Increasing salt tolerance in *Arabidopsis thaliana* through co-overexpression of *AtCLCc* and *PP2A-C5* genes

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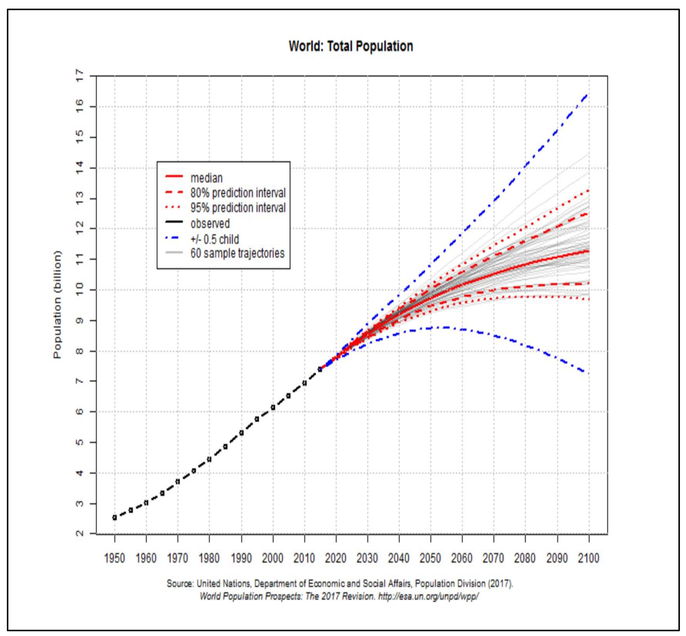
Abstract

Global environmental changes have caused an increase in salt-scarred farmlands and an increased need to fortify our agricultural practices. By manipulating the expression levels of individual genes, transgenic plants have shown much improved resistance to abiotic stresses, potentially leading to increased crop yield under stressful conditions. We are overexpressing two genes, AtCLCc and PP2AC5, in the model organism Arabidopsis thaliana to see if AtCLCc/PP2AC5 co-overexpressing plants perform significantly better than AtCLCc-overexpressing, PP2AC5-overexpressing, and wild-type plants under single stress, multiple-stress, and normal growth conditions. The AtCLCc gene encodes an antiporter that sequesters chloride anions into the cell's vacuole while exporting protons, thereby increasing plant stress tolerance under salt conditions. The PP2AC5 gene encodes a phosphatase that upregulates chloride channels such as AtCLCc, thereby increasing AtCLCc's activity. The AtCLCc/PP2AC5 co-overexpression construct was incorporated into the Arabidopsis genome utilizing Agrobacterium-mediated transformation, and homozygous transgenic plants were obtained. High-expression lines will be identified using RNA blot analysis and will be tested by physiological analysis under normal and salt conditions. The performance of AtCLCc/PP2AC5 co-overexpressing plants under high salinity conditions will be quantified by measurements of root length, shoot development, and overall yield and will be compared to both wild-type and single overexpression transgenic plants. If this project is promising, we will introduce these two genes into a more practical organism, such as cotton, to test if we can increase crop yields on a larger scale under high salinity conditions.



Methods

Agrobacterium-mediated gene transformation was used to incorporate the construct into the *Arabidopsis* genome.

Plants were identified from the initial testing and were screened for single T-DNA insertion lines and for homozygosity using a kanamycin resistant gene as a marker.

Seeds were sterilized with 70% ethanol/15% bleach before plating.

Agar plates contain nutrients and 75 mM concentration of NaCl.

Photos were taken after 10 days after plating.

PP2AC5-ClCc

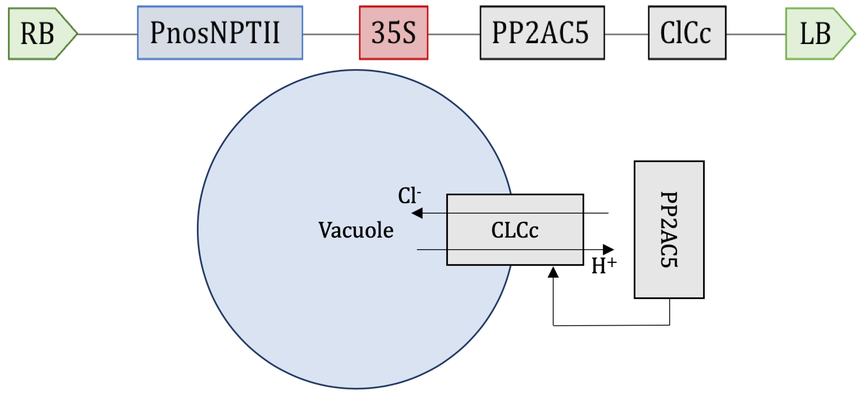


Figure 1. Genetic construct and intracellular interaction of *AtClCc* and *PP2AC5* overexpression in a plant cell

