Effects of indole-3-butyric acid and 1-naphthaleneacetic acid on *in vitro* rooting and the substrate mixing ratio on growth during the nursery stage of Mai vang (*Ochna integerrima*) HD01 line

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ABSTRACT

Identifying suitable plant growth regulators for the rooting stage and substrate mixing ratio for seedlings in the nursery remains a significant challenge, particularly in relation to Mai vang. This plant existed in culture and tradition for a long time and was considered a symbol of the traditional Tet. The experiments were conducted on Mai vang HD01 line, which was selected from Huu Duc Mai garden in Binh Loi apricot village, known for its exceptional characteristics. The objective of this study was to determine the optimal Indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) concentrations appropriate for root formation from shoot samples in in vitro condition and the optimal substrate mixing ratio appropriate for the growth of Mai vang HD01 line during the nursery stage. The study including two experiments were arranged in completely randomized design with one-factor and two-factor. For rooting induction, the culture medium supplemented with concentrations of IBA combined with concentrations of NAA was used, while to grow, Mai vang HD01 plants were planted in a substrate of coconut fiber, sand, rice husk ash, and vermicompost with different mixing ratios. The results showed that Mai vang HD01 shoots were cultured on Murashige and Skoog medium supplemented with 0.5 mg/L IBA combined with 2 mg/L NAA suitable for rooting and creating complete plants. The number of roots, root length, plant height and number of leaves were 6.9 roots; 3.5 cm; 2.3 cm and 5.9 leaves, respectively on day 60. Mai vang HD01 plants in the nursery stage were suitable for planting on a substrate with a mixing ratio of 1 coconut fiber:1 sand:1 rice husk ash:1 vermicompost with a 100% survival rate. They grew quickly to a height of 5.1 cm which was higher than that of plants planted on other substrate mix ratios on day 40.

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1. Introduction

Each flower has its own color and beauty. Among them, Mai vang plant is a wild plant, growing in the mountains and forests with a natural and attractive appearance. Over time, Mai vang plant was discovered and domesticated by humans. The plant is considered a symbol of the traditional Tet, representing spring. In Vietnam, Mai vang is a popular ornamental plant in the South and Central regions. Among them, the 'HD01' line selected at Huu Duc Mai garden in Binh Loi Mai Village has many outstanding characteristics, such as large flower diameter (up to 4 - 6 cm), extended flowering period, and darker petal and stamen color than other lines.

In vitro propagation techniques have the advantage of high multiplication as well as high uniformity of seedlings, so they have been applied to propagate many plant species with different economic values (Tran, 2005). Up to now, both domestically and internationally, there have been a few studies on in vitro propagation of Mai vang flowers, but there have been few studies on root formation as well as the seedling stage outside the nursery. Furthermore, although there has been some success in in vitro propagation of Mai vang flowers, each stage needs to be adjusted, optimizing the environment as well as adjusting the appropriate substrate mixing ratio for seedlings to grow well.

Among the plant growth regulators, auxin plays an important role in plant *in vitro* morphogenesis, especially root formation. The two most commonly used auxins were indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) which had been used individually or in combination in many studies on rooting in tissue culture (Phan et al., 2014; Irshad et al., 2018). For the seedling stage outside the nursery, commonly used pseudosynthetics are coconut fiber, sand, rice husk ash and vermicompost, which were known to grow well and have a high survival rate (Huynh, 2007; Phan et al., 2017). Therefore, in this study, we focused on studying the most suitable combination to form roots from Mai vang HD01 shoots and the most suitable substrate mixing ratio for growing plants in the nursery stage.

2. Materials and Methods

2.1. Time and place of the study

The study was carried out from July to October 2023, at the tissue culture lab and the plant nursery of the Department of Physiology and Biochemistry, Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam.

2.2. Experimental design

2.2.1. Experiment 1: Effects of IBA and NAA concentrations on the rooting process of Mai vang HD01 plant *in vitro*

Experiment 1 had two factors: IBA concentrations: 0 mg/L; 0.5 mg/L; 1 mg/L; 1.5 mg/L, and NAA concentrations: 0 mg/L; 1 mg/L; 2 mg/L; 3 mg/L. The experiment consisted of 16 treatments, with 3 replications, arranged in a completely randomized design. Each treatment in a replication had 5 flasks. Each flask had 3 samples.

