

**The effect of different compounds of selenium and iodine on selected biochemical and physiological characteristics in common buckwheat and pumpkin sprouts**

Vpliv različnih oblik selena in joda na izbrane biokemijske in fiziološke lastnosti pri kalicah navadne ajde in buč

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**Abstract:** There is little data about possible interactions between selenium and iodine on plants. Se is essential for I metabolism in the thyroid in mammals. Thus, it is of great importance to carry out the research with simultaneous application of both elements in plant cultivation that are used for human consumption. Seeds of common buckwheat and pumpkins were soaked in solutions: 10 mgSe/L in the form of selenite or selenate, and 1000 mgI/L in the form of iodide or iodate and their combinations. The content of chlorophyll *a* and *b*, and carotenoids were measured. Further, the measurements of fluorescence of chlorophyll *a* were performed. Control buckwheat sprouts and sprouts from seeds soaked in Se(VI) and Se(VI)+I(-I), had the lowest and similar amount of chlorophyll *a* and carotenoids. There was little effect of different treatments on potential photochemical efficiency of photosystem II (PS II) in common buckwheat sprouts. In pumpkin sprouts neither of treatment affected the amount of photosynthetic pigments, as well as potential photochemical efficiency of (PS II) which was around 0.8.

**Key words:** sprouts, common buckwheat, pumpkins, selenium, iodine

**Izveček:** Zelo malo podatkov obstaja o interakciji med selenom in jodom pri rastlinah. Selen je bistvenega pomena za delovanje ščitnice. Zato je pomembno, da preučujemo hkraten vpliv obeh elementov na rastline, ki jih uporabljamo za hrano ljudi. Seme navadne ajde in buč smo namakali v različnih raztopinah Se in I; selenata in selenita ter jodida oz. jodata ter vseh njenih kombinacij. Merili smo sledeče fiziološke in biokemijske lastnosti kontrolnih in obravnavanih rastlin: fotokemično učinkovitost fotosistema II (FS II) ter vsebnost fotosinteznih barvil (klorofil *a*, klorofil *b* in karotenoidi). Kontrolne kalice ajde in kalice, zrastle iz semen, namakanih s Se(VI) in se(VI)+I(-I), so imele najnižjo in podobno vsebnost klorofila *a* in karotenoidov. Obravnavanja so malo vplivala na potencialno fotokemično učinkovitost fotosistema II pri kalicah navadne ajde (kalice iz semen, namakanih v Se(IV)+I(-I), so imele najnižjo potencialno fotokemično učinkovitost fotosistema II). Nobeno obravnavanje ni vplivalo na vsebnost fotosinteznih barvil in potencialno fotokemično učinkovitost

fotosistema II pri kalicah buč. Potencialna fotokemična učinkovitost fotosistema II pri kalicah buč je bila okoli 0,8.

**Ključne besede:** kalice, navadna ajda, buče, selen, jod

## Introduction

Several minerals and trace elements e.g., iodine, iron, selenium, and zinc, are essential for normal thyroid hormone metabolism (Zimmermann and Köhrle 2002). Iodine (I) and selenium (Se) are not essential nutrients for plants but both play important roles in human and animal organisms (Smoleń et al. 2014). Plant roots can take up Se as selenate, selenite or organoselenium compounds, such as selenocysteine (SeCys) and selenomethionine (SeMet) (White et al. 2004). Selenite is rapidly converted to organoselenium compounds in the root, whereas selenate is delivered to the xylem and transported to the shoot, where it is assimilated into organoselenium compounds and redistributed within the plant (Terry et al. 2000). Role of Se is beneficial in plants capable of accumulating large amounts of this element. It acted as an antioxidant, inhibiting lipid peroxidation in ryegrass and increased yield under ambient radiation conditions in pumpkins (Hartikainen et al. 2000, Germ et al. 2005). There is scarce information about the effect of iodine on plants neither possible interaction with selenium fertilization. Uptake of iodine from the soil to the plants depend from adsorption–desorption processes in the soil (Zia et al. 2014). Leaf vegetables have higher absorption capacity than fruit vegetable in ten chosen plants in the study from Weng et al. (2013). Plants take up iodine through the root system, preferably as iodide (Fuge 2005, Smoleń et al. 2011). Dai et al. (2006) evidenced that iodide (I(-1)) and iodate ( $\text{IO}_3^-$ ) added to the soil, do not significantly affect spinach biomass production.

