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Research article

Effect of Post-harvesting with Different Photoperiods under Artificial Light Sources on Nitrate and Vitamin C Contents in Hydroponic Green Oak Lettuce

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Abstract: Green oak lettuce (*Lactuca sativa* L.) is a popular vegetable for consumers, but it is concerned about nitrate contamination that may harm human health. However, light can affect nitrate reduction and contribute to the accumulation of vitamin C in vegetables. Therefore, this study focused on the effects of post-harvesting with different photoperiods under artificial light sources on vitamin C and nitrate content in hydroponic green oak lettuce. Green oak lettuces were grown in the NFT system, harvested, and post-harvested under Bulb-LED (Experiment I), Bar-LED (Experiment II), and fluorescent lamp (FL) (Experiment III) for 6, 12, and 24 h photoperiods and replaced the nutrient solution with tap water. The nitrate content was significantly reduced after post-harvesting for 12 h photoperiods under FL (9,012 µg NO_3^- -N/g dry weight) followed by Bulb-LED (13,985 µg NO_3^- -N/g dry weight) and 24 h photoperiods for Bar-LED (10,727 µg NO_3^- -N/g dry weight). Vitamin C content was highest after post-harvesting for 24 h photoperiods under Bar-LED (45.47 µg/ml), followed by Bulb-LED (44.73 µg/ml) and FL (35.40 µg/ml). Post-harvesting with artificial light sources for 12 to 24 h photoperiods can improve hydroponic green oak lettuce quality.

Keywords: Green oak lettuce; nitrate; vitamin C; hydroponic system; photoperiods; post-harvest; artificial light source

1. Introduction

Recently, vegetables have been playing an important role in promoting human health and helping people avoid COVID-19, which affects human health worldwide [1]. Because vegetables contain phytochemicals such as flavonoids, carotenoids, soluble sugars, proteins, vitamin E, and vitamin C, suitable for human health [2,3]. In particular, vitamin C is key in promoting human immunity by supporting several innate immune system cellular functions [4]. In plants, vitamin C is an abundant component in all cell compartments. A biosynthetic of the vitamin C begins from D-glucose-6-P (product of photosynthesis), D-fructose-6-P, GDP-mannose, GDP-L-galactose, L-galactose, and L-galactono-1,4-lactone. Photosynthesis plays a major role in the biosynthetic of vitamin C.

Alternatively, light is the key factor in promoting vitamin C content in the plant [4]. Dowdle et al. [5] reported that vitamin C could be increased 20-fold by exposure to high light intensity for 24 h. Laing et al. [6] found that photon flux density levels (PFD) affect vitamin C content after 2 days.

Lettuce (*Lactuca sativa* L.) has a high nutritional value for humans; it is rich in minerals and vitamins. Aćamović-Djoković et al. [7] researched vitamin C content in several lettuce varieties, and it was in the range of 3.50–9.60 mg/100 g of fresh weight. However, lettuce is not always good for consumer health as people have concerned about excessive nitrate content, especially in hydroponic lettuce.

Nitrate is a nitrogen source that is the essential plant nutrient, a main constituent of chlorophyll, protein, and genetic material [8]. However, high intake of nitrate content by consumers may cause several types of cancer, such as bladder cancer [9], gastric cancer [10], and prostate cancer [11]. After consuming excessive nitrate, saliva, and gastric juice in the human body include nitrate reductase that can reduce nitrate to nitrite [12-13]. Nitrite has formed N-nitroso compounds, which are carcinogenic and can cause a variety of cancers [12–14]. For the plant, nitrate is needed, and it is taken up through the root hair, xylem, mesophyll cells, and cell walls. Then nitrate is reduced into nitrite, ammonium, glutamine acid, and other amino acids [15]. The process of nitrate reduction in plants needs energy from the photosynthesis system. The energy from photosynthesis has activated the suitable light wavelength [8].

