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Acta Bot. Croat. 75 (1), 17–24, 2016
DOI: 10.1515/botcro-2016-0016

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Selenium uptake and Se compounds in Se-treated buckwheat

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Abstract – In field experiments, tartary buckwheat and hybrid buckwheat were foliarly sprayed with an aqueous solution of sodium selenate (20 mg Se L^{-1}). In treated plants, the selenium content was significantly higher than in controls, irrespective of the plant part and taxon of buckwheat. The highest average Se concentrations in hybrid and tartary buckwheat were found in seeds. The main Se species found in seeds was Se-methionine. Selenium-sprayed plants had higher photochemical efficiency of photosystem II in both taxa and higher electron transport system activity in hybrid buckwheat, suggesting a positive effect of Se on physiological characteristics. Because of the concentration of Se in both buckwheat taxa and selenomethionine as the dominant species of Se, Se-enriched buckwheat is a potential source of dietary Se for animals and humans.

Keywords: hybrid buckwheat, selenite, SeMet, speciation, tartary buckwheat

Abbreviations: ETS – electron transport system, F_v, F_m, F_0 – variable, maximal and minimal chlorophyll fluorescence level from dark-adapted leaves, PSII – photosystem II, Se – selenium, SeMet – Se-methionine

Introduction

Buckwheat is becoming a very important alternative crop in Europe because it is well suited to ecological cultivation, and its use in the diet has many positive effects on the health and nutritional status of consumers (Bonafaccia et al. 2003b). It has favourable medicinal properties, because of its antioxidative (Holasova et al. 2002), anti-inflammatory and anti-carcinogenic effects and because it can increase the strength of blood vessels. Buckwheat also has significant health benefits for individuals with diabetes, obesity and constipation (Li and Zhang 2001). The buckwheat herb, especially tartary buckwheat (*Fagopyrum tataricum* Gaertn.), known to be a rich source of flavonoids, is used in herbal medicinal products, for green buckwheat tea, for producing buckwheat green leaf flour as an additive to some food products. In addition the fresh green plant parts are used as a vegetable (Fabjan et al. 2003, Kreft et al. 2006). Tartary buckwheat seeds contain proteins with a high biological value and a well-balanced amino acid composition, relatively high crude fiber content and vitamins B_1, B_2 and B_6 (Bonafaccia et al. 2003b). Its seeds are also an important dietary source of Zn, Cu and Mn (Bonafaccia et al. 2003a, Pongrac et al. 2011) and of phenolic compounds with antioxidant properties, such as rutin, quercetin and quercitrin (Fabjan et al. 2003). Tartary buckwheat is grown and used in mountainous regions of south-west China (Sichuan), in northern India, in Bhutan and Nepal (Bonafaccia et al. 2003b). In Europe, tartary buckwheat is currently grown as a crop only in a small area of north-west Europe (Bonafaccia et al. 2003a), and recently in Slovenia and Sweden.

Under the name *Fagopyrum hybridum* the most productive progenies F10 and later generations of hybrids *F. tataricum* (4x = 32) × *F. giganteum* are united. The taste and other characteristics of hybrid buckwheat grain are mostly similar to those of tetraploid bitter buckwheat. It possesses a rough grain of intermediate type with a high fraction of hulls (Fesenko and Fesenko 2010). Since hybrid buckwheat is a new buckwheat taxon, recently obtained in Orel, Russia, by interspecific crossing (Fesenko and Fesenko 2010), very little is known about its characteristics and properties.

Selenium (Se) is an element essential in humans and animals for normal functioning of a number of Se-dependent antioxidant enzymes, such as glutathione peroxidase and thioredoxin reductase (Brown and Arthur 2001). It also exerts beneficial effects in cancer prevention and can posi-

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tively influence many functions by reducing inflammation, and heart disease and regulating blood pressure (Brozmanová et al. 2010). This element, however, can also be toxic in higher amounts, its toxicity depending on its chemical form, concentration and other environmentally regulating variables. Worldwide, deficiency of Se in human diet is common and natural supplements have been recommended to increase daily Se intake (Pyrzynska 2009).

