Study on Cold Tolerance of Different Rootstocks of Melon Seedlings

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Abstract. The higher compatibility rootstocks of melon were selected and the influence of different rootstocks on increasing the cold resistance of grafted melon seedlings was investigated. The influence of different rootstocks on chilling tolerance of grafted melon seedlings and its physiological mechanisms were studied, including chilling injury index, content of chlorophyll, MDA, and electrolytic leakage under the different temperature (13°C/8°C, 9°C/4°C, 25°C/15°C) conditions. At the same time, Pumpkin ‘Japanese Cedar F1’, ‘Japanese Developed Root Sprouts Wood’, ‘Alam’ was used as rootstock for melon ‘Haojie 6’ in this experiment, and the own-root seedling was used as control. The results showed that Pumpkin ‘Alam’ used as rootstock material could improve the survival rate of grafted seedlings and the chilling tolerance of melon seedlings significantly.

1. Introduction

Melon (Cucumis melo L.) is an economically important species of the Cucurbitaceae family, and it is also the one of the global top ten economic cultivated fruits[1]. For the nice appearance, delicious sweetness and high nutrient quality with flavor, the melon gains high popularity from consumers. For the past few years, due to the continuous improvement of people’s living standards, the global demand for muskmelon growing to promote the rapid development of muskmelon production, only in 2005, China’s melon acreage has more than 40% of the world’s melon-growing area, the total output accounted for more than 50 percent of world production[2]. However, the melon is thermophilic plants, which main producing areas have been limited to the dry climate, sufficient sunlight and large temperature region for a long time, as for its cold intolerance. With the growing number of melon cultivation area, especially the area of the facility is increased at an alarming rate, the influence of plant disease, cold injury and continuous cropping obstacles were more and more serious. For example, General incidence of 20% to 30%, serious block to achieve the 80% to 90% in China[3]. Low temperature to become one of the main obstacle factors of the cultivated melon.

With the development of agricultural technology, in order to achieve high yield, grafting technology is increasingly used in vegetable cultivation. Numerous studies show that grafting could be overcome cropping obstacles, improve the cold hardness of seedlings[4], prevent the hazards of wilt effectively[5]. Therefore, we can take advantage of the grafting technique to improve seedling cold resistance, so as to achieve the goal of normal growth and production of seedlings under low temperature stress. There are few researches on the study of muskmelon rootstocks screening, especially on the study of the cold resistance of the grafted seedlings. For the reason that study on cold tolerance of different rootstocks melon seedlings have great significance.
The objective of this study was to analyze if the grafting technique can be useful for improving the behavior of the melon cultivars grafted onto cold-resistant rootstocks, and select the higher compatibility rootstock of melon and investigate the influence of different rootstocks about increasing the cold resistance of grafted melon seedlings, provide a theoretical basis for the breeding and production and cultivation of the melon resilience. To achieve this, we compared the scion ‘Haojie No.6’ directly grafted onto the Pumpkin ‘Japanese Cedar F1’, ‘Japanese Developed Root Sprouts Wood’, ‘Alam’ and the own-root seedling was used as control. The influence of different rootstocks on chilling tolerance of grafted melon seedlings and its physiological mechanisms were studied.

2. Materials and methods

2.1. Plant materials and sampling

Scion cultivar (Cucumis melo L. cv. Haojie6) is very popular early maturing variety. Rootstocks (pumpkin cv. ‘Japanese Cedar F1’, ‘Japanese Developed Root Sprouts Wood’ and ‘Alam’) were grown in a sand/soil/organic matter (1:1:2 by volume) mixture. Water soaking method was used to seed germination and seedlings were cultivated in the plastic pots (with upper diameter, base diameter and height of 10, 8 and 10 cm, respectively) in Teaching and research park-7 solar greenhouse (Patent right possessed by Sichuan Agricultural University, China). For grafting, rootstock seeds were sown for 7 days to produce seedlings with approximately the same size of hypocotyls as that of muskmelon. Cleft grafting was carried out at the two-leaf stage of growth. Own root seedlings (self-rooted control), ‘Haojie 6’/‘Alam’, ‘Haojie 6’/‘Japanese Cedar F1’ and ‘Haojie 6’/‘Japanese Developed Root Sprouts Wood’ graft combinations were named as CK, GA, GC and GD for short abbreviation, respectively. Dark treatment seven days after grafting, the humidity was controlled more than 90%, and the temperature was controlled at 25±1°C.

