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Changes in Growth Characteristics and Functional Components of *Lactuca indica* L. 'Sunhyang' Baby Leaf Vegetable by Light Intensity and Cultivation Period

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Abstract

Indian lettuce (*Lactuca indica* L.) 'Sunhyang' is wild plant bred by crossing Indian lettuce and 'Dragon's tongue'. It has wider leaf width and less bitterness than Indian lettuce. This study was conducted to determine the optimum light intensity for growth and functional components of *Lactuca indica* L. 'Sunhyang', considered as a new wild baby leaf vegetable throughout the year. Plants were cultivated in controlled environment at temperature of 24 - 25°C and relative humidity (RH) of 60 ± 5% from seedling to harvest. 16 days after sowing, plants were treated with four different light intensities of PPFD (photosynthetic photon flux density) 50, 100, 250 and 500 using white LED light for 18 days. Growth characteristics, leaf color, and functional components were investigated three times (6th, 12th, and 18th day) after PPFD treatment. At light intensity of PPFD 50, 100 and 250, it took 12 days after treatment (DAT) to reach optimum size to harvest (plant height 12 - 14 cm). Leaf width, leaf number, and leaf area increased more in PPFD 100 and 250 than PPFD 50. At PPFD 500, it took 18 DAT to reach appropriate size to harvest. Dry weight and dry mass ratio of shoot increased with increasing light intensity, whereas specific leaf area (SLA) decreased. Chlorophyll relative value (SPAD) and leaf color differed depending on light intensity. SPAD was the lowest in PPFD 50. Hunter a* value was the highest for PPFD 250 and 500 at 12DAT. The highest relative growth rate (RGR) was observed at 0 to 6DAT for PPFD 500, 6 to 12DAT for PPFD 50 and PPFD 250, and 12 to 18DAT for PPFD 250. Parameters for root growth of Indian lettuce were affected significantly by light and DAT. Total root length was longer in PPFD 250 and 500 than that in other treatment from 6DAT. Average root diameter and volume were the largest in PPFD 500. Anthocyanin contents, total phenolic contents, and free radical activity (DPPH) were increased with increasing light level. However, levels of functional components decreased at 18 DAT. These results suggest that *Lactuca indica* L. 'Sunhyang' can grow the best when it is kept at PPFD 250 at 12 DAT for optimal growth, leaf color, and functional components.

Additional key words: anthocyanin, indian lettuce, leaf color, plant height, root growth

Introduction

Light is one of the most important environmental factors as source of photosynthesis. Light factors such as light intensity, quality, and photoperiod can affect physiological, morphological, and anatomical responses of plants directly or indirectly (Son, 2016). Among these factors, light intensity can affect plant photosynthesis and enhance carbohydrate accumulation (Kwack et al., 2015a). Generally, when light intensity increases, photosynthetic rate also increases until light saturation point. However, very high light intensity can cause photoinhibition phenomenon (Bowes et al., 1972). Also when cultivated at low light condition, plants show morphological and anatomical changes such as increased specific leaf area (SLA) and plant height to absorb light for photosynthesis (Fan et al., 2013).

Vegetables and fruits are known to possess natural antioxidants such as anthocyanin and other phenolic compounds. Recently, consumers have increased consumption of certain foods containing functional components (You et al., 2011; Craver et al., 2017). It has been found that functional components can be accumulated by inadequate growth condition (Pérez-López et al., 2018). Environmental stress can lead to generation of reactive oxygen species (ROS) that can damage plant DNA, RNA, proteins, chlorophyll, and so on. Plants can also produce antioxidant to decrease or eliminate ROS (Edreva, 2005; Oh et al., 2009). To increase levels of functional components, various studies have been conducted by focusing on environmental factors such as light intensity and quality (Li and Kubota, 2009; Kwack et al., 2015a; Petrella et al., 2016; Son, 2016; Li et al., 2019), temperature (Oh et al., 2009; Lee et al., 2015), water deficit (Oh et al., 2010), and compositions of nutrient solution (Kwack et al., 2015b).

