

FAGOPYRIN AND RUTIN CONCENTRATION IN SEEDS OF COMMON BUCKWHEAT PLANTS TREATED WITH Se AND I

VSEBNOST FAGOPIRINA IN RUTINA V SEMENIH NAVADNE AJDE TRETIRANE S SELENOM IN JODOM

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ABSTRACT

Fagopyrin and rutin concentration in seeds of common buckwheat plants treated with Se and I

Very little data about impact of simultaneous addition of selenium and iodine on plants exists. Plants of common buckwheat *Fagopyrum esculentum* Moench. were grown outdoors and were foliarly treated at the beginning of the flowering with different forms of Se (selenite, selenate) and I (iodide, iodate) and their combinations. Plants were harvested and seeds were collected and grounded, and the concentrations of fagopyrin and rutin were determined. Nor Se, nor I, alone or their combination had an influence on the concentration of fagopyrin. Selenium, iodine and their combination affected the amount of rutin in common buckwheat grain.

Key words: fagopyrin, rutin, common buckwheat, seeds, Se, I

IZVLEČEK

Vsebnost fagopirina in rutina v semenih navadne ajde tretirane s selenom in jodom

Zelo malo je znanega o sočasnem vplivu elementov selen in joda na rastline. Rastline navadne ajde (*Fagopyrum esculentum* Moench.) gojene na prostem, smo v začetku cvetenja tretirali z raztopinami selen (selenit, selenat) in joda (jodit, jodat) ter njihovimi kombinacijami. Zrnje tretiranih rastlin je bilo zmleto in izmerili smo koncentracije fagopirina in rutina. Na koncentracijo fagopirina v zrnju ni vplivalo niti tretiranje rastlin s selenom niti z jodom, pa tudi ne kombinacija obeh elementov. Na koncentracijo rutina v zrnju pa so značilno vplivala tako posamična tretiranja z raztopinami obeh elementov kot tudi kombinirano tretiranje.

Ključne besede: fagopirin, rutin, navadna ajda, semena, selen, jod

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1 INTRODUCTION

Se and I are essential elements for proper function of human thyroid (ZIMMERMANN & KÖHRLE 2002, WHITE & BROADLEY 2009). Essentiality of Se and I for plants is still under debate. However, it has been evidenced that Se has many positive effects on plants, capable of accumulating this element (GOLOB et al. 2016). Recently there were some reports about combined effects of Se and I on plants (ZHU et al. 2004, SMOLEŃ et al. 2014, 2016, GERM et al. 2015, OSMIĆ et al. 2017, JERŠE et al. 2017). However, according to our knowledge, the effect of Se and I on buckwheat grain has not been studied. Common buckwheat (*Fagopyrum esculentum* Moench) is a very important alternative crop in Europe due to possibility of its ecological cultivation, furthermore, its use in the diet has many positive effects on health and nutritional status of consumers (SKRABANJA et al. 1998, 2000, 2001, KREFT et al. 2002, BONAFACCIA et al. 2003, KREFT 2016). Buckwheat grain contain high amounts of rutin. The role of rutin is scavenging free radicals and anti-fungal activity (SUZUKI et al. 2015). Besides flavonoids, buckwheat also contains trace elements, like Zn, Cu, Mn and Se (CUDERMAN & STIBILJ 2010). Buckwheat has many favourable medicinal properties for humans, because of its antioxidative (HOLASOVÁ

et al. 2002, KREFT 2016), anti-inflammatory and anti-carcinogenic effects and it can also increase the strength of blood vessels (BROZMANOVÁ et al. 2010). LI & ZHANG (2001) reported about significant health benefits of buckwheat for individuals with diabetes, obesity and constipation. Common buckwheat originates from Southwest China and it has been gradually spread to other continents (KREFT 1995). Buckwheat contains fagopyrins, phototoxic naphthodianthrones, similar to hypericin (BROCKMANN et al. 1952). The highest levels of fagopyrins were determined in flowers and leaves (STOJILKOVSKI et al. 2013). KREFT et al. (2013) evidenced that levels of fagopyrins increase gradually in sprout growth, especially in the light. Many researchers (TAVČAR BENKOVIĆ et al. 2014, TAVČAR BENKOVIĆ & KREFT 2015) reported that buckwheat sprouts contain fagopyrins that can cause photosensitization manifested as a skin irritation after exposure to sunlight. Phototoxic effects caused by fagopyrins are known as fagopyrism (WENDER et al. 1943). THEURER et al. (1997) reported that fagopyrin was less phototoxic than hypericin. Aim of our research was to find out if the simultaneous addition of Se and I affected the amount of fagopyrins and rutin in the common buckwheat seeds.

