Carotenoids Variability of Potato Tubers in Relation to Genotype, Growing Location and Year

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Abstract

In this study, three cultivars of potato and seven tetraploid breeding lines with different flesh colour were cultivated for three years at four locations in Poland. The objectives of this study were to quantify the levels of total (TC) and individual carotenoids (IC) and to determine the influence of genotype, farming system, growing location, years, and environment on the contents of these components. The aim of this study was also to identify genotypes with stable level of TC and IC. The mean total carotenoids content of tubers ranged between 5.57 and 20.20 mg kg⁻¹ FW and was influenced by location, year, genotype, and their interactions. Mean values of lutein ranged from 2.92 to 6.66 mg kg⁻¹ FW and zeaxanthin from 1.44 to 3.05 mg kg⁻¹ FW. The most stable level of total carotenoids content was observed in tubers of breeding line TG-97-403. The most unstable level was found in tubers of cv. Jelly. The ranking of other genotypes depended on the used method. The presented study showed that the contents of carotenoids in potato tubers are significantly affected by environmental factors that cannot be controlled, because these are usually related to the year of cultivation.

Resumen

En este estudio, se sembraron tres variedades de papa y siete líneas de mejoramiento tetraploides con diferente color de pulpa durante tres años en cuatro localidades en Polonia. Los objetivos de este estudio fueron cuantificar los niveles totales (TC) e individuales (IC) de carotenoides, y determinar la influencia del genotipo, sistema de cultivo, localidad, años, y el ambiente, en el contenido de estos componentes. La meta de este estudio también fue la de identificar genotipos con niveles estables de TC e IC. La media del contenido total de carotenoides de los tubérculos fluctuó entre 5.57 y 20.20 mg kg⁻¹ de peso fresco y fue influenciada por la localidad, el año, el genotipo, y sus interacciones. Los valores medios de luteína variaron de 2.92 a 6.66 mg kg⁻¹ en peso fresco, y la zeaxantina de 1.44 a 3.05 mg kg⁻¹ de peso fresco. El nivel más estable del contenido de carotenoides totales se observó en tubérculos de la línea TG-97-403. El nivel más inestable se encontró en tubérculos de la var. Jelly. Las cifras de otros genotipos dependieron del método usado. El presente estudio mostró que los contenidos de carotenoides en tubérculos de papa se afectan significativamente por factores ambientales que no pueden controlarse, porque generalmente están relacionados por el año de su cultivo.

Keywords *Solanum tuberosum* · Potato · Carotenoids contents · Lutein · Zeaxanthin · Stability · Tetraploid breeding lines

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Introduction

Potato is one of the major crops in the world (<http://faostat.fao.org>), which is due to the fact that its tubers are a valuable source of energy and compounds important in human diet. Potato tubers supply mainly carbohydrates, but are also a relatively rich source of amino acids, vitamins, dietary fibre and some minerals. In the potato tubers other components can also be found, among which are the carotenoids and phenolic compounds. These compounds determine flesh colour, which may be red or blue due to the presence of anthocyanins or

yellow or orange due to carotenoids. In the human diet, phenolic compounds and carotenoids have an antioxidant activity. As pointed out by Brown (2005), potato is not considered as a food rich in antioxidants, but there are some indications that breeding work may lead to increased concentrations of these compounds. In addition, Tatarowska et al. (2014) indicated that the organic farming conditions might favor more active carotenogenesis in potato tubers. Enhanced levels of antioxidants in potato tubers could be significant in those regions of the world, where production of potatoes is growing, because of their importance in the daily diet. On the other hand, obtaining potatoes with a higher content of antioxidant compounds may be especially beneficial for organic production, because the resulting product would be even more valuable in terms of nutritional value.

The color of fresh potato flesh is determined by varying contents of blue or red anthocyanin pigments or results from the accumulation of yellow and orange carotenoids (Brown et al. 2005). The carotenoids of potato are primarily oxygenated carotenoids, also known as xanthophylls (Brown et al. 1993). White-fleshed potatoes have 5–10 mg kg⁻¹ FW of total carotenoids, while yellow-fleshed potatoes have 10–35 mg kg⁻¹ FW. Dark-yellow potatoes may contain up to 100 mg kg⁻¹ FW, while the highest recorded contents reached up to 260 mg kg⁻¹ FW (Brown et al. 1993, 2005, 2006, 2007; Brown 2005, 2008 a, b; Lu et al. 2001; Nesterenko and Sink 2003; Reyes et al. 2005; Van Eck et al. 2007). Lutein, zeaxanthin, violaxanthin and neoxanthin are the major carotenoids present in potatoes whereas β -carotene is present in trace amounts (Haynes et al. 2011). Lutein and zeaxanthin are components of the human retina (Landrum and Bone 2001), which must be obtained from the diet in order to prevent the age-related macular degeneration (AMD). Green leafy vegetables represent a good dietary source of lutein but a poor source of zeaxanthin, which is found in significant concentrations in a small number of foods. Therefore, potato may be a strategic source for lutein and zeaxanthin, especially as staple food.

The concentration of total and individual carotenoids in potato tubers is affected by several factors such as genotype, agronomic factors, postharvest storage, cooking and processing conditions (Ezekiel et al. 2013; Hamouz et al. 2016; Hejtmánková et al. 2013; Kotíková et al. 2007; Valcarcel et al. 2015).

