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Pumpkin Rootstocks Improve the Low-temperature Resistance of Bitter Gourd through Physiological Regulation

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Abstract. **Bitter gourd (*Momordica charantia* L.) is one of the Cucurbitaceae species that is sensitive to low temperatures. Grafting onto pumpkin rootstock is an effective technique to increase the chilling tolerance of the Cucurbitaceae species upon exposure to low-temperature stress. However, research on the mechanism by which pumpkin rootstock increases chilling tolerance of bitter gourd remains limited. In this study, bitter gourd 'Baoyu No.2' was used as the scion, and pumpkin 'Dongfang Changsheng' served as the rootstock; self-rooted seedlings are used as a control. The grafted and self-rooted bitter gourd seedlings were exposed to low-temperature stress (10 °C) for 10 days and allowed to recover for 1 day at 25 °C/20 °C (D/N). Changes in chilling damage index, osmotic regulatory substance contents, antioxidant enzyme activity, and chlorophyll fluorescence induction kinetics were determined. We found that the malondialdehyde (MDA) and proline (PRO) levels in the leaves of both self-rooted and grafted bitter gourd seedlings, as well as the activity of antioxidant enzymes, including superoxide dismutase (SOD) and peroxidase (POD), tended to increase overall with increasing duration of low-temperature stress. After 10 days of low-temperature stress, the PRO content of grafted seedlings increased by 312.77% compared with the self-rooted seedlings. The SOD activity increased by 46.19%, and the POD activity increased by 4.66%. Under low-temperature stress, the chlorophyll fluorescence induction kinetics (OJIP) curves of both self-rooted and grafted seedlings clearly changed. As the low-temperature stress duration increased, the maximum fluorescence intensity (P) decreased compared with 0 day, and the J-I-P curve tended to flatten. However, the decline and flattening for grafted seedlings were weaker than those for self-rooted seedlings. After 3 days of low-temperature stress, the F_v/F_m of the self-rooted seedlings reached its lowest point of 0.63, which was 24.10% lower and 18.18% lower than that noted at 0 day and for the grafted seedlings, respectively. The PI_{ABS} of the self-rooted seedlings reached its lowest point of 0.61, which was 86.76% lower than that noted at 0 day and 68.88% lower than the grafted seedlings. Furthermore, after 3 days of low-temperature stress, the absorption per active reaction center (*ABS/RC*) of the self-rooted seedlings increased by 25.19% compared with 0 day and 26.54% compared with the grafted seedlings. Moreover, the dissipation energy per active reaction center (DI_o/RC) of the self-rooted seedlings was 179.43% of that noted at 0 day and 108.45% greater than that of the grafted seedlings.**

Bitter gourd (*Momordica charantia* L.), also known as bitter melon or balsam pear, belongs to the Cucurbitaceae family and is one of the major vegetable crops worldwide. Bitter gourd is widely distributed in tropical, subtropical, and temperate regions, including parts of East Africa, Asia, the Caribbean, and South America (Abbas et al. 2020; Cui et al. 2020; Guo et al. 2020; Zheng et al. 2023). Bitter gourd is highly popular among consumers worldwide because of its unique flavor and nutritional value (El-Khayat et al. 2024; Yan et al. 2019), being rich in minerals, vitamins, antioxidant substances, and other nutritionally important trace elements (Bortolotti et al. 2019). In addition, aromatic glycosides, flavor-related glycosides, and other alkaloid extracts from bitter gourd stems, leaves, and fruits have been confirmed to have significant pharmacological effects, including antidiabetic, anthelmintic, antitumor, and anti-inflammatory effects (Deshaware et al. 2018; Zhang et al. 2018).

Bitter gourd prefers warm temperatures and is very sensitive to cold environments (Cui et al. 2017). The most appropriate temperature for bitter gourd is 24 to 27 °C (Solankey et al. 2021). Temperatures below 10 °C lead to slow growth and the formation of rigid seedlings, affecting the yield and its availability in the market and severely restricting the expansion of the cultivation range of bitter gourd (Liang et al. 2023; Yang et al. 2024). Furthermore, when bitter gourd is planted in early spring in China, it experiences continuous low temperatures, leading to stress and cold damage, which reduces the yield of bitter gourd and causes serious economic losses to farmers.

