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THE INFLUENCE OF ZINC ON GROWTH AND DEVELOPMENT OF *DENDROBIUM KINGIANUM* BIDWILL IN *IN VITRO* CULTURE

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ABSTRACT

The influence of increased zinc content in Murashige and Skoog solid medium on the growth and development of the orchid *Dendrobium kingianum* Bidwill was studied. Sterile explants of pseudobulbs were used for micropropagation of the orchid plant on MS (1962) regeneration medium supplemented with 0.5 mg dm^{-3} NAA and 1.0 mg dm^{-3} kinetin. Zinc (as $ZnSO4 \cdot 7H2O$) was added to all combinations in concentrations 2, 4, 8 and 16 times bigger (17.2, 34.4, 68.8, 137.6 mg dm^{-3}) than the standard content (8.6 mg dm^{-3}) in MS-medium (control). The obtained results showed that treatment with 2 times increased zinc concentration stimulated the plant growth and development. After six and twelve months in *in vitro* culture the biggest number of shoots and roots, length of shoots and roots and the biggest fresh weight of plants were obtained in media with 2 times greater zinc content than in control (standard content of zinc in MS-medium). However in media with 8 and 16 times bigger zinc content, negative influence of zinc on the biometrical features was noted.

Key words: orchids, tissue culture, micropropagation, zinc sulphate.

ABBREVIATIONS

BA – 6-benzylaminopurine, kinetin – 6-furfurylaminopurine, IAA – indolyl-3-acetic acid, NAA – naphthalene-acetic acid, MS – Murashige and Skoog medium

INTRODUCTION

Dendrobium kingianum Bidwill can be found in Australia (New South Wales and Queensland). These extremely variable, lithophytic plants often grow in large masses. *Dendrobium kingianum* was discovered and described by J. C. Bidwill in John Lindley's Botanical Register in 1844. Bidwill sent the plants to Europe in the same year [4].

Vegetative propagation of orchid plants is very slow, hence the interest in *in vitro* techniques for their micropropagation [17, 18, 2, 10]. Many morphological factors (e.g. culture media, growth regulators and stimulators, sucrose concentrations, seed disinfestations, metals, micronutrients, light intensity) control the processes of plant growth and development in *in vitro* culture [1, 3, 8, 20, 22, 23, 24, 25]. Such factors can include micronutrients such as zinc (Zn). Zinc is an essential factor in the normal course of some metabolic processes such as enzymatic activity, auxin synthesis, gene expression, protein synthesis, structural and functional integrity of biomembranes and phytosynthetic carbon metabolism [5]. Zinc activity also controls many physiological processes, such as pathogen pressure, drought or heat that result in higher resistance of cultivated plants to abiotic and biotic stresses [14, 15].

The main purpose of this study was to investigate the influence of 2-, 4-, 8- and 16-fold increase in zinc content in MS-medium on growth and development of the orchid *Dendrobium kingianum* Bidwill in *in vitro* culture.

METHODS

Dendrobium kingianum Bidwill plants were collected from a plant growth chamber of Faculty of Agricultural Sciences in

Zamość, University of Life Sciences in Lublin, in August 2010. For the development of an aseptic culture, orchid pseudobulbs (2-3 cm long) with two terminal leaves were surface sterilized with 0.1% $HgCl_2$ solution for 3 minutes and transferred to an initial Murashige and Skoog solid medium (MS) [19] supplemented with IAA at 0.5 mg dm^{-3} and BA at 1.0 mg dm^{-3}. The pH of the medium was adjusted to 5.2. MS medium contained 3% sucrose and 0,8% Difco bacto-agar. Test glass culture vessels (100 ml) with Magenta B-caps as closures were dispensed with 20 ml medium respectively. The cultures were maintained at 22-24°C with 60% relative humidity and exposed to light 16 h per day at 54 µmol m^{-2} s^{-1} from fluorescent lamps. After two months, all newly formed shoots with two terminal leaves were separated, weighed on high precision scale in laminar flow chamber for *in vitro* and individually transferred to a multiplication MS-medium (3 shoots per vessel, and 30 per treatment) supplemented with NAA at 0.5 mg dm^{-3} and kinetin at 1.0 mg dm^{-3} [23]. Zinc (as $ZnSO_4 \cdot 7H_2O$) was added to all treatments at concentrations 2, 4, 8 and 16 times bigger (17.2, 34.4, 68.8, 137.6 mg dm^{-3}) than the standard content in MS-medium (8.6 mg dm^{-3}). The number and length of shoots and roots and the fresh weight of the plantlets were analysed after six- and twelve-month growing (after 3 and 6 passages) in 12-15 plantlets from every treatment. After twelve-month growing *in vitro* plantlets were taken from vessels (Photo. 1) and gently washed with distilled water. They were then transplanted into wet pieces of bark in small plastic pots at a temperature of 22-24°C and 16 h light: 8 h dark photoperiod provided by white fluorescent tubes (200 µmol m^{-2} s^{-1}). The pots were covered with polyvinylidene chloride film to avoid desiccation. Between 2-4 weeks after the start of acclimatization, the film was gradually removed. Experiment was repeated 2-times. Statistical analysis was done with the Tukey test.