Baby leaf vegetables are generally rich in antioxidants such as minerals, anthocyanin, flavonoids, and phenolic compounds (Martínez-Sánchez et al., 2008). They are cultivated for 20 - 40 days from sowing to harvest when plant height reaches about 10 cm. Consumer preference for baby leaf vegetables is changing fast due to their concern of their health and well-being. However, there are very limited kinds of baby leaf vegetables available limited such as lettuce, (red) tat-soi, amaranth, kyona and so on. It is time to find new varieties of vegetables with abundant nutrition and high quality.

Indian lettuce (*Lacutca indica* L.) grows wild in fields and mountains of South Korea. It has a bitter flavor. However, it is good for improving health condition, soothing, perspiration, and liver function. Indian lettuce 'Sunhyang' is bred by crossing Indian lettuce and 'Dragon's tongue' (Korea seed & Variety Service, No. 02-0091-2007-1). Leaves of 'Sunhyang' are softer, wider and less bitter than wild Indian lettuce and have red leaf vain involving anthocyanin (Noh et al., 2014). Its germination rate is also higher. Thus, 'Sunhyang' is suitable diversification of baby leaf vegetable. However, research data about optimum environment conditions for its year-round production are lacking. Therefore, it was of interest to determine suitable conditions for sustainable production of 'Sunhyang' as new wild baby leaf vegetable. This study was conducted to determine the optimum light intensity on plant growth and levels of functional components of Indian lettuce 'Sunhyang'.

Materials and Methods

Plant Material and Culture Conditions

The Indian lettuce 'Sunhyang' (*Lactuca indica* L.), bred in Gangwon Agricultural and Extension services, were used as plant material in this experiment. Environment conditions for cultivation were kept at air temperature of 24 - 25°C with relative humidity of 60 ± 5% in a growth room. The bar type of white LED light (ZVAS, Sunghyun Hightech Co. Ltd., S. Korea) was used and photoperiod was set at 16h light/ 8h dark periods. The six white LEDs were fixed at 35cm from the bottom(W 120 × L45 cm) and light intensity was adjusted based on the plug tray. The Indian lettuce seeds were sowed in a 128-hole plastic tray (Hole size: W2.8 × L2.8 × H4.0 cm) filled with horticultural substrate (HS, Baroker, Seoul bio Ltd., Korea). Light intensity was 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetic photon flux density (PPFD). Sixteen days after sowing, seedlings (plant height 6.4 ± 0.3 cm, number of leaves 3.0 ea.) were transplanted into a 72-hole plastic tray (Hole size: W4.0 × L4.0 × H5.0 cm) filled with a HS and treated light intensity treatments. The plants were irradiated with four different light intensities, PPFD 50, PPFD 100, PPFD 250, and PPFD 500 $\mu mol \cdot m^{-1} \cdot s^{-1}$, using the LED dimming controller (NES-350-24, Mean Well Enterprises Co. Ltd., Taiwan) 18days. Water was irrigated using overhead tap water about 500 ml per plug tray.

Analysis of Growth Characteristics

Growth characteristics of Indian lettuce were measured three times every six days, at 6, 12, and 18 days after treatment (DAT). For the shoot part, we measured plant height, the largest leaf length and width, number of leaves, leaf area (Li-3100c, Li-cor Inc., USA), shoot fresh weight (SFW), shoot dry weight (SDW), chlorophyll content (SPAD relative value, SPAD-502, Minolta Co. Ltd., Japan), and Hunter a value of leaf color (Tes-135a, Tes Electric Corp., Taiwan). Total length, average diameter, and volume of root were measured with WinRHIZO Program 09 (Regent Instrument Inc., Canada). Shoot dry mass ratio, relative growth rate (RGR), and specific leaf area (SLA) were then calculated using the following equations (Eq. 1, Eq. 2, and Eq. 3).

$$ShootDrymassratio(\%) = \frac{shoot\ fresh\ weight}{shoot\ dry\ weight} \times 100 \tag{1}$$

$$RGR(g \cdot g^{-1} \cdot day^{-1}) = \frac{\{\ln(W_2) - \ln(W_1)\}}{T_2 - T_1} \tag{2}$$

$$SLA(cm^2 \cdot g^{-1}) = \frac{Leaf\ area}{Dry\ weight} \tag{3}$$

Where W_1 and W_2 were shoot dry weight at harvest times T_1 and T_2, respectively.