Deficiency of Se is associated with many diseases and with deterioration in the health status of animals and humans. The Se supply in almost all European countries is below the recommended daily intake, which is 30–70 μg Se per day for adult Europeans (German Nutrition Society 2002). In two separate locations around the Rivers Drava and Sava in eastern Croatia, the mean content of selenium in foods was measured and the authors concluded that the dietary Se intake in both areas is likely to be between suboptimal and adequate (Klapec et al. 2004). In another study in Croatia, Matek et al. (2000) produced a report on the estimated dietary Se intake, which showed that Se intake for the observed group of females from the Zagreb area is lower than in the majority of European countries, and lower than the value recommended by the World Health Organisation. The authors claimed however that the risk for the inhabitants of the zone of developing Se deficiency is low. Se bioavailability strongly depends on the chemical form of Se found in the diet. Organic Se compounds are more bioavailable than inorganic forms. Se-enriched plants seem an ideal source of Se for animals and humans, because they contain many different chemical forms of Se, many of which are highly bioavailable (Navarro-Alarcon and Cabrera-Vique 2008).

The Se content in vegetables from many areas of the world reflects low levels in soil, and hence crops and food enriched with Se during cultivation could be an effective means of producing Se-rich foodstuffs (Pyrzynska 2009). Foliar spraying of buckwheat plants with a solution of sodium selenate at the beginning of flowering has a beneficial effect on the Se content in all above-ground plant organs, such as seeds, leaves, and stems (Smrkolj et al. 2006b, Vogrinčič et al. 2009). As plants are the main source of dietary Se, metabolism of Se in plants is important for the Se nutrition of humans and animals. Knowledge of locations of Se accumulation and its speciation is important because the anti-carcinogenic effects of Se depend on the species (Carey et al. 2012).

The essential nature of Se to plants remains unclear. Even though there is some evidence for its positive effect at low concentrations on many plant processes (Germ et al. 2007a), at higher concentrations it can induce negative effects on plant physiology (Hartikainen et al. 2000). Some research work has proposed that Se at low concentrations can increase the tolerance of plants exposed to various abiotic stressors (Germ et al. 2007a, Djanaguiraman et al. 2010).

Thus the purpose of the current study was to determine the effect of foliar spraying with Se-containing compounds on the physiological responses of plants determined by measurements of primary and terminal flow of electrons, on the content and the species of Se in the above-ground parts of the new and promising hybrid buckwheat and to compare the results with those in tartary buckwheat. Because of the potential toxicity of Se we will examine the plant vitality and the end of vegetative phenological phase by measuring electron transport activity. We hypothesized that Se treatment would significantly benefit physiological plant responses, including photochemical efficiency of photosystem II (PSII) and respiratory potential, in tartary as well as in hybrid buckwheat. We also hypothesized that Se treatment would increase the content of bioavailable Se in seeds of both buckwheat taxa.

Material and methods

Plant material and growth conditions

Tartary (*F. tataricum*) and hybrid (*F. hybridum*) buckwheat seeds were sown on a 10 m^2 experimental plot of the Biotechnical Faculty of the University of Ljubljana, Ljubljana. The experiment lasted from late June to late September, during which time the mean temperature was 21.1 °C and the mean humidity 67% (http://meteo.arso.gov.si/met/sl/ archive/). The control and treated groups of both buckwheat taxa were grown under the same conditions. At the beginning of flowering, half of the plants, the treated groups, were sprayed with a sodium selenate solution containing 20 mg L^{-1} of Se and a detergent (0.2 mL L^{-1} Triton X-100, Sigma), while control groups were sprayed only with detergent. Two weeks after Se spraying, chlorophyll fluorescence and terminal electron transport system (ETS) activity were measured. From the date of spraying with Se to the date of measurement of chlorophyll fluorescence and ETS activity the mean temperature was 24.5 °C and the mean humidity 62.3%. In that time there was a total of 181 hours of sunshine (http://meteo.arso.gov.si/met/sl/ archive/). Thirteen weeks after sowing, when the majority of grains reached their ripe phase, samples of both taxa and both groups were collected. For each taxon and treatment one sample of plants (10–15 plants) was taken for determination of Se content and Se speciation. Plants were air-dried and separated into individual parts: stems, leaves, seeds and husks and lyophilized using a Christ Alpha freeze dryer, then homogenized in an agate planar micro mill at 2600 rpm (Fritsch, Pulverisette 7, Idar-Oberstein, Germany).

