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Factors affecting micropropagation in Vanilla (*Vanilla × Tahitensis* J. W. Moore) from node explants

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Abstract. This study aims to develop a comprehensive micropropagation protocol for the Vanilla plant (*Vanilla × tahitensis* J. W. Moore). *In vitro* shoots of Vanilla were cultured on the MS (Murashige and Skoog) medium supplemented with growth regulators and gelling agents to assess shoot multiplication rates and root formation capability after 10 weeks. The MS medium with 1 mg/L BAP, 0.5 mg/L Kn, and 10% coconut juice produced 4.98 shoots per sample, the best shoot multiplication rate. Shoots reached an average height of 3.98 cm, with 4.25 leaves per sample. Gelrite was the most effective gelling agent for shoot development, resulting in the most significant shoot height (4.04 cm) and the most leaves (7.67 leaves per sample). The MS medium with 1 mg/L IBA and 0.5 mg/L NAA optimised root formation, producing 3.11 roots per sample, with an average root length of 5.11 cm. After 3 weeks of cultivation in decomposed coconut fibre, all seedlings survived with an average plant height of 8.39 cm and an average of 6.52 leaves per plant.

Keywords: Vanilla, in vitro, growth regulator, gelling agent, acclimatisation

1 Introduction

The Vanilla plant, or Vanilla orchid, is a perennial climbing plant, growing monopodially with green, thick, cylindrical, and succulent stems. Its fruit produces a unique aroma. This species has significant economic value and is extensively researched and developed [1, 2]. The Vanilla, originated in the tropical areas of South America, belongs to the genus Vanilla with about 140 species [3, 4], but only a few of them are cultivated for trading, such as *Vanilla planifolia* Andrews and *Vanilla × tahitensis* J. W. Moore. These two species could generate vanillin, which is the most expensive spice in the market [5, 6]. Unlike the other orchids, the Vanilla plant possesses the most economically important part in its fruit. Vanilla fruit produces vanillin (4-hydroxy-3-methoxybenzaldehyde) through a curing process (includes fermentation and drying). Vanillin makes up about 1 to 2% of the fruit extract, and it is widely used in the food, perfumery, and pharmaceutical industry [7–9].

Numerous previous studies on the micropropagation of vanilla orchids have been published. In particular, these researchers have utilised both direct and indirect organogenesis strategies for *in vitro* propagation of vanilla [10–15]. Furthermore, several studies have examined the effects of growth hormones and coconut juice on shoot formation ability and shoot development of the vanilla plant *in vitro* [1, 16, 17]. Besides the micropropagation method utilising solid and semi-solid media, both the Temporary Immersion Systems (TIS) and double-phase culture system have also been implemented for micropropagation of vanilla [18, 19].

In Vietnam, research on vanilla micropropagation is relatively limited [20–23]. In a published study in 2018, Ha et al. [21] successfully established a micropropagation protocol for the *Vanilla planifolia* variety in their laboratory. By 2024, Lai et al. [22] had completed the micropropagation process for this Vanilla species up to the nursery stage. At the same time, Uyen et al. [23] perfected a micropropagation protocol at a commercial scale, enabling the provision of adequate materials for planting *Vanilla planifolia* in Vietnam. However, most *in vitro* propagation studies focused on the *Vanilla planifolia* cultivar. This cultivar brilliantly adapts to the highland area of Vietnam, such as Dak Lak and Lam Dong Provinces, where the climate is not overly hot and humid. Meanwhile, no research on the micropropagation of the *Vanilla* × *tahitensis* species, which adapts well to the hot and humid tropical climate (typical climate of Vietnam) has been found in the literature. In this context, our study focuses on developing a comprehensive protocol of micropropagation for the *Vanilla* × *tahitensis* J. W. Moore species. It aims to optimise the shoot multiplication, root induction, and acclimatization. This protocol can help us to provide disease-free plant material, enhance its economic value, and conserve the genetic resources of this orchid in Vietnam.

