

# Quality of supplementary LED lighting effects on growth and photosynthesis of two different *Lactuca* recombinant inbred lines (RILs) grown in a tropical greenhouse

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## Abstract

LED lamps with various combinations of red (R) and blue (B) wavelengths were used to supplement sunlight for the growth of a heat-resistant (HR) and heat-sensitive (HS) recombinant inbred lines (RIL) of lettuce. The RB-LED ratios were 100R:0B (0B), 92R:8B (8B), 84R:16B (16B), and 76R:24B (24B) with an equal PPFD of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The greatest leaf expansion rates were observed at 8B for both genotypes. All HR-RILs had similar values of growth parameters and specific leaf area (SLA). However, higher values of growth parameters were observed in HS-RIL with 0B, 8B, and 16B than that under 24B and sunlight. Furthermore, HS-RIL had higher SLA under 0B compared to other conditions. Photosynthetic light-use efficiency and maximal oxygen evolution rate were the lowest under 8B for both genotypes. The quality of LED lighting, if provided, seemed to implicate genotype dependence, probably as a result of their different sensitivities to heat stress.

*Additional key words:* electron transport rate; leaf expansion; light- and CO<sub>2</sub>-saturated net photosynthetic rate; photochemical and nonphotochemical quenching; pigment; stomatal density.

## Introduction

Located near the Equator, warm temperatures throughout the year are typical of a tropical environment that allow for growth and production of vegetables all year round. However, in Singapore, plants are normally exposed to a few clear sunny days, followed by days of cloudy weather (He *et al.* 1996). In recent years, Singapore has been more frequently experiencing increasingly unpredictable environmental conditions of cloudy and hazy weather (Nobre *et al.* 2016). Since 1982, Singapore has been experiencing hazy weather almost annually due to large-scale forest fires that occur in the neighbouring countries. This is a concern as the resulting lowered light intensity compromised the productivity of crop plants (Jones 2006), resulting in longer growing periods. For instance, He *et al.* (2011a) reported that in Singapore, when lettuce plants were grown under low light during the haze episodes or under simulated haze conditions in the greenhouse, lower

photosynthetic rate and stomatal conductance ( $g_s$ ) were measured. Furthermore, plant productivity was lower with an increase in a nitrate concentration when compared to plants grown under higher light. Similar results were obtained with *Brassica alboglabra* (Chinese broccoli) (He *et al.* 2015a). In order to circumvent the problem of insufficient sunlight for cultivation of vegetable crops, this research implements the use of light-emitting diode (LED) light to supplement low sunlight intensity, whilst optimizing the use of existing natural irradiance.

With their high photoelectric conversion efficiencies, low thermal output and narrow band spectra (Yeh and Chung 2009), LED lamps have recently been preferred for use in crop production. As such, LEDs, which have the ability to enhance plant growth and photomorphogenesis, can be used to irradiate plants (Yorio *et al.* 2001, Watanabe 2011, Goto 2012, He *et al.* 2015b, Hernández and Kubota

Received 9 June 2017, accepted 7 February 2018, published as online-first 21 June 2018.

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*Abbreviations:* B – blue; Car – carotenoids; Chl – chlorophyll; DM – dry mass; ETR – electron transport rate; FM – fresh mass; HR – heat-resistant; HS – heat-sensitive; LED – light emitting diode; NPQ – nonphotochemical quenching;  $P_{Nmax}$  – light- and CO<sub>2</sub>-saturated net photosynthetic rate;  $q_p$  – photochemical quenching; R – red; RIL – recombinant inbred lines; SD – stomatal density; SL – sunlight; SLA – specific leaf area.

*Acknowledgement:* This project was funded by Singapore Millennium Foundation, Singapore (SMF-Farming System), and Monetary Academic Resources for Research, Nanyang Technological University, Singapore. We thank the Michelmor Lab at the UC Davis Genome Centre for providing us with the RIL seeds.

2016, Wang *et al.* 2016). Sager and McFarlane (1997) have previously used McCree's curve to explain how the use of R wavelengths (600–700 nm), being so close to the absorption peak of chlorophyll (Chl), can most efficiently power photosynthesis as they are maximally absorbed by plant pigments and phytochromes (Muneer *et al.* 2014). In comparison, B wavelengths of light perform photomorphogenic roles, such as phototropism (Blaauw and Blaauw-Jansen 1970), affecting water relations and carbon dioxide exchange, stem elongation (Cosgrove 1981), and stomatal control (Schwartz and Zeiger 1984, Hernández and Kubota 2016, Wang *et al.* 2016). Thus, using peak wavelengths of B (460 nm) and R (660 nm) quality, matching the active spectra of the plant's photoreaction system, Johkan *et al.* (2010) successfully cultivated red leaf lettuce. Using 50R:50B LED irradiation, with PPFD at  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the leaf shape and colour of the lettuce plants were similar to those grown under fluorescent light. Studies have reported that higher plant fresh mass (FMs) were obtained when grown with B light (Yorio *et al.* 2001, Matsuda *et al.* 2008). Bula *et al.* (1991) and Hoenecke *et al.* (1992) also found that insufficient intensity of B wavelengths of light resulted in elongation of leaf stems and internodes.

