Quality Control Techniques and Related Factors for Hydroponic Leafy Vegetables

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Additional index words. lettuce, oilseed rape, supplementary light, fertilization, nutritional value

Abstract. Hydroponics has been an increasingly important field of vegetable production. However, a big issue with hydroponics is that certain crops can quickly accumulate high levels of nitrate-N ($\text{NO}_3 \pm \text{N}$) from the hydroponic system. The objective of this research was to decrease NO_3 accumulation and increase the nutritional value and yield of vegetable crops using lettuce and oilseed rape as a model under hydroponic production. In this study, two technologies were applied to leafy vegetable production: 1) using supplementary lighting (blue-violet diode) by manipulating illumination and 2) removing fertilization before harvest for a short term (3 or 5 days), thus providing a practical experiment for improving yield and edible qualities of hydroponic leaf vegetable production. Illumination was applied 4 hours a day (0500–0700 HR and 1700–1900 HR) during good weather, or 12 hours a day during bad weather with insufficient natural light (<2000 lux) during the autumn and winter seasons. Results showed that the lettuce cultivar Ou-Luo and the oilseed rape cultivar Ao-Guan Pakchoi had increased yield (50.0% and 88.3%, respectively), decreased NO_3 content (26.3% and 30.8%, respectively), and increased total soluble solids (24.1% and 30.6%, respectively). The 5-day fertilizer-free treatment before harvest resulted in 19.2%, 6.4%, and 16.5% yield increases; and 26.0%, 24.3%, and 47.8% NO_3 decreases in oilseed rape cultivar Ao-Guan Pakchoi and lettuce cultivars Da-Su-Sheng and Ou-Luo, respectively.

Hydroponics is an increasingly important field for counterseason vegetable production because of its efficiency in fertilization, water, and space use. Furthermore, it can overcome the disadvantages of soil culture, such as continuous cropping obstacles, diseases, and pests (Sardare and Admane, 2013). According to the market research report by Transparency Market Research (2018), the global hydroponics market is anticipated to reach a value of US\$12.1 billion by the end of 2015 from US\$6.9 billion in 2016 (Mordor

Intelligence, 2018). The market is likely to register a promising 6.50% compound annual growth rate between 2017 and 2025. Green-leaf vegetables are considered to be a good source of ascorbic acid (vitamin C), beta carotene, iron (Fe), calcium, folate, and fiber; they are also low in calories and sodium; and all varieties are free of fat and cholesterol (Jones, 1982). Compared to hydroponic fruit, green-leaf vegetables are easy to plant and their production is low in cost because of the relatively short cultivation period (35 d) and simple cultivation facilities (Jones, 2016). Therefore, this segment is projected to lead the global market in coming years.

However, fast accumulation of high level of nitrate ($\text{NO}_3\text{-N}$) in plants from mineral

fertilizer is a big issue with hydroponic vegetable production (Colla et al., 2010). Human uptake of NO_3 is mainly derived from the consumption of raw vegetables (80%) and may be detrimental to one's health (Rathod et al., 2016). NO_3 itself is relatively harmless, because the fatal adult dose is considered to be ≈ 100 -fold greater than the acceptable daily intake of $\text{NO}_3\text{-}$ set by the European Union. Contrary to the relatively nondeleterious effect of the nitrate ion, when nitrite accumulates in the human body to a certain extent, it can form a strong carcinogen—nitrosamine—which may lead to carcinogenesis of the digestive system (Mensinga et al., 2003). The direct contribution of vegetables, fruits, and herbs to nitrite intake is relatively low (Riens and Heldt, 1992); however, the reduction of NO_3 to nitrite is ubiquitous in the organism when it was mediated by the endogenous: about 5% of the NO_3 is converted to nitrite after being ingested (Santamaria, 2006). Therefore, the accumulation of NO_3 was considered to be a crucial factor in reducing the edible qualities of some vegetables. The NO_3 content in leafy vegetables is related mainly to species and varieties, followed by environmental factors (e.g., light, soil, and moisture) and management (e.g., water, fertilization, and harvest) (Colla et al., 2018; Santamaria, 2006).

Minimizing NO_3 levels and increasing nutritional value, such as soluble sugar and vitamin C content, in plants has never failed to fascinate researchers (Resh, 2016). Finding out some specific means of regulating the weight of substances in plants would improve their edible qualities dramatically (Anjana and Iqbal, 2007; Cavauiolo and Ferrante, 2014). Nowadays, artificial environment management is a hot spot of agronomic system research (Jones, 2016). The relationship between light intensity and NO_3 accumulation in vegetables has been reported in several types of research. NO_3 accumulation in vegetables varies with season and tends to be stimulated during autumn and winter, with lower intensities than in spring (Santamaria et al., 1999). Human-made illumination has been widely applied in facility agriculture to compensate for insufficient natural lighting—especially during foggy, hazy autumns and winters—by extending time and enhancing intensity (Feng Tian, 2016). With the progress of artificial lighting, especially light-emitting diodes (LEDs) (Takemiya et al., 2005), illumination technologies in the hydroponic vegetable industry are being used more widely, which increases the yield and nutritional value of products significantly (Li and Zhou, 2013). Nitrogen (N) fertilization is the primary source of NO_3 for edible crops (Donner and Kucharik, 2003). Usually, application of high-level nitrogenous fertilizer results in an increase in NO_3 content in plants (Donner and Kucharik, 2003). Excessive applications of NO_3 in fertilizers during the late stages of vegetative growth have more impact on NO_3 accumulation in leafy vegetables than when applied during early stages because requirements for N

Received for publication 31 Dec. 2018. Accepted for publication 11 Feb. 2019.

