

Physiological Responses to Mineral-Excessive Conditions: Mineral Uptake and Carbohydrate Partitioning in Tomato Plants

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The shortage or surplus of minerals directly affects overall physiological metabolism of plants; especially, it strongly influences carbohydrate metabolism as a primary response. We have studied mineral uptake, synthesis and partitioning of soluble carbohydrates, and the relationship between them in N, P or K-excessive tomato plants, and examined the interaction between soluble carbohydrates and mineral elements. Four-weeks-old tomato plants were grown in a hydroponic growth container adjusted with excessive N (20.0 mmol L⁻¹ Ca(NO₃)₂·4H₂O and 20.0 mmol L⁻¹ KNO₃), P (2.0 mmol L⁻¹ KH₂PO₄), and K (20.0 mmol L⁻¹ KNO₃), respectively, for 30 days. Shoot growth rates were significantly influenced by excessive N or K, but not by excessive P. The concentrations of water soluble N (nitrate and ammonium), P and K were clearly different with each tissue of tomato plants as well as the mineral conditions. The NPK accumulation in all treatments was as follows; fully expanded leaves (48%) > stem (19%) = roots (16%) = petioles (15%) > emerging leaves (1). K-excessive condition extremely contributed to a remarkable increase in the ratio, which ranged from 2.79 to 10.34, and particularly potassium was dominantly accumulated in petioles, stem and roots. Fresh weight-based soluble sugar concentration was the greatest in NPK-sufficient condition (154.8 mg g⁻¹) and followed by K-excessive (141.6), N-excessive (129.2) and P-excessive (127.7); whereas starch was the highest in K-excessive (167.0 mg g⁻¹) and followed by P-excessive (146.1), NPK-sufficient (138.2) and N-excessive (109.7). Soluble sugar showed positive correlation with dry weight-based total N content (p<0.01) whereas was negatively correlated with soluble P (p<0.01) and dry weight-based total P (p<0.01). On the other hand, starch production was negatively influenced by total N (p<0.001), but, it showed positive relation with total K concentration (p<0.05). This study shows that uptake pattern of NPK and production and partitioning of soluble carbohydrate were substantially different from each mineral, and the relationship between water soluble- and dry weight-based-mineral was positive.

Key words: Carbohydrate partitioning, Mineral excess, Mineral uptake, Tomato, Water soluble minerals

Correlation analysis between water soluble (WS) NPK, dry weight (DW) NPK and soluble carbohydrates in fully expanded leaves of tomato plants grown under excessive N, P or K condition.

	WS-NO ₃ -N	WS-NH ₄ -N	WS-P	WS-K	DW-N	DW-P	DW-K	Soluble sugar	Starch
WS-NO ₃ -N	-								
WS-NH ₄ -N	0.85***	-							
WS-P	-0.33	-0.59**	-						
WS-K	0.80***	0.98***	-0.62**	-					
DW-N	0.59**	0.38	-0.60**	0.41	-				
DW-P	-0.41	-0.65**	0.99***	-0.69***	-0.61**	-			
DW-K	0.58**	0.87***	-0.54*	0.89***	0.02	-0.60**	-		
Soluble sugar	-0.08	-0.04	-0.65**	-0.01	0.62**	-0.60**	-0.19	-	
Starch	-0.31	0.07	0.04	0.09	-0.76***	0.04	0.50*	-0.43	-

Our data report that excessive status of minerals greatly affects the accumulation of soluble carbohydrates, and the relationship between them was mineral-dependent.

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Introduction

Physiological and biochemical approaches have been used to determine the specific roles of nitrogen (N), phosphorus (P) and potassium (K) in responses to plant growth and crop yields. It is a well-known basic concept in terms of plant nutrition that plant growth and yield reach the peak between critical and optimal ranges of mineral supply (Marshner, 1995). Unfortunately, however, the level showing excessiveness and toxicity of mineral elements is extremely dependent upon each element and, therefore, it is difficult and not clear to find out visible toxic symptoms of plants suffering from excessive mineral supply.

