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Non-Invasive Micro-Test Technology and Reciprocal Grafting Provide Direct Evidence of Contrasting Na^+ Transport Strategies between *Cucurbita moschata* and *Cucurbita maxima*

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Abstract: Cucurbita species are widely used as rootstocks for cucumber, watermelon, and other plants for the restriction of toxic Na^+ transport from root to shoot. Previous studies have found distinct salt tolerance strategies between *Cucurbita moschata* and *Cucurbita maxima*; the former accumulates a large amount of Na^+ in the root, while the latter accumulates Na^+ in the shoot. To further study the mechanism of Na^+ transport in plants, four reciprocal grafts were made between MB (*C. moschata*) and JHL (*C. maxima*), denoted as MB/MB, JHL/JHL, MB/JHL, and JHL/MB (scion/rootstock). The results showed that using MB as the rootstock effectively reduced the accumulation of Na^+ in the scion. Conversely, JHL rootstock tended to transfer more Na^+ to the scion. To clarify this phenomenon, the velocities of Na^+ flows on the root surface, stem, and vein of grafting combinations were measured using non-invasive micro-test technology. Compared with the seedlings using JHL as rootstock, the grafted combination with MB as rootstock had a higher root Na^+ efflux and lower Na^+ fluxes in the stem and vein. qRT-PCR analyses revealed the critical roles of salt overly sensitive 1 and high-affinity potassium as components of the mechanism enabling Na^+ exclusion from the root and Na^+ unloading from the stem xylem. Compared with the seedlings using MB as rootstocks, the JHL-grafted plants showed more rapid stomatal closure and decreased transpiration rate in the first three hours after salt stress but maintained a higher level under prolonged salt treatment (120 h). The tissue tolerances of JHL and MB were assessed using the isolated leaves under NaCl to exclude the influence of the root and stem. The results showed that the salinity inflicted more serious damage to MB leaves than to JHL leaves. qRT-PCR analyses indicated that the intracellular Na^+/H^+ transporter in the leaf vein was involved in this process. All these findings indicated that *C. moschata* and *C. maxima* adopted different strategies for regulating Na^+ transport, and grafting can be used as a tool to create more salt-tolerant plants.

Keywords: salinity; grafting; Na^+ transport; non-invasive micro-test; *Cucurbita*

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1. Introduction

Soil salinity is a global challenge affecting crop production. More than 800 million hectares of agricultural land are affected by soil salinity; most of these are found in arid or semi-arid regions of Africa, Asia, and Latin America [1]. Cucurbitaceae plants, such as melon, watermelon, and cucumber, are a group of glycophytes that serve as major fruit vegetables, and most Cucurbitaceae species are sensitive to salinity [2]. Increased Na^+ concentration in shoots remarkably contributes to salinity stress. Excess Na^+ in cell interferes with the transport of potassium and the activation of key enzymes involved in photosynthesis [3].

Grafting with salt-tolerant rootstocks is an important agronomic measure to deal with soil salinization in horticultural crops [4–7]. Numerous studies have shown that

Cucurbita has a stronger salt tolerance than other Cucurbitaceae crops. *Cucurbita* species are extensively used as rootstocks for cucumber, watermelon, and other cucurbit plants because of their strong salt tolerance.

The Na^+ contents in the shoots and leaves of crops grafted onto pumpkin rootstock are considerably lower than those of non-grafted crops [8,9]. This directly accounts for the better salt tolerance of pumpkin-grafted seedlings. Hence, considerable work has been performed on screening salt-tolerant *Cucurbita* species with a strong restricting ability on Na^+ transport to scions [8,10]. The lower Na^+ concentration in the scion is due to the stronger Na^+ exclusion in the root of the rootstock [11,12] and Na^+ unloading from the xylem [13]. The regulation of Na^+ influx, efflux, and compartmentation is important for plants to cope with Na^+ accumulation in the shoots. This process involves several transporters that participate in ion uptake and transport along the plant vascular system. Among these processes, Na^+ efflux is the first defense line for plants to exclude excess Na^+ from the root surface into the external medium. This process is mediated by the salt over-sensitive (SOS) pathway, in which the plasma membrane (PM) Na^+/H^+ antiporter pumps Na^+ out of the cell [14]. *Arabidopsis* plants overexpressing salt overly sensitive 1 (SOS1) have a remarkably increased salt tolerance [15,16]. This strategy has also been found in *Cucurbita* [9,17]. In addition to root Na^+ efflux, Na^+ delivery from root to shoot through the withdrawal of Na^+ from the xylem sap also plays a vital role [3]. High-affinity K^+ transporter-1 (HKT1) encodes a Na^+ preferential transporter considered to be involved in the process. The ectopic expression of CmHKT1 in cucumber decreases Na^+ accumulation in plant shoots [13]. When excess Na^+ arrives at the shoot, plants reduce the damage caused by toxic ions to the photosynthesis system by Na^+ compartmentalization in vacuoles. In our previous study, transcriptome analyses revealed the critical roles of intracellular Na^+/H^+ (NHX6) transporters as components of the mechanism enabling Na^+ sequestration in the leaf vein of *Cucurbita* species. Scanning electron microscopy and energy-dispersive X-ray microanalysis indicates that the root cortex is the main site for storing excess Na^+ [3,11].

