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Comparative proteomic analysis of floral color variegation in peach

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ABSTRACT

Variegation in flower is a special trait in ornamental peach (*Prunus persica* L.). To investigate the mechanism of color variegation, we used a combination of two dimensional gel electrophoresis and mass spectrometry to explore the proteomic profiles between variegated flower (VF) and red flower (RF) buds of the peach cultivar 'Sahong Tao'. More than 500 highly reproducible protein spots ($P < 0.05$) were detected and 72 protein spots showed a greater than two-fold difference in their values. We identified 70 proteins that may play roles in petal coloration. The mRNA levels of the corresponding genes were detected using quantitative RT-PCR. The results show that most of the proteins are involved in energy and metabolism, which provide energy and substrates. We found that LDOX, WD40, ACC, and PPO II are related to the pigment biosynthetic pathway. The activity of PPO enzyme was further validated and showed the higher with significant differences in RF compared with the VF ones. Moreover, the four UCH proteins are involved in protein fate and may be important in post-translational modifications in peach flowers. Our study is the first comparative proteomic analysis of floral variegation and will contribute to further investigations into the molecular mechanism of flower petal coloration in ornamental peach.

1. Introduction

Flower color is an important trait in ornamental plant. Variegation in flowers often attracts consumer attention and variegated plants are generally of high value in the ornamental markets. This unstable phenotype has been observed in petunia, snapdragon, and other plant species [1]. Flavonoids, carotenoids and betalains are major floral pigments. Flavonoids, especially anthocyanidins, contribute to the range and type of colored pigments in plants. Anthocyanidin is further modified with glycosyl, acyl, or methyl groups catalyzed by glycosyltransferase, acyltransferase and methyltransferase [2]. These morphological, physiological and biochemical changes associated with ornamental traits are inevitably the result of differential expression of a series of regulatory genes. Flower variegation is usually due to a group of colored cells descended from a single ancestral cell in which a somatic mutation from the recessive white to the pigmented revertant allele has occurred. Variegation patterns are determined by the somatic mutation frequency and timing during petal development [3].

In recent years, the proteomic approach has become widely recognized as a powerful tool applied to investigating and identifying protein changes in an organ, tissue or cell culture to characterize biochemical networks and to establish functional correlations between genotype and phenotype [4]. Two-dimensional gel electrophoresis (2-DE) is used to obtain high protein resolution for an in-depth analysis of protein maps. Comparative proteomics has the potential to screen many metabolic pathways simultaneously for alterations at the protein level. More recently, proteomic approaches have been used to investigate flower development in several plant species [5]. The comparison proteomes of flower and bud have suggested that sucrose generation derived by up-regulated phosphoglucomutase and down-regulated glycoprotein could induce the expression of flavonoid and anthocyanin-related genes, which is important for petal growth and color development in mature flowers [6].

Prunus persica, which belongs to the genus *Prunus* of Rosaceae, is an important ornamental plant [7] that originated in China and has a cultivation history spanning more than 3000 years. The ornamental peach exhibits an immense diversity of color in flowers. Thus, it is used as an attractive ornamental plant for landscape use. However, very little information is available on the molecular mechanism of the regulation of these traits, which limits the scope of rational selection to achieve improvements in ornamental peach.

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To investigate this trait, we performed comparative proteomics using a combination of 2-DE and mass spectrometry (MALDI-TOF/TOF MS) to identify the differentially expressed proteome between the variegated floral buds and the red ones in the same tree.

2. Materials and methods

2.1. Plant materials

We separately collected flower buds variegated (VF) and red (RF) petals in the pink stage (expanded but unflushed variegated and red variegated flower buds) [8]. The two kinds of flower buds, with the uniform genetic backgrounds, were from the same tree of cv 'Sahong Tao' grown on the campus of Nanjing Agricultural University, Nanjing, Jiangsu Province, China in March 2014 (Fig. 1). Both the two samples for 2-DE petals were without ramentum. Three biological replicates were performed for each sample. All of the samples were immediately frozen in liquid nitrogen and stored at −70 °C until use.

2.2. Protein extraction and 2-DE

Flower bud samples were ground into powder with 10% PVP in liquid nitrogen. Protein extraction was performed according to the trichloroacetic acid (TCA)/acetone precipitation method described by Gu et al. (2013) [9] with some modifications. About 0.6 g of powder was homogenized in 7 mL of cold acetone (containing 10% TCA and 0.07% DTT) and then precipitated 1.5 h at −20 °C. The protein powder was weighed and then dissolved in lysis buffer and shaken vigorously at 4 °C for 3.5 h. Insoluble material was removed by centrifugation at 15,000 × g for 30 min at 4 °C. After extraction, the protein concentration was determined by the Bradford method (1976) [10].

