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Research Article

Root growth of Eleuthero (*Eleutherococcus senticosus* [Rupr. & Maxim.] Maxim.) seedlings cultured with chitosan oligosaccharide addition under different light spectra

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Abstract

Eleuthero (*Eleutherococcus senticosus* [Ruprecht & Maximowicz] Maximowicz is an important understory species as well as a source of natural products in Asia. The natural source of Eleuthero has been over exploited for pharmacological use, but the manual restoration to rehabilitate its natural distribution has not yet been established. The root is one of the main underground organs in Eleuthero for the acquisition of pharmaceutical ingredients. Root growth also determines the seedling quality of this species for ecological restoration. In this study, Eleuthero seedlings were cultured for one growing cycle with chitosan oligosaccharide (CO) addition at rates of 0 and 25 ppm under artificial lightings by high-pressure sodium (HPS) lamps and two light-emitting diodes (LEDs) with photosynthetic photon flux density set to 94±5 μmol m^{-2}. Light spectra of red (R): green (G): blue (B) ratios were measured to be 43.7:54.6:1.7, 43.8:47:9.2, and 72.7:13.3:14 for the HPS, LED-1, and LED-2 treatments, respectively. In autumn, no interactive effect was found between CO addition and light spectra treatments on any root parameters. Compared to the HPS treatment, the LED-1 treatment resulted in a greater root dry weight and morphology by over 30%. The CO addition caused an increase of root growth by 40-70%. Fine roots in diameter between 0-0.4 mm were the main root part that contributed to the length, surface area, and root tip number. Therefore, LED lighting with the R/G/B spectrum of 43.8:47:9.2 was suggested for the culture of Eleuthero seedlings with the purpose of promoting dry weight and morphology of fine roots.

Keywords: artificial illumination; Ciwujia; natural product; Northeast China; underground organ

Introduction

The *Eleutherococcus* Maxim. [Acanthopanax (Decne. et Planch) Witte] genus comprises about 40 species, most of which are naturally distributed in Eastern Asia and Far East Russia (Wan *et al.*, 2016; Zaluski and Smolarz, 2016). *Eleutherococcus senticosus* (Eleuthero) is the only species from the *Eleutherococcus* family that was documented by the European Pharmacopoea and American Herbal Pharmacopoeia monographs

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(Zaluski and Smolarz, 2016). Forests in Northeast China account for a large area of the natural Eleuthero reserves (Guo *et al*., 2019). Eleuthero naturally distributes as a long-lived shrub in broad-leaved and mixed broadleaf-conifer forests. Underground organs of Eleuthero contain multiple antioxidants and pharmacological ingredients (Zhai *et al*., 2017). However, the over-exploitation of Eleuthero has almost wiped out its natural reserves in Northeast China (Zhai *et al*., 2017). Climate change has also contributed to the shift and decline of its natural distribution (Wan *et al*., 2016; Guo *et al*., 2019). Therefore, it is necessary to restore the natural source of Eleuthero through artificial restoration.

The seedling quality can be measured by attributes at the end of culture and can be used to forecast transplant performance (Duan *et al*., 2013; Wei *et al*., 2013a). A larger root system can elicit better root growth potential which is achieved through prolonged photoperiod by high-pressure sodium (HPS) lamps (Wei *et al*., 2013b; Zhu *et al*., 2016; Zhao *et al*., 2017; Li *et al*., 2017a). Light emitting diode (LED) technique can further promote seedling quality using designed spectrums (Li *et al*., 2017a, 2018; Zhao *et al*., 2019; Wei *et al*., 2020). The LED spectrum can induce growth and biomass accumulation in underground organs of vegetation. For example, red (R; 600-700 nm) light was found to depress root growth in tomato (*Solanum lycopersicum* L. cv. SV0313TG) seedlings (Li *et al*., 2017b) but favour root biomass in larch (*Larix principis-rupprechtii* Mayr.) (Zhao *et al*., 2019) and pine (*Pinus koraiensis*) seedlings (Wei *et al*., 2020). Evidence also exists that root biomass in *Dalbergia odorifera* is not responsive to light spectra (Li *et al*., 2018). These reports demonstrate the need to test spectra effect on root growth in Eleuthero.