Although we have demonstrated that the three above-mentioned strategies coexist in pumpkin plants, we do not know which is more important in reducing leaf Na^+ content in grafting combinations. Pumpkins with different Na^+ accumulation patterns are suitable materials for research. In previous studies, we found distinct Na^+ accumulation strategies between two *Cucurbita* species. Compared with *Cucurbita moschata*, *Cucurbita maxima* is more salt-tolerant but accumulates more Na^+ in the shoot [18,19]. This strategy in *C. maxima*, termed the "tissue tolerance" mechanism, has been observed in halophytes [20,21] and glycophytes [22–24] and is considered an efficient and less energy-consuming salt tolerance strategy. Conversely, *Cucurbita moschata* tends to store a large amount of Na^+ in its roots and is thus considered for use as a traditional salt tolerance strategy [13].

Grafting is widely used to provide salt tolerance in vegetables and is a tool used to study ion transport or long-distance signal transmission in plants [25]. Reciprocal grafting using different genotypes as rootstocks and scions to construct grafting combinations is a common strategy to study the interaction between aboveground and underground parts [12,26,27]. Non-invasive micro-test technology (NMT) is a method for obtaining ion information across membranes in plants and is an efficient tool for measuring the ion fluxes and movement direction [28]. In the present study, we constructed grafting combinations with *C. moschata* and *C. maxima* using reciprocal grafting and then used non-invasive micro-test technology (NMT) to detect the dynamic ion flow rates in different parts of the grafting combinations. We aimed to elucidate the mechanism of the salt tolerance of pumpkins with different genetic backgrounds at the whole-plant level and explore the possibility of creating new salt-tolerant plants using grafting technology.

2. Materials and Methods

2.1. Reciprocal Grafting of Two Pumpkins

Experiments were performed in a greenhouse at Jianghan University, Central China (longitude: 114.2, latitude: 30.5). Two pumpkin species, named "Miben" (abbreviated as

MB, *C. moschata*) and “Jinghongli” (abbreviated as JHL, *C. maxima*), were used as the scion or rootstock (materials were provided by the Huazhong Branch Center of National Vegetable Improvement Center, Huazhong Agricultural University). Four grafting combinations were used, namely, MB grafted onto JHL (MB/JHL, scion/rootstock), JHL grafted onto MB (JHL/MB), self-grafted MB (MB/MB), and self-grafted JHL (JHL/JHL).

Seeds were soaked in distilled water for 8 h and incubated in darkness at 28 °C until germination. Rootstocks were sown 3 days earlier than plants that were used for scions. When rootstock seedlings developed one true leaf, the scion seedlings were grafted onto them using the hole-insertion grafting method [25]. After 10 days, the grafted seedlings were transferred into plastic containers (six seedlings per container) containing 8 L of full-strength Hoagland’s solution. The nutrient solution was replaced every 3 days and continuously aerated. The experiment was conducted at a mean temperature of 28 °C/18 °C (day/night) and mean relative humidity of 60%/85% (day/night). The average daily photosynthesis active radiation inside the greenhouse was 26 mol m^{-2} day^{-1}, with a maximum radiation of 1650 mol m^{-2} s^{-1}. At the four-leaf stage, the grafted plants were used for subsequent experiments, except for the Na^+-flux experiment, which was conducted with two-leaf-stage seedlings.

2.2. Determination of Dry Weight and Na^+ and K^+ Contents

NaCl was added into the growth media to obtain a final concentration of 75 mM. After 5 days, whole plants were placed in a forced-air oven at 70 °C for 48 h to determine the dry weight. The dried roots, stems, and leaves of four grafting combinations were ground using a mortar and pestle. About 0.1 g of powder of the dried tissue was digested with 5 mL of 1 mol L^{-1} nitric acid for 3 h. Finally, the Na^+ and K^+ concentrations were analyzed using an atomic absorption spectrophotometer (Varian spectra AA 220, Varian, Palo Alto, CA, USA). The experiment was conducted in a completely randomized design, with five replicates (five seedlings) for each treatment (grafting combinations).

2.3. NMT Measurement of Ion Fluxes in Root, Stem, and Leaf Vein

Net Na^+ fluxes were measured using NMT (Younger USA LLC, Amherst, MA, USA). Four types of grafting combinations and two types of non-grafted seedlings were treated with 75 mM NaCl for 24 h, leading to substantial Na^+ accumulation in roots and stems and activating the Na^+ efflux system. Roots and stems from salt-treated plants were rinsed with distilled water and transferred into the measuring solution (0.1 mM KCl, 0.1 mM $CaCl_2$, 0.1 mM $MgSO_4$, 0.1 mM NaCl, and 0.3 mM MES; pH 6.0) [28]. Plant specimens were immobilized in the middle of poly-l-lysine-coated coverslips (2 cm × 2 cm) in the measuring chamber. The measuring sites in roots and stems are shown in Figure S1. The measuring site in the root was 400 μm from the root tip, which corresponded with the elongation zone and in which a vigorous Na^+ efflux had been observed in our previous study [11]. The experiment was conducted in a completely randomized design, with six replicates (six seedlings) for each treatment (grafting combinations).