Many studies have been performed to understand harmful and toxic effects, and physiological and biochemical responses caused by excessively-supplied mineral elements to plants as follows; ammonium in higher plants (Bendixen et al., 1997), phosphorus for *Polygonum hydropiper* (Huang et al., 2012), potassium for *Jatropha curcas* (Rodrigues et al., 2013), iron for rice (Becker and Asch, 2005), nickel for cucumber (Khoshgoftarmanesh and Bahmanziari, 2011), zinc for Chinese cabbage (Stuiver et al., 2014), boron for *Jatropha curcas* (Simon et al., 2013) and kiwifruit (Therios and Dimassi, 2003), and cadmium for maize (Janda and Szalai, 2006). A simple model elucidating the close relation between mineral status and carbohydrates was well documented that an increase in carbon (carbohydrates) concentration leads to an increase in biomass partitioning towards the roots, whereas an increase in mineral concentration favors biomass partitioning towards the shoot (Thornley, 1972). A limited supply of minerals led to an increase in both carbon allocation to roots and in the root-shoot dry weight ratio (Cakmak, 1994; Cakmak et al., 1994a; Ericsson and Kähr, 1995; Fredeen et al., 1989; Lewin et al., 1989; Peuke et al., 1994), and induced an accumulation of huge amount of soluble carbohydrates (Sung et al., 2013), however, carbon allocation under excessive or toxic mineral conditions was not clear.

Tomato (*Lycopersicon esculentum*) is one of the most popular vegetable crops cultivated commercially around the world and film houses-based cultivation area (the 5th in vegetable crops) throughout South Korea has been expanded year by year. In our previous study, we demonstrated uptake and distribution of mineral (N, P and K), and soluble carbohydrates partitioning in tomato plants grown under N, P or K-deficient conditions (Sung et al., 2013). There is little information on the relation between minerals, particularly water soluble mineral, and soluble carbohydrates, and on the differences by plant organs. The objective of this study was to examine uptake and distribution of mineral elements, production and partitioning of soluble carbohydrates, and the interaction between mineral element and soluble carbohydrate in high N, P or K-fed tomato plants.

Materials and Method

Plant materials and growth conditions The present study was performed at a green house in National Academy of Agricultural Science in Suwon, South Korea in 2012 for tomato (*Lycopersicon esculentum* cv. Seonmyoung). Seeds were germinated in a tray filled with perlite supplied with distilled-deionized water. Seedlings were transplanted into aerated containers with 1/3 strength of Hoagland nutrient solution. Four-weeks-old tomato plants were subjected to 12 holes-aerated 20 L capacity containers with Hoagland nutrient media [5 mmol L⁻¹ Ca(NO₃)₂·4H₂O, 5 mmol L⁻¹ KNO₃, 2 mmol L⁻¹ MgSO₄·7H₂O, 0.5 mmol L⁻¹ KH₂PO₄, 1.5 mmol L⁻¹ Fe-EDTA, 1 mmol L⁻¹ NH₄NO₃, and 1 mL of micro-elements mixture (H₃BO₃, 2.86 g L⁻¹; MnCl₂·4H₂O, 1.81 g L⁻¹; ZnSO₄·7H₂O, 0.22g L⁻¹; CuSO₄·5H₂O, 0.051 g L⁻¹; H₂MoO₄·4H₂O, 0.09 g L⁻¹]. In order to make N, P or K excessive environment, nutrient solution was adjusted as followed; N (20.0 mmol L⁻¹ Ca(NO₃)₂·4H₂O and 20.0 mmol L⁻¹ KNO₃), P (2.0 mmol L⁻¹ KH₂PO₄), and K (20.0 mmol L⁻¹ KNO₃). Tomato plants were constantly exposed for 30 days at average day temperature of 30 ± 3°C and night temperature of 20 ± 3°C. Mid-day photosynthetic photon flux density was 800-1,200 μmol m⁻² s⁻¹. The nutrient solution was replaced every 3 days in order to ensure a consistent nutrient supply. Plants were harvested to determine growth rates at 1, 5, 15 and 30 days after treatment (DAT) and, at 15 and 30 DAT, immediately separated into leaves, petioles, stems and roots for further analysis.

