Improving Productivity and Quality of Low-potassium Lettuce in a Plant Factory with Artificial Lighting

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人工光型植物工場における低カリウムレタスの生産性および品質向上に関する研究

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Chapter 1

General Introduction

Lettuce is an annual plant belongs to the Asteraceae (or Compositae) family. Its scientific name, *Lactuca sativa*, derived from the Latin word for milk, an allusion to the white substance that is often found when you cut the stem. Lettuce has a long and distinguished history, and its origin traced back to the eastern Mediterranean basin and western Asia. The earliest recorded depictions of the cultivated lettuce appeared in ancient Egyptian tombs dating back to at least 2500 BC (Harlan, 1986). The paintings show lettuce with thick stems and long, narrow pointed leaves (Ryder, 1999). At about the same time, the lettuce also evolved an oilseed form which had long narrow leaves and produced seeds for production of cooking oil (Ryder, 2002). Various types of lettuce began to exist and were quite popular in the Greek and Roman civilizations. The stem type lettuce, likely introduced from Persia, appeared in China between 600 and 900 AD. Lettuce was also been grown in Western Europe since the 15th century and was possibly brought to the America by Columbus during the second voyage in 1494. Many varieties of lettuce subsequent spread to the Western Hemisphere, and the consumption of lettuce had spread across the world since the late 20th century.

Lettuce is a variable species and comprises seven main types (crisphead, romaine or cos, butterhead, leaf, Latin, stem, and oilseed; Ryder 1999) differing morphologically (Figure 1-1). Crisphead lettuce forms a densely packed head with thick crisp leaves and flabellate leaf venation. Romaine lettuce has a large, erect, loaf-shaped head with oblong rigid leaves, and the midrib almost reaches the apex of

the leaf. Butterhead lettuce forms a small, round, loosely formed head with broad, soft, and supple leaves. Leaf lettuce does not form a compact head and all leaves branch from a central stalk. The leaves have various shape (e.g., broad, elongated, lobed, and curled) and various colors (e.g., red and green). Latin lettuce has a small, loose head with thick and crispy leaves and has dark green color. Stem lettuce has swollen elongated stalk and narrow leaves. The stalk can be eaten raw or cooked like asparagus, and the leaves can be eaten raw in a very young stage. Oilseed lettuce has large seeds and usually used for production of a cooking oil.

Nowadays, lettuce is commercially produced in many countries, including Asia, Europe, and North America. Statistically, China is the largest lettuce-producing country in the world. In 2008, the total world lettuce production was 23.5 million metric tons, where China accounted for more than half of the production (12.5 million metric tons). The United States was the second largest lettuce-producing country with the value of 4.11 million metric tons and Spain ranked third with 1.11 million metric tons. The world consumption of lettuce is rising because of its fresh appearance, pleasant aroma, and high nutritional value (Martínez-Sánchez, et al., 2011). In the United States, from the year 2000 to 2013, the per capita consumption of fresh lettuce (romaine and leaf lettuce) increased from 7.8 to 10.6 pounds. Having a salad (mainly consists of lettuce) before a meal is becoming a common habit which is good for people's health.

Lettuce adapts to many growth conditions, but it grows well in a relatively cool season (e.g., spring or fall). Traditionally, lettuce can be grown under open fields or protected cultivation in greenhouses. Temperatures between 15°C to 20°C are ideal for

lettuce growth. Well-drained, cool and loose soil with a slightly acidic pH of 6.0 to 6.5 is the favorite of all lettuce types.

In the 20th century, hydroponic has emerged as a new method of growing plants and is developing rapidly in the recent several decades. Hydroponic is a subset of hydroculture (Figure 1-2A), which can grow plants with nutrients and water, and without soil. In hydroponic agriculture, water is enriched with nutrients, creating a perfectly balanced, pH adjusted nutrient solution; it leads to quicker and more vigorous growth than that of soil-based agriculture. Currently, climate changes (e.g., heavy rains, heavy snows, floods, droughts, and abnormally high or low temperature), air pollutions (e.g., smog and acid rain), and soil pollutions (e.g., salinity and heavy metals) are threatening the worldwide food productions. The 2011 Great East Japan Earthquake and tsunami caused catastrophic damage to the farmlands. It is still difficult to grow plants on farmland because of the high salinity and tsunami deposits. In 2012, the record-breaking drought harmed more than half of the continental United States agriculture. Thus, protected cultivation methods with hydroponic systems are causing more and more attentions since they can protect crops from the adverse environmental conditions, and the hydroponic systems are easy to manage as compared to the soil-based agriculture.

One of the important plant production facilities is “plant factories with artificial lighting” (PFALs), which can produce high quality vegetables with high yield in controlled environment by using less water, nutrients, land, and labor than is possible with conventional agriculture (Table 1-1; Kozai, 2013a; Yamori et al., 2014). PFALs have been already used for commercial production of several hydroponic crops (e.g.,

lettuce, strawberry, herbs, and seedlings) in Japan. Lettuce is one of the foremost leafy vegetables grown in PFALs (Figure 1-2B). The largest PFAL in Japan (Spread Inc., Kyoto, Japan) produces 21,000 lettuce heads per day, and the largest one in Taiwan (Yasai Corp., Taiwan) produces 40,000 per day. Furthermore, in 2016, Japanese vegetable production company Spread will build a lettuce factory in Kyoto which can produce 30,000 heads of lettuce per day, and most of the work will be automated except the germination and seeding processes. However, PFAL, as a new emerging facility, many improvements still need to be done to maximize the PFAL yield and profitability.

Chronic kidney disease (CKD), a progressive loss in kidney function over a period of months or years, is becoming a major public health problem worldwide. According to the recent researches, the amount of Americans aged 30 or older who have some degree of CKD is projected to increase from 13.2% in 2015 to 16.7% in 2030 (Figure 1-3A; Hoerger et al., 2015), and every year millions die prematurely of cardiovascular complications related to CKD. The dietary potassium intake for patients suffering chronic kidney is very restrictive. Vegetables, especially green leafy varieties, are rich in potassium. Therefore, dialysis patients are suggested not to take raw vegetables, and eating raw leafy vegetables (e.g, a salad) is like a dream to them. Fortunately, hydroponic technology has given the dialysis patients a chance to consume raw lettuce since growers could cultivate lettuce with low potassium content by decreasing potassium level in nutrient solution (Figure 1-3B). In Japan, some companies are committed to producing low-potassium lettuce in PFALs. However, there are some difficulties in production, including inconsistent quality of plants, low

plant growth rate, and sodium settling to the bottom of the nutrient solution tank. Potassium, one of three major nutrients required for normal plant growth, is involved in many physiological processes, including photosynthesis, transpiration, and growth and development (Marschner, 1995; Pettigrew, 2008). Decreases in potassium supply to lettuce will absolutely affect plant growth, leading to reductions in plant yield and quality. But few studies have been conducted to analysis the physiological reaction of lettuce to low potassium conditions. It should also be noted that different lettuce types may have different performances under low potassium conditions, due to differences in plant form (Smith et al., 2011), plant vigor (Wallace et al., 2012), and leaf morphology (Mou, 2008). In order to improve the production method of low-potassium lettuce, it is necessary to investigate the response of plant growth and photosynthesis to low potassium conditions in lettuce.

In a PFAL, the nutrient management in hydroponic is to adjust electrical conductivity (EC) of the nutrient solution. This nutrient management maintains relatively high concentration of nutrients, providing plants more nutrients than is necessary. It can lead to excess ion uptake and hence to reduce yield and quality (e.g., high contents of nitrate and potassium in leafy vegetables). Also, the unabsorbed nutrient remained in the residue will cause serious environmental impacts. A quantitative nutrient management method employing a low concentration has been applied to the spinach cultivation, indicating that the nitrate content in leaves can be reduced without decreasing plant growth. Moreover, the nutrient solution at low concentration reduced emissions. It thus provides a possibility to produce

low-potassium lettuce with high quality and to reduce environmental pollution, using the quantitative nutrient management method.

Moreover, because of the vertical cultivation pattern, the mass production of vegetables within a small space is facilitated. A PFAL with 10 tiers of plants can have an annual production capacity of leafy vegetables that is roughly 100-fold that of an open field (Kozai, 2013a). However, the high plant density in the PFALs creates suboptimal conditions; leaves beneath the plant canopy (i.e., outer or lower leaves) suffer from low light conditions owing to shading by the upper leaves and by neighboring plants and therefore senesce faster (Figure 1-4). The senescent leaves become visibly yellow (chlorotic) and wilted (McCabe et al., 2001) from lack of chlorophyll and photosynthetic proteins. This decreases yield and increases labor costs for trimming. Thus, it is important to establish a cultivation method to retard senescence of outer leaves to improve PFAL yield.

In the present study, therefore, I conducted a series of experiments and aimed to explore possible ways to improve the productivity and quality of low-potassium lettuce in a PFAL.

In chapter 2, three lettuce types (i.e., green leaf lettuce, Boston lettuce, and romaine lettuce) with different morphological characteristics were hydroponically grown in nutrient solutions with reduced potassium. The reactions of photosynthesis to reduced potassium were assessed in both mature and newly expanded leaves. I also measured the growth analysis parameters to evaluate the relationship between plant growth and leaf photosynthesis among the different lettuce types.

In chapter 3, I analyzed the growth dynamics, biomass accumulation dynamics,

and nutrient (i.e., nitrogen, phosphorus, potassium, calcium, and magnesium) absorption dynamics of lettuce grown in a PFAL. A quantitative nutrient management method with a modified nutrient recipe was examined on the lettuce yield and quality as compared to the traditional EC-based method.

In chapter 4, lettuces were hydroponically grown in an environment-controlled cultivation room equipped with downward-facing white LEDs, red LEDs, or blue LEDs, same supplemental upward lighting were supplied in the late growth period of lettuces. The effect of light color on photosynthesis and plant growth of lettuce was evaluated. The effect of supplemental upward lighting from underneath the plant on leaf senescence in the outer leaves was analyzed. I also analyzed the economic benefit of this technique.

Table 1-1 Advantages of a plant factory with artificial lighting (PFAL).

Advantages of a PFAL
1. Mass produce vegetables year-round with vertical cultivation pattern.
2. Stable production unaffected by environmental, regional, seasonal limitations.
3. Produce high quality plant with high yield under controlled optimized environment.
4. Shorten the production cycle compared with conventional agriculture.
5. Reduced use of water, nutrients, land, and labor.
6. Produce pesticide-free vegetables.
7. Can cultivate and utilize transgenic plants.

(Kozai, 2013; Yamori et al., 2014)

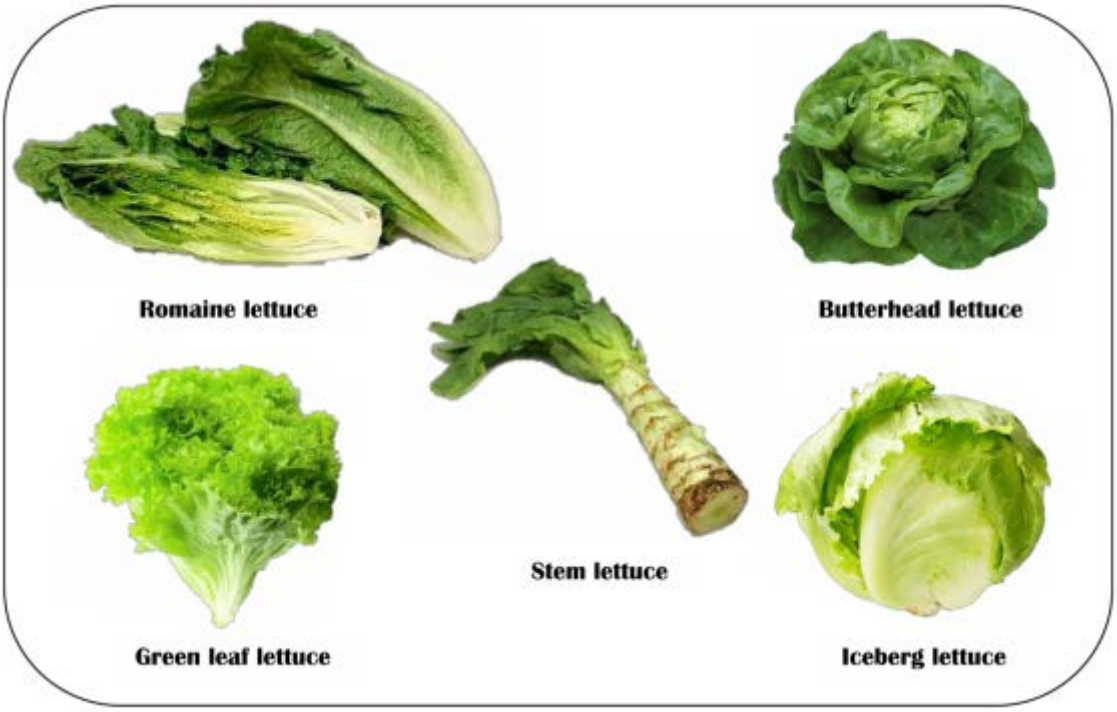

Figure 1-1 Several types of lettuce (*Lactuca sativa*) (Pictures available online: https://www.douban.com/note/515620260/).

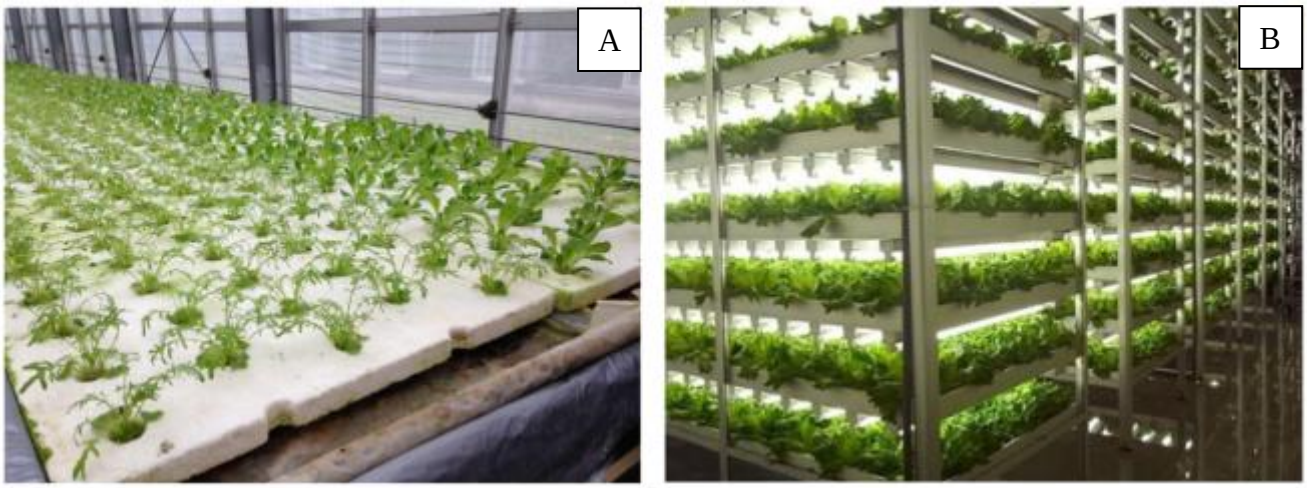

Figure 1-2 Hydroponic leafy vegetables in a greenhouse (A) and in a plant factory with artificial lighting (B).

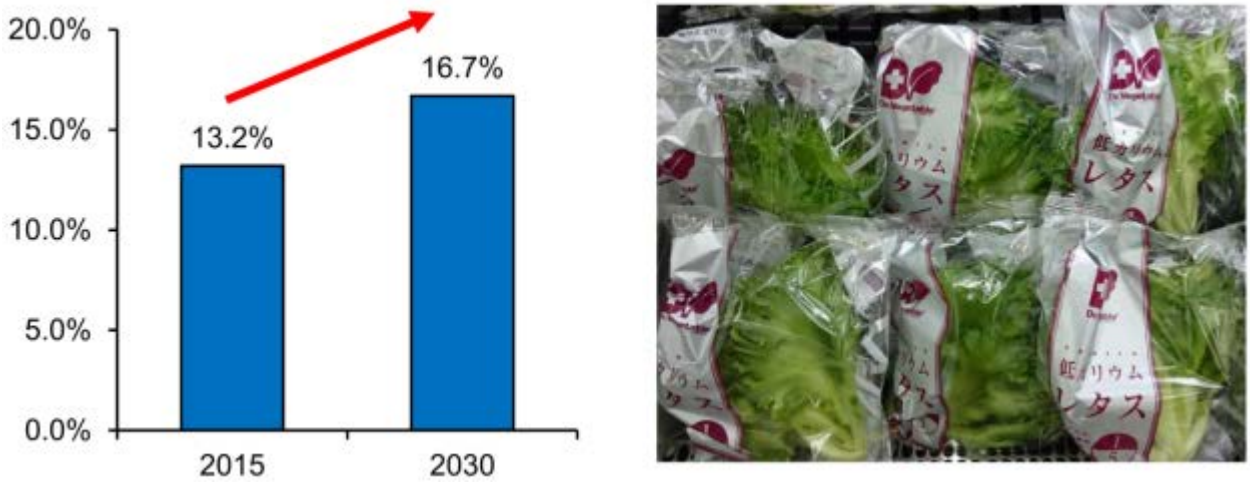

Figure 1-3 Estimated chronic kidney disease (CKD) prevalence in U.S. population (A) (Hoerger et al., 2015) and fresh lettuce with low potassium level (B).

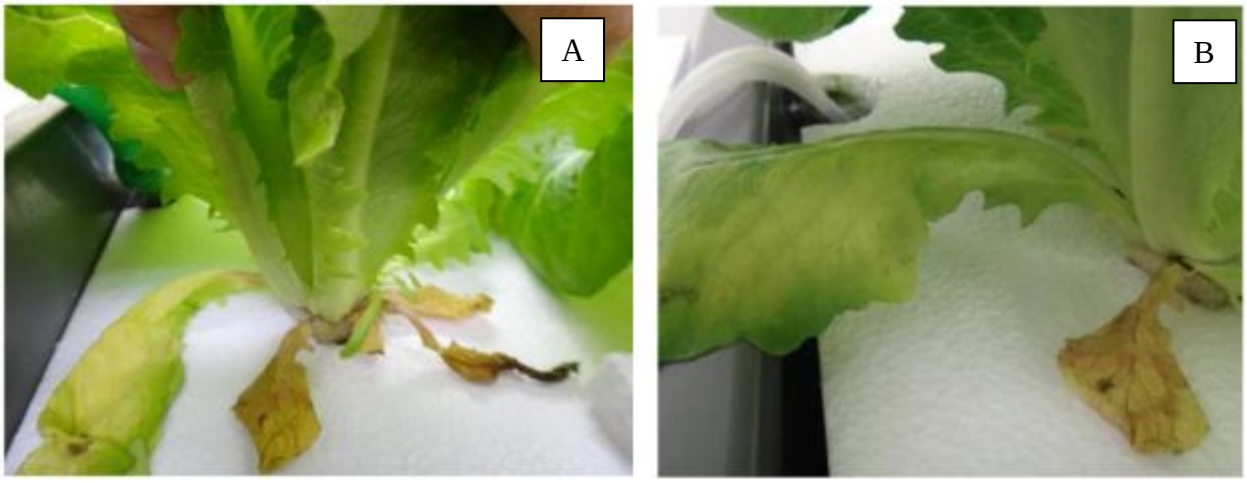

Figure 1-4 Outer leaf senescence in a plant factory with artificial lighting.

