Agro Bali : Agricultural Journal
Vol. 9 No. 1: 132-145, March 2026
e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

Thiamine and Foliar Hyponex Improve Acclimatization of *Coelogyne rochusseni* Plantlets in an NFT System

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Article history: submitted: September 19, 2025; accepted: March 19, 2026; available online: April 9, 2026

Abstract. The *Coelogyne rochusseni* orchid, an endemic orchid from the island of Sumatra, has beauty and potential for development. The purpose of this research is to explain the physiological mechanisms in increasing the growth and adaptation of orchid plants at the acclimatization stage using the NFT hydroponic system by spraying thiamine and hyponex fertilizer. The design used in this study was a factorial complete randomized design (CRD) consisting of two factors, spraying thiamine and hyponex fertilizer, each treatment consisting of 4 treatment levels, thiamine 0, 1, 2, 3 mg L^{-1} and hyponex 0,1, 2, 3 mg L^{-1}. 48 total plantlets analyzed and 3 replicates. The results of the research on acclimatization of the *Coelogyne rachusseni* orchid plantlets can be concluded that spraying thyamin and hyponex fertilizer as a single factor significantly affected all growth parameters, plant height (cm), leaf length (cm), number of roots (n),percentage of plantlets life (%) with an optimal concentration of 2 mg L^{-1} and in terms of interaction factors significantly affected the parameter of Percentage of live plantlets with an optimal concentration being a combination of 2 mg L^{-1} thiamine and mg L^{-1} hyponex.

Keyword: ~~a~~cclimatization; adaptation mechanism of orchid; hydroponic; hyponex; thiamine

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1. Introduction

Orchids are plants that have a very high level of diversity. It is estimated that there are around 35,000 species identified worldwide, and 5,000 in Indonesia (Heriansyah et al. 2025). The high diversity of orchid species in Indonesia is a wealth of biodiversity, with potential natural resources that can be utilized for various human interests, one of which is the *Coelogyne rochusseni* orchid.

The *Coelogyne rochusseni* orchid has a very attractive beauty for every connoisseur of beauty. Its beauty and distinctive aroma make this flower attractive, so it is widely hunted in its habitat. In addition, threats come from the high rate of deforestation and climate change, which reduce the population of this orchid plant in nature (Heriansyah and Marlina 2021). To save this orchid, ex-situ conservation efforts are carried out through tissue culture. The most crucial stage in tissue culture is acclimatization, as environmental conditions can threaten the process's success.

Orchid acclimatization as the final stage of tissue culture propagation techniques has a high threat of failure due to lack of nutrient intake which was originally provided in full by temporary tissue culture media must be interrupted with limited environmental conditions (Ferreira et al. 2022), Research on the acclimatization of orchids in the Coelogyne Genus has been carried out by Nugroho, Raden, and Kartanegara (2021), and Utami et al. (2022). However, there has been no acclimatization study on *Coelogyne rochusseni* orchids. The acclimatization stage of this orchid is experiencing problems due to insufficient nutrient intake, as plant roots are not functioning properly. A strategic solution is to acclimate using the hydroponic NFT system.

The use of the hydroponic NFT system has problems, as the transfer from the in-vitro environment to the in-vivo environment causes high plant stress. To overcome this problem, you can spray thiamine, a vitamin that reduces plant stress levels caused by environmental displacement (Vahl et al. 2025). The application of this thiamine has only been applied to conventional plant acclimatization, as studied by Yustitia (2017), Noventa and Ramadiana (2014), but

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

it has not been studied using the hydroponic method.

Increasing plant resistance from post-transplant stress also causes plants to not optimally absorb nutrients through the roots, so it is necessary to add nutrients through spraying foliar fertilizers, one of which is hyponex. Hyponex fertilizer contains macro- and micro-nutrients, which plants can obtain through the stomata on their leaves. So that it will be able to support the nutrient needs of plants ahead of the optimal functioning of the roots (Hwang et al. 2024).

The purpose of this research is to explain the first hydroponic acclimatization study on this species *Coelogyne rochusseni*, and the first combined thiamine and hyponex foliar nutrient factorial.

2. Materials and Methods

Plant Materials and Treatments

This research was carried out at the Hydroponic House of the Agrotechnology Study Program, Faculty of Agriculture, Universitas Islam Kuantan Singingi, at 0.50794° S, 101.50544° E, and 25 meters above sea level, over 5 months from April to August 2022.

The tools used in this research include hydroponic installations (pipes, machines, boxes [reservoirs]), EC meters, washing containers, stationery, and hydroponic installation assembly equipment. The materials used in this study were plantlets ready for acclimatization from the BBI Pekanbaru Tissue Culture Laboratory, AB-Mix solution, Thiamine, hyponex fertilizer, rockwoll, fungicide, UV plastic, and other washing materials. The orchid plantlets used were 1 year old, the average temperature in the greenhouse was 25-29^{0}C, with 80% lighting shade, the EC of the NFT solution was 500 and 1000 ppm, the shelves used were pipes, and the planting medium used was fern roots.

