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Nitrate accumulation, productivity and photosynthesis of *Brassica alboglabra* under low light with supplemental LED lighting in a tropical greenhouse

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ABSTRACT

In this project, all plants were grown hydroponically in full nutrients under prevailing greenhouse conditions for 20 days (full sunlight). Thereafter, plants were subjected to three different light treatments for 12 days: full sunlight, shade and shade supplemented with LEDs. The average midday photosynthetic photon flux density (PPFD) during the light treatment periods were 220 µmol m^{-2} s^{-1} (full sunlight), 55 µmol m^{-2} s^{-1} (shade) and 220 µmol m^{-2} s^{-1} (shade supplemented with LEDs). Shoot nitrate (NO_3^-) concentration increased significantly in plants grown in the shade. However, shoot NO_3^- concentration was reduced when plants were supplemented with red- and blue-LED lighting. Photosynthetic CO_2 assimilation, stomatal conductance and productivity also improved in these plants. Our results suggest that supplemental red- and blue-LED lighting in a tropical greenhouse during periods of cloudy and hazy weather could improve productivity and nutrient quality of Chinese broccoli.

Keywords: Hazy weather, LED lighting, NO_3^- concentration, Photosynthetic CO_2 assimilation, Productivity

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INTRODUCTION

Vegetables are the largest source of nitrate (NO_3^-) in our diet (Knight et al. 1987, Correia et al. 2010). The microflora in our oral cavity and enzymes in our digestive tract converts NO_3^- to nitrite (NO_2^-). As NO_2^- is toxic to humans (Correia et al. 2010, Zhou, Liu, and Yang 2013, Kmecl, Knap, and Žnidarčič 2017), it is undesirable to consume vegetables with high NO_3^- content. To reduce NO_3^- accumulation in vegetables, lesser NO_3^- could be supplied to the vegetables when they were subjected to low growth irradiance (Gruda 2005, Weightman et al. 2006). However, this practice may not be the most appropriate as plants require large amounts of NO_3^- and it is the only source of nitrogen (N) in hydroponic cultures in commercial farming (He 2010, Parks, Irving, and Milham 2012, Kmecl, Knap, and Žnidarčič 2017).

Although the warm climate allows for production of vegetables all year round in Singapore, vegetables grown in a tropical greenhouse have been more frequently experiencing increasingly unpredictable cloudy and hazy weather (Nobre et al. 2016). For instance, it was found that when lettuce plants were grown under low light during the haze episodes in the greenhouse, they had lower photosynthetic rate and stomatal conductance with higher shoot NO_3^- accumulation compared to those grown during sunny days (He, Cheok, and Qin 2011). Similar results were obtained with *Brassica alboglabra* (Chinese broccoli) (He, Lim, and Qin 2015.). Except for lettuce, higher NO_3^- accumulations has also been reported in other leafy vegetables such as cabbage, kale and spinach at low growth irradiances (Weightman et al. 2006, Parks, Irving, and Milham 2012, Kmecl, Knap, and Žnidarčič 2017).

Artificial light has been used by vegetable farmers, to enhance productivity and improve nutritional quality of vegetables for almost 150 years (Pfeiffer 1926). LED lighting is a preferred light source over fluorescent and high pressure sodium lamps (Massa et al. 2008) due to its low cost and durability. Further, growers can select specific wavelengths of light emitted by LED to enhance photosynthetic capacity and thus maximize productivity (Kim et al. 2004, He et al. 2017, Metallo et al. 2018). In a tropical greenhouse, our team has recently reported that higher shoot productivity and higher photosynthetic rates were observed in lettuce plants supplemented with different combinations of red- and blue-LEDs compared to those grown solely under prevailing sunlight (Choong et al. 2018). It was reported that NO_3^- accumulation can be reduced in lettuce under optimal combinations of red and blue light (Urbonavičiūtė et al. 2007) or red, blue, and white LEDs (Lin et al. 2013) or green, blue and red LEDs (Bain et al. 2018). Further, it was also observed decreased NO_3^- concentration in leafy vegetables when plants were subjected to short-term pre-harvest treatment of narrow-bandwidth red-LED (Samuolienė and Urbonavičiūtė 2009, Samuolienė et al. 2011). We have previously found that in a tropical greenhouse in Singapore, aeroponically grown Chinese broccoli (*B. alboglabra)* subjected to low light (cloudy days or haze) had the higher NO_3^- accumulation in the shoot compared to those grown under high light. Low light also had an inverse effect on total reduced N content (He, Lim, and Qin 2015).

