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The effect of selenium and iodine on selected biochemical and morphological characteristics in kohlrabi sprouts (*Brassica oleracea* L. var. *gongylodes* L.)

Vpliv selena in joda na izbrane biokemijske in morfološke lastnosti pri kalicah kolerabice (*Brassica oleracea* L. var. *gongylodes* L.)

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Abstract: Selenium (Se) and iodine (I) are essential elements for humans and animals, while their essential role for plants has not been established yet. There is also very little information about the interaction between selenium and iodine in plants. The aim of our research was to determine the effect of different forms of Se, I and their combinations on selected biochemical and morphological characteristics of the kohlrabi sprouts (*Brassica oleracea* L. var. *gongylodes* L.). Sprouts were grown from seeds, which were soaked in different solutions of selenite, selenate, iodide, iodate and their combinations. We measured the content of chlorophyll *a* and *b*, carotenoids, anthocyanins, and UV-A and UV-B absorbing substances. We also measured potential photochemical efficiency of photosystem II (PS II). At the end of the experiment the weight and height of the sprouts were measured. In order to compare the results the entire experiment was carried out twice. Different chemical forms of Se and I, and combinations did not significantly affect the number of sprouts that germinated from seeds. The various chemical forms of Se and I, and combinations differently affected on the amount of pigments in the kohlrabi sprouts. Potential photochemical efficiency of PS II was close to theoretical maximum 0.83.

Keywords: kohlrabi, sprouts, selenium, iodine

Izvleček: Selen (Se) in jod (I) sta esencialna elementa za ljudi in živali, medtem ko njuna esencialna vloga za rastline še ni dokazana. Obstaja tudi zelo malo podatkov o interakciji med Se in I pri rastlinah, zato je pomembno, da preučujemo hkraten vpliv obeh elementov na rastline, ki jih uporabljamo za prehrano ljudi. Z raziskavo smo želeli ugotoviti, ali različne oblike Se in I posamezno ali v kombinaciji vplivajo na izbrane biokemijske in morfološke lastnosti pri kalicah kolerabice (*Brassica oleracea* L. var. *gongylodes* L.). Kalice smo vzgojili iz semen, ki smo jih namočili v osem različnih raztopin z različnimi kombinacijami in oblikami Se in I ter v kontrolno raztopino. Ostale raztopine so poleg dH_2O vsebovale posamezno dodan selenit (SeO_3^{2-}) oz. selenat (SeO_4^{2-}) s koncentracijo 10 mg Se/L, jodid (I^-) oz. jodat (IO_3^-) s koncentracijo 1.000 mg I/L in kombinacije različnih oblik Se in I (SeO_3^{2-}+ I^-, SeO_3^{2-}+ IO_3^-, SeO_4^{2-} + I^-, SeO_4^{2-} + IO^{3-}). Selen je bil dodan v obliki natrijevega selenita (Na_2SeO_3) oz. natrijevega selenata (Na_2SeO_4), I pa v obliki kalijevega jodida (KI) oz. kalijevega jodata (KIO_3). Različne kemijske oblike Se in I ter njune kombinacije niso statistično značilno vplivale na število kalic, ki so vzklile iz semen. Različne kemijske oblike Se in I ter njune kombinacije so različno vplivale na koncentracijo barvil pri kalicah kolerabice. Potencialna fotokemična učinkovitost fotosistema II je bila blizu teoretičnega maksimuma 0,83.