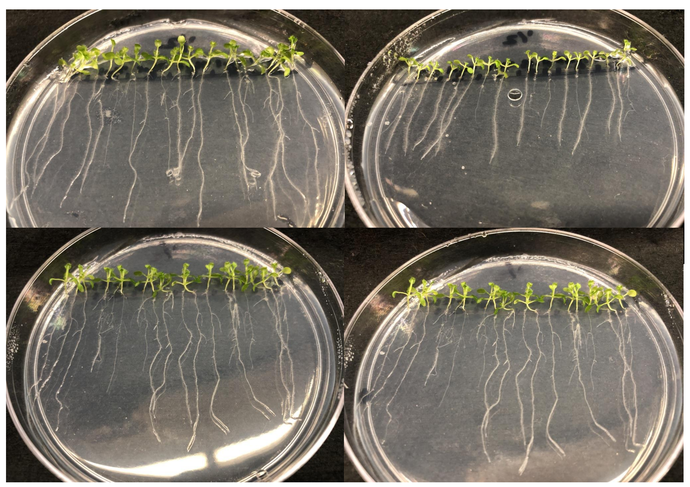
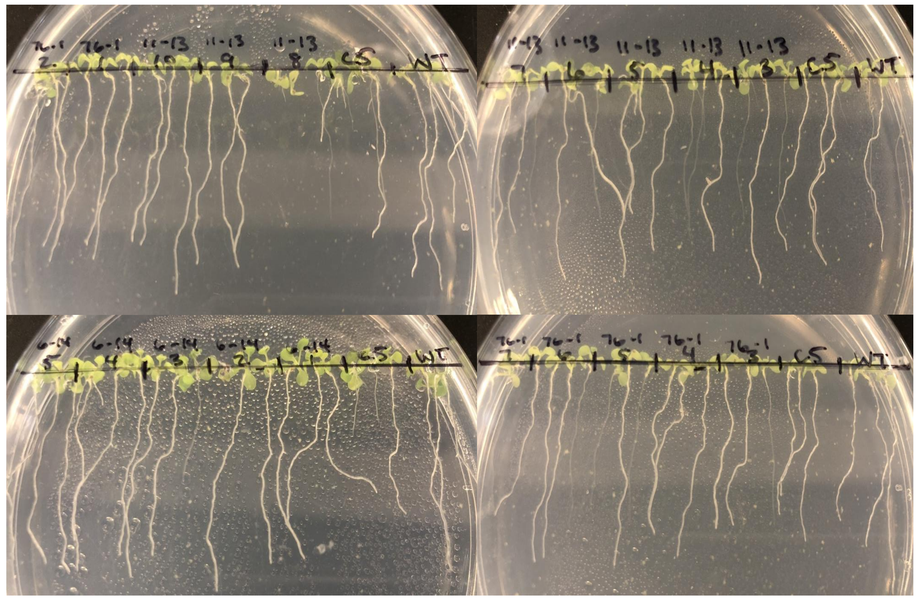


Figure 2. Photos taken on ½ MS 0 mM NaCl control plates 10 days after sterilization and plating. In each photo, left-most line is WT line. From top left to bottom right: lines 6-14:1-5, 76-1:1-7,11-13:3-10.

75 mM NaCl



150 mM NaCl

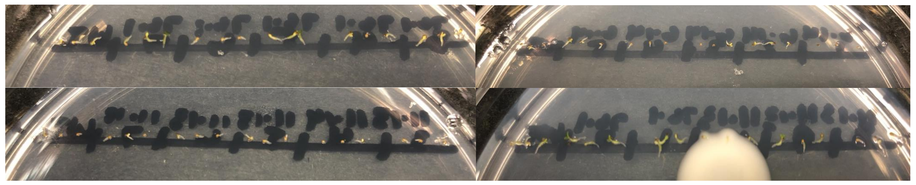


Figure 3. Photos taken of *Arabidopsis* plants plated on 75 mM (top four) and 150 mM (bottom four) ½ MS agar plates 10 days after sterilization and plating

Future Plans

High expression lines will be identified by using RNA blot analysis and phenotypic changes will be quantified. We expect that these multigene transgenic plants will perform much better physiologically with features including, but not limited to, better root quality, improved shoot development, and increased biomass when compared to control plants in a controlled environment as well as under multiple stress conditions.

If successful, this study will open the door to new varieties of crops that will flourish in traditionally harsh agricultural conditions, without a reduction in quality or yield.

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