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EFFECT OF VARIOUS STRONTIUM CONCENTRATIONS ON ITS UPTAKE AND THE CONTENT OF ISOFLAVONES IN SOYBEAN SPROUTS

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Metal ions can modify plant metabolism and change the level of biologically active components. In the present study, the impact of short-term exposure to strontium on the accumulation of the metal as well as the content of isoflavones in soybean sprouts was investigated. The seeds were germinated in hydroponics with 0, 1, 1.5, 2.5, 5.0, or 10.0 mM of Sr for 72 hours. The content of strontium was assessed using flame atomic absorption spectrometry and the amount of isoflavones was determined with high performance liquid chromatography. Dose-dependent accumulation of Sr and a linear correlation between the Sr concentration in the growth medium and the content of the element in the plant samples were observed. The largest changes in the isoflavone content, compared to the control, were noted in soy sprouts germinated in the presence of 5 and 10 mM of strontium. Daidzin, genistin, malonyldaidzin, and malonylgenistin were the dominant isoflavones and their content increased by approx. 28, 44, 34, and 47%, respectively, compared to the control. Low amounts of aglycones were found; moreover, their content decreased by ca. 19–30%. Our research can be important for obtaining a natural product enhanced with strontium and isoflavones, which contribute to prevention of osteoporosis associated with endogenous oestrogen deficits.

Keywords: *Glycine max* (L.) Merr., isoflavones, soy, sprouts, strontium

INTRODUCTION

Soy (*Glycine max* (L.) Merr.) is an annual plant from the legume family (Fabaceae, Lindl.) (http://www.theplantlist.org). It originates from China and Manchuria, but cultivation thereof gradually spread to other countries. Soybeans are a rich source of oil and protein and they are used to obtain soybean meal and vegetable oil. Soy products are extremely important for vegetarians and vegans, as they can be an alternative to animal-based foods, due to the complete composition of amino acids (Wójciak-Kosior et al., 2016a; Messina and Messina 2010).

In recent years, fresh germinated soybean has gained popularity. Soybean sprouts are widely consumed in many countries and regarded as a valuable component of a healthy diet due to their beneficial nutritional properties.

Many scientific publications have reported the nutritional value of soybean sprouts. They contain valuable nutrients such as proteins, unsaturated fatty acids, amino acids, minerals, vitamins, and dietary fibre, as well as biologically active secondary metabolites such as phenolic compounds, saponins, and phytosterols (Wójciak-Kosior et al., 2016a; Gu et al., 2017; Jiang et al., 2000). Among them, isoflavones representing the so-called phytoestrogens have aroused scientists' interest. They have been applied for prevention and treatment of hormonal disorders in postmenopausal women. In this period of life, the level of endogenous oestrogens declines, which increases the risk of development

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of osteoporosis and other disorders such as coronary heart disease or atherosclerosis. With their structural similarity to B-oestradiol, isoflavones exhibit affinity to oestrogen receptors and can be applied in hormone replacement therapy to alleviate the symptoms of menopause and prevent diseases associated with a decreased level of oestrogen (Watanabe et al., 2000; Watanabe et al., 2002; Kocjan et al., 2011).

It has been evidenced that the concentration of plant metabolites depends on a number of environmental factors. For instance, the addition of metal ions to the growth medium disrupts metabolic pathways, thereby affecting the biochemical profile of the plant. This can result in an increase (elicitation) or a decrease in the level of some plant metabolites (Murch et al., 2003; Michalak, 2006; Rai et al., 2005). Therefore, the influence of strontium ions added to the growth medium on the isoflavone content in soybean sprouts was investigated in the present study. Strontium was chosen because, due to its chemical and physical similarity to calcium, it can partially replace calcium, which is essential for plant development. Moreover, strontium formulations are applied to prevent postmenopausal osteoporosis, and enhanced production of isoflavones combined with accumulation of strontium in plant materials may increase the health potential of soybean sprouts.

MATERIAL AND METHODS

The isoflavone standards, solvents, and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was deionized and purified by ULTRAPURE Milipore Direct-Q® 3UV–R (Merck, Darmstadt, Germany). *Glycine max* (L.) Merr. soybeans were purchased in a local market (series PL230/12/210/L518. 2/2016).

