

Effect of growth regulators and culture conditions for micropropagation of Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv) on a temporary immersion system

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ABSTRACT

Ngoc Linh ginseng is a valuable medicinal crop recognized for its anti-amnesia, anti-inflammatory, hepatoprotective, and anti-cancer properties. This study was to investigate the variation effect of growth regulators and culture conditions for micropropagation of Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.) on a temporary immersion system. After ten weeks of cultivation, the best callus induction rate in a solid Murashige and Skoog (MS) medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) 1.5 mg/L was 90%. Callus cultured on MS medium (indole-3-butyric acid (IBA) 0.7 mg/L) supplemented with Thidiazuron (TDZ) 0.8 mg/L resulted in the highest embryogenesis rate, reaching 69.29%, with an average of 11.25 embryos per explant. The MS medium supplemented with 6-benzylaminopurine (BAP) 1 mg/L and α -naphthalene acetic acid (NAA) 1 mg/L was shown to be appropriate for shoot growth from Ngoc Linh ginseng embryos. The temporary immersion system showed that with a 6-benzyladenine acid (BA) concentration of 1.5 mg/L for 3 min of immersion, the greatest value for shoot number was 6.00 shoots/explant, and the height was 2.70 cm. Roots developed best at a treatment with a 5 min of immersion and NAA 1 mg/L concentration, root formation frequency, and root length reaching values of (71.25%; 1.61 cm). As a result, the temporary immersion system (TIS) system can be used for high-efficiency *in vitro* propagation of Ngoc Linh ginseng plants.

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1. Introduction

In 1985, Ha & Grushvitsky (1985) discovered and identified Ngoc Linh ginseng as a new medicinal species with the scientific name *Panax vietnamensis* Ha et Grush. Since its discovery, Ngoc Linh ginseng (NLG) has been the focus of comprehensive investigation by domestic and

international institutions, particularly regarding its chemical constituents, therapeutic properties, and breeding techniques. Especially, Duong et al. (2013) have successfully cultivated NLG tissue *in vitro* in multiple investigations. However, cultivating NLG tissue is still complicated. The ability to multiply shoots is poor, and the

culture to produce a plant suitable for growing in the wild is still limited. Many scientists are continuing to develop the optimum methods for growing Ngoc Linh ginseng *in vitro*. Growth and development are determined by plant growth regulators (PGRs), which are responsible for cell division and growth. The PGRs (auxin and cytokinin) regulate the development of different plant organs during *in vitro* growth. Auxins and cytokinins also improve cellular division and induce the formation of adventitious shoots by decreasing apical dominance (Pierik, 1988). Temporary immersion systems (TIS) allow for control of contamination, adequate nutrient and oxygen supply, mixing, relatively infrequent subculturing, ease of medium changes, and limited shear damage. The TIS results in increased biomass with less labor and faster than conventional (semi-solid) techniques. Consequently, they have been adopted by many propagation and breeding programs to save costs and accelerate the production of plantation units of elite clones and/or the release of new cultivars (Watt, 2012).

Recent research by Jekayinoluwa et al. (2019) has demonstrated the use of automated TIS, which is less expensive and provides an ideal environment for *in vitro* plant cell, tissue, and organ cultures with high productivity. The TIS offers a flexible and perspective-oriented cultivation technology that could be adopted for the needs of large-scale production of secondary metabolites by plant *in vitro* shoot cultures. The usefulness of TIS and their positive effects in one or more stages of plant tissue proliferation have been reported for several easily multiplying species, such as *Ananas comosus* (Escalona et al., 1999), *Eucalyptus spp.* (Alister et al., 2005), *Psidium guaiava* (Vicchez & Albany, 2014), *Sorbus domestica* (Valeria & Francesco, 2024),

In TIS, the immersion times vary significantly, according to the research results of Maciel et al. (2016), who showed that long immersion times (1 h every 6 h) were efficient for potatoes, whereas very short immersion times (1 min every 12 h) stimulated somatic embryo production in *Coffea arabica* and *rubber* and increased *grapevine* shoot propagation (Pérez, 2013). Nevertheless, at the moment, to the best of our knowledge, there is a scarcity of studies using the TIS system on Ngoc Linh ginseng. The majority of studies focus on hair root biomass (Ha & Duong, 2017). Nguyen et al. (2020) used the TIS system to survey the effectiveness of embryo multiplication, shoot germination, and small taproot in a $1/2$ SH (Schenk & Hildebrandt) liquid medium. Simultaneously, no research has investigated the effects of the combination of growth regulators and immersion time on Ngoc Linh ginseng shoot propagation and rooting in an MS liquid medium on TIS.

2. Materials and Methods

2.1. Plant materials, chemicals, and reagents

The initial material used for the experiments was a rhizome of a 5-year-old Ngoc Linh ginseng provided by the Medicinal Plant Research Laboratory, Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City.

