Grafting and Silicon Improve Photosynthesis and Nitrate Absorption in Melon (Cucumis melo L.) Plants

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ABSTRACT

Grafting of Cucurbitaceae species to some rootstocks seems to be especially beneficial for the nitrogen nutrition of these plants. Moreover, melons (Cucumis melo L.) have been considered among those plants that could benefit from the addition of Silicon (Si) in the Nutrient Solution (NS). Thus, two experiments were carried out in the following order: (i) it was investigated how grafting affects nitrate absorption (following the disappearance of nitrates from NS), leaf water relations, leaf gas exchange, chlorophyll parameters and antioxidant activity, and (ii) it was also assayed which of the aforementioned physiological factors could be associated with Si supply in melon plants at early developmental stage when metabolism is intense. Results revealed that grafting and Si supply could improve photosynthesis, nitrate absorption, and biomass production in melon plants with respect to non-grafted or plants not receiving extra Si. Grafting melon on Cucurbita rootstock improved photosynthetic performance associated with higher antioxidant activity in melon leaves. Silicon supplementation results lend support to an active role of Si in biochemical processes at chloroplast level in melons. Increased assimilation rates in grafted and Si treated plants (20 to 35%), resulted in higher nitrate depletion from the medium (17 to 18%), which boosted shoot biomass production (23 to 26%) compared to the control plants. Our results suggest that grafting and Si supply in melon plants may lead to a better crop performance and a lower environmental impact of greenhouse fertigation effluents with respect to nitrate leaching, in some instances.

Keywords: Antioxidant activity, Biomass production, Gas exchange, Hydroponic melons.

INTRODUCTION

Melons (Cucumis melo L.) are one of the most commercially important crops in the Mediterranean area and production of Galia-type melons in soilless systems could be an alternative crop for greenhouse growers. Optimizing plant growth while minimizing the NO$_3^-$ transport to ground and surface waters remains a major challenge in greenhouse horticulture in these countries.

Vegetable grafting is becoming a common practise in Mediterranean basin and grafting of Cucurbitaceae to some rootstocks seems to be especially beneficial for the nitrogen nutrition of these plants (San Bautista et al., 2011). Savvas et al. (2010) revealed that certain Cucurbitaceae rootstocks enhance not only the uptake of NO$_3^-$ and translocation to the shoot but also its utilization by the plant through a more intensive assimilation into amino acids and proteins. This notion was also supported by Ruiz and Romero (1999) and Colla et al. (2010) studying N metabolism in grafted melon plants in hydroponics and open field conditions. In this sense, other authors (Salehi et al., 2010) reported that grafting achieves an increase in ion influx to the scion, resulting in the increase of light energy transformation efficiency, CO$_2$ conductivity, dark reaction activity, and photosynthetic rate in the scion. However, few published data are available concerning which physiological and biochemical factors

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could be associated with N efficiency in melon plants particularly at early developmental stage when metabolism is intense (San Bautista et al., 2011).

Photosynthesis is a core function and its functional status has been considered an ideal physiological activity to monitor plant growth (Larcher, 1980). Since grafting can influence photosynthesis and, as a consequence, the availability of carbon based substrates (Colla et al., 2010), is of paramount importance to better understand photosynthetic responses of melon plants to grafting. However, comparing results from different studies are not always feasible since significant differences may exist due to experimental layout (Bolla et al., 2009). San Bautista et al. (2011) reported that grafting melon plants did not affect net photosynthetic values, whereas double grafting increased mineral and water absorption, light photosynthetic reaction, and biomass production. In this word context, Salehi et al. (2010) reported that grafting melon plants did not affect net CO₂ assimilation rate significantly, but the grafted plants had more net CO₂ assimilation rate than un-grafted ones. Despite the importance of a better understanding of the photosynthetic metabolism of grafted plants, few studies are concerned about photosynthesis performance and protective antioxidant responses of grafted plants (He et al., 2009).