The experiments were carried out in three variants and a control. In the first step, the seeds of soybeans were placed in distilled water (variant I) or in a water solution of strontium nitrate at a concentration of 1 mM, 2.5 mM, 5 mM, and 10 mM (variant II and III) for 24 h at 30°C. In the second step, the swelling seeds were moved to water (variant III) or to a water solution of strontium nitrate at an appropriate concentration (variant I and II). The samples were placed in a growth chamber at a temperature of 28°C, relative humidity 70%, and photosynthetic photon flux density of 150 μmol × m^{-2} × s^{-1} and incubated for 72 h. The control sample was placed and incubated in distilled water for 96 h in the same conditions as in the Sr treatments. The scheme of the experiments is shown in Fig. 1.

Next, the samples were washed with distilled water, dried, micronized, and subjected to high performance liquid chromatography (HPLC) and atomic absorption spectrometry (AAS) analysis.

DETERMINATION OF ISOFLAVONES

0.5 g of each sample was defatted with petroleum ether and extracted three times with 20 mL of methanol in an ultrasonic bath (3 × 15 min) (Wójciak-Kosior et al., 2016b). The extracts were combined, concentrated to 10 mL, and filtered. The analysis of isoflavones was performed using HPLC equipment (VWR Hitachi Chromaster 600, Merck), and the data obtained were analysed using EZChrom Elite software (Merck). The extracts were separated on an RP-18e LiChrospher 100 column (Merck) (25 cm × 4.0 mm i.d., 5 μm particle size) at 30°C. A mixture of acetonitrile (A) and water (B) with 0.025% of trifluoroacetic acid at a flow rate of 1.5 mL/min was used as a mobile phase. The following gradient program was used: A 15%, B 85% during 0–5 min; A 20%, B 80% during 5–15 min, and A 25%, B 75% during 15–40 min (Wójciak-Kosior et al., 2016c). The chromatograms were recorded in the range of 200–400 nm wavelength. The identity of the compounds was established by comparison of retention times and UV-Vis spectra with standards.

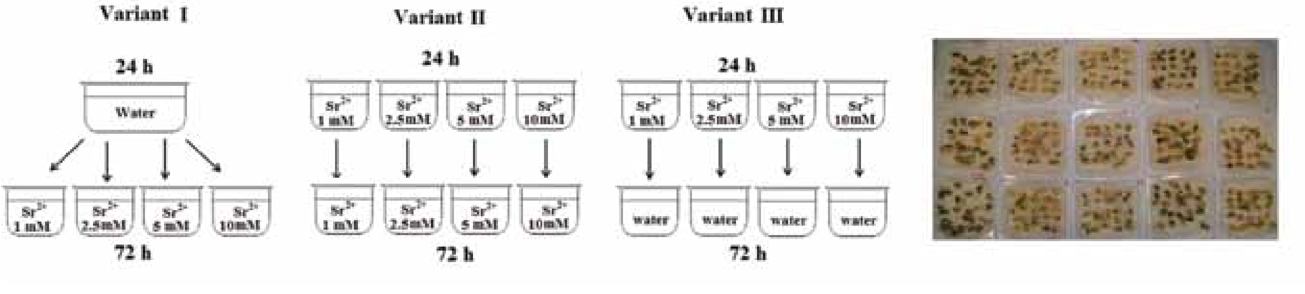

Fig. 1. Scheme of the experiment and an example of a photo of soy cultivated at different strontium concentrations.

STRONTIUM ACCUMULATION MEASUREMENT

0.5 g of each sample was mineralized in a closed microwave-assisted high-pressure digestion system (TOPWave, Analytik Jena, AG. Germany) with the use of a mixture containing 4 mL of 65% nitric acid and 10 mL of deionized water at 90% of the power generator for 40 min. The strontium contents were determined by flame atomic absorption spectrometry at $\lambda = 460.733$ nm (High-Resolution Continuum Source atomic absorption spectrometer ContrAA 700, Analytik Jena) (Sowa et al., 2014).

STATISTICAL ANALYSIS

All data were analysed by STATISTICA ver.10 (StatSoft, Inc, USA). Using the analysis of variance, the effects of Sr and the number of variants (two-way ANOVA) on isoflavones and Sr accumulation were analysed. The significance of differences was examined using Tukey's HSD test ($p < 0.05$). Additionally, a t-test was used to compare changes in the isoflavone accumulation between the control and the treatments at a 0.05 probability level. The experiments involved 12 treatments and the control with 3 pots and 24–26 seeds per pot. The whole experiment was repeated three times in the same conditions.

