Chill-Induced Decrease in Capacity of RuBP Carboxylation and Associated H₂O₂ Accumulation in Cucumber Leaves are Alleviated by Grafting onto Figleaf Gourd

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INTRODUCTION

In higher plants, organs that contribute to the organization of the whole plant body have different functions and the growth of the organs depends on interactions among the different organs. Roots change the mineral and water states in leaves by ion and water uptake and influence leaf growth and stomatal functioning by the root-sourced chemicals such as abscisic acid (ABA) (Sauter et al., 2001, 2002; Sobeih et al., 2004). It has been shown that the transpiration stream or xylem sap contains compounds such as ABA and cytokinins (Incoll et al., 1990; Beck and Wagner, 1994; Pospisilova, 2003). ABA has been implicated in a number of studies as the likely chemical substance used for root-to-shoot signalling especially under drought conditions, while cytokinins are known to be involved in the regulation of the tolerance is unknown. Here, cucumber plants grafted onto figleaf gourd (Cucurbita ficifolia), a chilling-tolerant species were used to study the role of roots in the regulation of shoot functioning and the associated root-to-shoot communication.

Key Results and Conclusions Grafted plants showed a significantly higher light-saturated rate of CO₂ assimilation (Aₘ) than own-rooted plants when roots were gradually cooled, but no differences were detected when shoots were cooled. Chill at 7°C irreversibly reduced Aₘ, and significantly decreased maximum carboxylation activity, Rubisco content and initial Rubisco activity. However, grafted plants showed weaker inhibition, together with decreased electron flux in the water–water cycle. Higher activity of antioxidant enzymes with less ROS production was found in grafted plants. In addition, ABA concentration increased by 48.4-fold whilst cytokinin concentration decreased by 91.5 % in the xylem sap of own-rooted plants after exposure to a 7 °C chill. In comparison, ABA and cytokinin concentrations increased by 10.5-fold and 36.9 %, respectively, for the grafted plants. Improved plant growth was also observed in grafted plants after the chill. These results suggest that some signals coming from chilling-resistant roots (i.e. ABA and cytokinins) protect leaf photosynthesis in shoots of chilling-sensitive plants.

Key words: Chilling, chlorophyll fluorescence, Cucumis sativus, electron transport flux, grafting, photosynthesis, reactive oxygen species, water–water cycle.

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Like other thermophilic plants grown in an unheated greenhouse, chilling under low light is a common episode during its growth (Yu et al., 2002b; Zhou et al., 2006). In this case, accumulation of the reactive oxygen species (ROS) both in shoots and roots occurred frequently, together with photosystem I (PSI) photoinhibition and an inhibited CO$_2$ assimilation in chilled leaves, and a reduction in the activity of plasma membrane H$^+$/ATPase in chilled roots (Terashima et al., 1994; Sonoike, 1995, 1996, 1997; Lee et al., 2004a, b). In a previous study, it was found that chilling under low light significantly decreased the capacity of RuBP carboxylation but increased H$_2$O$_2$ accumulation in cucumber leaves (Zhou et al., 2004, 2006). In comparison, figleaf gourd (Cucurbita ficifolia) is unique among cucurbit species with an optimal root temperature at approx. 14°C, 6°C lower than that of cucumber roots (Tachibana, 1982; Ahn et al., 1999). In this regard, it would be interesting to use figleaf gourd as the rootstock of cucumber plants to study the role of roots in the regulation of shoot functioning and the associated root-to-shoot communication. Here, the changes in leaf CO$_2$ assimilation, electron flux, the ROS-scavenging activities in the leaves, ABA and cytokinins in xylem sap exudation were examined in cucumber plants grafted onto figleaf gourd and in own-rooted cucumber plants during the chill and subsequent recovery. The mechanism by which the rootstock of figleaf gourd increases the chilling tolerance of the photosynthetic apparatus of cucumber leaves is also discussed.

**MATERIALS AND METHODS**

**Plant material and treatments**

Two species, cucumber (Cucumis sativus L. ‘Jinyan No. 4’) and figleaf gourd (Cucurbita ficifolia Bouché) were used. Seeds of both species were sown directly in vermiculite and grown for 7 d in a non-heated greenhouse at Zhejiang University, China. Average day/night temperatures were 25/20°C. The two species were then grafted together (top approach grafting), using figleaf gourd as the rootstock and cucumber as the scion. Groups of grafted seedlings were then transplanted into a container (40 × 25 × 15 cm) filled with 8 L of Enshi nutrient solution (Yu and Matsui, 1997), and maintained at a relative humidity of 95–100 %, a photosynthetic photon flux density (PPFD) of 600 μmol m$^{-2}$ s$^{-1}$, and at 25–30°C for 6 d. When the leaves began to grow, seedlings were transferred to a growth chamber with the following environmental conditions: 12-h photoperiod, temperature of 25/18°C (day/night) and PPFD of 600 μmol m$^{-2}$ s$^{-1}$.

