Improving Peel Quality of California Citrus Fruit

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High quality peels are a critical component of high value fresh market citrus fruit. Peel quality is defined by the expression of specific gene sets during development that result in the development of the external finish of fresh fruit, a genetic phenotype that is a key factor for marketing of the product. Our study has two specific objectives: (1) To survey the pattern of genes expressed in peel tissues that determine quality of citrus fruit, and (2) To identify and validate genes associated with the development of fruit quality and peel related external finish disorders.

To survey the pattern of genes expressed in peel tissues that determine quality of citrus fruit: To survey the pattern of gene expression in Citrus peels, we have sampled the expression profile both spatially in different tissues types and temporally at different time points during fruit development. This sampling pattern is represented in 10 cDNA libraries that have been constructed by us.

We have sampled 27,648 cDNAs from all 10 of our libraries, run the DNA sequence information though our cDNA analysis pipeline, and deposited an excess of 80% of these in GenBank. We have also routinely sampled all of the entries in the public database for Citrus (GenBank, NCBI) deposited by other groups working on Citrus. We have made our analysis web accessible to all Citrus researchers through the Core Genomics facility (CGF) website (http://cgf.ucdavis.edu/) available by clicking on the citrus icon.

We have currently analyzed 118,910 ESTs that correspond to a unigene set of 34,937 (29.4% discovery rate) genes with a majority (19,208; 16.2%) being singletons (represented once in our database) and 15,729 (13.2%) of these being 'contigs' (represented more than once in our online database). We have begun the process of grouping genes based upon function by analyzing the 'contig' fraction of the profile.

A digital analysis of roughly half of the contigs has yielded 324 contiguous sequences differentially expressed specifically in peel tissues during fruit development. Of these 256 are non-redundant UniGenes. Using InterProScan (EMBL-EBI) we have functionally categorized these with various biological processes (see Figure). Simultaneously, we placed the target sequences corresponding to these specific sequences on a Combimatrix (Mukilteo, WA) oligonucliotide array along with genes with known function (carotenoid pathway genes). Results of this analysis have shown that expression patterns are similar within related pathway genes, with instances of novel genes exhibiting similar pattern of expression.

To identify and validate genes associated with the development of fruit quality and peel related disorders: The rationale for the second objective of our study is to further investigate specific set of genes that we discover in objective 1 and to carry out a more in-depth analysis that could lead ultimately to the development of diagnostics and therapeutics.

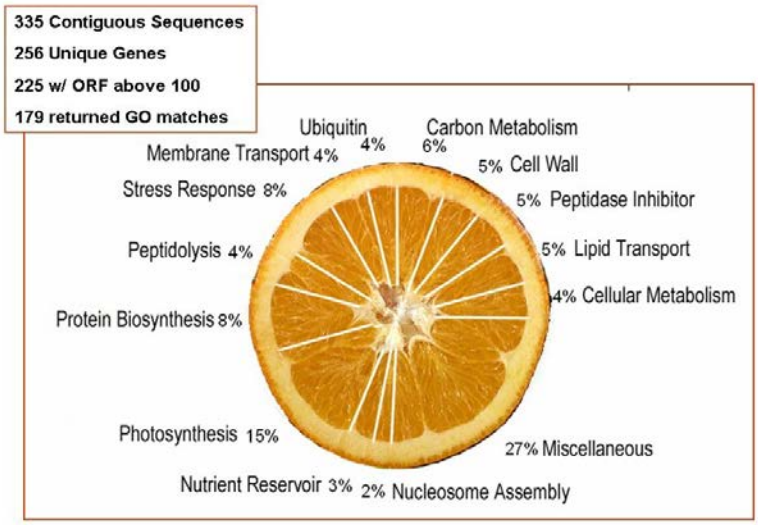

Figure 1. Two hundred and fifty six citrus peel specific unigenes were assigned gene ontology (GO) categories for biological process via InterProScan. Of these 179 were identified. The graph shows which biological categories were significantly represented

We have been successful in cloning 10 genes that are involved in the various steps in the carotenoid biosynthetic pathway that determine the color of Citrus fruit. We have examined the expression pattern of the genes and the expressed proteins to further establish their function in carotenoid biosynthesis.

Quantitative analysis of mRNA levels using gene specific TaqMan systems has revealed that four of the genes show a steady increase in expression during peel development. One of these genes, Chy-b, responsible for the synthesis of the carotenoid zeaxanthin, shows a maximum increase by day 165 after full bloom (165 DAFB) and then its expression is level through fruit maturation. Interestingly, we found five genes whose expression decreased during fruit maturation.

We are also examining the gene function analysis of the carotene pathway by color complementation using specific strains of *E.coli* that have been engineered to synthesize the appropriate carotenoid precursors. We have confirmed catalytic activities of Psy, Lcy-b, and Chy-b. Additionally, since the carotenoids accumulate in a specific subcellular compartment, namely plastids, we have been examining the localization of these proteins by protein import assay using isolated chloroplasts. We confirmed that Chy-b is targeted to the chloroplast membranes, whereas Lcy-b is mainly localized to the stroma.