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Antioxidant Activity, *S*-alk(en)yl-L-cysteine Sulfoxide and Polyphenol Content in Onion (*Allium cepa* L.) Cultivars Are Associated with their Genetic Background

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Abstract

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Six onion cultivars Bingo, Dormo, Elenka, Elbrus, Spirit, and Sturon grown in the Czech Republic for commercial purposes were analysed to investigate the content of health-promoting compounds. The results showed that at harvest time, cysteine sulfoxide content varied from 32.38 to 44.16 g/kg of dry weight, polyphenol content was between 2.66 and 3.37 g/kg of dry weight, and antioxidant activity ranged from 0.75 to 0.83 g/kg. Cv. Bingo had the highest level of the analysed compounds. The cultivars were concurrently analysed by DNA (microsatellite) markers. Dendrograms based on the chemical composition and DNA analysis were almost identical. This finding confirms the dependence of the secondary metabolite content on onion genotype.

Keywords: Alliaceae; cysteine sulfoxides; simple sequence repeat (SSR) marker; genotypes; secondary metabolites

The onion (*Allium cepa* L.) is one of the most valuable vegetables in the world and was known already in ancient times. The onion is one of the classic examples of *Allium* species used not only for culinary preparations but also for medicinal purposes. Compounds in onions have been reported to exhibit a range of health benefits, including anticancer properties, antiplatelet activity, antithrombotic activity and antibiotic effects (Suleria *et al.* 2015). These benefits are associated with sulphur compounds called cysteine sulfoxides (Starkenman *et al.* 2011). Onions also synthesise significant amounts of other metabolites including fructo-oligosaccharides (FOS), flavonoids, and saponins that contribute to their taste and health benefits (Pérez-Gregorio *et al.* 2014).

Several studies have indicated that the secondary metabolite content in *Allium* vegetables is associated both with environmental factors and with the genetic background of the respective cultivars (Huchette *et al.* 2005; Crosby *et al.* 2007). Perner *et al.* (2008) provided clear evidence that antioxidant activity, quercetin glycosides and organosulphur compounds can be increased in onion plants sufficiently supplied with nitrates or mycorrhizal fungi. Jabbes *et al.* (2012) described an association between organosulphur compounds and yield. The yield is generally considered to be genotype and environment dependent. Bystricka *et al.* (2015) observed differences between cultivars and a statistically significant increase of quercetin formation in all cultivars during vegetation. Also Bernaert *et al.* (2012) reported differences in *S*-alk(en)yl-L-cysteine sulfoxide (ASCO) content in various garlic cultivars. Lee *et al.* (2015) showed differences in secondary metabolite content

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between numerous onion cultivars. It has also been reported that the content of secondary metabolites in *Allium* species changes after harvest (Yamazaki *et al.* 2010; Bernaert *et al.* 2013). Such information is important for consumers.

The onion is one of the most commonly grown vegetables in the Czech Republic. In the second half of the 1990s, domestic production amounted to approximately 75% of the consumption of onions. The domestic production of vegetables has generally decreased and now accounts for less than a third of the total consumption. Low self-sufficiency is common in such vegetables, and the Czech Republic has suitable conditions for cultivation. Onion represents such an example. Regardless, onion breeding has a long tradition in the Czech Republic. It is to note that domestic farmers currently use seeds of foreign cultivars (Buchtová 2013).

Onions represent an important part of the human diet, so the characterisation of the cultivars currently available on the market from domestic sources was the purpose of the experiment. Here, the first report combining the genetic analysis of basic onion types grown in the Czech Republic with the chemical analysis of their health-promoting compounds is presented.

MATERIAL AND METHODS

Plant materials and genomic DNA extraction. Six short-day onion genotypes were used (Table 1). The onions were planted in mid-October and harvested in early May from the field in the central part of the Czech Republic (50°31'N, 14°59'E) with sandy loam soil. The onions were managed according to the standard farming practice. Leaves for DNA analysis were collected from the fields in March. When the plants matured and the foliage fell over, the onions were harvested and transported to the laboratory for analysis. The bulbs were stored in the dark at room temperature until processing.

