

Optimization of vanilla (*Vanilla planifolia* Andrews) growth with auxin and *Trichoderma harzianum* combination treatment

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Abstract

Vanilla is a type of spice plant with high economic value. The availability of healthy vanilla seedlings is now one of the conditions for successful large-scale vanilla cultivation. Conventional cultivation of vanilla generally uses a vegetative method (cuttings), in which the growth of roots and shoots is slow, so it needs to be accelerated. Application of the combination of plant growth regulator and biological inoculant is expected to stimulate root growth and development. This study used a completely randomized design (CRD) with two factors, namely auxin, which consisted of 4 concentration levels, namely: A0 (0 ppm), A1 (50 ppm), A2 (100 ppm), A3 (150 ppm); and *T. harzianum* which consisted of 4 concentration levels, namely: T0 (0 g), T1 (2 g), T2 (4 g), and T3 (6 g). Data analysis used quantitative methods. The results showed that there was no interaction between *T. harzianum* and auxin. The addition of *T. harzianum* could increase the vegetative growth of vanilla cuttings in the parameters of shoot growth time, plant height, number of leaves, fresh weight, and root length while the application of auxin affects the increased in the number of roots of vanilla cuttings.

Key words: *Vanilla planifolia*, root, auxin, *Trichoderma harzianum*

Introduction

Vanilla (*Vanilla planifolia*) is a spice plant with high economic value as an export commodity. Vanilla cultivation in Indonesia is mostly developed in West Java, East Java, North Sumatra, Lampung, Bali, West Nusa Tenggara, East Nusa Tenggara, South Sulawesi, North Sulawesi, Central Sulawesi, and to a lesser extent Papua (Wulandari, 2021). Vanilla exports in Indonesia for the last 4 years have increased from 217,8 tons in 2019 to 395,2 tons in 2022 (BPS, 2020; BPS, 2023).

The problem with vanilla cultivation in Indonesia is that productivity and quality are still low. Vanilla quality is affected by disease, harvest age, pod length, and post-harvest processing. Stem rot disease, caused by the fungus *Fusarium oxysporum* f. sp. *vanillae*, is a major obstacle in vanilla cultivation in Indonesia (Pinaría *et al.*, 2010; Wulandari, 2021). Poor cultivation techniques, such as a lack of pre-treatment during vegetative propagation, also contribute to a loss in vanilla yield, as it takes a long time for shoots from vanilla plant cuttings to mature in nurseries. Vegetative planting faces numerous challenges, including the amount of time required for root and shoot growth from cuttings (Wiriyana *et al.*, 2021). Therefore, it is necessary to use growth regulators to stimulate the growth of cuttings, both root and shoot growth. Auxin is a plant hormone that plays important role in plant growth. Auxin functions as a stimulator of cell division, which improves the root system so that plants can increase the absorption of nutrients into plant cells, it stimulates the elongation of plant cells thereby promoting stem elongation (Immanen *et al.*, 2016; Jamil *et al.*, 2021).

Endophytic fungi such as *Trichoderma harzianum* can increase vanilla plant resistance and growth (Mehetre and Mukherjee, 2015). *Trichoderma* produces harzionolide secondary metabolites,

mycotoxin antibiotics, and viridian, which can protect plants from disease attack and can function as growth promoters in plants (Cai *et al.*, 2013; Singh *et al.*, 2019). *T. harzianum* also increases the absorption of minerals and nutrients from the soil, thereby increasing the availability and absorption of nutrients for plants (Singh *et al.*, 2014). Growth regulators in vegetative propagation of plant cuttings can stimulate growth. In addition, the application of endophytic fungi can increase growth and disease resistance (Mehetre and Mukherjee, 2015). This study aims to analyze the effect of the combination of auxin and *Trichoderma harzianum* on optimizing the growth of vanilla (*Vanilla planifolia* Andrews) cuttings.

Materials and methods

Experimental design: A Completely Randomized Design (CRD) was employed, incorporating two factors: auxin (Clonex) at four concentration levels (A0: 0 ppm, A1: 50 ppm, A2: 100 ppm, A3: 150 ppm) and *Trichoderma harzianum* at four concentration levels (T0: 0 g, T1: 2 g, T2: 4 g, T3: 6 g). Each treatment level was replicated four times.