It has been reported that Silicon (Si) exerts beneficial effects on soilless-grown plants, and melons have been considered as one of the plants that could benefit from the addition of Si in the nutrient solution (Adams, 2002, Sonneveld and Voogt, 2009). Savvas et al. (2007) revealed that the stimulation of growth by Si may be either indirect, owing to the protective effects of Si against pathogens, or direct, originating from implications of Si to both morphological changes and physiological processes in plants. Kamenidou et al. (2009) also revealed that one of the most controversial Si benefits is its decrease of transpiration and increase of photosynthesis associated with foliar accumulation of Si. In cucumber, one of the protective effects of Si in photosynthesis has been considered to be associated with the antioxidant enzymes activity (Zhu et al., 2004). Currently, although the inclusion of Si in the nutrition scheme in soilless culture of some plant species is recommended (Sonneveld, 2002; Savvas et al. 2007), our knowledge regarding the hypothesized role for Si in plant physiology is still insufficient.

In view of the above, the present two experiments aimed to: (i) investigate how grafting affects nitrate absorption, leaf water relations, leaf gas exchange, chlorophyll parameters and antioxidant activity, and (ii) assay which of the aforementioned parameters could be associated with Si supply in the nutrient solution of melon plants.

**MATERIALS AND METHODS**

**Plant Material**

Two experiments were conducted at Agricultural Research Institute, Cyprus (34°44′N, 33°19′E) during the period 25 April-15 June 2013. A netted cultivar (cv. Dikti F₁, Rijk Zwaan, De Lier, Netherlands) of melon (*Cucumis melo* L.) plants was either Non-Grafted (N-GR) or Grafted (R-GR) onto the *Cucurbit* rootstock ‘RS 841’ (*C. maxima × C. moschata*) in a commercial nursery. The grafting combination was widely used by the local growers. Seeds of both melon and squash were sown on 2 March 2013 in trays filled with peat-based substrate. Grafting was made on 1 April 2013 using the procedure of “cleft grafting” joining the melon scion and the rootstock, using the tongue approach, with a clip and cutting off the scion 15 days later just below the graft union (Lee, 1994). The plants were transplanted in a mixture of peat (Shamrock moss peat) and perlite (1:1, v:v) in 7 cm square black plastic pots and used for Experiments 1 and 2. Tap water was used for irrigation.
Experiment 1

Rootstock-Grafted (R-GR) and Non-Grafted (N-GR) melon plants at the stage of two true leaves (8-10 cm height) were transferred for hydroponic culture in a floating system into a culture room with 22±2°C temperature, 55-65% relative humidity, 16 hours photoperiod and a light intensity of 150 µmol m⁻² s⁻¹ from April 25 to May 15, 2013. Floating system consisted of 12 individual polystyrene boxes (40 cm length×27 cm wide×17 cm deep) each containing 16 L of aerated nutrient solution. Six propagation pots, each containing one plant, were floated on each polystyrene box. Each box was considered as experimental unit arranged in a Completely Randomised Design (CRD) with six replications. Plants were harvested three weeks after transplanting (15 May 2013). The NS initially introduced into the system was identical in all experimental units, and the composition was as follows: 5.79 mM K⁺, 5.79 mM Ca²⁺, 2.07 mM Mg²⁺, 1.10 mM NH₄⁺, 15.43 mM NO₃⁻, 0.80 mM H₂PO₄⁻, 2.97 mM SO₄²⁻, 25 μM Fe, 5 μM Mn, 7 μM Zn, 1.0 μM Cu, 50 μM B, and 0.5 μM Mo. Corresponding Electrical Conductivity (EC) value of NS was 2.5 dS m⁻¹ and pH value was adjusted to 5.6–5.7.

Experiment 2

R-GR and N-GR melon plants were pruned to a height of 12 cm (four true leaves) and transferred into a culture room with 24±2 °C temperature, 55-65% relative humidity, 16 hours photoperiod and a light intensity of 150 μmol m⁻² s⁻¹ from May 23 to June 15, 2013. Floating system consisted of 18 individual polystyrene boxes and experimental setup was as in Experiment 1. R-GR and N-GR plants were supplemented with Si (Na₂SiO₃) at three rates (0, 0.5 and 1 mmol L⁻¹) in the NS. Each box was considered as experimental unit arranged in a Completely Randomised Design (CRD) with three replications. Plants were harvested three weeks after transplanting (15 June 2013). The NS initially introduced into the system was identical in all experimental units, and the same as in Experiment 1.