**Experiment I.** This experiment was designed to compare the difference of grafted plants and own-rooted plants in response of gas exchange to short-term fluctuations in root zone and aerial temperatures. Every 18 plants of own-rooted and grafted plants were used for this experiment. Shoots were gradually cooled from 35°C to 30, 25, 20, 15, 10 and 5°C and maintained at each temperature for 2 h while the root zone temperature was maintained at 25°C throughout the experiment. The following day the root zone temperature was gradually cooled from 25°C to 20,

15, 10 and 5°C and maintained at each temperature for 2 h while the air temperature was maintained at 25°C. Light-saturated rate of CO$_2$ assimilation ($A_{sat}$) was measured on four randomly selected leaves 2 h after the maintenance of each desired temperature under 1050 μmol m$^{-2}$s$^{-1}$.

**Experiment II.** This experiment was designed to compare plant growth, ABA and cytokinins concentration in xylem sap, leaf water potential, gas exchange, chlorophyll fluorescence, electron flow, Rubisco content and activity, and antioxidant system in response to long-term chill of grafted plants and own-rooted plants. Eighteen grafted and own-rooted plants at the four-leaf stage were exposed to 100 μmol m$^{-2}$ s$^{-1}$ at 14°C or 100 μmol m$^{-2}$ s$^{-1}$ at 7°C for 7 d, respectively. Thereafter, all plants were set for recovery at 25/18°C at 600 μmol m$^{-2}$ s$^{-1}$ PPFD for 7 d. However, plants for the hormone analysis were allowed to recover under clement conditions for 24 h before xylem sap was collected. Throughout the experiment, leaf water potential, gas exchange and chlorophyll fluorescence measurements were made and biochemical assays were performed on four randomly selected leaves per treatment. Each treatment included 18 plants with three replicates.

**Plant growth and leaf water potential**

The plastochron index (PI), which measures the developmental age of cucumber plants regardless of biomass parameters, was calculated following Coleman and Greyson (1976), with a reference leaf length of 60 mm. PI is defined as $n + [\ln(L_n) - \ln(R)]/[(\ln(L_n) - \ln(L_{n+1}))], where n is the number of the leaves longer than the reference length R, and $L_n$ and $L_{n+1}$ are the leaf lengths of the leaf numbers n and (n + 1), respectively.

Leaf water potential ($\psi_w$) was determined on intact excised leaves from chilled plants using a dew point microvoltmeter (HR-33T; Viescor, UT, USA).

**Gas exchange measurements**

For gas exchange and chlorophyll fluorescence analysis, chilled plants were transferred to a growth room at 25°C under a PPFD of 600 μmol m$^{-2}$ s$^{-1}$ for 2 h before measurements. Leaf gas exchange was measured using an infrared gas analyser (model CIRAS-1; PP System, Herts., UK) on the second true leaf of each seedling (Yu et al., 2002b). The leaf light-saturated rate of CO$_2$ assimilation ($A_{sat}$), intercellular CO$_2$ concentration ($C_i$) and stomatal conductance ($g_s$) were calculated by maintaining air temperature, air relative humidity, CO$_2$ concentration and PPFD at 25°C, 80–90%, 350 μmol mol$^{-1}$ and 1000 μmol m$^{-2}$ s$^{-1}$, respectively.

$A/C_i$ curves were also measured at a leaf temperature of 25°C and a PPFD of 1200 μmol m$^{-2}$ s$^{-1}$ (von Caemmerer and Farquhar, 1981). The maximum velocity of RuBP carboxylation by Rubisco ($V_{cmax}$) and maximum potential rate of electron transport contributing to RuBP regeneration ($I_{max}$) were estimated by fitting a maximum likelihood regression below and above the inflection of
the A/C₃ response as described by Ethier and Livingston (2004).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence parameters were measured with a pulse amplitude fluorimeter (Hansatech Instruments Ltd, Norfolk, UK) in the same leaves as those used for gas exchange measurements. Minimal (Fₐ), maximal (Fₘ), steady state (Fₛ) and light-adapted maximum (Fₘ) fluorescence were measured as reported by Yu et al. (2002a). Fluorescence parameters were calculated by FMS-2 on the basis of the dark-adapted and light-adapted fluorescence measurements. The maximal photochemical efficiency of photosystem II (PSII) (Fₘ/Fₘ), the quantum efficiency of PSII (ΦPSII) and the efficiency of excitation capture by open PSII centres were calculated as (Fₘ – Fₐ)/Fₘ, (Fₘ – Fₛ)/Fₘ and Fₛ/Fₘ, respectively. The photochemical quenching coefficient (qP) was calculated as described by von Kooten and Snel (1990).

