Effects on Growth and Metabolism of Difference between Day and Night Temperatures (DIF) and Supplementation with Rare Earth Elements (REE) in Micropropagated Dendrobium aphyllum (Roxb.) C. E. Fischer

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Abstract: Dendrobium aphyllum (Roxb.) C. E. Fischer is an ornamental plant with certain medicinal values from the Orchidaceae family. Currently, micropropagation is the main means through which it is propagated. In this research, the effects of different daytime and nighttime temperatures (DIF) and medium supplementation with rare earth elements (REE) on the growth and metabolism of D. aphyllum during a micropropagation period were investigated. Three kinds of REE nitrates, La(NO3)3, Ce(NO3)3 and Nd(NO3)3, at four concentrations, 50, 100, 150, and 200 µM, were added to the culture medium. Three DIFs, 0, 6 and 12 °C, with an identical mean daily temperature of 20 °C, were used with photoperiod/dark period temperatures of 20/20 °C, 23/17 °C and 26/14 °C, respectively. After four weeks of culture, both supplemental REE and DIF treatments promoted growth of the plant compared with the control group. With increased REE concentration and DIF, the growth of the plants was suppressed and symptoms of stress response occurred. At the same concentration, Ce(NO3)3 had a more significant promotional effect on plant growth. In general, the medium supplemented with 100 µM Ce(NO3)3 combined with a 6 °C DIF was the most optimal for the vegetative growth of the plant. A 12 °C DIF promoted synthesis of more primary metabolites such as total proteins and polysaccharides. From the perspective of improving the medicinal values of this plant, increasing the DIF for an optimal growth environment is a valuable reference. This study can provide a technical basis for the propagation and production of Dendrobium aphyllum in the future.

Keywords: abiotic stress; bioactive compound; enzyme activity; polysaccharide; plant quality

1. Introduction

Dendrobium (‘Shihu’ in Chinese) is the second largest genera in the Orchidaceae family, and is widely distributed throughout Asia, Europe and Australia. There are about 80 Dendrobium species in China, some of which display immunomodulatory, anti-tumor, and antioxidative properties [1,2]. In China, the fresh or dried stems of about thirty Dendrobium species are collectively regarded as a famous herbal medicine, and are used in tea or soups to improve health. ‘Shihu’ is listed as a “superior grade” medicinal herb in “Shen Nong’s Herbal classic,” which is one of the earliest herbal pharmacopeias in the world [3]. Dendrobium aphyllum is used as a common source of ‘Shihu’, and is grown in large areas of Southwest China [4]. It is known to protect the stomach, improve eyesight, relieve throat inflammation, and promote bodily functions in traditional Chinese medicine [5,6]. Due to its high medicinal values, the demand for D. aphyllum is increasing, which is leading to over-exploitation, which poses a threat to wild resources [7]. In order to simultaneously protect wild resources and meet the increasing market demand, Chinese producers have developed micro-propagation technology to induce D. aphyllum. It has since become
important to scientifically investigate how to improve the quality of micropropagated D. aphyllum.

Rare earth elements (REEs) are presented in the lanthanide series on the periodic table, which include lanthanum (La), neodymium (Nd), and cerium (Ce) [8]. The REEs are reported to be widely used in medical, agricultural, and industrial applications [9]. Previous research reported that REEs promoted cell growth and metabolite syntheses in plants [10–12]. The appropriate concentration of REEs may promote the growth of plants by reacting with some enzymes in plant cells and cell membranes, resulting in effects on enzyme function and membrane permeability. These changes in turn may improve nutrient absorption and use, and stimulate plants growth [13–17]. Recently, Fan et al. [18] reported that REEs promoted plant growth and enhanced metabolite production in Salvia miltiorrhiza. However, as far as we know, the effects of REEs on the growth of tissue-cultured D. aphyllum have not yet been investigated.

Temperature is an important environmental factor that affects plant growth and development. The difference between the day temperature (DT) and night temperature (NT), abbreviated as the DIF, influences the growth and development of plants, the effects of which are reflected in parameters such as plant height [19,20], dry matter, leaf area in rice [21], and plant architecture and root activities in tomato [22,23]. As an abiotic inducer, the DIF can promote the accumulation of metabolites [24] and directly influence the photosynthesis and polysaccharide accumulation in D. officinale [25]. However, to the best of our knowledge, there has been no research on the effects of the DIF on the growth and development of D. aphyllum.

Polysaccharides are the major active components in ‘Shihu’, and have many advantages, such as nontoxic, immunomodulatory, anti-tumor, antidiabetic, antioxidant, and hepatoprotective qualities [26–28]. Studies have proven that polysaccharides and alkaloids are the major constituents of Dendrobium [29]. Zhao et al. [4] reported that D. aphyllum yielded three polysaccharides with evident immunostimulant activities. Previous studies have reported that the polysaccharide contents in ‘Shihu’ cultured in farmlands or greenhouses were equal to or even greater than in those growing in the wild [30,31]. Therefore, from the perspective of polysaccharide production, culturing Dendrobium plantlets in “plant factories” is a feasible alternative [32].

There have been many studies on the effects of temperature, humidity, light, and nutrients on Dendrobium growth. As both DIF and REE have significant effects on the growth and metabolism of the plant, this experiment was planned to begin with these two factors, along related studies, in order to clarify the influence of these two factors on the growth and metabolism of D. aphyllum and whether there is an interaction between those two factors. This study was set up to investigate the effects of nitrates of lanthanum (La), cerium (Ce), and neodymium (Nd) as inducers of the growth and metabolism of tissue-cultured D. aphyllum under different DIFs, in order to determine an optimal REE supplement method and DIF and thereby improve the growth and medicinal metabolites and provide a scientific basis for artificial, large-scale cultivation of high-quality plantlets to meet the increasing demand for D. aphyllum.

2. Materials and Methods

2.1. Plant Materials and Culture Conditions

In vitro Dendrobium aphyllum (Roxb.) C. E. Fischer plants were donated by the Fairy Lake Botanical Garden Shenzhen and the Chinese Academy of Sciences. Single node segments (1.0–1.5 cm) with two leaves were selected as the plant material for this study. The Murashige and Skoog (MS) medium with 3% (w/v) sucrose and 0.65% (w/v) agar containing 2 mg L\(^{-1}\) 6-benzylaminopurine (6-BA) was used as the basal medium. Three different REEs (La, Ce and Nd) were added to the medium from their trivalent nitrates: lanthanum nitrate [La(NO\(_3\)]\(_3\)], cerium nitrate [Ce(NO\(_3\)]\(_3\)], and neodymium nitrate [Nd(NO\(_3\)]\(_3\)], at concentrations of 50, 100, 150, 200 µM. Different REE concentrations are denoted as ‘element symbol + concentration’ in the text hereafter. The medium without any REEs was
set as the control group. All media were adjusted to pH 5.8 and autoclaved at 121 °C for 20 min.

Each treatment had three replicates, with five explants each planted in 50 mL culture media. All cultures were placed in an environmentally controlled growth chamber (JMRC-600B, Jinwen Instrument Co., Ltd., Shanghai, China) at 75 ± 5% relative humidity, with 300 µmol·m⁻²·s⁻¹ photosynthetic photon flux density (PPFD) of light intensity which was measured at the horizontal position at the top of the plants. The light source was provided by cool white light emitting diodes (LEDs) (OPPLE Lighting Co., Ltd., Shanghai, China) with a 12:12 h of light: dark cycle. Three DIFs were set: 20/20 °C (DIF0), 23/17 °C (DIF6), and 26/14 °C (DIF12) day/night in different growth chambers. The control groups in each of the three different DIFs were abbreviated as ‘Control-0’, ‘Control-6’ and ‘Control-12’.

2.2. Growth Parameters

After four weeks, the growth parameters such as length (from the aerial part to the shoot tip), diameter (the thickest part), number, node number, leaf number, and fresh weight of the new shoots directly induced from explants were measured. The length and diameter were measured using a digital vernier caliper (SA150C, Shanghai Tool Works Co. Ltd., Shanghai, China), and the weight was measured using a scientific balance (FB224, Shanghai Sunny Hengping Scientific Instrument Co., Ltd., Shanghai, China).

2.3. Chlorophyll Content

The chlorophyll content was assessed with an ultraviolet-5500 spectrophotometer (UV-5500, Metash Instruments Co., Ltd., Shanghai, China) following the method of Qiu et al. [33]. In brief, in vitro leaves (100 mg, 1 mm segments) were soaked with 2 mL of dimethyl sulfoxide (DMSO) and boiled at 65 °C until the leaves turned white. After cooling, the total volume was brought to 10 mL with 80% acetone. The absorbance of the extract was recorded at 663.6 nm and 646.6 nm, and the chlorophyll a, chlorophyll b, and the total chlorophyll concentrations (mg·L⁻¹ FW) were calculated by following formulas:

\[ C_a = 12.27A_{663.6} - 2.52A_{646.6} \]
\[ C_b = 20.10A_{646.6} - 4.92A_{663.6} \]
\[ C_{total} = 7.35A_{663.6} + 17.58A_{646.6}. \]

A = absorptivity (L·g⁻¹·cm⁻¹) × cuvette thickness (cm) × solution concentration (g·L⁻¹)

2.4. Soluble Protein

The total protein content was determined according to the method of Braford [34]. Briefly, 1.0 g of fresh leaves were homogenized in a 10 mL sodium phosphate buffer (PBS, pH 7.0), and the supernatant was used after two steps of centrifugation (12,000× g for 6 min and 26,900× g for 16 min). The supernatant (100 µL) was then added to the 5 mL Coomassie brilliant blue G250 reagent, and after 2 min the absorbance of the mixed solution was recorded at 595 nm using a UV spectrophotometer. The soluble protein contents were estimated using bovine serum albumin (BSA) as a standard.

