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EFFECTS OF NITROGEN SOURCE AND WAVELENGTH OF LED-LIGHT ON ORGANOGENESIS FROM LEAF AND SHOOT TIP CULTURES IN *LYSIONOTUS PAUCIFLORUS* MAXIM.

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Abstract

Effect of nitrogen sources on *in vitro* culture of *Lysionotus pauciflorus* Maxim. was investigated using 4 variants of Murashige and Skoog (MS) medium, each with varied nitrogen composition and concentration: MS (1650 mg l^{-1} NH_4NO_3 + 1900 mg l^{-1} KNO_3), MS3B (825 mg l^{-1} NH_4NO_3 + 995 mg l^{-1} KNO_3), MS4 (1900 mg l^{-1} KNO_3), and MS5 (1900 mg l^{-1} KNO_3 + 1751 mg l^{-1} $NaNO_3$). When leaves were cultured on these variants of medium each containing 1 mg l^{-1} BAP and 0.5 mg l^{-1} NAA, adventitious shoot regeneration frequencies of MS, MS3B, MS4, and MS5 were 66.7, 53.3, 33.3, and 0%, respectively, and mean number of adventitious shoots was 8.9, 5.0, 0.9, and 0, respectively. When shoot tips were cultured on MS, MS3B, and MS4 without supplementation of any plant growth regulator, they elongated to give node numbers of 4.4 - 4.6, whereas MS5 gave less growth with node number of 2.4. Consequently, means of shoot length were 23.6, 28.7, 27.8, and 4.7 mm in MS, MS3B, MS4, and MS5, respectively. Effect of light quality on *in vitro* culture of leaf explants was also examined on MS containing 0.5 mg l^{-1} NAA in combination with or without 1 mg l^{-1} BAP by incubating at 25 ± 2°C under continuous light from light emitting diodes (LEDs) with peak wavelengths of 470 nm (blue), 590 nm (orange), 625 nm (red), and from white LEDs at 40 µmol m^{-2} s^{-1} or darkness. Adventitious shoots were regenerated from all leaf explants cultured under LED-light, whereas adventitious shoot regeneration was completely inhibited under the dark condition. The highest number of regenerated shoots was 30.4 when cultured on medium supplemented with 1 mg l^{-1} BAP and 0.5 mg l^{-1} NAA under red LED-light. The highest frequency of long adventitious shoot (> 5 mm) formation was also obtained under red LED-light. Under orange LED-light, shoot differentiation was suppressed as compared with that under the other peak-wavelength LED-light.

Key words: adventitious root regeneration, adventitious shoot regeneration, Gesneriaceae, lighting

INTRODUCTION

Lysionotus pauciflorus Maxim. (Gesneriaceae) is a plant with high ornamental value due to its beautiful attractive flowers. Moreover, it has attracted high attention as a medicinal plant, which has been used for lymph node tuberculosis, cough with tachypnoea, and rheumatic pains (Liu et al. 1996, 1998) by Yi minority of Yunnan Province in China rather than as an ornamental plant. Recently over collection for medicinal purposes is becoming a threat to the natural habitats. Therefore, it is necessary to produce large amount of nursery plants through *in vitro* culture to fulfill the demand in China. To date, micropropagation of this species by tissue culture technique has been reported only on plants native to Japan (Haruki and Inamura 2003) and Yunnan Province, China (Lu et al. 2006, Godo et al. 2010).

For improving productivity of nursery plant by tissue culture technique, many researchers have studied the culture conditions such as type of basal medium, combination and concentration of plant growth regulators, type and concentration of sugars, culture temperature, and illumination. Nitrogen is one of the main

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elements contributing to the plant growth. The form of nitrogen, as NH_4^+ or NO_3^-, has a dramatic influence on the morphogenic response of plant tissue culture (Bhojwani and Razdan 1996). Halperin and Wetherell (1965) reported that wild carrot calluses induced on a medium with KNO_3 as the sole source of nitrogen failed to form somatic embryo. Hassan et al. (1991) described that little or no shoots regenerated from leaf explants of pear on media lacking NH_4^+. In contrast, Ivanova and Van Staden (2009) described negative effects of NH_4^+ on regeneration and growth of new shoots of *Aloe polyphylla*.