It was also reported that the optimal combination of R and B light is species-dependent. For instance, R/B light ratios were 7/3 in strawberry plantlet (Nhut *et al.* 2003) and 1/3 in rapeseed plantlets *in vitro* (Li *et al.* 2013),

## Materials and methods

**Plant materials and culture methods:** The *Lactuca* RILs were obtained from crossing *L. sativa* L. 'Salinas' and *L. serriola* accession UC96US23 (Argyris *et al.* 2005). The seeds of previously identified HR- and HS-RILs (Choong *et al.* 2013) were germinated on moist filter paper in a petri dish, in the laboratory. Insertion of seedlings into polyurethane cubes, soaked in water, was carried out five days after germination. These seedlings were left to acclimatize to ambient tropical greenhouse conditions for 7 d before being transplanted into the aeroponic system (Lee 1993). The shoots of plants were exposed to 100% prevailing irradiances at a maximum PPFD of  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  on sunny days, with fluctuating ambient temperatures of 28–38°C and relative humidity of 65–95%. The roots were misted with full strength Netherlands Standard Composition (Douglas 1985) nutrient solution (EC 2.2 mS, pH 6.5), for 30 s between 5 min intervals and root-zone temperatures were kept at  $25 \pm 3^\circ\text{C}$  for the entire period of plant growth.

**Supplementary LED-irradiance treatments:** Plants were either grown under only natural sunlight (SL) or supplemented with one of the four RB-LED combinations for a continuous 10-h photoperiod (*i.e.*, 08:00–18:00 h) at mean PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  from the day of transplantation. The RB-LED lighting combinations were

respectively. However, it was 9/1 for *Arabidopsis* (Ooi *et al.* 2016), cucumber seedlings (Hernández and Kubota 2016), lettuce (Yorio *et al.* 2001, Stutte *et al.* 2009), and spinach (Yorio *et al.* 2001). As such, appropriate spectral quality needs to be identified for optimal plant growth as the optimal wavelengths seemed to be crop-specific (Hanyu and Shoji 2002, Massa *et al.* 2008, He *et al.* 2015b).

RB-LED lamps have a great potential as a light source to drive photosynthesis. Since different vegetable crops respond distinctly to RB light combinations (Yorio *et al.* 2001, Nhut *et al.* 2003, Stutte *et al.* 2009, Li *et al.* 2013, Hernández and Kubota 2016, Wang *et al.* 2016), this research studied this effect on two different thermotolerant *Lactuca* RILs previously selected (Choong *et al.* 2013). Using HR-RIL and HS-RIL, our team has reported that shoot and root FM of HR-RIL were significantly higher than that of HS RILs that were grown in the tropical greenhouse under ambient conditions due to their lower photoinhibition of PSII reaction center during midday (He *et al.* 2013). Recently, we have confirmed further that HR-RIL grown in the tropical greenhouse showed lesser photoinhibition as compared to HS-RIL (Lai and He 2016). Thus, we hypothesised that the two different genotypes, HR-RIL and HS-RIL, may exhibit different responses to supplemental light quality when they were grown in the tropical greenhouse. Here we reported results of the influence of supplemental light quality on two different thermotolerant lettuce RIL lines.

100R:0B (0B), 92R:8B (8B), 84R:16B (16B), and 76R:24B (24B). The spectral flux density of the various light treatments is shown in Fig. 1.

**Growth parameters:** Three or more plants of each genotype were harvested 28 days after transplant. Plant height was measured, leaf numbers were counted, and shoot and

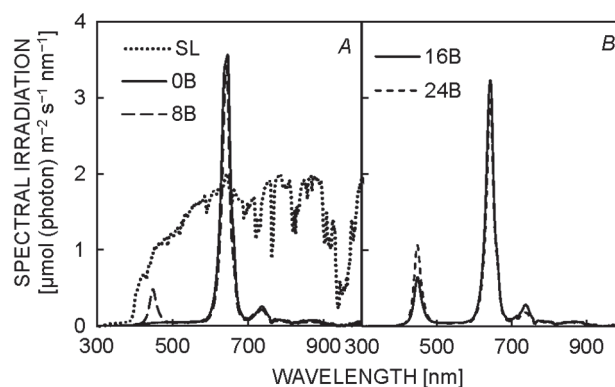


Fig. 1. Light spectral measurements with a spectroradiometer (PS300, Apogee Instruments, USA). Lettuce plants were grown under natural sunlight (SL) and four different supplementary RB-LED light combinations (0B, 8B, 16B, 24B), with PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ . A: SL, 0B, and 8B; B: 16B and 24B.

root FM were weighed after harvest. The shoot/root ratios were calculated using their FMs. Images of all leaves were captured and total leaf area was analyzed using *WinDIAS 3* image analysis system (*Delta-T Device*, UK). SLA was calculated by dividing the area of ten one-cm-diameter leaf discs with their dry mass, after drying for 5 d at 65°C in an oven.