Harvesting and Handling

At final harvest, half of the shoots (aerial part of plants) from each plot was frozen and stored at −30°C for total phenolics and antioxidant activity analysis and the other half was dried in a forced-air oven at 65°C to constant weight for shoot biomass determination.

Measurements

To calculate nitrate (NO₃⁻) absorption, both the input and output of the NS were determined and the NO₃⁻ absorption was determined from the changes in the solution volume and nitrate concentrations (disappearance of nitrates from the solution). The changes in water volume corresponded to plant water uptake since water losses from the system were negligible. The NO₃⁻ ion concentrations in NS were measured by UV/VIS spectroscopy at 220 nm (Eaton, 2005).

Before final harvest, the last functional fully expanded leaves on three melon plants per plot were used between 07:00–09.00 am to measure the following: (i) gas exchange parameters [i.e., net CO₂ assimilation (A), stomatal conductance (gs), intercellular CO₂ concentration (Ci), and rate of transpiration (E)] using a LI-6400 (LI-COR, Lincoln, NE, USA); and (ii) Water Use Efficiency (WUE) as A/E. LI-6400 measurements of photosynthesis and transpiration are based on differences in CO₂ and H₂O in an air stream that is flowing through leaf chamber. The system was calibrated prior to measurements. Subsequently, leaves were sampled and used to measure: (i) leaf water potential (Ψw) and osmotic potential (Ψπ) using SKPM 1400 pressure chamber (Skye
Instruments Ltd, Llandrindod, UK) and HR-33T microvoltometer (Wescor Inc., Logan, UT, USA), respectively; (ii) leaf turgor pressure ($\Psi_p$) as $\Psi_p - \Psi_z$; (iii) Relative Water Content (RWC) as described by Larcher (1980); (iv) chlorophyll contents according to Lichtenthaler (1987); (v) chlorophyll fluorescence (maximum efficiency of photosystem II; $Fv/Fm$ ratio) using OS-30p fluorometer (Opti-Sciences, Hudson, NH, USA), and (vi) Electron Transport Rate (ETR), following Baker (2008).

Total phenolic content, Ferric-Reducing Antioxidant Power (FRAP assay) and radical scavenging activity (2,2-DiPhenyl-1-PicrylHydrazyl; DPPH assay) of shoots were measured following Neocleous et al. (2014). For silicon analyses, each tissue from each Si treatment was analyzed in triplicate. Dry tissue was microwave digested (MARS 240/50, CEM Microwave Corporation, NC, USA) and Si content was measure by ICP-OES (Teledyne Leemans Labs, Prodigy Spec., NH, USA).

**Statistical Analysis**

Analysis of variance was evaluated by SAS (Version 9.2; SAS Institute Inc., Cary, NC, USA). Means were compared using Duncan’s multiple range tests at $P \leq 0.05$. Figures were drawn using GraphPad Prism (Version 5.0; GraphPad Software; San Diego California, USA).

**RESULTS**

**Experiment 1**

Grafting increased dry shoot biomass by 28% compared to non-grafted plants as shown in Figure 1. Similar to biomass production, the net assimilation of CO$_2$ (A), stomatal conductance ($g_s$), transpiration rate (E), and Water Use Efficiency (WUE) were significantly higher in rootstock-grafted plants (Figure 1). However, internal CO$_2$ (Ci) remained unaffected by tested treatments (Figure 1). Grafting had no effect either on leaf water relations, i.e. leaf water potential (avg. -1.04 Mpa), leaf osmotic potential (avg. -1.26 Mpa), leaf turgor pressure (avg. 0.22 Mpa) and relative water content (avg. 93%) or chlorophyll parameters, i.e. chlorophyll fluorescence in the form of $Fv/Fm$ ratio (avg. 0.80) and chlorophyll content (avg. 1.43 mg g$^{-1}$ FW).

