For. Stud. China, 2008, 10(2): 88–94
DOI 10.1007/s11632-008-0016-8

RESEARCH ARTICLE

Identification of differentially expressed genes associated with bud dormancy release in tree peony (*Paeonia suffruticosa*) by suppression subtractive hybridization

HUANG Xin[1,2], ZHENG Guo-sheng[2*], DAI Si-lan[1*], GAI Shu-peng[2]

[1]College of Landscape Architecture, Beijing Forestry University, Beijing 100083, P. R. China
[2]College of Life Science, Qingdao Agricultural University, Qingdao 266109, P. R. China

Abstract A subtractive cDNA library was developed to study genes associated with bud dormancy release in tree peonies. In order to identify genes that are highly expressed in buds released from dormancy, 588 clones were examined by differential screening. Of these, 185 clones were selected to be sequenced. A total of 37 unique sequences were obtained of which only 31 sequences have matches in the NCBI database or the *Arabidopsis thaliana* protein database. Semi-quantitative RT-PCR was used to confirm further the expression profiles for 12 transcripts identified within the subtractive cDNA library. Gene ontology analyses indicated that many of the different genes identified have unknown or hypothetical functions while it is speculated that other genes play different molecular roles. In our study, genes involved in bud dormancy release were growth-related or stress-responsive, while low-temperature-induced ribosomal proteins may also play a role in bud dormancy release. Our results provide interesting information for further understanding of the molecular mechanism of bud dormancy release in tree peonies.

Key words cDNA clone, dormancy, subtractive hybridization, tree peony (*Paeonia suffruticosa*)

1 Introduction

The tree peony is one of the most popular landscape plants which moves through a continuous cycle of dormancy and growth. The mixed buds, which finish differentiation in July or August each year, requires a chilling period over winter before it begins to grow (Wang, 1986). It is this period that is considered to be dormancy in tree peonies. This kind of dormancy is referred to as endo-dormancy, which is controlled by internal bud signals and prevents untimely growth during seasonal transitions, when environmental conditions often fluctuate between those permissive or inhibitory to growth (Lang and Martin, 1987).

The signals that induce or break endo-dormancy are reasonably well characterized. Light and temperature both play significant roles in the induction and breaking of endo-dormancy. In deciduous trees, shortening day lengths will increase cold hardiness, cease cell division and induce dormancy in the terminal meristem (Li et al., 2003). Extended chilling or freezing are often required to break the endo-dormant state (Henzell et al., 1991; Anderson et al., 2005; Chao et al., 2006). As in many perennials, sufficient chilling breaks endo-dormancy in tree peonies. Endo-dormancy imposed growth arrest is one of the key characteristics that prevent tree peonies from flowering well (Zhao et al., 2000).

More recently, there has been an effort to characterize molecular changes directly as regulated during onset and breaking of endo-dormancy. Many genes that are perhaps associated with endo-dormancy have been isolated from grape (Toni et al., 2003), potato (Falvre-Rampant et al., 2004), birch (Welling et al., 2004) and other plants (Clark et al., 1996; Brunel et al., 2002; Faye et al., 2003). Most of these genes are related to oxidative processes, abscisic acid signals and stress responses within the cell. These results all indicate that even in the dormant state, gene expression in the buds is still high, but genes that are directly involved in the dormancy-related process have not yet been identified in tree peonies. In some plant species (e.g. poplar and potato), progress has been made to identify markers for quantitative trait loci (QTL) associated with dormancy in buds (Freyre et al., 1994; Frewen et al., 2000). However, QTL analysis is not suitable for tree peonies because of their poorly defined genetics and lack of linkage or genetic maps.

Suppression subtractive hybridization (SSH) technique allows comparison between two populations of mRNA and identifies genes that are differentially expressed in the two populations. This technique has been widely used to isolate a large number of differentially expressed genes (Diatchenko et al., 1996;

*Author for correspondence. E-mail: gs-zheng@hotmail.com, silandai@gmail.com

Sangeeta and Anil, 2005; Zhang et al., 2005; Jia et al., 2006). In order to understand the molecular mechanism of bud dormancy release in tree peonies we constructed, in our research, a subtractive cDNA library which contains genes highly expressed in buds released from dormancy by using SSH. From the subtractive cDNA library, 37 unique sequences were obtained. Many genes were related to growth regulation or were stress-responsive. This study will bring us closer to understanding the molecular mechanism of bud dormancy release in tree peonies.