**Leaf expansion:** Young leaves were selected from at least four plants of each genotype and treatments. The images of the selected leaves were captured for four consecutive days, starting from 11 d after transplantation. Their areas were then analyzed using the *WinDIAS 3* software (*Delta-T Device*, Led, UK) (Tay *et al.* 2015).

**Stomatal density (SD):** An epidermal impression was obtained by coating the abaxial leaf surface with clear nail varnish. The dried nail varnish was peeled off and adhered to a microscope slide using clear sticky tape. The peel was viewed under the microscope at  $\times 400$  magnification. Three different fields of view were counted per peel and at least two peels were obtained per genotype and light treatment. The diameter of the field of view was measured using a ruler and the area calculated. SD was then calculated as the number of stomata per square millimeter.

**Light- and CO<sub>2</sub>-saturated net photosynthetic rate ( $P_{Nmax}$ ):** Newly mature lettuce leaves were obtained, using an oxygen electrode system (*Model LD2*, *Hansatech Ltd.*, King Lynn, England) at 25°C (Ball *et al.* 1987), 24 d after transplantation. The leaves were harvested at about 09:00 h local time (*i.e.*, within 2 h after sunrise) from at least three different plants per genotype and light treat-

ment. A 10-cm<sup>2</sup> leaf disc was cut from the leaf and placed at a chamber containing saturated CO<sub>2</sub> (1% CO<sub>2</sub> from a 1 mol L<sup>-1</sup> carbohydrate/bicarbonate buffer, pH 9). Photosynthetic O<sub>2</sub> evolution rates were measured under PPFDs of 1,000; 1,200; 1,500; and 1,900  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . It was found that a PPFD 1,500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  saturated both HR-RIL and HS-RIL leaf photosynthesis. The leaf disc was illuminated with PPFD of 1,500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  till a steady state of photosynthetic O<sub>2</sub> evolution rate was obtained.

**Photosynthetic pigments:** Four one-cm-diameter leaf discs were obtained from newly expanded leaves of three different plants. The leaf discs were left for 48 h in the dark, at 4°C, in 1.5 ml N,N-dimethylformamide. Absorption of four replicates was read at 480, 647, and 664 nm, using a spectrophotometer (*UV-2550*, *Shimadzu*, Japan) and Chl *a*, Chl *b*, total Chl, and carotenoids (Car) concentrations were calculated using the Wellburn (1994) method.

**Photochemical light-use efficiency:** Leaves were harvested at 09:00 h for Chl fluorescence analysis, where photochemical quenching ( $q_p$ ), nonphotochemical quenching (NPQ), and electron transport rate (ETR) of four detached newly expanded leaves from four different plants were measured at 25°C in the laboratory, using the *Imaging-PAM* Chl fluorometer (*Walz*, *Effeltrich*, Germany) (He *et al.* 2011b).

**Statistical analysis:** A mixed-model nested analysis of variance (*ANOVA*) was performed using *SPSS (Version 20, 2011)* to test for significant effects of variation between genotypes and their response to light treatments, using post-hoc *Tukey's* pairwise test, with significance at  $\alpha = 0.05$ .

## Results

**Leaf expansion:** HR-RIL demonstrated the fastest leaf expansion rate under 8B, followed by 16B and 24B, LED-lighting treatment (Fig. 2A). Leaf expansion under SL and 0B were similar but distinctly lower (Fig. 2A). The highest leaf expansion rate in HS-RIL was observed under 8B followed by 16B. HS-RIL had similar lower leaf expansion rates under SL, 0B, and 24B conditions (Fig. 2B).

**Plant height, leaf number and productivity:** In terms of other productivity parameters, such as plant height (Fig. 3A), leaf number (Fig. 3C), shoot FM (Fig. 3E), and root FM (Fig. 3G), all values were quite similar across the different RB-LED combinations for HR-RIL. On the other hand, plant height (Fig. 3B), shoot and root FMs (Fig. 3F,H) were lower under SL and 24B than the rest of the

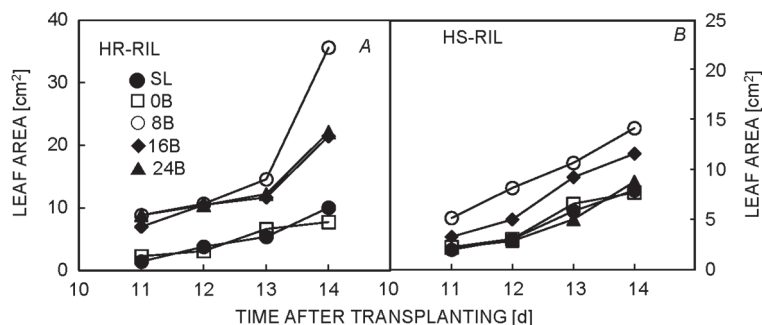


Fig. 2. Leaf expansion of HR-RIL (A) and HS-RIL (B). Lettuce plants were grown under SL and four different supplementary RB-LED light combinations (0B, 8B, 16B, 24B), with PPFD of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Each value is the mean of at least three different plants ( $n \geq 3$ ).