The objectives of this study was to quantify the level of total and individual carotenoids (lutein and zeaxanthin) in various potato genotypes and to determine the influence of genotype, farming system, growing location, years and environments on the content of these components. The additional aim of this study was to distinguish genotypes with stable level of total and individual carotenoids in potato tubers growing in various environments. This was done by applying two statistical

methods, the comparison of which was another additional goal of the work.

Methods

Plant Material

For field experiments three cultivars and seven tetraploid breeding lines were used. The tested cultivars were table ones, mid-early Satina and Tajfun, and late cultivar Jelly. All three cultivars, the German cultivars Satina and Jelly and the Polish cultivar Tajfun, have yellow tuber flesh. The breeding lines were obtained in a crossing programme performed at the Plant Breeding and Acclimatization Institute – National Research Institute, Młochów Research Center. All lines are complex hybrids that include introgression from multiple sources of tuber bearing *Solanum* species. Three of the tested breeding lines were white-fleshed and four were yellow-fleshed. From among seven breeding lines used in the study, the tetraploid breeding line TG-97-403, is characterized by a contribution of *Solanum phureja* in its pedigree, which theoretically is 15.6%. *S. phureja* was the donor of resistance to *Phytophthora infestans* in TG-97-403 (Śliwka et al. 2006), and is known as the source of higher carotenoids content of tubers (Bonierbale et al. 2009).

The other six breeding lines used in this work have similarly complex species composition, with the exception of a high contribution of *S. phureja*. They were derived from crossings involving single pollen parent, which had elevated levels of total carotenoids in tubers (29 mg kg⁻¹ FW), as compared to their seed parents (up to 6.5 mg kg⁻¹ FW). The exception was the line 13-VIII-45, which seed parent have also a high TC concentration in tubers (28 mg kg⁻¹ FW). The parentage of breeding lines and TC content in parents (with exception of TG-97-403) are given in Table 1.

Field Trials

The field trials were carried out during three years (2015–2017) at four locations in central Poland at Młochów (M), Chwałowice (C) and Radzików (R), and in south-eastern Poland at Boguchwała (B). At locations C and R, the organic cultivation system was applied and in locations M and B, the integrated system was applied. The details on the geographic location, soil and fertilizer doses for each location are shown in Table 2. In the ecological locations with organic farming, approved pesticides were applied during the vegetation period, i.e. copper fungicides against late blight and pyrethrin (plant extract) or Novodor against Colorado beetle. The Polish company AGRO BIO TEST certified both organic farms. The company implemented a quality system in 2002 according to the norm EN 45011 and received accreditation from the Polish Centre of Accreditation (AC 096).

Table 1 Parentage of breeding lines used in the study and total carotenoid content in their parents (data not published)

Genotype		Total carotenoid content (mg kg ⁻¹ FW) of tubers in ^a	
		Seed parent	Pollen parent
13-VIII-10	07-VIII-264 × Nr 10	6.5	29.0
13-VIII-27	07-VIII-264 × Nr 10	6.5	
13-VIII-45	M-62887 × Nr 10	28.0	
13-VIII-49	M-62910 × Nr 10	6.2	
13-VIII-50	M-62910 × Nr 10	6.2	
13-VIII-60	M-62910 × Nr 10	6.2	
TG-97-403	DG 92–515 × CEC-69	ne	ne

^a Data not published

ne not evaluated

The experimental design was a randomized complete block. In each of two blocks (= repetitions), cultivars and breeding lines were planted in 6 hill plots. Before planting, seed tubers weight was standardized (700 g per hill plot). Tubers were planted in the end of April and harvested after 130 days. Weather conditions during the field experiments in 2014, 2015 and 2016 are shown in Fig. 1.

Total Carotenoid Content (TC)

TC was estimated for tubers obtained in all field experiments. For each potato breeding line/cultivar and each repetition in each location in each year, three tubers were collected. Tubers were cut into 8 parts. Two fragments were selected for an analysis. Next, these fragments were cut into small cubes and frozen in liquid nitrogen, lyophilized and milled. Total carotenoids were isolated according to the procedure described in PN-90/A-75101/12 standard with modifications. The procedure has been shortened and limited to extraction with hexane: TC were extracted from 3 g of freeze-dried tissue in a glass flask with 50 ml of hexane for 24 h in the dark. Total carotenoids concentrations in the solvent were evaluated

spectrophotometrically with a UV-Vis HITACHI U-1900 spectrophotometer at 450 nm. All analyses were made with three technical repetitions each.

Individual Carotenoid Content (Lutein and Zeaxanthin) (IC)

IC was estimated for tubers obtained in field experiments for each breeding line/cultivar and each repetition in each location, but only in 2014 and 2015. Carotenoids were extracted from 2 g of freeze-dried tissue in the 50 ml falcon tube with 5 ml of ethanol (for HPLC) in 24 h in the dark. Then, 2 ml of the eluent was centrifuged on the filtration column (A&A Biotechnology, Poland) at 600 RPM for 30 s.

