Effects of Grafting on Sugar Metabolism of Melon (Cucumis melo L.) Seedling under Copper Stress

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Abstract. The experiment was carried out with pumpkin ‘JingXinZhen NO.3’ as the rootstock, thin-skin melon ‘IVF09’ as the scion, and self-rooted seedling as the control. The effect of grafting on the sugar metabolism of melon seedlings under copper stress was studied. The results showed that under the condition of copper stress, the growth of melon seedlings was inhibited, the sugar content of leaves decreased, and the sucrose content decreased significantly, and the total activity of sugar metabolism related enzymes changed. However, under the same conditions, the biomass of the grafted seedlings is greater than that of the self-rooted seedlings, and the greater the stress concentration, the greater the grafting promoting effect; Glucose and fructose in grafted leaves were significantly higher than those in self-rooted seedlings, raffinose and stachyose were significantly lower than self-rooted seedlings, the highest ratio of glucose to fructose increased by 5.26% and 32%; and the total activity of sucrose synthase (SS) in the leaves of grafted seedlings was lower than that of self-rooted seedlings. The total activity of sucrose phosphate synthase (SPS), neutral invertase (NI) and acid invertase (AI) was greater than that of self-rooted seedlings. The results showed that grafting could change the sugar activity of seedling leaves by changing the total activity of sugar metabolism related enzymes in melon seedling leaves, and then change the sugar metabolism of melon seedlings to reduce the toxicity of copper stress on melon seedlings.

1. Introduction
At present, due to the rapid development of industry and agriculture, the mining of copper-bearing minerals, the discharge of three wastes from smelters, the long-term use of copper-containing fertilizers and pesticides have made soil copper pollution increasingly serious[1-2]. When the soil copper pollution is serious, it not only causes heavy metal toxicity of plants, but also affects animals[3], microorganisms[4] and soil enzymes[5], causing ecosystem imbalance and even threatening human food safety.

Grafting is an old and emerging agronomic measure. It can promote the root absorption and photosynthesis of plants; improve the ability of osmotic adjustment, regulate ion absorption, etc.; thereby improving the physiological characteristics of plants against diseases, insects, salt and sputum. At the same time, it has been reported that grafting is an effective way to improve the tolerance of horticultural crops such as cucumber[6-7] to heavy metals such as copper and cadmium. However, whether grafting will increase the tolerance of melon seedlings to copper stress is unclear.

In this experiment, the white seed pumpkin ‘JingXinZhen NO.3’ was used as the rootstock and the thin-skin melon ‘IVF09’ was used as the scion to explore the effect of grafting on the sugar metabolism of melon seedlings under copper stress. The changes of sugar content and total activity of sugar metabolism related enzymes in melon seedlings under copper stress were discussed, and helps to
explain the stress resistance of copper metabolism to copper stress, in order to provide a theoretical basis for studying the physiological mechanism of grafting which may improve the tolerance of melon seedlings to heavy metals.

2. Materials and methods

2.1 Plant materials

The rootstock for the test was the pumpkin ‘JingXinZhen NO.3’, and the test scion was a thin-skinned melon ‘IVF09’.

2.2 Experiment design

Select a full, neat and consistent ‘JingXinZhen NO.3’ seed to be sown in a composite matrix (2:1 ratio of peat to vermiculite). After 2 days, the melons were sown, and the leaves of the stalks were exposed. The roots of one of the roots were grafted by the top plug. After the grafted seedlings survived for 5 days, the seedlings with robust and consistent growth were selected to be irrigated with treatment solution. Set up 6 treatments: U0: melon seedlings control; G0: melon grafted seedlings control; U400: melon seedlings + 400μmol/LCu²⁺; G400: melon grafted seedlings + 400μmol/LCu²⁺; U800: melon seedlings + 800μmol/LCu²⁺; G800: Melon grafted seedlings + 800μmol/LCu²⁺. It was adjusted with CuSO₄·5H₂O, and the treatment liquid was poured once every 2 days, and 40 mL was poured each time. 30 strains per treatment were repeated 3 times. When measured in 15 d growth index (plant height, stem diameter, leaf area, biomass), sugar content (glucose content, fructose content, sucrose content, raffinose content and stachyose content), total activity of glucose metabolism enzyme (SS, SPS).