2 Material and method

2.1 Material

The material used in this study was selected from the nursery house of the Biotechnology Institute of Tra Vinh University. The plant samples were washed in a detergent solution for 10 minutes and rinsed under running tap water for 2 minutes. Then, they were washed 3–5 times with distilled water. After that, the plant samples were cut into node segments of 1.2 to 2 cm in length. These surface nodes were sterilised with 70% ethanol for 5 minutes and then rinsed three times with sterile distilled water. Finally, the node samples were further sterilised with a 3% sodium chlorite solution for 10 minutes and rinsed again five times with sterile distilled water. The damaged parts from two sterile sites of the node segment were removed and then cultured in the culture medium.

2.2 Method

Experiment 1: *Effects of BAP and KN on Vanilla shoot multiplication*

The stem nodes were cultured on the MS medium [24] supplemented with 30 g/L sucrose, 3 g/L gelrite, and different concentrations of BAP (0, 1, 2, 3 mg/L) and KN (0, 0.5, 1, 1.5 mg/L). pH was adjusted to 5.8. Each treatment had 18 samples, and the data were recorded after 10 weeks of culture. The evaluated parameters are the number of shoots, the number of leaves per sample, and shoot height.

Experiment 2: *Effects of coconut juice on Vanilla shoot multiplication*

The stem nodes were cultured on MS medium supplemented with 1 mg/L BAP, 0.5 mg/L KN, 30 g/L sucrose, 3 g/L gelrite, and different concentrations of coconut juice (0, 100, 200, 300 mL/L). pH was adjusted to 5.8. Each treatment had 18 samples, and the data were recorded after 10 weeks of culture. The evaluated variables are the number of shoots and leaves per sample, shoot height, and fresh and dry weight of shoots.

Experiment 3: *Effects of gelling agents on Vanilla shoot multiplication rate*

The stem nodes are cultured on MS medium supplemented with 100 mL/L coconut juice, 1 mg/L BAP, 0.5 mg/L KN, 30 g/L sucrose, with different gelling agents (3 g/L gelrite, 3 g/L phytagel, and 8 g/L agar). pH was adjusted to 5.8.

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Each treatment had 18 samples, and the data were recorded after 10 weeks of culture. The observed parameters include the number of shoots and leaves per sample, shoot height, fresh weight, and dry weight.

Experiment 4: *Effects of IBA and NAA on Vanilla root formation capability*

The *in vitro* shoots were cultured on MS medium supplemented with 30 g/L sucrose, 1 g/L activated charcoal, 3 g/L gelrite, combined with different concentrations of IBA (0, 1, 2 mg/L) and NAA (0, 0.5, 1 mg/L). pH was adjusted at 5.8. Each treatment had 18 samples, and the data were recorded after 10 weeks of culture. Observed parameters include roots and leaves per sample, root length (cm), and plant height (cm).

Experiment 5: *Survival rate of in-vitro vanilla plantlets in coconut coir pellets under greenhouse conditions*

Ten-week-old rooted plantlets were separated from the culture medium, washed gently under running water to remove agar residues and then transferred to coconut coir pellets for cultivating. The cultivating area needs to be shaded with a black net and watered 2–3 times a day [25]. The survival rate, shoot height, and total leaf number were recorded after 7 days, 14 days, and 21 days of transferring.

2.3 Experimental condition

The medium was adjusted to a pH of 5.8 ± 0.2, and then sterilised at 121 °C, under a pressure of 1 atm for 15 minutes. The cultures were incubated at a temperature of 22–25 °C, a humidity of 70–80% with a 16-hour photoperiod under light (with a light intensity of 1500–2000 lux provided by white fluorescent lamps) and 8 hours in the dark.

2.4 Data analysis

All data obtained from the experiments were analysed with the Excel software and the MiniTab21 tool. One-way ANOVA and Tukey's test were used to validate the data at $p < 0.05$.

3 Results and discussion

3.1 Effects of BAP and KN on Vanilla shoot multiplication

In this experiment, we aimed to determine the optimal concentration of BAP and KN for the proliferation of Vanilla shoots in the culture medium. Different concentrations of these two growth regulators in the MS medium were surveyed, and the results are presented in Table 1.

