Effects of Yellow, Green, and Different Blue Spectra on Growth of Potato Plantlets In Vitro

Ruining Li¹, Wenwen Huang, Xiaoxiao Wang, Xiaoying Liu, and Zhigang Xu²

College of Agronomy, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

Additional index words. potato plantlets in vitro, yellow spectrum, green spectrum, blue lights of different wavelengths, strong plantlets culture

Abstract. The objectives of this study were to determine the effects of yellow light (Y), green light (G), and two blue lights (B) at different wavelengths in conjunction with red light (R) on the growth and morphogenesis of potato plantlets in vitro. Randomized nodal explants were cut into 1.0–1.5 cm pieces and were grown under five different light conditions: fluorescent white light (FL); the combined spectra of R, Y, and B at 445 nm (R₆₃₀B₄₄₅Y); the combined spectra of R, G, and B at 445 nm (R₆₃₀B₄₄₅G); the combined spectra of R, Y, and B at 465 nm (R₆₃₀B₄₆₅Y); and the combined spectra of R, G, and B at 465 nm (R₆₃₀B₄₆₅G). Morphogenesis and physiological parameters were investigated. The results showed that R₆₃₀B₄₄₅Y and R₆₃₀B₄₆₅Y increased the fresh weight (FW), dry weight (DW), stem diameter, blade number, leaf area, specific leaf weight (SLW), and the health index of potato plantlets in vitro; root activity increased significantly; and soluble sugar, soluble protein, and starch also increased. The addition of Y to the combined spectra of R and B contributed to the growth, development, and morphogenesis more than the combined spectra of R and B with G, and B at 445 nm was more effective at promoting plant growth than was B at 465 nm.

Potato (*Solanum tuberosum* L.) is an annual dicotyledonous herbaceous tuber crop that grows best in cool temperate climates under full sunlight, moderate daytime temperatures, and cool nights (Hawkes and Harris, 1992). However, potato plants are likely to be attacked by viruses, resulting in germplasm degradation and yield and quality decline, severely affecting the production of potato. The technology of virus-free microtubers and minitubers can remove the restriction of virus infection effectively. Therefore, vigorous potato plantlets grown in vitro are needed to produce microtubers and minitubers for cultivation. As a physical environmental factor, light (intensity, photoperiod, and spectrum) plays a vital role in regulating photosynthesis, metabolism, and morphogenesis of potato plantlets in vitro.

As a component of light, light spectrum is one of the most important environmental factors for the growth and development of potato plantlets in vitro. It provides not only energy for photosynthesis but also signal stimuli for physiological activities (Gao and Zhang, 2002). The light spectrum activates

a series of physiological and biochemical metabolic processes through photoreceptors in photomorphogenesis, which then control the growth and morphogenesis of potato tissues and organs in vitro (Eskins, 1992; Eskins et al., 1996; Mortensen and Stromme, 1987; Seabrook, 2005; Seabrook and Douglass, 1998). Charles et al. (1992) reported an increase in leaf surfaces of potato plantlets in vitro because of enrichment in red wavelengths, and Chang et al. (2009) reported that blue light-emitting diode (LED) was beneficial to increase dry matter content of potato plantlets in vitro. Seabrook and Douglass (1998) reported that plantlet height increased when blue spectrum was removed, and the addition of B restrained stem elongation (Aksenova et al., 1994). Wu et al. (2007) reported that R spectrum emission is near the point of maximum absorption by chlorophylls (Chls) and phytochromes and it is important for photosynthetic apparatus development and for starch accumulation, and that B is relevant for chloroplast development, Chls formation, and stomata opening. Jao and Fang (2001) reported that the combined spectra of R and B LEDs promoted the growth and development of potato plantlets in vitro, and in their subsequent experiment, these authors found that the combined spectrum of 45% R + 55% B was optimum for the accumulation of fresh and dry mass of potato plantlets in vitro (Jao and Fang, 2004). However, these authors did not investigate the effects of different wavelength blue LEDs on potato plantlets in vitro. The wavelengths of B range from 440 to 480 nm. LEDs with

peak at 445 and 465 nm are now commercially available, and the effect of these two wavelengths of blue LEDs on plants has not been explicitly investigated. We were interested in which wavelength of blue LED is more favorable for potato plantlets in vitro.

