論文題目

Study on the quantitative nutrient management in low-potassium lettuce production

2020 年 7 月

千葉大学大学院園芸学研究科
環境園芸学専攻生物資源科学コース
Xu Hongjia

（千葉大学審査学位論文）

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Chapter 1. General introduction

Chronic kidney disease

Chronic kidney disease (CKD) is a common disorder to the elderly people, and its prevalence is increasing globally. Japan is turning into a super-aged society and the number of CKD patients with dialysis treatment in Japan is now over 310,000, which is the second largest population in the world (Talukder et al., 2016). According to Japanese Society of Nephrology, the number of CKD patients is estimated about 13.3 million (one in eight adults) in Japan.

The kidneys normally excrete more than 90% of daily body K but patients with CKD can't completely excrete it, and thus, residual K accumulates in the body (Spital and Stems, 1988). The serum concentration of K is usually 3.5 to 5.0 mEq L^{-1} in a normal person. However, this value will be 2-3 times in the patient with CKD (Putcha and Allon, 2007). The accumulation of K in the blood disorders the metabolism of human body, threatening life (Barsoum, 2006; Zhang and Rothenbacher, 2008).

Hyperkalemia is an electrolyte disturbance, which K is too much in the extracellular space (serum K levels > 6.0 meq L^{-1}) , occurring with increased frequency among patients with chronic kidney disease, diabetes, heart failure and used of certain medications such as renin angiotensin aldosterone system inhibitors and nonsteroidal anti-inflammatory drugs (Kovesdy, 2017). Renal failure, and/or failure to augment distal tubular K secretion, is largely responsible for the maintenance of hyperkalemia. Hyperkalemia can induce or worsen cardiac arrhythmias, and it is associated with significantly increased mortality (Hayes et al., 2012).

Low-K lettuce

A detailed review of the various treatments, such as dialysis, resin adsorbent, diuretic medication and so on, applied in the context of acute management of hyperkalemia is beyond the scope of this research (Kovesdy, 2014). However, an important way to reduce the incidence of hyperkalemia is to prevent or support treatment through dietary adjustments. As a primary control measure, doctors restrict foods with high K content such as fresh vegetables, seaweed, bean and fruits including melon, strawberry, banana and kiwi (Montford and Stuart, 2017; Weiner and Wingo, 1998). Moreover, as K dissolves easily in water, CDK patients with dialysis are advised to cut these K-rich fruits and vegetables into small pieces and boil or soak them in a large volume of water prior to eating (Burrows and Ramer, 2008). Meanwhile, other nutrients in vegetables will be lost with heating, such as dietary fiber, ascorbic acid, phenolic compound and so on (Podsedek, 2007; Zia-ur-Rehman et al., 2003). In a word, the life quality of these patients decreases greatly. Therefore, supplementation of vegetables containing less amount of K than usual would be a useful preventive method.

The low-K vegetable cultivation is mainly researched in Japan. Many vegetables with low K content have been produced, such as spinach, lettuce, melon, tomato, carrot， and strawberry (Asao et al., 2013; Mondal et al., 2016; Nishikawa et al., 2016; Ogawa et al., 2012; Tsukagoshi et al., 2016; Yoshida et al., 2014). Almost all cultivation methods are to reduce the KNO_3 concentration in the hydroponic nutrient

solution and replace it with other nitrates such as $NaNO_3$, to reduce the K supply while ensuring an enough N supply. Among them, reducing the K content of melon and other fruit vegetables was insufficient, as when the plants were deficient in K, K would flow from the leaves to the fruit (Asao et al., 2013). And for leafy vegetables, the feasibility was higher. In spinach, low-K treatment could reduce K content by 79% without limiting plant fresh weight (Ogawa et al., 2012). Low-K lettuce has even been produced in plant factory with a large scale.

Lettuce is a popular K rich vegetable usually eaten raw in salad, about 490 mg 100 g^{-1} FW (Talukder et al., 2016). In Japan, a type of lettuce with low-K content has been marketed where K concentration was below 100 mg 100 g^{-1} FW. There was also no significant difference in other nutritional contents expect for higher Na and lower N concentration. Taste evaluation revealed that low-K lettuce has lower bitterness and higher saltiness than the normal leaf lettuce (Yoshida et al., 2014). However, the physiological reason was not clear.

Quantitative management method

Quantitative management (QM) method is based on the amount of nutrients required by the plant and the fertilizer is quantitatively added to the nutrient solution, not based on the concentration. In hydroponics, in case the inorganic ions in the solution are fully flowing, plants can absorb the nutrients they need at an infinitely low concentration (Japan Protected Horticultural Association, 2015). It has been proved that plants with QM method could grow with the same speed as the electrical

conductivity (EC) control method in hydroponics (Kidono and Suzuki, 2006; Nakano et al., 2010; Takei and Suzuki, 2013). Several vegetables have been cultivated successfully using the QM method, such as tomato (Kageyama, 1991; Kidono and Suzuki, 2006; Nakano et al., 2010), spinach (Maruo et al., 2001; Takei and Suzuki, 2013), melon (Pardossi et al., 2002), and chrysanthemum (Kageyama et al., 1995; Shima et al., 1995).

Recent years, soil pollution due to excessive fertilization was a major global problem (Rubio et al., 2017; Yan et al., 2008). The QM method could maximize the fertilizer utilization efficiency. Meanwhile, the advantages of the QM method had also been discovered as follow: (1) improved efficiency of nutrient usage, (2) reduction of negative environmental impacts, (3) facilitation of growth control in fruit vegetable production, (4) reduction of nitrate content in leaf vegetables, and (5) reduction of physiological disorder through effects on the ionic balance (Li et al., 2013; Matsuda et al., 2011; Sago and Shigemura, 2018).

In the low-K cultivation of lettuce, the high concentration of Na in the leaves was undesirable (Talukder et al., 2016). This would reduce the quality of low-K lettuce and had an adverse effect on dialysis patients (Kelly et al., 2017; Mills et al., 2016). We predict that using the QM method could reduce the Na concentration in low-K lettuce.

In the present study, therefore, I conducted a series of experiments and aimed to explore the application of QM cultivation method in low-K lettuce and its

physiological changes.

In chapter 2, lettuces (Lactuca sativa L.) were cultivated with 2 low-K management method in an environment control system. One method was based on EC management and the K supply was stopped at the end of cultivation, named LKEC. The other method was based on the nutrient quantitative management, and the nutrients amount required for low-K lettuce was quantitatively supplied, named LKQM. Meanwhile, lettuce with normal K concentration was cultivated with EC management as the control, named CK. I tried to reduce the sodium concentration in low-K lettuce by the QM method and studied the effect of low-K treatment on the absorption of other inorganic ions.

In chapter 3, low-K lettuce was cultivated with LKEC and LKQM treatments. I analyzed the photosynthesis, chlorophyll fluorescence, concentrations of soluble sugar, phenol, flavonoid and some secondary metabolites (SMs). I wanted to study the effects of low-K treatment on the physiology of lettuce from photosynthesis to primary and secondary metabolites, and compared the differences between LKEC- and LKQM-treated lettuce.

In chapter 4, I used the QM method to grow lettuces with 4 gradient of K, 400, 200, 100 and 50 mg $plant^{-1}$ respectively. I analyzed the soluble sugar of inner and outer leaves and the relative expression of sugar transporter genes to study the sugar transport in low-K lettuce.

Chapter 2. Application of quantitative management method in low-K lettuce cultivation

Abstract

In this study, two cultivation methods were used to cultivate the lettuce (*Lactuca sativa* L.) with low K concentration. One method was based on EC management and the K supply was stopped at the end of cultivation, named LKEC. The other method was based on the nutrient quantitative management, and the nutrients amount required for low – K lettuce was quantitatively supplied, named LKQM. Meanwhile, lettuce with normal K concentration was cultivated with EC management as the control, named CK. Compared with CK, both low K treatments limited the yield by nearly 20% without any visual deficiency symptoms. There was no significant difference between LKEC and LKQM in terms of plant growth. LKEC–treated lettuce contained lower Na and required less fertilizer than that of LKQM. Moreover, the plant adapted to the K deficiency stress by absorbing more cations to maintain osmotic pressure. N declined with the decreasing K. Herein, LKQM method was considered the practicable and better one in low K cultivation than LKEC method.

Introduction

Lettuce (*Lactuca sativa* L.) is a common leafy vegetable in Japan and a type of lettuce with low-K has been marketed where K concentration was below 100 mg 100 g^{-1} FW (Tsukagoshi et al., 2016). The low K vegetable cultivation is researched mainly in Japan, and all researchers adopt hydroponics. The cultivation of low K

vegetables often is achieved by reducing K in a nutrient solution. However, K usually is supplied as KNO_3; not adding KNO_3 leads to the absence of N. The synthesis of plant amino acids requires N, which involves almost all the physiological reactions in plants (Sung et al., 2015; Vidal and Gutierrez, 2008). Thus, other N sources should be added. Additionally, K is a macronutrient for plant growth, and its deficiency affects the functions of ion homeostasis, osmotic regulation, enzyme activity, membrane polarization, and various metabolic processes (Amtmann et al., 2005; Gattward et al., 2012; Pettigrew, 2008; Schachtman and Shin, 2007). Limiting K supply may cause a biomass decline. To malady this, some researchers proposed using Na instead of K because Na can replace some nonspecific physiological functions of K (Wakeel et al., 2011). Thus, replacing KNO_3 with $NaNO_3$ can limit K in solution without any N loss. Several low K vegetables have been successfully cultivated similarly (Asao et al., 2013; Ogawa et al., 2007; Zhang et al., 2017). However, Na has also reported to be harmful to dialysis patients (Kelly et al., 2017; Mills et al., 2016).

The QM method is usually used to reduce nitrate concentration in vegetables, control plant nutrient absorption, control plant growth and so on (Li et al., 2014). We hope that this method could reduce Na concentration in low-K vegetables. Moreover, different plant species, different growth environments, and different cultivation purposes will affect the element amount of different growth stages of the plant. So, the nutrient recipe for the QM method, which is the element amounts required by plants at different growth stages, should be prepared for the cultivation start.

In this study, two methods were used to cultivate “low-K lettuce”. One was based

on EC management and the K supply was stopped at the end of cultivation, named LKEC. The other was based on the nutrient quantitative management, and the nutrients amount required for low-K lettuce was quantitatively supplied, named LKQM. Meanwhile, lettuce with normal K concentration was cultivated with EC management as the control, namely CK. The element absorption of lettuce and residual fertilizer in a nutrient solution was compared. Moreover, to cultivate low-K lettuce with low Na level, we didn't add extra Na during LKQM method.

Materials and methods

Designation of nutrient recipe for low K lettuce production

In the LKQM treatment, in order to add fertilizer quantitatively, the element amount required by low-K lettuce at different growth stages was needed. The low-K recipe was designed using commercial low-K lettuce plants (Lactuca sativa L. cv. Frillice). The commercial low – K lettuces were cultivated in an environment – controlled plant factory with 1000 ppm CO_2 concentration, 21^{o}C air temperature, 12–h photo–period with 200 μmol m^{-2} s^{-1} photosynthetic photon flux density. The nutrient solution used the Otsuka–A formula (OAT Agrio Co., Ltd, Tokyo, Japan; EC: 1.45 dS m^{-1}; pH: 6.5). At 7 days before harvest, all K in the nutrient solution was replaced by Na. The growth period was 35 days. At the growth stages of 14, 28, and 35 days after seeding (DAS), 10 lettuce plants were bought separately from a lettuce production company in Japan. Fresh weight (FW), dry weight (DW), and concentrations of N, P, K, Ca, Mg, and Na were measured at each stage. The plant

growth and elements concentrations are described in tables 2-1 and 2-2. Based on this, we calculated the element amount required by low-K lettuce at these 3 growth stages (Table 2-3). Then we designed an inorganic fertilizer formula and added them to the nutrient solution in LKQM treatment quantitatively.