Application of high doses of I(-1) to lettuce has a phytotoxic effect on plant physiology. In contrast,  $\text{IO}_3^-$  treatments increased the biomass of the plants which showed an elevated photosynthetic rate, stomatal conductance, and transpiration comparing to control plants. Blasco et al. (2010) reported about the response of lettuce to iodine biofortification. They found out that application of  $\text{IO}_3^-$ , in contrast to I(-1), increased biomass

production, stimulated  $\text{NO}_3^-$  reduction and  $\text{NH}_4^+$  incorporation and optimised the photorespiratory process. Zhu et al. (2003), who studied the effect of iodine on spinach found out that iodine is not beneficial to the growth of spinach (*Spinacia oleracea*), but level of iodide above 10  $\mu\text{M}$  was detrimental to yields, while iodate had little effect on the biomass production. Authors suggest that the detrimental effect of iodide on plant growth is probably due to its excessive accumulation in plant tissues. Voogt et al. (2010) reported that when they treated lettuce with I(-1) and  $\text{IO}_3^-$  no impact on plant biomass or quality was found, and accumulation of I(-1) was more effective than  $\text{IO}_3^-$ . Iodine was mainly distributed to the outer leaves in lettuce. Landini et al. (2011) treated tomato (*Solanum lycopersicum*) with I(-1) and found out that tomato plants were no sensitive to high levels of iodine, stored both in vegetative tissues and fruits. They also reported that iodine was taken up better when supplied to the roots using hydroponically grown plants. However a considerable amount of iodine was also stored after leaf treatment that indicated that iodine is transport also through phloem.

Buckwheat is a good source of nutritionally important elements (Ikeda et al. 2006). It contains proteins that have a better balance of amino acids compared with cereals (Yoon et al. 2009). Buckwheat sprouts appeared some years ago as a new vegetable (Kim et al. 2004). Seeds of buckwheat and pumpkins can be used as additives to improve the quality of bread or other products (Stibilj et al. 2004). Pumpkin seeds have been used for centuries in traditional medicine, mainly in cases of problems of the kidney or the urinary tract (Kreft et al. 2002).

Despite its importance, studies focused on the effect of Se and I on plant physiological and biochemical characteristics are scarce (Zhu et al. 2004; Smoleń et al. 2014).

The aim of the study was to determine the possible simultaneous effect of iodine and selenium on the biochemical and physiological characteristics

on common buckwheat and pumpkin sprouts from seeds soaked in Se and I solution.

## Materials and methods

Common buckwheat and pumpkin seeds were soaked in solution for 4 h in 200 mL distilled water (MilliQ) (control), or in solutions of sodium selenate (10 mg Se(VI) /L), sodium selenite (10 mg Se(IV) /L), potassium iodide (1000 mg I(-1) /L), potassium iodate (1000 mg I(V) /L) and combinations: 10 mg Se(VI) /L+ 1000 mg I(-1) /L; 10 mg Se(VI) /L+ 1000 mg I(V) /L; 10 mg Se(IV) /L+ 1000 mg I(-1) /L; 10 mg Se(IV) /L+ 1000 mg I(V) /L. After soaking seeds were distributed in plastic bowls, which were covered with filter paper. During germination, seeds were watered with tap water as needed. Sprouts were grown in controlled conditions in the growth chamber with constant temperature 22°C and 65–70% relative air humidity. Measurements were done after 12 and 10 days of growing common buckwheat and pumpkin sprouts, respectively.

Contents of chlorophyll *a* and *b* and carotenoids were determined using a UV/VIS Spectrometer System (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). The total chlorophyll content was determined as described in Lichtenthaler and Buschmann (2001a, 2001b).

Fluorescence measurements were performed on the cotyledons of randomly selected sprouts using the fluorometer (PAM 2500 Portable Chlorophyll Fluorometer, WALZ). Prior to measurements samples were dark adapted for 20 min. The fluorescence parameters recorded included minimal ( $F_0$ ) and maximal ( $F_m$ ) chlorophyll fluorescence that were provided by dark-adaptation clips. The difference between  $F_m$  and  $F_0$  is called the variable fluorescence  $F_v$ .  $F_v/F_m = F_m - F_0/F_m$ .  $F_v/F_m$  ratio is common parameter used in fluorescence which reflects the capacity to trap electrons by the photosystem (PS) II reaction centre (Schreiber et al. 1995).