2.4. Determination of Gas Exchange Parameters

Gas exchange parameters were measured between 8:00 and 11:00 AM using an open gas exchange system (Li-6400, Li-Cor, Inc., Lincoln, NE, USA) after NaCl treatment. The second fully expanded leaf was selected for measurements. Stomatal conductance (Gs), transpiration rate (Tr), intercellular CO_2 concentration (Ci), and net photosynthesis rate (Pn) were determined. During measurements, the leaf chamber was controlled to maintain the leaf temperature at 25 °C, the CO_2 concentration at 360 μmol mol^{-1}, and the photosynthesis photon-flux density at 800 μmol m^{-2} s^{-1}. The experiment was conducted in a completely randomized design, with six replicates (six seedlings) for each treatment (grafting combinations).

2.5. Comparison of Salt Tolerance of Pumpkin Leaves In Vitro

The in vitro leaf experiment is based on a previous experiment on tomato leaves, with slight changes [29]. The leaves of the JHL and MB at the four-leaf stage were cut into squares (1 cm^2) with scissors. The leaves were then placed in Petri dishes containing 75 and 150 mM NaCl solutions with distilled water as the control. The culture medium was changed once a day, and the chlorophyll fluorescence parameter Fv/Fm was measured with a PAM-2500 chlorophyll fluorometer (Heinz Walz, GmbH, Effeltrich, Germany) after 3 days. The experiment was conducted in a completely randomized design, with six replicates (six seedlings) for each treatment (genotype of pumpkins).

2.6. Total RNA Extraction and Gene Expression Analysis

Total RNA was isolated from four parts of the un-grafted seedling (root, stem, vein, and mesophyll) using TransZol reagent (TransGen Biotech, Inc., Beijing, China) in accordance with the manufacturer's protocol. After extraction, the total RNA was dissolved in diethylpyrocarbonate-treated water. The cDNA template for quantitative real-time PCR (qRT-PCR) was synthesized from 1 μg of total RNA using HiScript II Q Select RT SuperMix for qPCR (Vazyme, Piscataway, NJ, USA). PCR was performed using an ABI 7000 machine (Applied Biosystems), and the cycling conditions were as follows: denaturation at 94 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 55 °C for 15 s, and extension at 72 °C for 15 s. The $2^{-\Delta\Delta ct}$ method was used to calculate relative gene expression values (NaCl/control). The specific primers (Table S1) were designed based on the published mRNA in the Cucurbit Genomics Database (http://cucurbitgenomics.org), URL (accessed on 25 May 2022) by using Primer 5 (V5.00) software. All RT–qPCR reaction results were obtained from three independent replicates.

3. Results

3.1. Dry Weight Reduction under Salt Stress

The salt-induced dry weight reduction was considerably stronger in MB/JHL than in the other three combinations (MB/MB, JHL/MB, and JHL/JHL; Figure 1). Compared with the control, salt treatment reduced the dry weight of MB/JHL by 48%. By contrast, no remarkable reductions were found in the other three combinations.

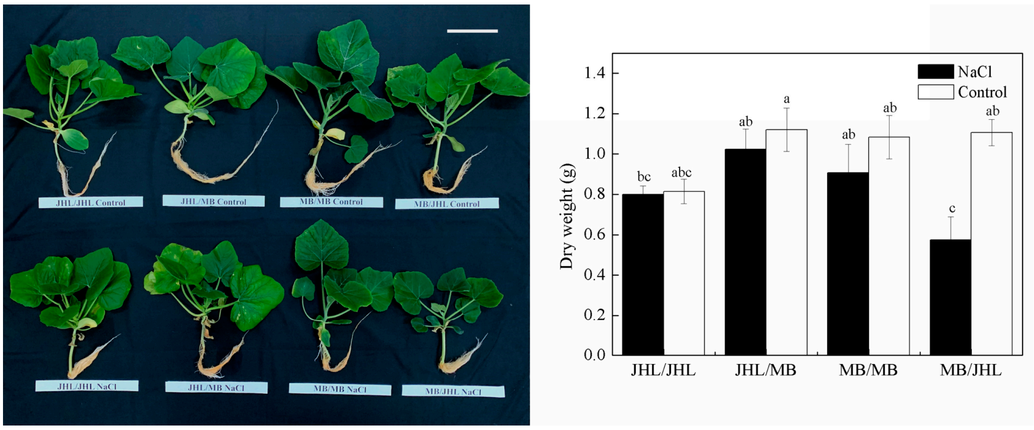

Figure 1. Effects of 75 mM NaCl on the growth and whole plant dry weight of four grafted combinations, namely, JHL/JHL (*C. maxima* self-grafted), JHL/MB (*C. maxima* scion grafted onto *C. moschata* rootstock), MB/MB (*C. moschata* self-grafted), and MB/JHL (*C. moschata* scion grafted onto *C. maxima* rootstock). Data are mean ± SE ($n = 5$). Columns with different letters significantly differ at $p < 0.05$. Scale bar: 10 cm.

3.2. Scion Na^+ Concentration

Na^+ concentrations were higher in the roots than in the stems and leaves in all four grafting combinations (Figure 2A). In grafting combinations JHL/JHL, JHL/MB, MB/MB, and MB/JHL, the Na^+ concentration in leaves was only 32%, 18%, 7%, and 19% of the Na^+ concentration in roots, respectively. MB as rootstock resulted in considerably lower stem Na^+ concentration than JHL as rootstock. The trend of K^+ concentration was consistent with that of Na^+. The grafting combinations with higher leaf Na^+ concentration also had higher K^+ concentration (Figure 2B).