A volume of 8 µl of filtered sample was separated on a Shimadzu Prominence HPLC (Shimadzu, Japan) using a reversed-phase Phenomenex Luna 4.6 µm 100A 150 × 4.6 mm HPLC at 50 °C column temperature. The flow rate was 1 mL min⁻¹ using mobile phase A: methanol/tetrahydrofuran/water = 45/10/45 (v/v/v) and B: tetrahydrofuran. The gradient was as follows: 0% B increasing to 50% over 25 min, equilibrated to 0% B for 5 min and held at 0% for

Table 2 Geographic, soil, fertilization and fore crops data for locations and years

Location	Geographic coordinates	Type of soil	Year	Doses of fertilizers kg ha ⁻¹
B	49° 58' 59" N 21° 56' 23" E	Cambisols	2014	120 N, 60 P ₂ O ₅ , 180 K ₂ O + 15 t composted manure + foliar fertilization Adob Cu, Adob Mn, Adob S, Basfoliar 36 Extra
			2015	120 N, 60 P ₂ O ₅ , 180 K ₂ O + foliar fertilization Basfoliar 36 Extra i Basfoliar 12–4–6 + S + amino
			2016	120 N, 60 P ₂ O ₅ , 180 K ₂ O + foliar fertilization Basfoliar 36 Extra, Basfoliar 12–4–6 + S + amino
C	51° 10' 56" N 21° 18' 17" E	Cambisols	2014	25 t/ha composted manure
			2015	
			2016	
M	52° 3' 0" N 20° 46' 7" E	Podzols	2014	90 N, 110 P ₂ O ₅ , 180 K ₂ O
			2015	90 N, 110 P ₂ O ₅ , 180 K ₂ O
			2016	90 N, 110 P ₂ O ₅ , 180 K ₂ O
R	52°13'38"N 20°36'55 "E	Phaeozem	2014	25 t/ha composted manure
			2015	
			2016	

Fig. 1 Precipitation (total monthly in mm) and temperature (monthly average in °C) during the 2014, 2015 and 2016 potato growing season in Boguchwała (B), Chwałowice (C), Młochów (M) and Radzików (R)

5 min. The UV/Vis absorbance detector collected data at 452 nm. Lutein and zeaxanthin quantifications were performed by using a standard curve ranging from 0.5 to 4 $\mu\text{g ml}^{-1}$. The standard curve was prepared for lutein and zeaxanthin standards (LGC Standards, UK). Quantification was performed using LC solution software (Shimadzu, Japan). All analyses were made with three technical repetitions each.

Flesh Colour Measurement

From each potato breeding line/cultivar, five tubers in two replications were evaluated. Before evaluation, tubers were removed from cold storage and allowed to warm overnight to reduce condensation. Tubers were sliced in half from the apical to the distal end, blotted dried, and evaluated for yellow-flesh intensity using a Colorimeter. For each potato clone/cultivar 60 measurements were taken. Intensity of potato flesh coloration was assessed using the Minolta CR-400 Colorimeter (Osaka, Japan)). The yellowness index was calculated according to the formula:

$$YI_{E313} = \frac{100(C_x X - C_z Z)}{Y}$$

where C_x and C_z are illuminant- and observer- specific constants; X , Y , Z - trichromatic values (ASTM 2005).

Statistical Analysis

Firstly, for TC, IC and yellowness index the three-way ANOVA was performed to reveal significance of fixed effects

of genotype (G), location (L) and year of experiment (Y) and their interactions. The design was fully balanced and genotypes were nested in combinations of years and locations. The TC values did not fulfill the assumptions of homogeneity of variances and were log transformed before ANOVA. In addition, in tables, the re-transformed mean values of TC content are shown. In the case of lutein, some measurements of its content were below the limit of quantification (LOQ). Discarding such observations from data analysis may lead to biased estimates, incorrect standard errors and incorrect inferences. To avoid such problems, the procedure of multiple imputation was applied before ANOVA.

The stability analyses for TC and lutein content were performed with two methods. The phenotypic stability of the quantitative trait can be explored by the use of many of statistical methods allowing the analysis of genotype by environment interaction. However, these methods may produce different results (Fasahat et al. 2015). The first one was AMMI (additive main effects and multiplicative interaction) analysis (Gauch 2006), which is widely used in stability analyses. This is a two-step method. The first step is the procedure of fitting genotypes and environments effects by usual ANOVA. In the second step, principal components analysis (PCA) fits multiplicative effects for the genotype by environment ($G \times E$) interaction. The PCA section of AMMI requires that $G \times E$ effects are arranged on a two-way matrix. In this publication such an arrangement was achieved by defining environments as combination of location and year ($E = Y \times L$).

The AMMI extracts new variables (PCs). Those of the PCs that explain the largest part of the interaction induced variability are graphically represented (these are usually the first two PCs) and are used for constructing a biplot. The AMMI model

does not provide quantitative measures useful for ranking genotypes, but this can be done by using the AMMI stability value (ASV) (Purchase et al. 2000). The ASV is the distance between a point corresponding to a particular genotype and the origin of the biplot using the first two PCs.

The other measure of stability of the TC content was based on the Scheffé-Caliński model, in which genotype means are fixed, whereas environmental effects are random (mixed model) (Mađry and Kang 2005). In this procedure, the ANOVA is the first step. Then, the evaluation of significance of individual genotypes contribution into interactions effects is performed. The interactions include those between genotype (G) and year (Y), genotype and location (L) and the highest order interaction between G, Y and L ($G \times Y \times L = G \times E$). For each genotype, the size of these interaction effects are measured by appropriate F statistic. The stable genotype has a low contribution to overall $G \times E$ interaction (the $G \times E$ effect for a specific genotype is statistically insignificant or, what is equivalent, the respective value of $F_{G \times E}$ statistics is accordingly low). The further steps are joint regression analysis, which determines to what extent the regression explains $G \times E$ and canonical variables analysis, which is essentially similar to principle component analysis (including graphical output in the form of biplots), which are not presented.

The ANOVA and AMMI were done with the R program (De Mendiburu 2015; R Core Team 2015). The analysis based on the Scheffé-Caliński model was generated in the program Sergen 3 (Caliński et al. 1998). In the case of lutein, the procedure of the multiple imputations was performed by R package Hmisc (Harrel et al. 2016). For graphs, ggplot package of R was used (Wickham 2009).

