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Optimal condition for Propagation and Growing of *Dendrobium thyrsiflorum*

Nguyen Thi Oanh[1], Nguyen Thi Diem[1], Nguyen Huu Tho[1,2], Nguyen Thi Kim Cuc[1,*]

[1]Institute of Biotechnology, Hue University, Road 10, Phu Thuong, Phu Vang, Thua Thien Hue, Vietnam
[2]College Electro-Mechanics, Construction, and Agro-Forestry of Central Vietnam, QL1A, Cat Hanh, Phu Cat, Binh Dinh, Vietnam

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ABSTRACT

Dendrobium thyrsiflorum is a medicinal orchid that is being gradually destroyed due to over-exploitation. This research focuses on *in vitro* production of *D. thyrsiflorum* plantlet under optimal acclimatization conditions. The results of the study showed that the best medium for seed germination was MS basal medium (full-strength MS nutrient plus 100 ml L^{-1} coconut water; 7 g L^{-1} agar, 30 g L^{-1} sucrose, and 0.5 g L^{-1} active charcoal) supplemented with 60 g L^{-1} mashed sweet potatoes, which induces 97.8% of seed germination. The shoot was well developed in MS basal medium supplemented with 60 g L^{-1} mashed sweet potatoes and 0.4 mg L^{-1} 6-Benzylaminopurine (BA) and, corpulent and green in shoot morphology. The shoot multiplication rate was greatest on MS basal medium supplemented with 0.4 mg L^{-1} BA and 0.4 mg L^{-1} Kinetin with 4.53 times, and the shoot height was reported at 3.45 cm after 8 weeks of subculture. Further, The shoot was 100% rooting, with an average of 4.51 roots/shoot and 5.34 cm per root in length when the shoot was implanted on MS basal medium plus 60 g L^{-1} mashed sweet potatoes and 0.75 g L^{-1} active charcoal. Especially, plantlets after transplanting to the orchid net house reached a 94.8% survival rate on tree fern after 24 weeks. Hence, the results of the study suggested a successful production of *D. thyrsiflorum* plantlet on the selected media compositions.

* Corresponding author
E-mail: ntkcuc.huib@hueuni.edu.vn (T.K.C. Nguyen)

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1 Introduction

Vietnam is a tropical country with diverse natural resources, especially medical plants (Tran 1998). Among the medicinal herbs, orchids are one of the most important groups that have been researched so far. Different medicinal orchids such as *Dendrobium* species, *Gastrodia elata, Bletilla striata, Anoectochilus formosanus, A. koshunensis, Cremastra appendiculata, etc.,* were successfully identified (Bulpitt et al. 2007) and are used as a herbal medicine in Taiwan, Korea, Japan, China, Vietnam. etc., for treatment of various diseases (Pant 2013). Notably, some orchid species like *D. aphyllum, A. setaceus,* and *D. nobile* were successfully cultured under *in vitro* conditions that not only provided disease-free seedlings but also help in preserving these medicinal herbs. Recently, *D. thyrsiflorum* has been reported as a valuable medicinal plant (Bhattacharyya et al. 2015) since several phytochemicals including coumarins, polysaccharides, scoparone, and ayapin have been successfully isolated from this orchid (Yan et al. 2009; Liu et al. 2013; Tikendra et al. 2018) which help protect smooth muscles, blood vessels, and have anti-cancerous properties (Yan et al. 2009).

D. thyrsiflorum is considered abundantly distributed in China, India, Thailand, Myanmar, Laos, and Vietnam, previously (Li et al. 2013; Phan and Nguyen 2017; Dang et al. 2018). However, several research groups, recently, suggest that *D. thyrsiflorum* has been dramatically destroyed under its natural habitat, mainly due to the low rate of natural seed germination (<5%), human deforestation, and excessive illegal exploitation (Martin and Madassery 2006; Hossain et al. 2013; Da Silva and Ng 2017). To preserve and develop this species, some research groups tried to generate quality seedlings by *in vitro* propagation that focused on germination and *in vitro* production of plantlets in test tubes (Da Silva and Ng 2017; Chu and Dao 2018; Adhikari and Pant 2019; Maharjan et al. 2019; Lin et al. 2020) which is still far from a practical application.