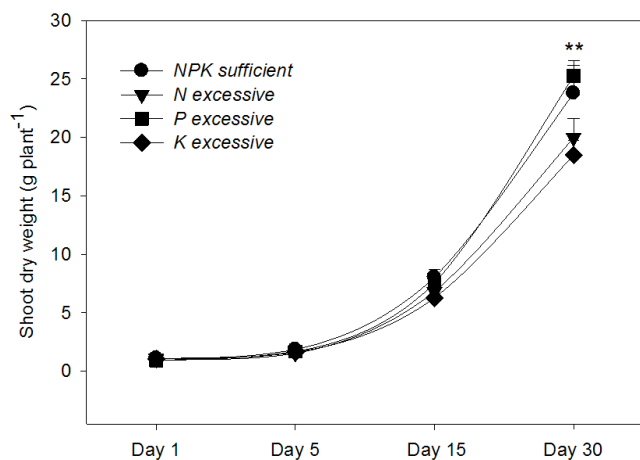
Measurement of nutrients Fresh plants were separated into young leaves, fully-expanded leaves, petioles, stems and roots, and then washed with tap water followed by distilled water. One gram of separated fresh samples was ground with 20 mL of dH₂O, passed through a membrane filter (0.45 μm), and used as a sample for measuring water soluble minerals. The oven-dried samples (0.3 g) which were at 80°C for 48 h were soaked in 5 mL of 368 mmol⁻¹ L salicylic acid in 84.7 % sulfuric acid (H₂SO₄) for 24 h then digested in a digestion system, heated to 300°C for 3 h, followed by several drops of hydrogen peroxide (H₂O₂). The extracted solution was transferred to 100 mL volumetric flasks and then diluted to 100 mL with deionized water for mineral assays. The N concentration was colorimetrically determined using the automatic flow injection analyzer (BRAN LUBBE, Germany). The P concentration was measured using the molybdate-blue colorimetry method (UV-2450, Shimadzu, Japan) and cation concentrations were determined with ICP-OES (INTEGRA XMP, GBC, Australia). The uptake ratio of each N, P, and K of each treatment was calculated as a ratio of each nutrient concentration in each treatment to that in NPK sufficient condition.

Measurement of soluble carbohydrates Soluble sugars from fresh leaves and roots were determined by the reaction of 1.0 mL of the alcoholic extract with 2.0 mL fresh 0.2% anthrone in sulfuric acid (w/v); the absorbance was read at 630 nm. After the extraction of the soluble fractions, the solid fraction was used for starch analysis. Starch was extracted with 9.3 mol L⁻¹ perchloric acid followed by 4.6 N. The extracts were combined and starch concentration was determined after reaction with the anthrone reagent. Glucose was used as the standard for soluble sugars.

Statistical analysis The analysis of variance (ANOVA) was conducted to find effects of treatments. Least significant difference (LSD) was performed to determine the significance of the difference between the means of treatments. *Pearson's* correlation coefficient analysis was performed to determine the relationship between minerals. An α value of 0.05 was chosen to indicate statistical significance. All statistical analysis was performed using version 9.01 of SAS (SAS Institute Inc, Cary, NC).

Results and Discussion

Dry weight accumulation Significant difference was found



between the dry weights of tomato shoots with increasing growth period under different mineral environments (Fig. 1). The reduction in growth was observed at 30 days after treatment (DAT), and growth rates under N- and K-excessive conditions were lower than that of the NPK-sufficiency, but not presented in high P. Higher minerals accumulation under N, P or K-excessive growth condition was also observed from the cross-sectioned tomato plants (Fig. 2), and the white-colored minerals were intensively deposited at the edge of stem. It is well-documented in the generalized plot of plant growth in response to changes in the concentration of a nutrient in the tissues which means the retarded growth in deficient zone, maximal growth in adequate zone, and decreased growth in toxic zone. Excessive mineral supply conditions on the growth have been reported previously (Cechin and Fumis, 2004; Egilla et al., 2001; Fredeen et al., 1989). From these results, it can be also deduced that plants have a big potential to resist four times stronger conditions, and the buffer ability is greatly dependent upon each mineral element. Furthermore, concerning water soluble NPK data, it is suggested that cations including K play a critical role to accumulate minerals in the stem.

Concentration in water soluble- and dry weight based-NPK and NPK uptake The concentration of water soluble

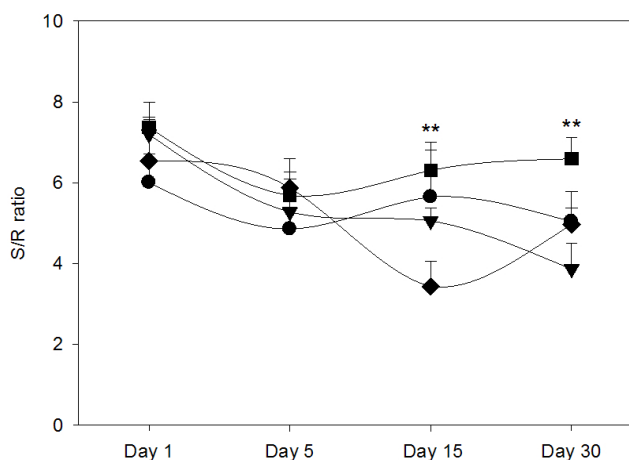


Fig. 1. Temporal changes in growth rates and S/R ratio of tomato plants grown under excessive N, P or K condition. Four week-old tomato seedlings were exposed to four times stronger Hoagland nutrient solution for 30 days. Different symbols (*, **, and ***) indicate significant differences ($p < 0.05$, 0.01, and 0.001, $n=5$) between treatments according to ANOVA test.



