
Title	Microclimate control to increase productivity and nutritional quality of leafy vegetables in a cost-effective manner
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Microclimate control to increase productivity and nutritional quality of leafy vegetables in a cost-effective manner

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Abstract: Food security is one of the key global challenges in this century. In Singapore, our research team has been using novel aeroponic technology to produce fresh vegetables since 1997. Aeroponic systems allow for year-round production of not only tropical, but also sub-tropical and temperate fresh vegetables, by simply cooling the roots suspended in aeroponic systems while the aerial parts grow under tropical ambient environments. It has also been used to investigate the impacts of root-zone CO₂ on vegetables by enriching root-zone CO₂ while their aerial portions were subjected to constant atmospheric CO₂. To compensate for the lack of available land, Singapore also needs to develop a farming system that can increase productivity per unit land area by many-fold. Over the past 10 years, my research team has established a commercially viable LED integrated vertical aeroponic farming system to grow different leafy vegetables under different LED spectra, intensities, and durations in the tropical greenhouse. The results demonstrate that it is possible to increase shoot production and rate of shoot production of leafy vegetables by increasing light intensity and extending the photoperiod under effective LED lighting. Furthermore, temperate vegetable crops such as lettuce were able to acclimate to high light intensity under supplementary LED lights to natural sunlight in the greenhouse. Supplementary LED lightings promote both leaf initiation and expansion with increased photosynthetic pigments, higher Cyt b₆f and Rubisco protein contents on a per area basis and thus improve photosynthetic capacity and enhance productivity. Plants sense and respond to changes in their immediate environments (microclimate), manipulating the root zone temperature (RZT) and water supply will impact not only their growth and development but also their nutritional quality. Our on-going research aims to investigate if the nutritional quality of leafy vegetables could be improved under suboptimal RZT and mild water deficit through deficit irrigation. If substantial energy and water savings in urban farming can be achieved without substantial yield penalty but with higher nutritional quality, the amount of water and energy saved can bring substantial benefits to society.

Keywords: leaf vegetables, LED lighting, microenvironment, nutrient spraying intervals, nutritional quality, photosynthetic performance, productivity, root-zone temperature

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1 Introduction

Singapore is a city-state country with the highest urban population in Southeast Asia. The total land used for land-based food farms is less than 1%^[1]. Furthermore, the tropical climate of Singapore is hot and humid all year round. Plants are poikilotherms and thus high temperatures have serious implications for vegetable production. As a result, more than 90% of Singapore's needs in subtropical and temperate vegetable crops, for instance, Chinese broccoli and lettuce are imported from other countries. In the future, temperature and light intensity may be very different from where we can grow vegetable crops today. Climate change further affects our ability to farm^[2]. For decades, pressure has been placed on Singapore's food security due to not only climate change but also population growth and many countries with large populations stockpiling their food reserves^[3]. The COVID-19

pandemic has placed unprecedented stresses on food supply chains, especially in Singapore. To ensure continuous food supply, Singapore should reduce dependency on a single food source through diversification. To further strengthen its food security, the Singapore Food Agency has set a goal of achieving '30 by 30', which is to develop the capability and capacity of agri-food industry to locally produce 30% of Singapore's nutritional needs by 2030^[3]. Land-scarce Singapore is also considered to be one of the most water-stressed countries in the world^[4]. To achieve '30 by 30' in Singapore, technology is the key enabler to help farms 'grow more with less'. Food production depends on land and water. The maintenance of food security, in terms of both quantity and quality, is an increasing challenge for Singapore.

Aeroponic farming systems have been adapted by us for growing high value subtropical and temperate vegetable crops in the tropics through manipulation of root environment such as root-zone temperature (RZT) control in order to save the power energy since 1994^[5]. Aeroponic farming systems have also been used to enrich RZ [CO₂] to study its interactive effects with RZT on the productivity and physiological performance of temperate lettuce grown indoors and in the greenhouse^[6-10]. Using the aeroponic growing systems, subtropical and temperate can now be grown in Singapore without cooling the whole tropical greenhouse as long as the roots of the plant are cooled^[2]. Cooling the RZ independently of hot ambient temperature promoted root growth