D. thyrsiflorum accumulates different bioactive compounds especially, coumarins which have different pharmacological properties (Wu et al. 2009). Further, in *D. thyrsiflorum*, coumarins accumulated in different tissue from stems to roots as well as being present at different ages (Yan et al. 2009). Therefore, to preserve and prepare high-quality plantlets at different stages for comprehensive research to analyze different phytoconstituents in *D. thyrsiflorum* in Vietnam, the current study was carried out. The aim of this study was to rapid multiplication of *D. thyrsiflorum* under in vitro seed germination on different media compositions and to acclimatize the developed plantlets of *D. thyrsiflorum* after transplanting in a net house.

2 Materials and Methods

2.1 Plant materials and aseptic culture

Seed pod of six months old *D. thyrsiflorum* collected from Nam Tra My forest, Quang Nam, Vietnam was used as a sample in this study. All experiments were carried out at the Laboratory of Cells-Institute of Biotechnology, Hue University from November 2020 to October 2021; under the controlled condition with a temperature of $25^{o}C \pm 2^{o}C$, 8 hours of light photoperiod, and between 1000 and 2000 lux light intensity. The culture media used in this study were adjusted to pH 5.8 before autoclaving and then poured into special Polypropylene (PP) plastic bags with square bottoms with approximately 70 mL media/bag (Le et al. 2022). These bags were kept aseptic on the clean bench with paper clips until used.

2.2 Methods

2.2.1 Seed germination and protocorm formation

Six-month-old fruits of *D. thyrsiflorum* were gently washed twice with cotton wool and disinfected with soap under tap water. Further, in a sterile incubator, the fruits were thoroughly washed twice in sterile distilled water for about 90 seconds. Then, the fruits were dipped in 96% alcohol and shaken for 20 seconds before being burned over an alcohol lamp flame for three seconds (Le et al. 2022; Nguyen et al. 2022).

After sterilization, the fruits were placed in a petri dish before being cut into two parts longitudinally to collect seeds. The seeds were then spread evenly over the surface of the prepared basal MS medium (MS nutrition + 100ml L^{-1} coconut water + 30g L^{-1} sucrose + 0.5 g L^{-1} activated carbon + 6.5 g L^{-1} agar) or Knops basal medium (Knops nutrition + 100ml L^{-1} coconut water + 30g L^{-1} sucrose + 0.5 g L^{-1} activated carbon + 6.5 g L^{-1} agar) supplemented with 60 g L^{-1} sweet potatoes or potatoes (Nguyen et al. 2014; Nguyen et al. 2016).

There were four different combinations of media used in this experiment, each medium had three replications and each replication had 10 culture medium bags containing sterilized seeds. The germination and contamination rate was analyzed after 6 weeks of culture; the protocorm morphology was assessed after 8 weeks of inoculation, and the optimal medium in this experiment was used for subsequent experiments. The percentage of seed germination was calculated by (a/b)*100, where a is the number of culture medium bags containing germinated sterilized seeds, and b is the total bags of particular treatment. The formulated four different combination media are

Medium 1: Basal MS medium + 60 g L^{-1} potatoes

Medium 2: Basal MS medium + 60 g L^{-1} sweet potato

Medium 3: Basal Knops medium + 60 g L^{-1} potatoes

Medium 4: Basal Knops medium + 60 g L^{-1} sweet potato

2.2.2 Shoot formation from protocorm

The protocorm was inoculated on the optimal medium from the "seed germination and protocorm formation" experiment and added with BA at a concentration of 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg L^{-1} to evaluate the ability of shoot formation from protocorm. The percentage of shoot formation, shoot height, number of leaves/shoot, and morphological characteristics of the shoot were obtained after 6 weeks of culture. The percentage of shoot formation was calculated by (a/b)*100, where a is the number of shoots induced, and b is the total number of protocorms. The shoot height was measured using a tape measure and the average number of leaves per shoot was calculated. There were three replications for each treatment and each replication had 10 samples.

2.2.3 Shoot multiplication

In this experiment, the shoots were cultured on the optimal medium supplemented with the best concentration of BA from the "shoot formation from protocorm" experiment in combination with concentrations from 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg L^{-1} of KIN to evaluate shoot multiplication after 8 weeks of culture. The monitoring indicators include: shoot multiplication coefficient, shoot height, and morphological characteristics of shoots.

