

Identification of Differentially Expressed Genes during Initial Floral Development in *Oncidium Pseudobulb*

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ABSTRACT

We used suppression subtractive hybridization (SSH) and microarray techniques to isolate genes that increased abundantly in the pseudobulb of *Oncidium Gower Ramsey* during initial inflorescence development. A subtraction cDNA library was generated by using the cDNA from pseudobulbs in two different stages: the ones with initial inflorescence (2-3 cm) and the other with longer inflorescence (7-8 cm), and the former was subtracted against the latter. In this work, a total of 425 clones with differential expression were obtained. With microarray analysis, forty-five clones which have expression levels up regulated two-fold in the initial inflorescence stage were obtained, and they shared the average insert size of 328 bp. Thirty-one ESTs showed homology to the known genes. Eight out of 31 genes were related to stress responses, including peroxidase, mannose-binding lectin precursor, metallothionein, and RD22. It is interesting that the appearance of peroxidase gene related to flowering time. Ten genes were involved in regulation, including latex-like protein, dicarboxylate transporter, profilin, allantoinase, ADP-ribosylation factor, and heat shock protein. One gene was related to cell wall metabolism, which is GTP-binding protein Rac. One gene encoded a structural

protein, alpha-tubulin. Four genes were involved in protein synthesis and degradation, including ribosomal protein and ubiquitin. Seven genes were related to photosynthesis. Additionally, fourteen genes showed no homology with previously-reported sequences.

Key words: *Oncidium Gower Ramsey*, Suppression subtractive hybridization (SSH), Microarray, Flower initiation-associated genes, Pseudobulb.

花序發育初期於文心蘭假球莖中具差異性表現基因之選殖

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摘要

利用抑制扣除雜交與微陣列技術，選殖花序發育初期於文心蘭假球莖中特異表現的基因。利用花序發育初期(花序長2-3公分)的假球莖，扣減具較長花序(7-8公分)的假球莖，獲得425個差異表現基因片段。又經微陣列分析，其中45個基因片段在花序發育初期的表現高於另一個時期兩倍以上，而基因片段平均大小為328 bp。再經序列分析，其中31個基因片段與已知蛋白質序列相似，分別參與逆境反應、細胞生理調節、細胞壁新陳代謝、構造蛋白質及光合作用，其中也出現與開花時間相關的過氧化氫酶基因片段。但其餘14個基因片段並未與任何已知蛋白質序列相似。

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INTRODUCTION

Oncidium Gower Ramsey is an economically important cut flower orchid in Taiwan. It produces flowers through most of the year with a major peak in September-November and a minor peak in May-June. However, the cut flower with the highest price is produced in January-March, which is also the period of lowest productivity (Chou 2002). For this reason, it has prompted the research community to concentrate its efforts on regulation of the flowering time of *Oncidium*.

The pseudobulb is the storage organ of *Oncidium*, and it emerges from the stem between node one and zero. The dormant buds at nodes 1 and -2 often develop into flowering buds. During the plantlet stage, when the new vegetative bud is about 15 to 25 cm long, dormant flower buds appear at nodes 1 and -2. When the dormant flower bud is about 0.8 cm, it already has eight differentiated scales. Although an axillary bud is present at the base of the fifth scale, it has not yet differentiated into a flower primordium. In the unsheathing stage, pseudobulb unsheathing accompanies the differentiation of the flower bud, and the scale and lateral branch primordia were observed. At the end of unsheathing stage, when the pseudobulb was about 10 cm long, the dormant flower bud has developed into a new inflorescence. The unsheathing stage is believed to be a key stage in flowering initiation (Li 2002). Finally, the pseudobulb reaches about 10 cm long, and the inflorescence reaches about 100 cm long.

Many factors affect the flowering of orchids, such as plant age, the nutrient conditions, and the interaction between daylength and temperature. An *Oncidium* Gower Ramsey tissue culture seedling requires about one and a half year to two years to enter reproductive growth. An *Oncidium* Goldiana flowering adult must contain at least two back shoots (Yong and Hew 1995).

Oncidium is a day-neutral plant, *i.e.*, indifferent to daylength (Hew and Yong 1997). However, long day treatment (16 hours) before the appearance of inflorescence stimulates the flowering of *Oncidium* Aloha Iwanaga, while

short day treatment (10 hours) delays it (Shyu 1997). The flowering season of the *Oncidium* Goldiana has also been reported (Hew and Yong 1997). The period of high flower production is followed by a period of low flower production, which is in turn followed by a period of high flower production. The daylength effect on the floral initiation may be depletion of storage.

