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ORIGINAL ARTICLE

Stem elongation and somatic embryogenesis under red light-emitting diode and subsequent growth of tuberous begonias (*Begonia* × *tuberhybrida* Voss) plantlets on medium containing cobalt nanoparticles

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Abstract

The stem elongation, somatic embryogenesis of Tuberous begonias (*Begonia* × *tuberhybrida* Voss) plantlet under different lighting (fluorescent lamps - FL, blue light-emitting diode - LED, red LED and blue to red LED ratio), and subsequent growth on medium containing cobalt nanoparticles were investigated. The results showed that shoots (1.5 cm in length) cultured under the red LED condition produced higher values of shoot length (6.13 cm), number of internodes per shoot (6.00 internodes), fresh and dry weights (640.34 mg and 78.25 mg, respectively) compared to those under the other lighting conditions after 60 days of culture. Meanwhile, the S-tTCL explants cultured under red LED achieved higher somatic embryogenesis (64.71%), number of somatic embryos (46.67 embryos) and percentage of somatic embryos with torpedo-shape (34.39%) and cotyledon (65.61%) as compared to those under the other lighting conditions after 60 days of culture. For plantlet formation and subsequent growth, somatic embryos (cotyledon shape) cultured on medium containing 0.0465 µg/L CoNPs enhanced plantlet growth, acclimatization and flowering of plantlets in the greenhouse.

Key message

Red light-emitting diodes enhanced stem elongation and somatic embryogenesis of tuberous begonia
Medium containing cobalt nanoparticles enhanced plantlet growth and their acclimatization

Keywords Light-emitting diodes · Somatic embryo · Transverse thin cell layer · Tuberous begonias

Abbreviations

B	Blue LED
CoNPs	Cobalt nanoparticles
FL	Fluorescent lamps
LED	Light emitting diodes
PGRs	Plant growth regulators
R	Red LED
SE	Somatic embryogenesis
S-tTCL	Internode explant cut tTCL

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Introduction

The spectral properties of light emitting diodes (LEDs) could influence morphological, anatomical and physiological processes such as shoot regeneration, somatic embryogenesis, root formation, changes in leaf anatomy and photosynthesis of plants cultured in vitro (Gupta and Jatothu 2013; Nhut et al. 2015; Gupta 2017), as well as have effects on acclimatization in the nursery (do Nascimento Vieira et al. 2015). Acclimatization is an important and often limiting stage in plant micropropagation. The stress caused by the plantlets being transferred into ex vitro conditions can reduce plantlet survival (Kaur and Sandhu 2015). In recent years, LED manufacturing techniques have been improved, and several studies on many plants reported that LEDs are the ideal light source in culture rooms (Nam et al. 2022). All publications focused on the effects of LEDs singly or in combination on the plant tissue culture. The increase in growth parameters such as fresh weight, and dry weight was observed when the plants were cultured under the conditions of blue LED and red LED in combination better than that of LED alone (Xu et al. 2020; Razzak et al. 2022). Meanwhile, the red LED stimulated the stem elongation of strawberries, eucalyptus, orchids, and bananas (Nhut et al. 2018; Tung et al. 2022a); the combination of red and far-red LEDs stimulated embryogenesis in *Dianthus caryophyllus, Phalaenopsis* (Aalifar et al. 2019; Naderi Boldaji et al. 2021). The role of light quality in plant morphogenesis is still unclear, it depended on wavelength, type of plant, plant growth stages and culture conditions such as light intensity, environmental composition or aeration conditions (Kulus and Woźny 2020; Fan et al. 2022).

Tuberous begonias (*Begonia* × *tuberhybrida* Voss) is a flower that is very popular because of its variety of colors, and one that is suitable for decorative planting in buildings and offices in big cities without green spaces. Therefore, this is a flower with very high economic value and always requires a good quality plant source. With the increasing demand for potted *Begonia* flowers there have been many studies aimed at regeneration and micropropagation for producing a large number of plantlets, such as shoot regeneration of four *Begonia* genotypes (Espino et al. 2004), micropropagation for commercial purposes (Nhut et al. 2005, 2010), and the use of explants and plant growth regulators (PGRs) in order to improve regeneration and micropropagation efficiency (Rowe and Gallone 2016). Moreover, this plant has been selected as the subject for physiological studies under in vitro condition, such as research examining the effects of PGRs on three types of in vitro explants (Rowe and Gallone 2016), plant regeneration from the petal explants of four *Begonia* cultivars (Velasco Martínez et al. 2018), and assessments of the genetic fidelity of *Begonia* plantlets for propagation (Aswathy and Murugan 2019).

Somatic embryogenesis (SEs) plays an important role in gene conservation and micropropagation based on biotechnological tools that have been successfully applied to many plant species (de Almeida et al. 2022; Machado et al. 2022). In micropropagation, culture media, plant growth regulators (PGRs), and culture conditions are considered essential for this process (Yue et al. 2022). Furthermore, SEs can be obtained in a direct or indirect pathway significantly dependent on the signalling of auxin and cytokinin (Phong et al. 2023). In addition, several factors that have a positive effect on the SEs induction and proliferation such as silver and copper nanoparticles are also being investigated in many plant regeneration processes (Mahendran et al. 2018; Bao et al. 2022). However, each plant species had a different response to influencing factors such as amino acids, polyamines, PGRs, carbohydrates, and light conditions (Tung et al. 2022a; Yue et al. 2022). Therefore, to be able to successfully plant regeneration through SEs, the selection of explants, PGRs, culture conditions as well as a number of other positive factors are essential. In addition, the SEs for plant regeneration in *Begonia* have been investigated, but their works mainly focused on explant types, PGRs, nutrient sources, amino acids, TCL culture, clinostat conditions, and similar factors on somatic embryos (Awal et al. 2008; Khai et al. 2021; Bao et al. 2022). However, the study of lighting conditions on stem elongation and somatic embryos on *Begonia* is still very limited.

