



**CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY**

Name(s) Saumya R. Keremane	Project Number 31571
Project Title Biochemical Analysis of Color Development in Citrus: Year 2	
Abstract Objectives/Goals Pigments such as lycopene, beta carotene, and phytoene provide immense health benefits. A better understanding of the mechanisms of carotenoid biosynthesis may lead to the rapid development of improved crop varieties. The goal of this project is to understand the biochemical basis of color development in citrus fruits. Methods/Materials Four varieties of citrus with different flesh colors were selected for the study: Oroblanco grapefruit (white), Moro blood orange (red), Star Ruby grapefruit (pink), and Washington Navel orange (orange). The sequences of phytoene desaturase and lycopene beta cyclase genes involved in carotenoid biosynthetic pathway were obtained for initial primer design based on the Arabidopsis genome database (TAIR) and the EST database of citrus. Fruit albedo and juice sac RNAs were reverse transcribed, coding regions of the two genes were amplified by PCR, cloned and sequenced. SYBR green based quantitative PCR was used to analyze expression levels of these two genes. Transcripts of phospholipase-D (Ankyrin), a house keeping gene were used for normalization. Results Alignment of the coding region sequences of both phytoene desaturase and lycopene beta cyclase genes showed amino acid differences in eleven positions each. Real time qPCR assays showed that in dark pigmented Moro blood orange and Star Ruby grapefruit, expression of both phytoene desaturase and lycopene beta cyclase was at least five-fold higher than the expression in Washington navel orange and Oroblanco grapefruit. Conclusions/Discussion Blood oranges are known to have originated as somatic mutants of sweet orange; sequence variability and differential gene regulation involved in the carotenoid biosynthetic pathway may cause small, yet crucial differences leading to changes in fruit color pigments. The study reveals a considerable increase in the gene expression levels of two major enzymes in the carotenoid biosynthetic pathway in the dark colored fruits of Moro blood orange and Star Ruby Grapefruit. The significance of the observed amino acid differences requires further elucidation.	
Summary Statement Citrus fruits with darker pigments had up-regulation of the carotenoid biosynthetic pathway genes, and single nucleotide polymorphisms leading to amino acid differences.	
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