The first dimension was performed on 17 cm linear immobilized pH gradient (IPG) strips, with a pH gradient of 4–7. The extracted proteins were diluted to a final concentration of 2.2 mg of total proteins in 350 μl of lysis buffer. The strips were rehydrated at 19 °C, 50 V for 13 h passively. Isoelectric focusing was performed in a PROTEAN®i12™IEF Cell (Bio-Rad, Hercules, CA, USA) (19 °C, 250 V 1 h, 500 V 1 h, 1000 V 1 h, 2000 V 1 h, 10,000 V 4 h and then run at 10,000 V for until the final volt-hours reached 65 kVh). After isoelectric focusing (IEF), the strips were equilibrated for two periods of 15 min. Following equilibration, the strips were sealed with molten 0.5% (w/v) agarose (0.001% bromophenol blue was added to the agarose solution) on 12% precast gels and then run on the Ettan™ DALT-12 system separation unit (GE Healthcare Piscataway, NJ, USA) in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS) at 16 °C with a cooling device (Ettan™ DALT-12 system power supply and control unit; GE Healthcare). The gels were run at 1 W per gel for the first 1.5 h followed by 15 W per gel until the blue line reached the bottom of the gel. Gel staining with Coomassie brilliant blue G-250 was done as described by Zhuang et al. (2013) [11]. Gel images were scanned using a Versa Doc IMAGING SYSTEM 4000MP (Bio-Rad). Each sample was performed in three repetitions for further analysis. Spot detection on the stained gels was performed using PDQuest software Version 8.0.1 (Bio-Rad, USA) and spot detection was manually refined. Quantitative comparisons of the averaged gels in each sample were used to determine significantly differentially expressed spots, and only the spots that showed at least a two-fold change and which were statistically significant in a one-way ANOVA ($P < 0.05$) for reproducible changes in the three analytical replicates were considered for further analysis.

2.3. In-gel digestion

Protein spots of interest were excised from the gels and cleaned with double-distilled water before being transferred into sterilized Eppendorf tubes. In-gel digestion was performed according to Katayama et al. (2001) [12]. Peptide MS and MS/MS were performed on an ABI 5800 MALDI-TOF/TOF Plus mass spectrometer

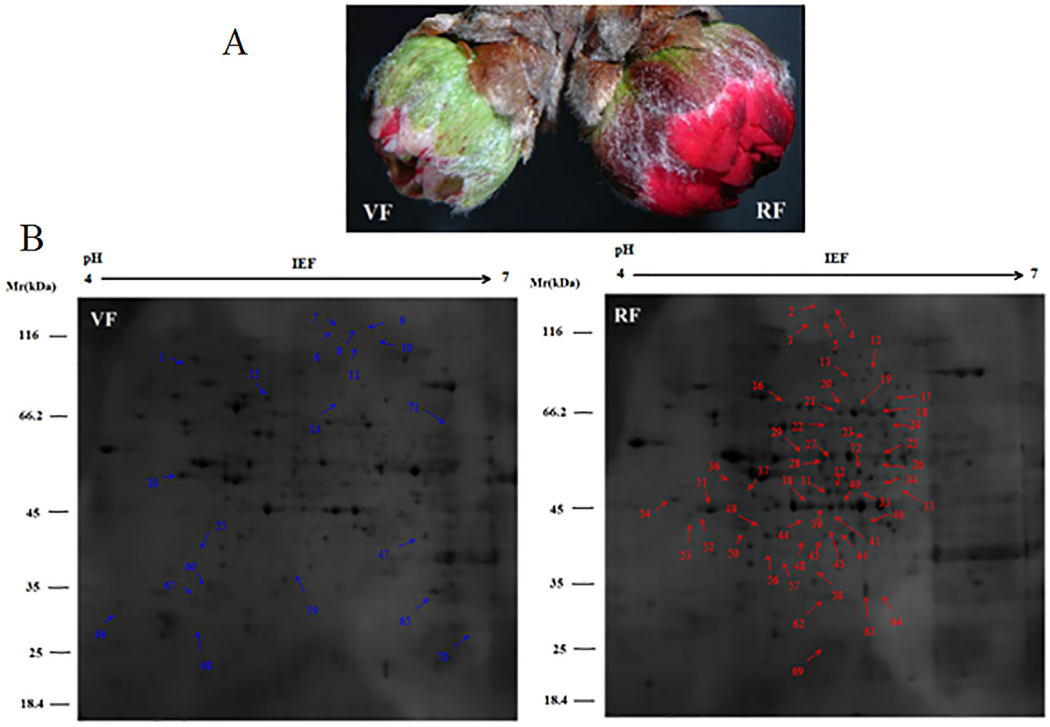

Fig. 1. (A) Example of the samples used in this study. (B) Profiles of the identified proteins. Numbers with arrows indicate the differentially expressed protein spots.