CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
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Project Number
S0407

Project Title
Biochemical Analysis of the Mechanisms of Cold Tolerance in Citrus

Abstract

Objectives/Goals
The long term goal of this 7-year project is to understand the biochemical basis of cold tolerance in citrus, and develop methods to combat the problem.

Methods/Materials
Partial sequences of putative cold tolerance genes were obtained by Blast search of expressed sequence tags database. A quantitative real time PCR assay was developed for analysis of differential expression of these genes in plants kept under warm and cold conditions. Available sequences were used for phylogenetic analyses using different computer programs.

Results
Six cold tolerant and four cold sensitive citrus varieties were used in this study. The cDNA concentrations were normalized using expression levels of a house keeping gene. These cDNAs were then used to study differential expression levels of two genes, ABF2 and ABF4. Increased expression of ABF2 was observed only in cold acclimated tissues of all known cold tolerant. Phylogenetic analysis of ABF2 gene sequences showed formation of distinct clades of cold tolerant and sensitive varieties, well supported by bootstrap analysis. A rapid non-destructive assay for screening large numbers of plants was developed.

Conclusions/Discussion
This is the seventh year of my project aimed at understanding biochemical mechanisms of cold tolerance in citrus. In the first phase, an anti-apoptotic gene was shown to confer cold tolerance in transgenic plants (published). In the second phase, four putative cold tolerance genes were analyzed for differential expression upon cold acclimation, and ABF3 gene was shown to express at higher levels in cold acclimated plants. External application of abscisic acid increased ABF3 gene expression in all plants. Two forms of ABF, ABF2 and ABF4 were identified this year. Detailed analysis by real time PCR in normalized cDNAs showed association of only ABF2 gene with cold tolerance. Phylogenetic studies supported the results. A rapid non-destructive assay was developed using cut-shoots instead of live plants for screening for cold tolerance. Breeding programs generate large numbers of hybrids every year with an objective of developing improved varieties. The rapid cold tolerance assay developed here may now be useful for screening these hybrids.

Summary Statement
A rapid non-destructive molecular assay was developed to screen citrus varieties for cold tolerance based on a gene that shows increased expression only in tolerant varieties upon exposure to cold.

Help Received
Dr. Lee supervised my research.