Table 1. Effects of BAP and KN on Vanilla shoot multiplication after 10 weeks of cultivation

Treatment	Concentration (mg/L)		Number of Shoot/sample (shoot)	Shoot height (cm)	Number of leaf/sample (leaf)
	BAP	KN			
Control	0	0	2.80 ± 0.67^{cdef}	2.71 ± 0.61^{cd}	4.22 ± 0.67^{fg}
V1	1	0	3.78 ± 0.67^{abc}	4.94 ± 0.64^{a}	5.44 ± 0.53^{de}
V2	2	0	3.67 ± 0.71^{abc}	3.44 ± 0.66^{bc}	5.56 ± 0.53^{cd}
V3	3	0	2.78 ± 0.67^{cdef}	3.45 ± 0.89^{bc}	4.56 ± 0.53^{ef}
V4	1	0.5	4.44 ± 0.88^{a}	4.25 ± 0.42^{ab}	7.67 ± 0.87^{a}
V5	1	1	3.89 ± 0.60^{ab}	4.64 ± 0.79^{a}	6.78 ± 0.67^{ab}
V6	1	1.5	3.44 ± 0.53^{abcd}	4.91 ± 0.68^{a}	6.44 ± 0.53^{bc}

Treatment	Concentration (mg/L)		Number of Shoot/sample (shoot)	Shoot height (cm)	Number of leaf/sample (leaf)
	BAP	KN			
V7	2	0.5	3.00 ± 0.50^{bcde}	2.49 ± 0.75^{cde}	3.44 ± 0.53^{gh}
V8	2	1	2.78 ± 0.97^{cdef}	1.81 ± 0.42^{def}	2.56 ± 0.53^{hi}
V9	2	1.5	2.44 ± 0.53^{defg}	1.81 ± 0.30^{def}	2.00 ± 0.71^{i}
V10	3	0.5	2.00 ± 0.42^{efg}	1.62 ± 0.51^{ef}	1.67 ± 0.50^{i}
V11	3	1	1.89 ± 0.60^{fg}	1.40 ± 0.37^{f}	1.67 ± 0.50^{i}
V12	3	1.5	1.56 ± 0.53^{g}	1.71 ± 0.72^{ef}	1.89 ± 0.79^{i}
Average			**2.96**	**3.01**	**4.14**
Standard deviation			**0.23**	**0.18**	**0.12**
CV%			**7.77**	**5.98**	**2.72**

Different letters (a–i) in the column indicate the significant differences among treatments (Duncan, $p \leq 0.05$). Data were presented as mean ± SD.

The experimental results show that the average number of shoots between treatments was from 1.56 to 4.44. In treatments without Kinetin (KN) in the medium (V1, V2, V3, and control), the optimal concentration of BAP for shoot proliferation was around 1 to 2 mg/L (with 3.78 and 3.67 shoots, respectively). Higher concentrations of BAP reduced the formation ability of vanilla shoots. 1 mg/L BAP in the medium was also the best concentration for shoot growth, with an average shoot height of 4.94 cm and an average number of leaves of 5.44 per sample (Table 1). These results are consistent to those reported by Abebe et al. [26], where 1 mg/L BAP in the MS medium optimally facilitated Vanilla shoot formation.

In micropropagation, the combination with an appropriate ratio of growth regulators would increase shoot multiplication as well as the quality of new shoots more than using only one type [27]. In this paper, the maximum average number of shoots was 4.44 in the MS medium supplemented with 1 mg/L BAP and 0.5 mg/L KN (Table 1, Treatment V4). The shoots were very healthy, green, and strong. This ratio was also optimal for the growth of Vanilla shoots in terms of number (4.44 shoots), height (4.25 cm), and average leaf number per shoot (7.76 leaves). When the concentration of BAP and KN in the medium was higher, the shoot formation ability decreased (Table 1). Unlike *Vanilla planifolia* in the results reported by Abebe et al. [26], *Vanilla* × *tahitensis* in our experiment requires lower KN concentration for optimal multiplication (0.5 mg/L compared with 1.5 mg/L in Abebe et al.'s report) [26].

The average leaf number per sample tended to increase when the BAP concentration in the medium increased from 1 to 2 mg/L (5.44 and 5.56 leaves, respectively) but declined at 3 mg/L of BAP in the medium (4.56 leaves). We could see that the leaf number was 7.67 per sample in Treatment V4 (the highest number of leaves) when the MS medium was supplemented with 1 mg/L of BAP and 0.5 mg/L KN. The leaf number then decreased as the BAP concentration increased from 2 to 3 mg/L, combined with the KN concentration from 0.5 to 1.5 mg/L in the culture medium. The leaf number was the lowest when 3 mg/L of BAP and 1 mg/L of KN were added to the MS medium (1.67 leaves only).