Analysis of Functional Components

Anthocyanin content was determined according to the method described by Rabino and Macinelli (1986) with minor modifications. Briefly, fresh leaf (0.5 g) was used to extract anthocyanin with 10 ml of 1% HCl-MeOH at 4°C for 48 hours in dark conditions. Absorbance of the extract solution was measured at wavelength of 530 nm with a UV spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan). Total phenolic content (TPC) was determined according to the Foiln-Dennis method described by Li and Kubota (2009) with gallic acid as standard. Fresh leaf(0.5 g) was used to extract total phenolic compounds with 10 ml of 80% MeOH at 4°C for 24 hours in dark conditions. Then 1 ml of the extract solution was mixed with 3 ml of distilled water, 1 ml of Folin & Ciocalteau's phenol reagent, and 1 ml of water-saturated sodium carbonate solution. After shaking, absorbance of the mixed solution was measured at wavelength of 735 nm using a UV spectrophotometer. Free radical activity (DPPH) was determined according to the method describe by Lee (2003) with minor modifications. 0.5 g of fresh leaf was used for extraction with 10 ml of MeOH at 4°C for 24 hours in dark conditions. Then 0.9 ml of extract solution was mixed with 2.7 ml of 0. 3mM DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution. After incubation for 50 min in dark condition at room temperature, absorbance of the solution was measured at wavelength of 517 nm using a UV spectrophotometer. DPPH was calculated using the following Eq. 4.

$$DPPH\ radical\ scavenging\ assay(\%) = \left(1 - \frac{Sample\ absorbance}{blank\ absorbance}\right) \times 100 \tag{4}$$

Statistical Analysis

The experiment was replicated four times for all treatments using a completely randomized design. Data were obtained from 7 plants for plant growth characteristics and 4 plants for root growth characteristics and functional components of each treatment. SAS package (statistical analysis system, version 9.4, SAS Institute Inc., Cary, NC, USA) was used for ANOVA (analysis of variance) and Duncan's multiple range test (DMRT) at 5% for data analysis.

Results and Discussion

Plant height of Indian lettuce 'Sunhyang' was found to be significantly different under different light intensity from six DAT (Fig. 1). Difference in plant height increased as DAT was lengthened. At 18 DAT, the highest plant height was observed in PPFD 50 and PPFD 100 (18.4 - 19.3 cm) whereas the lowest plant height was observed in PPFD 500 (14.1 cm). Plant height was significantly affected by DAT, PPFD and DAT × PPFD interaction. Fan et al. (2013) reported that plant height is decreased under high light intensity during tomato seedling cultivation. Steinger et al. (2003) also reported that low light level can lead to plant height increase to maximize the capture of light for adaption. Our results showed the similar effect of light intensity on plant height. Indian lettuce was harvested at 12th days from PPFD 50, 100, and 250

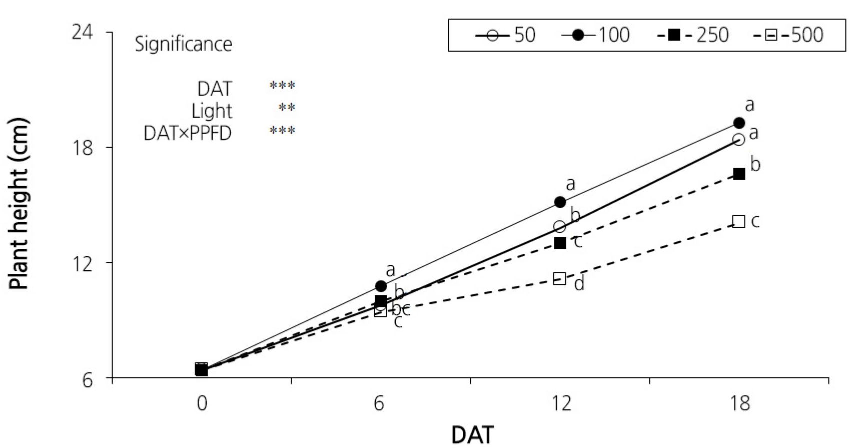

Fig. 1. Effect of light intensity on plant height of Indian lettuce 'Sunhyang' grown in controlled system. DAT means days after treatment. Means with different letters in each column are significantly different by DMRT at $p < 0.05$ (n = 7). ** and *** means significant at $p < 0.001$ and 0.001, respectively.

treatments or 18th days from PPFD 500. Upon harvest, plant height was about 12cm, for which is appropriate as baby leaf vegetables because the growers cut off about 2cm above ground.