The experiment was conducted using a Nutrient Film Technique (NFT) hydroponic system. Each NFT channel consisted of PVC pipes with an inner diameter of 5 cm and a length of 150 cm, installed at a 2% slope. A submersible pump delivered the nutrient solution at a constant flow rate of 1.5 L min^{-1} per channel, which is within the recommended operational range for NFT systems.

A 60-L nutrient reservoir was used, and the solution was replaced every 7 days. The nutrient solution was maintained at pH 5.8–6.2, and EC 1.5–2.0 mS cm^{-1}, and its pH and EC were monitored twice daily using a calibrated pH/EC meter. Greenhouse environmental conditions were maintained at 26 ± 2 °C, 65–75% RH, and a PPFD of 300 ± 20 µmol m^{-2} s^{-1} under a 16 h light/8 h dark photoperiod. Plants were rooted in fern root medium (70% organic fiber, 20% mineral particles, 10% coarse fragments) to ensure adequate aeration and moisture retention.

A 4 × 4 factorial design was implemented using four concentrations of Thiamine (0, 1, 2, and 3 mg L^{-1}) and four concentrations of green Hyponex fertilizer (0, 1, 2, and 3 mg L^{-1}). These levels reflect common low-dose foliar and nutrient supplementation ranges for early seedling and vegetative growth.

The experimental unit was defined as one plant, with five biological replicates per treatment combination, resulting in 4 × 4 × 5 = 80 plants in total. All plants were arranged in a randomized complete design within the NFT channels (Table 1).

Foliar spray solutions were prepared according to the concentrations listed in the treatment matrix. Each plant received 20 mL per application applied with a fine-mist sprayer. A 0.1% (v/v) Tween-20 surfactant was added to enhance leaf wetting.

Sprays were applied until just before runoff, ensuring uniform coverage of both leaf surfaces. Foliar applications were conducted twice weekly (every 3–4 days), starting 7 days after transplanting. All applications were performed after 16:00 to reduce evaporation and light-induced degradation.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

Table 1. Complete treatment matrix (Thiamine × Hyponex Green)

Treatment ID	Thiamine (mg L^{-1})	Hyponex Green (mg L^{-1})	Biological replicates	Experimental unit
T1	0	0	5	1 plant
T2	0	1	5	1 plant
T3	0	2	5	1 plant
T4	0	3	5	1 plant
T5	1	0	5	1 plant
T6	1	1	5	1 plant
T7	1	2	5	1 plant
T8	1	3	5	1 plant
T9	2	0	5	1 plant
T10	2	1	5	1 plant
T11	2	2	5	1 plant
T12	2	3	5	1 plant
T13	3	0	5	1 plant
T14	3	1	5	1 plant
T15	3	2	5	1 plant
T16	3	3	5	1 plant

Observation of Plant Growth and Adaptability

Plant height (cm) was measured by removing the plants from the netpot and recording the distance from the root neck to the tallest leaves with a ruler. Plant height was measured at the end of the study, which was 12 weeks after planting

Leaf length (cm) was measured by measuring plant leaves with a ruler, starting from the base to the tip. The length of plant leaves was measured at the end of the study, which was 12 weeks after planting.

The number of roots (n) was determined by removing the plants from the net pot containing the medium, then cleaning the remaining medium to facilitate counting the roots. The number of roots was monitored 12 weeks after planting.

Percentage of plantlets life (%), Observation of the percentage of life plantlets was observed from the beginning of the planting period to the end, the results of the percentage of life were totaled at the end of the study, dead plants were categorized by the characteristics of the leaves, bulbs, and roots of plants that had turned brown and withered.

This study was an experimental study using a factorial completely randomized design (CRD) consisting of two factors, namely the addition of thiamine and green hyponex fertilizer, each treatment consisting of 4 treatment levels, Thiamine 0, 1, 2, 3 mg L^{-1}, and the addition of green Hyponex fertilizer, 0.1, 2.3 mg L^{-1}. The total study comprised 16 research units with 3 replications, each unit consisting of 4 plants, of which 3 were sample plants; 192 plants were obtained.

Statistical Analysis

A two-way ANOVA with interaction was used to evaluate the effects of Thiamine, Hyponex, and their interaction. Normality of residuals was tested using the Shapiro–Wilk test, and homogeneity of variances using Levene's test. When necessary, data were transformed (log or square-root) to meet assumptions.

ANOVA results included F-values, p-values, partial η^2, and 95% confidence intervals. Significant differences ($p < 0.05$) were further analyzed using Tukey's HSD. Analyses were conducted in R version 4.3.1 using aov() and shapiro.test(),

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

car::leveneTest(), and means for post-hoc tests. Outputs were cross-validated in SPSS version 28.

3. Results and Discussion

The results of observations on the increase in plantlet growth of *coelogyne rochusseni* orchids, especially plant height variables by spraying thiamine and hyponex fertilizer, showed a significant increase based on ANOVA analysis, as a single factor showed a significant increase, but had no significant effect on the interaction between thiamine and hyponex, as shown in Table 1. Spraying thiamine as a single factor resulted in a significant increase in plantlet height, with the treatment showing the highest yield of 2 mg L^{-1} with a plantlet height of 6.34 cm. Then spraying hyponex fertilizer as a single factor showed a significant effect on increasing plantlet height with the best treatment at a concentration of 2 mg L^{-1}, with a plantlet height of 5.73 cm.