2 Materials and methods

2.1 Plant materials

Mixed buds were collected from tree peonies (*Paeonia suffruticosa* 'Lu He Hong') every ten days during the period of bud dormancy release (from November 7, 2005 to January 17, 2006) in Qingdao, Shandong Province, China. About 30 mixed buds were collected each time. The anatomical changes of mixed buds were observed in order to ascertain the stages of dormancy by using about 10 buds each time. Buds at the dormant and breaking stages were used to isolate RNA for the establishment of subtractive cDNA libraries and confirming gene expressions by semi-quantitative RT-PCR.

2.2 Subtractive library construction, differential screening and sequencing

Total RNA was isolated from growing and dormant buds using standard CTAB extraction and lithium chloride precipitation (Chang et al., 1993). Suppression subtractive hybridization (SSH) (Diatchenko et al., 1996) was performed to create forward and reverse subtracted cDNA libraries using the PCR-Select cDNA Subtraction Kit (Clontech). The Poly A^+ RNA fractions from each plant sample were isolated using the Oligotex mRNA Purification Midi Kit (Qiagen). For the forward library, the "driver" cDNA was synthesized from the mRNA isolated from dormant buds and the "tester" cDNA was produced from mRNA isolated from the growing buds, which had been exposed to a chill treatment. The reverse subtraction was made by reversing tester and driver cDNAs, where the products were only used as probes in differential screening. Purified secondary PCR-amplified products (40 ng) of the forward library were cloned into the pMD-18-T vector (Promega). Originally, the forward library contained about 4,000 independent cDNA clones. About 1,000 recombinant colonies were randomly selected to confirm the quality and quantity of inserts by PCR using a forward (5′-TCGAGCGGCCGCCCGGGCAGGT-3′) and a reverse (5′-AGCGTGGTCGCGGCCGAGGT-3′) primer.

High quality PCR products, in large amounts (> 100 $\mu g \cdot \mu L^{-1}$) from 588 colonies, were selected for further differential screening with the PCR-Select Differential Screening Kit (Clontech) to identify genes that are highly expressed in the breaking buds. One microlitre of each denatured PCR product was spotted onto a Hybond N^+ membrane (Amersham Biosciences) in four duplicate replications. All the Hybond N^+ membranes were dried at room temperature overnight and stored at –20°C for future use. In order to normalize the expression of all the clones in dormancy release, the PCR products of the constitutively expressed genes (elongation factor) were also spotted onto each Hybond N^+ membranes and used as control. During differential screening, four probes were used: the forward- and reverse-subtracted probes, and the forward- and reverse-unsubtracted probes. The first two probes were made from the same subtracted cDNA used to generate the forward subtracted and reverse subtracted cDNA libraries. The forward- and reverse-unsubtracted probes were made from unsubtracted "tester" (growing buds) and "driver" (dormancy buds) cDNA. The four differential probes (about 25 ng for each) were all ^{32}P-labeled using the Primer-a-Gene Labeling System (Promega) and hybridized with the four duplicate membranes respectively. The results of arrays were scanned and recorded with a Packard Instantimager (Packard Instrument Co.).

Clones showed stronger (more than 4-fold) hybridization signals when forward-subtracted probes were used than when other probes were used, probably due to the differentially expressed genes in growing buds. All these clones were selected and sequenced at the Huada Gene Research Center (Beijing, China) using M13 universal primers. Contig and sequence analysis were carried out using the Lasergene 6.0 sequence analysis software (DNASTAR).

2.3 Semi-quantitative RT-PCR analysis

The expression levels of many cDNA clones selected by differential screening were further tested by semi-quantitative RT-PCR. Total RNAs isolated from dormant buds and growing buds were treated with DNaseI (Invitrogen), and then reverse transcriptions were performed using a SuperScript First-Strand Synthesis Kit (Invitrogen) to produce total cDNA. From each of these unique sequences, different annealing temperatures and cycles (less than 35) were examined to obtain a linear range of amplification before performing PCR with at least two sets of biological replicates. Elongation factors served as the internal controls at the same time. Different primers and PCR conditions were not shown. DNA bands on ethidium bromide stained gels were recorded using the BioRad Imaging System.

