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EFFECT OF ROOTSTOCK ON THE GROWTH, PHOTOSYNTHETIC CAPACITY AND OSMOTIC ADJUSTMENT OF EGGPLANT SEEDLINGS UNDER CHILLING STRESS AND RECOVERY

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Abstract

Increasing evidences have shown that grafting can confer enhanced the resistance of many plant species to various biotic and abiotic stresses, but there is little information about the effects of grafting on the growth of eggplant plants under cold stress conditions. In this study, the eggplant (Jiza long eggplant) seedlings were grafted onto 'Hiranasu' and 'Taibyo' rootstocks, treated with low temperature (10/5ºC, day/night), and then recovered under normal conditions (25/18ºC, day/night). Meanwhile, we compared analysis of several physiological parameters among the non-grafted and two kinds of grafted plants. Our data revealed that cold stress resulted in a remarkable decrease in photosynthetic parameters of two kinds of grafted seedlings ('Taibyo'and 'Hiranasu' as rootstocks) and non-grafted seedlings. However, the reduction in photosynthetic parameters was observed lower in the grafted plants with the cold-tolerant rootstocks ('Hiranasu') than those with the cold-sensitive rootstocks ('Taibyo'). It was observed that these parameters were recovered more rapidly in the grafted plants with 'Hiranasu' rootstocks under normal conditions. Therefore, it can be concluded that grafting promotes the growth of eggplant seedlings with increased photosynthetic efficiency under cold stress and recovery as compared to non-grafted plants. Moreover, the grafted plants with the cold-tolerant rootstocks displayed better growth traits under cold stress.

Key words: Grafted eggplant seedlings, Growth, Photosynthetic characteristics, Carboxylation efficiency, Osmotic adjustment, Cold tolerance.

Introduction

Eggplant (*Solanum melongena* L.) is an economically important vegetable crop in Asia and Africa (Frary *et al*., 2007; Wang *et al*., 2015). It originated in India and is the thermophilic vegetable. However, eggplant is often cultivative, and its yield and quality are poor, especially in winter cultivation in unheated greenhouses in temperate regions, such as in North China (Wu *et al*., 2013). It has recently been shown that the tolerance of plants to abiotic stress is significantly improved by grafting (Ribeiro *et al*., 2016). It is hence believed that grafting will be a potential way to obtain cold-tolerant plants for agricultural sustainability.

To adapt cold stress, plants have evolved a series of mechanism at biochemical, physiological, and morphological levels (Liu *et al*., 2015; Borjas *et al*., 2016). One of the important strategies is to adjust osmotic-related stress, which maintains the photosynthetic capacity and growth of plants. When subjected to cold stress, several important osmoregulation substances have been found to accumulate abundantly in plant tissues. These metabolites can be employed as non-enzymatic antioxidants that help plants counteract adverse effects of reactive oxygen species (ROS) provoked by various abiotic stress (Gill & Tuteja, 2010), which play a role in the protection of membrane integrity (Chen *et al*., 2014). Previous studies have shown that plant photosynthesis is much sensitive to cold stress, which negatively impacts the photosynthetic apparatus in tropic and subtropical plants (Tan *et al*., 2008; Holá *et al*., 2008; Santos *et al*., 2011). Cold-treated plants often display decreased the rates of CO_2 fixation and photosystem II (PSII) electron transport (Liu *et al*., 2012) .The photosynthetic process changes further disrupted the uptake and translocation of mineral nutrients (Waraich *et al*., 2012).

Importantly, mechanisms for plant adaptation to cold stress can be favored through natural selection (Chen *et al*., 2014). Efficient utilization of stress-resistant accessions is a very important way for developing stress-tolerant plants. It has recently been indicated that the stress-tolerant accessions can be used as rootstocks to improve the ability of plants to adapt abiotic stress (Liu *et al*., 2013). Hitherto, grafting has become a critical strategy to enhance abiotic stress tolerance in plants. The interactions between graft, vegetable plants, and cold stress have been well studied in many plants such as in pepper, tomato and cucumber (Jang *et al*., 2013; Venema *et al*., 2008), whereas there is no researches on physiological alterations of eggplant plants after grafting and exposure to cold stress.

