Contents of Mineral Elements and Cytokinins in Xylem Sap of Two Oriental Melon Cultivars Affected by Rootstocks

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ABSTRACT Contents of mineral element and cytokinin in the xylem sap of 'Keumdongee' and 'Tongilhwang' oriental melons were compared with those in oriental melons grafted onto 8 rootstocks. The effect of grafting on the fruit quality of oriental melon was also investigated. Flesh firmness varied with rootstocks. Soluble solids contents in the placenta tissue of grafted 'Tongilhwang' were higher than that in the 'Keumdongee'. Electric conductivity of the xylem sap in own-rooted plants was higher in 'Keumdongee' than in 'Tongilhwang', but it increased in 'Tongilhwang' once they were grafted. The sap volume per plant was greater in 'Keumdongee' than in 'Tongilhwang'. The mineral concentrations varied considerably depending on the rootstock used. Xylem sap of grafted oriental melons contained a higher amount of mineral ions, especially NO_3^- and PO_4^-, than did the sap in own-rooted plants. The increase in the mineral levels in sap due to grafting was most apparent in 'Tongilhwang'. Xylem sap from both 'Keumdongee' and 'Tongilhwang' contained *trans*-zeatin (*t*-Z), *trans*-zeatin riboside (*t*-ZR), and dihydrozeatin riboside (DHZR). Small amounts of isopentenyl adenine (IPA) and isopentenyl adenine riboside (IPAR) were also detected. *Trans*-zeatin riboside was the most abundant, followed by *t*-Z. Cytokinin concentration in 'Keumdongee' was not significantly influenced by rootstock type used, although the highest concentration of cytokinins in 'Keumdongee' was obtained with 'Chamtozwa' rootstock. However, the cytokinin concentration in 'Tongilhwang' increased with grafting irrespective of rootstock type used.

Additional key words: electric conductivity, grafting, immunoaffinity column, interspecific hybrid squash

Introduction

Oriental melon is commonly grafted to cucurbitaceous plants which are resistant to soil-born diseases or nematodes, and are tolerant to adverse environmental conditions such as low temperature (Lee, 1994). Commonly it is grafted to interspecific hybrid squash (*Cucurbita maxima* × *C. moschata*) or pumpkin (*Cucurbita moschata*), whereas gourd and squash have been used as rootstocks for watermelon. However, there are some problems such as low sugar content in fruit, large numbers of fruits with internal breakdown, and excessive shoot growth when squash or pumpkin are used as rootstocks. The deterioration of fruit quality in grafted plants has been associated with the differential uptake of mineral nutrients by the rootstocks (Kato and Lou, 1989; Masuda et. al., 1986; Yamasaki et. al., 1994).

Concerning root function, xylem sap has been analyzed to assess the ability of the root system to absorb and transport nutrients to shoots (Clarkson, 1991; Widders and Lorens, 1982). Another important function of roots is the synthesis of plant hormones such as gibberellins, cytokinins, and abscisic acid (Itrai and Birnbaum, 1991). It is well known that cytokinins are principally synthesized in the roots and transported to the shoots through the xylem (Letham, 1994). Since various environmental factors such as light or rhizosphere condition induce noticeable changes in the level of cytokinin in xylem sap (Borisova et al., 1990; Kuraishi et al., 1991; Segreeva et al., 1990), this hormonal fluctuation is considered to reflect the physiological condition of the roots.

Despite the importance of the grafted seedlings in vegetable cultivation, there are few studies of the movement of hormones and minerals. The purpose of this research therefore was to determine the mineral composition and the levels of endogenous cytokinins in the xylem sap of own-rooted and grafted oriental melons, and to provide some basic information on grafting and the relationship between scions and rootstocks.

