Plant Physiology and Biochemistry 71 (2013) 37–48

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Overexpression of *PeHA1* enhances hydrogen peroxide signaling in salt-stressed *Arabidopsis*

Meijuan Wang [a,1], Yang Wang [a,1], Jian Sun [a,b], Mingquan Ding [a,c], Shurong Deng [a], Peichen Hou [a,d], Xujun Ma [a], Yuhong Zhang [a], Feifei Wang [a], Gang Sa [a], Yeqing Tan [a], Tao Lang [a], Jinke Li [a], Xin Shen [a], Shaoliang Chen [a,*]

[a] College of Biological Sciences and Technology, Box 162, Beijing Forestry University, Beijing 100083, PR China
[b] College of Life Science, Jiangsu Normal University, Xuzhou, Jiangsu 221116, PR China
[c] College of Agricultural and Food Science, Zhejiang Agricultural and Forestry University, Hangzhou, Zhejiang 311300, PR China
[d] National Engineering Research Center for Information Technology in Agriculture, Beijing 100097, PR China

ARTICLE INFO

Article history:
Received 16 February 2013
Accepted 20 June 2013
Available online 3 July 2013

Keywords:
Populus euphratica
NaCl
PM H^+-ATPase gene
K^+/Na^+ homeostasis
Ion flux
NMT
Antioxidant enzymes

ABSTRACT

The plant plasma membrane (PM) H^+-ATPase plays a crucial role in controlling K^+/Na^+ homeostasis under salt stress. Our previous microarray analysis indicated that *Populus euphratica* retained a higher abundance of PM H^+-ATPase transcript versus a salt-sensitive poplar. To clarify the roles of the PM H^+-ATPase in salt sensing and adaptation, we isolated the PM H^+-ATPase gene *PeHA1* from *P. euphratica* and introduced it into *Arabidopsis thaliana*. Compared to wild-type, *PeHA1*-transgenic *Arabidopsis* had a greater germination rate, root length, and biomass under NaCl stress (50–150 mM). Ectopic expression of *PeHA1* remarkably enhanced the capacity to control the homeostasis of ions and reactive oxygen species in salinized *Arabidopsis*. Flux data from salinized roots showed that transgenic plants exhibited a more pronounced Na^+/H^+ antiport and less reduction of K^+ influx versus wild-type. Enhanced PM ATP hydrolytic activity, proton pumping, and Na^+/H^+ antiport in *PeHA1*-transgenic plants, were consistent to those observed *in vivo*, i.e., H^+ extrusion, external acidification, and Na^+ efflux. Activities of the antioxidant enzymes ascorbate peroxidase and catalase were typically higher in transgenic seedlings irrespective of salt concentration. In transgenic *Arabidopsis* roots, H_2O_2 production was higher under control conditions and increased more rapidly than wild-type when plants were subjected to NaCl treatment. Interestingly, transgenic plants were unable to control K^+/Na^+ homeostasis when salt-induced H_2O_2 production was inhibited by diphenylene iodonium, an inhibitor of NADPH oxidase. These observations suggest that *PeHA1* accelerates salt tolerance partially through rapid H_2O_2 production upon salt treatment, which triggers adjustments in K^+/Na^+ homeostasis and antioxidant defense in *Arabidopsis*.

1. Introduction

The plasma membrane (PM) H^+-ATPase establishes an electrochemical gradient of protons to drive secondary transport of ions and metabolites [1]. The proton pump has a central function in the regulation of a variety of key physiological processes, such as stomata opening, phloem loading, root ion uptake, and salt tolerance [1]. The H^+-ATPase is encoded by a multigene family, and the expression of isogenes is differentially regulated according to tissue type and developmental stage [2]. Gene expression of the PM H^+-ATPase was previously shown to be altered by various environmental stimuli, including salinity [3], heavy metals [4], mechanical stress [5], and externally applied hormones [6]. In addition to genetic regulation, the activity of the PM H^+-ATPase may be modulated at the post-translational level, mainly via reversible phosphorylation [7].

Active Na^+ extrusion to the apoplast or the external environment is essential for sustaining intracellular Na^+ homeostasis in salt-treated plants [8]. This process depends on electrochemical H^+ gradients generated by the PM H^+-ATPase in various plant species [2,3,7–9]. In addition to controlling Na^+ homeostasis, the PM H^+-ATPase also plays an important role in the reduction of salt-induced K^+ loss [9,10]. This contribution is mainly due to the up-regulation of the H^+ pumps, which preserves a less-depolarized membrane potential and thus restricts K^+ efflux through depolarization-

* Corresponding author. Tel.: +86 10 62338129; fax: +86 10 62336164.
E-mail address: Lschen@bjfu.edu.cn (S. Chen).
[1] These authors contributed equally to this work.

