

***DENDROBIUM ANTENNATUM* LINDL. MICROPROPAGATED UNDER THE INFLUENCE OF VARIOUS CONCENTRATIONS OF FERBANAT L[®]**

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Abstract

*Orchids are among the most sought-after cut and potted plants in the 21st century. Dendrobium antennatum Lindl. is widely used as potted plant or as a cut flower. Due to this aspect, breeders need new propagation methods. In the present study, green antelope orchids were micropropagated using axillary buds at different concentrations of Ferbanat L[®] medium (0.1, 0.5, 1, and 5%) to assess its positive or negative effects regarding the plant's growth and development compared to control. The plants' survival rate was the highest when 5% Ferbanat L[®] was added to the medium. In the case of the chlorophyll *a + b* and total carotenoids no significant changes were observed, in the case of the peroxidase activity significant changes were observed by the different treatments. From the study, it can be concluded that control and 5% Ferbanat L[®] results were almost similar, however the treated plants (5%) recorded a higher survival rate, as well as the healthiest and most size-optimal plants. The results of the present research could positively improve the propagation of the *D. antennatum*.*

Key words: biostimulators, green antelope orchid, growth medium, propagation, tissue culture.

INTRODUCTION

Cultivating ornamental plants is one of the developing, most profitable, diverse and fast-changing sector of plant production (Volckaert, 2010; Datta, 2022; Chandel et al., 2022; Gabellini and Scaramuzzi, 2022). Ornamental plants include diverse group of whole plants or parts of plants, usually produced for decorative reasons (Adebayo et al., 2020). According to data provided by the world's largest flower exchanges, Royal Flora Holland, 11.7 billion products were traded in 2021, an increase of 3% in comparison with the previous year (Royal FloraHolland Annual Report, 2021). Decorative plant producers have implemented contemporary cultivation technologies; nonetheless (Salachna, 2022), many future improvements remain to be made since the output of decorative plants is increasing and consumer expectations are constantly changing (Volckaert, 2010; Proietti et al., 2022).

The Orchidaceae is among the angiosperms' largest family, with their origin dating back to 120 million years ago (Lam et al., 2015).

Dendrobium Sw. genus belongs to the family Orchidaceae, it is a pseudobulbous epiphyte and can be found primarily in the wet tropical biome. It is native in Papuasia and North Queensland (POWO, 2023), with 1545 accepted species (WFO, 2023), which are commonly used as a traditional tonic herb or food (Lam et al., 2015; Xu et al., 2013; Hinsley et al., 2018; Cheng et al., 2019). Among orchids, *Dendrobium* is one of the most well-known and significant blooming ornamental plants in the world (Mirani et al., 2017; Herastuti and Ek, 2020).

Dendrobium antennatum Lindl. commonly known as green antelope orchid is an ornamental potted plant, used also as cut flowers, with the flowering period occurring between March and December (Utami and Hariyanto, 2016). According to Nugroho et al. (2019), the green ante-lope orchid is an endangered species due to the conversion of forests; hence, conservation measures are required to preserve the plant in its natural habitat. Moreover, the *D. antennatum* is listed as endangered under the Nature Conservation Act 1992 (Queensland), Schedule 1 of the Endangered Species Protection Act

1992 (Cwlth), and Environment Protection and Biodiversity Conservation Act 1999 (Cwlth) (EPBC Act) (19. Approved Conservation Advice, 1999). For its conservation, another method could be the propagation of *D. antennatum* in higher quantities, to be restored in the wilderness. As previously mentioned, these orchid species are highly admired for commercial purposes, thus the propagated plants could also satisfy the flower market.

The *D. antennatum* can be propagated generatively and vegetatively. The plants in their native regions can easily produce fruits with an abundance of seeds, unfortunately only a small quantity of viable seeds is able to germinate (Nugroho et al., 2019). According to Herawati et al. (2020), orchids can reproduce less than 1% in natural environment. A main reason for this could be the small size of the seeds and the lack of endosperm, and as well the insufficiency of suitable mycorrhization. Therefore, new methods are needed to produce a high quantity of, healthy and size-optimal plants. Plant tissue culture allows growing plants from organs, tissues or cells in controlled incubated conditions. Moreover, this method supplies the necessary energy, nutrients and water for the plants (Phillips and Garda, 2019) and also produce clean, disease and virus-free new plants. In addition, tissue culture is an important propagation method in plant breeding (Custódio et al., 2022), the most highly exploited version of it being micropropagation (Kulus and Miler, 2021). Plant development can be manipulated by adding natural or synthetic plant growth regulators, which can have a positive influence on the growth and resistance of the newly propagated plants. Micropropagation is an intensely used propagation technique for orchid conservation (Tikendra et al., 2019). The new alternative strategy involves using plant growth regulators and phytohormones in agricultural practices to boost plants' immune-protective abilities against various viruses and pests as well as their ability to better adapt to challenging environmental conditions (Phillips and Garda, 2019).

