Front. Agric. China 2008, 2(4): 467–473
DOI 10.1007/s11703-008-0076-5

RESEARCH ARTICLE

Shangyin CAO, Qiuming ZHANG, Zhiyong ZHU, Junying GUO, Yuling CHEN, Huabai XUE

Preliminary proteomics analysis of the total proteins of flower bud induction of apple trees

Abstract Apple is one of the most important fruit trees in the world. Nevertheless, mainly due to its long juvenile period, its breeding work constantly falls far behind other crops. So the aim of this study is to reveal the mechanism of apple flower bud differentiation, shorten the juvenile period and accelerate its breeding process. Proteomics technology (including two-dimensional gel electrophoresis (2-DE), biomass spectrometry and bioinformatics) was applied to work on the specific protein of flower bud and leaf bud after the brachyblasts of 'Fuji' stopped growth for 3–9 weeks. The results showed that the morphodifferentiation of flower bud did not begin until the seventh week after the brachyblast stopped growth. Furthermore, compared with the leaf bud, flower bud had significant changes in the expression of 283 protein spots in quality and quantity on 2-DE maps. Among the 283 protein spots, four protein spots (16.4, 30.2, 40.3 and 65.1 kD) were characteristic of the flower bud in the archae-stage (initial inflorescence appeared) at the beginning of flower-bud differentiation, three (39.3, 60.2 and 66.3 kD) in the post-stage (Lateral-flower appears) and one (77.1 kD) in the sepal stage on 2-DE maps. Analysis by peptide mass fingerprinting and matrix-assisted laser desorption ionization time of flight mass spectrometry also identified and forecasted functionally by blasting different databases. In the four specific proteins, it was found that spots No. 256 (16.4 kD) and 298 (30.2 kD) were unknown proteins, spot Nos. 327 (40.3 kD) was identified as the synthesis enzyme protein and spot No. 367 (40.3 kD) was identified as a RNA-binding protein involved in transcription. When flower bud started to differentiate morphologically, we detected four specific proteins which were 16.4, 30.2, 40.3 and 65.1 kD. Three specific proteins 39.3, 60.2 and 66.3 kD were observed at side flower-appearing stage. When calyx began to emerge, there was one specific protein: 77.1 kD. The proteins 16.4 kD and 30.2 kD were two unknown specific proteins. The 40.3 kD protein was related to methionine synthetase while 65.1 kD protein, a RNA-binding protein, was related to transcription.

Keywords apple tree, flower-bud, proteomics, specific protein, 2-DE, peptide mass fingerprinting

1 Introduction

Slower development of fruit tree breeding than other crops is attributed to its longer juvenile period. For a long period of time, fruit tree breeders have been trying their best to find all kinds of ways to solve this problem. However, at present, they have made little progress in it. In our study, differential proteomics was employed to study protein changes of floral bud and gemmaceous meristem in flower bud induction period; moreover, the specific proteins related to flower bud induction were observed and determined. This study can significantly help reveal molecular essence of apple flower bud differentiation, demonstrate methods of expression from gene to trait and accelerate the breeding process. Many researches began to focus on flower physiological functions when Klebs proposed C/N theory in 1904. Before 1970s, flower physiology was mainly studied. Specifically, ''Blossom Amoxicillin'' theory by Chelason and the verified existence of photofrin were two extremely important milestones for plant blossom research; but certain substance with Blossom Amoxicillin characteristic was not confirmed, and the further function of photofrin after receiving environmental signal remained a hypothesis.

Translated from *Scientia Agricultura Sinica*, 2007, 40(10): 2281–2288 [译自: 中国农业科学]

Shangyin CAO (✉), Junying GUO, Yuling CHEN, Huabai XUE
Zhengzhou Fruit Research Institute, the Chinese Academy of Agricultural Sciences (CAAS), Zhengzhou 450009, China
E-mail: s.y.cao@163.com

Qiuming ZHANG
College of Horticulture, Hunan Agricultural University, Changsha 410128, China

Zhiyong ZHU
College of Horticulture, Jiangxi Agricultural University, Nanchang 330045, China

