Journal of Plant Physiology 219 (2017) 1–11

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

Domain-switch analysis of PeNHX3 from *Populus euphratica* reveals the critical role of the transmembrane domain 11 in Na^+ and Li^+ transport

Ting Pan, Yafen Liu, Xiaomeng Su, Lizhe An, Quan-Sheng Qiu*

MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou, Gansu, 73000, China

ARTICLE INFO

Keywords:
Populus euphratica
PeNHX3
TM11
Salt stress
Li^+ toxicity
Arabidopsis

ABSTRACT

Populus euphratica, the well-known tree halophyte, tolerates the stress of high levels of salt. We previously showed that the transmembrane domain 11 (TM11) of PeNHX3, a Na^+,K^+/H^+ antiporter from *P. euphratica*, was crucial for Na^+ and Li^+ transport in a yeast growth assay. Here, we examined the role of TM11 in catalyzing Na^+ and Li^+ transport in transgenic *Arabidopsis*. We found that PeNHX3 localized to the tonoplasts in *Arabidopsis*. Overexpression of PeNHX3 in *Arabidopsis* improved seedling growth and enhanced salt tolerance and Li^+ detoxification. However, overexpression of PeNHX3 did not improve *Arabidopsis* growth at KCl concentrations higher than 0.1 mM, suggesting a low K^+ transport activity for PeNHX3 in plants. We performed in planta domain-switch analysis by replacing the C-terminal domain of AtNHX1 with a C-terminal segment of PeNHX3 containing the TM11 domain. We demonstrated that TM11 was critical for the Na^+ and Li^+ transport activities by PeNHX3. Taken together, PeNHX3 plays an important role in salt tolerance and Li^+ detoxification in plants. TM11 controls the Na^+ and Li^+ transport activities of PeNHX3 in *Arabidopsis*.

1. Introduction

Na^+,K^+/H^+ antiporters play an important role in plant salt tolerance. Na^+,K^+/H^+ antiporters transfer the Na^+ or K^+ across a membrane in exchange for protons (H^+), thus these antiporters are critical to maintain low levels of salt in the cell (Blumwald et al., 2000; Counillon and Pouysségur, 2000; Padan et al., 2001; Pardo et al., 2006). The *Arabidopsis* genome encodes eight Na^+,K^+/H^+ antiporters, which can be divided into three subclasses: vacuolar (AtNHX1-AtNHX4), endosomal (AtNHX5 and AtNHX6), and plasma membrane (AtNHX7/SOS1 and AtNHX8) (Brett et al., 2005; Pardo et al., 2006; Chanroj et al., 2012).

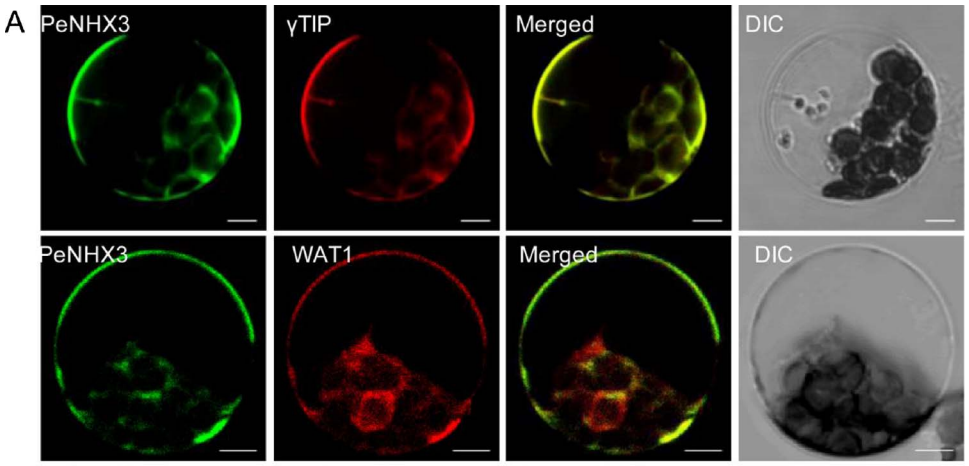
Significant progresses have been made toward understanding the molecular mechanism underlying Na^+ exclusion across the plasma membrane or sequestration into the vacuole by the Na^+,K^+/H^+ antiporters (Bassil et al., 2012; Yamaguchi et al., 2013; Bassil and Blumwald, 2014). The plasma membrane Na^+,K^+/H^+ antiporter *SOS1* was isolated from a genetic screen for identifying cellular components contributing to salt tolerance in *Arabidopsis* (Wu et al., 1996). *sos1* mutant was extremely sensitive to salt stress and accumulated more Na^+ (Shi et al., 2000, 2002), while overexpression of *SOS1* enhanced salt tolerance in *Arabidopsis* (Shi et al., 2003). Transport assays showed that *sos1* plants had a reduced Na^+/H^+ exchange activity across the plasma membrane (Qiu et al., 2002, 2003). Both the genetic and biochemical studies showed that SOS1 was a target of the SOS pathway that is composed of SOS2 (a serine/threonine kinase) and SOS3 (a Ca^{2+}-binding protein) (Shi et al., 2000; Qiu et al., 2002; Quintero et al., 2002).