RESULTS

In the present study, the effect of cultivation of soybean sprouts at different concentrations of strontium ions on the isoflavone content and accumulation of the element in plants was investigated. Furthermore, the impact of strontium on soybean germination was assessed.

Our investigation has revealed that strontium exerts a negative effect on germination. In all samples treated with strontium ions, a statistically significant decrease in the number of germinated seeds was observed, compared to the control. The percentage of seed germination in water was 77.7 ± 11.5%, whereas the mean values for samples incubated in the strontium solution were 56.8 ± 7.1, 58.8 ± 5.3, 60.8 ± 5.8, and 35.8 ± 10.1% for the concentration of 1, 2.5, 5, and 10 mM, respectively. As can be seen, there were slight differences in the concentration range of 1–5 mM; however, at 10 mM of Sr, the decrease in the germinating power was significant.

Next, the methanolic extracts from soybean sprouts were analysed for the isoflavone content using HPLC. Six compounds were identified (Fig. 2) and the phytochemical profile was in agreement with literature data (Quinhone and Ida, 2015).

Daidzin (daidzein 7-O-glucoside), malonyldaidzin, genistin (genistein 7-O-glucoside), and malonylgenistin were the dominant isoflavones in all extracts. Their amounts in the studied sprouts were 75.49–125.13, 255.86–406.65, 169.65–277.23, and 57.79–103.76 μg/g, respectively. Low contents of aglycone forms, i.e., in the range of 25.20–48.69 for daidzein and 23.60–72.28 μg/g for genistein, were also found. The amounts of the investigated compounds obtained in the particular variants of the experiments are shown in Figure 3.

As can be noted, the level of isoflavones fluctuated depending on the strontium concentration and the experimental variant. Compared to the control, the largest changes were observed in soy sprouts exposed to strontium (variant II and III) at the concentration of 5 and 10 mM. Generally, the amount of glycoside and malonylglycoside forms increased; in turn, the content of aglycone forms, especially genistein, decreased. This suggests that stress conditions promote bonding of aglycones into derivatives. The percentage changes in the isoflavone content, relative to the control taken as 100%, are summarized in Table 1. The greatest differences were obtained in the pre-treated soybean sprouts cultivated in the strontium solution (variant II) at the concentration of 5 and 10 mM. An increase in the bonded forms up to 28.4, 43.9, 33.9, and 47.3% was noted for daidzin, genistin, malonyldaidzin, and malonylgenistin,

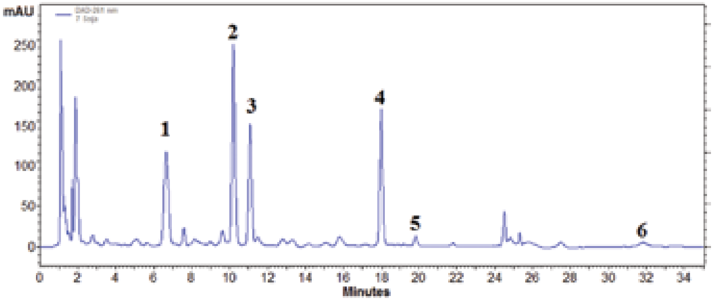

Fig. 2. Example of a chromatogram of the extract obtained from soybean sprouts: 1 – daidzin, 2 – genistin, 3 – malonyldaidzin, 4 – malonylgenistin, 5 – daidzein, 6 – genistein.

