Changes in Leaf Variegation and Coloration of English Ivy and Polka Dot Plant under Various Indoor Light Intensities

Jongyun Kim¹, Seung Won Kang², Chun Ho Pak³, and Mi Seon Kim^{4,5}

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Summary. Variegated foliage plants are often used in interiorscaping in low light environments. The changes in leaf morphology and coloration of two variegated foliage plants, english ivy (Hedera helix 'Golden Ingot') and polka dot plant (Hypoestes phyllostachya), under various light intensities [photosynthetic photon flux (*PPF*) at 2.7, 6.75, 13.5, 67.5, and 135 μ mol·m⁻²·s⁻¹] were investigated to elucidate their optimum indoor light environment. Digital image analysis was used to quantify the changes in variegation area and color in CIELAB color space. The changes in leaf morphology (thickness, length:width) and coloration were different between the two species. In general, growth of both species increased with increasing PPF. English ivy showed no significant changes in leaf variegation under different PPF. Under low PPF (≤13.5 µmol·m⁻²·s⁻¹), newly developed leaves of polka dot plant had reduced leaf variegation (44%, 72%, and 85% variegation loss under 13.5, 6.75, and 2.7 μ mol m⁻² s⁻¹, respectively). Anthocyanin content in leaves of polka dot plant also decreased with decreasing *PPF*, which reduced plants' aesthetic quality. English ivy leaves under high *PPF* (≥67.5 µmol·m⁻²·s⁻¹) displayed high brightness (L*) and yellowish green color (hue angle < 108°), which diminished its aesthetic value. Smaller leaf size and narrower shape of polka dot plant leaves under high PPF (≥67.5 µmol·m⁻²·s⁻¹) also diminished its aesthetic value. Overall, english ivy performed well in a PPF range from 2.7 to 13.5 μmol·m⁻²·s⁻¹, and polka dot plant required a PPF of at least 13.5 µmol·m⁻²·s⁻¹ to maintain its redpurple variegation in the indoor environment.

Interest in interiorscaping with plants has increased rapidly over the past four decades with the need for green in the urban environment (Manaker, 1997). House plants provide people with aesthetic appreciation and other beneficial effects. Plants can effectively improve the indoor air quality by reducing volatile organic compounds, such as formal-dehyde, benzene, toluene, ethylene, and xylene (Thomsen et al., 2011; Wolverton, 1988), thus reducing the risk of sick building syndrome (Kim et al., 2011). House plants also fulfill

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psychological needs of people by providing green color and comfort and enhance the indoor environment to make it more aesthetically pleasing (Bringslimark et al., 2007).

Foliage plants are often used as house plants because of their attractive foliage and their ability to survive and grow under limited indoor light (Chen and Henny, 2008). Among the characteristics of foliage plants, variegation is an important trait, which provides unique visual appearance and aesthetic variation in interior design, making it one of the considerations of consumers' preferences in purchasing decisions (Chen et al., 2004). Variegation is the occurrence of patterns,

especially as a result of differences in the amount or composition of the green pigment chlorophyll, although other pigments such as anthocyanin and carotenoids may also be involved in a wide variety of multicolored leaf patterns. Variegation can occur through differential gene expression, leaf blisters, virus, or genetic mosaics such as chimeras (Marcotrigiano, 1997), and the variegation appearance can be altered by environmental factors, particularly light intensity (Tilney-Bassett, 1986).

Among the environmental conditions for indoor plants, the most limiting factor for plant growth is reduced light intensity (Manaker, 1997). Although the light intensity outdoor is generally higher than 1000 μ mol·m⁻²·s⁻¹ of *PPF* at sunny days, typical indoor light intensity is less than 40 µmol·m⁻²·s⁻¹ (Manaker, 1997). Because plant growth depends on light (i.e., photosynthesis), plants require a particular light environment for proper growth and development (Maloof et al., 2001). However, plants also can adjust to varying light environments through physiological and morphological changes (i.e., acclimation), resulting either in increased light capture or improved light utilization. General acclimation responses to low light include higher shoot to root ratio (whole-plant level), increased leaf size per unit leaf dry weight, increased total chlorophyll content, and a decrease in the chlorophyll a:b ratio (individual leaf level) (Allard et al., 1991; Evans and Poorter, 2001; Nemali and van Iersel, 2004). However, the effect of low light intensity on variegation is not consistent among species (either with increase, decrease, or no changes in variegation), thus the information of appropriate light levels is critical for producing and maintaining variegated foliage plants with their best performance in the interiorscape (Chen and Henny, 2008; Li et al., 2007).

Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
6.4516	inch ²	cm ²	0.1550
28.3495	OZ	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
$(^{\circ}F - 32) \div 1.8$	°F	°Č	$(1.8 \times {}^{\circ}\text{C}) + 32$

¹Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742

²BET Research Institute, Chung-Ang University, Anseong 456-756, Korea

³Division of Biotechnology, Korea University, Seoul 136-713. Korea

⁴Floriculture Research Division, National Institute of Horticultural and Herbal Science, Suwon 440-706, Korea

⁵Corresponding author. E-mail: kimms290@korea.kr.

Previously, several attempts for measuring variegation were made (Marcotrigiano and Hackett, 1993; Shen and Seeley, 1983; Smith et al., 1988). Shen and Seeley (1983) measured leaf variegation using leaf area meter, and Marcotrigiano and Hackett (1993) measured leaf variegation using a photocopy machine, transparencies, and a portable area meter. However, these methods were timeconsuming and appeared relatively unreliable (Kwack et al., 1998; Li et al., 2007). Kwack et al. (1998) and Li et al. (2007) used a commercial scanner and digital image analysis software (Photoshop; Adobe Systems, San Jose, CA) to quantify the variegation, and they reported this method to be reliable and effective compared with previous methods.

Although color is one of the most important visual plant characteristics, the analysis of color was often neglected mostly because of lack of an appropriate method (McGuire, 1992; Voss, 1992). Digital image analysis also provides the ability to quantify coloration of the leaves, which can improve understanding of changes in variegation (Kwack et al., 1998). CIE L*a*b* (CIELAB) value is the most complete color space specified by the Commission Internationale de l'Éclairage (1978). CIELAB color space is suitable for standardization of colorimetric practice in science, which is numerically coordinated to locate individual colors, where L* represents darkness and brightness, a* represents green and red, and b* represents blue and yellow, as the value increases from negative to positive, respectively (Voss, 1992). McGuire (1992) indicated that chroma [C* (degree of departure from gray toward pure chromatic color)] and hue angle [h° (red, green, blue, and yellow)] calculated from a* and b* are somewhat analogous to color saturation or intensity and describe a more straightforward and appropriate measurement of color for horticultural research.

The objective of this study was to investigate the optimum light intensity for two foliage plants in an indoor light environment. Along with the morphological changes in the leaves of two foliage plants, digital image analysis could give a good indication of the changes in variegation and coloration under different light intensities. These results will improve

understanding of the light requirements for better performance of foliage plants under limited indoor light environments.

Materials and methods

PLANT MATERIAL. Rooted cuttings of english ivy ('Golden Ingot') and polka dot plant were obtained from a local garden center in Seoul, Korea. All the seedlings were transplanted into 3-inch-diameter round plastic pots filled with vermiculite (30% by volume) and soilless substrate [70% by volume (Sunshine Organic Mix2; Sun Gro Horticulture, Vancouver, BC, Canada)]. After acclimatization for 2 weeks in the greenhouse with shade at Korea University, Seoul, Korea, all the plants were moved to an indoor laboratory at a temperature of 25 °C. Plants were hand-watered every other day.