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decrease as plants mature (Blom-Zandstra and Lampe, 1983). For this reason, fertilization management during preharvest is an effective way to reduce NO_3 accumulation and increase nutritional value without loss in yield (Borgognone et al., 2016; Malagoli et al., 2004).

In hydroponic crops, relative research on manipulating light and fertilization has increased significantly, and systematical studies have provided integrated and elaborate information to enlighten and guide hydroponic production (Colla et al., 2018). However, most studies were conducted in a laboratory or at a small scale, lacking reports of researching experiments were carried out on real and large-scale production conditions, because of the limit in labor, time, and facilities (Craker and Seibert, 1983; Kitaya et al., 1998; Li and Kubota, 2009; Rajapakse and Shahak, 2008).

Therefore, in our study, we implemented operability improvement on the management technologies of lighting and preharvest fertilization in the actual production process of commercial greenhouses to obtain practical and feasible measures for controlling the accumulation of NO_3 and total soluble solids in hydroponic vegetables. We used lettuce (*Lactuca sativa* L.) and oilseed rape (*Brassica napus* L.) as model species in the research, which are the most commonly grown hydro-

ponic leafy vegetables in North American and East Asia, respectively (Fitt et al., 2006; Resh, 2016). The objective of our research was to decrease NO_3 accumulation and increase vitamin C and soluble sugar content, without losing yield in vegetable crops, by manipulating lighting and using two short-term (3 d or 5 d) fertilization breaks before harvest. About 18,480 lettuce and 5280 oilseed rape plants (including controls) were involved in our study, thus providing a theoretical basis for improving the qualities of hydroponic leaf vegetables in a practical case.

Materials and Methods

One oilseed rape cultivar, Ao-Guan Pakchoi; and two lettuce cultivars, American Da-Su-Sheng and Ou-Luo were used as examples of leaf vegetable crops in our study (Fig. 1A). The nutrient film technique (NFT) was applied to the experiments as hydroponic technology. The seedlings of the three cultivars were planted in the cultivation tank using the intensive plug-seeding method (Han, 2016), and the nutrient solution was circulated and flowed on the bottom of the container so the root system could absorb nutrients and water continuously, with a sufficient oxygen supply.

The experiments were conducted in the greenhouse of Hebei Academy of Agriculture

and Forestry Sciences, Shijiazhuang, China, using a randomized complete block design. Two seeds were planted in each well of the 72-well (hole) trays and contained cottonseed waste:meteorite at 2:1 (v/v). After 25 d, the uniform seedlings were selected and transplanted to a horizontal shelf in the NFT hydroponics system. The nutrient solution [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 600.00 $\text{mg} \cdot \text{L}^{-1}$; KH_2PO_4 , 180.00 $\text{mg} \cdot \text{L}^{-1}$; KNO_3 , 436.32 $\text{mg} \cdot \text{L}^{-1}$; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 900.00 $\text{mg} \cdot \text{L}^{-1}$; Fe-ethylenediaminetetraacetic acid, 23.00 $\text{mg} \cdot \text{L}^{-1}$; NaNO_3 , 5.0 $\text{mg} \cdot \text{L}^{-1}$; H_3BO_3 , 3.5 $\text{mg} \cdot \text{L}^{-1}$; Na_2MoO_4 , 0.24 $\text{mg} \cdot \text{L}^{-1}$; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.66 $\text{mg} \cdot \text{L}^{-1}$; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 2.01 $\text{mg} \cdot \text{L}^{-1}$; and NaCl , 0.88 $\text{mg} \cdot \text{L}^{-1}$] was set to run for 60 min with a 40-min break in each cycle using a Siemens Smart Line computer system (Siemens, Beijing, China). The electrical conductivity of the nutrient solution was limited to 1.5 to 3.0 $\text{mS} \cdot \text{cm}^{-1}$ and was measured using a conductivity meter (SX731; Sanxin, Shanghai, China). Each section of NFT hydroponics had 220 cells, and each cell contained four plants for lettuce and two plants for oilseed rape. After 30 d, all the plants were harvested for yield detection. Thirty-three cells (15%) were selected randomly from each section and were measured for their growth and edible qualities with three replications. In this way, a total of 396 plants (33 cells $\times$ 4 plants $\times$ 3 repeats) and 198 plants for oilseed rape (33 cells $\times$ 2

Fig. 1. (A) The varieties of leafy greens used in the experiment (from top to bottom): lettuce of Da-Su-Sheng, oilseed rape of Ao-Guan Pakchoi, and lettuce of Ou-Luo. (B) The plants and light-emitting diode (LED) arrangements. SL3, single light 75 cm from the vertical light (VL) hole; SL2, single light 50 cm from the VL hole; SL1, single light 25 cm from the VL hole; OL1, overlapping light at 25 cm from the VL hole; OL2, overlapping light at 50 cm from the VL hole; OL3, overlapping light at 75 cm from the VL hole. (C) Light intensities in different positions. SW, southwest; NW, northwest; SE, southeast; NE, northeast.