2.5. Plasma Membrane Permeability

The plasma membrane permeability was assessed with a modified version of the protocol described in McClendon [35] and was expressed in terms of the relative conductivity (%), which is defined as original conductivity/total conductivity × 100%. The primary and total conductivities were assayed with a conductometer (DDS-11A, Aipli Measuring Instrument Co. Ltd., Zhejiang, China).

After four weeks of growth, leaves of aseptic D. aphyllum were rinsed with deionized water and then put into test tubes with 20 mL deionized water. After that, they were vacuumed (WXZ-2, Yangguang Manufacturing Co., Ltd., Shanghai, China) for 20 min and placed at room temperature for 30 min before the original conductivities of the super-
natants were determined. The test tubes were heated in a boiling water bath at 100 °C for 20 min until the leaves turned brown, and the total conductivities of the supernatants were measured after cooling to room temperature.

2.6. Activity of Antioxidant Enzymes

To estimate the antioxidant enzyme activities, fresh leaves (1.0 g) were homogenized in a mortar with a 10 mL ice-cold extraction solution (50 mM phosphate buffer, pH 7.5, 1 mM EDTA, 20% (v/v) glycerol, 5 mM magnesium sulfate and 1 mM dithiothreitol). The homogenate was centrifuged (Sorvall ST 8R, Thermo Fisher Scientific Co., Ltd., Shanghai, China) at 10,000 × g for 15 min at 4 °C. The supernatant was decanted into 1.5 mL microcentrifuge tubes for determination of the enzyme activities.

The superoxide dismutase (SOD) activity was measured by the change in the absorbance at 560 nm during incubation in a 3 mL reaction mixture containing 75 μM nitroblue tetrazolium (NBT), 100 μM ethylene diamine tetra-acetic acid (EDTA), and 13 mM methionine, 0.4 mM riboflavin in a 50 mM phosphate buffer (pH 7.8) (200 μL), and 80 μL enzyme extraction. The SOD activity was determined by the formula $(A_{ck} - A_e)/(A_{ck} \times 50\% \times W)$, where $A_{ck}$ is the OD value of the control, $A_e$ is the OD of extracts, and $W$ is the sample weight [36].

The peroxidase (POD) activity was assayed using the guaiacol oxidation by measuring the increase of absorbance at 470 nm for 1 min, following the method of Zhang et al. [37]. The reaction mixture contained PBS (100 mM, pH 7.0), 20 mM guaiacol, 10 μM magnesium sulfate, 1 μM EDTA, 100 mM H₂O₂ and 100 μL enzyme extraction. The POD activity was defined as the formation of tetraguaiacol catalyzed by guaiacol peroxidase (POD) per minute and fresh weight.

The catalase (CAT) activity was quantified by the change in the absorbance at 240 nm during incubation of the extracts (100 μL) at 25 °C with 100 mM H₂O₂ in a 100 mM phosphate buffer (pH 7.0) in a total of 3 mL [38].

The ascorbate peroxidase (APX) activity was assayed using ascorbic acid (AsA) oxidation by measuring the decrease of the absorbance at 290 nm for 1 min, according to Nakano and Asada [39]. The reaction mixture contained 200 μL enzyme extraction, 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, and 5 mM ascorbic acid, to which 0.1 mM H₂O₂ was added in the end. The APX activity was defined as the formation of monodehydroascorbic acid (MDAsA) catalyzed by APX per minute and fresh weight.

2.7. Polysaccharide Content

Polysaccharides were extracted according to the method of Huang [40]; 80 mg of the dried sample powder was accurately weighed and extracted for 2 h in boiling water. Filtration was conducted after cooling and shaking. The solution (2 mL) was transferred to a 15 mL centrifuge tube where 10 mL of ethanol (100%) was added. After shaking and cooling for 1 h, the solution was centrifuged at 1500 × g for 20 min. After precipitation, the precipitate was washed twice with 8 mL ethanol (80%). The sample solution was obtained after centrifugation and dissolved in hot water.

The polysaccharide content was determined with phenol-sulfuric acid according to Huang [40]. The absorbance was measured at 490 nm using a UV spectrophotometer with a 3mL solution containing distilled water, 5% phenol solution, and sulfuric acid as a blank, and the test was performed three times in parallel. The calibration curve was prepared from the glucose reference, and the regression equation was $Y = 6.0389X + 0.1037$ ($R^2 = 0.9849$), where $Y$ is the absorbance and $X$ is the concentration. Polysaccharide quantification was performed on the basis of linear calibration plots of the absorbance versus the corresponding concentration.

2.8. Statistical Analysis

The statistical analyses of the data were performed using the SPSS software (version 16.0, SPSS Inc. Chicago, IL, USA). A parametric one-way analysis of variance (ANOVA)
with Duncan’s multiple-comparison test was conducted in order to examine the significant
differences among treatments at a 95% confidence limit \((p = 0.05)\). A randomized complete
block with three replications and five explants in each replication was applied. Based on
the measured parameters of length, diameter, fresh weight, number, node number, leaf
number, and total chlorophyll content of the new shoot, a rank sum test was conducted to
select the most suitable treatment for \(D. aphyllum\). The evaluation system of the rank sum
test used in this study is based on the significant differences of Duncan’s multiple range
test. All variables measured were considered equivalent, ranked in order, and subsequently
graded with the plant quality.

3. Results

3.1. Growth Parameters

As shown in Table 1, by measuring the length of new \(D. aphyllum\) shoots it was found
that the new shoot length with 100Ce in DIF12 was the greatest. Although this treatment
was not significantly different from 100Ce in DIF0, and 100Ce, 150Ce, 100Nd, 150Nd,
200Nd in DIF12, its influence on new shoot length was significantly higher than that of the
other treatments. Through the F-test, it was found that the DIF and REE concentrations
had significant effects on the new shoot length of \(D. aphyllum\), while the REE type had no
significant effects; there was no interaction among the three treatment factors. The average
new shoot length with DIF12 was the greatest (15.67 mm), followed by that with DIF0
(13.50 mm), and the lowest was with DIF6 (13.10 mm). In terms of the REE concentration,
the 100 \(\mu\)M REE treatment resulted in the longest length of new shoot (16.42 mm), followed
by 150 (14.35 mm), 200 (13.59 mm) and 50 (12.82 mm).

It was observed that 100Ce in DIF6 resulted in the greatest new shoot diameter. Although this treatment was not significantly different from 100La, 100Ce, 150Ce, 100Nd in DIF0, 100La, 100Nd, 200Nd in DIF6, and 100La in DIF12, its influence on the new shoot
diameter was significantly higher than that of the other treatments. Through the F-test, it
was found that the DIF and REE concentration also had a significant effect on the diameter
of new \(D. aphyllum\) shoots, while the REE type had no significant effects. There was a
certain interaction between the DIF, REE type, and REE concentration, but the interaction
among the three was not particularly significant. The average new shoot diameter with
DIF0 and DIF6 was 2.03 and 2.06 mm respectively, but it was significantly reduced to 1.64
mm with DIF12. The 100 \(\mu\)M REE treatment led to the greatest diameter of new shoots
(2.22 mm), followed by 150 (1.90 mm), 200 (1.86 mm) and 50 (1.74 mm) \(\mu\)M.

The results showed that 100Ce in DIF6 led to the greatest new shoot fresh weight.
Although its influence on the new shoot fresh weight was not significantly different from
that of 100Nd in DIF6 and 100Ce in DIF12, it was significantly higher than that of the other
treatments. In general, the DIF, REE type, and REE concentrations had significant effects
on the fresh weight, but there was no interaction among the three factors. The average
fresh weight with DIF6 and DIF12 was 0.39 g, and that with DIF0 was 0.28 g. The fresh
weight with the La(NO\(_3\))\(_3\) and Ce(NO\(_3\))\(_3\) treatments was higher (0.37 g), while that with the
Nd(NO\(_3\))\(_3\) and in the control group was lower (0.33 g). From the perspective of REE
concentration, the highest fresh weight was observed with the 100 \(\mu\)M treatment (0.43 g),
followed by 0.34 g with 150 \(\mu\)M, and 0.33 g with 50 and 200 \(\mu\)M.
Table 1. Effect of the DIF and REE supplementation on growth and development of *D. aphyllum* shoots.

