Effects of Nutrient, NaCl and Heat Stresses on the Major Cation Contents in Tomato Seedlings

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The effects of nutrient, NaCl and heat stresses on the major cation (K, Ca and Mg) contents were studied in tomato (*Lycopersicon esculentum* Mill. cv. House Momotaro) seedlings. The purpose of the present work was to explore the similarity of cation responses under the above-mentioned stress conditions. Nutrient stress was applied by splitting the seedling roots into two parts, one of which was grown in 1/2 strength Enshi standard nutrient solution (ESNS) and the other was immersed in tap water. NaCl stress was conducted by treating the seedlings with 1/4 strength ESNS containing 150 mM NaCl for 1 week (1/4 strength ESNS without NaCl as control). Heat stress was imposed by treating the seedlings at light 38°C and dark 33°C for 1 week (light 25°C and dark 20°C as control). It was found that leaf K content was significantly decreased under both nutrient and NaCl stresses, while it was significantly increased under heat stress. Leaf Ca content was unaffected under both nutrient and NaCl stresses, but significantly increased under heat stress. Leaf Mg content was significantly increased under nutrient, NaCl and heat stresses.

Keywords : tomato, hydroponics, split-root system, nutrient stress, Mg accumulation

INTRODUCTION

Cation contents of tomato plants are affected by several environmental stresses, such as salinity stress (Mitchell et al., 1991 ; Atta-Aly et al., 1992 ; Bolarín et al., 1995), heat stress (Choluj, 1996), and nutrient stress (Sonneveld and Voogt, 1991). In a previous study, we found that nutrient stress by split-root system significantly increased leaf Mg content (Zhu and Ito, 2000). Whether this phenomenon also occurs under NaCl and heat stresses remains unknown.

K content in tomato tissues, expressed on a dry weight basis, declines as the Na-salinity in the root media is increased (Pérez-Alfocea et al., 1996 ; Song and Fujiyama, 1996), while Ca content remains unaffected in tomato leaves and stems (Carvajal et al., 1999). Although salinity studies have analyzed plant tissues for Mg content, most of the salinity-nutrition studies have directed little attention to Mg nutrition as affected by salinity. Faiz et al. (1994) reported that Mg content in tomato shoots was increased with increasing salinity, but in their saline treatment, NaCl, MgCl₂ and MgSO₄ were included.

Under high root zone temperature, K content was increased, Ca content was decreased and Mg content was unaffected in tomato leaves (Nkansah and Ito, 1995). Heat stress, however, increased Ca translocation into tomato fruits (Starck et al., 1994). The objective of

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this paper is to explore the similarity of cation responses in tomato plants under nutrient, NaCl and heat stresses.

MATERIALS AND METHODS

Plant material. Seedlings of *Lycopersicon esculentum* Mill. cv. House Momotaro (Takii & Co., Ltd., Japan) were used in this study.

Nutrient stress experiments. Two hydroponic culture experiments were carried out in the greenhouse of the Faculty of Horticulture, Chiba University.

Experiment 1 was conducted in winter of 1997. The seedling raising method, the design of treatments, and the management of nutrient solution during seedling raising and treatment periods were the same as described in our previous report (Zhu and Ito, 2000). On October 30, seeds were sown in rice husk charcoal. Seven days after sowing, seedlings with fully expanded cotyledons were transplanted to polyurethane substrates. On November 28, seedlings with four expanded leaves were transplanted into 1/2 000 a Wagner's pots, with one seedling in a pot. Plants were aerated by air bubbles. Two treatments were designed as follows. 1) All roots of the seedling were grown in 1/2 strength Enshi standard nutrient solution ($EC = 1.46 \text{ dS} \cdot \text{m}^{-1}$, ESNS). 2) The roots of the seedling were evenly split into two parts, and one part was grown in 1/2 strength ESNS and the other in tap water ($EC = 0.36 \text{ dS} \cdot \text{m}^{-1}$). Five plants were grown in each treatment. On December 30, when the first truss flowered, the plants were harvested.