Therefore, plant blossom researches were limited to physiological phenomenon and ran into a predicament in the mechanism of blossom induction, signal transmission, determination and initiation. After 1970s, focus was given to genetic mechanism study of blossom course. Thus, blossom specific genes were identified and cloned using molecular biology and classical genetic analysis, specifically the cloning of blossom homeotic genes (Meyerowitz, 1998; Weigel, 1995; Weigel and Nilsson, 1995; Sung and An, 1997; Sung et al., 1999, 2000; Yao et al., 2001; Leandro et al., 2001). However, study on gene expression at intermediate product–mRNA level can not displace the study at protein level (ultimate expression product of gene). Furthermore, mRNA has low dependability with expression protein ($P < 0.5$), so accurate study of blossom gene function still needs to go back to the protein itself which can execute life function. With the full speed development of proteome technology, this long-standing predicament has been broken and the study of plant blossom mechanism has been accelerated enormously (Anderson and Seilhamer, 1997; Gygi et al., 1999; Wilkins et al., 1995). On the base of the study on apple flower bud differentiation period and mechanism over many years (Cao et al., 2000, 2001, 2003, 2005), we utilized modern proteomics technology to directly compare proteome difference between apple brachyblast apical meristems at floral bud induction period to provide experimental base for searching for genes which can encode specific proteins of floral bud pregnancy. Using proteomics technology to isolate, identify and understand the specific proteins related with floral pregnancy, we can establish a foundation of sequencing specific gene cDNA, blast sequence similarity, deduce amino acid total length sequence, and construct floral bud gene promoter, so as to finally reveal floral pregnancy mechanisms.

2 Materials and methods

2.1 Material sampling and treatment

The materials used in this study were taken from QIWU apple orchard in Anyang city, Henan Province, China. 75 healthy juvenile 'Fuji' (15 years old) grafted on HUAILAI begonia were selected and randomly divided into Group I, Group II and Group III, respectively. One week after flower drop (2004-04-26), brachyblasts which meanwhile stopped growth (when the last foliage exactly flattened) were marked (A), brachyblasts of Group I with three foliages were marked, which could not form flowers, and brachyblasts of the rest two groups with eight foliages were marked, of which above 85% could form flowers (Cao et al., 2000). At least 150 brachyblasts were marked on each tree. The spare marks were prepared in 15 days (2004-05-11) using the same method. The experiment was repeated in 2005.

From the third week (2004-05-17) when the brachyblasts stopped growth, to the third week when floral bud morphodifferentiation initiated, three hundred marked brachyblast tips were randomly selected and preserved into a nitrogen canister. Then their apical points were cut respectively with a stereo-microscope. After floral bud morphodifferentiation, according to different differentiation period, floral buds were divided into samples at different developmental stages and stored in a freezer ($-80^{\circ}C$).

2.2 Major equipments and agents

A series of electrophoresis devices purchased from Amersham Pharmacia Biotech Company were Image Scanner (electrophoresis scanning analysis system), EPS1001 (power source), Hoefer SE600 (cooling vertical electrophoresis system), IPGphor (isoelectric focusing electrophoretic apparatus), high speed refrigerated centrifuge, powerful assembly purified water apparatus (Millipore). SDS, acrylamide, bisacrylamide, aminoacetic acid, CHAPS, DTT, urea and IPG prefabricate gum strip. iodoacetamide, TEMED and low molecular weight standard protein were purchased from Sigma Company, GIBCO and Fermentas Company, respectively. Balance buffer (storation liquid) was mixed with 0.05 $mol \cdot L^{-1}$ Tris-HCl at pH = 8.8, 6 $mol \cdot L^{-1}$ urea, 30% glycerine, 2% SDS, and 0.25% bromchlorphenol blue solution. Balanced solution 1 was prepared by adding 100 mg DTT to 10 mL balanced solution; Balanced solution 2 was prepared by adding 400 mg iodo-acetamide to 10 mL balanced solution.

2.3 Protein extraction, two-dimensional electrophoresis and gel image analysis

2.3.1 Protein extraction

Twenty mg dry protein acetone powder and 0.5 mL sample lysate [9.5 $mol \cdot L^{-1}$ urea, 2% (volume fraction) Nonidet P-40, 0.2% (volume fraction) Ampholine (pH 3.5–10), 0.4% (volume fraction) Ampholine (pH 5–7 and pH 6–8) and 5% β-Me (β-Mercaptoethanol)] were accurately weighed out. After vortex and vibration, it was put into 37°C water, kept for 2 h and centrifuged at15000 $r \cdot min^{-1}$ for 5 min under room temperature, then four times (volume) of cold acetone was added into the supernatant, which was reprecipitated at room temperature for 1 h.The supernatant was removed after re-centrifugation at 15000 $r \cdot min^{-1}$ for 5 min and suitable IEF sample lysate was added to dissolve it again. After water bath at 37°C for 2 h and a centrifugation at 15000 $r \cdot min^{-1}$ for 5 min, 1:4 (v/v) $-20^{\circ}C$ precooling acetone was added to precipitate protein for 2 h under room temperature, and thereafter the above-mentioned sample lysate was added into the precipitate to re-centrifuge it. Finally, total protein

concentration of small quantity of the supernatant was determined in BCA method after the 37°C water bath for 2 h, and the rest was applied for protein electrophoresis analysis.