| DIF (°C) (D) (Photo-/Dark-Period Temp.) | REE (R) | Concentration (µM) (C) | Length (mm) | Diameter (mm) | Fresh Weight (g) | No. | No. of Nodes | No. of Leaves |
|----------------------------------------|---------|------------------------|-------------|---------------|-----------------|-----|--------------|--------------|
|                                        | Control-0 | 0                      | 10.3 ef     | 1.86 d–k      | 0.29 g–m        | 1.1 cd | 1.4 f        | 3.1 fg        |
| 0 (20/20 °C)                           | La(NO₃)₃ | 50                     | 11.7 ef     | 1.90 d–k      | 0.29 g–m        | 1.0 cd | 1.4 f        | 4.3 c–g        |
|                                        |         | 100                    | 15.4 b–e    | 2.29 a–d      | 0.38 c–g        | 1.2 b–d | 2.1 c–f      | 4.0 d–g        |
|                                        |         | 150                    | 14.1 b–f    | 1.70 g–l      | 0.31 f–l        | 1.2 b–d | 2.4 a–f      | 4.8 b–g        |
|                                        |         | 200                    | 12.9 c–f    | 1.80 e–l      | 0.27 h–m        | 1.3 b–d | 2.4 a–f      | 4.2 d–g        |
|                                        | Ce(NO₃)₃ | 50                     | 13.5 b–f    | 2.11 b–h      | 0.26 i–m        | 1.1 cd | 2.2 b–f      | 3.8 d–g        |
|                                        |         | 100                    | 17.2 a–d    | 2.55 ab       | 0.39 c–g        | 1.2 b–d | 2.4 a–f      | 4.2 d–g        |
|                                        |         | 150                    | 14.9 b–e    | 2.27 a–e      | 0.33 e–l        | 0.9 cd | 3.0 a–d      | 4.8 b–g        |
|                                        |         | 200                    | 10.5 ef     | 1.87 d–k      | 0.23 lm         | 1.1 cd | 2.1 c–f      | 4.3 c–g        |
|                                        | Nd(NO₃)₃ | 50                     | 12.5 d–f    | 1.76 f–l      | 0.25 j–m        | 1.0 cd | 2.2 b–f      | 4.6 b–g        |
|                                        |         | 100                    | 13.9 b–f    | 2.26 a–d      | 0.25 j–m        | 1.1 cd | 2.1 c–f      | 4.0 d–g        |
|                                        |         | 150                    | 14.6 b–e    | 2.18 b–g      | 0.24 k–m        | 1.3 b–d | 1.8 d–f      | 4.9 a–g        |
|                                        |         | 200                    | 14.0 b–f    | 1.81 e–l      | 0.21 m          | 1.1 cd | 1.8 d–f      | 3.7 d–g        |
| 6 (23/17 °C)                           | Control-6 | 0                      | 10.7 ef     | 1.66 h–l      | 0.34 e–k        | 0.8 d  | 1.6 ef       | 3.6 d–g        |
|                                        | La(NO₃)₃ | 50                     | 13.9 b–f    | 1.86 d–k      | 0.38 c–g        | 1.6 a–c | 1.8 d–f      | 3.6 d–g        |
|                                        |         | 100                    | 14.9 b–e    | 2.47 ab       | 0.47 b–d        | 1.9 ab  | 3.1 a–c      | 6.4 a–c        |
|                                        |         | 150                    | 13.0 c–f    | 1.97 c–j      | 0.34 e–j        | 1.3 b–d | 2.6 a–f      | 4.7 b–g        |
|                                        |         | 200                    | 13.4 b–f    | 1.93 c–k      | 0.39 c–g        | 1.3 b–d | 2.4 a–f      | 4.8 b–g        |
|                                        | Ce(NO₃)₃ | 50                     | 9.1 f       | 1.68 h–l      | 0.31 f–m        | 1.4 b–d | 2.4 a–f      | 3.6 d–g        |
|                                        |         | 100                    | 15.3 b–e    | 2.67 a        | 0.56 a          | 2.1 a  | 3.6 a        | 6.9 a          |
|                                        |         | 150                    | 11.6 c–f    | 1.77 f–l      | 0.38 c–g        | 1.3 b–d | 2.7 a–f      | 4.0 d–g        |
|                                        |         | 200                    | 14.2 b–e    | 2.00 c–i      | 0.39 c–g        | 1.1 cd  | 2.3 a–f      | 5.4 a–d        |
|                                        | Nd(NO₃)₃ | 50                     | 12.8 c–f    | 1.76 f–l      | 0.32 e–l        | 0.9 cd  | 2.8 a–e      | 4.3 c–g        |
|                                        |         | 100                    | 15.3 b–e    | 2.49 ab       | 0.49 ab         | 1.6 a–c | 2.7 a–f      | 6.7 ab          |
|                                        |         | 150                    | 12.4 d–f    | 2.17 b–g      | 0.34 e–j        | 1.3 b–d | 2.8 a–e      | 4.6 b–g        |
|                                        |         | 200                    | 13.3 b–f    | 2.39 a–c      | 0.35 e–j        | 1.4 b–d | 2.7 a–f      | 3.2 e–g        |
Table 1. Cont.

| DIF (°C) (D) (Photo-/Dark-Period Temp.) | REE (R) | Concentration (µM) (C) | Length (mm) | Diameter (mm) | Fresh Weight (g) | No. | No. of Nodes | No. of Leaves |
|----------------------------------------|---------|------------------------|-------------|--------------|-----------------|-----|--------------|--------------|
| Control-12                             | 0       | 13.3 b–f 1.49 j–l 0.37 d–h 1.0 cd 1.6 ef 2.9 g |             |              |                 |     |              |              |
| La(NO$_3$)$_3$                          | 50      | 12.9 c–f 1.77 f–l 0.40 b–f 1.2 b–d 2.0 c–f 4.3 c–g | 12.9 c–f 1.77 f–l 0.40 b–f 1.2 b–d 2.0 c–f 4.3 c–g |             |              |                 |     |              |              |
|                                        | 100     | 18.1 ab 2.23 a–f 0.43 b–e 1.6 a–c 2.8 a–e 4.3 c–g | 18.1 ab 2.23 a–f 0.43 b–e 1.6 a–c 2.8 a–e 4.3 c–g |             |              |                 |     |              |              |
|                                        | 150     | 12.9 c–f 1.85 d–l 0.37 d–h 1.0 cd 2.3 a–f 4.3 c–g | 12.9 c–f 1.85 d–l 0.37 d–h 1.0 cd 2.3 a–f 4.3 c–g |             |              |                 |     |              |              |
|                                        | 200     | 14.1 b–e 1.72 g–l 0.38 c–g 1.2 b–d 2.8 a–e 5.7 a–d | 14.1 b–e 1.72 g–l 0.38 c–g 1.2 b–d 2.8 a–e 5.7 a–d |             |              |                 |     |              |              |
| 12 (26/14 °C)                           |         |                       |             |              |                 |     |              |              |
| Ce(NO$_3$)$_3$                          | 50      | 14.4 b–e 1.49 j–l 0.39 c–g 1.2 b–d 2.6 a–f 4.2 d–g | 14.4 b–e 1.49 j–l 0.39 c–g 1.2 b–d 2.6 a–f 4.2 d–g |             |              |                 |     |              |              |
|                                        | 100     | 20.5 a 1.54 i–l 0.48 a–c 1.2 b–d 3.4 ab 5.3 a–e | 20.5 a 1.54 i–l 0.48 a–c 1.2 b–d 3.4 ab 5.3 a–e |             |              |                 |     |              |              |
|                                        | 150     | 17.8 a–c 1.46 kl 0.38 c–g 1.3 b–d 2.4 a–f 4.1 d–g | 17.8 a–c 1.46 kl 0.38 c–g 1.3 b–d 2.4 a–f 4.1 d–g |             |              |                 |     |              |              |
|                                        | 200     | 12.6 d–f 1.51 j–l 0.36 e–h 1.2 b–d 2.3 a–f 4.8 b–g | 12.6 d–f 1.51 j–l 0.36 e–h 1.2 b–d 2.3 a–f 4.8 b–g |             |              |                 |     |              |              |
| Nd(NO$_3$)$_3$                          | 50      | 14.6 b–e 1.37 l 0.36 e–h 1.3 b–d 1.9 c–f 4.0 d–g | 14.6 b–e 1.37 l 0.36 e–h 1.3 b–d 1.9 c–f 4.0 d–g |             |              |                 |     |              |              |
|                                        | 100     | 17.2 a–d 1.51 j–l 0.39 c–g 1.1 cd 2.3 a–f 4.9 a–g | 17.2 a–d 1.51 j–l 0.39 c–g 1.1 cd 2.3 a–f 4.9 a–g |             |              |                 |     |              |              |
|                                        | 150     | 17.8 a–c 1.71 g–l 0.35 e–j 1.3 b–d 2.4 a–f 5.2 a–f | 17.8 a–c 1.71 g–l 0.35 e–j 1.3 b–d 2.4 a–f 5.2 a–f |             |              |                 |     |              |              |
|                                        | 200     | 17.4 a–d 1.66 h–l 0.36 e–i 1.3 b–d 2.7 a–f 5.4 a–d | 17.4 a–d 1.66 h–l 0.36 e–i 1.3 b–d 2.7 a–f 5.4 a–d |             |              |                 |     |              |              |

F-test

| F-test     | D *** y | R NS | C *** | D × R NS | D × C ** | R × C * | D × R × C NS |
|------------|---------|------|-------|----------|----------|---------|--------------|

2 Mean separation within columns by the Duncan’s multiple range test at $p = 0.05$. * NS, **, ***. Not significant or significant at $p = 0.05$, 0.01, or 0.001, respectively.
According to the statistics on the number of new shoots induced, the treatment with the most average new buds was 100Ce in DIF6, the influence of which was not significantly different from that of 50La, 100La, 100Nd in DIF6 and 100La in DIF12, but which was significantly higher than that with the other treatments. The DIF significantly affected new shoot induction. The average number of new shoots with DIF6 was the greatest (1.4), followed by that with DIF12 (1.2) and DIF0 (1.1). The REE type had no significant effects on the number of new buds induced. However, the REE concentration had a significant effect on the number of new buds induced, although its influence was less than that of the DIF. The average number of new shoots induced was the highest at 100 µM (1.4), and the number of new shoots with the other three concentrations was the same (1.2); however, all were higher than that in the control group (1.0). There was no interaction among the three factors.

The highest average node number of new shoots was observed with 100Ce in DIF6. In general, the DIF had a significant effect on the node number, while the REE type had no significant effects. The influence of the REE concentration was greater than that of the DIF, and there was no interaction among the three factors. With the DIF6 treatments, the average number of nodes was the greatest, followed by that with DIF12 and DIF0. From the perspective of REE concentration, the maximum average number of nodes was 2.7 with 100 µM, followed by 2.5 with 150 µM and 2.4 with 200 µM. The lowest was 2.1 in the control group.