Table 1. Light intensities and increases at different positions under four lamps.

Treatment	Southwest lamp		Southeast lamp		Northwest lamp		Northeast lamp	
	Light (lux)	Increment (lux)	Light	Increment	Light	Increment	Light	Increment
SL3	1,806.2 e ^a	305.29	1,824.87 e	323.95	1,264.50 d	26.99	1,233.17 e	-4.34
SL2	2,038.39 e	537.48	1,994.82 de	493.91	1,551.19 c	313.68	1,472.22 cd	234.71
SL1	2,710.85 c	1,209.93	2,543.42 c	1,042.51	2,260.01 b	1,022.50	2,090.54 b	853.03
VL	3,491.23 a	1,990.32	3,022.15 a	1,521.23	2,827.79 a	1,590.28	2,726.60 a	1,489.09
OL1	3,079.15 b	1,578.24	2,762.78 b	1,261.86	2,247.26 b	1,009.75	2,287.36 b	1,049.85
OL2	2,278.92 d	778.00	2,119.70 d	618.78	1,583.03 c	345.52	1,648.08 c	410.57
OL3	1,909.69 e	408.77	1,838.45 e	337.53	1,279.00 d	41.49	1,311.06 de	73.55
Control	1,500.92 f	—	1,500.92 f	—	1,237.51 d	—	1,237.51 e	—

^aLowercase letters represent statistical significance at $P = 0.05$ for each parameter listed in the table.

SL3 = single light 75 cm from the vertical light (VL) hole; SL2 = single light 50 cm from the VL hole; SL1 = single light 25 cm from the VL hole; OL1 = overlapping light at 25 cm from the VL hole; OL2 = overlapping light at 50 cm from the VL hole; OL3 = overlapping light at 75 cm from the VL hole.

plants $\times$ 3 repeats) were tested for each treatment.

The bluish violet (370–480 nm) LEDs (LX1330B; Sampo, Shanghai, China) were arranged evenly above the hydroponic shelves, with 18 hydroponic cells per LED on average. When daytime natural lighting was more than 2000 lux, the LEDs were turned on from 0500 to 0700 HR each morning and from 1700 to 1900 HR in the evening (4 h/d). When daytime natural lighting was less than 2000 lux, the LEDs were turned on all day (0500–1900 HR). Light intensities were detected every hour at different positions under four LEDs: southwest, northwest, southeast, and northeast, as shown in Fig. 1B. The position of the LEDs and hydroponic cells have three lighting modes: single lighting (SL), vertical lighting (VL), and overlapping lighting (OL). Details of the illumination model from left to right in Fig. 1B are as follows: SL3, single light 75 cm from the vertical light (VL) hole; SL2, single light 50 cm from the VL hole; SL1, single light 25 cm from the VL hole; VL hole 40 cm below the LED perpendicularly; OL1, overlapping light at 25 cm from the VL hole; OL2, overlapping light at 50 cm from the VL hole; OL3, overlapping light at 75 cm from the VL hole.

After 30 d of growing, the growth traits and edible qualities of hydroponic vegetables were recorded to compare with the control groups, which were grown without illumination. Two durations (3 d and 5 d) of fertilization break were applied before vegetable harvest. The nutrient solution was replaced with water to cut all nutrient supplies. After harvest, growth traits and edible qualities were estimated for each treatment.

The growth traits included plant height, amount of chlorophyll, plant weight, yield, leaf length and width (supplementary light treatment only), and root volume (for fertilization-break treatments). Edible quality traits included the amount of NO₃, soluble sugar, vitamin C, and a total soluble solids (Beckles, 2012). Chlorophyll content was determined using a chlorophyll meter (SPAD-502; Generule, Shanghai, China (Ling et al., 2011)), NO₃ (Green et al., 1982) and the soluble sugar contents (Davies et al., 1998) were determined using a spectrophotometer (SP-1900 ultraviolet; Spectrum, Shanghai, China), vitamin C was determined by 2,6-dichlorophenol

indophenol titration, and soluble solid content was determined using a sugar meter (PAL-1; Atago, Shenzhen, China). All the monitoring and controlling of environmental conditions, including temperature, humidity, light, oxygen, and pH, were recorded automatically by a computer system (Siemens Smart Line).

Analysis of variance was performed using the general linear model of JMP Genomics 7. Student's t test at $\alpha = 0.05$ was used for multiple comparisons of the least square mean among the genotypes. The correlation coefficient for the traits was calculated using JMP Genomics 7 software.

