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Research article

Differential expression of ion transporters and aquaporins in leaves may contribute to different salt tolerance in *Malus* species

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ABSTRACT

Maintaining ion and water homeostasis in plants is an important defense strategy against salinity stress. Divergence in ion homeostasis between the salt-tolerant *Malus hupehensis* Rehd. and salt-sensitive *Malus prunifolia* 'yingyehaitang' was studied to understand their mechanisms for tolerance. Compared with the control on Day 15, plants of those two genotypes under high-salinity treatment had less K^+ in the leaves, stems, and roots. Contents were higher in the roots but lower in the leaves of *M. hupehensis* while levels in the stems were similar to those from *M. prunifolia*. For both genotypes, the sodium content increased after salinity treatment in all tissue types. However, the leaves from *M. hupehensis* had less Na^+ and maintained a lower Na^+/K^+ ratio. To understand the basis for these differences, we studied the ion transporters and regulation of aquaporin transcripts in the leaves. Transcript levels for both *MdHKT1* and *MdSOS1* were higher in *M. hupehensis*, implying that this species had better capacity to exclude sodium so that less Na^+ occurred in the leaves but more in the stems. *M. hupehensis* also had a greater amount of *MdNHX1* transcripts, which could have assisted in sequestering excess Na^+ into the vacuoles and sustaining a better cellular environment. A relatively higher level of aquaporin transcript was also found in *M. hupehensis*, suggesting that those plants were more capable of maintaining a better leaf water status and diluting excess ions effectively under high-salinity conditions. Therefore, these tested transporters may play important roles in determining how salinity tolerance is conferred in *Malus* species.

1. Introduction

Salinity affects large portions of the global land mass, and is a major threat to agricultural crop production. An excess of sodium ions is toxic to plants because of its adverse effects on K^+ nutrition, cytosolic enzyme activity, photosynthesis, and metabolism [1,2]. Intracellular Na^+ and K^+ homeostasis plays a vital role in the growth and development of higher plants [3]. The potassium macronutrient is necessary for several physiological processes, including the maintenance of membrane potential and turgor, activation of enzymes, regulation of osmotic pressure, stomatal movement, and tropisms [4]. Low cytosolic Na^+ contents and high K^+/Na^+ ratios aid in maintaining an osmotic and biochemical equilibrium in the cells. The influx and efflux of Na^+ and K^+ across membranes requires the functioning of trans-membrane Na^+ and K^+ transporters [5]. Several essential Na^+ transporters that detoxify elevated Na^+ contents have been researched, including *NHX1*, *SOS1*, and *HKT1*. Among the proteins involved in salt tolerance, Na^+/H^+ transporters appear to be of paramount importance [6]. Plant *NHX* proteins are critical for promoting the accumulation of Na^+ or K^+ inside the vacuoles [7]. *AtNHX1*, the most abundant vacuolar Na^+/H^+ antiporter in *Arabidopsis thaliana*, mediates the transport of those ions, thereby influencing plant development and contributing to salt tolerance [8]. Constitutive over-expression of that gene's homologues from other plants has been shown to confer significant salt-stress tolerance in a variety of species as a result of increased vacuolar sequestration of sodium ions [9]. The importance of *AtNHX1* to salt-stress tolerance has been further demonstrated with T-DNA insertional mutant *NHX1* 'knockout' plants, which lack a functional antiporter and are more salt-sensitive than wild-type *Arabidopsis* [10]. Furthermore, the *AtNHX1* and *AtNHX2* double knockout is characterized by significantly reduced growth, smaller cells, and shorter hypocotyls on etiolated seedlings. The *AtNHX5* and *AtNHX6* double mutant is also very sensitive to salt, indicating the possible involvement of *NHX* proteins in maintaining ion homeostasis under stress conditions [11,12].

Abbreviations: HKT, high-affinity K^+ transporter; K, potassium; MH, *Malus hupehensis* Rehd; MP, *Malus prunifolia* 'yingyehaitang'; Na, sodium; *NHX*, tonoplastic Na^+/H^+ antiporter; *PIP*, plasma intrinsic membrane protein; *SOS*, salt overly sensitive; *TIP*, tonoplast intrinsic membrane protein.

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We previously confirmed that over-expression of *MdNHX1* in *Malus domestica* M.26 rootstock enhances its tolerance under salinity stress [13]. *SOS1* encodes for a plasma membrane Na$^+$/H$^+$ antiporter, responsible for the exclusion of sodium in the apoplast. Over-expression of *AtSOS1* improves salt tolerance in *Arabidopsis* by limiting Na$^+$ accumulation in the cells [14]. Olías et al. have demonstrated that *Solanum lycopersicum* plants with reduced *SOS1* expression accumulate more Na$^+$ in the leaves, but less in the stems. This indicates that the *SlSOS1* antiporter is essential for maintaining ion homeostasis under salinity, and is critical for the partitioning of Na$^+$ in plant organs [15]. Members of the *HKT* gene family encode transporters in the plasma membrane that mediate the uptake of Na$^+$ or K$^+$ from the apoplast [16]. They can be classified into two subgroups, Class I and Class II [17,18]. Class II is found only with monocotyledonous species, and is involved in Na$^+$/K$^+$ co-transport or high-affinity Na$^+$ uptake. Class I transporters are selective for Na$^+$ [19]. They are important for cellular Na$^+$ and K$^+$ homeostasis, and they can retrieve Na$^+$ from the transpiration stream and so contribute to Na$^+$ exclusion from leaves [16,20]. *AtHKT1;1* null plants show severe Na$^+$ overaccumulations in the leaves, leading to chlorosis under salinity stress [21].

