Leaf Gas Exchanges and Mineral Ion Composition in Xylem Sap of Iranian Melon Affected by Rootstocks and Training Methods

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Abstract. Photosynthetic characteristics, concentrations of mineral elements in xylem sap, and some vegetative traits of ‘Khatooni’ melon were compared with those of melons grafted onto three Cucurbita rootstocks cvs., Ace, Shintozwa, and ShintoHongto, and trained with three methods: T1) no pinching and fruit thinning; T2) pinched to produce two lateral branches; and T3) pinched to two branches and all the flowers and lateral branches from lower nodes thinned. Internal CO2 and water use efficiency varied with rootstocks. Stem diameter of scions, aerial fresh and dry weights, mean fruit weight and yield, electric conductivity, pH, and sap volume per plant of grafted plants were higher in grafted melons than in the nongrafted ones. These traits were unaffected by training methods. Mineral concentrations varied considerably depending on the rootstocks and training methods used. Xylem sap collected from the decapitated stem base of grafted melons trained with T2 and T3 methods contained a higher amount of mineral ions, especially NO3-, PO4 3-, and K+, than did the sap from own-rooted plants. The increase in the mineral levels in sap resulting from grafting was most apparent in ‘Khatooni’ grafted onto ‘ShintoHongto’ rootstock.

Melons (Cucumis melo L.) are one of the important fruiting vegetable crops grown in Iran and are cultivated widely in temperate and subtropical climates. Iran is the third largest melon-producing country in the world with 1.2 million t/year (FAOSTAT, 2007). Melon cultivation is performed mostly under open fields and low tunnels for early production in eastern and central regions of Iran. Under Iranian conditions, ‘Khatooni’ melon (Inodorus group) is the most popular commercial cultivar grown in larger areas. To create conditions for maximum yield production of large and high-quality fruits, pruning and thinning offer the opportunity to adjust the fruit load of each individual plant according to its vegetative vigor (Papadopoulos, 1994). Iranian farmers have used pruning and thinning techniques to produce melons from ancient times. These techniques have been widely used to enhance fruit size, yield, and quality elsewhere (Baljee et al., 1982; Buitemelaar, 1987; Duwalda and Freeman, 1986; Kashi and Abedi, 1999). For selection of fruiting vines and maintaining two fruits per plant, the main stem tip is pinched when young and melons will be set at the sixth or seventh node of the two primary branches. These practices require too much time and labor for the farmers. Practices to produce large and high-quality fruits in this cultivar without pruning and thinning are needed.

Vegetable grafting is a common practice in Japan, Korea, the Mediterranean basin, and several European countries (Edelstein et al., 2004; Lee and Oda, 2003). Rootstocks are used in these countries to overcome soilborne diseases caused by successive cropping. In addition to disease resistance, grafted plants frequently exhibit improved yield and tolerance to low soil temperatures by increasing water and plant nutrients uptake (Ahn et al., 1999; Lee, 1994; Lee and Oda, 2003; Rivero et al., 2003; Yetisir and Sari, 2004). In some cases, the rootstock’s vigorous root system increases the efficiency of water and nutrient absorption and may also serve as a source of endogenous plant hormones, thus leading to increased yield in addition to disease control (Lee, 1994). Grafting influences absorption and translocation of phosphorus, nitrogen, magnesium, iron, and calcium ions. Absorption and translocation of other micronutrients such as iron and boron are also influenced by the rootstock (Rivero et al., 2004). Concentrations of nitrogen, phosphorus, calcium, and magnesium in xylem saps increased in grafted plants (Nie and Chen, 2000). Ion influx allows increased light energy transformation efficiency, CO2 conductivity, dark reaction activity, and photosynthetic rate in the scion (Sun et al., 2002). Increased photosynthesis can often be realized under suboptimal growing conditions such as weak sunlight and low CO2 concentration in solar greenhouses during winter months, allowing grafted plants to produce higher yields and sometimes improved fruit quality. The photosynthesis rate of grafted melon plants decreases drastically during late stages of fruit development. This decrease of photosynthesis may be responsible for low fruit quality (Xu et al., 2005). However, no studies are available on the photosynthesis performance of grafted plants for Iranian melons.

Despite the importance of the grafted seedlings in vegetable cultivation, there are few studies on the influence of training methods and movement of minerals in Iranian melons. Our hypothesis is that grafting may enhance overall plant growth and produce large fruits without rigorous pruning and thinning. To verify this hypothesis, a greenhouse experiment was carried out to test this hypothesis. Grafted and ungrafted melon plants were carefully evaluated in terms of leaf gas exchange and mineral composition in xylem sap.