Nanotechnology is considered the industrial revolution, promoting development in all fields, especially biomedicine, energy, environment, and information technology, and its impact on society as a whole. In addition, the advent of nanotechnology has provided the basic tools and technical foundation for the study of biological systems. The positive effects of metal nanoparticles on plants cultured in vitro such as increased seedling generation (Gopinath et al. 2014), enhanced metabolism of secondary compounds, nutrients, and agrochemicals, including phenolic compounds, flavonoids, etc. (Giraldo et al. 2014). Currently, some new roles of metal nanoparticles such as silver nanoparticles (AgNPs) as disinfectant agents for culture media replaced by autoclave sterilization; AgNPs replaced traditional disinfectants such as $HgCl_2$, $Ca(OCl)_2$; AgNPs added to the culture media as a growth promoter in some plants cultured in vitro (Ngan et al. 2020a; (Tung et al. 2021, 2022b; Cuong et al. 2021). Besides, iron nanoparticles (FeNPs) replaced Fe-EDTA chelate in the culture medium to increase the efficiency of iron element absorption for plants, thereby improving the growth of carnation in microponic culture (Ngan et al. 2020b). In which, cobalt nanoparticles (CoNPs) have been used as a factor that enhances nutrient absorption, seedling

germination, morphogenesis, and chlorophyll content of some plants cultured in vitro (Sarropoulou et al. 2016; Ngan et al. 2020a; Tung et al. 2022b). An important role of CoNPs in micropropagation was to inhibit the biosynthesis of ethylene gas and the activity of hydrolytic enzymes (cellulase and pectinase), thereby helping to overcome leaf abscission and improving the plantlet quality (Ngan et al. 2020a). So far, there have been no studies using CoNPs on *Begonia* plantlets. In this study, the effects of the combination of the blue to red LED on stem elongation, somatic embryogenesis, and plantlet formation on a medium containing CoNPs were studied.

In micropropagation, cobalt nanoparticles (CoNPs) have been used as a factor that enhances nutrient absorption, seedling germination, morphogenesis, chlorophyll content, and so on of some plants cultured in vitro (Sarropoulou et al. 2016; Hong et al. 2019; Ngan et al. 2020a; Tung et al. 2022b). An important role of CoNPs in micropropagation is to inhibit the biosynthesis of ethylene gas and the activity of hydrolytic enzymes (cellulase and pectinase), thereby helping to overcome leaf abscission and improving the plantlet quality (Ngan et al. 2020a). So far, there have been no studies using CoNPs on *Begonia* plantlets. In this study, shoots were cultured under different lighting conditions for stem elongation purpose; then, internodes cut into transverse thin cell layers and cultured under different lighting conditions to evaluate the efficiency of somatic embryogenesis, and subsequent growth of somatic embryos on medium containing CoNPs in order to establish an efficient protocol for the *Begonia* micropropagation.

Materials and methods

Plant material and CoNPs solution

A hundred shoots with three leaves isolated from 30-day-old in vitro Tuberous begonias (*Begonia* × *tuberhybrida* Voss) shoot clusters at Tay Nguyen Institute for Scientific Research (Dalat, Lam Dong, Vietnam) were used as initial materials.

CoNPs solution provided by the Institute of Environmental Technology (VAST, Vietnam) with a nanoparticle size ranging from 20 to 60 nm (Fig. 1) with a stock of 1000 ppm was made by the method of reducing the precursor $CoCl_2.6H_2O$ with the reducing agent being $NaBH_4$ and the reducing agent stabilizer being carboxymethyl cellulose (Ngo et al. 2014). $CoCl_2.6H_2O$ in MS medium (Murashige and Skoog 1962) was used as a control. Meanwhile, $CoCl_2.6H_2O$ was removed and CoNPs were added to the medium at different concentrations (1.55; 3.10; 4.65; 6.20; 12.40 µg/L – corresponding to a CoNPs content with a quarter, half, three quarters, one and two times $CoCl_2.6H_2O$ in MS medium); after that, all medium above was adjusted to pH 5.8 before autoclaving at 121 °C, 15 psi for 30 min.

Lighting and culture conditions

Fluorescent lamps (FL) with a wavelength of 320 to 800 nm (voltage of 220 V) and 1.2 m in length (40 W/T10) (Rang Dong Company, Hanoi, Vietnam) were used as control. Blue LED (B) with a wavelength of 450 to 470 nm (voltage of 3 V) and Red LED (R) with a wavelength of 650 to 665 nm (voltage of 2 V) (Super Bright LEDs Inc., Missouri, USA) were used as LEDs sources. The LED power was 0.1 W with

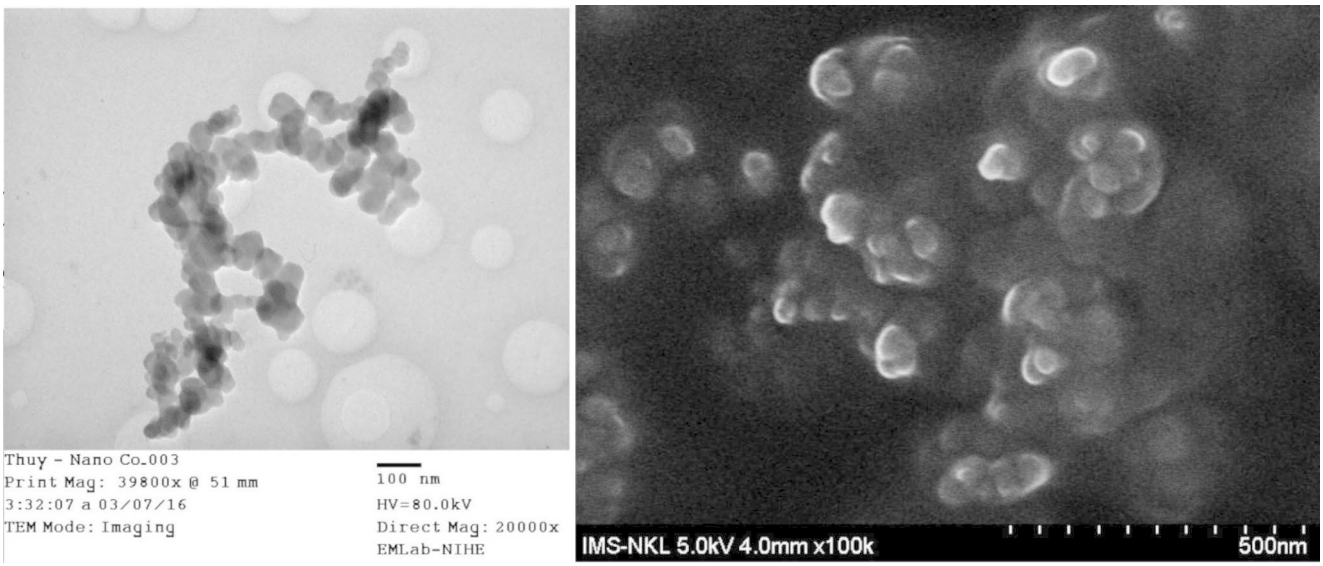

Fig. 1 Image of cobalt nanoparticles observed by transmission electron microscopy (TEM)