The same experiment was repeated in spring of 1998 (Experiment 2). The plant material and experimental methods were the same as those for Experiment 1, except for leaf sampling (see below). Seeds were sown on April 6 and seedlings were transferred to polyurethane substrates on April 17. On May 2 seedlings with four expanded leaves were transplanted into Wagner's pots as described above. On May 25, when the first truss flowered, the plants were harvested.

The daily maximum and minimum air temperatures were recorded with a maximum-minimum thermometer, and nutrient solution temperature was recorded at 9:00 A.M. throughout this experiment. During 1997 winter cultivation, the daily maximum and minimum air temperatures ranged from 20 to 29°C and 10 to 15°C respectively, and nutrient solution temperature varied from 14 to 20°C. There were 9 d with minimum temperature of 10°C. During 1998 spring cultivation, the daily maximum and minimum air temperatures ranged from 18 to 34°C and 12 to 20°C respectively, and nutrient solution temperature fluctuated between 15 and 25°C. There were 5 d with minimum temperature of 12°C. The overall temperatures in winter cultivation were lower than those in spring cultivation.

NaCl stress experiment. On November 15, 1999, seeds were sown in growing medium Serubaido (Sumirin Agro-Products Co., Ltd., Japan). Three weeks after sowing, seedlings with 2 leaves were transferred into plastic pots (10.5 cm in diameter), filled with growing medium Tsuchitaro (Sumirin Agro-Products Co., Ltd., Japan). The seedlings were raised in a plastic greenhouse. On January 25, 2000, when seedlings reached 8 leaves, NaCl treatment was started. Five seedlings were put into each plastic container (45×30×11 cm), two containers were used, one for NaCl treatment, the other for control. NaCl treatment was applied by adding 1/4 strength ESNS containing 150 mM NaCl into the container until reaching a depth of 2 cm. As control, only 1/4 strength ESNS was added into the other container. Both containers were placed in the same growth chamber. The nutrient solution in the containers was renewed every 2 d after the beginning of treatment. The environmental conditions of the growth chamber were set as follows: temperature was kept at 25 and 15°C during light and dark periods of 12 h each respectively, with light source of HPS lamps of

PPFD 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, relative humidity at 60%, and CO_2 concentration at 400 ppm. After 1 week of treatment, the plants were harvested.

Heat stress experiment. On October 27, 1999, seeds were sown in peat moss. Two weeks after sowing, seedlings with two leaves were transferred into plastic pots (5 cm in diameter), filled with rice husk charcoal. The pots were put into a plastic tank ($90 \times 60 \times 20$ cm), into which 1/8 strength ESNS was added. The management of nutrient solution was the same as described in nutrient stress experiments. On December 5, when seedlings reached 6 leaves, the plants were exposed to heat stress treatment. The pots were put into plastic containers with four pots in each container, into which 1/4 strength ($\text{EC} = 0.95 \text{ dS} \cdot \text{m}^{-1}$) ESNS was added until reaching a depth of 2 cm. For one treatment, two containers were put into each growth chamber. Two growth chambers were used, one for heat stress treatment (38 and 33°C during light and dark periods of 12 h each respectively) and the other for control (25 and 20°C during light and dark periods of 12 h each respectively). The plastic containers, the renewal of nutrient solution, and the environmental conditions of the growth chambers were the same as described in NaCl stress experiment, except for temperature. After 1 week of treatment, the plants were harvested.