Result

Supplementary lighting treatment and light intensities of different cells. As shown in Fig. 1B, under the four LEDs, the cell (hole) in the vertical light got the highest intensity, reached 1990.32 lux without natural light. Those cells farther away from the lights experienced gradually decreasing light intensity, and the value for each point is shown in Fig. 1C and Table 1. All light intensities for each cell in the illumination group were greater than the control.

Supplementary lighting impact on growth traits. To evaluate the lighting impact on growth and development of hydroponic vegetables, we evaluated the following growth traits: plant height, chlorophyll amount, leaf length, leaf width, fresh weight, and yield (Table 2). Plant height, chlorophyll amount, leaf width, and fresh weight of illuminated 'Ou-Luo' were greater than the controls, especially for the plants in VL cells, which had a 50.0% increase in yield. These values declined with a decrease in light intensity. Similar impacts were also found in 'Ao-Guan Pakchoi'. Plant height with supplemental lighting was significantly greater than the control. The best yield was 7751.7 kg/acre in VL cells, which was an 88.31% increase over the control. The gradient changes in growth traits in both lettuce and oilseed rape revealed that proper illumination could promote the growth and development of leafy vegetables.

Supplementary lighting impact on edible qualities. Under lighting conditions, 'Ou-Luo' displayed considerable variation in all nutrition traits compared to the control (Table 3).

NO₃ content decreased by 26.30%; total soluble solids and sugar contents increased as much as 24.05% and 33.5%, respectively; and vitamin C showed a gradual decline with light intensity increase. The NO₃ content in 'Ao-Guan Pakchoi' with supplemental lighting was reduced as much as 30.76% than plants with insufficient lighting. Total soluble solids and sugar contents increased by 30.6% and 30.5%, respectively; but vitamin C content decreased under supplemental lighting.

After being subjected to lighting treatments, the accumulation of NO₃ in lettuce correlated negatively with soluble sugar content and total soluble solids ($r = -0.699$ and $r = -0.787$, respectively); a positive correlation between NO₃ content and vitamin C content was seen ($r = 0.788$) (Table 4). The same phenomena were also seen in oilseed rape: NO₃ content correlated negatively with total soluble solids and soluble sugar content ($r = -0.956$ and $r = -0.813$, respectively); and a positive correlation between NO₃ and vitamin C content was seen ($r = 0.741$) (Table 4). These results indicate that lighting treatments can decrease NO₃ content and simultaneously increase total soluble solids and soluble sugar contents, but not vitamin C content.

Fertilization-break impact on growth traits. In our study, "fertilization break" was defined as cutting off all nutrient supply in the short term. The nutrient solution in the hydroponic system was replaced by pure water 3 d or 5 d before harvest. As shown in Table 5, the growth traits of 'Ao-Guan Pakchoi' with the fertilization break were greater than the control, especially plant height, chlorophyll amount, root volume, and plant weight. The two treatments (3 d and 5 d) improved yield by 10.7% and 19.2%, respectively.

Moreover, the growth traits of the 5-d treatment were slightly greater than the 3-d treatment. In 'Ou-Luo', the amount of chlorophyll, root volume, and plant weight increased significantly. Moreover, yield increased by 11.9% and 16.5%, respectively, with the 3-d and 5-d treatments. However, for 'Da-Su-Sheng', there was no significant difference between the control and the treatments in almost all traits. There was only a 2.0% and 6.4% increase in plant weight. These results confirm that a short-term fertilization break does not reduce plant

Table 2. Growth traits under different lighting treatments.

Treatment	Ht (cm)	Chlorophyll (%)	Leaf length (cm)	Leaf width (cm)	Fresh wt (kg)	Yield (kg/acre)	Yield increase (%)	Vegetable	Cultivar
SL3	16.6 bc ²	41 b	13.9 c	8.4a	0.11 a	5,880.6 e	10.00	Lettuce	Ou-Luo
SL2	15.95 bc	43.3 ab	15.3 abc	8.35a	0.12 a	6,415.2 d	20.00		
SL1	17.35 b	46.1 a	15.75 abc	7.9a	0.125 a	6,682.5 c	25.00		
VL	19.4 a	33.3 d	17.4 a	8.5a	0.15 a	8,019 a	50.00		
OL1	17.2 b	36.95 c	15.15 bc	9.15a	0.14 a	7,484.4 b	40.00		
OL2	17.3 b	35.45 cd	16.9 ab	8.9a	0.125 a	6,682.5 c	25.00		
OL3	17.35 b	42.8 b	15.3 abc	8.8a	0.105 a	5,613.3 f	5.00		
Control	15.267 c	35.7 cd	15.8 abc	7.733a	0.1 a	5,346 g	—		
SL3	14.2 e	41.6 a	9.8 b	6.5	0.1 a	5,078.7 e	23.38	Oilseed rape	Ao-Guan Pakchoi
SL2	15.4 de	38.25 d	10.4 ab	6.65	0.11 a	5,880.6 d	42.86		
SL1	18.6 a	40.45 b	10.95 a	6.85	0.12 a	6,147.9 c	49.35		
VL	16.4 bcd	36.8 e	10.25 ab	6.75	0.15 a	7,751.7 a	88.31		
OL1	15.95 cd	39.45 c	9.75 b	6.45	0.13 a	6,682.5 b	62.34		
OL2	17.8 ab	40.8 ab	10.1 ab	6.2	0.12 a	6,147.9 c	49.35		
OL3	17.2 abc	34.85 f	9.95 ab	6.65	0.11 a	5,880.6 d	42.86		
Control	11.83 f	37.33 de	10.63 ab	6.6	0.08 a	4,116.42 f	—		

²Lowercase letters represent statistical significance at $P = 0.05$ for each parameter listed in the table.