In tissue culture, the plant height, FW and DW of marigolds in vitro increased by 30% to 50% when the green (G) spectrum was removed (Klein, 1992). Kim et al. (2004) reported that the growth of lettuce was restrained when G ratio exceeded 50% but that the lettuce growth was promoted when G ratio was less than 24%. Ma et al. (2015) reported that the addition of G to the combined spectra of R and B contributed to stem length, stem diameter, leaf area, and the contents of Chl, soluble sugar, soluble protein, and starch of potato plantlets in vitro more than the combined spectra of R and B without G did. Because the wavelength range of the spectra was not uniform in some studies (spectra of 500–600 nm were classified as G), few studies on effects of Y (580–600 nm) (Y) on plant growth and development have been reported (Dougher and Bugbee, 2001). Al-Wakeel and Hamed (1996) reported that Y (with a peak wavelength at 595 nm) had a stronger inhibition on the growth of cucumber than G did (with a peak wavelength at 520 nm). Wang et al. (2009) reported that the DW, net photosynthetic rate, quantum yield of PSII electron transport, and Chl content of cucumber clearly decreased and that the parameters of stomatal conductance, total soluble sugars, sucrose, starch content, and Chl a/Chl b decreased under Y compared with those under white light at the same light intensity. Godo et al. (2011) reported the greatest rate of emergence of *Bletilla ochracea* under Y. Therefore, Y may play an important role in the process of growth and development of plants; however, the physiological significance of Y was not very clear in previous studies (Dougher and Bugbee, 2001). The combination of R and B is important for the growth of potato plantlets in vitro (Jao and Fang, 2004). However, the effects of the combination of R, B, and Y LEDs on potato plantlets in vitro have not been reported. As such, we are interested in whether the addition of Y to the combined spectra of red and blue is more conducive to the growth and development of potato plantlets in vitro than green.

LEDs are widely used in plant growth chambers and are useful in plant tissue culture because of their lower heat radiation, higher energy efficiency, and longer lifespan compared with fluorescent lamps (Ma et al., 2015; Nhut et al., 2003; Poudel et al., 2008). In our experiment, we used various LEDs to obtain B at two wavelengths and used Y, G, and R to design four combined spectra with uniform light intensity by modulating the electrical parameters of the LEDs. The objectives of this study were to investigate the effects of Y LED, G LED, and two B LEDs with R on the growth of potato plantlets in vitro and to identify the most effective light quality for the growth of potato plantlets in vitro.

Received for publication 3 Jan. 2018. Accepted for publication 5 Feb. 2018.

This research was supported by the National Natural Science Foundation of China (11674174), and the National R&D Program in Science and Technology of China (2017YFB0403903).

¹First author. E-mail: 2016201022@njau.edu.cn.

²Corresponding author. E-mail: xuzhigang@njau.edu.cn.

Materials and Methods

Plant materials. Potato cultivar ‘Shepody’ plantlets were grown in vitro using MS medium (Murashige and Skoog, 1962); they were provided by Jiangsu Polytechnic College of Agriculture and Forestry. The experiment was conducted at Nanjing Agricultural University. A tissue culture room with a relative humidity of $65\% \pm 2\%$ and a 16-h photoperiod was offered for culture of mother plants. In the tissue culture room, the daytime temperature was set to $25 \pm 2^\circ\text{C}$ and the night was $18 \pm 2^\circ\text{C}$. The photosynthetic photon flux density (PPFD) was $72 \pm 2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Stem segments ($\approx 1.0\text{--}1.5$ cm in length with one leaf) were dissected aseptically from potato plantlets in vitro, and they were inoculated vertically in MS medium with $30 \text{ g}\cdot\text{L}^{-1}$ sucrose and $8 \text{ g}\cdot\text{L}^{-1}$ agar. After being pre-cultured for 3 d under fluorescent white lamps, potato plantlets in vitro were grown under different light conditions for 30 d. For each treatment, 10 bottles were used and each bottle contained 10 randomized explants. Each experiment was repeated three times.

Light treatments. Light quality experiments were performed in a culture room. All LEDs are developed and offered by Opt-run Biotechnology Co., Nanjing, China. The peak wavelengths of LEDs used in the experiment were shown in Table 1 as follows:

1. FL (control): fluorescent lamp at broad wavelengths of 400–700 nm.
2. $\text{R}_{630}\text{:B}_{445}\text{:Y} = 6\text{:}2\text{:}1 = 48 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ R with a peak wavelength at 630 nm, $16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ B with a peak wavelength at 445 nm, and $8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Y with a peak wavelength at 590 nm.
3. $\text{R}_{630}\text{:B}_{445}\text{:G} = 6\text{:}2\text{:}1 = 48 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ R with a peak wavelength at 630 nm, $16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ B with a peak wavelength at 445 nm, and $8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ G with a peak wavelength at 520 nm.
4. $\text{R}_{630}\text{:B}_{465}\text{:Y} = 6\text{:}2\text{:}1 = 48 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ R with a peak wavelength at 630 nm, $16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ B with a peak wavelength at 465 nm, and $8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Y with a peak wavelength at 590 nm.
5. $\text{R}_{630}\text{:B}_{465}\text{:G} = 6\text{:}2\text{:}1 = 48 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ R with a peak wavelength at 630 nm, $16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ B with a peak wavelength at 465 nm, and $8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ G with a peak wavelength at 520 nm.

Total PPFD was recorded with a quantum sensor (LI-250A; LI-COR, Lincoln, NE) at $72 \pm 2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. All treatments were setup in a tissue culture room.