3 Results

3.1 Anatomical changes of mixed buds during bud breaking

In order to determine which developmental bud stage would be suitable for constructing the cDNA library, we observed anatomical bud changes during dormancy release. As shown in Fig. 1, the multi-petal flowers of 'Lu He Hong' are constituted of epigynous and hypogynous flowers which are differentiated from the same flower primordia. The center carpels of hypogynous flowers develop into the outboard petals of epigynous flowers. The morphological change of epigynous flowers can indicate the stages of dormancy release. We observed that the third stage (when epigynous flowers ceased to grow) was the time buds entered dormancy, while the sixth stage (when epigynous flowers resumed growth) was the time the buds burst.

3.2 Differential screening and sequencing

Differential screening results indicated that 185 out of 588 clones showed four-fold stronger hybridization signals when forward-subtracted probes were used, rather than other probes (Fig. 2). A total of 144 sequences were retained after removing low quality and vector sequences. Sequence analysis revealed that seven sequences (5%) were singletons. The other 137 sequences (95%) were assembled into 30 contigs, where each contig has 2–14 overlapping sequences. A putative mitochondrial phosphate transporter (MPT) appeared 14 times and a protein similar to an unknown protein (ABA46787) appeared ten times. Thus, from the original 144 sequences, a total of 37 unique sequences were obtained. These unique sequences have been submitted to GenBank with accession numbers EF607139, EF608929-43, EU072919-30, EU091152-54 and EU200687-92. All sequences can be accessed at the NCBI database (http://www.ncbi. nlm.nih.gov).

3.3 Blast searches of 37 unique sequences and classification of molecular function

Because of the methods used to develop the subtractive libraries, non-contiguous sequences could be produced from the same gene. These 37 sequences were in this way searched against the nucleotide or protein database of the NCBI and the *Arabidopsis thaliana* protein database at a cutoff e-value of 1e-05. The results showed that 31 sequences (84%) had one or more hits to 25 different genes. The remaining six sequences (16%) had no hits. Thus, there are 31 genes represented among the 37 clones, assuming that each of the six unmatched sequences represents an individual gene (Table 1).

For functional annotation, the top match at the *Arabidopsis thaliana* protein database was parsed and identifiers were used to search for gene ontology (GO) terms from the GO annotated *Arabidopsis* database of TAIR. The 25 matched sequences were manually categorized into eight molecular functional groups based on the GO Slim Classification for Plants developed at TAIR (htstage://www.arabidopsis.org/help/helppages/go_slim_help.jsp) while six unmatched sequences were classified into 'no matches' group. Of the annotated results generated, 11 clones (44%) were in a structural molecule activity group while the remaining clones were identified as belonging to other functional groups as follows: kinase activity (1 sequence, 4%), protein binding (2 sequences, 8%), other bindings (1 sequence, 4%), transporter activity (1 sequence, 4%), other enzyme activities (2 sequences, 8%), other molecular functions (1 sequence, 4%) and unknown molecular functions (6 sequences, 24%) (Fig. 3).

