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EFFECT OF CULTURE CONDITIONS ON THE GROWTH OF IN VITRO NGOC LINH GINSENG (*PANAX VIETNAMENSIS* HA ET GRUSHV.)

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SUMMARY

Ngoc Linh ginseng (*Panax vietnamensis* Ha *et* Grushv.) is a rare, precious, and endemic herb in Vietnam in terms of medicinal treasures. This herb's in vitro propagation has significant implications for both conservation and production. The promising research investigated how culture conditions can influence the growth of Ngoc Linh ginseng in vitro. Ngoc Linh ginseng plantlets have served as materials for research and culture. The effects of light intensities, day-night temperatures, and culture medium on the in vitro growth of plants received evaluation. The study found that the light intensity at 20 $\mu mol.m^{-2}.s^{-1}$, the day-night temperature of 23 °C–15 °C and the SH medium were optimal for the growth of Ngoc Linh ginseng in vitro. These results are essential for enhancing future in vitro Ngoc Linh ginseng growth.

Keywords: Ngoc Linh ginseng, light intensity, temperature, medium, in vitro propagation

Key findings: The latest study sheds light on certain primary culture conditions discovery directly affecting the growth of in vitro Ngoc Linh ginseng (*Panax vietnamensis* Ha *et* Grushv.) in Vietnam. The research successfully determined the optimal light intensities at 20 $\mu mol.m^{-2}.s^{-1}$, day-night temperatures of 23 °C–15 °C, and culture SH medium to grow in vitro Ngoc Linh ginseng. Furthermore, these findings could potentially improve the in vitro Ngoc Linh ginseng growth for production, preservation, and other extensive research in the future.

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INTRODUCTION

Ngoc Linh ginseng (*Panax vietnamensis* Ha *et* Grushv.), also known as Vietnamese ginseng, belonging to the family Araliaceae*,* is a rare, valuable, and indigenous species of ginseng in Vietnam. This ginseng has long been prominent and beneficial in traditional medicine as a precious herb with anti-stress, antioxidant, immune system boosters, cancer prevention, liver protection, and other therapeutic benefits. Based on many previous studies, Ngoc Linh ginseng contains various pharmaceutical compounds, of which saponins are the most significant components. In particular, dammarane saponin is abundant in Ngoc Linh ginseng compared to the other ginsengs. Furthermore, the M-R2 fraction in the ocotillol-type saponin group accounts for 50% of the total saponin content and has the most in *Panax* species (Hoang *et al*., 2012).

The earlier-mentioned characteristics have made Vietnamese ginseng a precious medicinal plant valued in Vietnam and worldwide. However, owing to overexploitation, Ngoc Linh ginseng has become among 250 high-risk extinction species in Vietnam's Red Data book, requiring protection (Duong *et al.,* 2013). Ngoc Linh ginseng grows primarily in the Ngoc Linh mountainous area of Tu Mo Rong district, Kon Tum province, and Nam Tra My district, Quang Nam province. This ginseng grows well when living in the natural forest canopy of the mountainous regions of Ngoc Linh, where it has the ideal conditions such as light intensity, day-night temperature, soil, and other factors (Nhut *et al.,* 2016). Ngoc Linh ginseng naturally grows at 1800–2000 m altitudes as a hygrophyte and shade-loving plant. It typically grows beneath the forest canopy, along moist streams on humus soil, and under a cloud cover that reaches 80% or more.

Moreover, Vietnamese ginseng is a native plant that prefers cooling climates. The average daytime temperature is from 20 °C to 25 °C, whereas the nighttime temperature ranges from 15 °C to 18 °C (Tordoff *et al.,* 2000; Dinh *et al.,* 2021). Furthermore, ginseng only grows well in Ngoc Linh mountainous areas, and harvesting takes a very long time, between five to eight years, given that ginseng roots need to contain sufficient active ingredients to meet plant demands (Vu *et al.*, 2009). The previously discussed natural living conditions of Ngoc Linh ginseng make it a difficult-to-grow herb plant species, especially when propagating in vitro.