TREATMENTS AND MEASURE-MENTS. To obtain suitable indoor light intensities, triband phosphor fluorescent lamps [Dulux L 36W (Osram Korea, Seoul, Korea) and FL40 EX-D (Kumho Electric, Seoul, Korea)] were used as the light source. Light intensity was measured using a quantum meter (LI-250; LI-COR, Lincoln, NE) and quantum sensor (LI-190; LI-COR) at the canopy of the plants and adjusted to ≈ 2.7 , 6.75, 13.5, 67.5, and 135 μ mol·m⁻²·s⁻¹ by altering the number of lamps that were energized and by using white, neutral filter paper. Light period was 16 h day and 8 h night.

All plants were harvested 9 weeks after treatments were initiated. At harvest, shoot height, longest root length, total fresh weight, and internode length were measured. To distinguish the effect of light intensity at the time of leaf formation, fully expanded leaves that formed before treatments were initiated [pretreatment expanded (PE) leaves] and newly formed leaves after treatments were initiated [recently expanded (RE) leaves] were analyzed separately, by measuring two or four leaves of each. Leaf size and leaf width:length, variegation (the relative amount of non-green leaf area per leaf), and coloration were measured through digital image analysis using Photoshop (Kwack et al., 1998; Li et al., 2007). Leaf thickness was measured with thickness gauge (MDC-SB; Mitutoyo Co., Kawasaki, Japan). Chlorophyll in

fully expanded leaves was extracted with 10 mL N,N-dimethylformamide solution per 0.2 g of samples and kept at 4 °C in dark condition for 48 h. The optical density (OD) value was measured by spectrophotometer (DU-650; Beckman Instruments, Fullerton, CA) at 647 and 664.5 nm wavelength, and total chlorophyll content and chlorophyll a:b ratio was calculated (Inskeep and Bloom, 1985). Anthocyanin content of fully expanded leaves of polka dot plant was extracted with 10 mL of 1% hydrochloric acid in methanol for 24 h at 4 °C. The OD value for anthocyanin was measured by spectrophotometer (DU-650) at the wavelength of 535 nm.

DETERMINATION OF COLORATION AND VARIEGATION. Sample leaves were scanned in a commercial scanner (Perfection 4180 photo; EPSON, Long Beach, CA) at 300 dots per inch (dpi). Leaf size and color were assessed from scanned image using Adobe Photoshop 5.5 (Adobe Systems). Each image of a leaf was selected using [magic wand] and [lasso] tools, and the selected area was reported in [histogram]. In [histogram], pixels are directly proportional to leaf size, where 13924 pixels are 1 cm² at 300 dpi scanning. To acquire the color information, RGB (red, green, blue) means in [histogram] were collected and converted into L*, a*, b* value through [color picker] in Photoshop. From acquired a* and b* value, C* and h° were calculated as below (McGuire, 1992):

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$$b^\circ = \frac{ATAN(b^*/a^*)}{6.2832} \times 360,$$
when $a^* > 0$ and $b^* \ge 0$

$$\hat{b} = \frac{ATAN(b^*/a^*)}{6.2832} \times 360 + 180,$$
when $a^* < 0$ and $b^* \ge 0$

Variegation was measured by [selection]–[color range] menu in Photoshop. This [color range] option selects the similar color with the range of [fuzziness]. We used the threshold level of [fuzziness] at 40 to select most of variegated area.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS. The experimental design was completely randomized

with five replications. Data were analyzed general linear models and regression in SAS (version 9.2; SAS Institute, Cary, NC). Most of the regression analyses were conducted with logarithmic transformations of *PPF*; however, when needed, *PPF* without transformation was used to determine the effect of *PPF* on plant responses.