2.2.2. Experiment 2: Effect of substrate mixing ratio on the growth of Mai vang HD01 plant in the nursery stage

Experiment 2 had single factor: only coconut fiber (G1-control), 1 sand:1 coconut fiber (G2), 1 sand:1 coconut fiber:1 rice husk ash (G3), 1 sand:1 coconut fiber:1 rice husk ash:1 earthworm compost (G4). The experiment consisted of 4 treatments, with 3 replications, arranged in a completely randomized design. Each treatment in a replication had 15 pots. Each pot had 1 plant.

2.3. Experimental method

The base medium in the experiment 1 was Murashige and Skoog (MS) medium (Murashige & Skoog, 1962), supplemented with 7 g/L agar, 30 g/L sucrose, and plant growth regulators according to treatments.

Figure 1. Mai vang HD01 shoot sample used in experiment 1.

In experiment 1, the shoot samples used were the samples from the best shoot multiplication treatment at the department's tissue culture room. Shoot samples were selected with a size of 0.8 to 1.0 cm and then all leaves and roots (if any) were cut off (Figure 1). Then, shoot samples were transplanted into MS medium supplemented with IBA and NAA at different concentrations and put under light 2500 ± 500 lux for 10 h per day and then recorded over a period of 60 days.

Figure 2. Mai vang HD01 plant sample used in experiment 2.

In experiment 2, the shoots were produced in large quantity following the best treatment in experiment 1 and these were cultured in MS medium for 3 weeks. Then, the plants that were 1.8 to 2.0 cm heigh, have 2 to 3 leaves and 3 to 4 roots, were chosen (Figure 2). The plants were grown on substrates with different mixing ratios and put under light intensity 3.755 ± 500 lux for 12 h per day. The plant parameters were recorded over a period of 40 days.

2.4. Parameters

2.4.1. Experiment 1

The number of roots was recorded every 10 days and these roots were counted when they reached a length of 0.5 cm. The plant height and number of leaves was also monitored every 10 days for 60 days. On day 60 of culture, the root length and stem diameter of 10 random shoots were measured.

2.4.2. Experiment 2

The monitoring indicators were recorded periodically every 10 days within 60 days. The survival rate of plants (%) was calculated by dividing the total number of living plants by the total number of plants grown in each treatment. The plant height was recorded from the root collar to the shoot tip. The number of leaves was recorded for leaves reaching a length of 1 cm or more. Five plants in each base plot were measured for the plant height and the number of leaves.

2.5. Data processing and analyses

Data were processed with Excel 2010 (Microsoft, USA). The R software (version 4.2.3) was used to perform ANOVA and post-hoc analysis with Duncan's multiple range test at a = 0.01 or a = 0.05. The number of roots and the root length in experiment 1 were transformed using the formula before statistical analysis (Gomez & Gomez, 1984).

3. Results and Discussion

3.1. Effects of IBA and NAA concentrations on the rooting process of Mai vang HD01 plant *in vitro*

3.1.1. Effect of IBA and NAA concentrations on the number root of Mai vang HD01 plant *in vitro*

The results in Table 1 showed that as early as day 40, when the root were distinguishable, significant differences among treatments due to the effects of each factor and the interaction between two factors could be observed, and these differences maintained until the end of the culture period (day 60). The trend in root number differences between treatments was also largely similar throughout the culture period. When BA and NAA were not used, no roots was formed, this proved the important role of auxin in root formation in Mai vang HD01 plant.

Table 1. Effects of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) concentrations on the number root of Mai vang HD01 plant *in vitro*

Days of culture	IBA concentration (mg/L)	NAA concentration (mg/L)				Average (I)
		0	1	2	3	
Day 40	0	0^{g}	1.1^{f}	1.8^{bcd}	1.2^{ef}	1.0^{C}
	0.5	1.5^{c-f}	1.9^{bcd}	3.6^{a}	2.11^{bc}	2.3^{A}
	1	1.6^{b-f}	1.5^{c-f}	1.7^{b-e}	2.13^{b}	1.7^{B}
	1.5	2.0^{bc}	1.4^{def}	1.9^{bcd}	1.3^{d-f}	1.7^{B}
Average (N)		1.3^{C}	1.5^{B}	2.3^{A}	1.7^{B}	
CV (%) = 5.6; $F_I = 62.6^{**}$; $F_N = 39.9^{**}$; $F_{I*N} = 18.9^{**}$						
Day 50	0	0^{f}	1.4^{e}	2.8^{bc}	1.5^{e}	1.4^{C}
	0.5	2.1^{b-e}	2.9^{b}	4.8^{a}	2.8^{bc}	3.2^{A}
	1	2.0^{cde}	2.1^{b-e}	1.9^{de}	2.8^{bc}	2.2^{B}
	1.5	2.7^{bc}	2.1^{b-e}	2.6^{bcd}	1.9^{de}	2.3^{B}
Average (N)		1.7^{C}	2.1^{B}	3.0^{A}	2.3^{B}	
CV (%) = 5.8; $F_I = 72.0^{**}$; $F_N = 42.5^{**}$; $F_{I*N} = 22.8^{**}$						
Day 60	0	0^{f}	2.1^{e}	3.9^{bc}	2.0^{e}	2.0^{C}
	0.5	3.1^{cd}	4.1^{b}	6.9^{a}	4.1^{b}	4.6^{A}
	1	2.9^{d}	3.1^{cd}	3.0^{cd}	4.1^{b}	3.3^{B}
	1.5	3.9^{bc}	2.9^{d}	3.9^{bc}	2.8^{de}	3.4^{B}
Average (N)		2.5^{C}	3.1^{B}	4.4^{A}	3.3^{B}	
CV (%) = 4.9; $F_I = 124.0^{**}$; $F_N = 72.6^{**}$; $F_{I*N} = 33.6^{**}$						