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Materials and Methods

Growing the Plants

Oriental melons of 'Keumdongee' and 'Tongilhwang' were used as scions and 7 squash cultivars and one melon cultivar were used as rootstocks. Scions were sown on February 2nd, 1998 and grafted onto squashes or melon on February 13th by tongue-approach grafting (Lee, 1994). On March 13th, both grafted and own-rooted plants were transplanted and cultivated in soil in a plastic house. Plants were pinched at the 8th node and four lateral shoots were allowed to grow as main vines. Two fruits were allowed to set and grow on each vine. Mature fruits were harvested on May 16th, 64 days after transplanting, and their characteristics were examined. The experimental design was a completely randomized design with three replications and 5 plants per each replication. The measurement data was analyzed using a SAS program.

Collection and Mineral Analysis of Xylem Sap

Xylem sap was collected 2 weeks after fruit harvest on May 30th (78 days after transplanting). Own-rooted plants were cut off at a position 25 cm above the ground, and grafted plants were cut off 20 cm above the position of the grafting. The few drops of bleeding sap from remaining stem base were discarded and the cut surface was washed with distilled water for 10 minutes. Xylem sap subsequently bled at the cut surface was collected in a flask precoated with silica and wrapped with aluminum foil. After a 12-hour period of collection, from 6:00 PM to 6:00 AM, the volume of xylem sap was recorded and the sap was stored at -20 ℃ for analysis. The mineral composition of the sap was analyzed using ion chromatography (DX-500, Dionex) after dilution 50 times with distilled water.

Quantification of Endogenous Cytokinins in Xylem Sap

The xylem sap was initially purified using XAD-2 Amberlite column to exclude mineral nutrients, and the elute was passed through immunoaffinity column (Lee et al., 1999). The immunoaffinity column was prepared with rabbit poly-

Table 1. Effect of different rootstocks on fruit growth characteristics of 'Keumdongee' and 'Tongilhwang' oriental melons[z].

Cultivar /Rootstock	Scientific name	Flesh firmness (kg · cm^{-2})	Fruit length (cm)	Fruit diameter (cm)	Width of placenta (cm)	Size of flower scar (mm)	Soluble solids content (%)	
							Placenta	Flesh
Keumdongee oriental melon								
Own-rooted	*Cucumis melo var. makuwa*	2.02 bc	12.3 ns	7.83 ns	4.00 b	3.67 ab	12.57 ns	7.43 ns
/UR-1	*C.*[y] *moschata* × *C. moschata*	2.08 abc	13.4	8.00	4.83 a	3.67 ab	11.43	10.00
/No. 2	*C. moschata* × *C. moschata*	1.99 c	13.1	7.53	4.17 ab	5.00 a	10.93	9.13
/No. 8	*C. moschata* × *C. moschata*	1.93 c	14.0	7.80	3.93 b	4.33 ab	11.50	9.67
/Chintozwa	*C. moschata* × *C. moschata*	2.28 ab	13.1	7.67	4.20 ab	3.67 ab	11.30	8.53
/Chinkyo	*C. moschata* × *C. moschata*	2.29 ab	13.1	7.93	4.30 ab	3.00 b	11.70	9.07
/Sintozwa	*C. maxima* × *C. moschata*	2.14 abc	12.5	7.67	4.10 ab	3.67 ab	11.70	8.73
/Chamtozwa	*C. maxima* × *C. moschata*	2.09 abc	12.7	7.67	4.07 ab	4.33 ab	11.67	9.40
/Melon	*Cucumis melo*	2.33 a	12.7	7.77	4.10 ab	3.33 ab	11.80	9.33
Mean		2.14	13.0	7.80	4.16	3.82	11.62	9.03
Tongilhwang oriental melon								
Own-rooted	*Cucumis melo var. makuwa*	2.63 b	14.7 a	8.80 ab	4.03 ns	4.33 ns	12.27 b	8.40 ns
/UR-1	*C.*[y] *moschata* × *C. moschata*	2.76 b	13.7 ab	8.00 bc	4.30	4.33	14.80 a	9.13
/No. 2	*C. moschata* × *C. moschata*	2.87 b	13.4 ab	7.90 c	4.13	3.33	14.47 a	8.53
/No. 8	*C. moschata* × *C. moschata*	2.87 b	14.1 ab	8.33 abc	4.07	4.00	14.50 a	8.87
/Chintozwa	*C. moschata* × *C. moschata*	3.15 a	13.0 b	7.93 bc	4.30	3.33	14.53 a	10.20
/Chinkyo	*C. moschata* × *C. moschata*	3.17 a	13.3 b	7.93 bc	4.03	3.17	13.33 ab	8.33
/Sintozwa	*C. maxima* × *C. moschata*	2.75 b	13.6 ab	8.37 abc	4.20	4.00	14.87 a	8.27
/Chamtozwa	*C. maxima* × *C. moschata*	2.85 b	14.4 ab	8.80 ab	4.27	4.67	15.13 a	9.00
/Melon	*Cucumis melo*	2.83 b	14.2 ab	9.00 a	4.17	4.00	13.60 ab	8.37
Mean		2.88	13.8	8.34	4.17	3.91	14.17	8.79