2.3 Statistical methods

All data were analyzed with statistical software SPSS version 22.0 (IBM Corporation). Comparisons of the means used the least significant difference (LSD) at P ≤ 0.05.

3. Results

3.1 Effects of Grafting on Growth of Melon Seedlings under Copper Stress

It can be seen from Table 1 that under non-stress conditions, the plant height, stem diameter, leaf area, fresh root weight of leaves and roots of melon grafted seedlings were greater than those of self-rooted seedlings. Under stress conditions, with the increase of Cu²⁺ concentration, the biomass of grafted seedlings and self-rooted seedlings decreased. The greater the Cu²⁺ concentration, the greater the growth-promoting effect of grafting. Under the stress of 400μmol/LCu²⁺, the graft height, stem diameter and leaf area of grafted seedlings increased by 6.88%, 29.01% and 27.62%, respectively. Under the stress of 800μmol/LCu²⁺, the graft height, stem diameter and leaf area of grafted seedlings increased by 17.65%, 95.12% and 43.32%, respectively. Under the same stress conditions, the fresh stem of the grafted seedlings is more important than the self-rooted seedlings. Under the stress of 800μmol/LCu²⁺, the difference was the most significant, and the dry weight of grafted seedlings increased by 11.11% compared with that of self-rooted seedlings.

| Treatment | Plant height (cm) | Plant diameter (mm) | Leaf area (cm²) | Shoot Fresh weight(g) | Dry weight(g) | Root Fresh weight(g) | Dry weight(g) |
|-----------|------------------|---------------------|----------------|-----------------------|---------------|----------------------|---------------|
| U₀        | 58.33±2.08ab     | 2.89±0.08d          | 41.39±1.55b    | 25.45±0.45b           | 3.02±0.07a    | 2.59±0.17a           | 0.17±0.02b    |
| G₀        | 60.33±4.73a      | 5.74±0.11a          | 51.43±0.54a    | 28.57±0.17a           | 3.14±0.04a    | 2.79±0.17a           | 0.20±0.04a    |

Table 1. Effects of Grafting on Growth of Melon Seedlings under Copper Stress
U400 53.33±2.08b 2.74±0.26bc 30.81±1.07d 20.85±0.15d 2.47±0.09bc 2.05±0.02b 0.14±0.02b
G400 57.00±2.65ab 5.18±0.16b 39.32±1.58bc 22.35±0.25c 2.60±0.05b 2.23±0.03b 0.18±0.01b
U800 39.67±4.73d 2.46±0.18e 25.53±4.23 e 19.62±0.15e 2.24±0.12d 1.79±0.04 b 0.10±0.01 c
G800 46.67±1.53c 4.80±0.35c 36.59±0.60c 20.95±0.05d 2.45±0.05b 2.06±0.03b 0.15±0.01b

Note: Different letters in the same columnment significant difference at 0.05 level. The same beow.

3.2 Effects of Grafting on Sugar Content in Leaves of Melon Seedlings under Copper Stress
As can be seen from Table 2, under non-stress conditions, grafting changed the level of sugar in the leaves. The content of glucose and fructose in the leaves of grafted seedlings was significantly higher than that in self-rooted seedlings. The content of sucrose, raffinose and stachyose was lower than that of self-rooted seedlings. Under the condition of Cu^2+ stress, the sugar content of grafted and self-rooted leaves decreased, but the glucose and fructose content of grafted seedlings were higher than that of self-rooted seedlings. The content of raffinose and stachyose was significantly lower than that of self-rooted seedlings, while sucrose showed High Cu^2+ stress was significantly higher than self-rooted seedlings. Under the stress of 400μmol/LCu^2+, the content of glucose and fructose in grafted seedlings increased by 5.26% and 32%, respectively, but the content of sucrose, raffinose and stachyose decreased by 16.02% compared with that of self-rooted leaves. 28.21%, 12.93%. Under the stress of 800μmol/LCu^2+, the contents of glucose, fructose and sucrose in grafted seedlings were increased by 4.11%, 9.10% and 54.41%, respectively, while the contents of raffinose and stachyose were decreased compared with those of self-rooted leaves 22.86%, 17.82%.

