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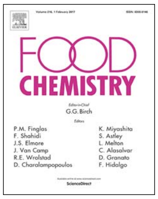

Tocotrienols and tocopherols in colored-grain wheat, tritordeum and barley

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ABSTRACT

Colored-grain spring and winter wheat, spring tritordeum and barley (blue aleurone, purple pericarp, and yellow endosperm) from the harvests 2014 and 2015 were evaluated for tocol contents by HPLC-FD. Higher content of total tocols was found in spring wheat varieties compared with winter varieties. Four tocols (β-tocotrienol, α-tocotrienol, β-tocopherol, and α-tocopherol) were identified in wheat and tritordeum varieties. Dominant tocols in purple- and blue-grained wheat and yellow-grained tritordeum were α-tocopherol and β-tocotrienol, whereas spring barley varieties differed from wheat and tritordeum by high α-tocotrienol content. Tocol content was significantly affected by genotype and in a lesser extent in some varieties and lines also by rainfall and temperatures during crop year. Higher rainfall and lower temperatures caused in most varieties higher tocol contents. Purple- and blue-grained wheat lines with higher tocol, anthocyanin and phenolic acids with health benefits may be useful for breeding new varieties.

1. Introduction

Tocols (vitamin E) comprise a chromanol ring with a C16 phytol side chain and are classified into two types in which the side chain is either saturated (tocopherols) or contains three double bonds at carbons 3, 7 and 11 (tocotrienols). They exist in eight forms (α-, β-, γ- and δ- both tocopherols and tocotrienols), which differ in positions of methyl groups on the chromanol ring (Shewry & Ward, 2012). Tocols (especially unsaturated tocotrienols) have antioxidant properties which may be responsible for their health benefits.

In cereals, the major lipophilic secondary metabolites with antioxidant properties include tocols and carotenoids (Atanasova-Penichon, Barreau, & Richard-Forget, 2016). Tocol composition of cereals includes tocopherols (α-, β-, δ- and γ-tocopherol) and tocotrienols (α-, β-, δ- and γ-tocotrienol) (Panfili, Fratianni, & Irano, 2003). The α-forms are predominant (Gutierrez-Gonzalez, Wise, & Garvin, 2013). Tocopherols are mainly present in the germ fraction while tocotrienols are present in the pericarp and endosperm fractions (Falk, Krahnstöver, van der Kooij, Schlensog, & Krupinska, 2004). In small-grained cereals such as oat, barley and wheat, tocotrienols are the main tocols and their concentrations range between 40 and 60 mg/kg DW, depending on the cereal type and the variety (Falk et al., 2004).

Wheat and barley are a good source of tocopherols and tocotrienols, which are known to reduce serum LDL cholesterol through their antioxidant action (Baik & Ullrich, 2008). Soft wheat was found to contain high total tocol level, similarly to barley (≅75 mg/kg DW) (Moore et al., 2005). β-Tocotrienol was the main vitamer found in hulled and dehulled wheats (33–43 mg/kg DW). High levels of tocols are characteristic for einkorn and emmer wheat (Lachman, Hejtmánková, & Kotíková, 2013; Hejtmánková, Lachman, Hejtmánková, Pivec, & Janovská, 2010). The most abundant tocol in einkorn wheat was β-tocotrienol (48.22 mg/kg DW), followed by α-tocotrienol (12.77 mg/kg DW), α-tocopherol (12.18 mg/kg DW), and β-tocopherol (4.79 mg/kg DW). Mean tocotrienol/tocopherol ratio in einkorn was estimated at 3.68 (Tsao, 2008). In small-grained cereals, e.g. bread wheat ZP Zemunska rosa or ZP Zlatna higher concentrations of total tocopherols (13.85 mg/kg DW and 32.65 mg/kg DW, respectively) were found when compared with other small-grained durum wheat varieties and breeding lines with different α-

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Abbreviations: α-T3, -tocotrienol; β-T3, -tocotrienol; γ-T3, γ-tocotrienol; δ-T3, δ-tocotrienol; α-T, α-tocopherol; β-T, β-tocopherol; γ-T, γ-tocopherol; δ-T, δ-tocopherol; CR, Czech Republic; DW, dry weight; HPLC-FD, high performance liquid chromatography with fluorescence detection; LOD, limit of detection

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tocopherol and (β + γ)-tocopherol contents (Žilić, Hadži-Tašković Šukalović, Dodig, Maksimović, Maksimović, & Basić, 2011). Within evaluation of fifteen diploid, tetraploid and hexaploid accessions belonging to different *Triticum* species, focused on chemical composition of wild and feral diploid wheats, the highest total tocol content was found in *T. thaoudar, T. aegilopoides, T. monococcum* and *T. urartu* (75.1 ± 3.95, 70.8 ± 3.35, 66.8 ± 3.82 and 63.9 ± 2.91 mg/kg DW, respectively) (Brandolini, Hidalgo, Gabriele, & Heun, 2015; Hidalgo, Brandolini, Pompei, & Piscozzi, 2006). The highest contents were characteristic for β-tocotrienol (in diploid wheats in range 37.90 ± 3.22–45.90 ± 2.31 mg/kg DW) followed by α-tocotrienol (9.1 ± 0.24–17.5 ± 0.85 mg/kg DW) and α-tocopherol (9.9 ± 0.01–12.6 ± 0.12 mg/kg DW). The lowest contents were reported for β-tocopherol (2.9 ± 0.04–5.5 ± 0.70 mg/kg DW). For durum, bread wheat and triticale, with a total tocol content of 33.75, 36.85 and 33.00 mg/kg DW, respectively, (β + γ)-tocotrienols was the major isomer, followed by α-tocopherol, α-tocotrienol and (β + γ)-tocopherols (Irakli, Samanidou, & Papadoyannis, 2011). In wheat the range of tocol concentrations in the HEALTHGRAIN was evaluated in common bread wheat, durum wheat, spelt, einkorn, and emmer 27.6–79.7 mg/kg, 40.1–62.7 mg/kg, 40.2–50.6 mg/kg, 29.0–57.5 mg/kg, and 29.0–57.5 mg/kg, respectively; in barley 46.2–68.8 mg/kg (Shewry et al., 2013).

On average, the raw kernels of einkorn had higher contents of total tocols and α-tocotrienol but lesser content of β-tocopherol than those of bread wheat. Unlike carotenoids, tocols are heat-resistant antioxidant compounds. Moreover, as already observed by Hidalgo, Brandolini, and Pompei (2010) in bread crust and water biscuits after baking, puffing led to a slight increase of α-tocotrienol and of β-tocopherol contents. As a consequence, total tocol content augmented only slightly, but significantly, both in bread wheat and einkorn. It has to be remembered that in wheat and other cereals tocols are esterified to lipids and other molecules. Drastic heat conditions, such as puffing, may break the bonds and increase the tocols extractable by the heat saponification method (Hidalgo, Scuppa, & Brandolini, 2016).

Tritordeum (*x Tritordeum martinii* A. Pujadas nothosp. nov.) is a new hexaploid hybrid derived from the cross between a South American wild barley (*Hordeum chilense* Roem. & Schult.) and durum wheat (*Triticum durum* Desf.), where tocol concentrations have not been reported yet.

Lipophilic and hydrophilic antioxidants are important wheat and other cereals constituents beneficial for human health. In wheat genotypes with purple pericarp and blue aleurone there are contained antioxidant pigments anthocyanins (Lachman, Martinek, Kotíková, Orsák, & Šulc, 2017). But so far no lipophilic tocotrienols and tocopherols were analyzed and reported in purple- and blue-grained wheat varieties and yellow-grained spring tritordeums. Therefore, the main aim of this study was the determination of tocols in purple- and blue-grained wheats and breeding lines and their comparison with standard varieties. Tocols were also determined in three tritordeum and three barley varieties.

2. Materials and methods

2.1. Materials

2.1.1. Analyzed cereals

Analyzed spring and winter wheat, spring tritordeum and barley varieties were obtained from the collection of Agricultural Research Institute Kroměříž, Ltd., Czech Republic, from the harvests in 2014 and 2015. Their major characteristics are described in Table 1. Field trials were sown in locality Kroměříž (N 49 °17′52″, E 17 °23′35″, 235 m a.s.l., beet production region, luvisol, average year sum of precipitation 599 mm and average year temperature 9.8 °C). The weather conditions during crop years 2013/2014 and 2014/2015 such as sum of rainfalls and mean monthly air temperatures are depicted in Table 2. The data are compared with long-term average for the location. Harvest was carried out using a plot harvester. Samples were stored after harvest in paper bags in a box in the dark at room temperature of 25 °C for 2 months before being analyzed.