a 330Ω resistor for R and a 220Ω resistor for B (TQCOM JSC., Hanoi, Vietnam). The 12 V power supply had a voltage of 5 A (AXT 450, Golden Field Firm). A circuit board (10 cm × 50 cm) with 480 LEDs included 10 LED bars with 48 bulbs divided into 16 circuits that consisted of three LED chips and one resistor connected in series. The mix of LEDs will be based on the number of R combined with B on the board. (Nam et al. 2016).

In vitro explants were placed in the tissue culture room with a temperature of 25 ± 2 °C and humidity of 55 to 60%, using FL and LEDs with an intensity of 40 to 45 $\mu mol.m^{-2}.s^{-1}$ [the light intensity was determined at 49 measuring points at a distance of 5 cm from each other on the shelf board (30 cm × 30 cm) by the light meter, and the distance between the light sources and explants was 30 cm (Nam et al. 2016)], and lighting time of 16 h/day. Meanwhile, plantlets were placed in the greenhouse of the Tay Nguyen Institute for Scientific Research (VAST, Dalat, Vietnam) with a temperature of 25 ± 2 °C during the day and 15 ± 2 °C at night, with an average humidity of 75 to 80% and using natural light with 50% shading by black net, soil pH 6.5.

Stem elongation of tuberous begonias shoots under LEDs

A hundred shoots (1.5 cm in length) were cultured on a nylon bag culture system (Nhut et al. 2022) containing 40 mL MS medium added to 30 g/L sucrose, 8 g/L agar and without plant growth regulators and placed under different lighting conditions (FL, R, 5B:5R and B) for elongated shoots (Fig. 2). The shoot length (cm), number of internodes per shoot, shoot fresh weight (mg) and shoot dry weight (mg) were obtained after 60 days of culture.

Somatic embryogenesis of tuberous begonias via S-tTCL culture under LEDs

A hundred internodes (1 cm in length) obtained from the elongated shoots cut into transversely thin cell layers (S-tTCL) with a size of 1 mm were cultured on petri dishes (6 cm in diameter) containing 10 mL ½ MS medium (MS medium reduced a half macro minerals) supplemented with 0.2 mg/L TDZ, 0.2 mg/L NAA, 30 g/L sucrose and 8 g/L agar (Nhut et al. 2005) and placed under different lighting conditions (FL, R, 9R:1B, 8R:2B, 7R:3B, 5B:5R and B) for somatic embryogenesis. The duration of somatic embryo induction (day), the percentage of somatic embryogenesis (%), number of somatic embryos (embryos), the percentage of somatic embryo shape (%) with globular, heart, torpedo

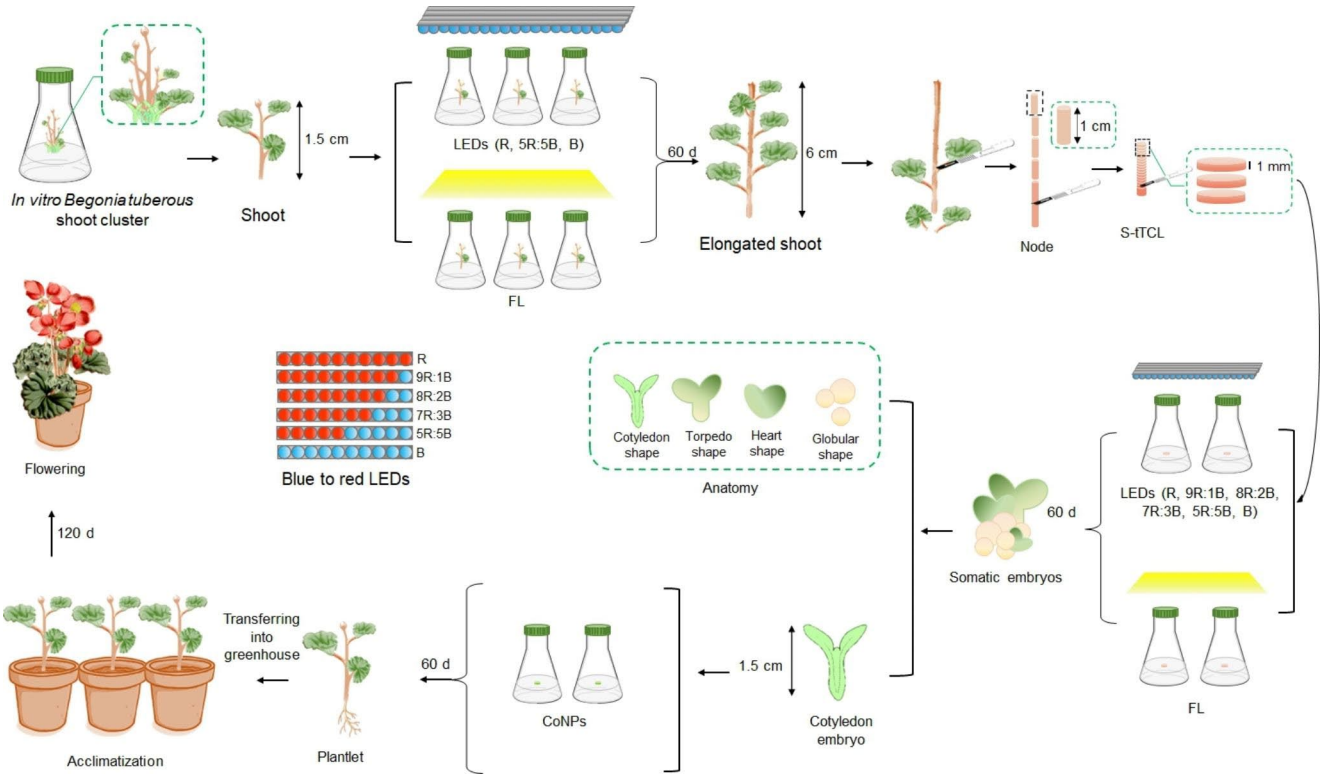

Fig. 2 Schematic illustration of Tuberous begonias (*Begonia* × *tuberhybrida* Voss) micropropagation

and cotyledon shapes, and cluster of somatic embryo fresh and dry weight (mg) were recorded after 60 days of culture.

