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Productivity and photosynthetic characteristics of heat-resistant and heat-sensitive recombinant inbred lines (RILs) of *Lactuca sativa* in response to different durations of LED lighting

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**Abstract**
This study investigated the impact of different photoperiods of red- and blue-LED lighting on the growth, as well as, photosynthetic characteristics of heat-resistant (HR) and heat-sensitive (HS) RILs of *Lactuca sativa* grown in a vertical farming system. Lettuce plants were grown under three different photoperiods (12, 16 and 18 h) of red- (85%) and blue- (15%) LED lighting, at a mean photosynthetic photon flux density (PPFD) of 300 µmol m\(^{-2}\) s\(^{-1}\). 27 days after transplanting, both HR- and HS-RIL plants had significantly higher shoot and root fresh (FW) and dry (DW) weights, leaf number and total leaf area (TLA) when subjected to extended photoperiods of 16 and 18 h than the normal 12 h photoperiod. Highest growth parameters were observed for the longest photoperiod of 18 h. However, photosynthetic properties were not greatly enhanced under the extended photoperiods of 16 and 18 h - only chlorophyll (Chl) a/b ratio was increased. Light saturated photosynthetic CO\(_2\) assimilation (\(A_{\text{sat}}\)) and stomatal conductance (\(g_{\text{s}}\)) among plants exposed to different durations of LED lighting showed no significant difference among the various photoperiods. Though longer photoperiods resulted in increased total carbon assimilation, this would have been due to the plants having a larger TLA. The decrease in soluble and insoluble sugar concentrations in lettuce leaves, under longer photoperiods, was most probably due to the dilution of carbohydrates as a result of increased leaf number and total leaf area. Thus, the results suggest a lack of feedback inhibition of photosynthetic end products in the leaves of lettuce plants, despite its exposure to longer photoperiods.

**INTRODUCTION**
Growing populations in modern societies create a perpetual demand for more food; climate change and limited land space, put further pressure on increasing crop yield. As vegetables contain most of the essential components required in human nutrition, they are an inevitable part of our diet. However, as perishables, they cannot be stocked. As such, space limited Singapore has to move towards using vertical farming systems to produce large quantities of high quality vegetable crops so as to ensure its growing population has a secured supply of vegetables (He, 2015; He et al., 2015).

Light-emitting diodes (LEDs) have been found to be a suitable source of artificial lighting for vegetable farming because they are energy-efficient, durable, and easily customized to the required light spectrum for optimal plant growth (Tamulaitis et al., 2005; Trouwborst et al., 2010; He et al., 2015). Kim et al. (2004) studied the effects of different LEDs on lettuce growth and found that red LED emits light that is closest to the absorption peak of Chl while blue LED is a good supplement due to its role in stimulating Chl synthesis.

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Other researchers also found that a combination of red- and blue-LED is most suitable for photosynthesis (Matsuda et al., 2007; Samuoliene et al., 2010; He et al., 2015). It was reported that short photoperiods under natural light, supplemented by low intensity LED lighting, resulted in plant characteristics similar to plants that were grown under long photoperiods (Pinho et al., 2012). Further, Yang et al. (2012) found that longer photoperiods can increase photosynthetic rate, enhancing vegetative growth. As such, altering photoperiod to increase crop yield is yet another aspect that can be explored.

There has been little research carried out to determine an optimal photoperiod for lettuce growth and explore the mechanism(s) responsible for increased productivity of lettuce plants grown under longer photoperiods. Thus, using a vertical aeroponic farming system within a tropical greenhouse, the objective of this study was to investigate the impact of exposing lettuce plants to different durations of red- and blue-LED lighting, on its vegetative production, photosynthetic properties and carbohydrate concentrations.