2.1.2. Chemicals and equipment used for analyses

Methanol, super gradient (min. 99.9%, Lach-Ner, Neratovice, Czech Republic), adjusted deionized ultra-pure (Type 1) water obtained with Milli-Q® Type 1 Ultrapure Water System (EMD Millipore SAS Corp., Molsheim, France), pyrocatechol, > 99.5% (Sigma–Aldrich, Inc., Saint Louis, MO, USA), and hexane, pure min. 95.0% (Penta, Prague, Czech Republic), potassium hydroxide, min. 85% (Lachema, Neratovice, Czech Republic) were purchased and used. DL-*α*-tocopherol, 98.2% (GC) and tocopherol set (Calbiochem, La Jolla, Canada) were used as standards.

A magnetic stirrer IKA RET control-visc C and the Vortex IKA MS 3 Basic (both from ILABO, Ltd., Kyjov, Czech Republic) were used for the homogenization of samples. Chromatographic determination was performed using a chromatographic system for HPLC – Dionex UltiMate 3000 RS (Thermo Fisher Scientific, Inc., Sunnyvale, CA, USA) with a fluorescence detector Dionex UltiMate 3000 RS (Thermo Fisher Scientific, Inc., Sunnyvale, CA, USA).

2.2. Methods

2.2.1. Sample preparation

Samples were prepared according to the method of Sánchez-Machado, López-Cervantes, and Ríos Vázquez (2006) with minor modifications. Briefly, approximately 0.4 g of homogenized wheat caryopses was weighed into long glass test tubes with plastic stoppers. Solutions of pyrocatechol (200 μL, conc. 2 mg/mL) and methanolic KOH (5 mL, 0.5 mol/L) were added to the wheat samples. The mixture was stirred on a Vortex apparatus to obtain complete homogenization (1 min). Then the test tube was placed into a water bath for 15 min at a temperature of 80 °C and it was shaken on the Vortex every 5 min (i.e. in 5, 10 and 15 min). Afterwards the test tube was rapidly cooled in a beaker with water and ice and after cooling 1 mL deionized water and 5 mL hexane were added. The mixture was stirred for 1 min on the Vortex and then 3 mL hexane aliquot was transferred into an evaporation flask and hexane was evaporated in a rotation vacuum evaporator at 30 °C. Dry residue was redissolved in 0.5 mL methanol and filtered through a syringe filter (nylon, 0.22 μm) into a dark vial for HPLC analysis. All steps were carried out at low light intensity (the windows were darkened with blinds with no direct lighting in the lab).

2.2.2. Tocopherol and tocotrienol chromatographic determination

HPLC-FD analyses were performed under the following conditions: analytical column Develosil® 5 μ RPAQUEOUS (250 × 4.5 mm), (Phenomenex, Torrance, CA, USA), mobile phase H_2O: methanol (3:97), (v/v), flow 1.0 mL min^{-1}, column temperature 30 °C, injection volume 10.0 μL (for samples with little analyte content it is possible to increase injection volume to 20.0 μL), time of analysis 30 min. The measurements were performed in three replicates. Conditions of detection: fluorescence detector, excitation wave length $\lambda = 292$ nm, emission wave length $\lambda = 330$ nm. Limits of detection (LOD) for individual tocols δ-T3, γ-T3, β-T3, α-T3, δ-T, γ-T, β-T and α-T were 0.056, 0.111, 0.111, 0.167, 0.056, 0.111, 0.111, and 0.167 μg/g, respectively. Chromatograms of blue-grained wheat EF02-54-9, V1-135-15, tritordeum HT 439 and barley cv. Lucius are given in Supplementary material (Figs. S1–S4). Repeatability of individual tocols α-T, β-T, γ-T, δ-T, α-T3, β-T3, γ-T3 and δ-T3 was 8.6%, 8.4%, 9.7%, 12.0%, 8.8%, 8.2%, 6.3% and 6.5%, respectively; percentage is dependent on their levels in measured samples.

Table 1
Description of investigated cereal genotypes.

*Genotype/variety	Species	Growth type	**State of origin	Variety status	Grain color
[1]Purple Feed	*Triticum aestivum* L.	spring	AUS	res. germplasm	purple pericarp
[1]Novosibirskaya 67			RUS	released variety	white grain
[1]UC 66049			USA	genetic resource	blue aleurone
[1]Tschermak's blaukörniger Sommerweizen			AUT	genetic resource	blue aleurone
[1]H-90-41			USA	genetic resource	blue aleurone
[1]EF02-54-9			USA	genetic resource	blue aleurone
[1]Xiao Yian			CHN	genetic resource	blue aleurone
[1]RU 687-12			CZE	breeding line	purple pericarp
[1]Konini			NZL	res. germplasm	purple pericarp
[1]Purple			CND	res. germplasm	purple pericarp
[2]ANK-28A			RUS	res. germplasm	purple pericarp
[1]ANK-28B			RUS	res. germplasm	purple pericarp
[1]TA 4024			ISR	genetic resource	yellow endosperm
[2]V1-107-15	*Triticum aestivum* L.	winter	CZE	breeding line	blue aleurone
[2]KM 53-14			CZE	breeding line	blue aleurone
[2]V1-126-15			CZE	breeding line	blue aleurone
[2]V1-133-15			CZE	breeding line	blue aleurone
[1]V1-135-15			CZE	breeding line	blue aleurone
[2]V1-141-15			CZE	breeding line	blue aleurone
[2]V1-131-15			CZE	breeding line	blue al./yell.end.
[2]Bona Vita			SVK	released variety	yellow endosperm
[2]Citrus			GER	released variety	yellow endosperm
[2]Akteur			GER	released variety	purple pericarp
[1]PS Karkulka			SVK	released variety	purple pericarp
[2]V2-68-15			CZE	breeding line	purple pericarp
[2]V1-176-15			CZE	breeding line	purple pericarp
[1]Bohemia			CZE	released variety	stand. (red grain)
[3]Rosso			AUT	released variety	purple pericarp
[2]V1-178-14			CZE	breeding line	purple pericarp
[1]V1-178-15			CZE	breeding line	purple pericarp
[1]HT 439	× *Tritordeum martinii* A. Pujadas nothosp. nov.	spring	ESP	breeding line	yellow endosperm
[1]JB 1			ESP	released variety	yellow endosperm
[1]JB 3			ESP	released variety	yellow endosperm
[1]AF Cesar	*Hordeum vulgare* L.		CZE	released variety	light yellow
[1]AF Lucius	*Hordeum vulgare* L.	spring, hull-less	CZE	released variety	light yellow
[1]Nudimelanocrithon	*H. vulgare* var. nudimelanocrithon		CZE	genetic resource	black grain

* Origins of selected materials: RU 687-12 = selection from *Triticum aestivum* L. var. *australianum* Udachin & Schachm.; V1-107-15 = BAUB 2786.2 × Skorpion; KM 53-14 = Skorpion × Ludwig; V1-126-15 = Skorpion × Magister; V1-133-15 and V1-135-15 = Skorpion × UC 66049; V1-141-15 = KM 824-1-01 × RU 440-5; V1-131-15 = (Skorpion × V1-702) × (Citrus × Bona Dea); Bona Vita = (SO-690 × Arida) × Arida; Citrus = (Sunnan × Monopol) × Stamm GI 912; PS Karkulka = ANK-28 A × PS 11; V1-178-14 = Meritto × ANK-28 A; V2-68-15 = (Indigo × Akteur) × (Skorpion × Bohemia); V1-176-15 = Blaucorn × Zappa; Bohemia = (540i × U6192) × (540i × Kontrast); V1-178-15 = Indigo × Mironovskaya 808.

** AUS Australia, AUT Austria, CHN China, CND Canada, CZE Czech Republic, ESP Spain, GER Germany, ISR Israel, NZL New Zealand, RUS Russian Federation, SVK Slovakia, USA United States of America; [1]Samples from the harvests 2014 and 2015 for the evaluation of year impact; [2]Samples from the harvest 2015; [3]Sample from the harvest 2014.