### Statistical analysis

The data were evaluated by ANOVA (Statgraphics Version 4) and the differences were tested using the Duncan test with a significance level of 0.05.

## Results

Amount of chlorophyll *a* in control buckwheat sprouts was lower and statistical significantly different from amount of chlorophyll *a* in sprouts from seeds, soaked in Se(IV), I(V) and Se(IV)+I(V). The lowest amount of chlorophyll *a* was measured in the sprouts from seeds, soaked in Se(VI) and statistically different from sprouts from seeds, soaked in Se(IV), I(-1), I(V), Se(IV)+I(-1), Se(IV)+I(V) and Se(VI)+I(V) (Fig. 1). The pattern was similar regarding chlorophyll *b* although not statistically significant (data not shown).

Amount of carotenoids in control buckwheat sprouts were statistically different from the amount of carotenoids from seeds, soaked in Se(IV), I(-1), I(V), Se(IV)+I(-1) and Se(IV)+I(V). The lowest amount of carotenoids was measured in the sprouts from seeds, soaked in Se(VI) and was statistically lower from sprouts from seeds, soaked in Se(IV), I(-1), I(V), Se(IV)+I(-1), Se(IV)+I(V) and Se(VI)+I(V) (Fig. 2).

Neither of treatment affected the amount of chlorophyll *a* (Fig. 3) and carotenoids (Fig. 4) in pumpkins sprouts. Similar pattern appeared for chlorophyll *b* (data not shown).

### Photochemical efficiency of PS II

There was little effect of different treatment on potential photochemical efficiency of photosystem II (PS II) in common buckwheat sprouts. The lowest potential photochemical efficiency of PS II was measured in common buckwheat sprouts from seeds, soaked in Se(IV)+I(-1). In pumpkin sprouts, neither treatment affected potential photochemical efficiency of PS II. Values were similar and around 0.8 (Table 1).

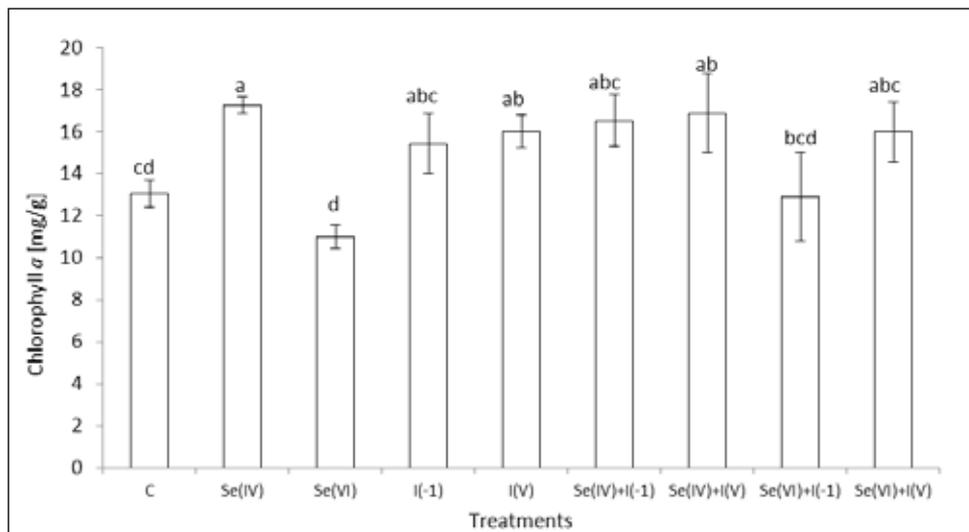


Figure 1: Concentration of chlorophyll *a* per DM in common buckwheat sprouts. Mean  $\pm$  SE,  $n = 6$ , C - control. Mean values, marked with the same letter, are not significantly different at  $p \leq 0.05$ .

Slika 1: Koncentracija klorofila *a* na SM v kalih navadne ajde. Predstavljene so povprečne vrednosti  $\pm$  SE ( $n = 6$ ). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri  $p \leq 0,05$ .