Nitrate can be reduced by pre-harvest or post-harvest, especially post-harvest. Post-harvesting reduces health risks while preserving and improving the quality of fruits and vegetables. Recently, light has been used as one of the options for post-harvest treatment tools because artificial light technology has been successfully developed in the horticulture industry. For example, post-harvest artificial light has affected the chemical composition and bioactive compounds of tomatoes, garlic, and lettuce [16,17]. Perera et al. [18] found that continuous lighting and photoperiod with UV-B and UV-C irradiation affect senescence and deterioration delays. Liu et al. [19] reported that irradiation of white light, red light, and UVC increases citrus quality after post-harvest. Nassarawa et al. [20] and Poonia et al. [21] found that LEDs can accumulate phytochemicals and antioxidants, reduce microbial spoilage, reduce senescence delay, extend shelf life, and increase disease resistance and nutritional quality. However, little is known about artificial light sources' vitamin C and nitrate content. Thus, this study mainly focused on the effects of post-harvesting with different photoperiods under artificial light sources on vitamin C and nitrate content in hydroponic green oak lettuce.

2. Materials and Methods

2.1 Plant materials and growth conditions

The green oak lettuce (*Lactuca sativa* L.) was used in this experiment. The lettuce plants were grown in a Nutrient Film Technique (NFT) hydroponic system with open air under 50% aluminum net shade for 28 days. The hydroponic nutrient solution was applied at an EC of 1.5–2.0 mS/cm and pH of 5.5–6.5. Formular A of nutrient solution contained Magnesium Sulphate ($MgSO_4{\cdot}7H_2O$, 500 g/10 L), Potassium Nitrate (KNO_3, 780 g/10 L), Mono Ammonium Phosphate ($NH_4H_2PO_4$, 130 g/10 L), Mono Potassium Phosphate (KH_2PO_4, 100 g/10 L), Manganese EDTA (MnEDTA, 8 g/10 L), Micro element (Boron EDTA, MnEDTA, MgO, CuEDTA, MoEDTA and FeEDTA, 10 g/10 L). Calcium Nitrate ($Ca(NO_3)_2\,4H_2O$, 1000 g/10 L) and Iron Chelate (FeEDTA, 10 g/10 L) were included in Nutrient Solution Formular B [22]. After 28 days of growth in the NFT system, lettuce plants were transferred into post-harvest treatments by lighting.

2.2 Light conditions and treatments

Lettuce plants were moved and placed into a post-harvest chamber with three shelves. The shelf has three cells [80 length x 40 width x 30 height (cm)] (More information is available in the previous experiment [23]). This post-harvest chamber was set up with three different artificial light sources 1); Bulb-LED (1:1:1 ratio of blue 460 nm: red 630 nm: red 660 nm, PPFD 2005.05 ± 53.62 μmol/m^2/s), 2); Bar-LED (2:1:1 ratio of blue 460 nm: red 630 nm: red 660 nm, PPFD 256.32 ± 11.56 μmol/m^2/s) and 3); fluorescence light (FL) (spectral wavelength 400 – 700 nm PPFD; 51.64 ± 11.20 μmol/m^2/s). The nutrient solution in the post-harvest chamber was replaced by tap water using DFT (Deep Floating Technique) system and supplied oxygen with an air stone bubbler.

Thus, the study was designed into three experiments, including 1) the effect of post-harvesting with different photoperiods under Bulb-LED source on vitamin C and nitrate contents in hydroponic green oak lettuce;

2) the effect of post-harvesting with different photoperiods under Bar-LED source on vitamin C and nitrate contents in hydroponic green oak lettuce; and 3) the effect of post-harvesting with different photoperiods under FL source on vitamin C and nitrate contents in hydroponic green oak lettuce. All three experiments were conducted using the same method, except for the light condition. These experiments were replicated three times and performed at the Laboratory of Urban Agriculture Technology, Division of Agricultural Technology, Department of Agricultural and Fishery Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus.

