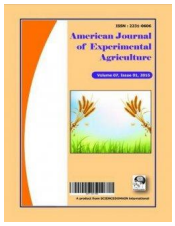

American Journal of Experimental Agriculture
9(2): 1-9, 2015, Article no.AJEA.18819
ISSN: 2231-0606

SCIENCEDOMAIN *international*
www.sciencedomain.org

Variability of Wheat Grain Image and Content of Phenolic Compounds and Carotenoids under the Impact of Selected Novel Plant Protection Treatments

Alicja Wasilewska[1*], Iwona Konopka[1], Małgorzata Tańska[1] and Urszula Wachowska[2]

[1]Department of Food Plant Chemistry and Processing, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland.
[2]Department of Ento and Phytopathology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland.

Authors' contributions

This work was carried out in collaboration between all authors. Author UW planned and realized biological treatment of wheat plants. Authors AW, IK and MT done all other experiments and wrote the first draft of the manuscript. Authors AW and MT performed the statistical analysis. Author IK managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2015/18819
Editor(s):
(1) Peter A. Roussos, Lab. Pomology, Agricultural University of Athens, Greece.
Reviewers:
(1) Petigrosso, Lucas Ricardo, Agricultural Engineer, National University of Mar del Plata, Argentina.
(2) Anonymous, Center University of Ain Temouchent, Algeria.
Complete Peer review History: http://sciencedomain.org/review-history/10121

Original Research Article

Received 11th May 2015
Accepted 20th June 2015
Published 10th July 2015

ABSTRACT

Aims: Variability of wheat kernel size and color as well as the content of selected phytochemicals in wheat grain under the impact of selected biological and fungicide crop protection agents was investigated.
Study Design: A randomized block method in quadruplicate. Plants sprayed with *Sphingomonas* sp. bacteria in a tillering phase and during the period of winter wheat heading stage.
Place and Duration of Study: Field experiments: Department of Ento and Phytopathology, Tomaszkowo, Poland during vegetation seasons of 2009-10 and 2010-11.
Analytical Part: Chair of Food Plant Chemistry and Processing, Olsztyn, Poland

**Corresponding author: E-mail: alicja.faron@uwm.edu.pl;*

Methodology: Wheat plants were protected using *Sphingomonas* bacteria or *Aureobasidium pullulans* yeast preparations, or using chemical fungicides and the impact of plant growth promoters was also measured. Image features of kernels were measured using digital image analysis, total contents of grain phenolic compounds, phlobaphenes, flavonoids, proanthocyanidins and carotenoids using spectrophotometric assays, and phenolic acids using high-performance liquid chromatography.
Results: Length was the most constant dimension of grain, independent of the type of crop protection, with the coefficient of variation (CV) 0.98%. In contrast, the width varied with CV = 3.13% and thicknesses with CV = 3.53%. The average kernel surface hue (26.44° +/- 0.75) and saturation (27.37% +/- 0.92) values were higher and the intensity of color (61.80% +/- 0.81) was lower than these of cross-section (23.85° +/- 2.12 and 19.06% +/- 1.27, 70.48% +/- 1.64, respectively). Total Folin-Ciocalteu reactive compounds occured in the largest quantities (1045 to 1507 μg g^{-1}). The least variable was content of phenolic acids (CV = 1.13%) and among this group – content of ferulic acid (CV = 0.73%).
Conclusion: It was found that the type of crop protection only slightly affected the variability of kernel dimensions and color. Kernels from control (unprotected) wheat plots were smaller and lighter, had less saturated color and were characterized by lower hue values as well as higher total phenolic compounds, and proanthocyanidins content. There were only minor differences between the biological and fungicide crop protection agents used.

Keywords: Plant protection; wheat grain; phytochemicals; digital image analysis.

1. INTRODUCTION

Size, shape, texture and color are the main kernel features used in botanical and technological classification, as well as in varietal/cultivar identification of cereal grain [1] The measurements of these features are increasingly performed using computer-aided vision systems, which are faster, more accurate and objective than the human eye [2]. In the digital image analysis (DIA) of grain samples their color, shape and dimensions are predominant subject of examination. These features are controlled by the structural and regulatory genes in plant [3]. Therefore wheats with blue, red or white grains [4,5], differing in size, shape and texture of the kernels are known [3,6].