Materials and Methods

Plant materials and grafting. The experiments were conducted in a plastic greenhouse during 2008 at the National Horticultural Research Institute of Rural Development Administration, located in Suwon, Republic of Korea. Seeds of ‘Khatooni’ melon (an open-pollinated cultivar) and the three Cucurbita rootstocks (Table 1) were sown simultaneously and separately in cells with 6 cm diameter and 4.5 deep (32-cell seedling trays) filled with a mixture (v:v) of coco peat (65% to 70%), peatmoss (8% to 12%), vermiculite (10% to 14%), zeolite (3% to 5%), and perlite (5% to 8%) on 4 Feb. 2008. The seedlings were grown in an environment-controlled...
greenhouse with 25/20 °C day/night tempera-
tures and were grafted on 11 Feb. by splice
grafting (Lee and Oda, 2003) at the cotyledon
stage, 7 d after sowing. Grafted plants were
transferred to a mist chamber for postgraft
care [greater than 95% relative humidity
(RH)] for 7 d, after which the RH was reduced
gradually for acclimatization.

Seedlings were transplanted to a plastic
greenhouse 1 month later. Soil texture was
loam clay. The plants were transplanted onto
raised beds spaced 2 m from center to center
and 50 cm between plants and grown horizon-
tally in the greenhouse. Three training methods
were applied to the grafted and nongrafted
melon seedlings after transplanting: T1) no
pinching and fruit thinning; T2) pinched to
produce two lateral branches and all the
flowers and secondary lateral branches were
preserved on two primary branches; and T3) pinched to two branches and all the flowers and lateral branches from lower nodes thinned and
fruits were maintained at the sixth or seventh
nodes of the two primary branches. The
experimental design consisted of randomized
blocks with three replicates with each subplot
being 8 m². Standard cultural practices, in-
cluding drip irrigation, were used. Water was
applied as required at ≈5-d intervals.

Leaf gas exchange measurements. Leaf
gas exchange was measured 70 d after trans-
planting between 1000 hr and 1400 hr using
a portable photosynthesis system with a 6-
cm² window–leaf chamber (LI-6400; LI-
COR Inc.). Measurements were taken from
the third expanded leaf from the stem tip of
three seedlings per treatment. Net photosyn-
thetic rate (A), stomatal conductance (gs), to
water vapor, transpiration, and leaf internal
CO₂ concentrations (Ci) were estimated by
the instantaneous values of humidity and CO₂
concentrations of air entering and leaving
the chamber were determined by the infrared
gas analyzer of the portable photosynthesis
system. Values of atmospheric CO₂ concentra-
tion (Ca), air temperature (Ta), and internal
photosynthetic photon flux densities (PPFD)
were obtained by the sensors of the equip-
ment. Intrinsic water use efficiency
(WUE) was calculated by the quotient be-
tween net photosynthesis/transpiration. The
mean values of vapor pressure deficit, Ta,
PPFD (measured by the external sensor of
the chamber), and Ca during the leaf gas
exchange measurements were 1.43 ± 0.15
kPa, 26 ± 0.2 °C, 460.5 ± 37.7 µmol photons/m²·s⁻¹ and 398.7 ± 0.3 µmol·mol⁻¹, respectively. Also fresh and dry biomass of
er upper organs in the grafted and nongrafted
plants were quantified.

Collection and mineral analysis of xylem
sap. Xylem sap was collected 85 d after trans-
planting (50 d after pollination). Own-rooted
plants were cut off at a position 25 cm above
the ground, and grafted plants were cut off 20
cm above the graft. The few drops of bleeding
sap from the remaining stem base were dis-
carded and the cut surface was washed with
distilled water for 10 min. Xylem sap sub-
sequently bled at the cut surface was collected
in a flask precoated with silica and wrapped
with aluminum foil. After a 24-h period of
collection, the volume of xylem sap was
recorded and the sap was stored at –20 °C
for analysis. Ions of NO₃– and NH₄⁺ were
analyzed by the Kjeldahl method (Kjeltec
Auto 1030 Analyzer, Foss Tecator, Hoganas,
Sweden). Total calcium (Ca⁺⁺), magnesium
(Mg²⁺), and potassium (K⁺) ions were analyzed
by an atomic absorption spectrophotometer
(AA-6800; Shimadzu Corp., Kyoto City,
Japan). Total phosphorus (P) was determined
by the vanadomolybdophosphoric calorimetric
method at 430 nm (ASC-5; Shimadzu Corp.).
Also, volume, electrical conductivity (EC), and
pH of bleeding xylem sap and some vegetative
growth characteristics such as stem diameter
of rootstock and scion, aerial fresh weight
(without fruits), and dry matter of grafted and
nongrafted plants were measured. Fruits were
harvested when fully mature (60 to 90 d after
anthesis) during June and July 2008. There
were five harvests at 7-d intervals. The fruits
from each plot were counted and weighed.