The results of observations on the increase in the growth of *coelogyne rochusseni* orchid plantlets, especially the leaf length variable by spraying thiamine and hyponex fertilizer, showed a significant increase based on ANOVA analysis, as a single factor showed a significant increase, but had no significant effect on the interaction between thiamine and hyponex, as shown in Table 1. Spraying thiamine as a single factor resulted in a significant increase in plantlet leaf length with the treatment showing the longest yield of 2 mg L^{-1} with a plantlet leaf length of 5.22 cm. Then spraying hyponex fertilizer as a single factor showed a significant effect on increasing the length of plantlet leaves with the best treatment at a concentration of 2 mg L^{-1}, with a length of 4.69 cm plantlet leaves.

The results of observations on the increase in the growth of coelogyne rochusseni orchid plantlets, especially the root number variable by spraying thiamine and hyponex fertilizer, showed a significant increase based on ANOVA analysis, as a single factor showed a significant increase, but had no significant effect on the interaction between thiamine and hyponex, as shown in Table 1. Spraying thiamine as a single factor resulted in a significant increase in the number of plantlet roots, with the treatment showing the highest yield of 2 mg L^{-1} with a total of 5.50 cm plantlet roots. Then spraying hyponex fertilizer as a single factor showed a significant effect on increasing the number of plantlet roots with the best treatment at a concentration of 2 mg L^{-1}, with several plantlet roots of 4.88 cm

The results of observations on the increase in adaptation of coelogyne rochusseni orchid plantlets showed a significant effect both as a single factor and as an interaction. Based on the results of the ANOVA analysis, spraying thiamine as a single factor increased plantlet adaptation at the best concentration of 2 mg L-1, with a plantlet survival percentage of 97.22%. Then spraying hyponex fertilizer as a single factor was able to significantly increase plantlet adaptation, but after being tested by a 5% tukey, the results were not significant between treatments. Furthermore, the interaction of spraying thiamine and hyponex showed a significant increase in plantlet adaptation with the best concentrations at 2 mg L^{-1} thiamine and 2 mg L^{-1} hyponex, resulting in a plantlet survival percentage of 98.55%, as shown in Table 2.

Plant height

The increase in the growth of *Coelogyne rochusseni* orchid plantlets at the acclimatization stage was strongly influenced by the plant's ability to respond to environmental stress after transfer from in-vitro conditions to an in-vivo environment (Khai et al. 2022). Environmental stress conditions induce plants to synthesize secondary metabolites, which are later used in plant defense mechanisms. This causes changes in assimilate allocation that affect growth (Jha, Nayyar, and Siddique 2022). Another physiological problem experienced by plantlets is the rate of transpiration which

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

causes plantlets to lose water due to changes in temperature and humidity in the environment (Limousin et al. 2022), to overcome this, plantlets need vitamins, especially thiamine, which plays a role in reducing plant stress so that the rate of transpiration and changes in assimilate allocation can be reduced (Jabeen et al. 2022).

Table 2. Different traits of thiamine and hyponex concentrations as affected by four levels to growth *coelogyne rochusseni* orchid plantlets