The effect of the day/night temperature on the flowering rate of *Oncidium* Gower Ramsey was investigated. *Oncidium* that grows at 25/20 and 20/15°C will reach a flowering rate of 100% , but those ones grow at 30/25°C do not (Li 2002). These results suggest that temperature only slightly affects the flowering rate. The flowering fluctuation is affected by both light and temperature, which affect the plants' vegetative growth and the timing of the development of new axillary buds.

Various other attempts have been made to implement known scientific approaches to induce the flowering of orchids, such as bud removal. In *Oncidium*, the developing inflorescence at a length of 5-10 cm could be removed to increase the flowering rate later (Lin 2002). In *Oncidium* Aloha Iwanaga, which has both upper and lower flower stalks, removal of upper flower stalk increases the quality of the lower flower stalk and brings forward flowering. The removal of the flower stalk at about 10 cm stimulates the production by the lower axillary bud of a second flower bud and delays the flowering time by three to four months. Removing the flower stalk when it is about 15 cm long reduces the depletion of nutrients in the pseudobulb, and stimulates new vegetative bud development. The new flower stalk flowering rate reached 100%, and the flowering time is delayed by six to seven months (Shyu 1997). These approaches are still in the experimental stage, and its application to industry still needs evaluation.

Carbon source is believed to be an important factor in the initiation of flowering. The carbon source includes photosynthesis products and storage organ. The pseudobulb is the storage organ of *Oncidium* (Yong and Hew 1995), and an *Oncidium* Goldiana flowering adult must at least have two back shoots (Yong and Hew 1995). We posit an interesting relationship between the nutritious state of the pseudobulb and the initiation of flowering. Therefore, SSH coupled

with microarray approach was applied to identify genes that have differentially expression level during the initial floral development.

MATERIALS AND METHODS

Plant Materials

According to Chang and Lee (1999), the unsheathing stage is the critical stage to determine the plant's flowering. And the major developmental stage of stalk node is when it is below 10 cm in length. In this work, we selected pseudobulbs in the early (2-3 cm) and the late (7-8 cm) stages of stalk node development to analyze the differential expression genes in the pseudobulbs.

Oncidium Gower Ramsey was obtained from a commercial grower's field, Shih-Dong Orchid Yard. Pseudobulbs (including node 1 to node-2) in two flowering stages were used. Pseudobulbs with initial inflorescence (2-3 cm) were used for tester mRNA extraction, and pseudobulbs with longer inflorescence (7-8 cm) were used for driver mRNA extraction. Pseudobulbs were collected, immediately frozen in liquid nitrogen, and stored at -70°C for RNA isolation.

RNA Isolation

Total RNA was prepared by modifying the method, described by Chang *et al.* (1993). About 5 g of grounded tissues were dissolved in 1.5 ml of extraction buffer (2% hexadecyltrimethylammonium bromide, 2% polyvinylpyrrolidone K30, 25mM EDTA, 2M NaCl, 0.05% spermidine, and 2% β -mercaptoethanol). It was centrifuged for 10 min at 12,000 g after standing at 65°C for 10 min, and the supernatant was collected. RNA was then extracted twice using the same volume of chloroform: isoamyl alcohol (24:1), and centrifuged for 10 min at 12000 g. The supernatant was collected. Following overnight precipitation in 2 M LiCl at 4°C, RNA was cleaned with 75% ethanol and dried briefly. Finally, it was dissolved in DEPC-H₂O, and stored at -70°C until needed. Poly (A)⁺ RNA was isolated from total RNA with the Oligotex mRNA kit (Qiagen, Valencia, CA) following the instructions of the manufacturer's protocol.

Subtraction cDNA Library Construction

The subtractive cDNA library was constructed from 3 μ g of poly (A)⁺ RNA isolated from the tester and the driver with a PCR-select cDNA subtraction kit (Clontech, Palo Alto, CA) following to the manufacturer's protocol. Subtracted cDNAs were ligated into the pGEM-T Easy Vector (Promega, Madison, WI), and then were transformed into *Escherichia coli* DH5 α competent cells. Putative transformants were checked for the presence of the inserted cDNA by colony PCR.