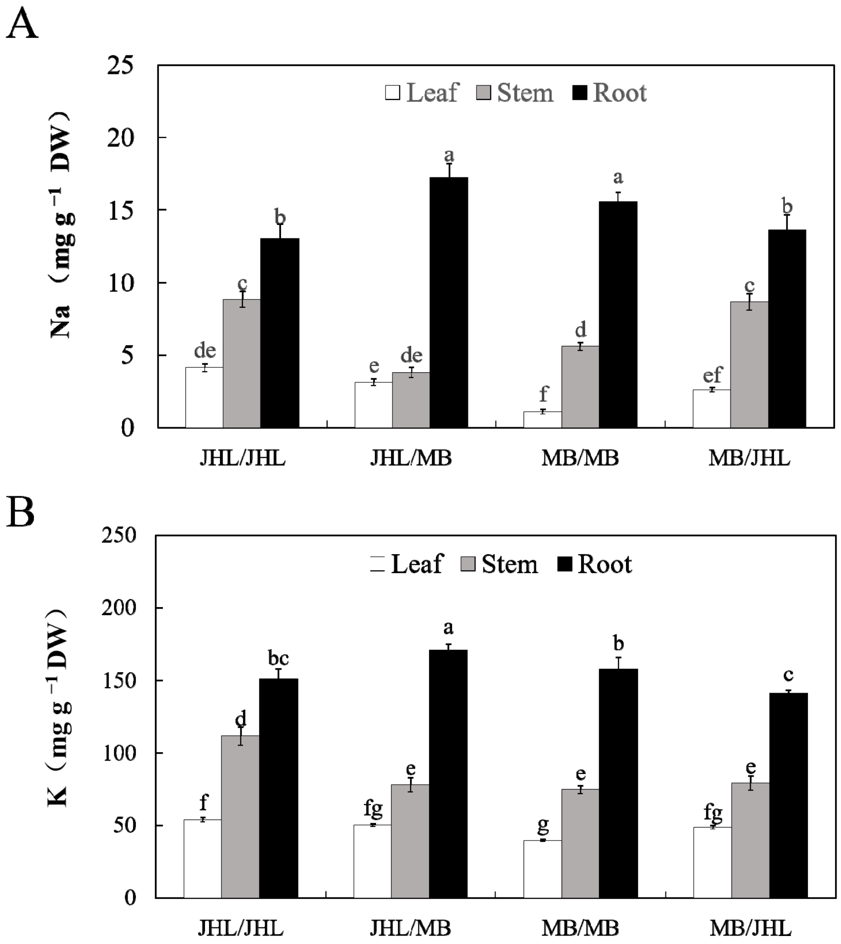

Figure 2. Effects of 75 mM NaCl on the accumulation of Na (**A**) and K (**B**) in leaf, stem, and root of four grafted combinations, namely, JHL/JHL (*C. maxima* self-grafted), JHL/MB (*C. maxima* scion grafted onto *C. moschata* rootstock), MB/MB (*C. moschata* self-grafted), and MB/JHL (*C. moschata* scion grafted onto *C. maxima* rootstock). Data are mean ± SE (n = 5). Columns with different letters significantly differ at $p < 0.05$.

3.3. Na^+-Flux Velocities in Stems and Veins

Massive Na^+ effluxes (higher than 10,000 nmol m^{-2} s^{-1}) in the apical maturation zone of the roots were recorded in six grafting combinations (including the non-grafted seedlings MB and JHL) (Figure 3A). Among the six materials, the non-grafted MB seedlings had the highest Na^+ efflux rate (17,940 nmol m^{-2} s^{-1}), and the JHL/JHL seedlings had the lowest efflux rate (12,360 nmol m^{-2} s^{-1}). On the whole, the Na^+ efflux of the material with MB as the root was higher than that with JHL as the root. No remarkable differences in Na^+ efflux were found among the materials with the same root genotype, and the shoot had little effect on root Na^+ efflux.