NO_3^- incorporation into reduced N compounds involves the reduction of NO_3^- to NO_2^- via the enzyme nitrate reductase (Foyer et al. 1998, Cookson,Williams, and Miller 2005, Bian et al. 2018). The accumulation of NO_3^- is closely related to the rate of NO_3^- assimilation in plants which depends on the light intensity (He 2010, He, Cheok, and Qin

2011). It was previously reported by our team (He, Lim, and Qin 2015) that after re-exposing the Chinese broccoli to another 10 days of full sunlight, the low-light grown vegetables demonstrated their ability to recover their photosynthetic rate, enhance productivity and reduce the NO_3^- concentration. However, there are very few researchers have studied the effects of supplemental LED lighting on *B. alboglabra*, a popular Chinese vegetable (Isabelle et al. 2010). Therefore, this study aimed to investigate the effect of supplementing of red- and blue-LED lighting to *B. alboglabra* grown in the tropical greenhouse in terms of moderating NO_3^- accumulation, improving photosynthesis and enhancing productivity.

MATERIALS AND METHODS

Plant materials and culture methods

Three days after germination, the *B. alboglabra* seedlings were inserted into polyurethane cubes soaked with water in trays. After allowing seedling establishment for 7 days, they were transplanted into hydroponic troughs. The nutrient solution used was based on full strength Netherlands Standard Composition. Solution pH was maintained at 6.5±0.2 and electrical conductivity of 2.2±0.2 mS cm^{-1}. The composition of full strength nutrient solution in mg l^{-1} was: K_2HPO_4, 187; $Ca(NO_3)_2.4H_2O$, 1237; $MgSO_4.7H_2O$, 609; K_2SO_4, 252; KNO_3, 293; FeEDTA, 20.52; $ZnSO_4.7H_2O$, 0.06; $CuSO_4.5H_2O$, 0.06; H_3BO_3, 0.59; $MnSO_4.H_2O$, 0.73; and $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.75. The plants were firstly grown under prevailing greenhouse conditions with average maximum midday photosynthetic photon flux density (PPFD) around 350 µmol m^{-2} s^{-1} for 20 days, and then subjected to three different light treatments described below. Ambient temperatures in the

greenhouse ranged from 26–36°C and relative humidity was between 65–95% during the experimental period.

Light treatments

The plants were separated into three groups and grown for another 12 days under different light treatments, namely prevailing sunlight, shade and shade supplemented with blue- and red-LED (shade + LED). Spectra of the three light treatments are shown in Figure 1. Black netting was used to provide shading. The supplementary LED treatment was applied from 07:00 h to 19:00 h. During the 12-day treatment period, PPFDs were measured at canopy level once every two days during midday. Average midday PPFDs were 220 (prevailing sunlight), 55 (shade) and 240 μmol m^{-2} s^{-1} (shade + LED). Natural haze was encountered after light treatment commenced when the PPFD levels were lower inside the greenhosue with average midday PPFD of 220 μmol m^{-2} s^{-1} compared to those (average midday PPFD of 350 μmol m^{-2} s^{-1}) before treatments. From 8 days after treatment, the natural haze reached high level where plants were respectively subjected to average midday PPFD of 90 (full sunlight), 30 (shade) and 200 μmol m^{-2} s^{-1} (shade + LED).

Measurements of shoot and root fresh weight (FW) and dry weight (DW)

After harvest, the plants were separated into leaf blades, petioles, stems and roots, respectively. The FW of the different plant parts were weighed separately. After taking the FW, all the tissues were wrapped with aluminum foil and dried at 80°C for 4 days to obtain DW.

Measurements of light-saturated CO_2 assimilation rate (A_{sat}) and light-saturated stomatal conductance ($g_{s\ sat}$)

A_{sat} and $g_{s\ sat}$ of attached fully expanded leaves (the 6th - 7th leaves from the base) were measured simultaneously every three days between 09:00 h to 11:00 h in the greenhouse from the intact plants using an open infrared gas analysis system with a 6-cm^2 chamber (LI-6400, Biosciences, U.S.). Readings were measured with a supplied LED light source at PPFD of 1000 µmol m^{-2} s^{-1}. The light source emitted in the wavelength ranged between 420 to 510 nm and 610 nm to 730 nm. The spectral output of the light source has one peak centred at about 465 nm and second peak centred at about 670 nm. Average ambient [CO_2] and relative humidity in the chamber were 410 ± 10 µmol·mol^{-1} and 70% respectively. Measurements were recorded when A_{sat}, and $g_{s\ sat}$ were stable. For each treatment, four measurements were made from four different leaves of four different plants (n = 4).