For morphological observation, the developing somatic embryos were cut by hand section, stained and observed under a optical microscope (Peterson et al. 2008). The embryo was cut into transversely (30–40 µm) and checked by Digimatic caliper (Model: 500-752-20, Mitutoyo Corporation, Japan), soaked in 10% Javel solution for 15 min and rinsed with distilled water. It was then soaked in acetic acid 10% for 10 min, and rinsed with distilled water about three times. Next, it was stained with a two-color dye solution (carmine red - iodine blue) for 5 min, then rinsed again with distilled water. Finally, the sample was placed on a glass slide with the addition of one drop of water or glycerin and covered with a lame. The sample was observed and the results recorded under an optical microscope (Model C-BD230, Nikon Instruments Inc., Japan).

Starch and glucose contents by spectroscopic analysis (UV - VIS)

SE cluster (0.3 g) (obtained from FL and optimal LED treatments) were first harvested by centrifugation at 8,750×g for 15 min and then washed and lyophilized before being disintegrated with a mortar and pestle for 5 min. The disintegration of SE was performed for 5 min, monitored under microscopic observation. The explants were removed from the mortar and pestle using acetone solvents. The starch content was quantified as described by Fernandes et al. (2012). The starch in the explant was hydrolyzed to maltodextrin by α-amylase (3000 U m/L) and then hydrolyzed to glucose by amyloglucosidase (3300 U m/L). The blank solution was prepared with 0.1 mL of distilled water with the addition of 3.0 mL of GOPOD (glucose oxidase-peroxidase). Total starch content (I) (% w/w DW) is determined by the formula: $I = A \times F/W \times 90$, in which A is the absorption coefficient of the test solution compared to the blank; F is the conversion factor from the absorbance value to the glucose value; and W is the mass of the test piece (mg).

On the other hand, SE cluster (0.3 g) (obtained from FL and optimal LED treatments) were peeled and cut into small pieces using a knife (0.2–0.3 cm of thickness) then were frozen and homogenized using a Bosch blender. All homogenized explants were stored in plastic bags at -18 °C. The sugar content was determined as described by Krivorotova and Sereikaite (2014). Glucose content in the explant was determined using the GOPOD reagent with maximum absorbance at 510 and D-glucose was used as a control. The sugar content of the explant was determined using the 3,5-dinitrosalicylic acid (DNS) reagent giving the product the maximum absorbance at 540 nm, while distilled water was used as the control. D-glucose was used to construct the calibration curve. The sugar content (S) (mg/g DW) is determined by the formula: $S = A \times n \times V/m$, in which A is the content of reducing sugar in the diluted sample solution (mg/g); n is the dilution factor; V is the standard solution volume (mL); and m is the mass of the test piece (g).

Plantlet formation on medium containing CoNPs

A hundred somatic embryos with cotyledon shape (1.5 cm in height) were cultured in a 500 mL bottle glass containing 40 mL MS medium supplemented with 0.1 mg/l BA, 0.5 mg/L NAA, 30 g/L sucrose, 1 g/L activated charcoal and 8 g/L agar (Nhut et al. 2005) and different concentrations of CoNPs for plantlet formation. The explants culture under FL lighting condition.The plantlet height (cm), number of leaves per plantlet, number of roots per plantlet, root length (cm), plantlet fresh, dry weight - determined by drying oven (MOV-112, Sanyo Electric Co., Ltd, Japan) at 60 °C to constant mass (mg), chlorophyll (nmol/cm^2) (checked by SPAD 502 Minolta Co., Ltd., Osaka, Japan) were obtained after 60 days of culture.

Ethylene accumulation

The ethylene gas collection process was carried out according to the Cristescu et al. (2012). Ethylene (1 mL) from a 250 mL culture bottle culture (capped with nylon film and covered with parafilm) was collected directly with a syringe and manually injected into the chromatograph with a leak-free syringe. The ethylene gas was then quantified by gas chromatography. A stainless steel column (3 m × 1.5 mm) filled with Porapack R (adsorbent) with a particle size of 80–100 Mesh was used. The detection temperatures of the column, injector, and ionizing flame were 60, 90, and 90 °C, respectively. Electrometer sensitivity 1 × 10–12 Am/V. Nitrogen gas (N_2) was used as the carrier gas (55 cm^3/min).

The subsequence growth of tuberous begonias plantlets in the greenhouse

A hundred plantlets derived from each treatment under different lighting conditions (FL, R, 9R:1B, 8R:2B, 7R:3B, 5B:5R and B) were washed to remove agar, treated with Antracol® 70WP and planted in plastic pots with a 15 cm top diameter, 12 cm bottom diameter and 20 cm height containing coir mixed with sand substrates in the ratio 2:1 and transferred to the greenhouse. The percentage of survival (%), plantlet height (cm), number of leaves per plantlet, number of roots per plantlet, root length (cm), plantlet fresh weight (mg), and chlorophyll (nmol/cm^2) were measured after 30 days. The plantlet height (cm), stem diameter (cm), number of inflorescences per plantlet, number of flowers per

inflorescence and flower diameter (cm) were then recorded after 120 days in the greenhouse.

Data analysis

All experiments were arranged in a completely randomized design, each experiment was repeated three times, and data were processed using SPSS 16.0 software (SPSS Inc., Chicago, TL, USA) with Duncan's test at a significance level of $p < 0.05$ (Duncan 1955) to determine the difference in the means.