The average number of leaves with 100Ce in DIF6 was the greatest, although there were no statistically significant differences with 150Nd in DIF0, 100La, 200Ce, 100Nd in DIF6, and 200La, 100Ce, 100Nd, 150Nd, 200Nd in DIF12, while it was significantly higher with the other treatments. In general, the DIF and REE type had no significant effect on the number of new bud leaves, while REE concentration had a significant effect. The number of leaves was the greatest in 100 µM treatments, followed by 4.6 in 150 and 200 µM, 4.1 in 50 µM, and 3.2 in the control group. There was a significant interaction between the DIF and REE concentration.

3.2. Chlorophyll Content

Chlorophyll a, chlorophyll b, and total chlorophyll contents, as well as the chlorophyll a/b ratio are presented in Table 2. With the increase in DIF, chlorophyll b content visibly increased (DIF0, 0.49 mg·g⁻¹; DIF6, 0.91 mg·g⁻¹; and DIF12, 1.05 mg·g⁻¹), while the chlorophyll a content did not change significantly. Therefore, the total chlorophyll content increased with the increase of DIF, while the chlorophyll a/b ratio decreased. The highest average chlorophyll a content was found with 50La in DIF6, which was significantly higher than that in all the other treatments. The highest average chlorophyll b content was found with 150Nd in DIF12, which was not significantly different from that with 50Nd and 200Nd in DIF12, but significantly higher than that of the other treatments. The total chlorophyll content with 150Nd in DIF12 was the greatest, which was not significantly different with that with 200Ce, 50Nd and 200Nd in DIF12. The highest chlorophyll a/b ratio was observed with 50La in DIF0, which was not significantly different from that with 100Ce in DIF0, but higher than that of all other treatments.
Table 2. Effect of the DIF and REE supplementation on leaf chlorophyll content of *D. aphyllum*.

| DIF (°C) (D) | REE (R) | Concentration (µM) (C) | Chlorophyll (mg g⁻¹) | Chlorophyll a/b |
|-------------|---------|------------------------|----------------------|-----------------|
|             |         |                        | a                    | b               | Total          |                   |
|             |         |                        |                      |                 | l-n            | 1.37             | cd               |
| 0 (20/20 °C)| Control-0| 0                      | 0.79 bc              | 0.57 lm         | 1.36           | l-n             | 1.37             | cd               |
|             | La(NO₃)₃| 50                     | 0.62 f-m             | 0.41 pq          | 1.03           | q-s             | 1.50 a           |
|             |         | 100                    | 0.56 l-n             | 0.43 o-q         | 0.98           | rs              | 1.30 de           |
|             |         | 150                    | 0.53 mn              | 0.41 pq          | 0.94           | s               | 1.27 e            |
|             |         | 200                    | 0.80 b               | 0.60 l           | 1.40           | k-m             | 1.34 c-e          |
|             | Ce(NO₃)₃| 50                     | 0.69 d-g             | 0.51 l-o         | 1.20           | n-p             | 1.35 c-e          |
|             |         | 100                    | 0.81 b               | 0.55 l-n         | 1.37           | lm              | 1.48 ab           |
|             |         | 150                    | 0.63 e-l             | 0.47 n-p         | 1.10           | p-r             | 1.36 c-e          |
|             |         | 200                    | 0.64 e-l             | 0.48 m-p         | 1.11           | p-r             | 1.34 c-e          |
|             | Nd(NO₃)₃| 50                     | 0.81 b               | 0.60 l           | 1.42           | j-m             | 1.35 c-e          |
|             |         | 100                    | 0.78 b-d             | 0.55 l-n         | 1.33           | m-o             | 1.41 bc           |
|             |         | 150                    | 0.68 e-j             | 0.51 l-p         | 1.18           | 0-q             | 1.34 c-e          |
|             |         | 200                    | 0.45 n               | 0.34 q           | 0.79           | t               | 1.33 c-e          |
|             | Control-6| 0                      | 0.70 c-g             | 1.03 c-e         | 1.72           | b-d             | 0.68 g-j          |
|             | La(NO₃)₃| 50                     | 0.98 a               | 0.71 k           | 1.68           | b-e             | 1.38 cd           |
|             |         | 100                    | 0.67 e-l             | 0.98 d-g         | 1.60           | d-i             | 0.64 g-k          |
|             |         | 150                    | 0.58 j-m             | 0.89 g-j         | 1.46           | i-m             | 0.65 g-k          |
|             |         | 200                    | 0.61 f-m             | 0.92 f-i         | 1.53           | e-l             | 0.66 g-j          |
|             | Ce(NO₃)₃| 50                     | 0.59 g-m             | 0.81 j           | 1.41           | k-m             | 0.73 fg           |
|             |         | 100                    | 0.67 e-k             | 0.84 ij          | 1.51           | f-l             | 0.80 f            |
|             |         | 150                    | 0.66 e-k             | 1.03 c-e         | 1.69           | b-e             | 0.65 g-k          |
|             |         | 200                    | 0.63 e-l             | 0.89 g-j         | 1.52           | e-l             | 0.71 g-i          |
|             | Nd(NO₃)₃| 50                     | 0.64 e-l             | 0.94 e-i         | 1.57           | d-k             | 0.68 g-j          |
|             |         | 100                    | 0.68 e-j             | 1.00 d-f         | 1.67           | b-g             | 0.68 g-j          |
|             |         | 150                    | 0.64 e-l             | 0.95 e-h         | 1.59           | d-i             | 0.68 g-j          |
|             |         | 200                    | 0.63 e-l             | 0.86 h-j         | 1.49           | h-m             | 0.73 f-h          |
Table 2. Cont.

| DIF (°C) (D) (Photo-/Dark-Period Temp.) | REE (R) | Concentration (µM) (C) | Chlorophyll (mg g⁻¹) | Chlorophyll a/b |
|----------------------------------------|---------|-------------------------|-----------------------|------------------|
|                                        |         |                         | a b Total             |                  |
| Control-12                             | 0       | 0.56 i–n 0.87 h–j 1.43 | i–m 0.64 h–k          |                  |
| La(NO₃)₃                               | 50      | 0.58 i–m 0.92 f–i 1.50 | g–m 0.63 i–k          |                  |
|                                        | 100     | 0.61 f–m 0.98 d–g 1.59 | d–j 0.62 i–k          |                  |
|                                        | 150     | 0.59 h–m 1.10 bc 1.69 | b–e 0.55 k            |                  |
|                                        | 200     | 0.57 k–m 0.91 f–j 1.48 | i–m 0.63 i–k          |                  |
| Ce(NO₃)₃                               | 50      | 0.64 e–l 1.03 c–e 1.68 | b–f 0.62 i–k          |                  |
|                                        | 100     | 0.71 c–f 1.08 b–d 1.78 | bc 0.66 g–j           |                  |
|                                        | 150     | 0.62 e–m 1.03 c–e 1.66 | c–h 0.60 jk           |                  |
|                                        | 200     | 0.68 e–h 1.14 b 1.82   | a–c 0.60 jk           |                  |
| Nd(NO₃)₃                               | 50      | 0.68 e–i 1.16 ab 1.84  | ab 0.58 jk            |                  |
|                                        | 100     | 0.60 g–m 0.98 d–g 1.58 | d–j 0.61 jk           |                  |
|                                        | 150     | 0.73 b–e 1.24 a 1.97   | a 0.58 jk             |                  |
|                                        | 200     | 0.68 e–h 1.16 ab 1.84  | ab 0.59 jk            |                  |

F-test

| D | NS ³ | *** | *** | *** |
| R | *    | *** | *** | *** |
| C | *** | *   | *** | *** |
| D × R | *** | *** | *** | *** |
| D × C | *** | *** | *** | *** |
| R × C | *** | *** | *** | *** |
| D × R × C | *** | *** | *** | *** |

² Mean separation within columns for each cultivar by the Duncan’s multiple range test at p = 0.05. ³ NS, *, ***, Not significant or significant at p = 0.05, 0.01, or 0.001, respectively.
3.3. Rank Sum

After summing all of the growth parameters with the different treatments for the new *D. aphyllum* shoots, the average rank sum of all treatments in DIF6 was the highest (3.44), followed by that of DIF12 (2.88) and DIF0 (2.15) (Figure 1). There were no significant differences among the three REE treatments. The mean rank sum of all treatments with Ce(NO$_3$)$_3$ was the greatest (2.96), followed by that of La(NO$_3$)$_3$ (2.83), and Nd(NO$_3$)$_3$ (2.82). All treatments had a higher rank sum than that of the control group (2.29). A comparison of the four REE concentrations showed that all treatments with 100 µM had the highest rank sum (3.68), followed in order by 150 µM (2.68), 200 µM (2.61), and 50 µM (2.49). They were also all higher than the rank sum of the control group (2.29). Among all the treatments, 100Ce in DIF6 achieved the highest rank sum (5.71), followed by 100Nd (5.56) and 100La (5.00) in the same DIF treatment. The lowest rank sum was observed with 200Nd in DIF0 (1.71).