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and development of subtropical and temperate vegetables and alleviated their limitation of photosynthesis in the tropics^[2,11,12]. By using aeroponically-grown temperate lettuce plants, the effects of elevated RZ [CO₂] on its productivity and physiological performance were also studied at different air and RZ temperatures in both controlled environment room and greenhouse. The results showed that elevated RZ [CO₂] reduced the negative impacts of high air temperature on photosynthesis, N metabolism, and growth of lettuce plants, and thus enhanced productivity^[7]. The effects of elevated RZ [CO₂] impact on productivity, nitrogen metabolism, and photosynthesis were also studied in a tropical greenhouse. Increased productivity and improved photosynthetic efficiency under elevated RZ [CO₂] at different RZTs were partially due to the alleviation of midday water loss, both dynamic and chronic photo inhibition as well as higher turnover of Calvin Cycle with higher Rubisco protein^[8].

Growing leafy vegetable crops vertically has become increasingly popular as it increases land use in Singapore. Light is a key factor in the success of such vegetable cultivation. A commercially viable LED integrated vertical aeroponic farming system has been established by our team to grow leafy vegetables both indoors^[13-15] and in the tropical greenhouse^[16,17]. Our results demonstrated that for leafy vegetables to be grown indoors, the optimal combination of red (R)- and blue (B)-LED is more effective than R-LED or B-LED alone in enhancing photosynthesis and thus productivity. When plants have the maximum light-interception per unit leaf area, shoot productivity is closely related to photosynthetic performance on a leaf area basis. Otherwise, light interception area and absorptance are important factors responsible for the whole plant photosynthetic capability that is associated with leaf growth and shoot productivity^[15]. However, the optimal combinations of different LED spectra are species-dependent. In land-scarce Singapore, indoor vertical vegetable farms are under pressure to produce more output per unit of land area. The success of LED - lit indoor vertical farming also depends on local cost of energy and other environmental factors such as temperature, relative humidity mineral nutrients and CO₂ level. To self-produce and to achieve the '30 by 30' goal in a 'grow more with less' manner, it is not possible to totally depend on high energy-cost vertical farming indoors, which requires a light and temperature-controlled environment. This mini review mainly discusses the impacts of LED spectral quality, intensity, and duration on different leafy vegetables aeroponically grown in the tropical greenhouse under cool-RZT to reduce cooling costs. Natural sunlight supplemented with LED lighting to improve vegetable production in the greenhouse is also discussed. Growing agricultural crops using indoor and greenhouse growing systems not only allow the growers to achieve higher productivity, but also enable them to manipulate the growth conditions to enhance the accumulations of nutritional compounds. The last part of this paper addresses the issues of how to achieve substantial yield but with higher nutritional quality in a cost-effective manner.

2 Productivity and photosynthesis of different leafy vegetables grown under different spectra, intensities and durations of LED lighting in the tropical greenhouse

High temperature generated in the tropical greenhouse reduced the growth and yield of subtropical and temperate vegetable crops if RZT is not controlled^[2]. The success of growing subtropical

and temperate vegetables using vertical farming systems in the tropical greenhouse also depends on providing effective and sufficient lighting to the plants to allow fast growth but with minimal energy utilization^[18-20]. Many studies have been done using different combinations of LED lighting, particularly at lower photosynthetic photon flux densities (PPFDs) on photosynthesis and productivity because photosynthesis is more efficient at lower PPFD in the range of 150-300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ^[14,17,21,22]. Not only light quality but also light intensity is the most critical environmental factor for plant growth and physiology^[23,24]. Shoot biomass accumulation, as well as photosynthetic performance, decreased in low light conditions^[24]. However, there are relatively few comparative studies to investigate LED spectral quality on plants under different light intensities.