MATERIALS AND METHOD

Culture of plant materials and light treatments

F9 generation of lettuce RILs with varying thermosensitivities were obtained by repeatedly crossing HR paternal L. serriola accession UC96US23 and HS maternal L. sativa Salinas (Argyris et al., 2005). Previously identified (Choong et al., 2012) HR-RIL#212 and HS-RIL#215 were germinated on moist filter paper in Petri dishes. After germination, seedlings were inserted into polyurethane cubes, soaked in water, and transported to the greenhouse for acclimatization. Eight days later, seedlings were transplanted into the polystyrofoam panels of the four-tiered vertical aeroponic farming system in the greenhouse. Lettuce plants were left to grow with roots suspended within the aeroponic troughs (He, 2010) and misted with nutrient solution, made up of Netherlands Standard Composition, for 30 s at 5 min intervals. pH and conductivity were maintained at 6.5 and 2.2 mS/cm, respectively. Rootzone temperature was maintained at 25°C while the aerial parts grew under fluctuating hot ambient temperatures (26 to 38°C) - temperate and subtropical vegetables can grow well by cooling only the rootzone to 15-25 ºC while their shoots were exposed to hot ambient temperatures (He and Lee, 1998). PPFD level was less than 10 µmol photon m⁻² s⁻¹ throughout the day within the vertical aeroponic farming systems. Hence, in this study, plants were subjected to three different durations of 12, 16 and 18 h of combined red- and blue-LED lightings with a mean PPFD of 300 µmol photon m⁻² s⁻¹. The 12 h photoperiod followed closely to the duration of natural light, from 0700 to 1900 hours. Photoperiods of 16 and 18 h started pre-dawn from 0300 to 1900 hours and 0100 to 1900 hours, respectively. The combination of red- and blue-LED lighting was set to 85% and 15%, respectively (Figure 1).

![Figure 1](image_url) Light spectra of red (85%) and blue (15%) LEDs were recorded at every 0.5 nm interval with a spectroradiometer (PS300, Apogee Instruments, USA).
Measurements of fresh (FW) and dry weights (DW)

Plants were harvested between 1000 and 1100 h, 27 days after transplanting (DAT). FW of shoot and roots were measured, using a weighing balance (Sartorius, Fisher General Specific Private Limited, Singapore). Plants were then dried in the oven at 80 °C for four days to obtain their DW.

Measurements of leaf expansion rate and total leaf area (TLA)

Newly emerged leaves were individually placed on a white background with a 5 cm scale drawn on it. Leaf images were taken with the scale clearly shown, at 12, 14, 16, 18 and 20 DAT. Leaf images were analysed using Image J (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA) to determine the leaf area. TLA was measured 27 DAT using WinDIAS3 (Dynamax Inc., Houston, USA).

Determination of photosynthetic pigments

Leaf discs were soaked in N,N-dimethylformamide and left in the dark, at 4 °C, for 48 h. Their absorption was read, using a spectrophotometer (UV-2550, Shimadzu, Japan), at 480, 647 and 664 nm. Concentrations for Chl, and carotenoids (Car) were calculated (Wellburn, 1994).

Measurements of light-saturated CO₂ assimilation rate (A_{sat}) and stomatal conductance (g_{s,sat})

Light-saturated CO₂ assimilation rate (A_{sat}) and stomatal conductance (g_{s,sat}) of attached youngest mature leaves were measured simultaneously between 0900–1100 h 27 DAT in the greenhouse using an open infrared gas analysis system (LI-6400, Biosciences, USA) in a 6 cm² leaf chamber. A_{sat} and g_{s,sat} were measured with a supplied LED light source, of wavelength between 660 to 675 nm, at PPFD of 1000 µmol photon m⁻² s⁻¹. Average CO₂ concentration in the leaf chamber was 400±5 µmol CO₂ mol⁻¹. Total CO₂ assimilation was calculated by multiplying the TLA and total duration of photoperiod with A_{sat} 27 DAT.

Determination of carbohydrates level

Newly mature leaves were harvest just before the end of the photoperiods 20 DAT. Both soluble and insoluble sugar concentrations were determined (Buysse and Merckx, 1993).

Statistical analysis

A two-way multivariate analysis of variance (MANOVA) was used to test for significant differences between the RILs under the three photoperiods, with means discriminated using Tukey’s multiple comparison tests when the main effects MANOVA was significant. Statistical analysis was carried out using Minitab, with significance level at p = 0.05.

RESULTS AND DISCUSSION

Productivity and leaf growth

HR-RIL exhibited significantly higher shoot FW, root FW and shoot/root ratio FW than HS-RIL (Figure 2). Higher shoot FW (Figure 2A) and root FW (Figure 2B) were observed in all lettuce plants subjected to 16 and 18 h photoperiods, compared to plants grown under 12 h photoperiod. Plants grown under 18 h photoperiod had the highest shoot FW (Figure 2A), root FW (Figure 2B) and shoot/root ratio FW (Figure 2C). For all lettuce plants, shoot DW, root DW and shoot/root ratio DW showed similar responses (data not shown). All lettuce plants subjected to 18 h photoperiod had the fastest leaf expansion (Figures 3A, 3B) compared to those grown under 12 and 16 h photoperiods. All plants
subjected to 18 h photoperiod had a significantly larger TLA (Figure 3C) and greater leaf number (Figure 3D) than plants grown under 12 and 16 h photoperiods.

