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# Effect of selenium, iodine and their combination on development of Tartary buckwheat sprouts

## Vpliv selena in joda ter njune kombinacije na razvoj kalic tatarske ajde

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**Abstract:** Tartary buckwheat (*Fagopyrum tataricum* Gaertn) is becoming more and more popular due to its health benefits for humans. It contains important fibres, vitamins, certain minerals and antioxidants as rutin. Sprouts are a hot trend in the food production and nutrition. Enrichment of sprouts with iodine (I) and selenium (Se) may prevent endemic deficiency of these elements for humans and animals. The aim of the study was to found out the effect of selenate (Se(VI)), iodate (I(V)) and their combination on morphological, physiological and biochemical properties of Tartary buckwheat sprouts. Tartary seeds were soaked in solutions with Se(VI) (20 mg/L), I(V) (1500 mg/L) or in Se(VI) + I(V) (20 mg/L Se(VI) + 1500 mg/L (I(V)). Experiment was performed in growth chamber in two repetitions. Measurements were performed three weeks after germination. The solution of iodate and combination of selenate and iodate lowered germination rate of sprouts. There was no effect of the treatments on the amount of chlorophyll *a*, anthocynins and UV absorbing compounds. The amount of rutin was the highest in control sprouts. According to physiological measurements, control sprouts and sprouts from treated seeds were not stressed by the treatments.

**Key words:** Tartary buckwheat, sprouts, selenium, iodine

**Izvleček:** Tatarsko ajdo (*Fagopyrum tataricum* Gaertn) v zadnjem času spet bolj pogosto sejemo zaradi njenih pozitivnh učinkov za zdravje ljudi. Ajda vsebuje pomembne vlaknine, vitamine, nekatere minerale ter antioksidativne snovi, kot je rutin. Kalice so trenutno zelo popularne v prehrani ljudi. Obogatitev kalic s selenom (Se) in jodom (I) lahko prepreči znake pomanjkanja obeh elementov pri ljudjeh in živalih. Cilj dela je bil ugotoviti vpliv selenata, (Se(VI)), iodata (I(V)) in njune kombinacije na morfološke, fiziološke in biokemijske lastnosti kalic tatarske ajde. Semena tatarske ajde smo izpostavili raztopinam, ki so vsebovale Se(VI) (20 mg/L), I(V) (1500 mg/L) ali Se(VI) + I(V) (20 mg/L Se(VI) + 1500 mg/L (I(V)). Kalitev in razvoj kalic smo spremljali v rastnih komorah v dveh ponovitvah. Po treh tednih smo poskus prekinili in opravili morfološke, fiziološke in biokemijske meritve na kalicah. Semena tatarske ajde, ki so bila namočena v raztopinah iodata in kombinaciji selenata in iodata, so imela nižjo kalivost. Obravnavanja niso vplivala na vsebnost klorofila *a*, antocianov in UV absorbirajočih snovi. Vsebnost rutina je bila najvišja v kontrolnih kalicah. Glede na rezultate fizioloških meritev, namakanje smen v raztopinah selenata, jodata ter njune kombinacije, ni delovalo stresno na kalice.

**Ključne besede:** tatarska ajda, kalice, selen, jod

## Introduction

Growing of buckwheat was in decline in the past century. However, recently cultivation has increased due to the growing interest in organic farming, alternative culture, old and traditional diets, outstanding nutritive properties and its positive effects on human health (Wieslander and Norback 2001). Cereals and pseudocereals are very important source of macronutrients and bioactive substances that have antioxidative activity. Buckwheat grains and groats contain numerous flavonoids and polyphenols, such as rutin, catechin, quercetin, p-coumaric acid, p-hydroxybenzoic acid and gallic acid (Dziedzic et al. 2018). Similarly to cereal crops, buckwheat seeds contain a lot of starch (Wronkowska and Haros 2014). Seeds, sprouts and fresh green parts of the plant are used in the diet (Golob et al. 2016). The presence of flavonoid rutin in buckwheat groats as well as in green parts of buckwheat plants is important due to its wide spectrum of pharmacological activities, including anti-inflammatory, anticancer, antiatherogenic, and antioxidant activity (Kreft et al. 2006, Starowicz et al. 2017).

