

PROJECT CONCLUDING-SUMMARY REPORT

## Characterization of Determinants in Citrus Fruit Development and Identification of Key Processes Affecting Fruit Quality

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Citrus fruit quality standards have been determined empirically, depending on species and on the particular growing regions. In general, the TSS (total soluble solids) to total acidity (TA) ratio determines whether citrus fruit can be marketed. The TSS/TA ratio in citrus fruits is dominated by two main components: (i) overall vacuolar juice cell acidity and the juice cell sugar content.

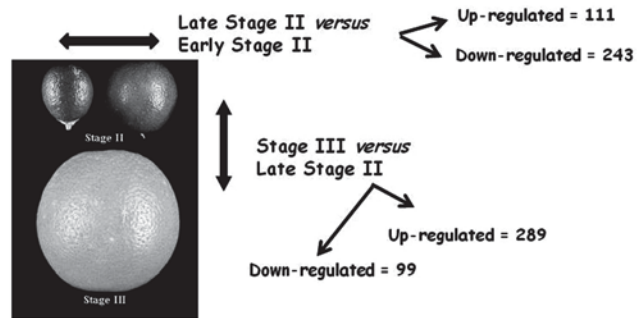
The objectives of our work are to characterize the physiological and biochemical components that promote and control the accumulation of TSS and TA in citrus fruits, using a multidisciplinary approach that combines cell biology, plant and fruit physiology, genomics, proteomics and metabolomics.

We are developing top-notch methodologies for the identification and characterization of key biochemical pathways in the citrus fruit. The biochemical characterization of the different fruit juice cell vacuole acid and sugar transporters and the sugar and citrate biosynthesis pathways will open many possibilities for fruit improvement. The understanding of the physiological and biochemical determinants for TSS and TA content in fruits will allow the enhancement of fruit quality during growth and pre and post-harvest practices, the improvement of citrus fruit sweetness and fruit acidity, the characterization of physiological disorders that depend on TSS and TA fruit content. (granulation, styler-end breakdown, etc.), and the response of citrus fruit to pathogen attack.

Our initial characterization of the juice cell Proteome (1,400 juice sac cell-specific proteins and enzymes, Katz et al., 2007) facilitated the identification of a large number of biosynthetic pathways associated with fruit development and the most important fruit quality traits. This work opened a wide array of possibilities for the validation of the ESTs collections developed under the sponsorship of CRB, and provided data that is essential to assess the role of the different processes associated with citrus fruit pre- and post-harvest methodologies.

One of the limitations that we encountered was the impossibility to compare quantitatively (or semi-quantitatively) the amounts of proteins within different samples. Using new technical advances and novel bioinformatics tools, we have now developed a label-free mass spectrometry method allowing the comparison of two different samples and the calculation of the differences in the different proteins in the samples using LC-MS-MS. This is of great importance because this method not only facilitates the mass-spectrometry-based comparison of fruit samples at different developmental stages but also makes possible the identification of the individual proteins/ enzymes changing during the different stages being compared and the calculation of the protein's abundance ratio.

Differential protein expression during citrus fruit development



As a first step towards the identification of key processes affecting fruit quality, we are focusing our work on: 1). Identifying the changes of key enzymes associated with the catabolism of plant growth regulators in order to fine-tune the use of plant growth regulators in the field; 2). Pre-harvest manipulation of fruit acidity; and 3). Characterizing the response of metabolic pathways to pathogen attack.

The importance of the characterization of the enzymatic network in citrus fruits in crop improvement is highlighted by the successful development of foliar treatments in the field, using compounds discovered by our proteomics/metabolomics efforts, that resulted in improved yield and fruit quality.

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