Composition of the culture medium used: The basic MS medium (Murashige & Skoog, 1962) was supplemented with 6-benzyladenine acid (BA), α -naphthalene acetic acid (NAA), Thidiazuron (TDZ), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), agar and sucrose for different culture experiments.

Origin of the temporary immersion system: In this study, the TIS system was designed according

to Escalona et al. (1999) with two glass containers of 1000 mL capacity. One for growing plants and a reservoir for liquid medium. The two containers were connected by silicone and glass tubes. The airflow was sterilized by passage through 0.2 μ m hydrophobic filters (Figure 1). Air pressure

from an air compressor pushed the medium from one container to the other to immerse the plants completely. The airflow was reversed to withdraw the medium from the culture container. Electronic timers controlled the frequency and length of the immersion period.

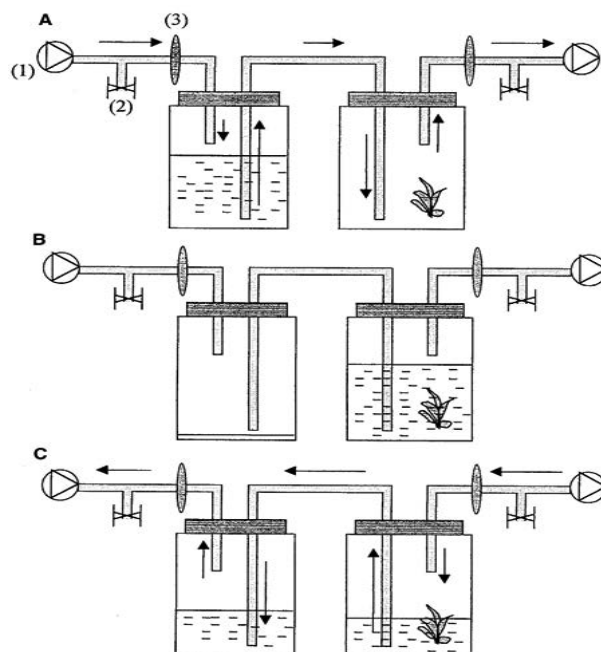


Figure 1. A–C Description of automated temporary immersion system. An (A) solenoid valve is opened, and compressed air forces medium into the plant container, immersing the plants (B).

(C) After a fixed time a second solenoid valve is opened, and air pressure forces medium back into the original container (A). 1 Air compressor, 2 solenoid valve, 3 hydrophobic filters (0.2 μ m) (Escalona et al., 1999).

2.2. Effect of 2,4-D and BAP on callus induction of Ngoc Linh ginseng

The initial material used for the experiments was a rhizome of 5-year-old Ngoc Linh ginseng. The rhizomes were sterilized and transplanted into MS medium using the sterile protocol described by Duong et al. (2010). Thereafter, the explants were used to conduct callus induction tests. The rhizome tissue was cut crosswise into thin, 1 mm-thick slices. Then it was cultivated in MS medium with 7 g/L agar and 3% sucrose. The experiment was set up completely randomized, with two

factors: concentrations of 2,4-D (0; 0.5; 1; 1.5 mg/L) and BAP (0; 0.5; 1.0; 1.5 mg/L). The experiment involved 16 treatments with 3 repetitions per treatment (9 explants per repetition). Explants were cultivated in an all-dark room.

2.3. Effect of TDZ concentrations on embryogenesis induction from callus tissue Ngoc Linh ginseng *in vitro*

Callus was cut into small pieces, transferred into a triangular vase containing MS medium (7 g/L) agar and 3% sucrose, and supplemented

with 0.7 mg/L IBA (indole-3-butyric acid). The experiment was completely randomized with one factor of TDZ at different concentrations (0; 0.2; 0.4; 0.6; 0.8 mg/L). The experiment involved 5 treatments and 3 repetitions (9 explants/repetition). All of the explants were cultured in a standard culture room at $22 \pm 2^\circ\text{C}$, illuminated at an intensity of 2000 lux for 10 h per day.

2.4. Effect of BA and NAA on shoot development of Ngoc Linh ginseng *in vitro*

The embryos from the embryogenesis induction experiment were continually transferred into a triangular vase containing MS medium, agar (7 g/L), and 3% sucrose, supplemented with BA at concentrations (0; 0.5; 1; 1.5 mg/L) and NAA (0; 0.5; 1; 1.5 mg/L). The experiment was arranged with 16 treatments and 3 repetitions (9 explants per repetition). All of the explants were cultured in a standard culture room at $22 \pm 2^\circ\text{C}$, illuminated at an intensity of 2000 lux for 16 h per day.

