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ANALYSIS OF TRANSGENIC COTTON ENGINEERED FOR HIGHER DROUGHT AND SALT-TOLERANCE IN GREENHOUSE AND IN THE FIELD

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Abstract

Drought and salinity are major environmental factors that limit crop productivity in Southwest of United States. Drought–caused loss in cotton yield is over $1 billion annually in Texas. One way to reduce cotton loss caused by drought and salt is to increase solute concentration in cotton root cells, so that the solute potential is more negative inside cells, which draws water to move into cells. The success of this approach was demonstrated by overexpressing the Arabidopsis gene *AtNHX1* that encodes a sodium/proton antiporter in various plants such as tomato and rapeseed. Overexpression of *AtNHX1* increases vacuolar uptake of sodium, which leads 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. These *AtNHX1*-expressing cotton plants are indeed more tolerant to salt treatment in greenhouse conditions. They also produced more bolls and fibers in the field conditions as tested in the fall of 2004 at Texas Tech's Experimental Farm in Lubbock, Texas.

Introduction

Drought and salinity limit crop productivity worldwide, and they are becoming more and more serious as global warming appears to be the trend for the climate of our planet. The drought–caused cotton loss in Texas is usually over 1 billion annually [Easton, 2000; Lee, 2000]. Saline water and soils in West Texas add further damages to cotton yield and fiber quality. To improve cotton's yield and quality, we must improve cotton's tolerance to drought and salt conditions. One effective way to achieve this goal is to increase solute concentration in the vacuoles of cotton root cells, so that solute potential is more negative inside, which then draws water to move into cells, therefore achieving better water retention and higher salt tolerance [Gaxiola et al., 2002]. One approach successfully used by Blumwald group at UC Davis in creating more salt-tolerant plants was overexpressing the 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 plants demonstrated higher vacuolar uptake of Na^+ than wild type plants did [Apse et al., 1999; Zhang and Blumwald, 2001; Zhang et al., 2001], which is responsible for increased salt tolerance. Overexpression of *AtNHX1* in native (i.e. Arabidopsis) or heterologous (i.e. tomato and Canola) systems leads to the same phenotype, i.e. increased salt tolerance, suggesting that this approach should work in most plants including cotton as well. We have therefore expressed the Arabidopsis *AtNHX1* in cotton to test if we could improve cotton's performance in the harsh conditions of West Texas.

Materials and Methods

Construction of *AtNHX1*-transforming vector

The wild type Arabidopsis gene *AtNHX1* was fused between the cauliflower mosaic virus 35S promoter and terminator in the intermediate vector pRTL2, and then inserted into the binary vector pCGN1578 [Yan et al., 2003]. The resulting binary vector was introduced into Agrobacterium for cotton transformation.

Cotton transformation

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

Salt treatment

Wild type (Coker 312) and transgenic cotton plants were germinated in soil for two weeks, and then transplanted into the 11-liter pots individually for another 5 days. Salt treatment was conducted in incremental manner: 50 mM 7 days, 100 mM 7 days, 150 mM 7 days, and finally 200 mM for 21 days before they were photographed.

Results

We have created over 30 transgenic cotton plants that express the Arabidopsis *AtNHX1* gene [He et al., 2003], and our PCR analysis and RNA blot analysis indicated that 12 transgenic lines expressed *AtNHX1* transcript at high levels [Zhang et al., 2004]. We then analyzed 6 transgenic lines for their salt tolerance in greenhouse conditions. Wild type cotton plants were severely inhibited by 200 mM of NaCl treatment (Fig. 1), whereas *AtNHX1*-expressing cotton plants were less inhibited by the same concentration of NaCl (Fig. 2). After 21 days under 200 mM of NaCl treatment, *AtNHX1*-expressing cotton plants were significantly bigger in size than that of wild type control plants (Fig. 3). We field-tested 7 transgenic lines in our experimental farm in Lubbock, and found that 6 of them produced higher fiber yield with an average increase of 20% per plant, and 5 of them had more bolls per plant than wild type control plants (data not shown). Unlike the salt treatment experiments that we repeated several times in greenhouse, we only did the field-testing experiment once. Therefore we plan to continue field-testing our *AtNHX1*-expressing cotton plants next year.

Discussion

AtNHX1-expressing cotton plants made in our laboratory prove to be more salt-tolerant as expected in greenhouse conditions. More importantly they appear to produce more fiber in the field condition, which could significantly improve cotton yield in regions where less annual rainfall and saline water and soils are major problems for agriculture such as West Texas.

References

Apse, M.P., G.S. Aharon, W.A. Snedden, E. Blumwald. 1999. Salt tolerance conferred by overexpression of a vacuolar N^+/H^+ antiport in Arabidopsis. Science 285: 1256-1258.

Bayley, C., N. Trolinder, C. Ray, M. Morgan, J.E. Quisenberry, and D.W. Ow. 1992. Engineering 2, 4-D resistant into cotton. Theor. Appl. Genet. 83: 645-649.