Determination of photosynthetic pigments

Fresh leaf samples (0.05g) were weighed and incubated in 5 ml of N,N-dimethylformamide in the dark for 48 h at 4 °C. The absorption of Chlorophyll (Chl) a, Chl b and carotenoids (Car) were measured at 647 nm, 664 nm and 480 nm respectively using spectrophotometer (UV-2550 Shimadzu, Japan). Chl and Car contents were calculated according to the method of Wellburn (1994).

Determination of NO_3^- concentration

Dried plant tissue was ground with deionised water and then incubated at 37°C for 2 h. Sample turbidity was removed by filtration through a 0.45 µm pore diameter membrane filter prior to analysis. NO_3^- was determined using a Flow Injection Analyser

(Model QuikChem 8000, Lachat Instruments Inc, Milwaukee, WI, USA). The principle of this method was to catalytically reduce NO_3^- to NO_2^- and measure the amount of NO_2^- present by a calorimetric reaction. NO_3^- is quantitatively reduced to NO_2^- by passage of the sample through a copperized cadmium column. The NO_2^- is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to analyze different variables crossed with different light treatments. Means were discriminated using Tukey's multiple comparison test when main effect ANOVA tests were significant. Statistical analyses were carried out using MINITAB software (Minitab, Inc., Release 16, 2010).

RESULTS

Productivity

For all plants, shoot FW increased for all treatments during the 12-day treatment period. The increase was greater under prevailing full sunlight and shade + LED treatments, but less under shade condition. At harvest, plants grown under shade + LED had the highest shoot and root FW while those grown under shade had the lowest shoot and root productivity (Figures 2A and 2B). Shoot/root ratio of plants subjected to shade was 1.7 and 2.3 times higher than those of plants exposed to prevailing full sunlight and plants subjected to shade + LED, respectively (Figure 2C). Similar trends were observed for DW of shoot, root and shoot/root DW ratio (data not shown).

A_{sat} and $g_{s\ sat}$

A_{sat} and $g_{s\ sat}$ of *B. alboglabra* plants grown under shade decreased from the start of light treatment (Figure 3). Plants grown under full sunlight and shade + LED had higher A_{sat} and $g_{s\ sat}$ compared to those grown under shade conditions on any given day. The values of A_{sat} and $g_{s\ sat}$ did not show significant difference between plants grown under full sunlight and shade + LED during the first nine days of treatment. At the end of treatment, plants grown under shade + LED had the highest values of A_{sat} and $g_{s\ sat}$ while plants grown in the shade had the lowest values of the parameters. Also, shade + LED plants had 1.3 times higher of A_{sat} (Figure 3A) and 2.8 times higher of $g_{s\ sat}$ (Figure 3B) than those of full sunlight plants.

Photosynthetic Pigments

Total Chl and Car contents, and Chl a/b ratio of *B. alboglabra* plants grown under shade decreased from the start of light treatment while total Car/Chl ratio remained constant during the treatment (Figure 4). Compared to shade grown plants, full sunlight grown plants had higher total Chl content (Figure 4A), total Car content (Figure 4B) and Chl a/b ratio (Figure 4C) but lower total Car/Chl ratio (Figure 4D) after six days of treatments. Total Chl, Car contents, Chl a/b ratio and Car/Chl ratio did not show significant difference between shade + LED grown plants and full sunlight grown plants during the first nine days of light treatments (Figure 4). At the end of treatments, there were no significant differences in total Chl and Car contents between shade + LED grown plants and shade grown plants (Figures 4A, 4B). However, shade + LED grown plants had higher Chl a/b ratio but lower Car/Chl ratio compared to those of shade grown plants (Figures 4C, 4D).

NO_3^- concentrations

NO_3^- concentrations of shoot, leaf, and petiole and stem for shade grown plants increased gradually during the 12-day treatment period and they were significantly higher after 12 days of treatments compared to those of full sunlight and shade + LED grown plants (Figures 5A, 5B, 5C). For full sunlight and shade + LED grown plants, NO_3^- concentration in all tissues remained constant. Compared to shade grown plants, NO_3^- concentrations were significantly lower in all aerial parts of full sunlight and shade + LED grown plants. No significant difference observed for root NO_3^- concentrations of all plants under different light treatments (Figure 5D).