2.3.2 The first dimension IEF electrophoresis

250 μL swelling sample solution was added into a 13-cm stripholder, and the anti-abrasion coating of IPG stripe was discarded, and put in the stripholder to soak the total stripe with gel facing down and eventually sufficient overlay oil was added. The stripe was fixed for 10 min utilizing 12.5% TCA (v/v) after electrophoresis, balanced for 20 min in balanced solution, and washed for 1 s with deionized water so as to eliminate the spare balanced buffer. Then the second dimension electrophoresis was directly executed or it was stored below −20°C.

2.3.3 Second dimension SDS-PAGE vertical electrophoresis

Perpendicular plate SDS-PAGE electrophoresis was adopted and isolating gel (gel concentration T = 12%) and condense gel (degree of cross linking C = 3.3%, 16 mL 30% ACR-Bis, 10 mL 1.5 mol·L^{-1} Tris-HCl, pH = 8.6, 1.3 mL glycerine, 400 μL 10% SDS, 160 μL TEMED, 300 μL AP, metered volume to 50 mL) were prepared respectively. Perpendicular plate etalon was 18 cm × 16 cm, and gel thickness was 1 mm. Balanced IPG stripe was placed on SDS-PAGE gel, with sufficient standard protein added at the one end, whose Mr was 116000, 66200, 45400, 35000, 25000, 18400 and 14400, respectively, and it was covered with 0.5% agarose. Electrophoresis condition was 20 mA (constant electrophoresis of each gel stripe at room temperature of 30°C). Electrophoresis was not completed till bromchlorphenol blue reached about 1 cm of the bottom of the gel.

2.3.4 Dyeing

The gel was fixed and stained in Coomassie Brilliant Blue staining solution of 0.3% Coomassie Brilliant Blue R250, 50% methanol, 10% glacial acetic acid and 39.7% water for 6 h, washed twice with double distilled water each for 3 min, then kept in the destaining solution of 2.5% methanol and 7.5% glacial acetic acid till background color completely vanished, followed by silver staining, washing the gel with double distilled water for 10 min, and treating it for 5 min in the sensibilizing solution of 400 mL ethyl alcohol, 20 mL 37% formaldehyde, and 2 mL 50% glutaric dialdehyde by metering the volume to1000 mL with double distilled water. Subsequently, after 40% ethyl alcohol treatment for 20 min, it was reduced using 0.6 mmol·L^{-1} $Na_2S_2O_3$ for 1 min, and washed with double distilled water for 2 min. It was rewashed with double distilled water for 1 min after being stained with 6 mmol·L^{-1} $AgNO_3$, and subsequently colorated in 240 mmol·L^{-1} Na_2CO_3 solution containing 14.8‰ (v/v) 37% formaldehyde in fresh and fixed with 5% (v/v) glacial acetic acid. Protein molecular weight and isoionic point were determined adopting 2-DIMENSIONAL calibration method (ID-Calibration).

2.3.5 Dried gel preparation

The dried gel was prepared according to Chen et al. (2001).

2.3.6 Gel image analysis

Image Master 2-D Elite was applied to decrease image background, detect spot ion and obtain spot location coordinate.

2.4 Peptide mass spectrogram finger print map analysis of protein spot on 2-DE gel

Peptide mass spectrogram finger print map analysis of protein spot was conducted according to the method by Courchesne and Patterson (1999). This map was compared with sequences of databases below, that is, MS-Fit (http://prospector.ucsf.edu) program was used in NCBInr.6.4.2003 and SwissProt.5.27.2003. And their index parameters were species defined as *Arabidopsis thalian*, minimum matches of 4 and nonmodified aminothiopropionic acid. According to wider molecular weight and isoionic point scope, the lowest mass tolerance was 100×10^{-6}, others were DNA Frame Translation:6, Considered modifications:|Peptide N-terminal Gln to pyroGlu| Oxidation of M| Protein N-terminus Acetylated|, Max. # Missed Cleavages:1, Peptide N terminus:Hydrogen, Peptide C terminus:Free Acid, Min Parent Ion Matches:1, MOWSE On:1, MOWSE P Factor:0.4, Sort Type: Score Sort, which were defaulted by factory. At the same time PeptIdent software (http://cn.expasy.org/) was used to search in the Swiss-Prot and TrEMBL database, and the species was defined as VIRIDIPLANTAE, the other index was the same as mentioned above. Protein function analysis was analyzed using the protein families database (Pfam) (http://www.sanger. ac.uk/Software/Pfam/).