Determination of physiological parameters

Fluorescence measurement, a non-destructive method, allows the rapid assessment of the quantum yield of electron flow through the PSII. This method has been widely used for detection of various stresses in plants. Murchie and Lawson (2013) reported that chlorophyll fluorescence can be used to determine the primary signals by active and passive methods. Chlorophyll fluorescence was measured *in situ* with a fluorometer (PAM 2500 Portable Chlorophyll Fluorometer, WALZ) in 6 vital plants (on the first fully developed leaf) from each treatment group and from both taxa of buckwheat. Measurements of minimal (F_0) and maximal (F_m) chlorophyll fluorescence were made after 15 min of darkness, provided by dark-adaptation clips. Fluorescence

was excited with a saturating beam of "white light" (PPFD = 8000 μmol m^{-2} s^{-1}, 0.8 s). The difference between F_m and F_0 is termed the variable fluorescence (F_v). The potential photochemical efficiency of PSII was evaluated in terms of the ratio F_v/F_m. The effective photochemical efficiency was determined after saturation with a 0.8 s pulse of white light (PPFD = 9000 μmol m^{-2} s^{-1}). The effective photochemical efficiency was calculated as $(F_m' - F)/F_m' = \Delta F/F_m'$, where F_m' is the maximum fluorescence signal of an illuminated leaf after a pulse of saturating light and F is the steady state fluorescence (Schreiber et al. 1996).

The respiratory potential of mitochondria was measured in the plants by terminal electron transport system (ETS) activity as described by Packard (1971) and modified by Kenner and Ahmed (1975). Fresh leaves of plants (0.006 – 0.012 g) were homogenized in a final volume of 4 mL of ice-cold homogenization buffer (pH = 8.4), followed by ultrasonic homogenization (4710, Cole-Parmer, Vernon Hills, IL, USA) for 20 sec at 40 W. The homogenates were centrifuged for 4 min at 0 °C at 10000 rpm (Sigma 2–16 PK, Germany). Within 10 min, 0.5 mL of supernatant (in triplicate) was added to a mixture of 1.5 mL substrate solution containing NADH, NADPH, Triton X-100, and 0.5 mL 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) reagent solution and incubated for 40 min at 20 °C. Formazan production was determined spectrophotometrically (Lambda 12, Perkin-Elmer, Norwalk, CT, USA) from the absorbance of the sample at 490 nm against the blank within 10 min of stopping the reaction with a 1:1 mixture of formaldehyde and phosphoric acid. ETS activity per dry weight (DW) was calculated according to Kenner and Ahmed (1975). ETS activity was measured on 5 plants from each treatment group and from both taxa of buckwheat.

Determination of total Se content

The total Se content was determined using hydride generation atomic fluorescence spectrometry (HG-AFS) in all plant parts of Se-treated and control buckwheat. We weighed 0.2 g of a lyophilized sample into a Teflon tube and Se content was determined in three aliquots. Digestion of samples was carried out in the closed tubes with a mixture of H_2SO_4, HNO_3, H_2O_2 and V_2O_5. HF was added only to samples containing fibers. Subsequently, the reduction of Se(VI) to Se(IV) was carried out by adding concentrated HCl and heating at 90 °C for 10 min. After digestion and reduction of samples, they were diluted with Milli-Q water and the Se content was determined by HG-AFS. Details of the method of digestion and optimal measurement conditions have been described in detail by Smrkolj and Stibilj (2004). The accuracy of the method was checked with the certified reference material spinach leaves (National Institute of Standards and Technology – NIST 1570a) and good agreement was found between the values obtained (115 ± 2 ng Se g^{-1}) and the certified value (117 ± 9 ng Se g^{-1}). Se in residues from the enzymatic extraction process was determined by the same procedure mentioned above. For total Se determination in supernatants from the enzymatic extraction process, 1 mL of concentrated HNO_3 was added to 0.5 g of supernatant. The mixture was heated for 30 min at 80 °C and then for 15 min at 160 °C in a closed Teflon tube. After cooling, 0.5 mL H_2O_2 was added followed by heating of the mixture for 10 min at 120 °C, and this procedure was repeated twice more. Afterwards the reduction of Se(VI) to Se(IV) was carried out by the addition of concentrated HCl and heating at 90 °C (Pograjc et al. 2012).