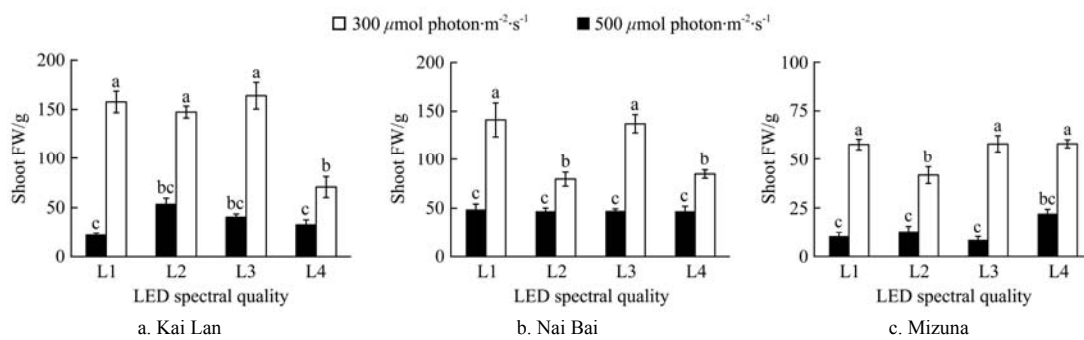
By using vertical aeroponic farming systems (Figure 1), comparative studies were carried out under different LED spectral qualities and quantities for three subtropical vegetables in the genus *Brassica* namely Kai Lan (*B. alboglabra*), Nai Bai (*B. chinensis* L.) and mizuna (*B. juncea* var. *japonica*) in the greenhouse. For all plants, their shoots were exposed to ambient temperatures ranging from 26°C to 34°C while the roots were sprayed with 25°C cool nutrient solution^[6]. Plants were grown under PPFD of 300 and 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ for 18 h supplied with each of the four red:blue:white (R:B:W) LED ratios: L1: 65R:0B:35W; L2: 65R:20B:15W; L3: 65R:25B:10W; L4: 65R:30B:5W for 3 weeks. Among the four different light conditions, the percentage of R-LED was kept constant at 65%. The variations of R:B:W LED ratios were designed between the differences in the percentages of B- and W-LED. Under a PPFD of 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$, no statistical difference in shoot fresh weight (FW) of Kai Lan was observed among L1, L2, and L3 while shoot FW was significantly lower under L4 with higher amount of blue lights than under other three conditions (Figure 2a). For Nai Bai plants exposed to a PPFD of 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$, shoot FW was similarly higher under L1 and L3 than under L2 and L4 (Figure 2b). Mizuna plants had significantly lower shoot FW under L2 compared to those under L1, L3, and L4 at a PPFD of 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. However, all plants had similar shoot FW regardless of LED spectral quality when exposed to a PPFD of 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ (Figure 2). Regardless of LED spectra, all plants had much higher shoot FW grown under a higher PPFD of 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ compared to those under 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. These results suggest that both spectral quality and light intensity are important to enhancing shoot productivity^[25]. The light-saturated photosynthetic CO₂ assimilation rate, A_{sat} , and stomatal conductance, $g_{s\ sat}$ of the three vegetable plants are shown in Figure 3. No statistical differences were observed in A_{sat} of Kai Lan plants grown under different LED spectral qualities at both PPFDs although the values of $g_{s\ sat}$ were significantly lower in plants grown under L1, L2, and L4 at a PPFD of 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ (Figures 3a and 3c). All Nai Bai plants had similar A_{sat} and $g_{s\ sat}$ (Figures 3b and 3e). Mizuna plants grown under L3 had the highest A_{sat} and $g_{s\ sat}$ compared to the rest of the plants (Figures 3c and 3f). Figure 3 shows that A_{sat} did not vary much on a leaf-area basis among Kai Lan and Nai Bai grown under different LED spectra at both PPFDs although the shoot FW of each species grown under L1, L2, and L3 was 3 to 4 folds higher under 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ than under 300 $\mu\text{mol}/\text{m}^2\cdot\text{s}$. These results indicate that higher shoot FW in plants grown under high LED does not link to photosynthetic rate on a leaf-area basis. For most plants, a slight change in light intensity leads to considerable changes in leaf morphology and structure^[26]. Enhanced shoot productivity of Kai

Lan and Nai Bai under $500 \mu\text{mol photon/m}^2\cdot\text{s}$ is closely linked to higher leaf number, larger total leaf area, and greater leaf thickness (data not shown). Therefore, the total amount of carbon fixed by plants under a high PPFD was much higher compared to those grown under a low PPFD. However, the morphological variations

in leaves beside higher leaf number under $500 \mu\text{mol photon/m}^2\cdot\text{s}$, were not clearly seen in mizuna plants between the two different light intensities. Some variations of A_{sat} (Figure 3c) among the different light treatments could be partially responsible for the differences in mizuna shoot FW (Figure 2c).

