Effect of Nutrient Quantitative Management on Potassium and Sodium Concentration in Low-potassium Lettuce

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Recently, the number of patients with chronic kidney disease has increased rapidly and kidneys with loss of the K-defecating function have been observed. Thus, providing vegetables with low potassium is an urgent unmet need. In this study, two cultivation methods were used to cultivate lettuce (Lactuca sativa L.) with low K concentrations. One method, dubbed LKEC, was based on electrical conductance management and the K supply was stopped at the end of cultivation. The other method, dubbed LKQM, was based on nutrient quantitative management, and the nutrients required for low-K lettuce were quantitatively supplied. Meanwhile, control lettuce with a normal K concentration, known as CK, were cultivated with electrical conductance management. Compared with CK, both low K treatments reduced the yield by nearly 20% without any visual deficiency symptoms. There was no significant difference between LKEC and LKQM in terms of plant growth. LKQM-treated lettuce contained lower Na and required less fertilizer than LKEC lettuce. Moreover, these plants adapted to K deficiency stress by absorbing more cations to maintain osmotic pressure. N declined with decreasing K. This suggested that the quantitative management method in low-potassium lettuce production reduced the potassium content in the lettuce plants to the same level as the EC management method, and significantly reduced the sodium content compared to EC management.

Key Words: cations, chronic kidney disease, fertilizer, osmotic pressure.

Introduction

Chronic kidney disease (CKD) is a common disorder in elderly people, and its prevalence is increasing globally. Japan is becoming a super-aged society and the number of CKD patients requiring dialysis treatment in Japan is now over 310,000, which is the second largest population in the world (Talukder et al., 2016). The kidneys normally excrete more than 90% of daily body potassium, but patients with CKD cannot completely excrete potassium, and as a result, residual potassium accumulates in the body; this is known as hyperkalemia. Hyperkalemia is an electrolyte disturbance, that can disorder human metabolism and is life-threatening (Barsoum, 2006; Zhang and Rothenbacher, 2008).

For CKD patients, dietary K is restricted to 2000–3000 mg·d⁻¹ (Bajwa and Kwatra, 2013). Raw vegetables, often rich in K, have not often been freely avail-
some researchers proposed using Na instead of K because Na can replace some nonspecific physiological functions of K (Abdul et al., 2011). Thus, replacing KNO₃ with NaN₃ can limit K in solution without any N loss. Several low K vegetables have also been successfully cultivated (Asao et al., 2013; Ogawa et al., 2007, 2012; Zhang et al., 2017). However, Na has also been reported to be harmful to dialysis patients (Kelly et al., 2017; Mills et al., 2016).

Recently, the quantitative management (QM) method was studied widely in response to environmental pollution caused by excessive fertilization (Rubio et al., 2017; Yan et al., 2008). The QM method is based on the amount of nutrients required by the plant and fertilizer is quantitatively added to the nutrient solution without being based on the concentration. In hydroponics, when inorganic ions in the solution are fully flowing, plants can absorb the nutrients they need at an infinitely low concentration (Tsukagoshi, 2015). Several vegetables have been cultivated successfully using the QM method, such as tomato (Kageyama et al., 1987; 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). Different plant species, different growth environments, and different cultivation purposes will affect the amounts of elements in different growth stages of the plant. Therefore, the nutrient recipe for the QM method, which is the amounts of elements required by plants at different growth stages, should be prepared before starting cultivation. Moreover, the QM method precisely controls nutrient absorption in plants (Li et al., 2014). We hope that this method will be suitable to reduce the Na concentration in low-K vegetables.

In this study, two methods were used to cultivate “low-K lettuce”. One was based on electrical conductance (EC) management and the K supply was stopped at the end of cultivation (LKEC). The other was based on nutrient quantitative management, and the amounts of nutrients required for low-K lettuce were quantitatively supplied (LKQM). Meanwhile, lettuce with normal K concentrations were cultivated with EC management as the control (CK lettuce). The element absorption of lettuce and residual fertilizer in each nutrient solution were compared. Moreover, to cultivate low-K lettuce with low Na levels, we did not add extra Na in the LKQM method.

### Materials and Methods

1) **Designation of the nutrient recipe for low K lettuce production**

In the LKQM treatment, in order to add fertilizer quantitatively, the amounts of elements required by low-K lettuce at different growth stages are needed. The low-K recipe was designed using commercial low-K lettuce plants (Lactuca sativa L. ‘Frillice’). The commercial low-K lettuces were cultivated in an environment-controlled plant factory with 1000 ppm CO₂ concentration, 21°C air temperature, 12-h photoperiod 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 seven days before harvest, all K in the nutrient solution was replaced by Na. The growth period was 35 days. At growth stages 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 element concentrations are shown in Tables 1 and 2. Based on these, we calculated the amounts of elements required by low-K lettuce in these three growth stages (Table 3). Then, we designed an inorganic fertilizer formula and added it quantitatively to the nutrient solution in the LKQM treatment.