Microarray

Preparation of *Oncidium* cDNA microarray: cDNA inserts were amplified from colonies by PCR. The 100 μ l reaction mixture contained 1X reaction buffer mixed with 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 μ M of each primer, 1U Taq DNA polymerase and 3 μ l bacterial culture template. PCR condition was 94°C for 5 min; 30 cycles of (94°C for 45s, 46°C for 30s and 72°C for 50s); and 72°C for 10 min. PCR products were visualized on 1.5% agarose gel to inspect the quality and quantity of PCR products. Then, the PCR products were dissolved in 50% DMSO printing buffer and spotted on glass slides. Each PCR product was spotted three times on each slide.

Probe preparation: Total RNA was extracted from tester and driver *Oncidium* pseudobulbs. Poly (A)⁺ RNA was isolated from total RNA using the Oligotex mRNA kit (Qiagen). Poly (A)⁺ RNA was visualized on 1% agarose/formaldehyde gels to inspect the quality. Two micrograms of mRNA of each stage were reverse-transcribed and labeled with cyanine 3 (Cy3). The microarray experiments were performed twice and the average gene expression was determined by the following formula. Finally, the normalized intensity ($m_{ij} L^{-1}$) was calculated as:

$$I = \sqrt{\frac{R}{C} \sum_{j=1}^C m_{ij}^2}$$

where m_{ij} is the adjusted intensity, *i.e.*, i is the number of rows and j is the number of columns; R is the total number of rows; and C is the total number of columns. The gene expression difference between pseudobulbs with initial and longer inflorescence was then calculated.

Microarray data acquisition and analysis: Genes that were expressed in the tester more than

twice as strong than in the driver were cloned, sequenced, and underwent BLAST analysis.

RESULTS

Construction of *Oncidium* SSH Library

Pseudobulbs with initial inflorescence (2-3 cm) and with longer inflorescence (7-8 cm) were used for subtractive library construction, and the former was subtracted against the latter. The total RNA yield was about 45 $\mu\text{g g}^{-1}$ of fresh tissue. The ratio of poly (A)⁺ RNA to total RNA was about 1.2%. Three μg of poly (A)⁺ RNA from each stage was used to construct the SSH library. After the second nest PCR, the products were cloned into pGEM-T Easy Vector and transformed into DH5 α *Escherichia coli* competent cells. The transformants were identified by blue/white color selection, and a total of 2,400 white colonies were obtained. Nineteen colonies were randomly picked and examined using colony PCR. The PCR products were visualized by agarose gel electrophoresis and 90% of the white colonies contained inserts from 400 bp to 900 bp in length. The differential expressions of the SSH library in various stages were verified again using a DNA microarray.

DNA Microarray

All of the SSH library colonies were examined with colony PCR. Four hundreds and twenty-five colonies produced PCR products with high quality and quantity. Finally, 318 PCR products were spotted on the glass slides. Each PCR product was spotted three times on a slide to confirm the consistency between spots. Each SSH library was spotted on two glass slides to check the consistency between slides. The *rca* (ribulose-1, 5-biophosphate carbohydrate/oxygenase activase) gene from rice was also spotted on the slide as an internal control between slides. After the poly (A)⁺ RNA in different stages were reverse-transcribed and labeled with Cy3, and each one was separately hybridized with SSH library microarray chip (Fig. 1). The hybridization image was scanned, and the fluorescence intensity between the slide duplicates was normalized by the *rca* gene. The normalized inflorescence intensity data was compared. The expression of 45 genes was doubled in the initial inflorescence stage and these cDNAs were cloned, sequenced, and searched for sequence homology using the BLAST program.