0981-9428/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.plaphy.2013.06.020

activated K^+ outward rectified channels and/or depolarization-activated non-selective cation channels [9,10]. Recent studies revealed that NaCl-induced H_2O_2 production contributes to K^+/Na^+ homeostasis via the PM H^+-ATPase [11–14]. However, the interaction between the PM H^+-ATPase and H_2O_2 signaling in salt adaptation in plants is still poorly understood.

H_2O_2 is a reactive oxygen species (ROS) that functions as an important regulator in various plant physiological processes such as root hair growth, ion transport, and stress response [15–17]. H^+ translocation derived by the PM H^+-ATPase is implicated in H_2O_2 signaling [12,17,18]. Increased PM H^+-ATPase activity in barley mesophyll cells was previously shown to correspond to exposure to powdery mildew [18]; the enhanced H^+ pumps caused apoplast acidification, which elevated H_2O_2 generation and led to a subsequent hypersensitive response in epidermal cells [18]. In *Arabidopsis thaliana* suspension cells, the PM proton pumps have also been implicated in hypo- or hyper-osmotic stress-induced production of an H_2O_2 wave [17]. Interestingly, H_2O_2 may be involved in the regulation of PM H^+-ATPase activity in herbaceous and woody species [11,19]. In *Vicia faba* guard cell protoplasts, abscisic acid inhibition of blue light-stimulated stomatal opening depends on the H_2O_2-induced dephosphorylation of the PM H^+-ATPase [19]. Therefore, the interaction between the PM H^+-ATPase and H_2O_2 signaling is crucial for plant growth, development, and stress adaptation.

Populus euphratica has been widely used as a model plant for the elucidation of the physiological and molecular mechanisms of salt tolerance in woody species [12,13,20–24]. Compared to salt-sensitive poplars, *P. euphratica* usually maintains a higher K^+/Na^+ ratio at both the tissue and cellular levels, which is mainly due to PM H^+-ATPase-dependent Na^+ extrusion and K^+ preservation [12,13,20–24]. Our previous microarray analysis revealed that *P. euphratica* leaves have a higher expression of PM H^+-ATPase than the leaves of the salt-sensitive poplar *Populus popularis* under both normal growth conditions and NaCl stress [3]. More rapid H_2O_2 production in response to NaCl salinity was observed in *P. euphratica* cells versus *P. popularis* [13]. A pharmacological investigation revealed that the PM H^+-ATPase may function as an ionic sensor to induce an early H_2O_2 burst, which contributes to the regulation of K^+/Na^+ homeostasis in *P. euphratica* cells [11–13]. However, there is no genetic evidence for this signaling cascade in *P. euphratica*.

The objective of the present study was to investigate the role of the H^+-ATPase in salt stress signaling and the control of ionic homeostasis. We cloned a putative PM H^+-ATPase gene, *PeHA1*, from *P. euphratica* and introduced it into the model plant *Arabidopsis*. *PeHA1* overexpression enabled *Arabidopsis* to retain K^+/Na^+ and ROS homeostasis under prolonged NaCl salinity. Our data reveal that H^+ pump-dependent ionic homeostasis control in transgenic *Arabidopsis* relies on a rapid burst of H_2O_2 after the onset of salt treatment. Our observations suggest that the PM H^+-ATPase functions as an ionic sensor and contributes to H_2O_2 signaling in higher plants.

2. Results

The gene *PeHA1*, encoding a putative PM H^+-ATPase, was cloned from the salt-resistant tree species *P. euphratica*. *PeHA1* contains the complete 2848-bp open reading frame encoding a polypeptide with 955 amino acids, which is predicted to be 104.8 kDa in size (Fig. 1A). Alignment of H^+-ATPases from several species indicates that PeHA1 harbors similar regulatory domains that are important for enzyme activity. Regions I and II in PeHA1 are two conserved auto-inhibitory domains, confirming the prediction that PeHA1 is an auto-inhibitory H^+-ATPase (Fig. 1A). The presence of a 14-3-3 binding domain suggests that the 14-3-3 protein interact with PeHA1 to regulate the enzyme's activity (Fig. 1A).