Light is also an important environmental factor affecting plant growth, and therefore, the effect of light on *in vitro* plant growth has been widely investigated. Since the light emitting diode (LED) has recently showed drastic development, it became possible to evaluate the effect of light quality on plant growth by using several types of LEDs, each with a different peak wavelength. However, much of the research on the light wavelength has been carried out so far by using only red and blue LEDs (Moreira da Silva and Debergh 1997, Lian et al. 2002, Poudel et al. 2008, Shin et al. 2008, Baque et al. 2010, 2011).

In the present study, we aimed to clarify effects of nitrogen source and wavelength of LED-light on tissue cultures as the basis for establishment of high productivity micropropagation system of *L. pauciflorus*.

MATERIALS AND METHODS

Plant materials

In vitro plants of *Lysionotus pauciflorus* native to Yunnan Province, China, were used as the source of explants in this study. They were maintained in glass tubes, with half-strength MS (Murashige and Skoog 1962) medium (half-strength MS micro- and macroelements, full-strength MS organic components, and 20 g l^{-1} sucrose) without supplement of any plant growth regulators (PGRs). The medium was solidified with 2 g l^{-1} gellan gum (Phytagel; Sigma Chemical Co., St. Louis, USA). These cultures were incubated at 25 ± 2ºC under 16 h cool white fluorescent lighting at a photon flux density of 40 µmol m^{-2} s^{-1}, daily.

Culture medium for nitrogen source experiment

Four variants of the medium, MS (1650 mg l^{-1} NH_4NO_3 + 1900 mg l^{-1} KNO_3), MS3B (825 mg l^{-1} NH_4NO_3 + 995 mg l^{-1} KNO_3), MS4 (1900 mg l^{-1} KNO_3), and MS5 (1900 mg l^{-1} KNO_3 + 1751 mg l^{-1} $NaNO_3$), all purchased from Duchefa Biochemie B.V., Netherlands, were used as basal ones. MS was original composition of Murashige and Skoog (1962) medium. MS3B, MS4, and MS5 were modified variants of MS medium with different combination and concentration of three nitrogen sources, NH_4NO_3, KNO_3, and $NaNO_3$ (Table 1). All variants of the medium were solified with 2 g l^{-1} gellan gum and adjusted to pH 5.8 before autoclaving at 121°C for 15 min.

Leaf culture for nitrogen source experiment

Leaves of about 1 cm in length excised from *in vitro* plants were individually placed onto 10 ml of MS, MS3B, MS4, and MS5 variants of the medium each supplemented with or without 1 mg l^{-1} 6-benzylaminopurine (BAP) and 0.5 mg l^{-1} α-naphthaleneacetic acid (NAA) in each glass tube (20 × 140 mm) covered with polypropylene cap. The cultures were incubated at 25 ± 2ºC under 16 h photoperiod with white fluorescent lamps (FF40SS EX-N/37-A; Hitachi, Tokyo, Japan) at 40 µmol m^{-2} s^{-1}. The experiment was conducted once using 15 leaves in each medium. Frequency of adventitious shoot regeneration, number of regenerated adventitious shoots, and frequency of roots, formed from leaves were counted after one month of culture.

Shoot tip culture for nitrogen source experiment

Shoot tips with two nodes of about 5 mm length were excised from *in vitro* plants maintained on half-strength MS medium without any PGRs, and six or seven shoot tips were placed on 100 ml of PGR-free MS, MS3B, MS4, and MS5 variants of the medium in a 450 ml glass bottle. The glass bottles were covered with a methylpentene cap and cultured at 25 ± 2ºC under 16 h photoperiod with fluorescent lamps at 40 µmol m^{-2} s^{-1}. The experiment was conducted once using more than 40 shoot tips in each medium. Node number and shoot length were measured after 10 weeks of culture.

