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Reduction of Potassium (K) Content in Strawberry Fruits through KNO_3 Management of Hydroponics

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The consumption of vegetables and fruits rich in potassium (K), such as melons and strawberries, is restricted in chronic kidney disease (CKD) patients. Therefore, we attempted to produce low-K strawberry fruits through management of a KNO_3 fertilizer in nutrient solution from anthesis to the harvest period. A general trend of decreasing K content in fruit was observed with the decrease of KNO_3 concentration in the nutrient solution. Among four strawberry cultivars, the fruit of the 'Toyonoka' exhibited a K reduction of about 64% when plants were grown in nutrient solution with KNO_3 at 1/16 of the normal level. Citric acid and ascorbic acid contents of 'Toyonoka' fruit were reduced with decreasing KNO_3 concentrations in the nutrient solution. Although the reduced NO_3^- of the nutrient solution was adjusted by using $Ca(NO_3)_2$ to obtain low-K strawberries, growth, yield, and quality did not vary with this adjustment. Compared with the typical level of K in strawberry fruit of 170 mg/100 g FW (Standard Tables of Food Composition in Japan, 2011), a 23.5% decrease (130 mg/100 g FW) in K was found in 1/32 level of KNO_3. The K contents of plant parts suggested that the low KNO_3 level was responsible for the low K absorption, which may have affected the translocation and accumulation of K into fruit. Therefore, 1/32 level of KNO_3 in nutrient solution lowers the fruit K content considerably.

Key Words: chronic kidney disease, *Fragaria* × *ananassa* Duch. 'Toyonoka', fruit potassium content, potassium nitrate, potassium translocation.

Introduction

Potassium (K) is a major electrolyte in the human body. It plays an important role in the contraction or relaxation of skeletal, smooth, and cardiac muscles, nerve impulse transmission, acid base equilibrium, enzymatic action, intracellular fluid tonicity, and renal function (Crawford and Harris, 2011; Russell, 2009). The average daily K intake is estimated to be 2000–3900 mg·day^{-1} (Choi and Ha, 2013; Kes, 2001; Putcha and Allon, 2007). About 77% of dietary K is excreted in the urine via the kidneys (Holbrook et al., 1984). A study found that higher K excretion is associated with a reduction in the risk of adverse renal outcomes (Smyth et al., 2014), but patients with chronic kidney disease (CKD) have limited ability to excrete K through the kidneys. As a result, the level of K increases in the blood serum. An elevated level of K in serum is called hyperkalemia, and is associated with an increased risk of chronic kidney disease progression and all-cause mortality in patients with chronic kidney disease (He et al., 2014). Mills et al. (2014) found that high dietary K was associated with an increased risk of cardiovascular disease among patients with CKD. In the United

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States, hyperkalemia causes 5 deaths/1000 person-years in patients with CKD (US Renal Data System, 2002). The number of CKD patients is continuing to increase globally. In Japan, approximately 13.3 million people were predicted to have CKD in 2005, which was about 13% of the Japanese adult population (Imai et al., 2007). Therefore, considerable focus should be placed on preventing this disease. Potassium concentration in blood serum and CKD are positively correlated, so management of potassium intake by changing the diet is one approach to control CKD prevalence. He et al. (2014) suggested that the mortality rate of CKD patients can be reduced with a moderate reduction in dietary sodium and potassium intake. Within the daily diet, fruits such as melons and strawberries and leafy vegetables are rich in K, which is essential for maintaining good health. However, the consumption of these food stuffs by patients with CKD is restricted, which negatively impacts their quality of life (QOL). CKD patients are often restricted to 1500 mg K a day (Committee for Revision of Diet Therapy Guideline, 2007). Therefore, the development of fruits and vegetables with low K content would be beneficial for the above-mentioned patients to maintain their QOL.

Potassium is also an essential major nutrient for plant growth and development. Plants absorb more K than any other element, with the exception of N (Britto and Kronzucker, 2008; Szczerba et al., 2009). Voogt and Sonneveld (1997) found that with increases in tomato plant growth, K absorption increases to a relatively greater extent than that of other nutrients. Recently, low K content lettuce (*Lactuca sativa* L.) has been established by K fertilizer management (Ogawa et al., 2007). Hydroponic culture systems have become widely used because they allow greater control over the root zone environment than soil culture, which makes nutrient management easy based on the plant requirements. Hydroponic culture of strawberries has gained in popularity for the commercial production of strawberries (Koshikawa and Yasuda, 2003). Generally, greenhouse-cultured fresh strawberries have a high K content of 170 mg/100 g FW of fruit (Standard Tables of Food Composition in Japan, 2011). Reducing this level in strawberry fruit would provide a good option in the diet for CKD patients. Asao et al. (2013) reported that a reduction in KNO_3 in hydroponic solution could reduce the K concentration in melon fruit. Therefore, the objective of the present study was to investigate the impact of reduced K concentration in nutrient solution on the fruit K content of strawberries, while maintaining normal growth, yield, and other indicators of fruit quality.

Materials and Methods

1. Strawberry cultivars

Micro-propagated vigorous, disease-free, and similarly sized plantlets of four strawberry (*Fragaria* × *ananassa* Duch.) cultivars 'Toyonoka', 'Akihime', 'Saga-honoka', and 'Ai-berry' plantlets were used for this study.