Chitosan is generated from chitin (β-1.4-N-acetylglucosamine) by deacetylation, which can be derived from the shells of marine animals. Chitosan oligosaccharide (CO) has been widely used as a plant growth modifier for several crop and woody species (Khan *et al*., 2002; Jeong and Park, 2005; Wang *et al*., 2017; Li *et al*., 2018). Rhizosphere moisture can be reserved by substrate and contributes to the application of CO addition through root soaking (Wang *et al*., 2017; Li *et al*., 2018). The CO addition to root has been found to promote new root number in over-year Buddhist pine (*Podocarpus macrophyllus*) seedlings (Wang *et al*., 2017), but it barely affects root biomass in fragrant rosewood (*D. odorifera*) seedlings unless it is paired with the manipulation of light spectra (Li *et al*., 2018). Therefore, the addition of CO may be used as a root-growth promoter for Eleuthero plants and should be tested with combined lighting spectra.

The purpose of the current study was to test the response of fine root growth of Eleuthero seedlings to combined treatments to the combined treatment of LED spectra and CO addition. We hypothesized that: (i) the root growth can be enhanced by combined effects of CO addition and light spectra, and (ii) fine root morphology with very fine diameters would respond.

Materials and Methods

Plant material

The study was conducted on Heilongjiang University of Chinese Medicine (45°43′34.66″ N, 126°38′44.12″ E), Harbin, Heilongjiang, China. Seeds of Eleuthero (*Eleutherococcus senticosus* [Ruprecht & Maximowicz] Maximowicz were collected from a seed orchard at Qitaihe (45°37′N, 31°15′E), Heilongjiang, China in the autumn of 2016. In early February of 2017, seeds were sterilized in potassium permanganate (0.5 %, w/w) for 5 min, soaked in distilled water for 12 h, and sowed in trays filled with sterilized sands for germination. Seeds were germinated in an incubation chamber where the condition was set at a range of 34/22 °C (max/min) for temperature with air humidity of 80% in a 16 h photoperiod (spectrum of 16.6% red, 75% green [G; 500-600 nm], and 8.4% blue [B; 400-500 nm] lights as 70 μmol m^{-2} s^{-1}) (Wang *et al*., 2018). Three months after germination, seedlings of similar size were transplanted into plastic pots (7 cm in height, 13 cm in diameter) which were filled with substrates (Mushro-Dust®, Zhiluntuowei Agric. S&T Ltd., Changchun, China) mixing peat, perlite, and controlled-release fertilizers (CRFs) (3:1:0.1, v/v/v). Each of the grouped pots was placed in one tank (35 cm × 55 cm) with the initial water table at the height of 6 cm.

Lighting spectra treatment

One week after transplant, potted seedlings were randomly arranged and placed into four replicated blocks (40 cm × 120 cm) on mobile seedbeds in a greenhouse. An LED lighting panel with an area of 0.66 m^2 (55 cm × 120 cm) was placed at the height of 80 cm. Two spectra were supplied by LEDs (LED-1, percent R/G/B, 43.8:47:9.2; LED-2, percent R/G/B, 72.7:13.3:14) with another by HPS (percent R/G/B, 43.7:54.6:1.7) as the control at a photosynthetic photon flux density (PPFD) at 94±5 μmol m^{-2} s^{-1} at 55 cm-height. Lighting was controlled in a 16-h photoperiod from 5:00 am to 21:00 pm. Throughout the experiment, relative humidity (RH) and temperature were monitored to be 70±10 % and 25/18 °C (day/night), respectively.