Leaf explant culture for LED lighting experiment

Two variants of MS medium supplemented with 1 mg l^{-1} BAP and 0.5 mg l^{-1} NAA, or 0.5 mg l^{-1} NAA alone, were used in the LED lighting experiment. The pH of all variants of the medium was adjusted to 5.8. After autoclaving at 121ºC for 15 min, fifty ml of the medium was placed in each Petri dish (9 cm diameter). Leaves excised from *in vitro* plants were cut perpendicularly into two segments at the midrib, and cultures were kept under light or dark.

After inoculating the explants as described above, Petri dishes were sealed with Parafilm™ (American Can Company, Chicago, IL, USA) and incubated at 25 ± 2°C under continuous light of 40 µmol m^{-2} s^{-1} from LEDs with peak wavelengths of 470 nm (blue), 590 nm (orange), 625 nm (red), and from white (sharp peak at 460 nm with a broad peak at 560 nm) LEDs or darkness (Fig. 1). The experiment was conducted once using ten leaf explants for lighting condition and twenty leaf explants for dark condition. Frequency of explants with adventitious shoot regeneration, number of regenerated adventitious shoots, and frequency of explants

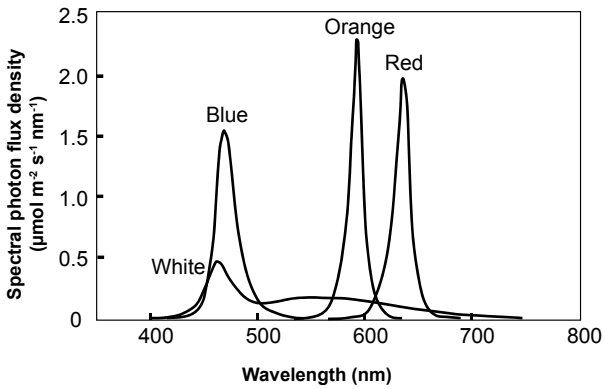

Fig. 1. Spectral profiles of white, blue, orange, and red light emitting diodes at 40 μmol m^{-2} s^{-1}.

with adventitious root regeneration were counted after 2 months of culture.

Statistical analysis

Data except regeneration frequencies of adventitious shoot and root were analyzed using ANOVA and correlation analysis was performed using software Statview (Abacus Concepts, Inc., Berkeley, CA, USA). Fisher's PLSD was used to compare means.

RESULTS AND DISCUSSION

Effect of nitrogen source on leaf culture

Adventitious shoot regeneration from leaf explants was observed within 2 weeks after initiation of culture. The frequencies of explants with adventitious shoot regeneration on PGR-free variants of medium MS, MS3B, MS4, and MS5, were 13.3, 6.7, 0, and 0%, respectively, and the means of adventitious shoot number were 1, 0.9, 0, and 0, respectively (Table 1). In contrast, on variants of medium containing 1 mg l^{-1} BAP and 0.5 mg l^{-1} NAA, the frequencies of shoot regeneration of MS, MS3B, MS4, and MS5 were 66.7, 53.3, 33.0, and 0%, respectively, and the mean number of shoots was 8.9, 5.0, 0.9, and 0, respectively (Table 1). These results suggest that the most suitable basal medium for adventitious shoot regeneration from leaf explants of *L. pauciflorus* was MS with original combination and concentration of nitrogen source (1650 mg l^{-1} NH_4NO_3 + 1900 mg l^{-1} KNO_3), regardless of presence of PGRs (Table 1). Tsai and Saunders (1999) also described that the mixed nitrogen source of MS was superior or equal to any single nitrogen source for shoot and leaf disk cultures of sugar beet.

The number and length of shoots regenerated from cotyledonary explants of mung bean were reduced by high concentration of NH_4^+ (Gulati and Jaiwal 1994). In the present study, however, both the regeneration frequency and the number of adventitious shoots produced on NH_4^+-lacking variants of the medium (MS4 and MS5) were low compare to those on NH_4^+-containing variants of the medium (MS and MS3B) (Table 1). Thus, NH_4^+ might be an important element for adventitious shoot regeneration from leaf explants of *L. pauciflorus*. Essential role of NH_4^+ on adventitious shoot regeneration has also been reported in apple (Fasolo et al. 1989, Predieri et al. 1989), garlic (Nagakubo et al. 1993), and pear (Hassan et al. 1991) although the responses of tissue culture to NH_4^+ varied from species to species.