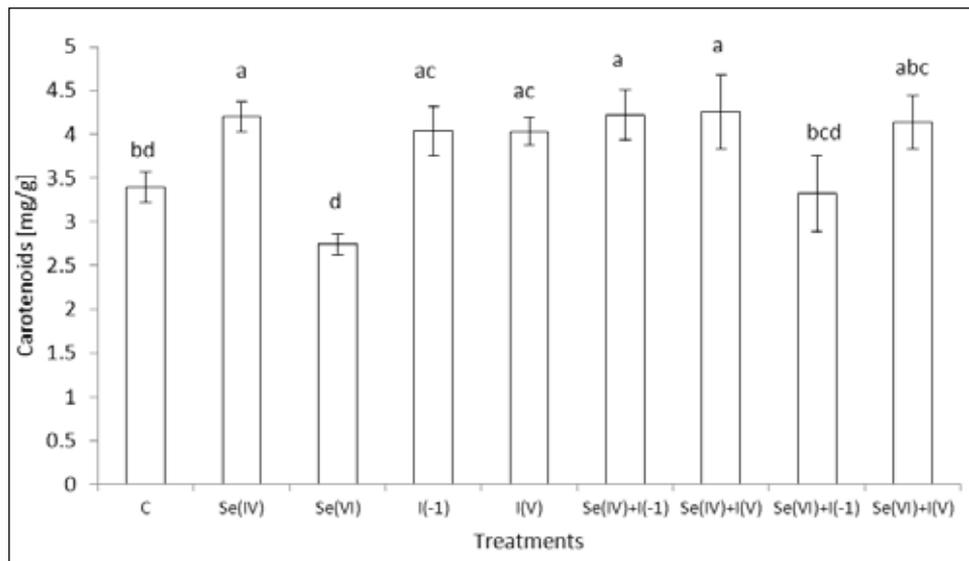


Figure 2: Concentration of carotenoids per DM in common buckwheat sprouts. Mean  $\pm$  SE,  $n = 6$ , C - control. Mean values, marked with the same letter, are not significantly different at  $p \leq 0.05$ .

Slika 2: Koncentracija karotenoidov na SM v kalih navadne ajde. Predstavljene so povprečne vrednosti  $\pm$  SE ( $n = 6$ ). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri  $p \leq 0,05$ .

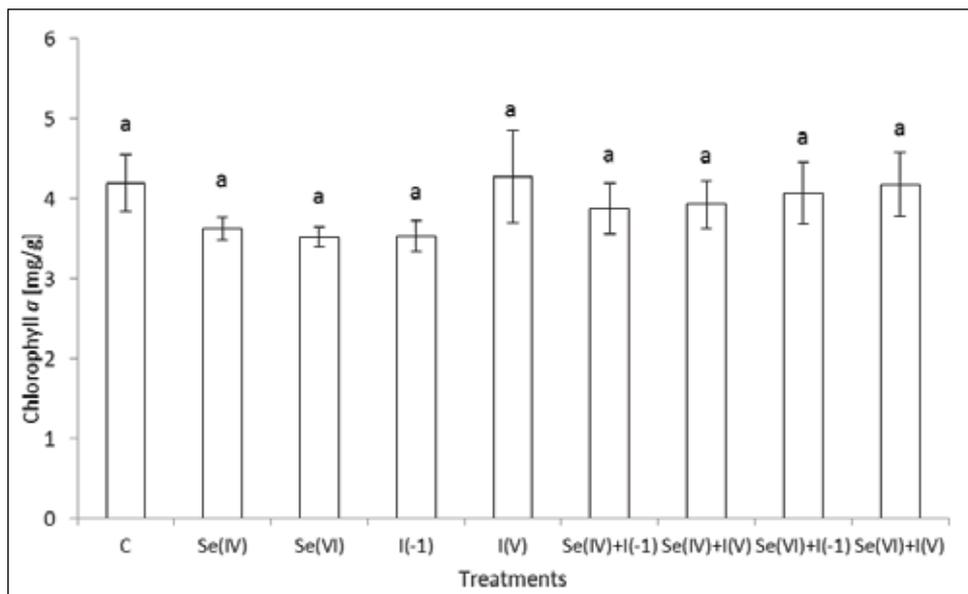


Figure 3: Concentration of chlorophyll *a* per DM in pumpkin sprouts. Mean  $\pm$  SE,  $n = 6$ , C - control. Mean values, marked with the same letter, are not significantly different at  $p \leq 0.05$ .

