Effect of low temperature stress on the growth and development of grafted pepino seedlings

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Abstract: Solanum muricatum (Pepino) is a solanaceous crop gaining popularity but susceptible to biotic and abiotic stress in cultivation. Grafting has been shown to improve the plant's adaptability to adverse conditions. The results showed that the In terms of The grafted cantaloupe eggplant showed stronger resistance to low-temperature stress, the increase in relative conductivity of cantaloupe eggplant autografted seedlings was significantly higher than that of grafted seedlings, and both MDA contents were significantly lower than that of cantaloupe eggplant autografted seedlings, and the low-temperature tolerance of the grafted seedlings became more pronounced with the prolongation of the stress time.When assessing resistance to low-temperature stress, grafted pepinos showed enhanced tolerance compared to non-grafted cuttings, likely due to synergistic effects between scion and rootstock during the grafting process. These results suggest that grafting can significantly improve pepino's low-temperature tolerance.

Keywords: Pepino; Grafting; Low Temperature Stress

1. Background

Pepino (Solanum muricatum) a plant from the Solanaceae family, is valued for its unique taste and nutritional benefits. However, its limited genetic diversity poses challenges in enhancing resilience to diseases and environmental stressors [1-3].Solanaceous crops face numerous diseases and environmental stressors that compromise their resilience when grown under natural conditions,Pepino closely related to tomatoes and potatoes, is similarly affected. While tomatoes have been extensively studied for grafting, with a substantial body of research on its molecular underpinnings[4,5], pepino's limited genetic diversity and germplasm resources pose challenges in enhancing resistance through genetic improvement. Cold stress is one of the key environmental factors that severely affect plant growth and development,especially for Pepino, Originating from South America,Pepino is sensitive to low temperatures. The best growing conditions for pepino are between 25 and 28 °C. When the temperature drops to 10 °C,Pepino stops growing and it even dies at temperatures below 1 °C,Extreme weather frequently occurs in China,especially during the seedling stage, such as late autumn, and early spring, in which pepino usually suffers cold stress[6].Thus, it is hard to grow the fruit and obtain high yields. Therefore, the study of how to improve cold resistance in pepino has become a vital part of pepino breeding projects. One way to improve cold tolerance is to graft plants onto rootstocks with higher cold tolerance. Grafting is a recognized method for improving resistance to biotic and abiotic stress,offering solutions to problems like continuous cropping, growth regulation, disease resistance,and yield and quality enhancement[7-9]. Furthermore, it has additional advantages in rootstock. Choosing the appropriate rootstock for Pepino cultivar has not received much attention from the scientific community,rootstocks affected plant vigor, nutritional status and overcoming biotic and abiotic stress[10, 11]The integration of rootstocks from diverse varieties through grafting can enhance growth, resistance, yield, and fruit quality. Despite the potential benefits,research into effective rootstock combinations for pepino grafting is sparse, particularly under suboptimal environmental conditions [12, 13]The goal is to use quality rootstocks for grafting to enhance disease resistance and promote plant growth. By identifying the best rootstock-scion combinations,we aim to study growth, resistance, and development patterns of grafted pepino under challenging conditions.

2.Materials and Methods

2.1 Plant materials

In this investigation,pepino cultivars from the Qinghai Academy of Agriculture and Forestry were studied: the oval-fruited "Qing-

ChanXiang" (LOF) and eggplant rootstock ("Moxi";E2).

These three rootstocks are commonly used for grafting Solanaceae vegetables. The research took place at the horticultural experimental station of Qinghai University's College of Agricultural and Forestry Sciences from June to December 2023. Pepino scions were grafted onto plants with lateral branches using the casing and sticking method, followed by a week of shading for healing. Grafted pepino plants were tested for by measuring leaf conductivity, levels, and enzyme activity.

3. Measurements

3.1 Leaf Nutrient Content

3.1.1 Photosynthetic Pigment Content

Chlorophyll content was extracted from fresh leaves using 95% anhydrous ethanol. The leaves were soaked in ethanol for 24 hours in darkness and absorbance was measured at 665 nm, 649 nm, and 470 nm

3.1.2 Antioxidant Enzyme Activity

The activity of antioxidant enzymes (POD, CAT, SOD, and MDA) was measured in the fourth fully expanded leaf from the apex of the grafted plants using Solarbio kits (BC00095, BC0205, and BC5156).