2.2.4 Rooting

Most of the plants required auxins to induce rooting. In the *in vitro* propagation process, authors widely used α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA) for shoot establish roots (Nguyen et al. 2012; Nong et al. 2016; Nguyen and Nguyen 2020). In addition, activated charcoal (AC) was added to the culture medium to improve cell growth as well as adsorb plant cell growth inhibitors. The role of AC has been demonstrated in the rooting of different plants (Nguyen et al. 2012). Therefore, in this experiment, we used the growth regulators, including Indole-3-acetic acid (IAA), NAA (Naphthalene acid acetic), and AC to determine the role of these substances in the rooting of *D. thyrsiflorum*.

After the shoot proliferation stage, shoots reaching a height of 2.5 to 3.0 cm were transferred to basal MS medium supplemented with IAA or NAA at a concentration of 0; 0.1; 0.3; 0.5 and 0.7 mg L^{-1} or AC with a concentration of 0.5; 0.75; 1 and 1.25 g L^{-1} for rooting. The rate of root induction in shoots, the number of roots, and the root length were identified after 6 weeks of culture.

2.2.5 Acclimatization plantlets

Plantlets with well-developed roots had 4.5-5 cm in height and 4-5 leaves which were removed under the controlled condition and acclimatized in the orchid net house for around two weeks to train gradually with the ambient environment before transplanting (Da Silva et al. 2017).

The orchid net house was about 60 m^2 in size and set up with an irrigation and shading system to avoid direct sunlight to plantlets and keep the temperature in the net house between 25^{o}C and 32^{o}C, light intensity was set between 1000 and 2000 lux, and about 12 to 16 hours light photoperiod.

In the transplanting process, plantlets were taken out from the culture bags and then washed thoroughly several times under tap water to remove the culture medium from the plantlet's roots before being grown on three different substrates to identify the survival rate and the development of plantlets after transplanting (Le at al. 2022). Plantlets were grown in pots (4x4cm) with a substrate consisting of coconut fiber/husk (1:1 ratio, w/w) (Nguyen et al. 2021), or mounted on milk apple wood (20x15cm) (Pradhan et al. 2014) or on tree fern (20x15cm) (Kang et al. 2020) with sphagnum moss.

Each treatment has three replications, each replication has 50 plantlets. The survival rate and growth indicator were analyzed after 24 weeks. Stem height was calculated from the base to the highest leaf using a tape measure.

2.3 Statistical analysis

Statistical analysis was performed using Microsoft Excel 2016, and by one or two ways analysis of variance (ANOVA) followed by Turkey's test, using the SPSS statistic 20.0 software (SPSS Inc., Chicago, IL, USA). Data represented significant differences as $p< 0.05$.

3 Results and Discussion

3.1 Germination of *D. thyrsiflorum* on Plant Growth Regulator-free medium

Results presented in Table 1 revealed that the starting medium had a significant effect on the germination of seeds after six weeks of sowing. The basal culture medium supplemented with mashed sweet potato or potato has a positive effect on *D. thyrsiflorum* seed germination (>80%) and the highest percentage of seed germination was 97.8% when seeds were cultured on basal MS medium (Medium 2) supplemented with 60 g L^{-1} mashed sweet potato. This figure was dramatically higher than those cultured on Medium 1 (86.0%), Medium 3 (85.7%), and Medium 4 (80.5%). Further, like seed germination, protocorm morphology was also

reported superior in Medium 2 and this was corpulent, round, and green in color (after eight weeks of sowing) which was better than those in other culture treatments (Figure 1b, c). Thus, Medium 2 is the most effective medium for *D. thyrsiflorum* orchid seed germination.