Water homeostasis is crucial to the growth and survival of plants under moisture-related stress. Plant aquaporins (AQPs), which play an essential role in plant–water relations [22], belong to a large superfamily of conserved proteins, i.e., major intrinsic proteins (MIPs). In our previous study with *Malus*, we cloned four full-length aquaporin genes: *MpPIP1*;*1*, *MpPIP2*;*1*, *MpTIP1*;*1*, and *MpTIP2*;*1*. Transcript analysis showed that all are involved in the response to drought. Many experiments have focused on the responses of aquaporins to salinity. Suga et al. [23] have shown that high salt concentrations increase the content of *RsPIP2-1* protein in radish seedlings whereas *RsPIP1-1*, *RsPIP1-2*, and *RsPIP1-3* proteins are not affected. Jang et al. [24] have demonstrated that, under various abiotic-stress conditions (including high salinity), the modulation of *A. thaliana* PIP genes is a complex process.

Despite all of these earlier discoveries, little has been known until recently about how salinity tolerance is acquired in *Malus* species. Nevertheless, understanding how individual mechanisms differ among rootstocks may provide a scientific basis for future genetic improvements in salt tolerance within that genus. We have previously evaluated the relative salinity tolerance of 15 apple rootstocks [25]. *Malus hupehensis* is salt-tolerant, while *Malus prunifolia* is quite salt-sensitive. Therefore, the aim of the present study was to characterize the response of ion transporters and aquaporins to salinity stress. We speculated that they have roles in maintaining a better cellular environment and influencing salt tolerance in differently tolerant genotypes.

2. Results

2.1. Effect of high salinity on Pn (net photosynthetic rate), EL (Electrolyte Leakage), total chlorophyll content, and biomass accumulation in two Malus genotypes

Although their rates of net photosynthesis decreased rapidly for both genotypes under salinity stress, *M. prunifolia* 'yingyehaitang' had a lower rate than *M. hupehensis* throughout the treatment period (Fig. 1A). Over 15 d, values for EL rose significantly for *M. prunifolia* compared with *M. hupehensis* (Fig. 1B).

Salinity caused chlorophyll contents to decline in both genotypes, although the reduction was less drastic for the salt-tolerant *M. hupehensis* (Fig. 1C).

After 15 d of treatment, we could confirm that the growth of *M. prunifolia* was more inhibited by salinity, as reflected by changes in its whole-plant fresh and dry weights. Respective increases were 6.94% and 18.4%, within the salt-tolerant *M. hupehensis* but only 2.86% and 5.7% within the sensitive *M. prunifolia* (Fig. 2A and B).

2.2. Accumulation of sodium and potassium in different plant tissues after high-salinity treatment

For both genotypes, the levels of sodium in leaves, stems, and roots rose significantly after 15 d of exposure to 100 mM NaCl (Fig. 3A–C). However, the average increase over the control was much greater for *M. hupehensis* (223.7% and 312.9% for roots and stems, respectively) than for *M. prunifolia* (159.9%, roots; 197.8%, stems). By contrast, in the leaves, the average increase over the control was much larger for *M. prunifolia* than for *M. hupehensis*, i.e., 1229.9% versus 929.8%.

Potassium contents decreased significantly in the stems of both genotypes after 15 d of treatment, whereas those declines were only minor in the roots and leaves (Fig. 3D–F).

High salinity significantly increased the Na$^+$/K$^+$ ratios in all tissues from both genotypes (Table 1). Although values peaked in stems from *M. hupehensis*, the leaves and roots from that species had lower ratios ($P < 0.05$) compared with *M. prunifolia*.

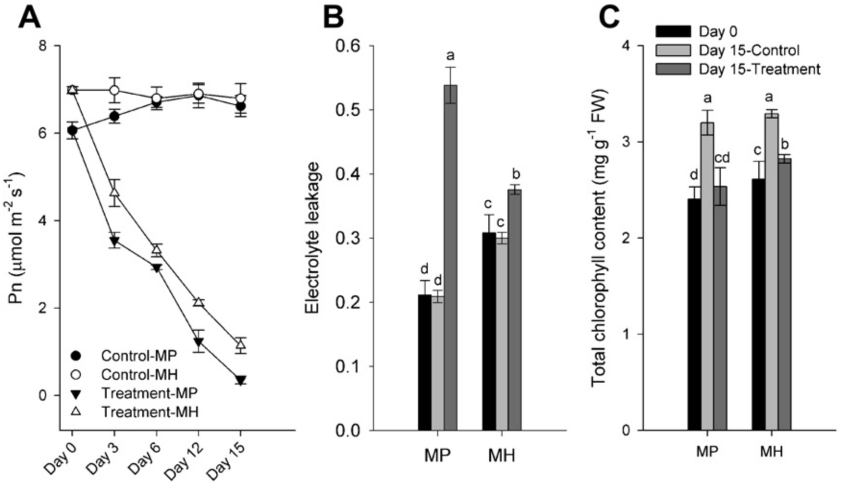

Fig. 1. Effect of salinity on Pn (net photosynthetic rate), EL (Electrolyte Leakage) and total chlorophyll contents in *Malus* genotypes. Pn data are means of 5 replicates ± SD. EL and total Chl content are means of 9 replicates ± SD. Different letters denote statistically significant differences by Duncan's multiple comparison tests ($P < 0.05$).