done.

Grain hardness and chemical analyses. Grain hardness and protein content were determined using a near-infrared reflectance (NIR) system Infratec™ 1241 Analyser (Foss, Denmark) fitted with a sample transport module and standard sample cups. Samples were scanned from 570 to 1 050 nm, and data were collected every 2 nm. Calibration set was supplied by the manufacturer of the equipment.

Carotenoids were determined according to Konopka et al. (2004). To each 10 g of fine-milled grain sample placed in a glass flask of 100 mL capacity, a 10 mL mixture of hexane, acetone, absolute ethanol and toluene (10:7:6:7 v/v/v/v), 2 mL of 40% potassium hydroxide solution (KOH) in methanol and 1 mL of 0.1% butylated hydroxytoluene (BHT) in ethanol were added. The solutions were vigorously shaken and left in the dark at room temperature for 16 h saponification. After saponification, 30 mL 10% Na_2SO_4 was added to each flask and extraction of carotenoids was performed four times with 10 mL of hexane. The collected extracts were evaporated to dryness at 40°C in a vacuum evaporator BUCHI R-200 (Switzerland). Finally, the dry extracts were re-dissolved in 1 mL of a methanol and dichloromethane mixture (45:55, v/v) and subsequently centrifuged (25 000 g for 10 min) in a centrifuge 5417R (Eppendorf, Germany). For the analysis, a set of high performance liquid chromatography 1200 series of Agilent Technologies, equipped with a photodiode array detector from the same company, was used. Separation was performed on an YMC C30 column 3 μm, 150 mm × 4.6 mm (YMC Europe GmbH, Germany) at 30°C. The chromatogram was recorded at a wavelength of 450 nm. The mobile phase was methanol and methyltert-butyl ether (89:11 v/v) at a constant flow rate of 1 mL min^{-1}. Carotenoids were identified based on characteristic spectra and retention times of four standards: β-carotene, α-carotene, zeaxanthin and lutein (Sigma-Aldrich, USA). The total content of carotenoids was calculated using a calibration curve of lutein, which was found as predominant homologue. Calibration curve was prepared in the pigment concentration range of 1–150 μg mL^{-1}, and the results are expressed in μg lutein per 1 g of grain dry matter (DM).

Phlobaphenes were determined using the method described by Pooma et al. (2002). To 100 mg of grain sample, 0.3 mL of concentrated hydrochloric acid (HCl) and 1.2 mL dimethylsulfoxide (DMSO) were added. Extraction was conducted for 20 min at a temperature of 20°C using an apparatus Thermomixer (Eppendorf, Germany). The tubes were centrifuged (25 000 g for 10 min), and the clear supernatant was mixed with methanol (to 20% of final concentration). Absorption of phlobaphene was measured at a wavelength of 510 nm and its content was expressed as μg of catechin per 1 g of sample DM. Calibration curve was prepared in the pigment concentration range of 20–400 μg mL^{-1}.

Statistical analysis. The differences between the grain features were determined using analysis of variance (*ANOVA*) with Duncan's test. The effects of the cultivar and environment (location, cultivation regimes) were determined using a three-way variance analysis with Wilks tests. The variation of cultivars was examined using principle component analysis (PCA). The calculations were performed at a significance level of $p \leq 0.05$ using software *STATISTICA*, v. 10 (StatSoft Inc., USA).

Results and discussion

The average values of grain length, width and thickness of the tested cultivars were 6.81–7.13, 3.53–3.73 and 2.52–2.72 mm, respectively (Table 3). Both the