2. Enshi nutrient solution

Strawberry plantlets were grown in 25% Enshi nutrient solution (pH 7.25 and EC 0.8 $dS \cdot m^{-1}$). Full-strength Enshi nutrient solution contains the following levels of salts per 1000 L of tap water: 950 g $Ca(NO_3)_2 \cdot 4H_2O$; 810 g KNO_3; 500 g $MgSO_4 \cdot 7H_2O$; 155 g $NH_4H_2PO_4$; 3 g H_3BO_3; 2 g $ZnSO_4 \cdot 7H_2O$; 2 g $MnSO_4 \cdot 4H_2O$; 0.05 g $CuSO_4 \cdot 5H_2O$; 0.02 g Na_2MoO_4; and 25 g NaFe-EDTA (Hori, 1966). In the present study, we reduced the amount of KNO_3 while keeping the levels of other nutrients constant in order to produce strawberry fruit with low K content through hydroponic culture. Although the pH of the nutrient solutions was not varied, the EC decreased gradually with gradual decreasing KNO_3. The EC values of 25% nutrient solutions with 1/4, 1/8, 1/16, and 1/32 levels of KNO_3 were 0.70, 0.67, 0.65, and 0.48 $dS \cdot m^{-1}$, respectively. The EC and pH of tap water used for this experiment were 0.22 $dS \cdot m^{-1}$ and 8.18, respectively.

3. Expt. 1. Selection of suitable cultivars for the production of low-K strawberries from plants grown in nutrient solution with 1/1, 1/4, or 1/16 the normal level of KNO_3

In this experiment, the above-mentioned four strawberry cultivars were evaluated for low K content in strawberry fruit using a hydroponic system inside a greenhouse (20 m × 5 m) of the Experimental Research Center for Biological Resources Science, Shimane University. The experiment was conducted from September 2009 to June 2010. The greenhouse had no heating system and its top windows opened at temperatures over 25°C. Micro-propagated strawberry plantlets were transferred into cell trays (48 cm × 24 cm × 4 cm, 72 cells/tray) with vermiculite substrate on 14 September, 2009, and kept there until 6 October, 2009, under controlled growth chamber conditions at 20°C/15°C (day/night), 60% relative humidity (RH), fluorescent light with intensity of 145 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a 12 h photoperiod for the formation of new roots and leaves. Twenty-five-percent standard Enshi nutrient solution was used for fertilization. Then, the cell trays were transferred into the greenhouse and kept there until 26 October, 2009. On 27 October, 2009, 6 four to five leaf-stage strawberry plantlets were transplanted into plastic containers (20 cm × 54 cm × 34 cm) with 50 L of 25% nutrient solution. Before transplanting, vermiculite adhering to the strawberry plantlet roots were washed gently by tap water in a bucket. A urethane foam block (23 mm × 23 mm × 27 mm) was used to hold the plants tightly with a floating board on the nutrient solution. Nutrient solutions were circulated for 24 h using pumps (KP-101; Koshin, Kyoto, Japan) The culture solutions

were renewed every two weeks throughout the entire growth period. Reduction of KNO_3 in the culture solution was started after the first anthesis of all plants. 25% standard nutrient solution with 1/1, 1/4, or 1/16 the normal level of KNO_3 was used during plant growth. In this experiment, the total number of strawberry plants (4 cultivars × 3 level of KNO_3 × 3 replications × 6 plantlets per treatment) was 216. Pollination was facilitated using a soft brush at two-day intervals. Fruit were harvested when they became about 80% red in color. At each harvest, the fresh weight of fruit was recorded and used for the final yield calculation. At final harvest on 14 June, 2010, the number of leaves and root length were recorded. Then, strawberry plant parts were separated into leaf crowns and roots, and dried in a constant temperature oven (DKN 812; Yamato Scientific Co., Ltd., Japan) for 72 h at 80°C. When the dry matter reached a constant weight, the dry weights of different plant parts were measured.

4. Expt. 2. Culture of F. × ananassa Duch. 'Toyonoka' in nutrient solutions with 1/1, 1/8, and 1/16 the normal level of KNO_3

This experiment was carried out in a controlled room of the plant factory support research lab of the Experimental Research Center for Biological Resources Science, Shimane University. From the earlier experiment, 'Toyonoka' was selected for this experiment. Plantlets were kept for 7 weeks from 29 October, 2010 to 19 December, 2010 in controlled conditions as maintained in Expt. 1 for flower bud induction. Then, five bud-formed plantlets were planted in a hydroponic culture bed using four urethane blocks as support filled with 50 L of nutrient solution at 25°C/20°C (day/night), 60% RH, under fluorescent light with intensity of 145 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a 12 h photoperiod on 20 December, 2010. Three beds were used for one treatment connected with a 150-L nutrient solution tank (Fig. 1). Nutrient solutions were recycled at 55/5 min (recycle/stop) using an automatic pump. Treatment included 25% standard Enshi nutrient solution with 1/1, 1/8, and 1/16 the normal level of KNO_3. In this experiment, the total number of strawberry plants (3 level of KNO_3 × 3 replications × 5 plantlets per treatment) was 45. Culture solution was renewed biweekly. Pollination was performed using the brush at two-day intervals. At maturity, the fresh weight of fruit was recorded and used for a final yield comparison among the treatments. At final harvest on 14 June, 2011, plant growth parameters were measured and dry weights of the different plant parts were measured after oven drying of the samples, as described for Expt. 1.

Fig. 1. Hydroponic system under a controlled environment where strawberry plants were cultured in bed with 50 L nutrient solution connected to a 150-L nutrient solution tank.