Results and discussions

MORPHOLOGY. Growth parameters such as shoot height, root length, and fresh weight were greater under higher light intensity for both species, except the fresh weight of polka dot plant (Fig. 1). Shoot height of english ivy showed a quadratic response to *PPF*, and polka dot plant showed linear response of shoot height to

log PPF. The response of shoot height was similar to the results with dracaena (Dracaena sanderiana 'Ribbon') grown under four shade levels [47%, 63%, 80%, and 91% (Vladimirova et al., 1997)]. Root length of both foliage plants had positive linear effect to the log*PPF* for both species (P < 0.001). English ivy had greater fresh weight under higher light intensity, but fresh weight of polka dot plant was not significantly affected across the light intensities (Fig. 1C). A general reduction in plant growth under lower light intensity was also reported in other foliage plants, such as pothos (Epipremnum aureum) (Nam et al., 1997), english ivy (Pennisi et al., 2005), dwarf umbrella tree (Schefflera arboricola) (Kubatsch et al.,

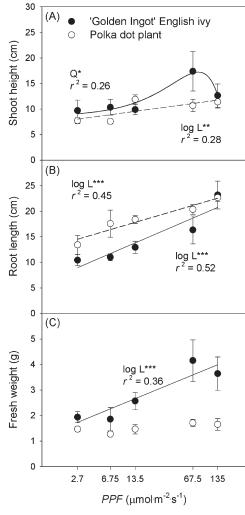


Fig. 1. Growth parameters of 'Golden Ingot' english ivy and polka dot plant 9 weeks after plants were placed under different indoor light intensities [photosynthetic photon flux (PPF)]. Error bar indicates mean \pm SE (n = 5). Q indicates quadratic response to PPF, and log L indicates linear response to log PPF. ***, **, and * indicate significance at P < 0.001, 0.01, and 0.05, respectively; 1 cm = 0.3937 inch, 1 g = 0.0353 oz.

2007), and coleus (Solenostemon scutellarioides) (Garland et al., 2010). A recent review article reported that shade induced stem elongation through phytohormone signaling, thus producing tall but thin plants as a shade avoidance response (Stamm and Kumar, 2010). However, english ivy and polka dot plant had no significant difference in the internode length across the light intensities (data not shown).

Previous research with three english ivy cultivars (Gold Child, Gold Dust, and Gold Heart) showed increased leaf area with increasing light level (Pennisi et al., 2005). Other researchers also reported the increase of leaf area with increasing light level in coleus (Garland et al., 2010), dwarf umbrella tree (Kubatsch et al., 2007), and wax begonia (Begonia semperflorenscultorum) (Nemali and van Iersel, 2004). However, in our experiment, leaf size of english ivy was not significantly different across PPFs (Fig. 2A). In polka dot plant, leaf size of RE leaves showed a quadratic response to logPPF, and PE leaves showed quadratic response to PPF (Fig. 2B) with the largest leaf size under 13.5 and 67.5 μ mol·m⁻²·s⁻¹, respectively. This quadratic response of leaf size to light intensity was similar to the response of dracaena (Vladimirova et al., 1997) and pothos (Nam et al., 1997).

Although both english ivv and polka dot plant had thinner RE leaves than PE leaves (P < 0.001), the change in leaf thickness in response to *PPF* was opposite between species (Figs. 2C and 2D). English ivy had thinner leaves under low PPF (P < 0.01), whereas polka dot plant had thicker leaves under low PPF (P < 0.001). Thinner leaves under low light have been reported as an acclimation process of shade plants with the higher specific leaf area [SLA (leaf area per unit leaf dry weight)] (Evans and Poorter, 2001). However, our results showed that polka dot plant decreased leaf thickness under high PPF (≥67.5 µmol·m⁻²·s⁻¹), and previous research reported that dwarf umbrella tree (Kubatsch et al., 2007) and dracaena (Vladimirova et al., 1997) showed no significant changes in leaf thickness in response to PPF, suggesting the changes in SLA or leaf thickness probably depend on the species and the acclimation period.