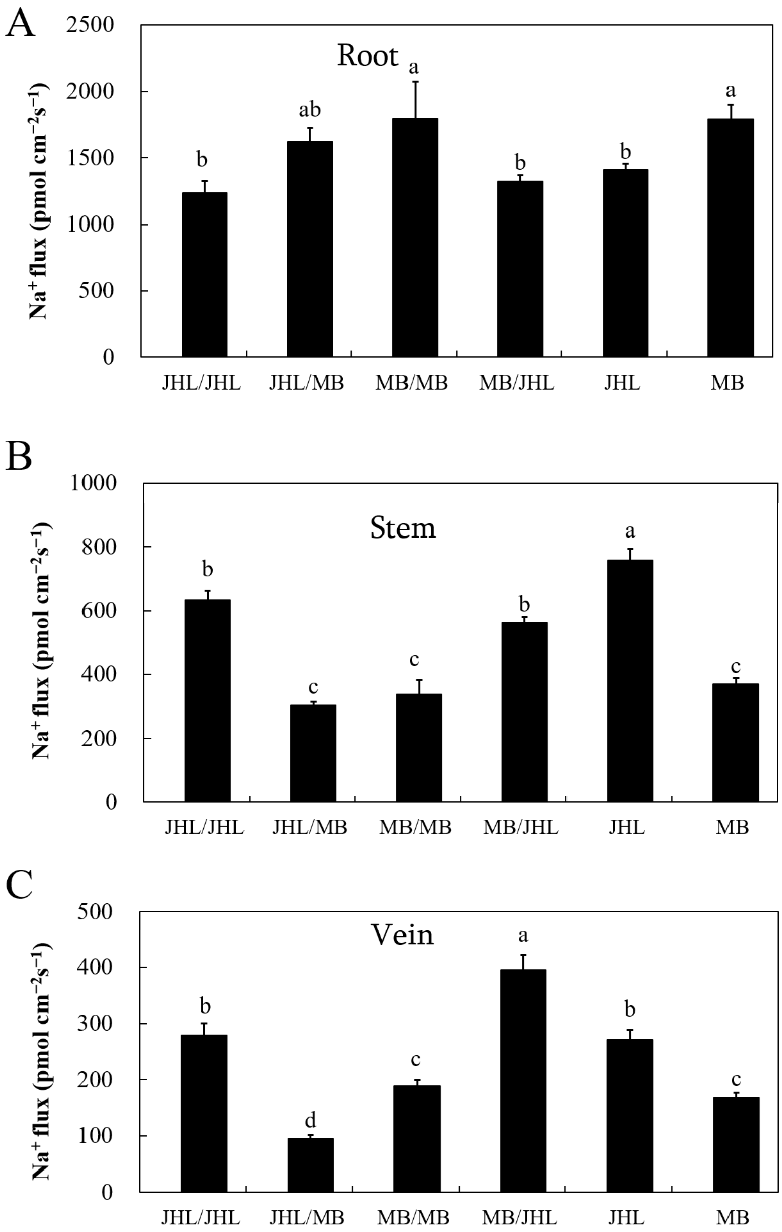

Figure 3. Net Na^+ fluxes measured from root (**A**), stem (**B**), and leaf vein (**C**) of four grafting combinations (JHL/JHL (*C. maxima* self-grafted), JHL/MB (*C. maxima* scion grafted onto *C. moschata* rootstock), MB/MB (*C. moschata* self-grafted), and MB/JHL (*C. moschata* scion grafted onto *C. maxima* rootstock)) and two non-grafted seedlings (JHL and MB) using the non-invasive micro-test technology (NMT). Data are mean ± SE ($n = 6$). Columns with different letters significantly differ at $p < 0.05$.

The ability of the whole root system to transfer Na^+ above ground was determined by measuring the Na^+ flux in the stem (Figure 3B). The monitoring point of flow velocity in the stem was located 1 cm below the graft healing point (or the cotyledon position of non-grafted seedlings; Figure S1). Net Na^+ effluxes of 6336 and 5619 nmol m^{-2} s^{-1} were detected in the JHL/JHL and MB/JHL combinations, which were twice those of MB/MB and JHL/MB (3372 and 3053 nmol m^{-2} s^{-1}, respectively), indicating a reduced Na^+ flux by the MB rootstock (Figure 3B).

The velocity of Na^+ efflux in leaf veins reflects the instantaneous rate of toxic ions reaching plant photosynthesis organs (Figure S1). Among the six combinations, MB/JHL had the highest Na^+-flux velocity (3946 nmol m^{-2} s^{-1}), and JHL/MB had the lowest Na^+-flux velocity (948 nmol m^{-2} s^{-1}; Figure 3C). Compared with the MB scion, JHL could remarkably reduce the Na^+ flux in the vein by using the same rootstock material. For example, the Na^+ flux in MB/MB was 1888 nmol m^{-2} s^{-1}, and that in JHL/MB was only 948 nmol m^{-2} s^{-1}.

3.4. Photosynthesis Parameters

Under salt stress for 120 h, the photosynthesis rates of the grafting combinations with JHL as the scion (10.7 and 10.9 μmol m^{-2} s^{-1} for JHL/JHL and JHL/MB, respectively) were considerably higher than those of the grafting combinations with MB as the scion (7.9 and 6.8 μmol m^{-2} s^{-1} for MB/MB and MB/JHL, respectively; Figure 4A). JHL/JHL and JHL/MB also showed a more rapid decline in photosynthesis rate and stomatal conductance than MB/MB and MB/JHL during the first 48 h after NaCl stress (Figure 4B,C). Transpiration rates and intercellular carbon dioxide concentrations had similar trends to stomatal conductance (Figure 4C).