Sampling and mineral content analysis. In nutrient stress Experiment 1, plants were divided into root, stem, lower 3 leaves, middle 3 leaves, and upper 3 leaves. In nutrient stress Experiment 2, plants were divided into root, stem, lower 4 leaves, middle 4 leaves, and upper 4 leaves. In heat and NaCl stress experiments, only the shoots were sampled. Shoots were divided into two parts, leaflets and stems with petioles. Samples of roots, leaves, and stems (or stems with petioles) were dried in an oven at 65°C for 1 week. The dried samples were milled and digested with H_2SO_4 -salicylic- H_2O_2 in flasks for mineral content determination (Walinga et al., 1995). Cations were determined by an atomic absorption spectrophotometer. Data were subjected to analysis of variance and the means were separated at $p < 0.05$ by Duncan's new multiple range test.

RESULTS

K, Ca and Mg contents under nutrient stress

Table 1 shows the effects of nutrient stress by split-root system on K contents of leaves and stems. In both winter and spring cultivation, K content was significantly decreased in lower leaves under nutrient stress. However, no significant difference in K contents was found in middle leaves, upper leaves and stems. In both winter and spring experiments, no significant difference in Ca contents of leaves and stems was observed between nutrient stress treatment

Table 1 Effects of nutrient stress by split-root system on potassium contents ($\text{mg} \cdot \text{g}^{-1} \text{ DW}$) of tomato leaves and stems.

Treatment	Leaves			Stems
	Lower	Middle	Upper	
1997 winter cultivation				
1/2 ESNS ^y + water	60.2 b ^z	71.5 a	70.7 a	116.8 a
1/2 ESNS (control)	70.8 a	76.9 a	75.8 a	125.0 a
1998 spring cultivation				
1/2 ESNS + water	62.7 b ^z	74.5 a	70.5 a	113.0 a
1/2 ESNS (control)	71.5 a	80.2 a	73.9 a	109.2 a

^z Different letters within short columns show significant differences by Duncan's new multiple range test at 5% level.

^y ESNS stands for Enshi standard nutrient solution.

and the control (data not shown).

Table 2 presents the effects of nutrient stress by split-root system on Mg contents of leaves and stems. In stems, nutrient stress did not significantly change Mg contents in both winter and spring cultivation. In winter cultivation, nutrient stress significantly increased Mg contents in all parts of leaves. In spring cultivation, however, this was observed only in middle leaves.

Effects of nutrient stress by split-root system on K, Ca and Mg contents of roots are given in Table 3. The pattern of K, Ca and Mg accumulation of roots in winter cultivation was similar to that in spring cultivation. K content of split roots either grown in 1/2 strength ESNS or immersed in tap water was significantly lower than that of the control. Ca content of split roots immersed in tap water was significantly lower than that of the control. However, this was not found in the split roots grown in 1/2 strength ESNS. The highest Mg content was observed in the split roots immersed in tap water.

K, Ca, Mg and Na contents under NaCl stress

Table 4 presents the effects of NaCl stress on K, Ca, Mg and Na contents of different parts of shoots. K contents of both leaflets and stems with petioles of NaCl treatment were significantly lower than those of the control. No significant differences in Ca contents of both leaflets and stems with petioles were found between NaCl treatment and the control. Mg content in leaflets of NaCl treatment was significantly higher than that of the control, showing no difference in stems with petioles. Na contents in both leaflets and stems with petioles of NaCl treatment were significantly higher than those of the control.

Table 2 Effects of nutrient stress by split-root system on Mg contents ($\text{mg}\cdot\text{g}^{-1}$ DW) of tomato leaves and stems.

Treatment	Leaves			Stems
	Lower	Middle	Upper	
1997 winter cultivation				
1/2 ESNS ^y + water	15.2 a ^z	12.5 a	9.5 a	6.3 a
1/2 ESNS (control)	11.8 b	9.2 b	7.7 b	4.6 a
1998 spring cultivation				
1/2 ESNS + water	17.9 a ^z	13.0 a	8.3 a	6.3 a
1/2 ESNS (control)	15.3 a	9.5 b	6.7 a	5.4 a

^{z,y} See Table 1.

Table 3 Effects of nutrient stress by split-root system on K, Ca and Mg contents ($\text{mg}\cdot\text{g}^{-1}$ DW) of tomato roots.