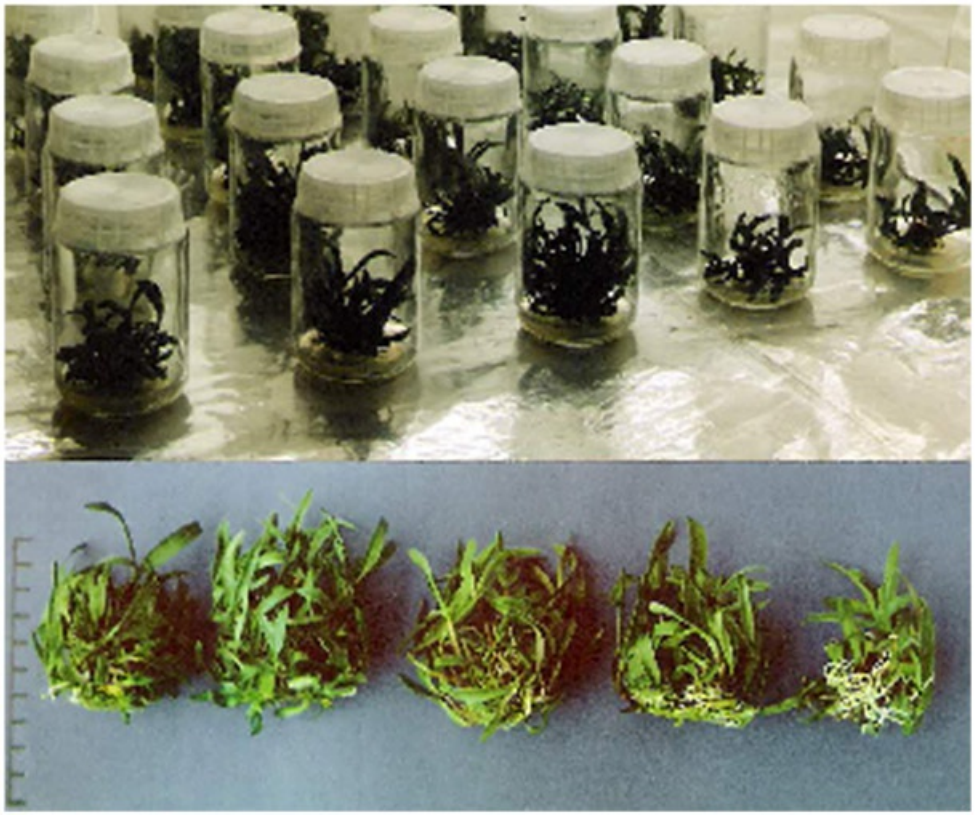

Photo. 1. *Dendrobium kingianum* Bidwill in *in vitro* culture (upper photo) and obtained plantlets (lower photo) after 12 months in MS medium supplemented with $ZnSO_4 \cdot 7H_2O$ (from the left): 8.6 (control), 17.2, 34.4, 68.8, 137.6 mg dm^{-3}

RESULTS

In this study, the formation of multiple shoots was successfully induced from single shoot explants of *Dendrobium kingianum* Bidwill. The number of multiple shoots was further increased with the supplementation of zinc at concentrations bigger than the standard content in MS-medium. A significant effect of the combination with 17.2 mg dm^{-3} Zn (as $ZnSO_4 \cdot 7H_2O$) on the growth and development of *Dendrobium kingianum* Bidwill was found. After 6 months of *in vitro* culture plantlets growing in treatment 2 times greater zinc content in MS medium had a significantly bigger number of shoots and roots, longer shoots and bigger fresh weight (Table 1).

