Gene Expression Profile During Apricot Fruit Growth, Using a Peach Microarray

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Abstract

There is a high degree of sequence conservation within the Family Rosaceae and, in particular, among the *Prunus* species. The first available peach microarray (µPEACH1.0) was employed for the investigation of the transcription profile during apricot (*Prunus armeniaca*) fruit growth and development. Fruits (cv. 'Goldrich') were harvested at two growth stages, corresponding to immature (6 weeks before harvest) and ripe (at harvest) stage. Overall, this preliminary transcriptomic approach suggests that peach microarray can be employed in a heterologous fashion. Amongst the 746 genes which could be statistically analysed, 287 were induced, while 260 were down-regulated. In addition to the well documented role of genes implicated in ethylene biosynthesis perception and transduction as well as those involved in cell wall metabolism, this study brings into light a putative role for genes encoding auxin-regulated proteins, and those responsive to stress conditions and to other biotic or abiotic stimuli. The data of the present study can also be used for comparative purposes within *Prunus* species, since for 203 and 189 genes that were up- and down-regulated respectively during apricot fruit development, data exist for their regulation during the last stages of peach fruit ripening. Future experiments should include analysis of more fruit stages and data validation for the genes of interest.

INTRODUCTION

Transcript-profiling methods are increasingly used to understand the biological basis of growth and development, and in the case of fruits, of their quality. Such methods provide information for thousands of genes, including those of still unknown function. Furthermore, high-throughput methodologies can be used for comprehensive transcriptome analyses, which may lead to further elucidation of fruit growth and development up to the ripening stage (reviewed in Bonghi and Trainotti, 2006).

Microarray technology is suitable for large-scale trancriptomic analyses, through the simultaneous monitoring of many genes in a single experiment, avoiding limitations of traditional molecular techniques, while it can be utilized in a heterologous fashion for gene discovery in species where few resources are available, thus making it an attractive genomic tool. To date, a tomato microarray was successfully employed for comparative studies in Solanaceae (tomato, pepper, eggplant) (Moore et al., 2005).

Considering the high degree of sequence conservation within the Family Rosaceae and, in particular, among the *Prunus* species, the first available peach microarray

(μPEACH1.0) was applied to investigate changes in gene expression during apricot fruit development. This microaray contains 4,806 oligoprobes (70 bases long) each corresponding to a single unigene, as indicated in a dedicated database (ESTree consortium, 2005). This unigene collection comes from EST sequences mainly obtained from libraries of ripening fruits, so it is biased towards this physiological process (Trainotti et al., 2006).

MATERIAL AND METHODS

Fruit material (cv. 'Goldrich') was collected in two distinct stages: (1) green – immature stage (S_1, 6 weeks before ripening), and (2) during the on-tree ripening (S_2, firmness values ~ 1 KgF). Such material was stored at -80°C until needed.

The RNA was isolated according to Bonghi et al. (1998). In order to remove contaminant DNA from the RNA samples, the nucleic-acid extract was treated with DNase, according to the manufacturer's instructions. The concentration of RNA was quantified by measuring the absorbance at 260 nm and its integrity was checked on agarose gels. The conversion of RNA into target cDNA, microarray hybridization and data analysis were carried out as analytically described by Trainotti et al. (2007). Genes showing $\log_2$ ratio either > 1 (up-regulated) or < -1 (down-regulated) were annotated following the Gene Ontology categories (GO) developed by TAIR. In addition, functional annotation for the genes differentially transcribed was done using the VirtualPlant 0.9 software.

RESULTS AND DISCUSSION

The distribution of up-regulated (287) and down-regulated (260) genes during the transition of 'Goldrich' apricot fruit from 'immature – green' to 'ripe – ready to eat' stage, based on their homology to the *Arabidopsis* proteome is depicted in Fig. 1. The data of the present study can be also used for comparative purposes within *Prunus* species, since for 203 and 189 genes that were up and down-regulated respectively during apricot fruit development, data exist for their regulation during the last stages of peach fruit ripening (ESTree consortium, 2005, Trainotti et al., 2006). In that case, however, data should correspond to similar developmental stages of the fruit in order to dissect common and/or divergent mechanisms within the Prunoideae sub-family.

