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Proteomic analysis of 'Zaosu' pear (*Pyrus bretschneideri* Rehd.) and its early-maturing bud sport

Xueting Liu[a,b], Rui Zhai[a,b], Wenting Feng[a,b], Shiwei Zhang[a,b], Zhigang Wang[a,b], Zonghao Qiu[a,b], Junke Zhang[a,b], Fengwang Ma[a,b], Lingfei Xu[a,b,*]

[a] College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Key Laboratory of Horticultural Plant Biology and Germplasm Innovation in Northwest China, Ministry of Agriculture, Yangling, Shaanxi 712100, China

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ABSTRACT

Maturation of fruits involves a series of physiological, biochemical, and organoleptic changes that eventually make fleshy fruits attractive, palatable, and nutritional. In order to understand the mature mechanism of the early-maturing bud sport of 'Zaosu' pear, we analyzed the differences of proteome expression between the both pears in different mature stages by the methods of a combination of two-dimensional electrophoresis (2-DE) and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis. Seventy-five differential expressed protein spots ($p < 0.05$) were obtained between 'Zaosu' pear and its early-maturing bud sport, but only sixty-eight were demonstratively identified in the database of NCBI and uniprot. The majority of proteins were linked to metabolism, energy, stress response/defense and cell structure. Additionally, our data confirmed an increase of proteins related to cell-wall modification, oxidative stress and pentose phosphate metabolism and a decrease of proteins related to photosynthesis and glycolysis during the development process of both pears, but all these proteins increased or decreased faster in the early-maturing bud sport. This comparative analysis between both pears showed that these proteins were closely associated with maturation and could provide more detailed characteristics of the maturation process of both pears.

1. Introduction

Fruit tree crops are agricultural commodities of high economic importance, while fruits also represent one of the most vital components of the human diet [1]. Unraveling the cellular mechanisms of fruit development has significant implications for our food supply, health, and nutrition [2]. The mature phenotype of fruit is a series of biochemical and physiological reactions that occur at the terminal stage of fruit such as the degradation of chlorophyll and starch, the enlargement of the fruit, the metabolism of sugars and acids, as well as hydrolysis of polysaccharides, pectin and modification of the cell wall structure [3–6].

For a long time, many genomic tools including mapping populations, mapped DNA markers and expressed sequence tag (EST) collections are available on study fruit development [3]. But gene expression levels may not predict the exact protein concentration and activity; these may be attributed to gene alternative splicing, mRNA degradation or inefficient translation, as well as protein post-translational modifications, processing and protein turnover [7]. Therefore, proteomics represents a powerful approach to characterize biochemical networks and to establish functional correlations between genotype and phenotype [8]. And it was also widely recognized as a useful tool in elucidating complex development features of tomato [9,10], apple [11], grape [12–14], orange [15], peach [16] and papaya [17]. In pear, previous proteomic studies focused on controlling the effect of atmosphere conditions on physiological disorder during storage [18–20], and the characterization of red skin bud mutation-related proteins [21]. However, there is no research about the protein changes during the maturation process of pears so far.

Abbreviations: 2-DE, two-dimensional electrophoresis; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; SSC, soluble solids content; TA, titratable acidity; ADK1T, adenosine kinase isoform 1T-like; CDC48, cell division control protein48; SAMs, S-adenosylmethionine synthetases; XTH, xyloglucan endotransglucosylase/hydrolase; α-Man, α-mannosidase; APX, ascorbate peroxidase; SOD, superoxide dismutase; MDHAR, monodehydroascorbate reductase; NADP-ME, NADP-dependent malic enzyme; cMDH, NAD-dependent malate dehydrogenase; Cyt-ACO, cytosolic aconitase; OECP, oxygen-evolving complex protein; OEEP, oxygen-evolving enhancer protein; TPI, triosephosphate isomerase; TKL, transketolase; PGK, phosphoglycerate kinase; XIM, xylose isomerase; GS, glutamine synthetase.