Intra-cultivar variability of grain image features is mainly the result of environment impact, especially climate and agriculture regimes during plant vegetation [7,8]. In response to environmental stresses, caused for example by the attack of insects and microbes, extreme conditions of temperature, water, sun lighting, soil composition and mechanical damage, plants produce grain with changed chemical composition. This grain usually deposits a higher content of compounds known as phytoanticipins or phytoalexins [9]. Many of them can be converted into phenolic compounds in a phenylpropanoid pathway [10]. For example, there is evidence for a higher impact of the growing conditions than the genotype on the accumulation of phenolic compounds in wheat grain [11,12]. According to Shewry and Ward [12] the most variable and susceptible to the effect of the environment are phenolic acids. The cited study also indicates that environmental factors influence mostly the free and conjugated acid contents (in about 60%), while the cultivar impact is much lower (up to 10%). Mpofu et al. [11] observed a similar relationship for the content of ferulic acid in wheat grain, which was 57% dependent on the environment and only 37% on the genotype. Environmental factors also determine the content of other groups of plant secondary metabolites, such as carotenoids [13], alkylresorcinols [14], and sterols [15].

In modern agriculture there is a growing trend for integration of standard pesticide treatments with pro-ecological methods [16,17]. These new methods of crop protection include biological methods, in which various species of microorganisms are utilized alone or in combination with other plant protection agents [18]. However, such treatments may affect the metabolic pathways in the plant tissues [19]. For example, Ronchi et al. [20] stated that fungicide tetraconazole affects phenylpropanoid-flavonoid biosynthesis, increasing the anthocyanin content in maize. The influence of microorganisms on biosynthesis or conversion of individual phytochemicals is still not well understood. Previous studies were mostly focused on the

effects of effective microorganisms (EM) on crop yield, e.g. wheat [21], maize [22] and rice [23].

The aim of this study was to determine the intra-cultivar variability of kernels image features as well as the content of total phenolic compounds, phenolic acids, phlobaphenes, flavonoids, proanthocyanidins and carotenoids in wheat grain from plants cultivated with the use of different biological protection agents in comparison with typically-used chemical fungicides and a control sample.

2. MATERIALS AND METHODS

2.1 Field Experiments

Field experiments were conducted from 2009-2011, in the north-eastern region of Poland. The plots with an area of 20-25 m^2 were sown with winter wheat (*Triticumaestivum L.* cultivar Bogatka). The experiment was established by a randomized block method with four replications. Plants were fertilized with nitrogen (N), potassium (K_2O) and phosphorus (P_2O_5) at doses of 100, 100 and 60 kg ha^{-1}, respectively. Growing plants were protected in the tillering phase BBCH 31 (the first node of at least 1 cm above node tillering) and during the period of winter wheat heading stage BBCH 55 (middle of heading, while half of inflorescence emerged) as shown in Table 1. In biological variants, the mixture of the *Sphingomonas* bacteria isolates (variant A2) and a mixture of the *Aureobasidium pullulans* fungus isolates (variant A3) were used. These microorganisms derived from grain or rhizosphere of winter wheat cultivar Tonacja, grown in the field conditions. In the chemical variants, fungicides set (variant A4) or plant growth stimulator (variant A5) were used. The unprotected plants were control (variant A1).

Wheat grain was harvested at maturity, dried to approx. 14%, and cleaned from broken kernels, dust and tailings in a ø200 mm, type LPzE-2e Multiserw laboratory vibrator. It was used to analyze kernel image features (dimensions and color) as well as phenolic compounds and carotenoids. Before chemical analyses, the grain was milled in a type A10 IKA Labortechnic mill to fine particles below 300 nm and hydrolyzed with 2 N NaOH for 4 h at room temperature. Hydrolizates were then neutralized (6 N HCl) and evaporated to dryness (type R-210 Buchi rotary evaporator).

2.2 Image Features of Kernels

The kernel dimensions: length, width and thickness (Fig. 1) and color of surface and cross-section were determined for 60 kernels using the digital image analysis according to Konopka et al. [24]. The images were acquired by a high resolution, low-noise CCD Nikon DXM-1200 color camera and analyzed by LUCIA G ver. 4.8 software. The results were presented in HSI (H-hue, S-saturation, I-intensity) color space, where H is expressed in degrees (in range 0-360°), and S and I in percentage (0-100% range).