The growth of herbal plant species, specifically in vitro ginseng, receives direct influences from light intensity, day-night temperature, and culture media. This is due to their impact on the biosynthesis of primary and secondary metabolites, which influences plant growth and development (Jie, 2003; Zoratti *et al.,* 2014). For the former, numerous studies have demonstrated that excessive or low light intensity affects different processes, including morphology, photosynthetic physiology, secondary metabolite production, and more. As a result, every plant has a spectrum of light intensities that is ideal for its growth and development (Ma *et al.*, 2015; Junqian and Baolin, 2016). Likewise, temperature is another vital environmental factor affecting plant growth and development. Every plant species has a suitable temperature range to grow and develop. In other words, certain species will only grow within a specific temperature range. A temperature that is too high or too low is harmful to plants. Particularly, excessive temperature will affect the chlorophyll content in leaves, which influences photosynthetic function, while low temperature could halt metabolic activity. Generally, night temperatures are lower than daytime temperatures, and this temperature change regulates a plant’s growth pattern and metabolism. Therefore, the difference in day-night temperature is a factor that needs controlling in herbal plants (Ohtaka *et al.,* 2020). Finally, media, particularly SH and MS medium, are vital elements that directly affect the growth and development of Ngoc Linh ginseng. Regarding SH, this medium comprises chief ingredients from the MS medium plus vitamins, amino acids, and inositol.

These ingredients in SH medium provide and balance more nutrients for difficult-to-grow and sensitive plants like ginseng. Numerous studies have shown that this medium can provide optimal conditions for

culturing Ngoc Linh ginseng and support the plant to adapt effectively to the microbial environment. The nitrogen concentration is the primary difference between MS and SH media (Duong *et al.,* 2013). The concentrations of $NO_3/NO_3^+NH_4$ in the SH medium were lower than those in the MS, and it is critical to consider that the MS had a larger ratio of ammonium and nitrate ions, which impacts plant growth.

In vitro, ginseng propagation demands similar cultured conditions as the natural conditions of the Ngoc Linh mountainous area, including light intensity, day-night temperature, culture media, and so on, for it to grow well. However, the previously mentioned culture conditions have no existing studies for in vitro ginseng. Based on the empirical issues discussed above, the objectives of this study sought to determine some vital culture conditions and culture medium that affect the growth of Ngoc Linh ginseng in vitro. The aim is to assist the plant in adapting to its natural conditions and having a high vitality, thereby contributing to the production and preservation of precious medicinal herbs for Vietnam.

MATERIALS AND METHODS

Plant material and procedure

In vitro Ngoc Linh ginseng (*Panax vietnamensis* Ha *et* Grushv.) provision by the Division of Plant Biotechnology - Biotechnology Center of Ho Chi Minh City, Vietnam comprised six months old, with two to three leaves measuring 1.5 to 2 cm.

Culture of Ngoc Linh ginseng in vitro and methods to evaluate effects of culturing condition commenced on five plantlets into an Erlenmeyer flask containing SH medium (Schenk and Hildebrandt, 1972, Duchefa, Netherlands, code: S0225), supplemented with kinetin (PGRs) (Cat#KB0745, Biobasic, Canada) at a concentration of 0.2 mg/L and 130 mL/L of coconut water. Growing the plantlets was for specific investigating conditions of light intensities, day-night temperatures, and culture media in the Percival CU-36L4 growth chamber (Percival Scientific, Inc., America). The completely randomized experiment had three replications, conducted by transplanting 15 plantlets into three Erlenmeyer flasks for each replication. After 12 weeks of culture, recording particular indicators included the number of leaves, roots, shoot height, and chlorophyll content.

Light intensity effect on growth of in vitro Ngoc Linh ginseng

The plantlets' cultivation consisted of varying light intensities (5, 10, 15, 20 $\mu mol.m^{-2}.s^{-1}$), 12 h of light per day, 23 °C day and 18 °C night temperatures, and 55%–65% day and night humidity. The completely randomized experiment with three replications on 15 plantlets transplanted into three Erlenmeyer flasks per replication had data recorded and analyzed for the number of leaves, roots, shoot height, and chlorophyll content after 12 weeks.

The number of leaves, roots, and shoot height calculation proceeded on the average of 15 plantlets for each replication. Measuring the height of a shoot of 12-week-old ginsengs began on the longest leaf in three replications. Chlorophyll content measurement employed the Chlorophyll Content Meter (Hansatech Instruments Ltd, Korean) on the second leaf with three replications.

Temperature effect on growth of in vitro Ngoc Linh ginseng

The plantlets cultured at day-night temperatures comprised 23 °C–18 °C, 23 °C–15 °C, and 23 °C–13 °C, with a light intensity of 20 $\mu mol.m^{-2}.s^{-1}$, 12 h of light per day, and a day-night humidity of 55%–65%, conducted with three replications of the completely randomized experiment. Some indicators described above also incurred recording and analysis after 12 weeks.

