

Influence of Nutrient Supply on Growth, Mineral Nutrients and Carbohydrates in Cucumber (*Cucumis sativus* L.)

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We investigated the growth of cucumber plants, the uptake and use of mineral nutrients, such as NO₃-N, NH₄-N, K⁺, Ca⁺⁺, Mg⁺⁺ and Na⁺, absorbed from media solution, and the synthesis and distribution of soluble sugars under nutrient-deficient condition. Difference in plant growth revealed after 20 days of treatment. Nitrate uptake in nutrient-deficient condition was significantly reduced compared with nutrient-normal treatment, and its distribution was primarily in petioles, stem, roots and less in leaves. In contrast, ammonium content was markedly predominated in fast growing organs, and it was significantly different in growing leaves, expanded leaves, and roots under similar growth conditions. K⁺ lack by deficient nutrient condition, was found in growing leaves. The Ca⁺⁺ content did not show significant difference between treatments and a substantial portion of Ca⁺⁺ remained in petioles. The Mg⁺⁺ content was significantly higher in the leaves of nutrient-normal condition compared with nutrient-deficient condition while significantly lower in stem and roots. The behavior of Na⁺ in plant was similar to K⁺ although its content was relatively little. The highest CO₂ assimilation was observed in fully expanded leaves of nutrient-normal condition, which was 1.7 times higher compared with nutrient-deficient condition. The instantaneous water use efficiency (A/E) and the A/gsratio, which is an index of leaf intrinsic water use efficiency for individual leaves, was 1.2 and 1.1 times higher, respectively. The total soluble sugar (TSS) contents were highest in leaves followed by petioles, stems and roots, and in younger leaves. The growing leaves contained about 7,200 mg kg⁻¹ of TSS in nutrient-normal condition whereas the TSS contents in nutrient-deficient condition were not significantly different between leaves. The Mg⁺⁺ and NH₄-N were positively correlated with the TSS whereas NO₃-N was negatively correlated.

Key words: carbohydrate, cucumber, growth, mineral nutrient, nutrient-deficiency

Introduction

Cucumber is one of the most popular vegetable crops grown commercially around the world, and according to agricultural statistic data (MIFAFF, 2008) its cultivation was sixth among vegetable crops in Korea. The recommended method on fertilization has been reported for constant-feed and for a subirrigation system but these studies have been concerned primarily with the level of

nitrogen fertilization.

A large number of studies have been performed on mineral nutrient deficiency in various crops since the consideration of mineral nutrients in plants. Most of them, however, were focused on the impact of plants under deficient conditions of individual element rather than overall nutrient deficiency. The main symptoms of nutrient deficiency in cucurbitaceous crops are as follows: N, growth reduction and leaf yellowing; P, stunted appearance and slower growth; K, older leaf chlorosis; Ca, spotted or necrotic leaves; Mg, leaf tip chlorosis; S, leaf yellowing; Fe, younger leaf chlorosis; Zn and Mn, interveinal chlorosis; Cu, crinkled leaf; B, shortened

internode and distorted leaf (Shear, 1975; Navarro and Locascio, 1979; Elamin and Wilcox, 1986; Lorenz and Maynard, 1988; Elamin and Wilcox, 1989; Frost and Kretchman, 1989). The nutrient deficiency symptoms mentioned above are definitely dependent on their mobility. In particular, mineral nutrients absorbed through roots are divided into two groups whether, fast and slow, and their fate whether to be a component of macro- or micro-molecule or to behave freely. Many studies were conducted regarding the distribution by plant organs of the contents of dry weight-based mineral nutrients, but the results were not enough to understand the mechanism related to the use of mineral nutrients. In addition, the associated metabolic features (e.g. related to carbohydrate metabolism), which could contribute to the understanding of the physiological background, have rarely been studied.

The objectives of this study are: 1) to investigate the distribution of water soluble mineral nutrients and carbohydrates in plant organs under deficient nutrient supply, 2) to know the correlation between the components analyzed, and 3) to determine whether fresh sample-based analysis can be the method in identifying nutritional disorder.