Leaf length under the PPFD 50 was the shortest at 6 DAT and there was no significant difference among the other three treatments (Table 1). However, leaves of Indian lettuce grown under PPFD 100 and PPFD 250 at 18 DAT were longer than PPFD 50 and 500. Leaf width showed difference among the four light intensity treatment at 12 DAT. Leaf width under the PPFD 100 was the largest among all treatments, although it was not significantly different among PPFD 50, 250, and 500 at 18 DAT. Leaf area was the smallest in PPFD 50 until 12 DAT and in PPFD 500 at 18 DAT. Leaf growth characteristics were significantly affected by DAT and light intensity ($p < 0.001$). On the other hands, leaf number and leaf area were significantly affected by DAT × PPFD. Plants show structural and physiological changes of leaves to adapt to the environment when they are cultured under inadequate light conditions (Lichtenthaler et al., 2007). In our study, PPFD 50 caused leaf growth retardation due to insufficient light intensity. Under PPFD 500, plants appeared to minimize the amount of light received by minimizing leaf area.

Chlorophyll relative value(SPAD) was not significantly different among treatments at 6 DAT, although lower SPAD relative values were found for PPFD 50 and 100 at 12 DAT (Table 2). SPAD relative values increased as light intensity increased, consistent with a previous study (Lee et al., 2001). When purchasing baby leaf vegetable, the color of leaf is a very important factor and also an indicator of antioxidant properties (Ali et al., 2009). Depending on light intensity, Indian

Table 1. Effects of light intensity on leaf length, leaf width, number of leaves, and leaf area of Indian lettuce 'Sunhyang' grown in controlled system

DAT[z]	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Leaf			
		Length (cm)	Width (cm)	Number	Area (cm^2)
0	-	3.3	1.9	3.0	10.5
6	50	4.4 b[y]	2.3 a	3.4 b	21.1 b
	100	5.5 a	2.5 a	4.3 a	28.2 a
	250	5.7 a	2.5 a	4.0 a	28.8 a
	500	5.4 a	2.5 a	4.0 a	29.2 a
12	50	6.6 c	2.9 a	5.3 b	37.4 b
	100	8.3 a	3.2 a	6.0 a	49.9 a
	250	7.5 b	3.2 a	5.7 a	52.5 a
	500	7.1 bc	2.8 b	6.0 a	47.3 a
18	50	9.0 b	2.8 b	6.4 c	72.5 b
	100	11.1 a	3.3 a	7.4 ab	96.4 a
	250	10.5 a	2.9 b	7.9 a	97.4 a
	500	9.4 b	2.9 b	7.0 bc	73.5 b
Significance[x]					
DAT		***	***	***	***
PPFD		***	***	***	***
DAT × PPFD		NS	NS	*	***

[z]DAT means days after treatment.

[y]Means with different letters in each column are significantly different by DMRT at $p < 0.05$ (n = 7).

[x]NS, *, *** means none significant, significant at $p < 0.05$ and 0.001, respectively.

Table 2. Effects of light intensity on chlorophyll content (SPAD) and Hunter 'a' value of Indian lettuce 'Sunhyang' grown in controlled system

DAT[z]	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	SPAD (Relative value)	Hunter a* (Value)
0	-	-	-10.9
6	50	35.8 a[y]	-9.8 c
	100	38.5 a	-9.8 c
	250	37.1 a	-5.3 b
	500	39.5 a	-2.0 a
12	50	32.7 c	-9.6 b
	100	35.5 b	-10.7 b
	250	42.2 a	-3.5 ab
	500	42.7 a	2.0 a
18	50	32.4 b	-10.7 b
	100	38.1 a	-12.9 b
	250	36.9 a	-3.3 a
	500	39.3 a	-0.0 a
Significance[x]			
DAT		NS	NS
PPFD		***	***
DAT × PPFD		*	NS

[z]DAT means days after treatment.
[y]Means with different letters in each column are significantly different by DMRT at $p < 0.05$ (n = 7).
[x]NS, *, *** means none significant, significant at $p < 0.05$ and 0.001, respectively.

lettuce 'Sunhyang' leaves showed difference in red expression (Table 2). Hunter a*value means chromaticity from green (-a) to red (+ a). From 6 DAT, Hunter a* value was high in PPFD 500 with high light intensity. The reddish leaf color appeared at 6 DAT in PPFD 500 and 12 DAT in PPFD 250.