### Table 1. Growth parameters of commercial low-K lettuce at three growth stages.

| DAS | Leaf FW (g) | Leaf DW (g) | Number of leaves | Leaf Area (cm²) |
|-----|-------------|-------------|------------------|-----------------|
| 14  | 3.4±0.4     | 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         |

* Each value is the mean±standard deviation (n = 10).

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

| DAS | Element concentration (mg·g⁻¹ FW) |
|-----|-----------------------------------|
|     | N       | P       | K       | Ca      | Mg      | Na      |
| 14  | 2.5±0.2 | 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 |

* Each value is the mean±standard deviation (n = 10).
Table 3. Nutrient amount required by low-K lettuce at different growth stages.

| DAS  | Nutrient amount (mmol/plant) |
|------|-----------------------------|
|      | 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 |

2) Experimental design

Experiments were performed in an environment-controlled chamber equipped with fluorescent tubes at the Chiba University Matsudo Campus from July 25 to August 29, 2018. Seeds of ‘Frillice’ lettuce were sown in urithane cubes (W 2.3 cm × D 2.3 cm × 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) with a 16-h photoperiod with 300 ± 10 μmol·m⁻²·s⁻¹ photosynthetic photon flux density, 20°C temperature, and 1000 ppm CO₂ concentration for seven days. Morphologically uniform seedlings were transferred into another growth chamber with the same conditions as the Nae terrace. The seedlings were transplanted onto foam boards with 26 holes floating (60 × 30 × 1 cm) on containers (60 × 30 × 11 cm) for the initial seven 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 (NO₃-N 0.8 mmol·L⁻¹, PO₄-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 the raw water for the nutrient solution. Fresh air was supplied to the nutrient solution with an air pump.

The lettuce plants were cultivated on three different nutrient solutions: (1) Half-strength Enshi formula solution (NO₃-N 8 mmol·L⁻¹, PO₄-P 0.67 mmol·L⁻¹, K 4 mmol·L⁻¹, Ca 2 mmol·L⁻¹, Mg 1 mmol·L⁻¹, Fe 3 ppm, B 0.5 ppm, Mn 0.5 ppm, Zn 0.05 ppm, Cu 0.02 ppm, Mo 0.01 ppm; Zhang et al., 2017) for the entire period (EC: 1.4 dS·m⁻¹; pH: 7.0; CK); (2) Half-strength Enshi formula solution from 7 to 28 DAS and half-strength Na replacing the K Enshi solution (All K was replaced by Na in the 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 7, 14, and 28 DAS, quantitative chemical fertilizers were added to the groundwater according to the new recipe for the LKQM method (Table 3). The water level of LKQM was adjusted every three days. The EC values of the CK and LKEC were determined every three days using a portable EC meter (EC Meter CM-31P; DKK-TOA Corporation, Tokyo, Japan). At the growth stages of 14 and 28 DAS, the nutrient solution was changed. The microelement concentrations in the nutrient solutions of these three treatments were the same; the experiments were repeated three times.

Additionally, the LKQM recipe in this experiment was not optimized because the cultivation environment was different from that of commercial low-K lettuce. However, since the purpose of this research was not to evaluate recipes, but to evaluate the usefulness of the QM method, the only thing to confirm was the consistency of the K concentration in lettuces in the LKEC and LKQM treatments.

3) 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 a minimum of 72 h and the leaf DW was measured.

4) Element concentrations in plants

The P, K, Ca, Mg, and Na concentrations were determined based on the methodology 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 Co., Ltd., Osaka, Japan). Nearly 250-mg DW of each plant was incinerated at 650°C for 72 h in a muffle oven (Muffle Furnace FO300; Yamato Scientific Co., Ltd., Tokyo, 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 before starting inductively coupled plasma optical emission spectrometry (ICP-OES, ICPE-9000; Shimadzu Corporation, Kyoto, Japan) analysis.

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

5) Element amounts in solution

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

The N concentration in the nutrient solution was obtained by adding the concentration of NO₃⁻ and NH₄⁺. The NO₃⁻ and NH₄⁺ concentration in the solution were determined based on the methodology by Chilund et al. (2016), 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 sampled. The unit was mmol/
Results

1) Plant growth

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

2) Nutrient concentrations in plants

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

3) Nutrient amounts in the solutions

The amounts of element in the LKEC solution remained unchanged from 7 to 28 DAS. N, P, K, Ca, Mg, and Na amounts in the solution were approximately 160, 13.7, 81, 68, 35, and 16 mmol/container, respectively. At 28 DAS, the amount of K dropped to the same level as the groundwater and the Na amount increased greatly due to the low K treatment. Nevertheless, N, P, Ca, and Mg levels remained unchanged in the solution (Fig. 3).