Table 2
Weather conditions during the growing seasons, September-August 2013/2014 and 2014/2015.

Growing season	Parameter	IX.	X.	XI.	XII.	I.	II.	III.	IV.	V.	VI.	VII.	VIII.
2013/14	Average temperature (°C)	13.4	10.8	5.7	2.3	1.3	3.8	8.1	11.3	14.0	17.8	21.0	18.0
	Deviation from long-term standard (°C)	**−0.9**	**+1.5**	**+1.7**	**+2.2**	**+2.6**	**+3.4**	**+3.8**	**+1.9**	**−0.5**	**+0.5**	**+1.8**	**−0.8**
	Monthly precipitation (mm)	94.1	36.8	26.1	9.4	23.8	19.3	5.2	15.5	74.9	63.9	78.1	69.4
	Per cent of long-term standard	**174**	**96**	**65**	**28**	**96**	**73**	**16**	**38**	**113**	**79**	**106**	**106**
2014/15	Average temperature (°C)	15.7	10.9	7.5	2.2	1.7	1.3	9.7	9.7	13.9	18.1	22.1	23.1
	Deviation from long-term standard (°C)	**+1.4**	**+1.6**	**+3.5**	**+2.1**	**+3.0**	**+0.9**	**+1.2**	**+0.3**	**−0.6**	**+0.8**	**+2.9**	**+4.3**
	Monthly precipitation (mm)	119.9	42.0	27.8	35.7	41.7	18.9	39.6	13.6	41.9	47.0	42.0	65.1
	Per cent of long-term standard	**221**	**109**	**70**	**107**	**167**	**71**	**121**	**33**	**63**	**58**	**57**	**99**

2.2.3. Statistical analysis

Statistical analyses were done with the software Statistica 7.0 (StatSoft, Inc., Tusla, Oklahoma, USA) and SAS computer program, version 9.1.3.(SAS Institute Inc., Cary, NC, USA. Differences between mean total tocol values in 2014 and 2015 were determined by a one-way ANOVA followed by the Student's *t*-test. Differences between spring and winter wheat, tritordeum and barley varieties in 2014 and 2015 were determined by a post hoc Scheffé's test, considering $\alpha = 0.05$ and the level of significance $P < 0.05$ as significant.

The principal component analysis (PCA) was used to reduce this complex data set to a lower dimension and reveal simplified structures and relation between individual wheat, tritordeum and barley varieties. The dominant individual tocopherols and tocotrienols have been included in the assessment of differences among the varieties (α-T, α-T3, β-T, β-T3). In our calculation, the code written for Matlab R2014a (The Mathworks, Inc., Natick, Massachusetts, USA) was performed, based on single value decomposition algorithm. The related properties of individual varieties were then sorted (grouped) after their scores in the z-score graph.

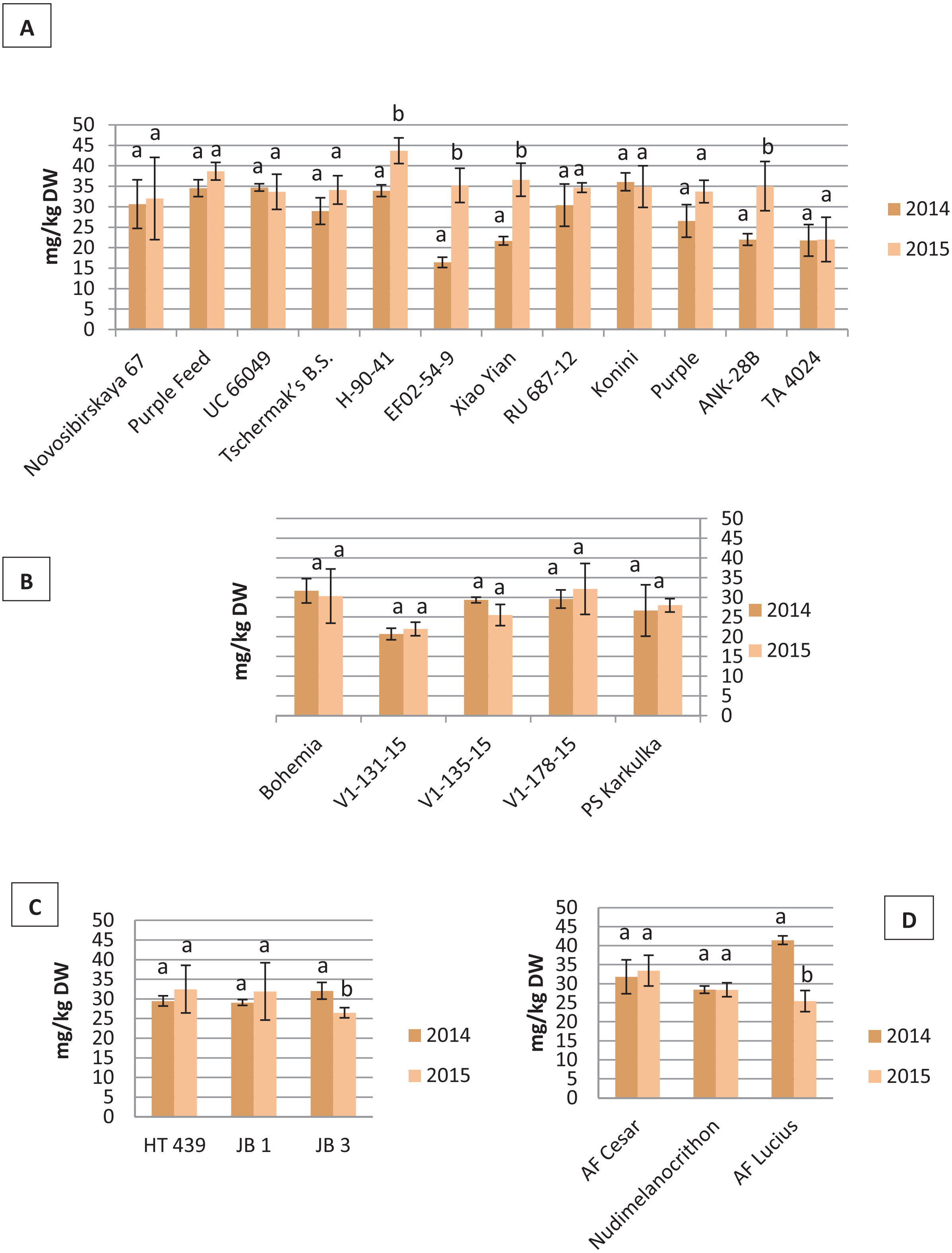

Fig. 1. Comparison of total tocol contents in spring (A) and winter (B) wheat, spring tritordeum (C) and spring barley (D) varieties and lines from the harvests in the years 2014 and 2015. Different letters within columns show statistically significant differences (Scheffé's test).

Table 3
Content of individual and total tocols in spring and winter wheat, tritordeum and spring barley varieties, harvests in 2014 and 2015 (mg/kg DW).