Tartary buckwheat *(Fagopyrum tataricum*) is a dicot pseudocereal and belongs to family Polygonaceae. Currently, buckwheat sprouts are used as a novel source of vegetables due to the presence of enormous nutraceutical properties. Buckwheat sprouts are popular as a health food in Korea and Japan because of their high flavonoids content (Kim et al. 2004, Ji et al. 2016), especially rutin, and also short biological cycle (7–10 days) (Suzuki et al. 2005). Tartary buckwheat sprouts attracted a lot of attention because of their beneficial effects on blood pressure (Nakamura et al. 2013).

Although Se is not involved in vital metabolic processes in plants, it could help the plants to reduce the damage caused by oxidative stress if it is used in small doses (Seppänen et al. 2003). Se is on the list of beneficial elements for plants: it is not required by all plants but can promote plant growth and may be essential for particular taxa (Pilon-Smits et al. 2009). Soil and foliar addition of Se fertilizers (agronomic biofortification) are often used to improve the concentration of Se in diet (Nawaz et al. 2017). Iodine is a trace element, essential for mammalian life. The goal of iodine biofortification of plants is to obtain food, rich in this element, which may increase its consumption by various populations (Piątkowska et al. 2016).

Iodine and selenium together enable important functional roles in organisms of humans and animals. Biofortification (enrichment) of plants with these elements is justified to prevent endemic deficiency of I and Se in humans and animals (Smoleń et al. 2015). However, knowledge about the effects of simultaneous fortification with Se in I on plant's physiology and metabolism is lacking. Besides knowing the amount of Se and I in the sprouts, effect of elements on biochemical characteristics and growth is also very important before growing of fortified sprouts for human consumption. We assume that simultaneous fortification with Se in I, as well as fortification with both elements alone, will have an effect on the growth and physiological characteristics of Tartary buckwheat sprouts.

## Materials and methods

Buckwheat seeds (cv. Darja) were soaked in solution for 8 h in 200 mL of distilled water (MilliQ) (control), or in solutions contained selenate ($SeO_4^{2-}$) with a concentration of 20 mg Se/L, iodate ($IO_3$-) with a concentration of 1500 mg I/L, and their combinations ($SeO_4^{2-}$ + $IO_3^-$) with a concentration 20 mg Se/L and 1500 mg I/L respectively. Selenium was applied in the form of sodium selenate ($Na_2SeO_4$), and iodine was applied in the form of potassium iodate ($KIO_3$). After soaking, seeds were distributed to the 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.

The contents of chlorophyll (Chl *a*, *b*) and carotenoids were determined following methods described by Lichtentaler and Buschman (2001a, b) and measured with a UV/Vis spectrometer. The anthocyanin contents were measured according to Drumm and Mohr (1978). The contents of UV-A and UV-B absorbing compounds were evaluated following the methodology of Caldwell (1968).

The potential photochemical efficiency of photosystem (PS)II was measured according to Schreiber et al. (1996) using a portable fluorometer

(PAM 2500 Chlorophyll Fluorometer; Heinz Walz GmbH, Germany). The respiratory potential of the mitochondria was determined *via* activity of terminal electron transport system (ETS) as described by Kenner and Ahmed (1975). For detailed description of the preparation of sprout tissue and extraction processes see Germ et al. (2005).

The normal distribution of the data was tested using Shapiro-Wilk tests. Differences between the conditions were tested using one-way analysis of variance followed by Duncan *post-hoc* tests. The level of significance was accepted at $p < 0.05$. The SPSS Statistics software, version 20.0 (IBM) was used for these calculations.

## Results

Germination rate was lower in sprouts from seeds soaked in solution of iodate and combination with selenate and iodate comparing to control groups and sprouts emerged from seeds, soaked in selenate solution. Dry mass of the sprouts was the lowest in sprouts developed from selenate treated seeds in the first experiment, while between control and other two treatments there were no differences in dry mass of the sprouts (Table1).