In the present study, two kinds of grafting rootstock were selected: one that was resistant and one that was sensitive to cold stress. The aim of this work is to explore the physiological responses to cold stress of eggplant plants grafted onto these rootstocks. Several physiological parameters were determined, including photosynthesis, chlorophyll fluorescence, and osmotic metabolites. Our study provided important evidence that grafting plants onto the cold-tolerant rootstocks will be an important tool against cold stress modulated by efficient osmotic adjustment.

Materials and Methods

The experiments were conducted in an unheated greenhouse from September to November 2014 at the Vegetables Experiment Station of Anhui Science and Technology University. The two eggplant rootstocks were 'Taibyo' (sensitive to cold) and 'Hiranasu' (resistant to cold) (Gao *et al.*, 2005), which were both introduced from Japan (BanTan Seed Company, Japan). The control was 'Jiza long eggplant' (Jinan Seed Company, Jinan, Chinese), a cold-tolerant variety used in winter cultivation that was provided by the Jinan Academy of Agricultural Sciences. In this experiment, the 'Jiza long eggplant' was the control (CK) and the grafted seedlings with 'Taibyo' and 'Hiranasu' as rootstocks were marked as T1 and T2, respectively.

Scion seeds were sown four days after the rootstock seeds were sown in order to obtain scion and rootstock with similar stem diameters. The seeds were directly sown into 0.6-L plastic pots filled with a 1:1 (v/v) growing substrate of perlite and vermiculite. The planted pots were kept in a climate chamber, maintained at a temperature of 25/18ºC (day/night). The grafting method of Lee (1994) was performed 7 weeks after the eggplant rootstock seeds were sown. Then, the grafted plant was placed in a high humidity room for subsequent healing. After 2 weeks of recovery and careful planting, plants at the 5th leaf stage were subsequently exposed to cold at a temperature of 10/5ºC (day/night) and a PPFD (photosynthetic photon flux density) of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 9 d. After the cold treatment, all plants were allowed to recover in the climate chamber at a temperature of 25/18ºC (day/night) and a PPFD of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 3 d.

Measurement of chlorophyll content: The content of chlorophyll (a+b) was determined from 1-cm in diameter leaf discs extracted with 2 ml 80% acetone (Arnon, 1949).

Analysis of chlorophyll fluorescence: Chlorophyll fluorescence was measured with a FMS-2 pulse modulated fluorometer (Hansatech, UK). After they were dark-adapted for 30 min, open leaves (which were uniformly exposed to light) were selected to measure the minimal fluorescence (Fo), the maximal fluorescence (Fm), PSII photochemical efficiency (Fv/Fm) and the quantum yield of PSII electron transport (Φ_{PSII}), with an initial light amount of 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a saturating light of 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and an action time of 1.4 s. More than fifteen leaves were measured in the light-adapted fluorescence parameters. The fluorescence parameters were all measured at illumination cultivation box conditions with a PPFD of approximately 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a temperature of 5°C.

Measurement of net photosynthetic rate at different PPFDs, CO_2 contents and temperatures: After recovery at a normal temperature for 2 hr, the 3rd functional leaf of low temperature stressed seedlings were collected to measure the net photosynthetic rate (Pn), stomatal conductance (Gs) and intercellular CO_2 concentration using a photosynthesis system (CIRAS-2, PPSYTEMS) with an ambient CO_2 concentration (Ca) of 380±4 $\mu L \cdot L^{-1}$, a temperature (Ta) of 25±0.5ºC and a photosynthetic photons flux density (PPFD) of 800±5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Then, controlling for two factors out of the aforementioned three, the photosynthetic response curves were measured for PPFD-Pn、Ca-Pn and Ci-Pn. From the PPFD-Pn curve, we could obtain the light compensation point (LCP) and light saturation point (LSP). From the Ca-Pn curve, we could obtain the CO_2 compensation point (CCP) and the CO_2 saturation point (CSP). According to the PPFD-Pn curve, we could calculate the apparent quantum efficiency (AQY) by beeline regression when the PPFD was less than 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and according to the Ci-Pn curve, we could obtain the carboxylation efficiency when the CO_2 concentration was less than 200 $\mu L \cdot L^{-1}$.