2.3 Determination of nitrate content

Lettuce leaves were harvested and washed with a running tap and distilled water three times. Then, the lettuce leaves were dried at 65 °C for 48 h. The samples were weighed to 1 g after being ground and blended, suspended in 10 mL of distilled water, allowed to stand at 45 °C for 1 h, and filtered through No. 40 Whatman filter paper. The extraction solution was added to 0.1 mL in a 50 mL tube with 0.4 mL of 5% (w/v) salicylic acid (Ajax Finechem, Australia). After that, the samples were left at room temperature for 20 min. Then, 9.5 mL of 2N sodium hydroxide (NaOH) solution was added to the samples. The absorbance of 412 nm (Biochrom, Libra S12, England) was used immediately for determination [24].

2.4 Determination of vitamin C content

The method of vitamin C determination was modified by Jagota and Dani [25]. Fresh samples were used, cut into small pieces, and weighed for 7 g. The samples were then homogenized and 30 ml of oxalic acid (0.5% w/v) was added. Moreover, homogenous extract solutions were filtered through No. 40 Whatman filter paper. 1 mL of homogeneous extract solution was mixed with 4 mL of 10% trichloroacetic acid, shacked, and placed on ice for 5 min. Then, the extract solution was centrifuged at 8,000 rpm for 5 min. After that, 3 mL of the extract solution was used, 0.2 mL of 0.2 M Folin-Ciocalteu reagent was added, and the extract solution was left at room temperature for 60 min. The absorbance at 760 nm was measured with a spectrophotometer (Biochrom, Libra S12, England).

2.5 Statistical analysis

The statistics of these studies were performed with a completely randomized design (CRD). A one-way analysis of variance (ANOVA) was used for data analysis with MS Excel software (version 7.0). Treatment means were analyzed by Least Significant Difference (LSD) at a confidence level of 95%.

3. Results

3.1 Experiment I: Effect of post-harvesting with different photoperiods under Bulb-LED on nitrate and vitamin C contents in hydroponic green oak lettuce

This experiment studied the effects of post-harvesting with different photoperiods under a Bulb-LED source on nitrate and vitamin C contents in hydroponic green oak lettuce. The nitrate content ranged from 13,984-27,016.46 μg NO_3^- -N/g dry weight, as shown in Figure 1A. The nitrate content decreased significantly after post-harvesting with Bulb-LED for 6, 12, and 24 h photoperiods, but 12 h treatment did not show a significant difference with 24 h treatment. The nitrate content was the lowest under post-harvested for 12 h (13,984 μg NO_3^- -N/g dry weight, 48% reduction), followed by treatments of 24 h (14,293.55 μg NO_3^- -N/g dry weight, 47% reduction), 6 h (16,248.29 μg NO_3^- -N/g dry weight, 40 % reduction) and control (27,016.46 μg NO_3^- -N/g dry weight) (Figure 1A).

In contrast, the vitamin C content was increased significantly under different photoperiods in hydroponic green oak lettuce after post-harvest with Bulb-LED for 12 (37.67 μg/ml, 22% increased) and 24 h (44.78 μg/ml, 45% increased) of photoperiod when compared with control treatment. However, the vitamin C content in the earlier photoperiod of 6 h (28.12 μg/ml) was decreased to 9%, which suggests that 12 h of photoperiod is necessary to enhance vitamin C content (Figure 1B).

3.2 Experiment II: Effect of post-harvesting with different photoperiods under Bar-LED on nitrate and vitamin C contents in hydroponic green oak lettuce

This experiment studied the effect of post-harvesting with different photoperiods under Bar-LED on nitrate and vitamin C content in hydroponic green oak lettuce. The nitrate content ranged from 10,727.02 to

27,016.46 μg NO_3^- -N/g dry weight in different photoperiods and it was significantly reduced by 53% to 60% when compared with the control treatment (27,016.46 μg NO_3^- -N/g dry weight) (Figure 2A). The lowest nitrate content was detected in 24 h of photoperiod (10,727.02 μg NO_3^- -N/g dry weight, 60% reduction) and followed by 12 h (12,544.58 μg NO_3^- -N/g dry weight, 54% reduction) and 6 h of photoperiod (12,647.46 μg NO_3^- -N/g dry weight, 53 % reduction), in which no significant difference was observed between them (Figure 2A).

