Effect of foliar application of selenium on the content of selected amino acids in potato tubers (*Solanum tuberosum* L.)

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ABSTRACT

Selenium (Se) is an important element associated with the enhancement of antioxidant activity in organisms. Potato is very suitable for fertilisation with Se (biofortification). The experiment was performed to examine the effect of foliar application of Se as sodium selenite (200 or 400 g Se/ha) at the tuberisation stage on a spectrum of amino acids in tubers of varieties. The trends of amino acids were consistent in both years of the study. Application of Se increased the relative content of total essential (EAA) and non-essential (NEAA) amino acids relative to the controls (Karin: EAA 16.81–21.73% and NEAA 14.18–18.63%; Ditta: EAA 4.71–13.00% and NEAA 5.78–6.49%). The increase in the content of phenylalanine (Phe) was particularly significant (up to 48.9%) when also the contents of aspartic acid (Asp), glutamic acid (Glu), threonine (Thr), and tyrosine (Tyr) increased significantly compared with the controls. The results of changes in the content of isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), valine (Val), alanine (Ala), arginine (Arg), proline (Pro), cysteine (Cys), glycine (Gly), histidine (His), and serine (Ser) were also discussed. The highest dose of selenium is shown as a stress factor. Its toxic effects resulted in a change of amino acid contents.

Keywords: functional foods; human health; glutathion-peroxidase

Selenium (Se) is an important element associated with the enhancement of antioxidant activity in plants, animals and humans (Rayman 2002). Beneficial effects of Se were reported explored in terms of plant protection against abiotic stress (Hartikainen and Xue 1999), plant protection against reactive oxygen compounds (Ríos et al. 2009), activator of the protective mechanism that reduces oxidation stress for example in chloroplasts (Seppänen et al. 2003), phloem-feeding aphids and herbivorous caterpillars (Hanson et al. 2004), and fungal diseases (Hanson et al. 2003). Selenium has a positive effect also on potato carbohydrate accumulation and possibly on yield formation (Turakainen et al. 2004).

The theoretical explanation for the antioxidative effects of Se on plants is the increased activity of the enzyme glutathion-peroxidase (GSH-Px) in selenium-treated plants. However, no Se-containing GSH-Px was identified in plants, in contrast to animals (Dayer et al. 2008, Ríos et al. 2009). Seppänen et al. (2003) discovered that Se reduced the activity of superoxide dismutase (SOD) and in some cases the amount of tocopherols.

Notwithstanding the above effects, high rates of Se are detrimental to plants and reduce biomass production. At adequate rates, which are different for individual groups of plants, Se is beneficial to plants (Ducsay et al. 2009). In experiments of Turakainen et al. (2004) potato yields increased even at a rate of 0.3 mg Se/kg of substrate. In plants of wheat, Se toxicity was not observed within the range of 4–200 g Se/ha (Lyons et al. 2005). Most species of cultivated plants, including potatoes, rank among the so-called non-accumulators of Se. Under standard conditions

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they contain 0.01 to 1.0 mg Se/kg of dry matter and are capable of accumulating Se in amounts of up to 25 mg/kg of dry matter.

Replacing the sulphurous amino acids Cys and Met with the selenium amino acids selenomethionine (Se-met) and selenocysteine (Se-cys) can disrupt biochemical reactions. There are several ways in which selenium may express its toxicity (Spallholz and Hoffman 2002). The selenium present in plants that are resistant to high rates of Se is a component of non-protein amino acids (Whanger 2002).

Turakainen et al. (2006) reported that most of the Se in potato tubers is a component of the protein fraction (49–65%) and that the concentration is lower than in leaves. Cuderman et al. (2008) reported that 30% of total Se in potato tubers is a constituent of protein. Munshi et al. (1990) discovered that selenite (SeO_3^{2-}) increased the protein content in tubers and reduced the content of free amino acids. Some Se is a component of the non-protein fraction in tubers (starch, water) and the starch content is positively correlated with the Se rate applied (Ferri et al. 2007).