Lipid peroxidation determination and the electrolyte leakage test: Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to Sun (2006). The conductivity as a solution of leaked electrolytes before and after boiling was determined using a DDS-11 A conductometer (Shanghai Dapu Instruments, Shanghai, China).

Determination of proline and soluble protein: Proline content was determined using Bates' method (1973). Soluble protein content was estimated using Bradford's method (1976). The results were expressed as micrograms per gram FW (fresh weight) according to a standard curve.

Results and Discussion

The influence of grafting on chlorophyll content of eggplant seedlings under cold stress and recovery: Low temperature is known to cause damage to chlorophyll due to photooxidation that consequently inhibits photosynthesis (Ying *et al*., 2000). Earlier reports also indicate that the stress-tolerant genotypes of a crop species maintain higher chlorophyll content under stress conditions (Yu *et al*., 2002). The chlorophyll content of young eggplant leaves decreased gradually with low temperature stress. On the 9th day of growth, the chlorophyll contents of the control, T1 and T2 were 0.876, 0.901, and 0.960 $mg \cdot g^{-1}$FW, which were 68.8%, 74.1% and 87.4% of that of the pretreated samples, respectively (Fig. 1). After recovery, the chlorophyll contents of the eggplant seedlings increased, but the recovery capacity of T2 was most rapid. There was no significant difference between T1 and the control, which implied that grafting with cold resistant rootstocks could reduce the extent of chlorophyll decomposition during low temperature stress.

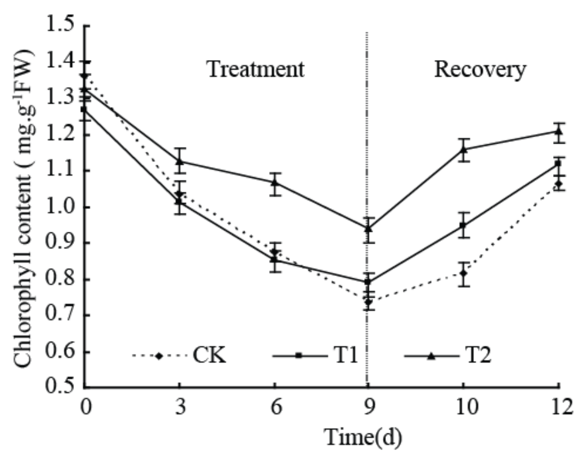

Fig. 1. Influence of cold stress (10^{o}C/5^{o}C) and recovery (25^{o}C/18^{o}C) on the chlorophyll content in grafted eggplant seedlings. CK, own-rooted seedlings, Jiza long eggplant; T1, grafted seedlings used Taibyo as rootstock; T2,

grafted seedling used Hiranasu as rootstock. The values are expressed as means ± SD for four plants per treatment.

The influence of grafting on the changes in the photosynthetic characteristics of eggplant seedlings under cold stress and recovery: The net photosynthetic rate (Pn) and stomatal conductance (Gs) measurements are commonly used to investigate the effect of seasonal climatic changes on the functioning of the photosynthetic system (Maxwell & Johnson, 2000; Öquist & Huner, 2003). Plants suffer damage when they are exposed to cold temperatures, especially if they are of tropical origin (Oliveira *et al*., 2004). Plant photosynthesis mainly relies on three physiology processes, the photosynthetic bottom CO_2 conductance, and the light and dark reactions of photosynthesis. The higher capacities of CO_2 conductance and the light and dark reactions of photosynthesis were the important physiological bases for the high photosynthetic rate of leaves. The effect of cold stress on plant photosynthesis was varied, and not only directly resulted in photosynthetic apparatus damage but also affected photosynthetic electron transfer, photosynthetic phosphorylation and enzymes related to the photosynthetic dark reaction (Liu *et al*., 2012). In cold stress, plant net photosynthetic rates decreased significantly (Liu *et al*., 2012; Liu *et al*., 2015).

