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GENETIC ENGINEERING COTTON FOR HIGHER DROUGHT- AND SALT-TOLERANCE

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Abstract

Drought and salinity are two major limiting factors in crop productivity. The drought–caused crop loss was over $1 billion annually in Texas, of which about half resulted from cotton loss. Besides drought, saline water and soils also contribute to the reduction in cotton yield and fiber quality in America's Southwest. One way to reduce cotton loss caused by drought and salinity is to increase solute concentration in the vacuoles of cotton cells, so that the solute potential is more negative inside cells, resulting in water to move into cells and avoiding accumulation of sodium ion to toxic level in cytoplasm, therefore better water retention and higher salt tolerance can be achieved. The success of this approach was demonstrated in various plants by overexpressing the Arabidopsis genes *AtNHX1* that encodes a sodium/proton antiporter and *AVP1* that encodes a proton pump. Overexpression of *AtNHX1* increases vacuolar uptake of sodium, whereas overexpression of *AVP1* generates higher proton electrochemical gradient (PEG) across the vacuolar membrane that energizes secondary transporters including AtNHX1, both of which lead to increased vacuolar solute concentration and therefore higher salt- and drought-tolerance in transgenic plants. In an effort to engineer cotton for higher drought- and salt-tolerance, transgenic cotton plants that express *AtNHX1* were created. Since AtNHX1 activity depends on PEG generated by proton pumps like AVP1, a coupled overexpression of *AtNHX1* and *AVP1* would potentially confer higher tolerance against drought and salt in transgenic plants. Therefore creating another transgenic cotton line that expresses the Arabidopsis *AVP1* gene is also underway. It is hoped that *AtNHX1*- and *AVP1*-double overexpression cotton will be more drought- and salt-tolerant.

Introduction

Environmental stresses such as drought and salinity are the major limiting factors in agricultural productivity. The drought–caused crop losses in Texas were over $2 billion for both 1996 and 1998, and $1.1 billion for 2000, with half the losses in cotton [Easton, 2000; Lee, 2000]. Besides drought, the saline water and soils of West Texas also contribute to the reduction in cotton yield and fiber quality. Increasing cotton's tolerance against drought and salt stress would improve cotton's yield and fiber quality, which will have an enormous impact on Texas' economy, since cotton is the most important crop in Texas. Furthermore, cotton-based products are estimated to generate additional $100 billions annually for the U.S. economy, making cotton the No. 1 value-added crop in the U.S. One way to increase drought- and salt-tolerance in plants is to increase solute concentration in the vacuoles of plant cells, so the solute potential becomes more negative inside cells, causing water to move into cells, increasing water retention and salt tolerance [Gaxiola et al., 2002]. Two approaches were used to increase solute contents in plant vacuoles (Fig. 1). The first approach was increasing the activity of antiporters on the vacuolar membrane that transport ions, sugars, or amino acids into vacuoles. The second approach was increasing the activity of proton (H^+) pumps on vacuolar membrane to move more H^+ into vacuoles generating higher proton electrochemical gradient (PEG). The Blumwald group at UC Davis used the first method to create salt- and drought-tolerant plants by overexpressing an Arabidopsis gene *AtNHX1* that encodes a vacuolar sodium/proton (Na^+/H^+) antiporter in transgenic plants [Apse et al., 1999; Zhang and Blumwald, 2001; Zhang et al., 2001]. Engineered transgenic Arabidopsis, tomato, and Canola expressed higher levels of AtNHX1 protein and demonstrated increased vacuolar uptake of Na^+ than wild type plants [Apse et al., 1999; Zhang and Blumwald, 2001; Zhang et al., 2001]. Gaxiola et al. used the second method to achieve higher drought- and salt-tolerance in transgenic plants by overexpressing the Arabidopsis gene *AVP1* that encodes a H^+ pump [Gaxiola et al., 2001], suggesting that increased H^+ pump activity could indeed lead to higher PEG that potentially further activates secondary antiporter activities, including AtNHX1, on the vacuolar membrane. Overexpression of *AtNHX1* alone could increase salt-tolerance, suggesting that the PEG generated by native proton pumps is enough to energize the increased AtNHX1 to function properly in native (i.e. Arabidopsis) and heterologous (i.e. tomato and Canola) systems. However, to further increase plant salt- and drought-tolerance, a coupled overexpression of both H^+ pumps and antiporters will probably be required.

Materials and Methods

Construction of *AtNHX1*- and *AVP1*-Transforming Vectors

Wild type *AtNHX1*, wild type *AVP1*, and a mutated *AVP1* genes were fused between the cauliflower mosaic virus 35S promoter and terminator in the intermediate vector pRTL2, respectively, and then inserted into the binary vector pCGN1578 [Yan et al., 2003]. The resulting binary vectors were transformed into agrobacteria for cotton transformation.

Cotton Transformation

The cotton transformation procedure established by Bayley et al. [1992] was followed with some modifications [Yan et al., 2003].