Table 2. Effects of grafting on glucose, fructose, sucrose, raffinose and stachyose content in leaf of melon seedlings under copper stress

| Treatment | Glucose content(mg/g) | Fructose content(mg/g) | Sucrose content(mg/g) | Raffinose content (mg/g) | Stachyose content (mg/g) |
|-----------|-----------------------|------------------------|-----------------------|-------------------------|-------------------------|
| U0        | 0.86±0.01b            | 0.84±0.02b             | 2.43±0.02a            | 0.94±0.01a              | 1.41±0.02a              |
| G0        | 2.34±0.03a            | 1.05±0.06a             | 1.82±0.07b            | 0.35±0.01c              | 1.12±0.01b              |
| U400      | 0.76±0.00d            | 0.50±0.01d             | 1.81±0.02b            | 0.39±0.01b              | 1.16±0.04b              |
| G400      | 0.80±0.01c            | 0.66±0.01c             | 1.52±0.02c            | 0.28±0.01d              | 1.01±0.06c              |
| U800      | 0.70±0.01f            | 0.44±0.01de            | 0.68±0.01d            | 0.35±0.01c              | 1.01±0.03c              |
| G800      | 0.73±0.00e            | 0.48±0.01e             | 1.05±0.08e            | 0.27±0.01d              | 0.83±0.01d              |

3.3 Effects of Grafting on Total Activity of SS and SPS in Leaves of Melon Seedlings under Copper Stress
It can be seen from Fig. 1 that under stress conditions, with the increase of Cu2+ concentration, the total activity of SS in melon grafted seedlings and self-rooted seedlings increased gradually, and the total activity of SPS decreased gradually. The total activity of SS in grafted seedlings was smaller than that of self-rooted seedlings, and the total activity of SPS was greater than that of self-rooted seedlings. Under the condition of 400μmol/LCu2+, the total activity of SS and SPS in the grafted seedlings was the most significant, which was 0.44 and 2.56 times of the self-rooted seedlings, respectively.
Effects of Grafting on Total Activity of NI and AI in Leaves of Melon Seedlings under Copper Stress

It can be seen from Fig. 2 that under stress conditions, the changes of total activity of NI and AI in the leaves of melon grafted and self-rooted seedlings are consistent, and gradually decrease with increasing Cu2+ concentration, but the total activities of NI and AI in grafted seedling leaves are always greater than that of the self-rooted seedlings. Under the stress of 800 μmol/L Cu2+, the total activity of AI and NI in the leaves of grafted seedlings was significantly higher than that of the self-rooted seedlings, which were 1.96 and 1.35 times of the self-rooted seedlings, respectively.

Fig 2. Effects of grafting on NI and AI total activity in leaf of melon seedlings under copper stress

4. Conclusions
In this experiment, due to the grafting of the scion and the rootstock, the growth and physiological characteristics of the rootstock were affected, and the inhibition of copper stress on the biomass of the melon seedling was weakened. Under the condition of copper stress, the sugar content in the leaves of grafted and self-rooted seedlings decreased, and the sugar synthesis ability of melon seedlings decreased. However, grafting promotes the conversion of sucrose, stachyose, and raffinose into small molecules of glucose and fructose, which enhances its osmotic adjustment ability, thereby improving the tolerance of melon seedlings to copper stress. Grafting can also regulate the sugar activity of melon seedling leaves by changing the total activity of sugar metabolism in melon seedling leaves, so as to reduce the toxic effect of copper stress on melon seedlings.

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