Fig. 2. Mineral deposition in outer layers of tomato stem observed at 30 days after N, P or K-excessive condition.

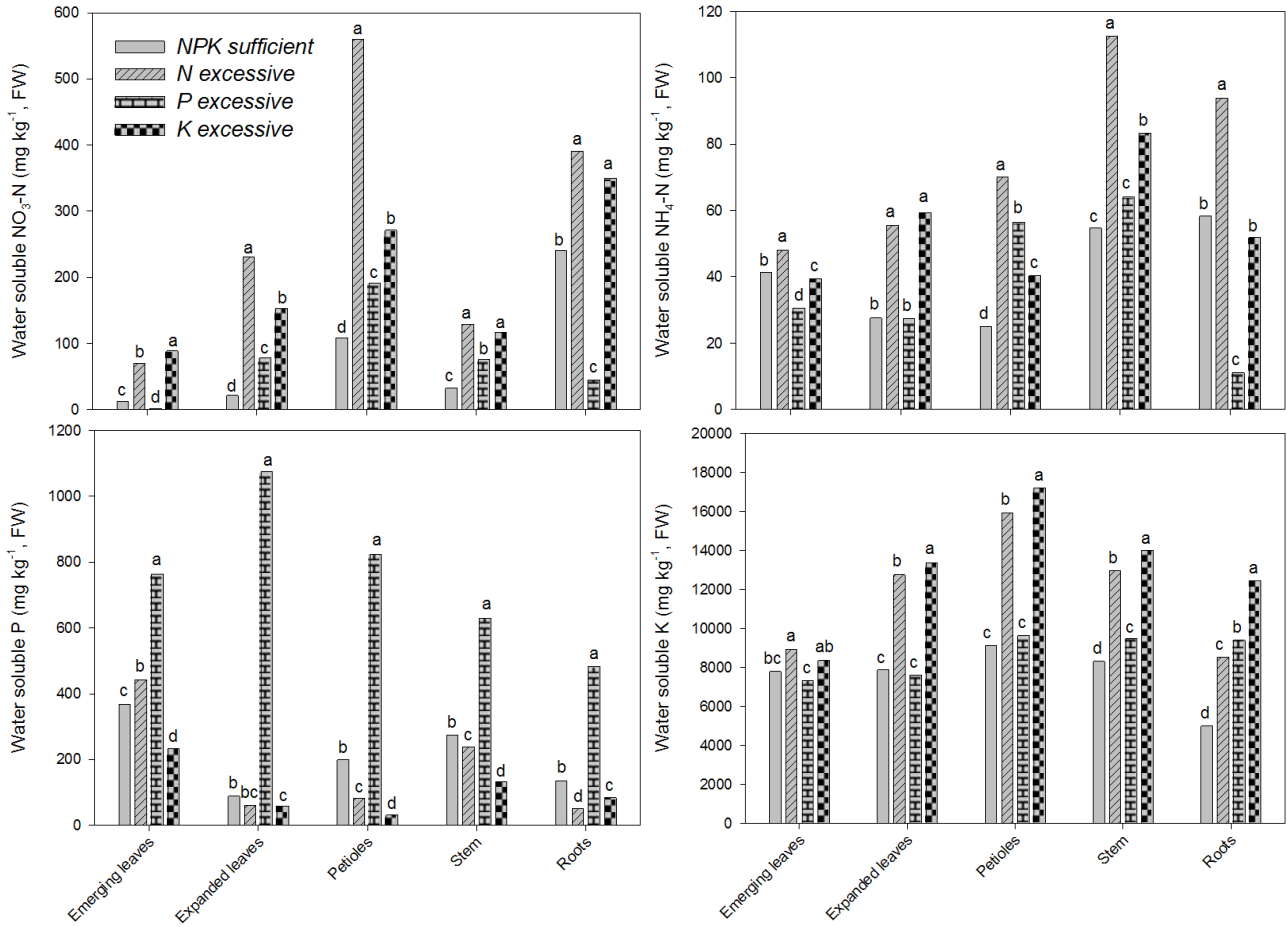


Fig. 3. Distribution of water soluble (WS) NO₃-N, NH₄-N, P and K by tomato tissues grown under excessive N, P or K condition. Different letter indicates significant differences between treatments according to LSD test (p < 0.05, n=5).