Materials and Methods

Plant material and growth conditions Cucumber seeds (*Cucumis sativus* cv. Ijoenbackdadagi) were sown in a germination tray filled with perlite supplied with a half strength of Ross nutrient solution ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 2.5 mmol L⁻¹ KNO₃, 2.5 mmol L⁻¹ MgSO₄ · 7H₂O, 1.0 mmol L⁻¹ KH₂PO₄, 0.5 mmol L⁻¹ Fe-EDTA, 0.5 mmol L⁻¹ H₃BO₃, 0.5 mmol L⁻¹ MnCl₂ · 4H₂O, 0.5 mmol L⁻¹ ZnSO₄ · 7H₂O, 0.5 mmol L⁻¹ CuSO₄ · 5H₂O, 0.5 mmol L⁻¹ H₂MoO₄ · 4H₂O, 0.5 mmol L⁻¹). Upon germination, 30 seedlings of uniform size were transferred to each of two plastic containers (120×50×20 cm) with continuously aerated hydroponic system containing same nutrient solution when the seeds were germinated. Plants were constantly grown for 4 weeks in a glass house with average day temperature between 25 and 30 °C and night temperature between 16 and 20 °C. Mid-day photosynthetic photon flux density was 900-1,000 μmol m⁻² s⁻¹. The nutrient solution was replaced every 7 days. After 4 weeks, plants were subjected to nutrient-deficient (one tenth strength of Ross nutrition solution) and -normal

condition (half strength of Ross nutrition solution), respectively, for 20 days. Plants were harvested between 13:00 and 14:00 at 10 and 20 days after treatment, and immediately separated into young leaves, old leaves, petioles, stem and roots. Fresh shoot weight of cucumber seedlings was measured and immediately ground with mortar and pestle with an addition of deionized-distilled water (1 : 5, w/v).

Mineral nutrient analysis Water soluble inorganic ions were extracted from the fresh materials with deionized-distilled water (1 : 5, w/v) at room temperature. Nitrate was reduced to nitrite by hydrazine in alkaline solution with a copper catalyst, after which it is reacted with sulphanilamide and NEDD (N-1-Naphthylethylenediamine di-HCl) to form a pink compound and absorbance was measured at 550 nm. Ammonium was added with salicylate and dichloroisocyanuric acid to produce a blue compound and measured at 660 nm. Nitroprusside was used as a catalyst. Water soluble nitrate and ammonium were analyzed by an Auto-analyzer 3 (BRAN LUBBE, Germany). Water soluble cations (potassium, calcium, magnesium and sodium) diluted with deionized-distilled water were analyzed by ICP-OES (INTEGRA XMP, GBC, Australia).

Photosynthesis determination A portable photosynthesis system (LCpro⁺, ADC, UK), equipped with a square (6.25 cm²) chamber, was used for CO₂ assimilation (*A*), transpiration rate (*E*) and stomatal conductance (*g_s*) measurements. Measurement of growing and upper-fully-expanded leaves was performed between 10:00 and 12:00.

Soluble sugar determination Total soluble sugar was determined according to Roe (1955). Fresh samples were extracted with 95 % EtOH (1 : 10, w/v), evaporated in 95 °C hot water and made up 10 ml with distilled-deionized water. The extract (1 ml) was mixed with 2 ml of fresh 0.2% anthrone in sulfuric acid (w/v) and the mixture was placed in boiling water for 10 min. After cooling on ice, the absorbance was measured at 630 nm (UV-2450, Shimadzu, Japan). Glucose was used as a standard for soluble sugars.