2.2. Determination of indicators and methods

The combinations of grafted seedlings were placed in the artificial climate chamber, processing in low temperature circumstances. Deal with day and night temperatures were: extremely low temperatures(9°C/4°C)[6-7], melon seedling roots inactivation of the critical temperature(13°C/8°C)[8], melon seedlings normal growth temperature(25°C/15°C). During the measurements the environmental greenhouse ranges were: relative humidity (60%); and intensity of illumination (1150μmol•m^{-2}•s^{-1}). There are three replicates in each treatment and 10 per repeat.

7 days after grafting, the graft survival rate of seedlings was statistical. Fully expanded foliage samples of melon grafted seedlings used to measure the chilling injury index, partial physiological and biochemical indexes(chlorophyll content, MDA, relative electric conductivity) were harvested in triplicate at 1 day, 4 days, 7 days after starting treatment and before processing with the solutions as described above.

2.3. Data Analysis

Microsoft Excel 2010 and DPS software were used for controlling and processing of data.

3. Results

3.1. The survival rate of grafted seedlings on different rootstocks

Graft compatibility is a prerequisite for successful grafting. Graft compatibility helps to further study the symbiotic affinity and explore the physiological conditions of grafted seedlings and fruit production status. As expected, the scion ‘Haojie 6’ on all pumpkin rootstocks showed good graft compatibility. The survival rate of grafted seedlings on different rootstocks more than 90% and the differences of survival rate were statistically insignificant (Table 1).
Table 1. The survival rate of grafted seedlings on different rootstocks

| Grafted seedling | Survival rate(%) |
|------------------|------------------|
| GA               | 94.87aA          |
| GC               | 92.31aA          |
| GD               | 95.12aA          |

Different lowercase letters indicate significant differences in the p = 0.05 level; capital letters indicate significant difference p = 0.01 level, the same below.

3.2. The chilling injury index of different rootstock seedlings at different temperatures

As shown in Table 2, basically, chilling injury index under different low temperature depicted the same decreasing trend in different graft combinations, was significantly lower than CK. Chilling injury index of CK was rising with time in the low temperature circumstances of 13°C/8°C. At the same treatment, seedlings GD showed chilling injury traits after 4 days low temperature treatment. There was no chilling injury on seedlings GA and GC. As shown in Figures under the low temperature circumstances of 9°C/4°C(Table 2), in the same processing time, the chilling injury index from smallest to largest were GA, GC, GD and CK. This set of data shows the three rootstocks can significantly improve the cold resistance of grafted melon seedlings. Meanwhile, it is noteworthy that rootstocks ‘Alam’ best for improving the performance of the grafted seedlings cold resistance from cold injury characters at low temperatures.

Table 2. The chilling injury index of different rootstock seedlings at different temperatures

| Processing time/Day | Grafted combinations | 9°C/4°C | 13°C/8°C | 25°C/15°C |
|---------------------|----------------------|---------|----------|-----------|
| 1                   | CK                   | 2.41aA  | 1.96aA   | 0         |
|                     | GD                   | 1.92abAB| 0bB      | 0         |
|                     | GA                   | 1.71bcAB| 0bB      | 0         |
|                     | GC                   | 1.27bc  | 0bB      | 0         |
| 4                   | CK                   | 3.12aA  | 2.46aA   | 0         |
|                     | GD                   | 2.54bb  | 1.07bb   | 0         |
|                     | GA                   | 2.53bb  | 0cC      | 0         |
|                     | GC                   | 2.02bc  | 0cC      | 0         |
| 7                   | CK                   | 4.00aA  | 2.75aA   | 0         |
|                     | GD                   | 3.47abA | 1.94bA   | 0         |
|                     | GA                   | 3.44abA | 0bB      | 0         |
|                     | GC                   | 3.21bA  | 0bB      | 0         |