Among the 287 up-regulated transcribed genes, 48 were related to stimulus response. Among them, 22 were expressed as response to hormones and more particular to auxin (Fig. 2a)**.** Members of the Aux/IAA family are highly up-regulated when ripening proceeds and their importance has been highlighted during the onset of peach fruit ripening (Trainotti et al., 2007). An up-regulation of genes encoding heat-shock proteins (HSP) was monitored (Fig. 2b), in accordance with data reported by Grimplet et al. (2005). Besides their well documented role as a response to stress conditions, HSP have been implicated in pectin depolymerization (Ramakrishna et al., 2003) and more recently were analysed in a proteomic level during tomato fruit development (Faurobert et al., 2007). In addition, a down-regulation of genes encoding for antioxidant enzymes (catalase, peroxidase, lipoxygenase, Fig. 2c) was monitored. A down-regulation of genes encoding for peroxidase and catalase has been also reported during the transition of apricot fruit from immature to mature stage (Grimplet et al., 2005). The regulation of other genes encoding some proteins of interest is depicted in Fig. 2.

Overall, results indicated that peach microarray can be successfully applied in related, yet phenotypically distinct, species belonging to the Sub-family Prunoideae. A thorough analysis of more fruit stages and data validation for the genes of interest should allow us to unravel the mechanisms underlying the ripening process in apricot and generally in stone fruits. Another future perspective should be the identification of genes differentially expressed during developmental stages of apricot fruit and their correlation with traits of agronomic interest.

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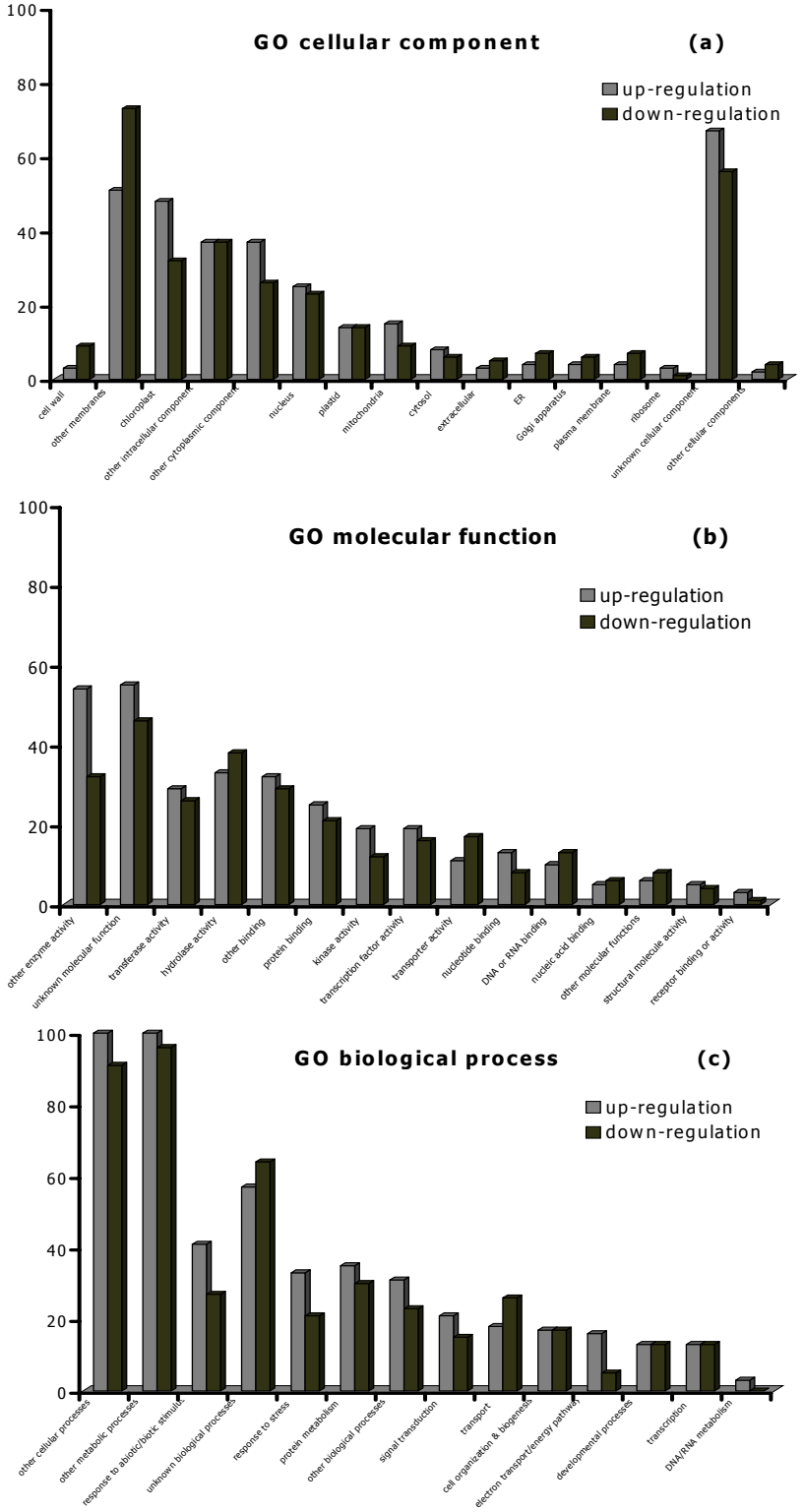

