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EFFECT OF FERRI(III)CITRATE AND POTASSIUM HEXACYANOFERRATE(III) ON GROWTH OF THE MOSS *BRYUM ARGENTEUM* HEDW. (BRYACEAE) *IN VITRO*

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Abstract — In order to examine the manner of iron uptake from the medium, *in vitro* culture of moss *Bryum argenteum* Hedw. (*Bryaceae*) was established. Under controlled conditions (16h light/8h dark, light intensity 47μmol $m^{-2}s^{-1}$, 25±2°C), the moss was grown on basal MS medium or on MS medium enriched with various concentrations of ferri(III)citrate or potassium hexacyanoferrate(III). It was expected that with the organic chelate complex, Fe(III) ion will be more available for the plant. Sixty days after establishing *in vitro* culture, the plants grown on MS medium enriched with the ferri(III)citrate complex were developed better than plants grown on media with the potassium hexacyanoferrate(III) complex. To judge from plant production *in vitro* and in view of the fact that the two compounds were the only source of Fe(III), it would appear that the citrate complex makes Fe(III) ions more available than potassium hexacyanoferrate(III). Further research will examine the concentrations of Fe ion uptake by plants and potential use of these tiny moss plants for the phytomining, phytoremedies and hyperaccumulating purposes.

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INTRODUCTION

Bryophytes are excellent indicators for a wide range of contaminants (Zechmeister *et al.*, 2002). This is a consequence of a series of morphological and physiological properties like the absence of cuticules and the existance of pronounced cationic exchange properties within the cell wall. Mosses have mainly been used as accumulation indicators especially for heavy metals, radionucleides, and toxic organic compounds. Sulfurous and nitrogen depositions can hardly be analyzed by methods of accumulation monitoring except by investigation of the frequency, distribution, fertility, and vitality of bryophyte species and populations. Similar methods are used for global change research, especially for analysis of climate warming and the influence of land use intensity on biodiversity.

Bryophytes show a limited range of anatomical or morphological features, but a wide range of physiological and dispersal adaptations to stress caused by natural or anthropogenic disturbance (Bates and Farmer, 1992).

The actual plant of a bryophyte is represented by the gametophytic generation, which is the most evolved haploid generation in the whole plant kingdom. Bryophyte spore germination and growth are very sensitive to all kinds of natural and human influences, and this sensitivity exceeds by far that of the green gametophore (Gilbert, 1968).

Mineral requirements of bryophytes are similar to those of vascular plants (Zechmeister et al., 2002). Mineral uptake by the cell is controlled by the semipermeabile membrane. The protonema and early stage of the gametophyte are attached to the substrate, and significant stocks of nutrients can be accumulated from the surface in this phase. Later on, many species (especially pleurocarpous ones) lose close contact with the substrate, and it is generally assumed that the atmosphere is main source of minerals for these species (Tamm, 1953; Bates and Bakken, 1998), though some elements (Ca, K, and P, for example) seem to arrive further via the substrate (Bates, 1992; Wells and Boddy, 1995; Brown and Brumelis, 1996; Brumelis and Brown, 1997).

Elements associated with well developed gametophores can be of four possible kinds (Brown and Bates, 1990; Bates, 1992): trapped particulated matter, soluble

intercellular matter, extracellular matter bound to the cell wall on charged exchange sites, or intracellular matter. Particulate matter and intercellular elements are unbound ions in the watery free space and can easily be removed by washing or mechanical treatment. Exchangeable cations are linked with possitively charged exchange properties of the cell wall, are fixed by a process mainly depending on physico-chemical processes, and are not physiologically active, whereas intracellular elements fulfil a physiological function.

Intracellular uptake in bryophytes is influenced by various aspects of plant metabolism. Entry to the cell plasma is determined by the affinity for an appropriate carrier, competitive elements, gradients in element concentration or energy status. Elements located within the cell influence cell metabolism. Uptake rates are in general much lower than at the extracellular sites (Zechmeister et al., 2002).

Non-physiological elements like heavy metals may also pass the limiting plasma membrane of the cell and affect cell metabolism. In consequence, these metals induce the production of thiol-containing peptides such as glutathiones, which therefore can be used as biomarkers for heavy-metal pollution (Bruns et al., 1999). Nevertheless, the cell wall is an efficient barrier against the penetration of heavy metals into protoplasma of the bryophyte cell (Shimwell and Laurie, 1972). Young shoots tend to have a more effective barrier than older ones (Lüttige and Bauer, 1968).

Some bryophyte species tolerate elevated concentrations of toxic elements on the physiological level (Url, 1959; Shaw, 1987).