3.1.3 Relative Conductivity

Relative conductivity of the leaves was assessed with a DDSJ-308F conductivity meter. Fresh leaf samples (0.1 g) were soaked in 10 mL deionized water for 12 hours. Initial conductivity (R1) was recorded, followed by a second reading (R2) post a 30-minute boiling water bath and cooling. Relative conductivity was calculated as: Relative conductivity= (R1/R2) *100%.

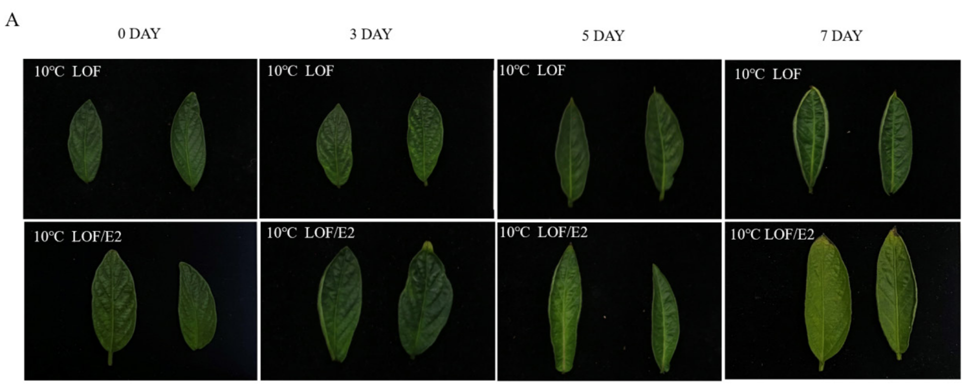
4.Statistical analysis

Data were analyzed using SPSS 20.0 software, with the t-test applied for significance analysis ($p < 0.05$). Correlation analysis employed the Pearson method. Bar charts were created using GraphPad Prism 8.0.

5.Results

5.1 Leaf biofilm permeability

Analysis reveals that after 7 days of low-temperature stress(Fig.1), pepino self-rooted seedlings exhibit a 1.2 times increase in relative conductivity compared to pre-stress levels, with grafted seedlings also showing a similar trend of rising conductivity. This suggests a significant increase in conductivity for self-rooted seedlings compared to grafted ones during low-temperature stress. The study indicates that grafting intensifies the impact of low temperatures on pepino production, with grafted seedlings demonstrating improved cold resistance as stress duration extends. Grafting appears to enhance pepino's ability to withstand low temperatures, as evidenced by the increased leaf curling in cutting seedlings compared to grafted ones after 7 days of 10°C treatment. This highlights the effectiveness of selected scion-rootstock combinations in enhancing cold resistance in pepino cultivation in regions like Qinghai. Overall, examining leaf cuticle permeability in grafted pepino seedlings under low-temperature stress holds practical significance for improving cultivation in cold climates.



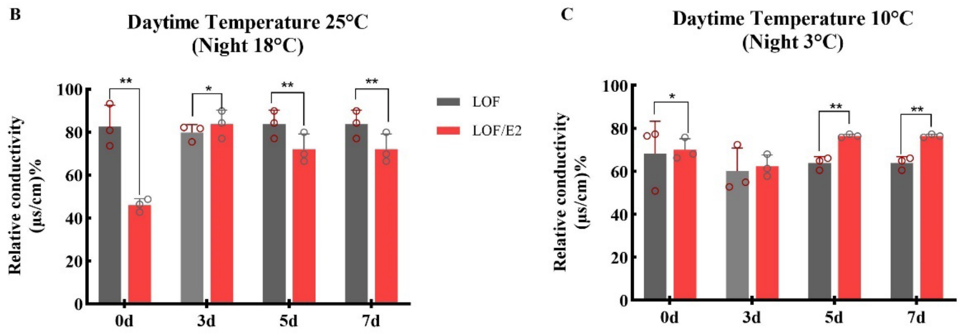


Figure 1. Response of relative conductivity in grafted pepino under low temperature stress. (A)Leaf morphology under low temperature treatment at 10°C; (B) Relative leaf conductivity of grafted pepino seedlings and cuttings under daytime temperature 25°C and nighttime temperature 18°C on the 3rd, 5th, 7th, and 9th day; (C) Relative leaf conductivity of grafted pepino seedlings under daytime temperature 10°C and nighttime temperature 3°C on the 3rd, 5th, 7th, and 9th day. "*" and "**" indicate significant difference and extremely significant difference ($P<0.05$, $P<0.01$).