BAP is a potent cytokinin and is often used to stimulate lateral shoot growth and increase shoot multiplication rates in micropropagation. BAP strongly promotes shoot proliferation;

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however, excessive concentrations can inhibit the growth and development of shoots because of hormonal imbalance. A paper by Janarthanam and Seshadri [28] on *Vanilla planifolia* indicates that BAP alone in the culture medium increases the shoot multiplication rate compared with the controls. Previous studies on different species (*Crossandra infundibuliformis, Geoderum purpureum,* and *Curculigo orchioides*) also demonstrate that BAP is more effective than KN in increasing shoot multiplication rates [29–31]. However, the combination of BAP and KN in a specific ratio can achieve optimal results. Our experiment on *Vanilla* × *tahitensis* determined that adding 1 mg of BAP and 0.5 mg of KN in one litre of MS medium was the optimal ratio for the shoot formation rate (Table 1). Higher concentrations of KN (combined with BAP) were ineffective and could even lead to the inhibition of shoot development in the culture medium. Competition or antagonistic interactions between cytokinin types may inhibit each other's bioactivity, leading to "hormonal confusion" in tissue and thus growth inhibition [32, 33].

3.2 Effects of coconut juice on Vanilla shoot multiplication

In this study, the results of Treatment V4 (MS medium supplemented with 1 mg/L BAP and 0.5 mg/L KN) from Table 1 were used as a control. We investigated the effect of coconut juice at different concentrations (10, 20, and 30%) on the shoot multiplication of Vanilla. The results are shown in Table 2.

Table 2. Effects of coconut juice on the shoot multiplication of Vanilla after 10 weeks of *in vitro* cultivation

Concentration (%)	Number of Shoot/sample (shoot)	Shoot height (cm)	Number of leaf/sample (leaf)	Fresh weight (g)	Dry weight (g)
Control	4.44 ± 0.88^{ab}	4.25 ± 0.42^{a}	7.67 ± 0.87^{a}	1.400 ± 0.443^{a}	0.106 ± 0.032^{a}
10	4.98 ± 0.93^{a}	4.15 ± 0.38^{ab}	4.25 ± 0.93^{b}	1.281 ± 0.172^{ab}	0.072 ± 0.004^{b}
20	3.89 ± 0.60^{b}	3.23 ± 0.49^{b}	3.33 ± 0.78^{bc}	1.074 ± 0.154^{b}	0.060 ± 0.004^{b}
30	2.67 ± 0.50^{c}	2.58 ± 0.64^{c}	2.83 ± 0.71^{bc}	0.702 ± 0.038^{c}	0.057 ± 0.005^{b}
Average	**3.97**	**3.51**	**4.52**	**1.114**	**0.074**
Standard deviation	**0.21**	**0.11**	**0.09**	**0.172**	**0.014**
CV%	**5.29**	**3.13**	**1.99**	**15.44**	**18.92**

Different letters (a–c) in the column indicate the significant differences among treatments (Duncan, $p \leq 0.05$). Data were presented as mean ± SD.

As can be seen in the table, coconut juice in the culture medium affected the shoot formation ability of the vanilla sample compared with the control. Among the three investigated concentrations of coconut juice, only the control medium supplemented with 10% of coconut juice had a positive effect on the shoot multiplication rate (4.98 shoots compared with 4.44 shoots of the control). However, 10% of coconut juice in the medium had a negative impact on other parameters compared with the control (Table 2, Figure 1). Coconut juice is known as a natural substance with a high concentration of zeatin and is usually utilised in micropropagation protocols. It provides zeatin to the culture medium and thereby enhancing cytokinin activity for shoot proliferation. Several reports have also revealed the positive effects of coconut juice (5–20%) on the development and growth of other orchid species, such as *Dendrobium, Vanda sanderiana*, and *Grandiflora* spp. [34].