Statistical analysis If two observations that were described in the tables and figures are different, this means

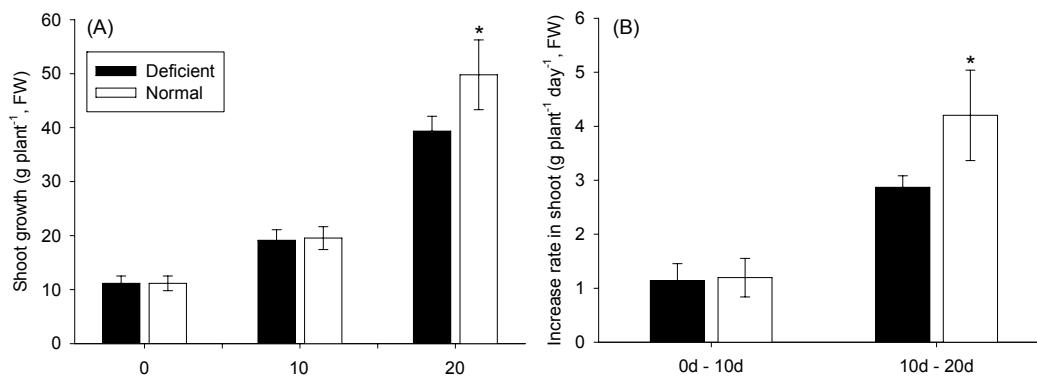


Fig. 1. Shoot growth and growth rate per day of cucumber plants measured at 0, 10 and 20 days after two nutritional treatments. Data represent mean \pm S. D. ($n = 5$).

that they are statistically significant by Student's *t*-tests (SAS, ver. 9.01). The correlation analysis was performed by Pearson's correlation coefficient test (SAS, ver. 9.01).

Results and Discussion

Cucumber growth Deficient nutrient condition slightly retarded the growth of cucumber plants after 20 days of treatment ($p < 0.05$), although there was no significant difference between both treatments by 10 days (Fig. 1A). The increase in growth rate was about 1.2 g (fresh) d⁻¹ by 10 days after the initiation and ranged from 3.0-4.5 g d⁻¹ from 10-20 days (Fig. 1B). Nitrogen and phosphorus were primarily involved in a favorable growth of crop than other nutrients and their influence was greater during long term cultivation (Lenka et al., 2006). It was considered that a cotyledon had a crucial role to supply the nutrients enough to cover early growth and as predicted under less nutrient condition, it did not show any great influence on plant growth.

Contents of mineral nutrients Nitrate uptake in nutrient-deficient condition was significantly reduced compared with nutrient-normal ($p < 0.01$ and 0.001, Fig. 2A). Nitrate in nutrient-normal condition was primarily distributed in petioles, stem and roots and less in leaves. However, it revealed that nitrate uptake of cucumber seedlings under nutrient-deficient condition was greatly reduced in roots. As a result, it appeared that nitrate existed in petioles (over 2,500 mg kg⁻¹ in nutrient-normal; 152 to 1,359 mg kg⁻¹ in nutrient-deficient) and its transport was limited into leaves (99 to 135 mg kg⁻¹ in nutrient-normal; 16 to 18 mg kg⁻¹ in nutrient-deficient) even-though plants suffered nutrient deficiency. In

contrast, ammonium content was markedly predominated in fast growing organs (Fig. 2B), and it was significantly different in growing leaves, expanded leaves and roots under both growing conditions ($p < 0.05$ and 0.01). This means that ammonium absorbed from roots is first