Culture media effect on growth of in vitro Ngoc Linh ginseng

Culturing of in vitro plantlets into an Erlenmeyer flask containing MS medium (Murashige and Skoog, 1962, Duchefa, Netherlands, code: M222) and SH medium

(Schenk and Hildebrandt, 1972) received supplements of kinetin at a concentration of 0.2 mg/L and 130 mL/L of coconut water. The plantlets culturing included 20 $\mu mol.m^{-2}.s^{-1}$, 12 h of light per day, day and night temperatures of 23 °C and 15 °C, respectively, and day-night humidity (55%–65%). The completely randomized experiment with three replications had the abovementioned indicators recorded and analyzed after 12 weeks.

Statistical analysis

The results were the mean of three repeated experiments. All the data underwent statistical analysis using the Statgraphics Centurion XV software. Statistical significance assessment used the one-way ANOVA Tukey-LSD at $P < 0.05$ considered significant.

RESULTS AND DISCUSSION

Light intensity effect on growth of in vitro Ngoc Linh ginseng

After 12 weeks, significant differences appeared in growth rates between ginseng plantlets cultured at different light intensities under day-night temperature conditions of 23 °C–18 °C. More specifically, indicators, such as the number of leaves, roots, shoot height, and chlorophyll content, rose gradually and peaked at 20 $\mu mol.m^{-2}.s^{-1}$ of light intensity. Moreover, in this study, the coefficient of variation (CV), the ratio of standard deviation to mean, is low, which means the estimate was more precise (Table 1). Under the light intensity of 20 $\mu mol.m^{-2}.s^{-1}$, the shoots grew well with dark green leaves and developed many roots with uniform sizes. Contrastingly, the plantlets cultured in lower light intensities exhibited less rooting and chlorophyll content, resulting in less chlorophyll and unequal shoot sizes (Figure 1). The plantlets developed more calli at a low light intensity. This result shows that the light intensity at 20 $\mu mol.m^{-2}.s^{-1}$ is suitable for the growth of Ngoc Linh ginseng.

Light is a vital environmental factor that affects plant morphogenesis and photosynthesis through plant receptors. It also participates in the biosynthesis of primary and secondary metabolites that influence plant growth and development (Jie, 2003; Zoratti *et al.,* 2014). Certain plant species, like bayberry trees, do not grow under high light intensity. Light irradiance decreases photosynthetic rates (Guo *et al.,* 2006). Consequently, all plants have relied upon their particular ranges of optimal light intensity for growth and development. In particular, irregular light levels influence several physiological processes, including morphology, photosynthetic physiology, and secondary metabolite production, to investigate the light intensity effect on ginseng growth. Jang *et al.*

Table 1. Effect of light intensities on growth of in vitro Ngoc Linh ginseng.

Light intensity ($\mu mol.m^{-2}.s^{-1}$)	Number of leaves	Number of roots	Shoot height (cm)	Chlorophyll content ($nmol/cm^2$)	Morphological characteristics
5	3.47^{b}	1.23^{c}	2.13^{b}	1.37^{ab}	Straight leaves; light green - dark green; short shoots; many calli.
10	2.8^{c}	1.4^{c}	2.13^{b}	$1,27^{b}$	Straight leaves; dark yellow - green; short shoots; many calli.
15	3.47^{b}	1.97^{b}	2.3^{ab}	1.3^{b}	Straight leaves; light yellow - green; tall shoots; few calli.
20	3.67^{a}	2.2^{a}	2.47^{a}	1.47^{a}	Straight leaves; green - dark green; tall shoots; almost no callus.
CV (%)	10.46	29.96	8.11	7.41	

Different letters (a, b, …) represent statistically significant differences at $P < 0.05$ (Tukey's test).

Figure 1. Growth of in vitro Ngoc Linh ginseng at different light intensities after 12 weeks. *A: 5 µmol.m^{-2}.s^{-1}; B: 10 µmol.m^{-2}.s^{-1}; C: 15 µmol.m^{-2}.s^{-1}; D: 20 µmol.m^{-2}.s^{-1}*

(2021) conducted a study on the morphogenesis and photosynthetic accumulation of ginseng of the genus *Panax* cultured under different light intensities from 25 to 150 µmol.m^{-2}.s^{-1}. The study findings indicated that the shoots flourished well at 25 µmol.m^{-2}.s^{-1} light intensity. Additionally, the ability of the plants to absorb chlorophyll was optimal at this light intensity and subsequently decreased as the light intensity increased. Therefore, it is evident that an overly intense light culture would likewise result in excessive light stimulation, harming the D1 protein that shields chlorophyll from absorption by the plant (Aro *et al.,* 1993).