Adventitious root formation was observed on all variants of the medium without applying any PGRs, but was inhibited on medium containing 1 mg l^{-1} BA and 0.5 mg l^{-1} NAA (Table 1). MS4, which included only KNO_3 as a nitrogen source, showed a high adventitious root formation frequency of 53.3%, and the frequencies on the other variants of the medium, containing NH_4NO_3 or $NaNO_3$, were low. These results suggest the negative effect of NH_4^+ and Na^+ on root formation of *L. pauciflorus*.

Table 1. Effects of type and concentration of nitrogen sources and plant growth regulators on regeneration of adventitious shoot and root from leaves of *Lysionotus pauciflorus* after one month of culture in 16 h lighting.

Medium	NH_4NO_3 (mg l^{-1})	KNO_3 (mg l^{-1})	$NaNO_3$ (mg l^{-1})	BAP (mg l^{-1})	NAA (mg l^{-1})	Explants with shoots (%)	No. of shoots	Explants with roots (%)
MS	1650	1900	-	-	-	13.3	1.0 ± 1.6 c	6.7
				1.0	0.5	66.7	8.9 ± 9.9 a	0.0
MS3B	825	950	-	-	-	6.7	0.9 ± 1.2 c	13.3
				1.0	0.5	53.3	5.0 ± 4.3 b	0.0
MS4	-	1900	-	-	-	0.0	0.0 ± 0.0 c	53.3
				1.0	0.5	33.3	0.9 ± 1.8 c	0.0
MS5	-	1900	1751	-	-	0.0	0.0 ± 0.0 c	13.3
				1.0	0.5	0.0	0.0 ± 0.0 c	0.0

Values represent the mean ± SD. Different letters among the means in the same column indicate significant differences at the 0.05 level.