3 Result and analysis

3.1 Observation of floral bud morphodifferentiation of Fuji apple

Table 1 shows that under natural condition, floral bud morphodifferentiation of Fuji apple began in the seventh week after brachyblast stopped growth, with floral buds

Table 1 Rate of flower bud in Fuji apple (%)

weeks of spur growth cessation/week		herein before 8 parcel leaf		herein after 3 parcel leaf	
A	B	A	B	A	B
3		0		0	
4	3	0	0	0	0
5	4	0	0	0	0
6	5	0	0	0	0
7	6	50.5a	0	0	0
8	7	75.7b	49.8a	0	0
9	8	86.1c	77.5b	0	0
	9		87.8c	0	0

Note: Values (average) with the same small letters represent differences at less than 5% significant level.

forming 42–49 days after the shoots stopped growth. It was found that twice marked brachyblasts shared the same rule, indicating the stable morphodifferentiation, which was in accordance with our previous works (Cao et al., 2000, 2001).

Floral bud morphodifferentiation progress of Fuji apple is shown in Fig. 1. Floral bud morphodifferentiation initiated in the seventh week after brachyblast stopped growth. Floral buds appeared in the early initial stage during the floral bud morphodifferentiation and some floral buds entered the late initial stage from the 49th to the 56th day after the brachyblast stopped growth, when lateral-flower began the floral bud morphodifferentiation. In the calyx period, floral bud appeared in the ninth week, when part of the buds were differentiated into calyx.

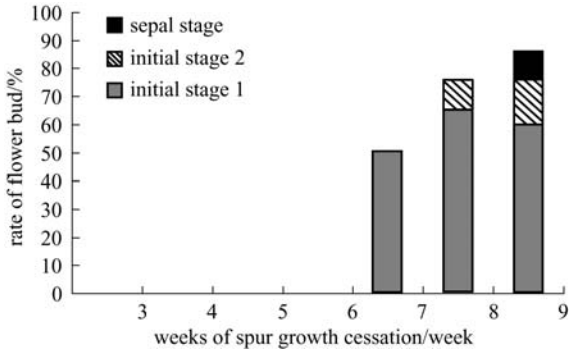

Fig. 1 Process of flower bud induction of Fuji apple

3.2 Protein changes of Fuji apple during floral bud induction

Image Master 2-D Elite was applied to analyze the floral bud and leaf bud protein 2-DE atlas of Fuji apple in the different stages after brachyblasts stopped growth. The results showed no difference in the protein electrophoresis atlas between floral bud and leaf bud from the third to sixth week (May 17 to Jun 7) after brachyblasts stopped growth. Furthermore, 655 protein spots were detected. However, floral bud and leaf bud protein electrophoresis atlas were obviously different from that of the seventh week (Jun 14) after brachyblasts stopped growth, and 705 protein spots were detected in floral bud and 655 protein spots in leaf bud in protein electrophoresis atlas. Apparently, the latter was obviously fewer than the former. Seven hundred and twelve and 716 protein spots were detected respectively in floral protein electrophoresis atlas from the eighth to ninth week after branchyblasts stopped growth and 655 protein spots were also detected in leaf bud protein electrophoresis atlas. When we compared soluble protein composition between floral bud and leaf bud meristem in Fuji apple, we found out that some floral bud proteins disappeared during the development process of brachyblast topbuds from vegetable growth to reproductive growth (from the sixth to seventh week after brachyblasts stopped growth) eg. 13.0, 17.1, 25.5, 27.3, 45.3, 131.1, 211.2 and 336.4 kD. Meanwhile, there were some new proteins that formed, eg. 16.4, 30.2, 40.3 and 65.1 kD. Furthermore, some proteins called coprotein existed in the floral buds and leaf buds (Table 2), eg. 5.4, 6.3, 10.0, 15.2, 20.2, 28.1 and 29.3 kD.