Total genomic DNA was extracted from frozen pooled leaves in parallel using the CTAB (cetyltrimethylammonium bromide) method, as described by Ovesná *et al.* (2014). DNA quality was examined by electrophoresis, and the amount was quantified by spectrophotometry. DNAs used for the Simple Sequence Repeat (SSR) analysis of all six cultivars were isolated from leaves.

DNA analysis by Simple Sequence Repeat. A set of 15 microsatellite markers (Table 2; Jakše *et al.* 2005) were used to establish the DNA profiles of the analysed onion cultivars.

PCR was performed as described by Mitrová *et al.* (2015). The amplification products were separated by capillary electrophoresis in an ABI PRISM 3130 sequencer (Applied Biosystems, Foster City, USA). A multiplexed configuration was used in one analysis. LIZ500 (Applied Biosystems, USA) was used as an internal size standard. Electrophoretograms were processed using the GeneMapper software (Applied Biosystems, USA). For each locus, the presence or absence of bands (markers) in each size category throughout all genotypes was scored. The data were set in a binary matrix.

Sample preparation for sulphur-containing amino acid analysis. For each cultivar, 20 g of tissue (mixture of 3 bulbs) was homogenised in 60 ml of buffer, consisting of 1.1 g *O*-(carboxymethyl)hydroxylamine hemihydrochloride (OCMHA) dissolved in 1 l of a methanol and deionised water mixture (4 : 1, V/V), and 2 ml of the internal standard norleucine, consisting of 2.5 g norleucine dissolved in 0.5 l of a methanol and deionised water mixture (4 : 1, V/V). The sample was homogenised for 1 minute. The re-

Table 1. List of analysed onion cultivars – contents of cysteine sulfoxides in the dry matter (average content of DM 12%), phenolic compounds and antioxidant activity measured in analysed onion samples

Cultivar	Maintainer	Alliin	Isoalliin	Methiin	Propiin	Phenolic compounds	Antioxidant activity
		ACSOs (g/kg DM)				(g/kg DM)	
Dormo	Cora Seeds Srl (Cesena, IT)	19.59 ± 1.92	12.34 ± 1.55	2.43 ± 0.25	0.34 ± 0.11	3.04 ± 0.30	0.75 ± 0.05
Spirit	Nickerson Zwaan BV (MADE, NL)	16.52 ± 2.12	13.48 ± 1.88	1.98 ± 0.24	0.39 ± 0.05	2.47 ± 0.35	0.68 ± 0.07
Bingo	BejoZaden BV (Warmenhuizen, NL)	23.38 ± 1.56	17.76 ± 1.63	2.4 ± 0.23	0.61 ± 0.07	3.32 ± 0.21	0.87 ± 0.09
Elbrus	SVS Holland BV (Enkhuizen, NL)	15.22 ± 1.95	17.19 ± 2.12	1.73 ± 0.12	0.34 ± 0.14	3.09 ± 0.17	0.82 ± 0.04
Sturon	Kees BroersenZaden (Tuitjenhorn, NL)	14.30 ± 1.89	27.74 ± 1.77	1.63 ± 0.29	0.41 ± 0.16	2.66 ± 0.16	0.76 ± 0.11
Elenka	Kees BroersenZaden (Tuitjenhorn, NL)	17.77 ± 1.42	14.38 ± 2.04	1.56 ± 0.12	0.67 ± 0.03	3.37 ± 0.14	0.83 ± 0.09

ACSOs – *S*-alk(en)yl-L-cysteine sulfoxides; DM – dry matter

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Table 2. List of SSR markers used in our study (Jakše *et al*. 2005) including their fluorescent labels, number of alleles detected per marker (Panel set of primers for multiplex reaction)