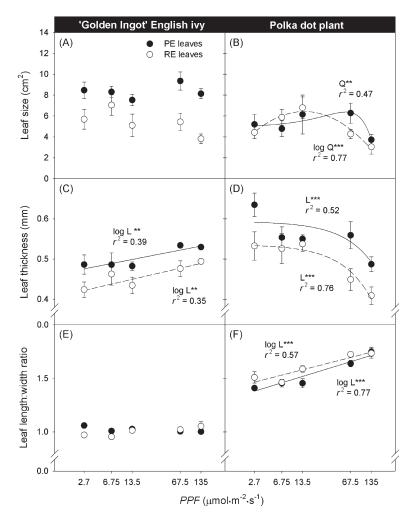


Fig. 2. Leaf morphology of 'Golden Ingot' english ivy and polka dot plant 9 weeks after plants were placed under different indoor light intensities [photosynthetic photon flux (PPF)]. PE leaves represent pretreatment expanded leaves and RE leaves represent recently expanded leaves after the treatment. Error bar indicates mean \pm SE (n = 5). L and Q indicate linear and quadratic response to PPF, and log L and log Q indicate linear and quadratic response to log PPF. ***, **, and * indicate significance at P < 0.001, 0.01, and 0.05, respectively; 1 cm² = 0.1550 inch², 1 mm = 0.0394 inch.

Leaf length:width of english ivy showed no significant difference across PPF levels (Fig. 2E). However, all polka dot plant leaves displayed narrower leaves under higher PPF with linear response to log PPF (Fig. 2F). Previous research suggested that the greater length: width contributed to larger light capture per unit leaf area (Takenaka, 1994). However, wider leaves of polka dot plant under low PPF are probably due to an acclimation to receive more light with wider area under limited light source. The smaller area and narrower shape of RE leaves of polka dot plant under high *PPF* (≥67.5 μ mol·m⁻²·s⁻¹) devaluate the aesthetic quality of the foliage plants.

VARIEGATION AND PIGMENTS. Changes in variegation by light intensity have been often investigated because of the importance of the aesthetic value of variegation in foliage plants. Previous research reported that variegated croton (Codiaenum variegatum 'Yellow Jade') and two pothos cultivars had less leaf variegation under low light intensity (Nam et al., 1997; Sul et al., 1997). On the contrary, dracaena and radiator plant (Peperomia obtusifolia) had more leaf variegation under low light intensity (Shen and Seeley, 1983; Vladimirova et al., 1997). Previous research with three cultivars of english ivy reported that the leaf variegation decreased under daily light integral (DLI) lower than

8.5 mol·m⁻²·d⁻¹ (Pennisi et al., 2005). Although our highest PPF treatment $(135 \,\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1})$ had a DLI of ≈ 7.8 mol·m⁻²·d⁻¹, 'Golden Ingot' english ivy had no significant change in variegation under different *PPF* levels (Fig. 3A). In polka dot plant leaves, only RE leaves decreased leaf variegation with decreasing PPF with linear response to logPPF (P < 0.001, Fig. 3B). RE leaves under PPF of 13.5 µmol·m⁻²·s⁻¹ reduced variegation by 44% of RE leaves of high *PPF* treatments (≥ 67.5 μ mol·m⁻²·s⁻¹), and the lowest *PPF* treatment had only 6% of leaf variegation. PE leaves of polka dot plant had no significant differences in variegation across the PPF, indicating no changes in variegation after the leaves were developed.

Total chlorophyll contents of both in PE and RE leaves of english ivy were higher under lower PPF with linear response to PPF (Fig. 3C). Chlorophyll a:b ratio of english ivy leaves were higher under lower PPF with quadratic response to log PPF (Fig. 3E). Higher chlorophyll contents under low light were also reported in other foliage plants such as philippine evergreen (Aglaonema commutatum) (Di Benedetto, 1991), spotted laurel (Aucuba japonica) (Andersen et al., 1991), variegated croton (Sul et al., 1997), and dwarf umbrella tree (Kubatsch et al., 2007). These responses are common in plants as an acclimation to improve photosynthetic efficiency under limited light (Evans and Poorter, 2001; Nemali and van Iersel, 2004). However, chlorophyll contents of polka dot plant showed no significant response to *PPF* (data not shown). Instead of the change in chlorophyll contents, both PE and RE leaves of polka dot plant had higher anthocyanin contents under higher *PPF* with linear response to PPF [P < 0.001] for both PE and RE leaves (Fig. 3D)], although PE leaves did not show any significant difference in variegation (Fig. 3B). Garland et al. (2010) indicated that the type of red variegation in coleus is due to nonclonal physiological changes in pigment concentration, and they reported less variegation under low light in coleus. This supports that this kind of variegation needs enough light to physiologically develop the pigment, anthocyanin. In particular, variegation resulting from changes in pigmentation other than chlorophyll (i.e.,