2.5. Effect of immersion time and growth regulators on shooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Shoots from the experiment (2.4) were about 0.5 cm in size and transplanted into the TIS system, which contained 150 mL of MS medium supplemented with 3% sucrose, two BA concentrations (0, 0.5, 1.0, and 1.5 mg/L), and a combination of immersion times (1, 3, and 5 min) with a 6-h immersion cycle. The study used 12 treatments and 3 repetitions (9 explants per repetition). Explants were cultivated in a standard culture chamber at $22 \pm 2^\circ\text{C}$, illuminated at 2000 lux for 16 h per day. Data on shoot formation was collected after 10 weeks of culture.

2.6. Effect of immersion time and growth regulators on rooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Shoots from the experiment (2.4) were about 0.5 cm in size and transplanted into the TIS system, which contained 150 mL of MS medium supplemented with 3% sucrose, two NAA concentrations (0, 0.5, 1.0, and 1.5 mg/L), and a combination of immersion times (1, 3, and 5 min) with a 6-h immersion cycle. The study used 12 treatments and 3 repetitions (9 explants per repetition). Explants were cultivated in a standard culture chamber at $22 \pm 2^\circ\text{C}$, illuminated at 2000 lux for 16 h per day. Data on root formation was collected after 10 weeks of culture.

2.7. Data analysis

All data were processed using Microsoft Excel software. Analyze One Way ANOVA and Two Way ANOVA with Minitab statistical processing software version 16.2.4 using Tukey's test method.

3. Results and Discussion

3.1. Effect of 2,4-D and BAP on callus induction

Studies in subjects of the genus *Panax* show that the callus initiation phase often involves a combination of cytokinins and auxins (Duong et al., 2010). Auxin, especially 2,4-D, plays an essential role in callus formation and proliferation at specific concentrations (Khan, 2019). The combination of BAP and 2,4-D as growth regulators have a positive effect on callus production (Hemmati et al., 2020).

After 6 weeks of culture (Figures 2a & 2c), the majority of the treatments began to exhibit the first signs of induction. Induction of callus formation occurs surrounding or on the surface of the explants. The callus is formed by cutting explants in contact with the medium supplemented with appropriate growth regulators (Yunita et al., 2021).

Table 1. Effect of 2,4-D and BAP on callus induction after 10 weeks of culture

Treatment	BAP (mg/L)	2,4-D (mg/L)	Callus induction frequency (%)
A1	0.0	0.0	-
A2		0.5	50.37 ^{ef}
A3		1.0	83.51 ^{ab}
A4		1.5	90.00 ^a
A5	0.5	0.0	-
A6		0.5	46.06 ^{fg}
A7		1.0	64.76 ^{cde}
A8		1.5	77.02 ^{abc}
A9	1.0	0.0	-
A10		0.5	43.94 ^{fg}
A11		1.0	57.12 ^{def}
A12		1.5	67.65 ^{bcd}
A13	1.5	0.0	-
A14		0.5	32.69 ^g
A15		1.0	54.94 ^{def}
A16		1.5	59.50 ^{def}
CV%			9.15

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. The percentage data were converted according to the formula $\arcsin\sqrt{(x/100)}$ before statistical processing. (-): The value of zero; 2,4-D: 2,4-dichlorophenoxyacetic acid; BAP: 6-benzylaminopurine.

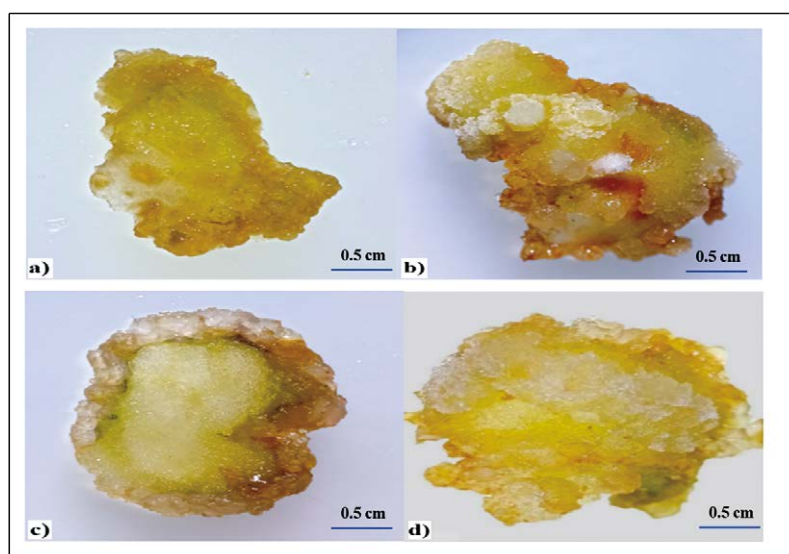


Figure 2. a, b) Callus after 6 and 10 weeks culture in treatment A8 (MS+ BAP 0.5 mg/L + 2,4-D 1.5 mg/L); c, d) Callus after 6 and 10 weeks culture in treatment A4 (MS + 2,4-D 1.5 mg/L). MS: Murashige & skoog; 2,4-D: 2,4-dichlorophenoxyacetic acid; BAP: 6-benzylaminopurine.