5. Expt. 3. Production of low-K strawberry fruits using culture solutions with 1/8, 1/16, and 1/32 the normal level of KNO_3 with or without adjustment of NO_3^- supply by $Ca(NO_3)_2$

This experiment was also carried out in a controlled room of the plant factory. On 4 April, 2012, 'Toyonoka' plantlets were kept for bud formation under controlled environmental conditions as described for Expt. 2. Five bud-formed plantlets were planted in a hydroponic culture bed on 15 June, 2012. Three beds were used for one treatment connected with a 150-L nutrient solution tank. Five types of nutrient solution were used as 25% standard nutrient solution with (i) 1/8 KNO_3, (ii) 1/16 KNO_3, (iii) 1/32 KNO_3, (iv) 1/16 KNO_3 with $Ca(NO_3)_2$ adjusted to the same NO_3^- levels as 1/8 KNO_3, and (v) 1/32 KNO_3 with $Ca(NO_3)_2$ adjusted to the same NO_3^- levels as 1/8 KNO_3. EC and the pH values of these nutrient solutions were 0.67, 0.65, 0.48, 0.54, and 0.53 $dS \cdot m^{-1}$ and 7.57, 7.60, 7.33, 7.55, and 7.53, respectively. In number (iv) and (v) types of nutrient solution, $Ca(NO_3)_2$ was used to recover the effects of reduced NO_3^- of KNO_3. Culture solutions were renewed biweekly. In this experiment, the total number of plantlets (5 level of KNO_3 × 3 replications × 5 plantlets per treatment) was 75. Pollination and fruit harvest were performed as mentioned for the earlier experiments. Harvested fruit were divided into three categories on the basis of pollination date, namely, stage 1 or early stage (fruit collected from 18/6/2012 to 22/6/2012

as the date of pollination), stage 2 or mid-stage (fruit collected from 16/7/2012 to 20/7/2012 as the date of pollination), and stage 3 or late stage (fruit collected from 13/8/2012 to 13/8/2012 as the date of pollination). On 28 September, 2012, growth parameters were measured at the final harvest. The dry weights of different plant parts were measured after oven drying of the samples as described for Expt. 1.

6. Measurement of soluble solid, titratable acid, and ascorbic acid contents of strawberry fruit

After harvest, the fruit was composited and frozen at −30°C for subsequent analysis of soluble solid, titratable acid, and ascorbic acid contents. Soluble solid content was measured by placing a fruit juice sample into a pocket digital refractometer (PAL-1; Atago Ltd., Tokyo, Japan). Titratable acid content was determined by diluting a 2 mL aliquot of strawberry juice to 10 mL with 8 mL of distilled water and adding 2–3 drops of phenolphthalein, followed by adjustment of the pH to 8.2 using 0.1 N (w/v) NaOH. The quantity of NaOH (mL) and the amount for appropriate acidity were converted into citric acidity (%). The ascorbic acid content was measured using 2,4-dinitrophenylhydrazine (DNP) colorimetry. Strawberry fruit juice (0.5 mL) was placed in a 50-mL test tube, to which 0.5 mL of 10% metaphosphoric acid solution, 1 mL of distilled water, 1 mL of 0.03% 2,6-dichlorophenol-indophenol (DCP), 2 mL of thiourea, and 1 mL of 2,4-DNP were added, followed by 3 h of incubation at 37°C in a water bath (BW400; Yamato Scientific Co., Ltd., Tokyo, Japan). After incubation, 5 mL of 85% H_2SO_4 was added while the samples were kept cool with iced water. After 30 min of cooling, the ascorbic acid content was measured at 520 nm using a spectrophotometer (U-2900; Hitachi High Technologies Corporation, Tokyo, Japan).

7. Determination of K and other nutrient concentrations in strawberry fruit

In order to measure the concentrations of K including Ca, Mg, Fe, and Na, fruit were kept in a 15-mL plastic test tube and left on ice for subsequent analysis of minerals. On the day of analysis, strawberry samples were taken out of the freezer for thawing. Then, the fresh weight of each fruit sample (8–10 g) was measured (Excellence XS Analytical Balance, XS204DRV; Mettler Toledo, Greifensee, Switzerland). Each sample was then placed in a 250-mL plastic bottle containing 200 mL of 1% HCl. The samples were next shaken in a Bio-Shaker (Bio-Shaker BR-43FL; TAITEC, Koshigaya, Japan) for 30 min at 150 rpm in order to liquefy the fruit flesh completely. The dissolved fruit samples were then filtrated (Advantec Grade No. 131, 185 mm; Advantec, Tokyo, Japan) and analyzed for the above minerals with a polarized Zeeman Atomic Absorption Spectrophotometer (Z-2310; Hitachi High Technologies Corporation).

8. Determination of K and other nutrient concentrations in strawberry plant parts

When the dry matter reached a constant weight, it was ground into powder with a mixer machine (MX-X53; National, Tokyo, Japan). Samples weighing 0.5 g were mixed with 8 mL of 97% HNO_3 and digested using a microwave sample preparation system (ETHOS1; Milestone S.r.l., Bergamo, Italy). After digestion, samples were brought up to a total of 50 mL in a volumetric flask by the addition of distilled water and then filtered with qualitative filter paper (Grade No. 131). The filtered sample solutions were analyzed for K, Ca, Mg, Fe, and Na using a Zeeman Atomic Absorption Spectrophotometer.

9. Statistical analysis

Analysis of variance was performed to test for significant effects of different KNO_3 levels in the nutrient solution on plant growth, fruit yield, and the quality and nutrients in parts of strawberry plants in all three experiments. Means were compared by Duncan's multiple range test (Mstat-C statistical software; Mstat Director, Department of crop and soil sciences, Michigan state University, USA) and Tukey's multiple range test (Statcel 2 Software; OMS Publications, Tokorozawa, Japan) at $P < 0.05$ and $P < 0.01$.

Results

1. Effects of reduced KNO_3 on the growth, yield, and fruit quality of four strawberry cultivars

It was cleared that strawberry cultivars significantly influenced the plant growth parameters while KNO_3 levels did not show any significant effects on those parameters (Table 1). Higher numbers of leaves were recorded for the 'Toyonoka' cultivar when plants were grown in the nutrient solution with 1/1 and 1/4 levels of KNO_3 and in 'Saga-honoka' with 1/1 and 1/16 levels of KNO_3. In terms of root length, longest and smallest roots were found in 'Toyonoka' with a 1/4 level of KNO_3 and 'Ai-berry' with 1/1 level of KNO_3. Among the four strawberry cultivars, higher and lower amounts of leaf dry matter were founded in 'Saga-honoka' and 'Ai-berry', respectively. From the interaction it was founded that 'Toyonoka' with a 1/16 level of KNO_3, 'Akihime' with a 1/1 level of KNO_3, and 'Ai-berry' with all levels of KNO_3 showed reductions in their leaf dry matter.