Results and discussion

Stem elongation of tuberous begonias shoot under LEDs

The results showed that different lighting conditions (R, B, 5R:5B and FL) affected the stem elongation of the shoots after 60 days of culture (Table 1). The shoot length (6.13 cm), number of internodes per shoot (6.00 nodes), fresh weight (640.34 mg) and dry weight (78.25 mg) under R were higher than those with other LEDs (Table 1; Fig. 3). In addition, shoot length and the number of internodes per shoot were two times higher, while the fresh and dry weights of shoots were one and a half times higher under the R condition as compared to shoots cultured under the FL condition.

Some studies have shown that LEDs affect plant growth and development via morphogenesis and stem elongation (Gupta and Jatothu 2013). Stem elongation was optimal in some plants under the R condition, such as Strawberries, *Eucalyptus*, *Cymbidium*, *Phalaenopsis*, Banana (Nhut 2002), Tomato (Hirai et al. 2006), Potato (Jiang et al. 2019a), and *Anthurium andraeanum* (Tung et al. 2021). However, plantlets under the R LED condition often had a slender stem, yellow leaves, lower chlorophyll content, reduced photosynthetic efficiency and decreased fresh and dry weight as compared to those under R combined with B conditions (Nhut et al. 2002). On the other hand, other studies have shown the opposite results, with stem elongation promoted under 70R:30B condition for *Paphiopedilum* (Nhut et al. 2007) and *Anthurium andraeanum* (Tung et al. 2021), or under B condition for *Paphiopedilum* (Luan et al. 2015).

In summary, light quality plays an important role in plant growth and development, but it needs to be studied in more detail. The effects depend on the type of light with different spectra (Tarakanov et al. 2021), or crops, explants and culture conditions, such as media composition (Schuerger et al. 1997), light intensity (Kurilčik et al. 2008), and aeration conditions (Hahn et al. 2000), to which plants respond differently. In this study, stem elongation was shown to be optimal under R condition. This has some important implications for the production of elongated shoots, and is a subject for further research.

Table 1 The growth of Tuberous begonias (*Begonia* × *tuberhybrida* Voss) shoots cultured under LEDs after 60 days of culture

Lighting condition	Shoot length (cm)	No. of stem nodes per shoot	Shoot fresh weight (mg)	Shoot dry weight (mg)
R	6.13a*	6.00a	640.34a	78.25a
B	2.73d	3.00c	415.29c	51.17c
5R:5B	4.47b	4.33b	459.30b	48.24ab
FL	3.27c	3.67bc	410.95 cd	33.23d

*Different letters shown in the same column represent significant differences at $p < 0.05$ in Duncan's test

Somatic embryogenesis via S-tTCL of tuberous begonias under LEDs

The R condition stimulated S-tTCL explants as early as 14 days through epidermal inflammation; meanwhile, the induction of S-tTCL explants was three to eight days later in the other LEDs and nine days later in the FL conditions (Fig. 4). Somatic embryogenesis, number of somatic embryos, and dry and fresh weight of somatic embryo clusters were highest under R condition after 60 days of culture (Table 2; Fig. 5). Furthermore, the embryogenesis efficiency decreased when the S-tTCL explant was cultured under a decreased R ratio and increased B ratio. These results proved that the B condition limited the somatic embryogenesis of S-tTCL explants. The efficiency of somatic embryogenesis under FL condition was lower than that seen in the R conditions.

Besides affecting SE, red LED condition also affected the activity of antioxidant enzymes. For the red LED-derived SE clusters, the antioxidant enzyme (CAT, SOD and APX) was higher than that of the FL-derived SE cluster after 60 days of culture. At the same time, the carbohydrate and starch content in the SE cluster derived from red LED was also higher than that of the control (FL) treatment (Table 3).

With regard to *Begonia* sp., Nhut et al. (2010) only described the shoot regeneration process directly from different explant sources. A more recent study by Khai et al. (2021) recorded and described somatic embryogenesis with a full shape under microgravity and in vitro conditions, and the explant source used was petiole sterilized with 1 g/L $HgCl_2$. However, how LED conditions affect somatic embryogenesis has not been examined in this plant. The process of somatic embryogenesis is influenced by several factors, such as plant growth regulators, nitrogen source, carbohydrates, explants, pH, lighting conditions,

Fig. 3 Stem elongation of Tuberous begonias (*Begonia* × *tuberhybrida* Voss) shoots under LEDs after 60 days of culture. **A**: Stem elongation of shoot under FL. **B**: Stem elongation of tuberous begonia shoot under R LED. **C, D, E**: Explants for cut transverse thin cell layer (S-tTCL)

and presence of polyamines or silver nanoparticles (Mo et al. 2020; Cuong et al. 2021, etc.). In the current study the R condition and thin cell layer culture gave a higher somatic embryogenesis efficiency compared with the control (FL condition). A comparison of the somatic embryo shapes also showed that the somatic embryos with cotyledon and torpedo shapes (65.61% and 34.39%, respectively) under the R condition were also higher than with the other LED and FL conditions (Table 2). The results showed that S-tTCL explant cultured under the R condition not only increased the efficiency of somatic embryogenesis, total number of somatic embryos, and fresh and dry weight of somatic

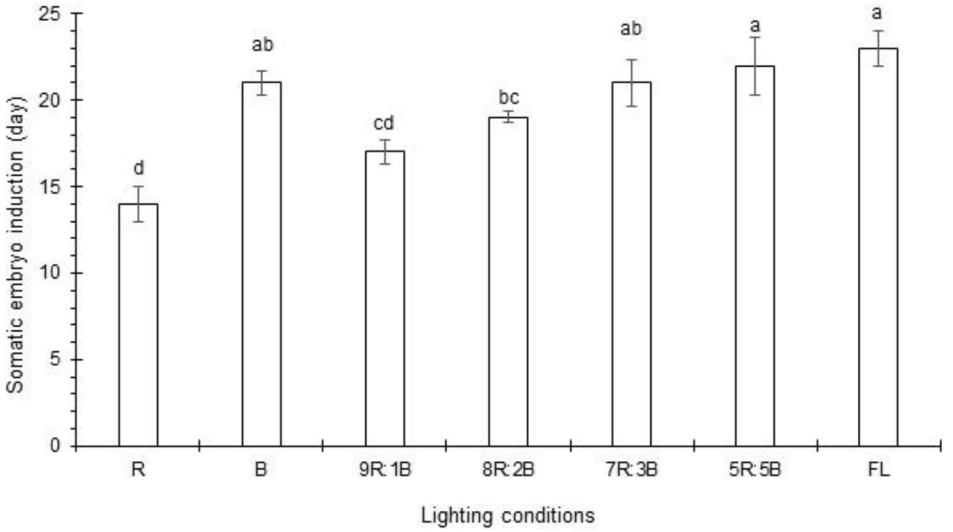

Fig. 4 Somatic embryo induction of Tuberous begonias (*Begonia* × *tuberhybrida* Voss) via stem nodes transverse thin cell layer (S-tTCL) under LEDs

