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A novel approach of LED light radiation improves the antioxidant activity of pea seedlings

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Abstract

Influence of light-emitting diode (LED) light on antioxidant activity of radiated pea seedlings was first studied using red (625–630 nm) and blue (465–470 nm) LED lights as light sources in an attempt to determine and compare the changes in chlorophyll and β-carotene contents, and Trolox equivalent antioxidant capacity (TEAC, μM). After radiation for 96 h, comparing to white light group, red light radiated seedlings displayed significant ($p < 0.05$) increases in stem length and leaf area, while blue light radiation significantly ($p < 0.05$) increased the stem length and seedling weight. Chlorophyll in leaves increased rapidly when seedlings were radiated by blue light but no significant ($p > 0.05$) difference was observed among light radiated seedlings after 96-h cultivation. β-Carotene content of LED radiated leaves was significantly ($p < 0.05$) higher in red light (54.47 ± 2.35 μg/g) group than in the others. TEAC value of ethanol and acetone extracts (50 mg/mL) of 240 pieces of red light radiated seedlings cultured for 96 h reached 106.48 and 81.68 μM, respectively, were higher than the other treatments. In conclusion, the contribution of red light to significant β-carotene expression and antioxidant activity for nutrition and health benefits and blue light to seedling weight and chlorophyll induction of radiated pea seedlings are emphasized.

Keywords: Light-emitting diode (LED); Light radiation; Antioxidant activity; Trolox equivalent antioxidant capacity; Radiated pea seedling

1. Introduction

Light is not only an essential energy source for plant but also an important signal influencing the transition from etiolated to de-etiolated state, a stimulus for plant development, biosynthesis of cell components and gene expression throughout the life cycle of a plant (Clouse, 2001; Erdei, Barta, Hideg, & Böddi, 2005). The integration, quality, duration and intensity of red light/far red light, blue light, UV-A (320–500 nm) or UV-B (280–320 nm) and hormone signaling pathways have a profound influence on plant by triggering on/off of physiological reactions and control the growth and development of plant (Briggs, Beck, Cashmore, Christie, & Hunghes, 2001; Briggs & Olney, 2001; Clouse, 2001; Kevin, 2000).

The advantages of using light-emitting diode (LED) as artificial light source for controlled-environment plant growth applications including high energy-conversion efficiency, using DC power, small volume, longer life, wavelength specific, light intensity/quality adjustable and low thermal energy output (Okamoto, Yanagi, & Kondo, 1997; Schuerger, Brown, & Stryjewski, 1997). Some crops and flowers have been cultured by LED light radiation such as lettuce (Hoenecke, Bula, & Tibbits, 1992; Okamoto, Yanagi, & Takita, 1996), pepper (Brown, Schuerger, & Sager, 1995), wheat (Goins, Yorio, Sanwo, & Brown,

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1997; Tripathy & Brown, 1995), spinach (Yanagi & Okamoto, 1997; Yanagi, Okamoto, & Takita, 1996), and banana (Duong, Hong, Watanabe, Goi, & Tanaka, 2002). Red light is important for photosynthetic apparatus development and may increase starch accumulation in several plant species by inhibiting the translocation of photosynthesis out of leaves (Saebo, Krekling, & Appelgren, 1995). Blue light is important for chloroplast development, chlorophyll formation and stomata opening (Senger, 1982).

Etiolated seedlings constitute a major component of human diets (including salad, sandwich, Chinese dish and drink), especially as breakfasts and for vegetarians, providing fiber, vitamins and phytonutrients. β-Carotene and chlorophyll synthesized by all plants are components of photosynthesis and serve critical functions in plant biology including light harvesting, quenching of photooxidation, coloring of plants and providing nutritional benefits as precursor of essential vitamins and antioxidants for human beings. Photoinhibition results in many fold increase in the activity of superoxide dismutase and ascorbate peroxidase for protecting the seedlings against photooxidative damage (Sankhalkar & Sharma, 2002). Menezes-Benavente, Kernodle, Margis-Pinheiro, and Scandalios (2004) indicated that expression of antioxidant defense genes would be triggered to defend the cells against oxidative damage.