Selenium species determination

Soluble Se species were extracted from samples by enzymatic hydrolysis. The sample (0.6 g) and a solution (8 g) containing 60 mg of the enzyme protease XIV were placed in a centrifuge tube and the mixture was shaken for 24 h in a water bath at 37 °C at 200 rpm. After extraction the samples were centrifuged at 11000 rpm at 4 °C for 60 min (5804R Eppendorf). The supernatants were separated from the residue and filtered successively through 0.45 μm and 0.22 μm Millex GV filters (Millipore Corporation). Supernatants and residues for total Se determination were stored at –20 °C prior to analysis. Working standard solutions in Milli-Q water of 100 ng g^{-1} of Se species containing selenite (Se(IV)), selenate (Se(VI)), Se-methionine (SeMet), Se-cystine ($SeCys_2$) and Se-methylselenocysteine (SeMeSeCys) were prepared daily by dilution of a stock solution (10 μg g^{-1}).

The concentrations of extracted Se species were determined using an ion-exchange high-performance liquid chromatography system coupled to an inductively coupled plasma mass spectrometry (HPLC-ICP-MS) apparatus (Agilent 7500ce, Tokyo, Japan). The Se species were separated on a Hamilton PRP-X 100 anion exchange column and a Zorbax 300-SCX cation exchange column. We used 1 mM and 10 mM citrate buffers in 2% methanol as the successive mobile phases for anion-exchange chromatography, and 3 mM pyridine buffer in 2% methanol as mobile phase for cation-exchange chromatography. The method and optimal operating conditions have been described in detail elsewhere (Cuderman et al. 2008).

The accuracy and precision of the results obtained for Se species determination were tested using the reference material wheat gluten (NIST 8418), for which SeMet data can be found in the literature. We found good agreement between our values (1.39 ± 0.08 μg g^{-1}) and the published values (1.53 ± 0.03 μg g^{-1}) obtained by Wolf and Goldschmidt (2007).

Statistical analysis

For statistical analysis SPSS Statistic software, version 20, (IBM) was used. The normal distribution of the data was tested with Shapiro-Wilk tests. Differences in ETS activity and in chlorophyll fluorescence between control and treated plants were evaluated by one-way ANOVA followed by Tukey's post-hoc multiple comparison tests. Differences at $p < 0.05$ were considered to be statistically significant. For Se concentration, only one sample was made for each treatment and each taxon, analysed in three aliquots, making it impossible to perform statistical analysis of the data. Results were given as means of three aliquots and standard deviation.

Results

Plant vitality after Se treatment

Hybrid buckwheat had a significantly higher F_v/F_m than tartary buckwheat in the control as well as in the Se-treated groups. Addition of Se significantly increased the potential photochemical efficiency in both taxa of buckwheat (Fig. 1). In addition to the potential photochemical efficiency, we also monitored the effective photochemical efficiency of PSII. However, there were no statistically significant differences in effective photochemical efficiency between control and treated groups of both taxa (data not shown).

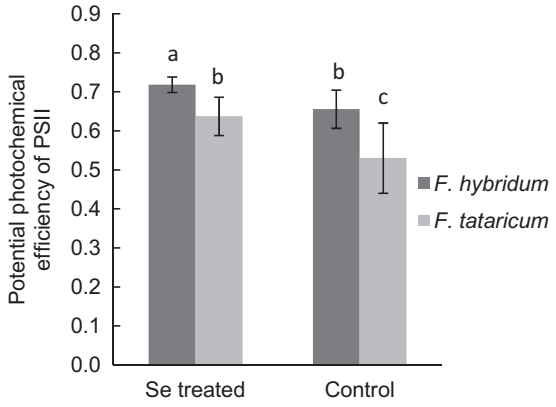

Fig. 1. Potential photochemical efficiency of photosystem II (PSII) in selenium-treated hybrid (*Fagopyrum hybridum*) and tartary (*F. tataricum*) buckwheat. Columns not sharing the same letter are significantly different ($p < 0.05$), ($n = 6$).

