CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)
Sravya R. Keremane

Project Number
S0409

Project Title
Biochemical Analysis of Mechanisms of Cold Tolerance in Citrus

Abstract

Objectives/Goals
To study the biochemical basis of cold tolerance in citrus by analysis of gene expression of selected genes in cold sensitive and cold-tolerant citrus varieties.

Methods/Materials
1. Selection of candidate genes by review of literature on cold tolerance reseaarch in other plant species;
2. Search for similar genes in citrus and design primers;
3. Cold acclimation of selected cold sensitive and tolerant citrus plants;
4. Sampling and extraction of RNA;
5. Test the quality of RNA and useability of primers by RT-PCR and gel analysis;
6. Quantitation of gene expression before and after cold acclimation by real-time PCR analysis;
7. Analysis of results

Results
Plants cold-acclimated for 2-8 days were analyzed for expression of three different genes by real-time PCR assay. Cold-sensitive varieties did not show any appreciable increased expression of ABF3 gene. Amongst the cold tolerant varieties, trifoliate oranges did not show any change, but sour orange and Cleopatra mandarin showed an increase in the level of ABF3 gene expression in cold acclimated plants. Upon cold acclimation for two days, an increase in trancripts of ICE1 gene was observed in the cold sensitive Mexican lime, cold tolerant sour orange and trifoliate orange. The CLTA gene expression increased only in cold tolerant trifoliate orange.

Conclusions/Discussion
The present study has shown that the study differential gene expression can be used in identifying cold tolerance genes, confirming the hypothesis. Three well studied cold tolerance genes from other plant systems, dehydrin, ABF3 and ICE1 were selected; the sequences of their homologues in citrus were identified and the information was used for designing primers.
A good correlation between cold tolerance and increased ABF3 gene expression was observed in cold tolerant Citrus varieties, sour orange and Cleopatra mandarin, but not in cold tolerant Poncirus trifoliata. These preliminary results suggest that two different mechanisms of cold tolerance may be present in the two genera, Citrus and Poncirus. This observation was further supported by results of analysis of plants exposed to longer periods of cold acclimation, in which all Citrus plants showed an increase in the level of ABF3 gene expression, but not in Poncirus. The present study has suggested a major role for ABF3 gene in cold tolerance in citrus.

Summary Statement
The ABF3 gene was shown to be upregulated in cold-tolerant citrus, but not in either cold sensitive citrus varieties, or in a related cold tolerant genus, Poncirus.

Help Received
The research was carried out at the USDA Citrus Germplasm Repository, Riverside.