Recently, far-infrared radiation was reported to facilitate the antioxidant activity of rice hulls (Lee, Kim, Jeong, et al., 2003) and raw and cooked turkey breast (Lee, Kim, Nam & Ahn, 2003), suggesting the light radiation was contributory to the enhancement of nutrients in foods. In this study, red and blue LEDs were first utilized as light sources to understand the effect of LED radiation on the changes of antioxidant activity of radiated pea seedlings.

2. Materials and methods

2.1. Materials

Peas (*Pisum sativum* L.), from Australia for seeding, were purchased from a local supermarket in Pingtung County. Ethanol of 95% (v/v) was the product of Taiwan Tobacco and Liquor Co., Taiwan. Each of acetonitrile, ethyl acetate, tetrahydrofuran, acetone, and acetic acid was HPLC grade and was purchased from Sigma (St. Louis, MO, USA); while trichloroacetic acid, potassium ferricyanide and $FeCl_2$ were from Merck (Darmstadt, Germany). β-Carotene from Calbiochem (Darmstadt, Germany) was used for HPLC analysis.

2.2. Lighting system and culture conditions

There is two parts in light-emitting set: easily detachable electric circuit for emitting and one DC power supply (DPS-3050) which was used to control the light intensity by electricity adjustment. LED light source was aligned in a rectangular way, it has 9 LED and 9 linked electricity conducting inhibitor to form a patch, and there are 12 patches, that is, 108 LED to form a rectangular light-emitting set. All the LED lamps were purchased from Chungjiang Electronic Supplier (Kaohsiung, Taiwan) and the types of LEDs are shown as following: Blue LED, LED-10M/AHH-BL; Red LED, LED-10M/HP-R; White LED, LED-10M/AHH-W.

The pea seeds (*P. sativum* L.) were soaked in distilled water for 7 h to induce sprouting, and transplanted to Bean-Sprout Cultivator (Kainet Co., Ltd, Taichung, Taiwan) for water culture in dark at temperature set at 25 ± 2 °C. After 4-day incubation, the seedlings were transferred for single wavelength light radiation, and then collected for further determinations. There were three treatments for LED light radiation: blue light (112.29 ± 6.78 lx), red light (128 ± 4.38 lx) and white light (135.86 ± 3.98 lx), the peak emission of blue and red LED were 465–470 nm and 625–630 nm, respectively. Sampling was conducted at 12, 24, 48, 72 or 96 h during the continuous radiation cultivation for the following determinations. Seedlings cultured for up to 4 days (96 h) are with suitable size for consumption in local markets.

2.3. LED wavelength determination

The wavelength of light source was determined by an Ocean Optics (USB2000 FLG, Ocean Optics Inc., FlA, USA) machine with an accuracy of up to 0.16 nm/channel. The power supply was set up at 5 V electric pressure and 100 Ω electric inhibiting condition for light intensity measurement.

2.4. Morphological characteristics determination

The items of morphology determination are as following:

(a) Stem height (cm): The length from seed (endosperm) to the top of the leaf measured by a Vernier Caliper.
(b) Stem diameter (mm): The length of internode diameter nearest to the seed (endosperm part) measured by a Vernier Caliper.
(c) Leaf area (length × width, cm^2): The length and the width on the top of the leaf measured by a Vernier Caliper.
(d) Plant weight (g): To measure the plant weight by an Electronic Balance.

2.5. Chlorophyll content determination

Sample (5 g), added with liquid nitrogen, was blended (cycle blender, Osterizer Co., Berlin, Germany), extracted with 5 volumes of 80% acetone for 1 h and then filtered through a filter paper (Whatman No. 2) to obtain the liquid portion. Chlorophyll extraction was conducted in dark place to avoid possible photo bleaching. The absor-

bance of the chlorophyll in acetone was measured at a wavelength of 652 nm with a spectrophotometer (model 7800, Jasco, Tokyo, Japan). The content of total chlorophyll was calculated by the following equation (Porra, Thompson, & Kriedelman, 1989):

$$\text{Total chlorophyll } (\text{mg/g}) = [D_{652} \times V] \times V/W$$

where V is the total volume of acetone extract (mL); W, the fresh sample weight (g).