Results

Tuber Flesh Colour

The yellowness index of the evaluated white-flashed potato breeding lines ranged from 37.1 to 42.7 and in yellow-flashed lines/cultivars ranged from 55.0 to 62.5 (Table 3). The positive correlation between yellowness index and TC was very high ($r = 0.93^{**}$), and between yellowness index and lutein content was lower and moderate ($r = 0.60^{**}$). The three breeding lines with white tuber flesh formed a distinct group with the yellowness indices significantly different from those found for yellow-flashed potatoes. However, the yellow-flashed group of lines/cultivars was not uniform in respect of this index (Table 3).

Total and Individual Carotenoids Contents of Potato Clones and Cultivars

Ten potato cultivars/breeding lines were analysed for their total and individual (lutein and zeaxanthin) carotenoid content. For the

Table 3 Yellowness index of ten genotypes grown in four locations in years 2014–2016

Genotype	Mean	±	sd		Tuber flesh colour
13-VIII-10	62.5	±	1.48	a	yellow
TG-97-403	60.3	±	1.98	ab	yellow
Satina	58.9	±	2.73	b	yellow
Jelly	58.0	±	2.95	bc	yellow
13-VIII-49	55.8	±	1.97	cd	yellow
13-VIII-60	55.5	±	2.63	cd	yellow
Tajfun	55.0	±	3.16	d	yellow
13-VIII-45	42.7	±	3.10	e	white
13-VIII-50	39.5	±	1.66	f	white
13-VIII-27	37.1	±	2.37	f	white

^a The higher value of YI the more yellow is tuber flesh. Mean values with the same letter are not different at $p < 0.05$

TC content, the three-way ANOVA revealed highly significant effects for location, year, genotype and their interactions (results not shown).

Mean values of TC for genotypes, locations and environments are presented in Table 4. The mean TC content of tubers ranged from $5.57 \text{ mg kg}^{-1} \text{ FW}$ (in 13-VIII-27) to $20.20 \text{ mg kg}^{-1} \text{ FW}$ (in 13-VIII-10). The higher levels of TC concentration were found for yellow-fleshed cultivars with an average mean value ranging from $9.23 \text{ mg kg}^{-1} \text{ FW}$ for cv. Tajfun to $20.20 \text{ mg kg}^{-1} \text{ FW}$ for breeding line 13-VIII-10. The TC level in white-fleshed potatoes varied from $5.57 \text{ mg kg}^{-1} \text{ FW}$ to $8.68 \text{ mg kg}^{-1} \text{ FW}$. The homogeneous groups of genotypes distinguished by the Tukey test are almost identical to those of the yellowness index.

The highest concentration of TC was found in tubers harvested in location B with integrated cultivation system ($14.05 \text{ mg kg}^{-1} \text{ FW}$) and in location C with organic farming ($13.33 \text{ mg kg}^{-1} \text{ FW}$), and the lowest concentration was in location M with integrated cultivation system ($8.74 \text{ mg kg}^{-1} \text{ FW}$). When environments (combinations of location and year) are considered, the concentrations of TC were the lowest in integrated system M_14 and organic R_14, and the highest in integrated system B_15 and B_16 (Table 4).

As for lutein, the three-way ANOVA showed highly significant or significant effects of genotype, location and all interactions. The exception was the insignificant effect of year (results not shown in tables). The highest levels of lutein were noted for yellow-fleshed breeding line 13-VIII-10 ($6.66 \text{ mg kg}^{-1} \text{ FW}$) and the lowest level for white-fleshed 13-VIII-27 ($2.92 \text{ mg kg}^{-1} \text{ FW}$). However, the group of seven genotypes with the lowest lutein content of tubers (Table 5) contains individuals with a high yellowness index (yellow fleshed 'Jelly') and with low values of this index (white fleshed 13-VIII-50).

The highest content of lutein was found in tubers harvested in integrated system location B ($5.42 \text{ mg kg}^{-1} \text{ FW}$) and

Table 4 Mean values of TC content (mg kg⁻¹ FW) of potato tubers from 10 genotypes grown in Boguchwała (B), Chwałowice (C), Młochów (M) and Radzików (R) in 2014, 2015 and 2016

Genotype	Integrated system						Organic system						Mean
	B_14	B_15	B_16	M_14	M_15	M_16	C_14	C_15	C_16	R_14	R_15	R_16	
13-VIII-10	23.12	25.23	25.42	13.64	16.21	18.83	27.74	23.35	18.85	16.98	23.71	15.19	20.20 a
Satina	23.81	20.07	22.26	8.49	11.53	13.25	28.38	12.17	31.51	12.98	17.54	29.37	17.79 ab
TG-97-403	12.31	25.93	22.97	5.97	19.31	19.38	13.92	20.61	23.28	10.20	22.16	22.70	16.95 ab
13-VIII-49	18.63	17.36	27.44	6.04	16.97	23.51	11.90	20.36	12.71	10.03	14.67	10.72	14.72 bc
Jelly	8.27	24.21	33.53	2.42	8.64	11.69	25.14	9.33	40.02	1.92	9.08	31.75	11.96 cd
13-VIII-60	6.91	16.23	18.66	2.61	13.36	21.04	5.55	15.92	27.24	6.08	16.67	17.09	11.67 cd
Tajfun	5.13	12.66	14.93	3.18	6.85	26.43	4.08	6.96	22.97	5.78	6.92	26.23	9.23 de
13-VIII-50	5.40	17.32	10.57	4.34	10.72	10.73	6.22	14.42	8.28	3.88	13.12	9.83	8.68 e
13-VIII-45	4.58	15.80	8.94	2.10	14.85	8.11	6.26	12.25	9.11	3.94	19.83	7.41	7.97 e
13-VIII-27	4.14	8.45	8.63	2.15	4.12	5.30	4.60	11.82	4.87	4.28	8.40	6.62	5.57 f
Environment mean	9.07d	17.45a	17.52a	4.18f	11.23bcd	14.23ab	10.29 cd	13.84abc	16.63a	6.31e	14.10abc	15.39ab	
Location mean	14.05			8.74			13.33			11.10			