Experimental design

Experiments were performed in an environmental-controlled chamber equipped with fluorescent tubes at Chiba university matsudo campus from July 25 to August 29, 2018. Seeds of lettuce "Frillice" were sown in urithane cubes (W 2.3cm × D 2.3cm × H 2.7 cm) on July 25th. The seedlings were cultivated in a growth chamber (Nae Terrace; Mitsubishi Chemical Agri Dream Co., Ltd., Tokyo, Japan) at 16-h photo-period with 300 ± 10 μmol m^{-2} s^{-1} photosynthetic photon flux density, 20°C temperature, and 1000 ppm CO_2 concentration for 7 days. The morphologically uniform seedlings were transferred into another growth chamber with the same condition of the Nae terrace. The seedlings were transplanted on foam boards with 26 holes floating (60 × 30 × 1 cm) on a container (60 × 30 × 11 cm) for the initial 7 days and the amount of nutrient solution was 18 L. Then, uniform lettuce plants were transplanted to foam boards with 6 holes (60 × 30 × 1 cm) in the same containers. Groundwater (NO_3-N 0.8 mmol L^{-1}, PO_4-P 0.006 mmol L^{-1}, K 0.06 mmol L^{-1}, Ca 1.0 mmol L^{-1}, Mg 0.8 mmol L^{-1}, Na 0.6 mmol L^{-1}) was used as raw water of nutrient solution. Fresh air was supplied to the nutrient solution with an air pump.

The lettuce plants were cultivated on 3 different nutrient solutions: (1) Half-strength of Enshi formula solution (NO_3-N 8 mmol L^{-1}, PO_4-P 0.67 mmol L^{-1}, K

4 mmol L^{-1}, Ca 2 mmol L^{-1}, Mg 1 mmol L^{-1}, Fe 3ppm, B 0.5 ppm, Mn 0.5 ppm, Zn 0.05 ppm, Cu 0.02 ppm, Mo 0.01 ppm; Zhang et al., 2017) during the whole progress (EC: 1.4 dS m-1; pH: 7.0; CK); (2) Half-strength of Enshi formula solution from 7 to 28 DAS and half-strength Na replacing K Enshi solution (All K was replaced by Na in Enshi formula) from 28 to 35 DAS (EC: 1.4 dS m^{-1}; pH: 7.0; LKEC); (3) Plants were transplanted to groundwater, and on the 7, 14, and 28 DAS, quantitative chemical fertilizers were added to the groundwater according to the new recipe of LKQM method (Table 3). The water level of LKQM was adjusted every 3 days. The EC value of the CK and LKEC was adjusted every 3 days by a portable EC meter (EC Meter CM-31P, DKK-TOA, Japan). At the growth stage of 14 and 28 DAS, the nutrient solution was changed. The microelement concentrations in nutrient solution of these three treatments were the same; three repeats were set in the experiment.

Additionally, the LKEM recipe in this experiment was not optimized, because the cultivation environment of this experiment is different from that of commercial low–K lettuce. However, since the purpose of this research is not the evaluation of recipes, but the evaluation of the usefulness of QM method, the only thing to be sure was the consistency of K concentration in lettuce with LKEC and LKQM treatments.

Plant growth measurements

Six plants were harvested at 14, 28 and 35 DAS. Number of leaves, total leaf area, and leaf FW were evaluated. Total leaf area was determined using a leaf area meter (LI-300; LI-COR, Lincoln, NE, USA). Then, plant tissues were dried at 80°C for minimum 72 h and the leaf DW were measured.

Element concentrations in plants

The P, K, Ca, Mg and Na concentrations were determined based on the methodology adjusted by Maillard et al. (2015). Plant dry samples were ground to a fine powder with inox beads in a grinder (Wonder Blender WB-1, Osaka Chemical, Japan). Nearly 250-mg DW of each plant was incinerated at 650^{o}C for 72 h in a muffle oven (Muffle Furnace FO300, Yamato, Japan). Ashes were dissolved using 3-ml 2 mol L^{-1} HCl, and the volume was fixed to 100 ml with deionized water. Then, the sample solutions were diluted 10 times to start the inductively coupled plasma optical emission spectrometry (ICP-OES, ICPE–9000. Shimadzu Inc., Japan) analysis.

The N concentration was determined based on the methodology adjusted by Liu et al. (2019). <150-mg of fine powder of each plant was placed in a nickel board. The total N concentrations in the samples were determined using a CN corder (CN Corder MTA-600, Yanaco, Japan).

Element amounts in solution

Fifty mL solution of each treatment was sampled every 7 days from the growth stage of 7 DAS. The samples of LKQM were collected before and after nutrient solution adjustment so there will be 2 values at 14 and 28 DAS.

The N concentration in the nutrient solution was obtained by adding the concentration of NO_3^- and NH_4^+. The NO_3^- and NH_4^+ concentration in the solution was were determined based on the methodology adjusted by Mario et al. (2015), using a reflectometer (RQflex plus; Merck, Darmstadt, Germany).

P, K, Ca, Mg, and Na concentrations in the solutions were analyzed by ICP-OES

without any dilution.

The amount of each element in a container was calculated by multiplying the concentration by the volume of the container (18 L). Therefore, the solutions in the container were full when sampling. The unit was mmol container^{-1}, because fertilizers were added quantitatively in LKQM treatment.

Statistical analysis

One-way analysis of variance (ANOVA) was calculated using SPSS 17.0 software (SPSS Inc., IL, USA). The mean values were compared using the Tukey's honestly significant difference (HSD) test ($P < 0.05$). Data were represented as the mean ± standard deviation (SD). Graphics was performed using the GraphPad Prism 5 (GraphPad, San Diego, CA 92108, USA).

Results

Plant growth

Until 28 DAS, all the plant samples exhibited the same growth. The morphological differences appeared at 35 DAS. There was no significant difference between LKEC and LKQM treatments in terms of all growth characteristics. Compared to the control, the FW, DW and leaf area of LKEC- and LKQM-treated lettuce plants significantly decreased about 25%, 20% and 17% respectively (Fig 2-1-A, B, D). There was no significant difference in the number of leaves between these three treatments (Fig 2-1-C).

Nutrient concentrations in plants

The N concentration in lettuce decreased by 6.4% and 6.9% in LKEC and LKQM treatments compared to the CK, respectively (Fig. 2-2-A). There was no significant difference between these three treatments in terms of P concentration (Fig. 2-2-B). K concentration in LKEC and LKQM treatments decreased nearly to 65% compared with the CK and no significant difference appeared between LKEC and LKQM treatment (Fig. 2-2-C). Ca concentration in lettuce plants increased in the LKEC and LKQM treatment compared with CK, whereas there was no significant difference between LKEC and LKQM (Fig. 2-2-D). LKQM-treated plants had the highest Mg concentration, followed by LKEC-treated plants. Mg concentration in CK was the lowest (Fig. 2-2-E). Na concentration of LKEC- and LKQM-treated plants was 507% and 107% higher than that of the control (Fig. 2-2-F).

Nutrient amounts in the solution

The element amounts in the solution of LKEC remained unchanged from 7 to 28 DAS. N, P, K, Ca, Mg, and Na amounts in the solution were nearly 160, 13.7, 81, 68, 35, and 16 mmol container^{-1}, respectively. At 28 DAS, the amount of K dropped to the level in groundwater and the Na amount increased greatly because of the low K treatment. Nevertheless, N, P, Ca, and Mg s remain unchanged in the solution (Fig. 2-3).

In LKQM treatment, the elements in the solution fluctuated violently. At 14, 28, and 35 DAS, before adding the fertilizer, the amounts of all the nutrients were close to those in the ground-water. At 35 DAS, N, P, K, Ca, Mg, and Na amount in the solution were nearly 11, 0.7, 3.8, 27, 16, and 14 mmol container^{-1}, respectively (Fig.

2-3).

Discussions

Low K treatments affected the plant growth, and element accumulations

The K concentration of lettuce treated with LKEC and LKQM was not significantly different from the commercial low – K lettuce, which met the standard of low – K lettuce. Compared with the commercial low – K lettuce, lettuces treated with LKEC and LKQM treatment owned different leaf numbers, Na concentration and other indicators (Table 2-1, 2-2; Fig. 2-1, 2-2), which were caused by different cultivation environments. However, there was no significant difference in the growth indicators of lettuce with LKEC and LKQM treatments (Fig. 1). Therefore, this study successfully cultivated low–K lettuce by the QM method.

K concentration in low K treatments decreased nearly to 65% to the CK, whereas FW and DW decreased nearly to 24% and 19%, respectively. However, no visual deficiency symptoms appeared (Fig. 2-4). The physiological action of K can be divided to specific and nonspecific physiological functions. The nonspecific physiological functions can be replaced by other ions (Jiang et al., 2001; Wang et al., 2012). In this study, as compared with the control, Ca, Mg, and Na concentrations in LKEC treatment increased to 0.15, 0.18, and 2.85 mmol 100 g^{-1} FW, whereas those in LKQM treatment increased to 0.21, 0.25, and 0.60, respectively (Fig. 2-1-D, E, F). This suggests that these cations replace some nonspecific functions of K. However, if the increased concentrations of these cationic charges are compared with the reduced concentration of K ions, i.e., nearly 5.4 mmol 100 g^{-1} FW in both low K treatments (Fig. 2-1-C), these excess cations cannot fill the decreased osmotic pressure caused by

K loss. Additionally, it has been reported that with K deficiency stress, soluble sugar is transported to the vacuole to maintain osmotic pressure, which is a high-energy-consumption process and a waste of photosynthetic products (Gerardeaux et al., 2010; Liu, 2016). This may also compensate for the vacancy in osmotic pressure after K deficiency.

In these two low K treatments, the metal element with the biggest increase was Na, also in LKQM treatment, as no extra Na was added (Fig. 2-1-F). LKQM-treated lettuce plants absorbed the Na from groundwater in response to K deficiency. Other studies have found the similar phenomena, even without the addition Na (Horie et al., 2007; Plett and Moller, 2010; Wakeel et al., 2010). The increase in Na concentration of plants in LKEC and LKQM treatments may be to compensate for osmotic pressure reduction caused by K deficiency (Wang et al., 2012).

Meanwhile, the Na concentration of commercial, LKEC and LKQM low–K lettuce were different. Sodium is not an essential element of plants (Abdul et al., 2011). Studies found that as the Na concentration in the nutrient solution increased, the sodium concentration in the plant also increased (Blom-Zandstra et al., 1998; Carbonell-Barrachina et al., 1997). We thought that the excessive Na concentration in commercial and LKEC low–K lettuce is a luxury absorption, because of the high concentration of Na in the K–free nutrient solution. In LKQM treatment, we didn’t add any Na, so the low–K lettuce in this treatment held lowest Na concentration

Concentrations of Ca and Mg were also increased in lettuces with LKEC- and LKQM-treated. This phenomenon was more obvious in LKQM treatment, which may be because of the low Na supply. Ca and Mg can also replace K to accomplish its nonspecific function, but there was a complex antagonistic relationship between these

cations. In different cases, they exhibit different antagonism or synergy (Li, 2007; Logunde et al., 1982).

Concentration of N in lettuce plant decreased by nearly 6% under the two low K treatments. K deficiency affects N metabolism and the NO3- transported in the xylem (Hu et al., 2015; 2017). Other plants have shown similar N reductions in the absence of K (Singh and Reddy, 2017, Walter and DiFonzo, 2007). In this research, K concentration in plants decreased nearly to 65% in two low K treatments, whereas nearly 6.5% decline appeared in N concentration; this may be because K cycling to absorb more nitrate for plants (Engels and Kirkby, 2001).