In crop production, agronomic practices such as chemical agent–mediated control and grafting have been widely applied to increase the cold resistance of crops. Among these techniques, grafting is a critical approach for improving the cold tolerance of cucurbit vegetables and has been widely applied in the production of watermelon (Lu et al. 2021, 2022), cucumber (Fu et al. 2023), and melon (Lang et al. 2024). However, few studies have investigated the results of grafting with appropriate rootstocks for cold tolerance in bitter gourd.

Du et al. (2016) reported that grafting could significantly improve the chilling tolerance of bitter gourd seedlings and alleviate the effect of low-temperature stress. Wang et al. (2018) reported that under low temperatures, the levels of antioxidant enzymes and antioxidants in grafted bitter gourd seedlings increased to maintain the stability of the ascorbic acid-glutathione cycle system. This helped decrease the accumulation of H_2O_2 and malondialdehyde (MDA), reducing damage from reactive oxygen species and enhancing the ability to resist low temperatures. Zou et al. (2018) reported that grafted seedlings effectively improved the contents of three osmoregulatory substances including soluble proteins, free proline (PRO), and soluble sugars under low temperature. Through the accumulation of these osmoregulatory substances, plants are able to maintain the water balance inside and outside the cells in a low-temperature environment and reduce ice crystal formation, thus enhancing their cold tolerance. Liang et al. (2023) reported that the use of pumpkin as rootstock can improve the low-temperature tolerance of bitter gourd through the regulation of sucrose and nitrogen metabolism.

Therefore, the purpose of this study was to explore the response mechanisms of grafted seedlings and self-rooted seedlings of bitter gourd under low-temperature stress and recovery conditions, with a focus on parameters such as the MDA content, PRO content, antioxidant enzyme activities, rapid chlorophyll fluorescence induction kinetics, and parameters associated with the response to low temperatures.

Materials and Methods

Plant materials. The experiments were carried out at the Vegetable Research Institute of Hunan Province in Changsha, China (28.19 N, 112.98 E), from Dec 2021 to Jun 2022, The bitter gourd variety 'Baoyu No.2' (early-maturing hybrid variety with good commercial qualities and large planting area) was used as the scion, and the pumpkin variety ‘Dongfang Changsheng’ (hybrid variety with strong cold resistance, widely used for early spring grafting seedlings of melon vegetables) was used as the rootstock. The rootstock seeds with full grains were selected, sterilized, and soaked for 6 h. Then, the seeds were germinated at 30 °C, and sown in a 50-well tray. On the fifth day after rootstock sowing, the bitter gourd seeds were soaked for 12 h, germinated at 30 °C, and then sown in a 50-cell tray. The seedling substrate was composed of peat:vermiculite:perlite at a ratio of 3:1:1 by volume. The plant growth chamber (JP19150, temperature range −6 °C to 40 °C, accuracy 0.1 °C, error ± 0.5 °C, JIUPO Biotechnology Co., Ltd., Fujian, China) was maintained at 25 °C/20 °C (D/N), with a 14 h photoperiod and a photosynthetic photon flux density (PPFD) of 300 μmol·m^{-2}·s^{-1}.

Grafting. Grafting was performed when the rootstock had one leaf, and the first two true leaves of the scion were unfolded. A “hole insertion grafting” procedure previously described by (Lee and Oda 2003) was used in this study. Grafted seedlings were managed as follows: 0 to 3 d, 28 °C/23 °C (D/N), 14-h photoperiod, PPFD 75 μmol·m^{-2}·s^{-1}, covered with plastic film; 4 to 6 d, 25 °C/20 °C (D/N), 14 h photoperiod, PPFD 150 μmol·m^{-2}·s^{-1}, plastic film opened for 1 to 2 h every 4 to 6 h during the 14 h of light exposure; and 7 to 8 d, 23 °C/18 °C (D/N), 14-h photoperiod, PPFD 225 μmol·m^{-2}·s^{-1}, plastic film opened during the 14 h of light exposure. On the ninth day after grafting, the plants were maintained at 25 °C/20 °C (D/N), 14-h photoperiod, PPFD 300 μmol·m^{-2}·s^{-1}.

Low-temperature treatment. At the third-leaf stage, the seedlings were exposed to 10 °C for 10 days and assessed for recovery at 25 °C/20 °C (D/N) for 1 day. The light settings remained as follows: 14-h photoperiod and PPFD 300 μmol·m^{-2}·s^{-1}. After the preliminary experiments, the temperature was set to 10 °C for the low-temperature stress test. There were two treatments, namely, grafted seedlings and self-rooted seedlings, with 50 plants in each treatment and three repetitions. The self-rooted seedlings experienced the same environmental conditions as the grafted seedlings before being subjected to low-temperature treatment.