Our results showed that the Pn and Gs of the eggplant seedling leaves decreased gradually with cold stress (Fig. 2). On the 9th day, the decreases in the range of CK, T1 and T2 were 82.6%, 79.5%, 53.5%, respectively. The Pn and Gs of T2 were 67.7% and 39.1%, which were higher than those of control, while those of T1 were similar to those of CK. After 3 d of recovery, the Pn and Gs were all increased and the T2 recovery capacity was highest. The trend of Ci was significantly different than that of Pn and Gs. During cold stress, the Ci increased after decreasing, and then decreased again during normal temperature recovery, which showed that the reason for the Pn decreasing in cold stress was non-stomatal limitation. In cold stress, the Pn decreasing of T2 was the least rapid, and the recovery capacity of T2 was the most rapid. These data demonstrated that grafted eggplant seedlings have a higher adaptation capacity of low temperature. Jang *et al*. (2011) found that the photosynthesis of grafted cucumber transplants during healing and acclimatization increased due to rootstock.

Influence of grafting on light requires changes in the characteristics of eggplant seedlings under cold stress and recovery: Recently, quantification of the light energy absorbed by leaves has become an important aspect in the study of photo-oxidative damage. Zhou *et al*. (2009) found that cold reduced light utilization. Our results showed that cold stress significantly reduced the apparent quantum yield of carbon assimilation (AQY) and the light saturation point (LSP) ($p<0.05$, Fig. 3), but cold increased the light compensation point (LCP) of eggplant seedlings. Under cold conditions, grafting significantly increased AQY for approximately 9 d; on the 9th day of cold stress, the AQY of grafted seedlings (T2) decreased by 32.5%, whereas the control decreased by 52.6%. After 3 days of recovery, the AQY of the grafted seedlings (T2) recovered to 97.2% of the pretreatment AQY. Cold stress increased the LCP of eggplant seedling leaves and decreased the LSP and AQY, which indicated that cold stress reduced not only the available light range but also the light utilization. However, the extent of the effect on seedlings that were grafted with cold-tolerant rootstocks was significantly less than that of ungrafted seedlings; therefore, we could conclude that grafted seedlings with cold-tolerant rootstocks increase light utilization under cold stress.

The influence of grafting on CO_2 requires changes in the characteristics of eggplant seedlings under cold stress and recovery: Consistent with the use of light energy, decreased the CO_2 assimilation of eggplant seedlings (Fig. 4), which showed that the CO_2 compensation point (CCP) of eggplant leaves in all treatments increased, but this effect was significantly alleviated by grafting; in particular, the seedlings with cold resistance rootstocks were affected less. After 3 days recovery, the CCP of the seedlings decreased, and their CO_2 saturation point and carboxylation efficiency increased. The recovery speed of T2 was the fastest, that of CK was the slowest, and that of T1 was in the middle. These results indicated that T2 had a higher ability for utilizing a wide CO_2 concentration range and that T2 exhibited a higher photosynthetic activity and CO_2 assimilation than ungrafted eggplant seedlings. A similar phenomenon was also observed in cucumbers following exposure to a suboptimal temperature (Zhou *et al*., 2009).