Planting and treatment: The research was conducted in a place shaded from the rain with a light intensity of 30-50%. Vanilla cuttings were obtained from 2-year-old broodstock. Vanilla cuttings were dipped for 5 minutes in auxin solution (Clonex) with four different concentrations: A0: 0 ppm, A1: 50 ppm, A2: 100 ppm, A3: 150 ppm for 5 minutes (Abha Manohar *et al.*, 2022). Cuttings were then planted in polybags with a size of 20x20 cm into the planting medium. After one week, *T. harzianum* was added to the media by making a hole beside the vanilla plant according to the treatment. Watering was done in the morning, and weeding was done when non-target weeds were found growing in the treatment polybags. Vanilla harvesting was done 90 days

after planting (DAP). Vegetative growth data collection is carried out every six days using quantitative data collection techniques for 90 days. Data collection on photosynthetic pigments was done after 90 DAP using quantitative data collection techniques.

Data analysis: Quantitative data obtained from this study were analyzed using analysis of variance (Two Way ANOVA) to determine the effect of treatment. Duncan's Multiple Range Test (DMRT) was used to determine significant differences between treatments at the 95% confidence level.

Results

Vegetative growth: After 90 days of the experiment, the ANOVA results indicated that there was no significant interaction ($P > 0.05$) between the application of auxin and *T. harzianum* concerning parameters such as shoot growth time, plant height, number of leaves, fresh weight, root length, and number of roots (Table 1 and 2).

Shoot growing time: The time for vanilla shoots to grow after applying *T. harzianum* ranged from 32.38-50.25 days. The growth time of shoots of the T0 and T3 treatments differed significantly from the T1 and T2 treatments, while the T0 and T3 did not show a significant difference (Table 1).

Plant height: After applying the *T. harzianum*, the vanilla plant height ranged from 4.55-6.04 cm. The T0 and the T1 treatments were not significantly different. The highest plant height was recorded in T2 and the lowest in T3 (Table 1). The highest plant height were obtained in the treatment of T2 and the lowest in T3 (Table 1).

Number of leaves: The highest number of leaves was obtained from T2. The lowest number of leaves was obtained from T3 and T0. The average number of leaves of T2 was significantly different from T3, while T0 and T1 were not significantly different (Table 1).

Fresh weight: The fresh weight of the T1 treatment showed a significant difference compared to T0. Additionally, the fresh weight of the T1 treatment significantly differed from both T2 and T3. Notably, the highest fresh weight of 18.48 g was achieved with the T1 treatment, contributing to an increase in vanilla's fresh weight (Table 1).

Root length: The root length of vanilla after addition of *T. harzianum* ranged from 19.5-31.17 cm. The highest root length

Table 1. Vegetative growth of vanilla 90 days after planting (DAP)

Treatment	Shoot growth time (days)	Plant height (cm)	Number of leaves	Fresh weight (g)	Root length (cm)	Number of roots
<i>Auxin</i>						
A ₀	38.31	5.70	1.67	16.29	25.63	3.67 ^a
A ₁	42.94	5.13	1.52	13.59	23.80	3.33 ^a
A ₂	41.38	5.37	1.65	15.46	24.30	3.17 ^a
A ₃	40.50	5.39	1.60	14.74	27.90	1.50 ^b
<i>T. harzianum</i>						
T ₀	50.25 ^a	5.13 ^{bc}	1.49 ^{bc}	13.67 ^b	31.17 ^a	2.58
T ₁	33.00 ^b	5.86 ^{ab}	1.76 ^{ab}	18.48 ^a	26.50 ^{ab}	2.75
T ₂	32.38 ^b	6.04 ^a	1.84 ^a	14.47 ^b	19.50 ^b	3.41
T ₃	47.50 ^a	4.55 ^c	1.34 ^c	13.48 ^b	24.42 ^{ab}	2.92

Note: Numbers followed by different letters for the same parameter show a significant difference from Duncan's test at the 95% level.



Fig. 1. Vanilla cuttings 90 days after planting (DAP).

was recorded in T0 and the lowest in treatment T2. Root length in treatments T1 and T3 were not significantly different from T0. Root length in treatment T0 was significantly different from T2 (Table 1).

Number of roots: The growth rate of vanilla roots after auxin addition ranged from 1.5-3.67 strands. The higher number of roots were obtained from the treatment of A0, A1, and A2. The growth of roots in the treatment of A0, A1, and A2 were significantly different from the A3 treatment (Table 1).