These results suggested that the *D. thyrsiflorum* seeds can germinate on the culture medium without plant growth regulator (PGR) supplemented, which will significantly contribute to reducing the cost of commercial plantlet production. In contrast to this, Tikendra et al. (2018) determined that the seeds of five-month-old *D. thyrsiflorum* germinated the best on the culture medium having different concentrations and combinations of BAP (BA), KIN, IBA (Indole-3-butyric acid), IAA, and NAA. In addition, previous studies have also identified that the PGR needs to be supplemented in the cultured medium to improve the seed germination rate, for example, *D. anosmum* had the best germination rate, reaching > 85% when cultured on MS basal medium supplemented with 1.0 mg L^{-1} BA and 0.2 mg L^{-1} NAA (Nguyen et al. 2021). Further, Le et al. (2020) reported that *D. adasatra* germinated on MS medium supplemented with 0.1 mg L^{-1} BAP at a rate of 98.5% after 6 weeks of culture, Asghar et al. (2011) reported that *D. noblie* germinated on the MS medium supplemented with 2.0 mg L^{-1} BAP. Hence, using MS basal medium supplemented by 60 g L^{-1} sweet potato could be an alternative medium for *Dendrobium* species seeds inoculation.

The microbial contamination rate was the most important figure to evaluate the success of the study. In this current study, this number was less than 5% which is an acceptable percentage in tissue culture (Cassells 1991).

Table 1 The influences of culture media on the seed germination and protocorm morphology

Time	6 weeks	8 weeks	Microbial contamination Rate (%)
Culture media	Germination rate (%)	Protocorm morphology (%)	
Medium 1	86.0[b]	Medium, light green	2.2
Medium 2	97.8[a]	Corpulent, green	2.2
Medium 3	85.7[b]	Corpulent, light green	2.2
Medium 4	80.5[c]	Medium, yellowest-green	4.4

Different letters on the same column indicate a statistically significant difference in the sample means with the p-value = 0.05 (Ducan's test)

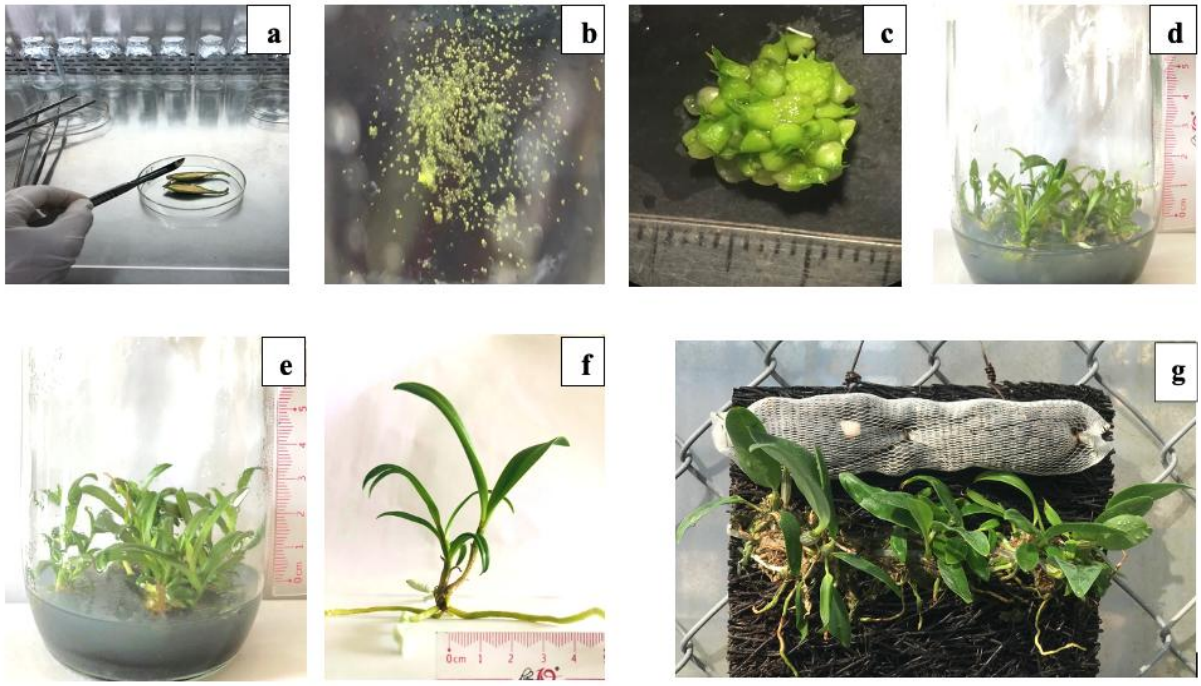

Figure 1 In vitro propagation of D. thyrsiflorum; (a) Collect seeds; (b), (c) Protocorm formation on medium 2; (d) Shoot development on medium containing 0.4 mg L^{-1} BA; (e) Shoot multiplication on medium adding 0.4 mg L^{-1} KIN and 0.4 mg L^{-1} BA; (f) Plantlets; (g) Plantlets growth in fern roots after 24 weeks.