Under post-harvest with Bar-LED of different photoperiods, the vitamin C content varied from 30.13 to 45.47 μg/ml. Although vitamin C content was significantly increased during the 12 h (35.82 μg/ml, 16% increase) and 24 h (45.47 μg/ml, 47% increase) photoperiods, it was decreased during the 6 h (30.13 μg/ml, 2% decreased) photoperiod (Figure 2B). The pattern of vitamin C content in Bar-LED treatment is similar to that of Bulb-LED treatment (Figure 1B, 2B).

3.3 Experiment III: Effect of post-harvesting with different photoperiods under FL on nitrate and vitamin C contents in hydroponic green oak lettuce

This experiment studied post-harvesting effects with different photoperiods under FL on nitrate and vitamin C content in hydroponic green oak lettuce. The nitrate content in different photoperiods ranged from 9,012.35 to 27,016.46 μg NO_3^--N/g dry weight and was significantly reduced from 22% to 67% compared to the control treatment (27,016.46 μg NO_3^--N/g dry weight), as shown in Figure 3A. The lowest nitrate content was detected in 12 h of photoperiod (9,012.35 μg NO_3^- -N/g dry weight, 67% reduction) which was almost three times the reduction and followed by 6 h (14,327.85 μg NO_3^- -N/g dry weight, 47% reduction) and 24 h (21,117.97 μg NO_3^- -N/g dry weight, 22 % reduction) than the control treatment (Figure 3A).

Hydroponic lettuces were post-harvested with FL and measured for vitamin C content in different photoperiods. The results showed that the vitamin C content increased by 6% to 15% in different photoperiods compared with the control treatment (30.87 μg/ml) and ranged from 30.87 to 35.40 μg/ml, as shown in Figure 3B. Unlike the post-harvest with Bar-and Bulb-LED lighting, the vitamin C content was significantly increased in hydroponic lettuce after being post-harvested with FL for 6, 12, and 24 h. The vitamin C content showed the highest under post-harvested for 24 h (35.40 μg/ml, 15% increase) followed by 12 h (34.56 μg/ml, 12% increase), 6 h (32.71 μg/ml, 6% increase) and control (30.87 μg/ml, 0% increased) in which 12 h photoperiod treatment was not a significant difference with 24 h treatment. It could be concluded that post-harvest treatment by Bar-LED, Bulb-LED, and FL decreased nitrate content in all different photoperiods, indicating that post-harvest by light can improve the quality of vegetables for human consumption. In addition, except for 6 h of photoperiod in the Bar-LED and Bulb-LED treatments, vitamin C content was significantly increased in other photoperiods and all of the FL, compared to the control treatment. This result also showed that post-harvest with different lights could improve the quality of lettuce by enhancing vitamin C content.

