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The Safety of Food Supplemented in Iron with Sprouted in Abiotic Stress Legumes Seeds - Heavy Metal Pollution

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Abstract. The objective of presented experiment was to examine how intense is the accumulation of metals contaminating culture media (lead, cadmium and chromium) from concentrated solutions of Fe^{2+}. Sprouting lupine seeds tolerate the presence in the medium 25 mM of Fe^{2+}, 25 mM Pb^{2+} and 35 mM Cr^{3+}. The tolerance for Cd^{2+} is very low (below 2.5 mM). The high overexpression of plant ferritin is observed in sprouted seeds, during their growth in the concentrated solutions of Fe^{2+} (20 mM). The obtained total iron content was ~975 mg/100 g of their dry matter. After the introduction of 1 mM Pb^{2+} ions into the medium, the difference in the iron content was not observed, but the content of lead increased 130-fold. The introduction of 5 mM Cr^{3+} into the medium resulted in almost 50-fold increase of its content and more than 45% decrease in iron content. Industrial production of bioactive food enriched in ferritin-iron from sprouted legumes seeds requires the use of solutions with a high chemical purity to prevent accumulation in plants other toxic metals.

Keywords: lupine germination, medium composition, iron, lead, cadmium, chromium

1. Introduction

Legume seeds sprouting in hydroponic cultures with high concentration of $FeSO_4$ cumulate high content of iron in form of ferritin [1], which makes them useful to bioactive food construction. Ferritin in these sprouted seeds may become a potentially excellent iron supplement, especially for people with disordered processes of absorption of divalent cations, as well as for vegetarians, who still are looking for a good source of iron in food of plant origin [2].

The large-scale cultivation of plants enriched in this bioactive ingredient creates many new problems, having no significance in the laboratory scale. One of such problems is to reduce the cost of media used for the growth of these sprouts. The easiest way to reduce this cost is replacement of pure chemical compounds and deionized water for the medium growth preparation with the chemicals of technical purity and using tap water. $FeSO_4$ with technical purity contains many different contaminants. Among the most common impurities of technical sources of $FeSO_4$ should be mentioned lead, cadmium and chromium.

Plants developed various systems of defense against heavy metal ions. At first some extracellular manners, such as mycorrhiza and excretion of some exudates. And also reduced uptake and efflux pumping at the plasma membrane, systems of chelation in cytosol, compartmentation in vacuoles and finally methods of repair stress-damaged protein [3]. On the basis of these different defense system, using the medium containing a high excess of iron and some contaminants, we could expect the following results. The first: some of contaminating metal ions, present in small concentration, could be absorbed by growing plants, without any impairments in dominating ion (i.e. iron) absorption. The second: some of these metals ions may be preferentially absorbed, limiting binding of iron ions. The third, the most optimistic situation, results from

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a huge excess of iron ions, which stimulate the plants mainly to their intoxification, leading to selective, high iron accumulation. Only the third of these situations enables us to replace the media components of the chemical purity for the technical grade ingredients. The problem of competitive metal ions binding were carried out, especially in the remediation field. It was proved, that lead, chromium and cadmium, frequent groundwater and soil pollution, are well absorbed from multi-metal solution [4], [5]. The object of presented experiment was to examine how intense is the accumulation of metals contaminating culture media (e.g. lead, cadmium and chromium) from highly concentrated solutions of Fe^{2+} and how the presence of another metal ions influence on iron accumulation.

2. Materials and Methods

2.1. Sprouted Lupine Preparation

Lupine seeds (*Lupinus luteus*, Zeus var.) from the Plant Breeding Antoniny (Poland), were used as a material for cultivation. The seeds were immersed every day over 7 days for a period of two hours in solutions of 0-35 mM $FeSO_4$, 0-35 mM $PbNO_3$, 0-35 mM $CdCl_2$ 0-35 mM $CrCl_3$, 5 mM $CrCl_3$+20 mM $FeSO_4$ and 1mM $PbNO_3$+20 mM $FeSO_4$.

The growing plants were placed in climate chambers (Adaptis 1/2000, Conviron), in the following conditions: temperature 23-24 ^{o}C, humidity 95-99%, illumination – 2 hours for the first six days of cultivation and 4 hours on the last day of the culturing.

Metal concentration tolerated by the plants was determined by the controlling the germination inhibition in the examined medium. A medium which reduced the germination more than 50% of seeds (while in water germinated ~ 82% of seeds) was considered as a medium not tolerated by the plant.

All salts used to prepare the solutions were chemically pure reagents.