Table 2 Effects of BA on the shoot formation from protocorm

BA (mg/L)	Shoot formation rate (%)	Shoot height (cm)	Number of leaves/shoot	Shoot morphology
0.0	64^{e}	1.17^{c}	2.40^{d}	Thin, light green
0.2	82^{d}	1.33^{b}	2.67^{cd}	Thin, green
0.4	98^{a}	1.53^{a}	3.67^{a}	Big, green
0.6	91^{b}	1.48^{ab}	3.27^{ab}	Big, green
0.8	84^{c}	1.29^{b}	3.07^{bc}	Medium, green
1.0	76^{d}	1.16^{c}	2.53^{d}	Thin, light green

Different letters on the same column indicate a statistically significant difference in the sample means with the p-value = 0.05 (Ducan's test).

Table 3 The effects of BA combined with KIN on the shoot multiplication capacity

BA (mg/L)	KIN (mg/L)	Numbers of shoots/shoot	Shoot height (cm)	Shoot morphology
0.4	0.0	2.40^{e}	2.24^{e}	Thin, green
0.4	0.2	3.67^{bc}	2.85^{c}	Medium, green
0.4	0.4	4.53^{a}	3.45^{a}	Big, green
0.4	0.6	3.93^{b}	3.02^{b}	Big, green
0.4	0.8	3.27^{bcd}	2.95^{bc}	Medium, light green
0.4	1.0	2.93^{d}	2.64^{d}	Thin, light green

Different letters on the same column indicate a statistically significant difference in the sample means with the p-value = 0.05 (Ducan's test)

3.2 Effects of BA on shoot formation from protocorm

As per the results of the first experiment, the basal MS medium containing 60 g L^{-1} sweet potato was used as the culture medium for this stage supplemented with various concentrations of BA to evaluate the formation and development of shoots from the protocorm. After six weeks of culture, protocorms were mostly induced to form shoots, and Table 2 clearly shows that the control medium (without BA addition) had the lowest average rate of shoot induction (64%) and the height of shoot length was only 1.17 cm, with 2.4 leaves/shoot, and light green shoots morphology while the increasing BA concentration from 0.2 to 0.6 mg L^{-1} in the medium led to a rise in the shoot formation rate.

Specifically, the concentration of 0.4 mg L^{-1} BA gave the best results on shoot growth and showed an average of 98% shoot formation, 1.53 cm of the average shoot height, and 3.67 leaves/shoot (Figure 1c). The shoot morphology in this medium was big and green. When gradually increasing the concentration of BA added to the culture medium from 0.8 to 1.0 mg L^{-1}, the percentage of shoot induction, the shoot height, and the number of leaves/shoot were reduced along with the smaller and light green color shoot body (1.0 mg L^{-1} BA). Maharjan et al. (2020) worked on the shoot proliferation in D. *chryseum* Rolfe and reported that ½ strength MS medium supplemented with 2.0 mg L^{-1} KIN has a significant effect on the shoot generation. Similarly, Nguyen (2021) identified that MS medium supplemented with 1.0 mg L^{-1} BA was good for shoot derived from *D. anosmum* protocorm. Meanwhile, Nguyen et al. (2016) combined three different PGRs in the medium culture (Basal Knops nutrient medium supplemented with 0.5 mg L^{-1} KIN + 0.3 mg L^{-1} BA + 0.2 mg L^{-1} NAA) to initiate shoot from the protocorm of *D. amabile* (Lour.) and suggested that each orchid species had a different requirement for their regeneration process.

3.3 Shoot multiplication

The combination of 0.0-1.0 mg L^{-1} KIN and 0.4 mg L^{-1} BA concentrations had a significantly positive effect on the shoot multiplication of *D. thyrsiflorum* (Table 3).