Table 1. Influence of increased zinc (as $ZnSO_4 \cdot 7H_2O$) content in MS solid medium on growth and development of *Dendrobium kingianum* Bidwill after 6 months *in vitro* culture (mean values for biometrical features per 1 explant)

Biometrical feature	Concentration of $ZnSO_4 \cdot 7H_2O$ (mg dm^{-3})					LSDp=0.05

	8.6(control)**	17.2 (2 x 8.6)	34.4 (4 x 8.6)	68.8 (8 x 8.6)	137.6 (16 x 8.6)	
Number of shoots	8.3	14.3*	12.2	9.1	7.5	5.06
Shoot length – mm	32.10	42.60*	35.63*	27.64*	23.80*	1.481
Number of roots	12.3	16.8*	15.5	12.5	11.1	4.10
Root length – mm	18.70	23.12*	19.35	15.44	4.86*	4.219
Fresh weight of plant – g (Increment – g)	1.09 (0.94)	2.56* (2.41*)	1.94 (1.77)	0.86 (0.73)	0.58 (0.46)	1.242 (1.288)

***- result significantly different in relation to the control at p=0.05**

**** – standard content of Zn in MS medium**

In the series with the 2 greater concentration of zinc in MS medium mean 14.3 shoots per 1 explant was obtained. It was a significantly bigger number of shoots than in control combination (mean 8.3 shoots per 1 explant). In the same series a significantly more roots per 1 explant than in the control was also noted - 16.8. The control plants formed 12.3 roots per 1 explant.

In the treatments 2 and 4 times greater zinc content in MS medium significantly longer and in the treatments 8 and 16 times greater zinc content significantly shorter shoots were noted than in the control. In comparison to fresh weight of a single explant, the increment of orchid plantlets fresh weight after six months of *in vitro* culture was a significantly bigger (2.41 g) in the case of the 2 greater concentration of zinc than in the control (0.94 g). In the treatment 2 times greater zinc content significantly longer roots were noted, but in the treatment 16 times greater zinc content the roots were shorter than in the control. In case of the treatments 4 and 8 times greater zinc content in MS medium more shoots and roots were obtained than in control but results were not statistically significant. The 16-fold increase in zinc content in MS medium led to a significantly lower length of shoots and roots, and 8-fold increase in zinc one led to a lower length of shoots than in the control.

The results after twelve months of *Dendrobium kingianum* Bidwill *in vitro* culture also show that 2-fold increase in zinc content in MS solid medium positively influenced on shoot and root development and plant fresh weight (Table 2). In the series with the 2 greater concentration of zinc in MS medium more shoots per 1 explant (31.7) were obtained than in control combination (17.4). In the same series more roots per 1 explant was noted (28.7) than in the control (18.8). In the treatments 2-, 4-fold increases in zinc concentration in MS-medium significantly longer and in the treatments 8-, 16-fold increases in zinc concentration significantly shorter shoots were noted than in the control.

Table 2. Influence of increased zinc (as $ZnSO_4 \cdot 7H_2O$) content in MS solid medium on growth and development of *Dendrobium kingianum* Bidwill after 12 months *in vitro* culture (mean values for biometrical features per 1 explant)

Biometrical feature	Concentration of $ZnSO_4 \cdot 7H_2O$ (mg dm^{-3})					LSDp=0.05
	8.6 (control)**	17.2 (2 x 8.6)	34.4 (4 x 8.6)	68.8 (8 x 8.6)	137.6 (16 x 8.6)	
Number of shoots	17.4	31.7*	24.1	15.8	12.5	10.39
Shoot length – mm	41.70	56.00*	47.60*	38.40*	25.50*	1.364
Number of roots	18.8	28.7*	20.6	19.9	14.1	7.80
Root length – mm	26.70	32.80	32.28	23.50	16.50*	6.412
Fresh weight of plant – g (Increment – g)	2.44 (2.29)	5.89* (5.74*)	4.00 (3.83)	2.76 (2.63)	1.65 (1.53)	2.083 (2.109)