2.2. Sample Preparation

Germinated seeds (both radicle and cotyledons) were dried at 45^{o}C, milled and subjected to the further analysis. Approximately 2-gram of each sample was weighed in quartz crucibles, subsequently ashed in the muffle furnace at 400 ^{o}C. Ash was dissolved in 1mol/l nitric acid (GR, ISO, Merck) and filled up in 25 ml volumetric polypropylene flasks to the mark by the same acid.

2.3. Determination of Metals Concentrations

The contents of iron, lead and cadmium in mineralizate solution were determined by the flame atomic absorption spectrometry method (F-AAS) using AAS-3 spectrometer (Carl-Zeiss, Germany). All the instrumental conditions applied for metal determinations were set in accordance to the general recommendations (wave length and gap width: 248.3 nm and 0.15 nm for Fe; 217.0 nm and 0.3 nm for Pb; 326.1 nm and 0.5 nm for Cd).

The chromium content was determined by the GF-AAS, with atomization in a graphite tube and background correction using spectrometer AAS-5 EA (Jenoptic, Germany) at wave length of 357.9 nm and gap width of 0.8 nm.

2.4. Statistical Analysis

All germination experiments were prepared in 9 repetitions. Obtained material were mixed and analyzed in 3 replications. The analysis of the results was conducted based on descriptive statistics (arithmetic mean and standard deviation), one-way analysis of variance and regression analysis using Statistica ver.8.0 software (StatSoft, USA).

3. Results and Discussion

The growth of all organisms is determined by the proper supply of metal ions. Plants have developed different mechanism of metal absorption from the soil as well as defense system against excessive ions concentration degrading cell functions. Generally, some of elements, such as Fe, Na, K, Mg, Ca, Mn, Mo, Co, Ni, Cu, P and B, are indispensable for plant growth and development. Another, as Pb, Se, Se, Cd, As, Li, are xenobiotic. And the others, which are not such toxic as lead and not essential as iron, and do not posses its own system of absorption in plants e.g. chromium [6], [7]. These elements induce oxidative stress and utilize other metals systems of absorption. The tolerance levels to these ions in the soil is different and depends on time, the botanical species and genus [3], [5], [8]. Also the function of elements in plants may depends on the species, e.g. cobalt becomes essential for the growth of legumes during relying upon atmospheric nitrogen [5], [6].

In order to enhance biofortification of iron in the sprouting lupine seeds, their tolerance on the presence of $FeSO_4$ in the medium during hydroponic culturing was determined. The lupine seeds quite well tolerated Fe^{2+} ions in concentrations between 0-25 mM, and accumulation of iron in sprouted lupine seeds from these kind of medium is listed in Table 1. The most useful concentration of iron ions in the medium for efficient germination of lupine seeds due to their biofortification in ferritin iron seems to be 20 or 25 mM. This concentration may be proposed for the preparation of these sprouts as a material for the production of iron supplements or special sorts of functional foods. The costs reduction of preparing these seeds enriched in ferritin iron could be achieved by using $FeSO_4$ with technical grade for preparing medium intended for sprouts culturing. However, this kind of medium will contain some contaminating elements, such as lead, cadmium and chromium.

Lead is nonessential, toxic element for plants. Lupine seeds tolerated the lead in the same concentration, as it was observed for iron, and the accumulation of this metal in the lupine tissue during the seven-day culture in the solutions of $PbNO_3$ at the concentration of 25 mM was 1453 mg Pb/100 g d.w. The tolerance of the cadmium by the germinating seeds was extremely low – 2.5 mM of Cd^{2+} strongly inhibited the growth of the plant in more than 50% of the plants. Cadmium is considered to be very mobile element, easily absorbed by roots. This kind of element is distributed in different plant organs equally [9], in contrast to lead, poorly mobile element, which concentration in tissues is variable, reaches the highest level in roots and the lowest in seeds [9]. In previous studies, in pea or bean seeds, cadmium delayed germination, membrane damage, impaired food reserve mobilization [10] as well as led to mineral leakage that resulted in nutrient loss [11]. In the mechanism of its toxicity is involved oxidative stress and it has been reviewed elsewhere [12].