In addition, Arabidopsis NHX1 was identified as a tonoplast Na^+,K^+/H^+ antiporter that sequesters Na^+ into the vacuole. Overexpression of NHX1 in *Arabidopsis* (Apse et al., 1999), tomato (Zhang and Blumwald, 2001), and *Brassica* (Zhang et al., 2001) increased salt tolerance and vacuolar Na^+ compartmentation. The Na^+/H^+ antiport activity of AtNHX1 can be regulated by SOS2 kinase and CaM (Qiu et al., 2004; Yamaguchi et al., 2005). AtNHX1 and LeNHX2 have a K^+/H^+ transport activity and facilitate K^+ transport into the vacuole (Venema et al., 2002; Rodriguez-Rosales et al., 2008; Leidi et al., 2010). *nhx1nhx2* double mutants displayed abnormal stamens and lacked silique formation as well as reduced growth, indicating their critical roles in cell expansion and flower development (Bassil et al., 2011a). *nhx1nhx2* was impaired in osmoregulation and turgor generation as well as creating the vacuolar K^+ pool, indicating their essential roles in vacuolar K^+ uptake, turgor regulation and stomatal movement (Barragán et al., 2012). Recently, the regulation of cellular Na^+ homeostasis and vacuolar trafficking by NHX5 and NHX6, the endosomal Na^+,K^+/H^+ antiporters in *Arabidopsis*, has been reported (Bassil et al., 2011b; Wang et al., 2015; Qiu, 2016).

Populus euphratica is a tree halophyte that tolerates high

* Corresponding author at: 222 South Tianshui Rd., Lanzhou, Gansu 73000, China.
E-mail address: qiuqsh@lzu.edu.cn (Q.-S. Qiu).

http://dx.doi.org/10.1016/j.jplph.2017.09.003
Received 30 May 2017; Received in revised form 12 September 2017; Accepted 13 September 2017
Available online 19 September 2017