Conversely, if the experiment's light intensity were too low, it would prevent photosynthesis, hindering the plant's ability to grow well and produce more calli. The stimulated cells from the division process generate the calli when lacking light. It is a fact that dark conditions support callus formation. Another example is *Epimedium,* which is a shade-tolerant species but depends critically on light intensity for its survival, early growth, photosynthetic apparatus development, and secondary metabolites production (Gottschalk, 1994; Wang *et al.,* 2007; Müller *et al.,* 2013). Therefore, certain plant species will reduce their photosynthetic ability under high light intensities and vice versa. Conversely, lower light will affect photosynthesis physiology; thus, each plant species will have an optimal light intensity (Junqian and Baolin, 2016).

Temperature effect on growth of in vitro Ngoc Linh ginseng

Another relevant environmental factor influencing plant growth and development is temperature. Since increasing temperatures significantly affect plant growth and development, it is critical to comprehend the mechanism underlying high-temperature effects on herbal plants. Plants regulate their growth depending on their environmental conditions. Temperature is a vital factor that directly affects plant growth. Each plant species has a suitable temperature range to grow and develop. Temperatures that are too high or too low can both cause a halt in metabolic activity and modify the amount of chlorophyll in the leaves, which, in turn, affects photosynthetic function. Therefore, a particular temperature range restricts the growth ability of certain species (Ohtaka *et al.,* 2020).

The number of roots and chlorophyll content reached the highest level at day-night temperatures of 23 °C–15 °C (Table 2). The

Table 2. Effect of day-night temperatures on growth of in vitro Ngoc Linh ginseng.

Day-night Temperature (°C)	Number of leaves	Number of roots	Shoot height (cm)	Chlorophyll content (nmol/cm^2)	Morphological characteristics
23-13	2.9^b	2.37^b	2.3^b	1.27^c	Straight leaves; light yellow - dark green; short shoots; no callus.
23-15	3.73^a	2.73^a	2.57^a	1.63^a	Straight leaves; green - dark green; tall shoots; almost no callus.
23-18	3.67^a	2.2^b	2.47^a	1.43^b	Straight leaves; green - dark green; tall shoots; almost no callus.
CV (%)	16.51	4.23	8.25	12.13	

Different letters (a, b, …) represent statistically significant differences at $P < 0.05$ (Tukey's test).

Figure 2. Growth of in vitro Ngoc Linh ginseng at various day-night temperatures after 12 weeks. *A: 23 °C - 13 °C; B: 23 °C - 15 °C; C: 23 °C - 18 °C.*

shoots cultivated at both day-night temperatures of 23 °C–18 °C and 23 °C–15 °C pairs showed healthy growth and dark green leaves. The plantlets cultured at 23 °C–15 °C had more developed and uniformly sized roots. More accurately, ginseng roots reached the highest and most significant difference at the day-night temperature of 23 °C–15 °C compared with other temperatures. Plantlets cultured at lower temperatures of 23 °C–13 °C grew unwell (Figure 2). No callus formation emerged in this experiment. This result indicates that the day-night temperature of 23 °C–15 °C is ideal for Ngoc Linh ginseng growth.

Temperatures within this range typically influence shoot growth, including leaf expansion and stem elongating and thickening. However, an increase in temperatures over the optimal range suppresses plant growth. The difference in day-night temperature also affects growth besides the absolute temperature. Generally, daytime temperatures are higher than night temperatures, and plants adapt their growth and metabolism to these temperature variations. One method to control plant growth in herbal plants is to alter the difference in day-night temperature (Ohtaka *et al.,* 2020). A recent study showed that each light quality, irradiance, and photoperiod

enables the production of plants with desired characteristics because it affects the proliferation and plantlet quality, including shoot length, fresh and dry weight, and photosynthesis (Cavallaro *et al.,* 2022). Similar to the results in this experiment, Wang *et al.* (2019) studied the effect of temperature on the roots of *Panax ginseng*. The authors cultured the roots of *Panax ginseng* in the dark at temperatures of 15 °C, 20 °C, 25 °C, and 30 °C, with the highest number of roots of ginseng at 15 °C and the lowest at 30 °C.