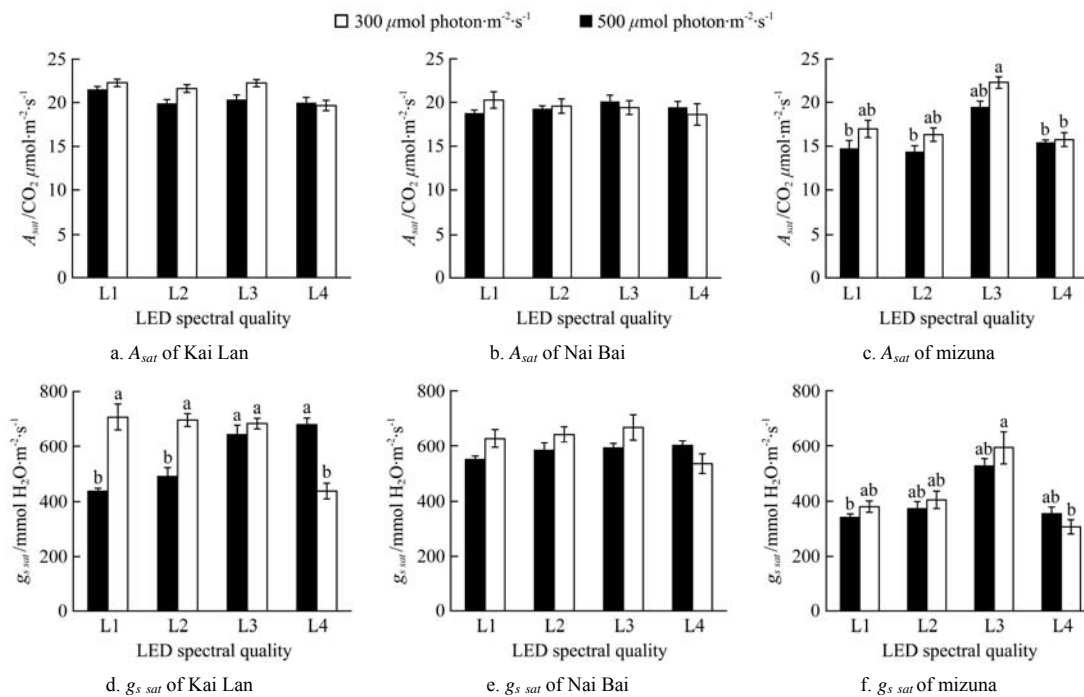


Figure 1 Vertical aeroponic farming systems in the tropical greenhouse of the National Institute of Education, Singapore



Note: L1: 65R:0B:35W; L2: 65R:20B:15W; L3: 65R:25B:10W; L4: 65R:30B:5W. Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$, $n=4$) as determined by Tukey's multiple comparison test (unpublished data).

Figure 2 Shoot FW of Kai Lan, Nai Bai, and mizuna grown under different LED spectra at 300 and 500 $\mu\text{mol photon/m}^2\cdot\text{s}$ (18 h photoperiod) for 3 weeks



Note: L1: 65R:0B:35W; L2: 65R:20B:15W; L3: 65R:25B:10W; L4: 65R:30B:5W. Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$, $n=4$) as determined by Tukey's multiple comparison test (unpublished data).

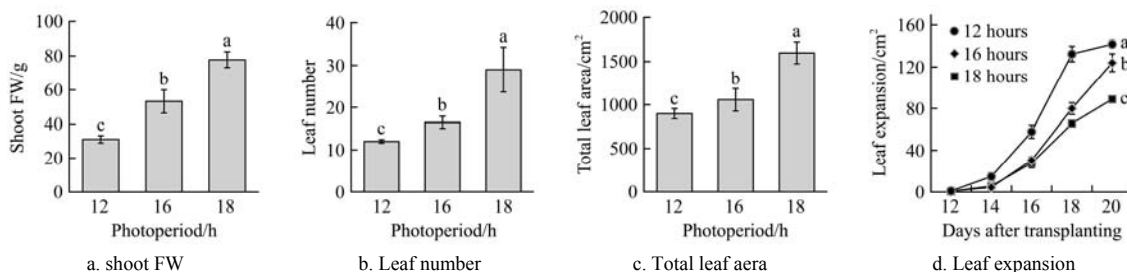
Figure 3 A_{sat} and $g_{s sat}$ of Kai Lan, Nai Bai, and mizuna grown under different LED spectra at 300 and 500 $\mu\text{mol photon/m}^2\cdot\text{s}$ (18 h photoperiod) for 3 weeks