Data were statistically analyzed using
SAS 9.1 software package (SAS Institute
Inc., Cary, NC) by analysis of variance and
d Duncan’s multiple range test to determine
significant differences between means.

Results

The photosynthetic characteristics of
‘Khatooni’ melon are shown in Table 2. No
differences were observed in net photosyn-
thesis, evaporation, or gs between nongrafted
and plants grafted onto different rootstocks.
Also, type of training did not show significant
differences in all of photosynthetic charac-
teristics (Table 2). Internal CO₂ (Ci) and WUE
were affected by rootstocks, and grafted plants
onto ‘Shintozwa’ rootstock had higher Cᵣ than
nongrafted ones, whereas WUE of grafted
plants was lower compared with own-rooted
plants (Table 2).

Xylem sap yield was ≈3-fold in ‘Kha-
tooni’ melon grafted on ‘Ace’ rootstock
(233.11 mL) compared with that of non-
grafted plants (74.78 mL). All of the grafted
plants tended to bleed greater amounts of
xylem sap than nongrafted plants (Table 3).
Significant differences were not found in the
xylem sap yield between controls and grafted
plants in mean values of training treatments
(Table 3). The average EC value of sap from
grafted plants was higher than that in sap
from own-rooted plants. Also, sap EC of
‘Khatooni’ melon in T1 training was lower
than other training methods. Sap acidity (pH)
ranged from 6.02 to 6.48 and was not influ-
enced much by the type of rootstocks or
training methods used (Table 4).

The use of grafting and training caused
differences in the mineral ion composition of
the scion of the cultivar Khatooni, principally
with regard to the ions NO₃–, NH₄⁺, PO₄³–,
and K⁺ (Table 3). With respect to NO₃–, the
grafted plants on all rootstocks presented
values exceeding those of the control plants
(960.30 mg L⁻¹), whereas the concentration
of NO₃– was not significantly different in the
plants grown with different training methods
(Table 3). In terms of NH₄⁺, the plants grafted
onto ‘Shintozwa’ showed higher concentra-
tion than control (265.87 mg L⁻¹ versus
106.67 mg L⁻¹) and other rootstocks (Table 3).
Significant differences were not found in the
NH₄⁺ concentration among training treat-
ments (Table 3).

In the case of PO₄³–, the higher concen-
trations were observed within grafted plants
(Table 3). Among the grafted plants, the highest
concentration of PO₄³– was recorded from the
graft combination Kh/Shintozwa, whereas
the lowest concentration was recorded by own-
rooted ‘Khatooni’ (Table 3). Significant differen-
ces were found in the PO₄³– concentration
among plants trained with different methods
(Table 3). The highest level of PO₄³– was

| Rootstock | Nature | Description | Seed production company |
|-----------|--------|-------------|------------------------|
| Ace (R1)  | Hybrid | Interspecific hybrid (C. maxima × C. moschata) rootstock, good fusarium resistance, high soil temperature tolerance, and high soil moisture tolerance | DongBu HiTek., Republic of Korea |
| Shintozwa (R2) | Hybrid | Interspecific hybrid (C. maxima × C. moschata) rootstock suitable for watermelon, cucumber, oriental melon; adapted to wide range of climatic condition with vigorous root system and good tolerance to fusarium disease | Nongwoo Bio, Republic of Korea |
| ShintoHongto (R3) | Hybrid | Interspecific hybrid (C. maxima × C. moschata) rootstock with vigorous root system, resistance to fusarium, and low temperature; also possesses excellent vigor and high fruit quality and good low-temperature tolerance | Syngenta Co., Republic of Korea |
| Scion Khatooni (Kh) (C) | Open-pollinated | Cucumis melo var. inodorus, produce large fruits and very susceptible to soilborne diseases and powdery mildew | Gold Seed Co., Iran |
obtained from the T2 training method. The plants trained with the T3 method showed lower PO$_4^{3-}$ values than did other plants (Table 3). The results of K$^+$ (Table 3) revealed significant differences between grafted plants and their respective controls, whereas the highest K$^+$ concentration was obtained with the 'ShintoHongto' rootstock. The lowest K$^+$ concentrations were found in ungrafted plants. K$^+$ levels showed significant differences in training treatments (Table 3); the highest concentration was found in T2 plants and the lowest levels of K$^+$ were recorded in the plants trained with the T1 method (Table 3).