The results showed that the numbers of strawberry fruits per plants were influenced by the cultivars, but not by KNO_3 levels (Table 2). Among the four cultivars, 'Toyonoka' with 1/1 level of KNO_3 produced the greatest number of fruits per plant, whereas the lowest number was found in 'Ai-berry' with a 1/16 level of KNO_3. Neither the cultivars nor the KNO_3 levels influenced the fruit weight per plant. However, 'Toyonoka' with a 1/1 and 'Akihime' with a 1/4 level of KNO_3 produced the greatest amount of fruit per plant. Larger fruit

Table 1. Effects of reduced KNO_3 on the growth of four strawberry cultivars grown in hydroponic nutrient solution.

Strawberry cultivars	KNO_3[z] treatments	Leaf numbers[y]		Root length (cm)		Dry weight (g)			
						leaves		crown	
Toyonoka		40.7	a[x]	47.7	a	26.0	ab	1.8	a
Akihime		23.4	b	38.5	a	25.9	ab	3.1	a
Saga-honoka		41.2	a	46.9	a	40.3	a	2.2	a
Ai-berry		25.5	ab	34.3	a	21.7	b	3.1	a
Significant level			**		NS		**		NS
	1/1	33.4	a	39.1	a	28.9	a	2.6	a
	1/4	34.5	a	43.4	a	30.0	a	2.6	a
	1/16	30.3	a	42.9	a	26.3	a	2.5	a
Significant level			NS		NS		NS		NS
Toyonoka	1/1	44.6	a	43.6	abcd	29.3	bc	1.6	f
	1/4	44.9	a	51.6	a	29.4	bc	1.8	ef
	1/16	32.7	abc	48.1	ab	19.4	c	1.9	def
Akihime	1/1	20.7	c	34.3	de	22.5	c	2.9	abcde
	1/4	25.8	c	40.8	bcde	28.5	bc	3.1	abcd
	1/16	23.7	c	40.3	bcde	26.9	bc	3.3	ab
Saga-honoka	1/1	41.9	a	45.4	abc	42.6	a	2.6	abcdef
	1/4	39.3	ab	45.4	abc	38.2	ab	2.1	bcdef
	1/16	42.4	a	49.8	ab	40.0	ab	2.0	cdef
Ai-berry	1/1	26.4	c	33.2	e	21.2	c	3.4	a
	1/4	27.9	bc	36.2	cde	23.9	c	3.2	abc
	1/16	22.3	c	33.6	de	19.8	c	2.7	abcdef
Significant level			*		*		*		*

[z] KNO_3 concentration in 25% standard Enshi nutrient solution.
[y] Parameters were measured on a per plant basis.
[x] Means within a column followed by different letters are significantly different according to Duncan's multiple range test at $P<0.05$ (n=18).
NS: not significant, * Significant at $P<0.05$, ** Significant at $P<0.01$.

were found for ta he 'Ai-berry' cultivar at all levels of KNO_3.

Fruit quality was also affected by the strawberry cultivars and reduced levels of KNO_3. Among the cultivars and KNO_3 level 'Toyonoka' with a 1/1 level of KNO_3 produced strawberries with higher ascorbic acid contents while it was lower in other cultivars with 1/16 of KNO_3 (Table 2). Although the cultivars and KNO_3 level did not significantly affect the soluble solid contents, 'Saga-honoka' fruit with 1/1 level of KNO_3 and 'Ai-berry' fruit with 1/16 KNO_3 produced the highest and lowest soluble solid contents, respectively. With the reduction in KNO_3 in the culture solution, K content in all cultivars decreased gradually (Table 2). Among the four strawberry cultivars, the lowest K was measured in 'Toyonoka' with 1/16 KNO_3. Compared with the control (1/1 level of KNO_3), the reduction was approximately 64%.

2. Culture of 'Toyonoka' in nutrient solution with 1/1, 1/8, and 1/16 levels of KNO_3

When strawberry plants were grown in nutrient solution with different reductions in the levels of KNO_3 in a controlled environment, the plant growth parameters significantly varied among the treatments (Table 3). Compared with the 1/1 level of KNO_3, leaf numbers were significantly reduced with KNO_3 at 1/16 the normal level. Root length was also affected by the reduced level of KNO_3. Compared with 1/1 level of KNO_3, smaller roots were found in plants grown at KNO_3 1/8 and 1/16 levels. Crown diameters of strawberry plants also decreased with the reduction in KNO_3 level in the culture solution. It was shown that, compared with the control, both 1/8 and 1/16 levels of KNO_3 reduced the fresh weight of leaves and crowns and the dry weight of leaves, crown, and roots.

Reduced KNO_3 concentration had significant effects on the yield and fruit quality of strawberries (Table 4; Fig. 2). Compared with the control, total fruit weight per plant was higher with KNO_3 at 1/8. Although the fruit numbers did not differ among the treatments, larger fruit were found at a 1/16 level of KNO_3. Fruit quality characteristics, such as citric acidity (%) and ascorbic acid content, were reduced with reductions in KNO_3 concentration. Compared with those at the standard KNO_3 concentration in the nutrient solution, soluble solids did not vary among the treatments.

Fruit K concentration was greatly influenced by the

Table 2. Effects of reduced KNO_3 on the yield and quality of strawberry fruit from four strawberry cultivars grown in hydroponic nutrient solution.

