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EFFECT OF PHOSPHORUS ON THE GROWTH AND PHOTOSYNTHETIC PIGMENTS CONTENT OF *HELICHRYSUM ARENARIUM* (L.) MOENCH PLANTLETS IN *IN VITRO* CULTURES

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Abstract

The plant material were plants of sandy everlasting (*Helichrysum arenarium* (L.) Moench) collected from natural locations in October 2012. Initial explants were apical buds enfolded into two leaves. Isolated explants were chemically sterilized. Reproducted shoots were divided and put into three types of mediums: medium MS (control) containing 37 mg $P \cdot dm^{-3}$, medium MS without additive of P (0,0 mg $P \cdot dm^{-3}$), medium MS with additional amound of P (74 mg $P \cdot dm^{-3}$). The aim of research was to specify the influence of phosphorus content in medium for choosen growth parameters and accumulation of assimilatory pigments: chlorophyll a, b, chlorophyll a+b, carotenoids. Analysis of these compounds were done spectrophotometrically. The made research, proved, that the richest in mentioned substances were microseedlings growing on the medium with increased amount of phosphorus. Phosphorus deficiency in medium MS had a statistically essential effect on changes in growth modifications of microseedlings of sandy everlasting (*Helichrysum arenarium* (L.) Moench). Additional amount of phosphorus in the medium had a statistically essential effect on increasing of chlorophyll a+b in comparison with plants growing on the medium MS with optimal phosphorus amount or without this element by accordinglly 36% and 23%. Moreover, it caused a growth of content of

chlorophyll a, b and carotenoids by accordinglly 18%, 32%, 20% in comparison with variant, where the medium MS without phosphorus was applied.

Key words: micropropagation, *Helichrysum arenarium*, phosphorus, bioactive substances

INTRODUCTION

Sandy everlasting (*Helichrysum arenarium* (L.) Moench) is a perennial plant of the *Asteraceae* family. In therapeutics the inflorescences of *Helichrysum arenarium* are used because of their content of biologically active compounds, which have choleretic, galley, hepatoprotective, diuretic, antithrombotic, capillary-sealing, detoxifying, antioxidative, antifungal, antiviral and antibactericidal properties (Aslan et al. 2006, Czinner et al. 2000, Lemberkovics et al. 2002, Pawlaczyk et al. 2009, Albayrak et al. 2010a, 2010b, Eroğlu et al. 2010, Stanojević et al. 2010). Cultivation of *Helichrysum arenarium* on a commercial scale using conventional methods is prohibited in Poland, because this species is under partial protection currently under the Regulation of the Minister of Environment of 9 October 2014 on the protection of plant species. One of the allowed methods for obtaining propagation material is the application of plant tissue and cell culture techniques (Sawilska and Figas 2006, Pawełczak and Bryksa-Godzisz 2008, Bryksa-Godzisz and Pawełczak 2010). In this way it is possible to win a significant amount of standard herbal material. The precise recognition of growth and development conditions of sandy everlasting will anable introduction of this species into field cultivation. Phosphorus is a necessery element for right functioning of each plant since it decides about quality and quantity of crop and cultivated plant. This element fulfills structural (phospholipidis), spare (fityna), regulating functions, takes part in process of inheritance (component of nucleic acid), energy storage (component of ATP), enzymes regulation. At the first stage of deficiency of that element in a plant there are growth modifications. Then irreversible metabolic changes, that delays plant blooming and fruiting (Marschner 1986, Ciereszko 2003, Gaj 2008, Bezak-Mazur and Stoińska 2013). In case of sandy everlasting inhibition of flowering herb lowers the quality of the material because of the lower yield obtained from the plant inflorescences.

The aim of the present research was to investigate the effect of phosphorus in MS medium for the selected parameters of growth and accumulation of certain bioactive substances: chlorophyll a+b, chlorophyll a, b, carotenoids.

MATERIAL AND METHOD

The plant material was collected from natural stands in Łosiny near Chojnice, Bory Tucholskie (N 53°37'13''; E 17°58'43'') in October 2012. Permission of sampling was granted by the Provincial Nature Conservation Office Bydgoszcz. Primary explants of *Helichrysum arenarium* (L.) Moench were initialised pical buds infolded into two leaves.