LKQM declined the sodium concentration in low-K lettuce and fertilizer usage

The growth between LKEC and LKQM treatment showed no significant difference, which means that the QM method can be used for the cultivation of low K lettuce without any decreased yield. QM method has been used in hydroponics for a variety of vegetables (Li et al., 2014; Takei and Suzuki, 2013), but to our knowledge, this is the first study to study the utilization in low K vegetables. This experiment opened the precedents and paves the way for the promotion of future QM method.

The biggest difference between LKEC and LKQM treatments was the Na concentration in plants. Na concentration in LKEC-treated lettuce was thrice that of LKQM-treated plants; 2000-mg Na is among the top priorities of the World Health Organization for the combat of chronic noncommunicable diseases (Humayun et al., 2011). In most patients with chronic kidney disease, habitual intake is too high despite medical supervision. Even at levels substantially above the recommended amount, a

moderately lower dietary Na is associated with a substantially better response to renin-angiotensin-aldosterone system blockade in short-term interventions and a substantially better renal and cardiovascular outcome in post-hoc analysis of difficult end-point studies (Humalda and Navis, 2014). So, in the present study, the decrease of Na concentration in low–K lettuce of LKQM treatment would contribute a lot to the treatment of chronic kidney disease, especially for people with high Na intake.

The unabsorbed nutrient remaining in the solution exhibited big difference between LKEC and LKQM treatments. In the LKEC treatment, all the elements amount in the solution remained unchanged, whereas in the LKQM treatment, those changed with a large fluctuation and were extremely lower than those in the LKEC treatment. At the end of each stage, the elements amounts were similar with those in the beginning (the groundwater). Another interesting phenomenon was that K amount in container of LKQM was less than that of LKEC at final stage eventhough no (LKEC) or only a small amount (LKQM) of K was added. The residual fromer nutrient solution could affect the solution composition. Potassium in LKQM treatment was almost absorbed by the lettuce plants at 27 DAS, so residual former nutrient solution at root and transplanted panel didn’t affect the K amount in solution at final stage. All the added nutrients were absorbed by the plants at every stage. It further suggests that the utilization rate of fertilizer under LKQM treatment reached 100%. Additionally, N, P, and K s of the LKQM solution were almost close to 0 in the end, which proffers a rational tool for the quantitative study of plant nutrition in the future. Ca, Mg, and Na s were at higher levels in the end than in the beginning because of the high

concentrations present in the groundwater. What's more, the Na amount remained in container at 35 DAS was higher than that of 28 DAS in the LKEC treatment (Fig. 3F). This might be because that Na absorption by plants was less than that of K (Fig. 2C; F) and Na gradually accumulated in the nutrient solution. Therefore, many companies with the low–K lettuce production by LKEC frequently update the nutrient solution including the high concentration of Na. This reduced the efficiency of fertilizer utilization in LKEC treatment.

Conclusion

Either the LKEC or LKQM treatments can cultivate low K lettuce, and the plant growth in these two treatments is almost same. LKQM method exhibited a lower Na concentration in plant and a higher fertilizer utilization efficiency than LKEC treatment and owned more advantage in the cultivation of low–K lettuce .

Tables and figures

Table 2-1. The growth parameters of the commercial low-K lettuce at 3 growth stages

DAS	Leaf FW (g)	Leaf DW (g)	Number of leaves	Leaf Area (cm^2)
14	3.4 ± 0.4[z]	0.15 ± 0.02	6.4 ± 0.5	84 ± 9
28	29.5 ± 2.6	1.23 ± 0.11	9.9 ± 0.8	485 ± 33
35	75.3 ± 4.4	2.72 ± 0.26	18.3 ± 0.7	1161 ± 51

[z] Each value is the mean ± SD (n=10).

Table 2-2. The N, P, K, Ca, Mg and Na concentrations of the commercial low-K lettuce at 3 growth stages

DAS	Element concentration (mg g^{-1} FW)					
	N	P	K	Ca	Mg	Na
14	2.5 ± 0.2[z]	0.51 ± 0.06	2.9 ± 0.2	0.43 ± 0.03	0.16 ± 0.01	0.19 ± 0.01
28	2.5 ± 0.2	0.55 ± 0.05	3.2 ± 0.2	0.42 ± 0.03	0.17 ± 0.01	0.16 ± 0.01
35	2.0 ± 0.1	0.39 ± 0.03	1.5 ± 0.1	0.47 ± 0.02	0.22 ± 0.01	0.54 ± 0.03

[z] Each value is the mean ± SD (n=10).

Table 2-3. Nutrient amount required by low-K lettuce at different growth stages

DAS	Nutrient amount (mmol $plant^{-1}$)				
	N	P	K	Ca	Mg
7 - 14	0.66	0.06	0.28	0.10	0.02
14 - 28	5.06	0.42	2.35	0.72	0.21
28 - 35	5.71	0.51	0.35	1.75	0.57

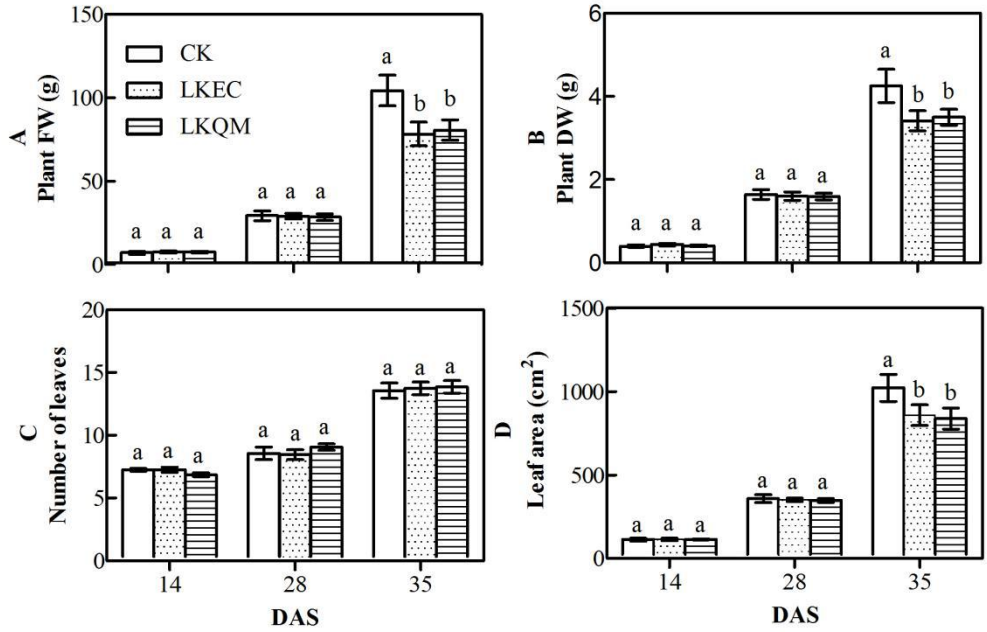

Fig. 2-1. Effects of LKEC and LKQM treatments on lettuce plant leaf fresh weight (FW, A), dry weight (DW, B), numbers (C) and areas (D) of leaves. Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

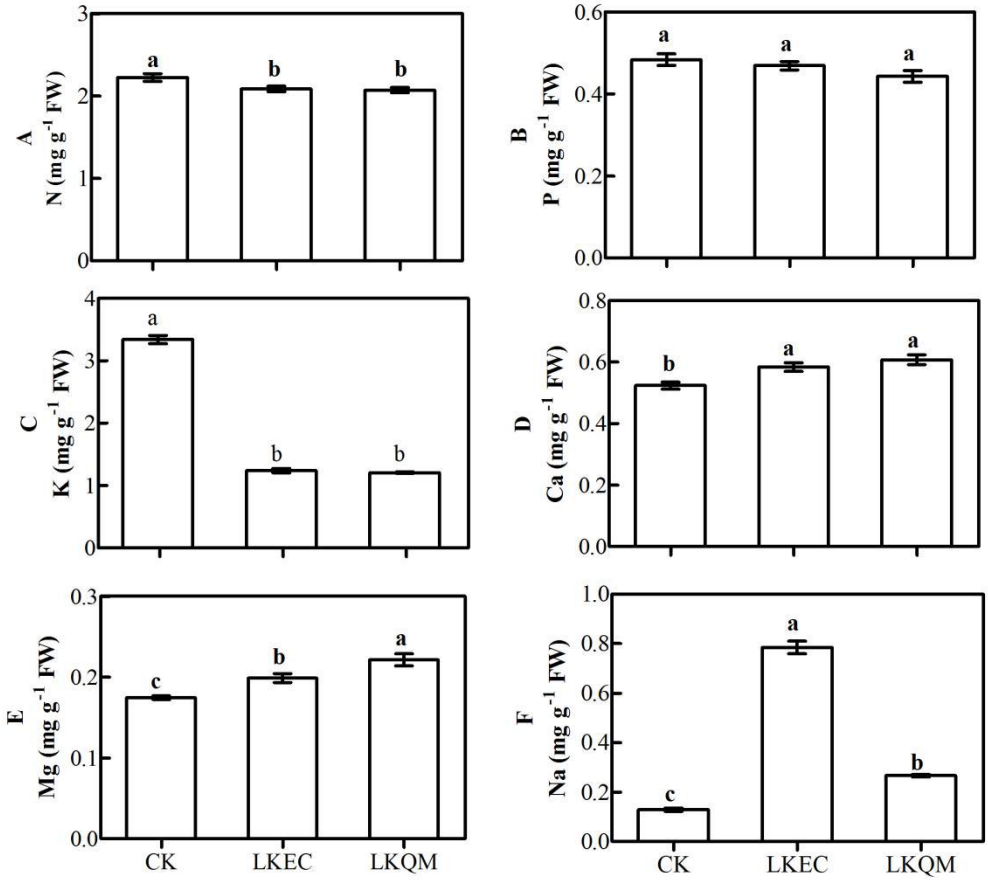

Fig. 2-2. Effects of LKEC and LKQM treatments on the concentration of N (A), P (B), K (C), Ca (D), Mg (E) and Na (F) in lettuce plant at 35 DAS. Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