Strawberry cultivars	KNO_3[z]	No. of fruits/plant	Fruit weight (g/plant)	Single fruit weight (g)	Soluble solids (%)	Ascorbic acid (ppm)	Fruit K (mg/100 g)
Toyonoka		27.0 a[y]	201.0 a	7.6 a	8.2 a	825.0 a	224.0 ab
Akihime		25.1 a	200.8 a	7.5 a	8.8 a	466.6 b	274.8 b
Saga-honoka		19.8 ab	148.0 a	7.4 a	8.7 a	494.3 b	235.5 ab
Ai-berry		14.7 b	151.9 a	10.8 a	7.4 a	448.7 b	198.8 b
Significant level		**	NS	NS	NS	**	**
	1/1	23.2 a	175.9 a	7.8 a	9.1 a	631.1 a	304.1 a
	1/4	21.9 a	183.4 a	8.6 a	8.1 a	583.5 ab	232.3 b
	1/16	20.4 a	166.9 a	8.6 a	7.6 a	444.6 b	163.4 c
Significant level		NS	NS	NS	NS	**	**
Toyonoka	1/1	29.8 a	217.7 a	7.3 b	9.1 ab	1038.1 a	311.1 ab
	1/4	27.8 ab	205.6 ab	7.5 b	8.2 abc	963.3 a	248.3 cde
	1/16	23.6 abcd	179.7 abcd	7.8 b	7.5 bc	406.1 b	112.7 h
Akihime	1/1	26.4 abc	186.5 abcd	6.8 b	9.3 ab	480.9 b	353.6 a
	1/4	22.7 abcde	214.5 a	7.6 b	9.0 ab	462.0 b	267.8 bcd
	1/16	26.1 abc	201.6 abc	8.1 b	8.2 abc	457.0 b	202.9 ef
Saga-honoka	1/1	20.7 bcdef	145.1 d	7.3 b	10.1 a	526.3 b	285.4 bc
	1/4	19.8 cdefg	154.5 cd	7.7 b	7.8 bc	471.6 b	229.9 def
	1/16	18.8 defg	144.1 d	7.3 b	8.5 abc	484.9 b	191.2 fg
Ai-berry	1/1	16.0 efg	154.4 cd	9.6 ab	8.3 abc	479.0 b	266.4 bcd
	1/4	14.8 fg	159.1 bcd	11.3 a	7.7 bc	436.9 b	183.0 fg
	1/16	13.2 g	142.2 d	11.3 a	6.3 c	430.2 b	146.7 gh
Significant level		*	*	*	*	**	*

[z] KNO_3 concentration in 25% standard Enshi nutrient solution.
[y] Means within a column followed by different letters are significantly different according to Duncan's multiple range test at $P<0.05$ (n=18).
NS: not significant, * Significant at $P<0.05$, ** Significant at $P<0.01$.

Table 3. Effects of reduced KNO_3 on the growth of strawberries 'Toyonoka' grown in renewed nutrient solution under controlled environmental conditions.

KNO_3[z]	No. of leaves[y]	Leaf		Root length (cm)	Crown diameter (cm)	Fresh weight (g)		Dry weight (g)		
		length (cm)	width (cm)			leaves	crown	leaves	crown	roots
1/1	60.8 a[x]	11.9 ab	16.2 a	45.0 a	1.3 a	255.7 a	21.5 a	44.5 a	1.5 a	1.4 a
1/8	51.9 ab	9.6 b	16.6 a	40.8 b	1.3 a	136.1 b	10.7 b	32.6 b	0.3 b	0.6 ab
1/16	44.7 b	12.5 a	16.4 a	37.5 b	1.0 b	91.5 c	9.9 c	26.7 b	0.2 b	0.4 b
	*	*	NS	*	*	*	*	*	*	*

[z] KNO_3 concentration in 25% standard Enshi nutrient solution.
[y] Parameters were measured on a per plant basis.
[x] Means within a column followed by different letters are significantly different according to Tukey's multiple range test at $P<0.05$ (n=15).
NS: not significant, * Significant at $P<0.05$.

reduction in KNO_3 concentration in the culture solution (Fig. 3). Compared with the 1/1 level of KNO_3, K content was decreased by 43% to 54% at 1/8 and 1/16 KNO_3, respectively. When the K concentrations of plant dry matter from different treatments were analyzed, significant differences were observed (Figs. 4–6). With the reduction in KNO_3 concentration in the culture solution, the K contents in leaves, crowns, and roots decreased gradually. Compared with that in the control, K content was reduced by 85% and 88% in leaves, 76% and 84% in crowns, and 86% and 89% in roots at 1/8 and 1/16 level of KNO_3, respectively.

3. Production of low-K strawberries through adjustment using $Ca(NO_3)_2$ in culture solution from 1/16 and 1/32 levels of KNO_3 to a 1/8 level of KNO_3

Compared to a 1/32 level KNO_3, leaves, crowns, and root dry matter of strawberry plants did not increased at

Table 4. Effects of reduced KNO_3 on the yield and fruit quality of strawberries ('Toyonoka') grown in nutrient solution under controlled environmental conditions.

KNO_3[z]	Fruit weight (g/plant)		Fruit no./plant		Single fruit weight (g)		Soluble solids (%)		Citric acidity (%)		Ascorbic acid (ppm)	
1/1	331.3	b[y]	50.9	a	6.5	b	7.8	a	0.39	a	460.5	a
1/8	431.0	a	57.3	a	7.5	a	8.1	a	0.28	b	435.1	b
1/16	379.9	b	48.6	a	7.9	a	7.7	a	0.23	b	427.9	b
		*		NS		*		NS		*		*

[z] KNO_3 concentration in 25% standard Enshi nutrient solution.
[y] Means within a column followed by different letters are significantly different according to Tukey's multiple range test at $P<0.05$ (n=15).
NS: not significant, * Significant at $P<0.05$.

Fig. 2. Strawberry fruits in a hydroponic system under a controlled environment.