![Figure 1. Effects of the DIF and REE supplementation on the growth and development of *D. aphyllum*, as assessed by rank sum test.](image)

3.4. Permeability of the Plasma Membrane

The plasma membrane permeability with 200Ce in DIF6 and 200Nd in DIF12 was significantly higher than that with all other treatments, followed by that with 150La and 200La in DIF12 (Table 3). In general, with the increase in DIF the plasma membrane permeability showed a gradual increase, with an average value of 18.29% for DIF0, 22.27% for DIF6, and 27.46% for DIF12. The REE type also had a significant effect on plasma membrane permeability: 22.53% with La(NO$_3$)$_3$, 22.92% with Ce(NO$_3$)$_3$, and 24.28% with Nd(NO$_3$)$_3$. However, with the increase in REE concentration, plasma membrane permeability increased significantly, and the average values of plasma membrane permeability with 50, 100, 150 and 200 µM were 18.70%, 21.68%, 22.57% and 30.02%, respectively, were all higher than the 15.82% observed in the control group. The F-test analysis showed that the DIF, REE type and REE concentration all had significant effects on the plasma membrane permeability, and there was an interaction among them.
Table 3. Effects of DIF and REE supplementation on the activities of antioxidant enzymes in *D. aphyllum*.

| DIF (°C) (D) (Photo-/Dark-Period Temp.) | REE (R) | Concentration (µM) (C) | Membrane Permeability (%) | SOD (U·mg⁻¹ protein) | POD (Δ470 g⁻¹ FW min⁻¹) | CAT (Δ240 g⁻¹ FW min⁻¹) | APX (Δ290 mg⁻¹ FW min⁻¹) |
|----------------------------------------|---------|------------------------|---------------------------|----------------------|--------------------------|--------------------------|--------------------------|
|                                        | Control-0 | 0 | 12.68 | r² | 45.64 | tu | 0.02 | qr | 0.01 | r | 3.12 | q |
|                                        | La(NO₃)₃ | 50 | 13.96 | qr | 53.25 | st | 0.15 | h-m | 0.11 | f-i | 3.52 | pq |
|                                        |          | 100 | 13.89 | qr | 70.77 | o-q | 0.13 | i-n | 0.11 | f-k | 8.35 | j-o |
|                                        |          | 150 | 16.17 | op | 56.78 | r-t | 0.26 | d-f | 0.23 | b | 14.01 | e-g |
|                                        |          | 200 | 21.41 | k-m | 91.52 | f-k | 0.35 | c | 0.30 | a | 19.74 | cd |
|                                        | Ce(NO₃)₃ | 50 | 12.67 | r | 66.78 | p-r | 0.03 | qr | 0.08 | i-o | 6.27 | m-q |
|                                        |          | 100 | 12.59 | r | 59.32 | q-s | 0.02 | r | 0.09 | g-n | 5.41 | n-q |
|                                        |          | 150 | 13.48 | qr | 81.76 | k-o | 0.01 | r | 0.18 | c | 6.89 | l-q |
|                                        |          | 200 | 16.53 | e | 126.28 | a | 0.09 | m-q | 0.18 | c-d | 21.62 | bc |
|                                        | Nd(NO₃)₃ | 50 | 17.74 | no | 40.02 | u | 0.18 | g-k | 0.06 | m-q | 8.75 | j-n |
|                                        |          | 100 | 22.16 | j-l | 80.34 | k-o | 0.02 | r | 0.07 | j-p | 8.96 | j-n |
|                                        |          | 150 | 32.56 | de | 88.31 | h-l | 0.01 | r | 0.11 | f-i | 15.25 | ef |
|                                        |          | 200 | 31.88 | e | 104.99 | b-e | 0.20 | f-h | 0.12 | f-h | 16.05 | ef |
|                                        | Control-6 | 0 | 17.02 | no | 49.49 | s-u | 0.11 | l-o | 0.04 | o-r | 3.42 | q |
|                                        | La(NO₃)₃ | 50 | 18.34 | n | 56.77 | r-t | 0.18 | g-j | 0.14 | d-f | 9.02 | j-n |
|                                        |          | 100 | 14.88 | pq | 50.41 | s-u | 0.27 | de | 0.13 | e-g | 11.38 | g-j |
|                                        |          | 150 | 17.31 | no | 85.89 | i-m | 0.48 | b | 0.17 | c-e | 7.85 | j-o |
|                                        |          | 200 | 22.55 | i-k | 101.06 | c-h | 0.51 | ab | 0.08 | h-o | 15.77 | ef |
|                                        | Ce(NO₃)₃ | 50 | 25.91 | gh | 59.39 | q-s | 0.12 | j-o | 0.01 | r | 6.55 | m-q |
|                                        |          | 100 | 29.17 | f | 71.32 | n-q | 0.11 | l-o | 0.10 | f-l | 7.44 | k-o |
|                                        |          | 150 | 20.61 | lm | 89.55 | g-l | 0.19 | g-j | 0.12 | f-g | 13.19 | f-h |
|                                        |          | 200 | 40.65 | a | 114.03 | b | 0.08 | n-r | 0.25 | b | 24.21 | ab |
|                                        | Nd(NO₃)₃ | 50 | 20.11 | m | 54.54 | r-t | 0.19 | g-i | 0.05 | n-r | 6.51 | m-q |
|                                        |          | 100 | 21.89 | j-l | 92.28 | e-k | 0.14 | h-n | 0.03 | p-r | 4.72 | o-q |
|                                        |          | 150 | 17.64 | no | 83.42 | j-n | 0.13 | i-n | 0.02 | qr | 10.46 | g-l |
|                                        |          | 200 | 23.39 | h-j | 135.95 | a | 0.10 | l-p | 0.11 | f-j | 26.95 | a |
| DIF (°C) (D) (Photo-/Dark-Period Temp.) | REE (R) | Concentration (µM) (C) | Membrane Permeability (%) | SOD (U mg⁻¹ protein) | POD (Δ470 g⁻¹ FW min⁻¹) | CAT (Δ240 g⁻¹ FW min⁻¹) | APX (Δ290 mg⁻¹ FW min⁻¹) |
|--------------------------------------|---------|------------------------|---------------------------|----------------------|-------------------------|-------------------------|-------------------------|
| Control-12                           | 12      | 0                      | 17.75                     | 49.59                | 0.13                    | 0.03                    | 7.27                    |
| La(NO₃)₃                             | 50      | 23.38                  | h-j                       | 77.41                | 0.28                    | d                       | 0.09                    | 9.67                    | h-m                    |
|                                      | 100     | 33.44                  | d                         | 66.88                | 0.25                    | 0.25                    | 0.07                    | 6.53                    | k-p                    |
|                                      | 150     | 37.05                  | b                         | 66.30                | 0.17                    | h-l                     | 0.06                    | 16.04                   | l-q                    |
|                                      | 200     | 37.94                  | b                         | 73.75                | 0.35                    | 0.24                    | 0.12                    | 19.68                   | 19.68                  |
| Ce(NO₃)₃                             | 50      | 21.78                  | j-l                       | 106.79               | 0.03                    | qr                      | 0.09                    | 9.20                    | i-n                    |
|                                      | 100     | 22.17                  | j-l                       | 103.95               | 0.03                    | p-r                     | 0.11                    | 14.08                   | e-g                    |
|                                      | 150     | 24.29                  | h                         | 95.02                | 0.05                    | o-r                     | 0.10                    | 16.37                   | d-f                    |
|                                      | 200     | 35.17                  | c                         | 95.58                | 0.19                    | g-j                     | 0.12                    | 22.03                   | bc                     |
| Nd(NO₃)₃                             | 50      | 14.37                  | q                         | 83.69                | 0.02                    | qr                      | 0.14                    | 11.53                   | g-j                    |
|                                      | 100     | 24.94                  | gh                        | 101.64               | 0.11                    | k-o                     | 0.14                    | 11.25                   | g-k                    |
|                                      | 150     | 24.00                  | hi                        | 109.37               | 0.04                    | p-r                     | 0.23                    | 11.33                   | g-j                    |
|                                      | 200     | 40.69                  | a                         | 96.43                | 0.21                    | e-h                     | 0.29                    | 17.17                   | d-e                    |
| F-test                               |         |                        |                           |                      |                         |                         |                         |                         |
| D                                    | ***     | y                      | ***                       | ***                   | ***                      | ***                      | ***                      |
| R                                    | ***     |                         | ***                       | ***                   | ***                      | ***                      | NS                      |
| C                                    | ***     |                         | ***                       | ***                   | ***                      | ***                      | ***                      |
| D × R                                 | ***     |                         | ***                       | ***                   | ***                      | ***                      | ***                      |
| D × C                                 | ***     |                         | ***                       | ***                   | ***                      | ***                      | ***                      |
| R × C                                 | ***     |                         | ***                       | ***                   | ***                      | ***                      | ***                      |
| D × R × C                            | ***     |                         | ***                       | ***                   | ***                      | ***                      | ***                      |

* Mean separation within columns by the Duncan’s multiple range test at p = 0.05. y NS, *, ***, Not significant or significant at p = 0.05, 0.01, or 0.001, respectively.
3.5. Activities of Antioxidant Enzymes

The activities of the four antioxidant enzymes are shown in Table 3. The SOD activities with 200Ce in DIF0 and 200Nd in DIF6 were significantly higher than those for all other treatments. In general, the SOD activity tended to increase with the increase of DIF, and the average SOD activity in DIF0, 6 and 12 were 74.29, 80.32 and 86.65 U·mg\(^{-1}\)·protein, respectively. The average SOD activity with Ce(NO\(_3\))\(_3\) and Nd(NO\(_3\))\(_3\) was 89.15 and 89.25 U·mg\(^{-1}\)·protein, respectively, while that with La(NO\(_3\))\(_3\) was 70.90 U·mg\(^{-1}\)·protein. The lowest level of 48.24 U·mg\(^{-1}\)·protein was found in the control group. With the increase of REEs, SOD activity gradually increased to 66.52, 77.43, 84.04 and 104.40 U·mg\(^{-1}\)·protein, respectively. Through the F-test analysis, it was found that the DIF, REE type, and REE concentration all had significant effects on SOD activity, and there was an interaction among the three factors.