In general, the rate of shoot proliferation in all experiments was more than 2.4 shoots/shoot. The shoot multiplication gradually increased with the rise of KIN concentration from 0.0 mg L^{-1} to 0.4 mg L^{-1} in the culture medium. Remarkably, combining BA with KIN at a concentration of 0.4 mg L^{-1} gave the best results with a shoot multiplication number of 4.53 shoots/shoot with an average shoot height of 3.45 cm, and the proliferate shoots are big and the leaves are green in color (Table 3, Figure 1e). However, the shoot proliferation, as well as the shoot height, decreased substantially when the KIN concentration increased up to 1.0 mg L^{-1} (2.93 shoots/shoot; 2.64 cm in shoot height) and the leaves were light green, and the shoots were small and thin.

The use of BA and KIN for *D. thyrsiflorum* shoot proliferation in this study (4.53 shoots/shoot) gave better results as compared to the Tikendra et al. (2018) study conducted on the same species collected from India when they used KIN and IAA for shoot multiplication (3.83 shoots/shoot). The number was also higher

than those of *D. chryseum* shoot multiplication (3.73) shoot/shoots. On the other hand, for rapid multiplication of *D. amabile* (Lour.) shoot, Nguyen et al. (2016) used a combination of all three PGRs including KIN, BA & NAA, and it led to rapid multiplication and it is up to 12.57 shoots/shoot.

3.4 Effect of IAA, NAA and AC on rooting

The shoots reaching a height of 2.5 to 3.0 cm in the proliferation stage were transplanted to the rooting medium to generate plantlets. The base of MS medium containing 60 g L^{-1} sweet potatoes was supplemented with IAA or NAA at a concentration of 0.1-0.7 mg L^{-1} or adjusted with AC with a concentration of 0.5-1.25 g L^{-1} to evaluate the rooting ability of *D. thyrsiflorum* collected in Vietnam.

The growth regulator IAA and NAA both had a concentration-dependent effect on the rooting ability of *D. thyrsiflorum* orchid (Table 4). With regard to IAA, the concentration of 0.5 mg L^{-1} IAA gave the highest rooting response of 93%, which was significantly higher than those in control (82.93%), 0.1 mg L^{-1} (85.20%), 0.3 mg L^{-1} (87.87%); and 0.7 mg L^{-1} (91.30%), correspondingly. However, this percent was considerably lower than that of *D. thyrsiflorum* shoot inoculation on culture medium supplemented with 0.5 mg L^{-1} or 0.7 mg L^{-1} NAA with 100% of shoot promoting root. The shoot height and the number of roots per shoot in culture medium supplemented with 0.5 mg L^{-1} NAA was the best with 5.31 cm in root height and 4.4 roots/shoot. These number was greater than those in culture medium fortified with 0.7 mg L^{-1} NAA (5.28 cm in root height, and 3.6 roots/shoot) and 0.5 mg L^{-1} IAA (4.3 cm in root height and 3.53 roots/shoot), respectively (Table 4).

Table 5 shows that when gradually adding the amount of AC in the medium, the percentage of rooting and the root length increased, but the number of roots tended to decrease. At a concentration of 0.74 g L^{-1} AC, the rooting rate reached the highest with 100%, the root height was 5.34 cm, and the roots per shoot were 4.51. The rooting rate was also 100% in the medium having 1.0 g L^{-1} AC and 1.25 g L^{-1} AC but the roots height and the number of roots per shoot were significantly lower (Table 5). Hence, the addition of 0.75 g L^{-1} AC to the culture medium was most effective, and it helped the plantlets to grow and develop well, positively affecting the rooting ability of the plant (Figure 1f).

Comparing the effect on the rooting ability of IAA, NAA and AC, the results of this study revealed that when media supplementing NAA with a concentration of 0.5 mg L^{-1} or AC with a concentration of 0.75 g L^{-1}, the rooting rates were similar (100%). However, compared with the cost and optimal environmental conditions, it is better to replace NAA with an AC growth regulator. The use of AC in the medium was also studied by Nguyen et al. (2022) on *D. anosmum* Lindl rooting The authors determined that 1.0 g L^{-1} AC was the best for *D. anosmum* Lindl shoot initiating root with 3.17 roots/shoot which was lower than that of this current study. Therefore, the optimal environment to create plantlets for *D. thyrsiflorum* was basal MS medium supplemented with 60 g L^{-1} mashed sweet potato and 0.75 g L^{-1} AC.