Phylogenetically, the amino acid sequence of PeHA1 exhibits the most similarity (98.7%) to the homologous sequence in *Populus trichocarpa* (NCBI Reference Sequence: XM_002330768.1; protein_id = XP_002330804.1; Fig. 1B). PeHA1 has a relatively high degree of similarity with AHA5 (NP_180028.1) in *Arabidopsis* (Fig. 1B). Evolutionary divergence is evident between PeHA1, *Arabidopsis* AHA7, and the PM H^+-ATPase genes from monocotyledon plants such as *Oryza sativa* and *Zea mays* (Fig. 1B).

The cellular localization of PeHA1 was determined by colocalization of the chimeric YFP::PeHA1 protein and the PM marker plasmid CFP::AtPIP2, which was transiently expressed in *Arabidopsis* mesophyll protoplasts under the control of a double CaMV 35S promoter. Fluorescence of YFP::PeHA1 was restricted to the PM of *Arabidopsis* protoplasts, without any detectable fluorescence in other parts of the cells (Fig. 2A). This observation was consistent with the fluorescence distribution of the PM marker, indicating the PM localization of PeHA1 (Fig. 2A).

We transformed *PeHA1* into WT *Arabidopsis* under the control of the CaMV 35S promoter. *PeHA1* expression and hydrolytic activity of the plasma membrane H^+-ATPase were examined in WT *Arabidopsis* and transgenic lines. Genomic DNA PCR and RT-PCR revealed *PeHA1* expression in the T3 lines of transgenic *Arabidopsis* (H1, H3, H8, and H9; Fig. 2B, C). Real-time PCR showed that the mRNA abundance of *PeHA1* was significantly higher in transgenic lines (especially H1 and H3) than in the wild-type (Fig. 2C). Using purified plasma membrane vesicles, hydrolytic activity of H^+-ATPase was measured in transgenic and wild-type *Arabidopsis*. Result showed that H1 and H3 exhibited a higher activity of ATP hydrolysis than H8, H9 and the wild-type (Fig. 2D).

Salt tolerance of wild-type and *PeHA1*-transgenic *Arabidopsis* were compared in this study. H1 and H3 showed higher germination rates at 100 and 150 mM NaCl versus WT (Fig. 3A, B). Therefore, H1 and H3 transgenic *Arabidopsis* lines were used for further analysis. Root elongation in H1 and H3 *Arabidopsis* was greater than in WT plants under both control and salinity conditions (Fig. 3C, D). Moreover, the dry weight of transgenic *Arabidopsis* plants was significantly higher than that of WT plants after 10 days of NaCl treatment (50 and 100 mM, Fig. 3E). Overall, our results indicate that ectopic expression of *PeHA1* improves salt tolerance in *Arabidopsis*. In our study, we found that the wild-type *Arabidopsis* and vector controls had not significant difference in either germination or root growth, irrespective of control or salt treatment (Supplemental Fig. S1).

We examined ion levels in wild-type *Arabidopsis* and *PeHA1*-transgenic lines under control and saline conditions. Long-term (21 days) exposure to 100 mM NaCl caused a significant rise in Na^+ levels in WT and transgenic *Arabidopsis*; however, a more pronounced effect occurred in WT seedlings (Fig. 4). NMT flux data revealed that Na^+ efflux in the apical regions of the roots was significantly increased in all genotypes under salinity conditions (50 and 100 mM NaCl; Fig. 5A). Notably, *PeHA1*-transgenic plants displayed 79–282% higher flux than WT plants (Fig. 5A). A net H^+ efflux was detected in the control roots of all tested genotypes (Fig. 5C). The H^+ extrusion was greater in H1 and H3 roots, resulting in a more acidic pH than wild-type (Fig. 5C, D). Salt treatment (50 or 100 mM NaCl) caused a net H^+ influx in WT plants (Fig. 5C), implying that H^+ efflux was consumed by Na^+ extrusion via an antiporter. This is consistent to Jayakannan et al., who found that NaCl-induced a H^+ influx from the mature root zone [25]. H1 and H3 roots retained a net H^+ efflux although the flux rate was reduced by exposure to 50 or 100 mM NaCl (Fig. 5C), suggesting that transgenic plants had created an increased proton motive force for Na^+/H^+ antiport. Proton transport activity and Na^+/H^+ antiport activity in membranes isolated from WT and transgenic plants were