SSR	Fluorescent label	No. of allels detected
Panel M		
ACM013	6FAM	2
ACM017	VIC	2
ACM018	NED	2
Panel N		
ACM004	6FAM	3
ACM066	VIC	1
ACM068	NED	1
Panel O		
ACM091	6FAM	2
ACM093	VIC	2
ACM094	NED	2
Panel P		
ACM105	6FAM	1
ACM112	VIC	3
ACM115	NED	2
Panel R		
ACM146	6FAM	2
ACM151	VIC	3
ACM170	NED	2

sulting slurry was transferred to a 50 ml centrifuge tube, centrifuged (5 min, 10 000 rpm, 20°C) and filtered through a microfilter (0.22 µm) into a fresh vial. The extract was used for further analysis. Samples prepared according to the procedures described above were kept refrigerated (4°C) until analysis. The analyses were run in three technical replicates.

To quantify the sulphur amino acids, solvent calibrations in the concentration range of 0.1–20 ng/ml were prepared. Standards (methiin, propiin, alliin, isoalliin) were provided by the University of South Bohemia, České Budějovice, Czech Republic.

LC-MS/MS sulphur-containing amino acid analysis. The measurement of sulphur amino acid content was performed by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (U-HPLC-ESI-QQQ-MS/MS) in a positive electrospray ionization mode.

The chromatographic analysis was conducted using an Acquity UPLC™ system (Waters Corp., Parsippany, USA) with an Acquity UPLC® BEH Amide column (100 mm × 2.1 mm i.d., 1.7 µm) at 40°C and a flow rate of 0.6 ml/minute. The mobile phase consisted of water with 0.4% formic acid (A) and acetonitrile with 0.2% formic acid (B) in a gradient elution: 0–3 min, 10–15% (A); 3–8 min, 15–60% (A); 8–12 min, 10% (A).

Mass spectrometry detection was performed on a QTRAP MS system (AB Sciex, Framingham, USA) equipped with an ESI source.

Antioxidant activity. The determination of antioxidant activity was performed spectrophotometrically using DPPH (2,2-diphenyl-1-picrylhydrazyl) (Carmona-Jiménez *et al.* 2014). A calibration curve was prepared using ascorbic acid in the concentration range from 0.1 to 1.7 µm.

To determine the antioxidant activity, the samples were diluted in deionised water at the 1 : 3 ratio (V/V). After one hour, the absorbance of each sample was measured at 517 nm against a blank prepared in the same way but without ascorbic acid.

Phenolic compounds. The determination of phenolic compounds was performed spectrophotometrically employing the Folin-Ciocalteu method (Sanchéz-Rangel *et al*. 2013; Vázquez *et al*. 2015). A calibration curve was prepared using phloroglucinol in the concentration range from 2 mg/l to 1000 mg/l. To determine the phenolic compounds, the samples were diluted in deionised water at the 1 : 3 ratio (V/V). After one hour, the absorbance of each sample was measured at 725 nm. The blank was prepared in the same way but without phloroglucinol.

Data analysis. An agglomerative hierarchical cluster analysis for both sets of markers used (SSR and antioxidant activity, ASCO content, and polyphenol content) was performed using the UPGMA algorithm for Euclidean distances. The Pvclust package (Suzuki & Shimodaira 2006) of the "R" software (R Core Team 2013) was used to assess uncertainty in the hierarchical clustering. For this purpose, the AU (approximately unbiased) *P*-values were calculated by multiscale bootstrap resampling (nboot = 10 000). We considered clusters to be stable and strongly supported by data if they had AU *P*-values greater than or equal to 70%. The robustness of the dendrograms was tested via the calculation of cophenetic correlation values for each dendrogram. The correlation between the original dissimilarity matrix and the cophenetic matrix was assessed using the Mantel test of matrix correspondence (Mantel 1967) with 10 000 permutations. The cophenetic values were also used to evaluate the similarity between the dendrogram based on SSR polymorphism and the data from Table 1 reflecting antioxidant activity, ASCO content, and polyphenol content. The cophenetic matrixes for both sets of markers were constructed, and the correlation between

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them was calculated using the Mantel test of matrix correspondence (Mantel 1967).