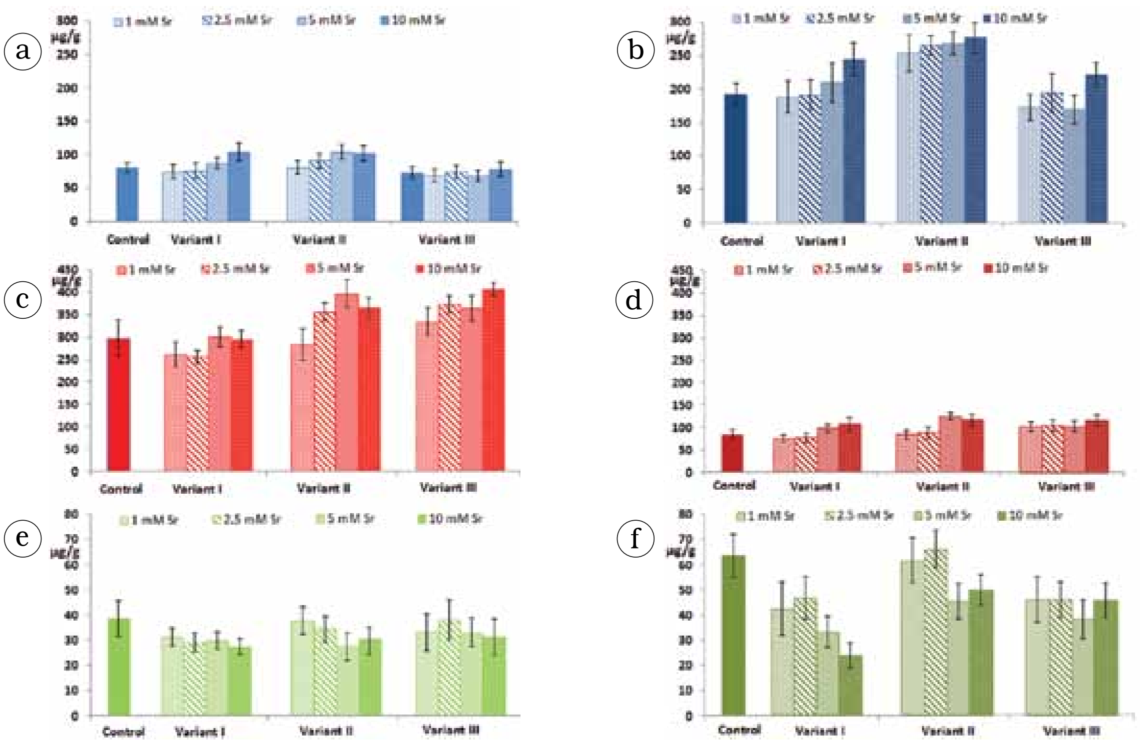

Fig. 3. Comparison of the isoflavone content in soybean sprouts obtained in the different variants of the experiment: (**a**) daidzin, (**b**) genistin, (**c**) malonyldaidzin, (**d**) malonylgenistin, (**e**) daidzein, (**f**) genistein.

TABLE 1. The percentage changes in isoflavones during cultivation at various strontium concentrations (the mean content of isoflavones in the control sample was taken as 100%). Variant I: 24h pre-incubation in water and cultivation in strontium, variant II: pre-incubation and cultivation in strontium, variant III: pre-incubation in strontium and cultivation in water.

Compoment	**variant I**				**variant II**				**variant III**			
	1 mM	**2.5 mM**	**5 mM**	**10 mM**	**1 mM**	**2.5 mM**	**5 mM**	**10 mM**	**1 mM**	**2.5 mM**	**5 mM**	**10 mM**
daidzin	-8,1	-6,0	7,8	**28.2***	-0,7	12,9	**28.4***	**26.5***	-14,7	-8,7	-15,5	-3,9
genistin	-1,9	-0,6	9,0	**27,4***	**32,1***	**38,1***	**39,7***	**43,9***	-10,2	1,1	-11,9	15,5
malonyldaidzin	-12,0	-13,8	1,4	-0,2	-4,5	19,9	**33,9***	**23,4***	12,3	**25,6***	**22,7***	**36,9***
malonylgenistin	-11,1	-9,5	15,1	**26,1***	-1,3	3,9	**47,3***	**36,8***	19,2	21,2	20,5	36,1*
daidzein	-18,1	-21,7	-19,2	-28,4*	-1,73	-10,3	-28,1*	-19,3	-11,7	-0,7	-13,5	-18,4
genistein	-34,5*	-27,5*	-48,9*	-63,3*	-4,43	-2,37	-29,8*	-22,7*	-28,6*	-28,8*	-41,1*	-29,2*

* – the value was statistically different comparing to the control ($p < 0.05$)

respectively; however, the amount of aglycones decreased by ca. 19–30%.

In the next step of our investigation, AAS analyses of the strontium content were carried out to assess the ability of four-day soy sprouts to accumulate Sr from the medium. The results are presented in Table 2. As expected, the highest concentration of Sr was found in the pre-treated samples incubated with the strontium solution (variant II). Moreover, we observed dose-dependent accumulation of Sr in all variants of the experiments and a linear correlation between the concentration of Sr in the growth medium and its content in the plant samples. The higher

TABLE 2. The content of strontium (mg/kg of dried plant material ±SD) and the bioconcentration factor in soybean sprouts cultivated at various strontium concentrations (n = 3). Variant I: pre-incubation in water and cultivation in strontium, variant II: pre-incubation and cultivation in strontium, variant III: pre-incubation in strontium and cultivation in water. Values followed by the same letters are not significantly different ($p < 0.05$, Tukey's test).

