

## Creating drought- and salt-tolerant cotton by overexpressing a vacuolar pyrophosphatase gene

Hong Zhang,<sup>1\*</sup> Guoxin Shen,<sup>2</sup> Sundaram Kuppu,<sup>1</sup> Roberto Gaxiola<sup>3</sup> and Paxton Payton<sup>4</sup>

<sup>1</sup>Department of Biological Sciences; Texas Tech University; <sup>4</sup>USDA-ARS Cropping Systems Research Laboratory; Lubbock, TX USA;

<sup>2</sup>Zhejiang Academy of Agricultural Sciences; Hangzhou, Zhejiang Province China; <sup>3</sup>School of Life Sciences; Arizona State University; Tempe, AZ USA

**I**ncreased expression of an Arabidopsis vacuolar pyrophosphatase gene, *AVPI*, leads to increased drought and salt tolerance in transgenic plants, which has been demonstrated in laboratory and field conditions. The molecular mechanism of *AVPI*-mediated drought resistance is likely due to increased proton pump activity of the vacuolar pyrophosphatase, which generates a higher proton electrochemical gradient across the vacuolar membrane, leading to lower water potential in the plant vacuole and higher secondary transporter activities that prevent ion accumulation to toxic levels in the cytoplasm. Additionally, overexpression of *AVPI* appears to stimulate auxin polar transport, which in turn stimulates root development. The larger root system allows *AVPI*-overexpressing plants to absorb water more efficiently under drought and saline conditions, resulting in stress tolerance and increased yields. Multi-year field-trial data indicate that overexpression of *AVPI* in cotton leads to at least 20% more fiber yield than wild-type control plants in dry-land conditions, which highlights the potential use of *AVPI* in improving drought tolerance in crops in arid and semiarid areas of the world.

Drought and salinity are major environmental factors that limit agricultural productivity in most parts of the world.<sup>1</sup> Climate change will likely make many places worse in terms of water availability and soil salinization,<sup>2</sup> which will have negative impacts on food production in world agriculture. Yet, the demand for more food will continue to rise because

of the growing world population that may reach 9 billion people by 2050.<sup>3</sup> Therefore, the primary challenge we face during this century is the production of more food under the constraints of limited water and fertilizer on marginal soils.

Many genes that respond to abiotic stresses have been identified in the model plant Arabidopsis,<sup>4</sup> and some of them were shown to play important roles in protecting plants under abiotic stress conditions.<sup>5</sup> The Arabidopsis vacuolar pyrophosphatase gene *AVPI* appears to be one of the most promising genes that may be used to improve drought- and salt-tolerance in crops.<sup>6</sup> Roberto Gaxiola's group first demonstrated that overexpression of *AVPI* could lead to significantly improved drought- and salt-tolerance in transgenic Arabidopsis plants.<sup>7</sup> Later when this gene was introduced into tomato<sup>8</sup> and rice,<sup>9</sup> similar tolerance phenotypes were observed. Overexpression of *AVPI* in cotton, not only improved drought- and salt-tolerance in greenhouse conditions, but also increased fiber yield in dryland field conditions.<sup>6</sup> *AVPI*-expressing cotton plants produced larger root systems and bigger shoot biomass than controls when grown under hydroponic conditions in the presence of up to 200 mM NaCl.<sup>6</sup> In the greenhouse, *AVPI*-expressing cotton plants also produced more root and shoot biomass than controls when grown under saline conditions or reduced irrigation.<sup>6</sup> The increased yield by *AVPI*-expressing cotton plants is due to more bolls produced, which in turn is due to larger shoot system that *AVPI*-expressing cotton plants develop under saline or drought conditions.<sup>6</sup>

