DOI: 10.1515/eces-2018-0042 ECOL CHEM ENG S. 2018;25(4):631-641

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SOYBEAN SEEDLINGS ENRICHED WITH IRON AND MAGNESIUM - IMPACT ON GERMINATION, GROWTH AND ANTIOXIDANT PROPERTIES

SIEWKI SOI WZBOGACONE ŻELAZEM I MAGNEZEM - WPŁYW NA KIEŁKOWANIE, WZROST I WŁAŚCIWOŚCI ANTYOKSYDACYJNE

Abstract: Iron (Fe) and magnesium (Mg) deficiency in human diets is a widespread problem observed in various regions of the world. Insufficient Fe uptake results in the development of iron dependent anaemia and depressed physical and intellectual performance. In turn Mg deficiency is associated with alterations in neuromuscular and cardiovascular systems. An emerging alternative to traditional supplementation of these elements in the form of pills, liquids or effervescent tablets, is introduction of fortified food products. In present study we show that preincubation of soybean seeds in Fe and Mg solutions leads to elevated content of these elements in the seedlings. Importantly the pretreatment did not affect germination rate, seedlings growth or, with an exception of Fe supplementation at highest concentration, antioxidant capacity. The obtained results indicate that preincubation of seeds in Fe and Mg solutions may be a promising method of obtaining enriched soybean sprouts.

Keywords: magnesium, iron, soybean, sprouts, fortification, biofortification, antioxidants

Introduction

Iron (Fe) is an essential element indispensable for proper functioning of organisms. In the case of humans it is found mainly in hemoglobin, myoglobin and other hem- and nonheme compounds. Its main functions include involvement in oxygen storage and

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transport, transfer of electrons in electron transfer chains and participation in oxidation-reduction reactions. Although Fe is abundant in the environment, it is found mainly in oxidized, not easily absorbed form. Reduced Fe levels in human organisms are associated with the development of iron dependent anaemia (IDA), alerted immunity mechanisms, reduced learning ability, hampered physical activity and increased mortality [1, 2]. According to World Health Organization anaemia affects over 1.6 billion of people. The most susceptible groups are woman in reproductive age (15-49 years) and children. The problem is generally more severe in the developing countries, particularly in Africa and South-East Asia. In these regions over 60 % of preschool age children and nearly 50 % of woman in reproductive age suffer from low hemoglobin levels indicating IDA. However, the anaemia is widespread public health problem and affects, to various degrees, nearly all countries of the world [3].

Another important element frequently deficient in human organisms is magnesium (Mg). Comparison of hypomagnesaemia studies carried out in various regions and population groups revealed that the prevalence ranges from nearly 2 % to over 36 % of general population. Some groups including elderly people, people suffering from kidney disorders, malabsorption and diabetes or subjected to intensive physical exercises are particularly susceptible to Mg depletion. It is worth highlighting that estimation of Mg status is not a routine practice. Moreover, it is commonly based on the measurements of Mg level in serum, which not necessary reflects the status of the whole body. Therefore, it is highly probable that the number of people affected by hypomagnesaemia is still underestimated [4, 5]. Magnesium is crucial for proper functioning of over 300 enzymes including adenylate cyclase, hexokinase, phosphofructokinase, protein kinases and phospholipase C. Additionally it is engaged in transport of ions and nerve conduction. Low Mg body levels are associated with alerted calcium and potassium homeostasis, osteoporosis, hypertension, heart arrhythmia and disfunctions of neuromuscular systems such as hyperexcitability, cramps, muscle weakening and tremor, nervousness, apathy and depression [5, 6].