* Corresponding author at: College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China. Tel.: +86 29 87082150; fax: +86 29 87082149.
E-mail address: lingfxu2013@sina.com (L. Xu).

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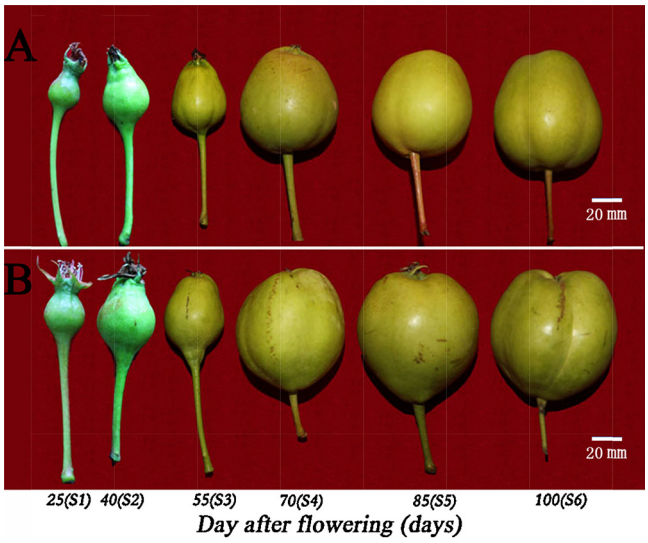

Fig. 1. Morphological changes in 'Zaosu' pear and its early-maturing bud sport during six growth stages (S1, S2, S3, S4, S5 and S6) according to the days after flowering. (A) The fruit development of 'Zaosu' pear; (B) the fruit development of the early-maturing bud sport.

'Zaosu' pear (*Pyrus bretschneideri* Rehd.) was a commercial Asian pear cultivar in China [22], and we found an early-maturing bud sport of 'Zaosu' pear in 2003 in Weinan, China. The early-maturing bud sport not only has better quality and larger size, but also earlier-maturation behavior, compared with 'Zaosu' pear (Figs. 1 and 2). Notably, the precocious characteristics of the early-maturing bud sport have been stable through observation and experiment after grafting on resource garden of Northwest A&F University, Shaanxi on July 12, 2009. Therefore, the bud sport is an excellent material for the study of protein variations during the maturation process.

In this study, to dig deeper into a detailed framework of pear fruit protein patterning and to explore the maturation mechanism of the early-maturing bud sport, a comparative proteomic investigation was conducted on pear fruits from 'Zaosu' pear and its bud sport at three maturation stages. A number of differentially expressed proteins were identified by means of 2-DE analysis and MALDI-TOF MS approach in both pears. Their putative physiological role in relation to the fruit maturation process is discussed.

2. Materials and methods

2.1. Plant materials

'Zaosu' pear (*P. bretschneideri* Rehd.) and its early-maturing bud sport, the tree of which had been ten years old at the germplasm resources orchard of Northwest A&F University, Shaanxi China (110°17′ E, 37°36′ N), were grafted on *P. betulifolia* Bunge rootstock and trained to the delayed-open central leader shape. The local climate was semiarid with deficits of plant available water during spring. Regular irrigation management was carried out according to the climate every year. At the same time, a commercially available compound fertilizer and urea fertilizer was added annually in equal amounts to each tree. Soil at the site is Earth-cumuli-Orthic Anthrosols, averaging 0.975% organic matter and pH 7.1 [23]. Under this condition, leaf and fruit ratio by flower/fruit-thinning of 'Zaosu' and its bud sport was approximately 30:1, and the yield was about 30 kg/tree.