3.3 Specific protein in floral bud pregnancy stage of Fuji apple

Reference gel and analysis gel were defined as 2-DE atlas of floral bud in the sixth week and seventh week after brachyblasts stopped growth, respectively. Two hundred and eighty-three protein spots showed evident changes in quantity and quality expression between the two atlases.

Comparing the 2-DE atlas of floral bud in the seventh week as reference gel with those in the eighth week after brachyblasts stopped growth as analysis gel, 2 specific protein spots including 1346 and 1365 protein spots were found in the latter, with isoelectric point (pI) being 5.73 and 6.70, and Mr being 21373 and 19983, respectively (Table 3).

The difference between the 2-DE atlas of floral bud in the eighth week and those in the ninth week after brachyblast stopped growth was that in the later there was only one specific protein spot of 1329, whose pI and Mr were 5.13 and 21821 respectively (Table 3).

From the above analysis, there were new proteins that formed in the floral buds during morphodifferentiation initiation and development process. And these proteins were related to the formation of inflorescence organs. In order to investigate the functions of specific proteins in floral bud differentiation program expression, the soluble proteins and their compositions of floral buds in the earlier development were analyzed respectively. Proteins only existing in certain floral or leaf bud development stage and not in other development stages were defined as floral bud development specific proteins. Common proteins were those existing in the whole development process. In the earlier period of floral bud development, a majority of common proteins existed but there still existed some

Table 2 Changes of soluble protein profile in Fuji apple during flower bud development

type	week after spur stopped growing	changes of soluble protein/kD
leaf bud	3–6	5.4, 6.3, 7.0, 9.3, 10.0, 11.2, 12.4, 13.0, 14.0, 15.2, 17.1, 18.0, 19.4, 20.2, 21.1, 22.0, 23.6, 24.3, 25.5, 26.0, 27.2, 28.1, 29.3, 31.0, 32.0, 33.5, 36.6, 38.3, 42.2, 44.7, 45.3, 47.0, 48.5, 49.6, 50.7, 53.9, 55.4, 57.2, 61.5, 70.3, 71.1, 72.4, 73.6, 84.4, 95.8, 98.1, 115.0, 131.1, 136.0, 174.2, 211.2, 336.4
flower bud	7	5.4, 6.3, 7.0, 9.3, 10.0, 11.2, 12.4, 14.0, 15.2, 16.4, 18.0, 19.4, 20.2, 21.1, 22.0, 23.6, 24.3, 26.0, 28.1, 29.3, 30.2, 31.0, 32.0, 33.5, 36.6, 38.3, 40.3, 42.2, 44.7, 47.0, 48.5, 49.6, 50.7, 53.9, 55.4, 57.2, 61.5, 65.1, 70.3, 71.1, 72.4, 73.6, 84.4, 95.8, 98.1, 115.0, 136.0, 174.2
flower bud	8	5.4, 6.3, 7.0, 9.3, 10.0, 11.2, 12.4, 14.0, 15.2, 16.4, 18.0, 19.4, 20.2, 23.6, 24.3, 26.0, 28.1, 29.3, 30.2, 31.0, 32.0, 33.5, 36.6, 38.3, 39.3, 40.3, 42.2, 44.7, 47.0, 49.6, 53.9, 57.2, 60.2, 61.5, 65.1, 66.3, 70.3, 72.4, 3.6, 84.4, 95.8, 98.1, 15.0, 136.0, 174.2
flower bud	9	5.4, 6.3, 7.0, 9.3, 10.0, 11.2, 12.4, 14.0, 15.2, 16.4, 18.0, 19.4, 20.2, 23.6, 24.3, 26.0, 28.1, 29.3, 30.2, 31.0, 32.0, 33.5, 36.6, 38.3, 39.3, 40.3, 42.2, 44.7, 47.0, 60.2, 65.1, 66.3, 72.4, 73.6, 77.1, 84.4, 95.8, 98.1, 115.0, 136.0, 174.2

Table 3 Changes of specific proteins in Fuji apple during flower bud development

week after spur stopped growing/week	newly emerged specific proteins/kD	disappeared specific proteins/kD
6		13.0, 17.1, 25.5, 27.2, 45.3, 131.1, 211.2, 336.4
7	16.4, 30.2, 40.3, 65.1	21.1, 22.0, 48.5, 50.7, 55.4, 71.1
8	39.3, 60.2, 66.3	49.6, 53.9, 57.2, 61.5, 70.3
9	77.1	

specific proteins; for example, 16.4, 30.2, 40.3 and 65.1 kD specific proteins began to appear in the seventh week when floral bud morphodifferentiation initiated after brachyblasts stopped growth, and 39.3, 60.2 and 66.3 kD began to appear in the later initial period of floral bud morphodifferentiation (in the eighth week after brachyblasts stopping growth) and 77.1 kD emerged in the ninth week (sepal stage).