Photosynthetic pigments content: The statistical analysis of chlorophyll a, chlorophyll b, and total chlorophyll content in vanilla leaves at 90 days after planting (DAP) indicates that the differences among the various treatments were not significant ($P > 0.05$). This suggests that the different combinations of treatments (A and T) do not substantially affect the chlorophyll content in the vanilla leaves. Specifically, chlorophyll a values ranged from 1.99 in treatment A2T3 to 2.83 in treatment A0T2, with a mean value of 2.53. Chlorophyll b values varied from 0.78 in treatment A0T3 to 1.13 in treatment A1T2, averaging 0.93. The total chlorophyll content ranged from 2.78 in treatment A2T3 to 3.87 in treatment A1T2, with an overall mean of 3.46. Despite these observed variations, the lack of statistical significance implies that the treatment combinations do not result in meaningful changes in chlorophyll content at this stage of plant growth (Table 2).

Table 2. The average content of chlorophyll a, chlorophyll b, and total chlorophyll in vanilla leaves at 90 days after planting (DAP)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
A0T0	2.44	0.96	3.39
A0T1	2.42	0.96	3.38
A0T2	2.83	0.98	3.8
A0T3	2.77	0.78	3.55
A1T0	2.55	0.98	3.54
A1T1	2.56	1.00	3.55
A1T2	2.74	1.13	3.87
A1T3	2.65	0.85	3.5
A2T0	2.70	0.97	3.67
A2T1	2.47	1.06	3.53
A2T2	2.69	1.06	3.75
A2T3	1.99	0.8	2.78
A3T0	2.29	0.79	3.07
A3T1	2.4	0.86	3.25
A3T2	2.39	0.83	3.22
A3T3	2.57	0.88	3.45
Mean	2.53	0.93	3.46
P= 0.05	0.61	0.96	0.81

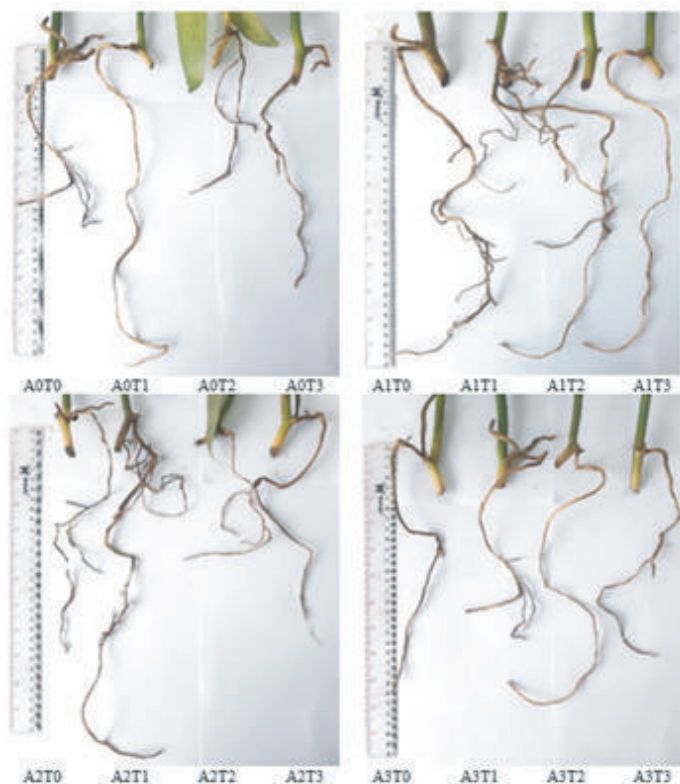


Fig. 2. Effect of treatments on root growth of vanilla cuttings at 90 days

Discussion

In our study, we conducted a comprehensive analysis to understand the effects of auxin and *T. harzianum* on the growth of vanilla plants. Our research aimed to investigate whether these two factors interacted to influence the growth of vanilla plants and elucidate potential mechanisms underlying any observed effects.

The results of statistical analysis showed that there was no interaction between auxin and *T. harzianum*. However, a solitary factor, stemming from the addition of auxin and *T. harzianum*, exhibited an effect. Our findings align with those of Lasmini *et al.* (2022), who noted no interaction between compost application and *Trichoderma* concerning shallot growth. The absence of interaction is likely due to the non-synergistic action of auxin and *T. harzianum*. The fungus *T. harzianum* possesses cellulase enzymes and secondary metabolites (Li *et al.*, 2015; Payne *et al.*, 2015). The production of secondary metabolites by *Trichoderma* fungi is influenced by environmental factors such as pH and temperature. (Nieto-Jacobo *et al.*, 2017).