The CO treatment

A tank was watered with CO solution (Qishanbao®, GlycoBio Co., Ltd., Dalian, China) at the concentration of 25 ppm, with distilled water in another tank. The CO treatment started at the same time with the lighting spectra treatment and was implemented every two weeks. Seedlings were cultured by commercial substrates wherein roots were fed by micro-nutrients (N-P_2O_5-K_2O, 10-4-7) from CRFs continuously for four months. Therefore, each seedling received 80 mg nitrogen (N) during culture, no extra nutrients were supplied.

Root sampling and measurement

Roots were sampled on the 15th September, 2017 at the end of culture. Four seedlings were randomly chosen and harvested from one combined treatment per block. Root samples were further divided by every first-order lateral root and scanned to obtain plane image (Epson Expression 10000XL, Japan). Scanned images were analysed using the WinRHIZO® software (Regent Instruments Inc., Canada) for average root diameter (mm), total root length (cm), total root surface area (cm^2), and number of root tips for the whole root system and in each of the 10 diameter classes (from 0 to 0.9 mm by every 0.1 mm and > 0.9 mm). Post-scanned root samples were oven-dried at 70 °C for two days and measured for dry weight.

Statistical analysis

Statistical tests and analyses were performed using the SPSS software (Ver. 19.0, IBM statistics, Chicago, U.S.A.) and graphs were generated using the SigmaPlot software (Ver. 11.0, Systat Software Inc., GmbH, Germany). All root data passed the normality test (Kolmogorov–Smirnov method). The two-way analysis of variance (ANOVA) was conducted on all root parameters to test the combined effects of lighting spectra and CO addition. When the interactive effect was detectable all results were compared by the one-way ANOVA with four replicates (n=4). Otherwise, the results were compared by the main effect of light spectra (n=8) or CO addition (n=12). The significance was identified at the P<0.05 level according to Duncan test. In order to analyse the relationship among root morphological parameters in different diameter classes, data of root length, surface area, and tip number were analysed by the principle component analysis (PCA) method. Coupled correlations were analysed between root dry weight and every fine root morphology.

Results

Effects of CO addition and light spectra on root growth

Effects of light spectra and CO addition had no combined effects on root biomass and morphology in Eleuthero seedlings (Table 1). Instead, either of the two treatments had a significant main effect on root parameters. Among the three light spectra, the LED-1 treatment resulted in better root growth than in the

HPS treatment. Compared to the HPS treatment, seedlings under the LED-1 spectrum had greater root length, surface area, diameter, tip number, and dry weight by 79%, 71%, 36%, 55%, and 77%, respectively (Figure 1). However, root growth in seedlings in the two spectra treatments had no difference. The CO addition increased root growth by 73%, 68%, 47%, 58%, and 65% in length, surface area, diameter, tip number, and dry weight, respectively (Figure 1).

Table 1. *F* values from ANOVA analysis of light spectrum (L), chitosan oligosaccharide (CO) addition, and their interaction (L×CO) on fine root morphology in Eleuthero (*Eleutherococcus senticosus* Ruprecht & Maximowicz] Maximowicz)

Source of variation	DF [a]	Root length	Surface area	Diameter	Tip number	Dry weight
L	2	25.31*** [b]	21.53***	13.18***	12.24***	27.16***
CO	1	36.99***	38.27***	18.94***	19.75***	40.62***
L×CO	2	2.43	2.32	1.96	2.26	0.50

[a]DF, degree of freedom; [b] ***, significance at the level <0.001.

