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Proteomic analysis of differentially expressed proteins in longan flowering reversion buds

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ABSTRACT

A proteomic approach was taken to compare the proteomes of normal flowering buds and flowering reversion buds in longan (*Dimocarpus longan* Lour. cv. Longyou). Two-dimensional gel electrophoresis (2-DE), coupled with mass spectroscopy and protein database searching, recognized 18 proteins that were differentially expressed in flowering reversion buds. Eleven of these were down-regulated, whereas seven were up-regulated. A subset of 13 proteins was identified by MALDI-TOF-TOF/MS and classified into regulatory proteins (kinase, 20S proteasome alpha 6 subunit, putative alpha 7 proteasome subunit, auxin-induced protein, and abscisic stress ripening-like protein), antioxidant-related proteins (Chain A, GDP-mannose-3′,5′-epimerase, putative lactoylglutathione lyase, and Chain A, ascorbate peroxidase), pollen fertility-related proteins (putative leucoanthocyanidin reductase 2, and putative isoflavone reductase), photosynthesis-related proteins (large subunit, ribulose-bisphosphate carboxylase–oxygenase), and molecular chaperones (disulfide isomerase). Among them, regulatory and antioxidant-related proteins accounted for almost two-thirds of these proteins, suggesting that they may play a more important role in bud differentiation. Identification of these proteins provides insights that may lead to a better understanding of the molecular basis for flowering reversion in longan.

1. Introduction

Flowering is an essential phenomenon in the life cycle of higher plants, being the main process for survival and reproduction of the species. In spermatophyte development, the change from vegetative to reproductive growth is called flowering transition. The latter is controlled by inheritance, environment, and other factors and is accompanied by various changes in morphology, cytology, physiology, and biochemistry. Upon completion of flowering transition, the apical meristem exhibits stable reproductive growth. However, plants may revert to vegetative growth, if circumstances are unsuitable for their reproductive growth. This process, called flowering reversion, is influenced by both internal and external factors, including temperature, photoperiod, light quality, light intensity, and herbicides (Battey and Lydon, 1990). Reversion of flowering occurs when production of vegetative structures is resumed in a meristem after floral development has been initiated; it is possible for leaf production to resume at any time during flower initiation as long as the shoot apical meristem has not yet become completely determined for flower formation (Lydon, 1998; Carolyn and Judith, 2000). There are two distinct types: inflorescence reversion, in which vegetative development occurs after, or intercalated within, inflorescence development, and flower reversion, in which the form of the flower itself is altered. The flower may be incomplete, with some parts replaced with leaves, or there may be proliferation after the formation of the normal complement of floral organs (Tooke et al., 2005).

In longan (*Dimocarpus longan* Lour. cv. Longyou), high temperature and moisture in winter are the major factors causing flowering reversion. During this process, normal flowers stop development halfway and form floral spikes with leaves (Fig. 1), and some even revert to vegetative branches. This phenomenon obviously decreases longan productivity in the following year. In this research, we have, for the first time, taken a proteomic approach to study longan flowering reversion. We have compared the proteomes of normal flowering buds and flowering reversion buds, allowing us to identify differentially expressed proteins that may play a significant role in flowering reversion and provide initial insights into the molecular basis for flowering reversion.

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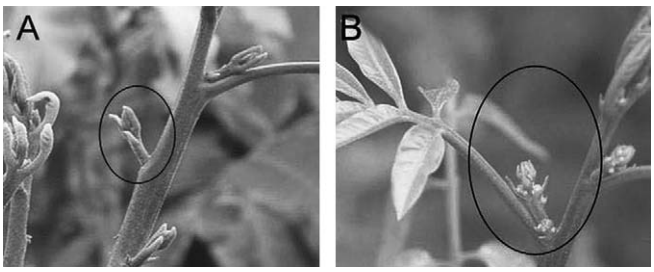

Fig. 1. (A) Longan normal flowering buds. (B) Longan flowering reversion buds.

2. Materials and methods

2.1. Plant materials

'Longyou' cultivar of longan (*D. longan* Lour.) grows in the orchard of the Putian Research Institute of Agricultural Sciences, Fujian Province, PR China. Samples, both normal and reversing flower buds, were collected from 3 different trees (triplicate) in April 2006. The buds were immediately immersed in liquid N_2 and then stored at −80 °C for later analysis.

2.2. Protein extraction

Protein extracts were prepared using the protocol of Carpentier et al. (2005). Longan buds (0.5 g) were ground into powder in liquid N_2 with 4 mL of precooled extraction buffer (100 mM Tris, 50 mM L-ascorbic acid, 100 mM KCl, 50 mM disodium tetraborate decahydrate, 1% Triton X-100, 2% β-mercaptoethanol, and 1 mM PMSF), and, after centrifugation at 15,000 × *g* for 10 min at 4 °C, the supernatant was carefully transferred to a new centrifuge tube. An equal volume of ice-cold Tris-buffered phenol (pH 8.0) was added, and the mixture was vortexed. After centrifugation at 20,000 × *g* for 10 min at 4 °C, the phenol phase was collected and precipitated overnight with five volumes of 0.1 M ammonium acetate/methanol at −20 °C. After 2 h, the mixture was centrifuged at 20,000 × *g* for 10 min at 4 °C. The pellet was washed once in ice-cold carbinol and then twice in ice-cold acetone containing 2% β-mercaptoethanol, before being allowed to air-dry at −20 °C.