inter- and intra-cultivar variation of main kernel axes was relatively small. The most varied was thickness, with an overall coefficient of variation equal to 6.2%, and the intra-cultivar variation from 4.41% ('Bogatka') to 7.59% ('Hybred'). The variation of kernel length and width did not exceed 3.22%. Grain of wheat hybrid cultivars was approx. 4% smaller in length and width than grain of cultivars and similar phenomenon was found for grain perimeter, area, and volume. Variability of circularity was from 0.81 to 0.83, while elongation index varied from 1.89 to 1.96 (Table 3). Data concerning differences of kernel dimensions within the cultivar samples are quite scarce. According to Dubey et al. (2006), the changes of length and width of grain of three wheat cultivars differing in sowing date were up to 5.7%. A higher variation of length and width (up to 6.5% and to 12.1%, respectively) among recombinant lines of wheat in relation to location and year was noted by Ramya et al. (2010). Hebda and Micek (2005) observed a high intra-sample variation of length, width and thickness of 8 wheat cultivars. Similar results were obtained by Zapotoczny (2011 a) based on analyses of 11 wheat cultivars differentiated by year of cultivation. The volume and circularity of grain in present study overlapped the ranges previously shown by Markowski et al. (2013). However, it can be emphasized that the circularity of the tested grain, with values from 0.81 to 0.83 in comparison to circularity from 0.69 to 0.76 of the wheat samples studied by Bergman et al. (2000), points to a more spherical grain which is more preferred by the market (Novaro et al., 2001).

Table 3. Intra- and inter-cultivar variability of analysed wheat grain samples

Feature	'Batuta'		'Bogatka'		'Hybred'		'Hymack'	
	x	CV	*x*	CV	*x*	CV	*x*	CV
Morphology								
Length mm	7.08 b	1.69	7.13 b	3.22	6.81 a	2.94	6.90 a	1.41
Width mm	3.70 b	3.13	3.73 b	2.99	3.62 a	1.52	3.53 a	2.06
Thickness mm	2.52 a	5.06	2.61 a	4.41	2.72 a	7.59	2.69 a	5.58
Perimeter mm	17.60 b	2.00	17.73 b	3.14	17.20 a	2.16	17.15 a	1.42
Area mm^2	20.49 b	4.44	20.78 b	5.53	19.11 a	3.64	18.98 a	3.49
Volume mm^3	41.78 b	7.24	43.05 b	10.10	40.33 a	6.22	39.85 a	4.06
Circularity	0.83 b	0.81	0.83 b	1.00	0.81 a	1.19	0.81 a	1.47
Elongation	1.92 a	1.58	1.91 a	1.02	1.89 a	3.12	1.96 b	1.51
Colour								
H (surface) °	25.39 a	3.89	25.89 b	1.12	27.09 c	3.13	27.47 d	2.02
S (surface) %	22.48 a	5.84	22.84 a	11.44	26.14 b	6.97	26.46 b	6.07
I (surface) %	71.14 b	2.43	70.58 b	3.34	67.64 a	2.84	67.68 a	2.31
He (endosperm) °	26.73 b	2.01	25.88 a	3.83	28.90 c	9.23	29.77 d	9.30
Se (endosperm) %	18.33 c	13.27	15.69 b	19.67	9.79 a	16.34	8.96 a	12.75
Ie (endosperm) %	73.04 a	5.04	75.03 a	5.59	83.03 b	4.72	84.74 b	3.32
Hardness								
NIR units	56.87c	32.47	44.33a	33.6	46.58b	43.72	46.43b	49.02
Chemical composition								
Protein % of DM	13.09 c	15.86	12.83 c	16.35	12.71 b	17.21	11.95 a	19.63
Carotenoids μg g^{-1} of DM	2.69 c	11.78	2.70 c	14.39	2.30 b	12.19	1.92 a	13.44
Phlobaphenes μg g^{-1} of DM	14.73 d	26.47	12.14 a	31.87	14.08 c	14.45	13.22 b	14.82

Note. x – mean value, CV – coefficient of variation (%); DM – dry matter; data with the different letters in the same line are significantly different ($p \leq 0.05$, one-way variance analysis with Duncan test).

An average colour of the tested kernels, with values approx. 27° for H, 24% for S and 69% for I (Table 3), was typical of common bread wheat grain (Knievel et al., 2009). Intra-cultivar variation of colour attributes showed that in the case of kernel surface, hue varied from 1.12% ('Bogatka') to 3.89% ('Batuta'), intensity from 2.31% ('Hymack') to 3.34% ('Bogatka') and saturation from 5.84% ('Batuta') to 11.44% ('Bogatka'). Endosperm colour was substantially more differentiated, especially in the case of saturation, in which intra-cultivar variation was from 12.75% ('Hymack') to 19.67% ('Bogatka'). Variation of hue and intensity did not exceed 9.30%. It is noteworthy that the hue of endosperm of cultivars was more constant than that of endosperm of hybrid cultivars. Analysis of variance showed that only the hue, both of the surface and endosperm, significantly differentiated the cultivars. However, grain of cultivars varied from that of hybrid cultivars in saturation and intensity. In general, hybrid wheats surface was of higher saturation and lower intensity (darker image), while endosperm was of lower saturation and higher intensity (brighter image).