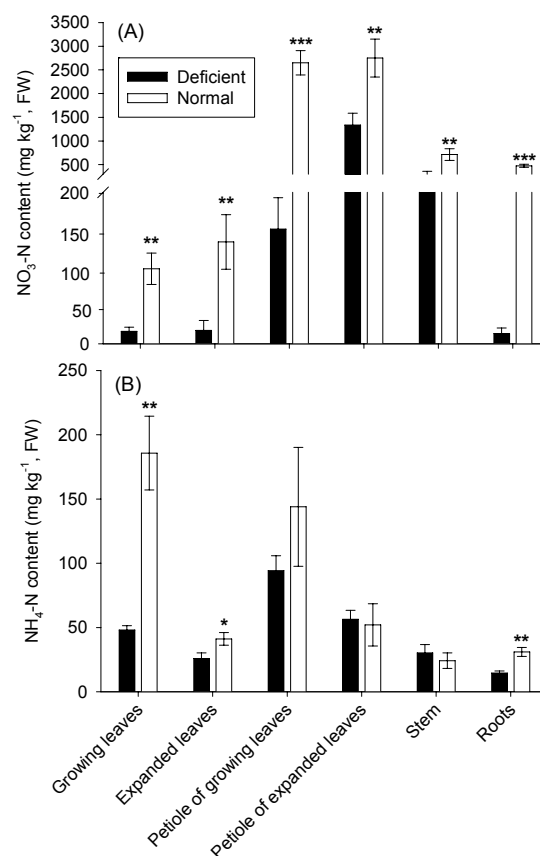


Fig. 2. NO₃-N (upper) and NH₄-N (lower) contents in different plant parts measured at 20 days after two nutritional treatments. Data represent mean \pm S. D. ($n = 5$). Different symbols (*, **, and ***) indicate significant differences ($P < 0.05$, 0.01, and 0.001) between treatments according to Student's *t*-test.

transported into plant parts requiring energy for cell growth where it is utilized. High N supply has a positive effect on the metabolic pathways related to N assimilation (Schible et al., 1997). $\text{NH}_4\text{-N}$ contents were significantly affected by tissue age, in that they were detected greater in younger plant parts (Lenka et al., 2006). In our experiment, it was concluded that the reduction in uptake of nitrate and ammonium from roots, the decreased assimilation rate of nitrate, and lower movement of nitrate into fast growing parts were major limiting factors to cucumber growth.

The distribution of major cations showed a similar tendency in both plant organs and growth conditions (Fig. 3). They were dominantly accumulated in petioles and strongly dependent on their own nature (movement-easy-element, upper-petioles; movement-difficult-element, lower-petioles). The cations, such as K^+ , Mg^{++} and Na^+ , which move readily in plant existed relative higher in fast growing tissues whereas the movement of Ca^{++} was considerably restricted due to its own nature. The K^+ ion showed significant difference between treatments in all organs except for stem ($p < 0.05, 0.01$ and 0.001 , Fig. 3A). In particular, the K^+ lack by deficient nutrient condition was found in growing leaves. Thereafter, it led to reduced leaf growth (data not shown) because of the failure of osmotic regulation such that K^+ is a prerequisite for turgor-pressure-driven solute transport in the xylem and the water balance of plants (Marschner, 1995). The Ca^{++} content did not show significant difference between treatments (Fig. 3B), and a substantial portion of Ca^{++} remained in petioles. Considering Ca^{++} content, it was presumed that an adequate level of free- Ca^{++} (water soluble) in plants was very low whereas most of Ca^{++} supplied from nutrient-normal condition seemed to be immobilized to form Ca-salt. Many plants produce Ca-oxalate as crystalline deposits, which can account for greater than 85 % of the dry weight of plant organs (Horner and Wagner, 1995; Webb, 1999; Nakata, 2003), and large amounts of Ca-oxalate crystals can be formed very rapidly (0.5-1 h Franceschi, 1989) and are associated with specialized subcellular structures, including membranes within the vacuole (Horner and Wagner, 1995). The Mg^{++} content was significantly higher in the leaves of nutrient-normal condition compared with nutrient-deficient ($p < 0.05$, Fig. 3C), while it was significantly lower in stem and roots ($p < 0.05$ and 0.001 , Fig. 3C). Although Mg-absent condition decreased leaf

chlorophyll, other parameters of leaf development and leaf Mg content were not significantly different from the plants with complete nutrients (Yeh et al., 2000). The behavior of Na^+ in plant was similar to K^+ although its content was relatively little (Fig. 3D). Na^+ was mainly allocated in the fast growing parts and it appeared that its transport was restricted as Na^+ supply was limited from the growth media.