Within the same group, mean values followed by the same letter indicate not significantly different; **: significantly different at 1% level.

On day 60, in terms of IBA concentration, Mai vang HD01 plant cultured in the medium supplemented with 0.5 mg/L IBA gave the highest number of roots, reaching 4.6 roots, a statistically significant difference compared to the remaining concentrations. Meanwhile, in terms of NAA concentration, Mai vang HD01 plant cultured in the medium supplemented with 2 mg/L NAA gave the highest number of roots, reaching 4.4 roots, a statistically significant difference compared to the remaining concentrations. In terms of the interaction between the two factors, the highest number of roots was 6.9 roots when Mai vang HD01 plant was cultured in the medium supplemented with 0.5 mg/L IBA combined with 2 mg/L NAA, a statistically significant difference compared to the number of roots of Mai vang plants cultured in the medium supplemented with other concentrations of growth regulators (Figure 3).

Figure 3. Mai vang HD01 plant of the treatments after 60 days of culture. I1-4: indole-3-butyric acid concentration corresponding to 0, 1, 2 or 3 mg/L; N1-3: 1-naphthaleneacetic acid concentration corresponding to 0; 0.5; 1 or 1.5 mg/L.

The results obtained in this research were consistent with the findings of Mai & Lam (2013), which indicated that Mai vang plants cultured on a medium with a combination of IBA and NAA effectively promoted root formation. However, the maximum number of roots produced by Mai vang plant reached only 1.9 roots after 8 weeks of culture, which was lower than the 6.9 roots observed in this study after 60 days. This demonstrates that auxin played a significant role in enhancing root formation in plant tissue and cell cultures (Nguyen, 2000), particularly in the in vitro study of Mai vang HD01 compared to conditions where auxin was not added to the culture medium. In contrast, the study by Ho et al. (2019) showed that in vitro Mai vang shoots did not depend entirely on growth regulators but were also affected by mineral concentration, shoot age, and other conditions such as the number of subcultures.

3.1.2. Effect of IBA and NAA concentrations on root length and stem diameter of Mai vang HD01 plant *in vitro*

On day 60, regarding IBA concentration, Mai vang HD01 plants cultured in the medium supplemented with 1.5 mg/L IBA exhibited the longest root length of 3.6 cm, which was statistically significantly different from the other concentrations. Meanwhile, concerning NAA concentration, Mai vang HD01 plants cultured in the medium supplemented with 2 mg/L NAA had the highest number of roots at 2.9 cm; however, this difference was not significant compared to the concentration of 3 mg/L NAA (2.8 cm), although it was statistically significant compared to the other concentrations. In terms of the interaction between these two factors, the longest root length of 4.0 cm was observed when culturing Mai vang HD01 plants on a medium supplemented with 1.5 mg/L IBA combined with 3 mg/L NAA. Although this difference was not significant compared to the root lengths of Mai vang HD01 plants cultured on media supplemented with 1 mg/L IBA combined with 3 mg/L NAA (3.6 cm), 0.5 mg/L IBA combined with 2 mg/L NAA (3.5 cm), and 1.5 mg/L IBA combined with 1 mg/L NAA (3.5 cm), as well

as 1.5 mg/L IBA (3.4 cm) and 1.5 mg/L IBA combined with 2 mg/L NAA (3.3 cm), it was statistically significant when compared to the root lengths of Mai vang HD01 plants cultured on media supplemented with other concentrations of growth regulators (Table 2). Thus, the results of this study further confirm the effectiveness of auxin on rooting in plants, demonstrating clear effects on root length (Nguyen et al., 2021).