Treatment	Plant Height (cm)	Leaf length (cm)	Number of roots (n)	Percentage of plantlets life (%)
Thiamine				
0 mg L^{-1}	4.35 ± 0.29 d	3.52 ± 0.19 d	3.66 ± 0.19 c	90.80 ± 0.43 d
1 mg L^{-1}	5.51 ± 0.28 c	4.19 ± 0.20 c	4.36 ± 0.33 b	92.83 ± 0.78 c
2 mg L^{-1}	6.34 ± 0.48 a *	5.22 ± 0.34 a *	5.50 ± 0.23 a *	97.22 ± 0.97 a *
3 mg L^{-1}	4.98 ± 0.30 b	3.58 ± 0.19 b	3.69 ± 0. 19c	93.25 ± 1.15 b
Hiponex				
0 mg L^{-1}	5.00 ± 0.80 d	3.55 ± 0.19 c	3.58 ± 0.19 c	93.33 ± 2.59 a
1 mg L^{-1}	5.29 ± 0.88 b	3.97 ± 0.28 b	4.27 ± 0.27 b	93.69 ± 2.85 a
2 mg L^{-1}	5.73 ± 0.97 a *	4.69 ± 0.35 a *	4.88 ± 0.26 a *	93.66 ± 3.40 a *
3 mg L^{-1}	5.17 ± 0.75 c	4.30 ± 0.35 c	4.47 ± 0.25 b	93.41 ± 2.31 a
Thiamine + Hyponex				
0 mg L^{-1} + 0 mg L^{-1}	4.06 ± 0.01 ns	3.00 ± 0.1 ns	3.00 ± 0.01 ns	90.44 ± 0.19 h
0 mg L^{-1} + 1 mg L^{-1}	4.2 ± 0.03 ns	3.33 ± 0.01 ns	3.66 ± 0.01 ns	90.66 ± 0.00 h
0 mg L^{-1} + 2 mg L^{-1}	4.73 ± 0.05 ns	4.00 ± 0.02 ns	4.22 ± 0.02 ns	90.66 ± 0.00 h
0 mg L^{-1} + 3 mg L^{-1}	4.42 ± 0.06 ns	3.78 ± 0.01 ns	3.77 ± 0.02 ns	91.44 ± 0.19 h
1 mg L^{-1} + 0 mg L^{-1}	5.13 ± 0.03 ns	3.33 ± 0.01 ns	3.33 ± 0.02 ns	92.33 ± 0.00 f
1 mg L^{-1} + 1 mg L^{-1}	5.51 ± 0.05 ns	3.89 ± 0.01 ns	4.33 ± 0.02 ns	92.44 ± 0.19 f
1 mg L^{-1} + 2 mg L^{-1}	5.81 ± 0.18 ns	4.89 ± 0.02 ns	5.00 ± 0.00 ns	92.55 ± 0.38 e
1 mg L^{-1} + 3 mg L^{-1}	5.6 ± 0.03 ns	4.67 ± 0.02 ns	4.77 ± 0.02 ns	94.00 ± 0.33 d
2 mg L^{-1} + 0 mg L^{-1}	6.00 ± 0.00 ns	4.67 ± 0.02 ns	4.77 ± 0.02 ns	96.55 ± 0.19 c
2 mg L^{-1} + 1 mg L^{-1}	6.32 ± 0.13 ns	5.22 ± 0.04 ns	5.55 ± 0.03 ns	97.33 ± 0.00 b
2 mg L^{-1} + 2 mg L^{-1}	7.04 ± 0.05 ns	5.89 ± 0.04 ns	6.22 ± 0.04 ns	98.55 ± 0.19 a *
2 mg L^{-1} + 3 mg L^{-1}	6.01 ± 0.01 ns	5.11 ± 0.04 ns	5.44 ± 0.03 ns	96.44 ± 0.19 c
3 mg L^{-1} + 0 mg L^{-1}	4.82 ± 0.13 ns	3.22 ± 0.01 ns	3.22 ± 0.01 ns	94.00 ± 0.57 d
3 mg L^{-1} + 1 mg L^{-1}	5.11 ± 0.09 ns	3.44 ± 0.01 ns	3.55 ± 0.01 ns	94.33 ± 0.33 d
3 mg L^{-1} + 2 mg L^{-1}	5.34 ± 0.01 ns	4.00 ± 0.00 ns	4.11 ± 0.02 ns	92.88 ± 0.19 e
3 mg L^{-1} + 3 mg L^{-1}	4.64 ± 0.06 ns	3.67 ± 0.01 ns	3.88 ± 0.01 s	91.77 ± 0.19 g

The column values with the same letters are statistical similar according to Tukey Test at $p < 0.05$

As shown in Figure 1, that thiamine at a concentration of 2 mg L^{-1} was able to increase plantlet height. This condition demonstrates that spraying thiamine is effective in helping plants control transpiration and changes in assimilate allocation under environmental stress (De Stefano et al. 2022). The results of this study are different from the results of research conducted by Latif, Syahbudin, and Siti,

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

(2020) which stated that giving thiamine at concentrations of 1.2, 3 and 4.5 mg L^{-1} had no effect on acclimatized growth of Dendrobium sp orchid plantlets, while in this study giving 2 mg L^{-1} of thiamine had an effect on increasing plantlet growth, this due to differences in orchid species, so they have different responses.

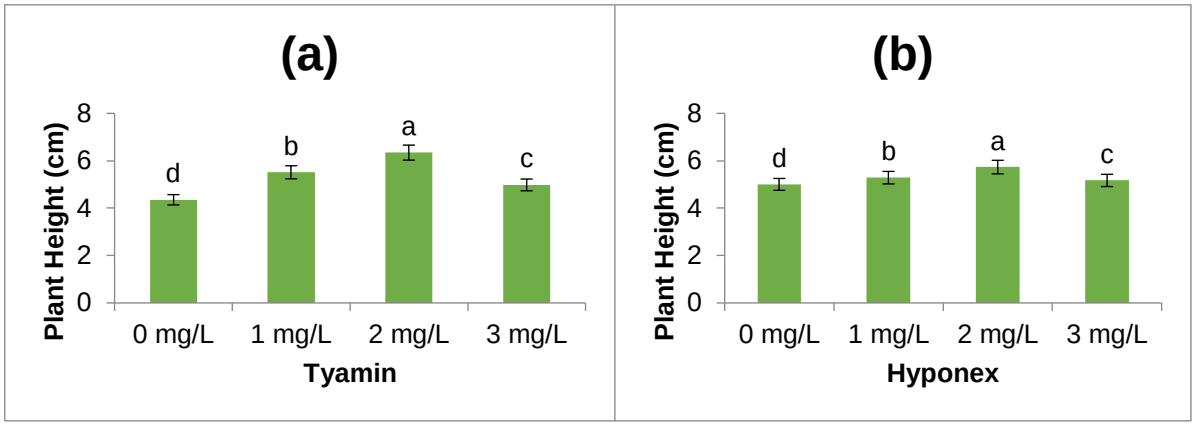

Figure 1. (a) Increase in plantlet height growth by spraying thiamine **(b)** Increase in plantlet height growth by spraying hyponex