[z]Mean separation within columns within a cultivar by DMRT at 5%.
[y]*Cucurbita.*

clonal antibodies to keyhole limphet hemocyanin (KLH)-cytokinin conjugates. Each polyclonal antibody was collected, titer determined and, after protein A column purification, coupled to an agarose ester support (Affi-Gel 10, BioRad) according to the procedure of Davis et al. (1986). Separate conjugations were performed for each antibody and column capacities were determined using authentic standards. The antibody support conjugates were then mixed proportionally to provide equivalent capacity for each type of antibody. Column bed volumes were approximately 0.5 mL. The elute from the immunoaffinity column was dried. After resuspension in 0.5 mL of 0.1 M acetic acid, the sample was analyzed and quantified through high performance liquid chromatography (HPLC; Waters 616 System) using Dedosil ODS-5 column (Lee et al., 1999).

Results and Discussion

Characteristics of Fruit and Bleeding Xylem Sap as Influenced by Rootstock

The fruit growth characteristics of two oriental melon cultivars are shown in Table 1. The type of rootstock used hardly influenced fruit characteristics in 'Keumdongee' oriental melon. In 'Tongilhwang', however, greater flesh firmness was obtained by using 'Chintozwa' or 'Chinkyo' rootstocks than by using any other rootstocks, or than was obtained for fruits from own-rooted plants. Soluble solids content was higher in fruits from grafted plants than in those from own-rooted plants. Shoot fresh weight differed considerably depending upon the cultivars and rootstocks.

However, such differences usually did not have any significance since all the plants were grown with frequent pruning and pinching to secure the uniform growth of fruits. The xylem sap yield was higher in 'Keumdongee' than in 'Tongilhwang'. Own-rooted plants of both cultivars tended to exude greater amounts of xylem sap

Table 2. Amount and electric conductivity of bleeding xlyem sap from 'Keumdongee' and 'Tongilhwang' oriental melon grafted onto different rootstocks[z].