Lettuce is an important temperate high valued leafy green that has been studied extensively in the tropical greenhouse by cooling their RZ^[2,11,12], elevating RZ [CO₂]^[6-11] as well as under different

LED light spectral quality in the cool growth room^[15] by our team. Controlling growth room temperature and supplemental lighting costs can be high. To reduce and offset costs, one of the strategies

is to grow the temperate lettuce in the tropical greenhouse by cooling only their roots under a long photoperiod at lower PPFD to shorten cropping cycles and increase the crop rotation per year. To achieve this, effective lighting and extending photoperiod could be used to accelerate plant growth^[27]. Figure 4 shows the effects of photoperiod on shoot FW, leaf number, total leaf area, and leaf expansion of a heat-resistant RIL lettuce (*Lactuca sativa*, HR-RIL#212), identified by our team previously^[28]. Lettuce plants were grown in the greenhouse aeroponically, where the RZT

was maintained at 25°C while the aerial parts were grown under fluctuating hot ambient temperatures (26°C to 34°C). Lettuce plants were subjected to three photoperiods of 12 h, 16 h, and 18 h of combined red- (85%) and blue-LED (15%) lightings with a mean PPFD of 300 $\mu\text{mol photon/m}^2\cdot\text{s}$. Plants subjected to 18 h photoperiod had more than 1.5 to 2 folds higher shoot FW, greater leaf number, larger total leaf area, and faster leaf expansion compared to those grown under 12 h and 16 h photoperiods (Figure 4).

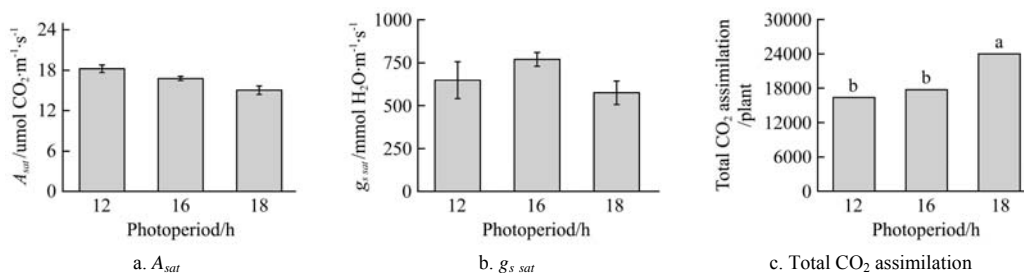


Note: Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$, $n=4$) as determined by Tukey's multiple comparison test (modified from He et al.^[16]).

Figure 4 Shoot FW, leaf number, total leaf area and leaf expansion of lettuce RIL after exposure to different photoperiods for 27 d

Longer photoperiods with the same PPFD resulted in increased daily light integral, and enhanced plant growth^[29]. However, no significant differences in A_{sat} and $g_{s\ sat}$ among lettuce plants exposed to different durations of LED lighting (Figures 5a and 5b). Higher shoot biomass accumulation in lettuce plants exposed to longer photoperiod could be related to photosynthesis on a whole plant basis^[30]. In our study, as the leaf number and the total leaf area (i.e., photosynthetic area) (Figures 4a and 4b) increased with extending photoperiod, a higher amount of carbon was fixed per plant. Although the photosynthetic rate is a critical factor for plant growth, whole-plant photosynthesis determined by leaf area and total light interception is directly related to overall crop

productivity^[30]. The total photosynthetic area could predict the whole plant carbon gain^[31]. For instance, total CO_2 assimilation of lettuce per plant under 18 h photoperiod was much higher (more than 2 folds) than under 12 h photoperiod (Figure 5c), despite similar A_{sat} on a leaf-area basis. In a vertical growing system, effective and sufficient lightings are crucial for plant growth performance, as well as for the sustainability of the production system^[32]. 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.



Note: Vertical bars represent the standard errors. Means with different letters are statistically different ($p < 0.05$, $n=4$) as determined by Tukey's multiple comparison test (modified from He et al.^[16]).