Optimization of *Coelogyne rochusseni* orchid plantlet growth during the acclimatization stage is also influenced by nutrient availability. The availability of these nutrients is a very important factor in supporting plant growth, especially plant height (Rianawati, Rahardjo, and Musalamah 2022). All essential nutrients affect plantlet height growth, especially the elements Nitrogen, Potassium, and Phosphorus; these three elements are macro elements needed in large quantities by plantlets. The roles of these three elements differ based on the plant's physiological system (Ferreira et al. 2022). Nitrogen increases cell permeability and is a basic ingredient that forms proteins in plantlets and affects chlorophyll formation in plants (Panjama et al. 2022). Potassium is a coenzyme that is used in optimizing enzymatic activity and used in optimizing absorption of other nutrients (Puri, Heriansyah, and Nopsagiarti 2022), while phosphorus is a nutrient that is a source of energy and is useful in the generative phase and formation of roots. Based on this study, spraying hyponex fertilizer containing N, P, and K increased plantlet growth during the acclimatization stage, as shown in Figure 1b.

Leaf Length (cm)

The growth of leaf length as a result of a physiological process in the form of a result of the process of photosynthesis (Fu et al. 2022). Leaves are organs where photosynthesis takes place, so it is very necessary for this organ to experience optimal growth (Orlov, Viktorova, and Eskov 2022). Optimization of leaf length gain is influenced by plant physiological conditions, especially plant stress conditions, plants that experience environmental changes from in-vitro conditions to in-vivo conditions (Taticharoen et al. 2023). Plant stress makes the process of photosynthesis experience obstacles, because there are changes in plant metabolism that lead to the formation of secondary metabolites as a plant defense mechanism, to neutralize and suppressing the process of formation of plantlet secondary metabolites requires thiamine to stimulate them again so that plants can maintain their physiological processes normally again (Bibi et al. 2022). This is in accordance with the results of the study shown in Figure 2b that the concentration of 2 mg L^{-1} thiamine is able to maintain the physiological conditions of plants in the normal phase, so that the growth of leaf length runs optimally.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

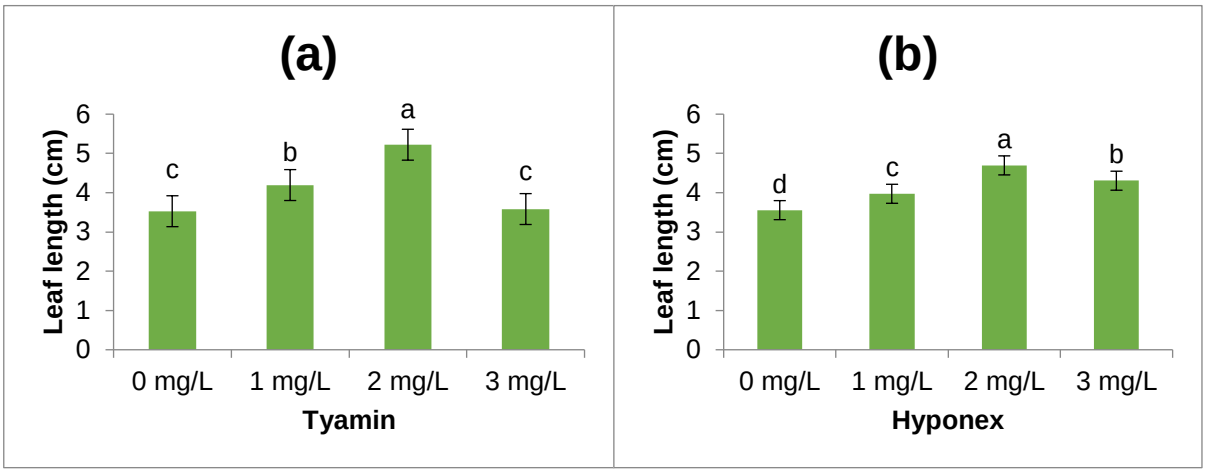

Figure 2. (a) Increase in plantlet leaf length growth by spraying thiamine **(b)** Increase plantlet leaf length growth by spraying hyponex fertilizer

Leaf length growth is influenced by nutrient availability, a basic requirement for photosynthesis. Plants in the acclimatization phase have difficulty absorbing nutrients through the roots because the roots have not yet reached full growth. (Mikavica et al. 2022). This condition will cause the plant to become nutrient-deficient, especially in macroelements. These elements will affect the growth of plant leaves, especially nitrogen nutrients. Nitrogen promotes plant metabolism, especially photosynthesis and chlorophyll formation, and increases cell permeability, enabling the perfect distribution of nutrients between cells. Spraying nutrients onto leaf stomata will accelerate nitrogen absorption, enabling plants to grow optimally, especially during leaf elongation (Panjama et al. 2022). This is in accordance with the results of this study in Figure 2b. The administration of 2 mg L^{-1} showed an increase in the growth of plantlet leaf length.