2.2. Protein extraction

Fruit samples were collected from six individuals (three 'Zaosu' and three early-maturing bud sport) at DAF (day after flowering) 25 (S1), DAF 40 (S2), DAF 55 (S3), DAF 70 (S4), DAF 85 (S5) and DAF 100 (S6). For each stage, about 8–10 fruits were harvested from each tree. Notably, fruit samples selected must be free from visible blemishes and disease. Flesh firmness, fruit weight, fruit polar and equatorial lengths, soluble solids content (SSC), and titratable acidity (TA) were measured immediately after picking. Fruit polar, equatorial lengths and fruit weight were measured with a vernier caliper. Pear samples for firmness, SSC and TA determinations were referring to the methods reported elsewhere [24]. On the other hand, a similar number of fruit samples including mesocarp and epicarp were cut into liquid N_2 and stored at −80 °C prior to being used for the measurement of starch content from S1 to S6, among which only S1, S3 and S5 had the protein and RNA extracted. The measurement of starch content is based on the methods reported by Stevenson et al. [25].

Protein mining was performed by means of a TCA/acetone precipitation, followed by a phenol extraction [26]. The pear tissue was ground to a fine powder in a mortar with liquid nitrogen. Samples containing 4 g of dried tissue were suspended in 10 ml of 10% (w/v) TCA/acetone, thoroughly vortexed for 2 min, centrifuged at 18,000 × *g* for 5 min at 4 °C, and removed of the supernatant. Samples were extracted with 10 ml of iced 100% methanol, containing 100 mM ammonium acetate and 10 mM DTT, and then with 10 ml of iced 80% acetone, in both cases following the vortexing and centrifugation steps reported above. After air drying at room temperature for 5 min, samples were added with 10 ml of Tris–phenol and shaken at room temperature for 20 min. Then samples were add with an equal volume of ice-cold extraction buffer (700 mM sucrose, 500 mM Tris–HCl pH 8, 10 mM EDTA, 4 mM ascorbic acid, 1 mM PMSF), extracted in a waring blender for another 20 min and then centrifuged at 13,000 × *g* for 15 min, at 4 °C. Proteins were precipitated from the phenol phase by addition of 5 vol of methanol solution, at −20 °C, overnight; they were pelleted at 15,000 × *g*, for 15 min, washed at first with iced 0.1 M ammonium acetate in methanol, then twice with cold acetone, finally with iced 80% acetone. It is noteworthy that a separate extraction was performed for each biological replicate.

2.3. Two-dimensional gel electrophoresis (2-DE)

For 2-DE analysis, protein samples were dissolved in IEF buffer (7 M urea, 2 M thiourea, 4%, w/v CHAPS, 0.5%, v/v Triton X-100, 65 mM DTT, 0.2%, w/v biolytes, Bio-Rad) for 2 h. Protein concentration of these samples was determined using BSA as standard by Bradford assay [27]. Small amounts of sample were used for quantification to minimize interference of IEF compounds in the Bradford assay. IPG strips (17 cm pH 4–7, ReadyStrip, Bio-Rad) were rehydrated overnight with 350 μl of IEF buffer containing 800 μg of total proteins. Proteins were focused using a Protean IEF Cell (Bio-Rad) at 17 °C, by applying the following voltages: S1 linear 250 V 30 min, S2 rapid 500 V 30 min, S3 rapid 1000 V 1 h, S4 linear 10,000 V 4 h, S5 rapid 10,000 V 100 kV h, S6 rapid 500 V 24 h. After IEF, the strips were equilibrated for 15 min in equilibration solution A (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2%, w/v SDS, 20%, v/v glycerol, 2%, w/v DTT) and in equilibration solution B (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2%, w/v SDS, 20%, v/v glycerol, 2.5%, w/v iodoacetamide) for another 15 min. Electrophoresis in the second dimension was carried out on 12% polyacrylamide gels (180 mm × 240 mm × 1 mm), using electrophoresis buffer (25 mM Tris–HCl pH 8.3, 1.92 M glycine and 1%, w/v SDS), with 80 V applied for 1 h, and 120 V until the dye front reached the bottom of the gel. 2-DE gels were stained with Coomassie Brilliant Blue G-250.