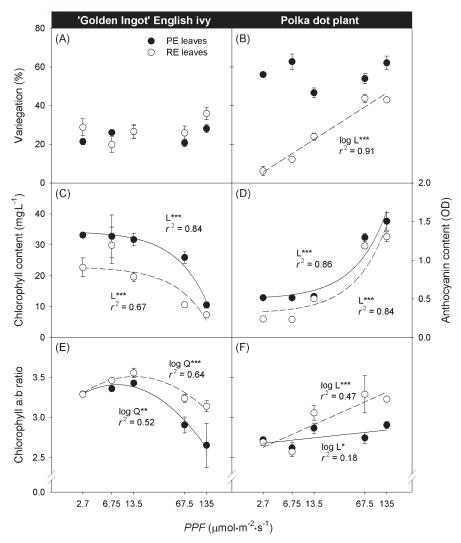


Fig. 3. Leaf variegation, chlorophyll content, chlorophyll a:b ratio, and anthocyanin content [optical density (OD)] of 'Golden Ingot' english ivy and polka dot plant 9 weeks after under different indoor light intensities [photosynthetic photon flux (PPF)]. PE leaves represent pretreatment expanded leaves and RE leaves represent recently expanded leaves after the treatment. Error bar indicates mean \pm SE (n = 5). L indicates linear to PPF, and log L and log Q indicate linear and quadratic response to log PPF. ***, **, and * indicate significance at P < 0.001, 0.01, and 0.05, respectively; 1 mg·L⁻¹ = 1 ppm.

carotenoid, anthocyanin) may need high light to support the leaf photosynthates to develop the pigmentation (Chen et al., 2004; Garland et al., 2010).

Changes in coloration of the Leaves. Although english ivy showed no significant difference in leaf variegation, the coloration of leaves was affected by PPF (Fig. 4). In english ivy, both PE and RE leaves had higher L* (i.e., brighter color) under higher PPF with a linear response to PPF (P < 0.001). a* of both PE and RE leaves also increased with increasing PPF with a quadratic response to PPF (P < 0.001); this indicates

diminishing green color. Chroma and hue angle calculated from a* and b* showed a significant decrease under high PPF (≥67.5 µmol·m⁻²·s⁻¹), which indicates less saturated green and pale green color (h° < 108) under high PPF. Interestingly, hue angle was not significantly different between PE and RE leaves. Green color value (L*, a*, and hue angle) was correlated with chlorophyll content (Fig. 4, P < 0.001), indicating lower chlorophyll contents under higher PPF (Fig. 3C) diminished the green color. In particular, hue angle showed a positive correlation with chlorophyll content (r^2 = 0.88 and 0.85 for PE and RE leaves, respectively) with no significant slope difference between PE and RE leaves (P = 0.82). This color analysis may be a good relative measurement of chlorophyll content.