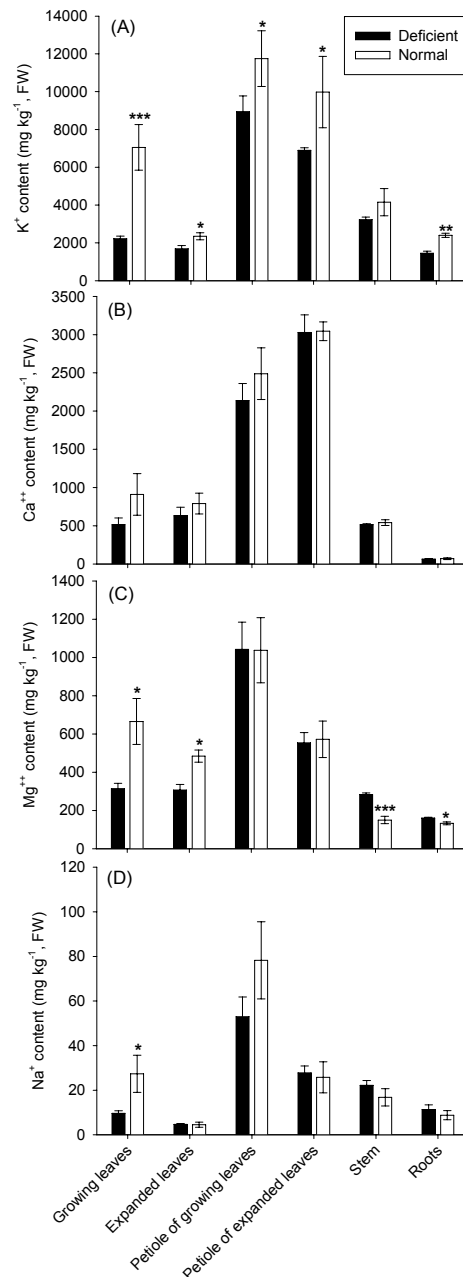


Fig. 3. Cations K^+ , Ca^{++} , Mg^{++} , and Na^+ contents in different plant parts measured at 20 days after two nutritional treatments. Data represent mean \pm S. D. ($n = 5$). Different symbols (*, **, and ***) indicate significant differences ($P < 0.05, 0.01$, and 0.001) between treatments according to Student's t -test.

Table 1. Transpiration rate (E), stomatal conductance (g_s), CO₂ assimilation (A), instantaneous water use efficiency (A/E) and intrinsic water use efficiency (A/g_s) on day 20 under different nutrient conditions.

	Growing leaves		T-test	Fully-expanded leaves		T-test
	Normal	Deficient		Normal	Deficient	
Intercellular CO ₂ ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$)	323 \pm 5	318 \pm 10	ns	302 \pm 26	319 \pm 8	ns
E ($\text{mmol H}_2\text{O s}^{-1}$)	5.91 \pm 0.17	4.97 \pm 0.41	*	5.89 \pm 0.09	4.89 \pm 0.35	**
g _s ($\text{mmol H}_2\text{O s}^{-1}$)	0.33 \pm 0.02	0.26 \pm 0.0.	*	0.32 \pm 0.02	0.24 \pm 0.04	*
A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	9.03 \pm 0.59	8.10 \pm 0.75	ns	12.85 \pm 3.00	7.51 \pm 1.16	*
A/E ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)	1.53 \pm 0.13	1.64 \pm 0.19	ns	2.18 \pm 0.49	1.53 \pm 0.18	ns
A/g _s ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)	27.68 \pm 2.36	31.52 \pm 4.75	ns	40.51 \pm 8.89	31.40 \pm 3.96	ns

Data represent mean \pm S. D. (n = 5). Different symbols (*, **) indicate significant differences ($P < 0.05$, 0.01) between treatments according to Student's t-test.