N (nitrate and ammonium), P and K was clearly influenced by each tissue of tomato plants as well as supplied mineral conditions (Fig. 3). Without a doubt, each mineral-excessive condition increased its mineral concentration in all plant tissues. Interestingly, greater N condition led to an obvious increase in water soluble K concentration except in roots, and greater K condition resulted in the significant elevation of water soluble nitrate and ammonium, by contrast, greater P condition greatly repressed an accumulation of water soluble N and K. The contents of mineral N, P and K were clearly influenced by N, P or K-excessive condition, respectively (Fig. 4). Among plant tissues under excessive N condition, the N concentration relative to dry matter was the greatest in emerging leaves and the lowest in stem. The excessive N also influenced the constant reduction in the contents of P and K in all plant parts. The ratio of N concentration relative to that in NPK sufficient condition was 109, 109, 125, 135 and 115% in emerging leaves, fully expanded leaves, petioles, stem and roots, respectively. The excessive P condition resulted in huge accumulation of P in all parts, particularly the fully expanded leaves, petioles and stem which showed 4.1, 6.5 and 6.7 fold greater than that of the NPK sufficient condition, and negatively affected the concentration of P and K. The K concentration

also revealed a significant increase under K-excessive condition, and was the greatest in petioles and roots and the lowest in emerging leaves. The ratio of K concentration relative to that in NPK sufficient condition was 115, 207, 267, 184 and 271% in emerging leaves, fully expanded leaves, petioles, stem and roots, respectively. The uptake of P and K of tomato plants was likely to equally respond to each mineral condition whereas the pattern in N was not clear (Fig. 5). In addition, the uptake seemed to be a little bit different from water soluble NPK concentrations. The NPK accumulation in all treatments was as follows; fully expanded leaves (48%) > stem (19%) = roots (16%) = petioles (15%) > emerging leaves (1%), although the percentage was greatly dependent upon each mineral condition. In general, plant growth was closely related with mineral uptake, and thus estimating the uptake rates of minerals elements can be helpful to comprehensively understand the status of plant growth, mineral concentration, etc (Cechin and Fumis, 2004; Egilla et al., 2001; Fredeen et al., 1989). The NPK taken up by tomato tissues was significantly different from each mineral. The NPK excess conditions revealed small changes in N uptake, whereas PK uptake was in directly responsive of each mineral supply rate. Our study demonstrated that tomato plants under excessive NPK condition showed not only the difference