The influence of grafting on chlorophyll fluorescence parameter changes of eggplant seedlings under cold stress and recovery: The chlorophyll fluorescence parameters using plant chlorophyll as an inter-probe provide abundant photosynthesis information and are sensitive to adversity. These parameters are an ideal method to study and probe the effect of low temperature on plant photosynthesis in a rapid, delicate and harmless manner (Khalekuzzaman *et al*., 2015). The changes induced in the Fv/Fm, $\Phi_{PS\,II}$ and qP of eggplant seedlings by cold stress and subsequent recovery are shown in Fig. 5; the chlorophyll fluorescence parameters were reduced, but the extent of the decreases were different between the grafted and ungrafted seedlings. On the 9th day under cold, Fv/Fm and $\Phi_{PS\,II}$ of grafted seedlings (T2) decreased by 4.4% and 16.4% of the pretreatment value, and those of CK decreased by 8.0% and 29.4%, respectively. After recovery at normal temperature for 3 days, the Fv/Fm and $\Phi_{PS\,II}$ of grafted seedlings (T2) almost reached to the normal level, but those of CK and T1 only reached 93.1% and 97.8%. The change in qP was similar to that in Fv/Fm and $\Phi_{PS\,II}$. In the present study, the Fv/Fm and $\Phi_{PS\,II}$ of all treatments decreased significantly under cold stress, which reflected the decrease in PSⅡ primary light energy conversion efficiency and the damage to the photosynthetic apparatus. This result was in agreement with the reports by Zhou *et al*. (2004), Wang *et al*. (2005) and Murata *et al*. (2007). However, the amount of change of grafted seedlings with cold-tolerant rootstocks was the least and that of control was most, which reflected that grafted seedlings had different cold resistance compared to control. The qP of grafted seedlings and control decreased under cold stress, which showed that the PSⅡ primary electron acceptor (QA) was translated less from the reduction state of QA (QA-); in other words, the activity of the PSⅡ electron transport was reduced. qN reflects the photon portion that was absorbed by the PSⅡ antenna pigment, and could not be used by photosynthetic electron transport. qN was dissipated as thermal energy, and the increase in qN may function to protect the photosynthetic apparatus (Toqun *et al*., 2004). In our study, the qP decreased the least and the qN increased most in eggplant seedlings grafted with high cold resistant rootstocks, which indicated that grafted seedlings had a higher capacity to utilize light and to reduce photoinhibition in cold stress. This was confirmed by a higher net photosynthetic rate in the grafted seedling leaves compared to control leaves at low temperature.

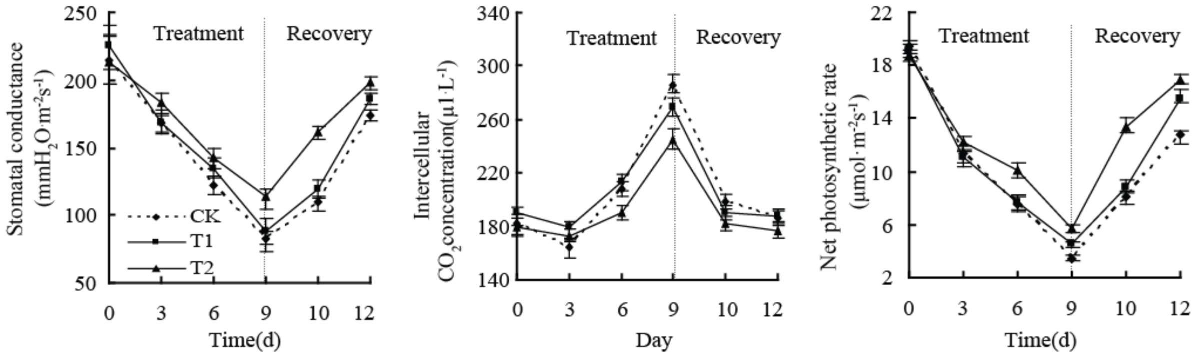

Fig. 2 Influence of grafting on the changes in leaf photosynthetic characteristics of eggplant seedlings under cold stress treatment (10°C/5°C) and recovery (25°C/18°C). All the data are presented as the mean values (±SE) of six independent measurements (n=6).