D. antennatum can have up to 130 cm pseudobulbs, a cluster holds up to 9-21 fragrant, long-lasting flowers, about 6 cm long, with white or greenish sepals, narrow and twisted

petals, with purple-veined lips. The cluster inflorescence is mostly erect, rarely longer than the leaves.

The composition of the medium in one of the most important factors in micropropagation (Tuwo et al., 2021). Some previous studies found that micropropagated orchids need nutrients and additional complex compounds to increase the macro and micro-nutrients content (Moraes et al., 2020). In addition, several studies reported the applicability of commercial or organic fertilizers to reduce the propagation costs, and induce faster callus formation (Moraes et al., 2020; Suryani and Sari, 2019; De Stefano et al., 2022; Sasmita et al., 2022). Tissue cultured orchid media can also be supplemented with banana flour, coconut water (Vilcherrez-Atoche et al., 2020), tomato or bean sprout extract (Dwiyani et al., 2022). Furthermore, it is reported that cytokinin 6-benzyladenine (BA) and auxin naphthaleneacetic acid (NAA) influenced the root initiation of *in vitro* propagated *Cymbidium aloifolium* seeds (Potshangbam and Nirmala, 2011).

In recent years, organic fertilizer has emerged as a beneficial substitute for reducing environmental damage. Zayed and Saber, (2020), reported that organic fertilizers increased the growth parameters and chlorophyll content of micropropagated date palm.

Biostimulants can be described as small amounts of organic and inorganic matter, which promote the plants growth and development (Kisvarga et al., 2022). Biostimulator microorganisms are important alternatives to promote plant production (Nephali et al., 2020; Sanó et al., 2022). Previous studies demonstrated that biostimulants positively influence the growth and development by micropropagated orchids (Gontijo et al., 2018; Paris et al., 2019; Bhattacharyya et al., 2021). Furthermore, it is also mentioned that biostimulants can influence the plants in a positive way in their adaptation to *ex vitro* conditions (Karpushina et al., 2021).

The aim of the present study was to investigate the effect of Ferbanat L[®] biostimulator on micropropagated *D. antennatum* Lindl. In the experiment the influence of Ferbanat L[®] was analyzed on the plant's growth, development parameters, on its chlorophyll and carotenoids concentration, and peroxidase enzyme activity.

We aimed to determine the appropriate concentration to get healthy, size-optimal and the largest quantity of new green antelope orchid plants, in order to decrease the loss of natural populations and to promote species conservation. The results of the present study are reported here for the first time.

MATERIALS AND METHODS

The experiment was conducted at the Hungarian University of Agriculture and Life Science, Department of Floriculture and Dendrology.

The axillary buds were obtained from the orchid collection of the ELTE Botanical Garden, Budapest. *D. antennatum* protocorms, were micropropagated from axillary buds, transferred to the media in a sterile chamber using sterile equipment. Axillary buds were surface sterilized with 70% ethanol for 1 min, 6 g/L Na-dihydroizocyanurate for 10 min and washed 3 times in sterile distilled water. The inocula were multiplied on 0.5 mg/L BA containing Knudson (KC) (Knudson, 1922) medium and were maintained on KC medium without plant hormones. The plants used for the experiment were maintained for two years. During the experiment the temperature was $22 \pm 2^\circ\text{C}$, light 16/8 h lighting with traditional fluorescent lamp, 2000 lux light intensity.

D. antennatum protocorms were introduced to the media. These contained different concentrations of Ferbanat L[®] (0% (as control), 0.1%, 0.5%, 1%, 5%). For each treatment 30 individual plants were selected as biological replicas. The preparation of the medium was a modified Vacin and Went medium (Vacin and Went, 1949) began with the accurate measurement of the required additives 500 mg/L $(\text{NH}_4)_2\text{SO}_4$, 200 mg/L $\text{Ca}_3(\text{PO}_4)_2$, 250 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 250 mg/L KH_2PO_4 , 525 mg/L KNO_3 , 25mg/L Na-Fe-EDTA and 75 mg/L MnSO_4 . These additives were completed with 20 g/L sucrose, 5.5 g/L Plant Agar (Duchefa). pH was adjusted to 5.7. The solution containing the required amount of culture medium was mixed with distilled water, and then all the stock solutions were pipetted into individual beakers, followed by the addition of sucrose, agar and Ferbanat L[®] at different concentrations levels (0.1%, 0.5%, 1%, 5%). All of this was eventually diluted to the desired volume. During the autoclaving

process, a temperature of 121°C and a pressure of 1.2 bar were applied during 30 minutes.