Estimation of the rate of alternative electron flow

The rate of electron transport through PSII [Jₑ(PSII)] was measured as reported by Harley et al. (1992). The rate of oxygenation by Rubisco (Vₒ) was estimated following von Caemmerer and Farquhar (1981) and the rate of carboxylation by Rubisco (Vₑ) was estimated as described by Miyake and Yokota (2000). In atmospheric conditions, the electron fluxes in the two cycles can be expressed as

\[ Jₑ = Jₐ(PCR) = 4 \times Vₐ \quad \text{and} \quad Jₑ(POC) = 4 \times Vₑ \]

(Krall and Edwards, 1992). An alternative flux, Jₑ, caused by electrons that are not used by the PCR or POC cycles in the total electron flux driven by PSII, can be estimated from Jₑ(PSII) – Jₑ(PCR + POC) (Miyake and Yokota, 2000). Jₑ(O₂-dependent) was calculated from the differences between Jₑ(20 % O₂) and Jₑ(2 % O₂) (Miyake and Yokota, 2000). Jₑ(O₂-independent) was then estimated from the differences between Jₐ and Jₑ(O₂-dependent) (Zhou et al., 2004).

Biochemical assays

Biochemical assays were carried out as reported by Zhou et al. (2004). The Rubisco content was determined spectrophotometrically by formamide extraction of the Coomassie Brilliant Blue R-250-stained subunit bands separated by SDS–PAGE (Makino et al., 1994) using calibration curves plotted from Rubisco purified from cucumber leaves. Rubisco activity was measured spectrophotometrically following Nakano et al. (2000). Protein content was carried out as described by Bradford (1976), using bovine serum albumin as standard.

Ascorbate peroxidase (APX) was measured as described by Nakano and Asada (1981), i.e. by monitoring the rate of ascorbate oxidation at 290 nm (E = 2.8 mm cm⁻¹). Glutathione reductase (GR) was assayed as reported by Foyer and Halliwell (1976), i.e. by monitoring the decrease in absorbance at 340 nm caused by NADPH oxidation (E = 6.2 mm cm⁻¹). Superoxide dismutase (SOD) was measured by the photochemical method as described by Giannopolitis and Ries (1977). The activities of catalase (CAT) and guaiacol peroxidase (GPOD) were assayed following Cakmak and Marschner (1992).

Ascorbic acid (AsA) was measured following Law et al. (1983). Glutathione (GSH) was determined as reported by Hissin and Hilf (1976). The content of H₂O₂ was measured by monitoring the A₄10 of titanium-peroxide complex following Patterson et al. (1984). The levels of malondialdehyde (MDA) were determined by the modified method of Heath and Packer (1968).

Xylem sap collection, ABA and cytokinin measurements

Xylem sap exudation was collected by cutting the stem 5 cm above the ground. It was stored at –82 °C until analysis (Tachibana, 1988). ABA and cytokinin concentrations in xylem sap were determined by enzyme-linked immunosorbent assay. Extraction and purification prior to immunoeassay have been described by Chen et al. (1997). The results of cytokinins were expressed as the combined concentrations of isopentenyladenine and isopentenyladenosine.

Statistical analysis

All treatments and assays were replicated four times. ANOVA was applied to assess the differences between treatments. Differences between means were established using the Duncan test (P < 0.05). Least squares linear regression analysis was performed to determine the correlation between Aₛₑat and other gas exchange and chlorophyll fluorescence parameters in all treatments. The data were analysed using Origin7.0 Software (OriginLab, Northampton, MA, USA).

RESULTS

Figure 1 shows the effects of aerial shoot and root zone temperatures on the light-saturated rate of CO₂ assimilation (Aₛₑat) in expt I. Both grafted and own-rooted plants showed their maximal Aₛₑat at 20–30 °C. There were no significant differences in Aₛₑat measured at all aerial temperatures between own-rooted and grafted plants. Although there was no difference in Aₛₑat measured under 20 and 25 °C of root-zone temperatures, the grafted plants, however, showed significant higher Aₛₑat than own-rooted plants at low root-zone temperatures. Aₛₑat for grafted plants at 15 °C, for example, was not different from that at 20 °C, but 43.8 % higher than that for own-rooted plants.

