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# Effect of green light on nitrate reduction and edible quality of hydroponically grown lettuce (*Lactuca sativa* L.) under short-term continuous light from red and blue light-emitting diodes

Zhonghua Bian[a], Ruifeng Cheng[b], Yu Wang[a], Qichang Yang[b,*], Chungui Lu[a,*]

[a] School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Brackenhurst Campus, NG25 0QF, UK
[b] Institute of Sustainable Development and Environmental Sciences in Agriculture, Chinese Academy of Agricultural Sciences, 100081, China

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ABSTRACT

Most leafy vegetables can accumulate large amounts of nitrate, which are often associated with harmful effects on human health. Nitrate assimilation in plants is determined by various growth conditions, especially light conditions including light intensity, light duration and light spectral composition. Red and blue light are the most important since both drive photosynthesis. Increasingly, recent evidence demonstrates a role for green light in the regulation of plant growth and development by regulating the expression of some specific genes. However, the effect of green light on nitrate assimilation has been underestimated. In this study, lettuce (*Lactuca sativa* L. cv. Butterhead) was treated with continuous light (CL) for 48 h by combined red and blue light-emitting diodes (LEDs) supplemented with or without green LED in an environment-controlled growth chamber. The results showed that nitrate reductase (NR) and nitrite reductase (NiR) related-gene expression and nitrate assimilation enzyme activities were affected by light spectral composition and light duration of CL. Adding green light to red and blue light promoted *NR* and *NiR* expressions at 24 h, subsequently, it reduced expression of these genes during CL. Compared with red and blue LEDs, green light supplementation significantly increased NR, NiR, glutamate synthase (GOGAT) and glutamine synthetase (GS) activities. Green-light supplementation under red and blue light was more efficient in promoting nutritional values by maintaining high net photosynthetic rates ($P_n$) and maximal photochemical efficiency ($F_v/F_m$).

## 1. Introduction

Nitrogen (N) plays an important role in plant growth and development (Wang et al., 2002). Nitrate is one of the most abundant N sources in natural and agricultural systems. It is absorbed in the root and mobilized to other organs. When the absorption of nitrate exceeds its assimilation, nitrate will accumulate in plants, particularly in hydroponic growing system. Excessive nitrate accumulation is known to be a common problem in most crops, especially in leaf vegetables (Bóbics et al., 2015; Cárdenas-Navarro et al., 1999).

It has been proved that consuming more vegetables every day can help people keep healthy, since phytochemicals (carotenoids and phenols, etc.) in vegetables are major sources of antioxidants in human diets and play important roles in alleviating age-relative diseases (Connor et al., 2005; Martínez-Sánchez et al., 2008; Mou, 2009). Along with tomatoes, lettuce is another major vegetable grown in greenhouses and is also the most important salad vegetable. Lettuce is most popular consumed as raw leaves due to its taste and high nutritional value, such as ascorbic acid, carotenoids and other antioxidant substances. However, lettuce is a hyperaccumulator of nitrates and easily accumulates high nitrates in its leaves (Escobar-Gutierrez et al., 2002). High levels of nitrates (usually nitrate level $\geq 700\ mg\ kg^{-1}$) in edible parts of vegetables have been implicated in increasing the risk of some diseases, such as methemoglobinemia and gastric cancer (Bruning-Fann and Kaneene, 1993). In order to prevent the risk of these diseases, the legal limit of nitrate to trade lettuce in European countries is 50–140 mg per day (Santamaria, 2006). Therefore, exceeded nitrate intake represents a risk for emergence of diseases which has aroused great concern. (Bian et al., 2015; Lin et al., 2013; Pérez-López et al., 2013; Samuolienė et al., 2012).

