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Microarray analysis of differentially expressed genes engaged in fruit development between *Prunus mume* and *Prunus armeniaca*

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ABSTRACT

Microarray analysis is a technique that can be employed to provide expression profiles of single genes and new insights to elucidate the biological mechanisms responsible for fruit development. To evaluate expression of genes mostly engaged in fruit development between *Prunus mume* and *Prunus armeniaca*, we first identified differentially expressed transcripts along the entire fruit life cycle by using microarrays spotted with 10,641 ESTs collected from *P. mume* and other *Prunus* EST sequences. A total of 1418 ESTs were selected after quality control of microarray spots and analysis for differential gene expression patterns during fruit development of *P. mume* and *P. Armeniaca*. From these, 707 up-regulated and 711 down-regulated genes showing more than two-fold differences in expression level were annotated by GO based on biological processes, molecular functions and cellular components. These differentially expressed genes were found to be involved in several important pathways of carbohydrate, galactose, and starch and sucrose metabolism as well as in biosynthesis of other secondary metabolites via KEGG. This could provide detailed information on the fruit quality differences during development and ripening of these two species. With the results obtained, we provide a practical database for comprehensive understanding of molecular events during fruit development and also lay a theoretical foundation for the cloning of genes regulating in a series of important rate-limiting enzymes involved in vital metabolic pathways during fruit development.

Introduction

Prunus mume and *Prunus armeniaca* are two genetically related plant species belonging to the sub-family Prunoideae within the Rosaceae family. These two species are native to China, the oldest and biggest center of origin for *Prunus* species. They have both been cultivated for thousands of years and there are abundant resources of wild and cultivated varieties in China. *P. mume* is classified into two groups, fruiting mei and flowering mei, based on their use as either fruits or ornamental flowers (Chu, 1999; Lu, 2000). Utilization of this plant is determined by the flowering patterns or the edible quality of fruits. Since *P. mume* has a sour taste when fresh, the fruits are often processed into salted mei, preserved sweetened mei, or wine and/or juice, all of which have high nutritional and medicinal value and are consumed in countries like China, Japan, and Korea (Li et al., 2010). In contrast, *P. armeniaca* fruits are sweet and most of them are grown for fresh or processed fruit and/or kernel products which are rich in vitamin E, plant sterols, fiber, and heart-healthy fats. Both of these *Prunus* species have played an important role in human diet and health since time immemorial and these attributes make them economically important and excellent fruit trees in China.

It has been reported that *P. mume* and *P. armeniaca* are genetically related and cross-compatible species. Inter- or intra-specific relationships between these two species have been well articulated (Hormaza, 2002; Fang et al., 2005, 2006a,b; Li et al., 2010). Despite this, there is a dearth of information on the differences in fruit development, physiology and biochemistry, especially exploiting and comparing the mechanisms of key genes affecting fruit development and ripening of both *P. mume* and *P. armeniaca* at the molecular level. Differences in these genes may be responsible for the induction of different fruit qualities in these fruit crops. This scarcity of knowledge contributes to difficulties in heterosis breeding, production, and post harvest handling of these fruits and their products. With the advent and application of molecular and genomic technologies to generate information, the understanding of genes and biochemical processes responsible for fruit growth

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and development has markedly increased over the last decade (Grimplet et al., 2005; Li et al., 2010). In order to have greater awareness and further understanding of relationships in the mechanisms of fruit development between *P. mume* and *P. armeniaca*, it is important to analyze the differences in expression of many important genes responsible for fruit development and their evolution in the plant kingdom using the known and valuable EST resources at mRNA level rather than using the botanical characteristics of these fruits.

The cDNA microarray-based technology was introduced to enable high throughput monitoring of gene expression (Duggan et al., 1999), and this has revolutionized gene expression studies by providing the means of simultaneously measuring mRNA levels of thousands of genes in complex biological samples (Cuzin, 2001). Besides this, microarray analysis has the technical merit of being highly sensitive to mRNA detection (Seki et al., 2001). The characteristics of high-throughput, miniaturization, continuity, automation, speed and accuracy in microarray technology has aroused great interest both internationally and domestically and is increasingly being applied in many research fields. Microarray technology is fluorescence based and is widely accepted as a fast and powerful tool for comprehensive characterization of different processes in diverse stages of plant development at the mRNA transcription level and is even available for use in almost any application requiring gene expression analysis (Aharoni and Vorst, 2002). It is also a validated method for true high-throughput analysis in investigating a variety of biomarkers and important genes in the development of plants (Alba et al., 2004).