Table 2. Effects of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) concentrations on root length and stem diameter of Mai vang HD01 plant *in vitro*

	IBA concentration (mg/L)	NAA concentration (mg/L)				Average (I)
		0	1	2	3	
Root length (cm)	0	0^{h}	1.1^{fg}	2.2^{de}	0.9^{g}	1.1^{C}
	0.5	2.6^{bcd}	2.4^{cd}	3.5^{abc}	2.5^{cd}	2.8^{B}
	1	1.8^{def}	1.6^{efg}	2.7^{bcd}	3.6^{ab}	2.4^{B}
	1.5	3.4^{abc}	3.5^{abc}	3.3^{abc}	4.0^{a}	3.6^{A}
Average (N)		2.0^{B}	2.2^{B}	2.9^{A}	2.8^{A}	
CV (%) = 6.7; $F_I = 109.4^{**}$; $F_N = 23.6^{**}$; $F_{I*N} = 11.2^{**}$						
Stem diameter (mm)	0	1.2	1.2	1.0	1.0	1.1
	0.5	1.2	1.1	1.3	1.2	1.2
	1	1.3	0.9	0.8	1.0	1.0
	1.5	1.0	1.2	1.1	1.1	1.1
Average (N)		1.2	1.1	1.1	1.1	
CV (%) = 16.4; $F_I = 2.8^{ns}$; $F_N = 0.9^{ns}$; $F_{I*N} = 1.7^{ns}$						

*Within the same group, mean values followed by the same letter indicate not significantly different; ns: not significantly different; **: significantly different at 1% level.*

Meanwhile, on day 60, Mai vang HD01 plant cultured in medium supplemented with different concentrations of IBA and NAA had stem diameters ranging from 0.8 mm to 1.3 mm, the difference was not statistically significant.

3.1.3. Effect of IBA and NAA concentrations on the height of Mai vang HD01 plant *in vitro*

The height of plant, which represents the growth of plant, showed gradual increase in some treatments, specifically treatments with 1.5 mg/L IBA combined with 2 mg/L NAA; 1.5 mg/L IBA; 1.5 mg/L IBA combined with 3 mg/L NAA; 0.5 mg/L IBA combined with 3 mg/L NAA; 1 mg/L IBA combined with 3 mg/L NAA; 0.5 mg/L IBA; 1 mg/L NAA (1.5 cm); 1.5 mg/L IBA combined with 1 mg/L NAA over the culture period from day 40 to day 60 (Table 3). In the rest of the treatments, plant height did not increase significantly, indicating that little plant growth occurred in these treatments.

Table 3. Effects of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) concentrations on the height of Mai vang HD01 plant *in vitro*

Days of culture	IBA concentration (mg/L)	NAA concentration (mg/L)				Average (I)
		0	1	2	3	
10	0	0.9	0.9	1.0	1.0	1.0
	0.9	1.0	0.9	1.0	1.0	1.0
	0.9	1.0	0.9	1.0	1.0	1.0
	1.0	1.0	1.0	1.0	1.0	1.0
Average (N)		0.9	1.0	1.0	1.0	
CV (%) = 14.9; $F_I = 0.5^{ns}$; $F_N = 0.8^{ns}$; $F_{I*N} = 0.3^{ns}$						
20	0	0.9	0.9	1.1	1.2	1.0
	0.5	1.0	1.1	1.1	1.2	1.0
	1	1.1	1.2	1.0	1.1	1.0
	1.5	1.2	1.1	1.1	1.0	1.0
Average (N)		1.1	1.1	1.1	1.1	
CV (%) = 14.2; $F_I = 0.9^{ns}$; $F_N = 0.7^{ns}$; $F_{I*N} = 1.2^{ns}$						
30	0	1.0	1.3	1.2	1.3	1.2^{B}
	0.5	1.2	1.2	1.4	1.3	1.3^{AB}
	1	1.2	1.2	1.1	1.2	1.2^{B}
	1.5	1.4	1.2	1.5	1.4	1.4^{A}
Average (N)		1.2	1.2	1.3	1.3	
CV (%) = 12.9; $F_I = 3.4^{*}$; $F_N = 1.0^{ns}$; $F_{I*N} = 1.3^{ns}$						
40	0	1.1^{g}	1.5^{a-g}	1.29^{d-g}	1.27^{efg}	1.3^{C}
	0,5	1.59^{a-f}	1.38^{b-g}	1.68^{a-d}	1.31^{c-g}	1.5^{AB}
	1	1.21^{fg}	1.32^{b-g}	1.22^{efg}	1.61^{a-e}	1.3^{BC}
	1.5	1.71^{ab}	1.41^{a-g}	1.8^{a}	1.69^{abc}	1.7^{A}
Average (N)		1.4	1.4	1.5		1.5
CV (%) = 10.7; $F_I = 13.5^{**}$; $F_N = 1.2^{ns}$; $F_{I*N} = 4.5^{**}$						
50	0	1.3^{d}	1.9^{ab}	1.6^{bcd}	1.4^{cd}	1.6^{B}
	0,5	2.0^{a}	1.8^{abc}	2.0^{a}	1.6^{bcd}	1.9^{A}
	1	1.5^{cd}	1.6^{bcd}	1.3^{d}	1.9^{ab}	1.6^{B}
	1.5	2.0^{a}	1.6^{bcd}	2.1^{a}	1.9^{ab}	1.9^{A}
Average (N)		1.7	1.7	1.8	1.7	
CV (%) = 8.7; $F_I = 20.1^{**}$; $F_N = 0.5^{ns}$; $F_{I*N} = 9.3^{**}$						
60	0	1.4^{g}	2.0^{a-d}	1.7^{c-g}	1.6^{efg}	1.7^{B}
	0,5	2.2^{a}	1.9^{a-e}	2.3^{a}	1.8^{b-f}	2.1^{A}
	1	1.69^{d-g}	1.7^{c-g}	1.5^{fg}	2.0^{a-d}	1.7^{B}
	1.5	2.13^{ab}	1.8^{b-f}	2.2^{a}	2.05^{abc}	2.1^{A}
Average (N)		1.9	1.9	1.9	1.9	
CV (%) = 7.4; $F_I = 24.9^{**}$; $F_N = 0.5^{ns}$; $F_{I*N} = 10.2^{**}$						