Ključne besede: kolerabica, kalice, selen, jod

Introduction

Selenium (Se) and iodine (I) are essential elements for humans and animals, while their essential role for plants has not been established yet (Hasanuzzaman et al. 2014). There is also scarce information about the interaction between selenium and iodine in plants. It is therefore important to study the combined effect of these two elements on plants which can be used for human consumption. Slovenia is a country with iodine deficiency, because of that fortification of salt with potassium iodide increased in 1999 to 25 mg KI per kg of salt. Later on recommended gradual decrease of salt in nutrition reduces this nutritional source of iodine. Lack of selenium in Slovenia soils is known as well (Pirc in Šajn 1997), that results to reduced selenium content in plants to the values below optimal to assure adequate nutritional supply from food of crop origin. Approximately 2/3 of the world's population has health problems associated with insufficient intake of Se and I with diet. One of the easiest ways to combat this problem is biofortification or enrichment of crops with Se and I, to increase the transfer of Se and I into the food chain (White and Broadley 2009). The primary rationale for this is that Se is essential for I metabolism in the thyroid. It was discovered that the deiodinase enzymes, which convert T4 (thyroxin) into T3 (triiodothyronine) and also T3 into T2 and thereby degrading it, are selenium enzymes. Plant roots can take up Se as selenate, selenite or organoselenium compounds, such as selenocysteine and selenomethionine. Plants are one of the main dietary sources of Se for humans and animals (Schiavon et al. 2017). Selenium is known to increase the tolerance of plants to UV-induced oxidative stress, regulate water status of drought exposed plants, delay senescence and promote the growth of ageing seedlings (Kuznetsov et al. 2003, Xue et al. 2001). In most soils, I is present in solution as iodide, although iodate can also be present under oxidizing conditions. The effect of I on biochemical and physiological process, has been scarcely evidenced (Blasco et al. 2011, Landini et al. 2011, Jerše et al. 2017). There is little data on combined effects of Se and I on physiological and biochemical characteristics and yield of plants (Zhu et al. 2004, Smolen et al. 2015, 2016). Our aims were to investigated the effect of addition of Se, I and I+Se on growth and physiological and biochemical characteristics of kohlrabi sprouts.

Materials and methods

Kohlrabi seeds were soaked in solution for 8 h in 200 mL of distilled water (MilliQ) (control), or in solutions contained selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}) with a concentration of 10 mg Se/L, iodide (I-) or iodate (IO_3-) with a concentration of 1000 mg I/L, and their combinations (SeO_3^{2-}+ I^-, SeO_3^{2-}+ IO_3^-, SeO_4^{2-} + I^-, SeO_4^{2-} + IO_3^-). Selenium was applied in the form of sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4), respectively. Iodine was applied in the form of potassium iodide (KI) and potassium iodate (KIO_3), respectively. After soaking seeds were distributed in plastic trays. Sprouts were grown in controlled conditions in the growth chamber with constant temperature 19°C and 60 % relative air humidity, and 160 µM $m^{-2}s^{-1}$ PAR, 16 h : 8 h. Measurements were done after 14 days of growing sprouts.

Contents of chlorophyll *a* and *b* and carotenoids were measured using a UV/VIS Spectrometer System (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). Chlorophyll content was determined as described in Lichtenthaler and Buschmann (2001a, 2001b). Content of anthocyanins was determined as proposed by Khare and Guruprasad (1993) and Drumm and Mohr (1978). Anthocyanins were extracted from weighed sprouts by homogenizing in a mortar and extracting with HCl:methanol = 1:99 (v/v). Absorbances of extracts were measured at 530 nm with a UV/VIS spectrometer (Lambda 25, Perkin-Elmer, Norwalk, CT, USA). Content of anthocyanins was expressed in relative units.

Content of UV-absorbing compounds was determined according to Caldwell (1968). Fluorescence of chlorophyll was performed on the cotyledons of randomly selected sprouts using the fluorometer (PAM 2500 Portable Chlorophyll Fluorometer, WALZ). Samples were dark adapted for 20 min prior to measurements. The fluorescence parameters that were recorded included minimal (F_0) and maximal (Fm) chlorophyll fluorescence and were provided by dark-adaptation clips. Fv is the variable fluorescence. Fv/Fm (Fv/Fm = Fm – Fo/Fm) 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).

Results

The percentage of germination of the individual treatments was 67 – 68%. It was in the same range in control and treated sprouts in both experiments (data not shown).

Sprouts from seeds, soaked in Se(VI), had in the first experiment statistically significantly lower concentration of chlorophyll *a* comparing to sprouts from seeds, soaked in Se(IV). Sprouts from seeds, soaked in I(-I), had statistically significantly lower concentration of chlorophyll *a* comparing to sprouts from seeds, soaked in I(V). Sprouts from seeds, soaked in Se(VI) and I(-I), had statistically significantly lower concentration of chlorophyll *a* comparing to sprouts from seeds, soaked in Se(VI)+I(V) and Se(IV)+I(-I). Sprouts from seeds, soaked in I(V), had statistically significantly higher concentration of chlorophyll *a* comparing to sprouts from seeds, soaked in Se(VI), I(-I) and Se(VI)+I(-I).