With respect to antioxidants, grafting increased shoot phenolic content and antioxidant activity as measured by FRAP and DPPH assays (Figure 2). Finally, R-GR plants absorbed significantly higher levels of nitrate from the medium compared to N-GR plants, as indicated by nitrate depletion from the nutrient solution (Figure 2).

**Experiment 2**

As shown in Table 1, grafting increased dry shoot biomass, net assimilation of CO$_2$ (A), stomatal conductance ($g_s$) and transpiration rate (E) compared to non-grafted plants. Plants received extra Si at 0.5 mM showed the highest values with respect to biomass production and A, whereas, plants not receiving extra Si showed the lowest values and the plants that received extra Si at 1.0 mM were in between (Table 1). Stomatal conductance ($g_s$), E and WUE remained unaffected by Si supplementation. However, Si supplemented plants showed lower internal CO$_2$ (Ci) than the other plants (Table 1). Neither grafting nor Si supply had a significant effect on leaf water relations, i.e. leaf water potential (avg. -0.91 Mpa), leaf osmotic potential (avg. -1.13 Mpa), leaf turgor pressure (avg. 0.22 Mpa) and relative water content (avg. 88%) or chlorophyll parameters, i.e. chlorophyll fluorescence $Fv/Fm$ ratio value (avg. 0.81) and chlorophyll content (avg. 1.50 mg g$^{-1}$ FW).

With respect to antioxidants, grafting increased shoot phenolic content and antioxidant activity as measured by FRAP and DPPH assays (Table 1). However, this was not the case in Si supplemented plants. Si supply decreased total phenolics in
Figure 1: Net CO₂ assimilation, shoot (aerial part) dry weight, stomatal conductance (gₛ), intercellular CO₂, transpiration rate and water use efficiency in hydroponically grown Rootstock-Grafted (R-GR) and Non-Grafted (N-GR) melon plants. Columns with different lower-case letters for each parameter differ significantly at $P \leq 0.05$ according to Duncan’s MRT. SE bars are shown.

shoots, while DPPH assay detected a reduction in antioxidant activity at high Si treatment. Either rootstock-grafted or Si supplemented melon plants absorbed significantly higher levels of nitrates from the medium than the other plants, as indicated by nitrate depletion from the medium (Table 1). Finally, grafting had no effect on tissue Si accumulation, whereas melon plants that received extra Si showed higher values compared to the plants which did not receive extra Si (Table 1).
Figure 2. Nitrate absorption, radical scavenging capacity, ferric reducing antioxidant power and total phenolics in hydroponically grown R-GR and N-GR melon plants. Columns with different lower-case letters for each parameter differ significantly at \( P \leq 0.05 \) according to Duncan’s MRT. SE bars are shown.