The results revealed that 2,4-D and BAP had an impact on the explant's development. After 10 weeks of culture, the whole explant's surface was covered with callus. However, for varying concentrations of plant growth regulators, the rate of callus formation varies with different treatments.

According to the data in Table 1, the frequency of callus formation in the medium supplied with 2,4-D differs significantly. When 2,4-D is added at concentrations ranging from 0.5 to 1.5 mg/L, the frequency of callus production steadily increases as the concentration increases. Based on observation, most calluses are white or yellowish-white. The growth regulators BAP and 2,4-D in the medium both impact the callus color. When auxin concentrations do not match cytokinins or the concentration of 2,4-D combined with BAP in the medium increases, the callus color changes, turning yellow to brown. The callus has a hard texture and an obvious brown surface. According to observations (Figures 2b & 2d), the callus induced with 2,4-D 1.5 mg/L had a yellowish-white and compact texture. More secondary metabolites can

accumulate in a compact callus texture (Indah & Ermavitalini, 2013) and the medium containing 2,4-D 1.5 mg/L had the highest frequency of callus development, at 90% (Table 1). Among all the growth regulators evaluated, 2,4-D gave the highest frequency of callus and somatic embryo formation in *Panax ginseng* (Zhong & Zhong, 1992; Arya et al., 1993) and *Panax Vietnamese ginseng* (Vu et al., 2015).

3.2. Effect of TDZ concentrations on embryogenesis induction from callus tissue Ngoc Linh ginseng *in vitro*

In addition to inducing shoot regeneration, cytokines also initiated somatic embryogenesis. It was reported in *Santalum album L.* that MS medium containing BA alone could induce a high frequency of somatic embryo differentiation (Akhtar & Shahzad, 2019). In recent years, there has been increased interest in the use of TDZ in the somatic embryogenesis regeneration of plants. TDZ, a synthetic phenylurea-type plant growth regulator, was initially thought to induce responses comparable to those generated by natural cytokinins. (Guo et al., 2011; Unal, 2018).

Table 2. Effect of TDZ on embryogenesis induction from callus of Ngoc Linh ginseng *in vitro* after 10 weeks of culture

Treatments	TDZ (mg/L)	IBA (mg/L)	Embryos induction frequency (%)	Average number of embryos (embryo/explant)
B1 (control)	0.0	0.7	33.33 ^c	3.17 ^d ± 0.43
B2	0.2	0.7	43.36 ^{bc}	4.33 ^{cd} ± 0.72
B3	0.4	0.7	54.89 ^{ab}	5.83 ^{bc} ± 0.43
B4	0.6	0.7	54.89 ^{ab}	6.25 ^{bc} ± 0.92
B5	0.8	0.7	69.29 ^a	11.25 ^a ± 0.55
B6	1.0	0.7	60.62 ^{ab}	7.50 ^b ± 0.58

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. TDZ: thidiazuron; IBA: indole-3-butyric acid.