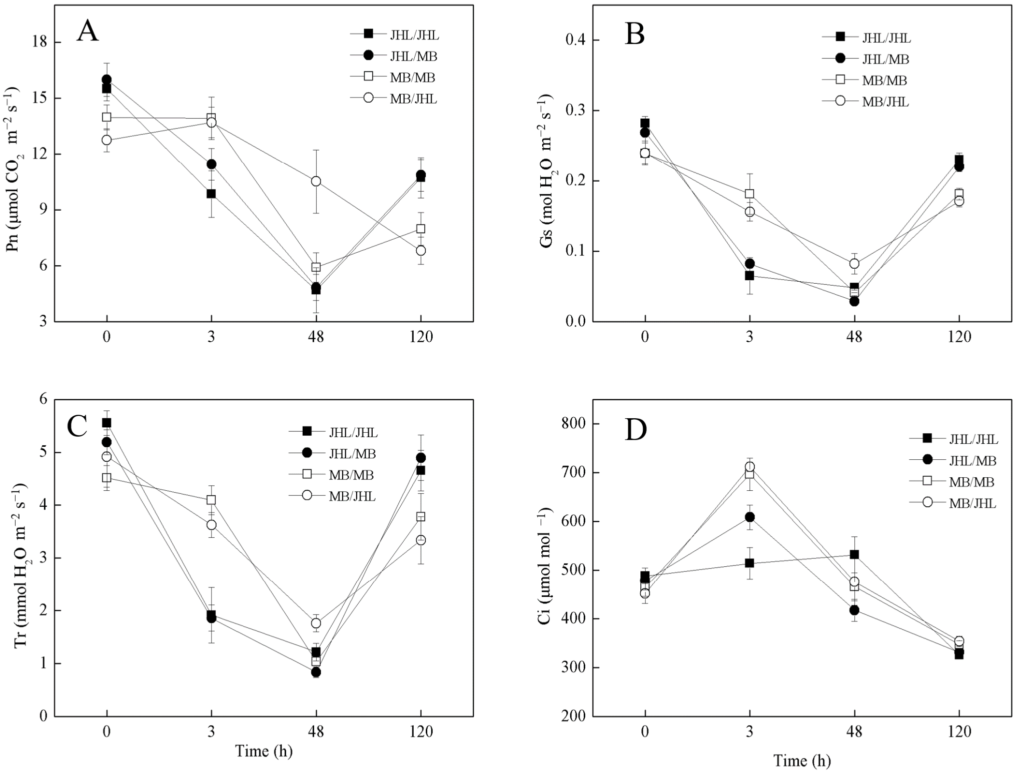

Figure 4. Time course of photosynthesis rate (**A**), stomatal conductance (**B**), transpiration rate (**C**), and intercellular CO_2 concentration (**D**) in four grafting combinations (JHL/JHL (*C. maxima* self-grafted), JHL/MB (*C. maxima* scion grafted onto *C. moschata* rootstock), MB/MB (*C. moschata* self-grafted), and MB/JHL (*C. moschata* scion grafted onto *C. maxima* rootstock)). Data are mean ± SE (n = 6).

3.5. Salt Tolerance of Pumpkin Leaves In Vitro

The isolated leaves of two pumpkin samples were treated with salt to exclude the influence of the root and stem on salt tolerance. The results showed no remarkable differences in the salt tolerance between the two species under 75 mM salt concentration (no significant difference in Fv/Fm values); however, 150 mM NaCl treatment seriously damaged the MB leaves, resulting in yellowed, destroyed leaves (Figure 5). According to the Fv/Fm, the photosystem of MB leaves was more seriously damaged under high-concentration salt stress than that of JHL leaves (Figure 5).

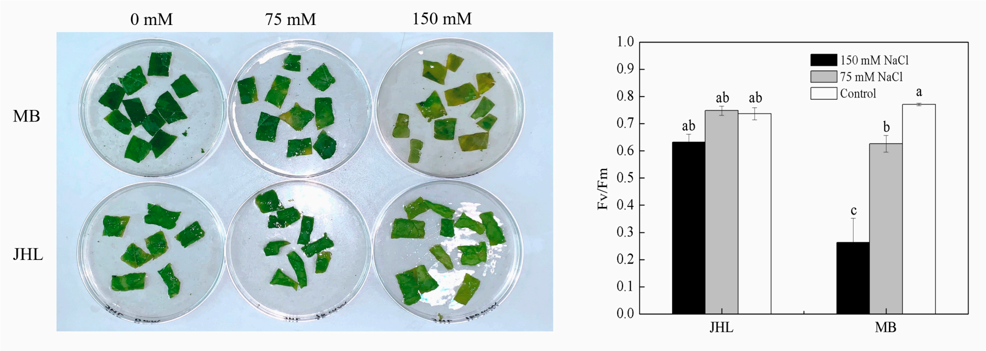

Figure 5. The isolated leaves of JHL (*C. maxima*) and MB (*C. moschata*) were treated with 75 or 150 mM NaCl for 3 days. The photochemical efficiency (Fv/Fm) of isolated leaves was measured with the PAM-2500. Data are mean ± SE (n = 6). Columns with different letters significantly differ at $p < 0.05$.

3.6. *NHX6* and *HKT1* Were Significantly Upregulated

The expression levels of three transporter genes involved in Na^+ transport were measured. The expression levels of *NHX6* in the mesophyll and vein of JHL was significantly higher than those in the stem and root (4.8-, 4.1-, 2.1-, and 2.6-fold, respectively). However, no difference existed in the four parts of MB regarding *NHX6* expression (Figure 6A). Unlike *NHX6*, a significantly higher expression level of *SOS1* was found in the MB root (2.0-fold) than in the mesophyll, vein, and stem (1.1-, 1.1-, and 1.0-fold) (Figure 6B). The expression levels of *HKT1* in the vein and stem was significantly higher than those in the mesophyll and roots in both genotypes (Figure 6C).