The described information demonstrate that Fe and Mg deficiency is a frequent problem occurring in developing as well as developed countries and that it leads to weakened physical and intellectual performances and decreased quality of life. Not surprisingly supplementation of diet with these essential elements is gaining increased attention. It is estimated that the whole dietary supplements market valued over USD 90 billions in 2013 with the greatest share of vitamins and minerals. In 2016 the global market of solely magnesium supplements was estimated to reach USD 92 millions [7, 8]. Emerging alternative to traditional supplements is food fortification or biofortification. Fortification strategy, which means industrial enrichment of food with minerals and vitamins, has been applied as mandatory practice in numerous countries to prevent public health problems. For instance obligatory enrichment of wheat flour, maize flour or rice with folate, introduced in over 80 countries, led to significant reduction of neuronal tube defects in new born children [9, 10]. Other successful programs include enrichment of salt with iodine preventing from the development of goiter and cretinism, supplementation of sugar with vitamin A in Guatemala leading to decline it vitamin A deficiency and decrease in anemia occurrence in children and woman of Costa Rica after mandatory fortification of milk and flour with iron [10, 11]. Beside mandatory fortification, there is an extensive list of mineral or vitamin enriched food products fortified voluntary by the producers. For example the list of vitamins (A, C and D), folate and/or iron enriched products in US includes milk, milk and fruit drinks, cereals, pasta dishes, pizza and sweet and salty snacks (biscuits, cookies, cracker, popcorn, pretzels etc.) [12]. In recent years increased attention is paid to biofortification described as increase in the content of nutrients in crop plants achieved through breeding, argonomic practices or genome manipulations. The HarvestPlus Program dedicated to the development and introduction of biofortified crops has released over 150 varieties in 30 countries. Recent research show that introduction of iron biofortified beans led to increased hemoglobin in university woman in Rwanda, consumption of iron rich pearl millet resulted in improved ferritin and total body iron in schoolchildren in India, while implementation of orange sweet potato and orange maize increased vitamin A levels in children from Uganda and Zambia [13].

In summary the fortification and biofortification is a promising method in prevention of vitamin and mineral deficiency and improvement of public health. The aim of present study was acquisition of Fe and Mg enriched soybean sprouts by pre-incubation of the seeds in Fe and Mg solutions. The conducted analysis included examination of the treatment impact on elements uptake and homeostasis, germination rate, seedlings growth and antioxidant activity.

Materials and methods

Treatment procedures and growth measurements

Soybean seeds, kindly supplied by Department of Genetics and Plant Breeding at the Poznan University of Life Sciences, were surface sterilized with 75 % ethanol for 5 min and afterwards with 1 % sodium hypochlorite for 10 min. Seeds were washed for 30 min under running water and soaked for exactly 2 h in 30 cm^3 of distilled water (control) or $FeSO_4$ with Fe at concentrations 25, 50, 100 and 500 mg/dm^3 or $MgSO_4$ with Mg at concentration 25, 50, 100 and 500 mg/dm^3. The incubation time has been chosen on the basis of preliminary studies showing high rate of seeds germination after 2 h of imbibition in distilled water. Thereafter the seeds were thoroughly washed and transferred to glass Petri dishes of 30 cm of diameter lined with two layers of lignin and one layer of blotting paper. The seeds were watered with 30 cm^3 of distilled water and germinated for 72 h in the dark at stable temperature of 21-22 °C. The germination rate was estimated after 24, 48 and 72 h. The length of the roots was measured after 72 h of germination.

Quantification of elements content

Mg, Fe as well as Na, K, Ca, Cl, Al, Mn, and Cu content in soybean was determined by means of neutron activation analysis at the reactor IBR-2, JINR, FLNP (Dubna, Russia). Characteristics of neutron flux density in the two irradiation channels equipped with the pneumatic system and registration of gamma spectra can be found elsewhere [14]. To determine elements with short-lived isotopes (Al, Ca, Cl, Cu, Mg, and Mn) samples were irradiated for 3 min and measured for 15 min. The concentrations of elements based on long-lived radionuclides: Na, K, and Fe were determined by irradiation for 4 days at neutron flux of $3.31 \cdot 10^{12}$ n cm^{-2} s^{-1}. After irradiation samples were re-packed, and measured twice using HP germanium detectors after 4 and 20 days of decay, respectively. The NAA data processing and determination of element concentrations were performed using software developed in FLNP JINR [15].