It is documented that the lack of iron in plants induces serious metabolic disfunctions, made easily visible by the presence of chloroses (in the absence of iron, chlorophylls are not synthesized). Iron lack induces many deffects in redox systems of plant cells, and inactivity of some enzymes (Nešković et al., 2003).

MATERIALS AND METHODS

The moss *Bryum argenteum* Hedw. was collected in November of 2000 in the Kalemegdan Fort Park (Belgrade). The voucher specimens are kept in the BEOU herbarium of Belgrade University.

Bryum argenteum was in the sporophyte phase, but without ripe opened capsules. *In vitro* culture was initiated from unopened capsules of almost mature spores (Sabovljević et al., 2002).

After collecting, sporophytes were separated carefully from gametophytes and rinsed with tap water for 30 minutes. Ten sporophytes of *B. argenteum* were then disinfected with 13% and 15% solutions of sodium hypochlorite.

After that, sporophytes were rinsed three times in sterile deionized water.

Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium that contained MS mineral salts and vitamins, 100 mg/l myo-inositol, 0.70% (w/v) agar (Torlak purified, Belgrade), and 0.1 M fructose instead of sucrose, was used as the basal medium.

In experiments where influence of ferri(III)citrate and potassium hexacyanoferrate(III) was observed, the basal medium was enriched with ferri(III)citrate or potassium hexacyanoferrate(III). Their concentrations were: 0.0001 mM, 0.001 mM, 0.01 mM, and 0.1 mM. Effects of exogenously applied ferri(III)citrate and potassium hexacyanoferrate(III) were determined.

The pH was adjusted to 5.8 before sterilization in an autoclave at 114°C and 108 kPa for 25 min.

The cultures were grown at 25±2°C under cool-white fluorescent light (47 μmol/m²s irradiance) and a day/night regime of 16/8 h.

To study morphogenesis of *B. argenteum* in culture *in vitro*, 10 mm long apical parts of shoots were used. For each concentration of applied compounds, 40 explants of moss shoots were cultivated in four petri dishes. Influence of ferri(III)citrate and potassium hexacyanoferrate(III) on the given moss species was quantified using an index of multiplication and secondary protonema diameter. The index of multiplication is represented by the number of newly grown shoots starting from one shoot explant.

RESULTS AND DISCUSSION

The results shown in Fig. 1 and Fig. 2 clearly indicate that uptake of the Fe^{3+} iron ion by the moss *B. argenteum* is better with the organic carrier ferri(III)citrate than with potassium hexacyanoferrate(III).

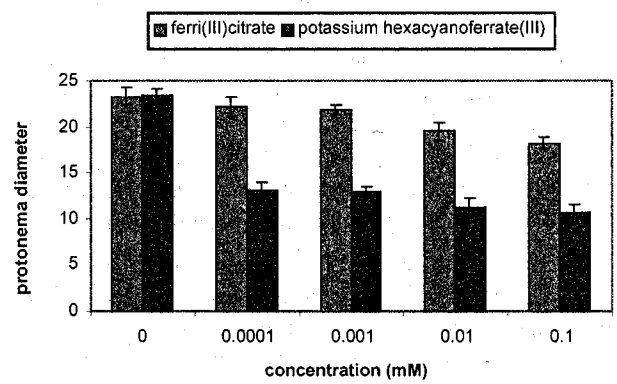

Fig. 1. Effects of ferri(III)citrate and potassium hexacyanoferrate(III) on protonema diameter in Bryum argenteum.

According to the obtained results, *B. argenteum* reacts differently to these two compounds. As for the effect of ferri(III)citrate, protonema diameter first increases at the optimum concentrations (0.0001 mM and 0.001 mM) and then slightly decreases (Fig. 1). However, in all cases protonema diameter in plants grown on media enriched with different concentrations of ferri(III)citrate is greater than protonema diameter in plants grown on media without this compound.

Potassium hexacyanoferrate(III) did not have a positive effect on protonema diameter in *B. argenteum*, and plants grown on the basal medium (not enriched with potassium hexacyanoferrate(III)) had the highest value of this parameter (Fig. 1). With increase of potassium hexacyanoferrate(III) concentration in the medium, protonema diameter slightly decreased. Protonema diameter in *B. argenteum* plants grown on media with ferri(III)citrate was greater than in plants grown on media enriched with potassium hexacyanoferrate(III). The indicated difference of this parameter appeared at the highest concentrations of these two compounds (0.01 mM and 0.1 mM).

Effects of ferri(III)citrate and potassium hexacyano-ferrate(III) on the index of multiplication in *B. argenteum* show different patterns. The index of multiplication in *B. argenteum* plants grown on media enriched with ferri(III)citrate is the highest at a concentration of 0.0001 mM, while any addition of exogenous potassium hexacyanoferrate(III) inhibits shoot formation (Fig. 2).