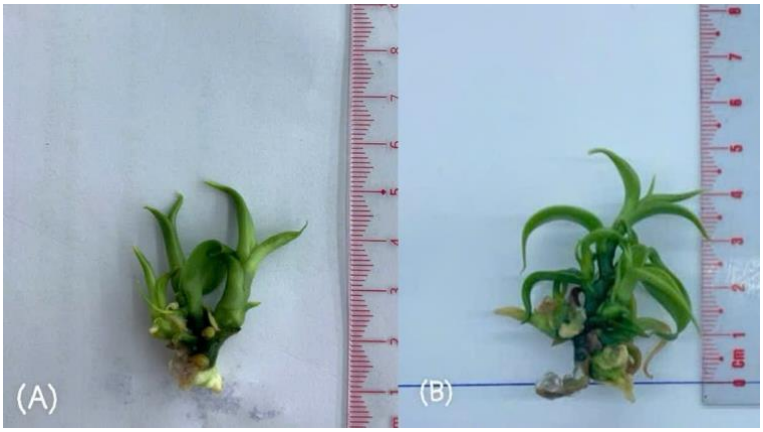

Fig. 1. Vanilla shoot regeneration in MS medium supplemented: 1 mg/L BAP + 0.5 mg/L KN (A); 1 mg/L BAP + 0.5 mg/L KN + 10% coconut juice (B)

3.3 Effects of gelling agents on Vanilla shoot multiplication rate

In this experiment, we investigated the effects of three different gelling agents, including plant agar, gelrite, and phytagel, on the shoot formation ability of the vanilla samples. The results in Table 3 indicate that among the three used gelling agents, gelrite (at the concentration of 3 g/L) produced the highest number of shoots per sample (4.67 shoots), followed by phytagel (4.44 shoots), while plant agar (8 g/L) only formed 2.33 shoots. This indicates that pure polysaccharide-based gelling agents like gelrite and phytagel are more effective than the others in supporting shoot formation. Gelrite and phytagel also had the same superior effect on other variables. Vanilla shoots cultivated in the gelrite medium developed the most significant average height (4.18 cm) and the most leaves per shoot (7.67 leaves), while the cultivation medium with phytagel produced much more biomass than the other (the fresh weight was 1.637 g and the dry weight was 0.102 g). This suggests that gelrite was better for shoot growth, but phytagel was better for biomass accumulation. Our results were more or less consistent with those reported by George and Ravishankar [35] on *Vanilla planifolia*. They also found the crucial function of gelrite in improving shoot multiplication of Vanilla. According to Patil and Neelannaver [36], alternative or mixed gelling agents like sago resulted in better shoot multiplication than agar. The clarity of gelrite is better than agar, which allows the light to penetrate deep into the culture medium. Furthermore, the very low iron content in gelrite also helps nutrients diffuse more effectively into the medium. These improved nutrient uptake as well increased shoot proliferation and rooting [37].

3.4 Effects of IBA and NAA on root formation capability of Vanilla

In this experiment, the standard-size shoots of vanilla from previous experiments were chosen from the shoot clusters and transferred to MS media supplemented with IBA and NAA at different concentrations to identify the optimal concentration for the induction of vanilla shoot. The results are presented in Table 4.

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Table 3. Effects of gelling agents on the shoot multiplication rate of Vanilla after 10 weeks of *in vitro* cultivation

Gelling agent	Concentration (g/L)	Number of shoot/sample (shoot)	Shoot height (cm)	Leaf number/sample (leaf)	Fresh weight (g)	Dry weight (g)
Plant Agar	**8**	2.33 ± 0.87^{b}	3.67 ± 0.58^{b}	4.11 ± 0.33^{c}	1.124 ± 0.045^{b}	0.052 ± 0.001^{b}
Gelrite	**3**	4.67 ± 0.87^{a}	4.18 ± 0.76^{a}	7.67 ± 0.87^{a}	1.475 ± 0.443^{a}	0.106 ± 0.032^{a}
Phytagel	**3**	4.44 ± 0.88^{a}	4.04 ± 0.42^{ab}	5.78 ± 0.67^{b}	1.637 ± 0.187^{a}	0.102 ± 0.021^{a}
Average		3.81	3.96	5.85	1.412	0.087
Standard deviation		0.009	0.17	0.27	0.20	0.01
CV%		0.24	4.29	4.61	14.16	11.49

Different letters (a–c) in the column indicate the significant differences among treatments (Duncan, $p \leq 0.05$). Data were presented as mean ± SD.