5.2 Chlorophyll content under low temperature stress

Low temperatures can accelerate chloroplast degradation and impede their synthesis, reflecting the degree of plant damage. With Figure 2, we find that with grafted seedlings showing notably higher levels than the rootstock and experiencing the smallest decrease. Additionally, the ratio of photosynthetic pigments changed after 3-7 days of low-temperature stress, resulting in varying degrees of decrease in carotenoids, chlorophyll a, and chlorophyll b. These findings indicate that low temperatures can degrade plant chlorophyll and hinder its synthesis. Grafted seedlings demonstrate stronger resistance to chlorophyll degradation compared to self-rooted seedlings, suggesting that grafting can enhance plants' adaptability to low temperatures and reduce chlor. This reduction in chlorophyll levels may negatively impact plant photosynthesis and energy conversion processes, further underscoring the beneficial role of grafting in enhancing plant resistance to lowtemperature

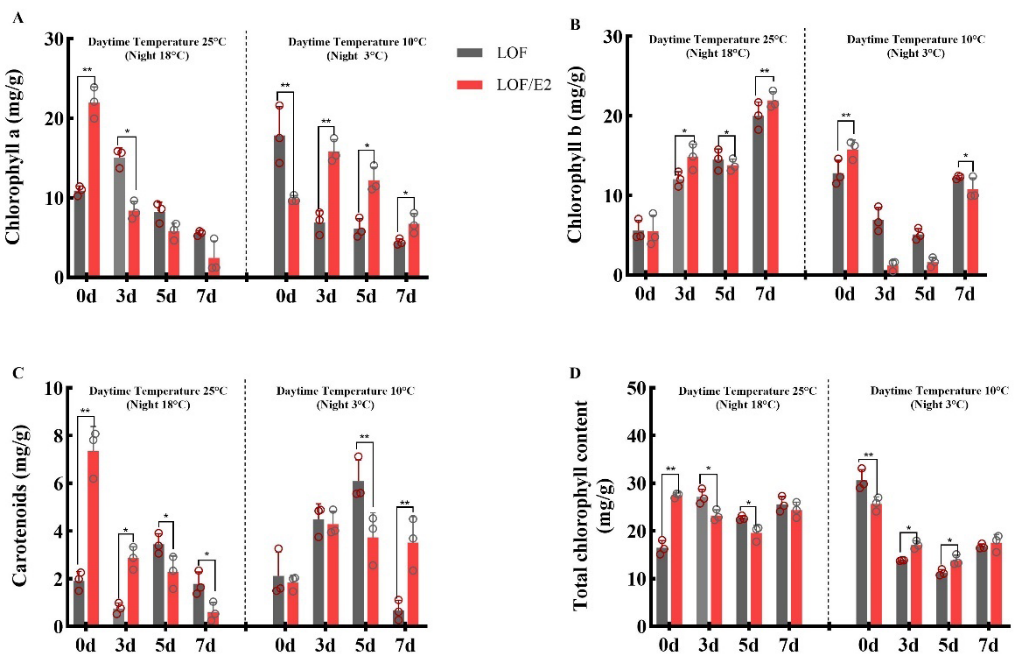


Figure 2. Response of photosynthetic pigments in grafted pepino under low temperature stress. (A) Chlorophyll a content of leaf of pepino cuttings and grafted seedlings on the 3rd, 5th, 7th, and 9th day; (B) Chlorophyll b(C) Carotenoid content (D) Total chlorophyll conten.

5.3Antioxidant enzyme activities

At low temperatures, the balance of reactive oxygen species metabolism is disrupted, posing potential harm to crops. Plant antioxidant enzymes such as SOD, CAT, and POD play a crucial role in eliminating excess reactive oxygen species and protecting plants from oxidative damage, forming the primary cold-resistant enzyme system in plants. When not exposed to low-temperature treatment, the activities of SOD, POD, and CAT enzymes in the leaves of both grafted and self-rooted seedlings are similar and consistent. However, after low-temperature stress, the activities of these three enzymes gradually decrease with prolonged stress time in both grafted and self-rooted seedlings. the MDA content in the leaves of both grafted and pepino self-root seedlings increases with days under low-temperature stress, with pepino self-root seedlings showing the lowest increase, significantly lower than grafted seedlings. This suggests that grafting pepino enhances the plant's ability to scavenge free radicals under adverse conditions.