The results of the Ca$^{2+}$ concentration reflected significant differences between grafted plants and their respective controls (Table 3). Grafted 'Khatooni' onto 'ShintoHongto' showed the lowest Ca$^{2+}$, whereas the greatest concentration was found in the combination Kh/ShintoZwa. Ca$^{2+}$ concentration in the xylem sap of the plants trained with the T1 method was lower than that from the plants trained with other methods (Table 3). No significant differences between the grafted and nongrafted plants in the Mg$^{2+}$ concentration of xylem sap were found with either rootstock (Table 3). Differences in Mg$^{2+}$ concentration were observed among training treatments (Table 3). The Mg$^{2+}$ concentration was higher for T2 plants than T1 and T3.

In relation to aerial fresh weight, expressed in kg/plant (Table 4), significant differences were found between controls and grafted plants 85 d after transplanting and harvesting time. Ungrafted ‘Khatooni’...
plants had the lowest aerial fresh weight at both times. ‘Ace’ gave the highest aerial fresh weight with scion ‘Khatooni’ (Table 4). Dry weight of aerial plants was significantly affected by grafting (Table 4). The highest aerial dry weight was recorded in ‘Khatooni’ onto ‘Ace’ rootstock with 271.34 g per plant, whereas the ungrafted plants had the lowest with 142.27 g 85 d after transplanting. These values increased to 554 g to grafted plants on ‘Ace’ rootstock and 401.30 g to ungrafted plants after final fruit harvesting time (Table 4). About fruits, the highest dry matter was obtained with ungrafted fruits (Table 5).

Significant differences were found between the diameter of scion stem in grafted and nongrafted plants (Table 4). The diameter of the scion stem was higher in grafted plants than own-rooted ones, whereas no significant differences were found in the diameter of rootstock stem among plants grafted on different rootstocks (Table 4). No significant differences between the training treatments in aerial fresh weight, aerial dry matter, diameter of rootstock, or scion of plants were found (Table 4).

Fruit yield varied among plants grafted onto the various rootstocks with different training methods. The variation was expressed mainly in the different numbers and size of fruits developed on the various rootstocks and training methods (Table 5). The use of ‘Ace’ and ‘Shintozwa’ rootstocks caused a significant increase in yield per plant of cv. Khatooni grown under greenhouse conditions. Also, the T3 training method showed the highest yield per plant (Table 5). The mean fruit weight was also significantly affected by grafting and training methods (Table 5).

In all measurements, the interactions between rootstocks and training methods were not significant.

Relative percentage of mineral ion compositions in xylem sap of grafted and nongrafted plants is shown in Figure 1. Among mineral ions, the concentration of NO$_3^-$ was the highest in the sap of all treatments and the graft combination Kh/Ace trained with the T3 method (R1T3) had the highest NO$_3^-$ concentration (54.53%). The lowest concentration of NO$_3^-$ (43.38%) was found in the combination Kh/Ace trained with the T3 method (R1T3). The Mg$^{2+}$ ion showed the lowest concentration in xylem sap among treatments (Fig. 1).

Discussion

In the present experiment, grafting did not affect net CO$_2$ assimilation rate significantly ($A$), but grafted plants had more net CO$_2$ assimilation rate than ungrafted ones. In the hetero-grafted plants, the increase was accompanied by a significant increase in $g_s$ and intercellular CO$_2$ concentration ($C_i$), implying that high $g_s$ was responsible for the increasing intercellular CO$_2$ concentration and net CO$_2$ assimilation rate by grafting. He et al. (2009) reported a significant reduction in the net CO$_2$ assimilation rate in ungrafted and self-grafted plants of tomato under salinity conditions resulting from stromatal limitation. WUE was the highest in ungrafted melon plants (Table 2) owing to the lowest of transpiration rate and $g_s$.