The highest mean value of POD activity (0.55 Δ470 g\(^{-1}\)FW·min\(^{-1}\)) was observed with 200La in DIF12, which was significantly higher than that with all other treatments, although no statistically significant differences were observed from that with 150La and 200La in DIF6. The highest mean value of POD activity in all treatments with DIF6 was 0.20 Δ470 g\(^{-1}\)FW·min\(^{-1}\), followed by 0.16 Δ470 g\(^{-1}\)FW·min\(^{-1}\) with DIF12; the lowest was 0.11 Δ470 g\(^{-1}\)FW·min\(^{-1}\) with DIF0. For the three different REEs, the highest average POD activity was with La(NO\(_3\))\(_3\) was 0.30 Δ470 g\(^{-1}\)FW·min\(^{-1}\), followed by that with Nd(NO\(_3\))\(_3\), which was 0.11 Δ470 g\(^{-1}\)FW·min\(^{-1}\). Ce(NO\(_3\))\(_3\) had the lowest average POD activity at 0.08 Δ470 g\(^{-1}\)FW·min\(^{-1}\), even lower than that of the control, 0.09 Δ470 g\(^{-1}\)FW·min\(^{-1}\). From the perspective of REE concentration, 200 µM REE resulted in the highest POD activity (0.25 Δ470 g\(^{-1}\)FW·min\(^{-1}\)), followed by 150 µM (0.15 Δ470 g\(^{-1}\)FW·min\(^{-1}\)), 50 µM (0.13 Δ470 g\(^{-1}\)FW·min\(^{-1}\)) and 100 µM (0.12 Δ470 g\(^{-1}\)FW·min\(^{-1}\)). The F-test analysis showed that the DIF, REE type, and concentration all had significant effects on POD activity, and there was an interaction among the three factors.

The 200La in DIF0 and 200Nd in DIF12 had the highest CAT activity, followed by 150La in DIF0, 200Ce in DIF6 and 200La, 150Nd in DIF12. Overall, DIF6 had the lowest average CAT activity (0.10 Δ240 g\(^{-1}\)FW·min\(^{-1}\)), and DIF0 was slightly higher and identical to DIF12 (0.13 Δ240 g\(^{-1}\)FW·min\(^{-1}\)). For different REEs, La(NO\(_3\))\(_3\) had the highest average CAT activity (0.14 Δ240 g\(^{-1}\)FW·min\(^{-1}\)), followed by Ce(NO\(_3\))\(_3\) (0.12 Δ240 g\(^{-1}\)FW·min\(^{-1}\)) and Nd(NO\(_3\))\(_3\) (0.11 Δ240 g\(^{-1}\)FW·min\(^{-1}\)). All REE treatments led to higher CAT activity values than that of the control group (0.03 Δ240 g\(^{-1}\)FW·min\(^{-1}\)). The CAT activity with 50 and 100 µM was the same (0.09 Δ240 g\(^{-1}\)FW·min\(^{-1}\)). The CAT activity gradually increased with the increase of REE concentration beyond 100 µM; 150 µM (0.14 Δ240 g\(^{-1}\)FW·min\(^{-1}\)) and 200 µM (0.19 Δ240 g\(^{-1}\)FW·min\(^{-1}\)). Through the F-test, it was observed that the DIF, REE type, and REE concentration all had significant effects on the CAT activity, and there was interaction among them, but the interaction between REE type and REE concentration was relatively weak.

The analysis of APX activity showed that 200Nd in DIF6 treatment had the highest APX activity, which was not significantly different from that with 200Ce in DIF6, but significantly higher than that with all other treatments. In general, the APX activity gradually increased with the increase in DIF, and the average APX activity with DIF0, 6 and 12 was 10.61, 11.34 and 13.73 Δ290·mg\(^{-1}\)FW·min\(^{-1}\), respectively. The three REE types had no significant effects on APX activity. The average APX activities with La(NO\(_3\))\(_3\), Ce(NO\(_3\))\(_3\) and Nd(NO\(_3\))\(_3\) were 12.33, 12.27 and 12.41 Δ290·mg\(^{-1}\)FW·min\(^{-1}\), respectively, which were all higher than that of the control group (4.60 Δ290·mg\(^{-1}\)FW·min\(^{-1}\)). The REE concentration also had a significant effect on APX activity, which showed an increasing trend with the increase in concentration. The average values of the APX activity with 50, 100, 150 and 200 µM were 7.22, 9.39, 12.38 and 20.36 Δ290·mg\(^{-1}\)FW·min\(^{-1}\), respectively. In addition to REE type, the other treatment factors all had significant effects on APX activity and interacted with each other.
3.6. Total Protein Content

By measuring the total protein content in *D. aphyllum*, it was observed that the average protein content with 150Nd in DIF6 was the highest (Figure 2). Although the were no significant differences in the total protein content with 150La, 200La, 100Ce, 150Ce, 100Nd in DIF6, and 100La, 150La, 200La, 100Ce, 150Ce, 200Ce, 50Nd, 100Nd, 150Nd in DIF12, the total protein was significantly higher than that in the other treatments. In general, the total protein content showed an upward trend with the increase in DIF: DIF0 3.94 mg·g⁻¹, DIF6 6.52 mg·g⁻¹, and DIF12 6.75 mg·g⁻¹. There were no significant differences in protein content among the different REEs, which was 5.68 mg·g⁻¹ with La(NO₃)₃, 5.94 mg·g⁻¹ with Ce(NO₃)₃, and 5.99 mg·g⁻¹ with Nd(NO₃)₃, respectively. All REEs resulted in a higher total protein content compared to that of the control group (4.13 mg·g⁻¹). The highest average total protein content was observed with 150 µM at 6.52 mg·g⁻¹, followed by 100, 200, and 50 µM, the total protein content of which was 6.03, 5.89, and 5.04 mg·g⁻¹, respectively.

![Figure 2](image-url)  
*Figure 2. Effects of DIF and REE supplementation on total protein content in *D. aphyllum*. The error bars represent the SEs of biological replicates (n = 3). Different letters within each panel indicate statistically significant differences at p = 0.05 using Duncan’s multiple range test.

3.7. Polysaccharide Content

The highest polysaccharide content was observed with 100Ce in DIF12, which was not significantly different from that with the 200Ce treatment in the same DIF but was sig-
nificantly higher than that of all other treatments (Figure 3). In general, the polysaccharide content increased significantly with the increase in DIF, and the average polysaccharide content with DIF0, 6 and 12 was 3.77, 5.78 and 8.02 mg g\(^{-1}\), respectively. DIF6 and DIF12 resulted in a polysaccharide content increase of 54% and 113%, respectively, compared to that with DIF0. The average polysaccharide content with La(NO\(_3\))\(_3\) (5.04 mg g\(^{-1}\)) and Nd(NO\(_3\))\(_3\) (5.27 mg g\(^{-1}\)) was not significantly different, but the polysaccharide content with Ce(NO\(_3\))\(_3\) (7.87 mg g\(^{-1}\)) was significantly higher than that with the other treatments; the lowest was observed in the control group (3.44 mg g\(^{-1}\)). The polysaccharide content in 100, 150, and 200 µM treatments was 6.59, 6.19, and 6.57 mg g\(^{-1}\), respectively. The 50 µM treatment led to a relatively low polysaccharide content at 4.89 mg g\(^{-1}\).

![Figure 3](image.png)

**Figure 3.** Effects of DIF and REE supplementation on polysaccharide content in *D. aphyllum*. The error bars represent the SEs of biological replicates (n = 3). Different letters within each panel indicate statistically significant differences at \( p = 0.05 \) using Duncan’s multiple range test.

3.8. Morphological Observations

Morphological observations are presented in Figure 4. In general, *D. aphyllum* grown with DIF6 had the greatest number of shoots and the highest plant quality, especially when 100 µM REE was added. The number of new shoots in DIF0 and DIF12 was relatively lower, and in DIF12 the plants were visibly thinner and more slender. It appears that the different REE types had no significant effects on the morphology, but there were significant
differences in response to the different REE concentrations. The biomass and growth of *D. aphyllum* in all 100 µM treatments were higher than those in the other treatments.