Growth parameters. After growing for 30 d, 15 potato plantlets in vitro from each treatment were randomly taken for growth analysis. The stem height was measured from the main stem base to the top of the plantlets using a ruler, whereas the stem diameter was obtained at the internodes above the penultimate leaf using vernier calipers (601-01; Links Inc., Harbin, China). The growth and morphology assessment of stem length, root length, stem diameter, SLW, blade number, leaf area, FW, DW, and health index was repeated three times with five plantlets in each treatment. The SLW of each plantlet was measured using the following equation:

$$\text{SLW} = \frac{\text{Leaf area}}{\text{Leaf DW}}$$

The shoot DW was measured by drying the shoot at 105°C for 15 min, then drying at 80°C until a constant mass was achieved, and then weighing by using an electronic balance (AU120; Shimadzu, Japan). The health index was determined using the following equation (Fan et al., 2013):

$$\text{Health index} = \frac{\text{Stem diameter}}{\text{Stem height}} \times \text{DW}$$

Root activity. The lateral root tips (0.5 g FW) from five random plantlets of each treatment were placed in 5 mL of 0.1% 2,3,5-triphenyltetrazolium chloride and 5 mL of 0.067 M potassium phosphate buffer. After mixing thoroughly, the roots were placed for 2 h (7) in a 37°C water bath. The reaction was ended with 2 mL of 1 M H_2SO_4 and the roots were removed and rinsed with distilled water. The samples were placed into a mortar with quartz sand and 10 mL of acetone (V) and ground until the root turned white. Optical density (ρ) was measured with a spectrophotometer (ultraviolet-1200; Jin Peng Inc., Shanghai, China) at 490 nm. Root activity was determined as the tetrazolium reduction intensity of the root at 1 g of FW per hour according to Li et al. (2010) as root activity = $\rho \times V/\text{FW} \times T$ ($\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$).

Content of Chl. Chlorophyll was extracted from the leaves of 10 plantlets at a similar position on the leaf in each treatment. Leaf samples (0.1 g; FW) were ground in a mortar, and two sequential extractions were performed for 2–6 h using 10 mL of 80% acetone until the

samples turned white. Absorbance was measured with a spectrophotometer (ultraviolet-1200; Jin Peng Inc.) at 663 nm (OD663) for Chl a and at 645 nm (OD645) for Chl b. The Chl concentrations were determined according to Lichtenthaler and Wellburn (1983).

Concentrations of carbohydrates and protein measurements. The contents of soluble sugar and starch were measured by the modified anthrone (Hushi Inc., Shanghai, China) method of Fairbairn (1953). The soluble protein content was determined by the Coomassie brilliant blue G-250 (Solarbio Inc., Beijing, China) method (Bradford, 1976).

Statistical analysis. Statistical analyses were conducted with Statistical Product and Service Solutions for Windows, Version 17.0 (SPSS, Japan). Data were analyzed by analysis of variance, and the statistical significance of differences between means was tested using Tukey’s test ($P < 0.05$).

Results

Agronomic traits. As shown in Table 2, compared with FL, the complex spectra of red and blue plus yellow significantly increased the health index (1.34 and 1.14, respectively), FW (296.67 and 256.67 mg, respectively), stem diameter (1.57 and 1.50 mm, respectively), blade number (6.67 and 7.33 p, respectively), and SLW (21.24 and 19.68 $\text{mg}\cdot\text{cm}^{-2}$, respectively) of potato plantlets in vitro (Table 2). This indicated that the addition of yellow LEDs to the combined spectra of red and blue was more beneficial to the vegetative growth of potato plantlets in vitro than FL.

The parameters of root length, stem diameter, SLW, blade number, FW, DM, and the health index of potato plantlets in vitro were significantly higher under $\text{R}_{630}\text{B}_{445}\text{Y}$ (127.93 mm, 1.57 mm, 21.24 $\text{mg}\cdot\text{cm}^{-2}$, 6.67 p, 296.67 mg, 53.67 mg, and 1.34, respectively) than under $\text{R}_{630}\text{B}_{445}\text{G}$ (119.43 mm, 1.27 mm, 15.06 $\text{mg}\cdot\text{cm}^{-2}$, 4.67 p, 231.00 mg, 42.33 mg, and 0.92, respectively) and were significantly higher under $\text{R}_{630}\text{B}_{465}\text{Y}$ (126.57 mm, 1.50 mm, 19.68 $\text{mg}\cdot\text{cm}^{-2}$, 7.33 p, 256.67 mg, 45.33 mg, and 1.14, respectively) than under $\text{R}_{630}\text{B}_{465}\text{G}$ (118.37 mm, 1.17 mm, 14.91 $\text{mg}\cdot\text{cm}^{-2}$, 4.67 p, 206.67 mg, 37.33 mg, and 0.69, respectively). These results revealed that the addition of yellow LEDs to the combined spectra of red and blue was favorable for the vegetative growth of potato plantlets in vitro.