DISCUSSION

Grafting increased net CO\(_2\) assimilation (A), stomatal conductance (\( g_s \)) and transpiration rate (E), whereas internal CO\(_2\) (Ci) remained unaffected. This may suggest that A was not simply dependent on stomatal factors. Given that E was enhanced while Ci was not altered, it could be suggested that melon plants modulated the width of stomatal pores to compromise water losses and CO\(_2\) requirements (Medrano et al., 2002; Neocleous and Savvas, 2013). Chlorophyll parameters and water relations cannot account for this influence in photosynthetic characteristics, since they remained unaffected by grafting. There is evidence that internal O\(_2\) concentrations are high during photosynthesis and chloroplasts are especially prone to generate Activated Oxygen Species (ROS) (Parida and Das, 2005). Once produced, superoxide (O\(_2^-\)) will rapidly dismutate to yield hydrogen peroxide (H\(_2\)O\(_2\)). In chloroplasts, H\(_2\)O\(_2\) can inhibit photosynthesis carbon assimilation by oxidizing enzymes of Calvin pathway (Ashraf, 2009). However, the production of ROS is ubiquitous during metabolism and all plants can cope with them. The question which arises is how efficiently melon plants control the rate of ROS production and ROS scavenging by producing different types of antioxidants. If grafting speeds up the production of antioxidants, it seems logical to conclude that a more efficient protection of photosynthetic apparatus will be allowed (He et al., 2009). Indeed, in the present study, R-GR showed increased antioxidant activity as measured by FRAP and DPPH assays, compared to N-GR plants. In addition, total phenolic content, a key-factor in non-enzymatic H\(_2\)O\(_2\) scavenging in plant cells (Blokhina et al., 2003), was also enhanced by grafting.
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Plant growth as biomass production is a measure of net photosynthesis (Parida and Das, 2005). In the present experiment, dry shoot biomass was enhanced in R-GR melon plants because of the increased photosynthetic capacity associated with biochemical functions at chloroplast level. Higher yield has been also observed in grafted cucumber plants due to their ability to maintain higher net CO$_2$ assimilation (Colla et al., 2010). Furthermore, grafted melon plants have been characterized by higher rates of nitrate (NO$_3^-$) reduction and higher demand for this nutrient, increasing its uptake from the medium compared with non-grafted plants (Ruiz and Romero, 1999). In fact, Savvas et al. (2010) revealed that Cucurbitaceae rootstock enhance not only the uptake of NO$_3^-$ and its translocation to the shoot but also its utilization by the plants through a more intense assimilation into amino acids and proteins. In view of the above mentioned reasons, it is suggested that, in the current study, increased photosynthetic rate increased carbon-based available substrates for nitrogen assimilation which entailed in higher shoot biomass production.

It is stated in the literature that grafting melon onto Cucurbita rootstock directly affects plant yield by interaction of some of the following processes: (i) increase of water and nutrient uptake as a result of the vigorous root system, (ii) enhanced production of endogenous hormones, or (iii) enhancement of scion vigour (He et al., 2009; Salehi et al., 2010). The results of this study let us suppose that the joint interaction of photosynthesis and antioxidant activity could explain the higher shoot biomass observed in melon plants grafted onto pumpkin rootstock. Since water relations and chlorophyll parameters remained unaffected by grafting, biomass accumulation in rootstock-grafted plants was mainly the result of a better nitrogen nutrition associated with enhanced CO$_2$ assimilation rate and antioxidant activity in leaves.
Plants that received extra Si in the medium showed higher values in shoot biomass production and CO\textsubscript{2} assimilation rate (A), while the internal CO\textsubscript{2} (Ci) was restricted with respect to the plants not receiving extra Si. Taking into consideration that stomatal conductance (g\textsubscript{s}) and transpiration rate (E) remained unaffected by treatment, lower Ci was probably the result of either more intense RuBP-carboxylase efficiency or morphological and metabolic modification in leaves, associated with foliar accumulation of Si (Savvas \textit{et al}., 2007; Mattson and Leatherwood, 2010). The results obtained in the current study imply that the stimulation of shoot biomass production possibly originated from the involvement of Si in the physiological processes of melon plants as indicated by advanced photosynthetic capacity. According to Adatia and Besford (1986), the activity of RuBP-carboxylase was 50% higher in the leaves of cucumber grown in recirculating nutrient solution enriched with Si. Furthermore, Epstein (1999) revealed possible implications of Si to biochemical processes. These results coincide with the notion that Si has an active role in plants physiology (Savvas \textit{et al}., 2007) and lend support to the incorporation of Si in the nutrition schemes of melons in some instances (Sonneveld, 2002). Although our results lend support to the active role of Si in photosynthetic functions, they somehow do not support an enhanced antioxidant activity as indicated in other crops such as barley and cucumber (Liang \textit{et al}., 2003; Zhu \textit{et al}., 2004). Moreover, Kamenidou \textit{et al}. (2009) cited some references on research results indicating decreases in the transpiration rate and increases of photosynthesis in Si treated plants, which was not the case in our study. It is possible that an imposed stress would have had unobserved responses, which was not studied in the present experiment. Last but not the least, Si application altered foliar Si accumulation in melon plants. Trace amounts of Si in the propagation substrate and irrigation water may be used to explain the accumulation of Si traces in plants not receiving extra Si. However, plants that received extra Si in the nutrient solution increased tissue Si concentration by 15 to 44% for the low and high Si treatments, respectively, indicating that melon plants may benefit by supplemental Si (Mattson and Leatherwood, 2010).