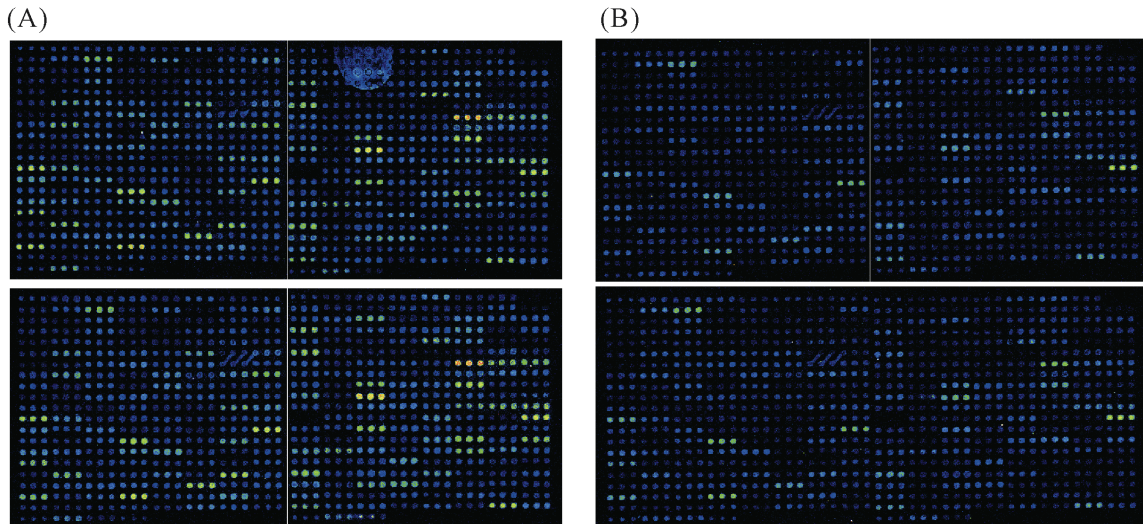


Fig. 1. Microarray image. The PCR products amplified from the subtractive library were arrayed induplicate on a microarray, and hybridized with Cy3-labeled probe of different inflorescence developmental stage: (A) 2-3 cm and (B) 7-8 cm. The brighter and darker spots indicate the higher and the lower expressions of those genes. Only those spots whose intensity ratios ≥ 2 were considered to be differentially expressed.

The average insert size of the 45 clones was 328 bp. Sequence analysis indicated that 31 ESTs were homologous with known proteins (Table 1), whereas 11 ESTs contained novel sequences. Eight out of 31 genes were related to stress responses, including peroxidase, mannose-binding lectin precursor, metallothionein, and RD22. Ten genes were involved in regulation, including latex-like protein, dicarboxylate transporter, profilin, allantoinase,

ADP-ribosylation factor, and heat shock protein. One gene was related to cell wall metabolism, which is GTP-binding protein Rac. One gene encoded a structural protein, alpha-tubulin. Four genes were involved in protein synthesis and degradation, including ribosomal protein and ubiquitin. Seven genes were related to photosynthesis. Additionally, fourteen genes showed no homology with previously-reported sequences (Fig. 2).

Table 1. Identification of genes differentially expressed in pseudobulb in initial inflorescence.

Clone	Sequence homology	Accession number	Sequence identity (%)	E value
Stress responses				
1f11	<i>Hevea brasiliensis</i> glutathione peroxidase	AAL55674	84	1e-08
1g06	<i>Nicotiana tabacum</i> secretory peroxidase	AAD33072	89	1e-20
3b02	<i>Gossypium hirsutum</i> peroxidase	AAA99868	71	2e-45
1f08	<i>Cymbidium hybrid</i> mannose-binding lectin precursor	S43463	54	1e-20
2d12	<i>Cymbidium hybrid</i> mannose-binding lectin precursor	S43464	54	6e-17
2g02	<i>Eichhornia crassipes</i> metallothionein	CAB53391	62	2e-13
3a03	<i>Eichhornia crassipes</i> metallothionein	CAB53392	45	2e-07
4a10	<i>Prunus persica</i> dehydration-responsive protein RD22	AAL26909	60	6e-16
Regulation				
2a12	<i>Arabidopsis thaliana</i> major latex-like protein	CAC83600	53	4e-10
3c01	<i>Arabidopsis thaliana</i> major latex-like protein	CAC83600	55	2e-10
1g10	<i>Arabidopsis thaliana</i> sodium sulfate or dicarboxylate transporter	BAA96091	77	4e-36
2f07	<i>Arachis hypogaea</i> profilin	AAD55587	80	4e-15
3c07	<i>Ctenocephalides felis</i> allantoinase	AAN72834	40	7e-14
3e02	<i>Glycine max</i> ADP-ribosylation factor	AAD17207	100	2e-15
1a11	<i>Oryza sativa</i> heat shock protein cognate 70	AAO65876	92	2e-41
3e12	<i>Euphorbia esula</i> dnaJ protein	AAF64454	65	1e-21
4b08	<i>Euphorbia esula</i> dnaJ protein	AAF64454	65	3e-18
4b01	<i>Pisum sativum</i> dnaK-type molecular chaperone	S44168	56	2e-15
Cell wall metabolism				
4e08	<i>Gossypium hirsutum</i> GTP-binding protein Rac 13	S57325	89	2e-22
Structural proteins				
2h09	<i>Hordeum vulgare</i> subsp. <i>valgare</i> alpha-tubulin 1	CAA67942	97	8e-43
Protein synthesis & degradation				
1g11	<i>Oryza sativa</i> 40S ribosomal protein S7	AAN04468	73	3e-38
3d06	<i>Oryza sativa</i> 40S ribosomal protein S7	AAN04469	72	2e-39
3h07	<i>Brassica rapa</i> ubiquitin/ribosomal protein	CAA80863	98	1e-37
4b05	<i>Drosophila melanogaster</i> ubiquitin S2	1212243D	52	1e-10
Photosynthesis				
2c04	<i>Oryza sativa</i> chlorophyll a/b-binding protein	T02125	81	9e-39
3c06	<i>Oryza sativa</i> chlorophyll a/b-binding protein	T02125	80	2e-27
4a03	<i>Pisum sativum</i> chlorophyll a/b-binding protein AB96	CDPM96	68	2e-28
2g11	<i>Pisum sativum</i> chlorophyll a/b-binding protein AB96	CDPM96	64	3e-11
3a08	<i>Lycopersicon esculentum</i> chlorophyll a/b-binding protein type I precursor	S16294	53	3e-19
3h01	<i>Hordeum vulgare</i> photosystem I chain IV precursor	F1BH4	88	1e-16
3b10	<i>Ginkgo biloba</i> light-harvesting complex b type 2, Lhcb2	AAB34067	95	5e-07