Longer photoperiods result in increased total integrated PPFD, where greater quantities of photosynthetic products are synthesized, resulting in enhanced plant growth (Demers and Gossenlin, 1998; Yan, 2012). Our findings showed that both HR-RIL and HS-RIL, grown under 18 h photoperiod, accumulated the highest biomass when compared to plants grown under shorter photoperiods. Shoot biomass was increased by more than twofold (Figure 2A), when compared to plants grown under 12 h photoperiods. This increase was mainly observed in the shoots, as shown from the highest shoot/root ratio FW (Figure 2C). Our results demonstrate that it is possible to increase shoot production and rate of shoot production of lettuce plants by extending the photoperiod to 18 h, when a constant moderate level of PPFD was provided. Plant growth and development are regulated by interactions among environmental, physiological and endogenous factors, especially hormones (Jouve et al., 1999). It was long reported that auxin was influenced by photoperiod (Bonner, 1940), which allowed more photosynthetic products to be utilised for leaf expansion in the earlier vegetative development of the plant. In this study, plants subjected to 18 h photoperiod showed a faster rate of leaf expansion and were fully expanded within 18 DAT, while plants grown under shorter photoperiods took more than 20 DAT (Figures 3A, 3B). TLA of plants was also proportional to the duration of photoperiod, where longer photoperiods resulted in larger TLA in both HS-RIL and HR-RIL (Figure 3C).

**Figure 2** Shoot FW (A), root FW (B), and shoot/root ratio FW (C) of HS-RIL and HR-RIL plants after exposure to various durations of LED lighting for 27 days. Values illustrated are the means of four measurements. Vertical bars represent standard errors. Different letters above the bar graphs denote statistical differences ($p < 0.05$) as determined by Tukey’s multiple comparison test.

**Figure 3** Leaf expansion of HS-RIL (A) and HR-RIL (B), total leaf area (C) and leaf number (D) of HS-RIL and HR-RIL after exposure to various durations of LED lighting for 27 days. Values illustrated are the means of four measurements. Vertical bars represent the standard errors and when they are not seen, they are smaller than the symbols. Different letters above the bar graphs denote statistical differences ($p < 0.05$) as determined by Tukey’s multiple comparison test.
**A_s sat, g_s sat and photosynthetic pigments**

HR-RIL had higher A_s sat and g_s sat compared to HS-RIL. This trend was observed across all photoperiods (Figures 4A, 4B). There were no significant differences in A_s sat and g_s sat among the different photoperiods within each RIL. However, total CO_2 assimilation increased with increasing photoperiod for both RILs (Figure 4C).

HR-RIL grown under 18 h photoperiod had significantly lower (p < 0.05) Chl and Car concentrations (Figures 5A, 5B) compared to those grown under shorter photoperiods. There were no significant differences in Chl and Car concentrations (Figures 5A, 5B) between lettuce plants that were subjected to shorter photoperiods of 12 and 16 h for both RILs. Plants grown under longer photoperiods of 16 and 18 h had higher Chl a/b ratios (Figure 5C) compared to that of plants exposed to 12 h photoperiod, for both RILs. All plants had a similar level of Chl/Car ratios (Figure 5D).

**Figure 4** A_s sat (A), g_s sat (B), and total CO_2 assimilation (C) of HS-RIL and HR-RIL after exposure to various durations of LED lighting for 27 days. Values illustrated are the means of four measurements. Vertical bars represent standard errors. Different letters above the bar graphs denote statistical differences (p < 0.05) as determined by Tukey's multiple comparison test.