Nitrate reductase (NR) is one of the key enzymes in regulating nitrate assimilation, which catalyses the reduction of nitrate to nitrite in

*Abbreviations:* CL, continuous light; LEDs, light emitting diodes; $F_v/F_m$, maximal photochemical efficiency of PSII; GS, glutamine synthetase; GOGAT, glutamate synthase; MDA, malondialdehyde; NR, nitrate reductase; NiR, nitrite reductase; NR, nitrate reductase related gene; NiR, nitrite reductase related gene; $P_n$, net photosynthetic rate

* Corresponding authors.
*E-mail addresses:* yangqichang@caas.cn (Q. Yang), chungui.lu@ntu.ac.uk (C. Lu).

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plants (Sivasankar et al., 1997). Regulation of NR involves a hierarchy of transcriptional and post-transcriptional controls (Yanagisawa, 2014). Light and carbohydrates influence NR at the transcription and translation levels. NR expression has been found to influence N uptake and reduction. For example, the AtSIZ1, in Arabidopsis, has been shown to control nitrogen assimilation by promoting sumoylation of NRs (Park et al., 2011). After nitrate reduction, nitrite is reduced to ammonium by the second enzyme of the pathway, the nitrite reductase (NiR). Previous studies have provided evidence that the activities of NiR, glutamate synthase (GOGAT) and glutamine synthetase (GS) can indirectly affect nitrate assimilation in plants (Barneix, 2007; Ruiz et al., 1999; Temple et al., 1998). Furthermore, the co-regulation of *NR* and *NiR* expression is not only important for nitrate assimilation but also a vital mechanism for preventing the accumulation of deleterious metabolic intermediates and energy saving for plant growth, especially under a biotic and/or abiotic stress environment (Małolepsza, 2007).

Light is one of the most important environmental factors in regulating plant growth and development (Kim et al., 2004; Li and Kubota, 2009). For plants, light is not only the driving force for photosynthesis but also the transduction signal to regulate gene expression via photoreceptors. Recently, light-emitting diodes (LEDs) have received considerable attention. LEDs now offer cheap, cool, controllable sources of light that can selectively and quantitatively provide different wavelengths. Previous studies have demonstrated that the combination of red (600–700 nm) and blue light (400–500 nm) is an effective lighting source for plant growth (Bian et al., 2015; Hogewoning et al., 2010). However, other light spectra, such as green light and far-red light, also have profound effects on plant procession via phytochromes and/or cytochromes (Folta and Maruhnich, 2007; Urrestarazu et al., 2016). Green light absorbed by anthocyanins can prevent photo-degradation of light-labile plant defence secondary metabolites, such as thiarubrine A, which is easily degraded after visible light or UV light exposure (Gould et al., 2010). In addition, green light can increase plant defence mechanisms via triggering special gene expression (Nagendran and Lee, 2015; Zhang and Folta, 2012).

We previously reported that adding green light to red and blue light had a positive effect on reducing nitrate content in lettuce under continuous light treatment. The suitable light spectral ratio for red, blue and green light is 4:1:1 (Bian et al., 2016). However, little is known regarding the NR and NiR gene expression and its enzyme activity in lettuce under different light spectra of short-term CL. Therefore, in this study we present (1) nitrate reduction enzyme activities and related gene expression and (2) the edible quality of lettuce under short-term CL by different LED light spectral composition. It also highlights effect of green light on nitrate reduction, edible quality of lettuce and expression of nitrate assimilation related genes under short-term continuous light with red and blue light. The result of this study could enable a better understanding of the effect of green light on nitrate reduction under short-term continuous light for producing high quality leaf vegetables in greenhouse and environment-controlled facilities.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

Lettuce (*Lactuca sativa* L. cv. Butterhead) seeds were sown in plastic trays filled with seed-peat mixture (1:1, v/v) substrate and germinated under fluorescent lamps (TL–D 36W, Philips) with 150 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD) of 12 h $d^{-1}$ in an environmentally controlled chamber. The day/night temperature, $CO_2$ level, and relative humidity in the growth chamber were 25/20 °C, 400 μmol $mol^{-1}$ and 75%, respectively. Water was added daily to maintain the moistness of the substrate and replenish evapotranspiration losses.