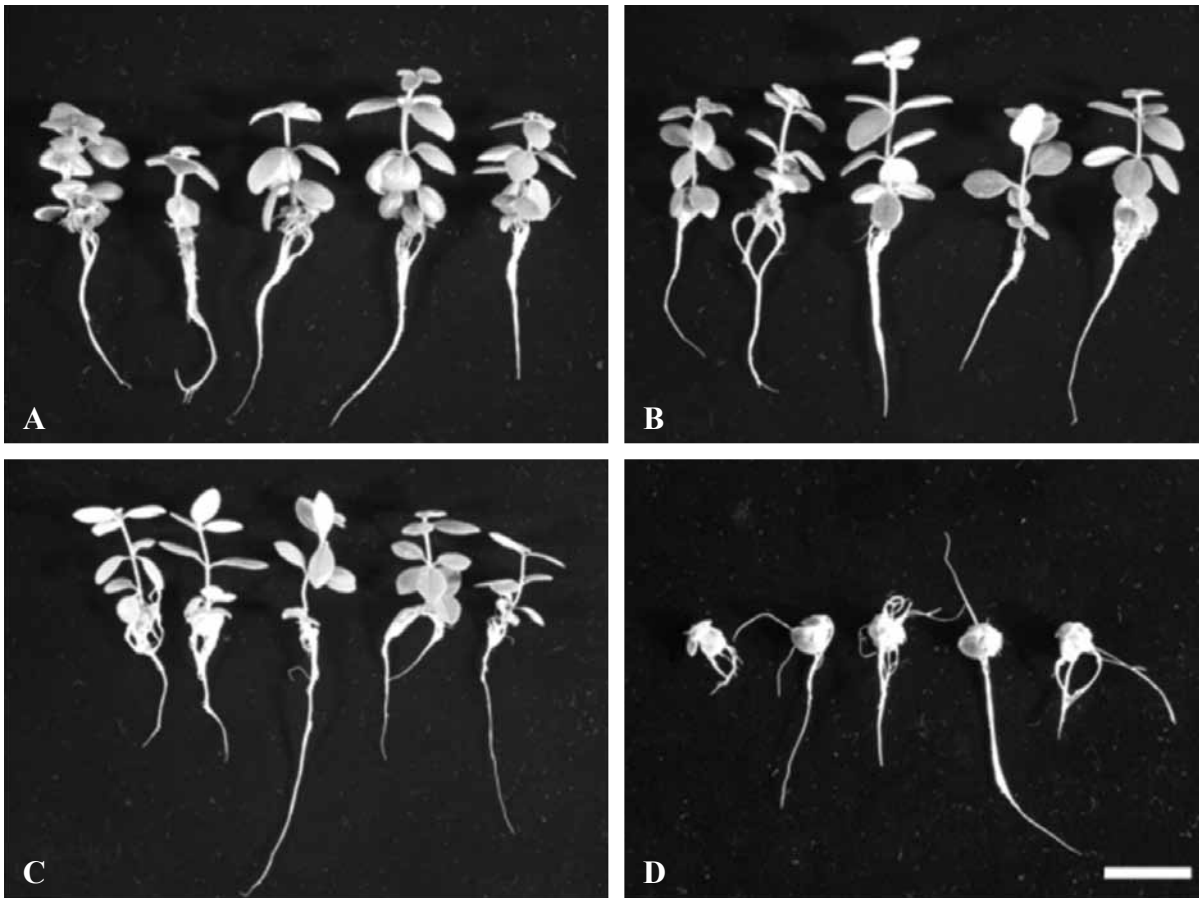

Fig. 2. Plantlets of *Lysionotus pauciflorus* ten weeks after shoot tip culture on four variants of the medium containing different type and concentration of nitrogen source without plant growth regulators. A) MS medium, B) MS3B medium, C) MS4 medium, D) MS5 medium. The bar indicates 2 cm.

Effect of nitrogen source on shoot tip culture

Shoot tips cultured on MS, MS3B, and MS4 elongated to give node numbers of 4.4-4.6, whereas MS5 gave less growth with node number of 2.4 after ten weeks of culture (Table 2, Fig. 2). The means of shoot length were 23.6, 28.7, 27.8, and 4.7 mm on MS, MS3B, MS4, and MS5, respectively (Table 2, Fig. 2). These results indicate that NH_4^+ is not indispensable for shoot growth of *L. pauciflorus* (Table 2) although it is an important element for shoot regeneration in leaf explants culture in this study (Table 1). The growth of shoots on MS5 was strongly inhibited. Since $NaNO_3$ was only used in MS5, it is possible that Na^+ strongly inhibited shoot growth of *L. pauciflorus*. Although the difference in adventitious root formation from leaf explants was observed among the culture variants of the medium (Table 1), shoot tips of 5 mm length with 2 nodes rooted easily within 2 weeks on all variants of the medium and developed enough root system, irrespective of the kind of nitrogen source (Fig. 2). In *Aloe polyphylla*,

Table 2. Effects of type and concentration of nitrogen sources on shoot tip growth of *Lysionotus pauciflorus* after 10 weeks of culture in 16 h lighting.

Medium	NH_4NO_3 (mg l^{-1})	KNO_3 (mg l^{-1})	$NaNO_3$ (mg l^{-1})	No. of node	Length of shoot (mm)
MS	1650	1900	0	4.6 ± 0.7 a	23.6 ± 7.5 b
MS3B	825	950	0	4.6 ± 1.0 a	28.7 ± 8.7 a
MS4	0	1900	0	4.4 ± 0.8 a	27.8 ± 4.4 a
MS5	0	1900	1751	2.4 ± 0.6 b	4.7 ± 1.0 c

Values represent the mean ± SD. Different letters among the means in the same column indicate significant differences at the 0.05 level.

shoots of good quality, high multiplication rate, and low hyperhydricity were produced on medium including NO_3^- as the sole nitrogen source (Ivanova and Van Staden 2009). In the present study, however, MS and MS3B variants of the medium showed good performance for shoot growth, regardless of the involvement of NH_4^+ as a nitrogen source (Table 2).