Ferbanat L[®], produced originally by the Turkish Ekosistem company, was allowed in Hungary, "Bistep conditioner" (Ördögh et al., 2019; Yaseen and Takácsné Hájos, 2021), which is a liquid humic extract made of bio-humus basis. It contains micro- and macro-elements, natural growth hormones, humic material, soil antibiotics, fito-vitamins, amino acids and useful soil microorganisms (Ferbanat Labs, 2023).

After six months, once the plants' vegetative components had fully matured, the size of the plants, root length, the number of leaves, roots, and ramifications were assessed. Furthermore, the plants survival rate was recorded at the end of the experiment. Plant length measurement was carried out under sterile conditions. During the experiment the plants were transplanted two times as this species (like many orchids) grows slowly.

The Chlorophyll $a + b$ and total carotenoids were measured from 120-150 mg of fresh material according to the modified method of Arnon and Cooper (1949). The material was ground to become a cooled mortar with ice-cold 80% (v/v) acetone and a small amount of pure quartz sand. The sample was completed for a final volume of 10 ml with 80% acetone. The prepared samples were centrifuged at 1350 rpm for 5 min at a temperature of 4°C . The absorbance of the supernatant was determined at 663 and 664 nm for chlorophyll $a + b$ and 480 nm for carotenoids with Thermo Scientific Genesys 10 vis (Thermo Fisher Scientific Inc., Finland) spectrophotometer.

Chlorophylls $a + b$ and carotenoid concentrations were calculated using the following equations:

$$\text{Chlorophyll } (a + b) \text{ } \mu\text{g/g fresh material} = (20.2 \times A_{644} + 8.02 \times A_{663}) \times V/w$$

$$\text{Carotenoid } \mu\text{g/g fresh material} = 5.01 \times A_{480} \times V/w$$

Where: V = volume of tissue extract (ml); w = fresh weight of tissue (g); $A_{1\text{min}}$ = absorbance, the light absorption at the wavelength of the blind. A means absorbance on the given wavelength. $A_{1\text{min}}$ shows the change of absorbance during 1 min. As the enzyme acts eliminates H_2O_2 , the color of orthodianizidine changes. The instrument measures the absorbance every 10^{th} second and calculates the

absorbance change during this period (1 min), against the blank.

For peroxidase enzyme activity 300-500 mg of fresh material was measured with analytical balance, and ground with 1200 μ l K-phosphate buffer (pH: 6.5) using clean quartz sand to an ice-cold mortar. After the material was centrifuged at 1350 rpm for 20 min at a temperature of 4°C, 1700 μ l of sodium acetate buffer (pH 4.5), 30 μ l H₂O₂ (1000x dilution (30%) of concentrated H₂O₂) and 20 μ l ortho-dianisidine (10 mg/ml) were added to 10 μ l of sample (Shannon et al., 1966). The absorbance of the supernatant containing the solution was determined at 460 nm, ten times/min and the absorbance changes were calculated by the Thermo Scientific Genesys 10 vis spectrophotometer (Thermo Fisher Scientific Inc., Finland). The enzyme activities were calculated using the following equations:

$$\text{enzyme activity (U/g)} = \Delta A_{1\text{min}} \cdot \text{dilution} / \varepsilon$$

Where: $\Delta A_{1\text{min}}$ = measured value; ε = molar extinction coefficient; $\varepsilon_{\text{ortho-dianisidine}} = 11.3$

$\Delta A_{1\text{min}}$ means the absorbance change of the solution within 1 min.

The significance of the differences between the treatments was tested by applying one-way ANOVA, at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's post hoc test was carried out to establish the statistically significant differences at $p < 0.05$. Heatmap and dendrograms were generated using the Euclidean distance based on Ward's algorithm for clustering. The analyses were performed using the Paleontological Statistics (PAST) software (Oslo, Norway) (Hammer, 2001).

RESULTS AND DISCUSSIONS

From the obtained data, on the one hand statistically significant differences were observed at the 0.1 and 0.5% treatments compared to the control, but 1 and 5% concentration treatments gave results similar to the control. On the other hand, 0.1 and 0.5% treatments had a detrimental effect on the micropropagated green antelope orchids, even preventing them from growing (Figure 1). Control, 1 and 5% concentration treatments produced a higher growth, with ~ 5 mm compared to the other two treatments (0.1 and 0.5%).