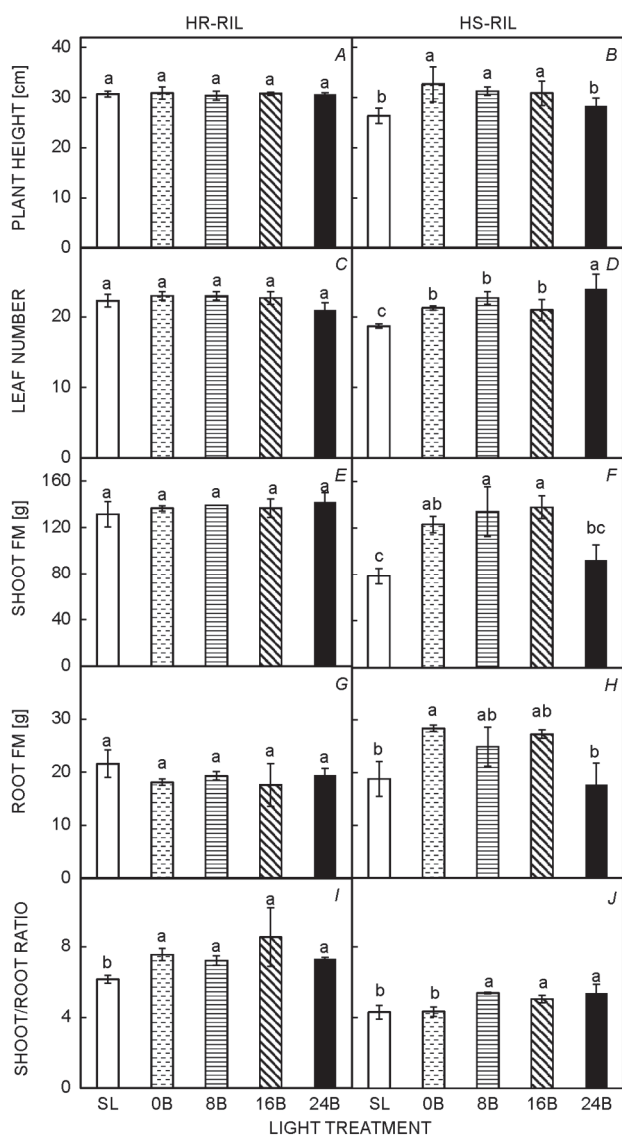


Fig. 3. Plant height (A,B), leaf number (C,D), shoot FM (E,F), root FM (G,H), shoot/root ratio (I,J) of HR-RIL (A,C,E,G,I) and HS-RIL (B,D,F,H,J) grown under SL and four different RB-LED light treatments (0B, 8B, 16B, 24B), with PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Parameters were measured 28 d after transplantation. Each value is the mean of at least three different plants ( $n \geq 3$ ). Means with different letters are statistically different ( $p < 0.05$ ;  $n \geq 3$ ) as determined by Tukey's multiple comparison test.

RB-LED-light treatments for HS-RIL. HS-RIL had the lowest leaf number when grown under SL and the greatest leaf number under 24B-light treatment (Fig. 3D). The shoot/root ratio of HR-RIL was lower under SL compared to all other RB-LED treatment. However, there was no significant difference in shoot/root ratios for HR-RIL between the different RB-LED treatments (Fig. 3I). Shoot/root ratios were similarly lower for HS-RIL under SL and 0B but much higher under 8B, 16B, and 24B (Fig. 3J).