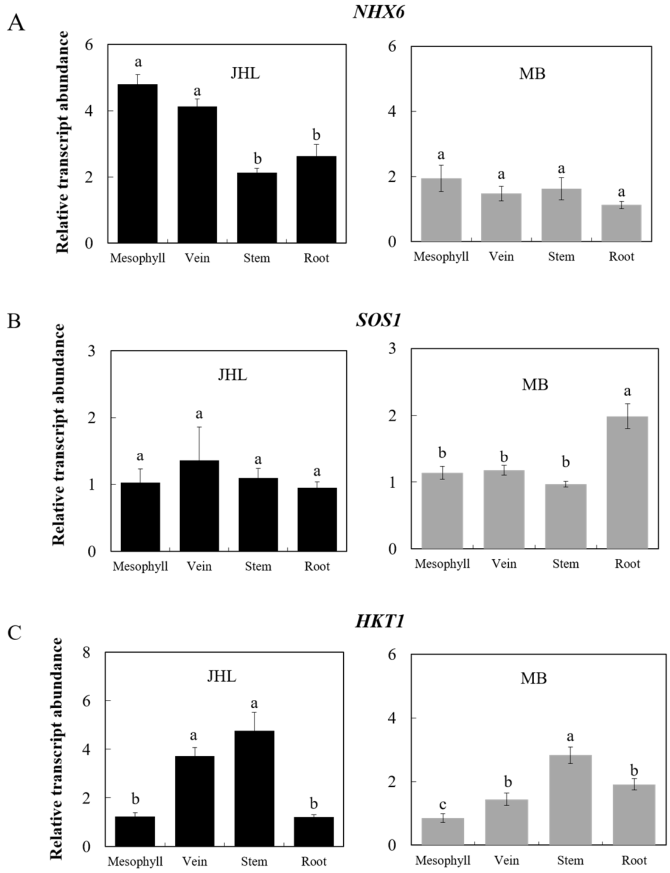

Figure 6. Relative expression (NaCl/control) of *NHX6* (**A**), *SOS1* (**B**), and *HKT1* (**C**) in the leaf mesophyll, leaf vein, stem, and root of JHL (*C. maxima*) and MB (*C. maxima*) for 24 h under 75 mM NaCl stress. Data are mean ± SE (n = 3). Columns with different letters significantly differ at $p < 0.05$.

4. Discussion

4.1. C. maxima and C. maxima Adopted Distinct Strategies to Limit Na+ Transport from Root to Shoot

Restricting the Na^+ content reaching the shoots is the key strategy for plants to cope with salt stress and is based on three main processes, namely, Na^+ efflux from the root surface, Na^+ unloading from xylem, and Na^+ compartmentation in the vacuole [30,31]. In this study, NMT was used to test the Na^+ fluxes among three key points: root, stem, and leaf vein. Although salt-tolerant cucurbits could limit Na^+ transport from root to shoot, the stage that is more important during this process is unclear [11,12,32]. The results of ion-flow-rate measurements suggested that root Na^+ efflux may not be the only factor affecting Na^+ transport to shoots in pumpkins; rather, the Na^+ unloading from the xylem in the stem or the Na^+ regionalization in root tissue may be more important. Lei [13] compared the hypocotyl ion flow rates of pumpkin and cucumber using NMT, and the results showed that Na^+ flux in the hypocotyl of pumpkin is smaller than that in cucumber. Consequently, aboveground Na^+ accumulation in pumpkin is significantly lower than that in cucumber. The gene expression patterns between JHL and MB under NaCl stress may provide clues for this phenomenon. *SOS1* is expressed in many tissues, particularly in the root epidermis and around the vascular tissue [31]. In the current work, *SOS1* was significantly upregulated in MB but had no change in JHL (Figure 6B).

When Na^+ reaches the area around the vascular bundle, the rate of Na^+ loading into the xylem becomes the key to controlling the accumulation of Na^+ in the aboveground parts. Very reliable data point to the important role of *HKT*-mediated transporters in controlling Na^+ translocation to the shoot by retrieving Na^+ from the xylem. In *Arabidopsis*, hkt1 mutations lead to the overaccumulation of Na^+ in shoots, rendering the plant hypersensitive to Na^+ [33]. In rice, *OsHKT1:5* is a PM Na^+ transporter expressed in parenchymal cells on the xylem that retrieves Na^+ from the xylem sap [34]. The expression level of *HKT1* in the stem and leaf vein was significantly higher than that in the mesophyll and root system, suggesting that xylem unloading plays a crucial role in the salt tolerance of *Cucurbita*. Notably, *HKT* expression also differed in the leaves of JHL and MB. These results were consistent with those found in *Sorghum bicolor* [35] and *Arabidopsis* [36]. This finding indicated that plant shoots also played a role in coping with salinity and that the unloading of Na^+ in the vascular system and its regionalization to peripheral tissues were the potential causes.

4.2. Cucurbitaceae Relied on the Tissue Tolerance Mechanism to Combat Salinity

The in vitro experiment and grafting combination experiment showed that JHL leaves had a stronger salt tolerance than MB leaves (Figures 2 and 5). This ability was reflected in the higher photosynthesis rate of the leaves when more Na^+ was transferred into the leaves. This strategy was similar to the mechanism of tissue salinity tolerance in halophytes that accumulated a large amount of Na^+ in the vacuole [37]. However, pumpkins are not real halophytes. The mesophyll cells in pumpkin cannot accumulate Na^+ in large quantities [18] but can store substantial Na^+ in the parenchyma surrounding vascular bundles [11,38]. This strategy has several explanations. The vacuolar region around the vascular bundle is closer to the site of Na^+ offloading and has a shorter route of transport. Cucurbitaceae plants also tend to have extremely long vines, which provide "volume" to accumulate and dilute the excess Na^+ in the stems and veins. Moreover, as one of the essential macronutrients, K^+ plays important roles in many fundamental physiological processes in plant cells. It is also essential as a counter-ion for the charge balance of ion transport across the plasma and intra-organelle membranes [38]. Therefore, higher K^+ retention is essential for plant function under saline conditions.