Effect of wavelength of LED-light on leaf culture

Adventitious shoots were regenerated from all leaf explants cultured under lighting from all types of LED, but shoot regeneration was completely inhibited under the dark condition (Table 3, Fig. 3). The negative effect of darkness on adventitious shoot regeneration was also reported in lettuce (Hunter and Burritt 2004) and *Cattleya* (Cybularz-Urban et al. 2007). More than 20 adventitious shoots were regenerated per leaf explant under white (460, 560 nm), blue (470 nm), and red (625 nm) LED-light, whereas shoot regeneration was suppressed under orange (590 nm) LED-light (Table 3). The highest number of regenerated adventitious shoots was obtained when leaf explants were cultured on medium supplemented with 1 mg l^{-1} BAP and 0.5 mg l^{-1} NAA under red LED-light. Similarly, the highest number of shoots per explant was also obtained under red light in two cultivars of lettuce (Hunter and Burritt 2004) and *Cattleya* hybrid (Cybularz-Urban et al. 2007), while significant difference was not recognized between red and white LED-light in the other two cultivars of lettuce (Hunter and Burritt 2004). The number of long adventitious shoot (> 5 mm) was also high under red LED-light, although these shoots showed abnormal morphology with spindly growth (Table 3, Fig. 3). Superiority of red LED-light over white or blue LED-light for shoot length was also reported in *Cattleya* hybrid (Cybularz-Urban et al. 2007), *Calanthe* hybrids (Baque et al. 2011), chrysanthemum (Kim et al. 2004), and grape (Poudel et al. 2008). Elongated adventitious shoots were easily rooted after transfering to half-strength MS medium without PGRs and abnormal morphology with shoot elongation

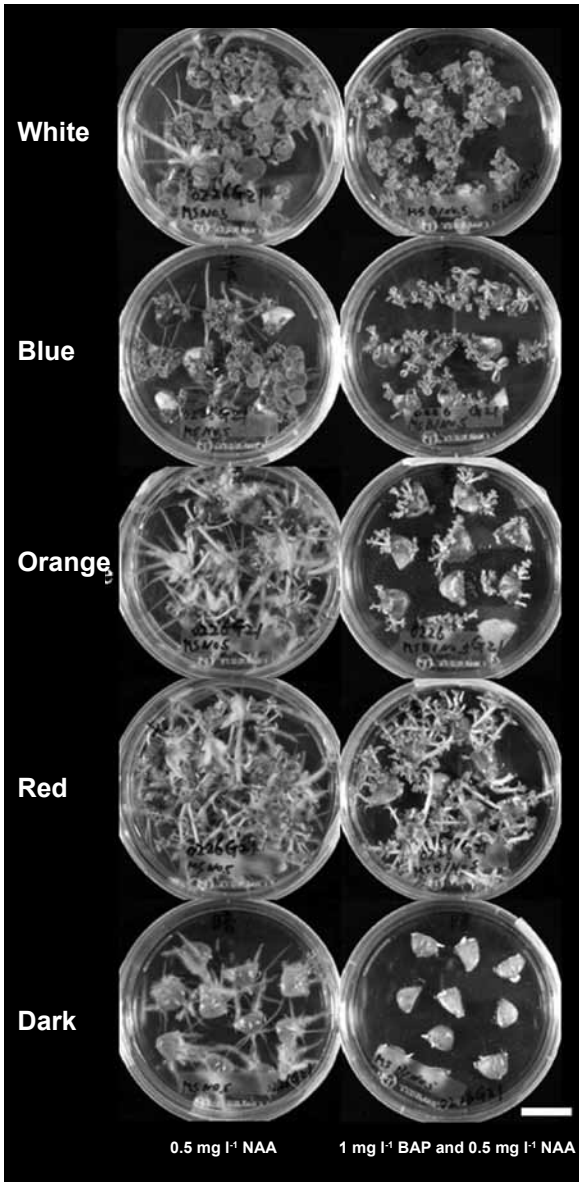

Fig. 3. Effect of wavelength of LED-light on adventitious shoot and root regeneration from leaf explants of *Lysionotus pauciflorus* after 2 months of culture. The bar indicates 1 cm.