Number of Roots (n)

Orchid plant roots are essential for orchid growth. The presence of roots will cause plants to undergo physiological changes and to carry out cell division and other metabolic activities (de Lima and Moreira 2022). For optimal nutrient absorption, plants must have sufficient roots. Roots are organs responsible for supporting plant growth, but when acclimatized, they experience stress, which eventually causes them to degrade functionally and no longer function as they should. (Maleva et al. 2022). Environmental stress during acclimatization causes changes in the allocation of water and plant nutrients, and forces plants to release secondary metabolites to maintain physiological conditions to remain stable. Thiamin, a vitamin that stimulates plants' physiological mechanisms to maintain plant conditions, remains stable (Fauziah, Setiari, and Saptiningsih 2022). This is in accordance with the results of this study in Figure 3a. Spraying thiamine increased the number of roots in orchid plants at an optimal concentration of 2 mg L^{-1}.

The ability of roots to regenerate is strongly influenced by the presence of nutrients in plant organs, which is the result of assimilate distribution distributed to all plant organs, this results in an increase in the plant's need for nutrient intake available both in the media and in the air (Kasutjianingati, Koesparwanti, and Eliyatiningsih 2022). The growth and increase in the number of plant roots are closely related to the element phosphorus, which functions to stimulate root formation. The existence of this phosphorus in plant organs can be taken from the media or absorbed from the air in the form of fertilization through the leaves. Giving hyponex fertilizer containing phosphorus

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

fertilizer can increase the number of roots of orchid plants (Haryuni et al. 2022). This is because spraying 2 mg L^{-1} is able to provide phosphorus, which increases the number of roots, and the appropriate concentration of phosphorus can increase the number of plant roots.

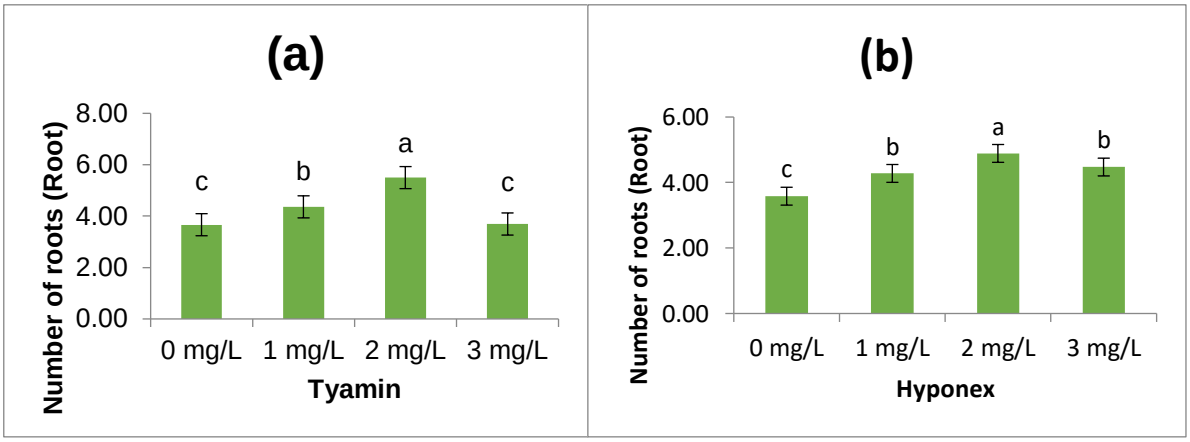

Figure 3. (a) Increasing the growth of the number of plantlet roots by spraying thiamine, **(b)** Increasing the growth of the number of plantlet roots by spraying hyponex fertilizer

Percentage of plantlets life (%)

The acclimatization stage is a critical phase for plants due to changing environmental conditions (Coopman and Kane 2018). If environmental changes cannot be neutralized by plants, then the plant will be nutrient-deficient and lose water, metabolic activity will decrease, and the plant will die (Kunakhonnuruk, Inthima, and Kongbangkerd, 2018). Vitamins are needed by plants to stimulate plants to maintain stability in plant cells to have enough water to continue metabolic processes. Vitamins, especially thiamine, accelerate the ability of plants by stimulating the work of enzymes and optimizing nutrient absorption, and plants easily get through environmental stress and can adapt (Sherif et al. , 2018). This was shown by spraying thiamine with at 2 mg L^{-1}, which was able to increase the percentage of plantlet survival. This is because the thiamine sprayed on the plantlets stimulates the plant's defenses, as shown in Figure 4a.

The percentage of life of plantlets is influenced by the availability of nutrients, as roots may not absorb nutrients effectively, posing a threat of death due to a lack of nutrients needed to carry out metabolic processes (Zhang, Zhang, and Fan 2022). Spraying hyponex fertilizer is able to provide nutrients for the orchid plantlets, because it is given through the plant's stomata so that the nutrients are still available even though the roots are not functioning perfectly. Spraying hyponex 2 g L^{-1} is the optimal concentration in increasing the percentage of plantlet life, because at high concentrations it can result in a decrease in the percentage of plantlet life, due to excess concentration which can damage stomata and cells on leaves.