Another advantage of this strategy is that the stomata do not have to be closed to limit Na^+ transport to the shoot because transport in the vascular bundle is powered by transpiration. Pumpkins such as JHL allow Na^+ to move toward the shoot with the transpiration pull. The benefit is that it can transport as much Na^+ as possible without affecting photosynthesis by maintaining a relatively high stomatal conductance under long-term salt stress. Meanwhile, the rapidly closed stomata found in JHL avoid “salt shock” in the first few hours after salt stress (Figure 4). However, the stomatal response depends only on the scion genotype and is unaffected by the rootstock genotype, thereby suggesting that the key stress signal is synthesized in leaves.

4.3. Grafting Can Be Used to Create More Salt-Tolerant Plants

Grafting is a traditional agronomic measure used to overcome soil-borne diseases, soil salinization, and heavy metal pollution [4,5,10]. The results of the present study showed that the process of salt tolerance in plants depended on the whole-plant level, i.e., the synergy between the underground and aboveground parts in controlling Na^+ transport. The salt tolerance of MB primarily depended on limiting Na^+ transport to the shoot, whereas JHL utilized shoot ion regionalization to reduce Na^+ damage to the photosynthesis system and a better “tissue tolerance” of leaf mesophyll. This has also been confirmed in previous studies on the growth and photosynthesis of the two materials under natural growth conditions [38]. Grafting these two pumpkins may create a more salt-tolerant material (JHL/MB) than using each pumpkin separately.

The salt tolerance mechanism of the rootstock-to-graft complex has been thoroughly studied, but the effect of the scion on salt tolerance is neglected [9,39]. The scions of some citrus [40–42], solanum [43,44], and other species have strong salt tolerance, and the advantages of these materials can be further retained in the grafting complex. Finally, grafting affinity is another important consideration in the construction of grafting complexes [45]. Many grafted seedlings grow slowly or even stop growing after graft healing [46,47]. Evaluating the affinity of grafting combinations is also a key work to be considered in creating salt-tolerant grafting complexes.

5. Conclusions

C. maxima (JHL) relied on a tissue tolerance mechanism to adapt salinity, whereas *C. moschata* species (MB) followed a Na^+-excluding strategy. Na^+ xylem unloading may be more important than root Na^+ efflux in Cucurbitaceae plants under salinity. Grafting can combine the advantages of the two types of plants to create a more salt-tolerant grafting complex (Figure 7).

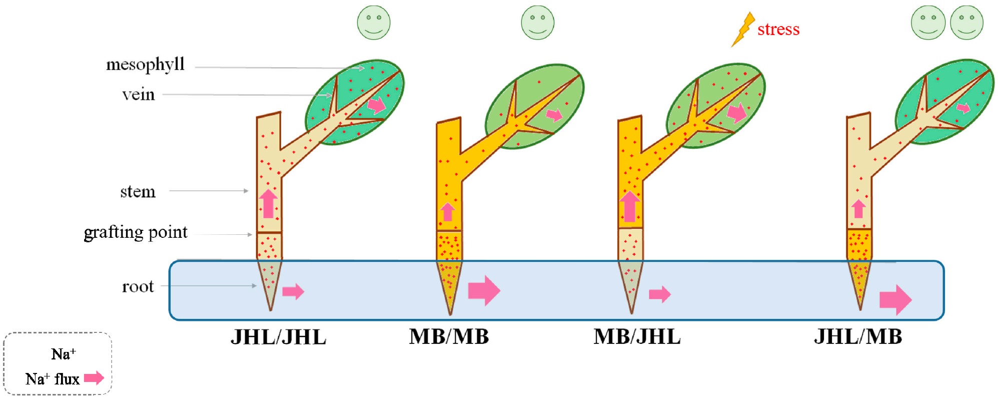

Figure 7. Schematic showing the salt tolerance mechanism of four different grafting combinations of pumpkins under NaCl stress. Different genotypes of rootstocks and scions are in different colors. Red dots represent the concentration of Na^+, and the arrows (their width) represent the velocity and direction of Na^+. Seedlings with MB as rootstock significantly reduced Na^+ concentration in shoot. Compared with MB, JHL leaves had stronger tissue salt tolerance to resist higher concentrations of Na^+ in mesophyll. MB/JHL performed worst because it transported excessive Na^+ into leaves but lacked strong tissue salt tolerance with the MB scion. JHL/MB had the strongest salt tolerance potential by combining the advantages of the two pumpkin genotypes.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy13071843/s1. Figure S1. Measurement position of ion flux in the roots, stems, and leaf veins of *Cucurbit*; Figure S2. A four-minute continuous Na^+ flux recording was conducted using non-invasive micro-test technology (NMT) with roots (A), stems (B), and leaf veins (C) of four grafting combinations (JHL/JHL (*C. maxima* self-grafted), JHL/MB (*C. maxima* scion grafted on *C. moschata* rootstock), MB/MB (*C. moschata* self-grafted) and MB/JHL (*C. moschata* scion grafted on *C. maxima* rootstock)) and two non-grafted seedlings (JHL and MB); Table S1. List of primer sequences used for qRT-PCR analysis.

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