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Reciprocal grafting between cucumber and pumpkin demonstrates the roles of the rootstock in the determination of cucumber salt tolerance and sodium accumulation

Yuan Huang, Zhilong Bie*, Pengyi Liu, Mengliang Niu, Ai Zhen, Zhixiong Liu, Bo Lei, Dongjun Gu, Chao Lu, Baitian Wang

College of Horticulture and Forestry, Huazhong Agricultural University/Key Laboratory of Horticultural Plant Biology, Ministry of Education, Wuhan, 430070, PR China

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ABSTRACT

The use of rootstock is a valid strategy in increasing cucumber salt tolerance by reducing sodium toxicity. However, the mechanism responsible for higher salt tolerance and lower Na^+ concentration in the shoot of grafted cucumber plants remains unclear. In this study, six combinations of cucumber (*Cucumis sativus* L., salt sensitive) and pumpkin (*Cucurbita moschata* Duchesne, salt tolerant): namely, ungrafted cucumber and pumpkin, self-grafted cucumber and pumpkin, cucumber grafted onto pumpkin, and pumpkin grafted onto cucumber, were exposed to 1 or 91 mM NaCl. The plant growth and Na^+ concentrations were measured at day 10 and day 30 after NaCl treatment. The results showed that when plants were exposed to 91 mM NaCl for 10 days, the shoot growth reduction in cucumber grafted onto pumpkin (29%) was lower than that in self-grafted cucumber (58%). Meanwhile, the reduction in pumpkin grafted onto cucumber (44%) was higher than that in self-grafted pumpkin (27%). The Na^+ concentration in the shoot of cucumber grafted onto pumpkin decreased by 69% compared with self-grafted cucumber, whereas the Na^+ concentration in the shoot of pumpkin grafted onto cucumber increased by 203% compared with self-grafted pumpkin. Quantitative analysis revealed that the pumpkin roots excluded 50.5% of Na^+, whereas nearly no Na^+ exclusion was observed in the cucumber roots. The Na^+ retention of plants with pumpkin roots decreased by an average of 15.9% in the shoot, whereas no retention of Na^+ was observed in the plants with cucumber roots. When the plants were exposed to 91 mM NaCl for 30 days, the average Na^+ concentration in the xylem sap of plants with pumpkin rootstocks decreased from 6.5 mM in the rootstock to 1.9 mM in the scion, decreased by 71%. However, the graft union was not a barrier for Na^+ transport when the cucumber was used as rootstock. These results suggest that pumpkin rootstock had higher capacity for Na^+ exclusion and Na^+ retention, which resulted in reduced Na^+ transport to the shoot and increased the salt tolerance of cucumber. In addition, the decreased Na^+ transport from rootstock to scion, which is required for cucumber salt tolerance, is primarily driven by the rootstock.

1. Introduction

Currently, more than 800 million ha of land worldwide contains salt deposits. This amount accounts for over 6% of the world's total land area. Of the 1500 million ha of land farmed by dry-land agriculture, 32 million ha are affected by secondary salinity. Of the 230 million ha of irrigated land, 45 million ha are salt affected (Munns and Tester, 2008). Hence, the salt tolerance of crops is needed to sustain the increasing demand in food production in many regions in the world.

The use of rootstock has been successfully demonstrated to increase salt tolerance of vegetable plants by reducing Na^+ toxicity. These vegetables include melon (Romero et al., 1997), tomato (Santa-Cruz et al., 2002; Chen et al., 2003; Estañ et al., 2005; Martinez-Rodriguez et al., 2008), and watermelon (Goreta et al., 2008). Using 'Chaojiquanwang' pumpkin as rootstock and 'Jinchun No. 2' cucumber as scion, Zhu et al. (2008) suggested that higher salt tolerance of grafted cucumber plants is associated with lower Na^+ concentration in the shoot. However, the mechanism responsible for lower Na^+ concentration in the shoot of grafted plants remains unclear.