Table 3. Effect of wavelength of LED-light on adventitious shoot and root regeneration from leaf explants of *Lysionotus pauciflorus* after two months of culture in continuous lighting.

LED	Peak wavelength (nm)	1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA				0.5 mg l^{-1} NAA			
		Explants with shoot regeneration (%)	No. of shoots per explant	No. of long (> 5 mm) shoots	Explants with root regeneration (%)	Explants with shoot regeneration (%)	No. of shoots per explant	No. of long (> 5 mm) shoots	Explants with root regeneration (%)
White	(460, 560)	100	20.4 ± 7.2 b	3.6 ± 1.4 b	0	100	23.1 ± 6.0 a	4.6 ± 2.1 b	100
Blue	470	100	23.6 ± 8.7 ab	3.4 ± 2.8 b	0	100	25.6 ± 8.8 a	0.8 ± 1.1 c	100
Orange	590	100	17.2 ± 8.6 b	1.7 ± 1.2 bc	0	100	11.6 ± 3.4 b	3.3 ± 1.9 b	100
Red	625	100	30.4 ± 7.5 a	8.1 ± 3.0 a	0	100	23.9 ± 6.4a	7.2 ± 3.9 a	100
Dark		40	5.4 ± 3.8 c	0.7 ± 1.2 c	100	5	0.1 ± 0.2 c	0.0 ± 0.0 c	100

Values represent the mean ± SD. Different letters among the means in the same column indicate significant differences at the 0.05 level.

turns to normal by moving to under fluorescent (data not shown). Consequently, red LED is a useful lighting source for micropropagation of *L. pauciflorus*.

Adventitious root regeneration was not observed in all explants cultured on medium supplemented with BAP and NAA except dark condition, but occurred in all explants cultured on medium supplemented only with NAA under light condition irrespective of wavelength of LED-light (Table 3, Fig. 3). In the culture under darkness, roots were regenerated from all explants on both treatments (Table 3).

The results of the present study suggest that NH_4^+ has a stimulating effect on adventitious shoot regeneration in leaf explant culture, but that NH_4^+ and Na^+ act inhibitory for adventitious root formation of *L. pauciflorus*. Red LED is a useful light source for multiplication of *L. pauciflorus*, whereas shoots regeneration was completely inhibited under the dark condition.

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REFERENCES

Baque M. A., Hahn E. J., Paek K. Y. (2010). Induction mechanism of adventitious root from leaf explants of *Morinda citrifolia* as affected by auxin and light quality. In Vitro Cellular & Developmental Biology - Plant, 46: 71-80.

Baque M. A., Shin Y. K., Elshmari T., Lee E. J., Paek K. Y. (2011). Effect of light quality, sucrose and coconut water concentration on the micropropagation of *Calanthe* hybrids ('Bukduseong' ×'Hyesung' and 'Chunkwang' ×'Hyesung'). Australian Journal of Crop Science, 5: 1247-1254.

Bhojwani S. S., Razdan M. K. (1996). Tissue culture media. *In*: Bhojwani S. S., Razdan M. K. (Eds). Plant Tissue Culture: Theory and Practice, A Revised Edition, Elsevier, Amsterdam: 39-62.

Cybularz-Urban T., Hanus-Fajerska E., Świderski A. (2007). Effect of light wavelength on *in vitro* organogenesis of a *Cattleya* hybrid. Acta Biologica Cracoviensia, Series Botanica, 49/1: 113-118.

Fasolo F., Zimmerman R. H., Fordham I. (1989). Adventitious shoot formation on excised leaves of *in vitro* grown shoots of apple cultivars. Plant Cell, Tissue and Organ Culture, 16: 75-87.

Godo T., Lu Y. X., Mii M. (2010). Micropropagation of *Lysionotus pauciflorus* Maxim. *In*: Jain S. M., Ochatt S. J. (Eds). Protocols for *In Vitro* Propagation of Ornamental Plants, Methods in Molecular Biology, Helsinki, Finland, Humana Press, 589: 127-139.