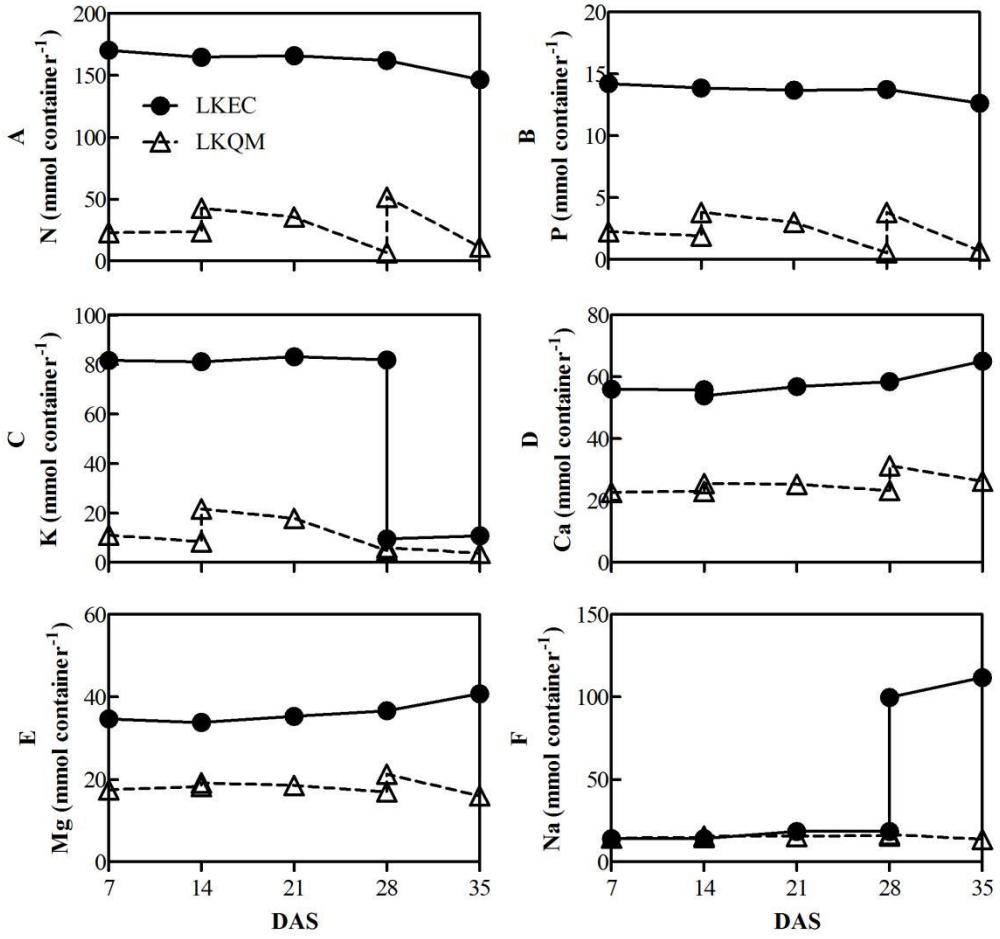

Fig. 2-3. Changes of the amounts of N (A), P (B), K (C), Ca (D), Mg (E) and Na (F) in the nutrient solution of LKEC and LKQM treatments at different stages.

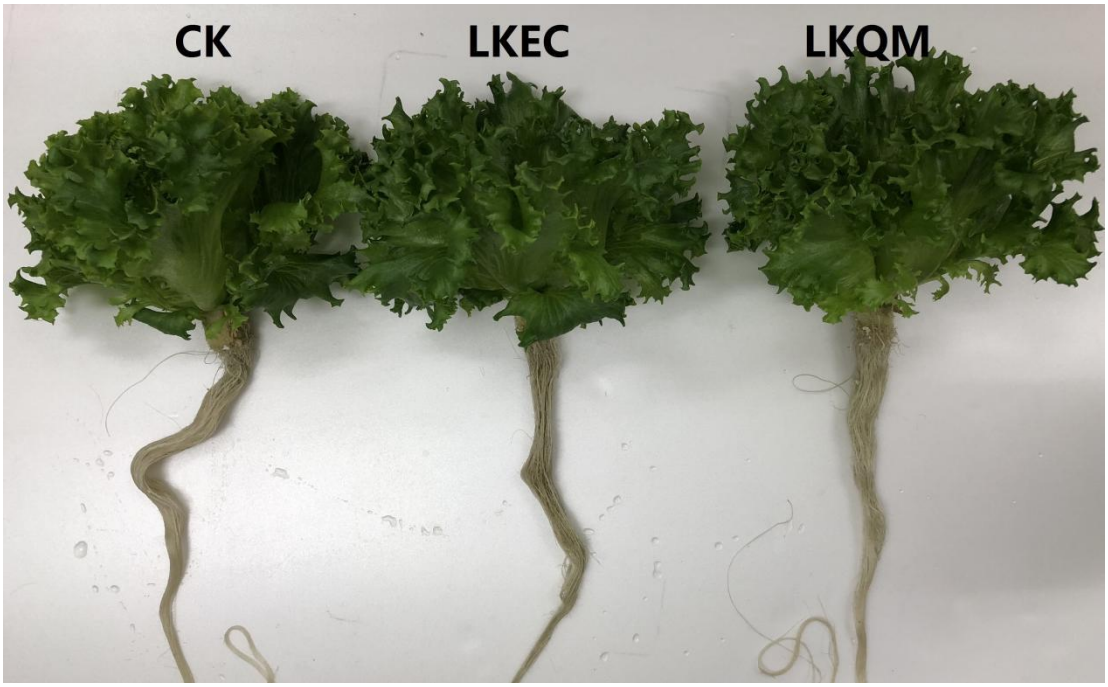

Fig. 2-4 Effects of LKEC and LKQM treatments on the lettuce morphology characteristics.

Chapter 3: Effects of low-K treatment on photosynthesis, primary and secondary metabolite concentrations in lettuce

Abstract

In order to learn more about the low-K vegetable, information is needed on the physiological differences between these vegetables and those with usual levels of potassium. In this study, lettuces (*Lactuca sativa* L.) were cultivated using two Low-K management methods in an environment-controlled system, LKEC and LKQM. Lettuce with normal K concentration was cultivated with EC management as the control (CK). Plant growth indices, leaf photosynthesis traits, chlorophyll fluorescence characteristics, concentrations of secondary metabolites (SMs), and antioxidant activity were examined to investigate the physiological effects of Low-K and high-Na concentrations during Low-K lettuce cultivation. Both low-K treatments significantly restrained the growth of lettuce and increased the concentration of soluble sugar. However, photosynthesis and fluorescence characteristics remained unchanged. This indicates that the biomass reduction of Low-K lettuce was due to the wasteful accumulation of carbohydrates rather than the decline in photosynthesis. Concentrations of SMs were increased in the low-K lettuce. In addition, higher concentrations of Na influenced the concentration of SMs, indicating that SMs were more sensitive to environmental stress.

Introduction

The traditional cultivation method was to replace all the K in the solution with Na in the later stage. In this method, the later nutrient solution contained a high concentration of Na, about 4 mol L^{-1}, which caused the lettuce to accumulate Na. High Na vegetables were not good for dialysis patients (Mills et al., 2016; Kelly et al., 2017). QM method decreased the Na concentration in low-K lettuce compared with the EC management, while no significant difference in plant growth. However, the physiological changes were still unknown.

Potassium is an essential macronutrient for plant growth (Pettigrew, 2008; Schachtman and Liu, 1999). K deficiency affects the functions of ion homeostasis, osmotic regulation, enzyme activity, membrane polarization, and various metabolic processes (Chérel et al., 2014; Lu et al., 2016b). Na is not an essential element in plants, but it is involved in non-specific functions such as plant growth stimulation and osmotic regulation (Wakeel et al., 2009). It is expected that limited supply of K and the Na accumulation in low-K lettuce would affect the lettuce growth, but the underlying physiological changes caused by this remain unknown.

Photosynthesis and chlorophyll fluorescence are essential for plant growth and development, which can partially explain the physiological response of plants after environmental stress (Sun et al., 2016; Yamori et al., 2016;). Studies have shown that K deficiency reduced the mesophyll conductance, thereby reducing plant photosynthesis (Lu et al., 2016a; Lu et al., 2019). High concentration of salt treatment decreased plant photosynthesis through osmotic stress and changes in related enzyme

activities (Kong et al., 2017), but information on the effects of low concentrations of Na is limited. Changes in photosynthesis and chlorophyll fluorescence could partially explain how K and Na affected the physiological response of low-K lettuce.

Soluble sugars are the primary metabolites of photosynthesis. It plays an important role in osmotic regulation with K and amino acid (Shabala and Cuin, 2007; Silva et al., 2010). In addition, the sucrose is the carbohydrate translocated from photosynthesizing tissues to non-photosynthetic sinks for use in metabolism and biosynthesis (Loka et al., 2018). The carbohydrate transport and distribution are important physiological factors affecting plant growth.

Lettuce is a popular healthy vegetable as it contains many bioactive phytochemicals (Vauzour et al., 2010). We called these secondary metabolites (SMs). SMs in lettuce are mainly phenolic compounds and flavonoids. They own the functions of antioxidant, antimicrobial, antifungal, antitoxic and radical scavenging properties, and affect the nutritional quality of plant (Hichri et al., 2011). The influence of mineral nutrition on concentrations of SMs in plants has been widely studied, but most studies have been focused on N and P. Phenolic compounds and flavonoids in lettuce are mainly the derivatives of caffeic acid and quercetin, and different varieties vary in their content of these compounds (Ouzounis et al., 2015; Yang et al., 2018), which have strong antioxidant properties and are an important indicator of lettuce quality.

In this study, two cultivation method, LKEC and LKQM, were used to cultivate the low potassium lettuce to explored the effects of K deficiency and Na accumulation on photosynthesis, primary and secondary metabolites concentrations.

Materials and method

Experiment design

Experiments were conducted in an environment-controlled chamber same to Chapter 1 at Chiba University, Matsudo Campus, from March 4 to April 9 in 2019. The cultivation of CK, LKEC and LKQM treatments were same to Chapter 1.

Plant growth analysis

Six plants were harvested from each treatment at 28 and 35 DAS. The number of leaves, total leaf area, and FW of the plants were immediately evaluated. Then, plant tissues were dried at 80°C for at least 72 h, and the DW of the plants were measured.

Potassium and sodium concentrations

The concentrations of K and Na was measured with the ICP-OES method same to Chapter 1.

Chlorophyll and carotenoid concentrations

Chlorophyll (Chl) and carotenoid (Car) concentrations were determined based on the methodology of Shen (1988). A leaf disk was taken from the third leaf from the outside of each plant using a puncher at 35 DAS and placed in a centrifuge tube. Then, 10 ml of a mixed extract (ethanol:acetone:water = 4.5:4.5:1) was added. The mixture was soaked in the dark to extract the pigments until the leaf disk was completely whitened. The absorption of the extract solution was measured at 440 nm, 645 nm, and 663 nm. The concentrations of Chl a, Chl b, and Cars were determined using three equations (mg dm^{-2}):

$$\text{Chl a} = (12.7 \times OD_{663} - 2.69 \times OD_{645}) \times V/A$$

$$\text{Chl b} = (22.9 \times OD_{645} - 4.68 \times OD_{663}) \times V/A$$

$$\text{Car} = [4.7 \times OD_{440} - 0.27 \times (\text{Chl a} + \text{Chl b})] \times V/A$$

where OD is the optical density, V is the volume of the extracting solution, and A is the area of the leaf disk (cm^2).

Gas exchange parameter

The measurement of gas-exchange parameters conducted is described by Yamori et al. (2011), using a portable photosynthesis system (Li-6400XT, Li-Cor Inc., Lincoln, NE, USA). The third leaf from the outside was picked, and the net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) were measured at 33 DAS. Light was provided by red and blue light-emitting diodes (6400-02B, Li-Cor Inc.). Photosynthetic photon flux density was measured at 300 μmol m^{-2} s^{-1}, and the leaf temperature, CO_2 concentration, and relative humidity were 20°C ± 1°C, 400 ± 5 μmol mol^{-1}, and 65% ± 5%, respectively.

Chlorophyll fluorescence parameters

Leaf chlorophyll fluorescence parameters were simultaneously measured at 34 DAS using a portable photosynthesis system (Li-6400XT, Li-Cor Inc.), with an integrated fluorescence fluorometer (Li 6400-40 leaf chamber fluorometer, Li-Cor Inc.), under ambient CO_2 concentration and 21% O_2. The maximum quantum yield of the PS2 primary photochemistry (Fv/Fm), efficiency of excitation energy capture by open PS2 reaction centers (Fv'/Fm'), quantum yield of PS2 electron transport (PhiPS2), and photochemical quenching (qP) were measured.