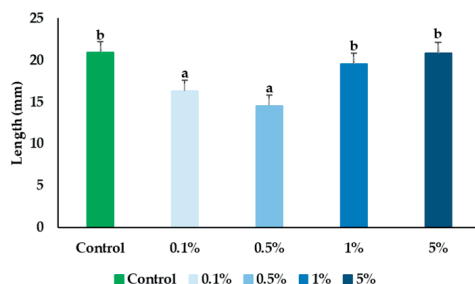


Figure 1. Effect of the Ferbanat L[®] on the length parameters of micropropagated *Dendrobium antennatum*. Plantlets under control conditions, and in the presence of the indicated concentration of Ferbanat L[®] (0.1, 0.5, 1 and 5%). Bars represent the means $\pm$ SE ($n = 30$). Different letters indicate significant differences between treatments ($p < 0.05$)

Under our experimental conditions, the root of the green antelope orchid was significantly influenced under the Ferbanat L[®] treatments (Figure 2). Root length recorded statistically significant differences under the treatments (Figure 3a). Under Ferbanat L[®] treatment, *D. antennatum* axillary buds presented significantly decreased root length compared to control.

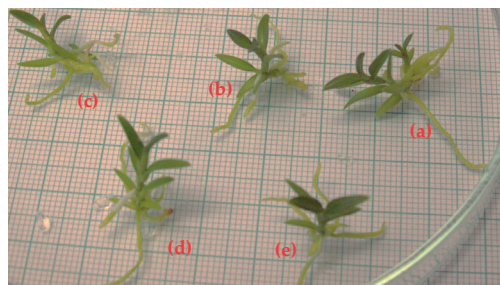


Figure 2. Root formation of *D. antennatum* under the effect of the Ferbanat L[®]. Control (a), 0.1% (b), 0.5% (c), 1% (d), and 5% (e)

Statistically significant differences were recorded when comparing the control to the buds treated with each of the four concentrations. However, the highest decreases were observed by the axillary buds treated with 0.5% of Ferbanat L[®], where ~ 14 mm differences were measured. Lower differences were reported between control and the other three treatments. The longest roots were determined by the control where 15.62 mm was the average length, followed by 5% with 11.12 mm. A smaller inhibition was observed at the 1% concentration where the average recorded length was 8.59

mm. Furthermore, a greater inhibition of average root length was reported at 0.1% (5.2 mm) followed by 0.5%, in this case only 1.86 mm was the average root length.

Similar changes were detected concerning the number of roots, as in the case of root length (Figure 3b). The 0.1, 0.5 and 5% treatments significantly decreased the root number compared to control. The 0.5% concentration treatment resulted in almost five times less roots formed compared to untreated *D. antennatum* axillary buds. However, in the case of the 1% concentration no statistically significant differences were determined in the number of roots compared to control. The greatest number of roots was reported under control conditions with an average of 5.1, which was followed by the 1% with an average of 4.2, by 5% with 3.73, and by 0.1% concentration level with 2.46 roots counted. The lowest number of roots was determined at 0.5 % concentration treatment, where only 0.5 were counted.

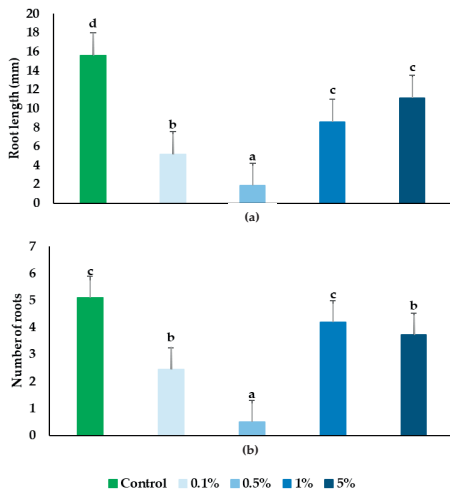


Figure 3. Effect of the Ferbanat L[®] on root length (a) and increment in root number (b) parameters of micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentrations of Ferbanat L[®] (0.1, 0.5, 1 and 5%). Bars represent the means $\pm$ SE ($n = 30$). Different letters indicate significant differences between treatments ($p < 0.05$)

Under our experimental conditions, no statistically significant differences were observed in leaves number appearance between the micropropagated *D. antennatum* axillary

buds when subjected different to treatments (Figure 4a). The average number of leaves was between 9 and 12.