In polka dot plant, the changes in coloration of RE leaves were greater than those of PE leaves (Fig. 4). The change in L* of PE leaves was by 5 with quadratic response, whereas L* of RE leaves increased by 23 with quadratic increase with decreasing PPF, indicating brighter leaf color under low PPF. Positive a* indicates red color, and a* of RE leaves significantly decreased greater than PE leaves under low PPF ($\leq 13.5 \, \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). This indicates that newly developed polka dot plant leaves had less red color (a* < 0) in the leaves under low $PPF (\le 13.5)$ µmol·m⁻²·s⁻¹), which agrees with less variegation resulting from lower anthocyanin contents. b* of RE leaves increased with decreasing PPF, which indicate enhanced yellow color, although there was no significant difference in b* value of PE leaves across *PPF*s. Hue angle of PE and RE leaves also increased with decreasing PPF, and RE leaves under low PPF (≤13.5 µmol·m⁻²·s⁻¹) had hue angle higher than 100°, indicating reduction of red color from variegation loss (Fig. 3D). Comparison between CIELAB values and anthocyanin contents showed good correlation between coloration and pigment in polka dot plant (Fig. 4). With increasing anthocyanin contents, brightness (L*) of the leaves was decreased and redness of color (a*) was increased in both PE and RE leaves, but more in RE leaves than in PE leaves (P <0.001). b* of RE leaves showed a negative correlation with anthocyanin contents, indicating more vellow color with lower anthocyanin contents. Overall, CIELAB showed that only newly developed leaves of polka dot plant changed color drastically with decreasing PPF, including brighter (higher L*), less red (lower a*), and more yellow color (higher b*).

Conclusions

General growth of both species increased with increasing *PPF*. However, two species showed different responses to light intensity in leaf morphology, such as leaf size, leaf thickness, leaf length:width ratio, variegation, and also in leaf physiology

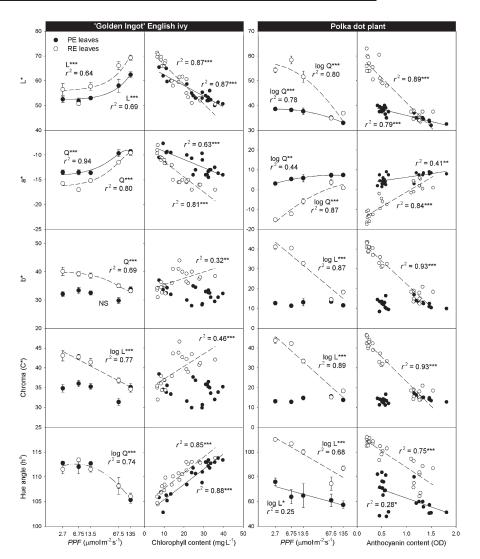


Fig. 4. Change in coloration (CIELAB color space; Commission Internationale de l'Eclairage, Paris) of 'Golden Ingot' english ivy and polka dot plant 9 weeks after various light intensities [photosynthetic photon flux (PPF)]. CIELAB values were obtained from digital image analysis using Photoshop software (Adobe Systems, San Jose, CA). Chroma and hue angle were calculated from a* and b*; L* represents darkness and brightness, a* represents green and red, and b* represents blue and yellow, as the value increases from negative to positive, respectively. PE leaves represent pretreatment expanded leaves and RE leaves represent recently expanded leaves after the treatment. Error bar indicates mean \pm SE (n = 5). L and Q indicate linear and quadratic response to PPF, and log L and log Q indicate linear and quadratic response to 1000, 1000, 1000, 1000, respectively; 1000,

with pigmentations. English ivy had thicker leaves with lower chlorophyll contents under high PPF, resulting in pale green color [low hue angle (<108°), high L*, and high a*]. This pale green color devaluated their physical appearance under high PPF, and the optimum range of PPF for english ivy was from 2.7 to 13.5 μ mol·m⁻²·s⁻¹.

Polka dot plant showed significant reduction in variegation (<56% of high *PPF* treatments) in RE leaves under low *PPF* (\leq 13.5 μ mol·m⁻²·s⁻¹)

along with reduction in anthocyanin contents. However, polka dot plant showed thin and narrow leaves under high *PPF* (≥67.5 µmol·m⁻²·s⁻¹). Therefore, polka dot plant may need a *PPF* of at least 13.5 µmol·m⁻²·s⁻¹ to maintain variegation. To better identify the optimum light level for polka dot plant, further research with various fertilizer level along with light intensity interaction may be needed.

Digital image analysis with scanned leaves effectively quantified the leaf variegation and leaf coloration as well. From this simple and effective analysis, more analysis of appearance with color may be quantified, such as chlorosis or pigment phenotype, as well as changes in variegation.

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