2 MATERIAL AND METHODS

Common buckwheat was sown on raised bed in the middle of July. At the beginning of flowering (28 days after sowing) plants were foliarly treated with solutions of sodium selenite (10 mg Se(IV)/L), sodium selenate (10 mg Se(VI)/L), potassium iodide (1000 mg I(-I)/L), potassium iodate (1000 mg I(V)/L), and combinations: 10 mg Se(IV)/L+1000 mg I(-I)/L; 10 mg Se(IV) /L+1000 mg I(V)/L, 10 mg Se(VI) /L+1000 mg I(-I)/L and 10 mg Se(VI)/L+1000 mg I(V)/L. After the ripening, we collected grains from the plants and measured the amount of fagopyrins and rutin.

Sample of seeds was homogenized using a mill (Kika® Werke M20, Germany). Fagopyrins were extracted with acetone/water (HPLC grade, Sigma-Aldrich). Dry samples (1 g) were suspended in 10 mL acetone/water (9/1) and thoroughly mixed for 1 min. The suspensions were then incubated for 20 h at 37 °C. Samples were mixed for 1 min and centrifuged at 1000 rpm for 5 min at 25 °C. Aliquots of the clear supernatants were dispensed into 2 mL plastic test tubes (TPP, Transdingen, Switzerland), and filtered through membrane

filters (Millex-GN filters; pore size = 0.2 µm, Millipore). The 1 mL of filtrate extracts was pipetted into glass vial and exposed 1 h to daylight and then were transferred and analyzed by HPLC. The HPLC system (Shimadzu Prominence) consisted of a system controller (CBM-20A), a column oven (CPO-20AC) and a solvent delivery pump with a degasser (DGU-20A5) connected to a refrigerated autosampler (SIL-20AC) with a photodiode array (PDA) detector (SPD-M20A) that monitored the wavelengths 190–800 nm and a fluorescence detector (LC-20AD XR, excitation wavelength = 330 nm and emission wavelength = 590 nm). The responses of the detectors were recorded using LC Solution software version 1.24 SP1. The chromatography was performed at 40 °C and a flow rate of 2 mL/min using a Phenomenex Kinetex® XB-C18 column (10 cm × 4.6 mm I.D., 2.7 µm particle size). The following gradient method using water (solvent A) and acetonitrile (solvent B), both containing 0.1 % trifluoroacetic acid, was utilized: 0.01–0.5 min 0 % B, 0.5–6.0 min 0–51 % B, 6.0–6.01 min 51 % B, 6.01–30 min 51–54 % B. The identity of

fagopyrins was known from previous research (TAVČAR BENKOVIĆ et al. 2014). The total content of fagopyrin was determined relative to the hypericin content, because its spectral characteristics and structure are similar to fagopyrin and fagopyrin as a standard reference compound was not available (HINNEBURG et al. 2005; OZBOLT et al. 2008; STOJILKOVIĆ et al. 2013). All fagopyrin peaks were integrated together (Fig. 1).

Rutin was measured according to the method described by MARKHAM (1989). Briefly: powdered plant material (1 g) was homogenized with extracting solvent (140:50:10 MeOH-H₂O-CH₃COOH, 20 mL) and filtered into volumetric flasks. Volumes were adjusted to 100 mL by addition of additional extracting solvent. To prepare the solutions for analysis, aliquots (2.5 mL) were transferred into 50 mL volumetric flasks and their volumes were made up with water. To each 10 mL of analysis solution, water (2 mL) and AlCl₃ reagent (133 mg crystalline aluminium chloride and 400 mg crystalline sodium acetate dissolved in 100 mL of extracting solvent, 5 mL) were added and absorbances

were recorded at 430 nm against a blank sample (10 mL of analysed solution plus 5 mL of water). The amount of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions, and expressed as g rutin/kg plant material.

Statistical analysis

The normal distribution of the data was tested using Shapiro-Wilk tests and homogeneity of variances was assessed using Levene's test. For the statistical analysis of the data the factorial analysis of variance (ANOVA) was used. Dependent variable was compared by two independent variables: selenium (Se) treatment (two groups - Se(IV) and Se(VI), iodine (I) treatment (two groups - I(-I) and I(V)), and their combination Se × I. The level of significance was accepted at $p < 0.05$.

A statistical analysis of the content of fagopyrin relative to different treatments with Se and I was carried out with one-way ANOVA by the Statgraphics XV program.