In the second experiment we determined in sprouts from seeds, soaked in Se(VI) and I(-I), statistically significantly lower concentration of chlorophyll *a* comparing to sprouts from seeds, soaked in Se(VI)+I(V), Se(IV)+I(-I) and I(V). Sprouts from seeds, soaked in Se(VI), had statistically significantly lower concentration of chlorophyll *a* comparing to sprouts from seeds, soaked in Se(VI)+I(-I) (Fig. 1).

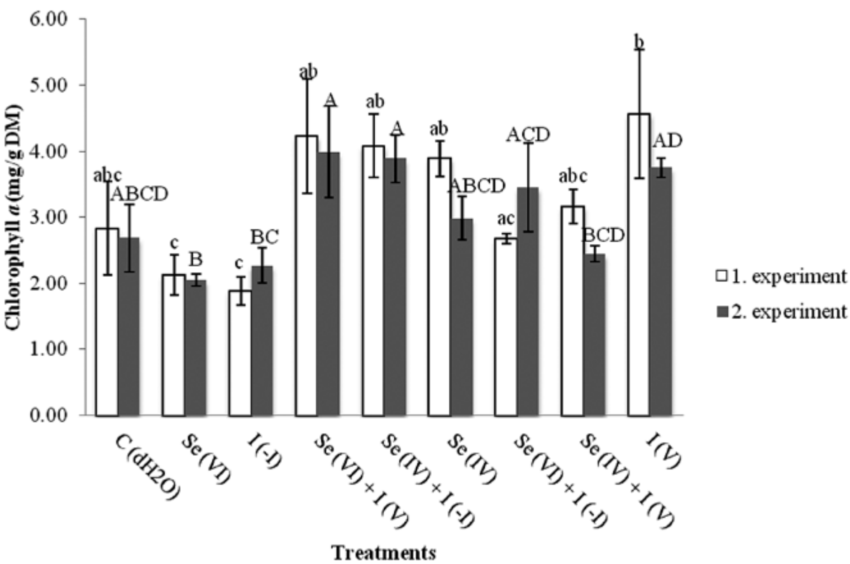

Figure 1: Concentration of chlorophyll *a* per DM in kohlrabi sprouts. Mean ± SE, n = 4, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 1: Koncentracija klorofila *a* na SM v kalicah kolerabice. Predstavljene so povprečne vrednosti ± SE (n = 4). C – kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0,05$.

In the first experiment had sprouts from seeds, soaked in Se(VI), statistically significantly lower concentration of carotenoids from sprouts from seeds, soaked in Se(IV). Sprouts from seeds, soaked in I(-I), had statistically significantly lower concentration of carotenoids comparing to sprouts from seeds, soaked in I(V). Sprouts from seeds, soaked in Se(VI) and I(-I) had statistically significantly lower concentration of carotenoids comparing to sprouts from seeds, soaked in Se(VI)+I(V) and Se(IV)+I(V). Concentration of carotenoids was similar in control and all treatments in the second experiment (Fig. 2).

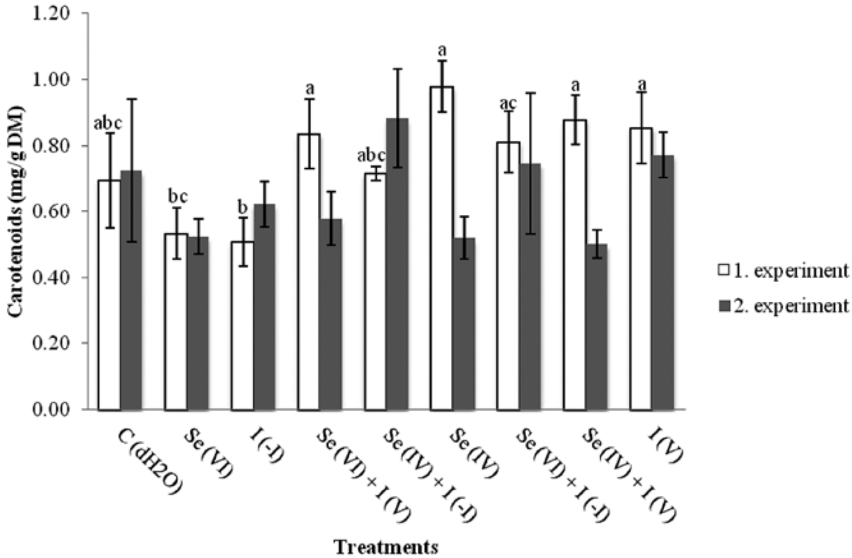

Figure 2: Concentration of carotenoids per DM in kohlrabi sprouts. Mean ± SE, n = 4, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 2: Koncentracija karotenoidov na SM v kalicah kolerabice. Predstavljene so povprečne vrednosti ± SE (n = 4). C – kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0{,}05$.