The response of orchid plantlets to spraying hyponex and thiamine showed an effect of increasing the percentage of plantlet life with the best concentration at 2 mg L^{-1} thiamine combined with 2 mg L^{-1} hyponex, this was due to the content of thiamine and nutrients in hyponex fertilizer causing orchid plantlets to be able to adapt to environmental conditions during acclimatization (Febriyani et al. 2019). As well as in the hydroponic system the NFT system is able to support the availability of water for plants so as to increase the percentage of plantlet life.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

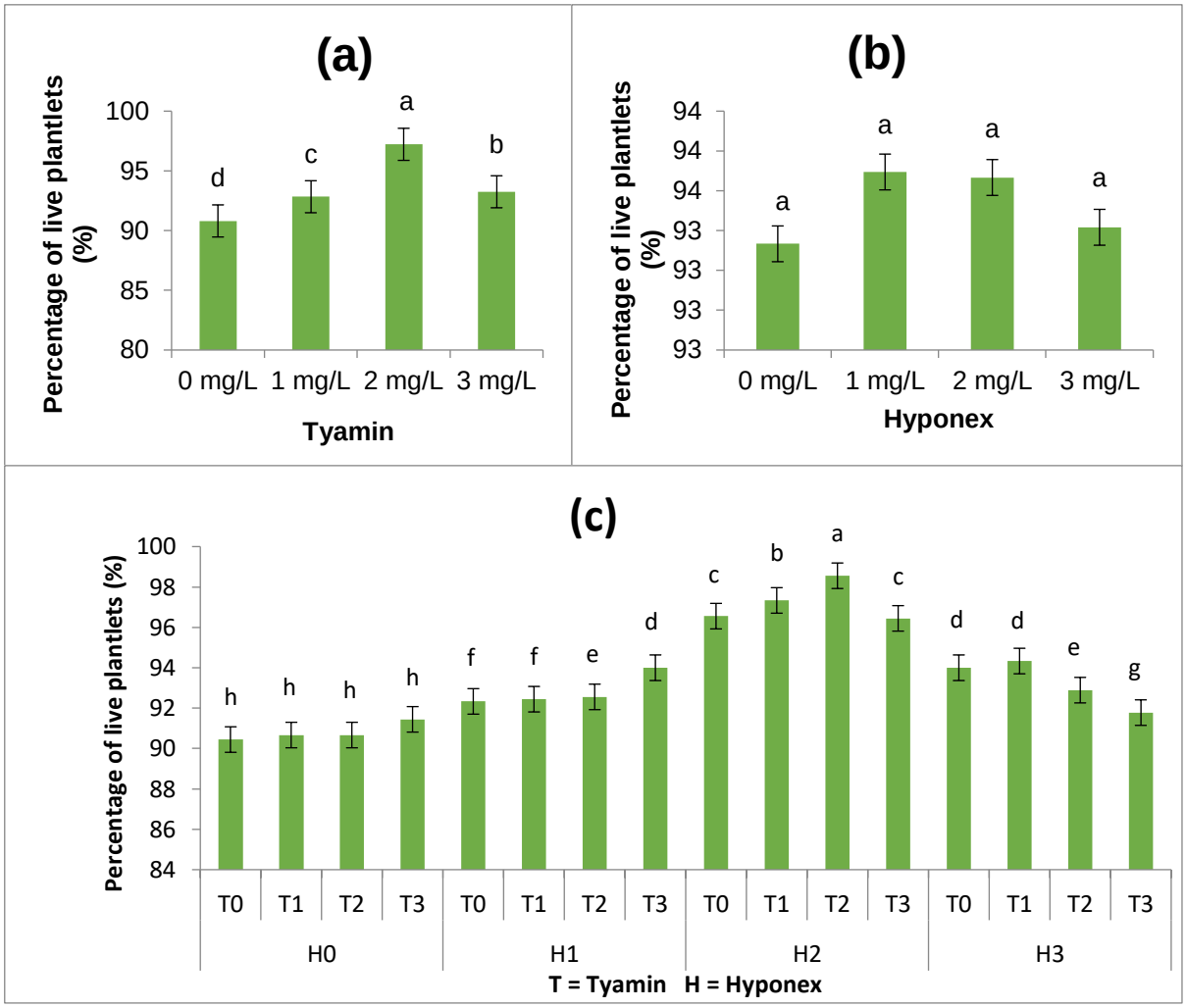

Figure 4. (a) Increasing the percentage of plantlet life by spraying thiamine, (b) Increasing the percentage of life of plantlets by spraying hyponex fertilizer, (c) Increasing the life percentage of plantlet explants by spraying thiamine and hyponex fertilizers.

Pearson's correlations between Thiamine and Hyponex

Thiamine and Hyponex fertilizer showed a strong positive correlation with all growth parameters. The highest correlations were found in root number (r = 0.75), leaf length (r = 0.76), plant height (r = 0.98), and plant survival rate (r = 0.68). These results indicate that nutrients and thiamine work together to support plant growth. Physiologically, plants often experience stress during the transition from in vitro to in vivo environments. During this process, thiamine plays a key role by signaling to help the plant absorb nutrients and maintain physiological balance, thereby reducing the risk of wilting and supporting successful adaptation (Figure 5).