Photosynthesis and carbohydrate synthesis Cucumber plants exposed to nutrient-deficient condition exhibited a significant decrease in photosynthesis as compared to nutrient-normal plants (Table 1). Intercellular CO₂ concentration was not significant between growth conditions, whereas transpiration, stomatal conductance and photosynthesis responded differently ($p < 0.05$ and 0.01). Transpiration rates and stomatal conductance in nutrient-normal condition were 1.2 and 1.3 times higher in comparison to nutrient-deficient, respectively. The highest CO₂ assimilation was observed in fully expanded leaves of nutrient-normal condition, which was 1.7 times higher compared with nutrient-deficient condition. The instantaneous water use efficiency (A/E) and the A/g_s ratio, which is an index of leaf intrinsic water use efficiency for individual leaves, was 1.2 and 1.1 times higher, respectively. Reduction of CO₂ assimilation rate can be partly related to stomatal closure (Pankovic et al., 1999; Issa et al., 2001), and lower transpiration rate was possibly related to a transiently low vapor pressure deficit, since transpiration rate is primarily a function of vapor pressure gradient and leaf stomatal conductance (Alexander et al., 1995). In contrast of other results (Moustakas et al., 1997; Syros et al., 2004), the reduction of water use efficiency (WUE), as determined by the A/E and the A/g_s, was not significantly different in this experiment, although the values was slightly higher in nutrient-normal condition.

The contents of total soluble sugar (TSS) were highest in leaves, followed by petioles, stems and roots, and also in younger than in older leaves (Fig. 4). The amount of TSS revealed significant differences in the leaves and petioles in response to nutrient supply ($p < 0.01$ and 0.001).

The growing leaves, which require substantial photosynthates for fast cell growth, contained about 7,200 mg kg⁻¹ (1.6 times higher than fully expanded leaves) of TSS in nutrient-normal condition whereas the TSS contents in nutrient-deficient condition were not significantly different between leaves. Also, the TSS was highly accumulated in petioles, and the nutrient-deficient condition affected adversely the accumulation process. TSS contents in stem and roots remained similarly in both conditions. The soluble carbohydrates were led toward fast growing tissues (Lenka et al., 2006), and a slower transport of carbohydrates from shoot to roots was documented in nutrient-rich (Kohl et al., 1998) and

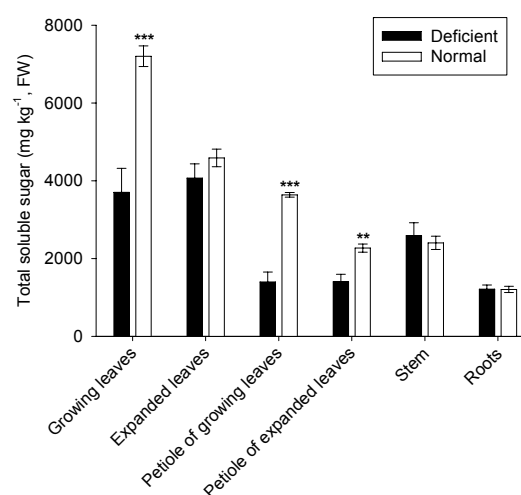


Fig. 4. Total soluble sugar contents in different plant parts measured at 20 days after two nutritional treatments. Data represent mean \pm S. D. (n = 5). Different symbols (*, **, and *) indicate significant differences ($P < 0.05$, 0.01, and 0.001) between treatments according to Student's t-test.**

Table 2. Correlation analysis between mineral nutrients and TSS.

	K	Ca	Mg	Na	NO ₃ -N	NH ₄ -N	TSS
K	-						
Ca	0.79***	-					
Mg	0.80***	0.76***	-				
Na	0.93***	0.62***	0.69***	-			
NO ₃ -N	0.71***	0.77***	0.45**	0.52**	-		
NH ₄ -N	0.68***	0.38*	0.76***	0.67***	0.24	-	
TSS	0.02	-0.14	0.35*	-0.04	-0.16	0.59***	-

nutrient-poor (Cizkova et al., 1996) conditions. These findings were in accordance with the carbohydrate metabolism found in our experiment.

Interaction between mineral nutrients and carbohydrate The correlation between mineral nutrients and TSS was analyzed (Table 2). All mineral nutrients analyzed had positive correlation ($p < 0.5, 0.01$ and 0.001). The Mg⁺⁺ and NH₄ - N were positively correlated with the TSS whereas NO₃ - N was negatively correlated ($p < 0.05$ and 0.001 , Fig. 5). Nitrogen (total N) content was positively involved in photosynthetic proteins and

carbohydrate concentration (Paul and Stitt, 1993). Our study demonstrated that NH₄- N content, among total N, had a positive influence on carbohydrate metabolism. In conclusion, the supply of deficient mineral nutrients resulted in an unfavorable plant growth, nutrient uptake and carbohydrate metabolism. Mineral nutrients and carbohydrates revealed the tendency to be concentrated toward fast growing parts, and carbohydrate metabolism was closely involved in the Mg⁺⁺ and NH₄ - N contents. Therefore, we suggest that fresh sample-based analysis can be done to determine nutritional disorder of plants.