Under clement conditions, grafted plants showed stronger growth vigour and the PI for grafted plants was significantly higher than that for own-rooted plants at the end of the experiment (Fig. 2A). Chill at both 14 °C and 7 °C decreased plant growth, although a slight increase in PI was also observed in grafted plants during the chill at 14 °C. Interestingly, the growth rate of grafted plants after a chill at 7 °C was almost identical to that of own-rooted plants after a chill at 14 °C. At the end of the experiment, the PI values of grafted plants were 17.5 % and 30.8 %
higher than those of own-rooted plants after chills at 14 °C and 7 °C, respectively (Fig. 2A).

For plants grown under clement conditions, grafting had little effect on $A_{\text{sat}}$, which remained between 14 and 16 μmol m$^{-2}$ s$^{-1}$ throughout the experiment (Fig. 2B). Chill at both 14 °C and 7 °C significantly decreased $A_{\text{sat}}$ and $g_s$ (Fig. 2B and C), and increased $C_i$. However, effects were less significant in grafted plants than in own-rooted plants. Chill at 14 °C for 7 d, for example, decreased $A_{\text{sat}}$ by 70.1 % and 86.4 % in grafted and own-rooted plants, respectively. Almost all plants recovered values close to control, except own-rooted plants chilled at 7 °C, which showed a reduced $A_{\text{sat}}$ value (38.5 %). In addition, stomatal limitation did not differ significantly throughout the experiment (data not shown).

The diurnal changes in leaf water potential ($\psi_w$) were recorded in the 2nd leaves during recovery. $\psi_w$ for grafted plants was not influenced by chill at 7 °C or 14 °C and $\psi_w$ remained between −0.85 and −0.65 MPa. Chill at 7 °C slightly decreased $\psi_w$ in own-rooted plants, which ranged from −1.0 to −1.2 MPa throughout the day (Fig. 3).

Chill at 14 °C and 7 °C all significantly decreased both the maximum velocity of RuBP carboxylation by Rubisco ($V_{\text{cmax}}$) and the maximum potential rate of electron transport contributing to RuBP regeneration ($J_{\text{max}}$) (Fig. 4). As compared with own-rooted plants, the decreases were less significant in grafted plants at both temperatures. $V_{\text{cmax}}$ in own-rooted plants decreased by 75.3 % after chill at 14 °C for 7 d, whilst it decreased by 63.0 % in grafted plants, $V_{\text{cmax}}$ and $J_{\text{max}}$ in own-rooted plants failed to recover to control values after 7 d chill at 7 °C. However, these

Fig. 1. Effects of (A) aerial temperature and (B) root-zone temperature fluctuation on CO$_2$ assimilation rate under saturated light ($A_{\text{sat}}$) in own-rooted and grafted plants. Open triangles, own-rooted and unchilled; closed triangles, grafted and unchilled. Measurement was made after 2 h at each temperature. Data are the means of four replicates with standard errors shown by vertical bars.

Fig. 2. Effects of (A) grafting and chilling on plastochron index (PI), (B) light-saturated rate of CO$_2$ assimilation ($A_{\text{sat}}$) and (C) stomatal conductance ($g_s$) in cucumber leaves. Open triangles, own-rooted and unchilled; closed triangles, grafted and unchilled; open circles, own-rooted and chilled at 14 °C; closed circles, grafted and chilled at 14 °C; open squares, own-rooted and chilled at 7 °C; closed squares, grafted and chilled at 7 °C. The vertical dashed line indicates the transfer of plants back to the control chamber. Data are the means of four replicates with standard errors shown by vertical bars.

Fig. 3. Diurnal time-courses of leaf water potential in own-rooted and grafted plants after 7 d chill. Open triangles, own-rooted and unchilled; closed triangles, grafted and unchilled; open circles, own-rooted and chilled at 14 °C; closed circles, grafted and chilled at 14 °C; open squares, own-rooted and chilled at 7 °C; closed squares, grafted and chilled at 7 °C. Data are the means of four replicates with standard errors shown by vertical bars.
values did not differ from those of control after 7 d of recovery in grafted plants.

Rubisco content and total and initial Rubisco activities were measured 3 d after recovery in clement conditions (Table 1). Chill at 14 °C had little effect on Rubisco content, total Rubisco activity and initial Rubisco activity except that the initial Rubisco activity for own-rooted plants decreased by 28.1% after the chill. Chill at 7 °C, however, significantly decreased Rubisco content, total Rubisco activity and initial Rubisco activity, especially in own-rooted plants. After the chill, Rubisco content, total Rubisco activity and initial Rubisco activity for own-rooted plants decreased by 48.7%, 69.5% and 72.8%, respectively, whilst these values for grafted plants decreased only by 16.1%, 35.4% and 32.1%, respectively.