RESULTS AND DISCUSSION

The onion is a reservoir of biologically active compounds. As individual cultivars differ in their content of such compounds, basic onion cultivars grown in the Czech Republic, which were provided by local commercial producers, were analysed. Seven days after harvest, the alliin content in onion bulbs varied from 14.3 to 23.38 g/kg of dry matter (DM), and the isoalliin content ranged from 12.34 to 27.74 g/kg of DM; these compounds were the most prevalent. The quantities of other ASCOs are listed in Table 1. The total amount of ASCOs varied from 32.38 to 44.16 g/kg of DM. Cvs Sturon and Bingo, maintained by Kees Broersen Zaden (Netherlands), contained the highest level of ASCOs. Cv. Bingo is a modern cultivar differing from the others as noted by the maintainer. This cultivar is suitable for mechanical harvest. Cv. Sturon is a traditional variety of the Rhineland onion type that has become a favourite for its stable yield. Other varieties contained 10% less ASCOs than cvs Sturon and Bingo.

At the time of harvest, alliin was the most prevalent ASCO in our samples, except for cv. Sturon, although other authors reported a prevalence of isoalliin (Yamazaki *et al.* 2010; Bernaert *et al.* 2012). Starkenmann *et al.* (2011) found a much higher level of isoalliin than alliin in onion tissues. Kubec and Dadáková (2008) did not detect even traces of alliin in the fresh tissue of onion bulb. However, they did not measure ASCO content immediately after harvest. The onion bulb is metabolically active due to the presence of enzymes. Therefore, the ASCO content during vegetation, harvest, and storage may vary, as can other metabolites. Some authors have suggested the possibility of ASCO content changes during storage in *Allium* species. Environmental conditions and farm practices influence the final composition of onion bulbs (Caruso *et al.* 2014; Kimura *et al.* 2014). Further work will therefore focus on the determination of ASCO content during storage.

The measured content of other constituents contributing to the health-promoting activities of the onion, represented by phenolic compounds, ranged from 2.66 to 3.37 g/kg of DM, with the maximum in cv. Elenka, a brown-skinned cultivar of Italian origin. Similar values were reported for a yellow cultivar by Bystricka *et al.* (2015), who used also the Folin-Ciocalteu protocol. In addition, the total antioxidant activity, which reflects the amount of compounds that can react with DPPH radicals (e.g. antioxidants) (Xie *et al.* 2014) and behave in the same way in the human body, was measured. The highest antioxidant activity was found in cv. Bingo (0.87 g/kg of DM) and the lowest value in cv. Spirit (0.68 g/kg of DM).