Variety	δ-T3	γ-T3	β-T3	α-T3	δ-T	γ-T	β-T	α-T	Σ
Spring wheat varieties 2015									
Novosibirskaya 67 (W)	< LOD	< LOD	10.46	2.38	< LOD	< LOD	5.26	13.90	32.00 ± 10.05ab
Purple Feed (B)	< LOD	< LOD	15.46	2.43	< LOD	< LOD	5.74	15.04	38.67 ± 2.15ab
UC 66049 (B)	< LOD	< LOD	12.63	2.74	< LOD	< LOD	4.36	13.91	33.64 ± 4.30ab
Tschermak's B. S. (B)	< LOD	< LOD	12.00	3.05	< LOD	< LOD	5.53	13.52	34.10 ± 3.42ab
H-90-41 (B)	< LOD	< LOD	14.44	3.37	< LOD	< LOD	6.16	19.70	43.67 ± 3.14a
EF02-54-9 (B)	< LOD	< LOD	13.02	2.19	< LOD	< LOD	5.12	14.88	35.21 ± 4.18ab
Xiao Yian (B)	< LOD	< LOD	13.04	2.33	< LOD	< LOD	6.05	15.17	36.59 ± 4.04ab
RU 687-12 (P)	< LOD	< LOD	13.70	3.39	< LOD	< LOD	4.58	12.99	34.66 ± 1.17ab
Konini (P)	< LOD	< LOD	13.20	2.74	< LOD	< LOD	4.89	14.08	34.91 ± 5.09ab
Purple (P)	< LOD	< LOD	16.16	2.68	< LOD	< LOD	4.06	10.82	33.71 ± 2.75ab
ANK-28B (P)	< LOD	< LOD	14.01	2.50	< LOD	< LOD	5.04	13.48	35.03 ± 6.01ab
TA 4024 (Y)	< LOD	< LOD	4.72	1.23	< LOD	< LOD	4.18	11.87	22.00 ± 5.42b
Average	< LOD	< LOD	12.74	2.59	< LOD	< LOD	5.08	14.11	34.52 ± 4.96
Spring wheat varieties 2014									
Novosibirskaya 67 (W)	< LOD	< LOD	15.62	3.58	< LOD	< LOD	3.15	8.29	30.64 ± 5.92a
Purple Feed (B)	< LOD	< LOD	13.392	2.00	< LOD	< LOD	5.77	13.36	34.52 ± 2.01a
UC 66049 (B)	< LOD	< LOD	15.58	2.82	< LOD	< LOD	4.18	12.15	34.71 ± 0.66a
Tschermak's B. S. (B)	< LOD	< LOD	10.01	2.46	< LOD	< LOD	5.01	11.46	28.93 ± 3.23ac
H-90-41 (B)	< LOD	< LOD	10.88	2.53	< LOD	< LOD	5.14	15.36	33.91 ± 0.46a
EF02-54-9 (B)	< LOD	< LOD	8.24	1.71	< LOD	< LOD	2.43	4.02	16.40 ± 1.23b
Xiao Yian (B)	< LOD	< LOD	8.34	1.36	< LOD	< LOD	4.01	7.96	21.67 ± 1.03bc
RU 687-12 (P)	< LOD	< LOD	11.44	2.73	< LOD	< LOD	4.85	11.36	30.38 ± 5.16a
Konini (P)	< LOD	< LOD	13.98	2.75	< LOD	< LOD	5.06	14.30	36.08 ± 2.07a
Purple (P)	< LOD	< LOD	12.10	2.43	< LOD	< LOD	3.06	8.93	26.53 ± 3.96c
ANK-28A (P)	< LOD	< LOD	11.19	1.93	< LOD	< LOD	2.12	4.47	19.72 ± 1.53bc
ANK-28B (P)	< LOD	< LOD	12.66	2.06	< LOD	< LOD	2.26	5.00	21.99 ± 1.29c
TA 4024 (Y)	< LOD	< LOD	6.13	1.17	< LOD	< LOD	4.36	10.12	21.78 ± 9.32bc
Average	< LOD	< LOD	11.50	2.27	< LOD	< LOD	3.95	9.75	27.48 ± 2.91
Winter wheat varieties 2015									
Variety	δ-T3	γ-T3	β-T3	α-T3	δ-T	γ-T	β-T	α-T	Σ
V1-107-15 (B)	< LOD	< LOD	9.98	1.89	< LOD	< LOD	2.17	11.15	25.19 ± 2.59ab
KM 53-14 (B)	< LOD	< LOD	8.06	1.82	< LOD	< LOD	3.20	9.48	22.56 ± 2.36ab
V1-126-15 (B)	< LOD	< LOD	8.70	2.59	< LOD	< LOD	2.67	11.28	25.24 ± 1.30ab
V1-133-15 (B)	< LOD	< LOD	10.10	2.24	< LOD	< LOD	3.63	12.92	28.88 ± 6.62ab
V1-141-15 (B)	< LOD	< LOD	7.33	1.66	< LOD	< LOD	2.68	10.67	22.34 ± 2.47ab
V1-131-15 (Y)	< LOD	< LOD	5.00	1.55	< LOD	< LOD	3.79	8.24	18.58 ± 3.41a
Bona Vita (Y)	< LOD	< LOD	2.11	1.18	< LOD	< LOD	4.14	11.77	19.20 ± 1.98ab
Citrus (Y)	< LOD	< LOD	5.54	1.41	< LOD	< LOD	2.80	8.00	17.75 ± 1.70a
Akteur (P)	< LOD	< LOD	13.46	1.91	< LOD	< LOD	2.52	7.36	25.25 ± 4.60ab
V1-178-14 (P)	< LOD	< LOD	8.77	1.95	< LOD	< LOD	2.13	7.18	20.03 ± 1.02ab
PS Karkulka (P)	< LOD	< LOD	12.31	1.81	< LOD	< LOD	3.79	10.08	27.99 ± 1.70ab
V2-68-15 (P)	< LOD	< LOD	10.93	1.97	< LOD	< LOD	4.27	13.68	30.85 ± 4.33ab
V1-176-15 (P)	< LOD	< LOD	11.15	1.89	< LOD	< LOD	2.26	6.93	22.23 ± 3.42ab
Bohemia (R)	< LOD	< LOD	11.89	2.07	< LOD	< LOD	4.54	11.84	30.34 ± 6.88ab
V1-131-15 (B)	< LOD	< LOD	6.72	1.45	< LOD	< LOD	3.67	10.15	21.99 ± 1.72ab
V1-135-15 (B)	< LOD	< LOD	10.18	1.45	< LOD	< LOD	2.71	11.19	25.53 ± 2.69ab
V1-178-15 (P)	< LOD	< LOD	11.78	2.86	< LOD	< LOD	4.57	12.95	32.15 ± 6.46b
Average	< LOD	< LOD	9.06	1.86	< LOD	< LOD	3.27	10.29	24.48 ± 4.44
Winter wheat varieties 2014									
PS Karkulka (P)	< LOD	< LOD	11.73	1.71	< LOD	< LOD	3.84	9.39	26.68 ± 6.52ab
Bohemia (R)	< LOD	< LOD	12.43	2.31	< LOD	< LOD	4.24	12.69	31.68 ± 3.09a
V1-131-15 (B)	< LOD	< LOD	7.504	0.94	< LOD	< LOD	3.05	9.22	20.71 ± 1.47b
V1-135-15 (B)	< LOD	< LOD	13.20	1.72	< LOD	< LOD	3.17	11.26	29.35 ± 0.71ab
V1-178-15 (P)	< LOD	< LOD	10.66	2.52	< LOD	< LOD	3.97	12.45	29.59 ± 2.31ab
Rosso (P)	< LOD	< LOD	8.00	1.94	< LOD	< LOD	4.06	10.28	24.27 ± 3.66ab
Average	< LOD	< LOD	10.59	1.86	< LOD	< LOD	4.76	10.88	28.08 ± 2.96
Spring tritordeum 2015									
Variety	δ-T3	γ-T3	β-T3	α-T3	δ-T	γ-T	β-T	α-T	Σ
HT 439 (Y)	< LOD	< LOD	13.30	7.64	< LOD	< LOD	2.40	9.12	32.46 ± 6.06a
JB 1 (Y)	< LOD	< LOD	12.32	5.64	< LOD	< LOD	2.85	11.07	31.88 ± 7.30a
JB 3 (Y)	< LOD	< LOD	11.67	5.44	< LOD	< LOD	1.81	7.53	26.45 ± 1.19b
Average	< LOD	< LOD	12.43	6.24	< LOD	< LOD	2.35	9.24	30.26 ± 3.32
Spring tritordeum 2014									
HT 439 (Y)	< LOD	< LOD	12.03	7.34	< LOD	< LOD	2.56	7.53	29.46 ± 1.30a
JB 1 (Y)	< LOD	< LOD	10.77	5.04	< LOD	< LOD	2.79	10.46	29.06 ± 0.74a
JB 3 (Y)	< LOD	< LOD	14.95	5.57	< LOD	< LOD	2.37	9.15	32.04 ± 2.13a
Average	< LOD	< LOD	12.58	5.98	< LOD	< LOD	2.57	9.05	30.18 ± 1.39
Spring barley 2015									
Variety	δ-T3	(β + γ)-T3		α-T3	δ-T	(β + γ)-T		α-T	Σ
AF Cesar (S)	< LOD	5.55		13.83	< LOD	2.53		11.52	33.43 ± 4.04a