Furthermore, chill at 14 °C and 7 °C all significantly decreased the Rubisco activation rate for own-rooted plants, while the Rubisco activation rate for grafted plants remained almost unchanged.

The maximal PSII efficiency ($F_v/F_m$) of own-rooted plants was not affected by chill at 14 °C but declined to 0.56 by chill at 7 °C (Fig. 5). However, $F_v/F_m$ rapidly recovered to a value close to control when the plants were Rewarmed. In comparison, $F_v/F_m$ did not vary significantly in grafted plants. Chill both at 14 °C and 7 °C decreased the relative quantum efficiency of PSII photochemistry ($F_{psii}$), the photochemical quenching ($q_P$) and the efficiency of excitation energy capture by open PSII reaction centres ($F_v/F_m$) in own-rooted and grafted plants, but its effects were less significant in grafted plants. $F_{psii}$, $q_P$ and $F_v/F_m$ all increased in grafted plants when chilled plants were allowed to recover under clement conditions for 7 d. However, the values of $F_{psii}$ and $q_P$ were still reduced in 7 °C-chilled own-rooted plants.

Chilling for 7 d significantly decreased the total electron flux in PSII ($J_e(PSII)$), the electron flux for photosynthetic carbon reduction ($J_e(PCR)$) and photorespiratory carbon oxidation ($J_e(PCO)$) (Table 2). Chill significantly increased the O$_2$-dependent alternative electron flux ($J_e(O_2$-dependent$)$), especially in own-rooted plants, which increased by 300% and 350% after chill treatments at 14 °C and 7 °C, respectively. The proportional increases were less significant in grafted plants: 6% and 18% of the $J_e(PSII)$ after a chill at 14 °C and a chill at 7 °C, respectively, than in own-rooted plants: 16% and 61%, respectively. Apparently, $J_e(O_2$-dependent$)$, which is related to the water–water cycle activity, accounted for most of the changes in alternative electron flux, since the O$_2$-independent alternative electron flux ($J_e(O_2$-independent$)$) was much lower than $J_e(O_2$-dependent$)$.

To find out if the increase in $J_e(O_2$-dependent$)$ was associated with higher activities of radical-scavenging enzymes or the levels of antioxidants at the end of chilling was tested (Table 3). In clement conditions, grafting had no significant effect on the activities of SOD, APX, GPOD, CAT or the contents of AsA, MDA and H$_2$O$_2$. Chill at 14 °C and 7 °C, however, increased the activities of SOD, APX, GR and CAT in most cases. In comparison, lower contents of AsA and GSH were found in grafted plants.

**Table 1. Effects of grafting and chill (for 7 d) on Rubisco content, total and initial Rubisco activity, and Rubisco activation rate of cucumber plants after recovery for 3 d**

| Treatment | Rubisco content (g m$^{-2}$) | Total Rubisco activity (μmol m$^{-2}$ s$^{-1}$) | Initial Rubisco activity (μmol m$^{-2}$ s$^{-1}$) | Rubisco activation rate (%) |
|-----------|-----------------------------|--------------------------------------------|---------------------------------------------|----------------------------|
| Grafting  | Chill | | | |
| ~ | ~ | 2.24 ± 0.18$^{ab}$ | 27.68 ± 3.13$^a$ | 13.12 ± 0.85$^b$ | 47.40 ± 2.63$^c$ |
| + | ~ | 2.35 ± 0.09$^a$ | 27.51 ± 2.40$^a$ | 13.58 ± 0.96$^a$ | 49.36 ± 1.34$^b$ |
| ~ | 14 °C | 2.10 ± 0.14$^{bc}$ | 24.92 ± 1.69$^a$ | 9.43 ± 0.29$^a$ | 37.87 ± 0.96$^b$ |
| + | 14 °C | 2.40 ± 0.18$^a$ | 27.98 ± 1.80$^a$ | 15.12 ± 1.20$^a$ | 54.03 ± 1.59$^a$ |
| ~ | 7 °C | 1.15 ± 0.07$^{bc}$ | 16.46 ± 1.67$^b$ | 3.56 ± 0.81$^b$ | 42.08 ± 1.55$^b$ |
| + | 7 °C | 1.97 ± 0.11$^c$ | 17.77 ± 1.64$^a$ | 9.22 ± 1.05$^b$ | 51.89 ± 1.92$^b$ |

Values are the means of three replicates with standard deviation.