Easton, P. 2000. Farm losses threaten to hit $1 billion. Associated Press, Oct. 6, 2000.

Gaxiola, R.A., G.R. Fink, and K.D. Hirschi. 2002. Genetic Manipulation of Vacuolar Proton Pumps and Transporters. Plant Physiol. 129: 967-973.

He, C., J. Yan, J. Wang, E. Blumwald, and H. Zhang. 2003. Creating salt- and drought-tolerant cotton by over-expressing the Arabidopsis antiporter protein AtNHX1. ASPB 2003 annual meeting, Abs# 163, Honolulu, HI.

Lee, S.H. (2000). Drought losses to Texas farmers pass $1 billion. The Dallas Morning News, Oct. 19, 2000.

Yan, J., J. Wang, C. He, A.S. Holaday, and H. Zhang. 2003. Overexpression of the Arabidopsis 14-3-3 protein GF14λ in cotton delays leaf senescence and improves drought tolerance. Plant Cell Physiol, in revision.

Zhang, H-X., and E. Blumwald. 2001. Transgenic salt tolerant tomato plants accumulate salt in the foliage but not in the fruits. Nature Biotech. 19: 765-768.

Zhang, H-X., J. Hodson, J.P. Williams, and E. Blumwald. 2001. Engineering salt- tolerant Brassica Plants: Characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. Proc. Natl. Acad. Sci. USA 98: 12832-12836.

Zhang, H., C. He, G. Shen, J. Yan, E. Blumwald, and R. Gaxiola. (2004). Genetic engineering cotton for higher drought- and salt-tolerance. In 2004 Beltwide Cotton Conferences, San Antonio, Texas, January 4-7, 2004.

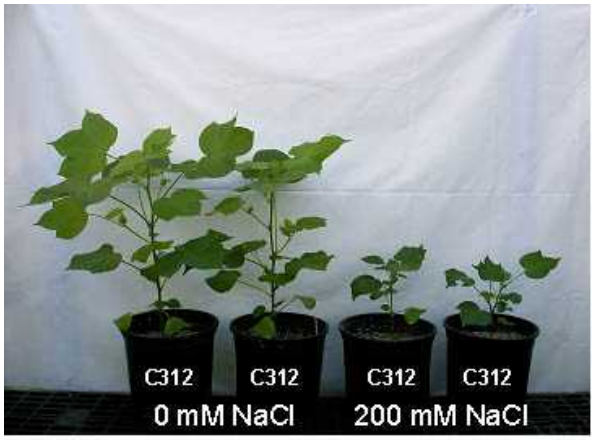

Fig. 1. Wild type plants (Coker312) under normal and salt treatment conditions. Plants were photographed after 21 days under 200 mM NaCl treatment.

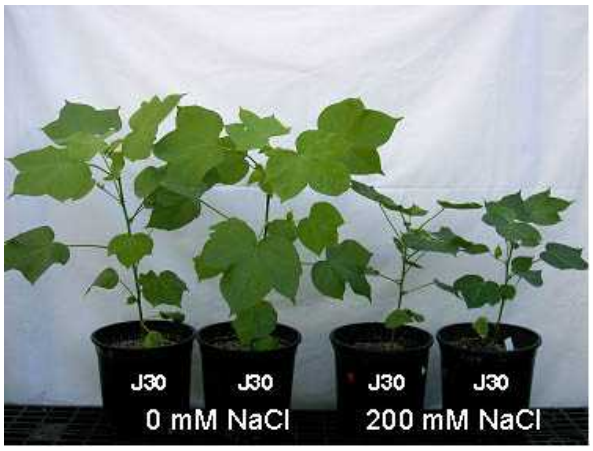

Fig. 2. Transgenic plants (line J30) under normal and salt treatment conditions. Plants were photographed after 21 days under 200 mM NaCl treatment.

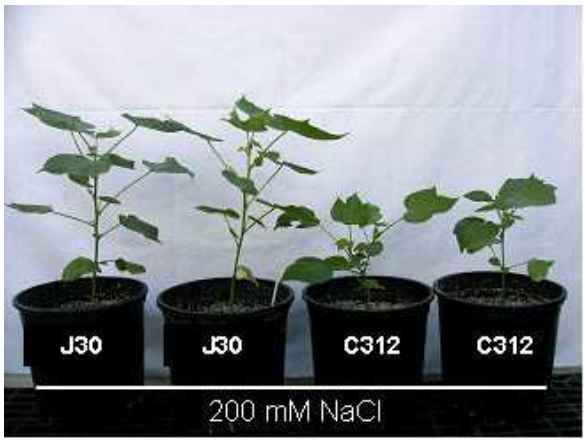

Fig. 3. Transgenic plants (line J30) and wild type control plants (Coker312) under salt treatment conditions. Plants were photographed after 21 days under 200 mM NaCl treatment.