In the first experiment sprouts from seeds, soaked in Se(VI), had statistically significantly higher concentration of anthocyanins comparing to sprouts from control and from seeds soaked in other treatments.

In the second experiment had sprouts from seeds, soaked in Se(VI), statistically significantly lower concentration of anthocyanins comparing to sprouts from seeds, soaked in Se(IV). Sprouts from seeds, soaked in I(-I), had statistically significantly higher concentration of anthocyanins comparing to seeds, soaked in Se(IV) (Fig. 3).

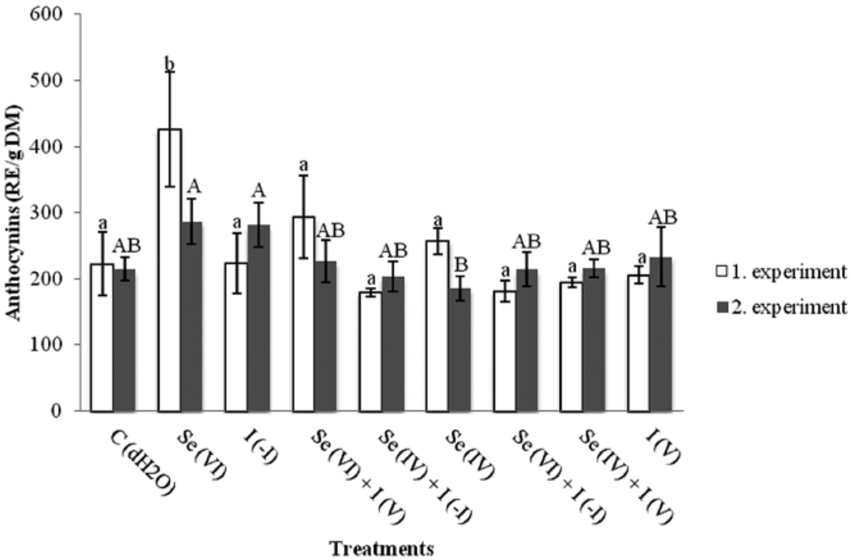

Figure 3: Concentration of anthocyanins per DM in kohlrabi sprouts. Mean ± SE, n = 4, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 3: Koncentracija antocianov na SM v kalicah kolerabice. Predstavljene so povprečne vrednosti ± SE (n = 4). C – kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0,05$.

Concentration of UV-B absorbing compounds was similar in control and treated sprouts in the first experiment.

In the second experiment had sprouts from seeds, soaked in I(V), statistically significantly higher concentration of UV-absorbing compounds comparing to sprouts from seeds, soaked in Se(IV)+I(-I) and Se(VI)+I(-I) (Fig. 4).

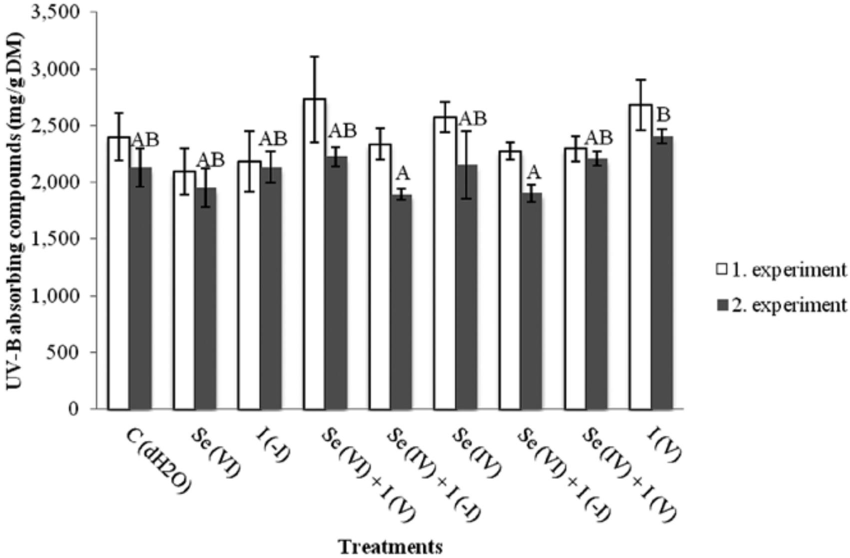

Figure 4: Concentration of UV-B absorbing compounds per DM in kohlrabi sprouts. Mean ± SE, n = 4, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

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

Potential photochemical efficiency of PS II was similar in control and treated sprouts in the first experiment.