Table 2 Somatic embryogenesis via stem nodes transverse thin cell layer of tuberous begonia under LEDs after 60 days of culture

Lighting condition	Somatic embryogenesis (%)	No. of somatic embryos	The percentage of somatic embryo shape (%)				Cluster of somatic embryo (mg)	
			Globular	Heart	Torpedo	Cotyledon	Fresh weight	Dry weight
R	64.71a*	46.67a	-**	-	34.39ab	65.61a	364.05a	33.43a
9R:1B	56.56ab	35.67b	-	22.43c	25.33c	52.34b	372.63a	35.56a
8R:2B	52.63b	21.33c	4.69e	9.38d	34.36ab	51.57b	257.26b	24.90bc
7R:3B	41.18c	12.33d	13.54d	10.79d	35.12ab	40.55c	219.75d	21.75c
5R:5B	31.25 cd	10.67de	25.02b	18.74c	32.03ab	34.21d	218.09e	21.12c
B	22.22e	9.67e	65.46a	34.54a	-	-	217.60e	21.49c
FL	29.42 cd	8.33e	20.00bc	12.00d	40.00a	28.00e	216.47f	21.21c

*Different letters shown in the same column represent significant differences at $p < 0.05$ in Duncan's test

** Data not recorded

embryo clusters, but also embryo the morphology in torpedo and cotyledon shapes (Fig. 5) were better.

Light quality affects the photo-oxidative properties of plants by modulating the antioxidant defense system, resulting in the rise of antioxidative enzyme activity. Enhanced antioxidant properties of many vegetables like pea, Chinese cabbage, kale, and tomato - have been observed as a response to the use of single-spectral or combined red (625–630 mm): blue lights (465–470 mm) when compared to with FL (Wu et al. 2007; Lee et al. 2016). Moreover, green (510 nm), yellow (595 nm), or even combined red: white LEDs also increase both antioxidant properties and anthocyanin accumulation (Dong et al. 2014; Lekkham et al. 2016). Such improvements in the antioxidant characteristics may arise due to the induction of β-carotene, glucosinolates, free radicals (e.g., DPPH; 1,1-diphenyl-2-picrylhydrazyl), scavenging activity, ROS-scavenging enzymes (e.g., superoxide dismutase), phenolic compounds, and vitamin C (Wu et al. 2007; Lee et al. 2016). Consuming antioxidant-rich fruits and vegetables can have health benefits. Therefore, it would be interesting to ascertain the health benefits of consuming LED-treated crops. Besides, the activity of the antioxidant enzymes peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) was enhanced in LED-treated broccoli. At the same time, the expression of the chlorophyll degrading genes, chlorophyllase II (BoCLH2), chlorophyllase III (BoCLH3), and pheophorbide an oxygenase (BoPAO) was suppressed in the early stages of storage. Furthermore, Jiang et al. (2019b) research demonstrated that irradiation of broccoli with red LED induced a series of physiological and molecular responses that extended postharvest quality and could be used to prolong the shelf-life of commercially produced broccoli. In *Rehmannia glutinosa in vitro* study, the total antioxidant capacity, reducing power potential, and DPPH radical scavenging capacity also revealed the enhancement of antioxidant capacity under both blue and red LED treatments (Manivannan et al. 2015). Although, blue LED is more effective in increasing the formation of antioxidant enzymes; however, in this study, blue LED did not have a positive effect on somatic embryogenesis. Meanwhile, red LED has a positive effect on somatic embryogenesis and also increases antioxidant enzyme content;