SL3 = single light 75 cm from the vertical light (VL) hole; SL2 = single light 50 cm from the VL hole; SL1 = single light 25 cm from the VL hole; OL1 = overlapping light at 25 cm from the VL hole; OL2 = overlapping light at 50 cm from the VL hole; OL3 = overlapping light at 75 cm from the VL hole.

Table 3. Edible qualities in different lights.

Treatment	Nitrate (mg·kg ⁻¹)	Decrease (%)	Soluble Solid (%)	Increase (%)	Vitamin C (mg·100 g ⁻¹)	Increase (%)	Soluble sugar (mg·g ⁻¹)	Increase (%)	Vegetable	Cultivar
SL3	2,718.65 a ²	4.45	4.25 bc	7.59	2.07 bc	-37.27	13.43 bcd	12.2	Lettuce	Ou-Luo
SL2	2,443.43 b	11	4.35 bc	10.13	2.49 b	-24.55	12.16 cd	1.59		
SL1	2,367.45 b	13.77	4.25 bc	7.59	1.89 c	-42.73	13.87 bc	15.87		
VL	2,023.52 d	26.3	4.9 a	24.05	1.68 c	-49.09	15.98 a	33.5		
OL1	2,107.91 cd	23.22	4.8 ab	21.52	1.78 c	-46.06	14.31 b	19.55		
OL2	2,097.37 cd	23.61	4.3 bc	8.86	1.98 c	-40	13.34 bcd	11.45		
OL3	2,311.63 bc	15.8	4.7 ab	18.99	2.08 bc	-36.97	14.88 ab	24.31		
Control	2,845.5 a	—	3.95 c	—	3.3 a	—	11.97 d	—		
SL3	2,381 b	20.21	3.7 a	15.63	30.56 d	-27.24	6.3 bc	3.28	Oilseed rape	Ao-Guan Pakchoi
SL2	2,162.9 cd	27.52	3.8 a	18.75	32.23 cd	-23.26	6.99 abc	14.59		
SL1	2,132.5 cd	28.53	4.05 a	26.56	32.71 c	-22.12	7.47 ab	22.46		
VL	2,066 d	30.76	4.18 a	30.63	36.52 b	-13.05	7.96 a	30.49		
OL1	2,111.6 cd	29.23	4.1 a	28.13	31.66 cd	-24.62	7.95 a	30.33		
OL2	2,141.7 cd	28.23	3.96 a	23.75	32.77 c	-21.98	7.21 abc	18.20		
OL3	2,218.5 c	25.65	3.87 a	20.94	33.25 c	-20.83	7.46 ab	22.30		
Control	2,984 a	—	3.2 b	—	42 a	—	6.1 c	—		

²Lowercase letters represent statistical significance at $P = 0.05$ for each parameter listed in the table.

SL3 = single light 75 cm from the vertical light (VL) hole; SL2 = single light 50 cm from the VL hole; SL1 = single light 25 cm from the VL hole; OL1 = overlapping light at 25 cm from the VL hole; OL2 = overlapping light at 50 cm from the VL hole; OL3 = overlapping light at 75 cm from the VL hole.

development or yield, but may increase them instead.

Fertilization-break impact on edible qualities. The results in Table 6 reveal that during the 3-d and 5-d treatments, the NO₃ content of ‘Ao-Guan Pakchoi’ decreased by 20.9% and 26.0%, respectively; followed by vitamin C content decrease of 19.9% and 19.6%, respectively; a total soluble solids content decrease of 2.6% and 8.7%, respectively; and soluble sugar decreased by 0.7% and 3.9%, respectively, compared with the control. The decreased NO₃ content was detected in ‘American Da-Su-Sheng’ (15.9% and 47.8%). The fertilization break also improved other edible qualities of ‘American Da-Su-Sheng’, especially during the 5-d treatment. The soluble sugar content increased by 54.0%, vitamin C content increased by 82.8%, and the total soluble solid content increased by 27.9%. We also noticed the 5-d treatment in ‘Ou-Luo’ improved edible qualities as well. The NO₃ content was reduced by 24.3%, the soluble sugar content increased by 88.6%, vitamin C

content increased by 16.7%, and total soluble solid content increased by 20%. These results indicate that a fertilization break decreased the NO₃ content and, at the same time, increased total soluble solids, soluble sugar, and vitamin C contents, especially during the 5-d treatment for the two lettuce cultivars, but not for oilseed rape.