The quality control of the analytical measurements was carried out using certified reference materials: NIST SRM 1547 - Peach leaves, NIST SRM 1633c - Coal fly ash, NIST SRM 2709 - Trace elements in soil, NIST SRM 2710 - Montana soil and BCR-667 - Estuarine sediment. The experimentally measured contents were in good agreement with the recommended values.

Measurement of antioxidant activity

Antioxidant activity was assessed according to the procedure described by Brand-Williams et al. [16]. Seedlings with similar fresh weight (200-300 mg) were homogenized in 2 cm^3 of 80 % methanol. The samples were transferred to Eppendorf tubes, incubated for 2 h at 37 °C and centrifuged 10 min at 12 000 rcf. Supernatants (100 mm^3) were transferred to mixtures containing 2 cm^3 of 80 % methanol and 250 mm^3 of DPPH. The blank contained 250 mm^3 of methanol instead of the DPPH. Straight after preparation of samples the absorbance was measured by λ = 517 nm, followed by 10 min incubation at room temperature in the dark and second spectrophotometrical measurement by the same light wave length. The antioxidant capacity was expressed as difference in DPPH quenching between the first and second specrophotometrical measurement.

Statistical analysis

The significant differences in relation to the control were calculated with the use of ANOVA by $p < 0.05$ (differences marked with **) and $p < 0.1$ (differences marked with *).

Results

Elements composition

The content of elements in seedlings grown for 72 h was quantified using neutron activation analysis (Fig. 1, Tables 1 and 2). Supplementation with Fe and Mg resulted in elevated content of these elements in the seedlings. In the case of Fe its content increased by approximately 100 % after incubation in concentration 50 and 100 mg/dm^3 and by over 500 % in the case of highest applied concentration (Fig. 1a). In the case of Mg the increase was less prominent. The level of this element was elevated by approximately 20 % in response to treatment with Mg at concentration 100 and 500 mg/dm^3 (Fig. 1b).

No differences in content of any other element between control and Fe or Mg treated seedlings were noted (Tables 1 and 2), with an exception of slightly alerted Al and Mn levels after incubation in Fe solution at highest applied concentration (Table 1).

Table 1

Content of Mg, K, Ca, Na, Cl, Al, Mn and Cu in control seedlings and seedlings grown from seeds incubated in Fe solutions after 72 h of germination

Element	Control	Fe 25	Fe 50	Fe 100	Fe 500
Elements content [mg/g d.m.]					
K	25 ±3	24 ±3	20 ±2	27 ±1	24 ±2
Ca	3.9 ±0.1	4.0 ±0.4	3.4 ±0.2	3.2 ±0.2	3.3 ±0.1
Elements content [μg/g d.m.]					
Na	282 ±33	303 ±71	361 ±88	336 ±17	297 ±29
Cl	155 ±8	171 ±16	160 ±13	143 ±12	147 ±5
Al	32 ±4	30 ±4	22 ±1*	27 ±3	25 ±1
Mn	33 ±4	34 ±4	27 ±1*	28 ±2	29 ±1
Cu	14 ±0.6	14 ±1	12 ±1	13 ±0.4	14 ±0.8

Table 2

Content of Mg, K, Ca, Na, Cl, Al, Mn and Cu in control seedlings and seedlings grown from seeds incubated in Mg solutions after 72 h of germination

Element	Control	Mg 25	Mg 50	Mg 100	Mg 500
Elements content [mg/g d.m.]					
K	17 ±0.6	16.5 ±0.9	17.1 ±0.7	19.1 ±0.7	16.9 ±0.9
Ca	3.8 ±0.3	4.0 ±0.1	3.8 ±0.1	4.1 ±0.2	3.4 ±0.2
Elements content [µg/g d.m.]					
Na	390 ±99	366 ±14	350 ±83	279 ±44	349 ±18
Cl	186 ±22	176 ±2	186 ±23	190 ±17	199 ±5
Al	38 ±1	42 ±4	35 ±3	48 ±5	53 ±8
Mn	27 ±1	26 ±1	27 ±2	31 ±2	27 ±2
Cu	14 ±1	14 ±1	15 ±0.5	17 ±1	15 ±0.5