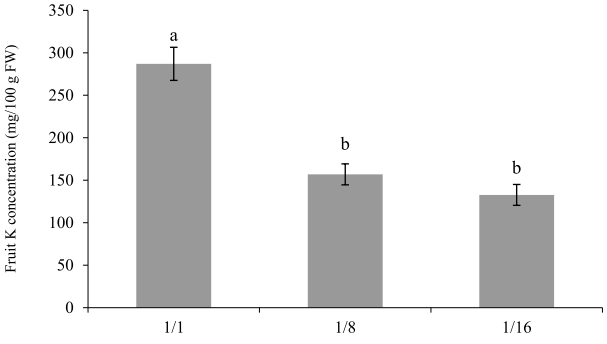

Fig. 3. Effects of reduced KNO_3 levels in culture solution on strawberry 'Toyonoka' fruit potassium content. Data are presented as means ± SE (n = 15). Different letters indicate significant differences between the treatments ($P < 0.05$).

a 1/32 level of KNO_3 with adjustment of the NO_3^- supply by $Ca(NO_3)_2$ (Table 5). There were no significant effects of $Ca(NO_3)_2$ adjustment on the yield and fruit quality, in terms of soluble solids, citric acidity, NO_3^-, and ascorbic acid concentration (Table 6). Compared with a 1/8 level of KNO_3, all levels of reduced KNO_3 in the nutrient solution reduced fruit K concentration in all three stages of harvested fruit (Table 7). K concentration in the strawberry fruits did not differ significantly in $Ca(NO_3)_2$ adjusted 1/32 level KNO_3 compared with a

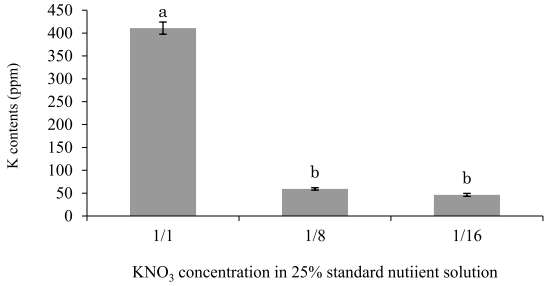

Fig. 4. Effects of reduced KNO_3 levels in culture solution on the potassium absorption by strawberry 'Toyonoka' leaves. Data are presented as means ± SE (n = 15). Different letters indicate significant differences between the treatments ($P < 0.05$).

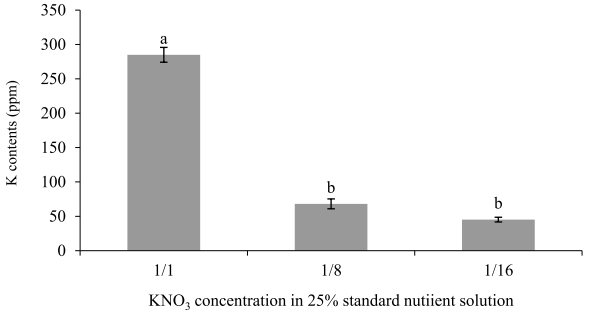

Fig. 5. Effects of reduced KNO_3 levels in culture solution on the potassium absorption by strawberry 'Toyonoka' crown. Data are presented as means ± SE (n = 15). Different letters indicate significant differences between the treatments ($P < 0.05$).

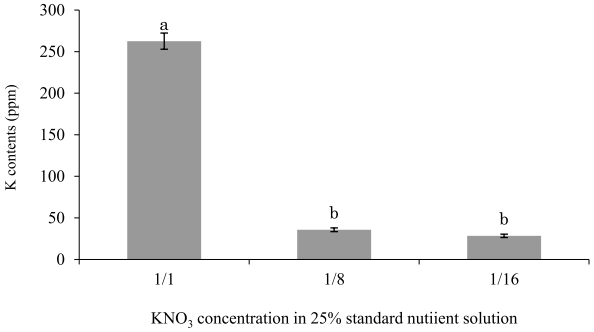

Fig. 6. Effects of reduced KNO_3 levels in culture solution on the potassium absorption by strawberry 'Toyonoka' roots. Data are presented as means ± SE (n = 15). Different letters indicate significant differences between the treatments ($P < 0.05$).

Table 5. Effects of $Ca(NO_3)_2$ adjustment for reduced NO_3^- of KNO_3 on the growth of strawberry plants.

KNO_3[z]	No. of leaves[y]	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Dry weight (g)		
					leaves	crown	roots
1/8	19.1 a[w]	241.8 a	166.9 a	501.6 b	12.8 a	1.5 a	4.3 a
1/16	14.3 b	222.3 ab	168.1 a	557.0 ab	12.5 a	1.2 ab	4.5 a
1/32	14.9 b	199.9 ab	145.7 b	556.0 ab	8.7 b	0.9 b	3.4 b
1/16 (adjusted)[x]	18.1 ab	219.3 ab	157.0 ab	600.3 ab	11.0 a	1.3 ab	4.0 ab
1/32 (adjusted)[x]	14.7 b	191.0 b	158.0 ab	546.5 ab	9.3 ab	1.0 ab	3.8 ab
	*	*	*	*	*	*	*

[z] 25% standard Enshi solution with KNO_3 conc. of 1/8, 1/16, and 1/32.
[y] Parameters were measured on a per plant basis.
[x] 1/16 and 1/32 KNO_3 with $Ca(NO_3)_2$ adjustment to 1/8 KNO_3.
[w] Means within a column followed by different letters are significantly different according to Tukey's multiple range test at $P<0.05$ (n=15).
* Significant at $P<0.05$.

Table 6. Effects of $Ca(NO_3)_2$ adjustment on reduced NO_3^- of KNO_3 on the yield and fruit quality of strawberry fruits.