Based on the results of a single-factor statistical analysis, there was an effect of auxin application on the number of roots ($P < 0.05$) (Table 1). We observed that the number of roots varied across different auxin concentrations. Specifically, treatments with 0 ppm auxin (A0), 50 ppm auxin (A1), and 100 ppm auxin (A2) yielded significantly different results compared to the treatment with 150 ppm auxin (A3). Clonex products contain IBA-type auxin (3.3 g/L). In this study, despite the significant effect of auxin on root growth, our study did not yield conclusive evidence that supplementing vanilla plants with exogenous auxin produced superior results compared to the control group. This raised the possibility that vanilla plants naturally possess an adequate supply of endogenous auxin to support their root growth.

According to Apriliani *et al.* (2015) plants naturally produce sufficient amounts of auxin. Endogenous auxins that are too high will trigger inhibition of cell elongation, impacting plant growth. However, excessively high concentrations of growth regulators under sufficient endogenous hormones will inhibit roots (Yunus *et al.*, 2016).

Auxin is known to play pivotal roles in various aspects of plant growth, including influencing differentiation, branching in roots, stem elongation, and stimulating fruit development (Kouassi *et al.*, 2013; Zhang *et al.*, 2022). The excess amount of auxin in plant tissue will be translocated to the base of the stem for root formation so that the increase in height is hampered (Kamila *et al.*, 2020). In a study by Wafia *et al.* (2021), adding IBA of 0 and 250 ppm to thymus stem cuttings for 5 minutes of immersion time can increase plant height and root length. This could be because the endogenous auxin in plants is sufficient to support the growth of cuttings. High concentrations of IBA can be toxic to plant cuttings, such as preventing root formation and adversely affecting shoot growth and development (Karimi and Yadollahi, 2012).

In contrast to the results observed with auxin, we found that the application of *T. harzianum* showed better results on almost all parameters of vanilla growth (Table 1). There was a significant effect on shoot growth time, plant height, number of leaves, fresh weight, and root length. These findings demonstrate the beneficial effects of *T. harzianum* on vanilla plant growth. Before such interactions occur, plants release root exudates, including peroxidase and oxylipin, into the soil as chemical signals to stimulate the growth of *Trichoderma*. When *Trichoderma* enters the plant, it is not recognized, thus triggering the activation of the plant's immune system locally and systemically. During the colonization of plant roots, *Trichoderma* grows between the intercellular spaces and inside the plant cells. *Trichoderma* releases elicitors such as Thph1 and Thph2, which are localized by root receptors ZmATG3 and ZmGLP, thereby triggering the activation of plant defenses against systemic diseases. To reduce the activity of the plant defense system, it is possible that *Trichoderma* uses effector molecules, including proteins and microRNAs, to suppress the host plant's immune genes so that they can remain in the plant roots (Rebolledo-Prudencio *et al.*, 2020).

One key factor contributing to the growth-promoting effects of *T. harzianum* is its ability to synthesize indole-3-acetic acid (IAA). Synthesis of IAA by *Trichoderma* promotes plant growth and the emergence of secondary roots, which increases the surface area of the roots and enhances the transport and absorption of nutrients. This occurs through direct contact between *Trichoderma* and plant roots or the excretion of volatile organic compounds (VOCs, *i.e.*, 6-PP and 2-heptanone) (Rebolledo-Prudencio *et al.*, 2020). Moreover, *Trichoderma* can act as an auxiliary agent in increasing and accelerating decomposition to maintain soil fertility (Zin and Badaluddin, 2020). According to Rebolledo-Prudencio *et al.* (2020), *Trichoderma* colonizes plant roots, regulating carbohydrate metabolism and genes related to photosynthesis. *Trichoderma* can increase the absorption and mobilization of nutrients (such as N, Fe, Cu, P, K) to optimize plant growth.

The T1 treatment resulted in the quickest shoot growth time. Specifically, adding 4 g of *T. harzianum* led to the fastest vanilla

shoot growth time, observed at 32.38 days after planting. According to Sandheep and Jisha (2013), *T. harzianum* increases nutrient absorption by secreting enzymes and insoluble nutrients, making them available to plants. According to Li *et al.* (2015), *Trichoderma* application can increase the amount of Fe and P available in the rhizosphere to 30 and 90%. In addition, the growth of shoots/shoots and roots, in response to *Trichoderma* inoculation, can increase the absorption of Cu, Na, and Zn as well as other micronutrients. Vanilla plant height ranged from 4.55-6.04 cm, with the highest value in T2. The symbiotic relationship between vanilla and *T. harzianum* can affect vanilla plant height. *Trichoderma* produces metabolites in the form of volatile compounds, capable of inducing resistance to plant pathogens, leading to improved plant health. *T. harzianum* produces precursor VOCs or participates in auxin signaling pathways (Lee *et al.*, 2016).