When lettuce seedlings had two true leaves, they were transplanted to 40–L containers of Hoagland solution (pH = 6.8 ± 0.2, EC = 1.9 ± 0.1 dS $m^{-1}$). These plants were randomly grown under a combination of red (R, peak at 660 nm) and blue LEDs (B, peak at 460 nm) (R:B = 4:1) or a combination of red/blue light with the addition of green light (G, peak at 530 nm) LEDs (R:B:G = 1:1:1). No-reflect black separators were placed between different light sources to avoid light contamination. To minimize any effects from uneven light between plants, the containers were systematically moved every other day. The PPFD was monitored daily by a spectroradiometer (Avaspec–2048CL, Avantes, Apeldoorn, The Netherlands) and was maintained at 200 μmol $m^{-2}$ $s^{-1}$ by adjusting the distance between the light sources and plant canopies. Other environmental factors were maintained at similar levels to those at the seedling stage. The nutrition solution was renewed every week.

**Table 1**
List of light spectral details and light duration applied for different light treatments.

| Treatments | Before light treatment (from transplanting until the light treatment) | | Light treatment (from the end of the dark period until 20 d after transplanting) | | |
|---|---|---|---|---|---|
| | Light sources | Light spectral ratio | Light sources | Light spectral ratio | Light duration |
| RB-control | RB LEDs | R:B = 4:1 | RB LEDs | R:B = 4:1 | 12 h light/12 h dark |
| RB-CL | | | | | 48 h light/0 h dark |
| RBG-CL | | | RBG LEDs | R:B:G = 4:1:1 | |
| rb-CL | RBG LEDs | R:B:G = 1:1:1 | RB LEDs | R:B = 1:1 | |
| rbg-CL | | | RBG LEDs | R:B:G = 1:1:1 | |

R, red light; B, blue light; G, green light; LEDs, light emitting diodes. RB and rb, combined R and B with a ratio at 4:1 and 1:1, respectively. RBG and rbg, combined R and B plus G with a ratio at 4:1:1 and 1:1:1, respectively. CL, continuous light. The light intensity of all treatments was 200 μmol $m^{-2}$ $s^{-1}$.

### 2.2. Light treatment

At the end of the dark period, 20 d after being transplanted, plants were transferred to environment-controlled growth chamber (temperature 25 °C) under PPFD of 200 μmol $m^{-2}$ $s^{-1}$. There were five treatments. The details of these treatments are summarized in Table 1. The plants grown under red and blue LEDs were treated with CL (RB-CL) or were treated with supplemental green LEDs (RBG-CL). The light ratios for RB-CL and RBG-CL were 4:1 and 4:1:1, respectively. The plants grown under red and blue LEDs (R:B = 4:1) with a photoperiod of 12 h were used as control (RB-control). Furthermore, plants grown under red, blue and green LEDs were randomly divided into two groups. One group was treated with CL using previous LED light sources (rbg-CL, r:b:g = 1:1:1), while the other received CL treatment by previous LED light sources but without green light LEDs (rb-CL, r:b = 1:1). rbg-CL was used to imitate the light spectra of white light–the most common standard illuminants used as target white points for RGB mixing calculations (Boray et al., 1989; Park et al., 2012). rbg-CL was used to further investigate if there is any different effect of green light on regulating nitrate metabolism when compared with rb-CL and RBG-CL. Adding rbg-CL treatment to this study could have an impact and value to the practical application. During the experiment, other environmental conditions were set as similar to those at the seedling stage. There were four replicates per treatment with 48 plants in total.

### 2.3. Measurements of net photosynthetic rate and chlorophyll fluorescence

The second-youngest and fully expanded leaves were used for monitoring the net photosynthetic rate ($P_n$) and chlorophyll fluorescence using a portable photosynthetic apparatus with a fluorescent