Hybrid buckwheat had a higher ETS activity than tartary buckwheat in both (control and treated) groups of plants (Fig. 2). Addition of Se significantly increased ETS activity in hybrid buckwheat but not in tartary buckwheat.

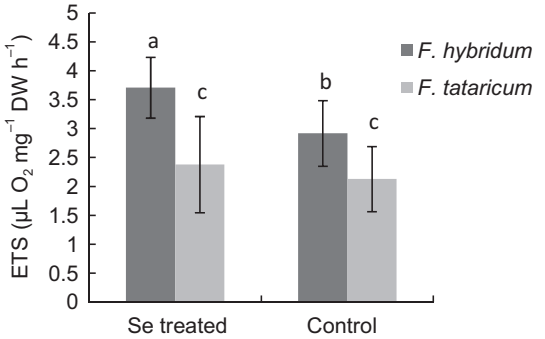

Fig. 2. Terminal electron transport system (ETS) activity in selenium-treated hybrid (*Fagopyrum hybridum*) and tartary (*F. tataricum*) buckwheat. Columns not sharing the same letter are significantly different ($p < 0.05$), ($n = 5$). DW – dry weight.

Concentration of Se in different plant parts

The Se content in the control groups of both buckwheat taxa was low (< 0.05 µg Se g^{-1}) in all plant parts. The measured values were near or below the detection limit, which was between 0.01 and 0.02 µg Se g^{-1} dry weight. The content of Se was about 30% lower in all plant parts of hybrid buckwheat in comparison to tartary buckwheat in Se-treated plants (Fig. 3). Foliar fertilization with sodium selenate increased the content of Se in all plant parts, especially in leaves and seeds where values in the treated groups were 186-fold (leaves) and 64-fold (seeds) higher than in the control groups for hybrid buckwheat and 83-fold (leaves) and 276-fold (seeds) higher for tartary buckwheat (Fig. 3). In Se-treated buckwheat the leaves and seeds within each taxon contained similar concentrations of Se, while in stems and husks lower concentrations of Se than in seeds and leaves were found (Fig. 3). A similar trend in the distribution of Se was also observed in both control groups of buckwheat (Tab. 1).

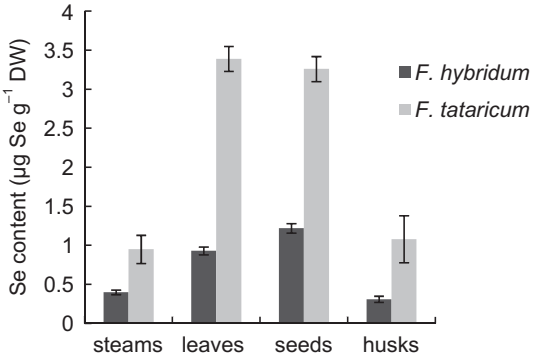

Fig. 3. Distribution of selenium in plant parts of selenium-treated hybrid (*Fagopyrum hybridum*) and tartary (*F. tataricum*) buckwheat. Data represents means ± standard deviations of three aliquots of composite sample. DW – dry weight.

Tab. 1. Concentration of selenium (µg g^{-1} DW) in controls and in selenium-enriched samples of hybrid and tartary buckwheat. Results are expressed as the average of three measurements of one sample ± standard deviation. DW – dry weight, Se – selenium.

Plant part	Hybrid buckwheat		Tartary buckwheat	
	control	Se-enriched	control	Se-enriched
stems	0.011±0.007	0.40±0.03	0.019±0.0007	0.95±0.18
leaves	0.005±0.001	0.93±0.05	0.041±0.002	3.39±0.16
seeds	0.019±0.004	1.22±0.06	0.013±0.006	3.59±0.52
husks	0.005±0.004	0.31±0.04	0.048±0.009	1.08±0.30

Selenium speciation

In Se-enriched buckwheat seeds, approximately 47% (hybrid buckwheat) and 44% (tartary buckwheat) of the total Se found in seeds was soluble (Tab. 2). To determine if losses of Se occur during enzymatic extraction, a mass balance was drawn up between the total Se in the lyophilized sample and the content of Se in the supernatant and residue. The mass balance for seeds and leaves of both taxa was approximately 100%, indicating that there was no loss of Se during extraction. The only soluble Se species identified in seeds by HPLC-ICP-MS was SeMet. SeMet in supernatant was confirmed by the standard addition method (Fig. 4). SeMet represented only 37% of the total Se content in seeds of hybrid buckwheat and 32% of the total Se content in