(continued on next page)

Table 3 *(continued)*

Variety	δ-T3	γ-T3	β-T3	α-T3	δ-T	γ-T	β-T	α-T	Σ
Nudimelanocrithon (BL)	< LOD	1.66		11.05	< LOD	3.31		12.39	28.40 ± 1.83ab
AF Lucius (S)	< LOD	4.56		10.64	< LOD	2.09		8.12	25.42 ± 2.78b
Average	< LOD 3.92			11.84	< LOD	2.64		10.68	29.08 ± 2.88
Spring barley 2014									
AF Cesar (S)	0.62	3.10[*]		17.76	0.29	0.63[*]		9.52	31.80 ± 4.46a
Nudimelanocrithon (BL)	[*] < LOD	1.84		13.09	< LOD	2.95		10.55	28.43 ± 0.96a
AF Lucius (S)	0.43	10.73		17.15	0.18	3.35		9.79	41.61 ± 1.12b
Average	0.36	5.22		16.00	0.17	2.31		9.95	33.99 ± 2.18

B – blue aleurone; P – purple pericarp; R – red grain; S – standard grain color; W – white grain; Y – yellow pericarp; BL – black grain; Tschermak's B.S. – Tschermak's blaukörnige Sommerweizen; values were an average of triplicates for each variety; ± standard deviation.

[*] In spring barley 2014 average values < LOD for δ-T3 were estimated to 0.028 and for β-T and β-T3 in AF Cesar 0.056 mg/kg DW (0.5 LOD); different letters within every analyzed set of wheats, tritordeums and barleys indicate statistically significant differences; spring barley and tritordeum samples are hull-less; line V1-131-15 was divided in two lines – V1-131-15 (Y) with yellow grains and V1-131-15 (B) with blue grains.

3. Results

3.1. Content of total and individual tocols in grains of spring and winter wheat, spring barley and tritordeum

Higher total contents of vitamin E were determined in the varieties of spring wheat harvested in 2015 in comparison with winter wheat varieties (harvest in year 2015, Fig. 1). The total content of vitamin E ranged from 22.00 mg/kg DW (TA 4024) to 43.67 mg/kg DW (H 90-41-9). The average value of all varieties was 34.52 mg/kg DW. Four of the eight forms of vitamin E were detected, namely β-T3, α-T3, β-T, and α-T, of which the highest concentration showed β-T3 (with values ranging from 4.72 to 16.16 mg/kg DW) and α-T (from 10.82 to 19.70 mg/kg DW). The contents of the other two detectable analytes were lower, namely α-T3 (from 1.23 to 3.39 mg/kg DW) and β-T (from 4.06 to 6.16 mg/kg DW, Table 3).

The highest average total tocol content in 2014 and 2015 harvest years was characteristic for blue-grained spring wheats (32.68 mg/kg DW) and purple-grained wheats (30.33 mg/kg DW), which is comparable with white-grained standard spring wheat Novosibirskaya 67 (32.00 mg/kg DW) and red-grained standard winter wheat Bohemia (31.01 mg/kg DW). Purple-grained winter wheat varieties showed lower tocol levels (26.56 mg/kg DW) as well as blue-grained winter wheat varieties (24.64 mg/kg DW). Low tocol levels were also found in yellow-grained spring wheat TA 4024 (21.89 mg/kg DW) and yellow-grained winter wheat varieties (18.51 mg/kg DW).

Among spring wheat varieties, statistically significant lower tocol content in the year 2014 was found in EF02-54-9 compared with content in variety Konini, Novosibirskaya, Purple Feed, UC 66049, and Tschermak's B.S. In the year 2015 statistically significant lower tocol content was determined in TA 4024 compared with tocol content in H-90-41 (Fig. 3). Within winter wheat statistically significant lower tocol content was characteristic for breeding line V1-131-15 (B) compared with tocol content in variety Bohemia in the year 2014. Statistically lower tocol contents were determined in V1-131-15 (Y) and Citrus compared with V1-178-15 tocol content in the year 2015.

Only four of the eight analyzed tocol homologues were identified in tritordeum (β-T3, α-T3, β-T, α-T), while the remaining tocols were below the detection limits. Major tocols were β-T3 and α-T. The contents of β-T3 ranged from 11.67 to 13.30 mg/kg DW and those of α-T from 7.53 to 11.07 mg/kg DW in 2015. Values α-T3 and β-T ranged from 5.44 to 7.64 mg/kg DW and 1.81 to 2.85 mg/kg DW, respectively. Between tritordeum varieties no significant differences in tocol contents were determined in both harvest years.

Among spring barley varieties from the harvest in 2014, all eight homologues of vitamin E were detected only in grain of the variety Lucius, while in varieties Nudimelanocrithon δ-T3 and δ-T, and Cesar β-T3 and β-T were below the LODs (Table 3). Total amount of tocols in barley ranged from 25.42 to 33.43 mg/kg DW (average 29.08 mg/kg DW) in 2015 and between 28.43 and 41.61 mg/kg DW (average 33.99 mg/kg DW) in 2014. Major tocols in barley were α-T3 and α-T (α-T3 ranged from 10.64 to 13.83 mg/kg DW in 2015 and from 13.09 to 17.76 mg/kg DW in 2014; for α-T from 8.12 to 12.39 mg/kg DW in 2015 and from 9.52 to 10.55 mg/kg DW in 2014, respectively). Tocol contents in AF Cesar and Nudilomelacrithon were significantly lower than tocol content in AF Lucius in the year 2014 (Fig. 3).

Principal component analysis (PCA) revealed groups, where within group similar wheat varieties could be identified (Fig. 2A–F). Winter wheat lines and varieties were divided in three groups in 2014 (Fig. 2A); the first group was represented by V1-178-15 (KM-178-15) and Bohemia varieties, the second one by V1-135-15 (KM-135-15) and PS Karkulka, the third one by V1-131-15 (KM-131-15) and Rosso varieties and breeding lines. Similarly, in winter wheat samples from 2015 three groups were identified (Fig. 2B). The first group was represented by the varieties V2-68-15 (KM-68-15), V1-178-15 (KM-178-15) and Bohemia; in the second group the V1-178-14 (KM-178-14), V1-176-15 and Akteur and in the third group V1-131-15 (KM-131-15) and Citrus with yellow endosperm were included. Completely different from other varieties was found the yellow-grained variety Bona Vita. Within spring wheats from 2014 two groups could be identified (Fig. 2C); in the first group the varieties Konini, Purple Feed, RU 687-12, and Tschermak's B.S. can be included, whereas ANK 28A and ANK 28B in the second group. Within spring wheats from 2015 only one group was identified including most varieties with exception of the TA 4024, H-90-41 and Purple varieties (Fig. 2D). In PCA of all analyzed cereals in 2014 and 2015 wheat, tritordeum and spring barley varieties differed from one another in both years (Fig. 2E and F). Grouping the varieties and lines can create the opportunity for the breeders to select appropriate parents for crossing.