Selenium metabolism is closely connected with the metabolism of nitrogenous substances in plants but particularly with amino acids. Against this background we studied the effect of selenium fertilisation in relation to the content of amino acids, depending on the variety and year. This is one of the ways of producing functional foodstuffs providing the human organism with a higher intake of this important element.

MATERIALS AND METHODS

A small-plot field experiment with foliar applications of Se in the form of sodium selenite (Na_2SeO_3) was carried out in the Czech Republic in 2007 and 2008, using divided plots at the experimental station of the Potato Research Institute Havlíčkův Brod in Valečov. The site lies in the main potato-producing region, at an altitude of 450 m. In 2007 the mean annual precipitation was 664.3 mm and mean annual temperature was 8.8°C, while the corresponding values for 2008 were 695.7 mm and 8.9°C. The experiment consisted of three treatments and four replicates. The treatments comprised a control; application of a single dose of 200 g Se/ha; and application of a split dose of 400 g Se/ha (200 + 200 g Se/ha, with 7 days between the doses). The Se was applied as a foliar application at the tuberisation stage. Two varieties of consumer potatoes were tested in the experiment – the early variety Karin and the semi-early variety Ditta. Agrochemical properties of the soil at the site, which is characterised as a Cambisol, are given in Table 1.

During the growing season the plots were kept free of weeds and protected against *Phytophthora infestans* Mont. and *Leptinotarsa decemlineata* Say. The tubers were harvested at the beginning of physiological maturity (Karin on 16 August 2007, on 18 August 2008; Ditta 7 days later). After harvest, the potato tubers were washed in water, peeled, cut into slices and dried at 60°C. Dry samples were then homogenised in a test mill.

Tuber samples for Se assessment were mineralised in a mixture of HNO_3 and H_2O_2 in the microwave Milestone MLS 1200 MEGA (Sorisole, Italy) and then analysed using the method of atomic absorption spectrometry on UNICAM 939 SOLAAR (Cambridge, UK) by means of the method of hydride production using the vapour system UNICAM VP 90 (Cambridge, UK).

The total content of amino acids was determined as described by Lošák et al. (2010). The results of the chemical analyses were processed statistically with the programme STATISTICA 8.0 using the multi-factorial variance analysis (MANOVA) and correlations were examined using the Pearson's correlation coefficient (*r*).

Table 1. Agrochemical properties of the soil at the experimental site

Evaluated using	Content of the element (mg/kg) and evaluation grade			
	2007		2008	
$P_{MehlichIII}$	71	satisfactory	151	high
$K_{MehlichIII}$	144	satisfactory	185	good
$Ca_{MehlichIII}$	1395	satisfactory	1568	satisfactory
$Mg_{MehlichIII}$	96	low	140	satisfactory
$S_{water\ soluble}$	18.2	–	12.9	–
$Se_{2\ mol/L\ HNO_3}$	< 0.10	low	< 0.10	low
pH/$CaCl_2$	5.4	acid	6.2	slightly acid