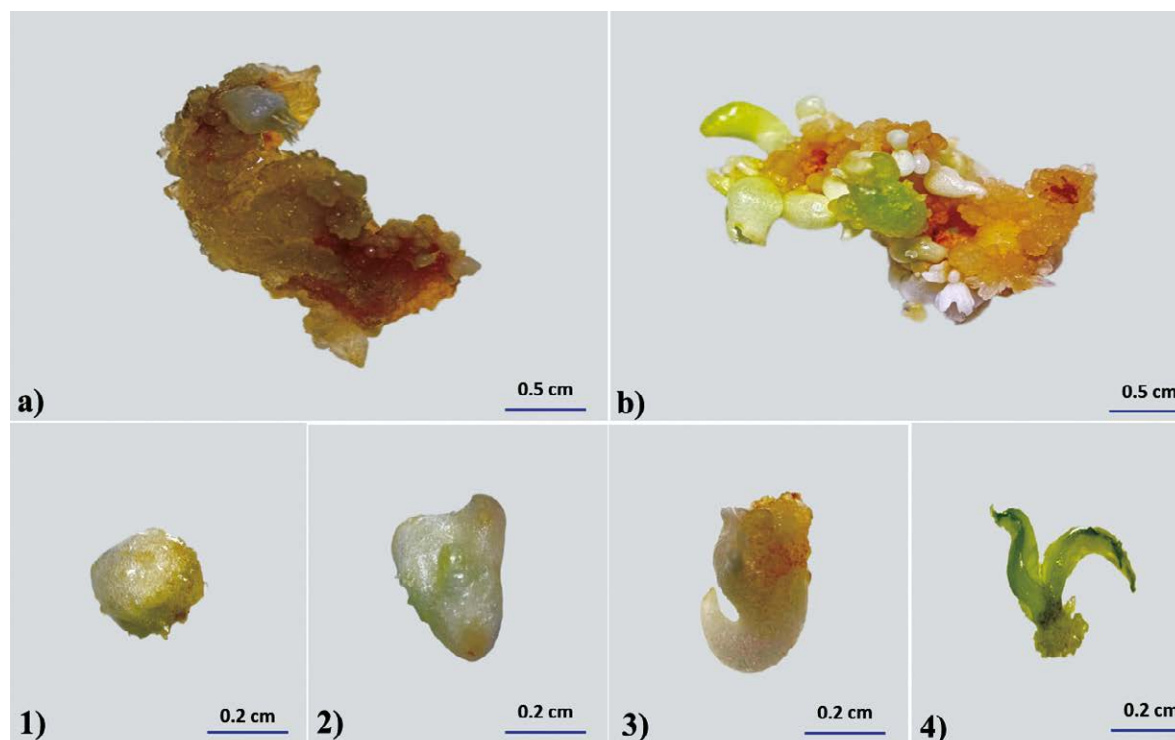


Figure 3. Embryogenesis of Ngoc Linh ginseng after 10 weeks culture. a) embryogenesis explant on medium MS + IBA 0.7 mg/L (control treatment); b) embryogenesis explant on medium MS + IBA 0.7 mg/L + TDZ 0.8 mg/L (1) Global shape, 2) Heart shape, 3) Torpedo-shaped, 4) Cotyledonary). MS: Murashige & skoog; TDZ: thidiazuron; IBA: indole-3-butyric acid.

After 10 weeks of culture, the results in Table 2 show that the embryogenesis frequency and average number of embryos in conditions supplemented with TDZ are significantly different from the control treatment. The embryogenesis frequency and average number of embryos increased with increasing TDZ concentration (0 - 0.8 mg/L), with the highest frequency observed in the B5 treatment. When MS medium was added with TDZ 0.8 mg/L and IBA 0.7 mg/L, the embryogenesis frequency reached 69.29%, with a number average of 11.25 embryos per explant. In B6 treatment (TDZ 1 mg/L and IBA 0.7 mg/L), the embryogenesis frequency and the number of embryos decreased. This shows that the auxin/cytokinin ratio is critical for embryogenesis

induction. At the same time, investigating embryo morphology in treatment B5 showed that the embryo developed through spherical, heart-shaped, torpedo-shaped, and cotyledon-shaped stages (Figure 3). In this study, the use of TDZ in the formation of embryos was achieved at low concentrations. This is in agreement with the study of Giridhar et al. (2004), which reported that low levels of TDZ stimulated direct somatic embryogenesis in coffee.

3.3. Effect of BAP and NAA on shoot development of Ngoc Linh ginseng *in vitro*

Cytokinins are substances that, when combined with auxin, promote the growth of young shoots and the formation of new shoot

apical meristems from the parenchyma. The growth regulator BAP is a cytokinin that helps increase cell growth when auxin is present in the environment. The frequency of shoot

induction varies according to whether the concentration of cytokinin supplied to the medium is appropriate (Bui, 2000).