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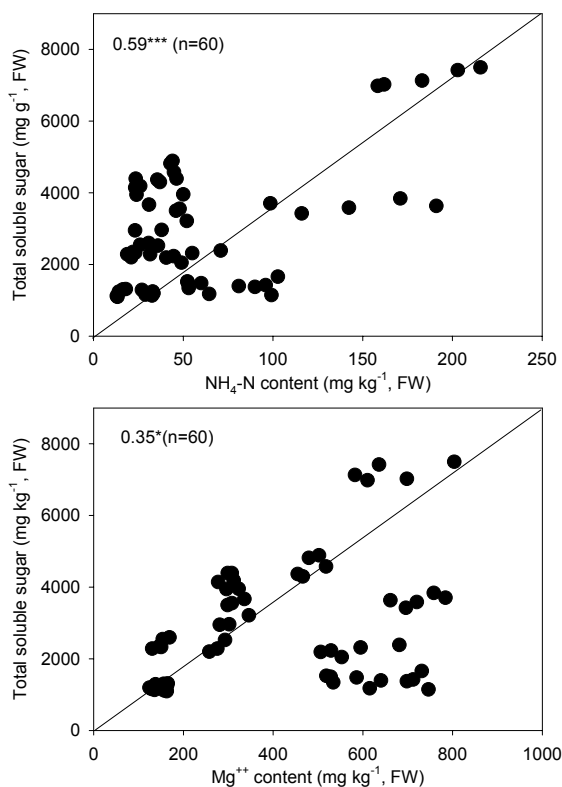


Fig. 5. Correlation analysis between total soluble sugar and ammonium or magnesium. Each dot represents six plant parts five replications two nutritional treatments (n = 60).

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무기영양액 농도 차이가 오이 생육, 무기성분 흡수 및 탄수화물 합성에 미치는 영향

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오이의 부위별 생체분석을 통하여 영양진단 가능성을 검토코자, 무기영양액의 양분농도가 결핍된 조건(1/10 Ross 용액) 하에서 오이의 생육, 무기성분의 흡수 및 탄수화물 합성에 관하여 조사하였다. 양분결핍에 의한 오이 생육저해는 처리 후 20일에 나타났다. 양분결핍조건에서 질산태 질소의 흡수는 정상조건에 비해 크게 감소하였으며, 질산태 질소함량은 잎보다 엽병과 줄기에서 높았다. 반면, 암모늄태 질소함량은 생장엽병 및 생장엽에서 뿌리보다 높았다. 양분결핍에 의한 칼리 부족은 생장엽에서 나타났다. 칼슘은 처리간에 유의적인 차이를 보이지 않았으며, 주로 엽병에 분포하였다. 마그네슘은 줄기나 뿌리보다는 잎에 다량 존재하였으며, 나트륨은 칼리와 유사한 경향을 보였으나 함량은 매우 낮았다. 이산화탄소 동화율은 정상 양분조건의 완전생장엽에서 가장 높았으며, 양분결핍조건과 비교할 때 약 1.7배 컸다. 수분이용효율은 처리간에 유의적인 차이는 보이지 않았으나, 정상 양분조건에서 약간 높았다. 수용성 당 함량은 잎에서 가장 높았고, 엽병, 줄기 및 뿌리의 순으로 나타났으며, 오래된 조직보다는 어린 조직에서 높았다. 정상 양분조건의 생장엽에서 측정된 수용성 당 함량은 약 7,200 mg kg⁻¹ 이었다. 무기성분과 수용성 당과의 상관관계를 알아본 결과, 마그네슘과 암모늄태 질소가 수용성 당과 고도의 정의 상관관계를 보였다.