The addition of 4 g *T. harzianum* increased the number of vanilla plant leaves. According to Elkeshi *et al.* (2020), the fungus *T. harzianum* can enhance plant growth by increasing chlorophyll synthesis and absorption of essential ions, including nitrogen, phosphorus, and potassium. The growth in the number of leaves is influenced by factors such as the availability of water, nutrients (N, P, K), and sunlight to carry out the photosynthesis process (Syifa *et al.*, 2020). *Trichoderma* grows between the spaces between cells, regulates carbohydrate metabolism and genes related to photosynthesis. *Trichoderma* also regulates the signaling and synthesis of phytohormones, including auxin, ethylene, jasmonic acid, and cytokinins (Rebolledo-Prudencio *et al.*, 2020). The best fresh weight (18.48 g) was obtained from 2 g *T. harzianum* (T1). Applying *Trichoderma* sp. to the soil can increase the ability of plant roots to absorb nutrients. Amiroh *et al.* (2020) showed that adding *T. harzianum* increased the growth, fresh weight, dry weight, and seed weight of soybeans to increase the wet weight of the plants. *T. harzianum* also increase the absorption of nutrients in the soil (N, P, K), the optimum absorption of nutrients can increase growth and cutting biomass (Zhang *et al.*, 2019).

Nutrients and water play a role in influencing root growth, and roots are the entry point for nutrients and water. Phosphorus is useful for stimulating root growth which is influenced by the supply of photosynthate from the leaves. Photosynthate will expand the root development zone and stimulate the growth of new primary roots (Syifa *et al.*, 2020). *T. harzianum* affects the growth of plant roots. The morphology of plant roots is important for maximizing nutrient uptake because a root system with a high ratio will more efficiently explore volumes in the soil. Microorganisms such as endogenous fungi are important in plants, especially in terms of P uptake, because mycorrhizae can increase the ability of roots to explore a wider soil. Root hairs are the general root structure, and increasing root length is an adaptation of plants to increase P uptake and plant competition when plant P is limited for growth. The increase in P absorption in plants was obtained from the association with mycorrhiza (Wulandari and Hartatik, 2022).

The combination of auxin and *T. harzianum* had no significant effect on the content of chlorophyll a, chlorophyll b, and total chlorophyll of vanilla after 90 days of treatment ($P > 0.05$). There was no interaction between auxin and *T. harzianum* on the content of chlorophyll of vanilla plants, besides that there was no effect of single factor application of auxin and *T. harzianum*

on photosynthetic pigments of vanilla plants. *T. harzianum* does not supply high amounts of nitrogen for tissue growth, so the chlorophyll content does not increase (Mahato and Neupane, 2017). Nitrogen deficiency in plants causes a decrease in its chlorophyll content, weakens photosynthetic efficiency, and inhibits carbohydrate synthesis, which results in a decrease in crop yield (Gu *et al.*, 2016). *T. harzianum* did not significantly affect the chlorophyll content but could increase the growth of shoots and roots of vanilla cuttings. This is presumably because *T. harzianum* produces cellulase, an enzymes needed as a catalysts to initiate plant respiration reactions. Without enzymes, plant respiration will be difficult because it requires a high energy level and is difficult to achieve. Enzymes also accelerate respiration rate and can meet the energy needs of plants. Thus, plants get enough energy intake to grow and develop. According to Lorito and Woo (2015), plants treated with *T. harzianum* showed high sugar accumulation, causing root absorption and photosynthesis to be more optimal, thus increasing vegetative growth. While no significant interaction was observed between these treatments, *T. harzianum* emerged as a promising growth-promoting agent, enhancing shoot growth, plant height, and leaf development in vanilla plants. The application of auxin did not result in a significant increase in these growth parameters, suggesting that endogenous auxins within the plants may be sufficient. In addition, chlorophyll content was not affected by these treatments.

Our findings emphasize the potential of *T. harzianum* as a valuable biological agent in optimizing vanilla cultivation, addressing the need to enhance productivity and quality in this economically important crop. Further research can refine application methods and concentrations to utilize the full benefits of *T. harzianum* for vanilla production in Indonesia.

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