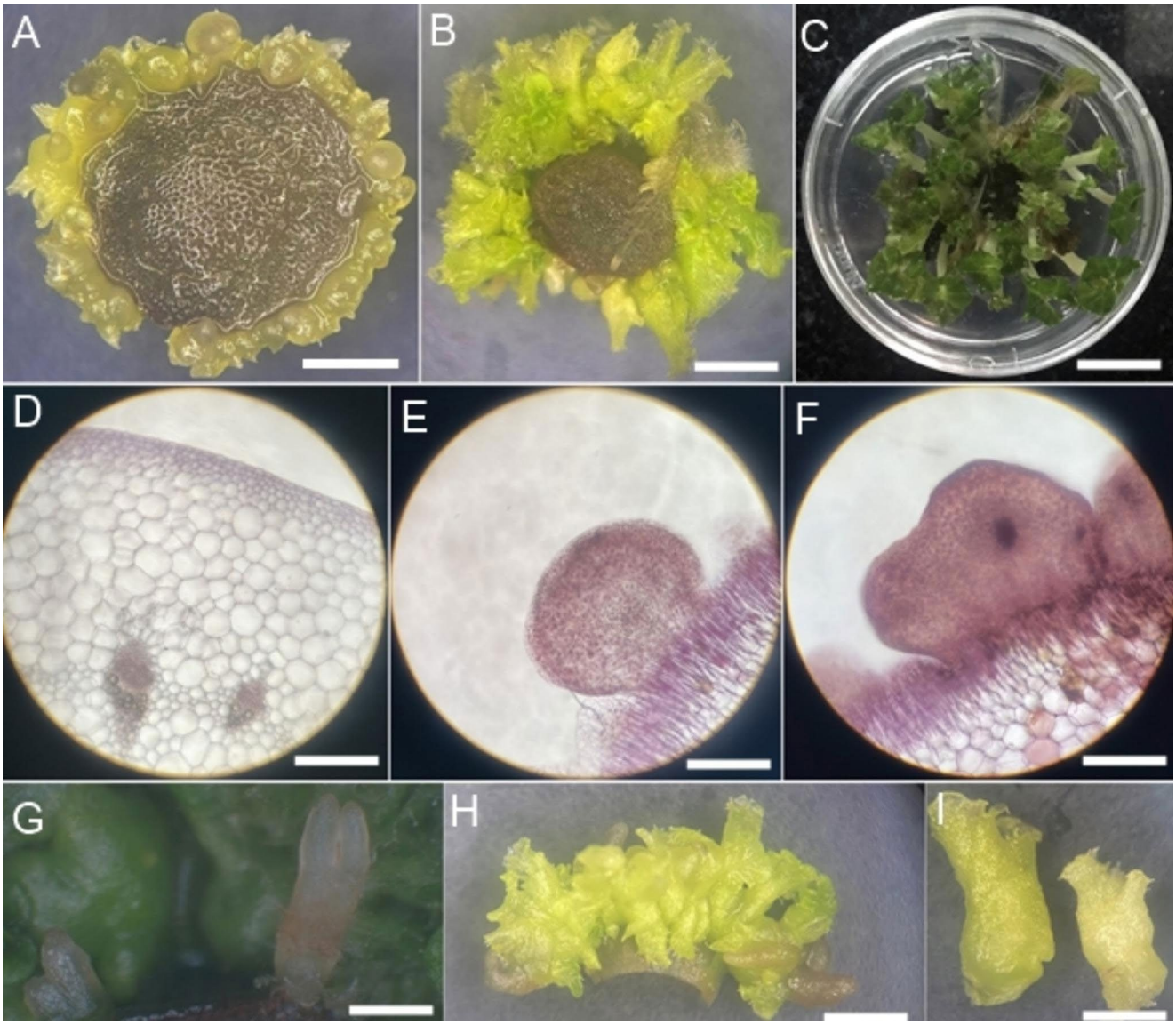

Fig. 5 Somatic embryogenesis via stem nodes transverse thin cell layer (S-tTCL) of Tuberous begonias (*Begonia* × *tuberhybrida* Voss) under red LED. **A**: Somatic embryos after 20 days (*Bar: 5 mm*). **B**: Somatic embryos after 40 days (*Bar: 5 mm*). **C**: Somatic embryos after 60 days (*Bar: 10 mm*). **D**: S-tTCL explant at day 0 (*Bar: 0.5 mm*). **E**: Somatic embryo with globular shape (*Bar: 0.5 mm*). **F**: Somatic embryo with later globular shape (*Bar: 0.5 mm*). **G**: Somatic embryos with heart and torpedo shapes (*Bar: 1 mm*). **H, I**: Somatic embryos with torpedo shapes (*Bar: 5 mm*)

Table 3 Antioxidant enzyme activity (CAT and APX), starch and glucose contents of *Begonia* × *tuberhybrida* Voss somatic embryonic clusters after 60 days of culture

Lighting condition	Starch (% w/w DW)	Glucose (mg/g DW)	CAT (U/g)	APX (U/g)	SOD (U/g)
FL	29.37 ± 0.23*	103.01 ± 0.11	224.06 ± 2.34	0.19 ± 0.01	35.25 ± 1.22
R	38.13 ± 0.10	154.43 ± 1.13	232.58 ± 3.63	0.29 ± 0.02	44.23 ± 2.49

*Mean ± Std. Deviation

therefore, the application of red LED in plant tissue culture is more optimal.

The process of inducing, differentiating, and developing somatic embryos involves changes in the content of carbohydrates such as starch and sugars, which serve as metabolic energy sources for tissue metabolism and growth. Multiple studies have indicated that high starch accumulation in explants promotes in vitro regeneration and the formation of meristems. In fact, starch accumulation is the first metabolic reaction in culture, where sucrose is converted to starch for tissue storage (Wu et al. 2007). In this study, the starch and sugar concentrations of somatic embryos derived from red

Table 4 Plantlet formation of tuberous begonia somatic embryo cultured on medium containing CoNPs after 60 days of culture

CoNPs (µg/L)	Plantlet height (cm)	No. of leaves per plantlet	No. of roots per plantlet	Root length (cm)	Plantlet fresh weight (mg)	Plantlet dry weight (mg)	Chlorophyll (nmol/cm^2)	Ethylene (ppm)
Control	6.07bc*	2.67d	5.00c	1.77c	277.07d	17.59bc	18.73 cd	1.35a
0.0155	5.24d	3.33bcd	2.00f	0.93f	254.20e	16.63d	14.03d	1.15b
0.031	6.25b	4.67b	6.33b	2.67b	370.36b	18.41b	22.15b	0.76c
0.0465	6.60a	6.00a	7.67a	4.83a	433.92a	21.21a	25.30a	0.52e
0.062	6.35b	4.33bc	3.67d	1.57d	342.63c	18.97b	20.04bc	0.61d
0.124	5.07de	3.67c	3.00e	1.27e	144.29f	13.09d	12.17de	0.84c

*Different letters shown in the same column represent significant differences at $p < 0.05$ in Duncan's test

Table 5 The subsequence growth of tuberous begonia plantlets after 30 days in the greenhouse

CoNPs (µg/L)	Survival rate (%)	Plantlet height (cm)	No. of leaves per plantlet	No. of roots per plantlet	Root length (cm)	Plantlet fresh weight (mg)	Chlorophyll (nmol/cm^2)
Control	73.33f*	6.27 g	4.33de	8.67f	1.90e	2650.54d	39.43d
0.0155	86.67d	7.13f	4.00bcd	11.33d	3.53c	3050.55bc	44.37c
0.031	80.00e	8.13d	6.00bc	15.67c	4.40b	2740.47d	43.90c
0.0465	96.67a	9.63a	8.00a	19.33a	7.10a	4110.21a	54.43a
0.062	93.33ab	9.00b	5.67bc	15.33c	4.20b	3660.37b	51.50b
0.124	90.00c	8.53c	5.00bc	17.33b	2.73d	3140.23c	50.93b

*Different letters shown in the same column represent significant differences at $p < 0.05$ in Duncan's test

LED were considered better, as evidenced by the higher starch and sugar content in the somatic embryo obtained from the red LED condition. Therefore, carbohydrates and starch are essential in somatic embryo generation, differentiation and development.