In the LKQM treatment, the elements in the solution fluctuated sharply. At 14, 28, and 35 DAS, before adding the fertilizer, the amounts of all the nutrients were like those in the groundwater. At 35 DAS, N, P, K, Ca, Mg, and Na amounts in the solution were 11, 0.7, 3.8, 27, 16, and 14 mmol/container, respectively (Fig. 3).

Discussion

1) Low K treatments affected plant growth and element accumulation

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

The K concentration in low K treatments decreased by almost 65% compared to the CK treatment, whereas FW and DW decreased by 24% and 19%, respectively. However, no visual deficiency symptoms appeared (Fig. S1). The physiological action of K can be divided to specific and nonspecific physiological functions. 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 the LKEC treatment increased to 0.15, 0.18, and 2.85 mmol/100 g FW, whereas those in the LKQM treatment increased to 0.21, 0.25, and 0.60, respectively (Fig. 2D–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 FW in both low K treatments (Fig. 1C), these excess cations cannot compensate for 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 gap in osmotic pressure after K deficiency.

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

Meanwhile, the Na concentrations of commercial, LKEC and LKQM low-K lettuce were different. Sodium is not an essential element for plants (Abdul et al., 2011). Studies found that as the Na concentration in a nutrient solution increased, the sodium concentration in plants also increased (Blom-Zandstra et al., 1998; Carbonell-Barrachina et al., 1997). We think that the excessive Na concentration in commercial and LKEC low-K lettuce is due to extra absorption because of the high concentration of Na in the K-free nutrient solution. In the LKQM treatment, no Na as added, so the low-K lettuce in this treatment had the lowest Na concentration.

Concentrations of Ca and Mg were also increased in LKEC- and LKQM-treated lettuces. This phenomenon was more obvious in the 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 is a complex antagonistic relationship between these cations. In different cases, they exhibit different antagonisms or synergies (Li, 2007; Ologunde and Sorensen, 1982).

Concentrations of N in lettuce plants decreased by 6% under the two low K treatments. K deficiency affects N metabolism and NO\textsubscript{3}\textsuperscript{−} transport 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 study, the K concentration in plants decreased by almost 65% in two low K treatments, but there was only a 6.5% decline in the N concentration; this may be because of K cycling to absorb more nitrate for the plant (Engels and Kirkby, 2001).

2) LKQM treatment reduced the sodium concentration and fertilizer usage in low-K lettuce

The growth in the LKEC and LKQM treatments showed no significant difference, which means that the QM method can be used for the cultivation of low K lettuce without any decrease in yield. The 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 its utilization in low K vegetables. This experiment paves the way for
the promotion of future use of the QM method.

The biggest difference between the LKEC and LKQM treatments was the Na concentration in plants. The Na concentration in LKEC-treated lettuce was three times that of LKQM-treated plants and 2000-mg Na is among the top priorities of the World Health Organization to combat of chronic noncommunicable diseases (Humayun and Mirza, 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). Therefore, in the present study, the decrease in Na concentration in LKQM treatment low-K lettuce could make a beneficial contribution to the treatment of chronic kidney disease, especially for people with high Na intake.

The unabsorbed nutrients remaining in the solution exhibited big differences between the LKEC and LKQM treatments. In the LKEC treatment, all the amounts of elements in the solution remained unchanged, whereas in the LKQM treatment, they changed with large fluctuations and were much lower than those in the LKEC treatment. At the end of each stage, the amounts of elements were like those at the start (the groundwater). Another interesting phenomenon was that the K amount in the LKQM container was lower than that of LKEC in the final stage, even though no (LKEC), or only a small amount (LKQM), of K was added. The residual nutrient solution could affect the solution composition. Potassium in the LKQM treatment was almost completely absorbed by the lettuce plants at 27 DAS, so the residual nutrient solution at the roots and transplanted panel did not affect the K amount in solution in the final stage. All the added nutrients were absorbed by the plants at every stage in the LKQM treatment. This suggests that the utilization rate of K under the LKQM treatment reached 100%. Additionally, N, P, and K of the LKQM solution were almost zero at the end; this implies 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 remaining in the container at 35 DAS was higher than that at 28 DAS in the LKEC treatment (Fig. 3F). This may be because Na absorption by plants was lower than that of K (Fig. 2C, F) and Na gradually accumulated in the nutrient solution. Therefore, many companies growing low-K lettuce using LKEC treatment frequently update the nutrient solution, including a high concentration of Na. This reduces the efficiency of fertilizer utilization in LKEC treatment.

Conclusion

Both the LKEC or LKQM treatments can cultivate low-K lettuce, and the plant growth with these two treatments is almost the same. The LKQM method resulted in a lower Na concentration in plants and a higher fertilizer utilization efficiency than the LKEC treatment and was superior for the cultivation of low-K lettuce.

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