These results indicate that the pattern of mineral absorption in ‘Khatooni’ was markedly influenced by the type of rootstocks and training methods used. Similar results have been reported with other crops grafted onto different rootstocks (Masuda, 1989; Masuda and Gomi, 1982). In the present study, the rate of exudation from grafted plants was higher than the nongrafted ones. These results suggest that the root activity of Cucurbita rootstocks may be higher than that of ‘Khatooni’ melon. Salehi et al. (2009) reported that the highest root activity (1.13 mg g$^{-1}$ DW/h formazan) was in nongrafted plants. Among mineral ions, only NO$_3^-$ and NH$_4^+$ failed to show significant differences.

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Table 5. Yield and yield components of ‘Khatooni’ melon as influenced by grafting and training methods.

| Treatment | No. of marketable fruit/plant | Mean fruit wt (kg) | Marketable yield (t/ha) | No. of unmarketable fruits/plant | Fruit dry matter (%) |
|-----------|-------------------------------|-------------------|-------------------------|----------------------------------|----------------------|
| Scion/rootstock |                               |                   |                         |                                  |                      |
| Kh/Ace    | 3.66 a*                        | 3.81 b            | 59.7 a                  | 4.66 b                           | 8.26 c               |
| Kh/Shintozwa | 3.66 a                        | 3.93 a            | 61.9 a                  | 5.33 a                           | 9.93 b               |
| Kh/ShintofHongto | 2.66 b                        | 3.70 c            | 39.2 b                  | 4.33 c                           | 9.81 b               |
| Khatooni (own-rooted) | 1.5 c                        | 3.25 d            | 24.3 c                  | 4.00 d                           | 11.22 a              |
|          | $P < 0.01$                     | $P < 0.01$        | $P < 0.01$              | $P < 0.01$                       | $P < 0.01$           |
| Training method |                               |                   |                         |                                  |                      |
| T1        | 2.75 b                         | 3.70 b            | 40.8 b                  | 5.00 a                           | 9.81 a               |
| T2        | 2.62 c                         | 3.73 a            | 38.8 c                  | 4.75 a                           | 10.05 a              |
| T3        | 3.25 a                         | 3.58 c            | 48.1 a                  | 4.00 a                           | 9.56 a               |

*Values within columns followed by different letters differ significantly by Duncan’s multiple range test at 5% or 1%.

**See Table 2 for details.**

NS = nonsignificant.
Plants trained with T2 and T3 methods showed the highest mineral composition in xylem sap. It is considered that in T2 and T3 treatments, earlier fruit set resulted to high absorption of ions such as PO$_4^{3-}$, K$^+$, Ca$^{2+}$, and Mg$^{2+}$. Yamasaki et al. (1994) reported that in watermelon/bottle gourd, only Ca concentration at 14 d after pollination was decreased by the two-fruited treatment at 14 d after pollination, but by harvest, all mineral concentrations except K decreased. In contrast, in watermelon/squash, despite a slight decrease in exudate yield, there was no significant decrease in Ca, K, and Mg, but a slight increase in NO$_3^-$ and P was observed in the two-fruited treatment at harvest.

The higher yield of melon from grafted plants observed in this study also had been reported earlier on tomato (Fernandez-Garcia et al., 2004), melon (Ruiz et al., 1997; Ruiz and Romero, 1999), and cucumber (Salehi et al., 2004). The lowest marketable yield recorded in self-grafted plants was associated with reduction in mean fruit weight and number of fruits per plant. These results coincided with the findings of Edelstein et al. (2004) and Traka-Mavrorna et al. (2000) who found that grafting melon onto Cucurbita rootstock enhances production by increasing both fruit number and weight. It was demonstrated that grafting directly affects plant yield (Nielsen and Kappel, 1996; Rivero et al., 2003) by interactions of some or all of the following processes: increase of water and nutrient uptake as a result of the vigorous root system of the rootstock (Lee, 1994; Ruiz et al., 1997; Salehi et al., 2009) enhanced production of endogenous hormones (Zijlstra et al., 1994) and/or enhancement of scion vigor (Leoni et al., 1990). The joint interactions of some or all of these processes could explain the higher yield in melon from hetero-grafted plants observed in the current study.

**Conclusion**

The results of the present work indicate that most photosynthetic characteristics were not influenced by rootstocks and training methods. Compositions as well as the relative amount of most cations and anions were influenced by the rootstocks and training methods, reflecting the significant variations in their xylem sap concentrations in scion plants onto ‘ShintoHongto’ and ‘Shintozawa’ rootstocks. However, the concentrations of NO$_3^-$ and NH$_4^+$ in xylem sap of grafted and nongrafted plants were unaffected by the training methods. Hetero-grafted plants showed the highest aerial fresh and dry weight, mean fruit weight, and yield.

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