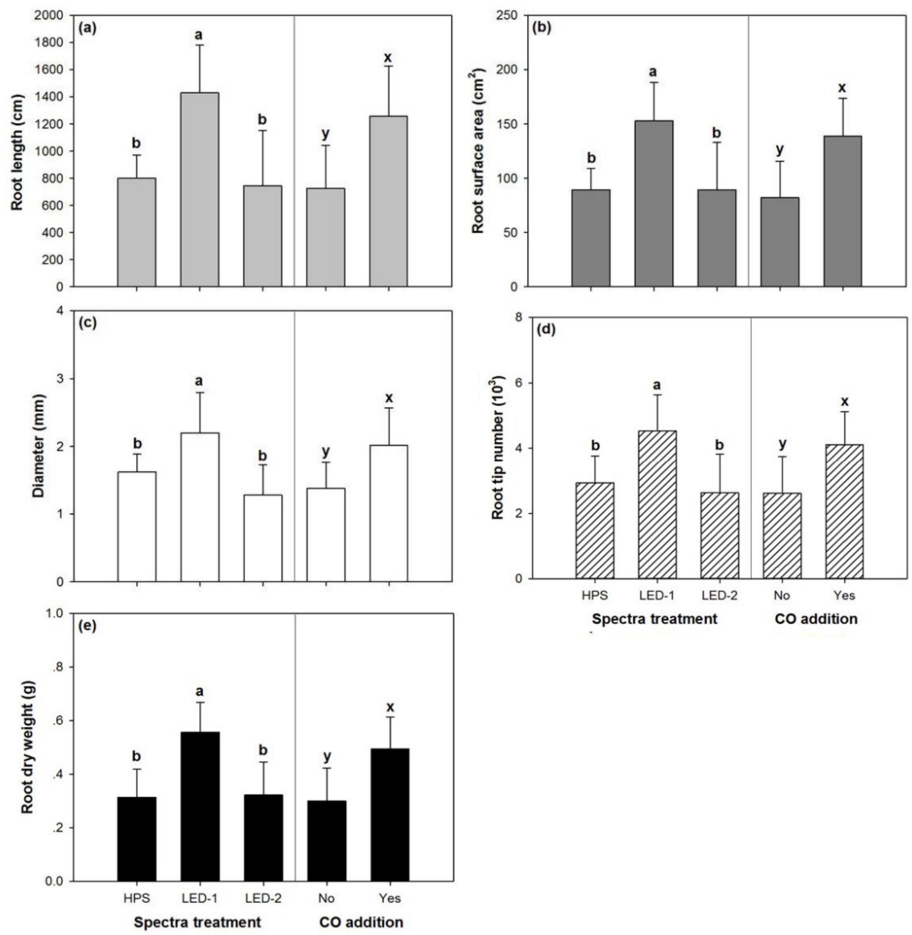

Figure 1. Root growth parameters of length (a), surface area (b), diameter (c), tip number (d), and dry weight (e) in Eleuthero (*Eleutherococcus senticosus* [Ruprecht & Maximowicz] Maximowicz) seedlings with chitosan oligosaccharide (CO) addition at rates of 0 (No) and 25 ppm (Yes) under light spectra from high-pressure sodium (HPS) lamps and two light-emit diodes (LEDs) panels. Different letters indicate significant difference among treatments. Lowercase letters of a, b, and c are labeled for light spectra treatment, and those of x and y are labeled for CO addition treatment

The response of fine root morphology in different diameter classes

Effects of light spectra and CO addition had no interactive effects on any fine root morphological parameters in any diameter classes (Figure 2). Similar to results about whole root system, fine root morphology was also higher with CO addition and highest in the LED-1 treatment among the three light spectra treatments, although significant results did not always persist in any diameter classes. Among the spectra treatments, fine root length was highest in the LED-1 treatment in all diameter classes except for the diameter of 0-0.1 mm (Figure 2a). However, CO addition increased fine root length in all diameter classes (Figure 2b). Root surface area was highest in the LED-1 treatment in all diameter classes except for the largest, which was more than 0.9 mm (Figure 2c). CO addition also increased surface area in all fine root diameter classes (Figure 2d). Root tip number only responded to light spectra in diameter classes between 0.1 and 0.5 mm (Figure 2e), while it responded to the CO addition only in diameter classes less than 0.3 mm (Figure 2f).