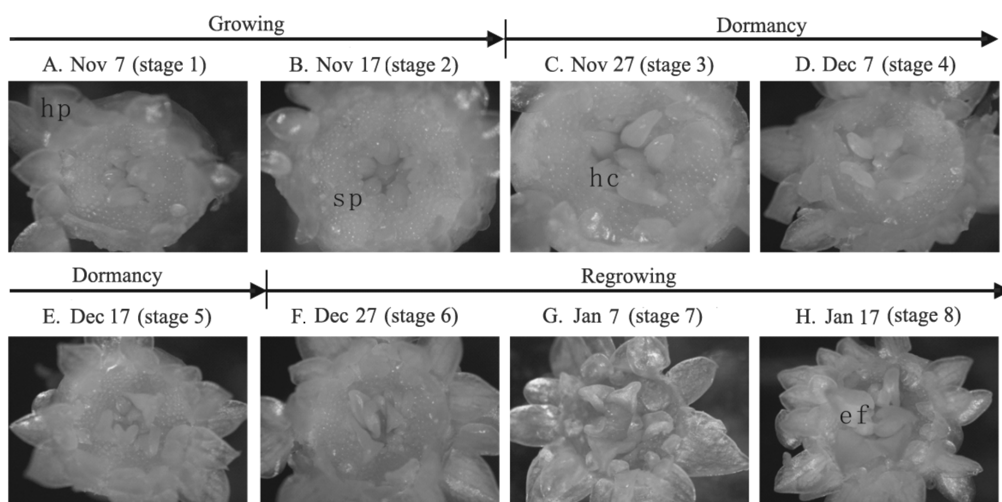

Fig. 1 Morphological change of mixed buds during dormancy release. Stage 3 (epigynous flowers cease growth) was considered as the time buds entered dormancy, while stage 6 (epigynous flowers resume growth) was the time of bud burst; hp: petals of hypogynous flowers; sp: stamen primordia; hc: carpels of hypogynous flowers; ef: epigynous flowers.

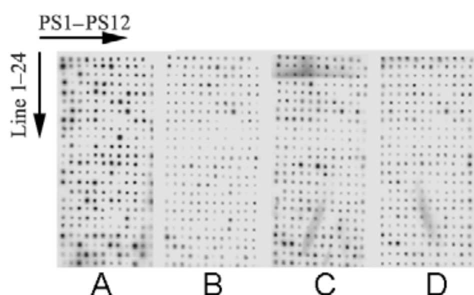

Fig. 2 Differential screening of a subtracted cDNA library from mixed buds of *Paeonia suffruticosa.* Four duplicate filter arrays of randomly selected clones from the library were hybridized with ^{32}P-labeled cDNA populations of 10^6 cpm·mL^{-1} activity made from (A) forward subtracted cDNA, (B) forward unsubtracted cDNA, (C) reverse subtracted cDNA and (D) reverse unsubtracted cDNA. Clones which have strong hybridization signals (more than four-fold) are representative of genes highly expressed in buds released from dormancy. The position of clones on the filter arrays is from left to right and from the top down. The clone at the top left corner is an elongation factor. There is only the hybridization result of 1–288 clones and the result of 289–588 clones is not shown.

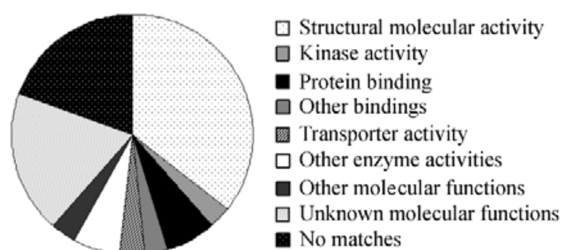

Fig. 3 Pie chart of molecular functional groups of candidate sequences in the subtractive cDNA library. The annotation results generated 11 clones (44%) in the structural molecular activity group, while the remaining clones were in other functional groups as follows: kinase activity (1 sequence, 4%), protein binding (2 sequences, 8%), other binding (1sequence, 4%), transporter activity (1sequence, 4%), other enzyme activity (2 sequences, 8%), other molecular functions (1 sequence, 4%) and unknown molecular functions (6 sequences, 24%).

3.4 Gene expression levels at dormancy and growing stages confirmed by semi-quantitative RT-PCR analysis

To confirm the differential screening results, semi-quantitative RT-PCR analyses were used to examine the expression levels of candidate genes at dormancy and growing stages. Among the 37 clones, 12 unique clones (PS18, PS43, PS53, PS60, PS68, PS125, PS138, PS142, PS199, PS527, PS557 and PS588) were selected on the basis of their putative molecular function. The RT-PCR analysis showed that PS60 and PS588 showed no detectable DNA bands while the other ten clones were detectable within 35 cycles. All the ten clones showed a higher expression level at stage 6 (growth stage) than at stage 3 (dormancy stage) (Fig. 4).