Regarding the number of ramifications, a few minor changes were recorded, however, no statistically significant differences were found (Figure 4b). The smallest number of ramifications was observed at the 1% concentration treatment (0.66), followed by the 5%, 0.5%, and control. The highest number was determined at the 0.1% concentration of Ferbanat L[®] where in this case an average of 1.93 was recorded.

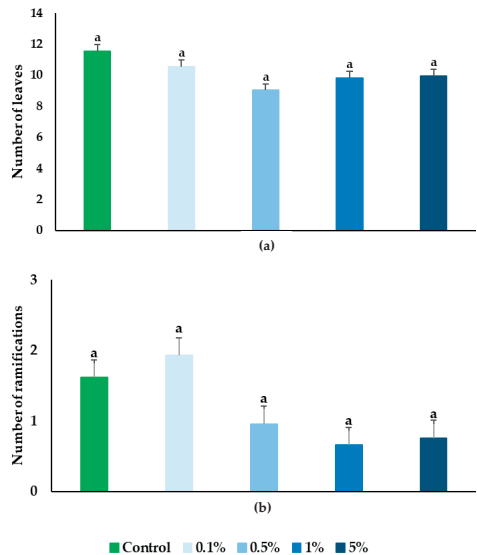


Figure 4. Effect of the Ferbanat L[®] on the number of leaves (a) and increment of ramifications (b) parameters of micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L[®] (0.1, 0.5, 1 and 5%). Bars represent the means $\pm$ SE ($n = 30$). Different letters indicate significant differences between treatments ($p < 0.05$)

The effect of Ferbanat L[®] is also confirmed by our results based on the presence of necrosis on plants parts (Figure 5). During the experiment, any plants that displayed necrosis on any of their organs were documented. It should be pointed out that all the axillary buds subjected to the 5% concentration survived the experiment (all 30 axillary buds). The treatment with 5% concentration was followed by the 1% concentration (63% of plants survived) and buds under control conditions (53% of plants survived). The highest plant loss was recorded

at the treatment with 0.1 and 0.5% concentration of Ferbanat L[®], in both cases only 30% of the axillary buds survived the propagation experiment (Table 1).

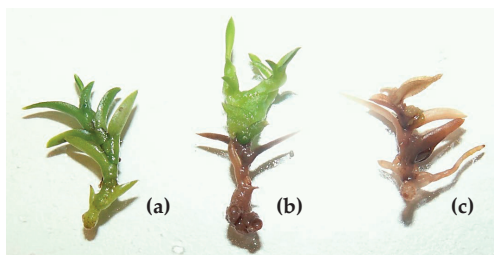


Figure 5. Plant health stage: healthy plant (a), starting of necrosis (b), necrosed (c). Plant health stage: healthy plant (a), starting of necrosis (b), necrosed (c)

Table 1. Effect of the Ferbanat L[®] on the plant survival rate of micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L[®] (axillary buds without treatment were considered as control, 0.1, 0.5, 1 and 5%)

Plant survival rate (%)				
Treatments applied				
Control	0.1%	0.5%	1%	5%
53	30	30	63	100

Photosynthetic pigments content of leaf - chlorophylls *a + b* (Table 1) and carotenoids (Table 2) - were determined in the selected *D. antennatum* plants, following the applied treatments.

Table 2. Photosynthetic pigment concentrations under the effect of the Ferbanat L[®]. Leaf content of chlorophyll *a + b* and total carotenoids in micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L[®] (0.1, 0.5, 1 and 5%)

Photosynthetic pigment concentrations (Chlorophyll (a+b) µg/g)				
Treatments applied				
Control	0.1%	0.5%	1%	5%
0.26 ± 0.02 a	0.26 ± 0.03 a	0.25 ± 0.02 a	0.25 ± 0.04 a	0.30 ± 0.03 a
Total carotenoids (µg/g)				
Treatments applied				
Control	0.1%	0.5%	1%	5%
0.06 ± 0.01 a	0.06 ± 0.01 a	0.06 ± 0.01 a	0.06 ± 0.01 a	0.07 ± 0.01 a

On the one hand, under the effect of 0.1, 0.5, and 1% Ferbanat L[®] the average values of chlorophyll were reduced, but the differences compared to control were not statistically significant. Furthermore, no statistically significant differences detected. Total carotenoid contents

were relatively low, with minimal variation between the various treatments. No statistically significant differences were reported between the control and the treated plants.