Isolated explants were chemically sterilized. In the first stage plant fragments after rinsing with tap water were dipped for 1 min. into a 70% ethanol solution for degreasing and surface pre-sterilization. Then the explants were treated with a 9% $Ca(OCl_2)$ solution with Tween 20 for 12 min. Finally, the buds were washed three times in bidistilled water. The so-sterilized explants of apical buds were inoculated on MS growth medium (Murashige and Skoog 1962) enriched with 1 $mg \cdot dm^{-3}$ KIN (kinetin). In addition, the medium comprised 3% sucrose as carbon source and was solidified by agar (0.8%). It was adjusted to pH 5.7 and autoclaved at a pressure of 0.5 MPa at a temperature of 121°C for 25 min.

In vitro culture of plants was conducted in a phytotron under controlled environmental conditions: temperature of 25 ± 2ºC, exposed to a 24-hour photoperiod (16 hours of light, 8 hours of dark), using fluorescent lamps Philips TLD 36W/54 emitting daylight. The quantum irradiation intensity was set up at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

After 8 weeks of culture under aseptic conditions axillary shoots were isolated and transplanted on the proliferation medium MS with additive of 4 $mg \cdot dm^{-3}$ KIN. After next 6 weeks of conducting the culture axillary shoots were isolated and transfered on the three variants of medium MS: medium MS (control) containing 37 mg $P \cdot dm^{-3}$, medium MS without additive of P (0,00 mg $P \cdot dm^{-3}$), medium MS with additional amount of P (74 mg $P \cdot dm^{-3}$). *In vitro* culture was carried out under the same growth conditions how in the first stage of experiment.

After this stage the lenght of roots and their number were measured and it was marked content of chlorophyll a, b, carotenoids in leaf tissue. The content chloroplast pigment were tested using the method of Arnon (1960). Level of chlorophyll pigments a and b as well as carotenoids were marked in acetone extract with spectrometer using (Eppendorf BioSpectometer). Material for tests was taken from three representative microseedlings of *Helichrysum arenarium* from each repetition of given experiment variants.

In each variant 50 explants were inoculated on the growth medium and the experiment was repeated three times.

The results for the length and number of roots and the plant height and the content of bioactive substances were subjected to statistical analysis. The results were exposed to the analysis of variance and the significance of differences between means was verified with the Tukey test at the significance of α=0.05.

Analysis of variance was performed using all the results applying 'Statistica for Windows Pl'.

RESULTS AND DISCUSSION

In this experiment the plantlets, that was growing on the medium MS without of phosphorus, are characterized by considerably lower growth and larger amount of side roots in comparison with plants from variants medium with standard amount of phosphorus (37.0 mg·dm^{-3}) and twice phosphorus (74 mg·dm^{-3}) (Table 1, Fig. 1). Ciereszko (2003) announces, that deficiency of phosphorus in a first stage of activities this stressor causes changes in morphology and metabolizm, that allow to adapt to the scarce conditions. Adaptation of the plants to the deficiency of phosphorus consists in growith stopping of elongation sprout and reduction of leaves surface. Also it changes in morphology of the root consisting in growth of mass and lenght of the under-earth part of the plant as well as amount of side roots and the lenght of trichomes (Halliwell et al. 2001, Koc and Skwierawski 2008).

Table 1. Influence of phosphorus on the height of micropropagation seedlings of *Helichrysum arenarium* (L.) Moench after 6 weeks of culture