4 Discussion and conclusions

4.1 Differential screening, analytically sequenced and differentially expressed genes

Differential screening was used to identify the positive clones from the subtractive cDNA library. From 588 clones, 185 positive clones (about 31.5%) were selected for further research. It was reported that the ratio of the positive cDNA clones obtained by SSH ranged from 10% to 90% (Diatchenko et al., 1996). After screening 588 clones and sequencing 144 clones successively, we identified 37 unique sequences from the subtractive cDNA library. The results indicate that most clones were attributed to highly redundant cDNAs. High redundancy may be a result of the nature of the samples subtracted against each other or over-expression of these genes in the samples. Large-scale library screening, using the redundant clones as probes before sequencing, reduced highly redundant clones and was an effort-saving approach to obtain differentially regulated genes. RT-PCR analysis of clones further confirmed that the cDNAs from the subtractive library were almost represented for genes highly expressed in the "tester" and indicated that the subtractive hybridization between "tester" and "driver" was effective.

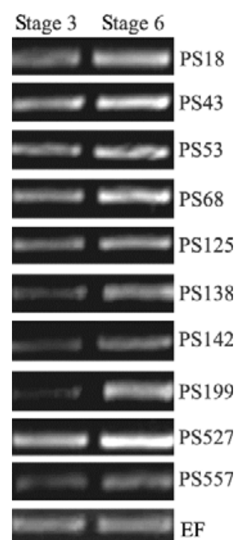

Fig. 4 The expression patterns of 12 clones (PS18, PS43, PS53, PS60, PS68, PS125, PS138, PS142, PS199, PS527, PS557, PS588) selected from the subtractive cDNA library at dormancy stage and growing stage by semi-quantitative RT-PCR. Total RNA was extracted from buds at stage 3 (dormancy stage) and stage 6 (growing stage). 10 genes appeared visible DNA bands within 35 cycles and PS60 and PS588 showed no detectable signal which was not shown. Elongation factor (EF) served as the internal control at the same time.

4.2 Gene expression at low levels

Blast searches revealed that 16% of the 37 unique sequences had no matches in the NCBI and *Arabidopsis thaliana* protein databases. The results indicate that many of the subtractive selected clones were representative of rare mRNA species. In fact, a high number of rare mRNA species may be reflected by the results of differential screening where radioactive signals in many hybridized clones were low. Moreover, in RT-PCR analysis, two sequences in 12 clones generated no detectable band within 35 cycles and designing new primer pairs did not improve the results. Secondary structures in the mRNA usually hamper PCR reactions, yet rare mRNA species can also be the cause of setbacks in PCR reactions. Thus, subtractive hybridization appeared to be a useful tool for isolating rare mRNA species that may be differentially regulated.

Table 1 Blast results in the NCBI database or the *Arabidopsis thaliana* protein database of 37 unique sequences and classification of their molecular functions

