

Identification of transcription factors potentially involved in the juvenile to adult phase transition in *Citrus*

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• **Background and Aims** The juvenile to adult transition (JAT) in higher plants is required for them to reach reproductive competence. However, it is a poorly understood process in woody plants, where only a few genes have been definitely identified as being involved in this transition. This work aims at increasing our understanding of the mechanisms regulating the JAT in citrus.

• **Methods** Juvenile and adult plants from Pineapple sweet orange (*Citrus sinensis*) and Rough lemon (*C. jambhiri*) were used to screen for differentially expressed transcription factors (TFs) using a 1.15K microarray developed on the basis of the CitrusTF database. Murcott tangor (*C. reticulata* × *C. sinensis*) and Duncan grapefruit (*C. paradisi*) were incorporated into the quantitative real-time reverse transcription-PCR validation in order to select those genes whose phase-specific regulation was common to the four species.

• **Key Results** A browsable web database has been created with information about the structural and functional annotation related to 1152 unigenes of putative citrus TFs (CTFs). This database constitutes a valuable resource for research on transcriptional regulation and comparative genomics. Moreover, a microarray has been developed and used that contains these putative CTFs, in order to identify eight genes that showed differential expression in juvenile and adult meristems of four different species of citrus. Those genes have been characterized, and their expression pattern in vegetative and reproductive tissues has been analysed. Four of them are MADS-box genes, a family of TFs involved in developmental processes, whereas another one resembles MADS-box genes but lacks the MADS box itself. The other three showed high partial sequence similarity restricted to specific Arabidopsis protein domains but negligible outside those domains.

• **Conclusions** The work presented here indicates that the JAT in citrus could be controlled by mechanisms that are in part common to those of Arabidopsis, but also somehow different, since specific factors without Arabidopsis orthologues have also been characterized. The potential involvement of the genes in the JAT is discussed.

Key words: *Citrus sinensis*, *C. jambhiri*, *C. reticulata*, *C. paradisi*, database, differential gene expression, juvenile to adult transition, juvenility, transcription factor, JAT.

INTRODUCTION

Development of higher plants has two distinct phases, juvenile and adult. In general, the juvenile phase is characterized by the inability to initiate floral development in response to floral-inductive cues (Zimmerman *et al.*, 1985).

Phase change in Arabidopsis and other herbaceous species involves the activation of new gene expression programmes. Genetic analyses have identified many genes that allow us to better understand how reproductive competence is regulated. Four genetic pathways exist that mediate the effect of hormones, photoperiod, light quality, temperature and other environmental factors in a complex way that involves an intricate network of signalling pathways (Amasino and Michaels, 2010).

One well-known class of regulators involved in floral transition is the MADS-box gene family (Becker and Theissen, 2003). Among them, *FLOWERING LOCUS C* (*FLC*), one of the central flowering regulators, and a small clade of closely

related genes called *MADS AFFECTING FLOWERING* (*MAF* genes), encode repressors of flowering and regulate the autonomous and vernalization pathways (Michaels and Amasino, 1999; Ratcliffe *et al.*, 2003). *FLC* expression is repressed by vernalization treatments, thereby promoting flowering after winter has passed (Michaels and Amasino, 1999). On the other hand, the second central regulator of the transition to reproductive phase, *CONSTANS* (*CO*), mediates the response to long-day photoperiod (Suarez-Lopez *et al.*, 2001). *FLC* and *CO* regulate the expression of genes responsible for the integration of the signals from the multiple flowering pathways (Simpson and Dean, 2002). One of these genes is *FLOWERING LOCUS T* (*FT*), a mobile flowering signal that acts as a floral promoter. *FT* activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), a MADS-box floral activator (Simpson and Dean, 2002), leading to induction of *LEAFY* (*LFY*), another MADS-box gene. *FT* is also responsible for the activation of the MADS-box gene *APETALA 1* (*API*), which is