Chapter 2: Plant growth and photosynthesis response to low potassium conditions in three lettuce types

Abstract

This study investigates the differences in plant growth and photosynthesis among three lettuce (*Lactuca sativa*) types with different morphological characteristics when grown in reduced potassium nutrient solutions. Lettuce was hydroponically grown in half-strength Enshi formula nutrient solution containing 100% (1/1 K treatment), 50% (1/2 K treatment), or 25% (1/4 K treatment) levels of potassium. Plant yield and relative growth rates (RGR) were lowest under a 1/4 K treatment for all three lettuce types. In green leaf lettuce, the reductions in both net assimilate rate (NAR) and leaf area ratio (LAR) led to a decline in RGR. In Boston lettuce and romaine lettuce, the reduction of RGR was mainly due to a reduction in LAR, and to a lesser extent caused by NAR. Reduced potassium in the nutrient solution had a greater effect on mature leaves than on newly expanded leaves for all three lettuce types. In green leaf lettuce and Boston lettuce, photosynthetic rates of mature leaves significantly decreased under reduced potassium treatments, with a steady or gradually increased intercellular CO_2 concentration; this indicated that non-stomatal factors suppressed the photosynthesis. In romaine lettuce, the photosynthetic rate was less influenced by reduced potassium levels in the nutrient solution, and the significant increase observed in leaf mass per area might contribute to maintaining photosynthesis in the leaf.

Introduction

Vegetables are an essential part of a person's daily diet. Lettuce is one of the most commonly consumed leaf vegetables, providing a variety of nutrients and vitamins (Galieni et al., 2015). Lettuce naturally absorbs a great amount of potassium, and has associated high potassium levels in leaves. Potassium is an essential nutrient for human health and any unused potassium is removed by the kidneys. In patients suffering chronic kidney disease, potassium uptake from food is restrictive. Dietary potassium is generally restricted to 2000–3000 $mg \cdot d^{-1}$ for patients requiring hemodialysis and 3000–4000 $mg \cdot d^{-1}$ for patients requiring peritoneal dialysis (Bajwa and Kwatra, 2013); dietary potassium intake should be limited when serum potassium is > 5 meq/L. In lettuce, potassium content can be partially reduced through boiling or soaking in water. Unfortunately, other nutrients, such as vitamin C and minerals, also lose their nutritional value during these processes. Owing to the recent rapid increase in kidney disease patients (Barsoum, 2006; Zhang and Rothenbacher, 2008), the demand for fresh lettuce with low potassium content has increased.

Potassium is one of three major nutrients required for normal plant growth. It is involved in many physiological processes, including photosynthesis, transpiration, and growth and development (Marschner, 1995; Pettigrew, 2008). Numerous studies suggest that plant growth and yield are strongly affected by different amounts of potassium fertilization (Jordan-Meille and Pellerin, 2004; Lebaudy et al., 2008; Sale and Campbell, 1987). For example, potassium supply is known to increase light utilization efficiency and CO_2 assimilation rate per unit leaf area (Collins and Duke,

1981; Epron et al., 2012), leading to increased plant yield. In contrast, potassium deficiency induces lower light absorption, a decrease in chlorophyll content, and a reduction in photosynthesis rate (Bednarz and Oosterhuis, 1999; Cakmak et al., 1994; Jin et al., 2011), thereby reducing plant yield and quality (Schwarz et al., 2013). Growth analyses, including relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) are used to understand inherent differences in a plant's response to environmental stresses, such as nutrient deficiency (Paul and Ayres, 1986), heavy metal toxicity (Vinit-Dunand et al., 2002), and high or low temperatures (Kurimoto et al., 2004). Few studies have focused on the physiological implications of reduced potassium supply on the growth and development of lettuce.

Generally, a plant grown under low potassium conditions has lower potassium content in the leaves (Asao et al., 2013; Terry and Ulrich, 1973) and the decreased potassium level in leaves is associated with decreased photosynthesis. Potassium plays an important role in the photosynthesis process, where it is involved in stomatal regulation, ATP synthesis, and enzyme activation (Amtmann et al., 2008; Lebaudy et al., 2008; Marschner, 1995; Tombesi et al., 1969). It has been suggested that the decrease in photosynthesis is closely connected with a reduced potassium content in the leaves (Jin et al., 2011; Weng et al., 2007). Many studies reported that potassium deficiency causes a reduction in photosynthesis and transpiration rates via actuation of stomatal closure (Peaslee and Moss, 1968; Römheld and Kirkby, 2010; Thiel and Wolf, 1997), thus suppressing plant growth. However, non-stomatal reductions of photosynthesis have been reported in potassium deficient plants, these are associated with a decline in chlorophyll content, inhibition of PSII activity, and electron transport

(Basile et al., 2003; Jin et al., 2011; Wang et al., 2012). Few studies have examined the effects of reduced potassium supply on photosynthesis in lettuce. Thus, it is hard to conclude whether photosynthesis will decline, and whether a stomatal or non-stomatal factor plays an important role in the photosynthesis reduction of lettuce exposed to a low potassium environment. Furthermore, inhibition of photosynthesis may be different depending on leaf age, as potassium is a mobile element inside plants (Marschner, 1995).

There are many types of lettuces including leaf lettuce (e.g., green leaf lettuce), butterhead lettuce (e.g., Boston lettuce), and romaine lettuce. The morphological characteristics of these three lettuce types are different. Most green leaf lettuce types feature frilly, wrinkled, or puckered leaves, with a tender leaf texture. Boston lettuce has leaves with a soft texture and a thin leaf structure. Romaine lettuce has long, broad, upright leaves, with a firm rib that almost reaches the tip of the leaf and the leaf texture is hard and crispy. These differences in lettuce type may result in different performances under low potassium conditions, due to differences in plant form (Smith et al., 2011), plant vigor (Wallace et al., 2012), and leaf morphology (Mou, 2008). It is therefore necessary to study how each lettuce type responds to reduced potassium in a nutrient solution in terms of plant growth and photosynthesis. In this study, the above three lettuce types were hydroponically grown in nutrient solutions with reduced potassium. First, the reactions of photosynthesis to reduced potassium were assessed in both mature and newly expanded leaves. Second, the relationship between plant growth and leaf photosynthesis among the different lettuce types was assessed by growth analysis at the whole plant level. This study aimed to investigate differences in

plant growth and photosynthesis responses to reduced potassium in nutrient solution among the three lettuce types, facilitating production of fresh lettuce with reduced potassium levels.

Materials and methods

Plant materials and growth conditions

The experiments were carried out from April to June 2015. Seeds of green leaf lettuce (*Lactuca sativa* L. cv. Green wave, Takii seed, Japan), Boston lettuce (*Lactuca sativa* L. cv. Okayama-saradana, Takii seed, Japan), and romaine lettuce (*Lactuca sativa* L. cv. Romana, Takii seed, Japan) were sown in urethane cubes (2.3 cm width, 2.3 cm depth, 2.7 cm height) and the seedlings were cultivated in a growth chamber at 20/17°C (day/night) at 350 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux for 12 h under cool white fluorescent lamps. 15 days after sowing (DAS), uniform seedlings were transplanted into a greenhouse of Chiba University, located in Matsudo city of Japan (long. 35°78′ N, lat. 139°90′ E) on 13 May 2015, and were harvested on 3 June 2015 for green leaf lettuce, on 5 June 2015 for Boston lettuce, and on 7 June 2015 for romaine lettuce. For the entire period of the experiment in the greenhouse, mean air temperatures were 25/20°C (day/night), relatively humidity 70%, and average light intensity (on the cultivation beds) 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants were grown in nutrient film technique hydroponics systems (77 $plants \cdot m^{-2}$, including the border plants) using half-strength Enshi formula nutrient solution (NO_3-N 8, PO_4-P 0.7, K 4, Ca 2, Mg 1 $mmol \cdot L^{-1}$; Asao et al., 2013). Reduced potassium treatments (keeping all other nutrients content constant) were conducted from the transplanting day, with $NaNO_3$

used to replace KNO_3 in nutrient solutions. The treatments were as follows: 1) 1/1 K (control): plants were cultured in half-strength Enshi formula nutrient solution containing 100% level of KNO_3, 2) 1/2 K: plants were cultured in half-strength Enshi formula nutrient solution containing 50% level of KNO_3, and 3) 1/4 K: plants were cultured in half-strength Enshi formula nutrient solution containing 25% level of KNO_3. Electrical conductivity of nutrient solution ranged from 1.47 to 1.54 $dS \cdot m^{-1}$ (pH: 6.8 ± 0.2). In each lettuce type, 5 randomly selected plants from 15 plants, except the border area in each treatment, were assessed for all measurements.

Determination of potassium

On the final harvest day, plants were separated into shoots and roots. Following determination of fresh weight, the shoot was immediately divided into outer and inner leaves. Counting from the lowest leaf, green leaf lettuce, Boston lettuce, and romaine lettuce had 5–6, 10–11, and 8–9 outer leaves, respectively. The remainder of the shoot part consisted inner leaves (green leaf lettuce, 10–12 leaves; Boston lettuce, 14–17 leaves; and romaine lettuce, 11–16 leaves). All plant tissues were dried in an oven at 80°C for at least 72 h, then ground into powder. Potassium content in plant tissues was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Thermo Fisher Scientific, Cambridge, UK). Approximately 0.05 g dry sample powder was dissolved in 8 mL nitric acid through a high performance microwave system (ETHOS One; Milestone, Sorisole, Italy). Following digestion, the dissolution was diluted to a 100 mL volume with deionized water, mixed thoroughly, and potassium content determined.

Gas exchange measurements

Leaf gas exchange parameters were recorded in each harvested plant just prior to the harvest by using a portable gas exchange system (LI-6400; LI-COR, Lincoln, NE, USA). Light was provided by red (peak wavelength: 665 nm) and blue (470 nm) light-emitting diodes (6400-02B; LI-COR, Lincoln, NE, USA). Photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were determined using mature (counting from the lowest leaf, the 4th leaf was used for all lettuce types) and newly expanded leaves (counting from the lowest leaf, the 8th leaf was used for green leaf lettuce, the 13th leaf for Boston lettuce, and the 11th leaf for romaine lettuce). Measurements were carried out between 09:00 and 16:00. The measurement conditions such as light intensity, CO_2 concentration, relative humidity and leaf temperature were 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 390 $\mu mol \cdot mol^{-1}$, 70 ± 5% and 25°C, respectively.

Plant growth measurements

Growth measurements and sampling were performed 15 DAS on the transplanting day and on the final harvest day (when control plant shoot fresh weight reached the commercial harvest weight (90–110 g): 37 DAS for green leaf, 39 DAS for Boston lettuce, and 41 DAS for romaine lettuce). Leaf number, total leaf area, shoot and root fresh weights, and shoot and root dry weights were evaluated on each sampling date. Total leaf areas were determined using a leaf area meter (LI-300; LI-COR, Lincoln, NE, USA) and leaf mass per area (LMA) calculated from leaf dry weight and total leaf area (leaf dry weight/total leaf area). Plant tissue dry weights were obtained after drying at

80°C for at least 72 h. Relative chlorophyll contents of mature and newly expanded leaves were determined using a SPAD analyzer (SPAD-502 Chlorophyll Meter; Konica Minolta, Tokyo, Japan).

Growth analysis parameters, including RGR, NAR, and LAR were calculated using the following equations:

$RGR = (1/W)(\Delta W/\Delta t) = [\ln(W_2) - \ln(W_1)]/(t_2 - t_1)$

where W_1 and W_2 are the total dry weights of the whole plant at times t_1 (i.e., 15 DAS for three lettuce types; the same below) and t_2 (i.e., 37 DAS for green leaf, 39 DAS for Boston lettuce, and 41 DAS for romaine lettuce; the same below),

$NAR = (1/L)(\Delta W/\Delta t) = [(W_2 - W_1)/(t_2 - t_1)] \times [\ln(L_2) - \ln(L_1)]/(L_2 - L_1)$

where L_1 and L_2 are the total leaf areas of the whole plant at times t_1 and t_2, and

$LAR = L/W = [L_1/W_1 + L_2/W_2]/2$

Ascorbic acid and nitrate content

Ascorbic acid and nitrate content in outer and inner leaves of the three types of lettuce were quantified with analysis strips using a reflectometer (RQflex plus; Merck, Darmstadt, Germany) for each treatment (Pantelidis et al., 2007).

Statistical analysis

Data are presented as means ± SD. Significant differences between three potassium treatments for each lettuce type was analyzed by using one-way ANOVA with Tukey's multiple comparison test using statistical software SPSS version 21.0 (at the $P < 0.05$ level).

Results

Plant growth

Reduced potassium in the nutrient solution significantly decreased plant biomass in all lettuce types, with a significant reduction in total leaf area, and relative chlorophyll content, and the 1/4 K treatment showed greater inhibition of plant growth than that of the 1/2 K treatment (Figure 2-1, Table 2-1). Shoot fresh weights of plants grown in the 1/4 K and 1/2 K treatments were significantly decreased, by 24.5% and 39.9% for green leaf lettuce, by 30.5% and 39.1% for Boston lettuce, and by 35.5% and 52.3% for romaine lettuce, respectively (Table 2-1). For plants grown in the 1/4 K treatment, a large reduction of relative chlorophyll content was observed in both mature and newly expanded leaves of green leaf lettuce, mature leaves of Boston lettuce, and the newly expanded leaf of romaine lettuce (Figure 2-1, Table 2-1). The root fresh and dry weights of plants grown in the 1/2 K and 1/4 K treatments decreased in green leaf lettuce and romaine lettuce (Table 2-1). Furthermore, a decline in leaf number and an increase in LMA were observed in romaine lettuce grown in all nutrient solutions with reduced potassium (Table 2-1). Leaf number, total leaf area, LMA, relative chlorophyll content, potassium content, and fresh weight and dry weight were significantly different among the three lettuce types. There was a significant interaction of potassium treatment and lettuce type for total leaf area and relative chlorophyll content.

Corresponding growth parameters were analyzed to evaluate any effects of reduced potassium on plant growth and development. The RGRs of reduced potassium

treatment plants were significant lower than in the control plants for all three lettuce types, and corresponded to final dry weight (Figure 2-2, Table 2-1). Decreases in NAR and LAR resulted in a lower RGR in green leaf lettuce, while the reduction of LAR solely explained the reduction in RGRs in Boston lettuce and romaine lettuce (Figure 2-2). For all three lettuce types, inhibitions of plant growth were more severe in the 1/4 K treatment.

Potassium content in different lettuce parts

In outer leaves, the potassium content of plants grown in the 1/2 K and 1/4 K treatments were reduced by 8.6% and 46.8% for green leaf lettuce, 9.4% and 41.7% for Boston lettuce, and 6.4% and 57.0% for romaine lettuce, respectively (Figure 2-3). In inner leaves, the potassium content of plants grown in the 1/2 K and 1/4 K treatments were reduced by 1.3% and 49.0% for green leaf lettuce, 5.5% and 53.7% for Boston lettuce, and 11.9% and 61.6% for romaine lettuce, respectively (Figure 2-3). For all three lettuce types, the potassium content in total leaves was significantly decreased in both 1/2 K and 1/4 K treatments, and the potassium content in the root was significantly decreased in 1/4 K treatments (Figure 2-3).

Photosynthetic parameters of mature and newly expanded leaves

In the mature leaf, photosynthetic rates of plants grown in the 1/2 K and 1/4 K treatments were reduced by 13.5% and 59.4% in green leaf lettuce, 23.5% and 40.4% in Boston lettuce, and 15.4% and 32.0% in romaine lettuce, respectively (Table 2-2). Stomatal conductance and transpiration rates of green leaf and Boston lettuce showed

similar trends, while intercellular CO_2 concentrations of mature leaves of green leaf lettuce were significant higher in the 1/2 K treatment (Table 2-2).

In the newly expanded leaf, photosynthetic rates of plants grown in the 1/2 K and 1/4 K treatments were reduced by 15.3% and 33.3% in green leaf lettuce, 3.9% and 31.8% in Boston lettuce, and 4.5% and 12.2% in romaine lettuce, respectively (Table 2-2). The transpiration rate of newly expanded leaves of green leaf lettuce showed a similar trend to the photosynthetic rate (Table 2-2). Stomatal conductance and transpiration rates of the newly expanded leaves of romaine lettuce showed the lowest values under a 1/2 K treatment (Table 2-2). The intercellular CO_2 concentration in newly expanded leaves was not affected by reduced potassium levels in the nutrient solution for all three lettuce types (Table 2-2). Photosynthetic rate of mature leaf and newly expanded leaf were significantly different among the three lettuce types. There was a significant interaction of potassium treatment and lettuce type for the photosynthetic rate of mature leaf and newly expanded leaf, stomatal conductance of newly expanded leaf, and intercellular CO_2 concentration and transpiration rate of mature leaf.

Ascorbic acid and nitrate content of lettuce

In outer leaves, the ascorbic acid content in green leaf lettuce grown in the 1/2 K and 1/4 K treatments were increased by 24.0% and 26.0%, respectively (Figure 2-4A). The nitrate contents of green leaf lettuce grown in the 1/2 K and 1/4 K treatments were reduced by 32.7% and 43.8%, respectively (Figure 2-4A). Ascorbic acid and nitrate contents of outer leaves in Boston and romaine lettuce were not affected by reduced

potassium levels in the nutrient solution (Figure 2-4A).

In inner leaves, the nitrate content of green leaf lettuce grown in the 1/2 K and 1/4 K treatments were reduced by 25.5% and 21.1%, respectively (Figure 2-4B). The nitrate content in inner and total leaves of Boston lettuce was lowest in the 1/2 K treatment (Figure 2-4B). Ascorbic acid content in inner leaves of the three lettuce types and the nitrate content in inner leaves of romaine lettuce were not affected by reduced potassium levels in the nutrient solution (Figure 2-4B).