RESULTS AND DISCUSSION

With further applications of Se its content in potato tubers increased as reported Turakainen et al. 2004. The content of Se was higher in the Karin tubers than in Ditta in both years of the study. The average content of Se in the dry matter of Karin tubers was 1.562 mg/kg at a rate of 200 g Se/ha and 2.027 mg/kg at a rate of 400 g Se/ha; in the dry matter of Ditta tubers the average Se contents ranged between 0.693 mg/kg at a rate of 200 g Se/ha and 1.129 mg/kg at a rate of 400 g Se/ha. Plant nutrition and fertilisation can significantly influence the amino acid content in plant tissues (Lošák et al. 2010, Pavlík et al. 2010). The general trends in the spectrum of amino acids in tubers of both varieties were similar in both years of the experiment. The total content of selected amino acids was higher in tubers of all Se-treated plants than in the controls (Tables 2 and 3). This confirms the findings of Munshi et al. (1990), although they also found that the total content of amino acids increased continuously with the increasing rate of soil applications of Se. Guo and Wu (1998) also reported an increase in the content of amino acids in *Melilotus indica* L. plants after Se treatment. In the present study, foliar application of a single low dose of Se (200 g Se/ha) had a more positive effect than a double dose (400 g Se/ha) on tuber amino acid content, increasing it by 19.8% in Karin (Table 2) and 8.4% in Ditta (Table 3) compared with the control plants. The increase in amino acid levels with the Se dose of 400 g/ha was 15.2% in Karin and 5.9% in Ditta compared with the controls. The results also showed that the response of both varieties comprised a different intensity of amino acid synthesis. This trend was similar in the case of essential and non-essential amino acids. After application of the higher dose of Se, a further small (< 1%) increase in the total content of non-essential amino acids was detected in Ditta. In contrast, Koutník (1996) discovered

Table 2. Relative and absolute content of amino acids in potato tuber DM of Karin variety

	Treatment					
	1		2		3	
	%	g/kg	%	g/kg	%	g/kg
EAA						
Ile	100.00	1.98[a]	109.32	2.17[a]	107.98	2.14[a]
Leu	100.00	2.97[a]	111.08	3.30[a]	107.06	3.18[a]
Lys	100.00	2.72[a]	109.95	2.99[a]	107.71	2.93[a]
Met	100.00	1.32[a]	98.07	1.29[a]	98.19	1.29[a]
Phe	100.00	4.45[a]	148.87	6.62[c]	138.80	6.18[b]
Thr	100.00	1.93[a]	142.01	2.74[b]	132.38	2.56[b]
Val	100.00	2.96[a]	108.03	3.20[a]	105.91	3.14[a]
NEAA						
Ala	100.00	2.37[a]	105.47	2.49[a]	102.07	2.41[a]
Arg	100.00	2.63[a]	107.46	2.82[a]	106.16	2.79[a]
Asp	100.00	9.01[a]	132.82	11.96[b]	126.11	11.36[b]
Glu	100.00	7.74[a]	113.71	8.80[b]	110.89	8.58[b]
Pro	100.00	2.01[a]	130.13	2.62[a]	124.84	2.51[a]
Cys	100.00	1.25[a]	116.78	1.46[a]	114.63	1.43[a]
Gly	100.00	2.08[a]	105.90	2.20[a]	98.59	2.05[a]
His	100.00	0.85[a]	105.40	0.89[a]	103.70	0.88[a]
Ser	100.00	1.63[a]	106.85	1.74[a]	98.68	1.61[a]
Tyr	100.00	1.58[a]	122.97	1.95[b]	121.89	1.93[b]
Σ EAA	100.00	18.33	121.73	22.32	116.81	21.42
Σ NEAA	100.00	31.13	118.63	36.93	114.18	35.55
Σ AA	100.00	49.47	119.78	59.25	115.15	56.96

1 – controls, untreated with selenium; 2 – 200 g Se/ha; 3 – 400 g Se/ha; EAA – essential amino acids; NEAA – non-essential amino acids; AA – amino acids

that the total content of amino acids decreased after foliar application of Se.

In terms of essential amino acids, the most intensive increase after Se treatment in both years of the experiment and in both varieties was in the content of Phe, which increased significantly (r = 0.586; P = 0.003). On application of 200 g Se/ha the average increase was by 46% higher than in the control, while on application of 400 g Se/ha it was higher by 31%. Munshi et al. (1990) also detected an increase in the Phe content after Se fertilisation. In their experiment, the Phe content in potato tubers increased from 20% to 53% with an increasing rate of Na_2SeO_3 application. Adámková et al. (2006) reported that one of the defence mechanisms of plants against stress is the production of phenylpropanoids, which are derived from Phe and Tyr. In our experiment the Tyr content increased in Se-treated potato tubers of both varieties. The metabolism of phenylpropanoids forms many substances that have significant antioxidant effects and biological significance (Harmatha 2005). The positive correlation observed here between Se application and Phe synthesis is probably another mechanism which is triggered under conditions of stress. The total anti-oxidative activity of potatoes is the combined result of the anthocyan and phenylpropanoic acid content (Lachman et al. 2005).