Edelstein et al. (2011) recently studied the Na^+ distribution in grafted melon and pumpkin plants. The authors found two mechanisms that could explain the decrease in shoot Na^+ concentrations in plants with pumpkin rootstocks: (1) Na^+ exclusion in pumpkin roots, and (2) Na^+ retention and accumulation within the pumpkin rootstock. However, they just used one salt concentration (5.9 mM Na^+, 7.0 mM Cl^-). The electrical conductivity (EC) was

* Corresponding author. Tel.: +86 27 87286908; fax: +86 27 87282010.
E-mail address: biezhilong@hotmail.com (Z. Bie).

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1.9 dS m^{-1} in the water used for irrigation. According to Munns and Tester (2008), soils are classified as saline when the EC is equal to or greater than 4 dS m^{-1}. This EC is equivalent to approximately 40 mM NaCl. Although this definition of saline soils is credible, the salinity stress response of crops also varies. In some crops, salinity stress is observed when irrigation water with 15.5 mM Na^+ is used (Edelstein et al., 2005). Therefore, the uptake and transport of Na^+ in grafted plants need further investigation at higher NaCl concentration.

As well known, almost all of the Na^+ which is transported to the shoot accumulates there, and several authors have also reported that Na^+ recirculation within the shoot is not negligible (Munns and Tester, 2008; Olías et al., 2009; Plett and Møller, 2010). Therefore, Na^+ accumulation in the shoot is the net result of Na^+ uptake and transport processes occurring in different plant organs. Each of these processes contributes to the salinity tolerance of a plant (Tester and Davenport, 2003). Cucumber (*Cucumis sativus* L.) is one of the most important vegetables in the world and is sensitive to salt stress (Huang et al., 2009). Previous studies have shown that the use of salt- tolerant pumpkin (*Cucurbita moschata* Duch.) rootstock can improve cucumber adaptation to salt stress (Zhu et al., 2008; Huang et al., 2011). This study aims to understand the mechanism involved in improving salt tolerance and decreasing the amount of sodium in the shoot of grafted cucumber plants using six kinds of plants, i.e., ungrafted cucumber and pumpkin, self-grafted cucumber and pumpkin, cucumber grafted onto pumpkin, and pumpkin grafted onto cucumber.

2. Materials and methods

2.1. Plant materials and growth conditions

The experiment was performed in a greenhouse in the experimental farm of Huazhong Agricultural University in Central China (30°27′ N, 114°20′ E). "Jinchun No. 2," a relatively salt-sensitive cucumber (*C. sativus* L.) cultivar (Tianjin Kerun Cucumber Institute, Tianjin, China), and "Chaojiquanwang" (*C. moschata* Duch.), a relatively salt-tolerant pumpkin cultivar (Tangshan Four Seasons Seed Industry, Hebei, China) (Zhu et al., 2008), were used as reciprocal grafting materials. Cucumber seeds were sown 10 days before those of the pumpkin in 50 seedling plug trays filled with a 1:1:1 (v/v/v) mixture of peat:vermiculite:perlite. Seven days later, grafting was performed using the approach method as described by Lee (1994). Four kinds of graft combinations were obtained: namely, self-grafted cucumber and pumpkin, cucumber grafted onto pumpkin, and pumpkin grafted onto cucumber. Ungrafted cucumber and pumpkin were used to investigate the effect induced by the grafting process itself.