Soluble sugar concentration

Total soluble sugar was measured using the method described by Ibrahim et al. (2012), with modifications. Samples of 50 mg leaf DW were placed in a test tube, to which 10 ml of 80% ethanol was added. This was placed in an 80°C water bath for 30 min and allowed to cool. Next, the supernatant was centrifuged at 4000 g for 3 min. This step was repeated twice, and the supernatant was combined. A measure of 10 mg of activated carbon was added to the supernatant and decolorized at 80°C for 30 min, and then the volume was adjusted to 25 ml. After filtration, the soluble sugar concentration was determined using the anthrone–sulfuric acid method at 620 nm.

Concentrations of total phenols and flavonoids

At 35 DAS, the third leaf from the outside was selected in this experiment. A sample of 0.5 g of lettuce sprouts was ground with liquid nitrogen, and 10 ml of 80% methanol was used for extraction. All samples were extracted in darkness for at least 90 min and were then filtered and stored at −20°C for the next determination.

The concentration of total phenols (CTP) was determined using the Folin–Ciocalteu colorimetric assay according to the methodology proposed by Singleton (1999), with modifications. A 0.5-ml extract was added to 2.5 ml of reaction solution, which contained 0.4 ml of Folin phenol, and was reacted for 3 min. Then, 10 ml of 10% Na_2CO_3 was added, and the absorption at 765 nm was measured. The results were expressed as mg chlorogenic acid equivalents (CGE) per gram FW.

The concentration of total flavonoids (CTF) was determined using the methods described by Zhishen (1999). A 0.5 ml extract was added to 1 ml of 5% $NaNO_2$, 1 ml

of 10% $Al(NO_3)_3$, and 4 ml of 2 mol L^{-1} NaOH and mixed and reacted for 15 min. Then, the absorption at 510 nm was measured. The CTF was expressed as mg of rutin equivalents (RTE) per gram FW.

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay

Antioxidant activity using the DPPH radical was determined based on the methodology of Viacava (2015). A 0.25 ml sample of extract was mixed with 1 ml of 100 μM methanol DPPH solution. The mixtures were immediately shaken and allowed to stand at a refrigeration temperature of 2°C in the dark. The decrease in absorption at 517 nm was measured after 60 min, and DPPH radical scavenging activity was expressed as mg of Trolox equivalents per 100 g FW.

Secondary metabolites concentrations

At 35 DAS, the third leaf from the outside were freeze-dried at 10°C for 5 days in a freeze-dry machine (DRC-1000, FDU-2100, EYELA Inc., Japan). The dried samples were crushed into powder and filtered through a sieve (1 mm). A 200 mg sample of a dry leaf was accurately weighed and transferred to a 5-ml Eppendorf tube (Hamburg, Germany). Then, 2.5 mL of methanol was added, mixed for 15 min at 1,000 rpm at 20°C using an Eppendorf ThermoMixer C, and centrifuged for 5 min. Next, 2.5 mL of methanol was added to the residue, and the same extract procedure was performed. The combined extracts were then transferred to a 5-mL volumetric flask and diluted with methanol to a 5 mL total volume. The solution was then filtered through a 0.22-μm nylon syringe filter (Shimadzu GLC Ltd., Tokyo, Japan).

Analysis of chicoric acid (CC) concentration was conducted using

high-performance liquid chromatography (HPLC). The HPLC system was composed of a Shimadzu LC-20A Prominence system equipped with a SIL-20AC autosampler and an SPD-20A PDA detector using LabSolutions software (Shimadzu, Kyoto, Japan). Specific HPLC conditions were followed for the analysis of CC: a 5 μm, 4.6 × 150 mm TSKgel ODS-80T$_M$ column (Tosoh, Tokyo, Japan) was used, at a temperature of 40°C, flow rate of 1.0 mL min^{-1}, run time of 15 min, detector wavelength of 330 nm, mobile phase of 15% acetonitrile/0.1% formic acid, and injection volume of 10 μL.

The concentrations of caffeic acid (CF), chlorogenic acid (CG), rutin (RT), and isoquercitrin (IQ) were measured using liquid chromatography-mass spectrometry (LC–MS), which was conducted according to Nguyen et al. (2019), with modifications. A LC–MS-2020 mass spectrometer equipped with an electrospray ionization (ESI) source, operating in negative mode, was used for the identification and quantification of CF, CG, RT, and IQ. The HPLC analysis of CF, CG, RT, and IQ was conducted using an XBridge BEH C18 column (3.5 μm, 2.1 × 150 mm, Waters, MA, USA) at a temperature of 35°C, flow rate of 0.2 mL min^{-1}, and an injection volume of 1 μL. The elution was conducted with a mobile phase consisting of solvent A (0.1% formic acid, *v*/*v*) and solvent B (100% acetonitrile) using a gradient flow of 10%–20% B at 0–5 min, 20% B at 5–10 min, and 10% B at 10–20 min. The eluent was passed to the ESI source, and a capillary voltage of 3.5 kV was used in the negative ion mode. Nitrogen was used as the drying gas with a flow rate of 15 L min^{-1} and nebulizing gas with a flow rate of 1.5 L min^{-1}. The desolvation line temperature

was set at 250°C. The ion trap was operated in full scan mode from *m*/*z* 50 to 1000 and selected ion monitoring mode with *m*/*z* 179, 353, 609, and 463 for a molecular ion $[M–H]^-$ of CF, CG, RT, and IQ, respectively.

Statistical analysis

A one-way analysis of variance was calculated using SPSS 17.0 software (SPSS Inc., IL, USA), and the mean values were compared using Tukey's honestly significant difference (HSD) test ($P < 0.05$). Data were represented as the mean ± standard deviation (SD). Graphics were created using GraphPad Prism 5 (GraphPad, San Diego, CA 92108, USA) and ChemDraw 19.0 (PerkinElmer Inc., USA).

Results

Plant growth

Both LKEC and LKQM treatments exerted negative effects on plant growth. However, no significant difference was found between the LKEC and LKQM treatments in measurements of plant growth. Compared with the CK, the LKEC and LKQM treatments decreased the plant FW, DW, and leaf area by about 24, 22, and 21%, respectively (Table 3-1). The number of leaves was not affected by the two low-K treatments.

K and Na concentrations

At 28 DAS, there was no significant difference in concentrations of K and Na between the CK, LKEC and LKQM treatments. At 35 DAS, there was no change in

the concentrations of K and Na in the CK, compared with the 28 DAS, while in the lettuce with LKEC and LKQM treatments, the K concentration decreased and Na concentration increased.

At 35 DAS, the K concentration in the plants with LKEC and LKQM treatments decreased by 65% compared with the CK approximately. No significant difference was found in the K levels between the two K treatments (Fig. 3-1-A). Compared with the CK, the LKEC and LKQM treatments increased the Na concentration by 245% and 103%, respectively (Fig. 3-1-B).

Photosynthesis related characteristics

The two low-K treatments did not affect the photosynthesis parameters, such as the Pn, Gs, Ci, and Tr (Table 3-2). In addition, there was no significant difference between the three treatments in chlorophyll fluorescence parameters, including Fv/Fm, Fv'/Fm', PhiPS2, and qP (Table 3-3).

The Chl a and b concentrations and the Chl a to Chl b ratio were also not affected by the low-K treatments, while the Car concentration was increased. No significant difference was found between the LKEC and LKQM treatments for photosynthetic pigment concentrations (Table 3-4).

Compared with the CK, LKEC and LKQM treatments increased the soluble sugar concentration by about 110% (Fig. 3-2).

Concentrations of total phenols and flavonoids

The CTP was increased by the low-K treatments. The LKEC treatment showed a more substantial increase, of about 20%, compared with the CK, while the CTP of the

LKQM treatment increased by about 11% (Fig. 3-3-A).

The CTF showed the same trend as CTP, with a higher gain: the LKEC treatment increased by 82%, while the LKQM treatment increased by 68% (Fig. 3-3-B).

Secondary metabolites concentrations

The lettuce under the LKEC treatment consistently contained the highest concentration of SMs, which were 67%, 92%, 23%, 32%, and 152% higher for RT, CG, CF, IQ, and CC, respectively, than the control. The RT, CG, and CC concentrations in the LKQM plants were also higher than the control by 36%, 28%, and 121%, respectively. The CF and IQ concentrations between the LKQM and CK treatments showed no significant difference (Table 3-5).

Antioxidant activity

Compared with the control, the antioxidant activity of the plants was significantly enhanced by the LKEC and LKQM treatments (Fig. 3-4). The Trolox equivalents in the LKEC treatment were 61% higher than the control, while in the LKQM treatment, they were 45% higher than the control.

Discussion

Effects of low-K treatments on photosynthesis and plant growth

The absence of significant differences in the growth indicators, photosynthetic indicators, and soluble sugars between the LKEC and LKQM treatments indicates that the two Low-K treatments had the same effect on the growth of the studied lettuce plants. Compared with the CK, the yield and K concentration in the lettuces from the

two Low-K treatments decreased by 20% and 65%, respectively, while the mean of Pn did not decline. However, the soluble sugar concentration in Low-K treatments increased by 110% compared with the control. Plants per unit mass fixed an equal amount of CO_2 through photosynthesis, and a large amount of soluble sugars existed in the Low-K lettuce, resulting in carbon not being fully used for cell growth, division, and differentiation. This might explain why the biomass of Low-K lettuce decreased with constant photosynthesis. A possible reason for soluble sugar accumulation in Low-K lettuce is that soluble sugars could have compensated for the osmotic pressure gap after K deficiency, as K is the main osmotic adjustment substance of plants (Cakmak et al., 1994; Hafsi et al., 2014; Talbott and Zeiger, 1996). In rice, when the K transporter-related gene *OsHAK1* is knocked out, the synthesis and transport of sucrose are reduced, but the concentration of sucrose in leaves was increased (Chen et al., 2018). In cotton, K deficiency also causes an increase in sucrose and starch in leaves (Hu et al., 2018). Therefore, the accumulation of soluble sugar might slow the growth of Low-K lettuce. In addition to soluble sugars, amino acids and other cations are also important components in maintaining plant vacuolar osmotic pressure, and they compensate for reduced osmotic pressure in the absence of K (Hu et al., 2018; Wakeel et al., 2011; Wang et al., 2012b). The increased sodium concentration in lettuce leaves in the LKEC and LKQM treatments indicated that Na was also involved in the compensation of osmotic pressure.

The leaf Pn can also be affected by K (Lu et al., 2016a; 2019). However, in this study, the mean Pn did not decline. Other critical components of photosynthesis, such

as Gs, Ci, Tr, Fv/Fm, Fv'/Fm', PhiPS2, qP, and the chlorophyll concentration, were also unaffected (Table 3 and 4). This finding might be because the reduced osmotic pressure, as a result of less K, was compensated for by substances such as soluble sugars, while K, which was controlled by stomatal conductance and related enzyme activity, was not affected. This phenomenon can be determined by the degree of K deficiency. Quantitative limitation analysis found that 0.98% K did not affect the net CO_2 assimilation rate, compared with 1.66% of K in the control treatment in *Brassica napus* L. (Lu et al., 2016b). In the soybean (cv. Spencer), K deficiency limited growth traits rather than the photosynthetic processes at a moderate K deficiency of 1% K concentration in the leaf (Singh and Reddy, 2017). These results were consistent with the finding of our study, that growth of Low-K lettuce was limited while the photosynthesis was constant. This might be because the decline of assimilation accumulation and biomass had already occurred before leaf photosynthesis was affected by K deficiency (Gerardeaux et al., 2010; Wang et al., 2012a).

Here we propose a hypothesis about the physiological response of Low-K lettuce. The K intake was decreased but photosynthesis was not reduced. The K concentration in the vacuole is reduced firstly, which leads to a decrease in the osmotic pressure of the cell. Therefore, the soluble sugar, amino acids, and cations are increased to maintain osmotic pressure, leading to a decline in the translocation of sucrose and amino acids, which inhibits the growth of lettuce plant.