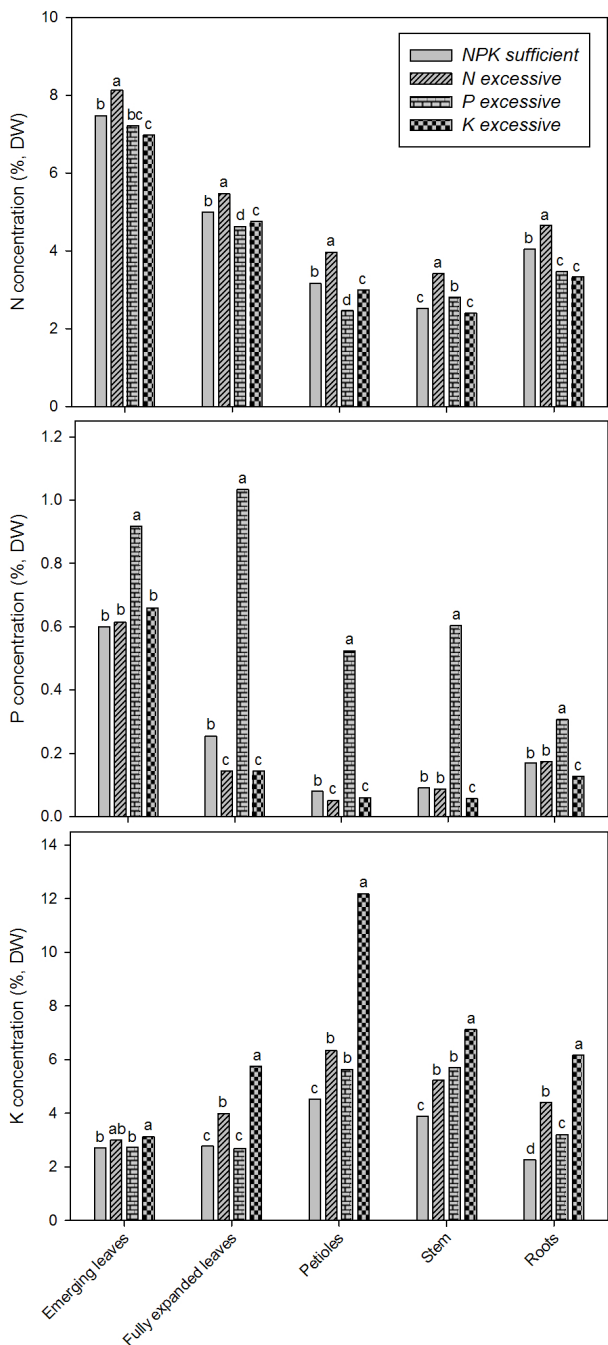


Fig. 4. Distribution of total N, P and K (dry weight-based, DWB) by organs of tomato plants grown under excessive N, P or K condition. Different letter indicates significant differences between treatments according to LSD test ($p < 0.05$, $n=5$).

in the uptake pattern of NPK, but also P and K could be absorbed by several times higher levels of physiological adequate range, and plants had a great potential to accumulate P and K absorbed excessively without any visible toxic symptom.

The ratio of mono-valent to di-valent cations The ratio of potassium (K) to the sum of calcium (Ca) and magnesium (Mg) was examined in each tissue of tomato plants grown under N, P or K-excessive conditions (Fig. 6), and the significant

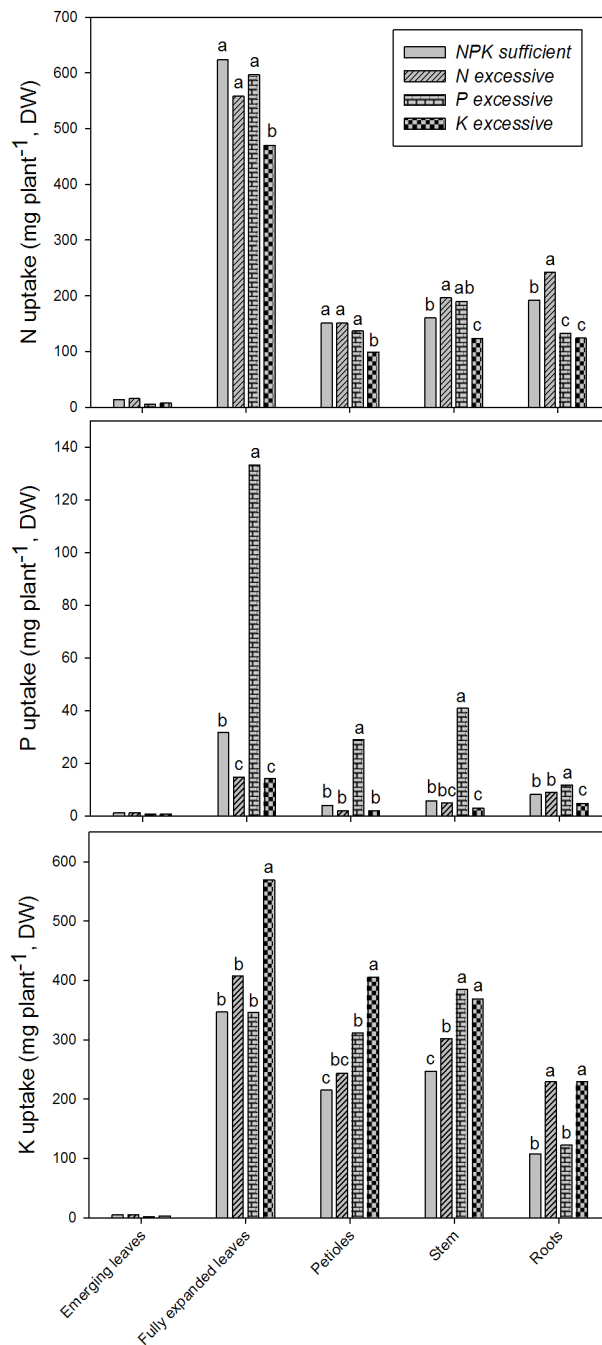


Fig. 5. Uptake of total N, P and K of tomato plants (dry weight-based, DWB) grown under excessive N, P or K condition. Different letter indicates significant differences between treatments according to LSD test ($p < 0.05$, $n=5$).