3 RESULTS AND DISCUSSION

The amount of fagopyrin ranged from 18.7 µg/gDM in Se(VI) treated plants to 33.9 µg/gDM in I(-I)+Se(VI) treated plants (Fig. 1). In the study of KOČEVAR GLAVIČ et al. 2017, where they measured the amount of fagopyrin in different parts of Tartary buckwheat, they determined 53.70 µg/gDM that was 1.8 fold higher than our results in common buckwheat. According to our knowledge, there are no reports about the effect of Se or I on the amount of fagopyrin in the plants in scientific literature. The amount of fagopyrins in the seeds of common buckwheat, foliarly treated with Se

and I, was not significantly different in control in comparison to treated groups (Fig. 1).

In conclusion, it was evidenced that in the studied material Se or I did not have any effect on the amount of fagopyrins in seeds in foliarly treated common buckwheat. However, in future investigations, the attention should be given on concentration of fagopyrins in the seeds of common buckwheat plants, treated with Se(VI). The fagopyrin concentration in Se(VI) plants is much lower in comparison to the control, but in present experiment, in the case of high variation of the results, it cannot be concluded to be significant.

In the grain from plants, treated with iodate, the addition of selenate and selenite had lowering impact to the amount of rutin (Table 1). In the grain of control plants and grain from plants, treated with iodide, neither treatment with the Se had an effect on the amount of rutin in the grains. If the plants were not sprayed with the solution of Se, iodate enhanced the amount of rutin in the grain, comparing to control. If the plants were treated with the solution of selenate, neither form of iodine affected the amount of rutin in the grain. Grains from plants, treated with iodide in combination with selenite, had higher amount of rutin comparing to grain from control plants and plants, treated with selenite in combination with iodate and with selenate in combination with iodate.

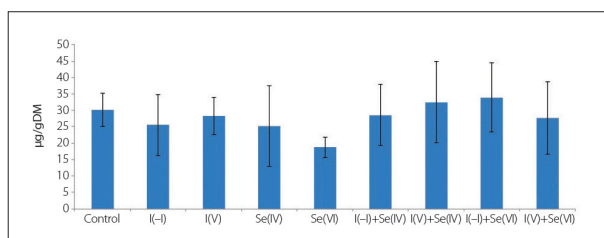


Figure 1: The effect of Se and I on the amount of fagopyrin in the seeds of common buckwheat. Data are given as mean value \pm standard error ($n = 4$ for each treatment).

Slika 1: Vpliv Se in I na koncentracijo fagopirina v zrnju navadne ajde. Podatki so srednje vrednosti \pm standardna napaka ($n = 4$ za vsako tretiranje).

Table 1: Concentration of rutin in grain harvested from plants treated with selenium and iodine**Preglednica 1: Koncentracija rutina v zrnju z rastlin tretiranih s selenom in jodom**

	Rutin (mg g ⁻¹)
<i>Selenium</i>	
0	2.42 ± 0.32 ^b
Se(IV)	5.80 ± 0.32 ^a
Se(VI)	1.91 ± 0.32 ^b
<i>Iodine</i>	
0	1.96 ± 0.32 ^b
I(-I)	5.78 ± 0.78 ^a
I(V)	2.39 ± 0.31 ^b
<i>Se × I</i>	
0+0	1.70 ± 0.17 ^c
Se(IV)+0	2.16 ± 0.36 ^{bc}
Se(VI)+0	2.02 ± 0.31 ^{bc}
0+I(-I)	2.3 ± 0.2 ^{bc}
0+I(V)	3.20 ± 0.3 ^a
Se(IV) + I(-I)	2.6 ± 0.4 ^{ab}
Se(IV) + I(V)	1.5 ± 0.3 ^c
Se(VI) + I(-I)	2.2 ± 0.2 ^{bc}
Se(VI) + I(V)	1.5 ± 0.2 ^c
ANOVA	
<i>Selenium (Se)</i>	***
<i>Iodine (I)</i>	***
<i>Se × I</i>	***

^a Data are given as mean value ± standard error (n = 4 for each treatment). Superscripted letters indicate significant differences between the different treatments (p < 0.05).

^a Navedeni podatki so povprečja ± standardna napaka (n=4 za vsako tretiranje). Različne nadpisane črke označujejo značilnost razlik med tretiranj (p < 0.05).