Fig. 1. Distribution of up- and down-regulated genes during the transition of 'Goldrich' apricot fruit from 'immature-green' to 'ripe' stage, based on their homology to the *Arabidopsis* proteome, and grouped according to the terms used in the (a) Gene Ontology (GO) cellular component, (b) GO molecular function and (c) GO biological process by TAIR (The Arabidopsis Information Resource).

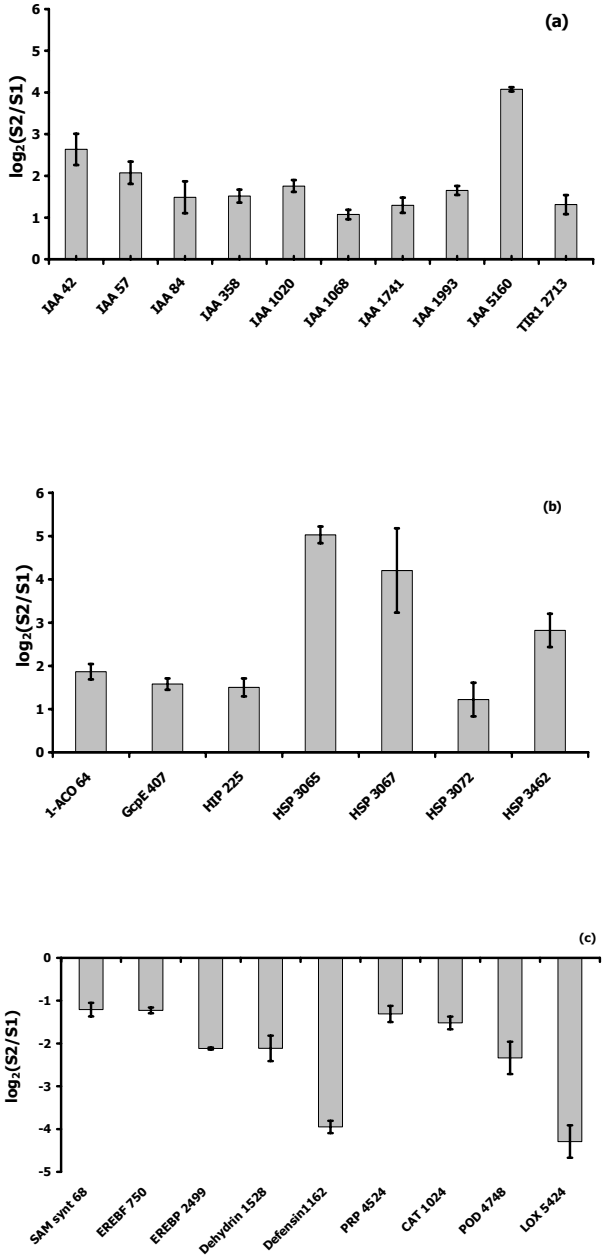

Fig. 2. Modulation of gene expression, measured by means of the μPEACH1.0 microarray, during the transition of 'Goldrich' apricot fruit from 'immature – green' to 'ripe' stage. The genes analysed code (a) for proteins putatively involved in auxin metabolism, (b) in response to stress conditions with up-regulation effect and (c) in response to stimuli with down-regulation effect. Numbers after gene acronyms indicate the peach contig name. Bars correspond to ± SD of the means. *IAA: auxin-induced or -regulated proteins, TIR1: transport inhibitor response 1-like protein, 1-ACO:1-aminocyclopropane-1-carboxylate oxidase, HIP:harpin-inducing protein, HSP:Heat-shock protein, SAM synt: S-Adenosylmethionine synthase, EREBF/P: ethylene-responsive element binding factor/protein, PRP:pathogenesis-related protein, CAT:catalase, POD:peroxidase, LOX:lipoxygenase.*