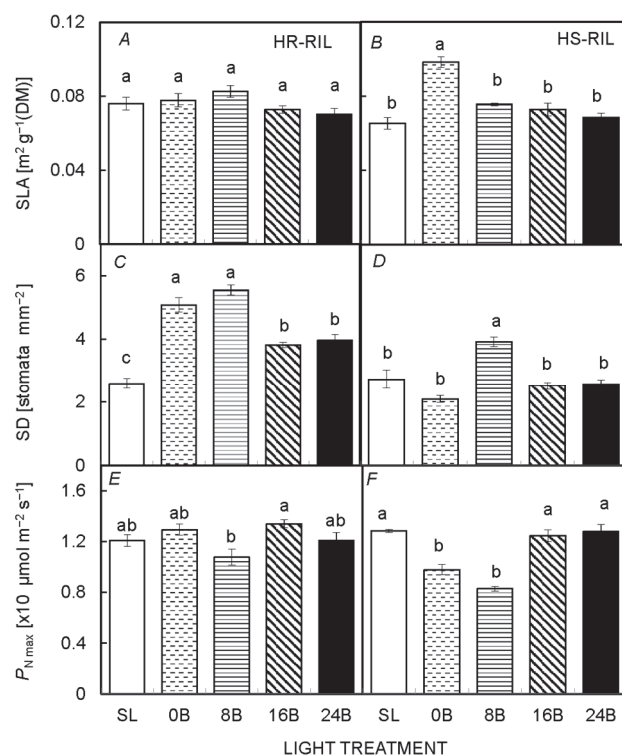


Fig. 4. SLA (A,B), SD (C,D), and  $P_{Nmax}$  (E,F) of HR-RIL (A,C,E) and HS-RIL (B,D,F) grown under natural SL and four different supplementary RB-LED light combinations (0B, 8B, 16B, 24B), with PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Measurements were made 28 days after transplant. Each bar graph is the mean of at least three different plants ( $n \geq 3$ ). Vertical bars represent the standard errors. Means with different letters are statistically different ( $p < 0.05$ ;  $n \geq 3$ ) as determined by Tukey's multiple comparison test.

**SLA, SD and  $P_{Nmax}$ :** SLA values (Fig. 4A) were highly similar regardless of light treatments for HR-RIL. However, for HS-RIL, SLA was distinctly higher under 0B light (Fig. 4B). Significantly greater SD was observed under 0B and 8B light treatments for HR-RIL (Fig. 4C) whereas SD for HS-RIL was only significantly higher under 8B light treatment (Fig. 4D). The significantly highest  $P_{Nmax}$  was observed under 16B for HR-RIL (Fig. 4E), whereas they were significantly higher for all light treatments of SL, 16B and 24B for HS-RIL (Fig. 4F).

**Photosynthetic pigments,  $q_p$ , NPQ and ETR:** Compared to plants grown under SL, even though concentrations of both Chl *a* and Chl *b* (data not shown) and total Chl (Fig. 5C) were greater at 0B, 8B, 16B and 24B, for HR-RIL, the Chl *a/b* ratios were similar across all light treatments (Fig. 5A). For HS-RIL, Chl *a* and Chl *b* concentrations (data not shown) and total Chl were the lowest under SL, but the Chl *a/b* ratio was significantly the greatest one (Fig. 5B). Though total Chl contents was

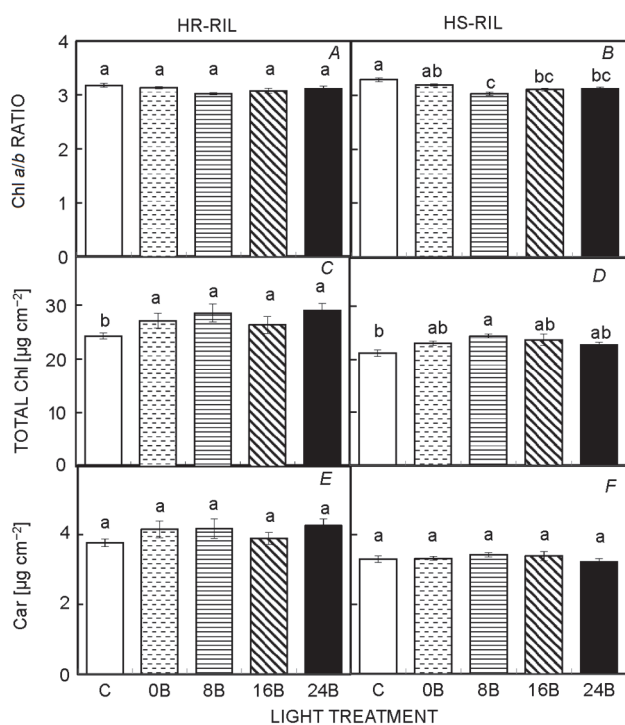


Fig. 5. Chl *a/b* ratio (A,B), total Chl content (C,D), and Car content (E,F) of HR-RIL (A,C,E) and HS-RIL (B,D,F) grown under natural SL and four different supplementary RB-LED light combinations (0B, 8B, 16B, 24B), with PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Each bar graph is the mean of at least three different plants ( $n \geq 3$ ). Vertical bars represent the standard errors. Means with different letters are statistically different ( $p < 0.05$ ;  $n \geq 3$ ) as determined by Tukey's multiple comparison test.

significantly lower under SL (Fig. 5D), for HS-RIL, the Car content was not significantly different under all light treatment (Fig. 5F). For HR-RIL, all plants also had the similar Car content (Fig. 5E). Fig. 6 shows the values of  $q_p$ , NPQ, and ETR measured under a PPFD of  $605 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The highest  $q_p$  and ETR were found at 24B-light treatment for both genotypes (Fig. 6A,B,E,F). The significantly lowest NPQ was observed at 24B-light treatment for both genotypes (Fig. 6C,D). Plants grown under 0B and 8B had lower  $q_p$  (Fig. 6A,B) and ETR (Fig. 6E,F) compared to other light treatments in both genotypes.