In the second experiment had sprouts from seeds, soaked in Se(VI)+I(-I), statistically significantly higher potential photochemical efficiency of PS II from sprouts from seeds, soaked in I(V). (Fig. 5).

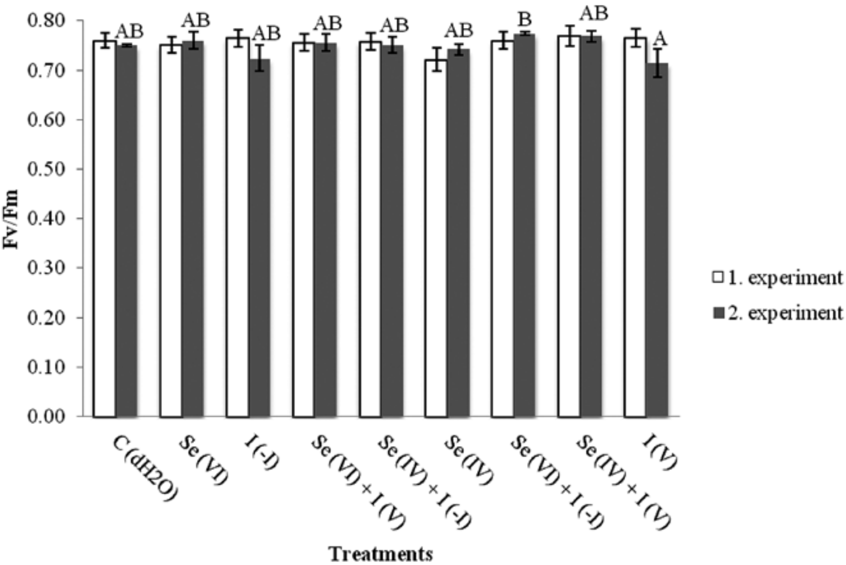

Figure 5: Potential photochemical efficiency of PS II in kohlrabi sprouts. Mean ± SE, n = 4, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 5: Potencialna fotokemična učinkovitost FS II v kalicah kolerabice. Predstavljene so povprečne vrednosti ± SE (n = 4). C – kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0{,}05$.

In the first experiment control sprouts showed statistically significantly lower dry mass comparing to sprouts from seeds, soaked in Se(VI), I(-I), Se(IV)+I(-I) and Se(VI)+I(-I). Sprouts from seeds, soaked in Se(IV)+I(-I), had statistically significantly higher dry mass comparing to sprouts from seeds, soaked in Se(VI), Se(VI)+I(V), Se(IV), Se(VI)+I(-I), Se(IV)+I(V) and I(V). Sprouts from seeds, soaked in I(-I), had statistically significantly higher dry mass comparing to sprouts from seeds, soaked in Se(VI)+I(V) and Se(IV)+I(V).

In the second experiment control sprouts showed statistically significantly lower dry mass comparing to sprouts from seeds, soaked in Se(VI)+I(V), Se(IV)+I(-I), Se(IV) and Se(IV)+I(V). Sprouts from seeds, soaked in Se(VI)+I(V) and Se(IV)+I(-1) had statistically significantly higher dry mass comparing to sprouts from seeds, soaked in Se(VI), I(-I), Se(VI)+I(-I) and I(V). Sprouts from seeds, soaked in Se(IV) and Se(IV)+I(V) had statistically significantly higher dry mass comparing to sprouts from seeds, soaked in Se(VI)+I(-I) and I(V) (Fig. 6).