difference showed in all tissues and treatments. K-excessive condition extremely contributed to a remarkable increase in the ratio, which ranged from 2.79 to 10.34, and particularly potassium was dominantly accumulated in petioles, stem and roots, which was the same response in the distribution of water soluble K. Excessive condition of N or P also influenced on the significant increase in the ratio. Despite the interrelationship between K, Ca and Mg in plant tissue, however, critical values of these elements are usually not seriously affected unless a

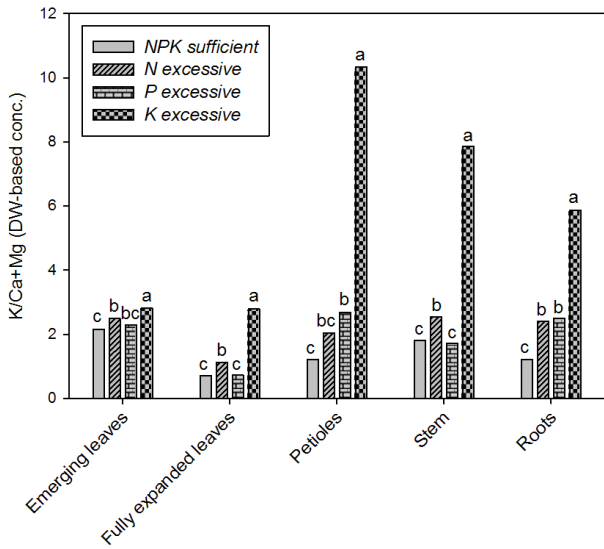


Fig. 6. The ratios of mono- (K) to di-valents (Ca + Mg) of tomato plants (dry weight-based, DWB) grown under excessive N, P or K condition. Different letter indicates significant differences between treatments according to LSD test ($p < 0.05$, $n=5$).

wide ratio exists between them. In our data, a K:Ca+Mg ratio was mostly increased by excessive N, P or K condition although the effect of K was distinct, and an over-absorbed K was predominantly accumulated in petioles, stem and roots which mean that plant minimizes the upward movement of K to avoid a toxicity on the leaves. Plants grown at greater K had a K:Ca ratio of 4 and 10, while K:Mg was 3 and 5 which is coincidence with our results (Egilla et al., 2001; Mills and Jones, 1996). It was suggested from the results that NPK excess leads to an increase in K:Ca+Mg ratio without growth reduction, and the ratio greatly depends on tissues.

Soluble carbohydrates distribution An increase or decrease in soluble carbohydrates was greatly dependent upon both plant tissues and mineral-fed conditions (Fig. 7). The supply of excessive N led to a constant decrease in soluble sugar and starch in all tissues. The excessive P supply resulted in slight decrease in soluble sugar in fully expanded leaves and petioles, whereas led to obvious increase in starch in petioles and roots. The K-excessive condition revealed greater accumulation of soluble sugar in stem and of starch in fully expanded leaves and stem, and there were a slight decrease or no change in other organs. Total content of soluble sugar per gram (FW-based) was the highest in NPK-sufficient condition (154.8 mg g^{-1}) and followed by K-excessive (141.6), N-excessive (129.2) and P-excessive (127.7). On the other hand, total starch content was the highest in K-excessive (167.0 mg g^{-1}) and followed by P-excessive (146.1), NPK-sufficient (138.2) and N-excessive (109.7). It is well defined that an increase in mineral nutrients results in a decrease in soluble carbohydrates, and K directly affects starch biosynthesis (Epstein and Bloom, 2005). Most