3.4 Database index

Protein spots (256, 298, 327 and 367 protein spots) that appeared latest were cut in two-dimensional electrophoresis atlas of the seventh week apical bud after Fuji apple brachyblasts stopped growth, enzymolyzed by trypase and determined on peptide quality fingerprint. According to a group of isotope peak values by mass spectrographic analysis, firstly we set species or taxon, followed by raising quality error gradually in wider molecular and isoelectric point scope, and using MS-Fit and PeptIdent to search them respectively (Table 4). These four protein spots were identified and analyzed on base data and initial results including protein abundance, molecular weight and isoelectric point experimental value, database and quality error, match peptide number, coverage fraction of peptide sequence to match protein, theoretical molecular weight and isoionic point, match protein name and GeneBank registration code, corresponding species and protein function. The lower quality error was set, the higher the retrieval result reliability was. When 100×10^{-6} quality error was set in this index, almost no match item appeared. However, when quality error was set at 200×10^{-6} or 0.25–1.0 Da, the retrieval achievement ratio increased. Because protein to be identified was from Rosaceae plant, whose genomic total sequence information has not been published at present, the reliability of match protein could be raised on the base of sequence similarity by setting the species within the plant or Rosaceae plant. In proteome study (Watson et al., 2003) on each lucerne organ in leguminous plant, the achievement ratio of protein identification was only 25% in the protein database (SwissProt and NCBInr); however, the average was 46% in EST database. Actually, mRNAs without poly (A+) tail such as chondriosome or chlorophyll corpuscle encoding mRNAs hardly appear in EST database. Therefore, it is necessary to use many different databases simultaneously so as to raise protein identification achievement ratio.

Table 4 Database querying result of spot Nos. 256, 298, 327 and 367

protein	peptide identity/%	sequence coverage/%	RMW	IP	species	protein name
256	43	27.0	28982	4.76	*Arabidopis thaliana*	unknown
298	37	9.0	26673	4.66	*Arabidopis thaliana*	unknown
327	48	37.3	21841	5.12	*Arabidopis thaliana*	RNAsynthetase
367	49	39.8	19823	6.49	*Arabidopis thaliana*	RNA conjugated protein

3.5 Protein identification

Protein identification using PMF was based on the following indexes: match peptide section numbers, quality accuracy (m/z accuracy), coverage fraction of peptide sequence to match protein total sequence (The lowest was 10%.), peptide spectra quality, match peak intensity (The minimum value was 18%–20%.), difference in molecular weight and isoionic point between experimental and theoretical value, and the species to which match protein (Wilkins et al., 1995) belonged. Protein function analysis was in database Pfam (http://www.sanger.ac.uk/Software/Pfam/). According to Plant Gene Functional Classification Program by Bevan et al. (1998) published in Nature, we could divide functional classification of match protein. When comparing retrieval results in databases and screening optimal record, we found that the protein spot No. 327 (a kind of RNA synthetase), was identified as the same protein in the two methods. Two hundred and fifty-six and 298 proteins were the most similar to the two unknown functional expression proteins in *Arabidopsis thaliana*. As to spot No. 367, for example (Fig. 2), in coherent index record, RNA binding protein (Acc.#:D96792) match was the best and the result is shown in Table 5. According to gene functional classification program in plant, the function of RNA binding protein was transcription and it was expressed in quantity (2.6% abundance) in apple brachyblast apical meristem, which was closely correlated with growth and development in full bloom.

In total, the coverage fraction of peptide sequences in four protein spots did not exceed 50%. The possible reason was that it was not from the same species. But if samples came from species whose total genomic sequences were known, accuracy rating and achievement ratio of protein identification with PMF would increase. With regard to human proteome study achievements, the ratio of protein identification was about 60% (Watson et al., 2003). Different retrieval methods yielded different results, so cascade connection mass spectrogram was applied to identify proteins exactly. Thus, this could raise achievement ratio of protein identification in binding use of PMF and peptide sequences.