Figure 4. Effects of DIF and REE supplementation on the morphology of *D. aphyllum* after four weeks of treatments.
4. Discussion

Data analysis revealed that the DIF had significant effects on all growth parameters except for the leaf number of new shoots and the chlorophyll a content in *D. naphyllum* (Table 1). DIF6 led to the shortest shoot length, the largest diameter and fresh weight, and the greatest number of new shoots and nodes. With the increase in DIF the total chlorophyll content increased, while the chlorophyll a/b ratio decreased (Table 1). The highest rank sum was obtained with DIF6 (Figure 1). The number of new *D. aphyllum* shoots with 6 °C of DIF was greater, and the new shoots were sturdier and more compact than those grown with 0 and 12 °C of DIF. The biomass of *D. aphyllum* grown with DIF0 was lower and the new shoots grown with DIF12 were more slender. In other words, the comprehensive quality of *D. aphyllum* shoots treated with DIF6 was higher from the perspective of propagation and ornamental values. Many similar findings have been reported in studies of different species of *Dendrobium* or other plants regarding the DIF; Chae et al. [41] studied the effects of different DIFs on growth and flag leaf occurrence in *D. nobile* and found that the stem length increased as the +DIF increased. The greatest stem diameter was obtained with a low +DIF condition with a high day temperature. The number of nodes and leaves also increased as the DIF increased. DIF had a great influence on internode elongation in many plant species, which can be explained by specific influences of plant hormones [42]. Shimizu [43] and Patel and Franklin [44] also mentioned in a review that DIF has an important influence on plant architecture. Greater elongation of stems and leaves is generally observed when the DIF is positive. In a study investigating how light source and DIF affect the growth and anthocyanin concentration in *Perilla frutescens*, Park et al. [45] found that plant height and fresh weight increased with an increase in the DIF, and the number of leaves with 8 °C of DIF was significantly higher than that with 0 °C of DIF. Matsuda et al. [46] found that a 10 °C DIF significantly increased the shoot dry weight of tomato compared to 0 °C DIF on the 49th day. A positive DIF (daytime temperature higher than night temperature) was observed to be able to increase the carbohydrate, free amino acid, and soluble protein contents in tomato [47]. Erwin et al. [48] found that the chlorophyll content in *Fuchsia* and *Dendranthema* decreased with decreasing DIF when calculated both on a per-unit-area and a per-gram-dry weight basis [49]. These findings are similar to the results obtained in our study; however, we found that the increase in total chlorophyll content was mainly caused by the increase of chlorophyll b. Vågen et al. [50] found that DIF treatments may influence phytochrome-controlled chlorophyll synthesis and chlorophyll a/b ratio. With an increase in the DIF, the chlorophyll a/b ratio decreased significantly because the chlorophyll b content decreased more than the chlorophyll a content. This may be attributed to an adaptation mechanism of plants under negative DIF in order to avoid capturing excessive light energy, as chlorophyll b is the main component of the light-harvesting complex protein [51]. Chen et al. [52] also found that 6 °C and 12 °C DIFs also increased tomato yield by 49.6% and 14.9%, respectively, compared to that with 0 °C DIF.

The permeability of the plasma membrane is very sensitive to stress. When plants are subjected to high temperatures, drought, salinity, and other stresses, the permeability of the plasma membrane will increase, and the inner membrane system of various organelles will swell, shrink, and even become damaged; this is mainly caused by membrane lipid peroxidation, membrane protein degeneration, and membrane lipid fluidity change [53]. There are many studies on the effects of extreme temperature stress on the plasma membrane permeability of plant materials, but there are few results on how DIF affects the plasma membrane permeability. Our study found that with the increase in DIF, the permeability of the plasma membrane also increased (Table 3); however, even with the highest DIF (DIF12), 26 °C during the day and 14 °C at night, this would not exert serious temperature stress on *D. aphyllum* because its suitable growth temperature is about 10–30 °C. This may suggest that a DIF up to 12 °C a day has triggered a temperature-adaptive response mechanism in the plant cells.
Metabolic responses in plants are a part of their stress and defense responses [11,54]. Polysaccharide synthethes are considered as one of the most important plant responses to stress [18]. Plants can prevent stress reaction by activating different antioxidative enzymes, such as super-oxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) [55]. Thus, the antioxidant contents in plants are a traditional standard for assessing the medicinal values of plants. Through analysis of the activities of four antioxidant enzymes (Table 3) that can reflect the contents of reactive oxygen species in plants, it was found that except for POD, the activities of the enzymes (SOD, CAT and APX) were the highest with DIF12. This also indicates that a higher DIF may cause D. aphyllum to produce higher levels of reactive oxygen species, which also increases the activity of antioxidant enzymes that can resist stresses. Muneer et al. [56] reached a similar conclusion when the effects of three different DIFs on tomato seedlings in the grafting stage were studied. The three DIFs were 23/23 °C (DIF0), 25/18 °C (DIF7), and 30/15 °C (DIF15). The results showed that treatments with a higher DIF led to a higher ROS content. The corresponding activities of three ROS-resistant enzymes, SOD, CAT, and APX, also significantly increased. A proteomic analysis was employed to compare the protein expression differences under the different DIFs. However, it remains unclear if such changes are specifically caused by a single high temperature in the daytime, a low temperature at night, or by the superposition of alternating temperature changes, which is worthy of future research.

As mentioned earlier, a positive DIF is known to be able to increase free amino acid and soluble protein content. Similar results were obtained in our study. The contents of proteins and polysaccharides increased with the increase in the DIF (Figures 2 and 3). The mechanism by which the DIF promotes the accumulation of organic matter is not complex. A higher daytime temperature can improve photosynthetic efficiency and promote the accumulation of photosynthetic products, while a lower nighttime temperature can reduce the respiration of plants, and therefore reduce consumption of the available assimilates [57–60]. However, in this study, it was found that although DIF12 resulted in the highest total protein and polysaccharide contents, these contents were not significantly higher than those obtained with DIF6. Therefore, we speculated that although 26 °C in the day was not a particularly high temperature, and 14 °C at night was not a particularly low temperature for D. aphyllum, DIF as high as 12 °C might exert a ‘DIF stress’ for plants. This stress may promote the synthesis of polysaccharides and stress-resistant proteins [61–67]. Yu et al. [68], in a study on the gene function of Dendrobium officinale, found that under abiotic stress the relative expression of the DoUGE gene was significantly increased in order to improve tolerance to abiotic stresses. The DoUGE gene, which is involved in the regulation of water-soluble polysaccharide contents in D. officinale, was positively correlated with DIF. Whether ‘DIF stress’ can lead to similar responses in D. aphyllum is a valuable avenue for future study. After comparing the main medicinal components of D. officinale in different growing environments, Yuan et al. [69] found that the contents of the medicinal components of D. officinale in wild environments were higher than those in the greenhouse. The DIF in wild environments is generally higher than that in greenhouses, so if this hypothesis is established it will provide a very valuable reference for environmental regulation of greenhouse medicinal cultivation of Dendrobium.

REE type only had significant effects on the fresh weight and chlorophyll content of the new shoots, while the REE concentration had significant effects on all growth parameters of D. aphyllum. In general, according to the rank sum test, the growth state of new Dendrobium shoots treated with Ce(NO$_3$)$_3$ was the greatest, while that with (NO$_3$)$_3$ was similar to that with Nd(NO$_3$)$_3$. Wang et al. [28] also found that cerium could improve the utilization of carbon and nitrogen sources in the protocorm-like bodies of D. huoshanense, significantly increase the contents of intracellular polysaccharides, nitrates and phosphates, enhance cell growth, and boost polysaccharide biosynthesis. There have been many studies on how REEs affect plant growth. For example, 0.63 mM lanthanum and cerium promoted the growth of the maize root system [70], and Lanthanum and cerium also increased wheat
yields [71]. Velasco et al. [72] found that 100 mg·kg\(^{-1}\) of cerium sulphate enhanced the root and shoot growth in *Phaseolus radiatus* and *Brassica pekinensis*, and they speculated that the effects of REEs on plant growth might be due to amelioration of H\(^+\) toxicity. Negatively charged cell surfaces in the roots accumulate toxic cations, and amelioration is affected by treatments that reduce the negativity of the cell-surface electrical potential by charge screening or cation binding. Polyvalent cations can cause charge reversals at the membrane surface [73,74]. It was also suggested that, at low levels, REEs can act as micronutrients, with toxicity occurring only at high concentrations [72]. We also found that the growth state and quality of plants were the greatest in the treatment with 100 \(\mu\)M REE, and the quality of plants decreased regardless of how the REE concentration increased or decreased. With increased REE concentrations, the permeability of the plasma membrane and the activities of four antioxidant enzymes (SOD, POD, CAT and APX) increased gradually. Generally, stress increases the production of reactive oxygen species (ROS); in order to minimize these toxic molecules, antioxidant enzyme activity also increases. Thus, we assume that a high REE concentration might cause slight salinity stress in *D. aphyllum*. The fresh weight and polysaccharide content of new shoots were the highest with the 100 \(\mu\)M REE treatments, and the total protein content was the greatest with the 150 \(\mu\)M REE treatment, which indicates that the addition of REEs could promote the growth and accumulation of the primary metabolites of *D. aphyllum*; however, an excessively high concentration could also inhibit their growth. Yuan et al. [69] found that for *D. officinale* in bionic cultivation mode, the total nitrogen was significantly and positively correlated with polysaccharide levels. The most important ecological factors affecting polysaccharide content are soil total nitrogen, maximum relative humidity, maximum temperature, and minimum relative humidity. All REEs used in this study were employed in the form of nitrates, so it is possible that the increase of nitrates also affected the growth and metabolism of the plants. However, trivalent nitrate REEs were selected for comparison in the design of this study, so the relative differences between the three REEs are still meaningful. Therefore, in any follow-up study it will be necessary to set up a control group to which no nitrogen is added.

From the overall perspective of this study, DIF12 and 100 \(\mu\)M of REEs were the turning point where treatment went from growth-prompting to stress-inducing. Therefore, DIF12 and 150, 200 \(\mu\)M REEs are considered as stress-inductive treatments. There was a synergistic effect between the REEs and DIF, mainly between the concentration of REE and DIF; with greater concentrations of REEs, the stress effects on the plants after the superposition of higher DIF were more obvious.

5. Conclusions

In conclusion, this study found that the medium supplemented with 100 \(\mu\)M Ce(NO\(_3\))\(_3\) at 6 °C DIF was the most optimal environment for the vegetative growth of *D. aphyllum*, and therefore the most suitable for the propagation stage. A 12 °C DIF can promote the synthesis of more primary metabolites such as proteins and polysaccharides. From the perspective of improving medicinal value, increasing the DIF on the basis of optimum growth environment is a valuable reference.