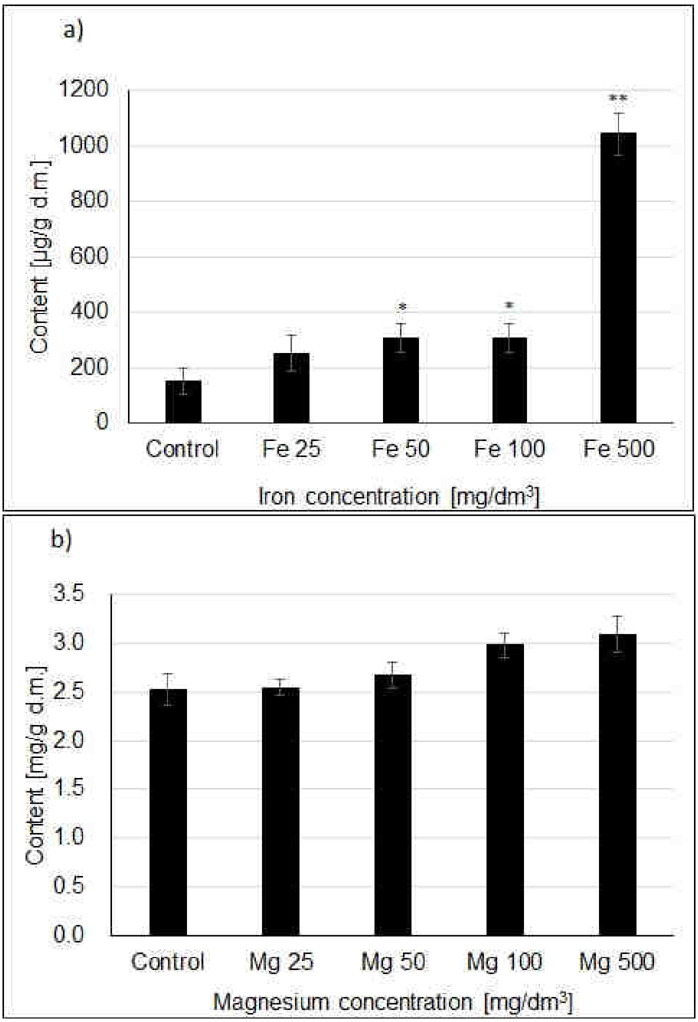

Fig. 1. Content of: a) Fe and b) Mg in seedlings grown from seed incubated in $FeSO_4$ and $MgSO_4$ solutions

Germination rate and seedlings growth

Incubation of seeds in Fe or Mg solutions did not affect germination rate in any of examined time periods (Fig. 2a and b). Metal treated and control seedlings germinated for 72 h showed also no differences in the terms of roots length (Fig. 3a and b). The results imply that preincubation in Fe and Mg solutions had neither inhibitory nor stimulatory influence on germination and seedlings growth.