During the fertilization break, the NO₃ content in ‘Ao-Guan Pakchoi’ correlated positively with vitamin C, total soluble solids, and soluble sugar contents ($r = 0.980, 0.846$, and 0.769 , respectively) (Table 7). This means the treatment reduced the NO₃ content and other edible qualities synchronously in similar degrees. Contrary results were observed in lettuce; NO₃ correlated negatively with soluble sugar, vitamin C, and total soluble solids contents ($r = -0.943, -0.981$, and -0.975 , respectively for ‘American Da-Su-Sheng’; and $r = -0.977, -0.930$, and -0.858 for ‘Ou-Luo’, respectively). The fertilization break increased soluble sugar, vitamin C and total soluble solids contents while reducing NO₃, especially in the 5-d treat-

ment. In addition, there were no negative influences on yield.

Discussion

Growth traits and edible qualities. In our study, we describe NO₃ content and total soluble solids (vitamin C and soluble sugar) as “edible qualities” to evaluate vegetable toxins and nutrition. It is worth noting that total soluble solids is a general term for all soluble substances (Kader, 2002). Because of limitations in labor and cost, we measured only two of them—vitamin C and soluble sugar—individually. Total soluble solids in our study is regarded as an index equaling vitamin C and soluble sugar used to assess nutritional value.

For two management strategies (supplementary light and fertilization), the growth traits we selected in the experiments were somewhat different. Often, insufficient lighting stimulates petiole development and elongation, which make leaves growing lengthwise; on the contrary, leaves elongate

Table 4. Correlations of nitrate, vitamin C, soluble sugar, and total soluble solid contents under light supplementation.

	Pearson	Nitrate	Vitamin C	Soluble sugar	Soluble solid	Vegetable	Cultivar
Nitrate	<i>r</i>	1	0.788	-0.699	-0.787	Lettuce	Ou-Luo
	<i>P</i>		0.02	0.054	0.02		
Vitamin C	<i>r</i>	0.788	1	-0.789	-0.731		
	<i>P</i>	0.02		0.02	0.039		
Soluble sugar	<i>r</i>	-0.699	-0.789	1	0.859		
	<i>P</i>	0.054	0.02		0.006		
Soluble solid	<i>r</i>	-0.787	-0.731	0.859	1		
	<i>P</i>	0.02	0.039	0.006			
Nitrate	<i>r</i>	1	0.741	-0.813	-0.956	Oilseed rape	Ao-Guan Pakchoi
	<i>P</i>		0.036	0.014	0		
Vitamin C	<i>r</i>	0.741	1	-0.323	-0.597		
	<i>P</i>	0.036		0.436	0.118		
Soluble sugar	<i>r</i>	-0.813	-0.323	1	0.908		
	<i>P</i>	0.014	0.436		0.002		
Soluble solid	<i>r</i>	-0.956	-0.597	0.908	1		
	<i>P</i>	0	0.118	0.002			

r = correlation coefficient; *P* = *P*-value.

Table 5. Growth traits in different fertilization-break treatments.

Treatment	Ht (cm)	Chlorophyll (%)	Aboveground fresh wt (g)	Below- ground fresh wt (g)	Root volume (mL)	Aboveground dry wt (g)	Below- ground dry wt (g)	Yield (kg/acre)	Yield increase (%)	Vegetable	Cultivar
Control	21.44 a ²	43.81 b	90 c	60.12 b	16.5 c	6.39 a	3.13 b	7,878.86 c	—	Oilseed rape	Ao-Guan Pakchoi
3 d	22.74 a	45.06 ab	100 b	60.83 b	17.62 b	6.92 a	3.19 ab	8,724.59 b	10.73		
5 d	23.58 a	47.3 a	110 a	70.0 a	20.31 a	7.16 a	4.08 a	9,392.29 a	19.21		
Control	30.04 ab	16.46 a	125.45 b	18.3 b	18.2 a	3.51 b	2.95 b	7,684.878 c	—	Lettuce	American
3 d	31.36 a	16.58 a	126.09 ab	20.51 ab	20.2 a	3.81 a	4.74 a	7,837.236 b	1.98		Da-Su-Sheng
5 d	29.7 b	15.3 a	131.16 a	21.73 a	20.6 a	3.92 a	4.87 a	8,173.487 a	6.35		
Control	25.52 a	34.04 ab	96.53 c	15.61 b	13.8 c	2.53 b	1.64 b	5,994.99 c	—		Ou-Luo
3 d	27.58 a	38.14 a	105.02 b	20.44 a	20.4 a	3.18 a	4.3 a	6,707.1 b	11.87		
5 d	27.7 a	32.86 b	111.47 a	19.14 a	17.6 b	3.46 a	3.23 a	6,982.36 a	16.47		

²Lowercase letters represent statistical significance at *P* = 0.05 for each parameter listed in the table.

Table 6. The edible qualities in different fertilization-break treatments.