As regards the essential amino acids, the content of Thr also underwent some noteworthy changes. In the variety Karin (Table 2), the Thr level increased significantly after application of 200 and 400 g Se/ha, by 42.0% and 32.4%, respectively, compared with the controls (r = 0.721; P = 0.008). However, when the variety Ditta was treated with these two rates of Se the Thr level decreased, although not by a statistically significant amount (Table 3). Thus in our case, the positive effect of Se application on the Thr content was connected with the variety. In their experiments with potatoes, Munshi et al. (1990) discovered that Se application had a positive effect on the Thr content. The contents of other essential amino acids in potatoes of both varieties treated with Se in the present study were similar

Table 3. Relative and absolute content of amino acids in potato tuber DM of Ditta variety

	Treatment					
	1		2		3	
	%	g/kg	%	g/kg	%	g/kg
EAA						
Ile	100.00	2.32[a]	103.50	2.40[a]	97.06	2.25[a]
Leu	100.00	3.67[a]	106.67	3.92[a]	102.41	3.76[a]
Lys	100.00	3.14[a]	106.83	3.35[a]	103.86	3.26[a]
Met	100.00	1.29[a]	101.18	1.30[a]	99.10	1.27[a]
Phe	100.00	5.26[a]	142.50	7.49[c]	123.84	6.51[b]
Thr	100.00	2.97[a]	98.11	2.92[a]	88.96	2.65[a]
Val	100.00	3.07[a]	102.93	3.16[a]	99.03	3.04[a]
NEAA						
Ala	100.00	2.85[a]	103.19	2.94[a]	96.18	2.74[a]
Arg	100.00	3.04[a]	96.53	2.94[a]	95.07	2.89[a]
Asp	100.00	10.77[a]	123.03	13.25[b]	128.58	13.85[b]
Glu	100.00	11.16[a]	96.34	10.76[a]	101.70	11.35[a]
Pro	100.00	2.65[a]	99.31	2.63[a]	85.88	2.28[a]
Cys	100.00	1.21[a]	98.26	1.19[a]	97.59	1.18[a]
Gly	100.00	2.21[a]	102.10	2.26[a]	95.31	2.11[a]
His	100.00	0.86[a]	101.00	0.87[a]	103.26	0.89[a]
Ser	100.00	1.87[a]	98.96	1.85[a]	91.69	1.72[a]
Tyr	100.00	1.66[a]	109.56	1.82[a]	106.58	1.77[a]
Σ EAA	100.00	21.72	113.00	24.55	104.71	22.75
Σ NEAA	100.00	38.31	105.78	40.53	106.49	40.80
Σ AA	100.00	60.04	108.39	65.07	105.85	63.55

1 – controls, untreated with selenium; 2 – 200 g Se/ha; 3 – 400 g Se/ha; EAA – essential amino acids; NEAA – non-essential amino acids; AA – amino acids

and the effect of Se application on their metabolism was not statistically significant. The lower dose of Se had a more positive effect on the content of the individual essential amino acids than the higher dose. Methionine was the limiting essential amino acid, as previously reported by Millard (1986) and Eppendorfer and Bille (1996). Application of Se to our plants did not affect the Met content in the potato tubers, confirming previous results reported by Koutník (1996). In contrast, Munshi et al. (1990) reported an increase in Met after Se application. The effect of the year on the Met content in our study was statistically significant only in the variety Ditta ($P = 0.027$).