Measurement of the chilling damage index. The chilling damage index (CI) was measured as described by (Xu et al. 2016). The degree of chilling tolerance was measured as follows: level 0, no symptoms; level 1, chlorosis or crinkling at the edge of old leaves; level 2, chlorosis or crinkling at the edge of less functional leaves; level 3, chlorosis or crinkling at the edge of functional leaves with healthy new leaves; level 4, chlorosis or crinkling and wilting of functional leaves with damaged new leaves; and level 5, severe damage to new leaves, wilting, or death. The CI was calculated according to the following formula: CI = (0 × number of plants with level 0 + 1 × number of plants with level 1 + 2 × number of plants with level 2 + 3 × number of plants with level 3 + 4 × number of plants with level 4 + 5 × number of plants with level 5)/total number of measured plants.

Measurement of chlorophyll fluorescence induction kinetics. Chlorophyll fluorescence was monitored with a FluorPen FP100 instrument (Photon Systems Instruments, Drasov, Czech Republic). Following the manufacturer’s protocols, after 30 min of dark adaptation, the Kautsky curve was recorded and analyzed according to the OJIP test via FluorPen 1.0.0.6 software (Kasampalis et al. 2021). The parameters, including the maximum photochemical efficiency (F_v/F_m), photosynthetic performance index (PI_{ABS}), specific energy flux per reaction center (RC) for energy absorption (*ABS/RC*), trapped energy flux (TR_o/RC), electron transport energy flux (ET_o/RC), and dissipated energy flux (*DIo/RC*) were obtained (Kalhor et al. 2018; Kasampalis et al. 2020). The saturation light intensity was set at 3000 μmol·m^{-2}·s^{-1}, and the actinic light intensity was set at 1000 μmol·m^{-2}·s^{-1}. Three seedlings were randomly selected for each treatment, with three replicates.

Measurement of osmotic regulatory substance contents and antioxidant enzyme activity. Leaf samples were collected at 0, 1, 3, 5, 7, and 10 d after low-temperature treatment and 1-day recovery (R1d) and stored at −80 °C until further analysis. Each treatment consisted of three replications, with three plants per replication. The malondialdehyde (MDA) content was determined using an MDA assay kit (TBA method), and the content of MDA was calculated using the difference in absorbance at 532 nm and 600 nm. The proline (PRO) content was determined using a PRO assay kit (ninhydrin method), The absorbance values were measured at 520 nm. The SOD activity was determined using a SOD assay kit (WST-8 method). For SOD activity, 50% inhibition of the xanthine oxidase reductase reaction was defined as one unit of enzyme activity. The absorbance values were measured at 450 nm. The peroxidase (POD) activity was determined using a POD assay kit (guaiacol method). For POD activity, a decrease in the absorbance at 470 nm by 0.01 within 1 min was taken as one unit of enzyme activity. All kits were purchased from Suzhou Keming Biotechnology Co., Ltd., in Suzhou, China. The measurement methods followed the manufacturer’s instructions.

Statistical analysis. Statistical analysis was performed via the statistical software DPS (version 7.05), and the differences between self-rooted seedlings and grafted seedlings were evaluated via *t* tests ($P \leq 0.05$).

Results

Effects of low-temperature stress on the CI of bitter gourd seedlings. As shown in Table 1, grafting had a significant effect on the CI of bitter melon seedlings under low-temperature stress. After 1 d of low-temperature stress, neither the grafted nor the self-rooted seedlings experienced apparent chilling jury. As the duration of low-temperature stress increased, the CI of the self-rooted and grafted seedlings gradually increased. Nevertheless, the increase of the grafted seedlings was significantly lower than the self-rooted seedlings. Notably, the CI of the grafted seedlings was significantly or highly significantly lower than the self-rooted seedlings after 3, 5, 7, and 10 d of low-temperature stress.