3.2. Comparison of tocol contents in growing seasons 2013/2014 and 2014/2015

When comparing identical analyzed spring wheat varieties from the harvests in 2014 and 2015, it is evident that majority of wheat varieties harvested in 2015 contained a higher level of vitamin E (Fig. 1, Table 3) The greatest difference was revealed in the variety EF02-54-9 (53.1%), whereas the lowest was in variety TA 4024 (0.9%). However, cvs. UC 66049 and Konini have shown lower total tocol levels in 2015 (33.64 and 34.91 mg/kg DW in 2015, 34.71 and 36.08 mg/kg DW in 2014, respectively). Statistically significant differences between years were not observed in winter wheat varieties, which were investigated in both the years. The greatest difference between years was found in V1-135-15 (13.0%), the lowest one in the variety Bohemia (4.4%). Higher levels of total tocols were found in the varieties Bohemia and V1-135-15 in the harvest 2014 (31.68 and 29.35 mg/kg DW in 2014, 30.34 and 25.53 mg/kg DW in 2015), unlike in the varieties V1-178-15 and PS Karkulka (29.59 and 26.68 mg/kg DW in 2014 and 32.15 and

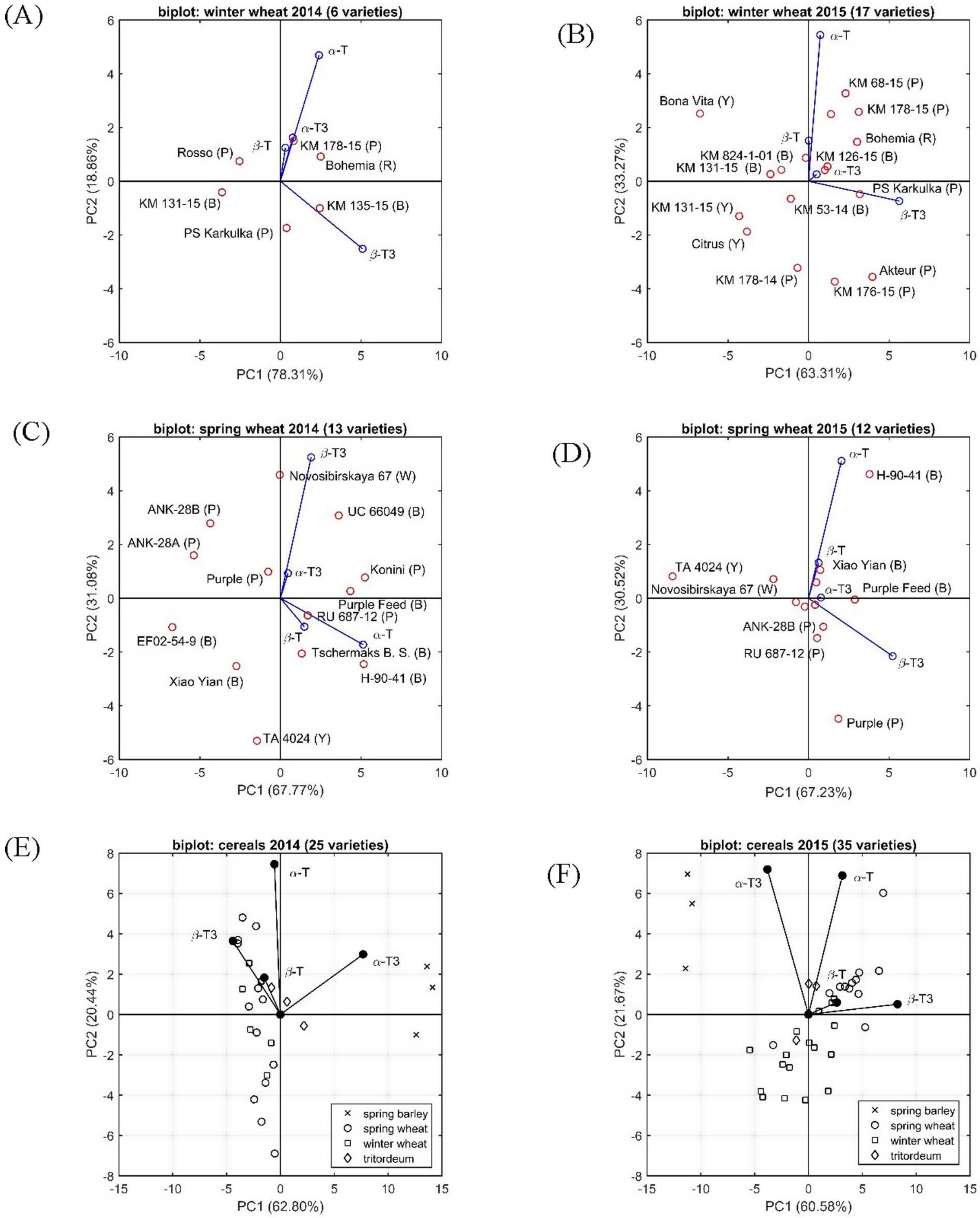

Fig. 2. Biplot between PC1 and PC2 showing contribution of various lines and varieties in variability of tocopherol (T) and tocotrienol (T-3) levels in grain of winter wheat varieties in 2014 (A) and 2015 (B), spring wheat varieties in 2014 (C) and 2015 (D), and all analyzed cereals (winter and spring wheat, spring tritordeum and barley in 2014 (E) and 2015 (F). KM means breeding lines originated from a program of Agrotest Fyto, Ltd., Kroměříž, CZE supposed for national tests: KM 131-15 = V1-131-15, KM 135-15 = V1-135-15, KM 178-15 = V1-178-15, V1-141-15 = KM 824-1-01 × RU 440-5, KM 68-15 = V2-68-15, KM 126-15 = V1-126-15, KM 178-14 = V1-178-14, KM 176-15 = V1-176-15.

27.99 mg/kg DW in 2015). Consequently, the average values of five analyzed varieties in both the years were identical (27.60 mg/kg DW).

Within tritordeum varieties from the 2015 harvest the content of vitamin E ranged from 26.45 mg/kg DW (JB 3) to 32.46 mg/kg DW (HT 439). The greatest difference between total tocol levels from the harvest 2014 and 2015 was found in the variety JB 3–17.2%, the lowest in the variety JB 1, namely 9.1%. In spring tritordeum, higher contents were found in 2015 than in 2014 in the varieties HT 439 (32.46 and 29.46 mg/kg DW, respectively) and JB 1 (31.88 mg/kg and 29.06 mg/kg DW, respectively). In contrast to this, in the variety JB 3 higher levels were found in 2014 (32.04 mg/kg DW) as compared to 2015 (26.45 mg/kg DW). It was found that there was no statistically