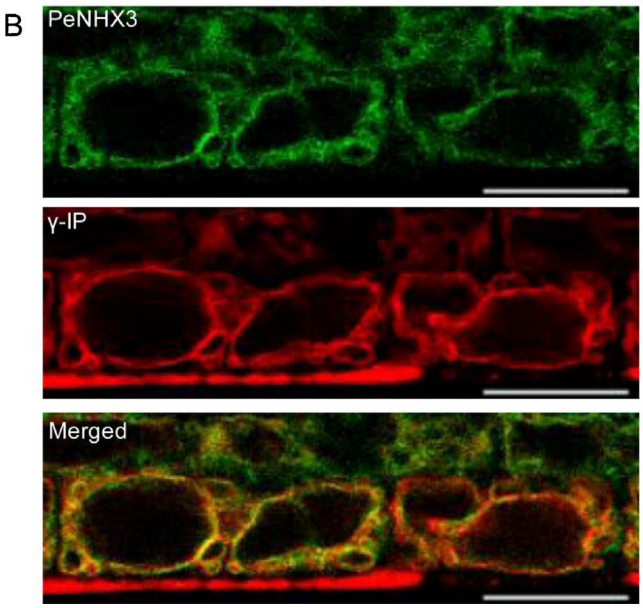


Fig. 1. PeNHX3 is localized in the tonoplasts.
(A) Transient co-expression of PeNHX3-GFP (green) with the tonoplast markers γTIP-RFP (red) and WAT1-RFP (red) in *Arabidopsis* protoplasts, respectively. Bars = 5 μm.
(B) Stable co-expression of PeNHX3-GFP (green) with the tonoplast marker γTIP-RFP (red) in *Arabidopsis* root cells. Bars = 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

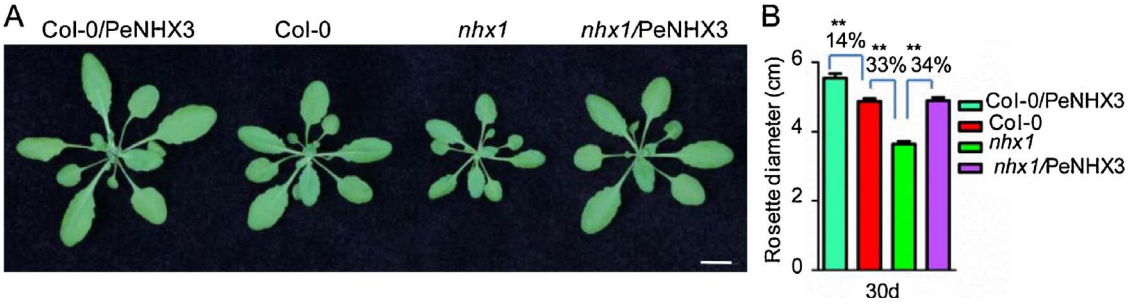


Fig. 2. PeNHX3 is critical for growth in *Arabidopsis*.
(A) The rosette of the seedlings. The Col-0, *nhx1* mutant, and the transgenic lines Col-0/PeNHX3 and *nhx1*/PeNHX3 were grown in soil for 30 days. Bar = 1 cm.
(B) Rosette diameters from A. Error bars represent SEM, **P < 0.01, by *t*-test.

concentrations of salt (Ottow et al., 2005; Brinker et al., 2010). A genomic study shows that maintaining low levels of salt in the cell is a strategy for *P. euphratica* to combat salt stress (Ma et al., 2013). The genome of *P. euphratica* has expanded genes encoding ion transporters, indicating the critical role of ion transporters in controlling salt homeostasis and hence salt tolerance in tree halophyte (Ma et al., 2013). *P. euphratica* has six *NHX* genes (*PeNHX1-6*) that have been shown to function in salt tolerance in a yeast system (Ye et al., 2009). Studies with electrophysiological assays found that *P. euphratica* was higher in Na^+/H^+ antiport than the salt-sensitive congener *P. popularis*, suggesting an important role of PeNHXs in salt tolerance (Sun et al., 2009). With the efforts of understanding the catalytic mechanisms governing the ion transport of PeNHX3, Wang et al. (2014) built a three-dimensional structure of PeNHX3 using the homologous modeling approach. Four conserved residues, including Tyr149, Asn187, Asp188, and Arg356, were identified in the TM4-TM11 assembly region of PeNHX3. Wang et al. (2014) found that PeNHX3 facilitated Na^+, K^+ and Li^+ transport in yeast. They further found that TM11 was crucial to salt tolerance and Li^+ detoxification by a domain-switch analysis (Wang et al., 2014). However, it is not clear whether or not PeNHX3 mediates Na^+, K^+ and Li^+ transport in plants. Also, the function of the TM11 in catalyzing ion transport needs to be verified in plants.