Discussion

Potassium is highly mobile inside plants (Marschner, 1995). Changes in potassium supply to a growing plant can cause significant alterations of potassium content in the plant tissues (Asao et al., 2013; Kaya et al., 2001). In the present study, potassium content in the outer leaves of three lettuce types was significantly decreased under a reduced potassium regime (Figure 2-3). In the mature leaves of all three lettuce leaf types, declines in photosynthetic rate showed the same trend as potassium content in the outer leaves, with all having significantly decreased photosynthetic rates in the 1/4 K treatments (Table 2-2). However, other photosynthetic parameters differed between the three lettuce types. The stomatal conductance of mature leaves of green leaf lettuce decreased with reduced potassium concentration, whereas intercellular CO_2 concentration gradually increased. In Boston lettuce, the stomatal conductance of mature leaves decreased at reduced potassium concentrations, while the intercellular CO_2 concentration remained steady. In romaine lettuce, both stomatal conductance and CO_2 concentration of mature leaves were not affected by a reduced potassium

concentration. These results suggested the major influence of reduced potassium supply on photosynthesis of mature leaves in green leaf lettuce and Boston lettuce was a result of non-stomatal limitations, such as a lower capacity of the CO_2-fixation cycle or a larger mesophyll resistance (Jin et al., 2011; Zhao et al., 2001). The reduction of relative chlorophyll content (Table 2-1) supported the contention that photochemical reactions of photosynthesis might be disrupted in green leaf lettuce and Boston lettuce under a 1/4 K treatment, leading to a decrease of light absorption and utilization in the leaves (Wang et al., 2012). Reductions of potassium content in the inner leaves of the three lettuce types showed similar trends as the outer leaves and with the exception of potassium content in the inner leaves of plants grown under the 1/2 K treatments, which were not significantly decreased in green leaf lettuce and Boston lettuce (Figure 2-3). This might be explained by old leaves translocating nutrients to newly developing leaves in response to environmental stresses (Greitner et al., 1994; Kozlowski et al., 1984). Correspondingly, reduced potassium in the nutrient solution had less of an impact on the photosynthesis of newly expanded leaves than in mature leaves for all three lettuce types. In green leaf lettuce and Boston lettuce, photosynthetic rates of newly expanded leaves were reduced under low potassium treatments, whereas stomatal conductance and intercellular CO_2 concentration were not affected. In romaine lettuce, the photosynthetic rate of newly expanded leaves was not affected by reduced potassium levels (Table 2-2). Plants have developed several ways to cope with unsuitable growth conditions. In romaine lettuce, the photosynthetic rate was maintained in mature leaves subjected to the 1/2 K treatment and the newly expanded leaves were not affected under both reduced potassium treatments (Table 2-2), even

though the potassium content in both mature and newly expanded leaves was significantly decreased in plants grown under both of the reduced potassium treatments (Figure 2-3). The maintenance of photosynthesis in romaine lettuce might be attributed to the observed increase in LMA of leaves (Reddy and Zhao, 2005), as leaves with higher LMAs perform better in resource-poor environments (Wilson et al., 1999).

At the whole plant level, reducing potassium concentration in the nutrient solution led to decreased potassium content in both total leaves and root for all three lettuce types, with the most severe effects observed under the 1/4 K treatment (Figure 2-3). Fresh and dry weights of shoots and roots in the three lettuce types showed similar trends to the potassium content in total plants, with plant biomass lowest under a 1/4 K treatment (Table 2-1). Regarding growth analysis, RGR was significantly decreased with reduced potassium levels in the nutrient solution in all three lettuce types, whereas each lettuce type showed a different response in terms of NAR and LAR. Plant biomass is strongly positively correlated to RGR, and plant growth analysis decomposes RGR into NAR and LAR (Evans, 1972). Therefore, it is important to evaluate NAR and LAR to investigate how physiological and morphological traits cause a reduction of RGR under reduced potassium treatments. In Boston lettuce and romaine lettuce grown under reduced potassium treatments, RGR was significantly decreased with reduced potassium, whereas no significant decrease of NAR was observed for the reduced potassium treatments, indicating that the reduction in RGR was mainly associated with a reduction in LAR, and to a less extent caused by NAR. Leaf area ratio, the amount of leaf area a plant develops per unit total plant mass (James and Drenovsky, 2007), is an important factor that determines inherent growth

rate. A lower LAR prevents a plant from fixing more carbon per unit plant weight (Nagai and Makino, 2009), resulting in a lower plant relative growth rate (Figure 2-2). In green leaf lettuce grown under reduced potassium treatments, the reduction of RGR was not only caused by a decrease in LAR but also by a decline in NAR. Since NAR is an index of leaf photosynthetic capacity, it indicated that the reduced potassium level in the nutrient solution also affected leaf photosynthetic capacity in green leaf lettuce (Vinit-Dunand et al., 2002), resulting in a decline in RGR (Figure 2-2).

The content of both ascorbic acid and nitrate was influenced by reduced potassium levels. Green leaf lettuce grown in the 1/4 K treatments had higher ascorbic acid and lower nitrate content (Figure 2-4) compared with the control plant. Previous studies demonstrated foliar potassium application increases plant qualities, such as increases in soluble solids, ascorbic acid, and β-carotene (Jifon and Lester, 2009; Lester et al., 2005). However, recent studies revealed potassium restriction during a plants' late growth period could increase ascorbic acid in lettuce and spinach (*Spinacia oleracea*) due to accumulation of ascorbate matrix and upregulation of L-galactono-γ-lactone dehydrogenase (Ogawa et al., 2014). Combined with the results of our experiment, it is possible to produce low potassium lettuce with increased functional components by reducing potassium levels in the nutrient solution. Moreover, a reduction of nitrate content was observed in green leaf lettuce (Figure 2-4B). This is in accord with a previous report that lettuce with low potassium content has low nitrate content (Yoshida et al., 2014). Since transpiration rate influences water and nutrient uptake by plants, the significant decrease in transpiration rates of mature and newly expanded leaves in green leaf lettuce under a 1/4 K treatment (Table 2-2) likely results

in lower nitrate content in leaves. Thus, growing green leaf lettuce in a nutrient solution with reduced potassium could prove useful in fulfilling the requirements of lettuce with low levels of potassium and nitrate.

In the present study, $NaNO_3$ was used to replace KNO_3 to reduce potassium levels in nutrient solutions. Previous studies (Flowers and Läuchli, 1983; Lindhauer et al., 1990; Marschner, 1971) have suggested that Na^+ has the potential to replace K^+ in certain non-specific physiological functions, such as osmoregulation. In this study, reduced potassium in nutrient solution inhibited plant growth and photosynthesis in three lettuce types (Figure 2-2, Table 2-2), suggesting that the Na^+ may only partially substitute K^+ in lettuce, which is consistent with the reports of Subbarao et al. (2003). Because this substitution ability is different between plants, further research is needed to better understand how Na^+ and K^+ affect plant growth and photosynthesis in different plants.

We concluded that reduced potassium in the nutrient solution decreased the growth and yield of three lettuce types. The decrease in RGR in green leaf lettuce was explained by the reductions in both NAR and LAR. The decrease in RGR in Boston lettuce and romaine lettuce was mainly due to a reduction in LAR. For all three lettuce types, mature leaves exhibited a significant decline in photosynthesis compared with newly expanded leaves under low potassium conditions. Non-stomatal factors suppressed the photosynthesis in green leaf lettuce and Boston lettuce, whereas the increased LMA might lead to the maintenance of photosynthesis in romaine lettuce. The quality of green leaf lettuce was increased by reducing the potassium level in the nutrient solution. However, the low yield of lettuce plants where the potassium level in

the nutrient solution is reduced right after transplanting suggests that the method used in the present study is unsuitable for low-potassium lettuce culture. In our future research, we will test quantitative nutrient management to improve the growth of low-potassium lettuce.

Table 2-1 Effect of using different potassium levels in the nutrient solution on leaf number, total leaf area, leaf mass per area (LMA), relative chlorophyll content (SPAD), shoot and root fresh weight, shoot and root dry weight of lettuce at final harvest time.

Lettuce type	Treatment[z]	Leaf number	Total leaf area (m^2)	LMA ($g \cdot m^{-2}$)	Relative chlorophyll content (SPAD)		Fresh weight (g)		Dry weight (g)	
					Mature leaf[y]	Newly expanded leaf	Shoot	Root	Shoot	Root
Green leaf	1/1 K	18.2 ± 2.7 a[x]	0.277 ± 0.029 a	18.9 ± 0.7 a	25.7 ± 2.0 a	26.2 ± 1.6 a	100.7 ± 11.2 a	10.7 ± 1.6 a	5.26 ± 0.69 a	0.688 ± 0.141 a
	1/2 K	16.2 ± 1.6 a	0.192 ± 0.020 b	20.2 ± 2.0 a	25.7 ± 2.7 a	25.0 ± 2.2 a	76.0 ± 8.3 b	6.2 ± 0.7 b	3.90 ± 0.76 b	0.466 ± 0.049 b
	1/4 K	15.2 ± 1.3 a	0.136 ± 0.020 c	20.7 ± 1.6 a	17.5 ± 1.8 b	20.7 ± 1.3 b	60.5 ± 9.2 b	5.0 ± 0.9 b	2.82 ± 0.58 b	0.358 ± 0.085 b
Boston	1/1 K	28.2 ± 3.4 a	0.332 ± 0.049 a	16.4 ± 1.6 a	30.9 ± 1.8 a	30.1 ± 1.6 a	97.9 ± 11.6 a	11.1 ± 1.8 a	5.23 ± 1.14 a	0.660 ± 0.184 a
	1/2 K	25.4 ± 1.6 a	0.171 ± 0.029 b	18.2 ± 1.6 a	28.8 ± 0.9 a	28.2 ± 0.9 a	68.0 ± 12.3 b	8.0 ± 2.2 a	3.10 ± 0.49 b	0.392 ± 0.087 a
	1/4 K	24.0 ± 0.9 a	0.169 ± 0.036 b	17.6 ± 1.3 a	26.1 ± 1.3 b	28.8 ± 1.6 a	59.6 ± 13.0 b	8.2 ± 2.2 a	2.94 ± 0.49 b	0.386 ± 0.083 a
Romaine	1/1 K	24.8 ± 1.6 a	0.181 ± 0.016 a	21.8 ± 0.7 b	34.4 ± 1.8 a	37.2 ± 1.6 a	95.4 ± 7.8 a	9.9 ± 0.7 a	3.96 ± 0.45 a	0.470 ± 0.060 a
	1/2 K	23.0 ± 1.6 a	0.118 ± 0.018 b	23.6 ± 0.9 a	34.3 ± 1.6 a	36.4 ± 1.6 ab	61.5 ± 11.0 b	8.4 ± 1.4 a	2.81 ± 0.54 b	0.374 ± 0.060 b
	1/4 K	20.2 ± 0.9 b	0.087 ± 0.011 c	24.0 ± 0.9 a	31.8 ± 1.8 a	33.3 ± 2.2 b	45.5 ± 6.0 c	5.4 ± 0.4 b	2.08 ± 0.22 c	0.294 ± 0.031 b

[z]1/1 K, 1/2 K, and 1/4 K denote plants grown in half-strength Enshi formula nutrient solution containing 100%, 50%, and 25% levels of KNO_3, respectively.

[y]Mature leaves were derived from outer leaves, whereas newly expanded leaves were derived from inner leaves.

[x]Values are means ± SD (n = 5). Different letters following values in rows under the same lettuce type refer to a significant difference between the three potassium treatments according to Tukey's HSD test ($P < 0.05$).

Table 2-2 Effect of using different potassium levels in the nutrient solution on photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate of lettuce.

Lettuce type	Treatment[z]	Photosynthetic rate ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)		Stomatal conductance ($mol\ H_2O \cdot m^{-2} \cdot s^{-1}$)		Intercellular CO_2 Concentration ($\mu mol \cdot mol^{-1}$)		Transpiration rate ($\mu mol\ H_2O \cdot m^{-2} \cdot s^{-1}$)	
		Mature leaf[y]	Newly expanded leaf	Mature leaf	Newly expanded leaf	Mature leaf	Newly expanded leaf	Mature leaf	Newly expanded leaf
Green leaf	1/1 K	15.5 ± 0.5 a[x]	14.4 ± 0.7 a	0.254 ± 0.012 a	0.286 ± 0.038 a	278.8 ± 3.1 b	295.7 ± 6.6 a	2.68 ± 0.05 a	3.37 ± 0.24 a
	1/2 K	13.4 ± 1.0 b	12.2 ± 0.7 b	0.222 ± 0.026 ab	0.230 ± 0.047 a	280.0 ± 14.7 b	290.3 ± 14.0 a	2.08 ± 0.24 b	2.98 ± 0.45 ab
	1/4 K	6.3 ± 0.5 c	9.6 ± 0.3 c	0.183 ± 0.033 b	0.212 ± 0.021 a	325.2 ± 13.1 a	306.2 ± 6.7 a	1.70 ± 0.33 b	2.37 ± 0.10 b
Boston	1/1 K	16.6 ± 0.7 a	15.4 ± 0.9 a	0.316 ± 0.024 a	0.273 ± 0.050 a	293.1 ± 10.9 a	283.5 ± 10.2 a	2.34 ± 0.38 a	3.15 ± 0.38 a
	1/2 K	12.7 ± 1.9 b	14.8 ± 0.2 a	0.281 ± 0.031 a	0.315 ± 0.055 a	305.2 ± 5.7 a	300.1 ± 15.1 a	2.52 ± 0.16 a	3.06 ± 0.45 a
	1/4 K	9.9 ± 0.5 b	10.5 ± 1.0 b	0.160 ± 0.026 b	0.220 ± 0.033 a	279.9 ± 17.0 a	301.8 ± 6.9 a	1.39 ± 0.17 b	2.30 ± 0.21 a
Romaine	1/1 K	16.9 ± 1.9 a	15.6 ± 1.0 a	0.258 ± 0.047 a	0.324 ± 0.028 a	268.3 ± 16.8 a	297.5 ± 11.6 a	2.35 ± 0.40 a	3.53 ± 0.21 a
	1/2 K	14.3 ± 1.2 ab	14.9 ± 0.7 a	0.250 ± 0.007 a	0.241 ± 0.038 b	286.0 ± 10.0 a	275.0 ± 15.7 a	2.33 ± 0.03 a	2.72 ± 0.36 b
	1/4 K	11.5 ± 0.5 b	13.7 ± 0.9 a	0.224 ± 0.059 a	0.288 ± 0.017 ab	293.3 ± 20.8 a	301.5 ± 7.3 a	2.22 ± 0.40 a	2.95 ± 0.10 ab

[z]1/1 K, 1/2 K, and 1/4 K denote plants grown in half-strength Enshi formula nutrient solution containing 100%, 50%, and 25% levels of KNO_3, respectively.

[y]Mature lettuce leaves were derived from outer leaves, whereas newly expanded leaves were derived from inner leaves.

[x]Values are means ± SD (n = 5). Different letters following values in rows under the same lettuce type refer to a significant difference between the three potassium treatments according to Tukey's HSD test ($P < 0.05$).

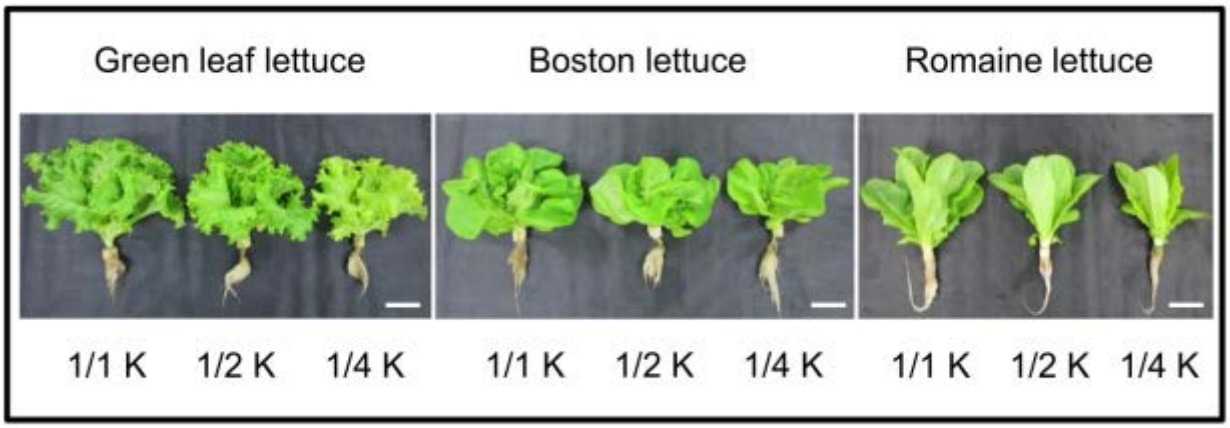

Figure 2-1 Green leaf lettuce, Boston lettuce, and romaine lettuce grown under different potassium treatments at final harvest time. 1/1 K, 1/2 K, and 1/4 K denote plants grown in half-strength Enshi formula nutrient solution containing 100%, 50%, and 25% levels of KNO_3, respectively. Bars indicate 10 cm.

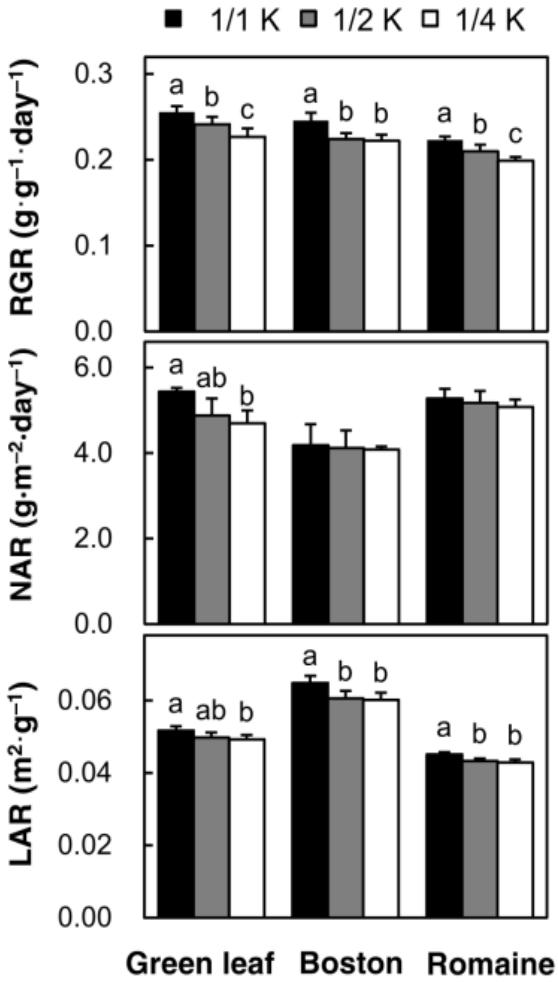

Figure 2-2 Effects of different potassium levels in the nutrient solution on relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) of lettuce plants grown under different potassium treatments. Black squares (1/1 K) denote plants grown in half-strength Enshi formula nutrient solution containing 100% level of KNO_3. Gray squares (1/2 K) denote plants grown in half-strength Enshi formula nutrient solution containing 50% level of KNO_3. Open squares (1/4 K) denote plants grown in half-strength Enshi formula nutrient solution containing 25% level of KNO_3. Values are means ± SD (n = 5). Different letters on top of bars under the same lettuce type indicate a significant difference between the three potassium treatments according to Tukey's HSD test ($P < 0.05$).