The root length, stem diameter, SLW, and blade number were not significantly different between $\text{R}_{630}\text{B}_{445}\text{Y}$ and $\text{R}_{630}\text{B}_{465}\text{Y}$ treated potato plantlets in vitro or between $\text{R}_{630}\text{B}_{445}\text{G}$ and $\text{R}_{630}\text{B}_{465}\text{G}$ treated plantlets. The parameters of FW, DW and the health index of plantlets in vitro were significantly higher under $\text{R}_{630}\text{B}_{445}\text{Y}$ (296.67 mg, 53.67 mg, and 1.34, respectively) than under $\text{R}_{630}\text{B}_{465}\text{Y}$ (256.67 mg, 45.33 mg, and 1.14, respectively) and were significantly higher under $\text{R}_{630}\text{B}_{445}\text{G}$ (231.00 mg, 42.33 mg, and 0.92, respectively) than under $\text{R}_{630}\text{B}_{465}\text{G}$ (206.67 mg, 37.33 mg, and 0.69, respectively) (Table 2).

Table 1. Different spectral conditions used in these experiments.

Light treatment	PPFD ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)					Total PPFD	
	R_{630}	B_{445}	B_{465}	G_{520}	Y_{590}	FL	($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
$\text{R}_{630}\text{B}_{445}\text{Y}$	48	16	—	—	8	—	72
$\text{R}_{630}\text{B}_{445}\text{G}$	48	16	—	8	—	—	72
$\text{R}_{630}\text{B}_{465}\text{Y}$	48	—	16	—	8	—	72
$\text{R}_{630}\text{B}_{465}\text{G}$	48	—	16	8	—	—	72
CK	—	—	—	—	—	72	72

FL = fluorescent white light; $\text{R}_{630}\text{B}_{445}\text{Y}$ = the combined spectra of R, Y, and B at 445 nm; $\text{R}_{630}\text{B}_{445}\text{G}$ = the combined spectra of R, G, and B at 445 nm; $\text{R}_{630}\text{B}_{465}\text{Y}$ = the combined spectra of R, Y, and B at 465 nm; $\text{R}_{630}\text{B}_{465}\text{G}$ = the combined spectra of R, G, and B at 465 nm. PPFD = photosynthetic photon flux density.

Table 2. Effects of Y, G, and two B in conjunction with R on the agronomic traits of potato plantlets in vitro.

Light treatment	Stem length (mm)	Root length (mm)	Stem diam (mm)	SLW (mg·cm ⁻²)	Blade no. (p)	Leaf area (mm ²)	FW (mg)	DW (mg)	Health index
R ₆₃₀ B ₄₄₅ Y	62.90 b	127.93 a	1.57 a	21.24 a	6.67 a	127.33 c	296.67 a	53.67 a	1.34 a
R ₆₃₀ B ₄₄₅ G	58.37 c	119.43 b	1.27 b	15.06 b	4.67 b	173.67 a	231.00 c	42.33 b	0.92 c
R ₆₃₀ B ₄₆₅ Y	59.77 c	126.57 a	1.50 a	19.68 a	7.33 a	112.33 c	256.67 b	45.33 b	1.14 b
R ₆₃₀ B ₄₆₅ G	63.17 b	118.37 b	1.17 b	14.91 b	4.67 b	151.00 b	206.67 d	37.33 c	0.69 d
CK	70.37 a	124.57 a	1.27 b	15.72 b	5.33 b	131.00 c	216.67 cd	44.33 b	0.80 cd

Letters (a–d) indicate statistically significant differences between the means ($P < 0.05$) using least significant difference with SPSS. FW = fresh weight; DW = dry weight; SLW = specific leaf weight.

These results showed that the combined spectra containing blue LEDs at 445 nm was beneficial for the vegetative growth of potato plantlets in vitro than blue LEDs at 465 nm.

Root activity. Figure 1 shows that the root activity (264.38, 202.65, 246.34, and 197.18 mg·g⁻¹·h⁻¹, respectively) of potato plantlets in vitro under all the LED treatments was significantly higher than under FL (173.12 mg·g⁻¹·h⁻¹) indicating that the addition of Y or G to the combined spectra of red and blue was beneficial for root growth.

The root activity under R₆₃₀B₄₄₅Y (264.38 mg·g⁻¹·h⁻¹) was significantly higher than that under R₆₃₀B₄₄₅G (202.65 mg·g⁻¹·h⁻¹), and the root activity was significantly higher under R₆₃₀B₄₆₅Y (246.34 mg·g⁻¹·h⁻¹) than under R₆₃₀B₄₆₅G (197.18 mg·g⁻¹·h⁻¹). This result indicated that the addition of yellow LEDs to the combined spectra of red and blue promoted the root activity of potato plantlets in vitro.

There was no significant difference in the root activity between R₆₃₀B₄₄₅Y and R₆₃₀B₄₆₅Y or between R₆₃₀B₄₄₅G and R₆₃₀B₄₆₅G. This indicated that the combined spectra containing blue LEDs at 445 nm had a uniform effect on the root activity of potato plantlets in vitro, as did blue LEDs at 465 nm.