DISCUSSION

In the present study, red- and blue-LED supplemented to plants grown under the shade enhanced shoot (Figure 2A) and root productivity (Figure 2B). Shade plants had lower shoot and root FWs and also less photoassimilate partitioned to the roots with higher shoot/root ratio FW compared those of plants grown under prevailing full sunlight and shade + LED (Figure 2C). Similar trends were observed from studies of partitioning patterns of shade-grown grapevine leaves compared to sun-grown grapevine leaves (Heuvel et al. 2002). Synthesis of sucrose in the cytosol for the phloem transport from the leaves to the roots requires energy and carbon sources, which can be affected by many environmental factors (Lemoine et al. 2013). However, these are limited at low light conditions due to the decreased A_{sat} (Figure 3A). During the experimental period, hazy weather occurred and reached high level from day 8 of light treatment. This contributed greatly to the decreases in A_{sat} and $g_{s\ sat}$ for plants grown under prevailing sunlight after

12 days of light treatment. However, supplementing red- and blue-LED to plants grown under shade enhanced A_{sat} (Figure 3A) and $g_{s\ sat}$ (Figure 3B). Similar results were also found in lettuce (Grude 2005, Choong et al. 2018) and spinach studies (Gruda 2005) where supplemental red and blue light were provided. Red and blue lights absorbed by plants allow for efficient photosynthetic performance while absorbed blue light contributes to additional stomatal opening (Kim et al. 2004, Li and Kubota 2009, He, Lim, and Qin 2015, He et al. 2017). It was reported that shaded plants, supplemented with LED, may result in higher photosynthetic electron transfer rate (Phyo and Chung 2013, Choong et al. 2018). Blue light also increased photosynthetic light use efficiency (Hogewoning et al. 2010, He, Lim, and Qin 2015, He et al. 2017, Choong et al. 2018). In this study, A_{sat} and $g_{s\ sat}$ of plants grown in shade + LED were highest and least affected by the hazy weather that was encountered since day 8 of light treatment (Figure 3). In this study, supplementary red- and blue LED lighting treatments changed not only light quantity but also light quality. Plants grown under blue light rich environment had increased stomatal conductance that resulted in greater net photosynthetic rate than those grown under blue light limited conditions (Hogewoning et al. 2010, He, Lim, and Qin 2015, Wang et al. 2016, He et al. 2017).

Blue light also regulated Chl synthesis (Mizuno, Amaki, and Watanabe 2011, He et al. 2017). Compared to shade grown plants, shade +LED and full sunlight grown plants had higher total Chl (Figure 4A) and Car (Figure 4B) contents, and higher Chl a/b ratio (Figure 4C) but slightly lower total Car/Chl ratio (Figure 4D) after 9 days of light treatments. Since green light enhances Chl and Car synthesis (Urbonavičiūtė et al. 2007), plants exposed to natural sunlight might have more Chl and Car than other plants because

1 they received more green light (Figure 1). In the study with spring barley plants
2 (*Hordeum vulgare* L. cv. Bonus), Materová et al. (2017) reported that green light
3 stimulated synthesis of Chl a. They also found that plants cultivated under green light
4 drastically increased concentration of geranylgeranyl reductase which is responsible for
5 the reduction of double bonds in the Chl synthesis pathway.

6 Vegetables are great sources of vitamins and minerals which are the essential foods
7 in healthy diet (Kmecl, Knap, and Žnidarčič 2017, Zhang et al. 2017). However,
8 vegetables also contain high level of hazard materials such as NO_3^- in the editable parts
9 (Colla et al. 2018, Zhang et al. 2018). Although clinical studies have not confirmed the
10 correlation between dietary NO_3^- and carcinogenesis, when exposed to excessive dietary
11 intakes of NO_3^-, vegetarians, infants and elderly could be at higher risk of developing
12 cancer (Colla et al. 2018). This is mainly due to the fact that a low level acute toxicity of
13 NO_3^- can be transformed into the NO_2^-, which has much higher acute toxicity. Health
14 concerns over NO_2^- presence in the human body is due to its association with gastric
15 and bladder cancers, and also infant methaemoglobinaemia syndrome (Kmecl, Knap, and
16 Žnidarčič 2017). NO_3^- concentration in plant tissues depends not only on the rate of
17 uptake by roots but also light availability, products of photosynthesis and phytochrome
18 (Cookson,Williams, and Miller 2005, Samuolienė and Urbonavičiūtė 2009, He 2009,
19 2010, He, Cheok, and Qin 2011, He, Lim, and Qin 2015). Higher NO_3^- concentrations of
20 shoot, leaf, and petiole and stem (Figure 5) found in plants grown under shade, than
21 grown under full sunlight, further supported that accumulation of NO_3^- in vegetables is
22 mainly regulated by light (Gruda 2005, He, Goh, and Qin 2014, He, Lim, and Qin 2015,
23 Bian et al., 2018). To reduce hazard NO_3^- in vegetables under low light, different