Slika 3: Koncentracija klorofila *a* na SM v kalicah buč. Predstavljene so povprečne vrednosti  $\pm$  SE ( $n = 6$ ). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri  $p \leq 0,05$ .

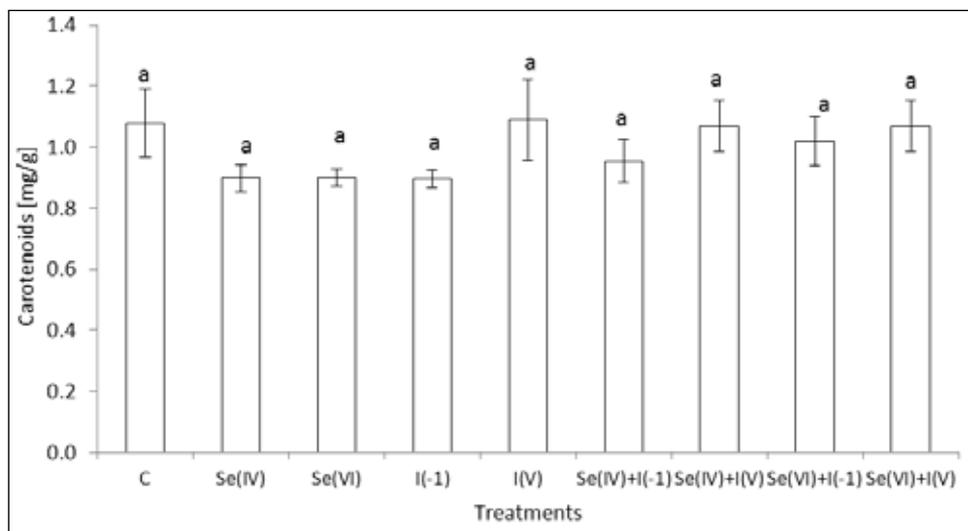


Figure 4: Concentration of carotenoids per DM in pumpkin sprouts. Mean  $\pm$  SE,  $n = 6$ , C - control. Mean values, marked with the same letter, are not significantly different at  $p \leq 0.05$ .

Slika 4: Koncentracija karotenoidov na SM v kalicah buč. Predstavljene so povprečne vrednosti  $\pm$  SE ( $n = 6$ ). C - kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri  $p \leq 0,05$ .

Table 1: Potential photochemical efficiency of PS II in common buckwheat and pumpkin sprouts. Mean values, marked with the same letter for each species, are not significantly different at  $p \leq 0.05$ , SE – standard error,  $n = 8$ .

Tabela 1: Potencialna fotokemična učinkovitost FS II v kalih navadne ajde in buč. Srednje vrednosti, označene z različnimi črkami, se med seboj statistično značilno razlikujejo pri  $p \leq 0,05$ , SE – standardna napaka,  $n = 8$ .

Treatment	Species			
	Pumpkins		Common buckwheat	
Treatment	mean	SE	mean	SE
Control	0.83a	0.006	0.72abc	0.02
SeIV	0.83a	0.003	0.75ab	0.02
SeVI	0.82a	0.002	0.70bc	0.02
I(-1)	0.83a	0.004	0.78ab	0.01
I(V)	0.83a	0.002	0.71abc	0.05
Se(IV)+I(-1)	0.83a	0.003	0.43d	0.06
Se(IV)+I(V)	0.83a	0.004	0.73ab	0.04
Se(VI)+I(-1)	0.83a	0.002	0.80a	0.01
Se(VI)+I(V)	0.83a	0.006	0.63c	0.03

## Discussion

### Biochemical response

Different forms of Se and I had different effect on the amount of chlorophyll *a*, *b*, and carotenoids. Control buckwheat sprouts and sprouts from seeds, soaked in Se(VI) and Se(VI)+I(-1) had the lowest and similar amount of chlorophyll *a* and carotenoids. There were no differences in the amount of photosynthetic pigments in pumpkin sprouts. Similar results were given by Mechora et al. (2011, 2014) who reported about similar amount of chlorophyll *a* and *b* in red cabbage and cabbage, treated with Se(VI). Cabbage was fertilized *via* the soil with an aqueous solution of Na selenate (33 mL per plant) containing  $2 \mu\text{g Se L}^{-1}$ , every second day for 2 months and second group was foliarly sprayed with  $20 \text{ mg Se L}^{-1}$  in the form of Na selenate ( $\sim 0.30 \text{ mL per plant}$ ) twice in the growing season. Red cabbage was as cabbage fertilized at a rate of 33 ml per plant, in the form of an aqueous solution containing Na selenate at a concentration of  $2 \mu\text{g Se L}^{-1}$ , every second day for two months. The second group of red cabbage was fertilized with  $0.5 \text{ mg L}^{-1}$  twice