Concentrations of pigments. Figure 2 shows that the Chl a of potato plantlets in vitro under R₆₃₀B₄₄₅G (6.14 mg·g⁻¹) was significantly higher than that under R₆₃₀B₄₄₅Y (5.20 mg·g⁻¹) and was significantly higher under R₆₃₀B₄₆₅G (5.96 mg·g⁻¹) than under R₆₃₀B₄₆₅Y (4.60 mg·g⁻¹). These results indicate that the addition of green LEDs to the combined spectra of red and blue was beneficial for the accumulation of Chl a in potato plantlets in vitro.

The Chl a content in potato plantlets in vitro was significantly higher under R₆₃₀B₄₄₅Y (5.20 mg·g⁻¹) than under R₆₃₀B₄₆₅Y (4.60 mg·g⁻¹) and was significantly higher under R₆₃₀B₄₄₅G (6.14 mg·g⁻¹) than under R₆₃₀B₄₆₅G (5.96 mg·g⁻¹). These results suggested that the combined spectra containing blue LEDs at 445 nm were more favorable for Chl a accumulation in potato plantlets in vitro than were those containing blue LEDs at 465 nm.

Concentrations of carbohydrates and proteins. As shown in Fig. 3, the parameters of the soluble sugars, soluble protein, and starch content in potato plantlets in vitro under R₆₃₀B₄₄₅Y (66.52, 7.32, and 23.46 mg·g⁻¹, respectively) were significantly higher than under R₆₃₀B₄₄₅G (51.68, 5.77, and 19.09 mg·g⁻¹, respectively) and were higher under R₆₃₀B₄₆₅Y (60.04, 6.06, and

Fig. 1. Effects of Y, G, and two B in conjunction with R on root activity of potato plantlet in vitro. Letters (a–c) indicate statistically significant differences between the means ($P < 0.05$) using least significant difference with SPSS.

Fig. 2. Effects of Y, G, and two B in conjunction with R on the pigment content of potato plantlet in vitro. Letters (a–d) indicate statistically significant differences between the means ($P < 0.05$) using least significant difference with SPSS.

Fig. 3. Effects of Y, G, and two B in conjunction with R on carbon and nitrogen metabolism of potato plantlet in vitro. Letters (a–c) indicate statistically significant differences between the means ($P < 0.05$) using least significant difference with SPSS. (A) Soluble sugar. (B) Soluble protein. (C) Starch.

21.79 mg.g⁻¹, respectively) than under R₆₃₀B₄₆₅G (49.50, 5.21, and 16.85 mg.g⁻¹, respectively). These results indicated that the addition of yellow LEDs to the combined spectra of red and blue was beneficial for the accumulation of carbohydrates and proteins in potato plantlets in vitro.

The parameters of soluble sugars, soluble protein, and starch content in potato plantlets in vitro were significantly higher under R₆₃₀B₄₄₅Y (66.52, 7.32, and 23.46 mg.g⁻¹, respectively) than under R₆₃₀B₄₆₅Y (60.04, 6.06, and 21.79 mg.g⁻¹, respectively) and were significantly higher under R₆₃₀B₄₄₅G (51.68, 5.77, and 19.09 mg.g⁻¹, respectively) than under R₆₃₀B₄₆₅G (49.50, 5.21, and 16.85 mg.g⁻¹, respectively). The results suggested that the combined spectra containing blue LEDs at 445 nm were more favorable for the accumulation of carbohydrates and proteins in potato plantlets in vitro than were those containing blue LEDs at 465 nm.

Discussion

The light spectrum is an important environmental factor that influences the morphogenesis

of plants. Johkan et al. (2010) reported that the shoot elongation of *Lactuca sativa* was promoted under G and argued that the inhibition effect of B for the stem elongation weakened under G. Ma et al. (2015) reported that the addition of green LEDs to the combined spectra of R and B proportionally reduced the amount of blue and likely alleviated the inhibition of stem elongation induced by B of potato plantlets in vitro. In our experiment, we observed that yellow LEDs added to the blue LEDs at 445 nm were more advantageous for stem elongation than were green LEDs; however, we also observed that green LEDs added to the blue LEDs at 465 nm were more advantageous for stem elongation than were yellow LEDs (Table 2).

The inhibition of stem elongation of potato plantlets in vitro was derived from the inhibition of cell elongation induced by blue fluorescent lamps (Wilson et al., 1993). Meijer (1971) reported a deficiency in B-induced stem elongation and considered that B was necessary for strong plantlet culture in terms of rooting of cucumber. Mcnellis and Deng (1995) considered that the inhibition of stem elongation of plants resulted from B at

short wavelengths. However, we observed that under the combined spectra of RBY, B at shorter wavelengths (445 nm) weakened the inhibition of stem elongation of potato plantlets in vitro more than B at longer wavelengths (465 nm) did. By contrast, under compound RBG spectra, B at shorter wavelengths (445 nm) strengthened the inhibition of stem elongation of potato plantlets in vitro more than B at longer wavelengths (465 nm) did (Table 2).