## Discussion

Over the past two decades, many studies have been carried out indoors to study application of LED lighting on vegetable production. It is well-known that light quality affects plant morphology and growth (Goins *et al.* 1997, Yorio *et al.* 2001, Matsuda *et al.* 2008, Cope and Bugbee 2013, Chang *et al.* 2016, Wang *et al.* 2016). Due to the regular cloudiness and several episodes of haze annually, most vegetables in Singapore are subjected to low greenhouse ambient light (He *et al.* 2011, 2015a). However, very little information is available on the supplementation of LED lighting to prevailing solar radiation in the tropical greenhouse for enhanced productivity of vegetable crops. This study used HR-RIL and HS-RIL of lettuce, which have different susceptibility to heat stress (Lai and He 2016) and photoinhibition (He *et al.* 2013) to study the impact of supplemental red- and blue-LED lighting on growth and photosynthesis in a tropical greenhouse in Singapore.

Studies have shown that the presence of B-LED lighting, in addition to R-LED lighting allows better and faster growth of plants (Yorio *et al.* 2001, Matsuda *et al.* 2008, Hogewoning *et al.* 2010, Darko *et al.* 2014, Muneer *et al.* 2014, He *et al.* 2015b, Wang *et al.* 2016, He *et al.* 2017). It can be observed that leaf expansion occurred at a distinctly higher rate of growth under 8B for both HR-RIL and HS-RIL (Fig. 2). This observation was also reported by Johkan *et al.* (2010) in red leaf lettuce, where larger leaf area was obtained at harvest under RB light quality. Supplementing only R light was ineffective as the leaf expansion rates for both genotypes were only equivalent to

that of plants growing under natural SL (Fig. 2). Evidence of crop specificity to quality of supplementary LED illumination can also be observed where leaf expansion at 24B-LED light treatment was similar to that at 16B, in HR-RIL (Fig. 2A), but in HS-RIL, they were more closely associated to SL and 0B instead (Fig. 2B). As such, HS-RIL exhibited higher sensitivity towards higher amounts of B light than HR-RIL. On the other hand, the leaf growth rate in HS-RIL (Figs. 2B) was much lower than that of HR-RIL. Similar results were also found in other HR-RIL and HS-RIL. For example, we found that optimal percentages of B light were different for different green and red leaf lettuce (*Lactuca sativa* 2326 and 2328) that have different sensitivities to heat stress and photoinhibition (unpublished data). Although there were some variations in the results due to the variations in greenhouse conditions and the optimal combination of R- and B-LED was species-dependent (Yorio *et al.* 2001, Nhut *et al.* 2003, Li *et al.* 2013, Hernández and Kubota 2016, Ooi *et al.* 2016), most experiments showed that supplemental LED lighting can increase crop productivity in periods of low light under any combination of R- and B-LED with B-LED fractions from 8 to 30%.

No significant differences between different light treatments could be detected amongst all growth traits for HR-RIL, suggesting that the intensity (*i.e.*, quantity) *per se* of supplementary RB lighting may impact the HR-RIL to a noteworthy extent (Fig. 3). HR-RIL had larger leaf area under 8B followed by 16B and 24B compared to SL and 0B (Fig. 2A). The PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  may be