Effects of low-temperature stress on the MDA and PRO levels of bitter gourd seedlings. Overall, the MDA content in the leaves of both self-rooted and grafted bitter gourd seedlings increased slightly with prolonged low-temperature stress (Fig. 1). After 1 d of low-temperature stress, the MDA content of the self-rooted seedlings peaked at 40.28 nmol/g and showed an increase of 17.72% compared with that at 0 d. After 5 d of low-temperature stress, the MDA content of the grafted seedlings peaked at 47.14 nmol/g and showed an increase of 21.67% compared with that at 0 d. In addition, the MDA content was 24.19% greater than that of the self-rooted seedlings. After 1 d of recovery, the MDA content of the grafted and self-rooted seedlings returned to the levels observed at 0 d of low-temperature stress. After 1 d of low-temperature stress, the PRO content of self-rooted and grafted bitter gourd seedlings rapidly increased and peaked. The PRO content of the self-rooted seedlings was 80.79 μg/g, demonstrating an increase of 88.77% compared with that at 0 d. In contrast, the PRO content of the grafted seedlings was 153.79 μg/g, which was 109.70% greater

Table 1. Chilling damage indices of bitter gourd seedlings after low-temperature stress.

	0 d	1 d	3 d	5 d	7 d	10 d	R1d
Grafted	0 ± 0	0 ± 0	0.01 ± 0.01	0.03 ± 0.01	0.09 ± 0.02	0.11 ± 0.02	0.11 ± 0.02
Self-rooted	0 ± 0	0.03 ± 0.03	0.08 ± 0**	0.18 ± 0.07*	0.28 ± 0.05**	0.47 ± 0.06**	0.47 ± 0.06**

*, ** Significant at $P < 0.05$ or 0.01, respectively.

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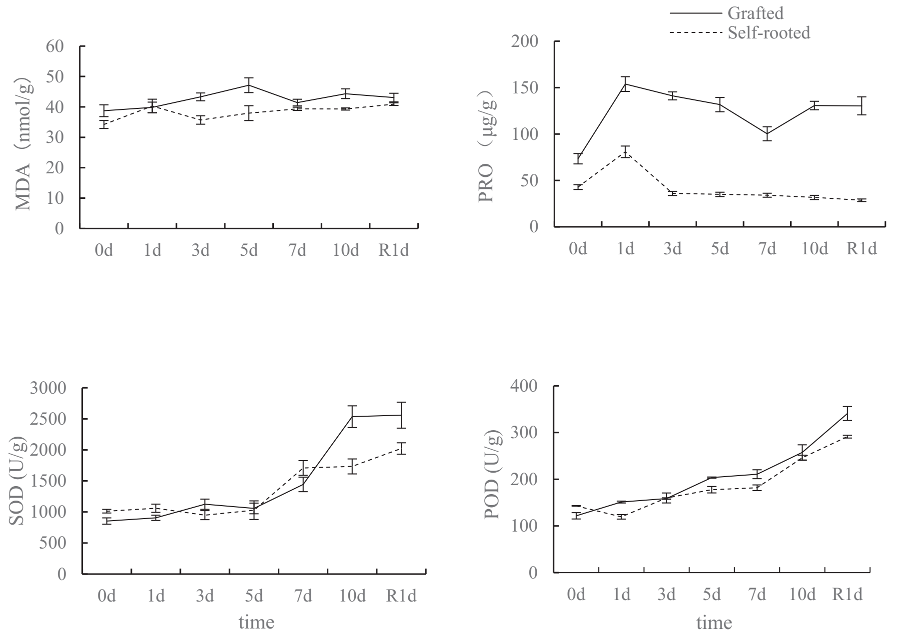

Fig. 1. Osmoregulatory substances and antioxidant enzyme activity of bitter gourd seedlings after low-temperature stress.

than that at 0 d and 90.69% greater than self-rooted seedlings. Compared with self-rooted seedlings, grafted seedlings consistently maintained significantly greater PRO levels. After 10 d of low-temperature stress, the PRO content of the self-rooted seedlings was 31.65 μg/g, while the PRO content of the grafted seedlings was 130.66 μg/g, which was 312.77% greater than that of the self-rooted seedlings. After 1 d of recovery, the PRO content in the self-rooted seedlings slightly decreased, returning to the level observed at 0 d, whereas the PRO content in the grafted seedlings remained relatively high.