Effects of K deficiency and Na accumulation on plant secondary metabolite concentrations

The concentrations of total phenol (Fig. 3-3-A) and flavonoid (Fig. 3-3-B) in Low-K treatments were higher than those of the control. In our study, the RT, CG, CA, IQ, and CC were also identified (Table 3-6). The concentrations of SMs in Low-K lettuce plants were higher than that of the control. Interestingly, Cars concentration in Low-K lettuce was also higher than that of the control (Table 3-4), as the Cars are also a kind of SMs providing protection when plants are overexposed to free radical detoxification (Lattanzio et al., 2006). Large number of experiments have been proposed, in which the nutrient deficiencies of plants were characterized by an accumulation of flavonoids, such as the anthocyanins (Yang et al., 2018a). In lupins (*Lupinus angustifolius*), K deficiency resulted in low-quality seeds with high alkaloid concentrations, such as angustifoline, lupanine, and 13-hydroxylupanine (Gremigni et al., 2001). In the *Chrysanthemum morifolium*, K deficiency increased the content of total flavonoids in leaves at the vegetative stage, while it decreased in leaves and flowers at the reproductive stage (Liu et al., 2010). A reasonable explanation might be that some metabolic enzyme activities were changed in Low-K plants, which caused the changing of metabolites. In addition, K was an essential element for plants, and this nutritional stress of K promotes the production of stress metabolites (Liu et al., 2010).

An interesting finding was that the concentration of SMs in lettuce plants in the LKEC treatment was higher than that in the LKQM treatment (Fig. 3-3, 3-4; Table 3-5). This finding might be due to the higher concentration of Na in the nutrient solution of LKEC, which was nearly 6 mM. Substantial research on the effects of Na

on concentrations of plant SMs has been conducted (Menezes-Benavente et al., 2004; Minh et al., 2016). However, most of this research has focused on salt stress, in cases where the Na concentration around the root zone reached 100–200 mM. It has been found that in the long-term treatment of 5 mM NaCl, the total carotenoids were increased, while the phenolic concentration remained stable in romaine lettuce (Kim et al., 2008). Salt stress often created both ionic and osmotic stress in plants, resulting in the accumulation or decrease of specific SMs in plants (Mahajan and Tuteja, 2005). In this research, the Na might only play its ionic function. What is more, the low concentration of Na in the solution did not affect plant growth, photosynthesis, and primary metabolites, but it affected the SMs, which proved that SMs were more sensitive to changes in the environment.

CG and CC were the main component of phenols in lettuce and were the CF derivatives (Ouzounis et al., 2015; Murthy et al., 2014). RT and IQ were the main flavonoids in lettuce and were the different products in the same metabolism (Verhoeyen et al., 2002). These products varied widely. CC concentration in lettuce was the highest among the individual phenolic compounds and the increase caused by K and Na was also the largest. CF and IQ were hardly affected by K deficiency, while they were affected by Na. These different changes indicate that K and Na affected not only the concentration of SMs but also the proportion of different metabolites. This might be because K deficiency and Na stress had different effects on different enzymes in the biosynthetic phenolic pathway (Wang et al., 2012b). In conclusion, both Na and K have important effects at the level of SMs.

The nutritional value of low-K

In either LKEC or LKQM treatment, as the K concentration decreased, other components were increased, such as soluble sugars, Na, phenols, and flavonoids (Fig. 3-1, 3-2, 3-3, and 3-4). These components also increased the burden on the kidney of dialysis patients (Nowak et al., 2017; Uribarri, 2018). The antioxidant activity of lettuce with LKEC treatment was highest and it is considered good for human health. However, most of the sources of this antioxidant activity are polyphenols, and they will accumulate and show the indubitable potentially toxic effects for dialysis patients (Malejane et al., 2018; Nowak et al., 2017).

General speaking, LKQM method was considered better because it reduced the Na concentration in low-K lettuce, as we expected. But the results on SMs were conflicting. So, as a functional food, the nutritional value of Low-K lettuce should be further evaluated.

Conclusion

After two low-K treatments (LKEC and LKQM) in this study, the yield of lettuce decreased without any photosynthesis changing. The soluble sugar concentration decreased, which was thought to be the main reason for the reduced biomass of low-K lettuce due to the decline in carbon source used for other organ growth. Potassium deficiency also increased the concentration of SMs in lettuce. What is more, higher concentration of Na in the nutrient solution of the LKEC treatment also caused an effect on the concentration of SMs, and Na and K had different effects on different

compounds.

Tables and figures

Table 3-1. Effects of LKEC and LKQM treatments on the lettuce plant fresh weight, dry weight, leaf number and leaf area.

Treatments	Plant FW (g)	Plant DW (g)	Number of leaves	Leaf Area (cm^2)
CK	106.8 ± 8.3 a[z]	4.01 ± 0.50 a	13.0 ± 0.8 a	1064 ± 55 a
LKEC	81.2 ± 6.3 b	3.41 ± 0.30 b	13.7 ± 0.5 a	844 ± 38 b
LKQM	81.1 ± 4.4 b	3.40 ± 0.17 b	13.6 ± 0.5 a	832 ± 43 b

[z] Each value is the mean ± SD (n=6). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

Table 3-2. Effects of LKEC and LKQM treatments on leaf photosynthesis

Treatments	Pn ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Gs ($mol\ H_2O\ m^{-2}\ s^{-1}$)	Ci ($\mu mol\ CO_2\ mol^{-1}$)	Tr ($mol\ H_2O\ m^{-2}\ s^{-1}$)
CK	5.54 ± 0.27 a[z]	0.201 ± 0.007 a	350 ± 5 a	1.74 ± 0.11 a
LKEC	5.47 ± 0.23 a	0.191 ± 0.009 a	355 ± 7 a	1.70 ± 0.06 a
LKQM	5.43 ± 0.10 a	0.189 ± 0.013 a	350 ± 2 a	1.68 ± 0.12 a

[z] Each value is the mean ± SD (n=6). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

Table 3-3. Effects of LKEC and LKQM treatments on leaf chlorophyll fluorescence

Treatments	Fv/Fm	Fv'/Fm'	PhiPS2	qP
CK	0.811 ± 0.013 a[z]	0.569 ± 0.021 a	0.222 ± 0.010 a	0.343 ± 0.010 a
LKEC	0.820 ± 0.005 a	0.565 ± 0.009 a	0.228 ± 0.007 a	0.349 ± 0.012 a
LKQM	0.823 ± 0.004 a	0.558 ± 0.033 a	0.211 ± 0.007 a	0.341 ± 0.018 a

[z] Each value is the mean ± SD (n=6). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

Table 3-4. Effects of LKEC and LKQM treatments on chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car) concentrations and chlorophyll a to chlorophyll b ratio (Chl a/ b) of lettuce

Treatments	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Car (mg g^{-1})	Chl a/ b
CK	1.85 ± 0.13 a[z]	0.92 ± 0.07 a	0.22 ± 0.02 b	2.02 ± 0.07 a
LKEC	1.82 ± 0.06 a	0.89 ± 0.07 a	0.26 ± 0.02 a	2.05 ± 0.09 a
LKQM	1.75 ± 0.07 a	0.89 ± 0.04 a	0.26 ± 0.01 a	1.97 ± 0.06 a

[z] Each value is the mean ± SD (n=6). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

Table 3-5. Effects of LKEC and LKQM treatments on Rutin (RT), Chlorogenic acid (CG), Caffeic acid (CF), Isoquercitrin (IS) and Chicoric acid (CC) concentrations (μg g^{-1} DW) in lettuce

Treatments	RT	CG	CF	IS	CC
CK	0.49 ± 0.03 c[z]	32.1 ± 1.7 c	0.35 ± 0.02 b	0.47 ± 0.02 b	144 ± 10 c
LKEC	0.82 ± 0.04 a	61.8 ± 1.8 a	0.43 ± 0.01 a	0.62 ± 0.01 a	362 ± 12 a
LKQM	0.67 ± 0.04 b	41.0 ± 2.0 b	0.36 ± 0.02 b	0.53 ± 0.02 b	317 ± 11 b

[z] Each value is the mean ± SD (n=6). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

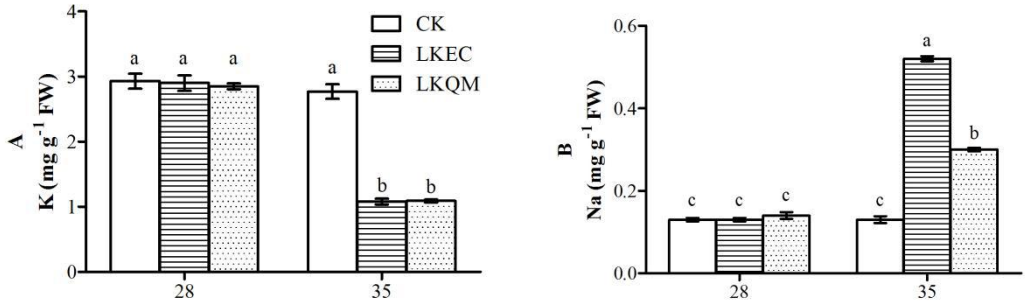

Fig. 3-1. Effects of LKEC and LKQM treatments on the K (A) and Na (B) concentrations of lettuce plant. Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

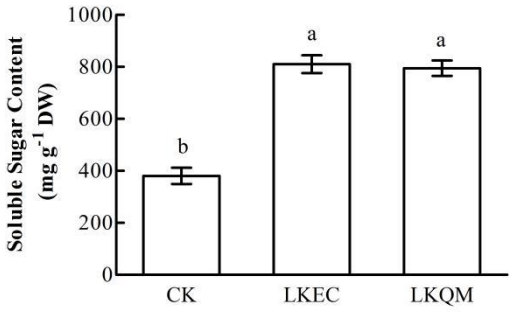

Fig. 3-2. Effects of LKEC and LKQM treatments on soluble sugar concentration of lettuce. Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

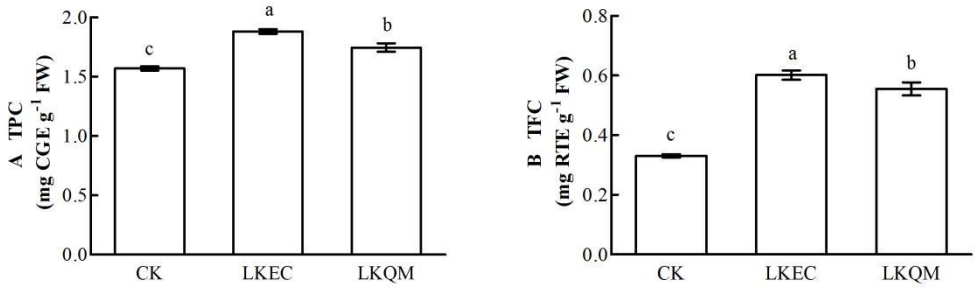

Fig. 3-3. Effects of LKEC and LKQM treatments on the concentrations of total phenols (CTP, A) and flavonoids (CTF, B) of lettuce. Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

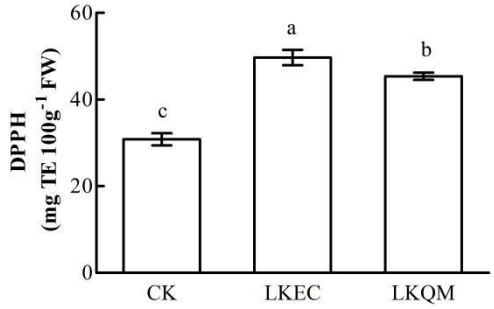

Fig. 3-4. Effects of LKEC and LKQM treatments on the antioxidant activity of lettuce. Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

Chapter 4: Effects of low-K treatment on sugar transport and related gene express in lettuce

Abstract

In order to study the sugar transport of lettuce under low-K environment, lettuce plants were cultivated with 4 gradient of K supply, which were normal amount and 1/2, 1/4, 1/8 amount of normal. The cultivation method was QM method. With the decrease of K supply, the FW, DW, K concentration in leaves and relative expression of *LsSUCs* were decreased, while the soluble sugar concentration was increased. This mean that with the K deficiency, soluble sugar transport in leaves was reduced and accumulated in cells, thereby limiting plant growth. Comparing K and soluble sugar concentrations in the outer and inner leaves, the ratio of K concentration and soluble sugar concentration in outer leaf to inner leaf were negatively correlated with the K concentration of whole plant. This indicate that efficient distribution of K and soluble sugar to the functioning plant tissues might be one of important mechanisms for K deficiency.