Values followed by different letters are significantly different at the 5% level.
after 7 d chill. Together with the changes in the antioxidant system, MDA and H$_2$O$_2$ accumulated significantly in 7 °C-chilled leaves. In contrast, grafted plants had lower H$_2$O$_2$ and MDA contents than own-rooted ones. For example, the H$_2$O$_2$ content in grafted plants after a chill at 7 °C was 53.6% lower than in own-rooted plants and did not differ significantly from that of unchilled plants.

The exudation rate of xylem sap for grafted plants did not differ from that of cucumber plants. The exudation rate significantly decreased after a chill at 7 °C (Table 4). Interestingly, chill increased ABA concentration in the xylem sap by 48.4-fold for the own-rooted and by 10.5-fold for grafted plants, with the increase in own-rooted plants being more significant. In contrast, chill decreased cytokinin concentration in the xylem sap of own-rooted plants by 91.5%, but increased its concentration by 36.9% for the grafted plants.

**DISCUSSION**

The present results show that both growth and CO$_2$ assimilation of cucumber plants were significantly inhibited by chill at 14 °C and 7 °C, respectively. However, this inhibition was effectively alleviated by grafting cucumber shoots onto roots of figleaf gourd (Figs 1 and 2). The improvement of root functioning in terms of ion and water uptake, H$^+$-ATPase activity and resistance to pathogen attack has been studied (Ahn et al., 1999; Pavlou et al., 2002); however, little is known about the effect of roots on the physiological functions, such as photosynthesis and antioxidant metabolism, of the aerial organs at low temperatures. Early studies showed that the root exerts its influences on shoot functioning by a root-to-shoot communication mechanism in droughted plants (Sauter et al., 2001, 2002; Sobeih et al., 2004). Certain scions of apple even show higher expression of a number of photosynthesis-related, stress-related genes (Jensen et al., 2003). While changing the aerial temperatures for cucumber plants did not result in any differences in $A_{sat}$ between own-rooted and grafted plants, chilling the roots, however, resulted in significantly higher $A_{sat}$ in grafted plants than that in own-rooted plants. Clearly, root-to-shoot communication was involved in the response of the plants to chill.

There is now strong evidence that ABA is strongly involved in the root-to-shoot communication and plays an important role in the regulation of stomatal behaviour of droughted plants (Sauter et al., 2001, 2002; Sobeih et al., 2004). Furthermore, some authors also found that cytokinins in xylem sap antagonized the effect of ABA on stomatal aperture, and an antagonistic interaction of cytokinins and ABA as non-hydraulic root-to-shoot signals has been proposed (Hansen and Dorffling, 2003). In agreement with these findings, a sharp increase in ABA content in the xylem sap, accompanied by decreased stomatal conductance and leaf water potential, were observed in plants after a chill at 7 °C, especially in own-rooted plants (Figs 2 and 3 and Table 4). The lower increase in ABA content in grafted plants suggests that the chill was less stressful as compared with the own-rooted plants. Interestingly, there was an increase in the content of cytokinins in xylem sap of grafted plants after the chill, in comparison to the decrease in the own-rooted plants (Table 4, Tachibana, 1988). However, it was not possible to attribute the large decrease in $A_{sat}$ only to the changes in ABA in chilled plants since the small differences in $g_s$ and $\psi_w$ in chilled plants are not likely to be the primary factors responsible for the
reduction of \( A_{\text{sat}} \). Meanwhile, changes in \( \psi_w \) and \( g_s \) were associated with an increased \( C_i \) in the present study (data not shown), suggesting that a non-stomatal factor was also involved in the reduced \( A_{\text{sat}} \), as observed in an earlier study (Zhou et al., 2004). Simultaneously, analysis of gas exchange and chlorophyll fluorescence parameters revealed that changes in \( A_{\text{sat}} \) are closely correlated to the changes in the maximum velocity of RuBP carboxylation by Rubisco \((V_{\text{cmax}}) (r = 0.98, P \leq 0.01) \) and electron flux for the photosynthetic carbon reduction, \( J_e(\text{PCR}) \) \((r = 0.99, P \leq 0.01) \). \( V_{\text{cmax}} \) may decrease owing to the inactivation or loss of Rubisco, while the reduction in \( J_{\text{max}} \) is associated with a diminution in other enzymes of the Calvin cycle such as FBPase and PEPcase (Bassham and Krause, 1969; Woodrow and Berry, 1988; Harrison et al., 1998; Nogués and Baker, 2000). After 5 d of recovery, \( V_{\text{cmax}} \) for grafted plants chilled at 7 °C was almost identical to that of the control, while \( V_{\text{cmax}} \) for own-rooted plants chilled at 7 °C was only 69.7 % of the control (Fig. 4). It is worth noting that a decrease in Rubisco activation rate occurred in own-rooted plants but not in grafted plants chilled at either 7 °C or 14 °C (Table 1). Furthermore, grafted plants showed higher Rubisco contents after 3 of recovery (Table 1).