4 Discussion

From floral bud morphology study in apple, apical buds transformed from vegetable to floral buds in 42–49 d (in the 7th week) after brachyblast stopped growth. In our study, soluble protein was analyzed during floral bud transformation period, and the result showed that the protein metabolism was very complex circa floral bud differentiation, accompanied with some protein disappearing and specific protein related with floral bud appearing, indicating that 16.4, 30.2, 40.3 and 65.1 kD protein existed from the seventh to the ninth week after brachyblasts stopped growth except in leaf bud. It was the reason that those were the key proteins of inflorescence

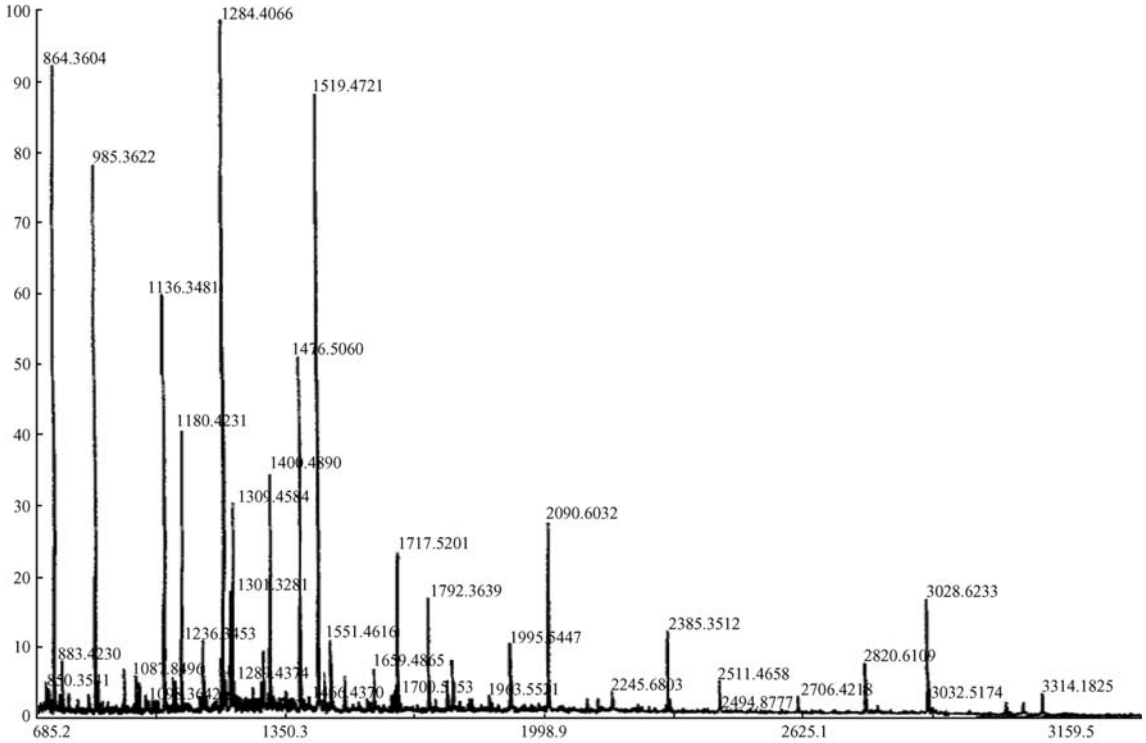

Fig. 2 PMF of spot No. 367 (TM-367) from 2-DE gel in Fuji apple

Table 5 Database query result of spot No. 367 (Acc1#:D93791)

m/z submitted	MH + matched	Delta Da	missed cleavages	peptide
1234.4070	1234.6501	−0.22	0	INHFHITSK
1354.4230	1284.2007	−0.23	1	ACADPTPIDGR
1284.4430	1384.7003	−0.21	1	RCADPTIIDGR
1392.3440	1752.9234	−0.54	0	VFVGLAWTQSETLR
2019.5970	2029.0602	0.44	0	QHEQYGELEAVVIDK
3112.2490	3301.7935	0.45	1	RNCNASLGRPRPLPAVIPNMPDLPR

differentiation expression; 39.3, 60.2 and 66.3 kD were the key proteins of sideway flower procedure expression, and 77.1 kD was the key protein of calyx procedure. Histochemistry study on floral bud differentiation in Fuji apple indicated that RNA in floral bud differentiation and bud meristem was correlated with protein synthesis ability. Strong ability of RNA and protein synthesis profited from penetrating development thus implemented in process expression in floral bud and vice versa. In this study, existing period of the specific proteins in floral bud in Fuji apple was definite. On this basis, these proteins could be isolated and purified further. Moreover, their structure and function could be further investigated whether biochemistry function was as that of structure proteins or as catalysis function. At the same time, additional proteins or regulating gene expression rejoining amynology method were investigated for their positioning study. Thus, we could understand whether specific rudimentum development differentiation procedure was expressed or degenerated lay in the appearance of some specific proteins.