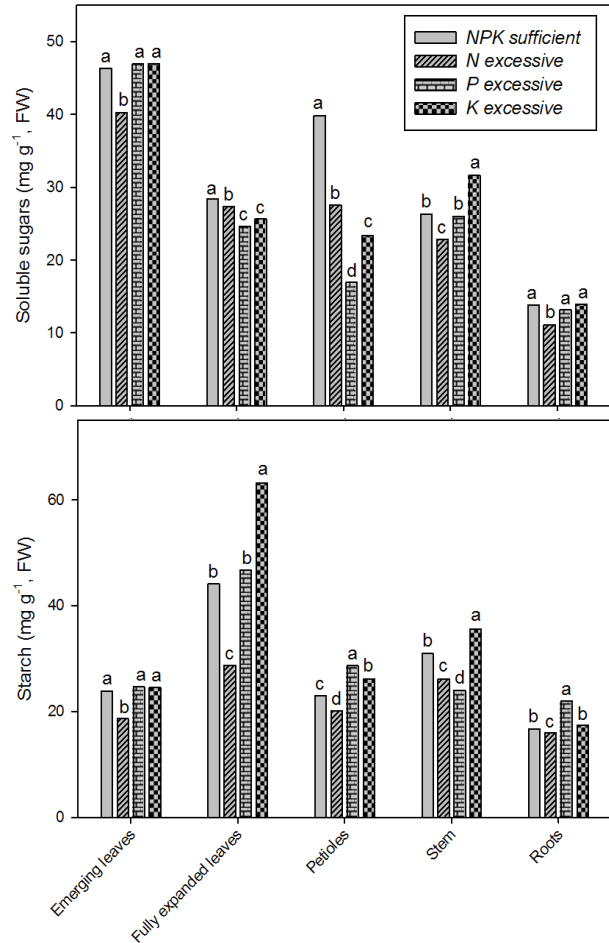


Fig. 7. Total soluble sugar and starch contents expressed as $\mu \text{mol g}^{-1}$ of glucose equivalents in leaves and roots of tomato plants grown under excessive N, P or K condition. Different letter indicates significant differences between treatments according to LSD test ($p < 0.05$, $n=5$).

researches in relation to the relationship between minerals and carbohydrates has been focused on the effects of mineral deficiency on the production and partitioning of soluble carbohydrates (Engels and Marschner, 1995; Fischer and Bremer, 1993; Fredeen et al., 1989; Marschner, 1995; Smith et al., 1990). Certainly, our results showed a constant decrease in soluble sugars under NPK excess conditions, whereas a tendency of starch accumulation was greatly influenced by each mineral which indicated a decrease in N excess, but an increase in K excess. Interestingly, soluble sugar and starch contents in emerging leaves were not affected by mineral excess without N. These results mean that the composition of soluble carbohydrates was strongly influenced by N status because of C:N metabolism, whereas excessive P and K showed little dependent response on carbohydrate metabolism.

Relationship between macro elements (N, P and K) and soluble carbohydrates The production of soluble carbohydrates seemed to be closely related with mineral status in fully expanded leaves of excessive N, P or K-fed tomato plants

Table 1. Pearson's correlation coefficient (n=100) between water soluble (WS) NPK, dry weight (DWB) NPK and soluble carbohydrates in fully expanded leaves of tomato plants grown under excessive N, P or K condition.

	WS-NO ₃ -N	WS-NH ₄ -N	WS-P	WS-K	DWB-N	DWB-P	DWB-K	Soluble sugar
WS-NO ₃ -N	-							
WS-NH ₄ -N	0.85***	-						
WS-P	-0.33	-0.59**	-					
WS-K	0.80***	0.98***	-0.62**	-				
DWB-N	0.59**	0.38	-0.60**	0.41	-			
DWB-P	-0.41	-0.65**	0.99***	-0.69***	-0.61**	-		
DWB-K	0.58**	0.87***	-0.54*	0.89***	0.02	-0.60**	-	
Soluble sugar	-0.08	-0.04	-0.65**	-0.01	0.62**	-0.60**	-0.19	-
Starch	-0.31	0.07	0.04	0.09	-0.76***	0.04	0.50*	-0.43

(Table 1), and the correlation between soluble carbohydrates and mineral elements (N, P and K) in other plant tissues except emerging leaves revealed a similar trend (data not shown). Soluble sugar showed positive correlation with dry weight-based (DWB) total N content ($p < 0.01$) whereas was negatively related with soluble P ($p < 0.01$) and dry weight-based total P ($p < 0.01$). On the other hand, starch production was negatively influenced by total N ($p < 0.001$), however showed positive relation with total K concentration ($p < 0.05$). In addition, our results revealed positive correlation between WS- and DWB-minerals ($p < 0.01$ and $p < 0.001$), and thus it is suggested that NPK status in plants through WS-concentration could be expected. Interestingly, N and K was the synergistic relation each other both WS- and DWB-forms, but antagonistic with P.

Conclusions

The responses of mineral uptake and carbohydrate partitioning to excessive N, P or K conditions were greatly different from those of mineral-deficient condition. The uptake pattern of NPK was substantially different with each mineral, and WS- and DWB-mineral was positively correlated. The influence of mineral excessive conditions on soluble carbohydrates was dependent upon each mineral. The present study provides some fruitful results to better understand the relationship between mineral and carbohydrates under mineral excessive conditions, and it is required to extend the knowledge of physiological-, biochemical- and molecular-responses to mineral excess in major crops, particularly greenhouse crops, to improve mineral acquisition and make more efficient use of minerals.

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