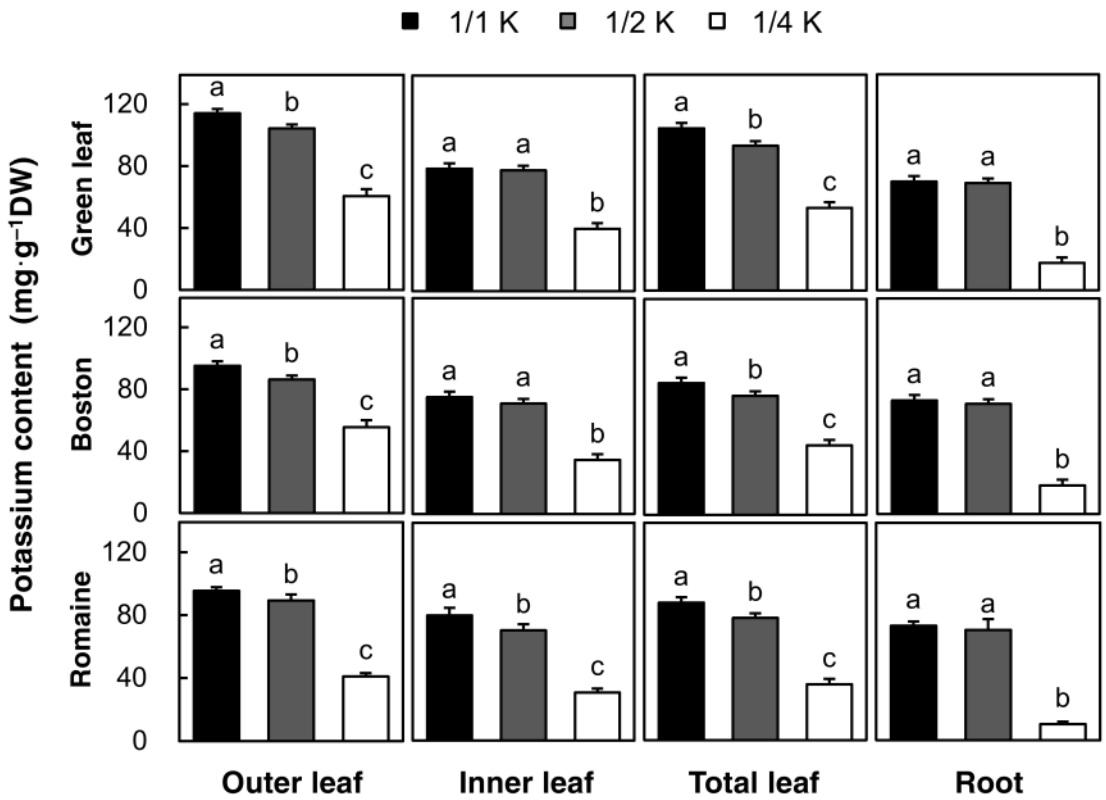

Figure 2-3 Potassium content in outer leaf, inner leaf, total leaf, and root of lettuce grown under different potassium treatments. Values are means ± SD (n = 5). Different letters on top of bars under the same lettuce type indicate a significant difference between the three potassium treatments according to Tukey's HSD test (P < 0.05). Abbreviations are the same as used in Figure 2-2.

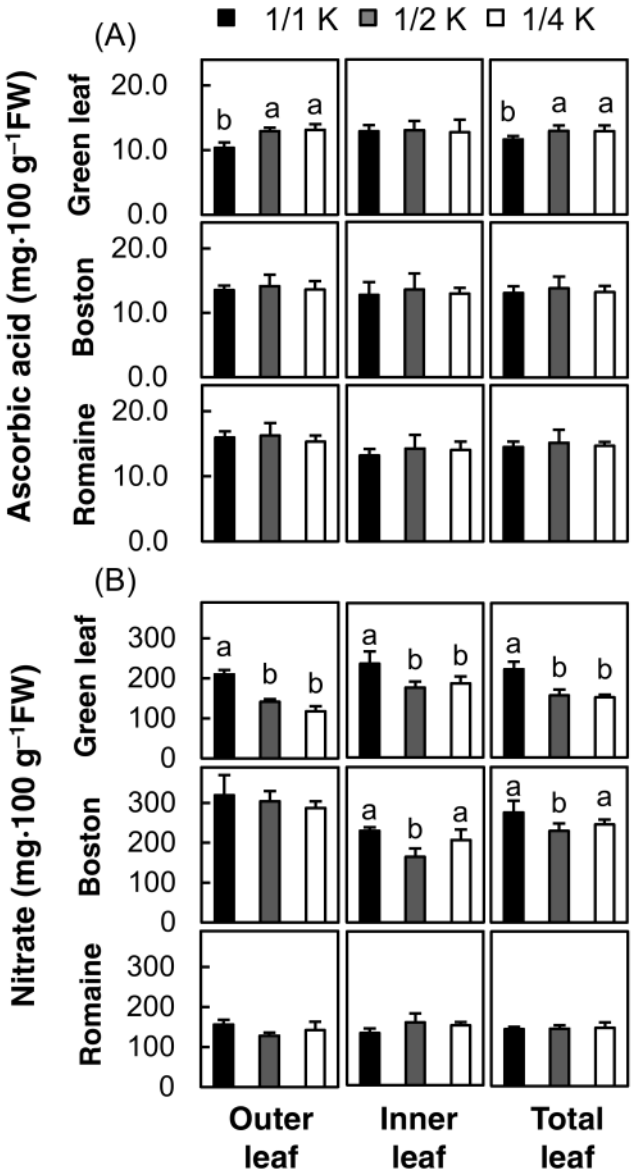

Figure 2-4 Ascorbic acid (A) and nitrate (B) content in outer, inner, and total leaves of lettuce plants grown under different potassium treatments. Values are means ± SD (*n* = 5). Different letters on top of bars under the same lettuce type indicate a significant difference between the three potassium treatments according to Tukey's HSD test ($P < 0.05$). Abbreviations are the same as used in Figure 2-2.

Chapter 3: Plant yield and quality of lettuce by using quantitative nutrient management in a plant factory with artificial lighting

Abstract

Climate change, air pollution, and soil pollution threaten current and future food production. Currently, a new type of facility, the “plant factory with artificial lighting” (PFAL), is being used to address these issues by growing plants under a controlled environment. Conventionally, EC-based hydroponic systems are used for plant cultivation in PFALs. However, the EC-based method has adverse effect on plant growth, reduces the efficiency of nutrient use, and the high level of nutrient residue in drained solution has serious environmental impacts. In the present study, a quantitative nutrient management method with a modified nutrient solution recipe was compared with the traditional EC-based method. The quantitative nutrient management reduced the nutrient supply without reducing plant growth, and remained low level of nutrient in the drained water. This method also reduced the potassium and nitrate content in lettuce leaves.

Introduction

In recent decades, global food production has faced threats from climate changes, air pollutions, and soil pollutions. A new plant production facility, the so-called “plant factory with artificial lighting” (PFAL) could realize stable plant production all year round unaffected by environmental, regional, seasonal limitations because it provides a controlled environment, including lighting, temperature, humidity, CO_2 concentration, and nutrients (Kozai, 2013a; Yamori et al., 2014). Currently, PFAL technology is being used for commercial production of leafy vegetables in Asian regions, including Japan, Korean, mainland China, and Taiwan (Hu et al., 2014; Kozai, 2013b).

In a PFAL, the nutrient management in hydroponic is based on the concentration of EC in nutrient solution. However, this conventional nutrient management maintains relatively high concentration of nutrients, providing plants more nutrients than they require for adequate growth, and this strategy seems not economic since excessive nutrients do not necessarily translate into higher yields (Rouphael and Colla, 2009). In contrast, it can lead to excess ion uptake and hence to reduce crop yield and quality (e.g., high nitrate content in leafy vegetables; Pardossi et al., 1994). The cost of establishing a PFAL is high (Kozai, 2013a) and the fertilizer prices have risen greatly. To reduce fertilizer use for PFAL production is critical to growers. Moreover, the unabsorbed nutrients will remain in the drained solution, cause waste of nutrients, and has serious environmental impacts (Maruo et al., 2001). Thus, the establishment of nutrient supply method to efficient use of nutrients in PFAL hydroponics is an important research goal to not only improve PFAL profitability but also reduce environmental pollution.

Some quantitative nutrient management method has been proposed for vegetable production in soilless culture (Maruo et al., 2001; Nakano et al., 2010; Kinoshita et al., 2016). Quantitative nutrient management is based on to control amount of nutrient

uptake rather than to maintain a concentration set-point (Maruo et al., 2001). Comparing with conventional EC-based nutrient management, quantitative nutrient management prevents over uptake of nutrient by a plant, increases nutrient-use efficiency, reduces the waste of nutrient, and prevents environmental pollution. Some plants, including spinach (Maruo et al., 2001) and tomato (Nakano et al., 2010; Kinoshita et al., 2016), have been successfully grown using quantitative nutrient management method without adverse effects on plant yield. In addition, the quantitative nutrient management increased ascorbic acid content and reduce nitrate content in the edible part of spinach plants (Maruo et al., 2001). In quantitative nutrient management, prediction of the amount of nutrient requirement for plant growth is very important. Several methods, such as the amount of water absorbed by plants (Nakano et al., 2006) and the leaf area of plants (Hosoi and Hosono, 2005), have been used to estimate the nutrient demands of plants.

In the present study, we proposed and test a different approach to predict the plant nutrient demand and to determine the nutrient supply. We aim to develop a quantitative nutrient management method that would be suitable for commercial production of low-potassium lettuce in a PFAL. In our approach we analyzed the nutrient absorption through total plant tissues and made a nutrient solution recipe. For cultivation, the amount of nutrients placed into water was determined by the number of plants. No further nutrient was supplied to control the EC value except to maintain a constant volume of nutrient solution via adding water. The plant yield did not differ significantly between the quantitative nutrient management method and the traditional EC-based method. However, the nutrient supply was greatly reduced, and it reduced the amount of nutrient residue in drained solution. In addition, the method increased ascorbic acid content and decreased potassium and nitrate content in leaves.

Materials and Methods

Experiment 1: plant biomass accumulation and nutrient absorption dynamics in a PFAL

The experiment was conducted in a PFAL located on the campus of the Center for Environment, Health and Field Sciences of Chiba University in Japan. Based on the production schedule of the Mirai Co., Ltd., the cultivation cycle was divided into three phases: 1) first seedling period: 0–14 day, 2) second seedling period: 15–25 day, and 3) final cultivation period: 26–35 day. Seeds of lettuce (*Lactuca sativa* L. cv. Frillice; Snow Brand Seed Co. Ltd., Sapporo, Japan) were sown in urethane cubes (2.3 cm width, 2.3 cm depth, 2.7 cm height). Seedlings were transplanted to cultivated panels (60 cm width, 30 cm depth, 1 cm height) at 3 days (1666.7 plants m^{-2}), 15 days (144.4 plants m^{-2}) and 26 days (33.3 plants m^{-2}) after sowing, respectively. Plants were grown in deep flow technique (DFT) hydroponic systems with Enshi formula nutrient solution (EC: 1.5 ± 0.1 $dS \cdot m^{-1}$, pH: 7.0 ± 0.5). Cool white fluorescent lamps were used to illuminate from above with a photosynthetically active photon flux density of 200 ± 10 $mol \cdot m^{-2} \cdot s^{-1}$. Temperature, photoperiod, relative humidity, and CO_2 concentration were controlled at 21 ± 0.5°C, 16 h, 70 ± 10%, 1000 $\mu mol \cdot mol^{-1}$, respectively.

Growth measurements were performed at plants sampled at 10 and 15–35 days after sowing. Shoot and root fresh weights of plants were measured immediately after harvesting, and the dry weights of shoot and root were measured after oven-drying at 80°C for 72 h. The nitrogen contents in the total plants were measured using a CNS- Analyzer (Vario EL; Elementar, Hanau, Germany). Elemental (phosphorus, potassium, calcium, and magnesium) contents in the total plants were quantified by inductively coupled plasma atomic emission spectrometry (ICP–OES) (iCAP 6000

Series; Thermo Fisher Scientific, Cambridge, UK). The experiment was independently performed three times and each replicate consisted of 5 plants.

Experiment 2: the quantitative nutrient management method using a modified nutrient recipe was compared with the traditional EC-based method

According to the data of experiment 1, we made a nutrient recipe (Table 3-1) for the final cultivation period of lettuce in a PFAL to realize quantitative nutrient management. Experiments were conducted to investigate the quantitative nutrient management method with a modified nutrient solution recipe on plant yield and quality during the final cultivation period, compared with traditional EC-based method. The cultivation procedure and growth condition were same as described in experiment 1. In the quantitative nutrient management treatment, the amount of nutrient placed into water was calculated by the number of plants, water was added to maintain the total volume of nutrient solution at 8L, and no further nutrient was added. In the EC-based treatment, the EC value was adjusted to approximately 1.5 $dS \cdot m^{-1}$, which was same value with that in experiment 1. Experiments were designed with three replicates for each treatment, and each replicate contained 6 plants.

Plants were harvest at 35 day after sowing, and the plant growth was measured. The nutrient uptakes by the plants during the final cultivation period were analysis. The chlorophyll, ascorbic acid, and potassium and nitrate contents were also measured.

Results

Experiment 1: plant growth and nutrient absorption dynamics

Plant showed an exponential growth curve throughout the cultivation cycle (Figure 3-1), indicating that the growth environment is suitable for plant development. The total fresh weight reached 52.8 g·plant^{-1}, with a total dry weight of 3.66 g·plant^{-1} (Figure 3-1). In the first seedling period, the dry matter accumulated to only 0.824% of the final total dry matter and gradually increased in the follow two periods, to 10.1% and 89.1% of the final total dry matter, respectively (Figure 3-1).

The macronutrient absorption dynamics followed an exponential curve (Figure 3-2). The highest nutrient accumulated in plants was N, followed by K, Ca, P, and Mg. The highest absorptions were found in the final cultivation period, where the values for N, K, Ca, P, and Mg were 10.3, 6.61, 0.659, 0.638, and 0.458 mmol·plant^{-1}, respectively (Figure 3-2).

Experiment 2: effect of the quantitative nutrient management method on plant yield and quality

There was no significant difference between the quantitative nutrient management method and traditional EC-based method in terms of plant yield and total leaf area (Table 3-2). However, the total amount of each nutrient supplied in the quantitative nutrient management method was greatly reduced in comparison with the traditional EC-based method (Table 3-3). The nutrient residue in drained solution showed significantly lower value in the quantitative nutrient management than that in the EC-based method (Table 3-3).

The potassium content and nitrate content in leaves were significant lower in quantitative nutrient management method than that in traditional EC-based method,

whereas the ascorbic acid content tended to be higher in quantitative nutrient management method (Table 3-4). No significant different was found in chlorophyll content between the quantitative nutrient management method and traditional EC-based method (Table 3-4).

Discussion

The objective of this study was to examine the quantitative nutrient management method with a modified nutrient solution recipe on plant yield and quality. In experiment 1, most of the dry matter accumulated in the final cultivation period (Figure 3-1), indicating that the final cultivation period is more important for the determination of final biomass compared with the first and second seedling periods. Also, the final cultivation period accounts for the largest use of space and nutrient during the production process of a PFAL. Thus, the final cultivation period was selected to test the quantitative nutrient management.

Comparing with the traditional EC-based method, the quantitative nutrient management reduced the nutrient supply to plants without causing any decrease in yield (Table 3-2). This is in accord with the previous studies in spinach (Maruo et al., 2001) and tomato (Nakano et al., 2010; Kinoshita et al., 2016), indicating that the excess nutrient has no contribute to the yield. This proposes that it is efficient to control the amount of nutrient supply that is adequate for plant growth rather than control the nutrient solution concentration. In addition, the nutrient residue in drained solution was lower in quantitative nutrient management method as compared to the EC-based method (Table 3-3), suggesting that quantitative nutrient management is a useful method to reduce the emission to the environment. On the other hand, the contents of potassium and nitrate were significantly reduced in quantitative nutrient management method

(Table 3-4). It proposes that lettuce with low nitrate content can be obtained through the application of quantitative nutrient management, since the excessive uptake of NO_3-N can be avoid by decreasing the nutrient supply. Quantitative nutrient management method also provides the possibility to reduce the potassium concentration in plants by optimizing the amount of potassium supplied in nutrient solution, realizing the production of plants with low potassium level for the kidney disease patients. However, additional researches are needed to determine whether such plants can be obtained through this approach in PFALs.

Table 3-1 The composition of modified standard nutrient solution used for quantitative nutrient management method.

Nutrient	$mmol \cdot L^{-1}$	$mg \cdot L^{-1}$
$Ca(NO_3)_2 \cdot 4H_2O$	1.3	307
KNO_3	6.4	647
$NH_4H_2PO_4$	0.6	72
$MgSO_4 \cdot 7H_2O$	0.3	79
$Mg(NO_3)_2 \cdot 6H_2O$	0.2	46

Table 3-2 Effects of nutrient management methods on the shoot fresh weight and dry weight, root fresh weight and dry weight, and total leaf area.

Nutrient management method	Fresh weight (g)		Dry weight (g)		Total leaf area (cm^2)
	Shoot	Root	Shoot	Root	
EC-based management	39.2 ns	10.3 ns	2.8 ns	0.71 ns	539 ns
Quantitative nutrient management	38.7	10.1	2.6	0.69	527

Significant difference between the EC-based management and quantitative nutrient management were examined by Student's *t*-test. No significant was found between two methods.

Table 3-3 Nutrient quantities of supply and residue in the EC-based method and quantitative nutrient management method.

	Nutrient management method	Nutrient (mg· plant^{-1})				
		N	P	K	Ca	Mg
Supply	EC-based management	233	32.7	416	86.7	20.0
	Quantitative nutrient management	140	19.6	250	52.0	12.0
Residue	EC-based management	138	18.8	61	45.7	11.4
	Quantitative nutrient management	8	3.5	9	11.1	3.9

Table 3-4 Effects of nutrient management methods on the potassium, chlorophyll, ascorbic acid, and nitrate content.

Nutrient management method	Potassium content (mg·100g^{-1} FW)	Chlorophyll content (SPAD)	Ascorbic acid content (mg·100g^{-1} FW)	Nitrate content (mg·100g^{-1} FW)
EC-based management	717.2	43.8 ns	63.0 ns	188.8
Quantitative nutrient management	501.0**	44.0	68.2	87.8**

Significant difference between the EC-based management and quantitative nutrient management were examined by Student's *t*-test. * Significant at P< 0.05, ** Significant at P<0.01, and ns means no significant.

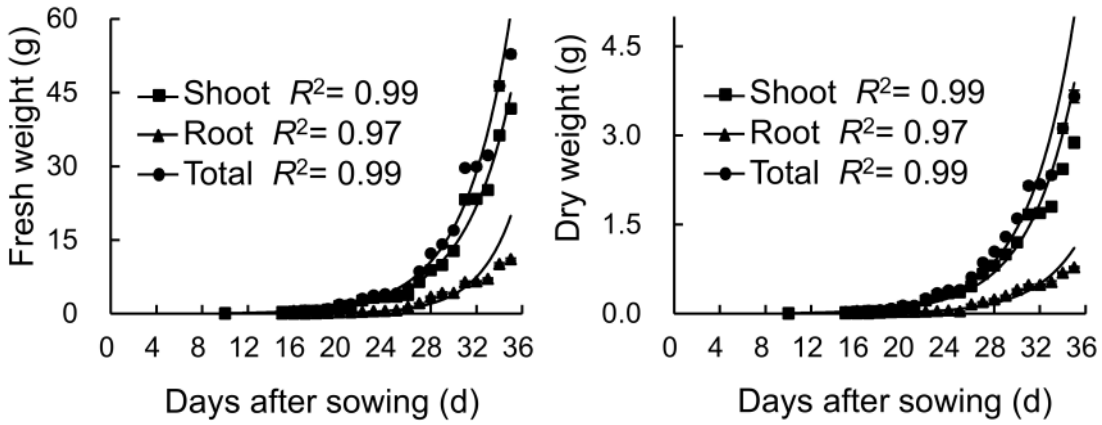

Figure 3-1 Plant biomass accumulation (fresh weight and dry weight) dynamics of lettuce, cultivated in a PFAL. Shoot part: filled square; root part: filled triangle; total plant: filled circle.