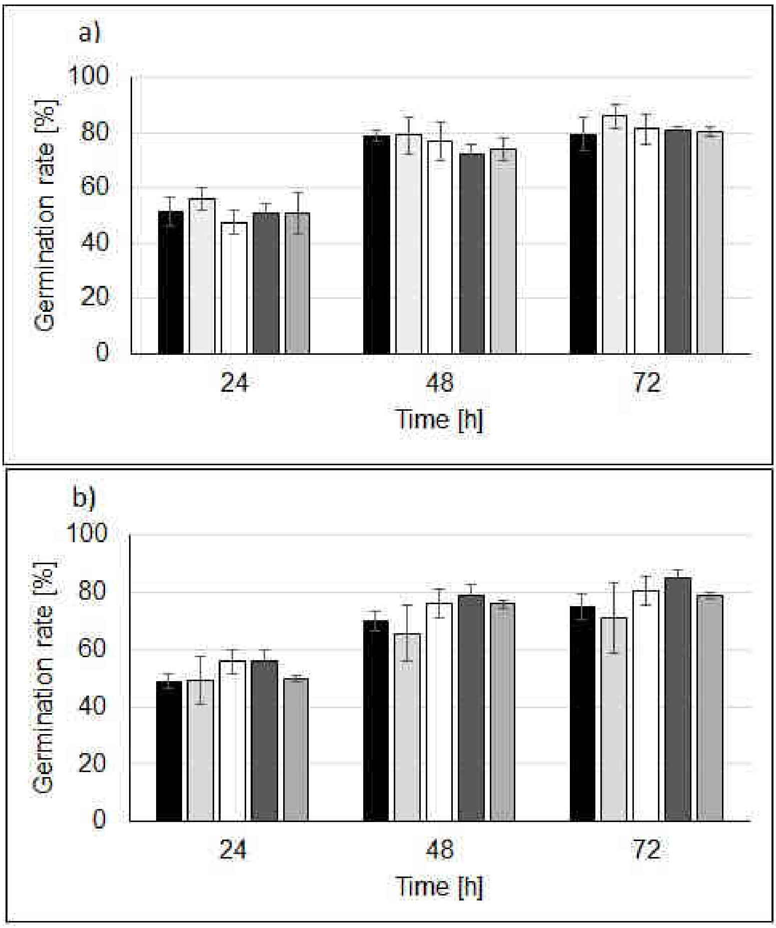

Fig. 2. Germination rate of seeds exposed to: a) $FeSO_4$ and b) $MgSO_4$ solutions with Fe or Mg at concentration 0 (control, black bars), 25 (cream-color bars), 50 (white bars), 100 (dark grey bars) and 500 mg/dm^3 (light grey bars) measured after 24, 48 and 72 h