Table 4 Effects of IAA and NAA on the rooting capacity of shoots

IAA (mg/L)	NAA (mg/L)	Rooting rate (%)	Root height (cm)	Number of roots/shoot
0		82.93^{e}	3.45^{c}	2.67^{b}
0.1		85.20^{d}	3.82^{b}	2.93^{b}
0.3		87.87^{c}	3.92^{b}	3.07^{ab}
0.5		93.00^{a}	4.30^{a}	3.53^{a}
0.7		91.3^{b}	4.05^{ab}	3.13^{ab}
	0.1	89.53^{c}	3.56^{d}	2.87^{c}
	0.3	95.13^{b}	3.90^{c}	3.20^{bc}
	0.5	100.00^{a}	5.31^{a}	4.40^{a}
	0.7	100.00^{a}	5.28^{ab}	3.60^{b}

Different letters on the same column indicate a statistically significant difference in the sample means with the p-value = 0.05 (Ducan's test)

Table 5 Effects of AC on the rooting capacity of shoots

AC (g/L)	Rooting rate (%)	Root height (cm)	Number of roots/shoot
0.50	83.33^{b}	3.54^{d}	2.73^{d}
0.75	100.00^{a}	5.34^{a}	4.51^{a}
1.00	100.00^{a}	5.02^{b}	3.86^{b}
1.25	100.00^{a}	4.05^{c}	3.33^{c}

Different letters on the same column indicate a statistically significant difference in the sample means with the p-value = 0.05 (Ducan's test).

Table 6 Effects of substrate cultures on the survival rate and growth indicator of plantlets

Substrate culture	Survival rate (%)	Stem height (cm)		Numbers of leaves/plantlets	
		Initial	After 24 weeks	Initial	After 24 weeks
SC1	82.2	4.5	7.3[b]	4	6.2[b]
SC2	87.8	4.5	7.4[b]	4	6.0[b]
SC3	94.8	4.5	7.9[a]	4	6.5[a]

Different letters on the same column indicate a statistically significant difference in the sample means with the p-value = 0.05 (Ducan's test)

3.5 The growth and development of plantlets after transplanting

After 24 weeks of growing on different substrate cultures, the plantlets were successfully acclimatized in the net house (Figure 1g**).** The survival rate was 94.8% when plantlets were grown on tree fern root mounts and mulched with sphagnum moss (SC3) which was 7% and 12.6% higher than those planted on pot substrate consisting of coconut fiber/husk (1:1 ratio, w/w; SC1), and on tree milk apple and mulched with sphagnum moss (SC2), respectively (Table 6). The stem height and the number of leaves per plant increased significantly when plantlets grown on SC3 were compared to the initial number and those in two other substrates. There was 7.9 cm in plant height and 6.5 leaves/plant on SC3 compared to 7.4 cm in plant height and 6.0 leaves/plant (SC2), and 7.3 cm in plant height and 6.2 leaves/plant (SC1) in turn.

The survival rate after transplanting in this study was substantially higher than that of Tikendra et al. (2018) study in this species with 91% after hardening in greenhouse conditions using a potting mixture of brick, charcoal species, and coconut husk (1:1:1). This identified that the substrate in this current study could be more efficient for *D. thyrsiflorum* plantlet growth than other substrates.

Conclusion

We have firstly investigated the optimal conditions for *in vitro* propagation of *D. thyrsiflorum* collected in Vietnam. The appropriate seeding medium was MS basal medium supplemented with 60 g L^{-1} sweet potato. The suitable medium for shoot development was MS basal supplemented with 60 g L^{-1} mashed sweet potato and 0.4 mg L^{-1} BA, the best rapid shoot multiplication was a combination of 0.4 mg L^{-1} BA and 0.4 mg L^{-1} KIN in the culture medium. 100% of the rooting shoot was identified in the medium with 0.75 g L^{-1} AC. Plants in the nursery on fern roots have a 94.8% survival rate.

Author contribution

TDN and TKCN designed the experiment. TDN, HTN, and TON carried out the experiments and performed data analyses. TDN and TON prepared all of the figures, and all authors contributed to data interpretation. TON wrote the first draft of the manuscript, and TDN and TKCN edited the draft. All authors reviewed the manuscript.

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Declaration of Competing Interest

The authors declare no competing interests.

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