In this study the peroxidase enzyme activity was determined from fresh leaf material (Figure 6). The enzyme activity changed in plants subjected to the Ferbanat L[®] treatment. No statistically significant differences were recorded by 0.1 and 0.5% concentration compared to control. Statistically significant differences were reported by treatments with 1 and 5% concentration when compared to control. With these treatments (1 and 5%) enzyme activity was reduced by half compared to control. The greatest decrease was determined by the 5% Ferbanat L[®] treatment with 0.762 U/g.

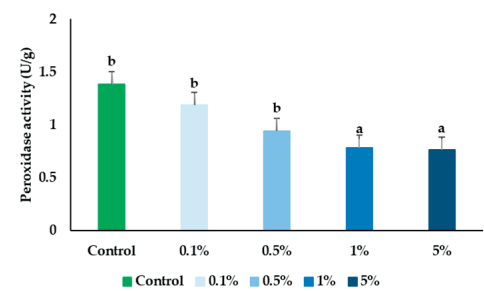


Figure 6. Leaf content peroxidase activity under the effect of the Ferbanat L[®] of micropropagated *D. an-tennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L[®] (0.1, 0.5, 1 and 5%). Bars represent the means ± SE (*n* = 30). Different letters indicate significant differences between treatments (*p* < 0.05)

The growth of the plants showed a strong positive correlation with root length (*r*=0.93), number of roots (*r*=0.93), plant survival rate (%) (*r*=0.79), and was moderately associated with number of leaves (*r*=0.57), chlorophyll content (*r*=0.52) and total carotenoids (*r*=0.43). Conversely, negative significant difference was recorded with number of ramifications (*r*=-0.19) and no association with peroxidase activity (U/g) (*r*=-0.01). Root length (mm) showed strong positive association with number of roots (*r*=0.93) and number of leaves (*r*=0.78), significant positive association with plant survival rate (%) (*r*=0.57), chlorophyll (*r*=0.38), peroxidase activity (*r*=0.32) and total carotenoids (*r*=0.27), but no significant association was noted with total carotenoids

($r=0.27$) and number of ramifications ($r=0.08$). Number of roots was found to have highly significant correlation with number of leaves ($r=0.74$) and moderate association with plant survival rate ($r=0.55$), chlorophyll ($r=0.25$), and peroxidase activity ($r=0.21$), though a weak association was recorded with total carotenoids ($r=0.16$) and number of ramifications ($r=0.03$). Number of leaves showed negative and weak association with total carotenoids ($r=-0.13$), positive and weak association with chlorophyll ($r=0.08$) and plant survival rate ($r=0.03$), though positive and significant association peroxidase activity ($r=0.78$) and number of ramifications ($r=0.67$). Number of ramifications showed negative and moderate significant association with plant survival rate ($r=-0.57$), and total carotenoids ($r=-0.42$), and negative significant difference was recorded with chlorophyll content ($r=-0.20$), whereas a strong and positive association was found with peroxidase activity ($r=0.89$). Plant survival rate (%) showed negative and significant association with peroxidase activity ($r=-0.53$), whereas a strong positive correlation was found with total carotenoids ($r=0.86$) and chlorophyll contents ($r=0.82$). The chlorophyll content was strongly and positively associated with total carotenoids ($r=0.97$), negative and intermediate significant association was recorded with peroxidase activity ($r=-0.31$), whereas the total carotenoids showed negative and significant association with peroxidase activity ($r=-0.51$).

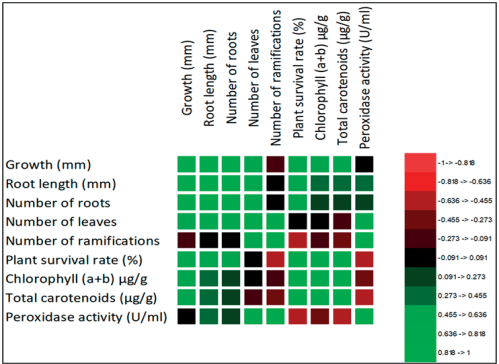


Figure 7. Correlation cluster heatmap illustrating the relationship among the evaluated traits of the micro-propagated *D. antennatum* revealed by XLSTAT. Note: Positive and negative correlations between the evaluated characteristics are displayed in red and green. A darker hue represents a strong correlation, whereas a lighter hue represents a weak correlation among the traits

Table 3. Correlation matrix (Pearson)