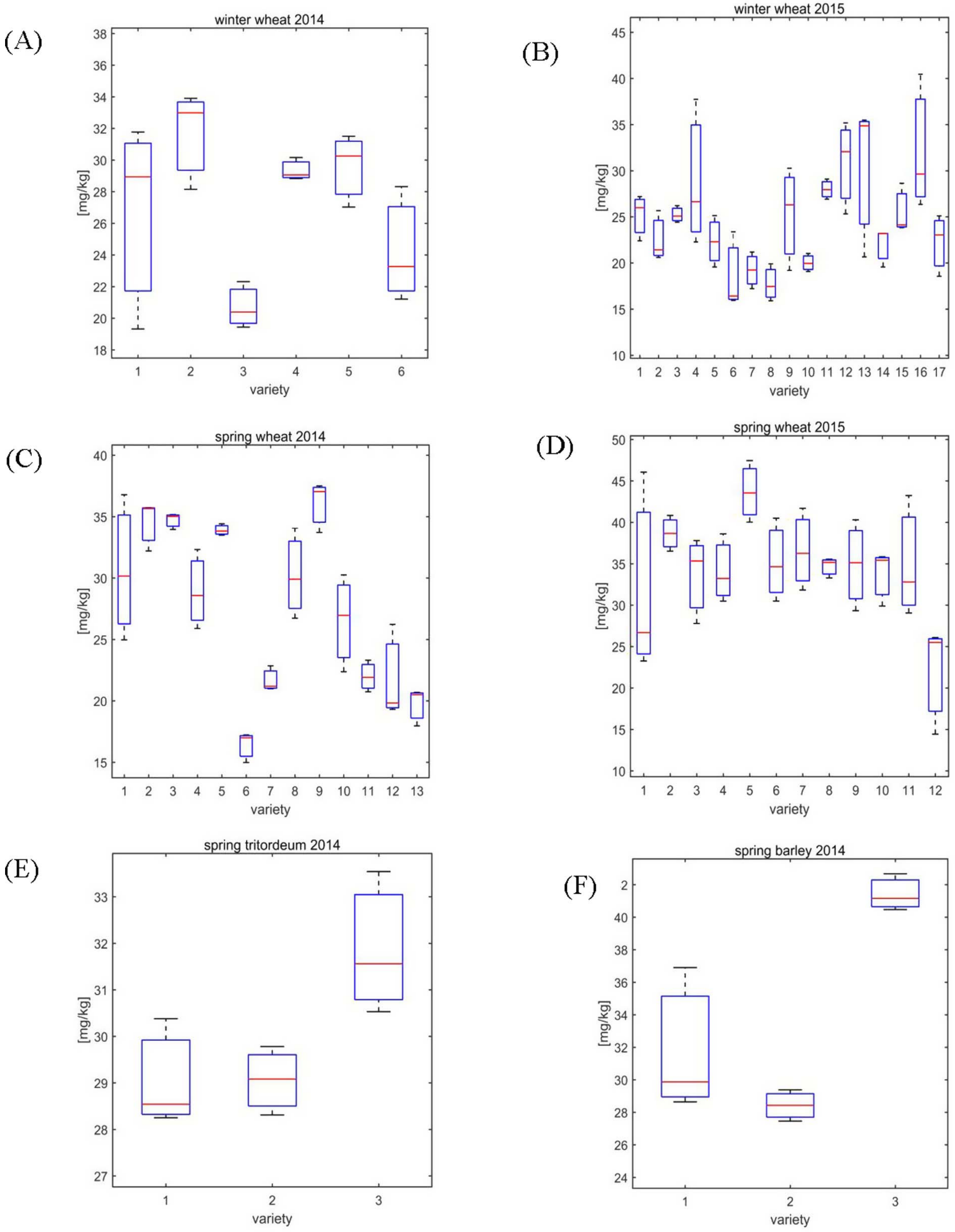

Fig. 3. Box plots of statistical evaluation by Scheffé's test of analyzed winter wheat in 2014 (A), winter wheat in 2015 (B), spring wheat in 2014 (C), spring wheat in 2015 (D), tritordeum in 2014 (E) and barley in 2014 (F). (A): 1 – PS Karkulka, 2 – Bohemia, 3 – V1-131-15 (B), 4 – V1-135-15, KM 178-15, 6 – Rosso; (B): 1 – V1-107-15, 2 – KM 53-14, 3 – V1-126-15, 4 – V1-133-15, 5 – V1-141-15, 6 – V1-131-15 (Y), 7 – Bona Vita, 8 – Citrus, 9 – Akteur, 10 – V1-178-14, 11 – PS Karkulka, 12 – V2-68-15, 13 – Bohemia, 14 – V1-131-15 (B), 15 – V1-135-15 (B), 16 – V1-178-15, 17 – V1-176-15; (C): 1 – Novosibirskaya 67, 2 – Purple Feed, 3 – UC 66049, 4 – Tschermak's B.S., 5 – H-90-41, 6 – EF02-54-9, 7 – Xiao Yian, 8 – RU 687-12, 9 – Konini, 10 – Purple, 11 – ANK-28B, 12 – TA 4024, 13 – ANK-28A; (D): 1 – Novosibirskaya 67, 2 – Purple Feed, 3 – UC 66049, 4 – Tschermak's B.S., 5 – H-90-41, 6 – EF02-54-9, 7 – Xiao Yian, 8 – RU 687-12, 9 – Konini, 10 – Purple, 11 – ANK-28B, 12 – TA 4024; (E): 1 – HT 439, 2 – JB 1, 3 – JB 3; (F): 1 – AF Cesar, 2 – Nudimelanocrithon, 3 – AF Lucius. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significant difference between HT 439 and JB 1 samples from 2014 to 2015, while the JB 3 variety has shown significant difference. The total average contents of three varieties were nearly identical (30.19 and 30.26 mg/kg DW in 2014 and 2015, respectively).

Comparing the same varieties of spring barley from the harvests in 2014 and 2015, a higher content of vitamin E was observed in 2014. The highest difference was found in the variety AF Lucius (48.8%), and the lowest in the black-grained variety Nudimelanocrithon, namely 11.6%. Higher rainfall and lower temperatures in the growing season of 2014 than of 2015, caused higher tocol concentration (mean 33.89 mg/kg DW in 2014 and 29.08 mg/kg DW in 2015). Statistically significant difference between the growing years was determined in the variety AF Lucius.

4. Discussion

4.1. Tocols in wheat and tritordeum

Among the analyzed colored-grain wheat varieties, mean higher content of total tocols was found in spring varieties (34.52 ± 4.96 mg/kg DW) than in winter varieties (24.48 ± 4.44 mg/kg DW). This is in accordance with typical ranges of tocols in wheat 28–80 mg/kg DW (Lampi, Nurmi, Ollilainen, & Piironen, 2010). In small-grained bread wheat (ZP Zemunska rosa or ZP Zlatna) higher concentrations of total tocopherols were found (13.85 mg/kg DW and 32.65 mg/kg DW, respectively) when compared with other small-grained durum wheat varieties and breeding lines with different α-tocopherol and (β + γ)-tocopherol contents (Žilić et al., 2011). However, in the evaluation of fifteen diploid, tetraploid and hexaploid accessions belonging to different *Triticum* species, with focus on chemical composition of wild diploid wheats, the highest total tocol contents were found in *T. thaoudar, T. aegilopoides, T. monococcum* and *T. urartu* (75.1 ± 3.95, 70.8 ± 3.35, 66.8 ± 3.82 and 63.9 ± 2.91 mg/kg DW, respectively) (Brandolini et al., 2015; Hidalgo et al., 2006). In our sets of analyzed wheat varieties such high contents were not determined; the highest total tocol level was determined in the spring variety H-90-41 43.67 ± 3.14 mg/kg DW. The total tocol levels in all wheat samples were lower than it was for soft wheat, containing a high total tocol level, similar to barley (≅ 75 mg/kg DW) (Moore et al., 2005). The content of the tocochromanols in the white flour (4.87–11.73 mg/kg) was much lower than that of whole flour of soft wheat (74.30 mg/kg) and durum wheat (60.60 mg/kg) as reported Labuschagne, Mkhatywa, Wentzel, Johansson, and van Biljon (2014).

Dominant tocols in purple- and blue-grained spring wheat were α-T (mean 14.11 mg/kg DW) and β-T3 (12.74 mg/kg DW). The same trend was observed in winter colored wheat varieties, where the mean levels of α-T and β-T3 were lower (10.29 and 9.10 mg/kg DW, respectively). β-Tocotrienol is reported the main vitamer found in hulled and dehulled wheats at 33–43 mg/kg DW (Moore et al., 2005). High levels of lipophilic antioxidant tocols are characteristic for einkorn and emmer wheat (Lachman et al., 2013; Hejtmánková et al., 2010). The most abundant tocol in einkorn wheat was β-tocotrienol (48.22 mg/kg DW), followed by α-tocotrienol (12.77 mg/kg DW), α-tocopherol (12.18 mg/kg DW), and β-tocopherol (4.79 mg/kg DW). Mean tocotrienol/tocopherol ratio in einkorn was estimated even to 3.68 (Tsao, 2008). However, in spring wheat samples, winter wheat, tritordeum and barley in this study this ratio was 0.798, 0.806, 1.611, and 1.183, respectively. In einkorn, emmer, Khorasan, durum, spelt, soft and hard wheat (total 22 varieties) the main tocols were β-tocotrienol ranging from 9.6 to 23.2 mg/kg, followed by α-tocopherol (5.5–11.9 mg/kg), α-tocotrienol (2.5–7.4 mg/kg) and finally β-tocopherol (2.0–6.6 mg/kg) in all analyzed wheat species (Abdel-Aal & Rabalski, 2008). Our results are in agreement with their data that between the dominant tocols of colored-grain wheats was observed β-tocotrienol, likewise as in hulled and dehulled wheats, or einkorn and emmer wheat species. However, the highest α-T content in most of wheat varieties with purple pericarp and blue aleurone seems to be different from other wheats, although the β-tocotrienol was estimated as the second most represented tocol. In Purple Feed, RU 687-12, Purple, ANK-28B, Akteur, V1-178-14, PS Karkulka, V1-176-15, and control variety Bohemia high levels were characteristic. Average content of total tocols in spring tritordeum was 30.26 ± 3.32 mg/kg DW with higher contents in cvs. HT 439 and JB 1 (32.46 ± 6.06 and 31.88 ± 7.30 mg/kg DW, respectively), which were similar to the levels in spring wheat. Likewise in wheat, the highest levels in tritordeum varieties found in our analyses were those of β-tocotrienol (12.43 mg/kg DW) followed by α-T (9.24 mg/kg DW). Interesting difference, as compared with tritordeum, was observed in barley, where α-T3 and α-T (11.84 and 10.68 mg/kg DW) were observed as the main vitamin E components. In lesser amounts β-T and α-T3 were quantified (in spring wheat 5.08 and 2.59 mg/kg DW, in winter wheat 3.27 and 1.86 mg/kg DW, in tritordeum 2.35 and 6.24 mg/kg DW). In all wheat and tritordeum lines no γ-isomers of tocopherol and tocotrienol were identified or quantified.