Plantlet formation and acclimatization

All CoNPs treatment and control samples achieved 100% in vitro rooting; however, CoNPs at appropriate concentrations stimulated plantlet growth better compared with the control (Table 3). The highest plantlet height (6.60 cm), number of leaves per plantlet (6.00 leaves), number of roots per plantlet (7.67 roots), root length (4.83 cm), fresh weight (433.92 mg), dry weight (21.21 mg), chlorophyll (25.30 nmol/cm^2) were obtained in 0.0465 mg/L CoNPs treatment. Moreover, the results showed that the ethylene gas content decreased significantly when using CoNPs. Especially at 0.0465 mg/L CoNPs, the ethylene gas content was lower compared to the other treatment and the control (Table 4).

Plantlet quality determined the subsequent growth of plantlets in the greenhouse. The percentage of survival (96.67%) of plantlets derived from 0.0465 mg/L CoNPs treatment was about 20% higher than that under control conditions after 30 days in the greenhouse (Table 5). The plantlet growth in terms of plantlet height, number of leaves per plantlet, number of roots per plantlet, root length, plantlet fresh weight and chlorophyll in the 0.062 mg/L CoNPs treatment was also increased compared to under the other conditions.

Table 6 The differences in the flowering stage of plantlets after 120 days in the greenhouse

CoNPs (µg/L)	Plantlet height (cm)	Stem diameter (cm)	No. of inflorescences per plantlet	No. of flowers per inflorescence	Flower diameter (cm)
Control	32.35d*	1.96 cd	-	-	-
0.0155	33.17d	2.02 cd	-	-	-
0.031	37.12c	2.33 cd	1.00c	1.00c	1.23c
0.0465	42.21a	3.00a	2.67a	3.67a	3.24a
0.062	39.34b	2.72ab	2.33ab	3.00ab	2.95b
0.124	40.03b	2.76ab	2.00b	3.00ab	2.56c

*Different letters shown in the same column represent significant differences at $p < 0.05$ in Duncan's test

After 120 days in the greenhouse, the CoNPs derived-plantlets began flowering; meanwhile, no buds or flowers appeared on the plantlets derived without CoNPs (Table 6; Fig. 6). The 0.465 µg/L CoNPs derived-plantlets achieved a greater plantlet height, stem diameter, number of inflorescences per plantlet, number of flowers per inflorescence and flower diameter than those in other CoNPs conditions.

When cultured, plantlets produced ethylene (a gaseous phytohormone) that regulates various growth and development processes (Abeles et al. 1992), complementary or antagonistic to other hormones (Muday et al. 2012). Ethylene biosynthesis involves a biochemical pathway with two steps starting from S-adenosyl-L-methionine (SAM), which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the ACC synthase enzyme (ACS). The ACC is further converted to ethylene by the enzyme ACC oxidase (ACO) (Chang 2016).

Fig. 6 The differences in the flowering stage of plant in the greenhouse after 120 days. **A**: Control. **B**: 0.062 µg/L CoNPs treatment

According to a recent study, cobalt (Co) plays an important role in inhibiting the formation and activity of ethylene gas. When there is an increase in Co concentration in the culture medium (1–10 µM) in *Swertia chirata*, the conversion of ACC to ethylene is significantly increased (Saha and Gupta 2018). Another study on *Zea mays* showed that reduced ethylene content (65%) was observed after treatment with 10 − 4 M cobalt nitrate, 70% of ACC oxydase activity was inhibited in *Pyrus communis* is related to the presence of 10 µM Co (Pereira-Netto 2001). This study has shown that CoNPs also play an essential role in inhibiting the formation and activity of ethylene gas. Similar results were also recorded in Rose and Gerbera; when adding 0.0465 mg/L CoNPs to the culture medium, the accumulated ethylene gas content in the culture flask was significantly lower compared with the other CoNPs treatments and the control (Ngan et al. 2020a; (Tung et al. 2022b). Furthermore, the effect of CoNPs on ethylene gas accumulation was superior to that of AgNPs. However, in our research and those two, the ethylene gas content also gradually increased as the concentration of CoNPs continued to increase (0.062–0.124 mg/L). This may be because the concentration of CoNPs is too high for the plantlet's needs, thereby inhibiting growth and causing stress to the plantlet.

The application of nanotechnology to plant micropropagation has shown that CoNPs can be a potential source of micronutrients to replace inorganic salts in culture medium (Fouad and Hafez 2018). CoNPs in the culture medium enhanced the formation, size and mass of microtubules in potato cultured in vitro (Hamza 2019). Another study on grape plants showed that the CoNPs enhanced shoot regeneration compared to that seen with the control (without CoNPs) (Shkopinskij et al. 2018; Hong et al. 2019) compared the effects of CoNPs produced in Vietnam and the US on soybean cultured at different stages showed similar efficiency. The results showed that CoNPs affected the growth, photosynthetic efficiency and chlorophyll a and b content at different stages in soybean leaves. A recent study by Tung et al. (2022b) on Gerbera showed that CoNPs improved the in vitro rooting, acclimatization, growth and flowering stages. As such, CoNPs have great potential to improve the growth, development and thus yield and quality of plantlets.

Conclusion

In this study, an established micropropagation protocol of Tuberous begonias (*Begonia* × *tuberhybrida* Voss), in which red LED was effective in increasing the stem elongation (shoot length, number of internodes per shoot, fresh and dry weights) and somatic embryogenesis (the percentage of SE, number of somatic embryos and percentage of

somatic embryos with torpedo-shape and cotyledon) via thin cell layer culture. In addition, somatic embryos cultured on medium containing 0.0465 µg/L CoNPs enhanced the plantlet growth, acclimatization and flowering of plants in the greenhouse.

Authors' contributions Bui Van The Vinh, Le The Bien and Hoang Thanh Tung acquired the data and wrote the manuscript. Hoang Dac Khai, Nguyen Thi Nhu Mai, Vu Quoc Luan, Do Manh Cuong, Hoang Thi Nhu Phuong, Ngo Quoc Buu and Nguyen Hoai Chau participated in performing the experiments, interpretation of data and revision of the content. Duong Tan Nhut and Hoang Thanh Tung conceptualized and designed the study. All authors discussed the results and contributed to the final manuscript.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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