Thiamine serves as a vital cofactor in the form of thiamine pyrophosphate (TPP), which plays a critical role in carbohydrate and amino acid metabolism (Bashir et al. 2023). It facilitates the decarboxylation of pyruvate into acetyl-CoA, a key step in cellular respiration and the utilization of photosynthetically derived energy, a process that also requires phosphorus (Li et al. 2022). Thiamine contributes to nutrient transport and enhances nutrient use efficiency. In thiamine-deficient plants, nitrogen utilization may be significantly impaired. Furthermore, thiamine has been shown to improve stress tolerance by stimulating antioxidant production and maintaining nutrient homeostasis (Mahmoud, Hefzy, and Zahran 2024). The combined application of thiamine

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

with essential nutrients such as calcium and magnesium can strengthen cell walls and mitigate stress-induced damage. Additionally, phosphorus (crucial for ATP synthesis) and potassium (essential for maintaining turgor pressure and activating enzymes) act synergistically with thiamine to promote vegetative growth and organ development in plants (Atif et al. 2024).

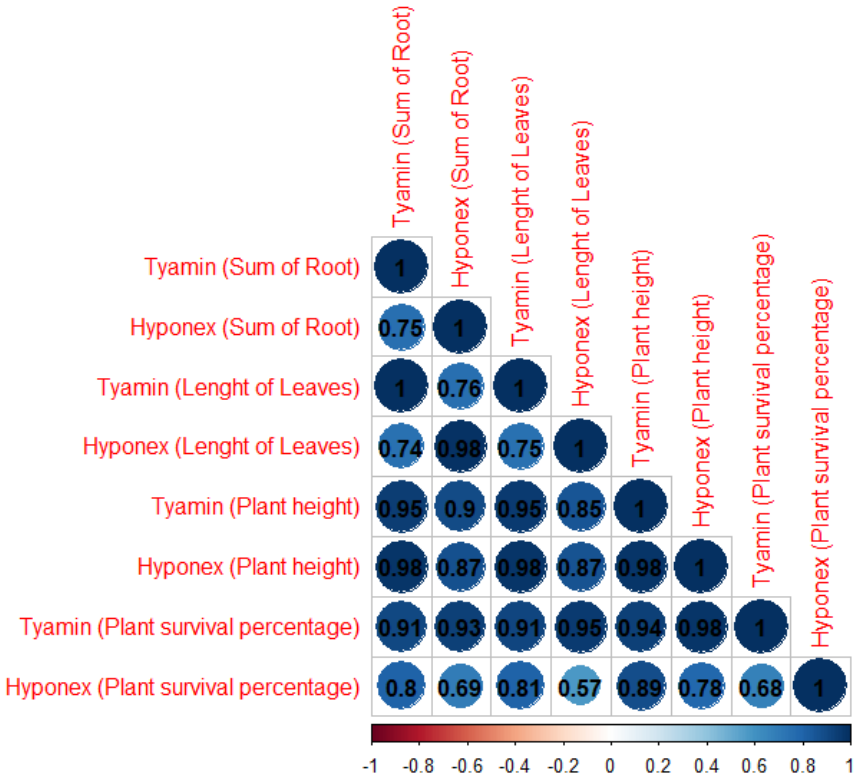

Figure 5. Pearson's correlations between Thiamine and Hyponex

4. Limitations and Future Directions

This study has several limitations that should be acknowledged. One of the primary limitations is the relatively small sample size used in the experiment, which may restrict the generalizability of the findings. A limited number of samples may not fully represent broader environmental conditions or plant responses. Therefore, the results of this study should be interpreted with caution when applied to wider contexts. Future research is recommended to involve a larger sample size to enhance the reliability and robustness of the findings. Additionally, further studies are encouraged to investigate the effects of a wider range of foliar fertilizers to obtain more comprehensive insights into their influence on plant growth and development. Expanding the range of foliar fertilizer treatments may contribute to a deeper understanding of the most effective fertilization strategies.

5. Conclusion

Based on the experimental results, the application of thiamine and Hyponex Green fertilizer significantly influenced the acclimatization of *Coelogyne rachusseni* orchid plantlets. As single factors, both treatments significantly affected the observed growth parameters, with the best response obtained at a concentration of 2 mg L^{-1}. A significant interaction between thiamine and Hyponex Green fertilizer was observed in plantlet survival, with the highest survival achieved at 2 mg L^{-1} thiamine and 2 mg L^{-1} Hyponex Green. From a practical perspective, the use of 2 mg L^{-1} thiamine, combined with 2 mg L^{-1} Hyponex Green

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v9i1.2537

fertilizer, is recommended to support short-term acclimatization of *Coelogyne rachusseni* plantlets under controlled greenhouse conditions. However, these findings should be interpreted cautiously because the results are limited to a single species and specific experimental conditions.

Future studies should validate these results through larger-scale nursery trials and multi-location experiments. Further research is also recommended to evaluate biochemical or physiological indicators of plantlet adaptation, monitor longer-term performance, including post-transplant survival and flowering, and assess the economic feasibility of the fertilization strategy.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used ChatGPT as an AI-assisted language tool to improve the clarity, grammar, and readability of the text. The AI tool was used solely for language editing and not for generating scientific content, data analysis, or interpreting results. All scientific content, analyses, and conclusions were developed by the authors. After using this tool, the authors carefully reviewed and edited the manuscript and take full responsibility for the final content of the publication.

Authorship Contribution Statement

Elfi Indrawanis: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing - original draft, visualization; Pebra Heriansyah: conceptualization, methodology, writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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