Variation of grain colour was reflected by changes of its chemical composition and hardness. Mean values of protein content varied from 11.95% ('Hymack') to 13.09% ('Batuta'), with high intra-cultivar variation from 15.86% ('Batuta') to 19.63% ('Hymack') (Table 3). Grain protein content is mostly affected by nitrogen fertilization (Park et al., 2014), and this was confirmed by our results for samples with extensive (10.84%) and intensive (14.45%) cultivation (Table 4). A concentration of carotenoids equalled: 1.92 μg g^{-1} ('Hymack'), 2.30 μg g^{-1} ('Hybred') and 2.69–2.70 μg g^{-1} ('Batuta' and 'Bogatka'), and was typical for bread wheats (Konopka et al., 2006; Lv et al., 2013; Ziegler et al., 2016; Lachman et al., 2017). Intra-cultivar variation of these pigments was similar between the tested cultivars and reached values from 11.8% to 14.4%. The cultivars 'Batuta' and 'Bogatka' were statistically identical in contents of protein and carotenoids. In contrast, deposition of phlobaphenes (red pigments) was a unique trait of the cultivars used, with the highest content (14.73 μg g^{-1}) in 'Batuta' and the lowest (12.14 μg g^{-1}) in 'Bogatka'. Intra-cultivar variation of phlobaphenes content (caused by cultivation regimes and growing location) was in a range of 14.45–14.82% for hybrid cultivars and in a range of 26.47–31.87% for self-pollinated cultivars. It can be emphasized that the

Table 4. Variability of analysed wheat grain samples under the impact of cultivation regime and growing location

Feature	Extensive cultivation		Intensive cultivation		Przecław		Nowy Lubliniec		Dukla	
	x	CV	*x*	CV	*x*	CV	*x*	CV	*x*	CV
Morphology										
Length mm	6.96 a	2.67	7.00 a	3.36	6.89 a	3.09	6.93 ab	2.80	7.12 b	2.22
Width mm	3.63 a	2.87	3.66 a	3.62	3.61 a	4.19	3.65 a	2.90	3.68 a	2.52
Thickness mm	2.61 a	7.29	2.66 a	5.18	2.64 ab	6.24	2.67 b	7.25	2.61 a	5.69
Perimeter mm	17.36 a	1.92	17.47 a	3.16	17.21 a	2.79	17.35 ab	2.33	17.70 b	1.99
Area mm^2	19.65 a	4.73	20.03 a	6.80	19.38 a	7.21	19.79 a	5.36	20.35 b	4.34
Volume mm^3	41.05 a	5.47	41.45 a	9.39	40.18 a	7.89	40.38 a	6.76	43.21 a	6.46
Circularity	0.82 a	1.96	0.82 a	1.24	0.82 a	2.37	0.82 a	1.18	0.81 a	1.04
Elongation	1.92 a	2.72	1.92 a	2.01	1.91 a	2.80	1.91 a	2.39	1.94 a	1.57
Colour										
H (surface) °	26.19 a	4.62	26.73 b	3.55	26.63 b	4.59	26.18 a	2.55	26.57 b	5.18
S (surface) %	24.23 a	10.98	24.72 b	10.43	26.28 b	9.19	23.38 a	7.29	23.78 a	11.42
I (surface) %	69.83 b	3.55	68.70 a	3.42	67.85 a	3.53	70.49 b	2.05	69.45 b	3.96
He (endosperm) °	28.15 a	10.81	27.49 a	6.54	26.04 a	5.81	28.84 b	10.25	28.59 b	6.53
Se (endosperm) %	11.87 a	31.51	14.65 b	34.38	13.76 b	33.77	12.87 a	33.49	13.16 ab	40.13
Ie (endosperm) %	81.09 b	6.47	76.83 a	8.44	76.30 a	7.24	79.91 b	7.29	80.67 b	8.60
Hardness										
NIR units	37.45 a	52.21	59.65 b	15.04	62.74 c	13.38	35.82 a	64.22	47.09 b	24.46
Chemical composition										
Protein % of DM	10.84 a	10.12	14.45 b	5.96	13.02 c	9.44	12.82 b	22.20	12.09 a	16.59
Carotenoids μg g^{-1} of DM	2.52 b	17.56	2.29 a	18.73	2.36 a	21.03	2.35 a	18.54	2.50 b	17.36
Phlobaphenes μg g^{-1} of DM	13.58 a	23.70	13.50 a	22.25	11.96 a	22.19	14.87 c	21.58	13.79 b	20.73