Buckwheat contains high level of rutin in nearly all organs, including grain, cotyledons, leaves, stems, and flowers. Rutin concentrations, which were detected in our experiment, are somewhat higher in comparison to the results reported by LEE et al. (2016) and DANILA et al. (2007), where rutin and other flavonoids measured in buckwheat grain and flour were analysed by HPLC. Amount of rutin was the highest in the grain, grown on foliarly treated plants with I(V). When Se was added in any form to iodate, the amount of rutin in the grain was reduced. Majority of other treatments did not differ from the control. Fortification of plants with selenite did not affect the amount of rutin content in buckwheat grain compared to control. Similarly, in the study of DONG et al. (2012) they found out, that concentration of rutin, an antioxidant with many interesting pharmacological effects (KREFT et al. 2002), was not significantly affected by selenium in the form of sodium selenite in *Lycium chinense*. On the contrary, in the study of TIAN & WANG (2008) they found out that Se application less than 1.0 mg per kg soil in the form of sodium selenite increases the accumulation and concentration of rutin in all organs of Tartary buckwheat.

POVZETEK

V času začetka cvetenja (28 dni po setvi na odprtem prostoru) so bile rastline tretirane z raztopinami Na selenita (10 mg Se(IV)/L), Na selenata (10 mg Se(VI)/L), K iodida (1000 mg I(-I)/L), K iodata (1000 mg I(V)/L) in s kombinacijami: 10 mg Se(IV)/L+1000 mg I(-I)/L; 10 mg Se(IV)/L+1000 mg I(V)/L, 10 mg Se(VI)/L+1000 mg I(-I)/L in 10 mg Se(VI)/L+1000 mg I(V)/L. Zrela semena so bila pobrana in zmleta. Izmerjene so bile koncentracije fagopirinov in rutina.

Koncentracija fagopirinov je bila od 18,7 µg/gDM v rastlinah, tretiranih s Se(VI), do 33,9 µg/gDM v rastlinah, tretiranih z I-I+Se(VI) (slika 1). Kolikor nam je znano, doslej ni literarnih podatkov o morebitnem vplivu selena ali joda na vsebnost fagopirinov v rastli-

nah. Pri tem poskusu se niso pokazale značilne razlike v koncentraciji fagopirinov med različno tretiranimi rastlinami, niti v primerjavi z netretirano kontrolo (slika 1)

Pri zrnju z rastlin, tretiranih z iodatom, selenatom in selenitom, je bilo ugotovljeno znižanje vsebnosti rutina (razpredelnica 1). Pri zrnju z rastlin, tretiranih z jodidom, nobeno od tretiranj z raztopinami selena ni pokazalo značilnih razlik v primerjavi z netretirano kontrolo. V primeru rastlin, ki niso bile tretirane z raztopina selena se je pokazal vpliv jodata na povečano vsebnost rutina v primerjavi s kontrolo. Pri rastlinah, tretiranih z raztopino selenata, nobena od oblik joda ni vplivala na koncentracijo rutina v zrnju. Zrnje z rastlin,

tretiranih z raztopinama joda v kombinaciji s selenom, je imelo višjo koncentracijo rutina v primerjavi s kon-

trolo in z rastlinami, ki so bile tretirane s selenitom in jodatom ali s selenatom v kombinaciji z jodatom.

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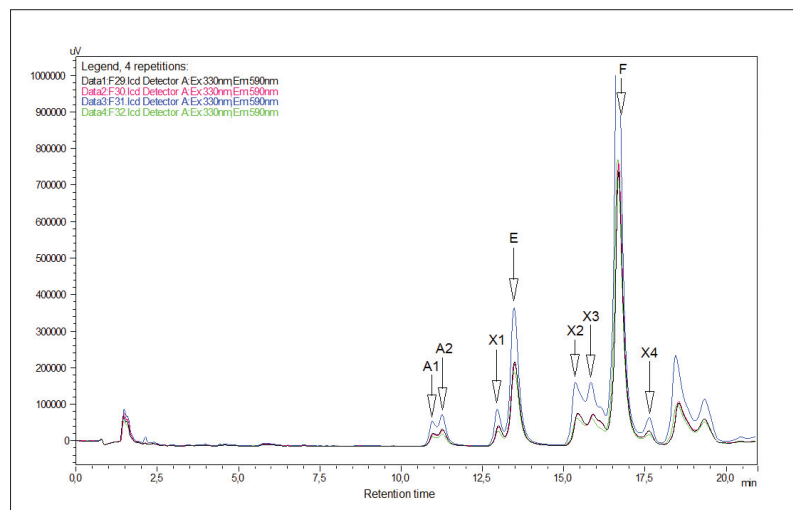


Figure 2: Chromatogram of fagopyrins obtained at HPLC analysis, peaks marked with A1, A2, X1, E, X2, X3, F and X4 show fagopyrins in seeds of common buckwheat

Slika 2: Kromatogram fagopirina s HPLC analizo, vrhovi označeni z oznakami A1, A2, X1, E, X2, X3, F in X4 kažejo fagopirine v zrnih navadne ajde