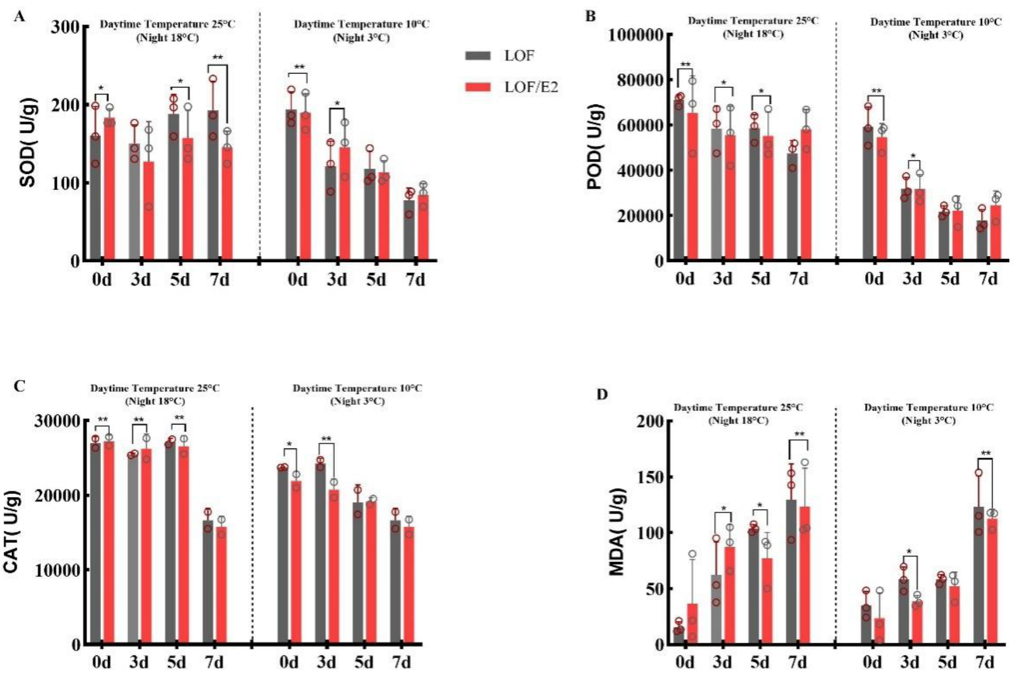


Figure 3. Response of antioxidant enzyme activity in grafted pepino under low temperature stress. (A) The SOD activity of the leaves of pepino cuttings and grafted seedlings on day 3, 5, 7, and 9; (B) POD ;(C) CAT (D)MDA

6.Discussion

6.1 Grafting Can Increase the pepino grafted seedlings's Cold Tolerance

Grafting is vital for enhancing plant cold resistance, especially in selecting stress-resistant seedlings for production. Grafting Solanaceae crops can boost stress tolerance, requiring resilient rootstocks for seedling growth. Research on enhancing cold tolerance in Solanaceae crops focuses on gene editing and transgenic methods, aiming to improve plant yield and quality[14,15]. Adversity or stress triggers plant defense systems, but excessive damage can lead to membrane and chlorophyll loss, impacting photosynthesis. Heterologous grafting has been shown to improve cold tolerance in plants by altering hormonal responseS[16-18]. Grafted pepino seedlings exhibit lower damage and higher chlorophyll content under low temperature stress compared to cuttings, indicating interaction between rootstock and scion enhances cold resistance. The stability of chlorophyll in pepino and dehydration contribute to this resistance. By studying grafted and self-rooted pepino seedlings, the relationship between grafted seedlings and rootstock/scion interaction can be analyzed. Interaction between scion and rootstock during grafting may lead to hybrid vigor in grafted seedlings.

7. Conclusions

The study shows that grafting can improve the cold resistance of plants. Through comprehensive evaluation of the physiological and growth indicators of different pepino scion combinations and self-rooting seedling propagation, it is concluded that the combination of eggplant as the rootstock and the elliptical fruit type "Light Oval Fruit (LOF)" as the scion of the main pepino variety exhibits the best pairing affinity. It also performs well during low-temperature stress and is the most suitable rootstock variety for grafting pepino, which can be promoted for use in pepino grafting.

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Abbreviations

LOF: Light oval fruit of pepino,as "QingChanXiang"; MDA: Malondialdehyde; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.