2.6. HPLC analysis of β-carotene

Sample (5 g) was first extracted with 40 mL of tetrahydrofuran (THF) (Sigma, St. Louis, MO, USA) in a cycle blender and then filtered through a Whatman No. 1 filter paper to obtain the filtrate. The residues were extracted twice with 10 mL of THF with the same procedure. The filtrates were combined and adjusted to 100 mL with THF in a volumetric cylinder, concentrated at 40 °C at a reduced pressure (10 mm Hg) by a rotary evaporator and then rehydrated to 2 mL by ethyl acetate. β-Apo-8′-carotenal (1.2 mg) (Sigma, Vienna, Austria) was added as internal standard. For β-carotene analysis, 10 μL extract was injected onto a reversed column (5u HYPERSIL® ODS, USA), which was eluted with a mobile phase of acetonitrile/methanol/ethyl acetate (740/160/100, v/v/v) (Murkovic, Mulleder, & Neunteuflw, 2002). Flow rate was controlled at 2.0 mL/min and the absorption of the effluent was monitored at a wavelength of 450 nm for β-carotene determination. Recovery of β-carotene spiked in sample matrices was determined to be 83–90%. Preparation of β-carotene stock solution (1000 μg/mL) was conducted following the method described by Murkovic et al. (2002) and the diluted stock solutions at various levels were used to construct the standard curve ($r^2 = 0.9993$).

2.7. Trolox equivalent antioxidant capacity (TEAC) assay

TEAC was conducted according to the procedures described by Miller and Evans (1996). To 7 mM ABTS (2,2′-azinobis[3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) solution was added potassium persulfate until it reached a final concentration of 2.45 mM. After being homogeneously mixed, the solution was stored at room temperature for 12–16 h to form the stable blue-greenish $ABTS^{\cdot+}$ free radical solution. Dilution of the thus obtained solution with water was conducted until the absorbance at 734 nm was 0.7. One milliliter of the diluted solution was mixed with various levels (5, 10, 15, 20, and 50 mg/mL) of ethanol or acetone sample extract, prepared with various numbers (24, 48, 72, 96, and 240 pieces, respectively) (on dried basis) of seedings and the change in absorbance at 734 nm was monitored by a Spectrophotometer. Trolox solutions at 15–150 μM were used to construct a calibration curve ($r^2 = 0.9966$) to estimate the scavenging activity on $ABTS^{\cdot+}$ radical.

2.8. Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS Institute, Inc., 1989) program package. Data were expressed as mean ±SEM. After an analysis of variance (ANOVA), significant difference among means were determined by Duncan's Test. Significance of differences was defined at $p < 0.05$. Three samples were each tested in duplicate.

3. Results and discussion

3.1. Effect of LED on pea sprout growth

Pea seedings are one of popular vegetables in oriental countries and the effects of radiation on pea sprout growth and nutritional changes are rarely studied. The seedlings were 4 days incubated in dark and transferred for different LED light radiation. No difference was observed at stem diameter among dark-grown and light radiated seedlings, but stem length, leaf area and seedling weight were greatly affected by light quality (Table 1). Light radiation induced color change and significantly ($p < 0.05$) retarded stem elongation of seedlings. Stem length of white light radiated seedlings was significantly ($p < 0.05$) shorter (about 16.38 cm) than the other radiated ones (about 21–23 cm). The leaf area of red light radiated seedlings was the largest (about 1.48 cm^2) and significantly ($p < 0.05$) different with other light radiation groups (about 0.52–1.1 cm^2). As on seedling weight, blue light radiated seedlings was the greatest (about 1.67 g) and significantly ($p < 0.05$) different with other light radiation groups (about 1.37–1.53 g). That is, the growth of leaf area and seedling weight is controlled by red light and blue light, respectively. Clouse (2001) revealed that light affected morphogenesis according to dark-grown seedlings have a greatly elongated hypocotyl. Red and far-red light influence greatly the growth and mor-

Table 1
Effect of LED lights on the morphological characteristics[a] of pea seedlings after radiation for 96 h

Light[b]	Stem length[c] (cm)	Stem diameter[c] (mm)	Leaf area[c] (cm^2)	Sprout weight[c] (g)
Dark	22.88 ± 1.70^{a}	$2.55 \pm 0.17^{a,b}$	0.52 ± 0.14^{c}	$1.50 \pm 0.12^{b,c}$
Blue	21.38 ± 1.91^{b}	$2.44 \pm 0.09^{a,b}$	0.91 ± 0.19^{b}	1.67 ± 0.27^{a}
Red	21.18 ± 1.05^{b}	2.62 ± 0.10^{a}	1.48 ± 0.27^{a}	1.53 ± 0.13^{b}
White	16.38 ± 0.30^{c}	$2.43 \pm 0.06^{a,b}$	1.11 ± 0.19^{b}	$1.37 \pm 0.16^{c,d}$