Cultivar /Rootstock	Shoot fresh wt (g)	Sap per plant (mL/12-hr)	Sap per 100g FW (mL/12-hr)	Sap EC (mS · cm^{-1})	Sap pH
Keumdongee oriental melon					
Own-rooted	464 bcd	111.5 a	24.1 ab	2.04 cd	5.46 b
/UR-1	370 c	88.0 ab	25.3 a	3.01 a	5.40 b
/No. 2	386 cd	71.7 abc	18.7 abc	2.47 b	5.49 b
/No. 8	458 bcd	57.4 bc	11.8 bcde	2.41 bc	5.51 b
/Chintozwa	632 a	41.5 cd	6.4 de	2.68 ab	5.44 b
/Chinkyo	498 b	64.5 bc	13.0 bcde	2.95 a	5.64 b
/Sintozwa	476 bc	78.8 abc	17.5 abcd	1.90 d	5.67 b
/Chamtozwa	473 bc	13.3 d	2.8 e	2.50 b	5.93 a
/Melon	456 bcd	45.0 cd	9.8 cde	2.36 bc	5.47 b
Mean	468.5	57.5	13.2	2.54	5.57
Tongilhwang oriental melon					
Own-rooted	272 d	43.2 b	16.2 a	1.47 c	5.59 b
/UR-1	694 a	65.6 a	9.7 bcd	4.22 a	5.50 b
/No. 2	510 bc	42.0 b	8.2 bcd	4.22 a	5.34 b
/No. 8	608 ab	38.7 bc	4.9 cd	2.71 b	5.63 b
/Chintozwa	340 cd	43.5 b	11.0 ab	2.82 b	5.64 b
/Chinkyo	340 cd	37.0 bc	10.9 abc	3.13 b	5.39 b
/Sintozwa	621 ab	18.8 c	3.3 d	3.01 b	5.72 b
/Chamtozwa	612 ab	34.0 bc	6.0 bcd	3.33 b	5.47 b
/Melon	283 d	49.5 ab	16.1 a	2.08 c	6.13 a
Mean	500.9	41.1	8.8	3.19	5.60

[z]Mean separation within columns within a cultivar by DMRT at 5%.

than grafted plants (Table 2). The average electric conductivity (EC) value of sap from 'Tongilhwang' was higher than that in sap from 'Keumdongee'. Grafting resulted in higher EC, although the EC value varied with scions and/or rootstocks. Sap acidity (pH) ranged from 5.23 to 6.13, and was not much influenced by the type of scion cultivars or rootstocks used.

Mineral Contents in Xylem Sap

Total mineral content in xylem sap was higher in grafted plants than in own-rooted plants. Increases in total mineral content due to grafting have been mainly attributed to increases in NO_3^- and PO_4^- concentrations as reported by Kim and Lee (1998). In scion cultivars, mineral content in 'Keumdongee' fluctuated considerably according to the rootstock, while in 'Tongilhwang' it increased due to grafting regardless of the type of rootstock used. The highest mineral levels in 'Keumdongee' were found in plants grafted onto 'Chintozwa' and 'Chamtozwa' rootstocks, and with 'Chintozwa' and 'Sintozwa' rootstocks in 'Tongilhwang' (Table 3). These results indicate that the pattern of mineral absorption in 'Keumdongee' was markedly influenced by the type of rootstock used, whereas in 'Tongilhwang' it was affected by grafting irrespective of rootstock cultivar. Grafting in 'Tongilhwang' also influenced absorption of cations such as K^+, Mg^{2+}, and Ca^{2+} in most rootstocks. Similar results have been reported with other crops grafted onto different rootstocks (Lee et al., 1999; Masuda, 1989; Masuda and Gomi, 1982). These results also suggest that effects of grafting on mineral content and mineral composition could be markedly modified depending upon the type of scion or rootstock cultivars used or their interaction with each other.

Cytokinins in Xylem Sap

Total cytokinin content in xylem sap is shown in Fig. 1 and in Table 4. With respect to scion cultivars, the average total cytokinin contents was 4.5 times higher in 'Tongilhwang' (37.66 ng) than that in 'Keumdongee' (8.22 ng). Concentrations of cytokinins varied considerably with rootstocks. For 'Keumdongee', cytokinin level in grafted plants was similar to that found in own-rooted plants, except for plants grafted onto 'Chamtozwa' rootstock, in which the level was the highest. In 'Tongilhwang', the levels of cytokinins in xylem sap increased as a result of grafting, especially when UR-1, No. 2, and 'Chamtozwa' rootstocks were used for the grafting. Five different cytokinins were identified in the xylem sap from oriental melons. *Trans*-zeatin riboside (*t*-ZR) was the most abundant, followed by *trans*-zeatin (*t*-Z) and dihydrozeatin riboside (DHZR). Isopentenyl adenine (IPA) and isopentenyl adenine riboside (IPAR) were also detected (Fig. 2). The cytokinin con-

Table 3. Mineral ion composition in bleeding sap of 'Keumdongee' and 'Tongilhwang' oriental melon as influenced by rootstocks[z].