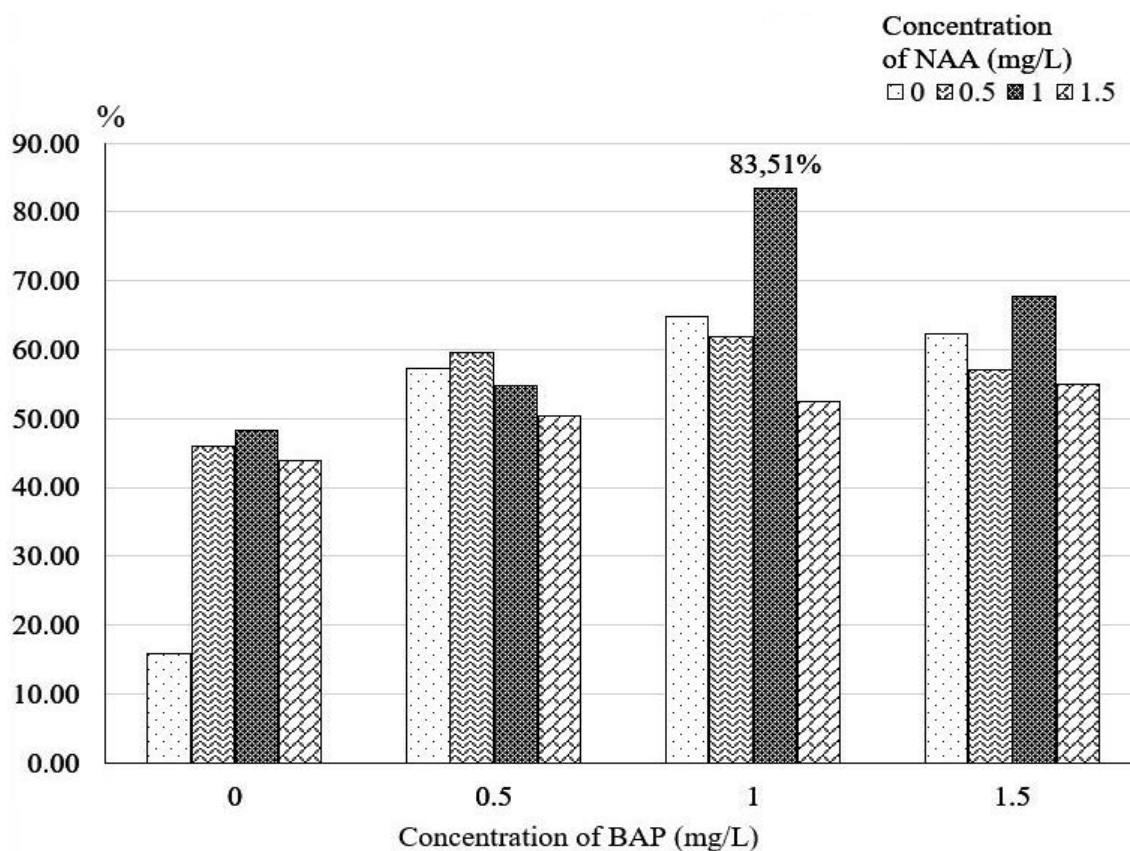


Figure 4. Frequency of shoot development from embryo explants of Ngoc Linh ginseng after 10 weeks of culture. NAA: α -naphthalene acetic acid; BAP: 6-benzylaminopurine.

Table 3. Effect of BAP (6-benzylaminopurine) and NAA (α -naphthalene acetic acid) on shoot development from embryo explants of Ngoc Linh ginseng in vitro after 10 weeks of culture

BAP (mg/L)	NAA (mg/L)	Number of shoots per explant	Shoot height (cm)
0.0	0.0	$0.30^h \pm 0.28$	$0.33^f \pm 0.29$
	0.5	$1.18^g \pm 0.28$	$0.63^f \pm 0.06$
	1.0	$1.44^g \pm 0.40$	$0.53^f \pm 0.06$
	1.5	$1.29^g \pm 0.06$	$0.67^f \pm 0.15$
0.5	0.0	$2.26^f \pm 0.17$	$1.23^e \pm 0.21$
	0.5	$2.59^{ef} \pm 0.07$	$1.47^e \pm 0.25$
	1.0	$3.00^e \pm 0.30$	$1.57^{de} \pm 0.12$
	1.5	$2.55^{ef} \pm 0.23$	$1.50^e \pm 0.10$
1.0	0.0	$4.44^{bcd} \pm 0.28$	$2.47^c \pm 0.15$
	0.5	$4.71^{bc} \pm 0.13$	$3.07^b \pm 0.12$
	1.0	$5.70^a \pm 0.24$	$3.57^a \pm 0.12$
	1.5	$4.85^b \pm 0.17$	$2.27^c \pm 0.25$
1.5	0.0	$4.00^d \pm 0.11$	$2.07^c \pm 0.12$
	0.5	$4.15^{cd} \pm 0.17$	$2.23^c \pm 0.06$
	1.0	$4.26^{bcd} \pm 0.23$	$2.37^c \pm 0.15$
	1.5	$3.85^d \pm 0.17$	$2.03^{cd} \pm 0.15$
CV		6.12	8.38

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$.

Shoot explants cultured on MS basal medium were used as controls; they showed signs of browning and failed to initiate shoots. Growth regulator application improved shoot responsiveness and induction. The frequency of shoot development, as well as the number and height of shoots, vary based on the concentration of plant growth regulators used. After 10 weeks of culture, the results of the experiment showed that increasing the concentrations of BAP and NAA from 0.5 to 1.0 mg/L increased the frequency of shoot development, the average number of shoots, and the average shoot height. This shows that cytokinin, when combined with auxin, increases the growth of young shoots and the production of new shoot apical meristems from the parenchyma. Several shoots were induced with the maximum frequency (83.51%) (Figure 4). The combination of both

growth regulators, BAP and NAA, yielded statistically significant differences in both the average number of shoots and the average shoot height. MS basic medium supplemented with the combinations BAP 1.0 mg/L and NAA 1.0 mg/L demonstrated the highest values, with 5.70 shoots/explant, and the maximum shoot height (3.57 cm) was observed (Table 3). It was most suitable for the shoot growth from the Ngoc Linh ginseng embryos. The result of this experiment is also similar to the findings of Duong et al. (2013), which reported that adding BA and NAA to the culture medium resulted in the highest number of shoots (6.3 shoots).