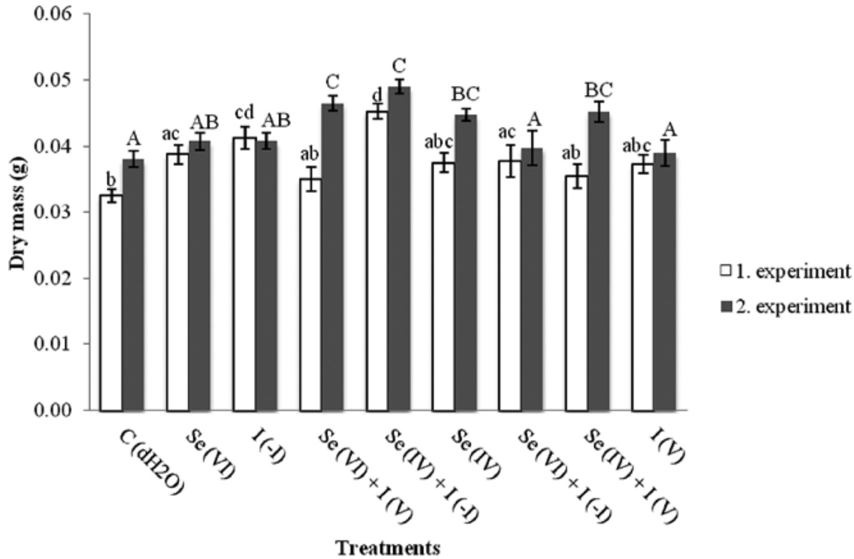

Figure 6: Dry mass of kohlrabi sprouts. Mean ± SE, n = 4, C - control. Mean values, marked with the same letter, are not significantly different at $p \leq 0.05$.

Slika 6: Suha masa kalicah kolerabice. Predstavljene so povprečne vrednosti ± SE (n = 4). C – kontrolne kalice. Stolpci, označeni z različnimi črkami, se med seboj statistično značilno razlikujejo pri $p \leq 0{,}05$.

Discussion

Seed germination is a critical stage in the plant life cycle. It starts with the imbibition, which means uptake of water of dry seed embrio (Herman et al. 2007). In our experiment different treatments did not affect germination of kohlrabi sprouts. Even though processes in seed germination and sprout development depend on environment factors and may be negatively affected by abiotic stress, such as high concentrations of minerals in soaking solution (Pongrac et al. 2016).

Increased chlorophyll levels indicate a greater potential for photosynthesis. In our study sprouts, treated with Se(VI), produced lower concentration of chlorophyll *a* and carotenoids comparing to sprouts developed from seeds which had been treated with Se(IV). Germ et al. (2015) conducted experiment with common buckwheat with the same concentrations and forms of Se and I as in the present study. Similarly as in the present study they found out that sprouts developed from seeds which had been soaked in Se(VI) had lower concentration of chlorophyll *a* and carotenoids comparing to sprouts from seeds, soaked in Se(IV). Sprouts from seeds, soaked in I(-I), had statistically significantly lower concentration of chlorophyll *a* comparing to sprouts from seeds, soaked in I(V). There are scarce information about the effect of iodine on the concentration of chlorophyll. In the study Krzepilko et al. (2016) found out that in comparison with the control, KI did not affect chlorophyll content of lettuce seedlings.

In the first experiment sprouts produced from seeds, soaked in Se(VI), had statistically significantly higher concentration of anthocyanins comparing to control seeds and seeds, soaked in other treatments. Results are in line with Hawrylak-Nowak (2008) who found out that in maize, selenate treatments at concentrations 7.9 mg Se/L increased the content of anthocyanins.

Potential photochemical efficiency of photosystem II was close to theoretical maximum (0.83) (Schreiber et al. 1995) in both control and treated groups. None of the treatments presented stress conditions for experimental plants.

Se and I added in any form and combination did not affect the synthesis of UV- absorbing compounds, the concentrations of which were similar in control and treated sprouts. Addition of Se may reduce the negative effects of UV-B radiation on seedlings of wheat possibly by increasing the amount and activity of the antioxidant enzymes (Yao et al. 2010, Yao et al. 2011) and by increasing the amount of anthocyanins and phenolic compounds (Yao et al. 2010), which also have an antioxidant effect.