**CONCLUSIONS**

The results of the present study showed that grafting and Si supply could improve photosynthesis, nitrate absorption, and biomass production in melon plants at early developmental stages when metabolism is intense compared with non-grafted or plants not receiving extra Si. Grafting melon on Cucurbita rootstock improved photosynthetic performance associated with higher antioxidant activity in plant tissue. Therefore, as a result of this superiority, grafted melon plants showed a higher demand for available nitrates in the medium, promoting biomass accumulation. Silicon supplementation results lend support to an active role of Si in melon plants physiology. Increased assimilation rates in Si treated melon plants, resulted in higher nitrate depletion from the medium as a function of higher availability of carbohydrates. As a general conclusion, grafting and Si supply may lead to a better crop performance and a lower environmental impact of greenhouse fertigation effluents with respect to nitrate leaching. Yet, there may be unobserved stress tolerance benefits from grafting or Si supplementations which were not tested in this study, therefore, the economic impact of these practices deserve further investigation.

**REFERENCES**

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پیوند زدن و مصرف سیلس در بیوه خریده (Cucumis melo L.) باعث بهبود فتوستوز و جذب نیترات می شود.

د. نیکلوس

چکیده

به نظر می رسد که پیوند زدن گونه های تیره کدوسان به برخی پایه ها اثر مفیدی دارد. به ویژه برای تغذیه نیترات این گیاهان همچنین، خریده ها (Cucumis melo L.) قلمداد می شوند که مصرف سیلس (Si) در محلول غذایی آن ها اثر مفیدی دارد. به این مقدار، دو آزمایش به ترتیب با اهداف زیر اجرا شد (1 بررسی چگونگی تاثیر پیوند زدن در جذب نیترات (کاهش نیترات در محلول غذایی)، روابط آبی برگ گیاه، نبادل گازی برگ، پاتامرهای کلویلی و فعالیت آنتی اکسیدانی (2) تعبیر این که کدام یک از پاتامرهای فیزیولوژیکی بیشتر گسترش یافته با تامین سیلس در مراحل اولیه رشد خریده که متاولیسم شدید است همراهی دارد. نتایج نشان داد که در مقایسه با خریده های پیوند نخورده و بیوه هایی که Si کافی دریافت نکرده پیوند زدن بیوه ها و مصرف Si به بیوه فتوستوز و افزایش جذب نیترات و عاملکرد بیوماس (زیست توده) می شود. پیوند زدن خریده روز پایه کدو سانان باعث بهبود عمل فتوستوز همراه با فعالیت آنتی اکسیدانی در برگ خریده شد. نتایج مصرف سیلس تکمیلی موجب نشان داد که عملکرد زیست توده این عصار در فرآیند های بیوشیمیایی در سطح کارپولاس در خریده است. در این آزمایش، افزایش نرخ ماده سازی (اسپیلازون) در بیوه های پیوند زده و آلیا هایی که سیلس دریافت کرده بودند به ترتیب 20% و 25% بود و منجر به کاهش برخی نیترات در محلول غذایی شد. به ترتیب 27% و 28% که این امر تولید زیست توده را در مقایسه با بیوه های شاهد به ترتیب 23% و 46% افزایش داد. به این ترتیب می توان گفت که پیوند زدن و تامین Si در خریده ممکن است منجر به عملکرد بهتر گیاه شده و در بعضی موارد اثرات زیست مخصوصی فاصله خروجی کود آبی را در گلخانه ها را که به تقویت عمیق نیترات می انجامد کاهش دهد.