Variables	Growth (mm)	Root length (mm)	No. of roots	No. of leaves	No. of ramifications	Plant survival rate (%)	Chlorophyll (a+b) µg/g	Total carotenoids	Peroxidase activity (U/ml)
Growth (mm)	1	0.935	0.938	0.572	-0.192	0.794	0.525	0.473	-0.014
Root length (mm)	0.935	1	0.935	0.780	0.082	0.575	0.388	0.278	0.324
No. of roots	0.938	0.935	1	0.742	0.033	0.553	0.258	0.167	0.211
No. of leaves	0.572	0.780	0.742	1	0.676	0.036	0.080	-0.133	0.785
No. of ramifications	-0.192	0.082	0.033	0.676	1	-0.575	-0.205	-0.426	0.891
Plant survival rate (%)	0.794	0.575	0.553	0.036	-0.575	1	0.820	0.866	-0.532
Chlorophyll (a+b) µg/g	0.525	0.388	0.258	0.080	-0.205	0.820	1	0.970	-0.316
Total carotenoids	0.473	0.278	0.167	-0.133	-0.426	0.866	0.970	1	-0.521
Peroxidase activity (U/g)	-0.014	0.324	0.211	0.785	0.891	-0.532	-0.316	-0.521	1

The results of the present study indicate that an appropriate percentage of Ferbanat L[®] can have a positive effect on *D. antennatum* micropropagation. Biostimulators can be used to increase the growth, the development and the survival rate of tissue cultured plants (Sanó et al., 2022; Vargas-Hernandez et al., 2017; Fei et al., 2019; Brazienė et al., 2019; Paradiković et al., 2019;

Ogunsanya et al., 2022). Moreover, biostimulant application could have a positive effect in stressful growth conditions (Paradiković et al., 2019), stimulate several physiological and molecular processes that lead to improved nutrient uptake and utilization efficiency (Rouphael and Colla, 2018; Meddich, 2022). Also, the medium in which the plants are tissue cultured is an important fact for achieve a higher success of *in vitro* propagation (Tharapan and Obsuwan, 2016). Nugroho et al. (2019) mentioned that the key fact of a successful micropropagation depends on the culture medium, the composition of the nutrients and the content of growth regulators. For instance, in a previous study it was reported that supplementing the Murashige and Skoog semi-solid medium with α -naphthalene acetic acid, 2,4-dichlorophenoxy acetic acid and 6-benzylaminopurine increased the development of the *Dendrobium longicornu* axillary buds, into plantlets (Dohling et al., 2012). Moreover, it was also reported that adding 1.5 mg/L BAP (benzylaminopurine) to the same Murashige and Skoog medium leads to a higher plantlet viability (Mullin et al., 2022). Regarding the plants survival rate the possibility of *in vitro* contamination is one of the main issues in micropropagation (Poobathy et al., 2019). From the data obtained it can be concluded that different concentration levels influence the growth of the subjected plants, in a positive way (1 and 5%), however by these two treatments no significant differences were observed when compared to control in the case of growth (in spite of the fact by this treatment the highest survival rates were recorded). Moreover, when the concentration level of the biostimulator was lower (0.1 and 0.5%) the plant growth was inhibited. Moraes et al., (2020) mentioned that commercial fertilizer supplemented with potato and banana pulp recorded better plant growth results by orchid micropropagation when compared with the organic fertilizer. Roots growth and development are vital for any plant. The goal of micropropagation is to generate plants with the most diverse root systems possible, which will allow them to adapt more effectively to environmental changes or stressors. The induction of adventitious roots is a crucial step during plant propagation (Koroch et al., 2002; Villafuerte et al., 2022). Regarding

the roots length of the micropropagated green antelope orchids it can be clearly observed that unfortunately the roots of treated plants were inhibited, under effect of the Ferbanat L[®]. The root length of untreated plants increased significantly compared with the treated plants. In a previous study it was determined that another type of biostimulator decreased the root and shoot values of *Hosta* 'Gold drop' (Ördögh et al., 2019). Considering the number of roots, here again the root formation was inhibited compared to control, at 0.1, 0.5, and 5%. No statistically significant differences were determined between control and 1% treatment. The greatest inhibition of the roots number was detected at the 0.5% concentration level of biostimulator. Contrary to our findings De Stefano et al., (2022) reported that, the organic supplements added to the growth medium showed greater values of plant length and number of roots compared with control. In a different study, the highest root number in *Dendrobium densiflorum* plants was obtained at full-strength Murashige and Skoog medium supplemented with 1.5 mg/L IBA (Pant et al., 2022). In yet another study it was reported that Murashige and Skoog medium supplemented with 1.5 mg/L indole-3-butyric acid produced the highest number of roots at *Pyracantha angustifolia* (Latunra et al., 2021).