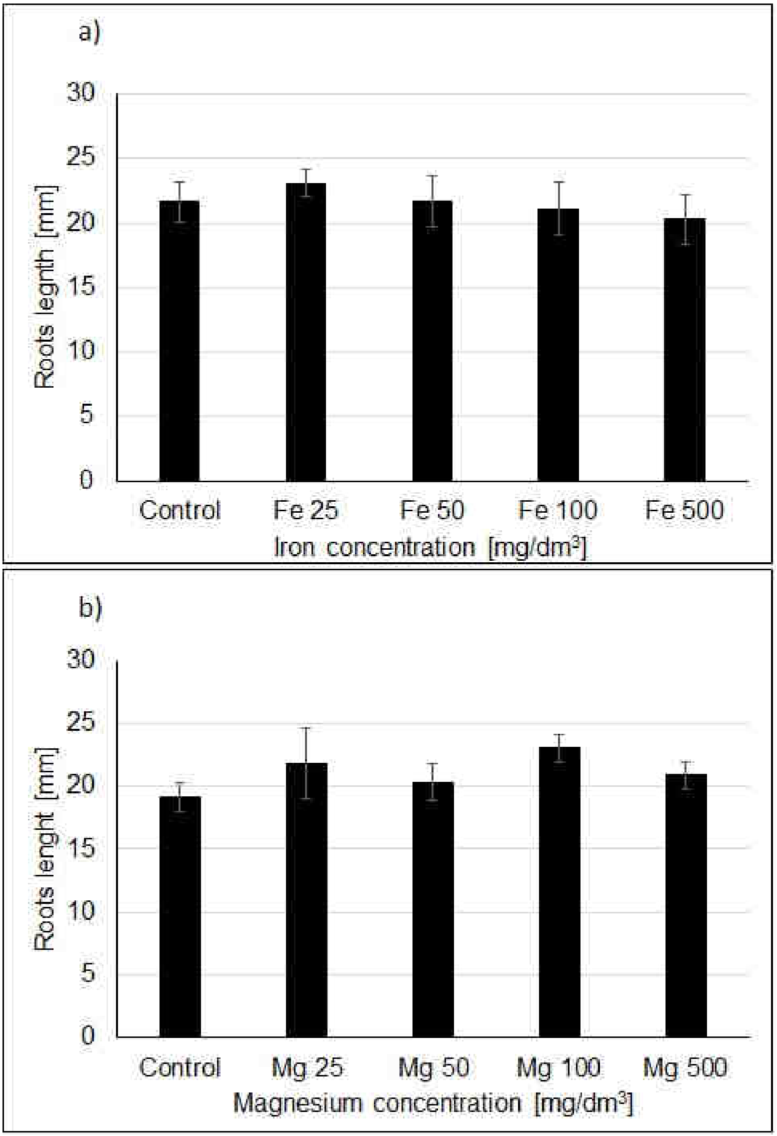

Fig. 3. Length of the roots of seedlings germinated for 72 h grown from seeds previously incubated in: a) $FeSO_4$ and b) $MgSO_4$ solutions

Antioxidant capacity

The experimental variants showing significant increase in Fe and Mg uptake, treated with metals at the concentration 100 and 500 mg/dm^3 (Fig. 1), were chosen for antioxidant activity measurements. In Fe-supplemented seedlings decrease in total antioxidant activity was noted after 72 h of germination in response to highest applied Fe concentration (Fig.

4a). In turn in response to Mg supplementation no differences were observed in any of the examined time points (Fig. 4b).

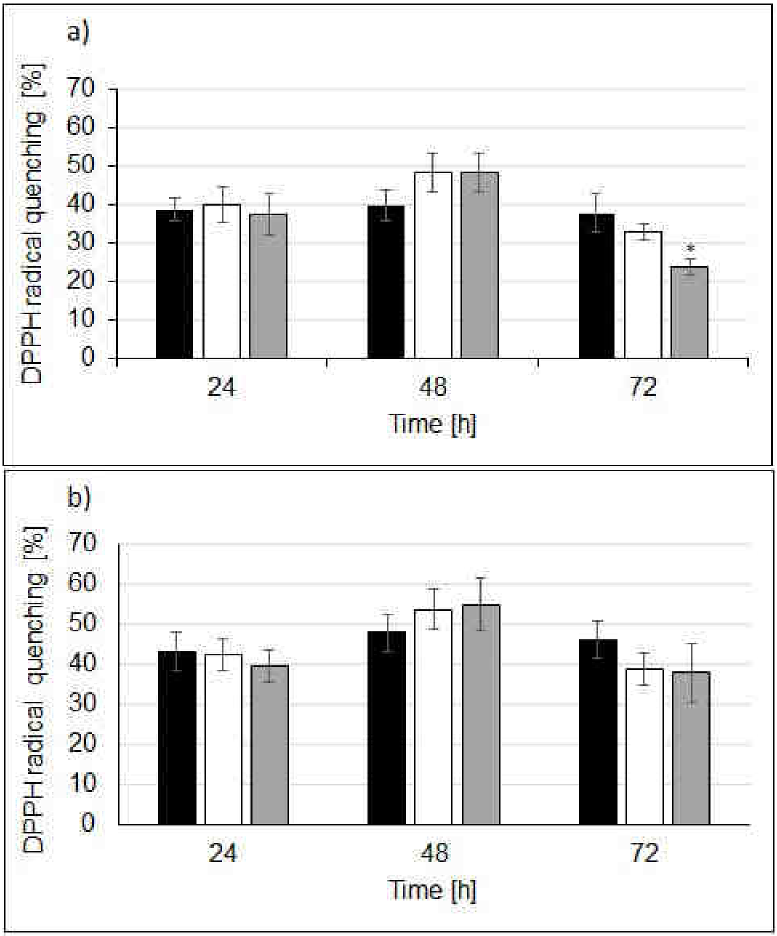

Fig. 4. Antioxidant activity of seedlings grown from seeds incuabated in: a) $FeSO_4$ and b) $MgSO_4$ solutions with Fe or Mg at concentration 0 (control, black bars), 100 (cream-color bars) and 500 $mg\cdot dm^{-3}$ (white bars) measured after 24, 48 and 72 h