Treatment	Sampled part ^x	K	Ca	Mg
1997 winter cultivation				
1/2 ESNS ^y + water	N	104.4 b ^z	9.3 a	5.3 b
	W	56.0 c	2.5 b	10.6 a
1/2 ESNS (control)	N	126.6 a	9.2 a	4.5 b
1998 spring cultivation				
1/2 ESNS + water	N	77.6 b ^z	5.2 a	9.2 b
	W	34.6 c	2.4 b	18.2 a
1/2 ESNS (control)	N	87.9 a	6.1 a	9.0 b

^{z,y} See Table 1.

^x For the treatment of 1/2 ESNS + water, there are two root samples, one of which is from nutrient solution (N), the other from tap water (W).

Table 4 Effects of NaCl stress on K, Ca, Mg and Na contents ($\text{mg}\cdot\text{g}^{-1}$ DW) of different parts of shoots in tomato seedlings.

Treatment	K		Ca		Mg		Na	
	L ^y	S+P ^y	L	S+P	L	S+P	L	S+P
NaCl	25.7 b ^z	51.1 b	7.9 a	3.6 a	11.6 a	10.1 a	94.8 a	74.1 a
Control	29.2 a	68.9 a	8.0 a	4.1 a	10.4 b	9.7 a	3.2 b	7.2 b

^z See Table 1.

^y L stands for leaflets, S+P for stems with petioles.

Table 5 Effects of heat stress on K, Ca and Mg contents ($\text{mg}\cdot\text{g}^{-1}$ DW) of different parts of shoots in tomato seedlings.

Treatment	K		Ca		Mg	
	L ^y	S+P ^y	L	S+P	L	S+P
Heat	39.6 a ^z	106.4 a	9.1 a	4.5 a	11.7 a	5.9 a
Control	28.6 b	107.7 a	7.1 b	4.1 a	8.7 b	5.6 a

^z See Table 1.

^y See Table 4.

K, Ca and Mg contents under heat stress

Table 5 shows the effects of heat stress on K, Ca and Mg contents of different parts of shoots. In leaflets, K, Ca and Mg contents of heat stress treatment were significantly higher than those of the control. In stems with petioles, no significant differences in K, Ca and Mg contents were found between heat stress treatment and the control.

DISCUSSION

Mg content of leaves increased under nutrient stress (Table 2), NaCl stress (Table 4), and heat stress (Table 5). When the split roots were immersed in tap water, K and Ca contents of roots were significantly reduced, but Mg content significantly increased (Table 3). Why was Mg content significantly increased? When tomato plants were subjected to nutrient stress, fruit vascular development was significantly promoted (Sanders et al., 1996). Smith (1973) found that in K-deficient barley leaves, putrescine was 13.3-fold greater than the control. This was confirmed in the leaves of barley, radish, pea, bean and spinach plants (Basso and Smith, 1974). Under nutrient stress, polyamine accumulation and ethylene evolution were enhanced in tomato leaves (Corey and Barker, 1989; Feng and Barker, 1993). Espartero et al. (1994) reported that under NaCl stress, SAM1 gene (encoding S-adenosyl-L-methionine, SAM synthetase) was expressed. SAM synthetase is required in lignifying tissue and vascular development (Espartero et al., 1994). SAM synthetase catalyzes the conversion of methionine and ATP to SAM. SAM is a precursor in polyamine and ethylene biosynthesis (Yang and Hoffman, 1984; Corey and Barker, 1989). It has been found that SAM synthetase requires an unusually high concentration of Mg^{2+} for optimal activity (Mudd and Cantoni, 1958; Markham et al., 1983). We postulate that the increased Mg contents in roots and leaves in the present work might be related to the increased activity of some enzymes.