*Within the same group, mean values followed by the same letter indicate not significantly different; ns: not significantly different; *: significantly different at 5% level ;**: significantly different at 1% level.*

Despite the gradual increase in plant height throughout the culture period, the trends in height differences among Mai vang HD01 plants cultured on media supplemented with various concentrations of growth regulators remained consistent from day 40 to day 60. The height of Mai vang HD01 plants reached its maximum in the medium supplemented with 1.5 mg/L IBA. Meanwhile, the average height of Mai vang HD01 plants in relation to NAA concentration was not significantly different. Regarding the interaction between these two factors, the maximum height of Mai vang HD01 plants was observed in the medium supplemented with 0.5 mg/L IBA combined with 2 mg/L NAA.

On day 60, regarding IBA concentration, the height of Mai vang HD01 plants reached its highest level of 2.1 cm in the medium supplemented with 1.5 mg/L IBA. Although this height was not significantly different from that of Mai vang HD01 plants in the medium supplemented with 0.5 mg/L IBA (which was also 2.1 cm), it was significantly different compared to the heights of Mai vang HD01 plants grown in media supplemented with other concentrations of IBA. Meanwhile, in terms of NAA concentration, the average height of Mai vang HD01 plant in the treatments reached 1.9 cm, the difference was not statistically significant. In terms of the interaction between the two factors above, the maximum height of Mai vang HD01 plant was 2.3 cm, cultured on a medium supplemented with 0.5 mg/L IBA combined with 2 mg/L NAA. Although the difference was not significant compared to the height of Mai vang HD01 plants grown on medium supplemented with 0.5 mg/L IBA (2.2 cm), 1.5 mg/L IBA combined with 2 mg/L NAA (2.2 cm), 1.5 mg/L IBA (2.13 cm), 1.5 mg/L IBA combined with 3 mg/L NAA (2.05 cm), 1 mg/L NAA (2.0 cm), 1 mg/L IBA combined with 3 mg/L NAA (2.0 cm), 0.5 mg/L IBA combined with 1 mg/L NAA (1.9 cm), the difference was significant compared to the height of Mai vang HD01 plants cultured on media supplemented with other concentrations of growth regulators.

3.1.4. Effect of IBA and NAA concentrations on the number of leaves of Mai vang HD01 plant *in vitro*

The number of leaves (Table 4) also showed that there were few changes in the differences among treatment from day 10 to 60, indicating that the effects of the growth regulator combinations were largely determined early in the culture period. This is despite the fact that the number of leaves still increased substantially from day 10 to day 60.