in the growing season. In addition, on the study, performed on hydroponically cultivated lettuce, the addition of Se(IV) (2–30  $\mu\text{M}$ ) and Se(VI) (2–60  $\mu\text{M}$ ) did not affected the amount of photosynthetic pigments (Hawrylak-Nowak 2013). In sweet basil foliarly treated with (1–50  $\text{mg Se} \cdot \text{dm}^{-3}$ ) selenite, no significant effect on the content of chloroplast pigments was detected (Hawrylak-Nowak 2008a). Similarly, the concentration of Se(VI) < 10  $\text{mg/kg}$  did not affect the amount of chlorophylls in *Lolium perenne* cultivated in a soil (Hartikainen et al. 2000). Foliarly spraying with higher concentration of I(-1) and I(V) in the solution as in this study and soil fertilization (15  $\text{mg I dm}^{-3}$ ) to the radish plants with I(-1) and I(V) did also not affect the amount of photosynthetic pigments (Strzetelski et al. 2010). On the other hand Xue et al. (2001) found out that the addition of Se(VI) in lower and higher concentration (0.1  $\text{mg/kg}$  and 1.0  $\text{mg/kg}$  soil respectively) induced synthesis of chlorophyll in young lettuce leaves, while in older leaves of lettuce, only higher concentration of Se(VI) induced the amount of chlorophyll. In younger leaves of *Lolium perenne* the addition of Se(VI) in concentration  $\geq 10 \text{ mg/kg}$  in the soil lowered the amount of chlorophylls while on the

other hand enhanced the amount of chlorophyll in older leaves (Hartikainen et al. 2000).

In hydroponic experiment barley seedlings were subjected to 2, 4, 8, 16 ppm Se in the form of Se(VI). Chlorophyll content of the seedlings was affected significantly in a dose dependent manner (Akbulut and Çakır 2010). Significant decrease was observed in the chlorophyll content at  $\geq 4$  ppm Se applications, similar concentrations as used in the present study. Decrease of the total chlorophyll concentration depending on the Se form (selenate or selenomethionine) and dosage (25, 50, and 100  $\mu\text{M}$  Se) were detected in hydroponically grown maize (Hawrylak-Nowak 2008b). Similarly in Tartary buckwheat, the foliar addition of Se(VI) (1 g Se/m<sup>3</sup>) at 10 times lower concentration in comparison to our used concentration of Se(VI), also lowered the amount of chlorophylls (Breznik et al. 2005). Under hydroponic cultivation in lettuce plants a marked decrease in photosynthetic pigments concentration was found after passing the toxicity threshold, which has been designated at a level of 15  $\mu\text{M}$  for selenite and 20  $\mu\text{M}$  for selenate (Hawrylak-Nowak 2013). In the study from Strzetelski et al. (2010), soil fertilization with iodine was carried out before radish sowing to the level of 15 mg I · dm<sup>-3</sup> soil. Foliar application of this element was performed twice using iodine solution in a concentration per pure element of 0.2%, in dose of 0.4 dm<sup>3</sup> · m<sup>-2</sup>. Iodine foliar and soil application in radish, regardless of iodine forms (I(-1), IO<sub>3</sub><sup>-</sup>), dose and application method, had no significant effect on the content of dry matter, as well as on the level of photosynthetic pigments in leaves. The objective of study from Blasco et al. (2011) was to determine the effect of the application of different doses (20, 40 and 80  $\mu\text{M}$ ) and forms of iodine (iodate [IO<sub>3</sub><sup>-</sup>] and iodide [I(-1)]) on photosynthesis and carbohydrate metabolism in lettuce plants. The Chl *a* content did not differ between I(-1)-treated lettuce plants and controls but was significantly reduced in plants treated with 80  $\mu\text{M}$  IO<sub>3</sub><sup>-</sup>.

#### *Physiological response*

Potential photochemical efficiency in common buckwheat (except sprouts from seeds, soaked in Se(IV)+I(-1)) and pumpkin sprouts was mainly around 0.7 and 0.8 respectively. Values, close to theoretical maximum 0.83 (Schreiber et al.