3.3. The physiological index of different rootstock seedlings at different temperatures

3.3.1. Chlorophyll

The results showed that the chlorophyll content of grafted seedlings was significantly higher than those of CK and seedlings GA chlorophyll content decreased the lowest especially. In the 13°C/8°C condition processing 7 days, seedlings GA, seedlings GC, seedlings GD and CK were 92.26%, 84.52%, 82.98%, 61.41% of the own-root seedling (In the 25°C/15°C condition processing 7 days). In the 9°C/4°C condition processing 7 days, seedlings GA, seedlings GC, seedlings GD and CK were 60.39%, 60.18%, 59.25%, 47.97% of the own-root seedling (In the 25°C/15°C condition processing 7 days) (Table 3). Comprehensive, at low temperature treatment, the chlorophyll content of the seedlings GA was significantly higher than the seedlings GC and seedlings GD, seedlings GC and seedlings GD were no significant differences. The differences of different grafted seedlings depend on the anti-cold properties of the rootstock and the affinity of rootstock and melon. ‘Alam’ for the stock effect is most obvious, ‘Japanese Cedar F1’ and ‘Japanese Developed Root Sprouts Wood’ followed by
Table 3. The Chlorophyll of different rootstock seedlings at different temperatures

| Processing time/Day | Grafted combinations | Chlorophyll (mg·g⁻¹) | 9°C/ 4°C | 13°C/ 8°C | 25°C/ 15°C |
|---------------------|----------------------|-----------------------|---------|-----------|-----------|
|                     |                      |                       |         |           |           |
| 1                   | GA                   |                       | 1.365ᵃᴬ | 1.533ᵃᴬ  | 1.552ᵃᴬ  |
|                     | GC                   |                       | 1.269ᵇᴮ | 1.457ᵇᴮ  | 1.550ᵃᴬ  |
|                     | GD                   |                       | 1.274ᵇᴮ | 1.453ᵇᴮ  | 1.544ᵃᴬ  |
|                     | CK                   |                       | 1.143ᶜᶜ | 1.317ᶜᶜ  | 1.549ᵃᴬ  |
| 4                   | GA                   |                       | 1.151ᵃᴬ | 1.477ᵃᴬ  | 1.550ᵏᴮᴬ |
|                     | GC                   |                       | 1.119ᵇᴮ | 1.384ᵇᴮ  | 1.563ᵃᴮᴮ |
|                     | GD                   |                       | 1.121ᵇᴮ | 1.383ᵇᴮ  | 1.563ᵃᴮᴮ |
|                     | CK                   |                       | 0.967ᶜᶜ | 1.430ᵃᴬ  | 1.550ᵏᴮᴬ |
| 7                   | GA                   |                       | 0.936ᵃᴬ | 1.430ᵃᴬ  | 1.550ᵏᴮᴮ |
|                     | GC                   |                       | 0.925ᵇ’B | 1.299ᵇ’B  | 1.537ᶜ’B  |
|                     | GD                   |                       | 0.926ᵇ’B | 1.297ᵇ’B  | 1.563ᵃᴮᴮ |
|                     | CK                   |                       | 0.757ᶜᶜ | 0.969ᶜᶜ  | 1.578ᵃᴱ  |

3.3.2. Malondialdehyde

Under low temperature stress, the photosynthetic rate decreased, causing the light excess, resulting in the strengthening of peroxidation and the destruction of lipid membranes and increasing malondialdehyde (MDA) content. For this reason, lipid membrane peroxidation level could weighed by MDA content under stress conditions. In this experiment, all melon seedlings in the low-temperature stress MDA content had increase, the largest increase in self-rooted, significantly higher than the three kinds of grafted seedlings, no significant difference between the grafted seedlings. Each treatment MDA decrease under different treatment temperatures, as time goes on, increased. under the conditions of 13°C/ 8°C the average decline of 60.68%, 9°C/ 4°C the average decrease of 79.51% (Table 4). The ability of undermine on the plasma membrane of seedlings, under low temperature of 9°C/ 4°C stress was greater than 13°C/ 8°C. There was a certain increase of three rootstocks on melon seedling resistance at low temperatures.