**Figure 5** Total Chl (A) concentration, total Car (B) concentration, Chl a/b ratio (C), and Chl/Car ratio (D) of HS-RIL and HR-RIL after exposure to various durations of LED lighting for 27 days. Values illustrated are the means of four measurements. Vertical bars represent standard errors. Different letters above the bar graphs denote statistical differences (p < 0.05) as determined by Tukey's multiple comparison test.
Other than the quantity and quality of light, the length of the photoperiod is also an important factor in the regulation of many plant physiological processes (Scheibe et al., 2005). Unfortunately, research work on the influence of photoperiod on metabolic processes, such as photosynthesis, is lacking in the tropical greenhouse. In this study, $A_{sat}$ and $g_{s sat}$ were higher in HR RIL than in HS RIL (Figures 4A, 4B). For each RIL, the values of these parameters were similar regardless of the photoperiod. However, as the TLA (i.e. photosynthetic area) and leaf number increase (Figures 3C, 3D), higher amounts of carbon was fixed per plant (Figure 4C). For instance, total CO$_2$ assimilation of HR-RIL grown under 18 h photoperiod was greater by more than two-fold than those grown under 12 h photoperiod (Figure 4C), despite similar $A_{sat}$ and $g_{s sat}$ measurements. Brazaitité et al. (2009) reported that, under LED lighting, higher content of photosynthetic pigment resulted in higher rates of photosynthesis of plants. In this study, total Chl and Car concentrations were significantly lower ($p <0.05$) in HR-RIL grown under 18 h photoperiod compared to those grown under shorter photoperiods. Lower Chl and Car concentrations could be due to the dilution of photosynthetic pigments, as a result of larger TLA (Figure 3C). Further, it is interesting to note that Chl a/b ratios were significantly higher under longer photoperiods of 16 and 18 h LED lighting, compared to 12 h photoperiod for both RILs (Figure 5C). This implies that Chl a, which absorbs violet-blue and orange-red light, was present in higher quantities in leaves of plants grown under longer photoperiods. Since Chl a/b ratios increase with increasing irradiance, at a given nitrogen availability (Kitajima and Hogan, 2003), the higher ratios obtained in this study could demonstrate that longer photoperiods are analogous to higher levels of light. As such, these plants seemed to have acclimatized and are already physiologically adapted to the various photoperiods within 27 DAT.

**Soluble and insoluble sugars**

Figure 6 shows the concentrations of soluble and insoluble sugars measured from leaves of both RILs that were harvested just before the end of the different photoperiods 20 DAT. HR-RIL had higher soluble sugar than that of HS-RIL regardless of photoperiod (Figure 6A). Both RILs had a similar level of insoluble sugar under 12 and 16 h photoperiods. However, insoluble sugar concentration was significantly lower in HR-RIL than in HS-RIL under 18 h photoperiod (Figure 6B) in the longer 18 h photoperiod. For both RILs, the 18 h photoperiod resulted in significant decreases of both soluble and insoluble sugar concentrations compared to those plants grown under 12 and 16 h of LED lighting.

![Figure 6](image_url) Soluble (A) and insoluble sugar (B) content of HS-RIL and HR-RIL after exposure to various durations of LED lighting for 27 days. Values illustrated are the means of four measurements. Vertical bars represent standard errors. Different letters above the bar graphs denote statistical differences ($p < 0.05$) as determined by Tukey's multiple comparison test.

When plants were exposed to longer photoperiods, high carbohydrate levels normally accumulate in the leaves (Boller and Nosberger, 1983). However, in this study, leaves subjected to 18 h LED lighting had lower amounts of soluble and insoluble sugars at
the end of the photoperiod (Figure 6), with HR-RIL exhibiting a greater difference. This could be attributed to transportation of carbohydrates to other parts of the plants, namely the shoot, for vegetative growth (Demers and Gosselin, 1998), since the increase in shoot growth was distinctly higher than that of the roots (Figures 2A, 2B), under the 18 h photoperiod. According to Sulpice et al. (2009), this negative correlation between shoot biomass and carbohydrate level resulted from the need to provide carbon for active plant growth. In the present study, when plants were exposed to a longer photoperiod of moderate PPFD of 300 µmol photon m⁻² s⁻¹, they were able to synthesize carbohydrates continuously at levels that could be efficiently and continuously transported to the active sinks. This resulted in the absence of feedback inhibition of photosynthetic end products on the photosynthetic process in the leaves, despite being subjected to a longer photoperiod (Zamiske and Schaffer, 1996), evident from the lower concentrations of both soluble and insoluble sugars at the end of the 18 h photoperiod. Over and above, lettuce plants exposed to longer photoperiods also had greater leaf numbers and larger TLA (Figure 2C, 2D) lending further support to the explanation that longer photoperiods promote the development of additional and larger sinks (Demers and Gosselin, 1998).

CONCLUSION

Although higher shoot and root productivities were observed in both HR-RIL and HS-RIL lettuce plants grown under longer photoperiods, we have found that $A_{sat}$ and $g_{s, sat}$ measured on 27 DAT were not the key drivers. Increased leaf numbers and TLA under longer photoperiods suggest that the development of additional and larger sinks were, instead, the main factors responsible for higher yield of the lettuce plants. Therefore, it is indeed possible to increase the yield of vegetable crops by extending the duration of moderate amounts of LED lighting, where photosynthetic products could be effectively transported out from the source leaves during the photoperiod.

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Literature Cited