Clone ID	Accession number	Blast in NCBI homolog gene accession number (e-value)	Blast in TIGR/TAIR Hit ID (e-value)	Functional annotation
Structural molecule activities				
PS353	EF608934	ABA40437 (4e-06)	AT1G48830 (3e-06)	40S ribosomal protein S7 (RPS7A)
PS401*	EF608933	ABF93903 (4e-14)	AT1G57860 (4e-16)	60S ribosomal protein L21
PS11	EF608936	ABK63942 (4e-06)	AT1G61580 (3e-06)	Structural constituent of ribosome
PS446	EF608935	Q9M5M7 (3e-21)	AT1G66580 (5e-21)	60S ribosomal protein L10
PS85	EF608937	ABD96966 (2e-47)	AT3G02560 (1e-47)	40S ribosomal protein S7 (RPS7B)
PS390*	EF608943	EAZ25517 (6e-16)	AT3G04400 (5e-18)	Embryo defective related
PS7	EF608929	Q9ZRS8 (3e-35)	AT3G10950 (9e-36)	60S ribosomal protein L37a
PS21	EF608930	AAR83877 (2e-40)	AT3G16780 (1e-38)	60S ribosomal protein L19
PS248*	EF608932	Q9LE95 (8e-08)	AT3G63490 (7e-10)	Ribosomal protein L1 family protein
PS188	EF608931	ABE88548 (3e-14)	AT4G16720 (2e-15)	60S ribosomal protein L15
PS128	EF608942	Q9ZRB2 (3e-74)	AT5G12250 (3e-75)	TUB6
Kinase activity, transferase activity and receptor binding or activity				
PS527	EU072923	BAD32780 (5e-66)	AT1G71830 (1e-65)	Somatic embryogenesis receptor kinase 1
Protein binding				
PS138/ PS420	EU091152/ EU072929	ABL85046 (1e-05)	AT1G80420 (4e-07)	DNA repair protein, putative (XRCC1)
PS557*	EF608939	CAB71090 (6e-14)	AT3G61600 (5e-16)	POZ/BTB containing-protein1
Other bindings				
PS142/ PS75	EU072922/ EU072930	CAH59632 (3e-09)	AT5G14040 (1e-12)	Mitochondrial phosphate transporter
Transporter activity				
PS173	EU072927	CAN67115 (6e-10)	AT5G64500 (1e-05)	Membrane protein-related
Other enzyme activities				
PS60*	EF607139	AAT92092 (2e-46)	AT5G51810 (3e-77)	GA20 oxidase
PS420	EU072928	CAC33768 (5e-14)	AT5G43330 (7e-12)	Malate dehydrogenase
Other molecular functions				
PS18	EU072920	AAC78332 (2e-55)	AT4G01900 (6e-51)	PII protein/glutamine synthetase B1
Unknown molecular functions				
PS125	EF698938	ABA46787 (5e-37)	AT1G26750 (2e-22)	Expressed protein
PS68	EU072919	AAX84678 (7e-23)	AT1G28330 (5e-22)	Dormancy associated protein1
PS57/ PS126	EF608940/ EU072924	NP_564931 (1e-12)	AT1G68490 (4e-14)	Hypothetical protein
PS199	EU072921	ABB89007 (6e-23)	AT1G47480 (6e-22)	Unknown protein
PS127	EF608941	AAN77145 (4e-06)	AT1G02750 (5e-06)	Drought-responsive family protein
PS43/ PS44/ PS190/ PS311	EU091154/ EU072925/ EU072926/ EU091153	AAX92687 (1e-05)	AT3G50980 (1e-05)	Dehydrin

* represents the sequences which are singletons. Six other sequences identified by differential screening but unmatched in *Arabidopsis thaliana* protein database are: PS52 (EU200688), PS53* (EU200689), PS218 (EU200687), PS416 (EU200690), PS452 (EU200691) and PS588* (EU200692).

4.3 Genes involved in bud dormancy release: growth regulation or stress-responsive

Based on sequence information, the potential function of some unique sequences may be postulated. For example, the deduced amino acid sequence of PS68 was very similar to that of anxin-repressed protein (ARP) and that of PS527 to somatic embryogenesis receptor kinase (SERK1). The ARP of strawberry and *Elaeagnus umbellate* proved to be associated with fruit growth and root nodule formation respectively (Reddy, 1990; Kim et al., 2007) while SERK1 in *Arabidopsis thaliana* (AtSERK1) was implemented during early embryo development (Hecht et al., 2001), and function in a common signaling pathway employed in both sporophytic and gametophytic cells (Kwaaitaal et al., 2005). In our study, these two genes showed higher expression at the growth stage than at the dormancy stage, so we regarded these genes maybe promote bud dormancy release and involve in the development of floral organs.

On the other hand, low temperature as an environmental signal also induced the expression of some stress-responsive genes. PS43 cDNA encoded a dehydrin which was found to be associated with dormancy and/or seasonal changes in woody plants (Arora and Rowland, 1997). It is apparently triggered by low temperatures and abscisic acid (ABA), bound water, which leads to frost protection and simultaneous deepening of dormancy (Faust et al., 1997). Rorat (2006) also proved that dehydrins (DHNs) accumulated in responsive to stress (e.g. drought, low temperature and salinity) which leads to cellular dehydration. The expression of dehydrin was upregulated at dormancy release, so we consider that dehydrin is response to low temperature and not to accelerate bud dormancy. At the same time, we found a gene (PS199) similar to carboxylesterase (CXE) which is a large family of enzymes that hydrolyzes esters of short-chain fatty acids and expressed higher at dormancy release. It has been proven that this gene plays a role in plant-pathogen interactions (Sean et al., 2003) and may be involved in signaling pathways (Stuhlfelder et al., 2002). According to the expression levels of these stress-responsive genes, we speculate that they all play important roles in increasing the resistance to low temperatures which is needed in bud dormancy release.