**Key words:** drought tolerance, proton pump, salt tolerance, transgenic cotton, vacuolar membrane

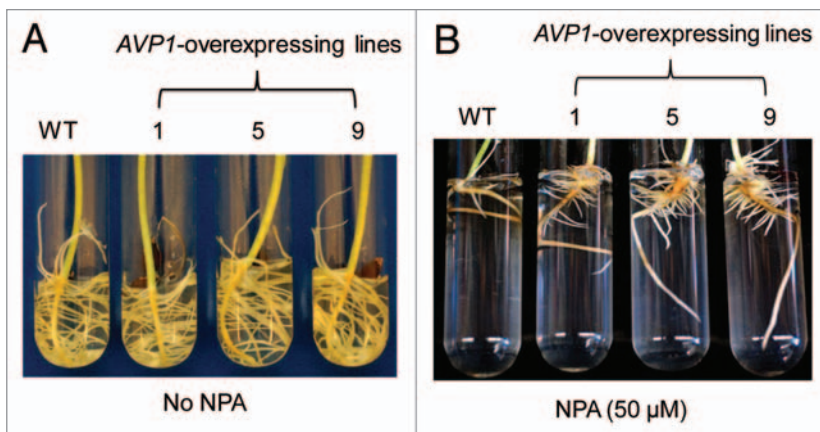
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\*Correspondence to: Hong Zhang;  
Email: hong.zhang@ttu.edu

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**Figure 1.** Root development of wild-type and *AVPI*-expressing cotton plants in the absence and presence of auxin transport inhibitor NPA. (A) Phenotype of cotton roots after 10 days of growth in the absence of NPA. WT, Wild-type; 1, 5, 9, three independent *AVPI*-overexpressing cotton lines. (B) Phenotype of cotton roots after 10 days of growth in the presence of 50 μM NPA.



**Figure 2.** Wild-type and *AVPI*-expressing cotton plants grown in the dryland field condition. Plants were planted in the middle of May 2009 and the picture was taken in the middle of July 2009 at the USDA Experimental Farm in Lubbock, Texas.

The larger root systems of *AVPI*-expressing cotton plants under saline and water-deficit conditions allow transgenic plants access to more of the soil profile and available soil water resulting in increased biomass production and yield. Li et al. showed that the larger root systems of *AVPI*-overexpressing *Arabidopsis* is caused by increased auxin polar transport in the root, which stimulates root development in *AVPI*-overexpressing *Arabidopsis* plants. Furthermore, a recent comparative study of transgenic *Arabidopsis* lines that produce enlarged leaves showed that auxin levels were increased by 50% in *AVPI*-overexpressing plants.<sup>11</sup> To test if altered auxin level is responsible for the observed

larger root systems in *AVPI*-expressing cotton plants, we germinated wild-type and *AVPI*-expressing cotton plants in the absence or presence of the auxin polar transport inhibitor Naphthylphthalamic acid (NPA). Both wild-type and *AVPI*-expressing cotton plants developed robust lateral root systems in the absence of NPA (Fig. 1A). The presence of 50 μM NPA resulted in nearly complete inhibition of lateral root development in wild-type plants, while lateral root development in *AVPI*-expressing plants was reduced, it was significantly greater than wild-type (Fig. 1B). These data indicate that *AVPI*-overexpression could overcome the inhibitory effects of NPA on root development

in *AVPI*-expressing cotton plants, suggesting that either increased auxin transport or higher auxin concentration in the root systems of *AVPI*-expressing cotton plants is responsible for the observed larger root systems, and eventually for the increased boll numbers and fiber yields under dryland field conditions.

Many genes that may play important roles under water-deficit conditions have been tested in laboratory conditions,<sup>4,5</sup> but very few have been tested vigorously in field conditions. A bacterial cold shock protein gene was shown to improve drought tolerance in maize based on multi-year and multi-place field trial experiments,<sup>12</sup> and it appears that this gene will likely gain approval for commercial release and become the first genetically engineered product that demonstrates improved drought tolerance in a major crop in the U.S. Another example of increased drought tolerance supported by multiple field trial experiments is through downregulation of farnesylation in transgenic canola plants.<sup>13</sup> Downregulation of farnesyltransferase by antisense or RNAi techniques in transgenic canola leads to increased sensitivity to abscisic acid, consequently resulting in smaller guard cell aperture under drought conditions. These transgenic canola plants lose less water through transpiration and are more drought resistant. Data from more than 5 years of field studies in Canada consistently proved that this approach can indeed increase drought tolerance in transgenic canola. Our study with *AVPI*-expressing cotton over the last several years in field conditions is another example that genetic engineering approach can be an efficient tool in generating drought-tolerant crops. *AVPI*-expressing cotton plants can establish a larger shoot mass in dryland conditions (Fig. 2), which results in increased boll numbers and fiber production. Our approach is likely applicable to other major crops as well.

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