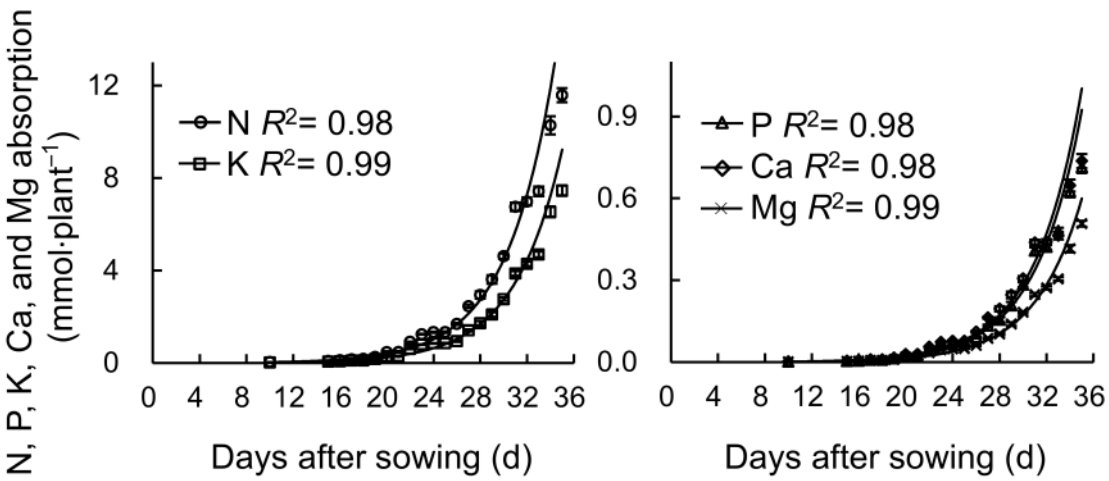

Figure 3-2 Macronutrient nutrient (N, P, K, Ca, and Mg) absorption dynamics of lettuce cultivated in a PFAL. N: open circle; P: open triangle; K: open square; Ca: open diamond; and Mg: cross.

Chapter 4: Supplemental upward lighting from underneath to obtain higher marketable lettuce leaf fresh weight

Abstract

Recently, the so-called “plant factory with artificial lighting” (PFAL) approach has been developed to provide safe and steady food production. Although PFALs can produce high-yielding and high-quality plants, the high plant density in these systems accelerates leaf senescence in the bottom (or outer) leaves owing to shading by the upper (or inner) leaves and by neighboring plants. This decreases yield and increases labor costs for trimming. Thus, the establishment of cultivation methods to retard senescence of outer leaves is an important research goal to improve PFAL yield and profitability. In the present study, we developed an LED lighting apparatus that would optimize light conditions for PFAL cultivation of a leafy vegetable. Lettuce (*Lactuca sativa*) was hydroponically grown under white, red, or blue LEDs, with light provided from above (downward), with or without supplemental upward lighting from underneath the plant. White LEDs proved more appropriate for lettuce growth than red or blue LEDs, and the supplemental lighting retarded the senescence of outer leaves and decreased waste (i.e., dead or low-quality senescent leaves), leading to an improvement of the marketable leaf fresh weight.

Introduction

Population growth has led to a steady increase in the demand for food, and now poses a threat to food security. In recent years, air pollution, rapid population growth, and resource shortages have focused increasing attention on food security. An emerging industry with the potential to alleviate some of these problems takes advantage of what have been called “plant factories”, which can produce high-yield and high-quality plants using less water, nutrients, land, and labor than is possible with conventional agriculture (Kozai, 2013a; Hu et al., 2014; Yamori et al., 2014). Plant factories with artificial lighting (PFALs) create an enclosed cultivation system that allows control of environmental factors such as lighting, temperature, humidity, and CO_2 concentration. Moreover, PFALs can overcome adverse conditions such as heavy rain, heavy snow, strong winds, and temperature extremes. PFALs have been already commercially used for the production of leafy vegetables in Japan, mainland China, and Taiwan (Kozai, 2013b).

Light sources such as fluorescent lamps, metal-halide lamps, and high-pressure sodium lamps are generally used for plant cultivation. They are used to increase the photosynthetic photon flux density (PPFD), but they also provide wavelengths that are not used efficiently or at all to support photosynthesis and plant growth (McCree, 1972; Björkman, 1981). In comparison, LED lighting systems have several advantages, including greater wavelength specificity (i.e., a narrow bandwidth), long operating lifetimes, and less heating. However, the optimal light wavelengths for plant cultivation remain unclear. Numerous studies have suggested that red and blue light are the most useful wavelength bands to drive photosynthesis, since chlorophyll has its maximum absorption in those bands (e.g., McCree, 1972; Okamoto et al., 1996), but blue light also

plays an important photomorphogenic role (e.g., suppresses hypocotyl elongation; Goins et al., 1997; Massa et al., 2008) in plants. However, it has been shown that the optimal light color for plant growth differs among plant species (Kim et al., 2006). For example, lettuce (*Lactuca sativa*) grown under red LEDs developed more leaves than lettuce grown under blue LEDs (Yanagi et al., 1996), but for spinach (*Spinacia oleracea* L.) or radish (*Raphanus sativus* L.) growth, the use of only red LEDs was unsuitable (Yorio et al., 2001). Other studies have found that light sources that contain blue light improved dry matter production and the photosynthetic capacity in pepper (*Capsicum annuum* L.; Brown et al., 1995), wheat (*Triticum aestivum* L.; Goins et al., 1997), and spinach (*Spinacia oleracea* L.; Matsuda et al., 2007). Thus, it remains unclear what light source would be most suitable for plant cultivation in a PFAL system. It is therefore necessary to identify the optimal light sources for various species to maximize plant yields.

On the other hand, although PFALs can produce high yields, the high plant density creates suboptimal conditions, because the outer or lower leaves are shaded and therefore senesce faster. Owing to shading by upper or outer leaves and by neighboring plants, leaves beneath the plant canopy suffer from low light conditions (Terashima et al., 2005). The leaf senescence that occurs at low light intensity is accompanied by chlorophyll loss, degradation of photosynthetic proteins, a decline in photosynthetic activity, and the remobilization of nutrients to younger tissues (Gan and Amasino, 1997; Weaver and Amasino, 2001; Brouwer et al., 2012). The senescent leaves become visibly yellow (chlorotic) and wilted (McCabe et al., 2001), leading to a reduction of the market price. Thus, these leaves must be removed, which can significantly decrease plant yield and increase labor costs. Therefore, establishing cultivation methods that retard senescence of outer leaves is an important goal to improve yield and profitability.

Since the main problem is the low light conditions experienced by shaded leaves, improving the light conditions of these leaves could delay senescence. Previous studies have shown that irradiation of both the adaxial and abaxial sides of a leaf can increase photosynthesis (Terashima, 1986; Soares et al., 2008), and different light colors have different effects on leaf senescence (Causin et al., 2006). However, no studies have examined the effects of supplemental upward lighting of the abaxial sides of leaves from underneath the plant to delay senescence of shaded leaves and improve plant growth. The present study therefore had two purposes: to study the effect of light color on photosynthesis and plant growth of romaine lettuce, and to analyze the effect of supplemental upward lighting from underneath the plant on leaf senescence in the outer leaves. We also analyzed the economic benefits of this technique. We found that white LEDs were more appropriate for romaine lettuce than red or blue LEDs, and that the supplemental upward lighting retarded senescence of the outer leaves and reduced waste, leading to an improvement in marketable leaf fresh weight; it also improved the nutrient quality of the plants.

Materials and Methods

Plant materials and growth conditions

The experiment was conducted in a commercial plant factory, which has two cultivating compartments: one is a nursery room and the other is a cultivation room. Romaine lettuce (*Lactuca sativa* L. cv. Romana; Takii Seed Co., Kyoto, Japan) seeds were sown in wet urethane cubes (2.3 cm width, 2.3 cm depth, 2.7 cm height), and the seedlings were grown in the nursery room at 20/17°C (day/night) under a PPFD of 350 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12 h provided by cool white fluorescent lamps. At 22 days after

sowing, the seedlings were transplanted into the cultivation room equipped with 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD of downward-facing white LEDs, red LEDs (peak wavelength 660 nm), or blue LEDs (peak wavelength 450 nm) (Figure 4-1A; red and blue LEDs were provided by Shibasaki Inc., Saitama, Japan; white LEDs were provided by ODC Co., Ltd., Kanagawa, Japan). Plants were grown in a deep-flow hydroponic system (37 $plants \cdot m^{-2}$) in Enshi formula nutrient solution with an electrical conductivity of 1.7 ± 0.1 $dS \cdot m^{-1}$ and a pH of 6.8 ± 0.2. The air temperature was maintained at 25/20°C (day/night), the relative humidity at 60%, the photoperiod at 16 h, and the CO_2 concentration at 1,000 ppm. The supplemental upward lighting treatments were performed from 15 days (when all outer leaves became shaded) or 22 days (when visible senescence of outer leaves began) after transplanting, with illumination at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD at the height of the outer leaves (4.0 ± 0.5 cm). The illumination was provided by supplemental LEDs placed on cultivation panels in order to direct the light upward from underneath the plants (Figure 4-2). The light colors of these supplemental LEDs were same to those of the LEDs used for downward lighting from above (Figure 4-1B).

Measurements of gas exchange and chlorophyll fluorescence

Gas exchange was measured with a portable gas exchange system (LI-6400; LI-COR, Lincoln, NE, USA) as described previously (Yamori et al., 2009, 2010). Plants were divided into six layers of leaves. Counting from the lowest leaf, the 1st to 3rd layers of leaves (white light, 10 to 11 leaves per plant; red light, 10 to 11 leaves per plant; blue light, 5 to 6 leaves per plant) were the outer leaves, and the 4th to 6th layers of leaves (white light, 9 to 10 leaves per plant; red light, 8 to 9 leaves per plant; blue light, 6 to 7 leaves per plant) were the inner leaves. After 30 min of illumination to

obtain steady-state photosynthesis, the net photosynthetic rate of the inner leaves (6th layer) and outer leaves (3rd layer) were measured.

To evaluate the degree of leaf senescence, we measured the maximum potential photochemical efficiency (the ratio of variable to maximum fluorescence, F_v/F_m) using an Imaging-PAM fluorometer (Walz, Effeltrich, Germany). Leaf discs (1.3 cm in diameter) were taken from the outer and inner leaves of each treatment (from the 1st to 6th layers), and were then vacuum-infiltrated in deionized water that included 0.005% Tween 20 (Shao et al., 2013). F_v/F_m was measured after 30 min of incubation in darkness.

Determinations of chlorophyll, carbon, and nitrogen contents

Right after the gas exchange and chlorophyll fluorescence measurements, leaf discs (0.85 cm in diameter) were taken from the outer and inner leaves (same leaves with the measurements of F_v/F_m) of each treatment. Chlorophyll was extracted in N,N-dimethylformamide and its content was determined using a spectrophotometer, according to the procedure of Porra et al. (1989). Leaf carbon and nitrogen contents were measured with a Vario EL III elemental analyzer (Elementar, Hanau, Germany) as described by Yamori et al. (2005).

Plant growth and nutritional quality of the romaine lettuce

At 53 days after sowing, plants were harvested, and the leaf and root fresh weights and leaf and root dry weights were measured. The dry weights of leaf and root were measured after oven-drying at 80°C for more than 72 h.

As a measure of the nutrient quality, the ascorbic acid and nitrate contents in the outer and inner leaves (all leaves in each group of layers were cut into pieces, and the 1

g fresh samples were used for measurements) of plants in each treatment were quantified by using an RQ Flex plus reflectometer (Merck, Darmstadt, Germany), following the method of Pantelidis et al. (2007).

Electricity consumption measurements

The consumption of electrical energy by each LED panel was measured with a multimeter and a clamp ammeter (Hioki 3169-01; Hioki E.E. Corporation, Nagano, Japan), and were used to evaluate the economics of the supplemental lighting.

Statistical analysis

Values were compared between illumination treatments by Tukey's multiple-comparison test (for photosynthetic rates without supplemental lighting, fresh weights, and wastage rates) or Student's *t*-test (for photosynthetic rates with supplemental lighting and nutrient contents) in SPSS statistical software v. 21.0 (SPSS, Chicago, IL, USA). Differences were considered significant at $P < 0.05$.

Results

Leaf characteristics

In plants grown under downward lighting but without upward lighting, the total chlorophyll content (Figure 4-3A) and maximum potential photochemical efficiency (F_v/F_m; Figure 4-3E) were highest in the most newly expanded leaves (6th layer), but gradually decreased from the inner leaves (4th to 6th layers) to the outer leaves (1st to 3rd layers). However, supplemental upward lighting maintained significantly higher

total chlorophyll content and F_v/F_m values in the outer leaves of plants grown under white LEDs (Figures 4-3B, F) and red LEDs (Figures 4-3C, G), but not under blue LEDs (Figures 4-3D, H), than in plants grown without upward lighting (Figures 4-3A, E). The 9-day supplemental lighting treatments maintained high total chlorophyll content and F_v/F_m in the outer leaves to some extent, but the 16-day treatments showed a more pronounced effect in retarding senescence of the outer leaves. White or red supplemental lighting also maintained the leaf nitrogen content in the outer leaves (Table 4-1). However, blue supplemental lighting (but not red or white) allowed a significant decrease in the leaf nitrogen content in the inner leaves.

Photosynthesis

In plants grown under downward lighting but without upward lighting, the photosynthetic rates of the most newly expanded leaves (in the 6th layer) were highest in plants grown under white or red LEDs, which did not differ significantly, and were significantly lower under blue LEDs (Figure 4-4A).

With supplemental upward lighting, the outer leaves showed positive net photosynthetic rates. In contrast, without upward lighting, plants had negative net photosynthetic rates in the outer leaves (Figure 4-4B). These results show that supplemental upward lighting could shift the carbon balance from negative to positive (i.e., it improved photosynthesis in the outer leaves).

Plant growth

Romaine lettuce showed distinct growth responses to the different light colors. In plants grown under downward lighting without upward lighting, the white LEDs yielded the highest total leaf fresh weights, and the blue LEDs produced the lowest total

fresh weight (Figure 4-5A); this corresponded to the results for photosynthetic rate in the most newly expanded leaves of lettuce grown only with downward lighting (Figure 4-4A). Moreover, marketable leaf biomass, which represents the remaining leaves after removal of the outer senesced leaves, showed the same trend as total fresh weight (Figure 4-5A). Root mass generally varied little between treatments, although it was significantly greater with white LEDs than with red or blue LEDs in the absence of supplemental lighting. The wastage rate, which equaled the difference between the total and marketable leaf fresh weights, was higher in plants grown under white LEDs than in plants grown under red or blue LEDs without supplemental lighting (Figure 4-6A).

White or red upward lighting increased the total leaf fresh weight compared with plants grown without upward lighting, and the difference was significant with 16 days of supplemental lighting (Figures 4-5B, C). Moreover, the white and red supplemental lighting significantly increased marketable leaf fresh weight, leading to significantly lower wastage rates, especially in the 16-day treatments (Figures 4-6B, C). However, blue supplemental lighting made no significant difference (Figures 4-5D, 4-6D).

Ascorbic acid and nitrate contents of the romaine lettuce

The ascorbic acid and nitrate contents were greatly influenced by the different light colors without upward lighting. The ascorbic acid content was generally highest under red LEDs, followed by blue and then white LEDs (Figure 4-7A). Conversely, the nitrate content of lettuce was significantly lower in leaves grown under red LEDs than in leaves grown under white or blue LEDs (Figure 4-7E).

Supplemental lighting significantly increased the ascorbic acid content in the outer leaves with white LEDs and in the outer and total leaves with red LEDs (Figures 4-7B, C), but did not significantly affect the nitrate content (Figures 4-7F, G). Supplemental

lighting with blue LEDs did not significantly affect either (Figures 4-7D, H).

Discussion

White LEDs had a greater effect on plant biomass production than red or blue LEDs

Romaine lettuces grown under white LEDs had significantly greater total and marketable leaf biomass than those grown under red or blue LEDs (Figure 4-5A), indicating that white light was a superior light source for the production of romaine lettuce in PFALs. Generally, there are two methods to generate white light: single-chip and multi-chip LEDs. Single-chip white LEDs include (1) a blue LED + a yellow phosphor (the most common type), (2) a blue LED + red and green phosphors, and (3) a near-ultraviolet LED + red, green, and blue phosphors. Multi-chip white LEDs include (1) red + green + blue LEDs and (2) blue + green + orange LEDs (Taguchi, 2003; Damilano et al., 2001). The white LEDs used in the present study combined a blue LED with a yellow phosphor (Figure 4-1A). The results with the white LEDs in the present study suggest that combining light wavelengths could have a greater impact on plant production than the use of red and/or blue LEDs, which have been believed to be useful for plant cultivation in PFALs (e.g., Lee et al., 2010; Son and Oh, 2013). This hypothesis is supported by previous studies which showed that the dry mass of lettuce and spinach grown under only red light was significantly lower than that under white light, which provided both red and blue wavelengths (Yorio et al., 2001), and leaf fresh weight of lettuce grown under combined red and blue LEDs was significantly lower than under a combination of red, blue, and white LEDs (Lin et al., 2013). An increasing number of white LEDs are being manufactured for use in home lighting, resulting in

reduced prices, and thus white LEDs might offer a good compromise between cost and optimal spectral characteristics as a light source for commercial plant productions in PFALs.

The different light colors influenced the ascorbic acid and nitrate contents in romaine lettuce (Figures 4-7A, E). Plants grown under red LEDs had the highest ascorbic acid content and the lowest nitrate content. If growers demand a higher ascorbic acid content or a lower nitrate content, red light could therefore help them meet their requirements. In PFALs, it is possible to match the light source to the production target. The levels of functional nutritional components such as ascorbic acid, alpha-carotene, and phenolic compounds can be increased by treatment with UV light (Xie et al., 2015) or red light (Bliznikas et al., 2012) during the late stages of cultivation. Therefore, in the future, it may be possible to achieve high plant yields with a high content of ascorbic acid or other nutrients by growing plants under white light and supplying red light during the late cultivation stage (e.g., 1 week before harvest) or directly under white light with supplemental red light.

Supplemental upward lighting can improve plant growth

Plant cultivation at the high density used inside PFALs increases the annual production capacity per unit area (Kozai, 2013b). However, the outer leaves of plants grown at this high plant density cannot receive sufficient light from above and thus senesce faster. Our results confirm this hypothesis: the chlorophyll content and the maximum potential photochemical efficiency (F_v/F_m) both decreased drastically in the outer leaves without supplemental upward lighting (Figures 4-3A, E), which are typical phenomena when leaves senesce (Thimann and Satler, 1979; Wingler et al., 2004). However, our results clearly show that the upward lighting maintained a higher

chlorophyll content (Figures 4-3B, C) and higher F_v/F_m (Figures 4-3F, G) in the outer leaves, indicating retardation of senescence. Moreover, it was apparent that the supplemental lighting promoted photosynthesis in the outer leaves, whereas the plants without supplemental lighting had a negative carbon balance (Figure 4-4B). This interpretation is supported by the higher nitrogen content in the outer leaves of plants with supplemental lighting (Table 4-1). Nitrogen is a major component of stromal enzymes and thylakoid proteins, so its availability strongly affects the plant's photosynthetic capacity (Evans, 1989; Yamori et al., 2011). These results demonstrate that supplemental upward lighting both retarded leaf senescence and improved photosynthesis in the outer leaves, and that this increased plant yields (Figures 4-5B, C). Given the higher plant production, we propose that a novel cultivation system should be developed that includes supplemental upward lighting from below the plants to optimize the light conditions in PFALs. However, it will be necessary to perform additional research to determine whether the best results can be obtained with a single light color or a combination of colors.