KNO_3[z]	Fruit weight (g/plant)	No. of fruits/plant	Soluble solids (%)	Citric acidity (%)	NO_3^- (ppm)	Ascorbic acid (ppm)
1/8	160.8 a[y]	28.3 a	8.4 a	0.32 a	589.0 a	237.1 a
1/16	172.0 a	25.0 a	8.4 a	0.32 a	759.3 a	233.1 a
1/32	169.0 a	26.1 a	7.7 a	0.29 a	546.9 a	250.1 a
1/16 (adjusted)[x]	165.7 a	27.1 a	8.8 a	0.32 a	851.0 a	244.2 a
1/32 (adjusted)[x]	167.9 a	25.4 a	7.5 a	0.29 a	754.6 a	255.4 a
	NS	NS	NS	NS	NS	NS

[z] 25% standard Enshi solution with KNO_3 conc. of 1/8, 1/16, and 1/32.
[y] Means within a column followed by different letters are significantly different according to Tukey's multiple range test at $P<0.05$ (n=15).
[x] 1/16 and 1/32 KNO_3 with $Ca(NO_3)_2$ adjusted to 1/8 KNO_3.
NS: not significant.

Table 7. Effects of $Ca(NO_3)_2$ adjustment on reduced NO_3^- of KNO_3 the mineral contents of strawberry fruits.

KNO_3[z]	K (mg/100 g)			Ca (mg/100 g)			Mg (mg/100 g)			Na (mg/100 g)			Fe (mg/100 g)		
	S1[y]	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
1/8	237 a[x]	199 a	183 a	85 a	89 ab	84 b	29 a	31 a	23 a	6.4 ab	6.8 a	3.4 a	2.4 a	2.5 a	1.9 a
1/16	181 b	160 b	161 b	77 a	73 b	83 b	31 a	38 a	25 a	8.0 a	5.1 b	3.1 a	1.9 a	1.9 a	1.7 a
1/32	168 bc	146 bc	130 c	70 a	78 b	90 ab	31 a	33 a	25 a	4.9 b	5.5 b	3.4 a	1.7 a	1.7 a	1.3 a
1/16 (adjusted)[w]	148 c	131 c	156 b	69 a	97 a	101 a	27 a	29 a	27 a	5.6 ab	5.6 b	3.7 a	1.6 a	1.3 a	1.6 a
1/32 (adjusted)[w]	149 c	135 c	126 c	79 a	104 a	97 a	28 a	32 a	27 a	5.2 b	7.3 a	3.5 a	1.6 a	1.4 a	1.4 a
	*	*	*	NS	*	*	NS	NS	NS	*	*	NS	NS	NS	NS

[z] 25% standard Enshi solution with KNO_3 conc. of 1/8, 1/16, and1/32.
[y] S1=stage 1 (fruit collected from 18/6/2012 to 22/6/2012 as the date of pollination), S2=stage 2 (fruit collected from 16/7/2012 to 20/7/2012 as the date of pollination), S3=stage 3 (fruit collected from 13/8/2012 to 13/8/2012 as the date of pollination).
[x] Means within a column followed by different letters are significantly different according to Tukey's multiple range test at $P<0.05$ (n=15).
[w] 1/16 and 1/32 KNO_3 with $Ca(NO_3)_2$ adjusted to 1/8 KNO_3.
NS: not significant, * Significant at $P<0.05$.

1/32 level of KNO_3. In terms of the calcium content of strawberry fruit, stage 1 fruit did not show any significant variation; however, in stage 2 and stage 3, calcium content increased significantly with $Ca(NO_3)_2$ adjustment for both 1/16 and 1/32 levels of KNO_3. Na content was increased in stage 2 fruit with $Ca(NO_3)_2$ adjustment of 1/32 level of KNO_3. Nutrient contents of strawberry leaves significantly varied among treatments of reduced KNO_3 and $Ca(NO_3)_2$-adjusted KNO_3 (Table 8). Compared with a 1/8 level of KNO_3, leaf K contents were decreased by about 37%, 56%, 46%, and 60% in plants grown with treatments of 1/16 and 1/32 levels of KNO_3, and $Ca(NO_3)_2$ adjustment of 1/16 and 1/32 KNO_3, respectively. Other nutrients in leaves, such as Ca, Na, and Fe, decreased with decreases in KNO_3 concentration in the nutrient solution. However, when the reduced NO_3^- was adjusted by using $Ca(NO_3)_2$, Ca content of leaves was increased with 1/16 level of

Table 8. Effects of $Ca(NO_3)_2$ adjustment on reduced NO_3^- of KNO_3 on the mineral contents of strawberry leaves.

KNO_3[z]	K (ppm) (×100)	Ca (ppm) (×10)	Mg (ppm) (×10)	Na (ppm) (×10)	Fe (ppm) (×1)
1/8	3.15 a[x]	5.19 a	0.43 b	0.43 a	1.36 a
1/16	1.98 b	4.69 b	0.53 a	0.40 ab	1.28 b
1/32	1.36 c	4.64 b	0.51 a	0.36 b	1.10 c
1/16 (adjusted)[y]	1.68 b	5.18 a	0.44 b	0.38 b	1.19 c
1/32 (adjusted)[y]	1.23 c	4.48 b	0.48 ab	0.33 b	1.16 c
	*	*	*	*	*

[z] 25% standard Enshi solution with KNO_3 conc. of 1/8, 1/16, and 1/32.
[y] 1/16 and 1/32KNO_3 with $Ca(NO_3)_2$ adjusted to 1/8 KNO_3.
[x] Means within a column followed by different letters are significantly different according to Tukey's multiple range test at $P<0.05$ (n=15).
* Significant at $P<0.05$.

KNO_3. The $Ca(NO_3)_2$ adjustment of reduced KNO_3 did not increase the Na or Fe contents of leaves. Mg content increased with reduced KNO_3 in the nutrient solution.