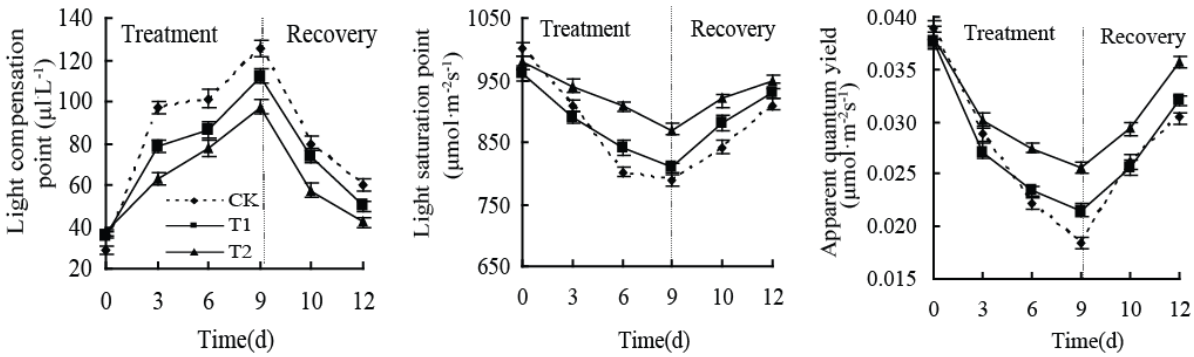

Fig. 3. Influence of grafting on the light requirement changes of eggplant seedlings under cold stress treatment (10°C/5°C) and recovery (25°C/18°C). All the data are presented as the mean values (±SE) of six independent measurements (n = 6).

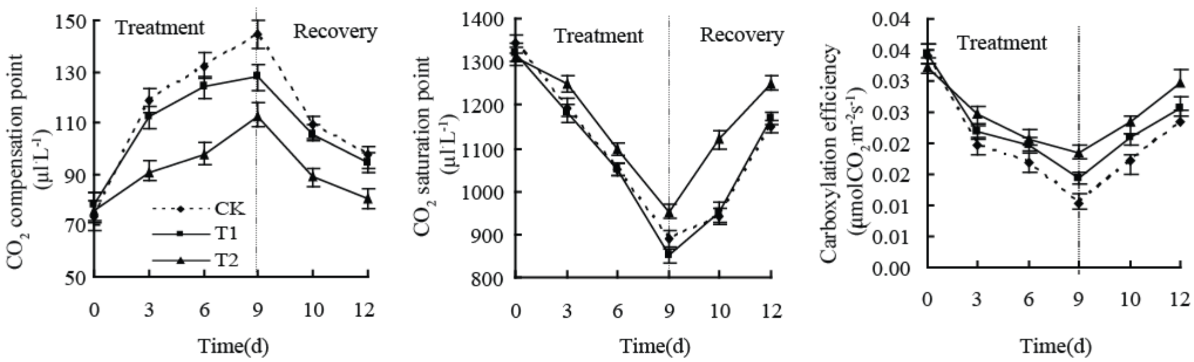

Fig. 4. Influence of grafting on the CO_2 requirement changes of eggplant seedlings under cold stress treatment (10°C/5°C) and recovery (25°C/18°C). All the data are presented as the mean values (±SE) of six independent measurements (n = 6).

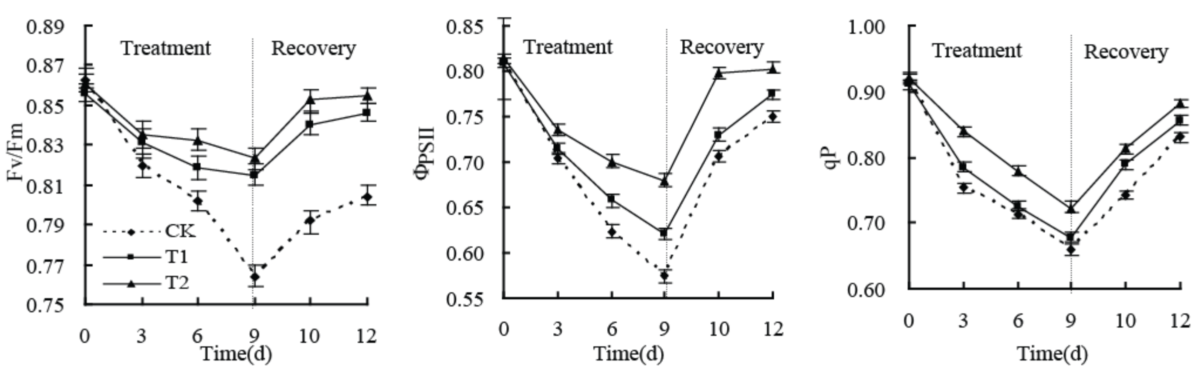

Fig. 5. Influence of grafting on the chlorophyll fluorescence parameter changes of eggplant seedlings under cold stress treatment (10°C/5°C) and recovery (25°C/18°C). All the data are presented as the mean values (±SE) of six independent measurements (n = 6).