Light treatments consisting of mixtures of RBY were more beneficial to the stem diameter of potato plantlets in vitro than were the FL treatment and other spectral combinations (Table 2). Ma et al. (2015) reported that green LEDs added to the combined spectra of R and B had a wider stem diameter than the combined spectra of R and B. In our experiment, when yellow LEDs were added to the combined spectra of R and B, we observed that yellow LEDs stimulated thicker stem diameter than did green LEDs. Johkan et al. (2010) reported that B inhibited lettuce stem elongation but promoted stem diameter. We also observed the same effect on the stem diameter of potato plantlets in

vitro between 445-nm blue LED light and 465-nm blue LED light (Table 2).

Plants that have a greater leaf number and a larger leaf area absorb more light energy, which results in more photosynthate and biomass accumulation (Liu et al., 1984). The combination of red and blue LED spectra provided sufficient and effective light for leaves (Kim et al., 2004) and was absorbed by leaves (Klein, 1992; Smith, 1994). Moreover, Liu et al. (2011b) reported that the addition of yellow LEDs to the combined spectra of R and B improved the photosynthetic efficiency of cherry tomatoes, which was consistent with our findings. Ma et al. (2015) reported that green LEDs added to the combined spectra of red and blue LEDs induced larger SLM, FW, DW, and leaf areas of potato plantlets. We observed that yellow LEDs added to the combined spectra of red and blue LEDs were more advantageous for the SLW, FW, DW, and blade numbers in potato plantlets than were green LEDs. In our experiment, we also observed under the combined spectra of RBG and RBY that B at 445 nm was more advantageous for the growth of potato plantlet leaves than was B at 465 nm (Table 2). We also observed that B at 445 nm was more conducive to the biomass accumulation of potato plantlets in vitro than was B at 465 nm (Table 2).

The root system was the main organ that absorbs water and nutrients. As the absorbance capacity of a root system with greater root activity strengthens, water and nutrient absorption increases, which further increased the biomass accumulation of plants (Lynch, 1995). Park et al. (2013) reported that white LEDs added to the combined spectra of red and blue LEDs promoted the root length of *Perilla*. Godo et al. (2011) reported that the most effective wavelength of light for *Bletilla* rhizoid formation was revealed to be in the range of 590 nm (Y) to 625 nm (R), which was similar to our findings. We observed that yellow LEDs added to the combined spectra of red and blue LEDs were more advantageous for root activity of potato plantlets in vitro (Fig. 1) and observed no significant differences in root activity between the treatments of R₆₃₀B₄₄₅Y and R₆₃₀B₄₆₅Y or R₆₃₀B₄₄₅G and R₆₃₀B₄₆₅G (Fig. 2).

The photosynthetic pigments of plants are responsible for the absorption, transfer, and transition of light energy. Chlorophyll is the basic material of photosynthesis in plants. The Chl content of leaves has a direct effect on the photosynthetic rate. The Chl content of lettuce plants under the combined spectra of R and B was reported to be significantly higher than that of plants under monochromatic and fluorescent light (Kim et al., 2004). Li et al. (2010) reported that the Chl content of upland cotton in vitro increased under the combined spectra of R and B, and the same result was observed in *Oncidium* plantlets in vitro (Liu et al., 2011a). In addition, Ma et al. (2015) reported that green LEDs added to the combined spectra of red and blue LEDs induced more Chl a in potato plantlets, which was similar to the results of our experiment.

Blue light promoted the formation of Chl, which significantly increased the photosynthetic pigment content of nonheading chinese cabbage and cotton (Li et al., 2012; Sæbø et al., 1995). In our experiment, we also observed that B at 445 nm was more advantageous for Chl a accumulation in potato plantlet leaves than was B at 465 nm.

The formation and distribution of carbohydrates and proteins play an important role in plants. The ecological adaptability, vegetative growth, reproductive growth, source-sink relationships, yield, and other physiological activities of plants were closely related to carbohydrate and protein concentrations (Osaki, 2001). Red light promoted starch accumulation in *Brassica napus* (Li et al., 2013). Conversely, B promoted protein generation in chrysanthemum plants (Kowallik, 1982; Kurilčik et al., 2008). Kowallik (1982) argued that B enhances dark respiration and that the organic acids synthesized amino acids during dark respiration, which therefore, leads to increased protein synthesis. Lin et al. (2013) reported that W added to the combined spectra of red and blue promoted soluble sugar accumulation in lettuce. Ma et al. (2015) reported that green LED added to the combined spectra of red and blue LEDs promoted carbohydrate and protein accumulation in potato plantlets than the combined spectra of R and B without G did. Because the wavelength range of 500–600 nm was classified as G in some studies, the effects of Y (580–600 nm) on plant growth and development were neglected. Therefore, the addition of green and yellow LEDs to the combined spectra of red and blue LEDs was to compare the effects of Y and G on growth and development of potato plantlet in vitro. We observed that the addition of yellow LEDs to the combined spectra of red and blue LEDs was more advantageous for carbohydrate and protein accumulation in potato plantlets than was the addition of green LEDs (Fig. 3). This may be the synergy of RBY better than RBG for the carbohydrates and proteins accumulation of potato plantlet.

