



**CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY**

Name(s) Saumya R. Keremane	Project Number S0599
Project Title Biochemical Analysis of Color Development in Citrus: Year 4	
Abstract Objectives/Goals Blood oranges are rich sources of anthocyanin shown to provide immense health benefits. The change of fruit flesh color from orange to blood red is cold weather dependent. The objective of the present study was to understand the changes in gene expression patterns during fruit development in blood oranges as compared to other citrus varieties. Methods/Materials Eleven citrus varieties with varying fruit colors, red, pink, orange and white, some mutants and hybrids were selected. Fruit was collected in 4 time points from Oct to Feb, and the anthocyanin was measured. Total RNA from fruit was extracted, DNased, and reverse transcribed using oligo dT primers. The absence of genomic DNA was checked by conventional PCR of malate dehydrogenase gene. Relative expression levels of 15 genes from two biosynthetic pathways were analyzed by SYBR green real time PCR assays. The expression levels of individual genes were normalized by using delta Ct method and the change in expression levels over time was calculated by delta delta Ct method. Results Higher level of anthocyanin was detected only in blood red fruits. Gene expression levels were compared between Oct and Feb in different varieties. In blood red colored fruits, the expression levels of anthocyanin biosynthetic pathway genes increased substantially from Oct to Feb. In pink and orange colored fruits, there was moderate increase mainly in carotenoid pathway genes. In light colored fruits, there was not much difference between Oct and Feb. Conclusions/Discussion Studies have shown that anthocyanins provide immense health benefits for combating obesity, cardiovascular diseases, diabetes and pancreatic cancer. Providing additional health benefits in commonly consumed food sources like oranges would be a good public health strategy. An understanding of the mechanisms of development of anthocyanins is a prerequisite for improvement of blood oranges. In this study, eleven varieties with and without anthocyanins in fruits were analyzed for expression levels of 12 different genes from two biosynthetic pathways. Blood red fruits showed very high levels of expression of four genes in anthocyanin pathway while orange and pink varieties showed a moderate increase of some genes mostly from carotenoid pathway, Very little change in expression of all 12 genes was observed in light colored fruits.	
Summary Statement Blood red citrus are rich in anthocyanins known to provide immense health benefits and development of these pigments during fruit development was associated with dramatic increase in levels of four genes in anthocyanin biosynthetic pathway.	
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