Gene expression products appeared in floral bud differentiation process, which played an important role in closing or inhibiting some genes, and ensured normal inflorescence rudimentum differentiation. Due to the limitation of plant genomic and protein sequences databases, and the differences in primary structure of identical functional protein among species, the above identification results need to be further verified and the concrete role of these proteins in apple floral bud induction is still waiting for further study.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 30671455), the National High Technology Research and Development Program of China (863 Program) (No. 2006AA100108), Special Project of Social Commonweal Research of the Ministry of Science and Technology of China (No. 2005DIA4J047) and Special Foundation of President of the Chinese Academy of Agricultural Sciences (CAAS) (No. 2004017).

References

Anderson L, Seilhamer J (1997). A comparison of selected mRNA and protein abundances in human. Electrophoresis, 18: 533

Bevan M, Bancroft I, Bent E, et al. (1998). Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. Nature, 391: 485–488

Cao S Y, Tang Y Z, Jiang A H (2001). Effects of PP333 and GA_3 on the mechanism of flower bud induction in apple tree. Acta Horticulturae Sinica, 28: 339–341 (in Chinese)

Cao S Y, Zhang J M, Wei L H (2000). Studies on the changes of endogenous hormones in the differentiation period of flower bud in apple trees. Journal of Fruit Science, 17: 244–248 (in Chinese)

Cao S Y, Zhang Q M, Guo J Y, Chen Y L (2005). Progress in proteomics technology and its application on fruit tree. Journal of Fruit Science, 22: 345–350 (in Chinese)

Cao S Y, Zhang Q M, Wu S (2003). Advances in research on the mechanism of flower-bud differentiation of fruit trees. Journal of Fruit Science, 20: 345–350 (in Chinese)

Chen W, Huang C M, Lu L X (2001). The improved method of two-dimensional electrophoresis of protein in recalcitrant plant litchi. Journal of Fujian Agricultural University (Natural Science), 30: 123–126 (in Chinese)

Courchesne P L, Patterson S D (1999). Identification of proteins by matrix-assisted laserdesorption/ionization mass spectrometry using peptide and fragment ion masses. In: Link AJ ed. 2-D Proteome Analysis Protocols. New Jersey, USA: Humana Press, 487–511

Gygi S P, Bochon Y, Franza B R (1999). Correlation between protein and mRNA abundance in yeast. Mol Cell Biol, 19: 1720–1730

Leandro P, Martin T M, Juaraz G (2001). Constitutive expression of *Arabidopsis* LEAFY or APETALA1, genes in citrus reduces their generation time. Bio Nature, 19(3): 263–267

Meyerowitz E M (1998). Genetic and molecular mechanisms of pattern formation in *Arabidopsis* flower development. J Plant Res, 111: 233–242

Sung S K, An G (1997). Molecular cloning and characterization of a MADS-box Cdna clone of the Fuji apple. Plant Cell Physiol, 38: 484–489

Sung S K, Yu G H, An G (1999). Characterization of MdMADS2, a member of the SOUAMOSA subfamily of genes in apple. Plant Physiol, 120: 969–978

Sung S K, Yu G H, Nam J, Jeong D H, An G (2000). Developmentally regulated expression of two MADS-box genes, MdMADS3 and MdMADS4, in the morphogenesis of flower buds and fruits in apple. Planta, 210: 519–528

Watson B S, Asirvatham V S, Wang L J (2003). Mapping the proteome of barrel medic (Medicagotruncatula). Plant Physiol, 131: 1104–1123

Weigel D (1995). The genetics of flower development: from induction to ovule morphogenesis. Annu Rer Genet, 29: 19–39

Weigel D, Nilsson O (1995). A developmental switch sufficient for flower initiation in diverse plants. Nature, 377: 495–500

Wilkins V C, Williams S J, Cerpa P A (1995). Progress with gene-product mapping of the Mollicutes: *Mycoplasma genitalium*. Electrophoresis, 16: 1090–1094

Yao J L, Dong Y H, Morris B A M (2001). Parthenocarpic apple fruit production conferred by transposon insertion mutations in a MADS-box transcription factor. Proc Natl Acad Sci USA, 98(3): 1306–1311