SFW was the lowest in PPFD 50. It was not significantly different among PPFD 100, 250, and 500 at 6 DAT or 12 DAT (Table 3). SDW and shoot dry mass ratio were the highest in PPFD 500 but the lowest in PPFD 50. SLA indicating leaf thickness showed a negative correlation with light intensity. It decreased with increasing light intensity. SFW and SDW were significantly affected by DAT, PPFD and DAT × PPFD ($p < 0.001$). Dry mass ratio showed significant correlation with PPFD and DAT × PPFD interaction and SLA only showed significant correlations with light intensity. Dry mass ratio and SLA were not affected by DAT. Plant produces and fixes carbohydrates through photosynthesis, thus increasing its dry weight. SDW and shoot dry mass ratio of Indian lettuce were increased when light intensity was increased. Fan et al. (2013) reported that SLA is always gradually decreased when light intensity is increased. Decreased SLA may reduce light absorption. Our research also showed that SLA value decreased with increasing light intensity. Leaf thickness appeared to be larger to control the amount of light reaching the mesophyll cell that contains many chloroplasts.

From 0 to 6 DAT, RGR was higher when high light intensity was increased (Fig. 2). However, the lowest RGR was at 0.09 $g \cdot g^{-1}$/day in PPFD 500 while it was not significantly different among the other three treatments (0.11 - 0.12 $g \cdot g^{-1}$/day at 6 to 12 DAT). From 12 to 18 DAT, the highest RGR value was at 0.12 $g \cdot g^{-1}$/day in PPFD 250 while the lowest one was

Table 3. Effects of light intensity on shoot fresh and dry weight, dry mass ratio, and specific leaf area (SLA) of Indian lettuce 'Sunhyang' grown in controlled system

DAT[z]	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Shoot weight (g/plant)		Dry mass ratio (%)	SLA ($cm^2 \cdot g^{-1}$)
		Fresh	Dry		
0	-	0.23	0.01	6.5	757.8
6	50	0.47 b[y]	0.03 d	5.4 c	841.6 a
	100	0.72 a	0.04 c	5.6 c	703.8 b
	250	0.81 a	0.06 b	7.0 b	516.1 c
	500	0.84 a	0.08 a	9.9 a	352.2 d
12	50	0.93 b	0.05 d	5.3 c	897.1 a
	100	1.29 a	0.08 c	6.0 c	658.0 ab
	250	1.49 a	0.12 b	7.8 b	453.7 bc
	500	1.48 a	0.14 a	9.5 a	340.3 c
18	50	1.80 d	0.09 c	4.9 d	830.6 a
	100	2.54 b	0.14 b	5.7 c	671.9 b
	250	2.84 a	0.25 a	8.7 b	399.2 c
	500	2.20 c	0.23 a	10.4 a	324.6 c
Significance[x]					
DAT		***	***	NS	NS
PPFD		***	***	***	***
DAT × PPFD		***	***	**	NS

[z]DAT means days after treatment.
[y]Means with different letters in each column are significantly different by DMRT at $p < 0.05$ (n = 7).
[x]NS, **, *** mean none significant, significant at $p < 0.01$ and 0.001, respectively.