approaches including clean production technology such as supplementary LED lighting had been adopted. For instance, using three different lettuce (*Lactuca sativa* L.) varieties, Samuolienė et al. (2011) reported that supplementary red- LED for 3 days before harvesting, reduced NO_3^- content in red and green leaf lettuce to 56.2% and 20.0%, respectively. It was reported that supplementation of red- and blue-LED lighting to lettuce resulted in lower leaf and petiole NO_3^- concentrations but higher soluble sugar concentrations (Zhou, Liu, and Yang 2013). Therefore, lowered leaf and petiole NO_3^- concentrations of *B. alboglabra* plants under shade + LED (Figure 5) might be accompanied with increased soluble sugar content. This increase in nutritional quality of the vegetable is desirable as leaves and petioles of *B. alboglabra* are edible plant parts. As such, effects of different light treatments on soluble sugar content of plant tissues merits our further study.

Beside NO_3^- concentration, nitrate reductase activity depends on light availability, products of photosynthesis and phytochrome (Samuolienė and Urbonavičiūtė 2009). Red light has the highest capacity in stimulating nitrate reductase activity as compared to other wavelengths of light (Samuolienė and Urbonavičiūtė, 2009). Therefore, lower NO_3^- concentrations measured in shoots of plants exposed to full sunlight and those grown in shade + LED suggest that nitrate reductase activities in the leaves of these plants could be higher than that of plants subjected to shade conditions.

CONCLUSION

As photosynthetic rate of plants grown in shade + LED increased, more energy, reducing power, precursors and carbon sources from photosynthesis were available for biosynthesis of organic molecules such as amino acids and nucleotides. Thus, these plants

had greater productivity than other plants. Supplementation of red and blue-LED lighting to *B. alboglabra* plants grown in the shade could reduce NO_3^- concentrations in edible plant parts, and improve photosynthesis and productivity. Therefore, commercial farmers may consider supplementing their *B. alboglabra* plants with red and blue-LED lighting during periods of cloudy and hazy weather so as to improve the productivity and nutrient quality of their crops.

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FIGURE CAPTIONS:

Figure 1. Spectral distribution (300 - 1000 nm) of light from full sunlight, shade, shade supplemented with red-and blue-LED, defined as Shade + LED (A) and red-and blue-LED (B). The spectral peaks of red- and blue-LED were 645 nm and 449 nm, respectively. Spectral scans were recorded every 0.5 nm with a spectroradiometer (PS300, Apogee Instruments, USA)

Figure 2. FW of shoot (A), root (B) and shoot/root ratio FW (C) of *B. alboglabra* under different light treatments. Each reading is an average of 4 plants. Vertical bars represent the standard errors. Means with different letters are statistically different ($P < 0.05$) as determined by Tukey's multiple comparison test.

Figure 3. A_{sat} (A) and $g_{s\ sat}$ (B) of *B. alboglabra* grown under different light treatments. Each reading is an average of 4 leaves from 4 different plants. Vertical bars represent the standard errors. Means with different letters are statistically different ($P < 0.05$) as determined by Tukey's multiple comparison test.

Figure 4. Total Chl content (A), total carotenoids content (B), Chl a/Chl b ratio (C) and total Car/Chl ratio (D) of *B. alboglabra* under different light treatments. Each reading is an average of 4 leaves from 4 different plants. Vertical bars represent the standard errors. Means with different letters are statistically different ($P < 0.05$) as determined by Tukey's multiple comparison test.

Figure 5. NO_3^- concentration of shoot (A), leaf (B), petiole and stem (C) and root (D) of *B. alboglabra* under different light treatments. Each reading is an average of 4 leaves from 4 different plants. Vertical bars represent the standard errors. Means with different

letters are statistically different ($P < 0.05$) as determined by Tukey's multiple comparison test.