[a] Average ± standard deviation of triplicate determinations.
[b] White light, 135.86 ± 3.98 lx; red light (625–630 nm), 128 ± 4.38 lx; blue light (465–470 nm), 112.29 ± 6.78 lx.
[c] Means in each column with the same letter are not significantly different ($p > 0.05$).

phology of potato plantlets (Miyashita, Kitaya, Kozai, & Kimura, 1995). In this case, we found that the morphology and growth of seedlings such as stem length, leaf area and seedling weight was controlled by the light quality of radiation. Hence, LED light can be selectively utilized for vegetable growth by using the photomorphogenic pigments that are responsible for photoperception and regeneration ability triggering (Lian, Murthy, & Paek, 2002).

Kim, Hahn, Heo, and Paek (2004) obtained the greatest stem elongation of chrysanthemum plantlet under red LED and Red + far-red than fluorescent light, blue, blue + red and blue + far-red LED. Miyashita, Kimura, Kitaya, Kubota, and Kozai (1997) revealed that red light significantly enhanced the elongation of stalk in pelargonium plantlets except the dry weight and the leaf acreage; on the contrary, the blue light inhibited the elongation of the stalk. The effect of red light seems inconsistent; red light caused a reverse effect on stem elongation in *Rehmannia glutinosa* plantlet (Tennessen, Singsaas, & Sharkey, 1994) and inhibited shoot elongation in marigold and salvia (Heo, Lee, Chakrabarty, & Paek, 2002). It seems that stem elongation is controlled by different synergistic interactions under light radiation according species, physiological recognition on the light signals and photochemical reactions are controlled by light quality. The receptor will catch up different wavelength of light, identify the signals, and translate into reactions in the cells, these phytochromes existed in the green plants are mainly chlorophyll and secondly carotene (Hopkins & Huner, 2004).

3.2. Effect of LED on chlorophyll and β-carotene content

Light radiation of etiolated pea seedlings greatly enhanced the expression of chlorophyll (Fig. 1) and significantly ($p < 0.05$) differed with the control seedlings under dark. Blue light caused a large increment of chlorophyll within 48 h, while white light induced a small increment and red light caused a gradual increment within 12–24 h. No significant ($p > 0.05$) difference of chlorophyll content among light radiations for 96 h (data not shown). Miyashita et al. (1997) also indicated that chlorophyll content in pelargonium plantlets is relevant to the ratio of red light during radiation cultivation, while higher ratio of blue light in the light source is related to the higher chlorophyll content in orchid tissue cultured seedlings.

As shown in Fig. 2, light radiation significantly ($p < 0.05$) enhanced the β-carotene content in leaves and stems of pea seedlings as compared with the dark treatment. In 96-h radiated seedlings, the β-carotene content of LED radiated leaf was significantly ($p < 0.05$) higher in red light group (54.47 ± 2.35 μg/g) than blue light group (47.39 ± 3.01 μg/g), white light group (44.65 ± 2.11 μg/g) and dark-grown seedling (6.16 ± 0.11 μg/g). However, no significant ($p > 0.05$) difference of β-carotene content was observed among radiated stems of seedlings (about 1.72–2.24 μg/g). The effect of light radiation on β-carotene formation has not been reported yet. However, light radiation such as ultraviolet or blue light radiation might control many kinds of reactions including: phototropism, immigration of chloroplast, day–night period control, genotype expression and the open/close of stomata (Masahiro et al., 2002). In this study, blue light was a good light source for chlorophyll