Tab. 2. Selenium (Se) speciation and selenium content (μg g^{-1} DW) in supernatants and residues of Se-enriched hybrid (*Fagopyrum hybridum*) and tartary (*F. tataricum*) buckwheat seeds and leaves. Results are expressed as the average of three measurements of one sample ± standard deviation; nd – not detected; * according to total Se content. DW – dry weight, SeMet – Se-methionine.

Plant part	Se content (μg g^{-1})		% of soluble Se	SeMet	
	residue	**supernatant**		(μg g^{-1})	(%)*
seeds					
F. hybridum	0.63±0.048	0.57±0.012	47.5	0.45±0.074	37.5
F. tataricum	2.10±0.044	1.70±0.08	44.7	1.22±0.018	32.1
leaves					
F. hybridum	0.99±0.048	0.07±0.006	6.6	nd	nd
F. tataricum	3.6±0.19	0.14±0.012	3.7	nd	nd

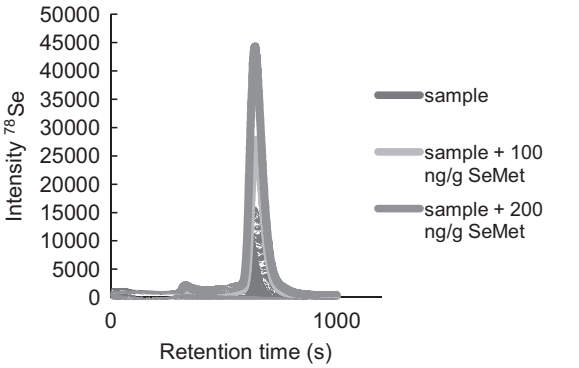

Fig. 4. Chromatogram of selenium (Se) species in enzyme hydrolysis extracts of the seeds of foliarly treated tartary buckwheat (SeMet, Se-methionine) on the anion column (PRP-X 100), with addition of standard solution of SeMet.

seeds of tartary buckwheat (Tab. 2). SeMet in the extract was stable, since the peaks of SeMet in extracts from both taxa two and four days after extraction were comparable.

After enzymatic hydrolysis of Se-enriched buckwheat leaves, only 6.6% of the Se was present in a soluble form in hybrid buckwheat and 3.7% in tartary buckwheat, but using HPLC-ICP-MS under the described conditions, we were unable to identify any of the Se species in the supernatants.

Discussion

Plant vitality after Se treatment

The control and treated groups of hybrid and tartary buckwheat had a lower potential photochemical efficiency (F_v/F_m) than the theoretical maximum value (Fig. 1), which ranges from 0.80 to 0.83 for a variety of unstressed dark adapted plants (Schreiber et al. 1996). The values of F_v/F_m reflect the maximum efficiency at which light absorbed by light harvesting antennae of PSII is converted to chemical energy (Baker and Rosenqvist 2004). The F_v/F_m ratio reflects photooxidative damage of PSII occurring during stress (Critchley 1998). Higher values of F_v/F_m in hybrid buckwheat in comparison to tartary buckwheat in the control as well as in the Se-treated groups could mean that the hybrid buckwheat has a better ability than tartary buckwheat to suppress photoinhibition reactions in the case of stressful environmental conditions, which would represent an advantage of this taxon in increasingly stressful environmental conditions.