Chang et al. (2009) reported that B was beneficial for protein accumulation in potato plantlets in vitro, and Johkan et al. (2010) argued that the shorter wavelengths of the same spectrum were favorable for protein accumulation, which was consistent with our results. We showed that B at 445 nm was more beneficial for protein accumulation in potato plantlets in vitro than was B at 465 nm (Fig. 3).

Conclusions

The present study shows that the addition of Y to the combined spectra of R and B LEDs promoted the growth of potato plantlets in vitro than G or FL. In addition, B₄₄₅ was more advantageous for the growth of potato plantlets in vitro than was B₄₆₅. Compared with other treatments, R₆₃₀B₄₄₅Y produced more vigorous potato plantlets in vitro. Therefore, it would be beneficial to use a R₆₃₀B₄₄₅Y spectrum for the cultivation of potato plantlets in the tissue culture room.

Literature Cited

- Aksenova, N.P., T.N. Konstantinova, L.I. Sergeeva, I. Macháčkova, and S.A. Golyanovskaya. 1994. Morphogenesis of potato plants in vitro. I. Effect of light quality and hormones. *J. Plant Growth Regulat.* 13(3):143–146.
- Al-Wakeel, S.A.M. and A.A. Hamed. 1996. Light-quality effect on growth and some biochemical aspects of mild-stressed *Cucurbita pepo* L. *Egypt. J. Bot.* 36:217–234.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
- Chang, H., Y.P. Wang, D. Wang, and F. Zhang. 2009. Effects of light quality on microtubule induction of *Solanum tuberosum*. *Chinese J. Appl. Ecol.* 20(8):1891–1895.
- Charles, G., L. Rossignol, and M. Rossignol. 1992. Environmental effects on potato plants in vitro. *J. Plant Physiol.* 139(6):708–713.
- Dougher, T.A. and B. Bugbee. 2001. Evidence for yellow light suppression of lettuce growth. *Photochem. Photobiol.* 73(2):208–212.
- Eskins, K. 1992. Light-quality effects on *Arabidopsis* development. Red, blue and far-red regulation of flowering and morphology. *Physiol. Plant.* 86(3):439–444.
- Eskins, K., K. Warner, and F.C. Felker. 1996. Light quality during early seedling development influences the morphology and bitter taste intensity of mature lettuce (*Lactuca sativa*) leaves. *J. Plant Physiol.* 147(6):709–713.
- Fairbairn, N.J. 1953. A modified anthrone reagent. *Chem. Ind.* 4:86.
- Fan, X.X., Z.G. Xu, X.Y. Liu, C.M. Tang, L.W. Wang, and X. Han. 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. *Scientia Hort.* 153:50–55.
- Gao, R. and H. Zhang. 2002. Advances of researches on photo regulation in plants. *J. Beijing For. Univ.* 24(5):235–243.
- Godo, T., K. Fujiwara, K. Guan, and K. Miyoshi. 2011. Effects of wavelength of led-light on in vitro symbiotic germination and seedling growth of *Bletilla ochracea* schltr. (orchidaceae). *Plant Biotechnol.* 28(4):397–400.
- Hawkes, J.G. and P. Harris. 1992. Biosystematics of the potato, p. 13–64. *The Potato Crop*. Springer, Netherlands.
- Jao, R.C. and W. Fang. 2001. Adjusting frequency and duty cycle to promote growth of potato plantlets in vitro using super-bright red and blue LEDs. *Proc. Intl. Symp. Design Environ. Control Trop. Subtrop. Greenhouses* 4:15–18.
- Jao, R.C. and W. Fang. 2004. Effects of frequency and duty ratio on the growth of potato plantlets in vitro using light-emitting diodes. *Hort-Science* 39:375–379.
- Johkan, M., K. Shoji, F. Goto, S. Hashida, and T. Yoshihara. 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience* 45:1809–1814.
- Kim, H.H., G.D. Goins, R.M. Wheeler, and J.C. Sager. 2004. Green-light supplementation for enhanced lettuce growth under red-and blue-light-emitting diodes. *HortScience* 39:1617–1622.
- Klein, R.M. 1992. Effects of green light on biological systems. *Biol. Rev. Camb. Philos. Soc.* 67(2):199–284.
- Kowallik, W. 1982. Blue light effects on respiration. *Plant Biol.* 33(33):51–72.