It should also be noted that the effect of supplemental upward lighting could differ among plant species and light colors, as well as being a function of the plant qualities (e.g., total biomass, nutrient content) the breeder prioritizes. In the present study, the plants grown under blue LEDs grew more erect (Table 4-2) and therefore could not efficiently absorb the upward lighting (Figures 4-3D, H & 4-5D). Thus, it is necessary to select suitable plant species and light sources for this cultivation method. Further research will be needed to optimize the cultivation system using supplemental upward lighting.

Economic benefit analysis of supplemental upward lighting

Because of the vertical cultivation pattern (Figure 4-2), a PFAL with 10 tiers of plants can have an annual production capacity of leafy vegetables that is 90 to 117 times the values that can be achieved in an open field (Kozai, 2013b). The largest PFAL in Japan (Spread Inc., Kyoto, Japan) can produce 21,000 lettuce heads per day, and the largest one in Taiwan (Yasai Corp., Taiwan) can produce 40,000 per day. Although these production rates are extremely high, yield losses caused by outer leaf senescence are also large. Based on total and marketable leaf fresh weights of 153.7 and 134.0 g, respectively, under white downward lighting without supplemental lighting (Figure 4-5), senescence of the outer leaves in these operations could cause losses of 413.7 kg FW/day in the Japanese PFAL and 788.0 kg FW/day in the Taiwanese PFAL. The present results clearly show that the wastage rate can be significantly decreased by the use of supplemental upward lighting (Figures 4-6B, C). Thus, supplemental lighting to delay senescence of outer leaves could be an attractive way to solve this problem while also decreasing labor requirements to remove the senesced leaves.

To analyze the economic benefits of the supplemental lighting, it is necessary to account for the energy cost of the lighting. Table 4-3 provides this comparison based on an electricity cost of 17.5 JPY/kW h, and the difference between the net selling price of the lettuce plants with and without supplemental lighting (i.e., the net income of the supplemental upward lighting). In the 9-day treatments, white LEDs (11.5 JPY/plant) produced a higher net income than red LEDs (1.6 JPY/plant) and blue LEDs (-2.3 JPY/plant). The results were similar for the 16-day treatment: the highest net income was again obtained with white LEDs (40.3 JPY/plant) followed by red LEDs (20.9 JPY/plant) and blue LEDs (-3.6 JPY/plant). White LEDs with 16 days of supplemental

white illumination yielded the highest biomass (158.0 g/plant) and the highest net income (40.3 JPY/plant). The net incomes calculated in this analysis don't include the savings that result from decreasing the labor cost for trimming of senesced leaves during packing, and thus the net benefit would be greater than our estimates. Although the 9-day supplemental treatments would reduce the electricity costs, plant biomass and net income increased less than in the 16-day treatments (Table 4-3). Thus, in order to increase production and net income, it is better to provide supplemental lighting for 16 days or even longer (e.g., from transplanting to harvest). However, determining the optimal duration and spectral characteristics of the supplemental light remains a challenge for future research.

Table 4-1 Effects of light color and duration of supplemental upward lighting on outer (3rd layer) and inner (6th layer) leaves properties (LMA, leaf mass per area; nitrogen content; C/N, the carbon to nitrogen ratio) in romaine lettuce.

Light source (LEDs)	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Supplemental upward lighting treatment (day)	Outer leaf			Inner leaf		
			LMA ($g \cdot m^{-2}$)	Nitrogen ($g \cdot m^{-2}$)	C/N ($g \cdot g^{-1}$)	LMA ($g \cdot m^{-2}$)	Nitrogen ($g \cdot m^{-2}$)	C/N ($g \cdot g^{-1}$)
White	200-0	0	13.2 ± 1.5 a	1.24 ± 0.27 b	10.05 ± 1.65 a	14.3 ± 4.2 ab	2.09 ± 0.57 a	6.09 ± 1.09 a
Red	200-0	0	12.3 ± 0.8 a	1.80 ± 0.36 a	6.79 ± 1.45 b	13.3 ± 1.1 b	2.13 ± 0.30 a	6.38 ± 0.96 a
Blue	200-0	0	12.8 ± 1.4 a	1.97 ± 0.31 a	6.12 ± 0.67 b	15.3 ± 0.8 a	2.48 ± 0.26 a	5.90 ± 0.34 a
White	200-0	0	13.2 ± 1.5 ns	1.24 ± 0.27 *	10.05 ± 1.65 ns	14.3 ± 4.2 ns	2.09 ± 0.57 ns	6.09 ± 1.09 ns
	200-40	16	13.9 ± 1.8 ns	1.58 ± 0.12 ns	7.90 ± 1.17 ns	16.0 ± 3.1 ns	2.56 ± 0.58 ns	6.56 ± 1.09 ns
Red	200-0	0	12.3 ± 0.8 ns	1.80 ± 0.36 ns	6.79 ± 1.45 ns	13.3 ± 1.1 ns	2.13 ± 0.30 ns	6.38 ± 0.96 ns
	200-40	16	12.6 ± 1.9 ns	2.04 ± 0.59 ns	6.05 ± 1.10 ns	14.0 ± 1.5 ns	1.92 ± 0.26 ns	6.99 ± 0.96 ns
Blue	200-0	0	12.8 ± 1.4 ns	1.97 ± 0.31 ns	6.12 ± 0.67 ns	15.3 ± 0.8 ns	2.48 ± 0.26 ns	5.90 ± 0.34 ns
	200-40	16	12.3 ± 1.2 ns	2.10 ± 0.27 ns	5.77 ± 0.65 ns	12.6 ± 2.9 ns	2.00 ± 0.28 *	5.92 ± 0.61 ns

Data represent means ± SD (n = 5). Data in the first three rows expressed the plants grown with illumination from above by white, red, or blue LEDs without any supplemental upward lighting, means followed by different letters differ significantly (Tukey's HSD test, $P < 0.05$) among three light colors. For the following rows, significant difference between two PPFDs were compared using Student's t-test. * means significant at $P < 0.05$, and ns means no significant.

Table 4-2 Light intensity which was received by the outer leaves of plants grown with illumination from above by white, red, or blue LEDs with supplemental upward lighting.

Supplemental upward lighting source (LED tapes)	PPFD at 4.0 cm distance from LEDs ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Shoot Angle (º)	PPFD at the height of the outer leaves ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
White	40	55.4 ± 0.5 b	37.2 ± 1.7 a
Red	30	43.5 ± 3.2 c	23.2 ± 1.7 b
Blue	40	70.6 ± 1.3 a	6.4 ± 1.1 c

The LEDs were located at the panels on the growth beds. The photosynthetic photon flux density (PPFD) was measured by R-2D color acetate film (R-2D, Taisei Chemical Industries, Tokyo, Japan) at the height of the outer leaves of lettuce grown with illumination from above by white, red, or blue LEDs with supplemental upward lighting. After 14 days of being illuminated by supplemental upward lighting, the R-2D color acetate films were collected for calculation of the light intensity by the equation: $[(833.3-416.7 \times (\log10D/Do \times 100)/(14 \times 24 \times 60 \times 60)] \times 106$, Do = 1.982 (value of film before exposing to the light), D represents the value of film after being exposed to the white, red, or blue supplemental upward lighting. Means followed by different letters differ significantly (Tukey's HSD test, $P < 0.05$) among three light colors.

Table 4-3 Evaluation of the feasibility of supplemental upward lighting with different durations.

Light source (LEDs)	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Supplemental upward lighting treatment (day)	Marketable leaf fresh weight (g)	(A) Selling price (JPY/plant)	(B) Electricity bill of supplemental upward lighting (JPY/plant)	Net selling price (JPY/plant)	Net income of the supplemental upward lighting (JPY/plant)
White	200-0	0	134.0 ± 8.2 b	268.0 ± 16.4 c	0.0 ± 0.0 c	268.0 ± 0.0 c	0.0 ± 0.0 c
	200-40	9	141.9 ± 6.7 b	283.8 ± 13.4 b	4.3 ± 0.0 b	279.5 ± 0.0 b	11.5 ± 0.0 b
	200-40	16	158.0 ± 11.4 a	315.9 ± 22.7 a	7.7 ± 0.1 a	308.2 ± 0.1 a	40.3 ± 0.1 a
Red	200-0	0	110.0 ± 6.2 b	219.9 ± 12.4 c	0.0 ± 0.0 c	219.9 ± 0.0 c	0.0 ± 0.0 c
	200-40	9	117.4 ± 7.2 b	234.7 ± 14.4 b	13.2 ± 0.0 b	221.6 ± 0.0 b	1.6 ± 0.0 b
	200-40	16	132.1 ± 8.4 a	264.2 ± 16.9 a	23.4 ± 0.1 a	240.8 ± 0.1 a	20.9 ± 0.1 a
Blue	200-0	0	69.7 ± 1.3 a	139.4 ± 2.7 a	0.0 ± 0.0 c	139.4 ± 0.0 a	0.0 ± 0.0 a
	200-40	9	71.2 ± 1.4 a	142.3 ± 2.7 b	5.3 ± 0.0 b	137.1 ± 0.0 b	- 2.3 ± 0.0 b
	200-40	16	72.6 ± 2.5 a	145.2 ± 5.0 b	9.4 ± 0.1 a	135.8 ± 0.0 c	- 3.6 ± 0.1 c

Based on local surveys, the selling price of lettuce was 200 JPY/100 g and the electricity bill was 17.49 JPY/KW h. (A) Selling price per plant was calculated as: A = marketable leaf fresh weight/100 × 200; (B) the electricity bill for supplemental upward lighting per plant was calculated as: B = electricity consumption of LED × 16/1000/12 × treatment days × 17.49, where 16 was the photoperiod and 12 was the number of plants illuminated by supplemental LEDs from underneath the plant; net selling price per plant was calculated as A minus B, and net income of the supplemental upward lighting per plant was obtained by the difference between the net selling price of the lettuce plants with and without supplemental lighting. Different letters follow the values of the rows within same light color refer to a significant difference between two PPFDs and three treatment durations (Tukey's HSD test, $P < 0.05$).

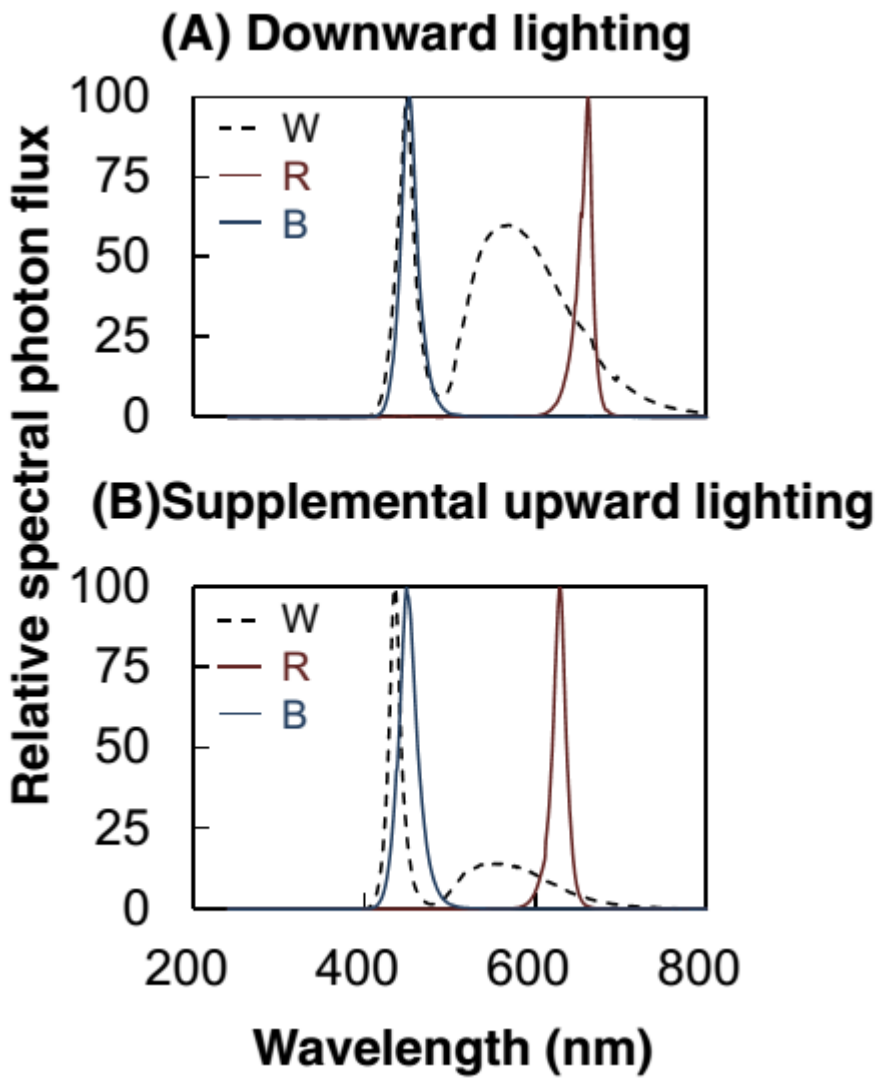

Figure 4-1 The relative spectral photon flux of (A) downward lighting (top LEDs) and (B) supplemental upward lighting (bottom LEDs). The wavelengths of light sources were recorded at 240-800 nm with a spectrometer (SR9910-v7, irradiant Ltd., Tranent, UK). W, R, or B denotes white, red, or blue LEDs.

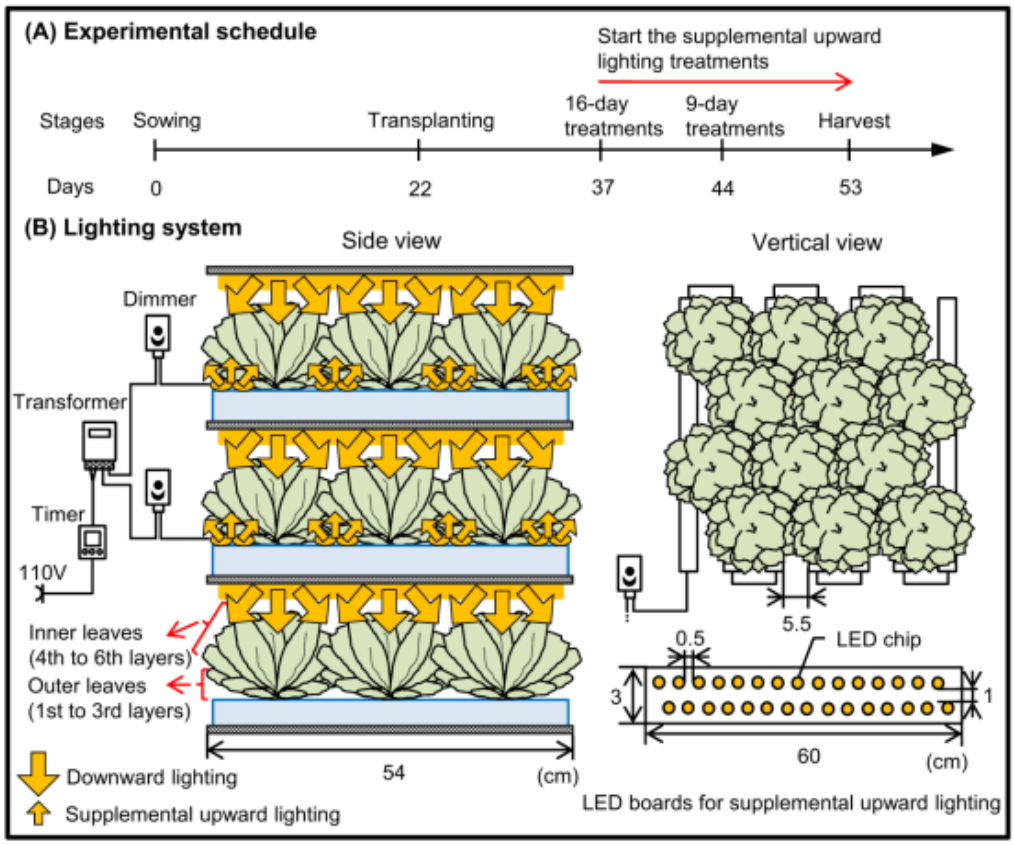

Figure 4-2 Schematic diagram of the experimental schedule (A) and the lighting system (B) used in the present study. (A) At 22 days after sowing, the seedlings were transplanted into growth chambers equipped with 200 μmol·m^{-2}·s^{-1} PPFD of downward-facing white, red, or blue LEDs and with light provided for 16 h per day. The supplemental upward lighting treatments were performed from 15 or 22 days after transplanting until harvest. (B) There were three vertically arranged cultivation beds for each light color. Lettuce grown in the top and middle layers received light from above (downward lighitng), with supplemental upward lighting for 9 and 16 days, respectively. Lettuce grown in the bottom layer received light only from above, without any supplemental upward lighting. The treatment arrangement was the same for each LED color: lettuce was grown at 37 plants·m^{-2}, with 22 LED boards (each board containing two rows of LED chips)·m^{-2} used for supplemental upward lighting. A digital timer, dimmer, and transformer were used to maintain the light period (16 h, the same as the downward lighting) and light intensity (40 μmol·m^{-2}·s^{-1} PPFD).

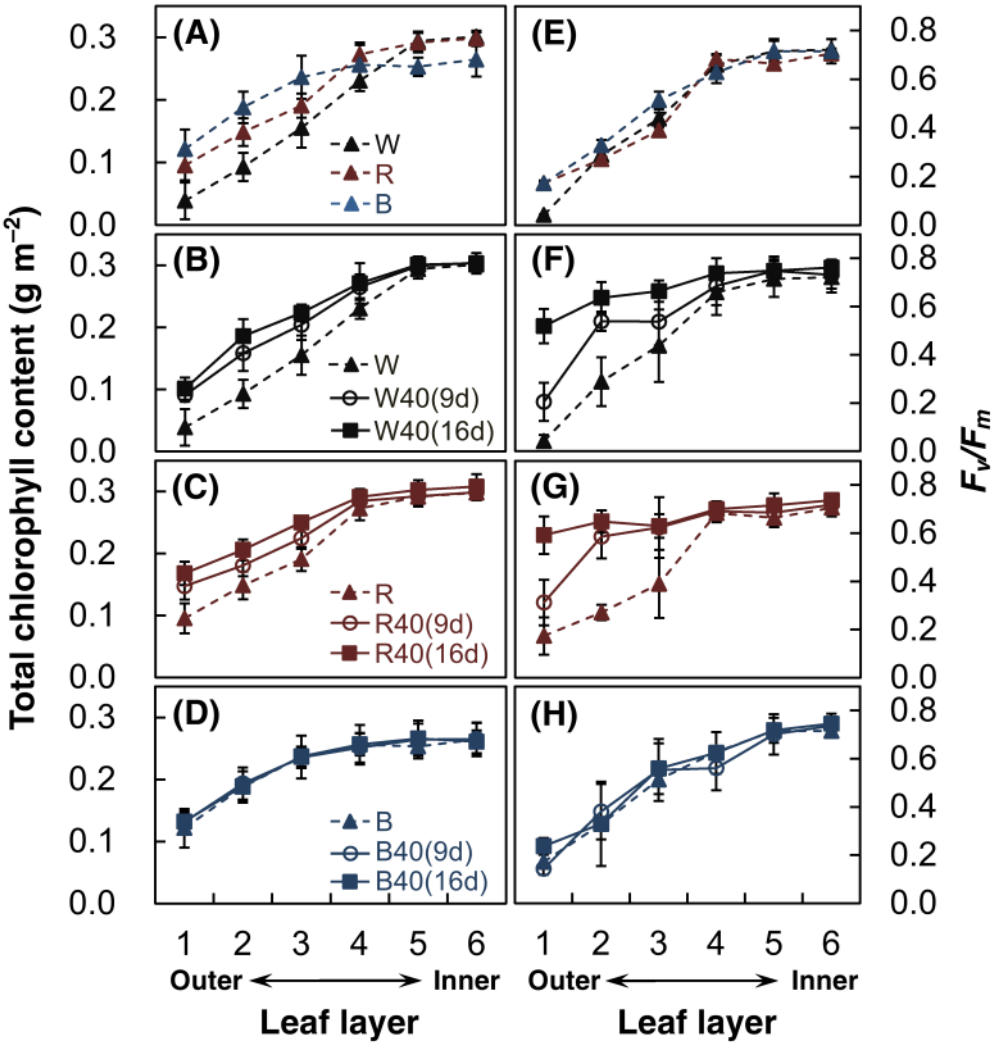

Figure 4-3 Total chlorophyll content (A–D) and maximum quantum yield (F_v/F_m) (E–H) in lettuce leaves from the six layers of leaves in plants grown under downward lighitng by white (W), red (R), or blue (B) LEDs, without or with supplemental upward lighting for 9 days ("9d") or 16 days ("16d") at 40 μmol·m^{-2}·s^{-1} PPFD. Data represent means ± SD (n = 3 to 5). (A, E) No supplemental upward lighting. (B–D, F–H) Same downward lighting as in (A), but with supplemental (B, F) white, (C, G) red, or (D, H) blue upward lighting.