4.4 Low-temperature induced ribosomal protein: involvement in bud breaking?

In our results, the sequences in structural molecule activity group were almost ribosomal protein. Kim et al. (2004) considered that induction of ribosomal protein genes might enhance the translation process or help proper ribosome functioning under low-temperature conditions and that ribosomal protein genes may be due to secondary signals during chilling treatment. Toorop et al. (2005) also found two ribosomal proteins which were associated with the germination of *Arabidopsis thaliana* seeds. The expression of the two genes were undetectable in dry seed, in low level dormant seed and in high levels under conditions that allowed completion of germination (Toorop et al., 2005). We thus presumed that all ribosomal proteins might be induced by low temperatures and played an important role in bud breaking.

In conclusion, we have identified and analyzed many genes of the tree peony during the period of bud dormancy release. These results provide evidence that suppression subtractive hybridization (SSH) may be of potential value for isolation of cDNAs expressed in the target tissues without any prior knowledge of the genes. The analyses of these genes indicate that although no outward signs are obvious, the expression of genes is high during dormancy. Many genes associated with bud dormancy release were growth-regulated or stress-responsive and ribosomal proteins may perhaps play important roles in dormancy release. Overall, our results open up new perspectives for clarifying the functional roles of various proteins and the mechanism of dormancy release in woody plants. Future research is ongoing to determine the regulation and function of these genes during bud dormancy release.

Acknowledgement

This study was supported by the Natural Science Foundation of Shangdong Province, China (Z2005D04).

References

Anderson J V, Gesch R W, Jia Y, Chao W S, Horvath D P. 2005. Seasonal shifts in dormancy status, carbohydrate metabolism, and related gene expression in crown buds of leafy spurge. Plant Cell Environ, 28: 1,567–1,578

Arora R, Rowland L J. 1997. Chilling-response dehydrins in blueberry: are they associated with cold hardiness or dormancy transition? Physiol Plant, 101: 8–16

Brunel N, Leduc N, Poupard P, Simoneau P, Mauqet J C, Viémont J D. 2002. *KNAP2*, a class I *KN1*-like gene is a negative marker of bud growth potential in apple trees (*Malus domestica* [L.] Borkh.). J Exp Bot, 53: 2,143–2,149

Chang S, Purgear J, Cairney J. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep, 11: 117–121

Chao W S, Serpe M D, Anderson J V, Gesch R W, Horvath D P. 2006. Sugars, hormones, and environment affect the dormancy status in underground adventitious buds of leafy spurge (*Euphorbia esula*). Weed Sci, 54: 59–68

Clark S E, Jacobsen S E, Levin J Z, Meyerowitz E M. 1996. The clavata and shoot meristemless loci competitively regulate meristem activity in *Arabidopsis*. Development, 122:

1,567–1,575

Diatchenko L, Lau Y F, Campbell A P, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov E D, Siebert P D. 1996. Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. PNAS, 93: 6,025–6,030

Falvre-Rampant O, Cardle L, Marshall D, Vlola R, Taylor M A. 2004. Changes in gene expression during meristem activation processes in *Solanum tuberosum* with a focus on the regulation of an auxin response factor gene. J Exp Bot, 55(397): 603–612

Faust M A, Erez L J, Rowland S, Wang Y, Norman H A . 1997. Bud dormancy in perennial fruit trees: Physiological basis for dormancy induction, maintenance and release. HortSci, 32: 623–629

Faye M R, Jennifer K H, Harry T H, Peter J D, David J H. 2003. Overexpression of a KNOTTED-like homeobox gene of potato alters vegetative development by decreasing gibberellin accumulation. Plant Physiol, 132: 106–117

Frewen B E, Chen T H, Howe G T, Davis J, Rohde A, Boerijan W, Bradshaw H D J. 2000. Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics, 154: 837–845

Freyre R, Warnke S, Sosinski B, Souches D S. 1994. Quantitative trait locus analysis of tuber dormancy in diploid potato (*Solanum* spp.). Theor Appl Genet, 89: 474–480

Hecht V, Vielle-Calzada J P, Hartog M V, Schmidt E D, Boutilier K, Grossniklaus U, de Vries S C. 2001. The *Arabidopsis* somatic embryogenesis receptor *kinase 1* gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol, 127: 803–816