Treatments did not affect the amount of chlorophyll *a*, anthocyanins, and UV-B and UV-A absorbing compounds, while the amount of carotenoids was the highest in sprouts from seeds, treated with combination of selenate and iodate in the first experiment. ETS activity was the lowest in control sprouts in the first experiment while there was no difference between control and treatments in the second experiment. There were no differences in the potential photochemical efficiency of PSII between control and sprouts from treated seeds in both experiments (Table 1).

The amount of rutin was the highest in control sprouts in the first experiment. In the second experiment, there was no difference between control and treated sprouts (Fig. 1).

**Table 1:** Biochemical and physiological responses of Tartary buckwheat sprouts grown from seeds previously soaked in solutions with addition of Se (Se(VI)) and I (I(V)) alone and simultaneous addition of Se and I (Se(VI) + I(V)).

**Tabela 1:** Biokemijski in fiziološki odziv kalic tatarske ajde zraslih iz semen, ki so bila predhodno namočena v raztopinah z dodatkom samo Se (Se(VI)), samo I (I(V)) in obeh elementov hkrati (Se(VI) + I(V)).

| | Treatment | | | | |
|---|---|---|---|---|---|
| **First experiment** | 0 | Se(VI) | I(V) | Se(VI) + I(V) | unit |
| germination | $74.2 \pm 4.4^{b}$ | $71.9 \pm 9.5^{b}$ | $61.6 \pm 1.5^{a}$ | $59.8 \pm 4.8^{a}$ | % |
| dry weight | $15.5 \pm 1.0^{b}$ | $13.8 \pm 0.7^{a}$ | $16.8 \pm 0.8^{b}$ | $16.4 \pm 0.7^{b}$ | % |
| chlorophyll *a* | $10.0 \pm 2.0^{a,b}$ | $8.5 \pm 3.1^{a}$ | $8.9 \pm 2.1^{a,b}$ | $13.1 \pm 3.0^{b}$ | mg/g d.w. |
| carotenoids | $1.2 \pm 0.2^{a}$ | $1.1 \pm 0.4^{a}$ | $1.2 \pm 0.3^{a}$ | $2.0 \pm 0.4^{b}$ | mg/g d.w. |
| anthocyanins per d.w. | $262 \pm 26^{a}$ | $206 \pm 66^{a}$ | $284 \pm 72^{a}$ | $287 \pm 75^{a}$ | rel. unit/g d.w. |
| UV-A abs | $46 \pm 4^{a}$ | $38 \pm 6^{a}$ | $41 \pm 10^{a}$ | $35 \pm 8^{a}$ | rel. unit/cm$^2$ |
| UV-B abs | $2.7 \pm 0.2^{a}$ | $2.2 \pm 0.3^{a}$ | $2.6 \pm 0.6^{a}$ | $2.1 \pm 0.5^{a}$ | rel. unit/cm$^2$ |
| $F_v/F_m$ | $0.8 \pm 0.02^{a}$ | $0.8 \pm 0.01^{a}$ | $0.8 \pm 0.02^{a}$ | $0.8 \pm 0.01^{a}$ | |
| ETS activity | $0.5 \pm 0.09^{a}$ | $0.7 \pm 0.14^{b}$ | $0.8 \pm 0.12^{b}$ | $0.7 \pm 0.12^{b}$ | µL $O_2$/mg d.w./h |