***- result significantly different in relation to the control at p=0.05**

**** – standard content of Zn in MS medium**

In comparison to fresh weight of a single explant, the significantly increment of orchid plantlets fresh weight in relation to the control was noted in 2-fold increase in zinc content combination (5.74 g). In this combination longer shoots and roots than in the control were also noted. In medium with 16 times bigger zinc content negative influence of zinc on the length of shoots and roots was noted. The treatments 8 and 16 times greater zinc content in MS medium led to the significantly shorter shoots of orchid plantlets and the treatment 16-fold increase concentration led to the significantly shorter roots than in the control.

DISCUSSION

The experiment showed that 2-, 4-fold increases in zinc concentration in MS-medium had a favourable effect on growth and development of *Dendrobium kingianum* Bidwill orchid plants in *in vitro* conditions. The increase in zinc concentration strongly stimulated shoot and root growth and development, and plant fresh weight increment.

Various investigators demonstrated the beneficial effect of Zinc on plants. Misra and Sharma [6] found that Zn improved plant height, leaf number and herbage weight of *Mentha arvensis*. Aziz et al. [3] reported, that plant growth parameters of *Cymbopogon citratus* increased significantly by increasing Zn application and 200 mg dm^{-3} of it gave the greatest increase in plant height, fresh and dry weight yield. Chakravarty and Srivastava [6] found that Zn is slightly less toxic than the other heavy metals (Al, Cd, Cu, Ni, Pb) at higher concentrations in*in vivo* and *in vitro* conditions. Zn was the only metal which could support callus growth of *Helianthus annuus* even at concentration as high as 6,5 mg dm^{-3}. Zn enhances growth at a 10-fold higher concentration than for example Cd. Zn is the least toxic of all the heavy metals tested, although very high concentrations show cytotoxic effects. It produced the smallest toxic effects on seed germination, cytotoxicity, and *in vitro* growth. Zn is required by the plant system in low quantities as an essential element for metabolic reactions [6].

It probably resulted from this that in higher plants zinc is either required for, or at least modulates, the activity of a large number of various types of enzymes – dehydrogenases, aldolases, isomerases and transphosphorylases. The role of zinc in DNA and RNA metabolism, cell division, and protein synthesis has been documented for many years. For example, a new class of zinc-dependent protein molecules (zinc metalloproteins) has been identified, which is involved in DNA replication, transcription and thus, regulation of gene expression. In these DNA-binding metalloproteins zinc is therefore directly involved in the translation step of gene expression and activation or repression of DNA elements [5, 13, 15]. The rate of protein synthesis and the protein content of zinc-deficient plants are drastically reduced whereas amino acids accumulate [15]. Zinc is a structural component of ribosomes and is essential for their structural integrity [21]. Hajiboland and Amirazad [12] reported that roots of red cabbage plants were more protected against reactive oxygen species imbalance under Zn deficiency conditions compared with leaves that was correlated well with the lower sensitivity of roots to low Zn supply. Zinc is absorbed through roots. After it reaches the xylem it is transported as a free ion. Normally Zn concentration is 10 or more times higher in root than in shoot [7, 11]. *Zn* is more mobile from roots to shoots than Cu and Ni [9].

Zinc is essential for growth regulation. Zinc improves chlorophyll function. It is essential for plant hormone balance, especially auxin (IAA) activity and electron transport [11]. Hence it is possible the stronger influence of zinc on orchid shoot than root growth and development.

CONCLUSIONS

1. The presence of 17.2 mg dm^{-3} $ZnSO_4 \cdot 7H_2O$ in the MS solid medium was the most efficient treatment for increasing shoot and root production during micropropagation of *Dendrobium kingianum* Bidwill orchid plants.
2. In the treatment 2-fold increase in zinc concentration in MS-medium (17.2 mg dm^{-3} $ZnSO_4 \cdot 7H_2O$), about 82% more shoots and 53% roots were obtained than in control after twelve months *in vitro* culture.
3. Zinc at a 2-fold higher concentration in MS-medium positively influenced fresh weight increment oforchid plants.

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