The mean values followed by the same letter do not differ significantly (according to Tukey test)

organic location C (4.27 mg kg⁻¹ FW) and the lowest – in organic R and integrated M (3.69 and 3.61 mg kg⁻¹ FW) respectively. The lutein content in environments ranged from 3.15 mg kg⁻¹ FW in R_15 to 5.78 mg kg⁻¹ FW in B_15 (Table 4). Lutein accounted on average for approximately 35% of the TC content and the concentrations of lutein and TC correlated highly ($r=0.87^{**}$ for years 2014 and 2015). However, in some clones proportion of lutein in TC was 46% and 55% as in case of white fleshed lines 13-VIII-27 and 13-VIII-50.

Due to a very large number of measurements below the LOQ, ANOVA for zeaxanthin content was not performed. Zeaxanthin content in potato genotypes ranged from 1.44 mg kg⁻¹ FW (in 13-VIII-60 in C_14) to 3.05 mg kg⁻¹ FW (in 13-VIII-50 in C_14) (Table 6). In many environments the zeaxanthin concentration was below the limit of quantification. Zeaxanthin content was low, but highly positively correlated with TC content ($r=0.92^{**}$, taking into account the years 2014 and 2015 as well as values above the limit of quantification). Zeaxanthin did not contribute significantly to

Table 5 Mean values of lutein content (mg kg⁻¹ FW) of potato tubers from 10 genotypes grown in Boguchwała (B), Chwałowice (C), Młochów (M) and Radzików (R) in 2014 and 2015

Genotype	Integrated system				Organic system				Mean ¹
	B_14	B_15	M_14	M_15	C_14	C_15	R_14	R_15	
13-VIII-10	10.13	5.32	3.66	5.32	8.54	6.35	10.41	3.56	6.66 a
Satina	8.19	7.75	3.16	3.91	10.66	3.81	6.04	4.66	6.02 ab
TG-97-403	5.41	11.13	2.75	5.76	4.54	5.41	3.44	3.88	5.39 b
13-VIII-50	3.16	10.03	3.82	3.41	3.13	2.60	2.60	2.91	3.81 c
13-VIII-49	4.41	3.82	2.75	3.44	3.82	3.91	4.10	2.60	3.59 c
Tajfun	loq ²	4.75	loq ²	3.22	2.47	2.66	loq ²	2.50	3.46 c
Jelly	2.38	4.82	2.91	3.16	5.50	2.53	2.81	2.66	3.25 c
13-VIII-60	3.69	3.63	loq ²	2.91	2.66	3.19	2.69	3.28	3.13 c
13-VIII-27	3.19	3.22	loq ²	2.56	2.94	2.85	3.78	3.38	2.94 c
13-VIII-45	2.60	3.38	loq ²	2.88	2.72	3.63	3.10	2.81	2.93 c
Mean ¹	5.05ab	5.78a	2.91d	3.65 cd	4.84b	3.69 cd	3.89c	3.15 cd	
Location mean	5.42		3.27		4.27		3.52		

¹ Due to presence values below the limit of quantification, the procedure of multiple imputation preceded further statistical analyses (ANOVA as well as calculation of mean values). The mean values followed by the same letter do not differ significantly (according to Tukey test)

² The values below the limit of quantification

Table 6 Mean values of zeaxanthin content (mg kg⁻¹ FW) of potato tubers from 10 genotypes grown in Boguchwała (B), Chwałowice (C), Młochów (M) and Radzików (R) in 2014 and 2015

Genotype	Integrated system				Organic system			
	B_14	B_15	M_14	M_15	C_14	C_15	R_14	R_15
TG-97-403	loq	1.84	loq	1.95	loq	1.80	loq	loq
13-VIII-50	2.96	loq	loq	loq	3.05	loq	2.02	loq
13-VIII-27	loq	loq	loq	loq	loq	loq	loq	loq
13-VIII-49	loq	loq	loq	loq	loq	1.98	loq	loq
13-VIII-45	1.72	loq	loq	loq	1.88	loq	2.06	loq
13-VIII-60	loq	loq	loq	loq	1.44	loq	loq	loq
Satina	2.38	1.98	loq	loq	2.48	loq	2.44	loq
13-VIII-10	1.75	1.73	loq	1.88	1.75	2.03	2.03	1.74
Tajfun	loq	1.96	loq	loq	loq	loq	loq	loq
Jelly	2.38	1.99	loq	loq	2.14	loq	2.23	loq

loq – values below the limit of quantification

the total carotenoid content in the tested genotypes. The lowest contribution was in tubers of Tajfun, Jelly and white fleshed clones (from 3.4 to 5.5%), and the highest in tubers of TG-97-403 (9%) and 13-VIII-10 (11.5%).