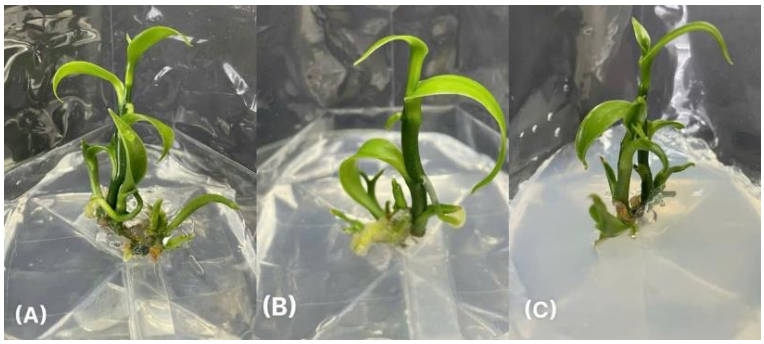

Fig. 2. Vanilla shoot regeneration in MS supplemented with gelling agent: 3 g/L gelrite (A); 3 g/L phytagel (B); 8 g/L plan agar (C)

Table 4. Effects of IBA and NAA on root formation capability of Vanilla after 10 weeks of *in vitro* cultivation

Treatment	Concentration (mg/L)		Number of root/sample (root)	Root length (cm)	Shoot height (cm)	Leaf number/sample (leaf)
	IBA	NAA				
R1 (Control)	0	0	1.55 ± 0.53^{bc}	2.56 ± 0.53^{c}	4.49 ± 0.29^{c}	3.52 ± 0.26^{d}
R2	1	0	2.00 ± 0.71^{b}	4.11 ± 0.33^{b}	5.02 ± 0.43^{bc}	4.54 ± 0.47^{bc}
R3	1	0.5	3.11 ± 0.78^{a}	5.11 ± 0.78^{a}	6.02 ± 0.40^{a}	5.63 ± 0.29^{a}
R4	1	1	1.89 ± 0.60^{bc}	3.78 ± 0.67^{b}	5.19 ± 0.33^{bc}	4.02 ± 0.42^{cd}
R5	2	0	1.39 ± 0.50^{bc}	3.67 ± 0.71^{b}	4.93 ± 0.42^{bc}	4.94 ± 0.69^{ab}
R6	2	0.5	1.44 ± 0.53^{bc}	3.67 ± 0.50^{b}	5.38 ± 0.69^{ab}	4.17 ± 0.67^{cd}
R7	2	1	1.11 ± 0.33^{c}	3.44 ± 0.53^{b}	4.45 ± 0.90^{c}	3.91 ± 0.66^{cd}
Average			1.58	5.21	3.67	3.86
Standard deviation			0.15	0.15	0.22	0.18
CV%			9.49	2.87	5.99	4.66

Different letters (a–d) in the column indicate the significant differences among treatments (Duncan, $p \leq 0.05$). Data were presented as mean ± SD.

Fig. 3. Vanilla shoot regeneration in MS medium supplemented with: 0 mg/L IBA + 0 mg/L NAA (A); 1 mg/L IBA + 0 mg/L NAA (B); 1 mg/L IBA + 0.5 mg/L NAA (C); 1 mg/L IBA + 1 mg/L NAA (D); 2 mg/L IBA + 0 mg/L NAA (E); 2 mg/L IBA + 0.5 mg/L NAA (F); 2 mg/L IBA + 1 mg/L NAA (G)

After 10 weeks of evaluation, the recorded results indicate that the presence of both IBA and NAA in the MS medium was better for the root formation process of vanilla shoot in terms of the root number and quality. We found that the MS medium supplemented with 0.5 mg/L of NAA and 1 mg/L of IBA was the best for the root formation (Treatment R3). The growth parameters of roots in this treatment were much more improved than in the others: 3.11 roots per shoot, 5.11 cm of root length, 6.02 cm of plant height, and up to 5.63 leaves per shoot (Table 4). Our findings are consistent with those reported in previous studies on the effect of growth regulators on root formation of Vanilla shoots [21–23, 38]. These studies confirmed that the optimal concentration of NAA for root stimulation was 0.5 to 1 mg/L in the medium.