The microarray technique has been used mainly to monitor changes in patterns of gene expression in different plant systems (Zheng et al., 2004; Keller et al., 2005; Stutte et al., 2006; Laitinen et al., 2007; Zhao et al., 2007; Hovav et al., 2008; Ludwików et al., 2009), and the first application of microarrays in a fruit crop was carried out in strawberry by Aharoni et al. (2000), who identified a gene involved in flavor biogenesis. Microarrays representing hundreds of cDNAs derived from a strawberry fruit cDNA library have been utilized for monitoring gene expression during fruit development and specifically in achene and receptacle tissue (Aharoni and O'Connell, 2002). The application of microarray technology has also focused on fruit development in different crops grown for their fruit, as exemplified by tomato (Frick and Schaller, 2002), pear (Fonseca et al., 2005), citrus (Shimada et al., 2005), grape (Waters et al., 2005; Terrier et al., 2005), apple (Degenhardt et al., 2005; Lee et al., 2007), banana (Xu et al., 2007) and peach (Ziliotto et al., 2008). However, hardly any literature discusses the nature of *P. mume* and *P. armeniaca* fruit development. There are a number of physical, chemical and physiological changes involved in the formation of fruit pigmentation, texture and aroma which in turn make the fruit more attractive and edible during development. It is worth noting that the sets of complex and interrelated mechanisms corresponding to the changes in phenotypic characteristics are the results of coordinated multi-gene expression and could also be regulated by exogenous factors. There was therefore a need to conduct an in depth analysis of the genes engaged in fruit development between *P. mume* and *P. armeniaca* so as to generate valuable information for use in studies on heterosis breeding, production, and post harvest handling of these fruits and their products.

Results

Clustering of differentially expressed genes

Fruit specific microarrays of *P. mume* and *P. armeniaca* containing a total of 10,641 ESTs, printed in duplicate spots, were used to identify genes activated during fruit development and ripening. All the data relating to these microarrays were submitted to NCBI (National Center for Biotechnology Information) GEO (GSE37950, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE37950). The ESTs with two-fold increases or decreases in expression were regarded as differentially expressed genes compared *P. mume* and *P. armeniaca*. From the entire set of ESTs, 1418 (13.33%) exhibited differentially expressed genes during fruit development or ripening. Comparative analyses between the *P. mume* and *P. armeniaca* samples showed that 707 genes were up-regulated while 711 were down-regulated (Table 2). There were 190 and 208 differentially expressed genes between two intra-specific varieties of *P. mume* and *P. armeniaca*, respectively. This indicates that expression of these genes is relatively consistent during fruit growth and development with minimal differences between these varieties. However, 1100–1900 differentially expressed genes were found between the inter-specific varieties which was about 7 times more than those in their intra-specific counterparts, demonstrating that similar genes are expressed differently in different species, or that expression of certain genes may be species specific. From our comparison results for the total number of genes, 10% of the differentially expressed genes were found between *P. mume* and *P. armeniaca*, an observation that indicates that these two species have the closest genetic relationships among all the *Prunus* species.

After sequence analysis and homology search, all unique sequences exhibiting changes in expression during fruit development were clustered. To divide the gene expression dataset into groups of observations that are similar to each other, agglomerative hierarchical clustering was applied. All the detailed information for differentially expressed genes detected by microarray analysis is as shown in Supplementary file 1, and the clustering diagram shows that the up-regulated or down-regulated genes in these two species were divided into a number of groups. Genes which have high similarity in a main cluster were further clustered into the sub-groups. A partial list of genes showing differential expression is as shown in Fig. 1.

Annotation and analysis of genes differentially expressed during fruit development

We used BLAST2GO to separately annotate our differentially expressed gene sequences. Annotation results showed that 472 sequences (66.8%) in the up-regulated gene group obtained 2253 GO numbers and 352 (49.8%) EC numbers, while 455 (64%) sequences in the down-regulated gene group had 2224 GO and 338 EC numbers, respectively. Each group was classified into three different categories belonging to biological process, molecular function and cellular components according to level 2 (Fig. 2).

On the categorization of biological process, 707 up-regulated and 711 down-regulated differentially expressed genes with known or predicted functions were classified into 21 groups based on their similarities as shown in Fig. 2. A large number of genes were shown to be involved in metabolic process (28%, 28%), cellular process (23%, 27%) and response to stimulus (13%, 11%). There were also genes involved in developmental process (6%, 3%), multi-cellular organism process (6%, 3%), localization (6%, 4%), biological regulation (5%, 7%), reproduction (4%, 1%), cellular component organization (2%, 4%), signaling (2%, 3%), multi-organism process (2%, 2%) and cell wall organization (1%, 2%). Only few differentially expressed genes were seen to be involved in growth, immune system, rhythm, death, viral reproduction, cellular component biogenesis, cell proliferation, and cell killing or locomotion, accounting for up to 1% of the functional genes. It is worth mentioning that based on the high similarities with known functional proteins, some genes not only belonged to one functional process as annotated above, but they could also be involved in two or more parts of a function as shown in the pie charts. The genes with