Under nutrient stress by split-root method, K contents of lower leaves (Table 1) and roots (Table 3) were significantly reduced. Under NaCl stress, Na contents in both leaflets and stems with petioles were significantly increased, while K contents in both leaflets and stems with petioles were significantly declined (Table 4). There is competition with K uptake by

other cations under stress conditions (Gadallah, 1996 ; Okubo and Utsunomiya, 1996). The reduction of K content under either nutrient stress or NaCl stress in the present work might result from Mg-K or Na-K antagonism

Ca content in leaflets was significantly increased under heat stress (Table 5). Ca is transported mainly through the xylem vessels and its distribution within a plant is affected by the transpiration stream and by IAA (Hanson, 1984). Although the transpiration rate of leaves was not determined in this study, high transpiration rate of leaves might occur under high temperature (38°C) with plenty of water supply. The increasing of Ca content under heat stress might result from the high transpiration rate of leaves.

In the present investigation, leaf Mg content (especially in middle leaves, Table 2) of tomato seedlings was increased under nutrient stress by split-root system. This result was in agreement with our previous report (Zhu and Ito, 2000). Sonneveld and Voogt (1991) reported that Ca stress (deprivation) significantly increased leaf Mg content in rockwool grown tomato. Under NaCl stress, K content was significantly decreased, Ca content was unaffected, and Na content was significantly increased in shoots (Table 4). This result coincided with reports of other researchers (Pérez-Alfocea et al., 1996 ; Carvajal et al., 1999). Leaf Mg content was significantly increased under NaCl stress (Table 4). Although Faiz et al. (1994) reported that Mg content in tomato shoots was increased with increasing salinity, but in their saline treatment, NaCl, MgCl₂ and MgSO₄ were included. Therefore, it was difficult to distinguish the effect of NaCl from that of MgCl₂ and MgSO₄ on the elevation of shoot Mg content. Under heat stress, leaf K, Ca and Mg contents were significantly increased (Table 5). Nkansah and Ito (1995) reported that under high root zone temperature, K content was increased, Ca content was decreased, and Mg content was unaffected in tomato leaves. Our result was partly different from that of Nkansah and Ito (1995). This might arise from different tomato cultivars and different experimental methods.

In conclusion, leaf Mg content was significantly increased under nutrient, NaCl and heat stresses. Leaf K content, however, was decreased under both nutrient and NaCl stresses, while it was increased under heat stress. Leaf Ca content was unaffected under both nutrient and NaCl stresses, but significantly increased under heat stress. These results demonstrated that the pattern of Mg response in leaves of tomato seedlings was very similar, while that of K and Ca was different under the stress conditions conducted in the present work.

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〈和文抄録〉

養分・NaCl および高温ストレスがトマト苗の主要カチオン含有率におよぼす影響

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養分・NaCl および高温ストレス条件でトマト (*Lycopersicon esculentum* Mill. cv. ハウス桃太郎) 苗の主要カチオン (K, Ca および Mg) 含有率を調査した。本研究の目的は、上記のストレス条件で、カチオン感応の類似性を検討することであった。養分ストレス区として、トマト苗の根を二つに分け、一つは園試処方 of 1/2 濃度に、もう一つは水道水にそれぞれ浸漬し、その対照区として、トマト苗を園試処方 of 1/2 濃度で水耕した。NaCl ストレス区として、トマト苗を 150 mM NaCl を加えた園試処方 of 1/4 濃度で 1 週間処理し、その対照区として、トマト苗を NaCl 無添加の同濃度培養液で生長させた。高温ストレス区として、トマト苗を明期 38°C・暗期 33°C で 1 週間処理し、その対照区として、トマト苗を明期 25°C・暗期 20°C で生長させた。葉の K 含有率は、養分・NaCl ストレス区で、有意に低下し、高温ストレス区で、有意に増加した。葉の Ca 含有率は、養分・NaCl ストレス区で、対照区と有意差が認められず、高温ストレス区で、有意に増加した。葉の Mg 含有率は、養分・NaCl および高温ストレス区で、有意に増加した。