



**CALIFORNIA STATE SCIENCE FAIR  
2007 PROJECT SUMMARY**

<b>Name(s)</b> <b>Sravya R. Keremane</b>	<b>Project Number</b> <b>S0409</b>
<b>Project Title</b> <b>Biochemical Analysis of Mechanisms of Cold Tolerance in Citrus: Effects of Abscisic Acid</b>	
<p style="text-align: center;"><b>Abstract</b></p> <p><b>Objectives/Goals</b> To study the effect of abscisic acid root drench on levels of expression of different cold tolerance genes. If transcription increases with addition of abscisic acid then it may be possible to use abscisic acid root drench during periods of freeze to prevent the death and loss of citrus plants. Freeze damages are common to citrus industries in California and Florida. California citrus industry lost about a billion dollars in the winter of 2007 because of freeze damages.</p> <p><b>Methods/Materials</b> Twenty Mexican lime and sour orange plants were treated with root drench of abscisic acid and placed in both 4<sup>o</sup> C and 24<sup>o</sup> C. Leaf samples were taken after 2 hours and 48 hours from both the treated and control plants. Total RNA was extracted using Qiagen RNeasy kit and cDNA was made using oligo dT primer and superscript II reverse transcriptase from Invitrogen. Quantitative real-time Polymerase Chain Reaction (qPCR) was done using SYBR greenER qPCR mix from Invitrogen. Expression of four genes were analyzed in separate PCRs using specific primers for detecting transcripts of abscisic acid binding factor 3 (ABF3), Inducer of CBF expression (ICE I), citrus low temperature a (CLTa), and a house keeping gene, aspartate trans-carbamylase (ATC) as an internal control. The results were analyzed using 2 hour and 48 hour samples from each plant. A standard curve was constructed using serial dilutions from a cloned plasmid of ABF3 transcript (Mexican lime). A fragment of ABF3 gene was amplified by PCR from both DNA and cDNA of Mexican lime and sour orange using specific primers. These products were cloned and sequenced. An alignment of DNA and cDNA was made using ChromasPro, Clustal and Gendoc programs.</p> <p><b>Results</b> Abscisic acid treatment increased the levels of expression of three different cold tolerance genes, ABF3, ICE1 and CLTa, in both greenhouse and cold conditions. Comparison of sequences of ABF3 in DNA and transcripts revealed the presence of an intron in cold susceptible Mexican lime, but not in cold tolerant sour orange.</p> <p><b>Conclusions/Discussion</b> Abscisic acid root drenching resulted in increased levels of expression of all three cold tolerance genes in both Mexican lime and sour orange. These results suggest the usefulness of abscisic acid in enhancing cold tolerance in citrus. Further confirmation of these results is required, but even a modest increase in cold tolerance would substantially reduce the loss due to cold damage.</p>	
<b>Summary Statement</b> Analyzing the effect of Abscisic Acid root drench on levels of expression of selected genes in citrus with the objective of understanding mechanisms of cold tolerance.	
<b>Help Received</b> Used lab facilities at the USDA Citrus and Date Germplasm Repository under supervision of Dr. Richard Lee.	