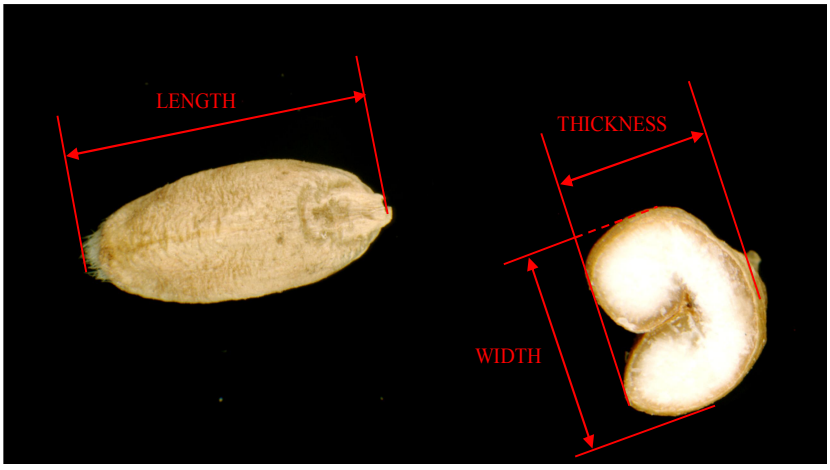

Fig. 1. Images of wheat kernel surface and cross-section with a indication of the dimensions measured by the digital image analysis

Table 1. Plant protection in field experiment

No.	Protection variant	BBCH 31*	BBCH 55
A1	Control	-	-
A2	Biological protection I	*Sphingomonas* sp.	*Sphingomonas* sp.
A3	Biological protection II	*Aureobasidium pullulans*	*Aureobasidium pullulans*
A4	Fungicide protection	propiconazole	fluoxastrobin propiconazole
A5	Plant growth stimulator	para-nitrophenolate ortho-nitrophenolate 5-sodium nitroguaiacolate	para-nitrophenolate ortho-nitrophenolate 5-sodium nitroguaiacolate

** BBCH-scale – (ger. Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) an European scale used to identify the phenological development stages of a plant*

2.3 Chemical Analyses

2.3.1 Determination of total content of folin-ciocalteu reactive compounds (TPC)

Extraction of Folin-Ciocalteu reactive compounds was done according to the method described by Ribereau-Gayon [25]. TPC were 2-fold extracted by the use of 80% methanol, extracts were mixed and then evaporated. TPC were determined spectrophotometrically and the color reaction was carried out by adding Folin-Ciocalteau reagent, 14% sodium carbonate and distilled water. After 60 min in darkness the absorbance of solutions was measured against the reagent sample (without the extract) at the wavelength of 720 nm, with a UNICAM UV/Vis UV2 spectrophotometer. TPC content was expressed as µg of D-catechin in 1 g of a sample dry matter.

2.3.2 Determination of phenolic acids (PA) content

PA content was determined based on the method described by Konopka et al. [26]. Phenolic acids were 3-fold extracted with the use of diethyl ether. Collected extracts were evaporated to dryness in a Buchi rotary evaporator type R-210. In the next step, dry extracts were re-dissolved in methanol (HPLC grade) and PA were determined by the RP-HPLC technique (Agilent Technologies 1200 series system). The mobile phase consisted of two solvents: A – 0.15% formic acid in acetonitrile (*v/v*) and B – 0.15% formic acid in water (*v/v*). The applied gradient was as follows: 0-7 min 10% of eluent A, followed by linear increase up to 100% of eluent A over 43 min. The flow rate was 0.2 ml/min and detection was performed at the 280 and 320 nm wavelengths. PA were identified based on comparison of absorption spectra to the reference phenolic acids. PA content was expressed as µg of ferulic acid equivalent in 1 g of a dry matter sample.