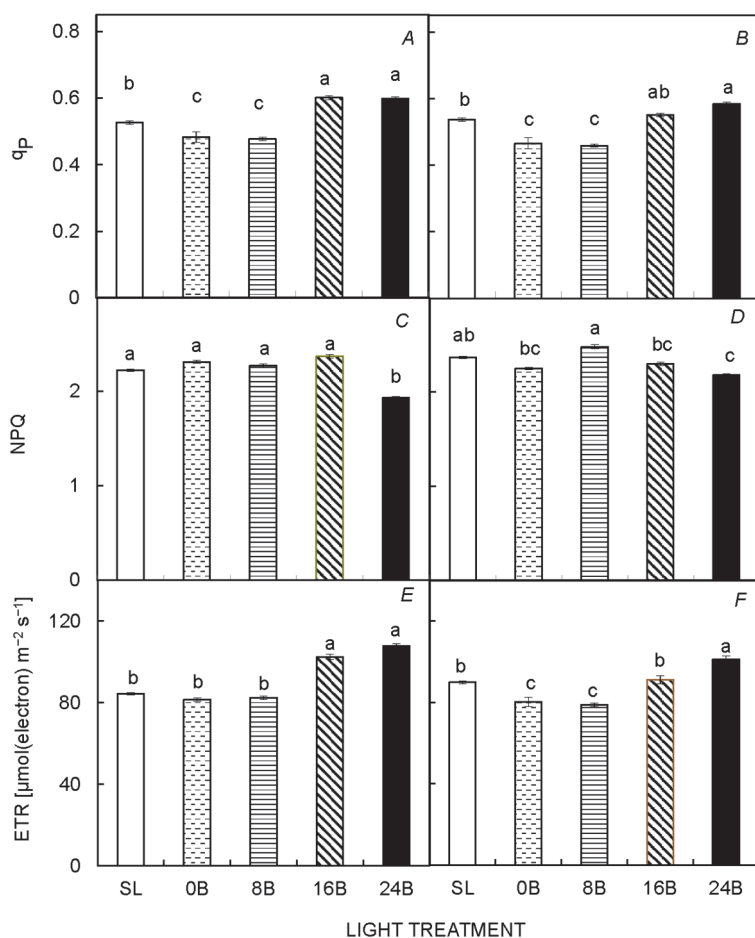


Fig. 6.  $q_p$  (A,B), NPQ (C,D), and ETR (E,F) of HR-RIL (A,C,E) and HS-RIL (B,D,F) grown under natural SL and four different supplementary RB-LED light combinations (0B, 8B, 16B, 24B), with PPFD of  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ . All measurements were made under a PPFD of  $605 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Each bar graph is the mean of at least three different plants ( $n \geq 3$ ). Vertical bars represent the standard errors. Means with different letters are statistically different ( $p < 0.05$ ;  $n \geq 3$ ) as determined by Tukey's multiple comparison test.

too low to cause any significant changes for HR-RIL. Christophe *et al.* (2006) reported that leaf appearance rate and petiole elongation of white clover (*Trifolium repens* L.) were strongly controlled not only by the changes of blue light portion but also its intensity. Larger leaf area normally captures more light to promote growth (Gifford *et al.* 1984, Koester *et al.* 2014). However, all HR-RIL plants had similar shoot FM (Fig. 3E) and leaf thickness measured by SLA (Fig. 4A) regardless of light treatments. In studies with *Arabidopsis*, the relationship between leaf area and plant biomass was found to be non-linear and variable depending on carbon partitioning (Weraduwage *et al.* 2015). The relationship between leaf area growth and growth of lettuce in terms of total FM may depend on how newly fixed carbon is partitioned among new leaf area, leaf mass, stem mass and root mass. In the present study, we did not separate the leaf mass and stem mass. Those plants with lower leaf growth could channel more biomass to their stem. The shoot/root ratios (Fig. 3J) of HR-RIL, were the lowest under SL and greatest under 16B. However, there was no significant difference in shoot FMs amongst the light treatments. The outstanding shoot/root ratio at 16B light treatment could be an indicator of the optimal R/B ratio for supplementary light treatment for HR-RIL.

Another crucial role of light quality in regulating plant growth is through leaf and stomatal development. Plants grown under B light supplemental to R light environment increased their SD resulting in greater photosynthetic capacity than those grown under B light-limited conditions (Hogewoning *et al.* 2010, Chang *et al.* 2016, Wang *et al.* 2016). In this study, all HR-RIL plants had similar shoot FM (Fig. 3E), but under 0B and 8B light treatments, SD values of HR-RIL were higher compared to other light treatment (Fig. 4C). Leaves grown under different light qualities involved changes in SD which could have contributed to differences in  $g_s$  (Schoch *et al.* 1980, Hogewoning *et al.* 2010, Wang *et al.* 2016). Hogewoning *et al.* (2010) reported that  $g_s$  and light-saturated  $\text{CO}_2$  assimilation rate ( $P_{\text{max}}$ ) of cucumber (*Cucumis sativus*) significantly increased with increasing blue light percentage, resulting from increasing number of stomata on adaxial leaf surface. Although the total stomatal pore area only takes up about 3% of the leaf surface (Willmer and Fricker 1996), the exchange of  $\text{CO}_2$  correlates well with SD (Tanaka *et al.* 2013). This is because stomata are essential for the uptake of  $\text{CO}_2$ , but not for  $\text{O}_2$ . Thus, it is difficult to correlate  $\text{O}_2$  exchange with SD due to the concentration gradient between the atmosphere and inside

the leaf for O<sub>2</sub> is much greater than the gradient for CO<sub>2</sub>. However, in studies with lettuce, Wang *et al.* (2016) found that both  $P_{\max}$  and  $P_{N\max}$  increased with increasing B/R light ratio until 1, associated with increased  $g_s$  and SD, but there was no positive correlation between photosynthesis and shoot dry mass accumulation. It was reported that the SD of the abaxial and adaxial surfaces of rapeseed leaves (*Brassic napus* L. cv. "Zhongshuang11) increased with increasing B/R light ratio from 0 to 100% but with the highest  $P_{N\max}$  under 25% of B light (Chang *et al.* 2016). In the present study, although we only measured  $P_{N\max}$ , not  $g_s$  and  $P_{\max}$ , our results supported the fact that an increase in SD does not always enhance photosynthesis, growth, and biomass accumulation (Kim *et al.* 2004, Chang *et al.* 2016, Wang *et al.* 2016). The effects of light quality on SD and the relationship between SD and  $g_s$  and photosynthetic capacities are species-specific. Although SD was lower at 16B and 24B (Fig. 4C), 16B-light treatment under which plants had higher photosynthetic rate, could indeed be the most optimal light quality for growing HR-RIL despite the lack of distinctive shoot and root FMs, most probably due to the low quantity of supplementary LED lighting.