Table 4. The Malondialdehyde(MDA) of different rootstock seedlings at different temperatures

| Processing time/Day | Grafted combinations | MDA(µmol·g⁻¹FW) | 9°C/ 4°C | 13°C/ 8°C | 25°C/ 15°C |
|---------------------|----------------------|-----------------|---------|-----------|-----------|
|                     |                      |                 |         |           |           |
| 1                   | GA                   |                 | 17.826ᵇᴮᴮ | 9.943ᵇᴬ  | 7.594ᵇᴮ  |
|                     | GC                   |                 | 16.648ᵇᴮ | 9.450ᵇᴬ  | 8.556ᵃᴮᴮ |
|                     | GD                   |                 | 16.855ᵇᴮ | 10.47₁ᵃᴮᴬ | 8.180ᵃᴮᴬ |
|                     | CK                   |                 | 19.736ᵃᴮ | 11.689ᵃᴮ | 8.848ᵃᴮ  |
| 4                   | GA                   |                 | 22.707ᵇᴮ | 11.564ᶜᶜ | 8.039ᵇ’B  |
|                     | GC                   |                 | 22.360ᵇ’B | 12.334ᵇ’Bᶜ | 8.535ᵇᴮᴮ |
|                     | GD                   |                 | 25.859ᵃᴮᴮ | 12.941ᵃᴮᴮ | 8.330ᵃᴮᴮ |
|                     | CK                   |                 | 27.292ᵃᴮ | 13.370ᵃᴮ | 8.830ᵃᴮ  |
| 7                   | GA                   |                 | 27.625ᵇ’B | 16.930ᵇᴬ | 8.150ᵃᴮ  |
|                     | GC                   |                 | 28.118ᵇ’B | 16.485ᵇᴬ | 8.926ᵃᴮ  |
3.3.3. **Relative electric conductivity**

Relative conductivity is physiological indicators, reflects the degree of plant membrane damage at low temperature. In this research, 7 days of low temperature stress, the grafted seedlings blades relative electrical conductivity was significantly lower than self-rooted seedlings. Self-root seedlings and grafted seedlings performance with chilling injury index were significant positively correlated ($r = 0.97^{**}$). Meanwhile, in the 13℃/ 8℃ condition, seedlings GA, seedlings GC, seedlings GD and CK were 7.78%, 10.22%, 10.59%, 30.22% of the own-root seedling (In the 25℃/ 15℃ condition processing 7 days). In the 9℃/ 4℃ condition, seedlings GA, seedlings GC, seedlings GD and CK were 12.29%, 15.42%, 16.35%, 44.30% of the own-root seedling (In the 25℃/ 15℃ condition processing 7 days) (Table 5). The relative conductivity difference between A and B, under low temperature stress, was not significant. However, it was significantly higher than the Aramaic grafted seedlings. The data illustrated rootstock ‘Alam’ could significantly improve the ability to protect the melon plant membrane at low temperatures.

| Processing time/ Day | Grafted combinations | Relative electric conductivity (%) |
|----------------------|----------------------|------------------------------------|
|                      |                      | 9℃/ 4℃  | 13℃/ 8℃  | 25℃/ 15℃ |
| 1                    | GA                   | 0.690c  | 0.661c   | 0.654ab  |
|                      | GC                   | 0.707b  | 0.673bc  | 0.642b   |
|                      | GD                   | 0.707b  | 0.679bb  | 0.658a   |
|                      | CK                   | 0.784a  | 0.695a   | 0.643b   |
| 4                    | GA                   | 0.697d  | 0.661c   | 0.648a   |
|                      | GC                   | 0.713c  | 0.673bc  | 0.637b   |
|                      | GD                   | 0.729b  | 0.679bb  | 0.644ab  |
|                      | CK                   | 0.891a  | 0.695a   | 0.637b   |
| 7                    | GA                   | 0.722c  | 0.693c   | 0.643a   |
|                      | GC                   | 0.740b  | 0.701c   | 0.636ab  |
|                      | GD                   | 0.741b  | 0.710bb  | 0.642aAB |
|                      | CK                   | 0.912a  | 0.823a   | 0.632bb  |

4. **Conclusions**

Grafting could improve the cold resistance of melon seedlings, overcome barriers of continuous cropping, and reduce disease. As the temperature decreases, conductivity, MDA content was positively correlated with chilling injury index. Chlorophyll in plant photosynthesis plays an important role to capture light energy, and its content directly affects the plant ability of photosynthesis.

From what has been mentioned above, selection of pumpkin ‘Alam’, as the rootstock of melon ‘Haojie 6’, could significantly improve its cold resistance, enhanced melon seedlings to low temperature adaptability.

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