2.3.3 Determination of proanthocyanidins (PRO) content

PRO were analyzed based on the method described for condensed tannins by Naczk et al. [27]. Proanthocyanidins were 2-fold extracted (30 min, 20°C) with 80% acetone. The combined extracts were then evaporated to dryness and dissolved in methanol. In the next step, after 1-butanol-HCl reagent addition, the solutions were heated for 2 h in sealed vials in a boiling water bath, and cooled to room temperature. Absorbance was measured at 550 nm, and PRO content was expressed as µg of D-catechin equivalent in 1 g of a dry matter sample.

2.3.4 Determination of phlobaphenes (PHLOB) content

PHLOB were determined according to Wilailak et al. [28]. Concentrated HCl and dimethyl sulfoxide (DMSO) were added to the ground samples and phlobaphenes were extracted for 20 min at 20°C. The samples were then centrifuged (25 000 × g, 10 min, 25°C) and supernatants were diluted with 20% methanol. The centrifugation was repeated and the absorbance of supernatants was measured at a wavelength of 510 nm. The PHLOB content was presented as µg of D-catechin equivalent in 1 g of a sample dry matter.

2.3.5 Determination of flavonoids (FLAV) content

FLAV were determined using the aluminum chloride colorimetric method by Lacko-Bartosova et al. [29]. Samples of ground wheat grain were mixed with 80% methanol and were placed in an ultrasonic bath for 15 min. They were then

centrifuged (25 000 × g, 15 min, 25°C). The supernatants were mixed with 80% methanol, followed by 10% aluminum chloride, 1 N potassium acetate and distilled water. The mixtures were incubated for 30 min at room temperature, centrifuged (25 000 × g, 10 min) and their absorbance was measured at 415 nm with a Unicam UV/Vis UV2 spectrofotometer. FLAV content was calculated using a standard calibration of D-catechin methanol solution and expressed as µg of D-catechin equivalent in 1 g of a dry matter sample.

2.3.6 Determination of total carotenoids (CAR) content

CAR were determined spectrophotometrically by the method described by Craft and Soares [30]. To this end, 2.5% extract solutions in hexane were prepared and their absorbance was measured at the wavelength of 454 nm (maximum of lutein absorption). The measurements were carried out with a UNICAM UV/Vis UV2 spectrophotometer. CAR content was calculated based on molar absorptivity coefficient (for lutein dissolved in hexane, it is equal to 147300 L mol^{-1} cm^{-1}) and the molar mass of lutein (equal to 568.87 g mol^{-1}). The results were presented as µg of lutein equivalent in 1 g of a dry matter sample.

2.4 Statistical Analysis

All analyses were made in triplicate. The results were analyzed statistically using variance analysis. For calculations, $p \leq 0.05$ was established as the level of significance. To isolate statistically homogeneous groups, a "post-hoc" Duncan test was used. Calculations were performed using STATISTICA version 10.0 PL (StatSoft, Inc.).

3. RESULTS AND DISCUSSION

3.1 Variability of Kernel Image Features

Dimensions of wheat kernels varied in the ranges of 7.09-7.26, 3.05-3.29 and 2.42-2.66 mm for the length, width and thickness, respectively (Table 2). The tested Bogatka cultivar was typical in this regard to other winter wheat cultivars [1,31]. Generally, the smallest were kernels of the control sample. The use of biological and chemical treatments significantly increased width and thickness. It was found that the most constant dimension of grain, independent of the type of crop protection, was length with the coefficient of variation 0.98%. In contrast, the width varied with CV = 3.13% and thicknesses with CV = 3.53%. Similarly, Breseghello and Sorrells [32] found that the cultivar is major source of variation for linear dimensions of kernels, while the area and perimeter of cross-section are location × population dependent. On the other hand, the phenotypic variation between kernels of the same cultivar is very low and it is determined mostly by environmental factors, particularly for self-fertile species [33].