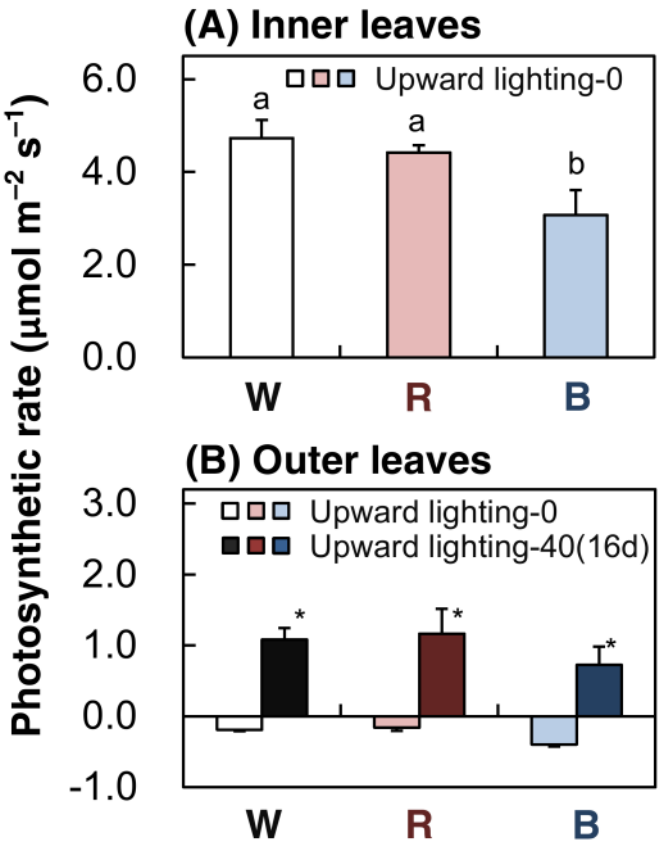

Figure 4-4 Photosynthetic rates of plants grown under downward lighting by white, red, or blue LEDs, with or without supplemental upward lighting. Data represent means ± SD (n = 3). (A) Photosynthetic rates were measured under ambient light conditions in the most newly expanded leaves (in the 6th layer) in plants grown without upward lighting. Bars labeled with different letters differ significantly (Tukey's HSD test, $P < 0.05$) among the three light colors. (B) Photosynthetic rates were measured under ambient light conditions in the outer leaves (in the 3rd layer) in plants grown under downward lighting by white, red, or blue LEDs, with or without supplemental upward lighting for 16 days. Bars labeled with "*" differ significantly for a given light color (Student's t test, $P < 0.05$). "Upward lighting-0" denotes plants grown without supplemental upward lighting; "Upward lighting-40 (16d)" denotes plants grown with 16 days of supplemental upward lighting at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD.

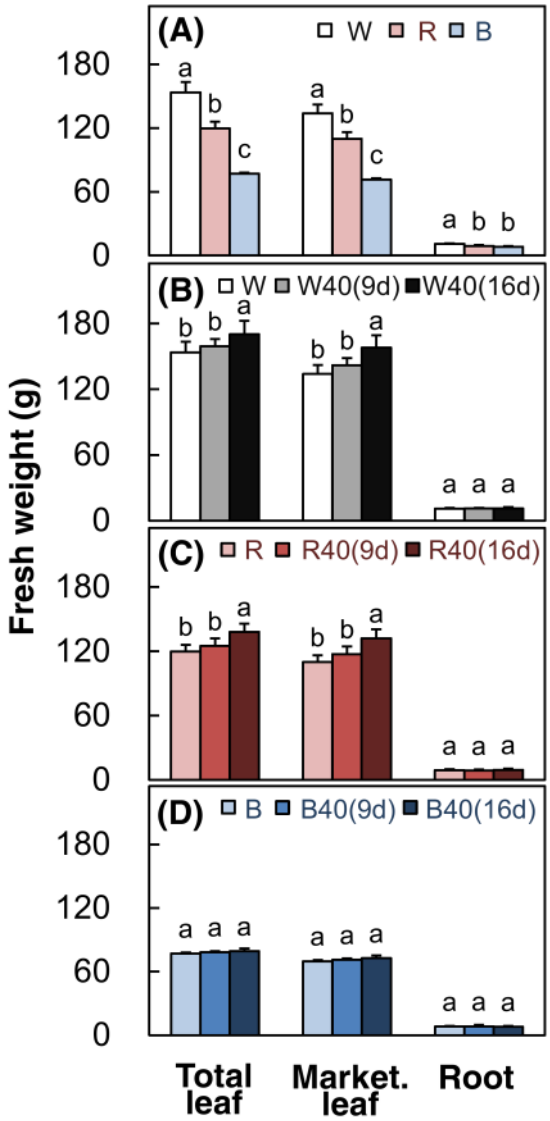

Figure 4-5 Total leaf fresh weights, marketable leaf fresh weights, and root fresh weights of plants grown under (A) white (W), red (R), and blue (B) LEDs without supplemental upward lighting, or under the same downward lighting from above with supplemental upward lighting from (B) white, (C) red, or (D) blue LEDs. Data represent means ± SD (n = 3 to 5). Supplemental upward lighting was provided for 9 days ("9d") or 16 days ("16d") at 40 μmol·m^{-2}·s^{-1} PPFD. Bars labeled with different letters differ significantly (Tukey's HSD test, $P < 0.05$).

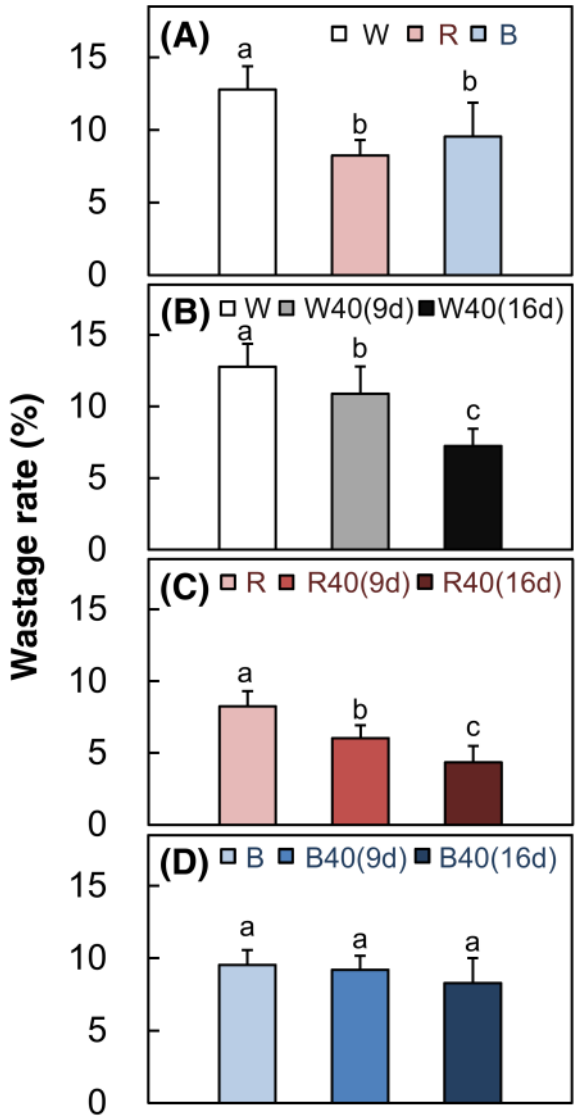

Figure 4-6 Wastage rates (total leaf fresh weight minus marketable leaf fresh weight) of outer senesced leaves of plants grown under downward lighting by white, red, or blue LEDs (A) without and (B–D) with supplemental upward lighting. Data represent means ± SD (n = 3 to 5). Supplemental upward lighting was provided for 9 days ("9d") or 16 days ("16d") at 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. Bars labeled with different letters differ significantly between treatments (Tukey's HSD test, $P < 0.05$).

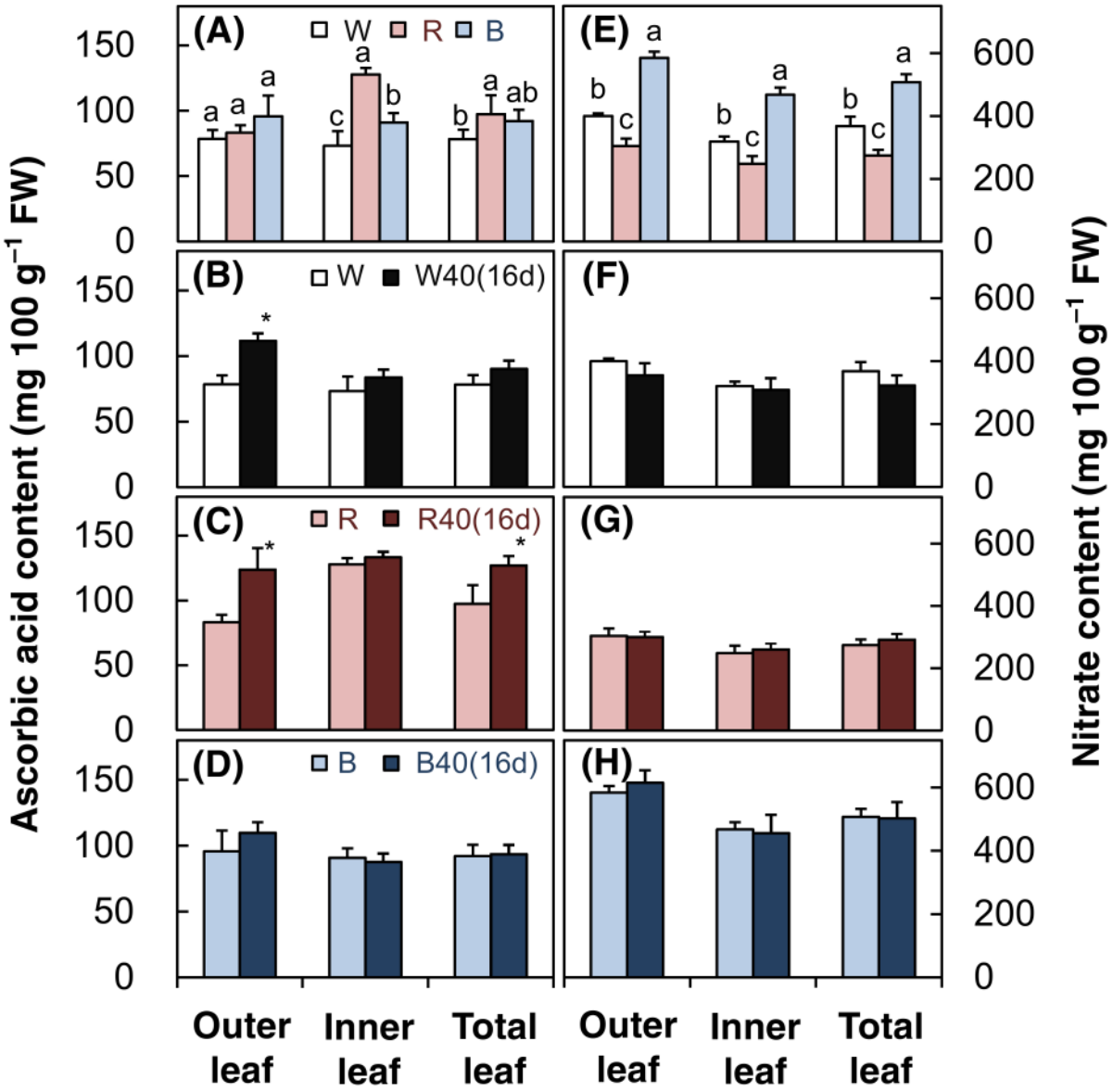

Figure 4-7 Ascorbic acid (A–D) and nitrate contents (E–H) in the outer leaves, inner leaves, and total leaves of lettuce plants grown under downward lighting by white (W), red (R), or blue (B) LEDs: (A, E) without or (B–D, F–H) with supplemental upward lighting at 40 μmol·m^{-2}·s^{-1} PPFD for 16 days ("16d"). Data represent means ± SD (n = 3 to 5). (A, E) Bars labeled with different letters differ significantly among the same plant parts (Tukey's HSD test, $P < 0.05$). (B–D, F–H) Bars labeled with "*" differ significantly between treatments (Student's t test, $P < 0.05$).

Chapter 5: Conclusions and prospects

Summaries of the studies

Lettuce (*Lactuca sativa*) is one of the most widely consumed vegetables throughout the world and hydroponic lettuce has become a very important part of the worldwide lettuce production. With the advantages of hydroponics, indoor cultivation can be realized. Currently, a new type of facility, the "plant factory with artificial lighting" (PFAL), has been used for the commercial production of lettuce in a controlled environment, using less water, nutrients, and labor. Since the rate of chronic kidney disease increases all over the world yearly, the demand for fresh lettuce with low potassium level is increasing. In Japan, some companies are committed to producing low-potassium lettuce in PFALs. However, they are having some difficulties such as inconsistent quality of plants, low plant growth rate, and sodium remaining in solution and tank. Therefore, establishing cultivation methods that can produce low-potassium lettuce with high yield and good quality is an important goal to improve profitability of a PFAL. The present study had two purposes: to investigate the reaction of lettuce to low potassium conditions in order to improve the production skills of low-potassium lettuce, and to explore the possible way to improve the yield and quality of low-potassium lettuce.

In chapter 2, I studied the responses of three lettuce types with different morphological characteristics to reduced potassium in nutrient solutions. Plant yield and RGR were lowest under a 1/4 K treatment for all three lettuce types. In green leaf lettuce, the reductions in both NAR and LAR led to a decline in RGR. In Boston lettuce and romaine lettuce, the reduction of RGR was mainly due to a reduction in LAR, and to a lesser extent caused by NAR. Reduced potassium in the nutrient solution had a greater effect on mature leaves than on newly expanded leaves for all

three lettuce types. In green leaf lettuce and Boston lettuce, photosynthetic rates of mature leaves significantly decreased under reduced potassium treatments, with a steady or gradually increased intercellular CO_2 concentration and this indicated that non-stomatal factors suppressed the photosynthesis. In romaine lettuce, the photosynthetic rate was less influenced by reduced potassium levels in the nutrient solution, and the significant increase observed in leaf mass per area might contribute to maintaining photosynthesis in the leaf.

In chapter 3, I analyzed the growth dynamics and nutrient (nitrogen, phosphorus, potassium, calcium, and magnesium) absorption dynamics of lettuce in a PFAL. Most of the dry matter and nutrients were accumulated in the final cultivation period, suggesting that this period is important to maximize plant yield. A quantitative nutrient management method with a modified nutrient solution recipe was investigated in deep flow technique lettuce during the final cultivation period. The plant yield of the quantitative nutrient management method was similar to that of EC-based method. However, the nutrient supply was reduced through the quantitative nutrient management method, and it also remained a lower level of nutrient residue in drained solution. In addition, the quantitative nutrient management method decreased the contents of potassium and nitrate in plants.

In chapter 4, I analyzed the effect of light color on photosynthesis and plant growth of romaine lettuce, and investigated the effect of supplemental upward lighting from underneath the plant on leaf senescence in the outer leaves. I found that white LEDs were more appropriate for lettuce growth than red or blue LEDs. Supplemental white and red upward lighting retarded the senescence of outer leaves, increased the net photosynthetic rate in outer leaves and decreased waste part of leaves, leading to an improvement of the marketable leaf fresh weight. The supplemental upward lighting from underneath for 16 days gained more yield and net profit

compared with that for 9 days. Moreover, the plants grown under blue LEDs grew more erect and could not efficiently absorb the upward lighting, suggesting that it is necessary to select suitable light quality for certain plant species when using this cultivation method.

Recommendations for future study

Potassium is one of the principle plant nutrients underpinning crop yield production and quality determination (Pettigrew, 2008). Since potassium is involved in many physiological processes, a low supply of potassium will cause a decline in plant productivity. The present work investigated the plant growth and photosynthesis response to low potassium conditions in three lettuce types, and examined the effects of quantitative nutrient management and supplemental upward lighting on lettuce yield and quality. Although the potassium content in lettuce leaves was reduced by using quantitative nutrient management in the present study, the potassium level was still high for kidney patients with kidney disease. Further researches need to be conducted to improve the quantitative nutrient management for low-potassium lettuce culture in PFALs. In addition, the next step is to verify whether low-potassium lettuce with high yield and good quality can be obtained by supplemental upward lighting from underneath the plants.

Plant growth is influenced by various environmental factors, including lighting, temperature, relative humidity, CO_2 concentration, and nutrient solution. The present study was focused on nutrient solution and lighting. Future studies are suggested to work on how to optimize other environmental factors for production of low-potassium lettuce, such as temperature and CO_2 concentration. It has been proved that low or high root-zone temperature reduces plant yields since it negatively affects several plant physiological processes, such as photosynthesis. However, the root-zone temperature is barely controlled, throughout the

cultivation in PFALs. Thus, it would be possible to increase plant yield by optimizing the root-zone temperature in the hydroponic system. Furthermore, CO_2 as the source of carbon is essential for plant growth. Elevated CO_2 concentration increases carbon assimilation and carbohydrate accumulation within source leaves, which has beneficial effects on plant yield. In PFALs, because of the closed cultivation environment, the CO_2 concentration can be increased to meet the requirements of plant growth, leading to a high yield. There is a need to carry out related works in future research to investigate the effect of CO_2 concentration on plant growth in a PFAL.

References

Amtmann, A., S. Troufflard, and P. Armengaud. 2008. The effect of potassium nutrition on pest and disease resistance in plants. Physiol. Plant. 133: 682–691.

Asao, T., M. Asaduzzaman, M. F. Mondal, M. Tokura, F. Adachi, M. Ueno, and T. Ban. 2013. Impact of reduced potassium nitrate concentrations in nutrient solution on the growth, yield and fruit quality of melon in hydroponics. Scientia Hort. 164: 221–231.