Determining the number of leaves is also an important factor in assessing plant growth. Under our experimental conditions the number of leaves appearance was not influenced in a statistically significant manner, with only small variations between various treatments. Similarly, to previous findings, the biostimulator had an impact on the number of ramifications, although no statistically significant variations were found. An experiment showed that liquid organic fertilizer had a positive effect on the number of shoots and number of leaves at *Vanda tricolor* Lindl. var. *suavis* protocorm (Nowakowska et al., 2022). Pant et al. (2022) revealed that, the greatest number of shoot formation at *Dendrobium densiflorum* protocorms was determined at a full-strength MS medium supplemented with 15% coconut water. Perhaps one of the most important results of the present experiment is be the plant survival rate. According to Nowakowska et al. (2022), although the tissue culture method is a hugely

popular, the growth conditions of *in vitro* propagation are the cause of some species' extremely low plant survival rates (i.e., medium containing high humidity, large quantities of nutrients and sugar which can be easily obtained by plants). Shoot necrosis is described as a physiological condition brought on by culture conditions and resulting in the loss of the shoot tip of *in vitro* plants (Teixeira da Silva et al., 2020). From the obtained data it can be clearly determined that, the greatest survival rate was recorded at a 5% concentration level of Ferbanat L[®]. With other concentrations, only 30% of the plants survived the trial with no necrosis observed, with the largest losses observed at 0.1% and 0.5%. Instead in the case of control and of the plants treated with 1% of biostimulant more than 50% of the plants survived. From these results, it can be concluded that using Ferbanat L[®] at a concentration of 5% helps in the alleviation of environmental stress factors. Healthy plants can be generated that are expected to perform better under any acclimation.

According to Clapa et al. (2022) the photosynthetic pigments content for *in vitro* cultivation seems to be more affected by the specificity of the plant than by the growing medium. Regarding, the photosynthetic pigments concentration (chlorophyll *a + b* and total carotenoids), no statistically significant differences were determined in the present experiment. Carotenoids are a group of natural tetraterpenoid pigments which are naturally occurring and are synthesized by bacteria, algae, fungi, and plants (Nisar et al., 2015; Sun et al., 2018). It was reported that carbon organic fertilizer improved the chlorophyll content of micropropagated date palm (Zayed and Saber, 2020). In a study by Shekhawat et al. (2022) it was determined that plants developed *in vitro* using liquid medium obtained higher content of chlorophylls and carotenoids. In a study conducted by El-Naggar et al., (2023) the chlorophyll content of blue *Hyacinthus orientalis* was increased when benzyl adenine, or kinetin was added to the medium. Photosynthetic pigments content of micropropagated *Cassia alata* plantlets was significantly higher in *ex vitro* conditions compared with *in vitro* conditions (Ahmed and Anis, 2014). In this matter, in comparison to *in vitro* circumstances, it was found that the pigment content greatly increased

when the *Calathea* 'Maui Queen' plants were transferred in *ex vitro* conditions (Van Huylbroeck et al., 2000).

According to the present experiment the peroxidase enzyme activity decreased at 1 and 5% concentration levels of biostimulator. It can be concluded that Ferbanat L[®] helps plants in reducing the negative effect of various environmental stressors, especially at the mentioned concentrations. A previously conducted study revealed that the peroxidase enzyme activity at *in vitro* cultivated *Hosta* 'Gold Drop' plants was reported the lowest if Ferbanat L[®] was added to the growth medium (Ördögh et al., 2019).

CONCLUSIONS

To our knowledge, this study presents the first report on the investigation of Ferbanat L[®] used on the micropropagation of *D. antennatum* orchid.

The present experiment provides data on the comparison of different concentration levels of Ferbanat L[®] used as biostimulant and their influence on growth and development parameters. Moreover, photosynthetic pigment concentration, and peroxidase enzyme activity levels of micropropagated *D. antennatum* plantlets were also determined. According to the obtained results, it can be concluded that by most of the examined factors no statistically significant differences were determined between the control and the treated plants. Root number and length of non-treated plants presented significantly larger quantities compared to the treated plants; however, the highest survival rate, the healthiest and most size-optimal plants were observed by the 5% Ferbanat L[®] treatment. Based on our experiment, Ferbanat L[®] is more important in preventing stress caused by micropropagation than in increasing the morphological parameters. Overall, it can be concluded that the proper dosage of Ferbanat L[®] can improve the development, growth, and survival rate of the examined plants, although more research is required.

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