1995) meant that different forms of Se and I and their combination did not damage photosynthetic apparatus. Similar results were given regarding common buckwheat (Breznik et al. 2005, Tadina et al. 2007) and Tartary buckwheat (Breznik et al. 2005), foliarly treated with Se(VI) (1 g Se/m<sup>3</sup>). In addition, in the experiment, where Se(VI) was added to red cabbage (Mechora et al. 2011) and chicory (Germ et al. 2007), potential as well as effective photochemical efficiency were similar in treated and control plants. In pumpkins foliar spraying with Na-selenate solution (1.5 mg Se L<sup>-1</sup>) did not influence the potential photochemical efficiency of PS II (Germ et al. 2005). A positive effect of Se on potential photochemical efficiency was reported for the strawberries, cultivated in soil enriched with Se (0.1 mg Se kg<sup>-1</sup>soil and 1 mg Se kg<sup>-1</sup> soil in the form of H<sub>2</sub>SeO<sub>4</sub>), but the same treatment had no positive effect on barley (Valkama et al. 2003).

## Conclusions

There is scarce information about the effect of different forms of I on plants and particularly with the combination with Se. Concentrations, used in the present study, mainly caused no negative effect on the biochemical and physiological characteristics of sprouts.

## Povzetek

Pri nas in po svetu se je močno povečalo zanimanje za ajdo, tudi zaradi izredno skladne sestave hranilnih snovi v njenih zrnih. Vsebuje zelo kakovostne beljakovine. V tradicionalni medicini se uporabljajo bučna semena že stoletja predvsem v primerih težav z ledvicami ali sečili. Kalice vsebujejo veliko beljakovin, vitaminov, ogljikovih hidratov, olj, rudninskih snovi in s tem pripomorejo k biološki polnovredni prehrani. Selen in jod sta elementa, ki sta ključnega pomena za pravilno delovanje ščitnice in sta nujno potrebna pri izgradnji tiroidnega hormona, njegovi aktivaciji in metabolizmu. Ker je Se neobhodno potreben za metabolizem I v ščitnici raziskovalci menijo, da je smiselno gojenim rastlinam sočasno dodajati oba elementa.

Kalice navadne ajde in buč smo gojili v rastni komori s stalno temperaturo 22°C in 65–70% r.z.v. Seme ajde in buč smo namakali v različnih raztopinah selenia in joda. Preučevali smo vpliv naslednjih obravnavanj: selenat (10 mg Se(VI)/L), selenit (10 mg Se(IV)/L), jodid (1000 mg I(-1)/L), jodat (1000 mg I(V)/L) in kombinacije 10 mg Se(VI)/L+ 1000 mg I(-1)/L; 10 mg Se(VI)/L+ 1000 mg I(V)/L; 10 mg Se(IV)/L+ 1000 mg I(-1)/L; 10 mg Se(IV)/L+ 1000 mg I(V)/L in K (kontrola – destilirana voda brez dodanega selenia in/ali joda).

Merili smo sledeče fiziološke in biokemijske lastnosti kontrolnih kalic in kalic, zrastlih iz obravnavanih semen: fotokemično učinkovitost fotosistema II (FS II) ter vsebnost fotosinteznih barvil (klorofil *a*, klorofil *b* in karotenoidov). Kontrolne kalice in kalice, zrastle iz semen, namakanih v Se(VI) ter Se(VI)+I(-1), so imele najnižjo in podobno vsebnost klorofila *a* in karotenoidov. Obravnavanja so imela majhen vpliv na potencialno fotokemično učinkovitost fotosistema

II pri kalicah navadne ajde. Namakanje semen v različnih raztopinah Se in I ni vplivalo na vsebnost fotosinteznih barvil in potencialno fotokemično učinkovitost fotosistema II pri kalicah buč.

Z raziskavo smo želeli ugotoviti, ali selen in jod, ki ju dodajamo hkrati, vplivata na kalice navadne ajde in buč. Raziskava je zanimiva zato, ker je malo podatkov o hkratnem delovanju selenia in joda na biokemijske in fiziološke parametre rastline in tudi zato, ker sta selen in jod elementa, ki sta zelo pomembna za delovanje ščitnice.

## 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) and projects J4-4224 and J4-5524.

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