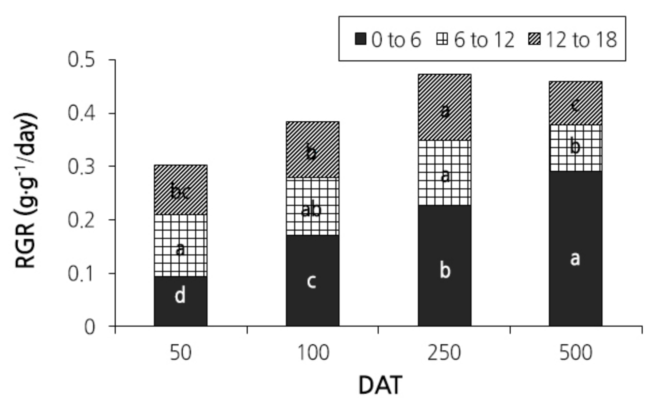

Fig. 2. Effects of light intensity on shoot relative growth grow (RGR) of Indian lettuce 'Sunhyang' grown in controlled system. DAT means days after treatment. Means with different letters in each column are significantly different by DMRT at $p < 0.05$ (n = 7).

at 0.08 $g \cdot g^{-1}$/day in PPFD 500. Seo et al. (2018) reported that RGR varies depending on crops. It tends to decrease as growth period elapses. In our results, RGR was the highest from 0 to 6 DAT in all treatments except PPFD 50.

Root growth characteristics of Indian lettuce were affected by light intensity and DAT (Table 4). Total root lengths were longer in PPFD 250 and 500 than those in the other two treatments from 6 DAT. At 6 DAT, average diameter of root was

Table 4. Effects of light intensity on total root length, root average diameter, and root volume of Indian lettuce 'Sunhyang' grown in controlled system

DAT[z]	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Root		
		Total length (cm)	Ave. diameter (mm)	Ave. volume (cm^3)
0	-	48.3	0.25	0.03
6	50	50.7 b[y]	0.39 a	0.06 a
	100	82.7 b	0.40 a	0.10 a
	250	125.2 a	0.56 a	0.29 a
	500	183.9 a	0.56 a	0.54 a
12	50	65.7 d	0.35 c	0.06 c
	100	165.6 c	0.39 c	0.21 c
	250	259.8 b	0.53 b	0.61 b
	500	305.0 a	0.67 a	1.12 a
18	50	173.5 c	0.43 b	0.25 c
	100	234.7 bc	0.65 b	0.81 c
	250	253.8 ab	0.83 a	1.46 b
	500	288.1 a	1.14 a	2.95 a
Significance[x]				
DAT		***	***	***
PPFD		***	***	***
DAT × PPFD		*	*	***

[z]DAT means days after treatment.

[y]Means with different letters in each column are significantly different by DMRT at $p < 0.05$ (n = 4).

[x]*,*** means significant at $p < 0.05$ and 0.001, respectively.

0.4 - 0.6 mm, showing no significant difference among treatments. However, after 12 DAT, the diameter of root increased with increasing light intensity. Average volume of root also showed similar tendency to root diameter. The total root length, average of root diameter and volume showed significant correlation with DAT, PPFD and DAT × PPFD. Root length extension also show high correlation with solar radiation accumulation (Nagel et al., 2006). Zha and Liu (2018) also reported that when cherry radish was cultivated at different light intensity and quality, root diameter and volume were increased at high light intensity. At 18 DAT, no further total root length increased in PPFD 250 and 500, indicating that the volume of the plug cell was limited due to increased diameter and volume of root.

Levels of functional components of Indian lettuce 'Sunhyang' were affected by DAT and light intensity (Fig. 3). Anthocyanin content decreased in PPFD 50 at 18 DAT. It was not significantly different among treatments at 12 DAT. One main cause of anthocyanin accumulation is for photo-protection (Logan et al., 2015). Thus, anthocyanin content was higher in PPFD 100, 250, and 500 than PPFD 50, consistent with results of Craver et al. (2017), in which anthocyanin content in baby leaf vegetable kohlrabi was high when it was grown with high light level. TPC and DPPH also showed similar tendency. At 6 DAT, TPC was 37.6 - 58.8 mg/100g FW, about 31 - 41% higher than that at 12 and 18 DAT. Oh et al. (2010) reported that TPC and antioxidant capacity of lettuce were the highest at 7th day after germination. They decreased when growing period became longer. TPC and DPPH were the lowest in the PPFD 50. Red leaf vegetables are known to be richer in total phenolic contents than green leaf vegetables because of the presence of anthocyanin (Llorach