Figure 5 A_{sat} , $g_{s\ sat}$, and total CO_2 assimilation of lettuce RIL after exposure to different photoperiods for 27 d

3 Effects of natural sunlight supplemented with LED lighting on productivity and photosynthetic performance in the tropical greenhouse

Although cooling the RZ allows year-round production of subtropical and temperate vegetable crops in Singapore's warm climate^[10], these crops have frequently experienced increasingly unpredictable cloudy weather. The natural sunlight (SL) is not often sufficient for plant plants in the greenhouse even for a horizontal single-layer growing system. It was reported that low light intensity under natural SL, supplemented by low intensity LED lighting, resulted in plant characteristics similar to plants that were grown under long photoperiods^[33]. He et al.^[34] investigated the responses of aeroponically grown temperate Cos lettuce (*Lactuca sativa* L.) to two different intensities of supplementary

LED lighting in the greenhouse from the middle of September to early December 2018. During that period, the average maximum PPFD inside the greenhouse was around 500 $\mu\text{mol/m}^2\cdot\text{s}$ only from 1200 to 1500 h on non-rainy days, which was 30% to 35% of full sunlight as there were frequent cloudy and rainy days. Bolting occurred in most lettuce plants when grown under low levels of natural SL. In our study, Cos lettuce grown under natural SL supplemented with LED lighting provided as a combination of red- (633 nm and 656 nm) and blue-LED (463.5 nm) lightings in the ratio of 9:1 at PPFD of 150 and 300 $\mu\text{mol/m}^2\cdot\text{s}$ (SL + 150 PPFD and SL + 300 PPFD). The photoperiod of LED lighting was 12 h from 07:00 to 19:00, which was similar to the daylight period. Since it is a temperate crop, the roots of Cos lettuce were misted with cool nutrient solution kept at 25°C for the entire growth period. With increasing supplementary LED light intensity, Cos lettuce plants had significantly higher shoot FW, higher leaf number, and

larger total leaf area but with similar specific leaf area compared to those grown under natural SL. Light intensity influences multiple aspects of plant functioning including leaf development and growth, biomass accumulation, and photosynthetic performance^[35]. In the same study, it was also found that higher light intensities drive greater A_{sat} with increased concentrations of total chlorophyll and carotenoids, and higher contents of Cyt b_6f and Rubisco protein^[34].

A number of factors may contribute to increased plant growth and photosynthetic performance in response to increased light intensity. Similar to our other studies with different leafy vegetables grown with different light intensities (Figure 2) and durations (Figure 4), increased shoot biomass of Cos lettuce grown under SL supplemented with two different levels of LED lighting resulted from both more leaves and individual bigger leaves^[34] for greater light interception and photosynthesis^[15]. In the previous study^[36] carried out with sweet potato (*Ipomoea batatas*) leaves in the same greenhouse grown under sunlight with supplemental LED lighting, it was found that high light intensity increased leaf thickness with decreased SLA. However, there were no significant differences in SLA of Cos lettuce grown under different light intensities^[34]. Thicker leaves generally accumulate higher amount of photosynthetic enzymes on a per area basis and thus contribute to greater CO₂ fixation capacity of high-light grown leaves^[37]. The results obtained from the sweet potato leaves grown under different supplemented LED light intensities in the greenhouse also supported those earlier studies^[36]. A_{sat} of Cos lettuce grown under SL + 150 PPFD and SL + 300 PPFD were respectively, 2.09- and 5.68-fold of those plants grown under SL alone even if all leaves had similar thickness. Supplementary LED lightings to natural SL improve photosynthetic capacity of Cos lettuce was further supported by higher photosynthetic O₂ evolution rates (P_N) measured under saturated CO₂ concentration at PPFD \geq 600 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ ^[34]. Measured at high PPFDs, increased P_N could be through the increases of Cyt b_6f concentrations as it is the component of photosynthetic apparatus which may be the site of the rate-limiting step in the electron transport chain^[38]. Lower Cyt b_6f content is the main rate-limited factor that determines light- and CO₂-saturated photosynthetic capacity of Cos lettuce grown under lower light intensities^[38,39]. Cos lettuce plant grown under high light enhanced the capacity of light utilization may also through increases in Rubisco content^[40]. In our study with sweet potato leaves^[36], natural SL inside the greenhouse was much higher (average maximum PPFD \sim 800 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) than that of the study with Cos lettuce (average PPFD \sim 500 $\mu\text{mol}/\text{m}^2\cdot\text{s}$)^[33]. How the levels of Cyt b_6f and Rubisco protein are regulated when LED lightings are supplemented to fluctuating natural SL inside the tropical greenhouse and thus the final yield, merit our further studies.