Medium effect on growth of in vitro Ngoc Linh ginseng

All indicators and parameters, such as the number of leaves, roots, shoot height, and chlorophyll content, were higher in cultured plantlets under the SH medium than in cultured plantlets under the MS medium (Table 3). Although the height was not significantly higher between the two media, the shoots in the SH medium developed better, with greener leaves, indicating higher chlorophyll and more rooting. However, the height is nonsignificantly different (Figure 3). Specifically, the average number of leaves in the MS medium is 2.93, while those in the SH medium are significantly higher at 3.73. Still, the shoot height in the MS medium is similar to the SH medium. The average number of roots in the MS is 2.73 compared with 2.47 in the SH. A marked disparity between the chlorophyll content of the leaf in the SH and MS medium occurred, with the former being higher than that of the latter. During the culture process, it was also evident that Ngoc Linh ginseng in the SH medium formed less callus than in the MS medium.

Ngoc Linh ginseng, a difficult-to-grow plant, requires special conditions to develop well. The SH medium includes basic ingredients from the MS medium plus vitamins, amino acids, and inositol. These ingredients provide and balance more nutrients for difficult-to-grow and sensitive plants, such as Ngoc Linh ginseng. Various studies have demonstrated that the SH medium, which assists in balancing the ratio of growth hormones, such as auxin and cytokinin, can maintain the growth and development of ginseng during process culture. This medium can provide optimal conditions for culturing Ngoc Linh ginseng and support the plant's good adaptation to the microbial environment. The prime difference between the MS and the SH media is nitrogen concentration, according to a study by Duong

Table 3. Effect of MS and SH medium on growth of in vitro *Panax vietnamensis***.**

Medium	Number of leaves	Number of roots	Shoot height (cm)	Chlorophyll content (nmol/cm^2)
MS	2.93	2.47	2.5	1.5
SH	3.73*	2.73*	2.57	1.63*

*significant difference at a 0.05 probability level.

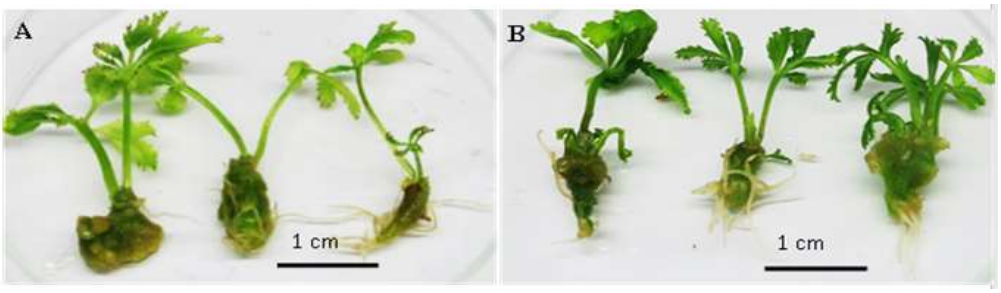

Figure 3. Growth of in vitro *Panax vietnamensis* in MS and SH medium after 12 weeks. *A: MS medium, B: SH medium.*

et al. (2013). The concentrations of ammonium and nitrate ions were lower in the SH medium than in the MS, and the ratio of $NO_3/NO_3^+NH_4$ of the MS was higher. It proved that these salt ions had changed the pH of the medium during the process of culturing on the MS medium, causing damage to plants.

Besides, the SH medium has also shown evidence to support well in rooting Ngoc Linh ginseng, which is crucial for the propagation and culture of mother plants to produce more seedlings. This result is consistent with previous studies, which showed that the SH medium is suitable for the growth of in vitro ginseng. Cuong *et al.* (2021) conducted an experiment wherein the culture of plantlets on the SH medium investigated the seedling quality. Following a 12-week culture period, the recorded indicators related to roots, including stem diameter (cm), length (cm), fresh weight (g), and SPAD (nmol/cm^2) of seedlings bore comparison. However, since this plant needs more optimal conditions to achieve the best culture and propagation efficiency, further research and optimization of the SH medium are essential.

CONCLUSIONS

In summary, this study initially determined certain suitable culture conditions for the growth of Ngoc Linh ginseng in vitro. These conditions include the light intensity of 20 µmol.m^{-2}.s^{-1}, day-night temperature of 23 °C–15 °C, at which Ngoc Linh ginseng can grow well, defining the SH medium as more appropriate. Furthermore, Ngoc Linh ginseng is a difficult-to-grow plant that requires special conditions identical to natural conditions to flourish. These results indicate potential in vitro Ngoc Linh ginseng growth for production, preservation, and further extensive research to meet the medicinal demand.

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