The average kernel color values were as follows: Hs = 26.44°, Ss = 27.37%, Is = 61.80% for the surface and Hc = 23.85°, Sc = 19.06%, Ic =

Table 2. Dimensions and color of wheat kernels

No.	Dimensions (mm)			Surface color			Cross-section color		
	length	width	thickness	Hs (°)	Ss (%)	Is (%)	Hc (°)	Sc (%)	Ic (%)
A1	7.09[a*]	3.05[a]	2.42[a]	25.15[a]	26.01[a]	63.13[a]	20.52[a]	18.87[ab]	71.76[ab]
A2	7.26[a]	3.20[b]	2.5a[b]	26.45[b]	27.70[bc]	61.43[b]	23.24[b]	19.84[a]	69.33[a]
A3	7.19[a]	3.18[b]	2.59[bc]	26.72[b]	27.34[b]	61.78[b]	24.24[c]	16.96[b]	72.63[b]
A4	7.12[a]	3.29[b]	2.59[bc]	26.87[b]	28.56[c]	60.98[b]	25.50[d]	20.19[a]	68.77[a]
A5	7.13[a]	3.28[b]	2.66[c]	27.03[b]	27.23[b]	61.68[b]	25.74[d]	19.42[a]	69.93[ab]
X	7.16	3.20	2.55	26.44	27.37	61.80	23.85	19.06	70.48
SD	0.07	0.10	0.09	0.75	0.92	0.81	2.12	1.27	1.64
CV [%]	0.98	3.13	3.53	2.84	3.36	1.29	8.89	6.66	2.33

** Mean values in the same column followed by different letters are significantly different ($P \leq 0.05$).*

Abbreviations: X – mean value, SD – standard deviation, CV – coefficient of variation, Hs, Ss, Is – hue, saturation and intensity of the kernel surface, Hc, Sc, Ic – hue, saturation and intensity of the kernel cross-section

70.48% for its cross-section (Table 2). Control kernels were generally less saturated and lighter and their hue values were lower by about 1-2° (for surface) and 3-5° (for cross-section) than of other samples. The most different from the control sample was grain from a plot treated with plant regulators (Hs and Hc values) and protected by fungicide (Ss, Sc, Is and Ic values). Konopka et al. [26] observed that H and S values increased by 1-3 units and, simultaneously, the I value decreased by about 2 units by applying organic fertilizers. In the present study, the tendency was the same, when both biological and chemical crop protection were used. The highest color variability between tested grain variants was noted for hue of cross-section (8.89%) and its saturation (6.66%). It has been found that the variability of surface color was about 2-3-fold lower than of cross-section. Konopka et al. [34] found a similar relationship for other 6 winter wheat cultivars. Wiwart et al. [33] stated that even though color and shape of kernels are weakly modified by environment conditions, these features can be regarded as varietal traits.

3.2 Variability of Grain Phytochemicals

On average, wheat grain contained 1324 μg g^{-1} of Folin-Ciocalteau reactive compounds (TPC), with CV = 13.11% (Table 3). Phenolic acids constituted up to 61% of TPC, with an average content and share equal to 629 μg g^{-1} and 47%, respectively, and with a very low variation (1.13%) between tested samples. Extensive research of 150 wheat genotypes conducted by Shewry and Ward [12] determined the variation of phenolic acids content to be from 326 to 1171 μg g^{-1}. The cited authors stated that the total content of phenolic acids is approximately to the same degree dependent on cultivar and environment (25 and 20%, respectively). A significantly higher impact of the environment (up to 57%) was stated in the work of Mpofu et al. [11]. According to Konopka et al. [26], the total polyphenol compounds content may be increased by up to 11% in organically fertilized grain.

Low variability of the total phenolic acid content (1.13%) was confirmed by the small fluctuations (0.73%) of the main member of this group – ferulic acid. This acid accounted for approximately 90% of all acids, with small amounts of p-cumaric, sinapic, vanilic, p-OH benzoic and protocatechuic acids (Table 4). Okarter et al. [35] observed a similar phenolic acid composition in 6 wheat cultivars. In their studies, the most common was ferulic acid, followed by p-cumaric. These authors also found small amounts of syringic and caffeic acids, which were not detected in our research. It was generally found that the share of less frequent acids, in comparison with ferulic acid, was more varied by plant treatments used with the highest CV (42.27%) for sinapic acid (Table 4). A similar phenomenon was noted by Mpofu et al. [11], who showed that environment mostly affects the content of vanilic and syringic acids.