| | Treatment | | | | |
|---|---|---|---|---|---|
| **Second experiment** | 0 | Se(VI) | I(V) | Se(VI) + I(V) | unit |
| germination | 85.7 ± 4.8$^{b}$ | 81.0 ± 7.9$^{b}$ | 68.5 ± 6.3$^{a}$ | 70.5 ± 2.5$^{a}$ | % |
| dry weight | 12.8 ± 0.5$^{a}$ | 13.2 ± 1.5$^{a}$ | 13.4 ± 2.0$^{a}$ | 16.5 ± 3.6$^{a}$ | % |
| chlorophyll *a* | 8.2 ± 1.0$^{a}$ | 7.9 ± 0.9$^{a}$ | 8.6 ± 1.3$^{a}$ | 7.8 ± 1.0$^{a}$ | mg/g d.w. |
| carotenoids | 1.1 ± 0.2$^{a}$ | 1.0 ± 0.2$^{a}$ | 1.0 ± 0.1$^{a}$ | 1.0 ± 0.1$^{a}$ | mg/g d.w. |
| anthocyanins per d.w | 439 ± 102$^{a}$ | 315 ± 103$^{a}$ | 294 ± 77$^{a}$ | 397 ± 156$^{a}$ | rel. unit/g d.w. |
| UV-A abc | 46 ± 4$^{a}$ | 38 ± 6$^{a}$ | 42 ± 10$^{a}$ | 35 ± 8$^{a}$ | rel. unit/cm$^2$ |
| UV-B abc | 2.8 ± 0.3$^{a}$ | 2.6 ± 0.2$^{a}$ | 2.8 ± 0.5$^{a}$ | 2.6 ± 0.7$^{a}$ | rel. unit/cm$^2$ |
| $F_v/F_m$ | 0.6 ± 0.02$^{a}$ | 0.7 ± 0.08$^{a}$ | 0.7 ± 0.03$^{a}$ | 0.7± 0.4$^{a}$ | |
| ETS activity | 1.0 ± 0.3$^{a}$ | 0.8 ± 0.1$^{a}$ | 1.1 ± 0.3$^{a}$ | 1.0 ± 0.3$^{a}$ | µL $O_2$/mg d.w./h |

Data are means ± standard deviation (n = 4 for each treatment). Different letters indicate significant differences between the treatments, (p <0.05; Duncan test). Abbreviations: d.w., dry weight; ETS, electron transport system activity; abc, absorbing compounds.
Rezultati so predstavljeni kot povprečje ± standardna deviacija (n = 4 za vsak tretma). Različne črke označujejo statistično značilne razlike med tretmaji (p <0,05; Duncan test). Okrajšave: d.w., suha teža; ETS, aktivnost elektronskega transportnega sistema; abc, absorbirajoče snovi.

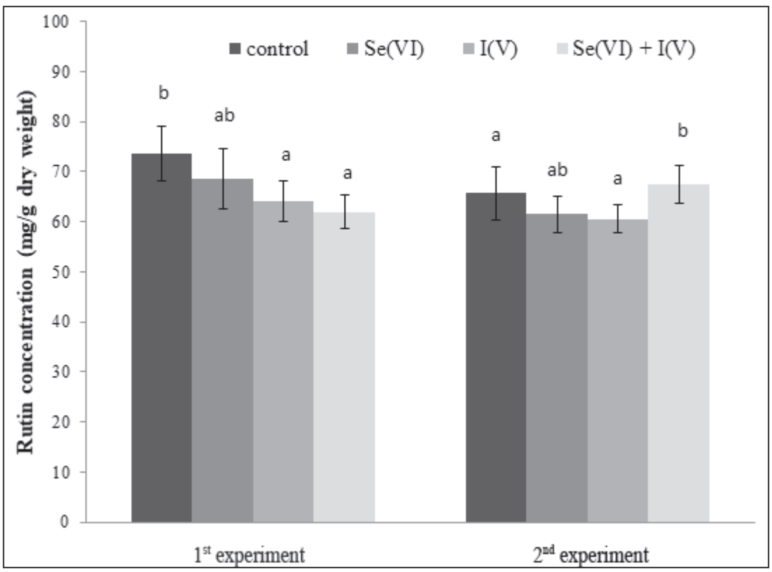


**Figure 1:** Rutin concentration in Tartary buckwheat sprouts grown from seeds previously soaked in solutions with addition of Se (Se(VI)) and I (I(V)) alone and simultaneous addition of Se and I (Se(VI) + I(V)). Different letters indicate significant differences between the treatments (p <0.05; Duncan test).