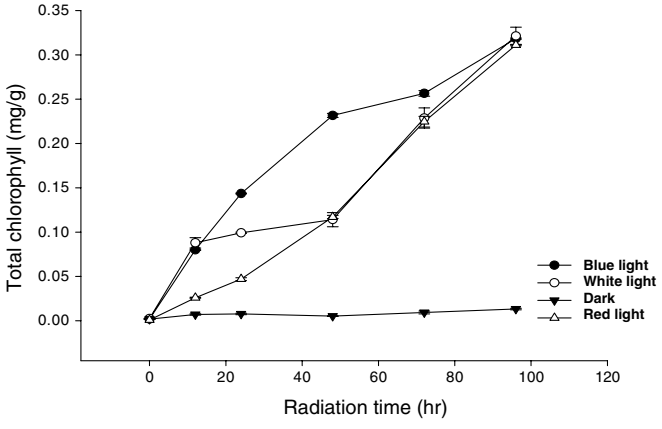

Fig. 1. Chlorophyll content in leaves of pea seedlings radiated by different light sources. Bars in the curve refer to standard deviation. Each value is the average of three determinations. White light, 135.86 ± 3.98 lx; red light, 128 ± 4.38 lx; blue light, 112.29 ± 6.78 lx.

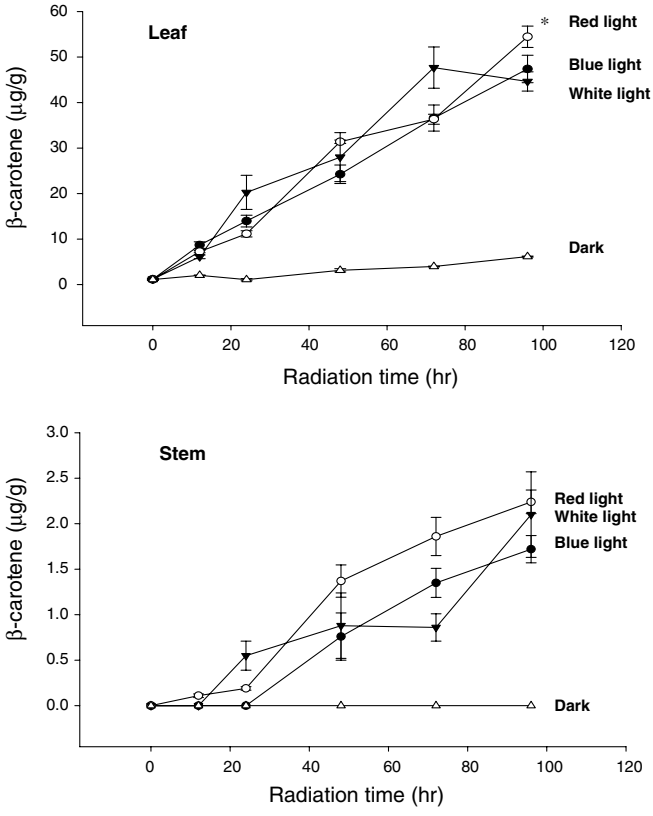

Fig. 2. Changes of β-carotene contains (μg/g) in leaves and stems of pea seedlings radiated by different light sources. Bars in the curve refer to standard deviation. Each value is the average of three determinations. White light, 135.86 ± 3.98 lx; red light, 128 ± 4.38 lx; blue light, 112.29 ± 6.78 lx. *, significant ($p < 0.05$) different from other values at the same radiation time.

Table 2
Trolox equivalent antioxidant capacity[a] (TEAC, μM) of pea seedings after 96 h-radiation by various LED lights[b]

Weight of sample (mg/mL)[c]	Dark	Blue light	Red light	White light
Ethanol extracts[d]				
50	58.23 ± 2.58^{d}	63.54 ± 2.84^{c}	106.48 ± 4.57^{a}	98.68 ± 4.35^{b}
20	34.86 ± 2.23^{c}	35.57 ± 1.54^{c}	59.62 ± 2.24^{b}	58.27 ± 2.4^{b}
15	22.16 ± 1.64^{e}	29.38 ± 1.89^{d}	54.62 ± 2.27^{b}	50.25 ± 2.35^{c}
10	19.94 ± 1.34^{e}	21.05 ± 1.74^{e}	37.16 ± 1.74^{c}	35.57 ± 1.32^{c}
5	8.91 ± 0.86^{f}	10.73 ± 0.64^{f}	$24.30 \pm 1.12^{d,e}$	20.49 ± 1.02^{e}
Acetone extracts[d]				
50	17.47 ± 0.84^{gh}	46.49 ± 2.24^{ghi}	81.68 ± 3.71^{a}	71.61 ± 3.42^{b}
20	15.10 ± 0.63^{fg}	$29.06 \pm 7.69^{d,e}$	51.13 ± 2.59^{b}	44.30 ± 3.51^{c}
15	12.64 ± 0.84^{gh}	16.92 ± 3.72^{fg}	37.00 ± 2.71^{c}	$32.72 \pm 1.49^{c,d}$
10	7.16 ± 3.69^{hij}	10.97 ± 2.35^{ghi}	$32.00 \pm 1.9^{c,d,e}$	$28.75 \pm 1.29^{d,e}$
5	4.14 ± 3.12^{j}	6.29 ± 4.75^{ij}	26.29 ± 1.04^{e}	19.14 ± 0.87^{f}