Effects of low-temperature stress on the SOD and POD activities of bitter gourd seedlings. Under low-temperature stress, both the SOD and POD activities increased (Fig. 1). From 0 d to 7 d of low-temperature stress, there was no significant difference in SOD activity between the self-rooted and grafted seedlings. After 10 d of low-temperature stress, the SOD activity of the grafted seedlings peaked at 2534.27 U/g, which was 46.19% greater than the self-rooted seedlings and 196.79% greater than that observed at 0 d. After 1 d of recovery, the SOD activity of the grafted seedlings remained unchanged, whereas the SOD activity of the self-rooted seedlings rapidly increased, coming closer to the value observed for grafted seedlings. After 1 d of low-temperature stress, the POD activity of the grafted seedlings slightly decreased by 16.42% compared with 0 d and continued to increase with increasing low-temperature stress duration. After 10 d of low-temperature stress, the POD activity of the self-rooted seedlings peaked at 246.00 U/g, which was 72.09% greater than that noted at 0 d. The POD activity of the grafted seedlings also peaked at 257.48 U/g, with levels 111.62% greater than that at 0 d and 4.66% higher than the self-rooted seedlings. After 1 d of recovery, the POD activity of the grafted and self-rooted seedlings continued to increase, with no significant difference between the two treatments.

Effects of low-temperature stress on the chlorophyll fluorescence-induced kinetic curve of bitter gourd seedlings. Figure 2 shows that under low-temperature stress, the shapes of the OJIP (rapid chlorophyll fluorescence induction kinetics) curves of leaves from both self-rooted and grafted bitter gourd seedlings changed significantly. With prolonged low-temperature stress, the maximum fluorescence intensity (P) decreased compared with that noted at 0 d, and the J-I-P curve tended to flatten. The decrease in amplitude and flattening trend of the grafted seedlings were weaker than the self-rooted seedlings. After 1 d of recovery, the OJIP curves of self-rooted and grafted plants reverted to the levels observed at 0 d.

Specifically, after 0 d of low-temperature stress, there was minimal difference in the OJIP curves between self-rooted seedlings and grafted seedlings (Fig. 3A). After 1 to 7 d of low-temperature stress, the P phase and J-I-P amplitude of the OJIP curve of self-rooted seedlings decreased, and the difference from grafted seedlings gradually increased (Fig. 3B–E). After 5 d of low-temperature stress, the P phase of self-rooted seedlings decreased to the lowest value, as did the J-I-P amplitude of the curve (Fig. 3D). In contrast, after 10 d of low-temperature stress, the P phase and J-I-P curve of the grafted seedlings decreased to the lowest level but were still significantly greater than the self-rooted seedlings (Fig. 3F), indicating that the grafted seedlings caused less damage to the photosynthetic apparatus under low-temperature stress. After 1 d of recovery, the OJIP curves of the self-rooted and grafted seedlings reverted to the levels observed at 0 d (Fig. 3G).

Effects of low-temperature stress on the maximum photochemical efficiency and photosynthetic performance index of bitter gourd seedlings. At baseline (0 d), the maximum photochemical efficiency (F_v/F_m) and the PI_{ABS} showed no significant difference between self-rooted and grafted bitter gourd seedlings (Fig. 4). With prolonged low-temperature stress, both the F_v/F_m and PI_{ABS} of self-rooted and grafted bitter gourd seedlings showed a "decrease-increase-decrease" trend. The F_v/F_m and PI_{ABS} of the grafted seedlings were consistently greater than those of the self-rooted seedlings under low-temperature stress. After 1 d of recovery, the F_v/F_m and PI_{ABS} recovered to or slightly exceeded the values observed at 0 d. After 3 d of low-temperature stress, the F_v/F_m of the self-rooted seedlings reached its lowest point of 0.63, which was 24.10% lower than that noted at 0 d and 18.18% lower than the grafted seedlings. However, the decrease of the grafted seedlings was not obvious, only decreasing to 0.77 at 3 d and 10 d, which did not significantly differ with that noted at 0 d. The PI_{ABS} of the self-rooted seedlings reached its lowest point of 0.61, which was 86.76% lower than that noted at 0 d and 68.88% lower than the grafted seedlings.