**Slika 1:** Vsebnost rutina v kalicah tatarske ajde zraslih iz semen, ki so bila predhodno namočena v raztopinah z dodatkom samo Se (Se(VI)**),** samo I (I(V)) in obeh elementov hkrati (Se(VI) + I(V)). Različne črke označujejo statistično značilne razlike med tretmaji (p <0,05; Duncan test).

## Discussion

Germination is complex process in which a new plant emerges from a seed and is affected by a variety of environmental conditions (Todirascu-Ciornea et al. 2016). Treatment with iodate and combination of selenate and iodate negatively affected the germination of Tartary buckwheat sprouts. Iodate exerted negative effect on germination on wheat in the study from Todirascu-Ciornea and Dumitru (2015), where potassium iodate ($KIO_3$) solutions in $10^{-3}$, $10^{-4}$ and $10^{-5}$ M concentrations were used. However, potassium iodate did not significantly influence the germination rate of the wheat seeds in the study from Todirascu-Ciornea et al. (2016) where two seed varieties, Putna and Gasparom, were treated with water (control) or $10^{-3}$, $10^{-4}$ and $10^{-5}$ M solutions of $KIO_3$. Older study point out the positive role of low iodine concentrations on plants (Borst-Pauwels 1962). The author identified the positive effect of iodine on the growth of spinach, clover, tomato, turnip, barley, wheat and mustard. There was no effect of iodine on the growth of buckwheat, and a negative impact on oats. Dai et al. (2006) showed in their study that biomass productions of spinach was not affected by the addition of iodate and iodide.

Treatments in the present study did not affect the amount of chlorophyll *a* in the Tartary buckwheat sprouts. Amount of chlorophyll *a* was also not affected in pea sprouts, soaked in Se and I solution (Jerše et al. 2017). Similarly, in the study of Krzepilko et al. (2016) authors reported that in comparison with the control, KI did not affect chlorophyll content of lettuce seedlings. Amount of anthocyanins, which are often synthesised under stress conditions (Hawrylak-Nowak 2008) did not differ between the control sprouts and sprouts from treated seeds, which is consistent with results from study on pea sprouts (Jerše et al. 2017).

In the study on wheat seedlings Todirascu-Ciornea and Dumitru (2015) reported that $10^{-5}$ M solution of $KIO_3$ exerted significant strong influences, stimulating the chlorophyll *a* synthesis in comparison with the control. On the other hand, $KIO_3$ in concentration of $10^{-3}$ M did not affect content of chlorophyll *a*, while the $KIO_3$ $10^{-4}$ M solution exerted even a slight inhibitory effect. Soaking seeds of common buckwheat in iodate solution increases the amount of chlorophyll *a* in common buckwheat while the treatment with selenate and combination of selenate and iodate also did not affect the amount of chlorophyll *a* comparing to control sprouts (Germ et al. 2015). The amount of UV-B and UV-A compounds were similar in control sprouts and sprouts from treated seeds as was previously evidenced for kohlrabi sprouts (Osmić et al. 2017).

Potential photochemical efficiency of photosystem II was close to theoretical maximum 0.83, especially in the first experiment which is typical for unstressed green plants (Schreiber et al. 1996) in both control and sprouts from treated seeds. Values were close to 0.7 in the second experiment and similar between control sprouts and sprouts from treated seeds. Values close to 0.83 was observed in the study from Germ et al. (2015) and Osmić et al. (2017) studying the effect of Se and I in different forms on common buckwheat (*Fagopyrum esculentum*) sprouts and kohlrabi sprouts (*Brassica oleracea* L. var. *gongylodes* L.) respectively. In addition ETS activity did not differ between control and treated groups in the second experiment. According to the results from potential photochemical efficiency of photosystem II, ETS activity and the amount of anthocyanins, none of the treatments presented stress conditions for Tartary buckwheat sprouts.