Stability of TC and Lutein Content

The stability of TC content was evaluated by using AMMI and the mixed model of Schaffe-Caliński. The first step of AMMI was ANOVA, based on the model with three sources of variation, G, E (combination of locations and year) and the interaction G × E, which accounted for 29%, 35% and 27% of total sums of squares, respectively (Table 7). The final output of AMMI is graphically represented in the form of the biplot

(Fig. 2), which have been completed by providing the quantitative measure of ASV. In the case of tested genotypes, their distance from the biplot origin was between two extremes: the highest was found for Jelly and the lowest for TG-97-403 (the highest and smallest ASV, respectively). This distance for Satina, Tajfun and 13-VIII-10 is much higher than for the rest of the genotypes, but no other genotype was as close to the origin as TG-97-403 (Fig. 2). The ASV and ranks of individual genotypes are given in Table 8.

In the biplot, the environments are presented as vectors, each corresponding to the combination of location and year (Fig. 2). The vectors have usually the same direction and angles between them are acute (indicating correlation), if those vectors represents the same year. It is clearly visible for environments from 2015 and 2016, in which outliers were B_15 and M_16. In the case of 2014, the environments formed the less related group and the most atypical environment was C_14. On the contrary, vectors for the same location and different years have obtuse angles or right angles with exception of B_16 and B_15, M_15 and M_16 or R_14 and R_15 (Fig. 2).

The second stability parameter was the value of the $F_{G \times E}$ statistic obtained from analysis of the Scheffé-Caliński mixed model. The initial step was ANOVA, in which the total sum of squares (SS) was partitioned differently as compared to AMMI (Table 7). From among environmental factors, Y accounted for 24% of total sum of squares, while contributions of L and E (= Y × L) were much lower (Table 6). As for interactions between G and Y or L, the dominant contribution in total SS had the interaction G × Y.

In the second step, it was found that for one genotype, Jelly, its contribution in G × E was significant (significant value of $F_{G \times E}$) (Table 8). This effect of Jelly was not explained by the regression. As for other interactions, only for the breeding

Table 7 Significance of variance parameters for total carotenoids (TC) and lutein for 10 genotypes grown in 4 locations in 3 years

Source of variation	ANOVA for Scheffe-Caliński model				ANOVA in AMMI analysis			
	TC		Lutein		TC		Lutein	
	Significance	% of SS	Significance	% of SS	Significance	% of SS	Significance	% of SS
Genotype (G)	*	30%	***	30%	**	29%	***	35%
Location (L)	ns	7%	***	11%	ni		ni	
Year (Y)	***	24%	ns	0%	ni		ni	
G × L	**	5%	***	13%	ni		ni	
G × Y	***	15%	***	11%	ni		ni	
Environment E(= Y × L)	**	3%	**	3%	**	35%	***	18%
G × E	**	8%	***	14%	**	27%	***	39%
error	na	8%	na	18%	na	8%	na	8%

*, **, *** - significant at $P = 0.05$ or 0.01 or 0.001 , respectively; ns not significant, ni the source of variation not included in the model, na not applicable SS sum of squares. Both ANOVA models differently partitions the variation. SS for E in AMMI analysis approximately corresponds to the sum for L, Y and E in mixed model ANOVA;

SS for G × E in AMMI corresponds to the sum for interactions G × L, G × Y and G × E in mixed model ANOVA

Fig. 2 Biplot of 10 potato genotypes and 12 environments (combinations of location and year) for TC concentration

lines 13-VIII-10 and 13-VIII-45 the significant contribution in the on $G \times Y$ was found.

The values of $F_{G \times E}$ and ASV were highly correlated (Spearman's rank correlation coefficient was $r_s = 0.70^*$).

The analysis AMMI was also conducted for lutein concentration (Table 7, Fig. 3 and Table 9). ANOVA revealed that genotypes, environment and their interactions accounted for 35%, 18% and 39% of total SS, respectively (Table 7). Compared to the TC analysis, these contributions were much smaller for the environment and much larger in the case of the interaction $G \times E$.

Table 8 ASV and $F_{G \times E}$ for TC (mg kg^{-1} FW) in potato tubers evaluated in 12 environments

Genotype	Mean TC content	ASV ^a		$F_{G \times E}$ ^b		Sum of ranks
		value	rank	value	rank	
TG-97-403	16.95	0.13	1	0.07	1	2
13-VIII-50	8.68	0.47	2	0.61	3	5
13-VIII-45	7.97	0.61	5	0.64	4	9
13-VIII-27	5.57	0.53	3	1.92	7	10
13-VIII-10	20.20	0.91	8	0.49	2	10
13-VIII-60	11.67	0.70	6	1.22	5	11
13-VIII-49	14.72	0.57	4	2.28	9	13
Satina	17.79	0.81	7	1.25	6	13
Tajfun	9.23	0.93	9	2.09	8	17
Jelly	11.96	1.59	10	7.22*	10	20

Significant: * at $P < 0.05$

^a ASV AMMI stability value

^b $F_{G \times E}$ – statistic F for interaction $G \times E$ (mixed model of Scheffe-Caliński)

After PCA, the genotypes showing the highest contribution in variability caused by $G \times E$ were found and these were located at larger distances from the centre of the biplot. These genotypes are 13-VIII-10, 13-VIII-50, TG-97-403, and Satina. The smallest contribution was found for Jelly and Tajfun (Fig. 3 and Table 9). The environments may be grouped according to year with exception of environments B_15 and C_14 (Fig. 3).

The second stability parameter was obtained by applying the Scheffe-Caliński model. ANOVA revealed that the main effects G, L and Y accounted for 30%, 11% and 0% of total SS, respectively. As for interactions involving G ($G \times L$, $G \times Y$ and $G \times E$), they accounted for 11% - 14% of total SS) (Table 7).