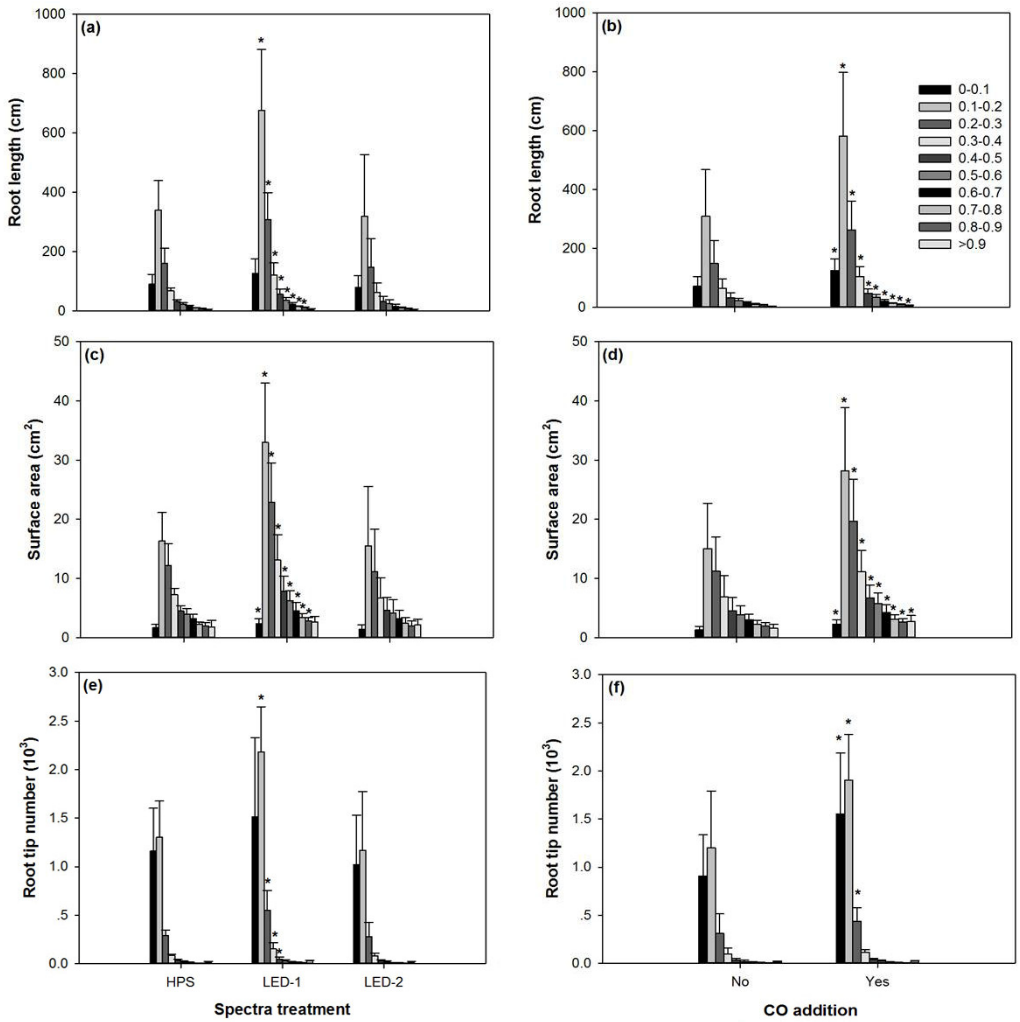

Figure 2. Fine root morphological parameters of length (a and b), surface area (c and d), and tip number (e and f) in different diameter classes (legend unit: mm) in Eleuthero (*E. senticosus* [Rup. & Max.] Max.) seedlings with chitosan oligosaccharide (CO) addition at rates of 0 (No) and 25 ppm (Yes) under light spectra from high-pressure sodium (HPS) lamps and two light-emit diodes (LEDs) panels. Asterisks indicate significant difference between HPS and LED-1 treatments or between with and without CO addition treatments

The relationship among root growth parameters

Coupled linear correlations indicated that any two root morphological parameters had a positive relationship between each other (Table 2). Also, positive correlation was found between root morphological parameter and dry weight. In detail, the relationship among fine root morphologies in different diameter classes can be described by the PCA model (Figure 3). The first PC accounted for 79.25% of the total data variation and the second PC accounted for 10.35%. In the axis of PC-1, data about fine root length and surface area in all diameter classes showed high tendency to each other (Figure 3). These data were also closely accompanied by high eigenvalues of root tip numbers in diameter classes from 0 to 0.4 mm. In the second PC axis, root tip number in diameter class of 0.5-0.6 mm was accompanied by negative eigenvalues for root tip number in diameter classes from 0.6 to 0.9 mm (Figure 3).