Note. x – mean value, CV – coefficient of variation (%); DM – dry matter; data with the different letters in the same line, separately for cultivation regimes and growing locations, are significantly different ($p \leq 0.05$, one-way variance analysis with Duncan test).

content of phlobaphenes in wheat grain determined in our study is difficult to compare, since the references are scarce and phlobaphenes in the wheat grain are usually identified as present or absent (Lukow et al., 2012). The authors cited detected them only in the red class of wheat grain, while phlobaphenes may be formed from chemically related proanthocyanidins (Young et al., 1985). Possible misclassification of both these groups of chemical compounds may be a reason for the lack of conformity concerning the content of proanthocyanidins in bread wheat (Stehno et al., 2011; Lukow et al., 2012; Žilić et al., 2013).

The average endosperm hardness of the hybrid cultivars, as well as of the 'Bogatka', was on similar level – approx. 44–46 NIR units, while the grain of the 'Batuta' was characterized by a substantially higher value – on average 56.87 NIR units (Table 3). These values classified the first group to the medium-soft category, while the 'Batuta' was classified as medium-hard (Hrušková, Švec, 2009). A detailed analysis showed that hardness was the most variable intra-cultivar feature, with variation from approx. 33% for self-pollinated cultivars to 49% for the 'Hymack'. In an extreme case ('Hymack'), environmental conditions shifted the cultivar category from extra soft (hardness below 12 NIR units) to hard (61–72 NIR units). The data presented in Table 4 showed that changes of hardness were the highest between grain samples cultivated in Przecław and Nowy Lubliniec (62.74 *vs* 35.82 NIR units) and between extensive and intensive cultivation regime (37.45 *vs* 59.65 NIR units). Variation caused by growing location probably resulted from differences of the physical and chemical properties of the soil, since the average precipitations and temperatures during vegetation were similar (Tables 1–2). Positive impact of intensive cultivation was related to increased content of grain protein (Table 4), and affected by increased fertilization with nitrogen and the use of plant protection agents. Similar predominant impact of the environment on the texture of 30 soft wheat cultivars was previously noted by Morris et al. (2005).

Grain dimensions and shape was only slightly cultivar- and environment-dependent (Table 5). In contrast, the cultivar was the main source of variation in the case of surface hue and intensity (50.49% and 41.11% of explained variance, respectively), as well as saturation of endosperm colour and carotenoid content (46.68% and 52.97% of explained variance, respectively). The location of plots mostly affected endosperm hardness (36.24%), while cultivation method affected hardness (36.37%) and protein content (78.36%). The summarized effect of interactions of the main factors was the highest for phlobaphenes (70.63%). This generally confirms the results of previous studies, in which morphology, colour, hardness / texture and chemical composition were only partly affected by the cultivar (Morris et al., 2005; Hrušková, Švec, 2009; Knievel et al., 2009; Stracke et al., 2009; Fratianni et al., 2013; Lv et al., 2013).