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References
1. Xing, X.; Cui, S.W.; Nie, S.; Phillips, G.O.; Goff, H.D.; Wang, Q. A review of isolation process, structural characteristics, and bioactivities of water-soluble polysaccharides from *Dendrobium* plants. **Bioact. Carbohydr. Diet. Fibre** 2013, 1, 131–147. [CrossRef]

2. Meng, Q.; Fan, H.; Xu, D. Superfine grinding improves the bioaccessibility and antioxidant properties of *Dendrobium officinale* powders. **Int. J. Food Sci. Technol.** 2017, 52, 1440–1451. [CrossRef]

3. Deng, Y.H.; Xu, K.P.; Tan, G.S. Advances in studies on chemical constituents and pharmacological activities of plants of *Dendrobium Sw. Chin. Tradit. Herb. Drugs* 2002, 25, 677–680. (In Chinese)

4. Zhao, Y.L.; Wang, S.L.; Li, X.Y. Study on polysaccharides from *Dendrobium* (Roxb.). **Plant Res. Yunnan** 1994, 16, 392–396. (In Chinese)

5. Shao, L.; Huang, W.H.; Zhang, C.F.; Wang, L.; Zhang, M.; Wang, Z.T. Study on chemical constituents of *Dendrobium aphyllum* (Roxb.). **China J. Chin. Mater. Med.** 2008, 33, 1693–1695.

6. Zhang, C.F.; Shao, L.; Huang, W.H.; Wang, L.; Wang, Z.T.; Xu, L.S. Study on chemical constituents of phenolics of *Dendrobium aphyllum* (Roxb.). **China J. Chin. Mater. Med.** 2008, 33, 2922–2925.

7. Liu, H.; Luo, Y.B.; Heinen, J.; Bhat, M.; Liu, Z.J. Eat your orchid and have it too: A potentially new conservation formula for Chinese epiphytic medicinal orchids. **Biodiv. Conserv.** 2014, 23, 1215–1228. [CrossRef]

8. Wang, X.; Lin, Y.S.; Liu, D.W.; Xu, H.J.; Liu, T.; Zhao, F.Y. Cerium toxicity, uptake and translocation in *Arabidopsis thaliana* seedlings. **J. Rare Earths** 2012, 30, 579–585. [CrossRef]

9. Hu, Z.Y.; Richter, H.; Sparovek, G.; Schnug, E. Physiological and biochemical effects of rare earth elements on plants and their agricultural significance: A review. **J. Plant Nutr.** 2004, 27, 183–220. [CrossRef]

10. Yuan, Y.J.; Li, J.C.; Ge, Q.Z.; WU, J.C. Superoxide anion burst and burst production induced by Ce⁴⁺ in suspension cultures of *Taxus cuspidate*. **J. Mol. Catal. B Enzym.** 2002, 18, 251–260. [CrossRef]

11. Wu, J.Y.; Wang, C.G.; Mei, X.G. Stimulation of taxol production and excretion in *Taxus* spp cell cultures by rare earth chemical lanthanum. **J. Biotechnol.** 2001, 85, 67–73. [CrossRef]

12. Zhang, C.H.; Li, Q.Q.; Zhang, M.X.; Zhang, N.; Li, M.H. Effects of rare earth elements on growth and metabolism of medicinal plants. **Acta Pharm. Sin. B** 2013, 3, 20–24. [CrossRef]

13. Chen, S.A.; Zhao, B.; Wang, X.D.; Yuan, X.F.; Wang, Y.H. Promotion of the growth of *Crocos sativus* cells and the production of crocin by rare earth elements. **Biotecnol. Lett.** 2003, 26, 27–30. [CrossRef]

14. Diatloff, E.; Smith, F.W.; Asher, C.J. Effects of lanthanum and cerium on the growth and mineral nutrition of corn and mungbean. **Ann. Bot.** 2008, 101, 971–982. [CrossRef]

15. Olivares, E.; Aguilar, G.; Colonnello, G. Rare earth elements in vascular plants: A review. **Interciencia** 2011, 36, 331–340.

16. Huang, G.; Wang, L.; Zhou, Q. Lanthanum (III) regulates the nitrogen assimilation in soybean seedlings under ultraviolet-B radiation. **Biol. Trace Elem. Res.** 2012, 151, 105–112. [CrossRef]
54. Peng, X.; Zhou, S.L.; He, J.Y.; Li, D. Influence of rare earth elements on metabolism and related enzyme activity and isozyme expression in *Tetrastigma hemsleyanum* cell suspension cultures. *Biol. Trace Elem. Res.* 2013, 152, 82–90.

55. Bowler, C.; Camp, W.V.; Montagy, M.V.; Inzé, D.; Asada, K. Superoxide dismutase in plants. *Crit. Rev. Plant Sci.* 1994, 13, 199–218. [CrossRef]

56. Muneer, S.; Ko, C.H.; Wei, H.; Chen, Y.; Jeong, B.R. Physiological and proteomic investigations to study the response of tomato graft unions under temperature stress. *PLoS ONE* 2016, 11, e0157439. [CrossRef]

57. McCree, K.J. An equation for the rate of respiration of white clover plants grown under controlled conditions. In *Prediction and Measurement of Photosynthetic Productivity*; Šetlík, I., Ed.; Centre for Agricultural Publication and Documentation: Wageningen, The Netherlands, 1970; pp. 221–229.

58. Bunce, J.A. Response of respiration of soybean leaves grown at ambient and elevated carbon dioxide concentrations to day-to-day variation in light and temperature under field conditions. *Ann. Bot.* 2005, 95, 1059–1066. [CrossRef] [PubMed]

59. Cheng, W.; Sakai, H.; Yagi, K.; Hasegawa, T. Interactions of elevated CO$_2$ and night temperature on rice growth and yield. *Agric. For. Meteorol.* 2009, 149, 51–58. [CrossRef]

60. Kanno, K.; Makino, A. Increased grain yield and biomass allocation in rice under cool night temperature. *Soil Sci. Plant Nutr.* 2010, 56, 412–417. [CrossRef]

61. Rezaeieh, K.A.P.; Gurbuz, B.; Uyanık, M. Biotic and abiotic stresses mediated changes in secondary metabolites induction of medicinal plants. In Proceedings of the Tibbi ve Aromatik Bitkiler Sempozyumu Conference, Antalya, Turkey, 4 March 2012; pp. 218–222.

62. Gabbish, A.A.; Klenwachter, M.; Selmar, D. Influencing the content of secondary metabolites in spice and medicinal plants by deliberately applying drought stress during their cultivation. *Jordan J. Biol. Sci.* 2015, 8, 1–10. [CrossRef]

63. Manikonda, P.K.; AbhyaniKarn, G.; Rao, K.V.; Reddy, V.D.; Subramanyam, C. Salt stress enhances daidzein production in hairy root cultures of *Psoralea corylifolia* L. (Fabaceae). *Proc. A.P. Akad. Sci.* 2009, 13, 35–49.

64. Mohammadi, H.; Hazrati, S.; Ghorbanpour, M. Tolerance mechanisms of medicinal plants to abiotic stresses. In *Plant Life under Changing Environment*; Academic Press: Cambridge, MA, USA, 2020; pp. 663–679.

65. Sharma, A. Gene expression analysis in medicinal plants under abiotic stress conditions. In *Plant Metabolites and Regulation under Environmental Stress*; Academic Press: Cambridge, MA, USA, 2018; pp. 407–414. [CrossRef]

66. Aghaei, K.; Komatsu, S. Crop and medicinal plants proteomics in response to salt stress. *Front. Plant Sci.* 2013, 4, 8. [CrossRef]

67. Afrin, S.; Huang, J.-J.; Luo, Z.-Y. JA-mediated transcriptional regulation of secondary metabolism in medicinal plants. *Sci. Bull.* 2015, 60, 1062–1072. [CrossRef]

68. Yu, Z.; He, C.; da Silva, J.A.T.; Zhang, G.; Dong, W.; Luo, J.; Duan, J. Molecular cloning and functional analysis of DoUGE related to water-soluble polysaccharides from *Dendrobium officinale* with enhanced abiotic stress tolerance. *Plant Cell Tissue Organ. Cult.* 2017, 131, 579–599. [CrossRef]

69. Yuan, Y.; Tang, X.; Jia, Z.; Li, C.; Ma, J.; Zhang, J. The effects of ecological factors on the main medicinal components of *Dendrobium officinale* under different cultivation modes. *Forests* 2020, 11, 94. [CrossRef]

70. Diatloff, E.; Smith, F.W.; Asher, C.J. Rare earth elements and plant growth: III. Responses of corn and mungbean to low concentrations of cerium in dilute, continuously flowing nutrient solutions. *J. Plant Nutri.* 1995, 18, 1987–2003. [CrossRef]

71. Meehan, B.; Peverill, K.; Skroce, A. The impact of bioavailable rare earth elements in Australia agricultural soils. In Proceedings of the First Workshop on Soil and Plant Analysis, Ballarat, VIC, Australia, 2–4 March 1993; pp. 36–41.

72. Velasco, J.R.; Domingo, L.E.; Lansangan, A.S.; Sierra, Z.N. Cultural studies on coconut Cadang: Reaction of plants to the rare earths, thallium and certain soil samples. *Philipp. J. Coconut Stud.* 1979, 4, 1–13.

73. Abe, S.; Takeda, J. Effects of La3+ on surface charges, dielectrophoresis, and electrofusion of barley protoplasts. *Plant Physiol.* 1988, 87, 389–394. [CrossRef] [PubMed]

74. Obi, I.; Ichikawa, Y.; Kakutani, T.; Senda, M. Electrophoresis, zeta potential and surface charges of barley mesophyll protoplasts. *Plant Cell Physiol.* 1989, 30, 129–135. [CrossRef]