Treatment	Nitrate (mg·kg ⁻¹)	Decrease (%)	Vitamin C (mg·100 g ⁻¹)	Increase (%)	Soluble solid (%)	Increase (%)	Soluble sugar (mg·g ⁻¹)	Increase (%)	Vegetable	Cultivar
Control	3,739.42 a ²		62.77 a		5.75 a		4.36 a		Oilseed rape	Ao-Guan Pakchoi
3 d	2,958.54 b	20.88	50.3 b	-19.87	5.6 a	-2.61	4.33 a	-0.69		
5 d	2,765.9 b	26.03	50.49 b	-19.56	5.25 a	-8.70	4.19 a	-03.90		
Control	3,271.32 a		0.58 b		3.05 c		1.88 b		Lettuce	American
3 d	2,752.56 b	15.86	0.65 b	12.07	3.5 b	14.75	1.871 b	-0.48		Da-Su-Sheng
5 d	1,709.21 c	47.75	1.06 a	82.76	3.9 a	27.87	2.913 a	54.95		
Control	2,590.83 a		1.14 a		3.25 c		2.488 c			Ou-Luo
3 d	2,569.78 a	0.81	1.04 a	-8.77	3.6 b	10.77	3.047 b	22.47		
5 d	1,961.66 b	24.28	1.33 a	16.67	3.9 a	20.00	4.692 a	88.59		

²Lowercase letters represent statistical significance at *P* = 0.05 for each parameter listed in the table.

sideways under adequate light (Muramoto et al., 1965; Pepper et al., 1994; van der Graaff et al., 2000). It has been reported that the shortage of nutrients in hydroponic solution could stimulate root development (Hodge et al., 2009; Trejo-Téllez and Gómez-Merino, 2012). Therefore, in our study, we tested leaf growth and root features of the plants to verify the effects of lighting and fertilization breaks. We found that leaf and root features are in accordance with growth in insufficient lighting and fertilization-free conditions. These two representative morphology changes can help researchers ensure rationality of the results quickly. We also measured chlorophyll content to evaluate the health of the plants, because abundant chlorophyll usually implies vigorous growth and development of plants (Chaerle and Van Der Straeten, 2001).

Supplementary lighting. Light is one of the most critical factors during plant growth. Despite photosynthesis, most plant characteristics are also influenced by the mode of light, including intensity, rhythm, period, and type (Kami et al., 2010; Takemiya et al., 2005). There are many theories which can explain the correlation between light and NO₃ content, and the lighting drove activity changes of NO₃ reductase can be one of the causes (Konstantopoulou et al., 2010). As others have reported, a reduction in light intensity is accompanied by a decrease in NO₃ reductase activity, which induces fast NO₃ accumulation in several important leafy vegetables (Fallovo et al., 2009; Pilgrim et al., 1993). Our research confirmed toxicity reduction with gradients in light intensity. Another explanation of NO₃ reduction in our experiment is the variation of NO₃ among different parts of every plant. The order of

NO₃ content has been listed by former researches as petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed (Santamaria et al., 1999). In general, the NO₃ concentration in the petiole is about two to five times greater than in leaf, depending on the vegetable species (Elia et al., 2000; Koh et al., 2012; Umar et al., 2007). During the development of the plant, the petiole is the basic part to form the leaf, root, and other storage organs, where NO₃ tends to accumulate, compared with other parts of the vegetable (Maynard et al., 1976; Santamaria et al., 1999). The greater growth of the petiole under insufficient lighting was recognized in our study, although it was not surprising to find high-level NO₃ accumulation in these plants.

Despite our encouraging results, we found some discrepancies with other work. With supplemental lighting, vitamin C decreased

Table 7. Correlation of nitrate, vitamin C, soluble sugar, and total soluble solid contents after nutrient break.

	Pearson	Nitrate	Soluble sugar	Vitamin C	Soluble solid	Vegetable	Cultivar
Nitrate	<i>r</i>	1	-0.943	-0.981	-0.975	Lettuce	American Da-Su-Sheng
	<i>P</i>		0.216	0.125	0.144		
Soluble sugar	<i>r</i>	-0.943	1	0.99	0.845		
	<i>P</i>	0.216		0.091	0.36		
Vitamin C	<i>r</i>	-0.981	0.99	1	0.912		
	<i>P</i>	0.125	0.091		0.269		
Soluble solid	<i>r</i>	-0.975	0.845	0.912	1	Lettuce	Ou-Luo
	<i>P</i>	0.144	0.36	0.269			
Nitrate	<i>r</i>	1	-0.977	-0.93	-0.858		
	<i>P</i>		0.138	0.239	0.343		
Soluble sugar	<i>r</i>	-0.977	1	0.829	0.949		
	<i>P</i>	0.138		0.377	0.205		
Vitamin C	<i>r</i>	-0.93	0.829	1	0.61	Oilseed rape	Ao-Guan Pakchoi
	<i>P</i>	0.239	0.377		0.582		
Soluble solid	<i>r</i>	-0.858	0.949	0.61	1		
	<i>P</i>	0.343	0.205	0.582			
Nitrate	<i>r</i>	1	0.769	0.980	0.846		
	<i>P</i>		0.441	0.128	0.358		
Soluble sugar	<i>r</i>	0.769	1	0.626	0.991	Oilseed rape	Ao-Guan Pakchoi
	<i>P</i>	0.441		0.569	0.083		
Vitamin C	<i>r</i>	0.980	0.626	1	0.722		
	<i>P</i>	0.128	0.569		0.486		
Soluble solid	<i>r</i>	0.846	0.991	0.722	1		
	<i>P</i>	0.358	0.083	0.486			

slightly with increasing light intensity, which does not reflect results from previous studies (Li and Kubota, 2009). Those studies claimed that, with a rapid increase in biomass under supplemental lighting, vitamin C should increase synchronously with the growth surge (Sørensen et al., 1994). This phenomenon may be caused by inconsistent NO₃ accumulation and vitamin C production during the whole growth period, which is affected easily by many factors (Chen et al., 2003; Lee and Kader, 2000).