Discussion

Iron and magnesium deficiency is a widespread problem observed in various parts of the world. Lowered Fe levels lead to iron dependent anaemia associated with hampered physical and intellectual performance and increase mortality. In turn hypomagnesia is accompanied by alterations in calcium and potassium homeostasis and disorders in neuromuscular and cardiovascular systems [1-6]. The proposed strategies for prevention of mineral deficiency in humans are introduction of supplements, industrial fortification of food products or biofortification of crops [9-10, 17]. In present study we aimed to acquire

Fe and Mg enriched soybean sprouts through preincubation of seeds in Fe and Mg solutions. The experiment design has been chosen for several reasons: soybean is one of the most important crops of the world, consumed worldwide due to high content of easily digested proteins, unsaturated fatty acids including 3-omega and numerous other biologically active compounds such as isoflavones (genistein, daidzein, glycitein) [18, 19]. Sprouting additionally improves the nutrition value of soybean through increase in amino acid, vitamin and isoflavones content and reduced levels of antinutritional compounds such as trypsin inhibitors or phytic acid, which interferes with mineral absorption [20]. The method of seed preincubation is cheap, fast and very easy in application. Additionally a throughout study of fortification with nine element of garden cress, sunflower, mung bean, soybean and lentil demonstrated that soybean and lentils possess the highest ability to accumulate supplied minerals [21].

In the present study we show that preincubation of seeds in Fe and Mg solutions resulted in higher amount of these minerals in the seedlings. The effect was greater in the case of Fe - at the highest applied concentration the level of this element in seedlings increased fivefold (Fig. 1a). In the case of Mg the highest applied concentrations resulted in rise by approximately 20 % (Fig. 1b). Sprout fortification with Fe and Mg has been already successfully applied in mung bean, alfalfa, broccoli and radish [22, 23]. Iron enrichment has been also described in the case of soybean and adzuki bean sprouts [24, 25]. Additionally present study shows that metal pretreatment did not affect the level of other analyzed elements (K, Ca, Na, Cl, Al and Mn), with exception of slight decrease in Al in Mg content in the case of preincubation in Fe solution (Tables 1 and 2).

Exposure of seeds to metals and other elements might alert the germination rate and growth of seedling. For instance excessive treatment with selenium resulted in inhibition of mung bean germination and hampered growth of sprouts. Similarly exposure to high Fe concentrations resulted in decrease in germination rate of wheat [26, 27]. In present research preincubation of seeds in Fe and Mg solutions had neither inhibitory nor stimulatory effect on seeds germination (Fig. 2). The pretreatment also did not affect the growth of seedlings indicated by roots length (Fig. 3). Thus it can be concluded that incubation of seeds in Fe and Mg solutions will not influence the productivity of soybean sprouts. Treatment with minerals might also affect plants antioxidant status. It has been demonstrated that Fe and Mg supplementation resulted in increase of reactive oxygen species (ROS) level and alterations in antioxidant machinery in mung bean, alfalfa, broccoli and sunflower sprouts. The response differed depending on plant species and applied mineral. For instance exposure to Fe resulted in increased phenolics level in broccoli, alfalfa and mung bean sprouts, while Mg treatment led to the opposite effect. Similarly, in broccoli the content of ascorbic acid increased in response to Fe and decreased in reaction to Mg supplementation [23, 24]. In the case of present research pretreatment with Fe at highest concentration resulted in decrease in total antioxidant activity after 72 h of germination (Fig. 4a). Otherwise no significant changes in antioxidant status were observed in any of the treatments and time variants (Fig. 4).

Conclusions

Present study shows that even short term incubation of soybean seeds in Fe and Mg solutions results in higher content of these elements in seedlings. Importantly, it does not affect germination rate or seedlings growth. However, a decrease in antioxidant activity has been noted in the case of supplementation with highest Fe concentration. It can be concluded that incubation of seeds in Fe and Mg solutions is a promising, fast, easy and cheap method of obtaining enriched sprouts. Additional studies would be needed to examine the bioavailability of the minerals and the effect of treatment on the level of other bioactive compounds.

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