4 Improved nutritional quality of leafy vegetables at suboptimal RZT and deficit irrigation for reducing energy and water use

It has been recently reported that environmental stress would have negative impacts on the yield of vegetables but the impacts on nutritional quality were mixed^[41]. Today, there is a major paradigm shift in how we perceive food, from the traditional concept of carbohydrate, protein, fat, and calories towards critical functional molecules such as total phenolic compounds (TPC) and ascorbic acid (ASC) which have health promoting properties^[42]. While optimizing ambient microclimate by using natural SL and LED lighting to improve yield and reduce energy consumption in

the greenhouse, we also aim to enhance nutritional quality through manipulating the RZT and nutrient spraying interval. In collaboration with one of the local vegetable farms, we have tested our hypothesis if vegetable yield can be sustained with enhanced nutritional quality under suboptimal RZT and deficit irrigation using aeroponic farming systems.

To investigate the impact of RZT on the nutritional quality, four different leafy vegetables namely Xiao Bai Cai (*B. rapa chinensis*), Hong Kong Cai Xin (*B. rapa var. parachinensis*), Tuscan Kale (*B. oleracea* 'Lacinato') and Lollo Bionda (*L. sativa* var. *crispa*) were tested. After 25 d of transplanting, all vegetable crops had the highest shoot FW when they were grown at 25°C-RZT followed by those at 28°C-RZT, and the lowest shoot FW was found in those grown at hot ambient-RZT (data not shown). Table 1 shows the concentrations of TPC and total ASC measured from the different vegetable crops with their roots grown at different RZTs while exposing their aerial shoots to hot fluctuating ambient temperature (26°C to 34°C) for 25 d^[2]. Although RZT had a certain impact on the accumulations of TPC in all four species, there are no generalizations for any specific degree of RZT effects on the respective parameters. For instance, TPC concentration was higher in all species at 28°C-RZT compared to those at 25°C-RZT and ambient-RZT. Grown at ambient-RZT, Hong Kong Cai Xin and Lollo Bionda had higher total ASC than at 28°C-RZT and 25°C-RZT. Although RZT affected the accumulation of TPC in all species and the concentration of total ASC in Hong Kong Cai Xin and Lollo Bionda, the changes were of relatively small magnitude. Thus, there are no recommendations to enhance nutritional quality by simply manipulating RZT. However, in the study of two temperate vegetable species, *L. sativa* (cv. Canasta) and *Eruca sativa* (cv. Arugula), we have previously found that RZ heat priming at 38°C during midday for one week minimized reduction in shoot FW with almost double amounts of TPC and total ASC after transferring to higher RZT of 42°C (unpublished data). This result supported the findings of another study with lettuce, which showed that heat treatment enhanced nutritional quality^[43]. Thus, it is feasible to sustain productivity and enhance the quality of subtropical and temperate vegetable crops at lower production costs through RZT manipulation.

Table 1 TPC, total ASC, and proline concentrations of aeroponically grown Xiao Bai Cai, Hong Kong Cai Xin, Tuscan Kale and Lollo Bionda at different RZTs for 25 d in the tropical greenhouse

Species	Parameter	RZT		
		25°C-RZT	28°C-RZT	Ambient-RZT
Xiao Bai Cai	TPC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	460.67 \pm 15.69 ^{ab}	478.55 \pm 15.90 ^a	437.52 \pm 16.72 ^b
	Total ASC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	328.01 \pm 20.22	342.60 \pm 21.55	362.47 \pm 22.61
Hong Kong Cai Xin	TPC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	493.39 \pm 3.27 ^b	541.88 \pm 8.78 ^a	475.13 \pm 6.45 ^b
	Total ASC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	443.24 \pm 16.81 ^b	451.91 \pm 19.52 ^{ab}	526.30 \pm 10.71 ^a
Tuscan Kale	TPC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	603.13 \pm 27.72 ^b	656.54 \pm 16.72 ^a	616.02 \pm 4.86 ^{ab}
	Total ASC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	590.01 \pm 1.91	584.92 \pm 2.23	588.40 \pm 2.87
Lollo Bionda	TPC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	128.86 \pm 1.65 ^b	144.98 \pm 6.52 ^{ab}	134.42 \pm 3.83 ^b
	Total ASC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	72.16 \pm 7.46 ^a	34.05 \pm 4.04 ^b	65.97 \pm 1.19 ^a

Note: Values are means \pm S.E. where different letters indicate significant differences ($p < 0.05$, $n=4$) as determined by Tukey's multiple comparison tests (unpublished data).