The last step was to assess the impact of individual genotypes on interactions. Only for three genotypes (13-VIII-10, 13-VIII-50 and Satina), the highly significant values of $F_{G \times E}$ were found, which indicated their significant contribution to the observed $G \times E$ (Table 9). None of the genotypes had a significant contribution to the interactions $G \times Y$ and $G \times L$.

The correlation between both stability parameters $F_{G \times E}$ and ASV was different from that obtained for TC and was only moderate ($r_s = 0.47$).

Discussion

Concentration of Carotenoids

The obvious statement is that the improvement of potato nutritional value of potato may be achieved by breeding methods. The prerequisite is the presence of variability in the trait of interest in the available breeding pool of potato. In the case of carotenoids such variability was found in various genetics pools (Andre et al. 2007; Brown et al. 1993;

Fig. 3 Biplot for 10 potato genotypes grown at 8 different environments for lutein concentration

Breithaupt and Bamedi 2002; Burlingame et al. 2009; Haynes et al. 2010; Iwanzik et al. 1983; Lu et al. 2001).

In the presented study, the six of evaluated breeding lines are derived from crosses involving pollen parent with increased TC concentration in tubers (in the case of line 13-VIII-45 both parents have increased level of TC). These lines had significantly increased concentrations of TC (up to 20.2 mg kg⁻¹ FW) as compared with the parent with the low TC (up to 6.5 mg kg⁻¹ FW), but only when the flesh of their tubers was yellow. The close relation between yellowness of tuber flesh and carotenoids level was found by many authors (Brown et al. 1993; Brown 2005; Brown et al. 2005, 2006, 2007, 2008; Nesterenko and Sink 2003; Van Eck et al. 2007) as well as in this study, as indicated by a very high correlation coefficient between TC and yellowness index.

It can be concluded that breeder may expect increases of TC concentration in tubers of progeny clones if at least one of their parents is characterised by an increased level of TC and individuals with yellow flesh are selected.

One of the tested clones TG-97-403 was derived from a different breeding program and had a significant contribution of *S. phureja*, which is a potential source of high concentration of carotenoids. Moreover, the tuber flesh of TG-97-403 was as yellow as tuber flesh of 13-VIII-10, which was outstanding in both YI value and TC concentration. Despite the fact that TG-97-403 has clearly yellow tuber flesh and *S. phureja* in its pedigree, the concentrations of carotenoids were lowered as compared to what could be found in *S. phureja* and the respective concentrations of TC, lutein and zeaxanthin were 16.95 mg kg⁻¹ FW, 5.39 mg kg⁻¹ FW and 1.55 mg kg⁻¹

Table 9 ASV and $F_{G \times E}$ for lutein concentration (mg kg⁻¹ FW) in potato tubers evaluated in 8 environments

Genotype	Mean lutein content	ASV ^a		$F_{G \times E}$ ^b		Sum of ranks
		value	rank	value	rank	
13-VIII-27	2.94	2.31	3	0.56	1	4
Tajfun	3.46	0.56	1	2.47	6	7
13-VIII-49	3.59	2.56	4	1.76	3	7
13-VIII-45	2.93	3.09	6	1.46	2	8
Jelly	3.25	0.78	2	3.04	7	9
13-VIII-60	3.13	2.85	5	2.32	5	10
TG-97-403	5.39	6.63	8	1.92	4	12
Satina	6.02	5.87	7	8.61**	8	15
13-VIII-50	3.81	7.24	9	10.13**	9	18
13-VIII-10	6.66	8.69	10	15.15**	10	20

Significant: * at $P < 0.05$ and ** at $P < 0.01$

^{a, b} see the footnote of Table 7

FW. The various clones or cultivars of *S. phureja* were quite extensively tested for carotenoids content (Bonierbale et al. 2009; Brown et al. 1993; Griffiths et al. 2007; Lu et al. 2001; Morris et al. 2004) and in some cases the levels total and individual carotenoids were very high. Bonierbale et al. (2009) identified *S. phureja* clones, in which the TC content exceeded 200 mg kg⁻¹ FW, zeaxanthin content exceeded 100 mg kg⁻¹ FW and lutein content reached 19 mg kg⁻¹ FW.

The number of individual carotenoids evaluated during the study was limited. However, lutein seems to be one of the major carotenoids in the examined tubers (on average 35% of TC) or may be even one of the dominant components of TC in white fleshed tubers (up to 55% of TC). A higher proportion of lutein in white fleshed potatoes was observed by Haynes et al. (2010). This was also found in cultivars in which tuber flesh color is determined by anthocyanin pigments (Hamouz et al. 2016; Hejtmánková et al. 2013). Brown et al. (2008) found the lutein as a main carotenoid in potatoes. However, the prevalence of lutein in TC content was also found in yellow fleshed tubers of *S. phureja* cultivars (Burmeister et al. 2011; Hejtmánková et al. 2013).

The second carotenoid was zeaxanthin, which variation cannot be evaluated; its concentration was low and very frequently below LOQ, which was observed mainly in white-fleshed breeding lines and in tubers grown in the conventional location M in 2014 (the lowest average TC content was recorded), but also in environments with not so low average TC content as in tubers from organic location R in 2015. The proportion of zeaxanthin in tested tubers was estimated to be rather low and the highest was found in breeding lines 13-VIII-10 and TG-97-403, both having the highest values of YI. The latter line has *S. phureja* in origin and in this species high concentrations of zeaxanthin were found (Brown et al. 1993; Breithaupt and Bamedi 2002; Bonierbale et al. 2009).