On day 60, in terms of IBA concentration, the number of leaves was highest, reaching 3.6 leaves, in the medium supplemented with 0.5 mg/L IBA, a statistically significant difference compared to the other three concentrations. Meanwhile, in terms of NAA concentration, Mai vang HD01 plant cultured in the medium supplemented with 2 mg/L NAA had the highest number of leaves, reaching 3.5 leaves, a statistically significant difference compared to the other three concentrations. In terms of the interaction between the two factors above, Mai vang HD01 plant cultured in the medium supplemented with 0.5 mg/L IBA combined with 2 mg/L NAA had the highest number of leaves, reaching 5.9 leaves, a statistically significant difference compared to the number of leaves of the Mai vang HD01 plants cultured in the medium supplemented with other concentrations of growth regulators.

Table 4. Effects of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) concentrations on the number of leaves of Mai vang HD01 plant *in vitro*

Days of culture	IBA concentration (mg/L)	NAA concentration (mg/L)				Average (I)
		0	1	2	3	
10	0	0.7^{bcd}	0.4^{e}	0.9^{ab}	0.3^{e}	0.6
	0.5	0.4^{e}	0.9	1.1^{a}	0.8^{b}	0,7
	1	0.7^{bcd}	0.9	0.4^{e}	0.8^{b}	0,7
	1.5	0.8^{b}	0.9^{ab}	0.5^{cde}	0.4^{e}	0,7
Average (N)		0.7^{AB}	0.6^{AB}	0.73^{A}	0.6^{B}	
CV (%) = 16.6; $F_I = 2.6^{ns}$; $F_N = 3.0^{*}$; $F_{I*N} = 21.0^{**}$						
20	0	1.0^{fg}	0.8^{gh}	1.3^{cde}	0.9^{fgh}	1.0^{B}
	0.5	0.8^{gh}	1.8^{a}	1.8^{a}	1.4^{cd}	1.2^{A}
	1	1.1^{ef}	0.7^{h}	0.7^{h}	1.1^{ef}	1.1^{AB}
	1.5	1.2^{de}	0.8^{gh}	0.8^{gh}	0.7^{h}	1.1^{AB}
Average (N)		1.0^{B}	1.2^{A}	1.2^{A}	1.0^{B}	
CV (%) = 9.3; $F_I = 6.3^{**}$; $F_N = 9.0^{**}$; $F_{I*N} = 52.9^{**}$						
30	0	1.51^{cde}	1.2^{ef}	1.7^{bcd}	1.3^{ef}	1.4^{B}
	0.5	1.1^{f}	1.2^{ef}	2.6^{a}	1.8^{bc}	1.7^{A}
	1	1.49^{de}	1.9^{b}	1.1^{f}	1.4^{def}	1.5^{B}
	1.5	1.47^{de}	2.4^{a}	1.3^{ef}	1.1^{f}	1.6^{AB}
Average (N)		1.4^{B}	1.7^{A}	1.7^{A}	1.4^{B}	
CV (%) = 8.1; $F_I = 9.1^{**}$; $F_N = 21.1^{**}$; $F_{I*N} = 54.0^{**}$						
40	0	2.0^{c}	1.4^{d}	2.6^{b}	1.5^{d}	1.9^{C}
	0,5	1.4^{d}	1.5^{d}	3.8^{a}	2.7^{b}	2.3^{A}
	1	2.1^{c}	2.8^{b}	1.3^{d}	2.1^{c}	2.1^{B}
	1.5	2.2^{c}	3.6^{a}	1.5^{d}	1.6^{d}	2.2^{A}
Average (N)		1.9^{B}	2.3^{A}	2.3^{A}	2.0^{B}	
CV (%) = 6.2; $F_I = 27.0^{**}$; $F_N = 29.7^{**}$; $F_{I*N} = 156.3^{**}$						
50	0	2.7^{d}	1.6^{e}	3.3^{c}	1.7^{e}	2.3^{B}
	0,5	1.6^{e}	1.7^{e}	5.0^{a}	3.5^{c}	3.0^{A}
	1	2.6^{d}	3.4^{c}	1.6^{e}	2.6^{d}	2.6^{B}
	1.5	2.7^{d}	4.1^{b}	1.7^{e}	1.8^{e}	2.5^{B}
Average (N)		2.4^{B}	2.7^{A}	2.9^{A}	2.4^{B}	
CV (%) = 9.6; $F_I = 13.0^{**}$; $F_N = 11.1^{**}$; $F_{I*N} = 78.3^{**}$						
60	0	3.1^{d}	2.1^{e}	4.0^{c}	2.0^{e}	2.8^{C}
	0,5	2.1^{e}	2.1^{e}	5.9^{a}	4.1^{c}	3.6^{A}
	1	3.1^{d}	4.0^{c}	2.0^{e}	3.2^{d}	3.1^{B}
	1.5	3.2^{d}	5.0^{b}	2.0^{e}	2.1^{e}	3.1^{B}
Average (N)		2.9^{C}	3.3^{B}	3.5^{A}	2.9^{C}	
CV (%) = 4.8; $F_I = 50.1^{**}$; $F_N = 48.4^{**}$; $F_{I*N} = 283.2^{**}$						

Within the same group, mean values followed by the same letter indicate not significantly different; *ns: not significantly different; *: significantly different at 5% level; **: significantly different at 1% level.*

The above results showed that Mai vang HD01 plant cultured on MS medium supplemented with 0.5 mg/L IBA combined with 2 mg/L NAA are suitable for root formation and complete plant formation.