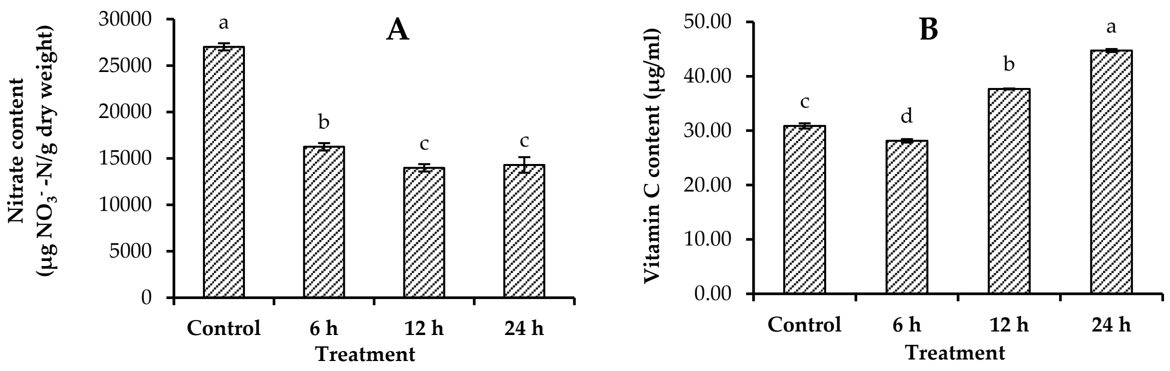

Figure 1. Effect of post-harvesting with different photoperiods under Bulb-LED on nitrate content (A) and vitamin C content (B) in hydroponic green oak lettuce. The error bars represent the standard deviation over three replications. The means denoted by the various letters differ significantly at p < 0.05, according to the least significant difference.

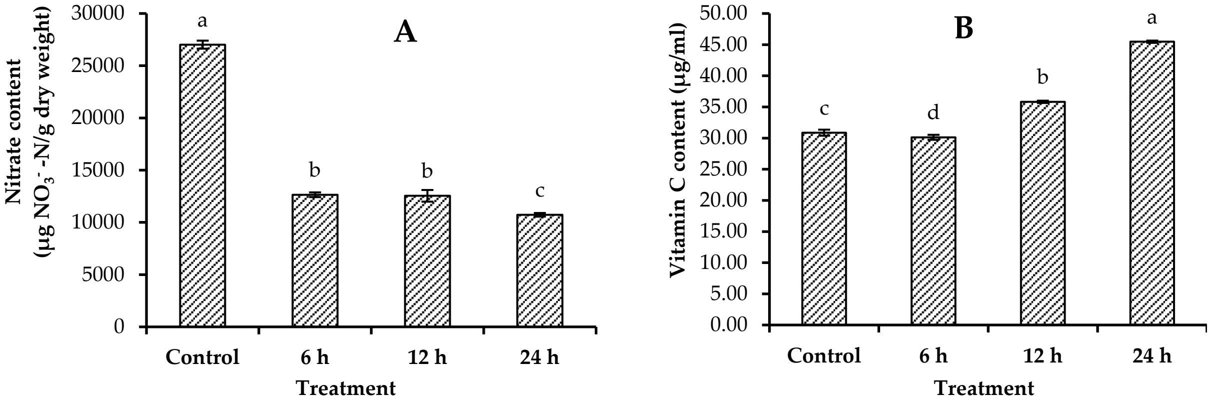

Figure 2. The effect of post-harvesting with different photoperiods under Bar-LED on nitrate content (A) and vitamin C content (B) in hydroponic green oak lettuce. The error bars represent the standard deviation over three replications. The means denoted by the various letters differ significantly at $p < 0.05$, according to the least significant difference.