The influence of grafting on osmoregulation substance changes of eggplant seedlings under cold stress and recovery: Free proline, soluble protein and soluble sugar are important substances involved in the osmoregulation of plants (Xi *et al*., 2013). In this study, proline and the soluble protein contents of the leaves increased in different eggplant seedlings under cold stress (10℃/5℃) and recovery (25℃/18℃) (Fig. 6). Grafting with the 'Hiranasu' rootstock (T2) increased the contents of proline and soluble protein by 13.9% and 40% under cold stress, compared to the control group, and its osmoregulation substance increased during recovery (25℃/18℃). Grafting with the 'Taibyo' rootstock (T1) had a lessened effect. Therefore, we can conclude that grafting enhanced the osmoregulation substance of eggplant seedlings during cold stress and recovery.

The influence of grafting on MDA and relative conductivity changes of eggplant seedlings under cold stress and recovery: Malondialdehyde (MDA) is an indicator of lipid peroxidation. The MDA content and relative conductivity of eggplant seedling leaves increased during cold stress (10℃/5℃) but then decreased during recovery (25℃/18℃). Compared to grafted plants, the MDA content and relative conductivity of ungrafted eggplant seedlings was significantly increased (Fig. 7); when measured on the 9th day of treatment under cold stress (10℃/5℃), the MDA content and relative conductivity of ungrafted eggplant seedlings were 16.7 nmol·g^{-1}FW and 29.2%, respectively. Grafting can promote the rapid recovery of membrane structure; furthermore, grafting with the 'Hiranasu' rootstock (T2) could restore 91.5% of the relative conductivity. These results are consistent with previous reports on two flooding-tolerant rootstocks (Arbona *et al*., 2008).

The influence of grafting on the growth of eggplant seedlings under cold stress: Zhou *et al*. (2009) found that grafting alleviated the growth inhibition of cucumber seedlings caused by cold stress. Compared to ungrafted plants, the plant height of all grafted eggplant seedlings was significantly increased (Table 1) when measured after 9 days of cold stress (10℃/5℃) and 3 days of recovery (25℃/18℃). Grafting with the 'Hiranasu' rootstock (T2) increased the plant height by 18.4%. The fresh weight of all grafted eggplant was variable under cold stress, compared with the control; the shoot fresh and root fresh weight of the T2 increased by 39.4 and 50.9%. There was no difference in the stem diameters of the eggplant seedlings. Grafting tended to increase the growth of eggplant seedlings under cold stress. Venema *et al*. (2008) demonstrated that grafting is associated with an increase in plant vigor that is observed via the increases in stem diameter and plant height.