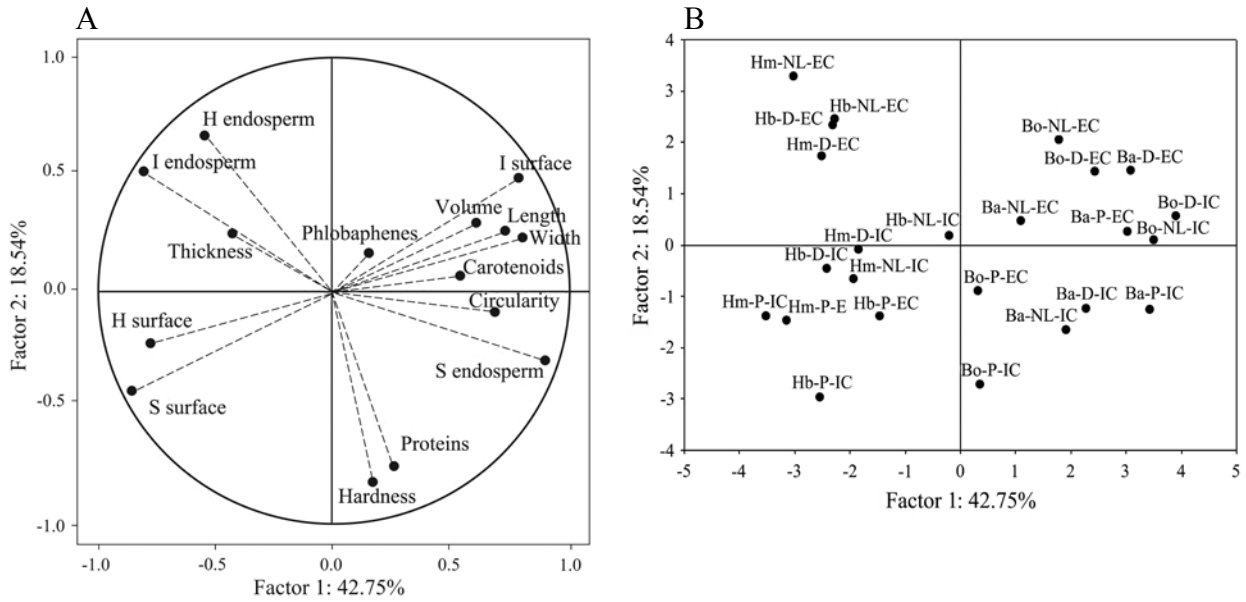
PCA loadings plot (Fig. A) showed the relationships between kernel dimensions and shape, colour, chemical compounds and hardness. The PC1 explained 42.75% of the variance and was mostly related to kernel width and colour, while PC2 explained next 18.54% of the variance and was related to grain hardness and protein content. The cumulative percentage of explained variance for PC1–PC4 was 83% (data not shown).

On the PCA score plot (Fig. B), a visible separation of self-pollinated cultivars from hybrid cultivars and an overlapping of samples within these two groups were observed. Changes in the location and cultivation regimes resulted in a shift of all tested wheat grain scores.

Table 5. Effect of cultivar and environment (% of explained variance) on wheat grain morphology, colour, hardness and tested chemical compounds

Feature	Cultivar	Location	Cultivation method	Interactions effect	Error
Length mm	27.74	ns	ns	ns	72.26
Width mm	27.27	ns	ns	15.66	57.08
Thickness mm	ns	15.33	ns	18.33	66.35
Volume mm^3	25.61	ns	ns	ns	74.39
Circularity	14.37	ns	5.15	15.30	65.18
H (surface) °	50.49	2.16	6.11	30.47	10.77
S (surface) %	27.88	9.41	12.98	22.60	27.12
I (surface) %	41.11	2.74	13.90	22.81	19.44
He (endosperm) °	29.18	28.66	ns	6.33	35.82
Se (endosperm) %	46.68	ns	7.83	14.29	31.19
Ie (endosperm) %	34.77	ns	9.53	18.12	37.58
Hardness NIR units	7.04	36.24	36.37	19.79	0.56
Proteins % of DM	4.35	3.84	78.36	13.32	0.13
Carotenoids μg g^{-1} of DM	52.97	2.55	6.72	32.72	5.04
Phlobaphenes μg g^{-1} of DM	10.22	15.67	ns	70.63	3.49

Note. The results are expressed as percent of total mean square, ns – effect not significant, three-way analysis with Wilks test, $p \leq 0.05$.