2.2. Short-term experiment (10 days of salt stress)

Fifteen days after grafting, the plants were transferred into 20 L plastic containers (eight plants per container) containing full-strength Hoagland solution (Hoagland and Arnon, 1950). At the three-leaf stage, the plants were treated with either 1 mM (tap water containing 1 mM NaCl) or 91 mM NaCl for 10 days. The EC of the nutrient solutions was 2.05 dS m^{-1} and 9.87 dS m^{-1}, respectively. The twelve treatments (six combinations of plants × two salt concentrations) were replicated three times, with eight plants in each replicate, and arranged in a randomized complete block design. During the greenhouse culture, the plants were grown at day maximal photosynthetic photon flux density (PPFD) 1051 μmol m^{-2} s^{-1} (average PPFD 289 μmol m^{-2} s^{-1}), day temperature between 17 °C and 36 °C (average temperature 26 °C), night temperature not lower than 14 °C, and day relative humidity between 50% and 90% (average relative humidity 76%). Five days after NaCl treatment, the gas exchange parameters were measured. After 10 days of NaCl treatment, the plant growth, and Na^+, Cl^-, and K^+ concentrations were measured.

2.3. Long-term experiment (30 days of salt stress)

Fifteen days after grafting, the plants were transplanted into plastic containers of polyethylene containing 8 L of substrate (1 mm to 5 mm in diameter, peat: vermiculite: perlite = 1:1:1, v/v/v), and each container contained one seedling. The plants were arranged at a density of approximately six plants/m^2. The plants were irrigated with normal nutrient solutions for the first five days after transplant (Hoagland and Arnon, 1950) and then treated with 91 mM NaCl. The twelve treatments were replicated three times and arranged in a randomized complete block design. Thirty six plots were used, and each plot consisted of six plants. The amount of irrigation solution applied for each plant was 0.2–1 L daily, depending on plant growth stage and environmental conditions. During the greenhouse culture, the plants were grown at day PPFD 1084 μmol m^{-2} s^{-1} (average PPFD 275 μmol m^{-2} s^{-1}), day temperature between 14 °C and 36 °C (average temperature 24 °C), night temperature not lower than 13 °C, and day relative humidity between 30% and 90% (average relative humidity 70%). After 30 days of NaCl treatment, the xylem sap was collected. The shoot growth and Na^+ concentrations were then measured.

2.4. Determination of plant growth

Three plants were harvested per treatment, and the roots were rinsed in deionized water. The plants were carefully blotted with tissue paper. In the short-term experiment, the plants were dissected into shoot and root. In the long-term experiment, only the plant shoot was harvested. The part above the grafted union was regarded as the "shoot", and the part below was the "root" in the grafted plants. For the ungrafted plants, the part above the cotyledon node was regarded as the "shoot", and the part below was the "root". The shoots and roots were placed in a forced air oven at 105 °C for 15 min and then at 70 °C for 3 days to determine the dry weights of the shoot, root, and whole plant (shoot and root).

2.5. Determination of Na^+, K^+, and Cl^- concentrations

After measuring the dry weights, the Na^+, K^+, and Cl^- from the roots and shoots were extracted and measured according to the procedure described by Xu et al. (2006). The Na^+ and K^+ concentrations were analyzed with atomic absorption spectrophotometer (Varian spectra AA 220, Varian, Palo Alto, CA, USA). The Cl^- concentration was determined by silver nitrate ($AgNO_3$) titration using a neutral indicator agent containing 4.2% (w/v) K_2CrO_4 and 0.7% (w/v) $K_2Cr_2O_7$. The average Na^+ and Cl^- concentrations of the whole plant were then calculated.

2.6. Calculation of Na^+ absorption factor (AF) and retention factor (RF)

The AF of an excluded element (such as Na^+ in this study) for a given plant, which is the percentage of the absorption of a non-excluded element (such as Cl^- in this study) by the same plant under the same growing conditions for the same period, can be calculated using the equation described by Edelstein et al. (2011), which is $AF = [(C_{ep} \times C_{fw})/(C_{ew} \times C_{fp})] \times 100$, where C_{ew} and C_{fw} (mM) are the average concentrations of Na^+ and Cl^- in the irrigation water, respectively, and C_{ep} and C_{fp} (mmol kg^{-1}) are the Na^+ and Cl^- concentrations of the whole plant, respectively. In this study, C_{ew} and C_{fw} are the same, whereas C_{ep} and C_{fp} were calculated using