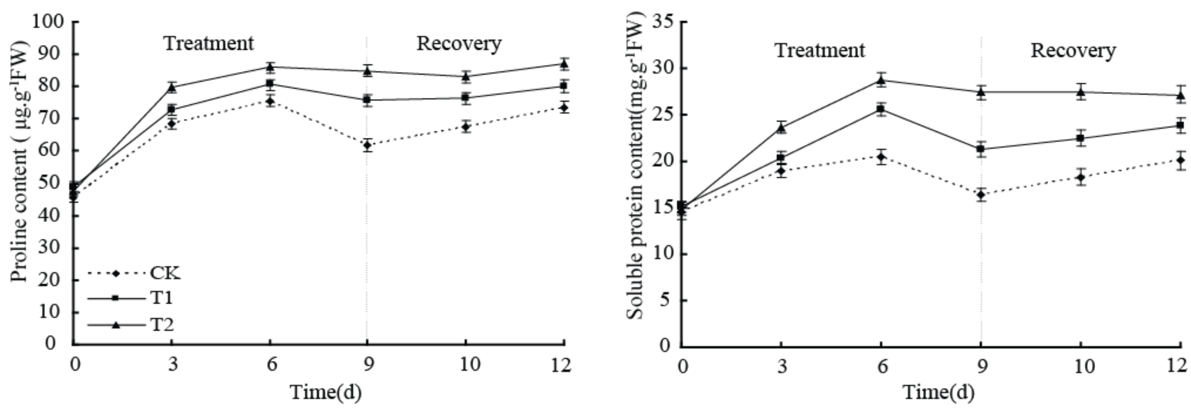

Fig. 6. Influence of grafting on proline and soluble protein content changes of eggplant seedlings under cold stress treatment (10°C/5°C) and recovery (25°C/18°C). All the data are presented as the mean values (±SE) of six independent measurements (n = 6).

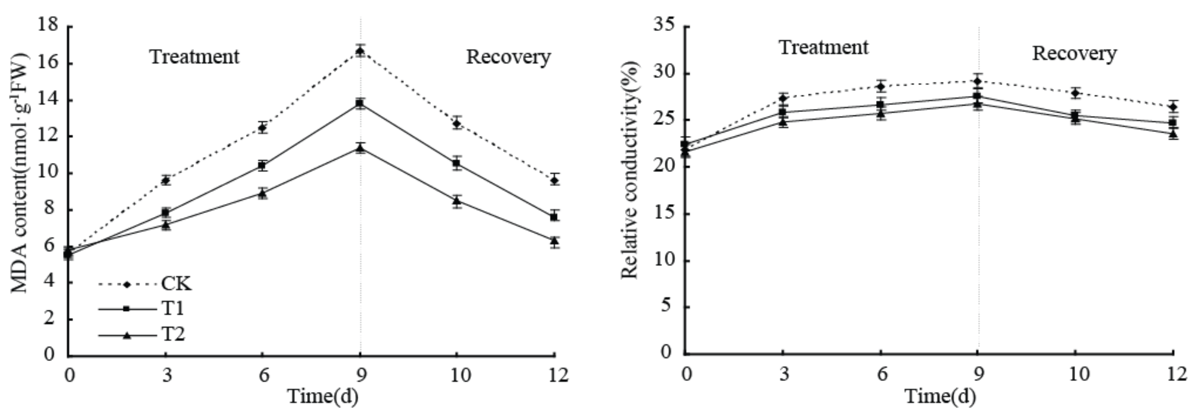

Fig. 7. Influence of grafting on MDA content and relative conductivity changes of eggplant seedlings under cold stress treatment (10°C/5°C) and recovery (25°C/18°C). All the data are presented as the mean values (±SE) of six independent measurements (n = 6).

Table 1. Effects of grafting on the growth of eggplant seedlings under cold stress (10°C/5°C) and recovery (25°C /18°C).

Treatment	Plant height (cm)	Stem diameters (cm)	Shoot fresh weight ($g\cdot plant^{-1}$)	Root fresh weight ($g\cdot plant^{-1}$)
CK	15.42c	0.35a	14.68c	4.48b
T1	17.34b	0.36a	18.24b	6.22a
T2	18.26a	0.36a	20.46a	6.76a

Note: Different letters following the data in the same column indicated a significant difference at the 0.05 level. The data were recorded during the treatments of the eggplant seedlings (28 days after grafting). The data are presented as the means ± SE (n=6).

Conclusions

Our results showed that grafting significantly alleviates the effects on the growth, photosynthetic system and osmoregulation substances under the cold stress (10/5°C) and recovery (25/18°C) of eggplant in the following ways. First, grafting promotes higher stomatal conductance and CO_2 assimilation in eggplant seedlings under cold stress. Second, grafting increases leaf chlorophyll content and the light utilization of eggplant seedlings under cold stress, especially in low light. Third, grafting enhances the osmoregulation substance of eggplant seedlings under cold stress and recovery; in particular, eggplant seedlings with cold-tolerant rootstocks exhibit higher photosynthetic activity and a lower proportion of energy dissipation compared with ungrafted eggplant seedlings. Therefore, grafting promotes the growth of eggplant seedlings under cold stress.

Acknowledgments

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