The Determinants of Sugar and Acid Content in Citrus Fruits and Citrus Fruit Proteomics

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The general objective of our work is the characterization of the physiological, biochemical and molecular components that control the accumulation of TSS (Total Soluble Sugars) and TA (Total Acidity) in citrus fruits. The understanding of the cellular/molecular determinants for TSS and TA content in fruits will allow the enhancement of fruit quality during pre- and post-harvest practices, the improvement of citrus fruit acidity and sweetness, and the characterization of fruit disorders that depend on TSS and TA fruit content.

During the last granting year we have focused in two aims: (1) the identification of all of proteins in the navel orange fruit; and (2) the molecular identification of the fruit juice sac cell vacuoles sugar transporters.

The navel orange Proteome: [Procedure – We isolated juice sacs from fruits at different growth states and used a high throughput LC-MS/MS (LTQ-FT) method for the identification of juice sac proteins using Citrus fruit juice sacs were collected and fractionated in order to isolate the soluble proteins and tonoplast-, plasma membrane-, ER/Golgi-, and mitochondria-enriched fractions (Katz, et al., 2007). The different protein fractions were digested by trypsin and analyzed by LC-MS/MS. After MASCOT search the data was statistically filtered by SCAFFOLD (http://www.proteomesoftware.com/) according to their Xcorr, probability of protein identification (99%), the number of unique peptides on which the protein identification is based (at least 2) and by minimum identification probability of a peptide from at least one spectrum (95%).]

The analysis of proteins extracts from citrus juice sacs resulted in the identification of 1,394 fruit proteins and assigned function to 1,247 proteins, and integrated these proteins in 84 biosynthetic known pathways (Katz et al., 2007). Our results clearly show that in during the acid decline of fruits, there is a significant amount of citrate used for the synthesis of proteins, and that there is sugar synthesis in the fruits. These results are of high relevance since they provide support to the notion of regulation of sucrose content by synthesis in the fruit.

These processes could provide important practical applications not only for the regulation of sugar content, but also to the development of treatments that could change the fruit sugar and acid balance and as a consequence represent targets for the manipulation of abscission processes, control of fruit size, fruit quality, etc.

The molecular identification of the fruit juice sac cell vacuoles sugar transporters: Vacuoles occupy up to 95% of the juice sac cell volume, and they are the site of accumulation of most sugars. Three full length cDNAs and genomic sequences have been obtained and analyzed. The predicted structures of these two putative vacuolar sugar transporters from citrus juice cells showed proteins comprising twelve transmembrane domains and a large central hydrophilic region. The expression of these genes increased during development and correlated well with sugar accumulation in the fruit. Heterologous expression of GFP-fused gene constructs in yeast revealed that both are localized to the vacuole (Figure 1). [Katz., E., Fon, M., Lee, Y.J., Phinney, B.S., Sadka, A, and Blumwald, E. (2007). The citrus fruit proteome: insights into citrus fruit metabolism. Planta: 226:989-1005]
Figure 1. Expression of citrus sugar transporters CX673451 and CTG1087794 GFP-fusion proteins in S. Cerevisiae. Yeast was transformed with GFP-fusion expression vectors. Fusion protein localized at the vacuole. (A) and (B): CTG1087794-GFP and CX673451-GFP fusion proteins localized at the vacuole. (C): Control- GFP protein localized in Cytosol. Original vector was pDR196 containing PMA1 promoter and ADH terminator for the gene expression and URA3 Gene as selective maker.