Cultivar /Rootstocks	Mineral ion concentration ($mg \cdot L^{-1}$)							
	Total	Cations			Anions			
		K^+	Mg^{2+}	Ca^{2+}	NO_3^-	PO_4^-	SO_4^{2-}	Cl^-
Keumdongee oriental melon								
Own-rooded	1330.9	81.8 abc	36.3 c	240.5 de	288.0 fg	559.6 d	48.8 d	42.7 c
/UR-1	2889.8	165.6 a	72.6 a	335.2 ab	752.3 cd	1532.1 a	63.9 d	76.1 c
/No. 2	2213.8	111.5 abc	46.0 bc	236.6 de	543.7 de	1157.8 b	61.3 d	56.8 c
/No. 8	1730.1	53.9 bc	33.1 c	184.1 e	494.2 de	826.0 c	62.5 d	66.4 c
/Chintozwa	3517.5	147.0 a	71.1 a	382.8 a	962.8 b	1723.1 a	112.9 c	113.5 b
/Chinkyo	3219.4	150.0 a	80.2 a	340.9 ab	1253.2 a	1169.1 b	42.7 de	178.2 a
/Sintozwa	1175.9	35.8 c	30.2 c	313.8 bc	167.3 g	550.9 d	37.0 e	30.6 c
/Chamtozwa	3314.2	145.5 a	77.6 a	359.6 ab	822.1 bc	1573.4 a	133.1 b	195.3 a
/Melon	2396.1	132.1 ab	67.2 ab	277.2 cd	392.3 ef	1267.2 b	178.7 a	65.3 c
Mean	2557.1	117.7	59.7	303.8	673.5	1224.9	86.5	97.8
Tongilhwang oriental melon								
Own-rooted	1334.5	103.3 cd	13.2 e	86.4 d	365.0 d	639.4 b	45.3 b	67.9 c
/UR-1	2625.4	156.3 bcd	62.3 d	308.9 bc	490.8 d	1468.5 a	65.1 b	73.5 c
/No. 2	2881.9	163.7 bc	67.0 cd	348.1 b	851.4 ab	1309.8 a	50.5 b	91.5 c
/No. 8	2749.2	192.0 b	100.5 ab	400.5 b	768.4 bc	1104.4 a	56.4 b	127.0 a
/Chintozwa	3036.0	212.4 ab	81.2 bc	325.0 b	884.6 ab	1298.1 a	71.4 b	160.9 ab
/Chinkyo	2331.8	70.5 d	84.5 bc	332.6 b	445.9 d	1231.1 a	78.1 b	82.5 c
/Sintozwa	3377.9	280.0 a	108.1 a	505.4 a	1015.1 a	1158.9 a	111.0 a	196.3 a
/Chamtozwa	2784.2	84.8 cd	92.2 ab	494.4 a	714.6 abc	1110.5 a	116.1 a	126.9 b
/Melon	2329.4	172.2 bc	20.8 e	229.6 c	582.8 cd	1094.6 a	115.2 a	100.8 c
Mean	2764.5	166.5	77.1	368.1	719.2	1222.0	83.0	119.9

[z]Mean separation within columns by DMRT at 5%.

Table 4. Cytokinin concentrations in xylem sap of 'Keumdongee' and 'Tongilhwang' oriental melon as influenced by rootstocks[z].