Influence of Environmental Effects

Purchasers of organic food assume that organic farming practice positively affects its nutritional properties. However, the findings of studies comparing concentration of specific nutrients between conventionally and organically grown foods usually are not conclusive (e.g. Bourn and Prescott 2002). There are two questions about nutritional value of organic food. The first is, whether the level of compounds important for nutritional value may be increased in organic conditions, which is crucial for proponents of organic farming. The second question is, whether the nutritional value of cultivars remains unchanged in a new cultivation system, taking into account that new potato cultivars are selected on the basis of field trials, which are still under traditional cultivation system.

The relationship between farming system and concentration of the health-promoting food ingredients is not well recognized. The available experimental evidence does not provide

unequivocal conclusions about the effects of organic farming on concentrations of such ingredients. The increase of carotenoids content due to applying organic cultivation was not observed (Murniece et al. 2012 in potato; e.g. Søltoft et al. 2011 in carrot) or, on the contrary, a decrease was found, as in the case of red pepper (Daoud et al. 2006) or potato (Wierzbicka and Hallmann 2013). In the presented study, the effect of cultivation system cannot be directly compared, because the different systems were not applied in the same locations. However, the observed variability of TC content did not show a clear pattern associated with the cultivation system, and in particular it was not shown that TC content increased under organic conditions. The highest TC content was observed in conventional location B in 2015 and 2016. In turn, in 2014, the TC concentration was lowered in all locations as compared to 2015 and 2016, and the lowest concentrations were found in tubers from both conventional and organic locations, M and R respectively.

With regard to lutein, the fluctuations of its content depended rather on location than year, which was confirmed by the ANOVA results (ANOVA according to the mixed model).

The effects of environmental factors on the accumulation of carotenoids in potato tubers were rarely evaluated. Usually, authors of these studies found that carotenoids concentration depend on genotype and various environmental factors (Haynes et al. 2010; Hejtmánková et al. 2013; Hamouz et al. 2016; Kotíková et al. 2007; Othman 2009; Payyavula et al. 2012; Reddivari et al. 2007; Tatarowska et al. 2014), but sometimes an influence of environment was not found (Brown et al. 2008). In the presented study, the environmental effects were slightly higher (in the case of TC) or clearly lowered (in the case of lutein) as compared to the effect of genotype. The mixed model of ANOVA enabled a more thorough analysis of environmental effects as compared to ANOVA in AMMI, since the impacts of years and locations were evaluated separately. In the case of TC, the predominant was the effect of year. With respect to lutein, the effect of location was most important, while the effect of year was insignificant.

The final step was the analysis of the $G \times E$ interaction by applying two methods, what lead to identifying genotypes with a stable expression of the tested traits. In the case of carotenoids, such analyses of $G \times E$ interaction were rarely carried out (Haynes et al. 2010; Tatarowska et al. 2014). A stable genotype is regarded a genotype with an insignificant contribution to this interaction. However, in each of the applied methods $G \times E$ is analyzed differently. In the case of the Scheffé-Caliński mixed model, the contribution of each genotype in the highest order interaction $G \times Y \times L$ was measured by F statistics, which are stability parameters. The lower order interactions involving G (i.e. $G \times L$ and $G \times Y$) were tested separately, and for most genotypes in presented study were insignificant for both TC (with two exceptions) and lutein. In the case of AMMI, the inference about stability is possible after PCA applied for the effects of $G \times E$.

Despite these differences, in the case of TC both methods turned out to give a fairly consistent ranking of genotypes in terms of stability. The most stable level of TC in various environments was expressed by the breeding line TG-97-403. This line is characterized by a significant proportion of *S. phureja* in its origin, in contrast to all other tested breeding lines and cultivars. The most unstable level of TC was found in tubers of Jelly, for which both indicators consistently pointed to instability.

In the case of stability of lutein concentration, both methods indicated almost the same most unstable genotypes. The only exception was TG-97-403, which was unstable according to AMMI and stable according to the mixed model. The ranking of other genotypes depends on the method. The variability of the ranking of genotypes depending on the applied method of stability assessment is often found (Adugna and Labuschagne 2003; Francis and Kannenberg 1978). The best solution is to use few methods and select the most stable genotypes on the basis of the sum of ranks (Ahmadi et al. 2015).

However, the most outstanding result obtained for the lutein, is that the ranking of genotypes is completely different from that obtained for TC concentration. The example of this is 'Jelly', which was the most unstable genotype as regards TC concentration and was among the most stable in the lutein content.

Conclusion

The presented study proved that the total content of carotenoids in potato tubers is significantly affected by environmental factors that cannot be controlled, because these are usually related to the year of cultivation. However, the level of lutein, carotenoid especially important for human health, was affected mainly by location. The direct evidence of the impact of the cultivation system is not provided, however, there are indications that the effects of the year and location play the most important role in controlling the accumulation of carotenoids in tubers. Despite the influence of these usually uncontrolled factors, it was possible to identify genotypes with an increased and stable level of TC or lutein in tubers. Although TC and lutein content were highly correlated, the analysis of stability indicated that genotypes stable in TC and in lutein content were not the same. The plant factors controlling the stable levels of various carotenoids may respond in different ways depending on type of carotenoids and environmental factors.

In terms of stable expression and content of total carotenoids, the outstanding was the breeding line, which originates from South American germplasm. This indicate how important is broadening of genetic base for improvement of nutrition value of potato.

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