The results for non-essential amino acids are summarised in Tables 2 and 3. The application of Se most markedly influenced the content of Aspartic acid (Asp), which increased significantly in Se-treated tubers of both varieties in both experimental years. Asp increased on average by 27.6% compared with the controls. In both varieties there was a strong positive correlation (r) between the application of Se and the Asp content; $r = 0.740$ ($P = 0.006$) and $r = 0.827$ ($P = 0.001$) in the varieties Karin and Ditta, respectively. The Asp content was also affected differently depending on the variety ($r = 0.548$; $P = 0.006$). Asparagine (Asn) results from amidation of aspartic acid (Asp). Munshi et al. (1990) discovered that the total content of Asn increased on average by 13% in Se-treated potato tubers. Aspartic acid and Asn are important in the transport and storage of nitrogen in plant tissues. They perform this function even under stress when the plant is not able to ensure the standard level of proteosynthesis (nutrient deficiency, drought, salting, heavy metal contamination, pathogen attack) (Lea et al. 2007). Aspartic acid is yet another amino acid the content of which was positively affected by Se application and which in plant metabolism is connected with reaction to stress. The elevated content of Tyr is also worth noting (Tables 2 and 3). In the variety Karin it increased by as much as 23% and in the variety Ditta by almost 10%. The correlation between the increased content of Tyr and Se rate was positive ($r = 0.501$) and the effect of the year was significant ($r = 0.585$; $P = 0.003$). These results are in agreement with Munshi et al. (1990), who found that the Tyr content increased only when the lowest rate of Na_2SeO_3 was applied. Together with Phe, Tyr is a precursor of phenylpropanoids (Adámková et al. 2006), which participate in the defence reactions of plants. The production of phenylpropanoids is triggered by stress factors, such as UV radiation; insufficient nitrogen, phosphorus and iron nutrition; low temperature; injury; and pathogen attack (Dixon and Paiva 1995).

The content of Pro, the free form of which is connected with the resistance of plants to drought and salinisation (Lea et al. 2007), was not statistically significantly influenced by Se application (Tables 2 and 3). The effect of the variety on the Pro content was noticeable; in the variety Karin it increased by as much as 30%, while in the variety Ditta the Pro content decreased by up to 14% after the Se treatment. Munshi et al. (1990) observed that after Se application the content of Pro increased and was positively correlated with the increasing rate of Se application. Other authors, e.g. Guo and Wu (1998), did not detect any significant change in the Pro content after Se application.

In Karin, the Glu content also increased significantly after the Se treatment. The content of Cys in Karin increased by 16.8% and 14.6% after application of 200 and 400 g Se/ha, respectively, whereas in Ditta the Cys content decreased after Se application. We were unable to prove statistically that Se application influenced the content of Cys. Its content was significantly affected in relation to the variety ($P = 0.001$). In experiments by Ríos et al. (2009) with lettuce plants (*Lactuca sativa* L.), the Cys content in leaves was influenced by the form of Se applied. The Cys content decreased with the increasing rate of selenite (SeO_3^{2-}) application and increased with the increasing rate of selenate (SeO_4^{2-}) application. Ramos et al. (2010) described different effects of various forms of Se on the metabolism of lettuce plants (*Lactuca sativa* L.). These results imply that the response of Cys metabolism to Se is the result of a combination of effects determined by plant species, variety and form of applied Se. In the group of non-essential amino acids, the lowest content detected in this study was of His.

We may conclude by saying that foliar application of Se had a positive effect on the metabolism of amino acids in both potato varieties tested. This was positively reflected in tuber yields, particularly of plants treated with a lower rate of Se, which confirms the findings of Turakainen et al. (2004). An important factor in Se fertilisation is an adequate level of nitrogenous nutrition and naturally also of other biogenic elements (Ducsay et al. 2009), which would be able to inhibit any toxic effect of Se on the plants. The increased intensity of amino acid metabolism may be another mechanism by which Se participates positively in a number of documented cases of increased resistance of plants to stress, the key amino acids probably being Phe and Asp.

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