After the experiment investigating the development of shoots from Ngoc Linh ginseng embryos, a large number of plantlets were created. The roots were removed from the

plantlets, leaving only the shoots. These shoots were used as materials for the next experiment on the TIS system.

3.4. Effect of immersion time and growth regulators on shooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Cytokinin promotes cell division and cell expansion and growth is stimulated by adding a

cytokinin to a proliferation medium. Cytokinin induces the initiation and capability of axillary meristems which result in shoot formation.

In this way, different concentrations of one of the common cytokinin types of plant growth regulator (BA) were applied to effect (Kadota & Niimi, 2003). The results after 10 weeks of culture are shown in Table 4.

Table 4. Effect of immersion time and growth regulators on shooting of Ngoc Linh ginseng *in vitro* on TIS (temporary immersion system) after 10 weeks culture

Immersion Time (min)	BA (mg/L)	Number of shoots (shoot/explant)	Shoot height (cm)
1	0.0	1.96 ^e ± 0.17	0.38 ^g ± 0.04
	0.5	2.93 ^d ± 0.17	1.44 ^d ± 0.05
	1.0	3.26 ^d ± 0.28	1.57 ^c ± 0.04
	1.5	4.04 ^c ± 0.17	2.36 ^b ± 0.13
3	0.0	2.04 ^e ± 0.17	0.49 ^{fg} ± 0.04
	0.5	4.07 ^c ± 0.23	1.50 ^c ± 0.03
	1.0	5.29 ^b ± 0.17	2.68 ^a ± 0.08
	1.5	6.00 ^a ± 0.11	2.70 ^a ± 0.04
5	0.0	-	-
	0.5	2.18 ^e ± 0.23	1.33 ^d ± 0.04
	1.0	1.89 ^e ± 0.11	0.82 ^e ± 0.03
	1.5	1.22 ^f ± 0.22	0.63 ^f ± 0.04
CV%		5.79	3.37

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. (-): the value of zero. BA: 6-benzyladenine acid.

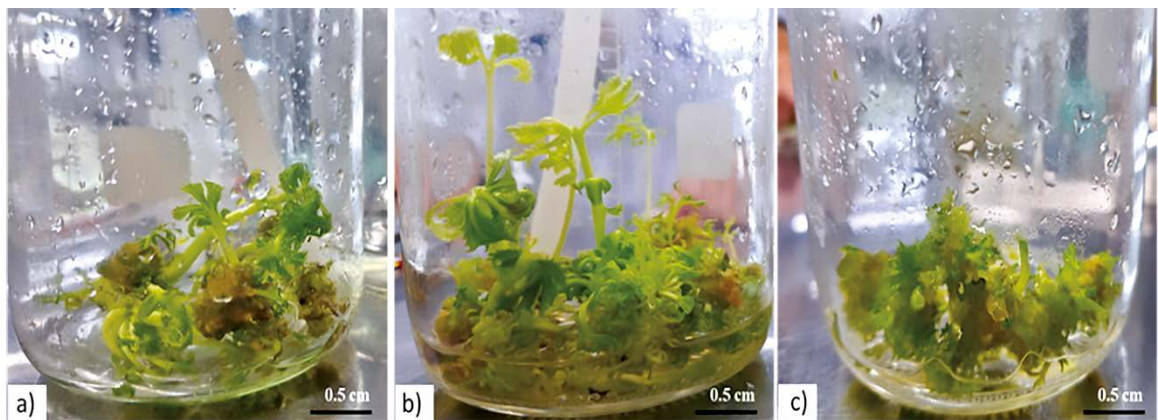


Figure 5. Shoots induction of Ngoc Linh ginseng at a concentration of 6-benzyladenine acid 1.5 mg/L combined with immersion time on temporary immersion system: a) 1 min; b) 3 min; c) 5 min.

The results in Table 4 and Figure 5 show that the number of shoots and shoot height at immersion times of 1 min and 3 min corresponded with additional BA concentrations ranging from 0.0 mg/L to 1.5 mg/L. Specifically, at BA 0 mg/L, there was no statistically significant difference between Ngoc Linh ginseng explants grown for 1 min or 3 min. However, as the BA concentration increased, the number of shoots and shoot height reached their highest at BA 1.5 mg/L within 3 min of immersion, giving 6.00 shoots and 2.70 cm. This observation aligns with the findings of Cung et al. (2007) research on the *Phalaenopsis hybrid*, suggesting that in the TIS system, the well-ventilated environment facilitates optimal growth due to continuous air exchange between the internal and external environments through the pump system and filter membranes. Conversely, at a 5-min immersion duration, the results demonstrated an inverse relationship with the increase in additional BA concentration. Both the number of shoots and shoot height declined compared to the 1-min and 3-min immersion times. Particularly in the absence of BA addition, no shoot formation occurred, with NLG shoots displaying signs of browning and failing to develop. This outcome parallels the findings of Silva et al. (2007), for the result observed the

highest number of shoots and shoot mass at a 3-min immersion duration in a 2-h cycle when investigating pineapple plant propagation on the TIS. The success of the TIS micropropagation protocol depends not only on the number of shoots obtained by explants but also on the morphological quality and vigor present in the seedlings produced (Martre et al., 2001).