Effects of low-temperature stress on the phenomenological energy fluxes per RC of bitter gourd seedlings. Under low-temperature stress, the ABS/RC and DI_o/RC of the grafted seedlings showed no significant changes, but the self-rooted seedlings increased obviously (Fig. 4). After 3 d of low-temperature stress, the ABS/RC of the self-rooted seedlings showed

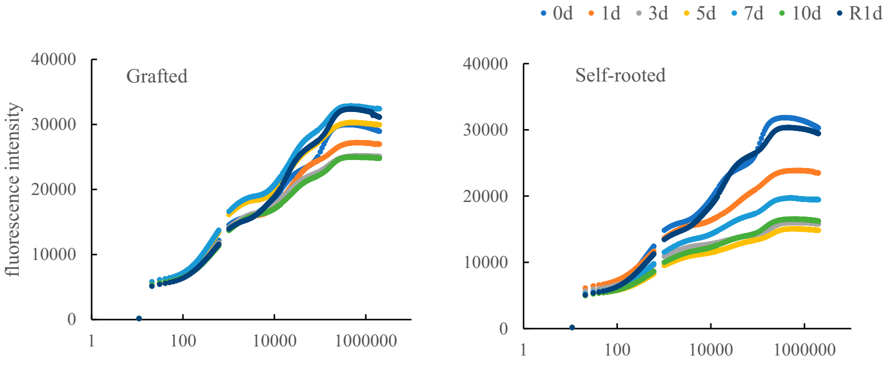

Fig. 2. Chlorophyll fluorescence-induced kinetic curve of bitter gourd seedlings after low-temperature stress(1).

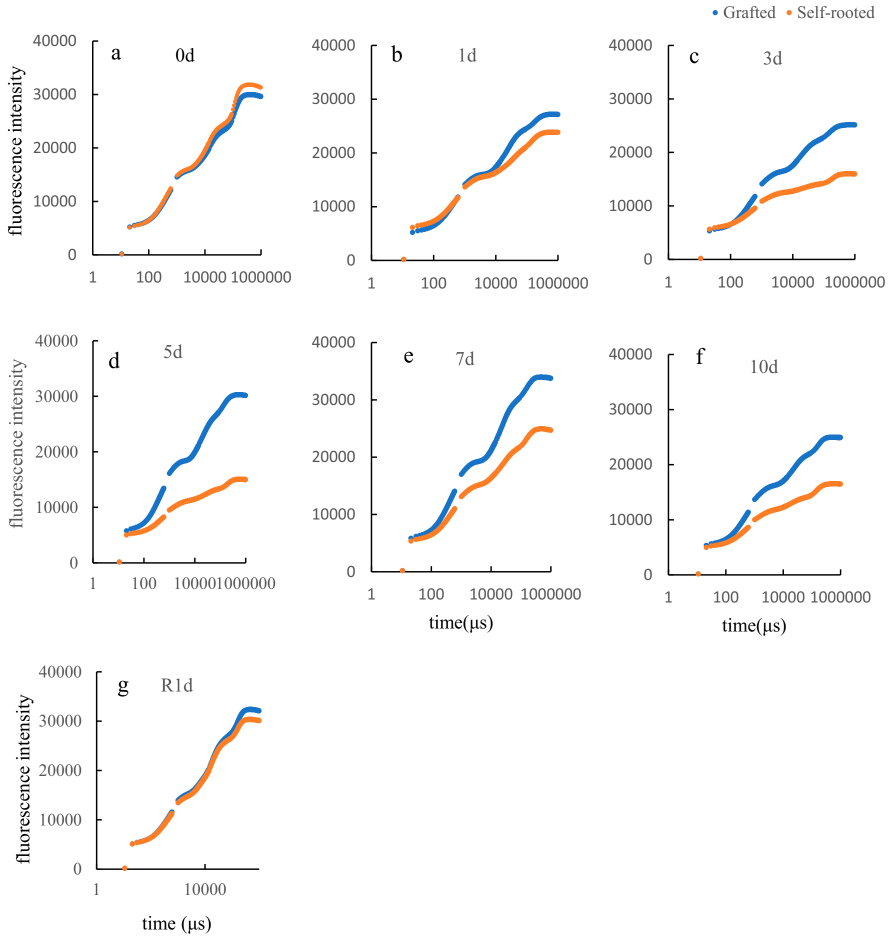

Fig. 3. Chlorophyll fluorescence-induced kinetic curve of bitter gourd seedlings after low-temperature stress(2).

a 25.19% increase compared with that noted at 0 d and a 26.54% increase compared with the grafted seedlings. Moreover, the DI_o/RC of the self-rooted seedlings showed a 179.43% increase compared with that noted at 0 d and a 108.45% increase compared with the grafted seedlings. Under low-temperature stress, the ER_o/RC and TR_o/RC of the grafted and self-rooted seedlings decreased. After 5 d of low-temperature stress, the ER_o/RC and TR_o/RC of the grafted seedlings were significantly greater than the self-rooted seedlings. After 1 d of recovery, all the indicators of the grafted and self-rooted seedlings returned to the levels noted at 0 d.