Previous studies found that chilling resulted in more significant reductions in \( \text{rbcL} \) and \( \text{rbcS} \) transcripts, Rubisco content and initial Rubisco activity (Zhou et al., 2004, 2006). Taken together, it is likely that the faster recovery of \( V_{\text{cmax}} \) and \( \text{CO}_2 \) assimilation for grafted plants was attributed to the higher activation state of Rubisco and Rubisco synthesis, and/or less Rubisco degradation (Sassenrath et al., 1990; Nakano et al., 2006; Zhou et al., 2006).

It would be interesting to elucidate the reason for the large differences in Rubisco content, total Rubisco activity and the initial Rubisco activity between own-rooted plants and grafted plants after exposure to a chill. The different response of cytokinin delivery in xylem sap after the chill may partly explain the changes in Rubisco content and Rubisco activity (Table 1). Compared with own-rooted plants, grafted plants delivered much higher amounts of cytokinins in response to the chill. Cytokinins can increase the mRNA levels of the large and small subunits of Rubisco and the activities of Rubisco and FBPase (Davies and Zhang, 1991). Cytokinins in xylem sap are also involved in chloroplast build-up and chlorophyll formation, and regulation of light-inducible nitrate reductase and the light-harvesting pigment–protein complex and shoot greening.

### Table 2. Effects of grafting and chilling on total electron flux in PSII \([J_e(\text{PSII})]\), electron flux for photosynthetic carbon reduction \([J_e(\text{PCR})]\), electron flux for photorespiratory carbon oxidation \([J_e(\text{PCO})]\), an \(O_2\)-dependent alternative electron flux \([J_{\text{a}}(O_2\text{-dependent})]\) and an \(O_2\)-independent alternative electron flux \([J_{\text{a}}(O_2\text{-independent})]\) in cucumber leaves

| Treatment | Unchilled | Chilled at 14 °C | Chilled at 7 °C |
|-----------|-----------|-----------------|----------------|
| Grafting  | Own-rooted | Grafted         | Own-rooted     | Grafted         |
| Chill     | \(a\) (\(\mu\)mol e\(^{-} m^{-2} s^{-1}\)) | \(a\) (\(\mu\)mol e\(^{-} m^{-2} s^{-1}\)) | \(a\) (\(\mu\)mol e\(^{-} m^{-2} s^{-1}\)) | \(a\) (\(\mu\)mol e\(^{-} m^{-2} s^{-1}\)) |
| \(-\)     | 89.2 ± 1.4\(^a\) | 18.1 ± 1.4\(^a\) | 6.6 ± 1.4\(^a\) | 0.8 ± 0.3\(^a\) |
| +        | 70.6 ± 1.4\(^a\) | 1.0 ± 0.2\(^a\) | 0.6 ± 0.1\(^a\) | 0.5 ± 0.1\(^a\) |
| \(+\)     | 20.0 ± 2.8\(^b\) | 3.8 ± 0.8\(^b\) | 3.2 ± 0.1\(^b\) | 0.5 ± 0.1\(^b\) |
| \(+\)     | 70.6 ± 1.4\(^a\) | 2.0 ± 0.1\(^a\) | 0.4 ± 0.4\(^a\) | 0.5 ± 0.1\(^b\) |
| \(+\)     | 70.6 ± 1.4\(^a\) | 2.0 ± 0.1\(^a\) | 3.0 ± 0.1\(^b\) | 0.5 ± 0.1\(^b\) |

Measurements were made after 7 d of chilling followed by 2 h at 25 °C and 600 \(\mu\)mol \(m^{-2} s^{-1}\) before the measurements. Values are the means of four replicates with standard errors.

### Table 3. Changes in the activities of antioxidant enzymes (SOD, APX, GR, CAT and GPOD), the content of antioxidants (ASA and GSH) and \(H_2O_2\) as well as lipid peroxidation (expressed as MDA content) in cucumber leaves as influenced by grafting and chilling treatments