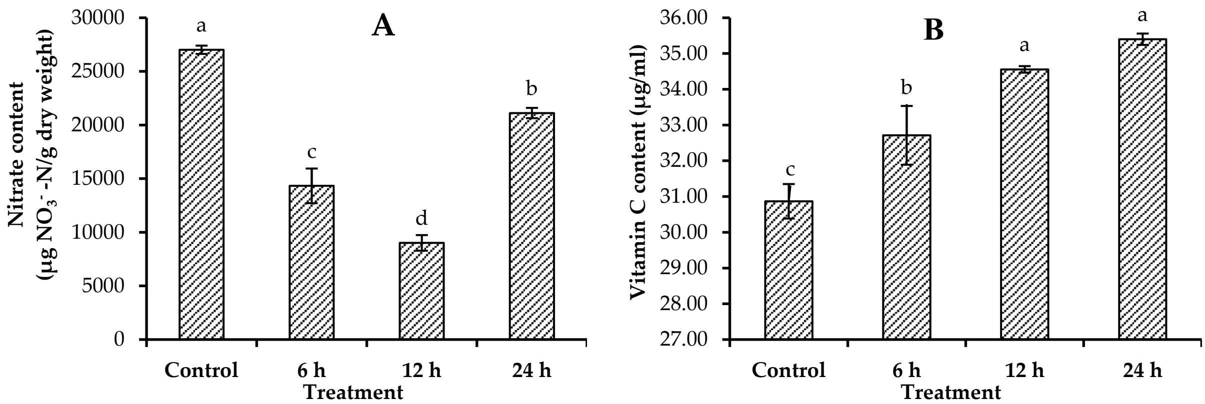

Figure 3. The effect of post-harvesting with different photoperiods under FL on nitrate content (A) and vitamin C content (B) in hydroponic green oak lettuce. The error bars represent the standard deviation over three replications. The means denoted by the various letters differ significantly at $p < 0.05$, according to the least significant difference.

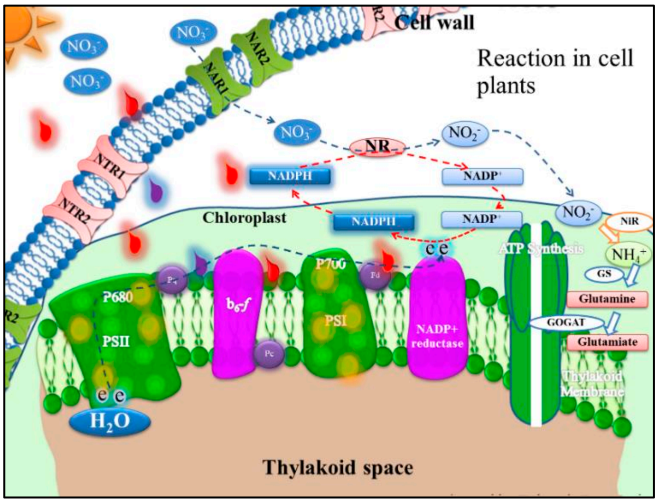

Figure 4. The mechanism of light affects nitrate reduction in plant cells (Adapted from Chow [26] and Sanz-Luque et al. [27]). Water molecules (H_2O) break down molecules and release two electrons in photosynthesis II (PSII P680). Two electrons move to photosynthesis I (PSI P700), and NADP+ reductase uses these two electrons to change NADP+ to $NADPH_2$. $NADPH_2$ carries two electrons from the chloroplast to the cytosol. Two electrons from $NADPH_2$ activate Nitrate Reductase (NR) in the cytosol for the conversion of nitrate molecules (NO_3^-) to nitrite molecules (NO_2^-). The nitrite molecule moves into the chloroplast and has been changed to ammonium by Nitrite Reductase (NiR). Then, ammonium is transformed into glutamin and glutamate acids by the glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (GOGAT), respectively.

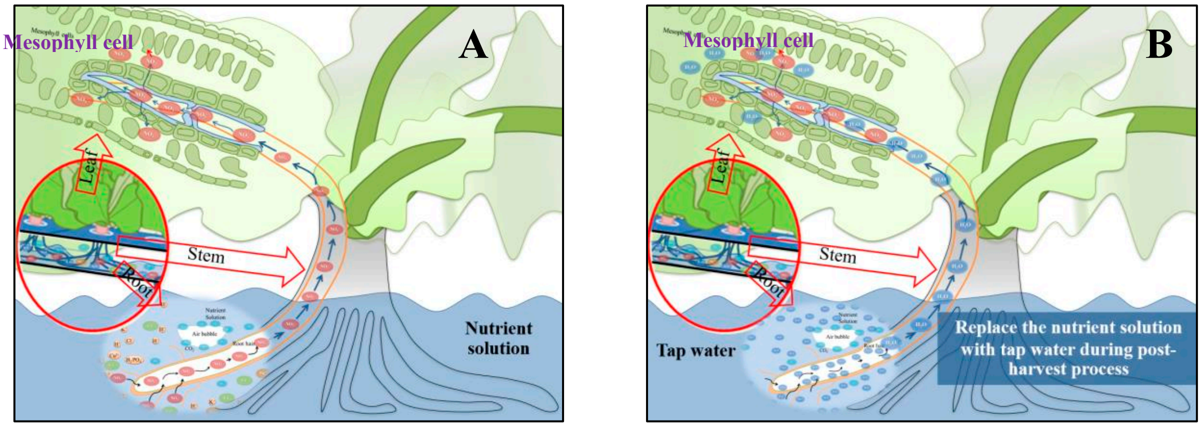

Figure 5. The root zone before post-harvest shows nitrate transportation via xylem vessel under conditions of nutrient solution (A). The root zone is shown during post-harvest with photoperiods and replacing the nutrient solution with tap water (B).