Less common in grain were flavonoids (41.01 μg g^{-1} – 3.1% of TPC), phlobaphenes (13.10 μg g^{-1} – 1.0%) and proanthocyanidins (1.40 μg g^{-1} – 0.1%). The rest of the TPC compounds were probably constituted mainly by alkylresorcinols, which are present in various cereals in amounts from 339 to 759 μg g-1 [36]. A significantly higher content of flavonoids (201 to 677 μg g-1) in the grain of six wheat cultivars was noted by Leoncini et al. [37]. Our results are much closer

Table 3. Content of total Folin-Ciocalteu reactive compounds (TPC), phenolic acids (PA), phlobaphenes (PHLOB), flavonoids (FLAV), proanthocyanidins (PRO) and carotenoids (CAR) in wheat grain

Variant	TPC	PA	PHLOB	FLAV	PRO	CAR
	μg ferulic acid g^{-1}		μg catechin g^{-1}		μg lutein g^{-1}	
A1	1507[a*]	634[a]	15.30[a]	41.83[a]	1.59[a]	2.21[a]
A2	1383[b]	622[b]	13.67[b]	40.05[a]	1.49[a]	2.62[b]
A3	1045[c]	636[a]	10.59[c]	40.80[a]	1.23[b]	2.40[c]
A4	1292[d]	633[a]	12.45[b]	36.42[b]	1.42[ac]	2.23[a]
A5	1392[b]	621[b]	13.51[b]	45.94[c]	1.27[bc]	2.27[a]
X	1324	629	13.10	41.01	1.40	2.35
SD	173.54	6.92	1.74	3.43	0.15	0.17
CV [%]	13.11	1.13	13.28	8.36	10.71	7.23

**Means in the same column, followed by different letters are significantly different ($p \leq 0.05$).*
Abbreviations: X – mean value, SD – standard deviation, CV – coefficient of variation

Table 4. Content of phenolic acids in wheat grain (μg g^{-1})

Variant	ferulic	p-cumaric	sinapic	vanilic	p-OH benzoic	protocate-chuic
A1	567.8[a*]	34.5[a]	2.7[a]	2.8[a]	4.5[a]	11.3[a]
A2	568.9[a]	26.9[b]	8.9[b]	2.9[a]	4.0[b]	10.7[b]
A3	571.1[a]	35.7[a]	9.9[c]	2.9[a]	4.6[a]	11.4[a]
A4	568.6[a]	31.6[c]	12.8[d]	3.1[a]	4.9[c]	11.8[a]
A5	560.3[b]	34.6[a]	9.7[c]	2.4[b]	3.9[b]	9.8[c]
X	567.3	32.7	8.8	2.8	4.4	11.0
SD	4.12	3.56	3.72	0.26	0.42	0.78
CV [%]	0.73	10.89	42.27	9.29	9.55	7.09

** Means in the same column, followed by different letters are significantly different ($p \leq 0.05$).*
Abbreviations: X – mean value, SD – standard deviation, CV – coefficient of variation

to those presented by Wijaya and Mares [38], in which apigenine content (main flavonoid) in the 70 wheat cultivars was determined in range of 43-167, with a mean value of 80 μg g^{-1}. References about other phenolics (proanthocyanidins and phlobaphenes) examined in our study are scarce. These compounds are end products of the phenylpropanoid pathway and are reported to be associated with a brown/red color of wheat grain [39,40]. Usually they are detected in wheat grain as present or absent in colorimetric tests, because of their difficulty in isolation from plant material [41].

The average carotenoid content was 2.35 μg g^{-1} with variability from 2.21 to 2.62 μg g^{-1}. These values are in the range of 1.3-4.0 μg g^{-1} given for wheat grain by other studies [26,34,35]. The reported variability of these compounds was 7.23%, indicating an essential effect of the growing conditions. It expands the conclusions of Stracke et al. [13], who showed that climate has a significant impact on the carotenoid concentration in grain.

Summarizing this part of study, it can be concluded that the control sample was the most abundant, both in TPC compounds, phlobaphenes and proanthocyanidins, but at least as rich in carotenoids. In regard to the plant treatments used, phlobaphenes and proanthocyanidins were the most varied among the tested phytochemicals, with a coefficient of variation equal to 13.28% and 10.71%, respectively.