- Kurilčik, A., R. Miklušytė-Čanová, S. Dapkūnienė, S. Žilinskaitė, G. Kurilčik, G. Tamulaitis, P. Duchovskis, and A. Žukauskas. 2008. In vitro culture of *Chrysanthemum* plantlets using light-emitting diodes. *Cent. Eur. J. Biol.* 3 (2):161–167.
- Li, H.M., C.M. Tang, Z.G. Xu, X.Y. Liu, and X.L. Han. 2012. Effects of different light sources on the growth of non-heading Chinese cabbage (*Brassica campestris* L.). *J. Agr. Sci.* 4(4):262–273.
- Li, H., C. Tang, and Z. Xu. 2013. The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis in vitro. *Scientia Hort.* 150(2):117–124.
- Li, H., Z.G. Xu, and C. Tang. 2010. Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets in vitro. *Plant Cell Tissue Organ Cult.* 103(2):155–163.
- Lichtenthaler, H.K. and A.R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11:591–592.
- Lin, K.H., M.Y. Huang, W.D. Huang, M.H. Hsu, Z.W. Yang, and C.M. Yang. 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. capitata). *Scientia Hort.* 150(2):86–91.
- Liu, M.X., Z.G. Xu, Y. Yang, and Y.J. Feng. 2011a. Effects of different spectral lights on *Oncidium* PLBs induction, proliferation, and plant regeneration. *Plant Cell Tissue Organ Cult.* 106:1–10.
- Liu, X.Y., S.R. Guo, Z.G. Xu, X.L. Jiao, and T. Tezuka. 2011b. Regulation of chloroplast ultrastructure, cross-section anatomy of leaves, and morphology of stomata of cherry tomato by different light irradiations of light-emitting diodes. *HortScience* 46:217–221.
- Liu, Z.Q., Z.Y. Liu, D.P. Ma, and S.F. Zeng. 1984. A study on the relation between chlorophyll content and photosynthetic rate of rice. *Acta Agronomica Sinica* 10(1):57–62.
- Lynch, J.P. 1995. Root architecture and plant productivity. *Plant Physiol.* 109:7–13.
- Ma, X., Y. Wang, M. Liu, J. Xu, and Z. Xu. 2015. Effects of green and red lights on the growth and morphogenesis of potato (*Solanum tuberosum* L.) plantlets in vitro. *Scientia Hort.* 190:104–109.
- McNellis, T.W. and X.W. Deng. 1995. Light control of seedling morphogenetic pattern. *Plant Cell* 7(11):1749–1761.
- Meijer, G. 1971. Some aspects of plant irradiation. *Symp. Electricity Artificial Light Hort.* 22:103–108.
- Mortensen, L.M. and E. Strømme. 1987. Effects of light quality on some greenhouse crops. *Scientia Hort.* 33(1–2):27–36.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* 15 (3):473–497.
- Nhut, D.T., T. Takamura, H. Watanabe, K. Okamoto, and M. Tanaka. 2003. Responses of strawberry plantlets cultured in vitro, under superbright red and blue light-emitting diodes (leds). *Plant Cell Tissue Organ Cult.* 73(1):43–52.
- Osaki, M. and T. Shinano. 2001. Plant growth based on interrelation between carbon and nitrogen translocation from leaves. *Photosynthetica* 39(2):197–203.
- Park, Y.G., H.J. Oh, S.J. Hwang, and B.R. Jeong. 2013. Growth and anthocyanin concentration of *Perilla frutescens* var. *acuta* Kudo as affected by light source and DIF under controlled environment. *Hort. Environ. Biotechnol.* 54 (2):103–108.
- Poudel, P.R., I. Kataoka, and R. Mochioka. 2008. Effect of red- and blue-light-emitting diodes on growth and morphogenesis of grapes. *Plant Cell Tissue Organ Cult.* 92(2):147–153.
- Sæbø, A., T. Krekling, and M. Appelgren. 1995. Light quality affects photosynthesis and leaf anatomy of birch plantlets in vitro. *Plant Cell Tissue Organ Cult.* 41(2):177–185.
- Seabrook, J.E.A. and L.K. Douglass. 1998. Prevention of stem growth inhibition and alleviation of intumescence formation in potato plantlets in vitro by yellow filters. *Amer. J. Potato Res.* 75(5):219–224.
- Seabrook, J.E.A. 2005. Light effects on the growth and morphogenesis of potato (*Solanum tuberosum*) in vitro: A review. *Amer. J. Potato Res.* 82(5):353–367.
- Smith, H. 1994. Sensing the light environment: The functions of the phytochrome family, p. 377–416. Springer, Dordrecht, The Netherlands.
- Wang, H., M. Gu, J. Cui, K. Shi, Y. Zhou, and J. Yu. 2009. Effects of light quality on CO₂ assimilation, chlorophyll-fluorescence quenching, expression of calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. *J. Photochem. Photobiol. B Biol.* 96(1):30–37.
- Wilson, D.A., R.C. Weigel, R.M. Wheeler, and J.C. Sager. 1993. Light spectral quality effects on the growth of potato (*Solanum tuberosum* L.) nodal cuttings in vitro. *In Vitro Cell. Dev. Biol. Plant* 29(1):5–8.
- Wu, M.C., C.Y. Hou, C.M. Jiang, Y.T. Wang, C.Y. Wang, and H.H. Chen. 2007. A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. *Food Chem.* 101 (4):1753–1758.