Hajiboland and Keivanfar (2012) investigated the influence of Se(VI) in oilseed rape (*Brassica napus*). Oilseed rape plants were 19 weeks foliarly sprayed with the Se(VI) at different concentrations: 0 (control), 0.01, and 0.02 mg Se/L. They found that the dry weight of the pods and seeds was significantly higher for the plants treated with Se(VI) compared with control plants. Foliar spraying with Se(VI) had no effect on the dry weight and height of above ground parts and on the dry mass of roots and seeds of two varieties of common buckwheat (Tadina et al. 2007). In contrast, foliar spraying of potatoes with Se(VI) at a concentration of 10 mg Se/L lowered the weight of the tubers (Germ et al. 2007). On the other hand in our experiment in the first experiment sprouts from Se(VI) treated seeds had a positive impact on the dry weight of sprouts. Addition of I(-I) in the form of KI at concentration of 10 mg I/L, significantly reduced the biomass of lettuce plants in comparison with plants from the control treatment, whereas the addition of I(V), in the form of KIO_3, increased biomass of plants, which reached a maximum value at a dose of 2.5 mg I/L (Blasco et al. 2011). In our experiment the soaking of seeds in I(V) solution did not affect the dry mass of sprouts. Foliar spraying radish plants (*Raphanus sativus*) with I(-I) respectively I(V) had no effect on the dry weight of leaves and roots (Strzetelski et al. 2010). Blasco et al. (2008) found that treatment of lettuce with I(-I) ($\geq$ 10 mg I/L) had toxic effects on the growth of plants due to excessive accumulation of this element in the plant tissue.

Smoleń et al. (2014) found out that treatment of lettuce plants with Se(VI)+I(V) had no effect on the average weight of the heads. Zhu et al. (2004) added Se(VI) and I(V) to the nutrient solution, where they grow spinach plants. Se(VI) was added in concentrations of 0.8, 1.6 and 4 mg Se/L, while the I(V) was added in concentrations of 1.25, 2.5 and 6.25 mg I/L. It has been found that the addition of Se (VI) at a concentration of 0.8 and 1.6 mg Se/L and I(V) at a concentration of 1.25 and 6,25 mg I/L respectively lowered the biomass of roots.

However in our second experiment I(V) treatment did not have any effect on the dry mass of sprouts in either of experiments.

Conclusions

The aim of our research was to determine the effect of different forms of Se, I and their combinations on selected biochemical and morphological characteristics of the kohlrabi sprouts (*Brassica oleracea* L. var. *gongylodes* L.). Response of sprouts to different chemical forms of Se and I, and combinations thereof differed between the measured parameters. Higher concentration of Se and I would probably have a greater impact on the kohlrabi sprouts.

Povzetek

Selen (Se) in jod (I) sta nujno potrebna elementa za ljudi in živali, medtem ko njuna esencialna vloga za rastline še ni dokazana. Cilj raziskave je bil ugotoviti, ali različne oblike Se in I posamezno ali v kombinaciji vplivajo na izbrane biokemijske in morfološke lastnosti pri kalicah kolerabice (*Brassica oleracea* L. var. *gongylodes* L.). Poskus je bil sestavljen iz devetih obravnavanj. Kalice smo vzgojili iz semen, ki so bila namočena v različne raztopine z različnimi kombinacijami in oblikami Se in I. Raztopine so vsebovale selenit (SeO_3^{2-}) oz. selenat (SeO_4^{2-}) s koncentracijo 10 mg Se/L, jodid (I^-) oz. jodat (IO_3^-) s koncentracijo 1.000 mg I/L in kombinacije različnih oblik Se in I (SeO_3^{2-}+ I^-, SeO_3^{2-}+ IO_3^-, SeO_4^{2-} + I^-, SeO_4^{2-} + IO^{3-}). Merili smo koncentracijo klorofila *a* in *b*, karotenoidov, antocianov, UV-A absorbirajočih snovi, UV-B absorbirajočih snovi ter potencialno fotokemično učinkovitost fotosistema II (FS II). Ugotavljali smo delež kaljivosti semen. Po koncu poskusa smo izmerili še maso kalic. Različne kemijske oblike Se in I ter njune kombinacije niso statistično značilno vplivale na število kalic, ki so vzklile iz semen. Različne kemijske oblike Se in I ter njune kombinacije so različno vplivale na koncentracijo barvil pri kalicah kolerabice. Potencialna fotokemična učinkovitost fotosistema

II je bila blizu teoretičnega maksimuma 0,83, kar kaže, da kalice niso bile izpostavljene stresnim razmeram. Predvidevamo, da bi imele večje koncentracije preučevanih elementov večji vpliv na rast in fiziološke lastnosti kalic.

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