Ba – 'Batuta', Bo – 'Bogatka', Hb – 'Hybred', Hm – 'Hymack'; P – Przecław, NL – Nowy Lubliniec, D – Dukla; EC – extensive cultivation, IC – intensive cultivation

Figure. Principle component analysis (PCA) loading (A) and score (B) plots, showing correlation of wheat grain features and differentiation of tested grain samples

Conclusions

1. The features of kernel dimensions and shape were only slightly affected by the tested sources of variation. The lowest value of intra-cultivar variation was noted for grain length (up to 3.22% of explained variance) and circularity (up to 1.47%); while the most variable were grain protein content (up to 19.63%) and endosperm colour saturation (up to 19.67%), phlobaphenes content (up to 31.87%) and hardness (up to 49.02%).

2. The cultivar effect had a predominant impact on hue of grain surface colour (up to 50.49%) and carotenoid content (up to 52.97%). In contrast, the method of cultivation mostly affected grain hardness (up to 36.37%) and protein content (up to 78.36%).

3. The principle component analysis (PCA) showed a possibility of discrimination of wheat cultivar from hybrid cultivars.

4. Further studies should be conducted to show the efficiency of vision and artificial systems to assess the accuracy of classification of such highly intra-cultivar differentiated grain samples.

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Paprastųjų kviečių grūdų morfologinių savybių, tekstūros ir spalvos priklausomumas nuo auginimo aplinkos ir vietos

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Santrauka

Kviečių grūdų klasifikavimo skaitmeninės sistemos daugiausia grindžiamos grūdų išvaizdos ir tekstūros savybėmis. Šie požymiai yra labai priklauso nuo aplinkos, todėl variacija tarp mėginių gali turėti įtakos klasifikuojant, rūšiuojant ir atskiriant grūdus. Tyrimo tikslas – nustatyti paprastųjų kviečių grūdų dydžio, formos, spalvos, endospermo kietumo ir kai kurių cheminių junginių (karotenoidų, flobapenų ir baltymų), susijusių su grūdų spalva, variaciją. Tyrimo metu keturių veislių: dviejų savidulkių 'Bogatka' bei 'Batuta' ir dviejų hibridinių veislių 'Hybred' bei 'Hymack', žieminiai paprastieji kviečiai buvo auginti skirtingose augavietėse, taikant intensyvų ir ekstensyvų auginimo režimą, trijose vietose, siekiant sukurti grūdų morfologinių savybių, kietumo ir cheminių junginių skirtumus. Grūdų spalva ir dydis matuoti panaudojus skaitmeninę vaizdo analizę, kietumas ir baltymų kiekis analizuoti naudojant artimosios srities infraraudonųjų spindulių atspindžio (NIR) spektroskopiją, karotenoidai ir flobafenai tirti taikant kolorimetrinį metodą. Tyrimo rezultatai parodė, kad kviečių grūdų matmenys ir forma buvo pastovūs. Didžiausia buvo nustatyta storio (iki 7,59 %) ir tūrio (iki 10,10 %) variacija. Paviršiaus spalva taip pat buvo santykinai pastovi, o labiausiai kintamas požymis (iki 11,44 %) buvo spalvos prisotinimas. Endospermo spalva atskleidė maždaug 2–3 kartus didesnį spalvos savybių varijavimą; labiausiai varijavo spalvos prisotinimas (iki 19,67 %). Iš tirtų cheminių junginių grūduose mažiausiai varijavo karotenoidų (iki 14,39 %), labiausiai – flobafeno (31,87 %) kiekiai. Tarp tirtų mėginių labiausiai kito grūdų kietumas, jis buvo didžiausias (49,02 %) veislės 'Hymac'. Grūdų spalvai (iki 50,49 % variacijos šaltinio) ir karotenoidų kiekiui (52,97 %) didžiausią įtaką turėjo genotipas. Auginimo aplinka turėjo didžiausią įtaką grūdų baltymų kiekiui (82,20 %) ir kietumui (72,61 %).

Reikšminiai žodžiai: aplinka, flobafenas, karotinoidai, kietumas, kviečių grūdai, spalva.