In the experiment where the effect of ferri(III)citrate on plant morphogenesis was observed, the index of multiplication first increases at the optimum concentration (0.0001 mM), slightly declines up to 0.01 mM, and then rapidly decreases at the highest concentration (0.1 mM). In *B. argenteum* plants grown on media with potassium hexacyanoferrate(III), the index of multiplication is very low on the highest concentration (0.1 mM). The index of multiplication in plants grown on media enriched with low potassium hexacyanoferrate(III) concentrations was slightly higher, but the highest value of this parameter was obtained on the basal medium (without potassium hexacyanoferrate(III)).

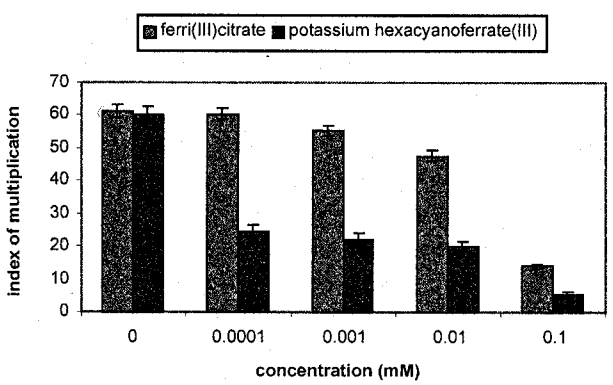

Fig. 1. Effects of ferri(III)citrate and potassium hexacyanoferrate(III) on index in multiplication of Bryum argenteum.

Although the manner of Fe ion uptake has been documented in vascular plants, there is no evidence for the bryophytes. Moreover, all evidence in vascular plants pertains to the roots, while bryophytes have no roots and uptake is performed by the whole plant surface. In view of the fact that vascular plants always uptake Fe (Fe^{2+}, Fe^{3+}) ions bound to some organic carrier (chelates or siderophores), it seemed logical to expect that bryophytes do the same. In fact, Fe uptake increased with the citrate carrier. This was as expected, since in vascular plants it has been demonstrated that citrate and malate production and their exudation into the rhizosphere increase Fe binding into chelate complexes (Kochien, 2000). Alternatively, grasses have developed their own chelating pathway for iron different of other vascular plants. It can be speculated that one of the above mentioned pathways, both of them, or some other mode of iron uptake are present in bryophytes. Also, the presence of the enzyme ferri-reductase or Fe ion transporters has not been documented for bryophyte cell membranes. Further investigations are needed.

CONCLUSIONS

In development of the moss *B. argenteum*, iron uptake is better in plants grown on media with ferri(III)citrate than in ones grown on media enriched with potassium hexacyanoferrate(III). These data can be used in developing biotechnology for remediation of substrata with high concentrations of irons.

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УТИЦАЈ ГВОЖЂЕ (III) ЦИТРАТА И КАЛИЈУМ-ХЕКСАЦИЈАНОФЕРАТА (III) НА РАСТЕЊЕ МАХОВИНЕ *BRYUM ARGENTEUM* HEDW. (*BRYACEAE*) У КУЛТУРИ *IN VITRO*

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In vitro култура маховине *Bryum argenteum* Hedw. (*Bryaceae*) је успостављена на подлози Murashige и Skoog (MS), при чему је праћен процес усвајања гвожђа из хранљиве подлоге. Културе *in vitro* су расле у условима дугог дана (16 h дан/8 h ноћ), на температури од 25±2°C, при влажности ваздуха од 60-70% и на светлости интензитета 47 μmol $m^{-2}s^{-1}$. Маховина *Bryum argenteum* је расла на MS базалној подлози (без додатка биљних хормона) или на MS подлози којој су додати гвожђе (III) цитрат или калијум-хексацијаноферат (III). Очекивано је да ће Fe^{3+} јон бити доступнији биљци у виду органског хелатног комплекса. Резултати су праћени 60 дана након успостављања *in vitro* културе. Биљке које су расле на подлози којој су додате различите концентрације гвожђе (III) цитрата боље су се развијале у поређењу са биљкама које су расле у присуству калијум-хексацијаноферата (III). На основу добијених резултата закључује се да биљке ефикасније усвајају Fe^{3+} јоне у облику цитратног комплекса. Будућа истраживања биће усмерена у правцу проучавања опсега концентрација Fe^{3+} јона које су биљке у могућности да усвајају као и о могућностима коришћења ове врсте маховине у процесима фиторемедијације и хиперакумулације.