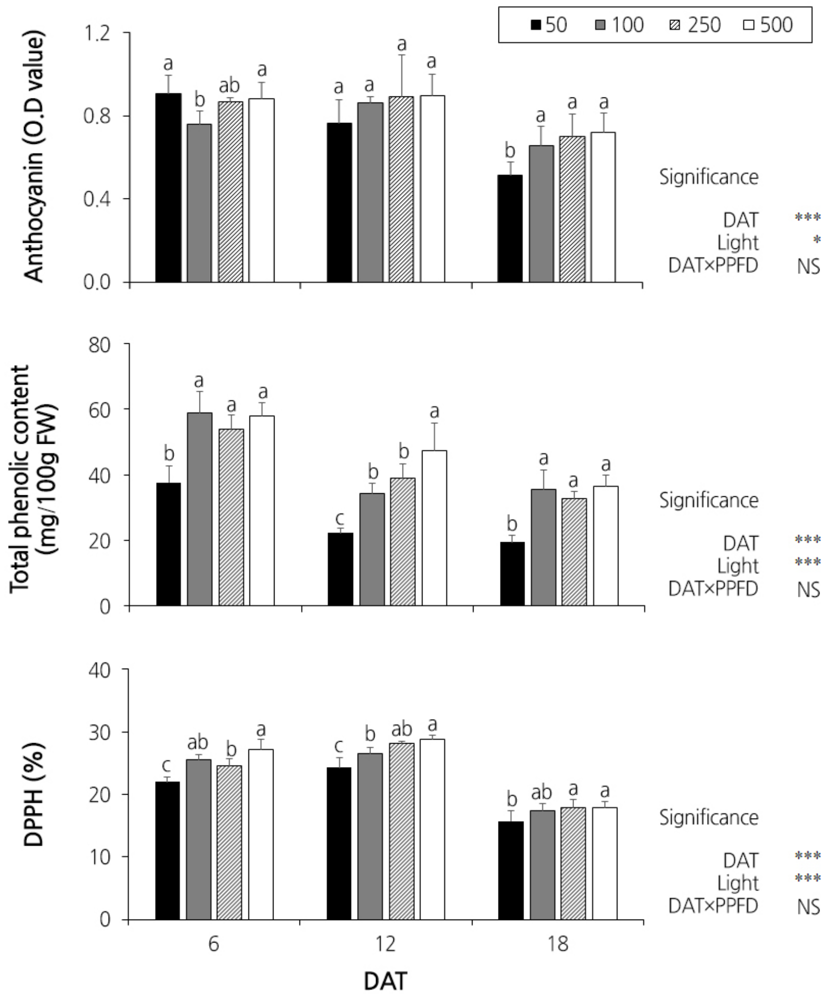

Fig. 3. Effects of light intensity on anthocyanin content, total phenolic content, and free radical activity (DPPH) of Indian lettuce 'Sunhyang' grown in controlled system. DAT means day after treatment. Means with different letters in each column are significantly different by DMRT at $p < 0.05$ (n = 4). NS, *, *** mean none significant, significant at $p < 0.05$ and 0.001, respectively.

et al., 2008). Our results showed that TPC was highly accumulated in PPFD 500 under which the color of leaves was reddish. Perez-Lopez et al. (2018) have reported that environmental stress such as high light intensity not only enhances phenolic contents, but also improves antioxidant capacity under ambient CO_2 condition (400 μmol·mol^{-1}).

In summary, light intensity influenced the growth characteristics of Indian lettuce 'Sunhyang', including shoot growth, root growth, and leaf morphology. Light intensity also can change harvesting time of Indian lettuce 'Sunhyang' cultivated in controlled environment for year-round production. 'Sunhyang' baby leaf vegetable is ready for harvest from 12 DAT in both PPFD 100 and 250 treatments. These two light conditions would be a good range of light intensity to produce proper 'Sunhyang' baby leaf vegetable because good growth characteristics and no difference in functional components compared to other treatments were obtained from these two light intensities. Since baby leaf vegetable growers look for red leaf vegetables to mix with other green baby leaf vegetables, Indian lettuce 'Sunhyang' grown at PPFD 250 will be useful to increase the value of the produce due to its red color of leaves and high quality of functional components.

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