Table 2. Pearson correlations between couples of root diameters in Eleuthero (*E. senticosus* [Rupr. & Maxim.] Maxim.) cultured under three spectrums with or without CO addition

	Root length	Surface area	Diameter	Tip number	Dry weight
Root length	-	-	-	-	-
Surface area	R=0.9967 P<0.0001	-	-	-	-
Diameter	R=0.9578 P=0.0026	R=0.9370 P=0.0058	-	-	-
Tip number	R=0.9487 P=0.0039	R=0.9548 P=0.0030	R=0.8660 P=0.0257	-	-
Dry weight	R=0.9866 P=0.0003	R=0.9894 P=0.0002	R=0.9224 P=0.0088	R=0.9606 P=0.0023	-

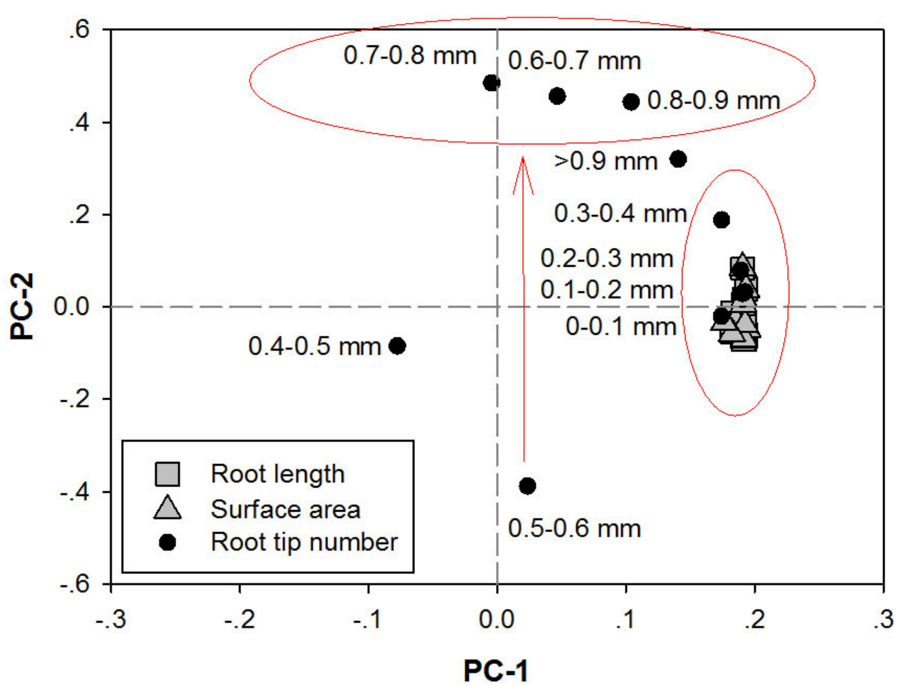

Figure 3. Principle component (PC) analysis on fine root morphological parameters of length, surface area, and tip number in Eleuthero (*E. senticosus* [Rup. & Max.] Max.) seedlings

Discussion

Our results disagreed with our first hypothesis. However, our results revealed significant main effects from either treatment on all root parameters. These results do not agree with Wang *et al*. (2017), who found an interactive effect of combined first-year CO addition and photoperiod on the second-year new-root-number in Buddhist pine seedlings. Authors therein demonstrated the nutrient dynamic across years and suggested that the over-year interactive effect on roots was caused by the inherent nutrient cycling. Further studies are suggested to continuously test the interactive effect of CO addition and light spectra on nutrient allocation during culture of Eleuthero seedlings to detect the possible mechanism of null interaction on root growth.