Table 1. The capacities for metal binding by sprouted lupine seeds [mg/100 g of dry matter] in hydroponic cultures from the medium containing: $FeSO_4$, $PbNO_3$, $CdCl_2$, $CrCl_3$

Ion in the medium	The content [mg/100 g d.m.] of investigated metal ion after culturing of lupine seeds in medium with concentration							
	0 mM	5 mM	10 mM	15 mM	20 mM	25 mM	30 mM	35 mM
Fe^{2+}	6.66 ±3.08 a	116.2 ±2.05 b	238.8 ±3.05 c	530.4 ±1.33 d	638.2 ±9.22 e	975.6 ±20.7 f	nt*	nt*
Pb^{2+}	0.99 ±0.01 a	350.9 ±14.9 b	550.0 ±23.0 c	890.0 ±41.2 d	1140 ±159.0 e	1453 ±8.20 f	nt*	nt*
Cd^{2+}	0.32 ±0.10	nt*	nt*	nt*	nt*	nt*	nt*	nt*
Cr^{3+}	0.60 ±0.04 a	60.34 ±0.04 b	134.6 ±0.05 c	190.5 ±0.07 d	273.7 ±10.3 e	356.0 ±0.10 g	340.9 ±0.12 f	335.9 ±0.10 f

Abbreviation: *nt - the concentration not tolerated by the plant in the medium; means on same line without common superscript differ significantly ($P < 0.05$)

Chromium, without causing the death of the lupine cells, was absorbed by the plants from the wide range of concentration (from 0 to 35 mM Cr^{3+}).

The toxicity of metal ions in prediction models (tested plant) is varied depending mainly on the plant species, the test used (e.g. root growth inhibition test, plant amount test, germination power) and the metal.

For example, decreased seed germination rate in *Arabidopsis* was reported in the order of Hg>Cd>Pb>Cu [13]. In the present study, the metal toxicity can be ordered as follows Cd^{+2}> Pb^{+2}=Fe^{+2}>Cr^{+3}.

Sprouted seeds obtained after culturing in the pure 20 mM $FeSO_4$ solution, contained lead and chromium, accumulated from the soil by the seeds, during their growth on a field. The content of these ions in sprouted seeds statistically not differed from their content in the seeds (data not presented).

For the next part of experiment, it was decided to introduce into the medium only 1 mM of $PbNO_3$ (which is a strong xenobiotic element for the plants), and 5 mM of $CrCl_3$ (which seems not to be as toxic as lead to germinating lupine seeds – tab. 1). In Table 2 the content of examined elements in sprouted seeds after the 7 day of culturing in these media were presented.

Table 2. Accumulation of metal ions in sprouted lupin seeds in the presence of other ions

Type of medium	Ion accumulation in the sprouted lupine seeds [mg/100 g d.m.]		
	Fe^{2+}	Pb^{2+}	Cr^{3+}
20 mM Fe^{2+}	975.6±20.7 b	1.42±0.20 a	0.70±0.10 a
1 mM Pb^{2+} +20 mM Fe^{2+}	969.0±101.7 b	189.5±20.6 b	nd*
5 mM Cr^{3+}+20 mM Fe^{2+}	664.4±109.0 a	nd*	32.1±1.7 b

Abbreviations: *nt - the concentration not tolerated by the plant in the medium; means in the column without common superscript differ significantly ($P < 0.05$)

The content of lead in the sprouted seeds after their growth in the medium composed from the 20 mM of $FeSO_4$ and 1 mM of $PbNO_3$ in Table 2 is presented. Even if lead concentration was small, the content of this element in sprouted seeds significantly increased (more than 130-fold) in comparison to the medium containing only iron ions. However, the presence of lead ion in the medium do not influenced the iron accumulation. The accumulation of these ions by the lupine seeds at this stage of the growth seems to be completely independent process. The mechanism of lead absorption explains this phenomenon, because this element is transported into the root cells via Ca^{2+}-permeable channels or via the apoplastic pathway [14].

After the cultivation of lupine in the solution of 20 mM $FeSO_4$ and 5 mM of $CrCl_3$, an unexpected inhibition of iron absorption was observed. The content of iron in sprouted seeds decreased more than 30%, while the content of chromium in the material increased by 4600%. The concentration of chromium in the medium was 4-fold lower than iron, and still its accumulation in sprouted seeds increased almost 50 times. The chromium accumulation from the multi-metal system is strongly competitive process to the iron absorption. This effect results from the loss of chromium specific transport system by plants, and exploiting to it absorption carriers of essential ions, as iron or sulfate [7].

4. Conclusion

Seeds germination in $FeSO_4$ solutions is an easy way to their enrichment in ferritin iron. However, due to the high efficient systems of ions absorption in roots, the culturing process must be carried out in the medium prepared using the chemicals with high purity. While lead is accumulated irrespectively of iron concentration, the strong competition between absorption of chromium and iron was observed. The extremely high iron ions excess inhibits absorption of another ions, but the inhibition is not sufficient to guarantee the safety of consumers.

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6. References

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