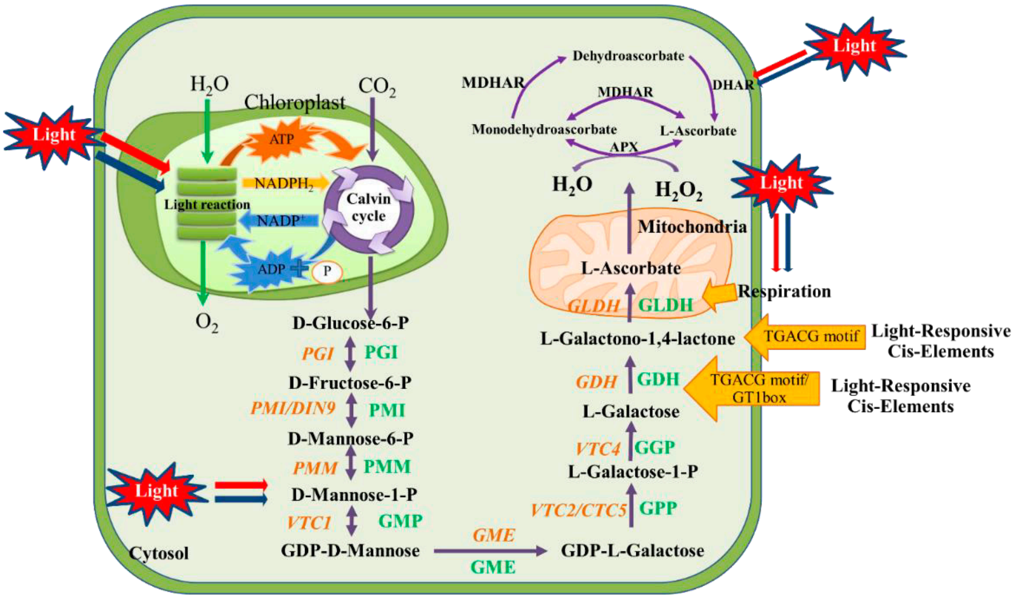

Figure 6. Summary of the effects of light on vitamin C (ascorbate) biosynthesis (adapted from Rosado-Souza et al. [32] and Paciolla et al. [31]). The genes of the pathway are highlighted in orange and written in italics. The enzymes are highlighted in green. Abbreviations: PGI, Phosphoglucose isomerase; PMI, phosphomannose isomerase; PMM, phosphomannomutase; VTC1, Vitamin C1; GMP, GDP-D-mannose pyrophosphorylase, GME, GDP-mannose-30-50-epimerase; GGP, GDP-L-galactose transferase; GPP, L-galactose-1-phosphate phosphatase; GDH, GDP-L-galactose transferase; GLDH, L-galactono-1,4-lactone dehydrogenase; APX, ascorbate peroxidase; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase, the details show in the text.

4. Discussion

Nitrate content has been reduced after post-harvest because light acts to produce electrons in the photosynthesis process. The electrons have been received by NADP+ and ADP+, which have been converted to $NADPH_2$ and ATP, respectively. $NADPH_2$ and ATP provide energy to activate Nitrate Reductase and Nitrite Reductase, which catalyze nitrate to nitrite and nitrite to ammonium. Ammonium then assimilates to glutamine and glutamate acids by GS and GOGAT (Figure 4) [27,28]. However, the nitrate assimilation process helps to assimilate nitrate to glutamate acid, but nitrate residue in the lettuce remains in the xylem vessel of the root, stem, petiole, and leaf veins [29]. As mentioned in materials and methods, these experiments were designed to replace the nutrient solution with tap water to clean up the xylem vessel of roots, stems, and leaves. After the nitrate assimilation process, tap water occurs in a xylem vessel, as shown in Figure 5. Thus, the results of these experiments indicated that nitrate was reduced after post-harvest light treatment. These results are similar to those of Guffanti et al. [30], Wanlai et al. [31], and Cometti et al. [32].