3.5. Effect of immersion time and growth regulators on rooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Roots are an essential component of the plant, absorbing water, mineral salts, and nutrients for growth and development. In contrast to wild plants, *in vitro* plants are unlikely to be able to form roots on their own due to a lack of phytohormones (Nguyen & Nguyen, 2011). Therefore, when constructing the cultivation process, particular emphasis should be placed on nurturing the plant's root system and establishing an optimal environment conducive to root formation and development. The importance of auxin in root development is demonstrated in *in vitro* culture techniques. In the medium containing only auxin, the cultivated tissue only forms roots (Vu et al., 2009). The results after 10 weeks of culture are displayed in the table following:

Table 5. Effect of immersion time and growth regulators on rooting of Ngoc Linh ginseng *in vitro* on temporary immersion system

Immersion time	NAA (mg/L)	Frequency of root induction (%)	Number root per explant	Root length (cm)
1	0.0	-	-	-
	0.5	19.47 ^{ef}	0.11 ^{de} ± 0.00	0.37 ^e ± 0.06
	1.0	30.50 ^{cde}	0.26 ^d ± 0.06	1.29 ^b ± 0.02
	1.5	39.63 ^{bcd}	1.18 ^b ± 0.06	0.73 ^d ± 0.03
3	0.0	-	-	-
	0.5	15.86 ^{ef}	0.11 ^{de} ± 0.11	0.18 ^f ± 0.16
	1.0	50.37 ^{bc}	1.26 ^b ± 0.13	1.20 ^b ± 0.04
	1.5	59.50 ^{ab}	1.93 ^a ± 0.13	0.94 ^c ± 0.02
5	0.0	-	-	-
	0.5	25.24 ^{de}	0.53 ^c ± 0.03	0.42 ^e ± 0.09
	1.0	71.25 ^a	1.85 ^a ± 0.13	1.61 ^a ± 0.09
	1.5	54.74 ^{ab}	1.07 ^b ± 0.06	0.71 ^d ± 0.01
CV%			8.55	6.85

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. (-): the value of zero. NAA: α -naphthalene acetic acid.



Figure 6. Roots induction of Ngoc Linh ginseng at a concentration of α -naphthalene acetic acid 1 mg/L combined with an immersion time of 5 min/ 6 h on temporary immersion system; a) After 6 weeks of culture, b) After 10 weeks of culture. 1), 2), 3) Root morphological different types emerged.

Table 5 and Figure 6 show that root initiation of Ngoc Linh ginseng explants began about 4 to 6 weeks after transferring the rooting medium. The highest rooting induction frequency after 10 weeks of culture. The TIS showed minimal callus at the base of roots and a well-developed root system. During immersion times of 1, 3, and 5 min, all NAA concentrations ranging from 0.5 to 1.5 mg/L produced roots. At an NAA concentration of 1 mg/L, both 3-min and 5-min immersion resulted in root number values with no significant difference; however, root length was greatest at the 5-min immersion (1.61 cm) and had the highest root induction frequency of 71.25%. Therefore, the treatment with a 5-min immersion time and NAA 1 mg/L concentration is the best for Ngoc Linh ginseng root induction on the TIS. Also with the TIS system, Marco & Lourdes (2016) evaluated three bioreactor systems for *V. planifolia* micropropagation (multiplication and rooting). After 4 weeks of culture on the TIS system, the number and length of roots increased (4.25 roots and 2.41 cm) with an immersion frequency of 2 min every 4 h.

Finally, by combining appropriate cultural ventilation and occasional contact between shoots and the liquid medium, the microenvironment inside the temporary immersion container was improved, which contributed to improved shoot and root formation of Ngoc Linh ginseng. Plantlets of Ngoc Linh ginseng obtained in this study would probably have positive effects on ex vitro rooting and transplanting in large-scale commercial production. These results agree with those obtained in the micropropagation of other plant species using TIS systems (Escalona et al., 1999; Bernal et al., 2008; Marco & Lourdes, 2016).

4. Conclusions

The results of our study demonstrated the utility of the TIS for micropropagation of Ngoc Linh ginseng with the increased number of shoots and roots. In the current investigation, immersion time was 3 min every 6 h with a concentration of BA of 1.5 mg/L during shoot propagation for the best number of shoots (6.00 shoots/explant) and 2.70 cm in height. Simultaneously, at 5 min of immersion time and a concentration of NAA of 1 mg/L, the results showed the highest frequency of root (71.25%) and root length (1.61 cm).

Conflicts of interest

The authors declare no conflicts of interest.

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