Bajwa, S. S. and I. S. Kwatra. 2013. Nutritional needs and dietary modifications in patients on dialysis and chronic kidney disease. J. Medical Nutr. Nutraceuticals 2: 46.

Barsoum, R. S. 2006. Chronic kidney disease in the developing world. N Engl J Med 354: 997–999.

Basile, B., E. J. Reidel, S. A. Weinbaum, and T. M. DeJong. 2003. Leaf potassium concentration, CO_2 exchange and light interception in almond trees (*Prunus dulcis* (Mill) DA Webb). Scientia Hort. 98: 185–194.

Bednarz, C. W. and D. M. Oosterhuis. 1999. Physiological changes associated with potassium deficiency in cotton. J. Plant Nutr. 22: 303–313.

Björkman, O. 1981. Responses to different quantum flux densities. p. 57–107. In: O. L. Lange, P. S. Nobel, C. B. Osmond and H. Ziegler (eds.). Physiological Plant Ecology I. Springer-Verlag, Berlin, Germany.

Bliznikas, Z., A. Žukauskas, G. Samuoliene, A. Viršile, A. Brazaityte, J. Jankauskiene, et al. 2012. Effect of supplementary pre-harvest LED lighting on the antioxidant and nutritional properties of green vegetables. Acta Hortic. 939: 85–91.

Brouwer, B., A. Ziolkowska, M. Bagard, O. Keech, and P. Gardeström. 2012. The impact of light intensity on shade-induced leaf senescence. Plant Cell Environ. 35: 1084–1098.

Brown, C. S., A. C. Schuerger, and J. C. Sager. 1995. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. J. Am. Soc. Hortic. Sci. 120: 808–813.

Cakmak, I., C. Hengeler, and H. Marschner. 1994. Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. J. Experimental Bot. 45: 1251–1257.

Causin, H. F., R. N. Jauregui, and A. J. Barneix. 2006. The effect of light spectral quality on leaf senescence and oxidative stress in wheat. Plant Sci. 171: 24–33.

Collins, M. and S. H. Duke. 1981. Influence of potassium-fertilization rate and form on photosynthesis and N_2 fixation of alfalfa. Crop Sci. 21: 481–485.

Damilano, B., N. Grandjean, C. Pernot, and J. Massies. 2001. Monolithic white light emitting diodes based on InGaN/GaN multiple-quantum wells. Jpn. J. Appl. Phys. 40: L918.

Epron, D., J. P. Laclau, J. C. Almeida, J. L. M. Gonçalves, S. Ponton, C.R. Sette, and Y. Nouvellon. 2012. Do changes in carbon allocation account for the growth response to potassium and sodium applications in tropical *Eucalyptus* plantations? Tree Physiol. 32: 667–679.

Evans, G. C. 1972. The quantitative analysis of plant growth. University of California Press, Berkeley, CA.

Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78: 9–19.

Flowers, T. J. and A. Läuchli. 1983. Sodium versus potassium: substitution and

compartmentation. p. 651–681. In: A. Läuchli, and A. Pirson (eds.). Encyclopedia of Plant Physiology. Springer-Verlag, Berlin, Germany.

Gan, S., and R. M. Amasino. 1997. Making sense of senescence (molecular genetic regulation and manipulation of leaf senescence). Plant Physiol. 113: 313.

Galieni, A., C. Di Mattia, M. De Gregorio, S. Speca, D. Mastrocola, M. Pisante, and F. Stagnari. 2015. Effects of nutrient deficiency and abiotic environmental stresses on yield, phenolic compounds and antiradical activity in lettuce (*Lactuca sativa* L.). Scientia Hort. 187: 93–101.

Goins, G. D., N. C. Yorio, M. M. Sanwo, and C. S. Brown. 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J. Exp. Bot. 48: 1407–1413.

Greitner, C. S., E. J. Pell, and W. E. Winner. 1994. Analysis of aspen foliage exposed to multiple stresses: ozone, nitrogen deficiency and drought. New Phytologist 127: 579–589.

Harlan, J. R. 1986. Lettuce and the sycomore: sex and romance in ancient Egypt. Economic Bot. 40: 4–15.

Hoerger, T. J., Simpson, S. A., Yarnoff, B. O., Pavkov, M. E., Burrows, N. R., Saydah, S. H., and Zhuo, X. 2015. The future burden of CKD in the United States: a simulation model for the CDC CKD Initiative. Amer. J. Kidney Dis. 65: 403–411.

Hosoi, N. and T. Hosono. 2005. Liquid cultivation of tomato plants by daily control of fertilizer application in which there are no N/P/K residues in the culture solution and the area of leaf groups is used as an indicator. Bull. Natl. Inst. Veg. Tea Sci. 4: 87–119 (In Japanese with English abstract).

Hu, M. C., Y. H. Chen, and L. C. Huang. 2014. A sustainable vegetable supply chain

using plant factories in Taiwanese markets: A Nash-Cournot model. Int. J. Prod. Econ. 152: 49–56.

James, J. J. and R. E. Drenovsky. 2007. A basis for relative growth rate differences between native and invasive forb seedlings. Rangeland Ecol. Mgt. 60: 395–400.

Jifon, J. L. and G. E. Lester. 2009. Foliar potassium fertilization improves fruit quality of field-grown muskmelon on calcareous soils in south Texas. J. Sci. Food Agr. 89: 2452–2460.

Jin, S. H., J. Q. Huang, X. Q. Li, B. S. Zheng, J. S. Wu, Z. J. Wang, and M. Chen. 2011. Effects of potassium supply on limitations of photosynthesis by mesophyll diffusion conductance in *Carya cathayensis*. Tree Physiol. 31: 1142–1151.

Jordan-Meille, L. and S. Pellerin. 2004. Leaf area establishment of a maize (*Zea mays* L.) field crop under potassium deficiency. Plant Soil 265: 75–92.

Kaya, C., H. Kirnak, and D. Higgs. 2001. Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorus in tomato cultivars grown at high (NaCl) salinity. J. Plant Nutr. 24: 357–367.

Kim H. H., R. M. Wheeler, J. C. Sager, G. D. Goins, and J. H. Norikane 2006. Evaluation of lettuce growth using supplemental green light with red and blue light-emitting diodes in a controlled environment-a review of research at Kennedy Space Center. Acta Hortic. 711: 111–120.

Kinoshita, T., Yamazaki, H., Inamoto, K., and H. Yamazaki. 2016. Analysis of yield components and dry matter production in a simplified soilless tomato culture system by using controlled-release fertilizers during summer–winter greenhouse production. Scientia Hort. 202, 17-24.

Kozai, T. 2013a. Plant factory in Japan—current situation and perspectives. Chron. Hort. 53: 8–11.

Kozai, T. 2013b. Resource use efficiency of closed plant production system with artificial light: concept, estimation and application to plant factory. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 89: 447–461.

Kozlowski, T. T. and S. G. Pallardy. 1984. Effect of flooding on water, carbohydrate, and mineral relations. p. 165–193. In: T. T. Kozlowski (eds.). Flooding and plant growth. Academic Press, New York, NY.

Kurimoto, K., D. A. Day, H. Lambers, and K. Noguchi. 2004. Effect of respiratory homeostasis on plant growth in cultivars of wheat and rice. Plant Cell Environ. 27: 853–862.

Lebaudy, A., A. Vavasseur, E. Hosy, I. Dreyer, N. Leonhardt, J. B. Thibaud, and H. Sentenac. 2008. Plant adaptation to fluctuating environment and biomass production are strongly dependent on guard cell potassium channels. Proc. Natl. Acad. Sci. 105: 5271–5276.

Lee, J. G., S. S. Oh, S. H. Cha, Y. A. Jang, S. Y. Kim, Y. C. Um, et al. 2010. Effects of red/blue light ratio and short-term light quality conversion on growth and anthocyanin contents of baby leaf lettuce. J. Bio-Environ. Control 19: 351–359.

Lester, G. E., J. L. Jifon, and G. Rogers. 2005. Supplemental foliar potassium applications during muskmelon fruit development can improve fruit quality, ascorbic acid, and beta-carotene contents. J. Amer. Soc. Hort. Sci. 130: 649–653.

Lin, K. H., M. Y. Huang, W. D. Huang, M. H. Hsu, Z. W. Yang, and C. M. Yang. 2013. The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. *capitata*). Sci. Hortic. 150: 86–91.

Lindhauer, M. G., H. E. Header, and H. Beringer. 1990. Osmotic potentials and solute concentrations in sugar beet plants cultivated with varying potassium/sodium ratios.

Zeitschrift für Pflanzenernährung und Bodenkunde 153: 25–32.

Martínez-Sánchez, A., J. A. Tudela, C. Luna, A. Allende, and M. I. Gil. 2011. Low oxygen levels and light exposure affect quality of fresh-cut Romaine lettuce. Postharvest Biol. Technol. 59: 34–42.

Marschner, H. 1971. Why can sodium replace potassium in plants? Potassium Biochem. Physiol. 50–63.

Marschner, H. 1995. Function of mineral nutrients: micronutrients. p. 313–404. In: R. Marschner (eds.). Mineral Nutrition of Higher Plants 2nd Edition. Academic Press, Cambridge, London.

Maruo, T., H. Hoshi, M. Hohjo, Y. Shinohara, and T. Ito. 2001. Quantitative nutrient management at low concentration condition in NFT spinach culture. Acta Hortic. 548, 133-140.

Massa, G. D., H. H. Kim, R. M. Wheeler, and C. A. Mitchell. 2008. Plant productivity in response to LED lighting. HortScience 43: 1951–1956.

Matsuda, R., K. Ohashi-Kaneko, K. Fujiwara, and K. Kurata. 2007. Analysis of the relationship between blue-light photon flux density and the photosynthetic properties of spinach (*Spinacia oleracea* L.) leaves with regard to the acclimation of photosynthesis to growth irradiance. Soil Sci. Plant Nutr. 53: 459–465.

McCabe, M. S., L. C. Garratt, F. Schepers, W. J. Jordi, G. M. Stoopen, E. Davelaar, et al. 2001. Effects of PSAG12-IPT gene expression on development and senescence in transgenic lettuce. Plant Physiol. 127: 505–516.

McCree, K. J. 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agric. Meteorol. 9: 191–216.

Mou, B. 2008. Lettuce, In Vegetables I, p. 75–116. In: J. Prohens and F. Nuez (eds.). Hdbk. Plant Breeding. Springer Science, New York, NY.

Nagai, T. and A. Makino. 2009. Differences between rice and wheat in temperature responses of photosynthesis and plant growth. Plant Cell Physiol. 50: 744–755.

Nakano, Y., H. Sasaki, A. Nakano, K. Suzuki, M. Takaichi. 2010. Growth and yield of tomato plants as influenced by nutrient application rates with quantitative control in closed rockwool cultivation. J. Jpn. Soc. Hortic. Sci. 79, 47–55.

Nakano, Y., S. Watanabe, H. Kawashima, and M. Takaichi. 2006. The effect of daily nutrient applications on yield, fruit quality, and nutrient uptake of hydroponically cultivated tomato. J. Japan. Soc. Hort. Sci. 75: 421–429 (In Japanese with English abstract).

Ogawa, A., S. Fujita, and K. Toyofuku. 2014. A cultivation method for lettuce and spinach with high levels of vitamin C using potassium restriction. Environmental Control Biol. 52: 95–99.

Okamoto K., T. Yanagi, S. Takita, M. Tanaka, T. Higuchi, Y. Ushida, et al. 1996. Development of plant growth apparatus using blue and red LED as artificial light source. Acta Hortic. 440: 111–116.

Pantelidis, G. E., M. Vasilakakis, G. A. Manganaris, and G. Diamantidis. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. Food Chem. 102: 777–783.

Pardossi, A., F. Malorgio, and F. Tognoni. 1994. Control of mineral nutrition in melon plants grown with NFT. Hydroponics and Transplant Production 396, 173-180.

Paul, N. D. and P. G. Ayres. 1986. The effects of infection by rust (*Puccinia lagenophorae* Cooke) on the growth of groundsel (*Senecio vulgaris* L.) cultivated under a range of nutrient concentrations. Ann. Bot. 58: 321–331.

Peaslee, D. E. and D. N. Moss. 1968. Stomatal conductivities in K-deficient leaves of

maize (*Zea mays* L.). Crop Sci. 8: 427–430.

Pettigrew, W. T. 2008. Potassium influences on yield and quality production for maize, wheat, soybean and cotton. Physiol. Plant. 133: 670–681.

Porra, R. J., W. A. Thompson, and P. E. Kriedemann. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta (BBA)-Bioenergetics 975: 384–394.

Reddy, K. R. and D. Zhao. 2005. Interactive effects of elevated CO_2 and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton. Field Crops Res. 94: 201–213.

Römheld, V. and E. A. Kirkby. 2010. Research on potassium in agriculture: needs and prospects. Plant Soil 335: 155–180.

Rouphael, Y. and G. Colla. 2009. The influence of drip irrigation or subirrigation on zucchini squash grown in closed-loop substrate culture with high and low nutrient solution concentrations. HortScience 44, 306-311.

Ryder, E. J. 1999. Lettuce, endive and chicory. CABI Publishing, Wallingford, UK.

Ryder, E. J. 2002. The new salad crop revolution. Trends in new crops and new uses. ASHS Press, Alexandria, VA, 408–412.

Sale, P. W. G. and L. C. Campbell. 1987. Differential responses to K deficiency among soybean cultivars. Plant Soil 104: 183–190.

Schwarz, D., G. B. Öztekin, Y. Tüzel, B. Brückner, and A. Krumbein. 2013. Rootstocks can enhance tomato growth and quality characteristics at low potassium supply. Scientia Hort. 149: 70–79.

Shao, L., X. W. Chen, Y. J. Chen, B. Y. Sun, W. Chow, and C. Peng. 2013. Differential

responses of photosystem II activity to photooxidation in red and green tissues of *Amaranthus tricolor* leaves. Pakistan J. Bot. 45: 1905–1912.

Smith, R., M. Cahn, O. Daugovish, and S. Koike. 2011. Leaf lettuce production in California. UCANR Publ., Richmond.

Soares, A. S., S. P. Driscoll, E. Olmos, J. Harbinson, M. C. Arrabaça, and C. H. Foyer. 2008. Adaxial/abaxial specification in the regulation of photosynthesis and stomatal opening with respect to light orientation and growth with CO_2 enrichment in the C_4 species *Paspalum dilatatum*. New Phytol. 177: 186–198.

Son, K. H. and M. M. Oh. 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48: 988–995.

Subbarao, G. V., O. Ito, W. L. Berry, and R. M. Wheeler. 2003. Sodium-a functional plant nutrient. Critical Rev. Plant Sci. 22: 391–416.

Taguchi, T. 2003. Present status of white LED lighting technologies in Japan. J. Light Vis. Environ. 27: 131–139.

Terashima, I. 1986. Dorsiventrality in photosynthetic light response curves of a leaf. J. Exp. Bot. 37: 399–405.

Terashima, I., T. Araya, S. I. Miyazawa, K. Sone, and S. Yano. 2005. Construction and maintenance of the optimal photosynthetic systems of the leaf, herbaceous plant and tree: an eco-developmental treatise. Ann. Bot. 95: 507–519.

Terry, N. and A. Ulrich. 1973. Effects of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol. 51: 783–786.

Thiel, G. and A. H. Wolf. 1997. Operation of K^+-channels in stomatal movement. Trends Plant Sci. 2: 339–345.

Thimann, K. V. and S. O. Satler. 1979. Relation between leaf senescence and stomatal

closure: Senescence in light. Proc. Natl. Acad. Sci. U.S.A. 76: 2295–2298.

Tombesi, L., M. T. Calè, and B. Tiborne. 1969. Effects of nitrogen, phosphorus and potassium fertilizers on the assimilation capacity of *Beta vulgaris* chloroplasts (I). Plant Soil 31: 65–76.

Vinit-Dunand, F., D. Epron, B. Alaoui-Sossé, and P. M. Badot. 2002. Effects of copper on growth and on photosynthesis of mature and expanding leaves in cucumber plants. Plant Sci. 163: 53–58.

Wallace, R. W., A. L. Wszelaki, C. A. Miles, J. S. Cowan, J. Martin, J. Roozen, and D. A. Inglis. 2012. Lettuce yield and quality when grown in high tunnel and open-field production systems under three diverse climates. HortTechnology 22: 659–668.

Wang, N., H. Hua, A. E. Eneji, Z. Li, L. Duan, and X. Tian. 2012. Genotypic variations in photosynthetic and physiological adjustment to potassium deficiency in cotton (*Gossypium hirsutum*). J. Photochemistry Photobiology B: Biol. 110: 1–8.

Weaver, L. M. and R. M. Amasino. 2001. Senescence is induced in individually darkened Arabidopsis leaves, but inhibited in whole darkened plants. Plant Physiol. 127: 876–886.

Weng, X. Y., C. J. Zheng, H. X. Xu, and J. Y. Sun. 2007. Characteristics of photosynthesis and functions of the water-water cycle in rice (*Oryza sativa*) leaves in response to potassium deficiency. Physiol. Plant. 131: 614–621.

Wilson, P. J., K. E. N. Thompson, and J. G. Hodgson. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytologist 143: 155–162.

Wingler, A., M. Marès, and N. Pourtau. 2004. Spatial patterns and metabolic regulation of photosynthetic parameters during leaf senescence. New Phytol. 161: 781–789.

Xie Z., M. T. Charles, D. Charlebois, D. Rolland, D. Roussel, M. Deschênes, et al. 2015. Preharvest exposure to UV-C radiation: impact on strawberry fruit quality. Acta Hortic. 1079: 589–592.

Yamori, W., T. Nagai, and A. Makino. 2011. The rate-limiting step for CO_2 assimilation at different temperatures is influenced by the leaf nitrogen content in several C_3 crop species. Plant Cell Environ. 34: 764–777.

Yamori, W., K. Noguchi, and I. Terashima. 2005. Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant Cell Environ. 28: 536–547.

Yamori, W., K. Noguchi, K. Hikosaka, and I. Terashima. 2009. Cold-tolerant crop species have greater temperature homeostasis of leaf respiration and photosynthesis than cold-sensitive species. Plant Cell Physiol. 50: 203–215.

Yamori, W., K. Noguchi, K. Hikosaka, and I. Terashima. 2010. Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. Plant Physiol. 152: 388–399.

Yamori, W., G. Zhang, M. Takagaki, and T. Maruo. 2014. Feasibility Study of Rice Growth in Plant Factories. Rice Res. Open Access 2: 119.

Yanagi T., K. Okamoto, and S. Takita. 1996. Effects of blue, red, and blue/red lights of two different PPF levels on growth and morphogenesis of lettuce plants. Acta Hortic. 440: 117–122.

Yorio, N. C., G. D. Goins, H. R. Kagie, R. M. Wheeler, and J. C. Sager. 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. HortScience 36: 380–383.

Yoshida, T., K. Sakuma, and H. Kumagai. 2014. Nutritional and taste characteristics of

low-potassium lettuce developed for patients with chronic kidney diseases. Hong Kong J. Nephrology 16: 42–45.

Zhao, D., D. M. Oosterhuis, and C. W. Bednarz. 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. Photosynthetica 39: 103–109.

Zhang, Q. L. and D. Rothenbacher. 2008. Prevalence of chronic kidney disease in population-based studies: systematic review. BMC Public Health 8: 117.