Cultivar /Rootstock	Endogenous cytokinin concentration ($ng \cdot mL^{-1}$ sap)				
	Trans-Z	*Trans*-ZR	DHZR	IPA	IPAR
Keumdongee oriental melon					
Own-rooted	0.27±0.02[y]	6.85±0.89	2.03±0.79	0.37±0.04	-
/UR-1	0.64±0.23	8.73±1.53	T	T	T
/No. 2	0.36±0.09	7.84±0.58	T	0.37±0.05	-
/No. 8	0.62±0.23	7.62±1.91	-	T	-
/Chintozwa	0.22±0.03	8.50±1.68	0.82±0.15	-	-
/Chinkyo	0.48±0.02	6.33±1.89	-	-	-
/Sintozwa	2.53±1.36	8.24±1.60	T	T	-
/Chamtozwa	6.49±1.93	10.18±2.18	-	-	-
/Melon	0.46±0.04	9.00±1.52	0.74±0.10	T	T
Mean	1.32	8.14	0.52	0.23	
Tongilhwang oriental melon					
Own-rooted	0.83± 0.06	11.45±0.82	6.54±1.06	0.79±0.07	0.78±0.09
/UR-1	2.07± 0.76	52.73±1.70	1.63±0.23	0.65±0.03	0.52±0.05
/No. 2	0.59± 0.05	56.31±2.40	1.43±0.04	1.11±0.09	0.62±0.04
/No. 8	0.73± 0.34	19.70±4.16	1.72±1.12	0	0
/Chintozwa	0.93± 0.46	34.78±3.06	1.55±0.61	T	T
/Chinkyo	0.51± 0.01	41.82±3.02	1.09±0.20	T	0.68±0.03
/Sintozwa	1.60± 0.20	27.32±2.99	T	1.20±0.10	1.57±0.09
/Chamtozwa	1.47± 0.11	50.90±2.14	1.52±0.20	1.13±0.13	0.95±0.13
/Melon	1.61± 0.24	17.87±1.38	6.16±0.64	1.26±0.17	1.12±0.21
Mean	1.15	35.23	2.54	0.77	0.83

[z]Z:zeatin; DHZR:dihydrozeatin riboside ; IPA:isopentenyl adenine ; ZR:zeatin riboside, respectively.
[y]Mean of 3 replicates±standard error.
T : Only trace amount was detected.

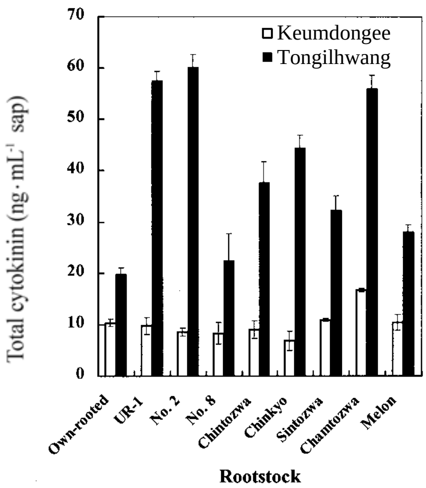

Fig. 1. Cytokinin contents in xylem sap of 'Keumdongee' and 'Tongilhwang' oriental melon as influenced by rootstocks.