Results

We have created over 30 transgenic cotton plants that express the Arabidopsis *AtNHX1* gene [He et al., 2003] (Fig. 2). Our PCR analysis of 30 independent transgenic lines using *AtNHX1*-specific primers indicates that 27 of them contain the introduced transgene (Fig. 3A). The RNA blot analysis indicates that 12 transgenic lines expressed *AtNHX1* transcript at high levels (Fig. 3B).

Discussion

AtNHX1-expressing cotton plants have been made in our laboratory and biochemical and physiological experiments are being conducted to test if they are more tolerant against drought and high salt conditions. Furthermore, transgenic lines that overexpresses the Arabidopsis *AVP1* gene is being constructed, and it is hoped that *AtNHX1*- and *AVP1*-double overexpression cotton will demonstrate even higher drought- and salt-tolerance. We expect to see increased cotton yield and longer fiber length from these transgenic cotton plants, because water plays a pivotal role during fiber cell elongation. A field test of *AtNHX1*-expressing cotton is planned for the incoming summer.

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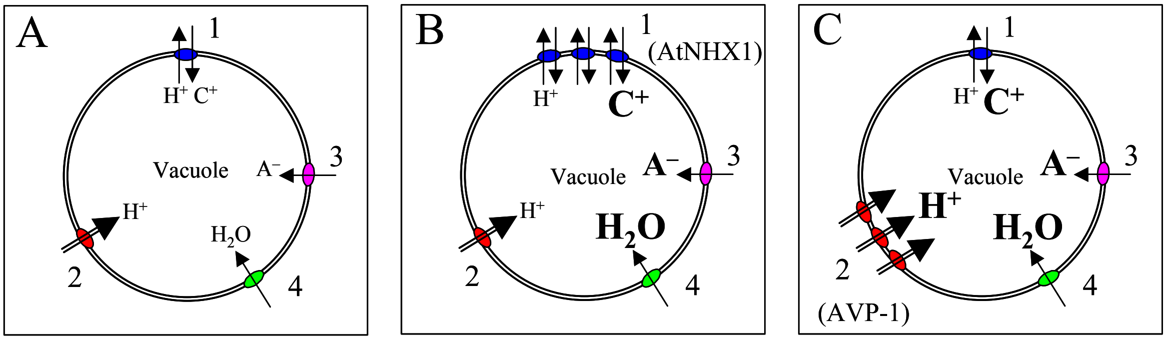

Fig. 1. Model of vacuolar antiporter, proton pump, anion channel, water channel, and their roles in water status in plant cell. 1, Na^+/H^+ antiporter; 2, H^+-pump; 3, anion channel; 4, water channel. **A**, proton pumps such as AVP1 generate PEG that drives all vacuolar antiporters including Na^+/H^+ antiporter, thereby keeping solute potential in plant vacuole low and water moving in; **B**, overexpression of a Na^+/H^+ antiporter, e.g. AtNHX1, increases Na^+ concentration inside vacuole, making vacuolar solute potential more negative and more water moving in; **C**, overexpression of a H^+ pump, e.g. AVP1, increases PEG and consequently activates all vacuolar antiporters including Na^+/H^+ antiporter, more water moving in.

Fig. 2. *AtNHX1*-expressing cotton plants grown in greenhouse.

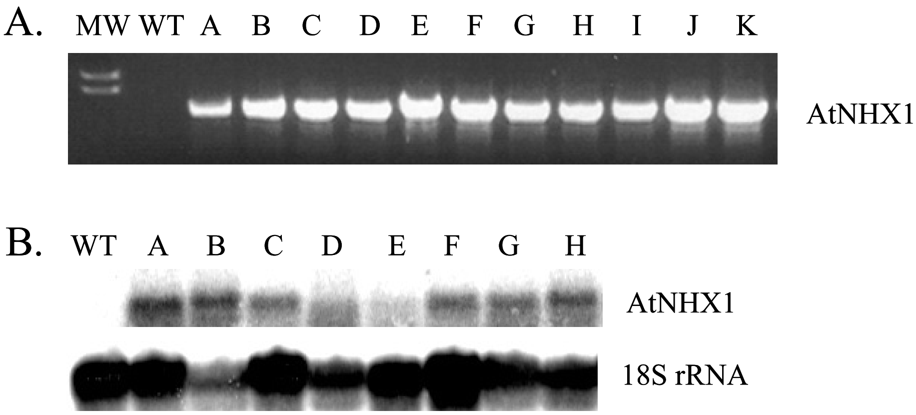

Fig. 3. Molecular analysis of *AtNHX1*-expressing cotton plants. **A**. PCR analysis of wild type and eleven independent transgenic cotton plants using *AtNHX1*-specific primers. MW, molecular weight markers (2.3.kb and 2.0 kb); WT, wild type; A to K, transgenic cotton plants that contain *AtNHX1* transgene. **B**. Northern blot analysis of wild type and eight independent transgenic cotton plants. An AtNHX1 cDNA fragment was used as probe and the 18S rRNA was used as the RNA loading control.