Introduction

Previously, we suspected that low potassium caused soluble sugars to accumulated in the cells, reducing the outflow of photosynthetic products, thereby limiting the growth of lettuce plants.

Potassium acts not only as an ionic osmoregulator (Mengel et al., 1982), but also affects photosynthesis in other way, including its control over the accumulation of

sugar in the chloroplasts, in the degradation of chlorophyll and in the synthesis of starch (Amtmann et al., 2005). These latter processes affect the distribution of photosynthetic products. When experiencing K deficiency, plants become less capable of maintaining the long distance transport of assimilate through the phloem (Cakmak et al., 1994). Usually, the research on the relationship between source and sink will be used in the experiment of K deficiency to study the distribution of carbohydrates, such as leaf and pistils (Hu et al., 2018), leaf and spikelet (Chen et al., 2018).

Lettuce as a leaf vegetable, the internal sugar transport is rarely studied. However, the inner and outer leaves of leaf vegetables varied greatly in various substances, such as sugar, protein, minerals and so on (Baslam et al., 2013; Kim et al., 2017). The inner and outer leaves are at the different growth stages, so there is a big difference in various metabolic processes between inner and outer leaves. With the K deficiency, the relationship between inner and outer leaves has not been studied.

In this study, lettuce plants were cultivated with 4 gradient of K, the normal amount (CK), 1/2, 1/4 and 1/8 amount of CK, by the QM method to explore the accumulation and distribution of carbohydrates under K deficiency.

Materials and methods

Experiment design

Experiments were performed in an environment-controlled chamber same to Chapter1 from October 1 to November 5, 2019.

The lettuce plants were cultivated with 4 different QM recipe as Table 4-1.

According to the elemental absorption data of ordinary lettuce in Chapter 1, the recipe of CK was made firstly. With the control of other elements unchanged, the K supply of each treatment was reduced to 1/2, 1/4 and 1/8 of CK respectively. These treatments were named 1/2, 1/4 and 1/8. The solution was changed at 14 and 28 DAS.

Plant growth analysis

Six plants were harvested from each treatment at 35 DAS. Lettuce plants were divided into two parts. The first three healthy leaves from outside were outer leaves. Other leaves were the inner leaves. The FW of the each part was immediately evaluated. Plant tissues were dried at 80°C for at least 72 h, and the dry weights of each part was measured.

K concentrations

The concentrations of K and Na was measured with the ICP-OES method same to Chapter 1.

Gas exchange parameter

The third leaf from outside was selected to measure the gas exchange parameter with the same method as Chapter 3.

Soluble sugar concentration

The soluble sugar concentrations of inner and outer leaves were measured with the Anthrone sulfate method same to Chapter 3.

Quantitative real time PCR (qRT-PCR)

The qRT-RCR protocol applied followed Gurdon et al (2019) to measure the expression levels of the *LsSUT*, *LsSUT3* and *LsSUT4* genes. Total RNA was isolated

using the NucleoSpin® RNA (Macherey-Nagel GmbH & Co. KG., Germany) according to the manufacturer's instructions. cDNA synthesis was performed from total RNA using the ReverTra Ace® qPCR RT Master Mix (Toyobo Co. Ltd, Japan), according to the manufacturer's instructions.

The qPCR reaction was performed using the TB Green® *Premix Ex Taq*™ II (Takara Bio, Japan), with 80 ng of cDNA and 0.4 μM of each forwards and reverse primers in a 25 μl final reaction volume on a StepOnePlus Real-Time PCR System (Applied Biosystems, UK), under following setting: 90°C for 30 seconds and 40 cycles at 95°C for 5 seconds, 60°C for 30 seconds. Melting curve analysis was conducted immediately after the qPCR reaction from 60-95°C in 0.3°C increments to confirm the absence of primer dimers, DNA contaminants and secondary products. Gene-specific PCR primers (Table4-2) were designed according to the cDNA sequences using the Beacon designer 7 software (Palo Alto, California). The *ACT* gene from *Lactuca sativa* was used as an internal control to normalize amounts of template cDNA. Relative expression was calculated following the suggestion of Li et al (2014). Three biological replicates of each parent were tested in duplicate for each gene target.

Statistical analysis

A one-way analysis of variance was calculated using SPSS 17.0 software (SPSS Inc., IL, USA), and the mean values were compared using Tukey's honestly significant difference (HSD) test ($P < 0.05$). Data were represented as the mean ± standard deviation (SD). Graphics were created using GraphPad Prism 5 (GraphPad,

San Diego, CA 92108).

Results

Plant growth

With the decrease of K supply, the FW and DW of lettuce plants also declined. Compared with the CK, the FW of lettuce plants treated with 1/2, 1/4 and 1/8 of K supply decreased by 13.6%, 23.8% and 36.9% respectively, while the DW decreased 9%, 18% and 28.4% (Table 4-3).

K concentration

Compared with the CK, the K concentration of lettuce plants with treatments of 1/2, 1/4 and 1/8 K supply decreased by 45%, 67% and 80% respectively (Fig. 4-1). Either the inner leaves or the outer leaves showed the same trend.

The outer/inner ratios of leaf K concentration and the K concentration of whole plant were analyzed. The outer/inner ratio was negatively correlated with the K concentration of whole plant (Fig. 4-2, $P < 0.01$).

Photosynthesis

There was no significant difference on the Pn, Gs, Ci and Tr between the treatments of CK, 1/2 and 1/4. The decline appeared at the 1/8 treatment, with the 98%, 40%, 13% and 29% decline of Pn, Gs, Ci and Tr compared with the CK, respectively (Table 4-4).

Soluble sugar concentration

Compared with the CK, the soluble sugar concentration of lettuce plants with

treatments of 1/2, 1/4 and 1/8 K supply increased by 50%, 93% and 138% respectively (Fig. 4-3). Either the inner leaves or the outer leaves showed the same trend.

The outer/inner ratios of leaf soluble sugar concentration and the K concentration of whole plant were analyzed. The outer/inner ratio was negatively correlated with the K concentration of whole plant (Fig. 4-4, $P < 0.01$).

Expression of LsSUCs

With the decrease of K supply, the relative expressions of *LsSUC*, *LsSUC3* and *LsSUC4* were decreased gradually (Fig. 4-5).

Discussion

As the K supply decreased, the soluble sugar concentration in lettuce leaves enhanced, while the relative expression of *LsSUCs* were decreased, which confirmed that K deficiency leaded to an increase in the accumulation of soluble sugar and a decrease in transport, thereby limiting the lettuce growth.

K deficiency caused a decrease in K concentration in both inner and outer leaves. Interestingly, the outer/inner ratios of leaf K concentration was increased as the level of K deficiency increased. This indicated that under low K stress, more of the limited K resources of lettuce were given to the outer. When different rice varieties suffer from K deficiency, the relative K concentration (Ratio of K concentration in leaf with K deficiency to normal plant) of the upper leaves is 20% - 100% higher than the lower leaves (Yang et al., 2004). This might indicate that efficient distribution of K to the

functioning plant tissues or organs might be one of important mechanisms for K internal use efficiency.

A surprising finding was that the soluble sugar concentration of inner leaves was much higher than that of outer leaves. It is possible that inside leaves would be still acting as a strong sink of sugars synthesized in outer leaves in order to supply the youngest leaves with carbohydrates for metabolic purpose (Baslam et al., 2013). Here, we tried to explain the sugar transport of lettuce under K deficiency by using the source-sink relationship. The soluble sugar concentration in either inner or outer leaves were increased with the K deficiency stress. However, the the outer/inner ratios of leaf soluble sugar concentration was increased as the level of K deficiency increased. This indicated that under K deficiency, although the content of soluble sugar in the inner and outer leaves increased, the relative accumulation (Ratio of soluble sugar concentration in leaf with K deficiency to normal plant) of soluble was higher in outer leaves (source). Other studies on the relationship between sink and source also show that when the plant was under low K stress, the concentration of soluble sugar in the leaf (source) increased, and the loading and flowing in the phloem decreased, resulting in the reduction of sugars in pollen, pistils, spikelet and so on (sink) (Chen et al., 2018; Hu et al., 2015; 2018).

Conclusion

With the K deficiency, soluble sugar accumulation was increased while its transport was reduced, thereby limiting plant growth. As the level of K deficiency increased,

lettuce plant distributed the K and soluble sugar to the functioning plant tissues.

Tables and figures

Table 4-1. The QM recipe of the CK, 1/2, 1/4 and 1/8 K-supplying treatments at different growth stages

DAS	Treatment	Nutrient amount (mmol plant^{-1})				
		N	P	K	Ca	Mg
7-14	CK	1.6	0.12	0.6	0.24	0.12
	1/2	1.6	0.12	0.6	0.24	0.12
	1/4	1.6	0.12	0.6	0.24	0.12
	1/8	1.6	0.12	0.6	0.24	0.12
14-28	CK	4.8	0.48	2.4	0.96	0.48
	1/2	4.8	0.48	2.4	0.96	0.48
	1/4	4.8	0.48	2.0	0.96	0.48
	1/8	4.8	0.48	0.7	0.96	0.48
28-35	CK	14.0	1.40	7.0	2.8	1.40
	1/2	14.0	1.40	2.0	2.8	1.40
	1/4	14.0	1.40	0.0	2.8	1.40
	1/8	14.0	1.40	0.0	2.8	1.40

Table 4-2. Primers of sucrose transporter genes used for qRT-PCR analysis

Gene	Primer ID	Primer sequences
LOC111911847 (LsSUC)	F(5'-3')	GTCGGTTTCTGGATTCTT
	R(5'-3')	GGATAAGTCAGCCAACAA
LOC111896097 (LsSUC3)	F(5'-3')	GAGGTTGAAGAAGAAGAT
	R(5'-3')	CACAATAAGCACTGAATG
LOC111894701 (LsSUC4)	F(5'-3')	GTGAGAAGAAGACGAGAG
	R(5'-3')	GACCTTGAGTAGCATTGT
LOC111889264 (Actin)	F(5'-3')	ATCCACGAGACGACTTAT
	R(5'-3')	CATCCTATCAGCAATTCCA

Table 4-3. Effects of CK, 1/2, 1/4 and 1/8 K-supplying treatments on the lettuce plant fresh and dry weight

Treatments	Plant FW (g)	Plant DW (g)
CK	102.9 ± 9.2 a[z]	4.01 ± 0.44 a
1/2	88.9 ± 2.9 b	3.65 ± 0.19 b
1/4	78.4 ± 2.6 c	3.29 ± 0.16 c
1/8	64.9 ± 6.3 d	2.87 ± 0.27 d

[z] Each value is the mean ± SD (n=6). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

Table 4-4. Effects of CK, 1/2, 1/4 and 1/8 K-supplying treatments on the lettuce leaf photosynthesis

Treatments	Pn (μmol CO_2 m^{-2} s^{-1})	Gs (mol H_2O m^{-2} s^{-1})	Ci (μmol CO_2 mol^{-1})	Tr (mol H_2O m^{-2} s^{-1})
CK	5.46 ± 0.37 a[z]	0.230 ± 0.011 a	350 ± 16 a	1.71 ± 0.11 a
1/2	5.21 ± 0.24 a	0.219 ± 0.009 a	356 ± 11 a	1.68 ± 0.06 a
1/4	5.13 ± 0.30 a	0.218 ± 0.010 a	343 ± 14 a	1.68 ± 0.11 a
1/8	3.06 ± 0.31 b	0.139 ± 0.013 b	304 ± 13 b	1.22 ± 0.12 b

[z] Each value is the mean ± SD (n=6). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.