Gulati A., Jaiwal P. K. (1994). Plant regeneration from cotyledonary node explants of mungbean (*Vigna radiata* (L.) Wilczek). Plant Cell Reports, 13: 523-527.

Halperin W., Wethrell D. (1965). Ammonium requirement for embryogenesis *in vitro*. Nature, 205: 519-520.

Haruki K., Inamura H. (2003). Propagation and growing of *Lysionotus pauciflorus* Maxim. Bulletin of the Shimane Agricultural Experimental Station, 34: 61-70.

Hassan A. Q., Skirvin R. M., Below F. E. (1991). Influence of nitrogen form and NH_4^+-N/:NO_3^--N ratios on adventitious shoot formation from pear (*Pyrus communis*) leaf explants *in vitro*. Plant Cell, Tissue and Organ Culture, 27: 315-319.

Hunter D. C., Burritt D. J. (2004). Light quality influences adventitious shoot production from cotyledon explants of lettuce (*Lactuca sativa* L.). In Vitro Cellular & Developmental Biology-Plant, 40: 215-220.

Ivanova M., Van Staden J. (2009). Nitrogen source, concentration, and NH_4^+: NO_3^- ratio influence shoot regeneration and hyperhydricity in tissue cultured *Aloe polyphylla*. Plant Cell, Tissue and Organ Culture, 99: 167-174.

Kim S. J., Hahn E. J., Heo J. W., Paek K. Y. (2004). Effects of LEDs on net photosynthetic rate, growth and leaf stomata of *Chrysanthemum* plantlets *in vitro*. Scientia Horticulturae, 101: 143-151.

Lian M. L., Murthy H. N., Paek K. Y. (2002). Effects of light emitting diodes (LEDs) on the *in vitro* induction and growth of bulblets of *Lilium* Oriental hybrid 'Pesaro'. Scientia Horticulturae, 94: 365-370.

Liu Y., Wagner H., Bauer R. (1996). Nevadensin glycosides from *Lysionotus pauciflorus*. Phytochemistry, 42: 1203-1205.

Liu Y., Wagner H., Bauer R. (1998). Phenylpropanoids and flavonoid glycosides from *Lysionotus pauciflorus*. Phytochemistry, 46: 339-343.

Lu Y. X., Godo T., Chin D. P., Mii M., Guan K. Y. (2006). Establishment of callus culture with high regeneration ability from leaf segments of *Lysionotus pauciflorus* Maxim. Propagation of Ornamental Plants, 6: 180-186.

Moreira da Silva M. H., Debergh P. C. (1997). The effect of light quality on the morphogenesis of *in vitro* cultures of *Azorina vadalii* (Wats.) Feer. Plant Cell, Tissue and Organ Culture, 51: 187-193.

Murashige T., Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Nagakubo T., Nagasawa A., Ohkawa H. (1993). Micropropagation of garlic through *in vitro* bulblet formation. Plant Cell, Tissue and Organ Culture, 32: 175-183.

Poudel P. R., Kataoka I., Mochioka R. (2008). Effect of red- and blue-light-emitting diodes on growth

and morphogenesis of grapes. Plant Cell, Tissue and Organ Culture, 92: 147-153.

Predieri S., Fasolo F., Malavasi F. (1989). High-frequency shoot regeneration from leaves of the apple rootstock M26 (*Malus pumila* Mill.). Plant Cell, Tissue and Organ Culture, 17: 133-142.

Shin K. S., Murthy H. N., Heo J. W., Hahn E. J., Paek K. Y. (2008). The effect of light quality on the growth and development of *in vitro* cultured *Doritaenopsis* plants. Acta Physiologiae Plantarum, 30: 339-343.

Tsai C. J., Saunders J. W. (1999). Evaluation of sole nitrogen sources for shoot and leaf disc cultures of sugarbeet. Plant Cell, Tissue and Organ Culture, 59: 47-56.