4. CONCLUSION

The methods of plant protection had a little effect on the variation of wheat kernel dimensions, although the differences between individual variants and control were significant. Protected plants were characterized by wider and thicker kernels, while the length was stable for all variants. A higher impact of treatments used was noted for kernel color, especially for its cross-section (with CV up to 8.87% for hue). It shows that utilization of grain color in cultivar classification of wheat may lead to erroneous results, especially in the case of inbred lines. The stated variation of grain color results mainly from changes of grain chemical composition. Among the studied groups of phytochemicals, TPC and phlobaphenes were the most variable, while the content of phenolic acids (PA), especially ferulic acid, was the most constant. It was found that the biological protection significantly influenced primarily phlobaphenes and carotenoids contents, while fungicides and growth stimulator – flavonoids and proanthocyanidins contents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Paliwal J, Jayas DS, Visen NS, White NDG. Quantification of variations in machine-vision-computed featuresof cereal grains. Can. Biosyst. Eng. 2005;47:7.1-7.6.
2. Du C-J, Sun D-W. Recent developments in the applications of image processing techniques for food quality evaluation. Trends Food Sci. Technol. 2004;15(5):230-49.
3. Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, et al. A genetic framework for grain size and shape variation in wheat. Plant Cell. 2010;22(4):1046-56.

4. Zheng Q, Li B, Li H, Li Z. Utilization of blue-grained character in wheat breeding derived from *Thinopyrum poticum*. J. Genet. Genom. 2009;36(9):575-580.
5. Guo Z, Xu P, Zhang Z, Guo Y. Segregation ratios of colored grains in F1 hybrid wheat. Crop Breed. Appl. Biotechnol. 2012;12(2):126-31.
6. Zapotoczny P. Discrimination of wheat grain varieties using image analysis and neural networks. Part I. Single kernel texture. J. Cereal Sci. 2011;54(1):60-68.
7. Altenbach SB, DuPont FM, Kothari KM, Chan R, Johnson EL, Lieu D. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. J. Cereal Sci. 2003;37(1):9-20.
8. DuPont FM, Altenbach SB. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. J. Cereal Sci. 2003;38(2):133-46.
9. Ribera AE, Zuniga G. Induced plant secondary metabolites for phytopatogenic fungi control: A review. J. Soil Sci. Plant Nutr. 2012;12(4):893-911.
10. Vogt T. Phenylpropanoid biosynthesis. Mol. Plant. 2010;3(1):2-20.
11. Mpofu A, Sapirstein HD, Beta T. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J. Agric. Food Chem. 2006;54(4):1265-70.
12. Shewry PR, Ward JL. Exploiting genetic variation to improve wheat composition for the prevention of chronic diseases. Food and Energy Security. 2012;1(1):47-60.
13. Stracke BA, Eitel J, Watzl B, Mäder P, Rüfer CE. Influence of the production method on phytochemical concentrations in whole wheat (*Triticum aestivum* L.): A comparative study. J. Agric. Food Chem. 2009;57(21):10116-21.
14. Bellato S, Ciccoritti R, Del Frate V, Sgrulletta D, Carbone K. Influence of genotype and environment on the content of 5-n alkylresorcinols, total phenols and on the antiradical activity of whole durum wheat grains. J. Cereal Sci. 2013;57(2): 162-69.
15. Alignan M, Roche J, Bouniols A, Cerny M, Mouloungui Z, Merah O. Effects of genotype and sowing date on phytostanol-phytosterol content and agronomic traits in wheat under organic agriculture. Food Chem. 2009;117(2):219-25.
16. Edwards SG. Influence of agricultural practices on Fusarium infection of cereals and subsequent contamination of grain by trichothecene mycotoxins. Toxicol. Lett. 2004;153(1):29-35.
17. Directive of the European parliament and of the council 2009/128/EC of 21 October 2009 establishing a framework for community action to achieve a sustainable use of pesticides.
18. Schisler DA, Khan NI, Boehm MJ. Biological control of Fusarium head blight of wheat and deoxynivalenol levels in grain via use of microbial antagonists. Adv. Exp. Med. Biol. 2002;504:53-69.
19. Lubbe A, Verpoorte R, Choi YH. Effects of fungicides on galanthamine and metabolite profiles in Narcissus bulbs. Plant Physiol. Bioch. 2012;58:116-23.
20. Ronchi A, Gandolfina F, Gozzoc F, Tonelli C. Effects of a triazolic fungicide on maize plant metabolism: Modifications of transcript abundance in resistance-related pathways. Plant Sci. 1997;130(1):51-62.
21. Javaid A, Bajwa R, Anjum T. Effect of heat-sterilization and EM (effective microorganisms) application on wheat (*Triticum aestivum* L.) grown in organic-amended sandy loam soil. Cereal Res. Commun. 2008;36(3):489-99.
22. Shah SH, Saleem MF, Shahid M. Effect of different fertilizers and effective microorganisms on growth, yield and quality of maize. Int. J. Agri. Biol. 2001;3(4):378-79.
23. Hussain T, Javaid T, Parr JF, Jilani G, Haq MA. Rice and wheat production in Pakistan with effective microorganisms. Am. J. Alternative Agr. 1999;14(01):30-36.
24. Konopka I, Kozirok W, Tańska M. Wheat endosperm hardness. Part I. Relationships to colour of kernel cross-section. Eur. Food Res. Technol. 2005;220(1):11-19.
25. Ribereau-Gayon P. Conspectus of the phenolic constituents. In: Plant Phenolics, Heywood VH. Hafner Publishing Co. New York, USA; 1972.
26. Konopka I, Tańska M, Faron A, Stępień A, Wojtkowiak K. Comparison of the phenolic compounds, carotenoids and tocochromanols content in wheat grain from organic and mineral fertilization. Molecules. 2012;17(10):12341-56.
27. Naczk M, Nichols T, Pink D, Sosulski F. Condensed tannins in canola hulls. J. Agric. Food Chem. 1994;42(10):2196-200.