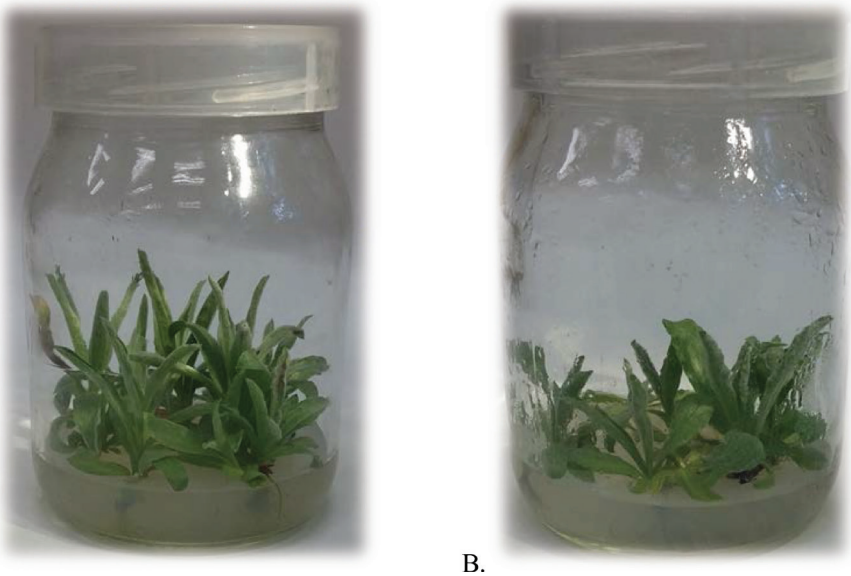
The content of phosphorus in the medium MS (mg·dm^{-3})	Mean height of the plants (cm)	Mean length of roots (cm)	Mean number of primary roots	Mean number of lateral roots
37.0 (MS)	3.70±0.45a	3.70±0.48a	3.80±0.80a	22.60±2.09b
74.0 (MS+P)	4.30±0.97a	2.50±0.38a	4.80±1.44a	24.40±3.13b
0.0 (MS-P)	2.50±0.56b	2.08±0.29a	4.60±1.01a	45.00±3.68a

Results are mean ± SD (standard deviation); means followed by the same letter do not differ significantly at α=0.05.

Phosphorus is the most important nutritional element in improvement of photosynthesis (Bisht and Chandel 1991). Phosphorus addition into the medium can in a direct and indirect way influence the growht of the key enzyme of photosynthesis – carboxydismutaze (also called RuBisCO – ribulose bisphosphate carboxylase-oxygenase) (Usuda and Shimogavara 1991, Rao and Terry 1995, Pieters et al. 2001) and thereby the photosynthetic activity of the plant.

The assimilatory pigments (chlorophyll and carotenoids) are chemical connections that influence intensity of photosynthesis so the production of biomass. In case of chlorophyll a, b there was not stated considerably diferences in extract from plantlets of sandy everlasting. In the carried out experiment the additional

amount of P in medium influenced increased of chlorophyll a+b. Its amount was higher comparison with the plants growing on medium MS with standard amount of phosphorus and without that element by accordinglly 36% and 23% (Table 2). Similar tendency in growth of chlorophyll as a result of application of phosphoric fertilization were observed in case of asparagus *Asparagus racemosus* (Willd.) belonging to the family *Asteraceae* (Vijay et al. 2009).



A. B.

Figure 1. Micropropagation seedlings of sandy everlasting (*Helichrysum arenarium* (L.) Moench) on MS medium with twice the amount of phosphorus (74 mg ·dm^{-3}) (A), and MS medium withaut phosphorus (B)

Table 2. Content of chlorophyll a, b, a+b and carotenoids in micropropagation seedlings *Helichrysum arenarium* (L.) Moench (mg·g^{-1} fresh matter) after 6 weeks of culture

The content of phosphorus in the medium MS (mg·dm^{-3})	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids
37,0 (MS)	1.261±0.042a	0.419±0.042a	1.682±0.078b	0.569±0.078a
74.0 (MS+P)	1.410±0,072a	0.871±0.023a	2.279±0.069a	0.720±0.057a
0.0 (MS-P)	1.201±0.021a	0.662±0.053a	1.851±0.043b	0.590±0.039a

Results are mean ± SD (standard deviation); means followed by the same letter do not differ significantly at α=0.05

In case of carotenoids it was not stated considerably differences in extract from plantlets of sandy everlasting. However, the analysis carried out showed, that the richest in these substances plantlets were these growing up on the medium with increased amount of phosphorus. Application of the additional amount of that macronutrient caused increase of carotenoids by 20% in comparison with variant, in that the medium MS without phosphorus was applied.

CONCLUSIONS

Phosphorus deficiency in the medium MS considerably influenced changes in growth modification of sandy everlasting (Helichrysum arenarium (L.) Moench). Microseedlings that were growing on the medium with additional amount of phosphorus contained considerably more of general of chlorophyll (a+b) in comparison with variant with standard amount of phosphorus and without that element, accordingly by 36% and 23%. Changeability of morphological and metabolic features of sandy everlasting that were result of phosphorus addition to the medium MS, shows higher requirements of that plant in relation to this macronutrient. It is necessery to continue that research, in order to take into account this tendency in moving of sandy everlasting to the field cultivation.

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