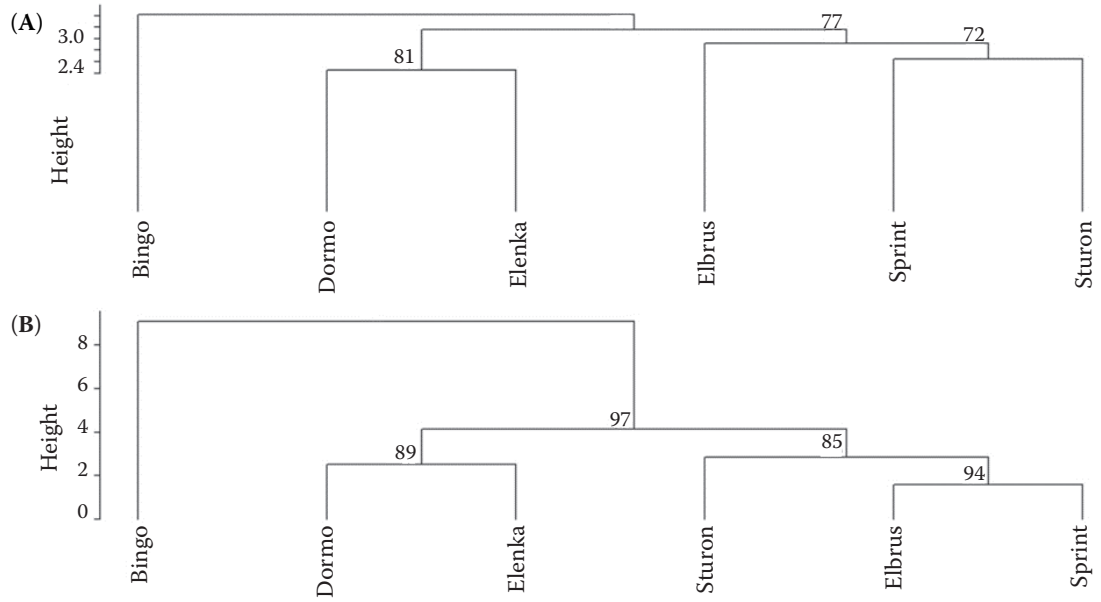

Figure 1. A dendrogram showing associations among 6 onion cultivars computed by Euclidean distances and average linkage (UPGMA) clustering method for (**A**) SSR polymorphism and (**B**) ASCO content, polyphenolic compound content and antioxidant activity. The numbers above each node represent approximately unbiased (AU) *P*-values, calculated by the multiscale bootstrap resampling (nboot = 10 000). The height indicates dissimilarity of each cluster

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The same set of cultivars was subjected to evaluation of DNA variability by the SSR length polymorphism analysis. DNA extraction was performed from leaves as identical results are obtained regardless of the tissue type used for analysis (Ovesná *et al.* 2014). The varieties were clearly identified, and each was assigned a typical DNA profile. The presence or absence of individual markers was used to visualise associations among the cultivars. Grouping of the cultivars by the UPGMA clustering method separated them into three groups (Figure 1A). The same procedure was applied to visualise associations based on antioxidant activity, ASCO content and polyphenol content in Table 1 (Figure 1B). Cultivars were clustered into the same groups in both analyses. Cv. Bingo did not associate with the other cultivars, which corresponds to the maintainer's claims about the specific characteristics of this cultivar. Two hybrid cultivars, cv. Dormo and cv. Elenka, were grouped together. Cvs Elbrus, Spirit, and Sturon, all developed by various Dutch companies, constituted the third cluster.

Cophenetic correlation values showed goodness of fit between the original data matrix and the resulting dendrograms. The Mantel test using 10 000 permutations revealed a positive and significant cophenetic correlation coefficient for SSR data ($r = 0.899$, $P \leq 0.01$) as well as for the chemical analysis data listed in Table 1 reflecting antioxidant activity, ASCO content and polyphenol content ($r = 0.800$, $P \leq 0.01$). The correlation between dendrograms based on SSR data and ASCO content, polyphenolic compound content and antioxidant activity (Table 1) was 0.825 ($P < 0.05$). This value indicates goodness of fit between the two sets of markers.

In a previous study (Ovesná *et al.* 2011) the analysis of 135 genotypes revealed that the total cysteine sulphoxide content is genetically variable in garlic (*Allium sativum* L.). Other authors have also reported correlations between the genetic background estimated by molecular markers and antioxidant capacity or ASCO content in *Allium* species (Kamenetsky *et al.* 2005; Tedeschi *et al.* 2014). Molecular markers, namely SSR, are widely used in plant breeding as they are associated with certain traits (Collard & Mackill 2008). Presented data confirmed that a previously developed SSR marker set (Mitrová *et al.* 2015) could be used not only for cultivar authentication but also as quality markers associated with a certain value of antioxidant capacity. A broader set of onion genotypes cultivated under different environmental conditions or farming practices has to be analysed to validate individual SSR markers as these factors may have impacts upon analysed traits.

CONCLUSIONS

Onion cultivars differ in their content of biologically active compounds. Alliin was the most prevalent ASCO at harvest. A good correlation between the genetic background of cultivars and antioxidant activity, ASCO content and polyphenol content was demonstrated. The study indicates the need for further monitoring of changes in the content of biologically active substances during onion storage.

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