Fertilization break. N is the necessary element during plant growth and development, but it is also the source of the NO₃ hazard (Mantelin and Touraine, 2004). An appropriate nutrient formula and management would minimize this harm without losses in yield or nutritional value (Bar-Yosef et al., 2009). Short-term fertilization break has been considered to be a reliable method for reducing the NO₃ hazard (Borgognone et al., 2016). Our results showed that the yield of all tested plants increased, and NO₃ contents declined, after the fertilization break treatment, which is consistent with the reports. As plants reach maturity, their requirements for N decrease (Blom-Zandstra and Lampe, 1983). Because the fertilization break removed all excess N supplies, plants demonstrated a dramatically reduced NO₃ content (Borgognone et al., 2016; Malagoli et al., 2004). In another aspect, the absence of fertilization could stimulate root development, which could consume a large amount of NO₃ stores in petioles. Because N mainly helps to form storage organs (roots, rhizomes, and tubers) (Alexander et al., 2008), the edible part (yield) of the vegetable would not be influenced by the treatments. The mechanism by which yield increased during the two fertilization-break treatments is still not clear yet.

Moreover, contrary to the lettuce cultivars used in our study, total soluble solids, soluble sugar, and vitamin C contents decreased with NO₃ decline in oilseed rape as reported by Oh et al. (2009). Although this result can be explained by content variations among species, according to Bell (1993), further research is still needed to elucidate why fertilizer-break treatment reduced the NO₃ content but increased yield, especially for those regional preference vegetables like oilseed rape.

Impacts and outlooks. Hydroponic systems have been used as one of the essential modes for facility agriculture in commercial production for several crops (Trejo-Téllez and Gómez-Merino, 2012). An ideal system must construct and manage all the facilities in an appropriate way to gain the expected profit (Jones, 2016). Considering the enormous input, a sustainable strategy including economy, health, and environmental friendliness are required for all facility agriculture systems (Zhang et al., 2015). Our research followed this strategy in three aspects: 1) enhanced the edible qualities and decreased toxicity in vegetables, 2) improved the vegetable yield in natural-light shortage seasons, and 3) reduced waste emission into the environment by our lighting and fertilization-break treatments. Moreover, opposed to laboratory or small-scale production, we mobilized considerable resources in facilities, labor, technology, and policies to support this research, which resulted in more than 23,000 experimental plants harvested for yield. Meanwhile, 15% of them were collected to assess growth traits and edible qualities. More important, hydroponic systems may boost the vegetable industry in developing countries where air and soil pollution causes one-third a reduction in value of autumn and winter vegetable pro-

duction in the greenhouse (Fallovo et al., 2009; Jackson et al., 2004).

Few studies compare with ours in terms of scale. However, we have to admit there were some weaknesses in our study. First, because of device and technique limitations, we had to use lux to measure the light intensity, which is preferred for evaluating intensity in humans over plants. Second, because of labor and technique limitations, we could only detect NO₃, total soluble solids, vitamin C, and soluble sugar to evaluate edible qualities, which are not comprehensive vegetable qualities (Shewfelt and Bruckner, 2000). Third, with such a large scale of vegetable production, we could not make sure equipment, facilities, and labor worked the same during the growth period, which may cause relatively large errors compared with laboratory experiments. For example, the measurement and recording of light intensities were conducted manually by different technicians who used hand-held detectors, all of which could be prone to errors. In the future, we will endeavor to improve the rigor and consistency of our experiments.

During long-term evolution, natural selection, and cultivation, the crop like lettuce and oilseed rape was “gaining” more and more redundancy genes that induce a lot of differences in plant behaviors, even under a same circumstance among species and varieties (Allard and Bradshaw, 1964; Burns et al., 2011). Therefore, regarding improvement in lighting and fertilization management for leafy vegetables, it is necessary to consider the mode of treatment, such as continuous or noncontinuous, short term or long term, and so on. The combination of several treatments also needs to be studied in future research, which may also influence the metabolic balance in plants (Mooney, 1972). Thus, it is possible, by changing the balance of

endogenous synthesis, especially in vivo substances, to control the development of plants (Li et al., 2017). Also, the impacts of lighting and fertilization break on the qualities and yield of other vegetables need further experimentation, which is warranted to assess the physiologic and molecular changes linked to these modifications and to identify treatments that can be applied strategically to reduce NO₃ accumulation in leafy vegetables.

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