The PPFD for Eleuthero seedlings in our study (94±5 μmol m^{-2} s^{-1}) was supplied continuously for 16 h every day. This light intensity was much lower than that (250 μmol m^{-2} s^{-1}) in the continuous lighting for Norway spruce (*Picea abies*) and Scots pine (*P. sylvestris*) seedlings but close to that (70-80 μmol m^{-2} s^{-1}) used as supplementary lighting for conifer species from temperate zones (Apostol *et al*., 2015) and that (72-73 μmol m^{-2} s^{-1}) used in continuous lighting for fragrant rosewood seedlings (Li *et al*., 2017a, 2018). Eleuthero is an undergrowth species and highly shade-tolerant; thus, a low light intensity is suitable for its growth and development.

Compared to the spectrum from HPS lamps, that from LED lighting resulted in larger root morphology and greater dry weight. In other studies, using HPS spectrum as the control, Riikonen *et al*. (2016) found that the LED spectrum of 75% R and 25% B can increase root to shoot biomass ratio compared to that from HPS. However, Riikonen (2016) reported greater root biomass in Scots pine seedlings under 25%B/R LED lighting than under HPS lamps. In addition, neither Apostol *et al*. (2015) nor Li *et al*. (2018) found significant change of root biomass under LED spectra compared to that under HPS lamps. Therefore, taking seedlings as materials, Eleuthero was more sensitive to light spectra in root growth than most of the tree species in current studies. Because root is one of the main organs with abundant pharmacological compounds for Eleuthero seedlings (Sun *et al*., 2011; Zhai *et al*., 2017), the LED lighting with the spectrum of 43.8%R/47%G/9.2%B can be considered for use with the purpose of promoting fine root elongation in this species.

Our results showed that the addition of CO can promote root dry weight and fine root morphology, which concur with former findings about plant root growth in response to CO addition (Aleksandrowicz-Trzcińska *et al*., 2015; Chamnanmanoontham *et al*., 2015; Zong *et al*., 2017). Nevertheless, Li *et al*. (2018) found no effect of CO addition on root dry weight in fragrant rosewood. These varied results are caused by the difference of plant species and the dose of CO. Future work is needed to detect the deeper mechanism for the effect of CO addition on root growth in Eleuthero seedlings.

Our results about correlation between fine root variables across diameters suggested that surface area grew with the increase of root elongation. Root length and surface area in transplanted Buddhist pine and Northeast yew (*Taxus cuspidata*) seedlings also showed high similarity (Wei *et al*., 2017). Similar results were also reported during the first-year culture of Northeast yew seedlings (Zhao *et al*., 2017). Root tip in diameter of 0-0.4 mm accounted for the main response among all fine roots. They also showed highly positive relationships with root length and surface area, suggesting that new egress of very fine roots contributed to the length and area growth in Eleuthero seedling roots. According to the study on morphological and anatomical relationship of loblolly pine trees, fine roots in diameter <0.5 mm were mostly white new roots and those in diameter between 0.5 and 1 mm mostly changed to be woody with orange or brown colour (McCrady and Comerford, 1998). In Eleuthero seedling roots, the root tip number varied depending on the relationship with length and surface area in different diameters likely because of their different functions. Therefore, we can accept our second hypothesis.

Conclusions

Treatments of CO addition and light spectra had no interactive effect on root growth parameters in Eleuthero seedling roots. Instead, either treatment had a main effect on root morphology and dry weight. Compared to the light spectrum (43.7% R:54.6% G:1.7% B) from HPS lamps, that of 43.8% R:47% G:9.2% B from LED resulted in greater root dry weight and morphology by over 30%. The CO addition would also increase root growth by 40-70%. Fine roots in diameter between 0-0.4 mm were the main root part that contributed to the length, surface area, and new root number. Therefore, LED lighting can be used for the culture of Eleuthero seedlings with the purpose of promoting dry weight and morphology of fine roots.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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