Most edible Kale cultivars contain high TPC and total ASC. In our recent study with Tuscan Kale, it was found that cooling the RZ to 28°C seemed to be the optimal RZT to achieve substantial shoot FW compared to those grown under hot ambient-RZT. However, there was no significant difference in total ASC among the Tuscan Kale grown at different RZT (Table 1). It was reported that crop yield and quality can be sustained under water deficits by manipulating root-to-shoot signaling processes^[44,45]. Such manipulation is necessary to maintain crop production while reducing the amount of irrigation water used in urban farming. For many crops, there is a poor understanding of how much water is required for achieving maximal production. Understanding water usage can not only save water but also arouse interest in the use of ‘deficit irrigation’ (the application of only a predetermined percentage of potential water loss). Deficit irrigation will not only greatly reduces water use, but enhances crop quality and the production of stress-responsive, plant-sourced chemicals which have positive benefits for human health^[45]. In the study with aeroponically grown common ice plants (*Mesembryanthemum crystallinum*), a facultative CAM plant, we have recently found that drought stress via deficit irrigation did not induce CAM photosynthesis but regulated photosynthetic performance and enhanced nutritional quality. For phytochemicals such as TPC and total ASC, but plants grown under 240 min nutrient spraying interval had significantly higher values than the other plants under shorter nutrient spraying intervals^[46]. Using Tuscan Kale, we are currently investigating if deficit irrigation could enhance the nutritional quality of this high valued leafy green through increasing nutrient spraying interval during the growth cycle or just prior to harvest without substantial yield penalty. Table 2 shows that increasing nutrient spraying interval from 5 to 30 min significantly decreased shoot FW by 19% but increased total ASC by 26%. However, Tuscan Kale plants grown under 5 min spraying interval for 28 d before transferring to 30 min spraying interval for 2 d had similar shoot FW but 22% higher total ASC compared to those grown under 5 min nutrient spraying interval. Longer nutrient spraying interval did not seem to affect the concentration of TPC. These results suggest that deficit irrigation could be used to enhance the total ASC concentration of Tuscan Kale without substantial yield penalty, the amount of water saved can bring substantial benefits to urban farms.

Table 2 Shoot FW, TPC, and total ASC concentrations of Tuscan Kale grown at different nutrient spraying interval for 30 d in the tropical greenhouse

Parameter	Nutrient spraying interval /min		
	5	30	*5→30
Shoot FW/g	11.23±1.08 ^a	9.09±0.78 ^b	12.74±1.05 ^a
TPC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	876.42±17.30	880.35±29.85	807.38±7.21
Total ASC/ $\mu\text{g}\cdot\text{g}^{-1}$ FW	1140.40±27.19 ^b	1438.42±39.43 ^a	1393.73±39.92 ^a

Note: *5 min for 28 d and 30 min for 2 d. Values are means ± S.E. where different letters indicate significant differences ($p < 0.05$, $n=4$) as determined by Tukey's multiple comparison tests (unpublished data).

5 Conclusions

By growing different leafy vegetables using a vertical aeroponic farming system, our results have demonstrated that it is possible to increase shoot production by increasing light intensity of LED and extending the photoperiod. It was also shown that temperate lettuce was able to acclimate to high PPFDs through supplementing LED light to natural SL in a tropical greenhouse. Supplementary LED lightings promote leaf initiation and

expansion with increased photosynthetic pigments, higher Cyt b_6/f , and Rubisco protein contents on a unit area basis and thus improve photosynthetic capacity and enhance productivity of lettuce. The preliminary results also suggest that nutritional quality of Tuscan Kale can be improved under manipulation of RZT and deficit irrigation, particularly prior to harvest, without substantial yield penalty. The amount of energy and water saved can bring substantial benefits to both the consumers and growers.

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