Rutin is a secondary metabolite that prevent herbivory by larvae of specific species of insects (Simmonds 2003). Rutin concentration in mature cotyledon in common buckwheat was very high in the study from Suzuki et al. (2005) which was much higher than other defence compounds of plant. Therefore, authors presumed that also in cotyledon rutin may have a role to prevent consumption by insects. Several researchers evidenced that rutin functions as a UV screen under high solar radiation (Mahdavian et al. 2008). However, in the study from Suzuki et al. (2005), rutin concentration was high even in buckwheat cotyledons that were grown under darkness. Authors proposed that this result suggests that rutin in buckwheat cotyledons may have other roles, such as enhancement of the defence system against cold stress or desiccation stress in Tartary buckwheat leaves in addition to protection against herbivory and UV screening. In the present study, the amount of rutin was the highest in control sprouts. Treatment with iodate and combination with selenate and iodate lowered the amount of rutin comparing to control in the first experiment. However, the pattern did not repeat in the second experiment. The practical view of the research would be the recommendation, if the amount of Se and I in the sprouts from treated seeds, would be suitable for human consumption. Thus, the amount of Se and I have to be measured in sprouts from treated seeds before recommended them as functional food for humans.

## Conclusions

The aim of our study was to found out effect of selenate (Se(VI)), iodate (I(V)) and their combination on morphological, physiological and biochemical properties of Tartary buckwheat sprouts. The soaking seeds in solution of iodate and combination between selenate and iodate lowered germination of sprouts. There was no effect of the treatments on the amount of chlorophyll *a,* anthocyanins and UV absorbing compounds. The amount of rutin was the highest in control sprouts. According to physiological measurements, the treatments did not impose stress to Tartary buckwheat sprouts. Biofortified plants with elements like Se are used in human diet. However, the amount of Se and I have to be measured in spouts from treated seeds before we can recommend them as food for humans.

## Povzetek

Semena tatarske ajde so bogata z aminokislinami, vitamini, minerali in s fenolnimi snovmi kot je rutin. Rutin se nahaja večinoma v cvetovih in v zelenih delih rastline. Z raziskavo smo želeli ugotoviti, ali izpostavitev semen tatarske ajde Se(VI) in I(V) ter Se(VI) + I(V), vpliva na rast in biokemijske lastnosti kalic tatarske ajde. Selen je esencialen element za človeka in živali, saj omogoča normalno delovanje od selena odvisnih encimov, hkrati pa ima pozitivne lastnosti protivnetnega delovanja in deluje proti nastanku nekaterih vrst raka in srčnih bolezni. Selen in jod sta v tesni biološki povezavi, saj so od selena odvisni nekateri encimi družine jodotironin dejodinaze; pomanjkanje selena lahko učinkuje na presnovo joda v organizmu. Interakcija selena z jodom med privzemom v rastline ali v presnovnih procesih znotraj rastline še ni popolnoma pojasnjena.

Semena tatarske ajde smo izpostavili Se(VI), I(V) ter Se(VI) + I(V). Ko so semena vsrkala raztopine, smo jih prestavili v rastno komoro. Po treh tednih rasti smo opravili morfološke in biokemijske meritve. Izvedli smo dve ponovitvi. Kalivost semen je bila najvišja pri kontroli in pri kalicah tatarske ajde, katerih semena so bila izpostavljena Se(VI), nižja kalivost je bila izmerjena pri kalicah iz semen, izpostavljenih I(V) ali Se(VI) + I(V). Iz izmerjenega razmerja $F_v/F_m$ sklepamo, da tretiranja semen tatarske ajde s Se(VI), I(V) ali Se(VI) + I(V) niso negativno vplivala na kalice, saj je bilo razmerje $F_v/F_m$ podobno vrednostim kontrolne skupine. Aktivnost elektronskega transportnega sistema (ETS) je bila najnižja pri kontrolni skupini v prvem eksperimentu, v drugem treatiranje semen ni vplivalo na aktivnost ETS pri kalicah. Obravnavanja niso vplivala na vsebnost klorofila *a*, antocianov in UV zaščitnih snovi. Vsebnost rutina je bila najvišja v kontrolnih kalicah. Glede na rezultate fizioloških meritev, kalice tatarske ajde niso bile izpostavljene stresnim razmeram.

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