2.3. Two-dimensional gel electrophoresis

The sample powder (10 mg) was solubilized in 300 μL of lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% Pharmalyte 3–10, and 40 mM DTT) and incubated at 37 °C for 2 h. Protein quantification was undertaken according to Bradford (1976).

First-dimensional electrophoresis was carried out using an IPGphor II (Amersham Biosciences) isoelectric focusing (IEF) system. IPG dry strips (pH 4–7, 24 cm length, linear) were rehydrated at 24 °C and 30 V for 12 h with 150 μL lysis buffer mixed with 300 μL rehydration solution (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG-buffer, 0.002% Bromophenol Blue, and 40 mM DTT). After rehydration, IEF was performed under the following conditions: 200 V for 1 h, 500 V for 1 h, 1 kV for 1 h, gradient 8 kV for 0.5 h, and 8 kV up to 40,000 Vhs. Before SDS-PAGE, the strips were equilibrated twice: (i) for 15 min in reducing equilibration buffer (50 mM Tris–HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 1.0% (w/v) DTT, and a trace of Bromophenol Blue), and (ii) for 15 min in equilibration buffer containing 2.5% (w/v) iodoacetamide instead of 1.0% (w/v) DTT. The strips were transferred onto vertical 12.5% SDS-PAGE self-cast gels. SDS-PAGE was performed at 18 °C using an Ettan DALT-six System (Amersham Biosciences): 15 mA for 30 min and 30 mA for 12 h for every strip. After 2-DE, gels were stained with Coomassie Brilliant Blue (CBB) R-250. To ensure data reliability, sample preparation and 2-DE were performed in triplicate.

2.4. Gel scanning and analysis

2D-electrophoresis gels were scanned with Image scanner EPSON PERFECTION 2480 PHOTO (clairvoyant scanning with 300 dpi optical resolution, windows default for contrast and brightness). Images were analyzed using the specialized software program PDQuest. The optimized parameters were as follows: saliency 2.0, partial threshold 4, and minimum area 50. The gel images were normalized according to the total quantity in the analysis set. Relative comparison of intensity abundance between normal flowering and flowering reversion (3 replicate samples for each group) were performed using Student's *t*-test. Expression intensity larger than 2.0 ($p \leq 0.05$) or smaller than 0.5 ($p \leq 0.05$) were set as a threshold indicating significant changes.

2.5. In-gel tryptic digestion

Protein spots were excised from gels and placed into a 96-well microtiter plate for destaining using 25 mM ammonium bicarbonate in 50% acetonitrile (30 min at 37 °C). Subsequently, gel pieces were washed twice with deionized water, shrunk by dehydration in acetonitrile, and then swollen for 30 min at 4 °C in a digestion buffer containing 25 mM ammonium bicarbonate and 12.5 ng/μL trypsin. After digestion at 37 °C for at least 12 h, peptides were extracted twice using 0.1% TFA in 50% acetonitrile.

2.6. Protein MALDI-TOF-TOF/MS analysis

Extracts were dried under N_2. For MALDI-TOF-TOF/MS, peptides were eluted onto the target with 0.7 μL of matrix solution (α-cyano-4-hydroxycinnamic acid in 0.1% TFA, 50% acetonitrile). Samples were allowed to air-dry before inserting them into the mass spectrometer.

The MALDI mass spectrometer (MS) was an ABI 4700 TOF-TOF Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) instrument. The UV laser was operated at a 200 Hz repetition rate with wavelength of 355 nm. The accelerated voltage was operated at 20 kV, and the mass resolution was maximized at 1500 Da. Trypsin-digested myoglobin was used to calibrate the mass instrument with internal calibration mode. All the spectra of the real samples were acquired using a default mode. Parent mass peaks with mass range 700–3500 Da and minimum S/N 20 were picked out for tandem MS/MS analysis. Combined MS and MS/MS spectra were submitted to MASCOT (V2.1, Matrix Science, London, UK) by GPS Explorer software (V3.6, Applied Biosystems) and searched using the following parameters: NCBInr database (release date: 27 June 2007), trypsin digest with one missing cleavage, no fixed modifications, MS tolerance of 0.2 Da, MS/MS tolerance of 0.6 Da, and possible oxidation of methionine. Known contaminant ions (keratin) were excluded. The MASCOT protein scores (based on combined MS and MS/MS spectra) of greater than 65 were considered statistically significant ($p \leq 0.05$).

2.7. Statistical analysis

A repeated measurement is given as the mean ± SD. The comparison of differences among the groups was carried out using the Student's test. Significance was defined as $p \leq 0.05$.

3. Results

3.1. 2-DE analysis of flower bud proteins in longan

To test whether the proteins of longan buds could be effectively separated by 2-DE, protein extracts were first examined within the pH range 3–10. Most flower bud proteins lay within the narrow