FLOWERING AND FRUCTIFICATION IN "VALENCIA" ORANGE: ANATOMICAL, PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR ASPECTS

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Orange orchards in São Paulo State show low yield efficiency and annual yield oscillation. Crop management, aiming to stabilize the annual orange yield, depends on the understanding of the interactions among different environmental factors and related physiological processes. As for all dicot plants, orange leaves are carbon providers (source) and, at least at one stage during its development, leaves, roots, branches flowers and fruits become carbon sinks, draining sucrose from the leaves. Leaves and stems are the main organs in plants accumulating photo-synthesized carbohydrates, and at a given moment in time, they make it available for other organs acting as sinks. During spring, after flower induction, the orange tree blossoms in São Paulo State followed by a period of intense flowering and young fruit dropping. Photosynthesis and carbohydrate reserves seem to affect flower fertilization, and fruit development. In the summer, the environmental conditions favour the production of energy derived from photosynthesis, and leaves maintain the carbohydrate supply to sustain plant growth and the accumulation of storage products. Fruits do not accumulate starch in the juice sacs but accumulate organic acids, establishing a link between respiratory cycle and sucrose translocation. Almost nothing is known about nitrogen metabolism in the fruits, compared with the information available for nitrogen metabolism in leaves.

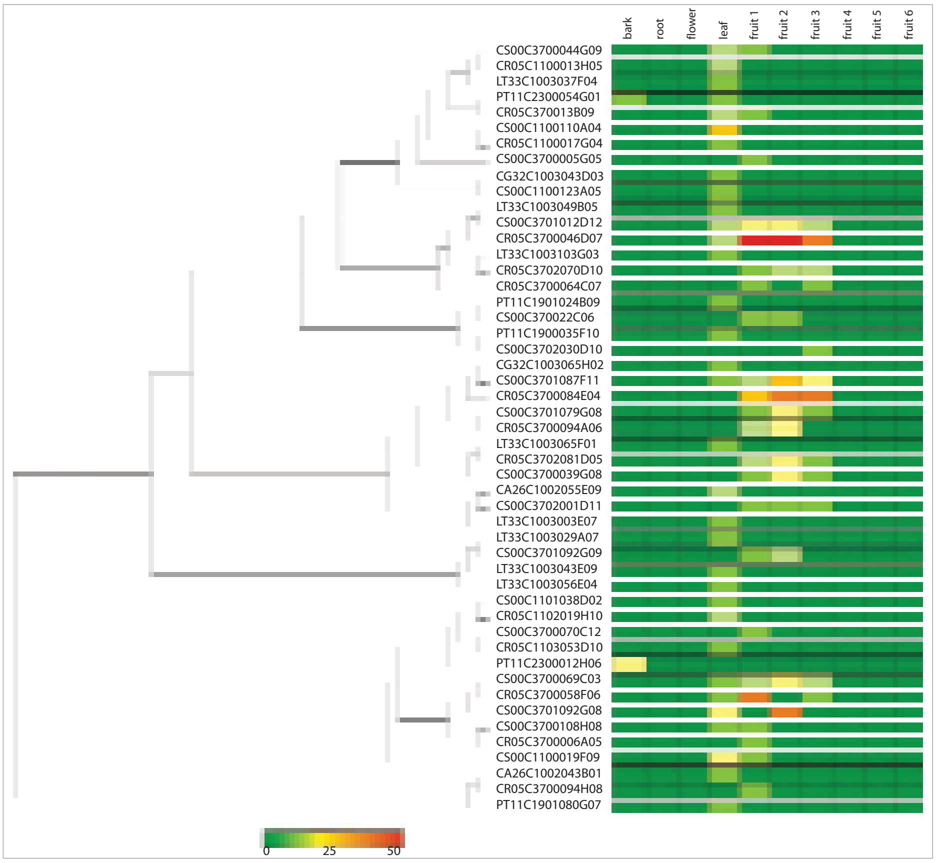

Figure 1. Expression profiles of the 49 Citrus putative terpene synthase EST clusters in selected cDNA libraries from the CitEST database. Data represent the relative number of reads from a specific library in each EST cluster after normalization. Each EST cluster is represented by a single row, and each library is represented by a simple column. The cladogram on the left represents the relatedness of all Citrus sequences and was build according to their relative genetic distances. CA refers to Citrus aurantium *sequences; CG refers to* Citrus aurantifolia *sequences; CR refers to* Citrus reticulata *sequences; CS refers to* Citrus sinensis *sequences; LT refers to* Citrus latifolia *sequences; PT refers to* Poncirus trifoliata *sequences*

The aim of this project is to analyse the biochemical, physiological and molecular aspects related to carbon partitioning in Valencia orange during flowering, fruit setting and maturation. Experiments with photosynthesis, hormones, nitrogen and carbohydrate compounds will be performed from blooming season until fruit maturation. At specific stages, gene expression analyses will be performed with candidate genes using microarrays and *in situ* hybridization techniques.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

To obtain a high production of citrus fruits in 2008, we induced heavy citrus flowering through the removal of fruits produced during the previous season (February/2007). These experimentally-prepared plants were compared with control plants bearing fruits. As expected, the experimental plants showed higher productivity than control plants in 2008. Several physiological and biochemical parameters were measured in the plants (sampling from August to December 2008).

Plant material was collected for microarray studies (based also on data previously obtained using the EST-Citrus databank, http://biotecnologia.centrodecitricultura.br/), and also for *in situ* hybrization to study genes related to flower bud differentiation. Other experiments were performed, in greenhouse conditions, to study the relation of water transport and flowering.

The field experiments have shown that the variation in carbon reserves in citrus depends on sink demand and photosynthesis, which may not be synchronized. Thus, under high carbon demand, carbon stored in the roots and stems may be remobilized. Also, this may be influenced by seasonal variation in climate and growth stages.

We observed significant changes in photosynthesis rates throughout the year in citrus plants. The photosynthesis rate, in warm and humid summer days, when intense vegetative and fruit growth occur, is 3 times the observed rate during winter. Our study showed that this might be related to the high and low carbon demand and that it is strongly related to temperature and water status. Sucrose exported from the leaves to the growing tissues clearly stimulates photosynthesis. On the other hand, this export is related to intense growth rates occurring during summer.

The *in situ* hybridization experiments, together with the morpho-anatomical description of samples showed the changes occurring in the lateral buds of branches, associating morphological modifications with gene expression patterns. Plants not bearing fruits have shown 15 days delay in the initiation of the reproductive development, followed of a delay in the expression of the *CsLFY* gene, responsible for the transition of the vegetative meristem to the flower meristem in Arabidopsis. Since *LFY* gene is the molecular activator of other floral development genes, it also has shown a delayed expression, as presented by the expression pattern of *CsAP1* and *CsPI*, homologous of *APETALA1* and *PISTILLATA*.

MAIN PUBLICATIONS

Dornelas MC, Mazzafera P. 2007. A genomic approach to the characterization of Citrus terpene synthase gene family. *Genetics and Molecular Biology*. **30**: 832-840.

Dornelas MC, Camargo RLB, Berger IJ, Takita MA. 2007. Towards the identification of flower-specific genes in *Citrus spp*. *Genetics and Molecular Biology*. **30**: 761-768.

Dornelas MC, Camargo RLB, Figueiredo LHM, Takita MA. 2007. A genetic framework for flowering-time pathways in *Citrus spp*. *Genetics and Molecular Biology*. **30**: 769-779.

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