3.2. Effect of substrate mixing ratio on the growth of Mai vang HD01 plant in the nursery stage

When bringing plants to the nursery, the substrate mixing ratio is one of the factors that directly affects the survival and growth rates of the plants. Therefore, a suitable substrate mixing ratio is decisive for the success of the in vitro propagation process (Lam & Nguyen, 2005).

3.2.1. Effect of substrate mixing ratio on the survival rate of Mai vang HD01 plant in the nursery stage

The results presented in Table 5 indicate that, on day 40, the survival rate of Mai vang plant grown on four types of substrates, namely, only coconut fiber (control), 1 sand:1 coconut fiber, 1 sand:1 coconut fiber:1 rice husk ash, 1 sand:1 coconut fiber:1 rice husk ash:1 vermicompost, reached an impressive survival rate of 100%.

Table 5. Effect of substrate mixing ratio on the survival rate (%) of Mai vang HD01 plant in the nursery stage on day 40

Type of substrate	Survival rate (%)
Only coconut fiber (control) (G1)	100
1 sand: 1 coconut fiber (G2)	100
1 sand: 1 coconut fiber: 1 rice husk ash (G3)	100
1 sand: 1 coconut fiber: 1 rice husk ash: 1 vermicompost (G4)	100

The findings of the above research align with the studies conducted by Stefanello et al. (2009) and Nguyen (2019), which demonstrated that plants grown on coconut fiber in combination with various other substrates significantly enhance survival rates during the nursery stage. This result was much higher than that reported by Ho et al. (2019) when growing Mai vang plant on a substrate containing sand, soil, coconut fiber, and rice husks in a ratio of 30:50:10:10, which resulted in a survival rate of 78%. This suggests that the choice of substrate plays a crucial role in promoting the successful establishment and growth of plants in vitro in the nursery stage.

3.2.2. Effect of substrate mixing ratio on the height of Mai vang HD01 plant in the nursery stage

Table 6 showed that the height of Mai vang HD01 plants increased; however, there was no change in trend from 10 to 40 days after planting. The tallest height was consistently observed in plants grown on a substrate with a mixture ratio of 1 sand:1 coconut fiber:1 rice husk ash:1 vermicompost.

Table 6. Effect of substrate mixing ratio on the height of Mai vang HD01 plant in the nursery stage

Type of substrate	Day 10	Day 20	Day 30	Day 40
G1	1.9^{b}	2.0^{b}	2.2^{b}	2.5^{b}
G2	2.0^{bs}	2.1^{b}	2.3^{b}	2.6^{b}
G3	2.0^{b}	2.1^{b}	2.2^{b}	2.6^{b}
G4	2.4^{a}	2.9^{as}	4.1^{a}	5.1^{a}
	CV (%) = 8.7 $F_{tính} = 4.4^{*}$	CV (%) = 8.0 $F_{tính} = 16.2^{**}$	CV (%) = 8.3 $F_{tính} = 50.5^{**}$	CV (%) = 10.4 $F_{tính} = 44.6^{**}$

*In the same column, mean values followed by the same letter indicate statistically insignificant differences; *: significantly different at 5% level; **: significantly different at 1% level.*

On day 40, Mai vang HD01 plant was grown on a substrate with a mixture ratio of 1 sand:1 coconut fiber:1 rice husk ash:1 vermicompost, giving the largest height of 5.1 cm, which was a statistically significant difference compared to the height of Mai vang HD01 plants grown on other mixture ratios (Figure 4).