Means in each row with the same letter are not significantly different ($p > 0.05$).
[a] Average ± standard deviation of triplicate determinations.
[b] White light, 135.86 ± 3.98 lx; red light (625–630 nm), 128 ± 4.38 lx; blue light (465–470 nm), 112.29 ± 6.78 lx.
[c] 50, 20, 15, 10, and 5 g of sample was prepared from 240, 96, 72, 48, and 24 pieces of seedings, respectively.
[d] Pea seedings were extracted with ethanol or acetone and the antioxidant capacity of different levels of extracts was referred to Trolox.

induction (Fig. 1), while β-carotene accumulation was stimulated by red light (Fig. 2). Cultivation of pea seedlings for 96 h gives the suitable size for consumption.

3.3. Effect of LED on TEAC value

TEAC value of ethanol extracts of pea seedlings after 96-h radiation under different LEDs was determined. It was observed that the TEAC value of red light radiated seedlings (106.48 μM) was significantly ($p < 0.05$) different with white light radiated group (98.68 μM), blue light radiated group (63.54 μM), and dark-grown (58.23 μM) seedlings at a concentration of 50 mg/mL extract. It decreased with the decreasing level (20–5 mg/mL) of ethanol extract of pea seedings. Therefore, seedings cultured with red light radiation appeared to be potent in antioxidant capacity.

TEAC value of acetone extracts of pea seedlings radiated for up to 96 h showed the same tendency as ethanol extracts (Table 2). TEAC value of acetone extract from pea seedlings under red LED was the greatest (81.68 μM) and significantly ($p < 0.05$) higher than that of white light group (71.61 μM), blue light group (46.49 μM), and dark group (17.47 μM). That is, the antioxidant capacity of pea seedlings was significantly enhanced by red light radiation. Total phenol compounds and free radical scavenging activity in methanol extract of rice hulls increased as a result of the far-infrared radiation on rice and led to the antioxidation of cooked turkey meat (Lee, Kim, Jeong, et al., 2003; Nam, Kim, Ahn, & Lee, 2004). In addition, volatile aldehydes such as hexanal, pentanal, and propanal in rice hulls were also reduced in far-infrared radiated rice (Lee, Kim, Nam & Ahn, 2003).

Reports on reducing power of light radiated vegetables are rare. Meir, Kanner, Akiri, and Philosoph (1995) indicated that strong reducing power in tissues would reduce peroxide level and was effective in enhancing antioxidant activity. Liu et al. (2004) demonstrated that down-regulated LeHY5 (positive regulator of fruit pigmentation) plants exhibit defects in light responses, including inhibited seedling photomorphogenesis, loss of thylakoid organization, and reduced carotenoid accumulation. In contrast, repression of LeCOP1LIKE (negative regulator of fruit pigmentation) expression results in plants with exaggerated photomorphogenesis, dark green leaves, and elevated fruit carotenoid levels. These above mentions suggested that light signal transduction genes such as LeHY5 and LeCOP1LIKE influence pigmentation, nutritional value and quality of fruits. In this study, red light (610–710 nm) is an important light for etiolated pea seedlings that affects the expression of β-carotene and antioxidant ability of seedlings, revealing that light quality affects the phenotype expression, physiological metabolism and antioxidant activity of etiolated seedlings. Therefore, it is necessary to apply proper LED light to meet different purpose and detailed studies are required regarding the application of LED light for seedling growth for economic utility, nutrition enhancement and the correlation between light quality and growth of dietary seedlings. And it is interested to study on the effect of light quality on gene expression of etiolated pea seedlings in the future.

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