In other treatments (R4, R6, R7), the presence of IBA and NAA in the MS medium at higher concentrations led to poorer results. As in Treatment R6, the MS medium supplemented with 2 mg/L of IBA and 0.5 mg/L of NAA resulted in the lowest number of roots (1.1 roots per sample), indicating that auxin at a concentration exceeding the optimal threshold in the medium inhibits root growth. Our results are consistent with those in previous research that reported that high auxin concentrations can lead to reducing root formation capability [39].

3.5 Survival rate of *in vitro* Vanilla plantlets in coconut coir pellets under nursery conditions

Following root induction, the complete plantlets of Vanilla from the culture medium were transplanted into coconut coir pellets and acclimatised under nursery conditions to evaluate their survival rate. The results show that after 3 weeks of acclimatisation, the survival of the plantlets was 100%, and all plantlets grew very well (Table 5).

The planting substrate plays a significant role in determining the survival, growth, and development of *in vitro* plantlets under nursery conditions. An appropriate substrate should provide sufficient porosity and moisture, stabilise pH, and maintain nutrients for root development.

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In our results, besides the complete survival rate of the plantlets, other variables, such as plant height and the leaf number, increased over time, from 6.99 cm (plant height) and 4.62 leaves at day 7 to 8.39 cm and 6.52 leaves at day 21 (Table 5, Figure 4). It demonstrates that the plants not only survived but also grew well morphologically in coconut coir pellets under nursery conditions. These results conform with previous findings [21–23]. *Vanilla planifolia* seedlings exhibit significant survival rates on a medium primarily composed of coconut coir mixed with other by-products (rice husk ash, peat, etc.) [14, 40].

Table 5. Survival rate of *in vitro* Vanilla plantlets in coconut coir pellets after 3 weeks under nursery conditions

Day after transfering	Survival rate (%)	Plant height (cm)	Leaf number (leaf)
Day 7	100	6.99 ± 1.80	4.62 ± 1.12
Day 14	100	7.39 ± 2.25	5.36 ± 1.24
Day 21	100	8.39 ± 2.75	6.52 ± 1.49
Average	100	7.67	16.5
Standard deviation	100	0.47	0.19
CV%	0	6.13	1.15

Different letters (a–c) in the column indicate the significant differences among treatments (Duncan, $p \leq 0.05$). Data were presented as mean ± SD.

Fig. 4. Vanilla seedlings after 3 weeks grown on decomposed coconut coir pellets: 7 days of acclimatization (A); 14 days of acclimatization (B); 21 days of acclimatization (C)

4 Conclusion

Our results indicate that the MS medium supplemented with 1 mg/L BAP, 0.5 mg/L KN, 100 mL/L coconut juice, and 3 g/L gelrite is the best for the regeneration of *Vanilla* × *tahitensis* shoots, with an average number of shoots per sample ranging from 4.44 to 4.98 and an average shoot height from 4 to 4.15 cm. Gelrite is considered the most suitable gelling agent with the optimal concentration of 3 g/L in the MS medium for shoot proliferation, producing an average of 4.44 shoots per sample. The MS medium supplemented with 1 mg/L IBA combined with 0.5 mg/L NAA is the most suitable for root formation of *in vitro* Vanilla shoots. After 3 weeks of acclimation on coconut coir pellets, the survival rate is 100%, and the plantlets achieve an average plant height of 8.39 cm and 6.52 leaves per plant. This is the first optimised protocol for

micropropagating *Vanilla* × *tahitensis*, and it is suitable for commercial production of this orchid variety in Vietnam. However, there are still numerous issues that need to be addressed. The occurrence of somaclonal variation during the propagation process has not yet been determined, and future studies should include ISSR markers to make it clear. Moreover, whether the vanillin content in tissue-cultured *Vanilla* × *tahitensis* orchids is higher than in conventionally propagated plants and also higher than in the *Vanilla planifolia* orchid still needs to be clarified in further studies.

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Author Contributions

NLNH, NKN, and TTKN collected and prepared samples, and performed experiments and data collection. NLNH, NKN and TNA analysed data, wrote and revised the manuscript. NKN supervised the project. All authors read and approved the final manuscript.

Declarations

All the authors have no interests to declare.

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