4.2. Tocols in barley

Average total tocols content of three analyzed barley varieties in both the years was 31.54 ± 2.53 mg/kg DW, with higher levels in light yellow-grained cv. AF Lucius (33.52 ± 1.95 mg/kg DW) and cv. AF Cesar (registered in CR in 2014, 32.61 ± 4.25 mg/kg DW). Black-grained Nudimelanocrithon (genetic resource, origin in Ethiopia) contained on average lesser tocol amounts (28.41 ± 1.40 mg/kg DW). These results are consistent with that reported by Benešová et al. (2012), who quantified total tocols using UPLC in barley cv. AF Lucius and Nudimelanocrithon 23.62 and 17.03 mg/kg in 2009 and 38.27 and 17.46 mg/kg in 2010, respectively, with the highest proportions of α-T3, α-T and (β + γ)-T3. Their given values are comparable with 20.29 mg/kg DW in genotype Jet but considerably lower than 102.43 mg/kg DW in genotype Harrington (Do, Cozzolino, Muhlhausler, Box, & Able, 2015). In another study the total tocol content of whole pearled grain barley varieties ranged between 40 mg/kg and 151.1 mg/kg (Temelli, Stobbe, Rezaei, & Vasanthan, 2013). Do et al. (2015) observed generally lower vitamin E content in hull-less or colored barley genotypes. Both genotype and location significantly influenced the level of tocols in the barley kernel, with genotype having the greater effect for most tocol homologues (Cavallero, Gianinetti, Finocchiaro, Delogu, & Stanca, 2004). Significant genotype x location interaction was observed for six out of eight homologues, but not for total tocotrienols and total tocols; the coefficient of determination for genotype was high for most homologues in the barley kernel. The hull-less trait negatively affected the content of total tocols, influencing both tocopherols (positively) and tocotrienols (negatively). A combination of high levels of α-T, δ-T3 and, particularly, α-T3 appears to be distinctive trait of hulled barley. Hull-less genotypes are mainly characterized by lower combined levels of these homologues but by higher levels of γ-T and δ-T.

In three analyzed hull-less spring barley varieties the number of identified and quantified tocols was higher, in cv. AF Cesar in 2015 six (only δ-T3 and δ-T were under the limits of detection, likewise as in other varieties AF Lucius and Nudimelanocrithon); in 2014 even all eight tocols in cv. AF Lucius could be identified and quantified. The proportion of tocols could be arranged as α-T3 > α-T > γ-T3 > γ-T > β-T3 > β-T. Do et al. (2015) found in barley genotypes also three tocotrienols and three tocopherols in descending order α-T3 > α-T > γ-T3 > β-T3 > γ-T > β-T. Their results showed higher β-T3 than γ-T. In our study, using HPLC with RP β- and γ-isomers were not completely separated due to coelution, so we expressed their contents as sum (β + γ) isomers (Table 3). Only black-grained variety Nudimelanocrithon differed in average of both years with higher α-T (11.47 mg/kg DW) and (β + γ)-T (3.13 mg/kg DW). Generally, only about 13% of the tocochromanols were found in the germ fraction, whereas the pericarp fraction contained about 50% and the endosperm fraction about 37% of the tocochromanols (Falk et al., 2004).

As reported by Temelli et al. (2013) and Idehen, Tang, and Sang (2017), α-T3 is the most represented tocol isomer in whole grain barley, contributing about 47.7% of the total tocol content, followed by α-T (17.7–33.9%), γ-T3 (10.4–20.2%), γ-T (1.9–9.2%), β-T3 (2.9–7.8%) and δ-T3 (2.7–6.7%). Barley was suggested be one of the richest tocol sources among cereal grains. However, even if the scale of contained tocols and tocopherols was complete, total tocol content was lower compared to spring wheats and tritordeum according to our results.

4.3. Effect of growing year on tocol contents in wheat, tritordeum and barley grains

In general, both the factors, genotypes and environmental conditions, had an impact on tocol contents in the analyzed wheat genotypes. Some of the genotypes were more sensitive to the effects of weather conditions in the growing cultivation year, whereas others were relatively stable. The average total content of tocols in spring wheat varieties was higher in 2015 (34.52 mg/kg DW) compared to 2014 (27.48 mg/kg DW). Statistically significant differences between the growing years were found in the varieties H-90-41, EF02-54-09, Xiao-Yian and ANK-28B. Differences in the total content could be partly explained by differences in grain sizes, and aligned to the increase in total tocol contents, which may also reflect the higher rainfall period and lower temperatures during spring growing season in 2015 and more favorable moisture and temperature conditions during early stages of plant development (Table 2). It might indicate a different sensitivity and reaction of genotypes and varieties. Accessions of einkorn grown in Italy during two years gave lower total tocol contents after a rainy period during growing, indicating the importance of rain on the quality of the seeds (Hidalgo, Brandolini, & Ratti, 2009). There was only 1.2 to 1.3-fold difference in the average total tocol content of the 26 winter and spring bread wheat genotypes grown in the four locations in 2007 (Lampi, Nurmi, & Piironen, 2010; Shewry, Piironen, Lampi, & Ward, 2010). There were large differences in environmental conditions with respect to geographical, soil, and climatic conditions (in Hungary, Poland, United Kingdom and France), which further challenged the stability of the genotypes. Similarly, higher tocol contents were reported in white flour from ten red hard wheat varieties from cool location with a high rainfall (Labuschagne et al., 2014). We observed in winter wheat insignificant differences between crop years with higher tocol contents in the crop year 2014/2015 in V1-178-15 and Karkulka varieties. A similar trend was found in the investigated tritordeum varieties with the exception of JB 3 (Fig. 1).

Within analyzed barley varieties, AF Cesar and Nudimelanocrithon showed insignificantly higher tocol content in 2015. The optimal temperature and moisture increased α-tocopherol content in three Latvian hull-less barley genotypes (Bleidere, Zute, Brunava, Bobere, and Jācobsone (2013), although some studies reported that organically and conventionally fertilized grain had similar levels of tocochromanols (Konopka, Tańska, Faron, Stępień, & Wojtowiak, 2012). However, in the variety AF Lucius analyzed in this study higher total tocol content was quantified in 2014 in relation to weather effect. Similarly, in recent study of Benešová et al. (2012), different reactions of barley varieties in 2009 and 2010 have been observed. In the study the varieties and lines Nudimelanocrithon, Xanadu, Annabel, AF Lucius, KM-1057 and KM-2283 showed opposite trend than the varieties Abyssinian and Wanubet.

5. Conclusions

Evaluation of tocol (tocotrienol and tocopherol) contents in wheat samples revealed their higher amounts in spring wheats as compared with winter wheats. Wheat varieties with blue aleurone or purple pericarp showed higher tocol contents than varieties with yellow or white grain, and thus may suitably enrich their antioxidant and health properties. In wheat and tritordeum samples there were quantified four tocols, with prevailing α-tocopherol and β-tocotrienol, and lesser levels of α-tocotrienol and β-tocopherol. Barley had lower tocol content than wheat and tritordeum, but six or all eight tocol homologues could be quantified. Unlike wheat and tritordeum, major quantified tocols in barley were α-tocotrienol, α-tocopherol and γ-tocotrienol. Higher rainfall and lower temperatures during early stages in 2015 than in 2014 caused in average of all analyzed samples higher tocol contents in spring wheat and tritordeum, however some winter wheat, tritordeum and barley varieties varied in their genotype response.

The results indicate that total tocol content in wheat varieties with colored grain is comparable with other wheat species like spring soft and winter hard wheat, spelt, einkorn and emmer wheat and may contribute to antioxidant complex of colored-grain wheats. There exist significant differences in bioactive compounds content among individual genotypes. This could be used in further breeding of new lines and varieties. In purple-grained and blue-grained wheats, tocols may contribute to anthocyanins and phenolic acids antioxidant activity. Although tocols content in yellow-grained wheat varieties is lower than in purple- and blue-grained genotypes, they can contribute together with high carotenoid content to the beneficial effects on human health.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.07.123.

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