The addition of Se significantly increased the potential photochemical efficiency in both taxa of buckwheat (Fig. 1), suggesting a positive effect of Se in reducing the photoinhibitory effects of environmental stressors. Potential photochemical efficiency was also higher in the foliarly Se-treated progeny of tartary buckwheat plants sprayed with 10 mg Se(VI) L^{-1} than the controls in the study from Kreft et al. (2013). A positive effect of Se on F_v/F_m was demonstrated in the cultivation of strawberries in soil enriched with Se (0.1 mg Se kg^{-1}soil and 1 mg Se kg^{-1} soil in the form of H_2SeO_4), but the same treatment had no positive effect on barley (Valkama et al. 2003). Zhang et al. (2014) found out that soil application of selenite (50 g Se h^{-1}) enhanced the activity of the photosynthetic system by increasing F_v/F_m. In the study by Diao et al. (2014) addition of 0.05 mM Se (Na_2SeO_3) in hydroponic experiments caused an increase in the maximum quantum yield of PSII (F_v/F_m) in two cultivars of tomato. Further, foliar spraying with Na-selenate solution did not influence the potential photochemical efficiency in pumpkins (1.5 mg Se L^{-1}) (Germ 2005), in chicory (1 mg Se L^{-1}) (Germ et al. 2007b), in common buckwheat (1 mg Se L^{-1}) (Breznik et al. 2005, Tadina et al. 2007), red cabbage (soil fertilized 30 times with Se (VI) 2 μg L^{-1}, or twice with 0.5 mg L^{-1} Se) (Mechora et al. 2011), or in cucumber plants (2–80 μM of Se) under hydroponic conditions (Hawrylak-Nowak et al. 2015).

The general metabolic activity of different organisms may be assessed by measurement of the ETS activity in mitochondria (Packard 1971). The addition of Se enhanced ETS activity in hybrid buckwheat but not in tartary buckwheat. Tadina et al. (2007) studied the effect of Se addition on two cultivars of common buckwheat, Pyra and Siva. Foliar spraying with selenate (1 mg L^{-1}) significantly increased ETS activity in the cultivar Siva and decreased it in the cultivar Pyra. However, Breznik et al. (2005) did not notice significant differences in ETS activity between control and Se treated (foliar application of Na-selenate solution at a concentration 1 mg L^{-1}) groups of tartary buckwheat or of common buckwheat. Foliar treatment with selenate (1.5 mg L^{-1}) had no effect on ETS activity in pumpkin plants (Germ 2005). In accordance with our results, the addition of Se increased ETS activity in chicory (foliarly sprayed with Na-selenate solution with concentra-

tion 1 mg L^{-1}) (Germ et al. 2007b), in foliarly sprayed young pea plants (10 mg L^{-1}) (Smrkolj et al. 2006a) and three weeks after germination in the Se-treated progeny of tartary buckwheat plants (Kreft et al. 2013). A possible explanation for the increased ETS activity in Se-treated buckwheat plants could be increased glutathione peroxidase (GPx) activity in mitochondria. Hartikainen et al. (2000) observed that Se exposure induced GPx activity in ryegrass and Hawrylak-Nowak et al. (2015) investigated the effect of selenite or selenate with different concentrations (2–80 μM) on cucumber plants. The root respiratory activity considerably increased with increasing selenite concentrations, suggesting the upregulation of mitochondrial dehydrogenases activity. Increased ETS activity in hybrid buckwheat by the addition of Se is in line with findings that the addition of Se to *Vigna radiata* enhanced respiratory as well as succinate dehydrogenase activity and the involvement of Se in mitochondrial membrane functions (Easwari and Lalitha 1995). Germ et al. (2009) also observed that Se is involved in activation of energy resources in green alga *Zygnema* sp.

Concentration of Se in different plant parts

The Se content in plants is largely dependent on the Se content in soil. Se concentrations in cultivated plants are usually low (< 0.1 μg Se g^{-1}) (Ihnat 1989). Our results revealed that hybrid and tartary buckwheat were moderate sources of Se (Tab. 1). For comparison, the Se content in seafood ranges from 0.4 to 1.5, in meat from 0.1 to 0.4 and in fruits and vegetables less than 0.1 μg Se g^{-1}, all on a dry weight basis (Navarro-Alarcon and Cabrera-Vique 2008).

All plant parts in both buckwheat taxa treated with Se showed the ability of buckwheat to accumulate high concentrations of Se. The highest concentration of Se in Se enriched plants was found in seeds and leaves while considerably lower concentrations were found in stems and husks in both taxa studied. Similar distribution patterns of Se in common buckwheat plants were observed by Vogrinčič et al. (2009), who used a lower concentration of Se for foliar spraying. Higher contents of Se in seeds and leaves than in stems was also observed by Ožbolt et al. (2008) in an experiment in which buckwheat was grown from seeds previously soaked in solutions of sodium selenate and sodium selenite.