Discussion

Among four strawberry cultivars, 'Toyonoka' and 'Ai-berry' plants exhibited reductions in the dry matter contents with 1/16 level of KNO_3 in the nutrient solution (Table 1). In another experiment, it was found that the dry matter contents of leaves, crowns, and roots significantly decreased with decreases in KNO_3 fertilizer in the nutrient solution (Table 3). This growth reduction was attributed to reductions in leaf number. Potassium is the cationic nutrient required at the highest level by plants, constituting up to 10% of plant dry matter (Leigh and Wyn Jones, 1984). This reduction in biomass because of K^+ deficiency is often accomplished by a reduction in leaf area (Jordan-Meille and Pellerin, 2004). Moreover, nitrate is generally the preferred source for crop growth (Miller and Cramer, 2004). Besides the KNO_3 reduction in the nutrient solution, the difference in cultivars also has an effect on the K uptake and utilization for the normal growth of plants. Woodend and Glass (1993) studied the uptake and utilization of K in wheat under conditions of K stress and found some variation among the varieties and genotypes.

Compared with the control (full strength of KNO_3), 'Ai-berry' exhibited decreases in fruit yield by about 8% with 1/16 KNO_3 in the nutrient solution (Table 2). It has been reported that K deficiency is related to reduced stomatal conductance, thereby impairing CO_2 fixation, which disrupts the conversion of light energy to chemical energy, and the phloem export of photosynthates from source to sink (Cakmak, 2005). In the case of maize and wheat production, when an insufficient level of K in soil was recovered using K fertilizer, the yield of those crops was increased (Ebelhar and Varsa, 2000; Singh and Sharma, 2001). However, when 'Toyonoka' was grown in a plant factory with a 1/8 level of KNO_3, its yield improved compared with that of the control (Table 4). This improved yield was attributable to a higher number of fruit. Luxurious vegetative growth was found in plants grown with control nutrient solution, leading to the lowest yield. Egilla et al. (2001) reported that an adequate amount of K can enhance the total dry matter accumulation of crop plants in comparison to lower K concentration.

The soluble solid contents of 'Saga-honoka' fruits were reduced with reductions of KNO_3 in the culture solution. In terms of ascorbic acid content, a similar trend was found in 'Toyonoka' cultivars (Table 2). Researchers have generally suggested that adequate K nutrition increases yield, fruit size, soluble solids, and ascorbic acid concentration, as well as improving fruit color, and increasing the shelf life and shipping quality of many horticultural crops (Kanai et al., 2007; Lester et al., 2005, 2006). Reduced KNO_3 in the nutrient solution with $Ca(NO_3)_2$ adjustment did not show any effects on the citric acid and ascorbic acid contents of strawberry fruit. Some studies have shown that there was little or no change in fruit quality with K fertilization in apples, cucumbers, mangoes, bell peppers, strawberries, and watermelons (Albregts et al., 1996; Hassanloui et al., 2004; Hochmuth et al., 1994; Locascio and Hochmuth, 2002; Perkins-Veazie et al., 2003; Rebolledo-Martinez et al., 2008; Umamaheswarappa and Krishnappa, 2004).

It was found that simple management with K fertilizer in the nutrient solution could reduce the level of K in strawberry fruit in all of the examined strawberry cultivars. Among these cultivars, 'Toyonoka' plants with a 1/16 level of KNO_3 exhibited a reduction of K of 64% in fruit compared with the control (Table 2). In two other independent experiments, the same level of KNO_3 reduced K by 54% and 32% in strawberry fruit (Fig. 3; Table 6). A general trend of decreasing K content in fruit was observed with decreasing KNO_3 concentrations in the nutrient solution. These results are in accordance with the study of Asao et al. (2013) in which the K of melon fruit decreased with decreasing KNO_3 levels in the nutrient solution. The reduction of K in the fruit may have been due to its low absorption by the

plants from the nutrient solution, low partitioning in different plant parts, and finally low accumulation in fruit. The total amount of K absorbed by the crops during the growing season depends upon the crop species being grown, the amount of native K^+ in the soil, the amount of K^+ applied to the fertilizer, its availability in the soil, the environmental conditions during the growing season, and management practices (Mullins and Burmester, 1998). In other research, it was found that K uptake also depends on plant-related factors, including genetics and the developmental stage, such as vegetative versus reproductive stages (Rengel and Damon, 2008). Pettersson and Jensen (1983) found differences among barley genotypes in terms of K influx and efflux, transport to shoots, and K use efficiency (dry matter produced per amount of absorbed K). On the other hand, the reduced level of KNO_3 also reduced the NO_3^- in the nutrient solution, which may have affected the K absorption. Blevins et al. (1974) studied the effects of NO_3^- on cation uptake. Fruit K reduction is also known to be related to the harvesting period. The lowest level of K in fruit was measured when harvesting at the end of the harvesting period (Table 6). It is possible that at this end stage plants take up less K from the nutrient solution and it accumulates at a low level in fruit. Na and Ca concentrations of strawberry fruits significantly varied with different treatments (Table 6). Asao et al. (2013) also found that a reduced level of KNO_3 in the nutrient solution increased the Na content in melon fruit. A reduced level of KNO_3 with $Ca(NO_3)_2$ adjustment increased the Ca contents of mid-and final-stage fruit and the Na content in mid-stage fruit (Table 6). This suggested that the increment of these ions compensated for the reduction in K.

A reduced level of K in nutrient solution was shown to lead to a drastic reduction in K accumulation in strawberry plant parts. When strawberry plants were grown with reduced KNO_3, K concentrations in leaves, crowns, and roots significantly decreased (Figs. 4–6; Table 7). These results indicated that strawberry plants grown with a nutrient solution containing a higher level of KNO_3 had a higher K concentration in plant parts at harvest, as we restricted the KNO_3 supply from formation to the anthesis stage, and therefore reduced the amount of K that translocated from the plant parts to fruit during the maturation stage.

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