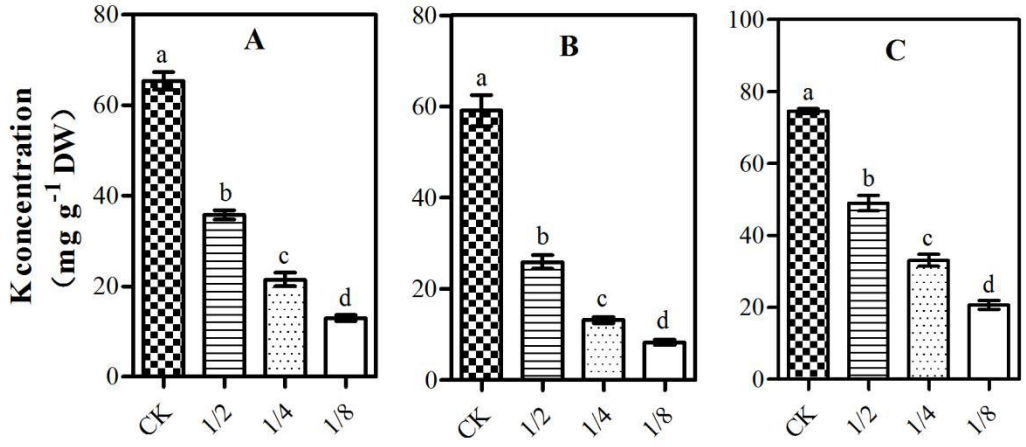

Fig. 4-1. Effects of CK, 1/2, 1/4 and 1/8 K-supplying treatments on the K concentration of whole plant (A), inner leaves (B) and outer leaves (C). Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

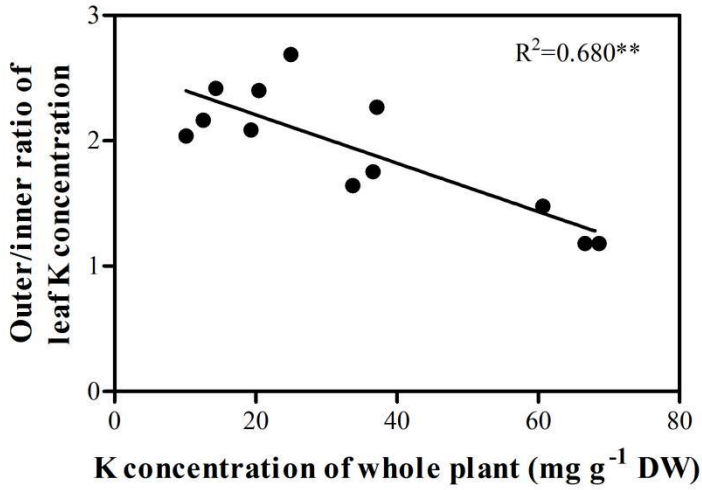

Fig. 4-2. The relationship between outer/inner ratio of leaf K concentration and K concentration of whole plant. Values are the average of three replicates. Regression coefficients and significance are shown when $P < 0.05$ (**$P \leqslant 0.01$).

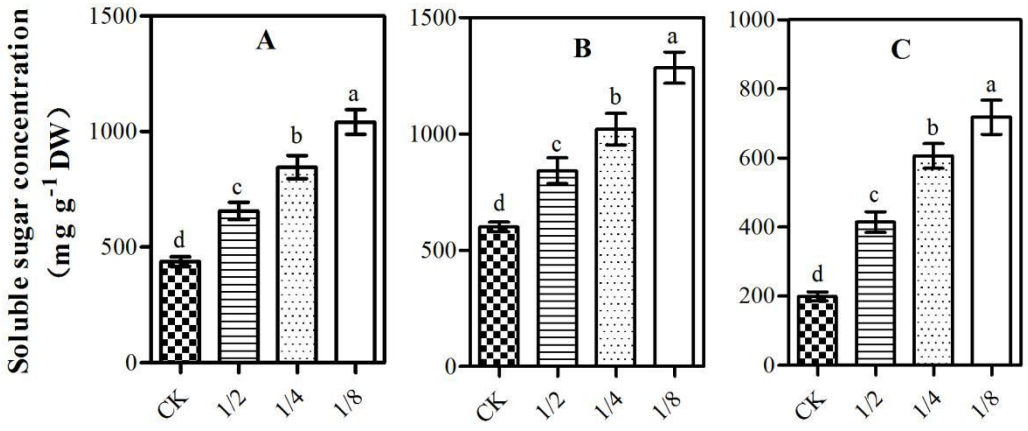

Fig. 4-3. Effects of CK, 1/2, 1/4 and 1/8 K-supplying treatments on the soluble sugar concentration of whole plant (A), inner leaves (B) and outer leaves (C). Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

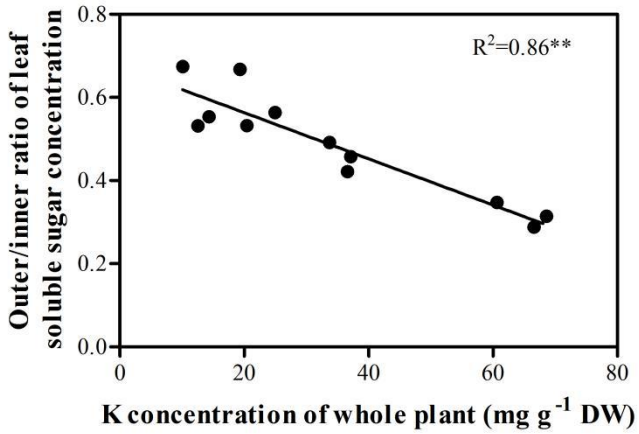

Fig. 4-4. The relationship between outer/inner ratio of leaf soluble concentration and K concentration of whole plant. Values are the average of three replicates. Regression coefficients and significance are shown when $P < 0.05$ (**$P \leqslant 0.01$).

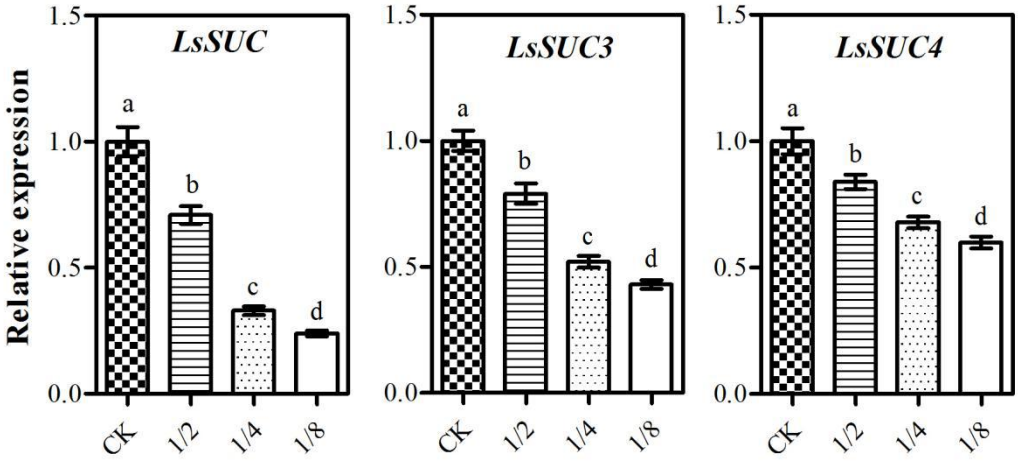

Fig. 4-5. Effects of CK, 1/2, 1/4 and 1/8 K-supplying treatments on the relative expression of *LsSUC*, *LsSUC3* and *LsSU4* genes. Columns and bars represent the means and SD (n = 6), respectively. Different letters indicate significant differences at the 0.05 level by Tukey's HSD test.

Chapter 5: Conclusions and prospects

Summaries of the studies

Since the rate of chronic kidney disease (CKD) increases all over the world yearly, the demand for low-K vegetable is increasing. Lettuce (*Lactuca sativa* L.) is one of the most widely consumed vegetables throughout the world. In Japan, a type of lettuce with low-K content has been marketed where K concentration was below 100 mg 100 g^{-1} FW. However, this low-K lettuce often accumulated a lot of Na, which was harmful to CKDs.

The research of nutrient quantitative management (QM) method has been going on for nearly 20 years. It can quantitatively control the plant element absorption. In this study, I tried to cultivate low-K lettuce with QM method to decrease the Na accumulation. Meanwhile, I studied the physiological changes of lettuce with K deficiency, to provide theoretical basis for the low-K cultivation.

In chapter 2, lettuces (Lactuca sativa L.) were cultivated with 2 low-K management method in an environment control system, LKEC and LKQM. Compared with CK, both low K treatments limited the yield by nearly 20% without any visual deficiency symptoms. There was no significant difference between LKEC and LKQM in terms of plant growth. LKEC-treated lettuce contained lower Na and required less fertilizer than that of LKQM, whcih was considered the practicable and better one in low K cultivation than LKEC method.

In chapter 3, lettuces (Lactuca sativa L.) were cultivated with LKEC and LKQM treatment. Low-K treatments significantly restrained the lettuce growth, with an

increased soluble sugar concentration and unchanged photosynthesis and fluorescence characteristics. This mean that the biomass reduction of low-K lettuce was due to the wasteful accumulation of carbohydrates rather than the decline in photosynthesis. Concentrations of SMs were increased in the low-K lettuce. In addition, the concentration of SMs in lettuces treated with LKEC and LKQM were also different. This meant that higher Na concentration in nutrient solution of LKEC also owned an effect on the concentration of SMs.

In chapter 4, lettuce plants were cultivated with 4 gradient of K supply, which were normal amount and 1/2, 1/4, 1/8 amount of normal. The cultivation method was QM method. With the decrease of K supply, the FW, DW, K concentration in leaves and relative expression of *LsSUCs* were decreased, while the soluble sugar concentration was increased. This mean that with the K deficiency, soluble sugar transport in leaves was reduced and accumulated in cells, thereby limiting plant growth. Comparing K and soluble sugar concentrations in the outer and inner leaves, the ratio of K concentration and soluble sugar concentration in outer leaf to inner leaf were negatively correlated with the K concentration of whole plant. This indicate that efficient distribution of K and soluble sugar to the functioning plant tissues might be one of important mechanisms for K deficiency.

Recommendations for future study

The QM method in low-K cultivation was considered better than the traditional EC management, due to the lower Na concentration in lettuce plants and less fertilizer use. In the future, the QM method should be applied to more cultivation purpose. This will

make a huge contribution to reducing fertilizer use.

The soluble sugar, Na and SMs concentrations in low-K lettuce were also changed with the K decline. These substances were also considered to affect CKDs. So as a functional food, the nutritional value of low-K lettuce should be further evaluated.

With the K deficiency, the outer leaves of lettuce plants would be allocated more K and soluble sugar. This means that removing the outer leaves will change the K and soluble sugar concentration of the lettuce. This is also an idea for future research.

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