The results indicated that selenate applied by foliar spraying was efficiently transported from leaves to seeds. Similar conclusions were obtained for tartary buckwheat in the study performed by Golob et al. (2015) who added Se to soil in concentrations of 0.002, 0.5 and 10 mg L^{-1} or sprayed plants with Se at concentrations of 0.5 mg L^{-1}. Successful transport of Se from leaves to seeds of a foliarly sprayed barley crop was also observed by Macleod et al. (1998). It was found that hybrid buckwheat had significantly lower concentrations of Se in stems, leaves, seeds and husks than tartary buckwheat. Hybrid buckwheat displayed a higher photochemical efficiency of PSII (Fig. 1) and a higher respiratory potential (Fig. 2) than tartary buckwheat in both control and treated groups. These results indicated that hybrid buckwheat was less sensitive to selenium spraying and was more metabolically active than tartary buckwheat.

Selenium speciation

In Se-enriched buckwheat seeds around 47% (hybrid buckwheat) and 44% (tartary buckwheat) of the total Se found in seeds was soluble (Tab. 2). For comparison, Smrkolj et al. (2006b) reported that the great majority of Se(VI) added to tartary buckwheat by foliar application was transformed to SeMet, 99% of the total Se in seeds. Vogrinčič et al. (2009) also observed a higher percentage of SeMet, 59% of the total Se content in common buckwheat seeds, compared to the present results. The lower percentage of soluble Se in seeds in the present study could be due to the use of other plant taxa, a different concentration of Se in the foliar solution or different growing conditions. The experiments of Smrkolj et al. (2006b) were carried out under controlled conditions in the laboratory, whereas our experiment took place outdoors in the field, where plants were exposed to varying weather and radiation conditions, and to other factors that can influence plant metabolism.

Good conversion of selenate to selenomethionine in seeds was also demonstrated in cereals. Stadlober et al. (2001) reported that SeMet is the predominant form of Se found in seeds of cereals (wheat, barley and rye) grown in soil to which fertilizer supplemented with selenate had been added.

The results showed that the majority of Se in leaves was insoluble, and as such could not be harmful to the plants, although in tartary buckwheat the concentration of Se was high, > 3 μg g^{-1}. These results are consistent with those of Smrkolj et al. (2006b), who found about 7% of soluble Se in buckwheat leaves which had been foliarly sprayed, (15 mg Se L^{-1}), of which only 3% was identified as Se(VI). Vogrinčič et al. (2009) also obtained similar results for the solubility of Se in Se-enriched common buckwheat leaves (foliar spraying, 10 mg Se L^{-1}). After enzymatic hydrolysis with protease XIV they found around 14% of soluble Se. Because of the concentration of Se in both buckwheat taxa and SeMet as the dominant species of Se, Se-enriched buckwheat is a potential source of dietary Se for animals and humans.

Hybrid buckwheat responded to enhanced levels of Se by higher photochemical efficiency of PSII and by enhanced potential respiratory activity, but tartary buckwheat only by higher photochemical efficiency of PSII. This revealed that Se at this concentration did not have negative effects on the physiological characteristics of buckwheat plants. On the contrary, additional Se increased the vitality of the plants. A large proportion of the soluble Se in Se-enriched buckwheat seeds was in the form of SeMet, which is known to be the major nutritional source of Se for animals and humans. All this indicates that Se-enriched buckwheat could form a potential source of supplementary dietary Se for animals and humans. In all plant parts of hybrid buckwheat we found about one third lower Se concentrations than in tartary buckwheat. The data presented in this study constitute the first report on potential and effective photochemical efficiency of PSII, on terminal electron transport system (ETS) activity in mitochondria and on Se uptake and distribution as well as Se speciation in plant parts for hybrid buckwheat.

Acknowledgments

This research was financed by the Ministry of Education, Science and Sport, Republic of Slovenia, through the programmes "Biology of plants" (P1-0212), "Young researchers" (34326), "Nutrition and Public Health" (P3-0395), and projects J4-4224 and J4-5524 and supported by the European project EUFORINNO (RegPot No. 315982) of the FP7 Infrastructures program. The authors are grateful to Anthony R. Byrne and Bill Milne for revising the English text.

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