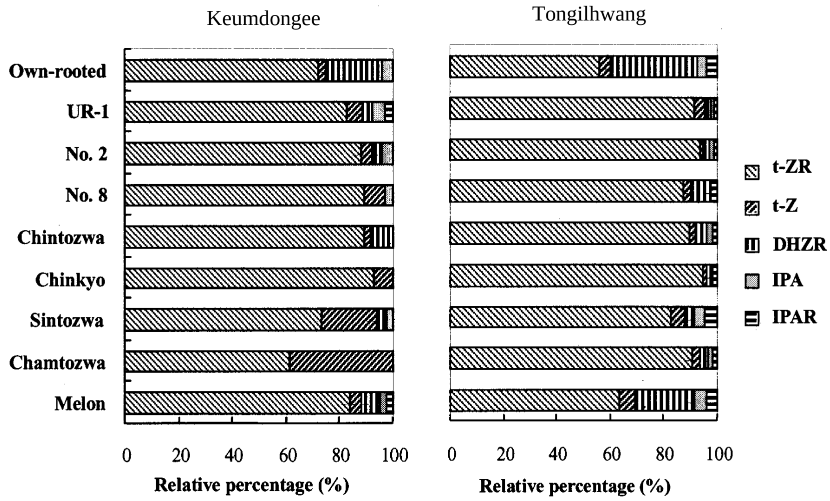

Fig. 2. Cytokinin compositions in xylem sap of 'Keumdongee' and 'Tongilhwang' oriental melon as influenced by rootstock.

centration per ml of sap varied depending upon scions and rootstocks (Table 4). The concentration of *t*-Z was the highest in the sap from 'Chamtozwa' rootstock in 'Keumdongee' and 'UR-1' rootstock in 'Tongilhwang', respectively (Table 4 and Fig. 2). The amount of DHZR was higher in both types of own-rooted plants as well as in 'Tongilhwang' grafted onto 'Melon', as compared to amounts in other rootstocks. IPAR rarely reached detectable level in 'Keumdongee', but was definitely identified in 'Tongilhwang' (Fig. 2 and Table 4). These results suggest that cytokinin contents in xylem sap could indeed be affected by the characteristics of scion cultivars as well as of different rootstocks. In addition, 'Keumdongee' grafted onto 'Chamtozwa' produced both the largest total number of fruits and the highest number of marketable ones (data not shown). Also the 'Chamtozwa' rootstock had a highest level of total cytokinin content (Fig. 1). These results suggest that cytokinin concentration in xylem sap may correlate with the fruit set or fruit yield as also suggested by Ogawa et al. (1990) and Yamasaki et al. (1994). But the involvement of cytokinin in xylem sap in the fruit production of scion is still unclear, and the relationship between fruit yield and cytokinin contents in ascending xylem sap requires further detailed investigation.

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참외품종과 대목종류에 따른 목부액내의 무기성분 및 시토키닌 함량

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초 록

8가지의 대목종류에 접목 또는 무접목한 '금동이' 참외와 '통일황' 참외의 목부액내 무기성분 및 시토키닌류 함량을 분석하여 비교하였으며, 이와 더불어 과실특성을 조사하였다. 과실의 경도는 대목 또는 접수에 따라서 차이를 보였으며, 과실의 태좌부의 당도는 '통일황'에서 높았다. 목부액의 전기전도도(EC)는 무접목묘에서는 '금동이'에서, 접목묘에서는 '통일황'에서 높았으며, 개체당 목부액의 분비량은 '금동이'에서 많았다.

목부액내의 무기성분함량은 접수 및 대목의 종류에 따라 차이를 보였고, 접목묘에서 무접목묘보다 높았는데 특히 NO_3^-와 PO_4^-의 함량에서 증가를 보였다. 이러한 접목에 의한 무기성분 함량의 증가 효과는 '통일황'에서 뚜렷하였다.

목부액내의 시토키닌류는 *trans*-zeatin(*t*-Z), *trans*-zeatin riboside(*t*-ZR), dihydrozeatin riboside(DHZR)와 소량의 isopentenyl adenine(IPA) 및 isopentenyl adenine riboside (IPAR)가 검출되었으며, 목부액내의 주요 시토키닌은 *t*-ZR로 가장 높은 비율을 차지하였고, 다음으로 *t*-Z가 풍부하였다. 시토키닌 함량은 '금동이'에서는 '참토좌'대목에서 함량이 가장 높았으나, 대목에 따른 현저한 차이를 보이지 않았던 반면에, '통일황'에서는 대목의 종류에 관계없이 접목에 의한 증가를 보였다.

추가 주요어 : 면역친화성컬럼, 전기전도도, 접목, 종간잡종호박