Discussion

Previous studies have suggested that grafting can improve the cold tolerance of plants, mainly because it can also increase the stability of cell membranes and osmotic regulation ability, increase antioxidant enzyme activity (Oustric et al. 2017), improve chloroplast stability, and increase photosynthetic adaptation to cope with low-temperature stress (Shi et al. 2019). Grafted seedlings have greater photosynthetic activity and a lower energy dissipation rate, that is, the electron flux in PSII is lower than the energy dissipation rate through light dependence or constitutive heat dissipation and fluorescence (Zhou et al. 2009).

Leaf CI can visually reflect the degree of damage to seedling leaves caused by low-temperature stress. In this study, we found that the CI of grafted seedlings was significantly lower than that of self-rooted seedlings after low-temperature stress. This phenotype revealed that grafting pumpkin rootstock onto bitter gourd can enhance the ability of bitter gourd seedlings to resist low-temperature stress.

MDA is a product of membrane lipid peroxidation in plant organs under low-temperature stress, indicating damage to plant cell membranes. In many plants, the MDA levels increase under low-temperature stress (Huang et al. 2018). In this study, it was observed that under 10 °C stress, the leaf MDA levels of self-rooted and grafted plants slightly increased with prolonged exposure to low temperatures. Further research is needed to determine the correlation between MDA levels and cold tolerance induced by pumpkin rootstock grafting in bitter gourd seedlings.

PRO is a multifunctional amino acid involved in numerous cellular signaling pathways in plants (Rehman et al. 2021). The main role of PRO is its involvement in osmoregulation, and the stabilizing of sub-cellular structures and scavenging of free radicals (Pociecha et al. 2008). It accumulates under a variety of stresses, and triggers or participates in cellular defense (Li et al. 2023). The PRO content is often used as an indicator of plant cold resistance, with higher levels indicating greater cold stress resistance (Guan et al. 2023). Resistant varieties tend to accumulate relatively high levels of PRO (Jiao et al. 2021). In this study, after 10 d of low-temperature stress, the PRO content in the leaves of grafted seedlings was 312.77% higher than that in self-rooted seedlings, indicating that grafting seedlings can effectively increase the content of PRO under low-temperature stress, thereby improving the cold resistance of bitter gourd seedlings. This finding is consistent with previous research in bitter gourd by Zou et al. (2018).

SOD and POD are key components of the plant membrane protection system, helping to eliminate reactive oxygen species and maintain active oxygen metabolism balance to enhance plant cold resistance (Jan et al. 2018; Wan et al. 2015; Wang et al. 2016). Studies on bitter gourd have shown that the levels of SOD and POD activities can be used as indicators of cold tolerance in bitter gourd (Chen et al. 2017; Niu et al. 2020). In this study, after 10 d of low-temperature stress, the SOD activity and POD activity in the leaves of grafted seedlings increased by 46.18% and 4.66%, respectively, compared with those in self-rooted seedlings. This finding suggests that pumpkin rootstock-grafted seedlings mainly increase the SOD activity under low-temperature stress, thereby enhancing the cold resistance of bitter gourd seedlings, aligning with the findings of Du et al. (2016) in bitter gourd.

The use of chlorophyll fluorescence dynamics measurement with a rapid and nondestructive probe for the assessment of function has become increasingly popular in studying plant photosynthetic function and the effects of various stresses on plant (Bednaříková et al. 2020). Chlorophyll fluorescence induction kinetic (OJIP) curves and their parameters can intuitively reflect the extent of plant damage under stress (Lefi et al. 2023; Yang et al. 2023). Among these parameters, the maximum photochemical efficiency (F_v/F_m) is one of the most frequently used parameters to assess plant health and photosynthetic performance (Shin et al. 2021). In healthy leaves, the F_v/F_m ratio typically remains stable at around 0.8 and is not influenced by species or environmental conditions. This ratio may decrease in response to stressors like drought, high temperature, and low temperature, but can about recover to its original level with appropriate recovery treatments (Chiango et al. 2021). PI_{ABS} is also a widely used metric for measuring nonbiological stress reactions and plays a crucial role in assessing the photosynthetic efficiency performance (Bano et al. 2021; Mendes et al. 2021; Sousaraei et al. 2021). In this study, it was found that the F_v/F_m ratio changed significantly under low-temperature stress; however, its fluctuation amplitude was less pronounced compared with PI_{ABS}. PI_{ABS} was demonstrated to be more sensitive to low temperatures than F_v/F_m. When used in