Sr in medium	**Variant I**		**Variant II**		**Variant III**	
	mg/kg ± SD	**BCF**	**mg/kg ± SD**	**BCF**	**mg/kg ± SD**	**BCF**
0 (control)	0.57 ± 0.16^{a}		0.64 ± 0.16^{a}		0.61 ± 0.17^{a}	
1.0 mM	30.06 ± 11.04^{b}	0.34	61.28 ± 14.22^{c}	0.70	46.55 ± 16.27^{b}	0.53
2.5 mM	71.17 ± 17.86^{c}	0.32	139.74 ± 16.75^{d}	0.64	103.50 ± 18.68^{e}	0.47
5.0 mM	155.33 ± 20.08^{d}	0.35	259.56 ± 23.10^{f}	0.59	194.12 ± 26.10^{g}	0.44
10.0 mM	278.34 ± 21.31^{f}	0.32	429.29 ± 27.69^{h}	0.49	320.77 ± 24.68^{i}	0.37

concentration of Sr obtained in variant III (pre-incubation in strontium and cultivation in water) compared to variant I (pre-incubation in water and cultivation in strontium) indicated that Sr was more effectively absorbed during seed swelling. The bioconcentration factor (BCF) (the ratio of the ion content in the material to its content in the environment), which indicates the ability to accumulate metals, decreased at the higher Sr concentration in the medium.

To sum up, the best results in terms of the seed germinating power, isoflavone content, and strontium concentration in the plant material were obtained when the soybean seeds were pre-incubated and cultivated at the 5 mM strontium solution. The higher Sr concentrations turned out to be toxic and the amount of germinated seeds was significantly lower than in the control.

DISCUSSION

Sprouts are a very attractive component of diet, as the intense biochemical processes occurring during sprouting increase the nutritional value of plants, e.g., they have an effect on the content of proteins, free amino acids, sugars, and vitamins, especially vitamin C (Quinhone and Ida, 2015; Sun et al., 2018). The enrichment of soybean sprouts with micro and macro elements during cultivation to obtain a health-enhancing product has recently received more researchers' attention (Diowksz et al., 2014; Wang et al., 2016; Lazo-Vélez et al., 2018; Zielińska-Dawidziak et al., 2018). However, the influence of additives on the level of plant secondary metabolites should also be taken into consideration, because they can disturb the metabolic pathways and thus alter the phytochemical profile. For instance, Wang et al. (2016) proved that supplementation with Ca improved the morphological parameters of soy sprouts and total isoflavone content. However, the impact of Ca on the particular forms of isoflavones differed significantly and, a decrease in the amount of aglycones was observed, as in our results. Similarly, Lazo-Vélez et al. (2018) noticed dose-dependent changes in the isoflavone level during fertilization of soy sprouts with selenium salt. On the other hand, iron ions did not have a statistically significant effect on the total flavonoid and total phenolic content up to day 4 of germination; however, a considerable increase was observed over a longer time of cultivation (Zielińska-Dawidziak et al., 2018).

Our previous experiments with longer (25 days) hydroponic cultivation of soy at a strontium concentration of 0.5–3 mM showed high accumulation of Sr in shoots (up to 1246 mg/kg of dried material) and no negative effect on soy growth parameters up to 2.0 mM Sr (Sowa et al., 2014). There was also an increase in the isoflavone content (Wójciak-Kosior et al., 2016b). Based on these findings, it can be claimed that the longer cultivation of soy at a lower Sr concentration was more beneficial.

CONCLUSION

Our investigations have indicated that strontium can be successfully used to functionalize soybean sprouts. However, the way of acquisition of plant material and the dose of strontium used in the cultivation experiments were extremely important. The most valuable plant material in terms of strontium and isoflavones was obtained when the pre-incubation and germination were conducted at a Sr concentration of 5 and 10 mM.

The content of daidzin, genistin, malonyldaidzin, and malonylgenistin were by approx. 28–47 % higher than in the control; in turn, the amount of aglycones decreased by ca. 19–30%.

AUTHORS' CONTRIBUTIONS

Study conception and design – MW, SD, IS, ML; performing experiments – SD, KŁ, MS, KZ; analysis of data – MW, SD, IS; drafting of manuscript – MW, SD, IS; reading and final approval of the version submitted to be published – all authors.

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