| Parameter | Own-rooted | Grafted | Own-rooted | Grafted | Own-rooted | Grafted |
|-----------|-----------|---------|-----------|---------|-----------|---------|
| SOD (unit \(m^{-1} \) protein) | 8.61 ± 0.43\(^a\) | 8.32 ± 0.21\(^a\) | 8.35 ± 0.34\(^a\) | 9.81 ± 0.92\(^a\) | 9.74 ± 0.71\(^a\) | 11.51 ± 0.70\(^a\) |
| APX (\(\mu\)mol \(m^{-1} \) protein) | 0.73 ± 0.13\(^a\) | 0.77 ± 0.10\(^a\) | 0.75 ± 0.14\(^a\) | 1.09 ± 0.11\(^a\) | 0.79 ± 0.12\(^a\) | 1.27 ± 0.11\(^a\) |
| GR (\(\mu\)mol \(m^{-1} \) protein) | 1.38 ± 0.20\(^a\) | 1.78 ± 0.17\(^a\) | 2.51 ± 0.12\(^a\) | 3.09 ± 0.12\(^a\) | 1.67 ± 0.08\(^a\) | 2.56 ± 0.21\(^a\) |
| CAT (\(\mu\)mol \(m^{-1} \) protein) | 11.02 ± 0.61\(^a\) | 10.22 ± 0.43\(^a\) | 6.84 ± 0.21\(^a\) | 8.61 ± 0.52\(^a\) | 5.13 ± 1.34\(^a\) | 8.23 ± 0.31\(^a\) |
| GPOD (\(\mu\)mol \(m^{-1} \) protein) | 0.41 ± 0.17\(^a\) | 0.41 ± 0.10\(^b\) | 1.02 ± 0.09\(^a\) | 0.97 ± 0.34\(^a\) | 0.52 ± 0.04\(^a\) | 0.53 ± 0.04\(^a\) |
| AsA (\(\mu\)mol \(g^{-1} \) FW) | 0.78 ± 0.50\(^a\) | 0.86 ± 0.05\(^b\) | 0.77 ± 0.10\(^b\) | 0.75 ± 0.05\(^b\) | 2.93 ± 0.18\(^a\) | 1.54 ± 0.07\(^a\) |
| GSH (\(\mu\)mol \(g^{-1} \) FW) | 0.83 ± 0.06\(^a\) | 1.06 ± 0.17\(^a\) | 0.79 ± 0.07\(^a\) | 1.37 ± 0.10\(^a\) | 2.71 ± 0.05\(^a\) | 1.01 ± 0.02\(^a\) |
| \(H_2O_2\) (\(\mu\)mol \(g^{-1} \) FW) | 9.39 ± 5.38\(^a\) | 9.43 ± 2.38\(^a\) | 100.3 ± 5.78\(^a\) | 83.4 ± 3.14\(^a\) | 189.8 ± 15.3\(^a\) | 88.8 ± 7.4\(^a\) |
| MDA (\(\mu\)mol \(g^{-1} \) FW) | 7.76 ± 0.51\(^a\) | 7.31 ± 0.54\(^a\) | 10.26 ± 0.31\(^b\) | 9.52 ± 0.09\(^a\) | 12.84 ± 0.21\(^a\) | 10.77 ± 0.34\(^a\) |

Measurements were made 7 d after chilling treatment. Values are the means of four replicates with standard deviation.

Data followed by different letters are significantly different at the 5 % level.
Accordingly, leaves of grafted plants, in addition to their ties than own-rooted plants after exposure to a chill. Grafted plants exhibited higher SOD, APX and GR activities. The reason for increased activities of SOD, APX and GR, and lower contents of AsA and GSH than the own-rooted plants after exposure to thermal stress and they argued that the ascorbate/glutathione cycle in grafted plant functions better by a continual oxidation/reduction process, leading to low levels in plant cells (Rivero et al., 2003).

Change in redox homeostasis is supposed to alter the function of photosystems. PSI was selectively photoinhibited in cucumber when exposed to chilling–light treatment, which is directly caused by ROS (Sonoike and Terashima, 1994; Terashima et al., 1994; Sonoike, 1995, 1996, 1997). Choi et al. (2002) proposed that Cu/Zn-SOD is the primary target of light–chilling stress, followed by subsequent inactivation of PSI by ROS. Accordingly, the lower ROS level in grafted plants is one of the possible reasons for less photoinhibition. Further work is needed to elucidate the relationship between the ROS-scavenging capacity and photoinhibition in chilled plants.

In conclusion, roots of cucumber plants have significant influence on the functioning of the photosynthetic apparatus by delivering chemicals such as the hormones ABA and cytokinins in root-to-shoot communication. Grafting cucumber shoot onto figleaf gourd would increase the rate of cytokinin to ABA in the xylem sap, resulting in higher Rubisco content and activity, higher ROS-scavenging enzyme activities and lower ROS accumulation in chilled leaves. The use of grafted plants under chilling stress conditions favours the plant through an intrinsic mechanism that improves photosynthesis, thereby promoting growth and development.

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