HS-RIL grown under SL and 24B were shorter (Fig. 3B). Since the leaf number, and shoot and root FMs were also similarly lower under SL than the rest of the light treatments, it could be due to these plants being exposed to lower light intensity as a result of the lack of supplementary lighting that resulted in smaller plants. As for HS-RIL at 24B (Fig. 3), lower shoot and root FMs could be due to the shorter plants. High blue light has been shown to reduce plant height (Islam *et al.* 2012, Nanya *et al.* 2012). It is interesting to note that the shoot/root ratio was greater after 8B, 16B, and 24B light treatments (Fig. 3I) for HS-RIL, despite the significantly lower shoot FM found at 24B, than at 8B. 8B had significantly higher SD than that of 24B (Fig. 3D), though the  $P_{N\max}$  was significantly lower at 8B than 24B. Cumulatively, all these growth parameters (Fig. 3) demonstrate that HR-RIL grew better under 16B whilst there is no distinctive evidence to demonstrate which light treatment was more optimal for HS-RIL. As such, it is noteworthy to recognize that they behaved differently under different qualities of supplementary light, despite their high genetic similarities. This result suggests that responses of HR-RIL and HS-RIL to supplementary LED light quality may also depend on other abiotic factors such as temperature, light intensity in the greenhouse. The responses of the two different lettuce RILs to these abiotic factors may be different.

It has been reported that B light effects on plant and photosynthetic performance could be due to its influence on photosynthetic pigments (Senger and Bauer 1987, López-Juez and Hughes 1995, Wang *et al.* 2016). However, in the present study, all photosynthetic pigments were highly similar across the light treatments, with the exception of lower total Chl content in both genotypes grown under SL, but higher Chl *a/b* ratios in HS-RIL grown under SL (Fig. 5). Wang *et al.* (2016) reported that

lettuce grown under a mixture of R and B light or B light had a higher Chl *a/b* compared to red-light treatment (Wang *et al.* 2016). All LED-light treatments including sole R light resulted in higher total Chl content for both genotypes. B light deficiency that was adverse to Chl biosynthesis has been reported in wheat (Tripathy and Brown 1995), spinach (Matsuda *et al.* 2008), and cucumber seedling (Hogewoning 2010, Hernández and Kubota 2016) and *Rosa × hybrida* (Terfa *et al.* 2013). In the present study, it seemed that B light from the background sunlight was sufficient for the synthesis of Chl. It was also reported that B light can be absorbed either by Chl or by Car for the formation of 'sun-type' chloroplasts (López-Juez and Hughes 1995). However, all plants had similar Car content (Fig. 5E,F).

When measured at a PPFD of 605  $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$ , which was the saturated light intensity for ETR according to the light-response curves for all the plants (data not shown),  $q_p$  and ETR values of HR-RIL grown under 16B and 24B were higher than those of plants grown under other lower level of B light or SL (Fig. 6A,E). These results indicate that plants under higher B light treatments utilized more light energy absorbed by Chl for photochemistry (Hemming 2011, Muneer *et al.* 2014, He *et al.* 2015b). Highest  $q_p$  and ETR, with correspondingly the highest and lowest NPQ (Fig. 6C), were found, respectively, at 16B and 24B light treatment for HR-RILs. Higher utilisation of light energy (*i.e.*, higher  $q_p$  and ETR) and higher capacity of heat dissipation (*i.e.*, lower NPQ) when grown in the tropical greenhouse (He *et al.* 2001, He and Lee 2004) further confirmed that 16B could be indeed the most optimal light quality for growing HR-RIL as mentioned earlier.

In a tropical greenhouse, plants cope with variation of light intensities daily. Excess absorbed light energy can be detrimental to PSII unless it is dissipated harmlessly in a process known as dynamic photoinhibition, which is a reversible down-regulation mechanism to reduce the light utilisation efficiency by diverting the excessive energy to the xanthophyll cycle so as to protect PSII reaction centers from photodamage (Demming-Adams and Adams III 1992, He *et al.* 2001, He and Lee 2004). The excess energy may cause damage to the PSII reaction center, leading to sustained photoinhibition or chronic photoinhibition (Barber 1995). Excessive light is usually associated with high temperature (He *et al.* 1996). Our previous study found that although dynamic photoinhibition measured by lower midday Chl fluorescence  $F_v/F_m$  ratio occurred in both genotypes with less dynamic photoinhibition in HR-RIL than in HS-RIL, chronic photoinhibition did not occur in either RIL (He *et al.* 2013). Similar results were also obtained in this study (data not shown). For HS-RIL, the highest  $q_p$  and ETR and the lowest NPQ were also found in plants grown under 24B. However, there were no significant differences in  $q_p$ , ETR, and NPQ between plants grown under 16B and SL. These results implied that the utilisation of light energy and capacity of heat

dissipation were different between HR-RIL and HS-RIL under different supplementary LED lightings.

**Conclusion:** The present study suggested that 16B supplementary light treatment is optimal for HR-RIL. However, with the high shoot FM and shoot/root ratio in HS-RIL at 8B, this seemed to be the optimal light treatment. Based on further examination of its leaf growth and photosynthetic parameters, there seemed to be some evidence to support

## References

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