Henzell R F, Briscoe M R, Gravett I. 1991. Improving kiwifruit vine productivity with plant growth regulators. Acta Hort, 297: 345–350

Jia Y, Anderson J V, Horvath D P, Gu Y Q, Lym R G, Chao W S. 2006. Subtractive cDNA libraries identify differentially expressed genes in dormant and growing buds of leafy spurge (*Euphorbia esula*). Plant Mol Biol, 61:329–344

Kim H B, Lee H, Oh C J, Lee N H, An C S. 2007. Expression of *EuNOD-ARP1* encoding auxin-repressed protein homolog is up regulated by auxin and localized to the fixation zone in root nodules of *Elaeagnus umbellate*. Mol Cells, 23:115–121

Kim K Y, Park S W, Chuang Y S, Chung C H, Kim J I, Lee J H. 2004. Molecular cloning of low-temperature-inducible ribosomal protein from soybean. J Exp Bot, 55: 1,153–1,155

Kwaaitaal M A C J, de Vries S C, Russinova E. 2005. *Arabidopsis thaliana* somatic embryogenesis receptor kinase1 protein is present in sporophytic and gametophytic cells and undergoes endocytosis. Protoplasma, 226: 55–65

Lang G A, Martin G C. 1987. Endo-, para-, and eco-dormancy: physiological terminology and classification for dormancy research. HortSci, 22: 371–377

Li C Y, Anneli V A, Tuula P, Olavi J, Pekka H, Palva E T. 2003. Ecotype-dependent control of growth. Dormancy and freezing tolerance under seasonal changes in *Betula pendula* Roth. Trees Struct Funct, 17: 127–132

Marshall S D, Putterill J J, Plummer K M, Newcomb R D. 2003. The carboxylesterase gene family from *Arabidopsis thaliana*. J Mol Evol, 57(5): 487–500

Reddy A S N. 1990. Molecular cloning and sequencing of a cDNA for an auxin-repressed mRNA: correlation between fruit growth and repression of the auxin-regulated gene. Plant Mol Biol, 14: 127–136

Rorat T. 2006. Plant dehydrins-tissue location, structure and function. Cell Mol Biol Lett, 11: 536–556

Sangeeta A, Anil G. 2005. Isolation and transcription profiling of low-O_2 stress-associated cDNA clones from the flooding-stress-tolerant FR13A rice genotype. Ann Bot, 96: 831–844

Sean D G M, Joanna J P, Kim M P, Richard D N. 2003. The carboxylesterase gene family from *Arabidopsis thaliana*. J Mol Evol, 57: 487–500

Stuhlfelder C, Lottspeich F, Mueller M J. 2002. Purification and partial amino acid sequences of an esterase from tomato. Phytochem, 60: 233–240

Toni P M, Kirsten S, Effie A, Scott T, Ada C, Robert H. 2003. Genes associated with the end of dormancy in grapes. Funct Integr Genom, 3: 144–152

Toorop P E, Barroco R M, Engler G, Groot S P, Hilhorst H W. 2005. Differentially expressed genes associated with dormancy or germination of *Arabidopsis thaliana* seeds. Planta, 221: 637–647

Wang L Y. 1986. Observations on the morphology of flower bud differentiation of cultivars of tree peony and the analysis on the formation of flower forms. Acta Hort Sin, 13: 203–208 (in Chinese)

Welling A, Rinne P, Annell V A, Sar K S, Heino P, Palva E P. 2004. Photoperiod and temperature differentially regulate the expression of two dehydrin genes during overwintering of birch. J Exp Bot, 55: 507–517

Zhang Y, Mian M A, Chekhovskiy K, So S, Kupfer D, Lai H, Roe B A. 2005. Differential gene expression in *Festuca* under heat stress conditions. J Exp Bot, 56: 897–907

Zhao H J, Zhang W T, Zheng G S, Wang Z Z. 2000. Characteristics on deep dormancy of *Paeonia suffruticosa* and ways of removing deep dormancy. Shandong For Sci Tech, 5: 44–46 (in Chinese)

(Received December 21, 2007 Accepted March 12, 2008)