28. Wilailak P, Christos G, Grotewold E. Transposon insertions in the promoter of the Zea mays a1 gene differentially affect transcription by the Myb factors P and C1. Genetics. 2002;161(2):793-801.
29. Lacko-Bartošová M, Kosík T, Kobida L. Free falvonoid and antioxidant activity of winter wheat in sustainable forming systems. J. Microb. Biotech. Food Sci. 2013;2(Special B):2099-107.
30. Craft NE, Soares JH. Relative solubility, stability, and absorptivity of lutein and beta-carotene in organic solvents. J. Agric. Food Chem. 1992;40(3):431-34.
31. Hebda T, Micek P. Zależności pomiędzy właściwościami geometrycznymi ziarna zbóż. IR 2005;6(66):233-41. (Polish).
32. Breseghello F, Sorrells ME. QTL analysis of kernel size and shape in two hexaploid wheat mapping populations. Field Crop. Res. 2007;101(2):172-79.
33. Wiwart M, Suchowilska E, Lajszner W, Graban Ł. Identification of hybrids of spelt and wheat and their parental forms using shape and color descriptors. Comput. Electron. Agric. 2012;83:68-76.
34. Konopka I, Kozirok W, Rotkiewicz D. Lipids and carotenoids of wheat grain and flour and attempt of correlating them with digital image analysis of kernel surface and cross-sections. Food Res. Int. 2004;37(5):429-38.
35. Okarter N, Liu Ch-S, Sorrells ME, Liu RH. Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. Food Chem. 2010;119(1):249-57.
36. Dykes L, Rooney LW. Phenolic compounds in cereal grains and their health benefits. Cereal Food World. 2007;52(3):105-11.
37. Leoncini E, Prata C, Malaguti M, Marotti I, Segura-Carretero A, Catizone P, et al. Phytochemical profile and nutraceutical value of old and modern common wheat cultivars. PLoS One. 2012;7(9):e45997. DOI:10.1371/journal.pone.0045997.
38. Wijaya GY, Mares DJ. Apigenin di-C-glycosides (ACG) content and composition in grains of bread wheat (*Triticum aestivum*) and related species. J. Cereal Sci. 2012;56(2):260-67.
39. McCallum AA, Walker JRL. Proanthocyanidins in wheat bran. Cereal Chem. 1990;67(3):282-85.
40. Himi E, Noda K. Red grain colour gene (R) of wheat is a Myb-type transcription factor. Euphytica. 2005;143(3):239-42.
41. Santos-Buelga C, Scalbert A. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. J. Sci. Food Agric. 2000;80(7):1094-117.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/10121