These experiments studied effect of post-harvesting effects with different photoperiods under artificial light sources on nitrate and vitamin C content in hydroponic green oak lettuce. We found that vitamin C content was increased by -2% to 47% when compared with the control treatment, and the results of vitamin C content ranged from 30.13 to 45.47 µg/ml, as shown in Figure 1B-3B. The vitamin C content significantly differed in hydroponic green oak lettuce after post-harvest with all light sources for 6, 12, and 24 h, except for Bar-LED and Bulb-LED for the 6 h treatment. The vitamin C content showed the highest under post-harvested Bar-LED for 24 h following Bulb-LED for 24 h, Bulb-LED for 12 h, Bar-LED for 12 h, FL for 24 h, FL for 12 h, FL for 6 h, control, Bar-LED for 6 h, and Bulb-LED for 6 h, respectively. According to the findings of these studies, light is an important factor in vitamin C biosynthesis [4]. Similarly, Dowdle et al. [5] and Laing et al. [6] found that vitamin C content can increase after exposure to high light intensity.

Light is essential for photosynthesis, which generates energy to stimulate the D-Glicose-6-P to D-Mannose/L-Galactose pathway of ascorbate biosynthesis in plants, as shown in Figure 6 [33,34]. Light is responsible for stimulating CO_2 to D-Glucose-6-P in the Calvin cycle by using ATP and $NADPH_2$, which are generated from the photosynthesis process in the chloroplast. Following that, D-Glucose-6-P was converted into D-Fructose-6-P, D-Mannose-6-P, D-Mannose-1-P, GDP-D-Mannose, and D-Fructose-6-P. GDP-L-Galactose, L-Galactose-1-P, L-Galactose, L-Galactono-1,4-lactone (cytosol), and L-Ascorbate (mitochondria) stimulate phosphoglucose isomerase (PGI), phosphomannose isomerase (PMI), phosphomannomutase (PMM), GDP-mannose-30-50-epimerase (GME), GDP-L-galactose transferase (GGP), L-galactose-1-phosphate phosphatase (GPP), L-galactose dehydrogenase (GDH), and L-galactono-1,4-lactone dehydrogenase (GLDH), respectively. Plant genes play the main role in the D-Mannose/L-Galactose pathway of ascorbate biosynthesis in plants, including PGI, PMI/DIN9, PMM, VTC1, GME, VTC2/CTC5, VTC4, GDH, and GLDH, respectively [34]. L-Ascorbate has been stimulated in mitochondria and transported into the cytosol. L-ascorbate has been used to reduce H_2O_2, a relative oxygen species (ROS), and it has been converted to monodehydroascorbate by the APX enzyme. However, the MDHAR enzyme can convert monodehydroascorbate to L-Ascorbate, or it can change to dehydroascorbate and then to L-Ascorbate by DHAR and MDHAR enzymes, respectively [33].

5. Conclusions

This study focused on the effect of post-harvesting with different photoperiods under artificial light sources on vitamin C and nitrate content in hydroponic green oak lettuce. The nitrate content was reduced lowest after post-harvested for 12 h photoperiods under Bulb-LED and FL and 24 h photoperiods under Bar-LED. Vitamin C content was the highest after post-harvested for 24 h photoperiods under Bar-LED, Bulb-LED, and FL. Thus, post-harvesting with artificial light sources for 12 to 24 h photoperiods can help to improve the quality of hydroponic green oak lettuce by increasing vitamin C and reducing nitrate content.

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