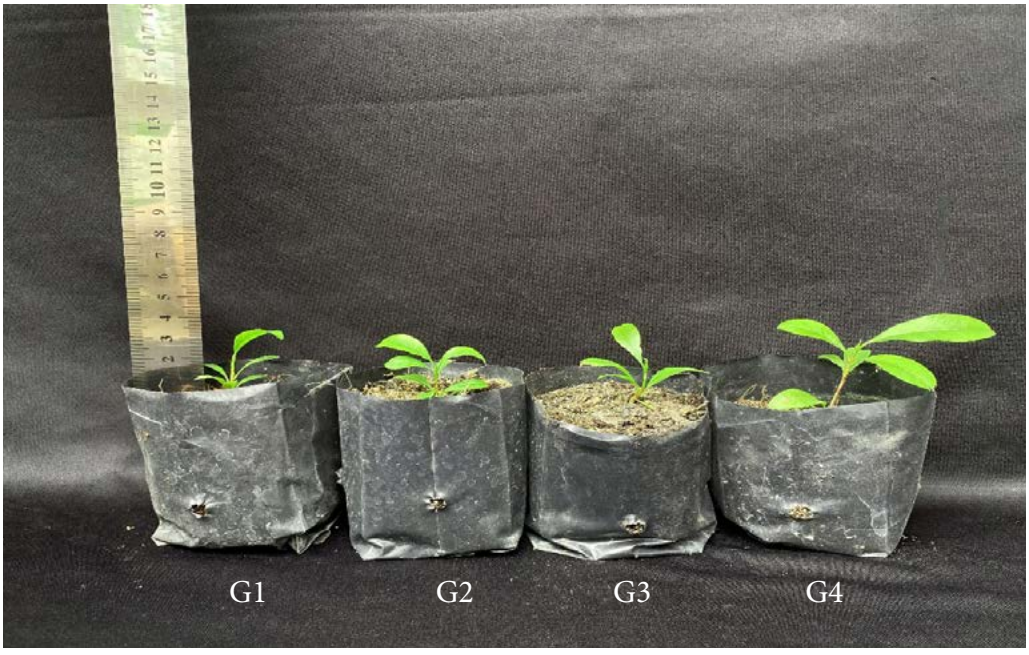

Figure 4. Mai vang HD01 plant of the treatments on day 40. G1: only coconut fiber; G2: 1 sand:1 coconut fiber; G3: 1 sand:1 coconut fiber:1 rice husk ash; G4: 1 sand:1 coconut fiber:1 rice husk ash:1 earthworm compost.

3.2.3. Effect of substrate mixing ratio on the number of leaves of Mai vang HD01 plant in the nursery stage

Contrary to height, the number of leaves of Mai vang HD01 plants grown on different mixture ratios of substrates varied from 10 to 40 days. However, the difference was not statistically significant (Table 7).

Table 7. Effect of substrate mixing ratio on number of leaves of Mai vang HD01 plant in the nursery stage

Type of substrate	Day 10	Day 20	Day 30	Day 40
G1	2.1	3.9	4.9	5.3
G2	2.9	4.1	5.0	5.8
G3	3.1	3.3	3.6	4.2
G4	2.9	4.0	5.1	6.1
	CV (%) = 14.2	CV (%) = 11.3	CV (%) = 17.2	CV (%) = 14.2
	$F_{tính} = 3.7^{ns}$	$F_{tính} = 2.3^{ns}$	$F_{tính} = 2.5^{ns}$	$F_{tính} = 3.6^{ns}$

ns: not significantly different.

The results presented above suggest that during the nursery stage, Mai vang HD01 plants are optimally suited for cultivation on a substrate with a mix ratio of 1 sand:1 coconut fiber:1 rice husk ash:1 vermicompost. This combination facilitates enhanced growth rates and height compared to plants grown with other substrate mixing ratios. The observed effects can be attributed to the addition of nutrient-rich vermicompost (Ramnarain et al., 2019). Vermicompost enhances microbial activity in the soil, increases oxygen availability, maintains optimal soil temperature, improves soil porosity and water permeability, and enriches nutrient content. These factors collectively contribute to enhanced plant growth, yield, and quality (Ansari & Ismail, 2012).

4. Conclusions

Mai vang HD01 plants were cultured on MS medium supplemented with 0.5 mg/L IBA combined with 2 mg/L NAA, which is suitable for root formation and overall plant development. The results indicated that the number of roots, root length, plant height, and number of leaves were 6.9 roots, 3.5 cm, 2.3 cm, and 5.9 leaves, respectively.

During the nursery stage, Mai vang HD01 plants thrived when grown on a substrate with a mixing ratio of 1 sand:1 coconut fiber:1 rice husk ash:1 vermicompost. This combination achieved a survival rate of 100% and promoted rapid growth, resulting in an average height of 5.1 cm, which was higher than that observed with other substrate mixing ratios.

Conflict of interest

I certify that this is my own research work. The data and results presented in the study are genuine and have never been published in any other journal.

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