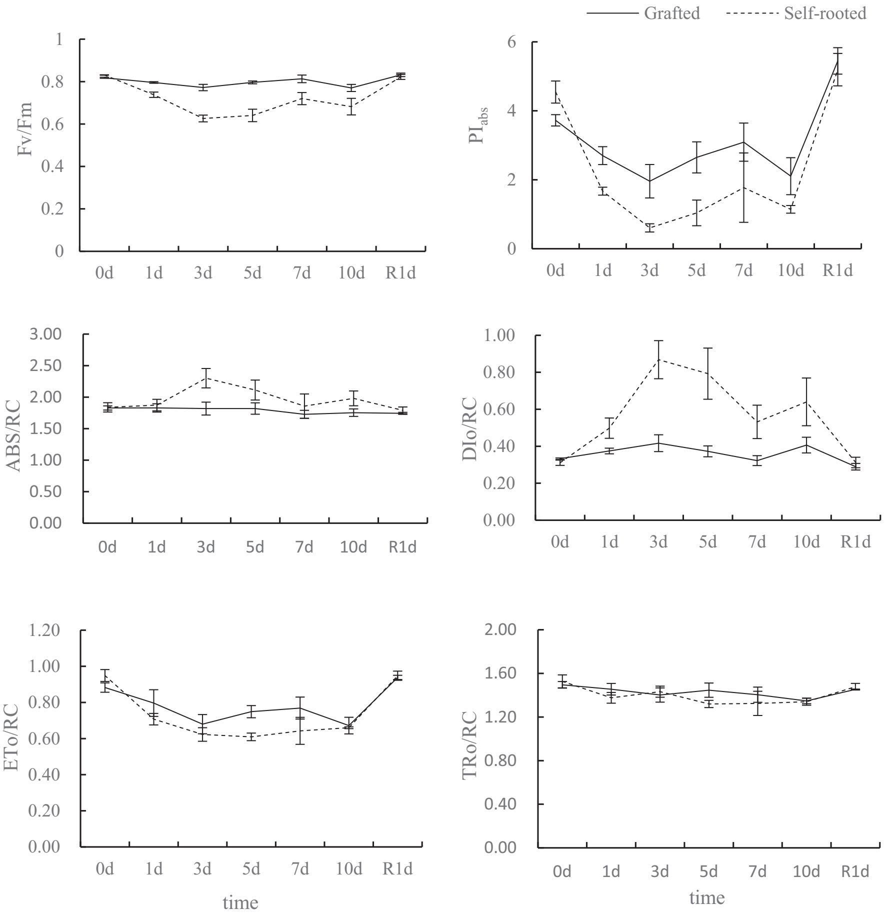

Fig. 4. Chlorophyll fluorescence parameters of bitter gourd seedlings after low-temperature stress.

conjunction, these metrics can provide a more comprehensive assessment of the plant's response to low temperatures. After 10 d of low-temperature stress, the decreases in F_v/F_m and PI_{ABS} in the leaves of grafted seedlings were significantly lower than self-rooted seedlings. This study also found that self-rooted seedlings exhibited a significant increase in DI_o/RC under low-temperature stress, suggesting the activation of defense mechanisms in reaction centers; this led to decrease in the energy share for electron transfer, resulting in inhibition electron transfer and increased heat dissipation. In contrast, grafted seedlings showed minimal changes in heat dissipation (DIo/RC) under the same stress conditions. Furthermore, the grafted seedlings exhibited significantly smaller changes in ABS/RC, ET_o/RC, and TR_o/RC compared with the self-rooted seedlings, suggesting that the photosynthetic performance of the grafted seedlings was more stable under low-temperature stress.

Conclusion

In summary, pumpkin rootstock grafting can improve the low-temperature resistance of bitter gourd seedlings by increasing the PRO content under low-temperature stress, increasing SOD activity, and stabilizing leaf photosynthetic performance.

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