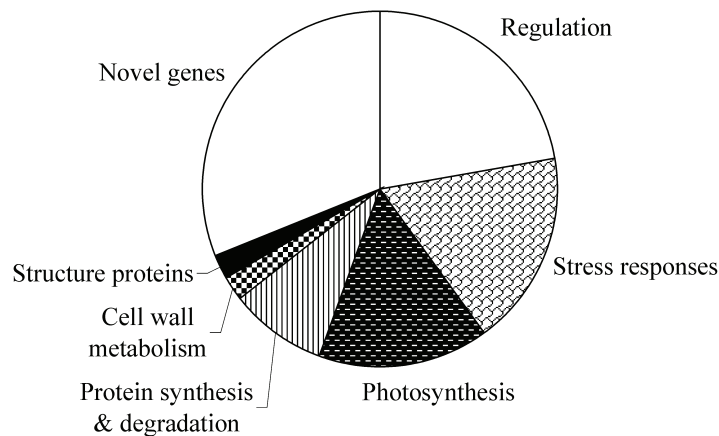


Fig. 2. Functional classification of 45 clones from the subtractive library. cDNA clones were classified according to their putative function. Novel genes: those with no hits to known sequences.

DISCUSSION

The sequences isolated from pseudobulbs with differential expression share a wide range of functions. The peroxidase superfamily consists of various groups of enzyme, including catalase, and animal and plant peroxidase. Glutathione peroxidase (GPX), belonging to the animal peroxidase family, can catalyze the reduction of hydrogen peroxidase, organic hydroperoxidases, and lipid hydroperoxidases, by reduced glutathione, and thereby protects cells against oxidative damage (Chaudiere and Tappel 1983). Although plant cDNAs that encode GPX-like proteins have been isolated from various plants, such as *Arabidopsis thaliana* and *Oryza sativa*, little is known of the function of GPX homologs in plants. In *Arabidopsis thaliana*, *AtGPX* genes are ubiquitous and regulated by abiotic stresses (Rodriguez *et al.* 2003). In *Oryza sativa*, *OsGPX1* is stress-inducible and protects cells against both metabolic and environmental oxidative stresses (Kang *et al.* 2004).

Peroxidase (class III peroxidases), belonging to the plant peroxidase family, can catalyze the reduction of hydrogen peroxidase by transferring electrons to various donor molecules such as phenolic compounds, lignin precursor, auxin or other secondary metabolites. Peroxidases participate in a wide range of physiological processes, such as lignification, suberisation, auxin catabolism, cross-linking of cell wall proteins, defense against pathogen attack, salt

tolerance, and oxidative stress (Hiraga *et al.* 2001, Penel *et al.* 1992). Peroxidases also participate in flowering. Transgenic tobacco plants that underexpress peroxidase flower sooner and those ones overexpress peroxidase flower later (Lagrimini *et al.* 1997).

In *Arabidopsis*, before flowering, the activity of ascorbate peroxidase tAPX shows a significantly negative correlation with flowering time (Lokhande *et al.* 2003), and reactive oxygen species (ROS) increases are associated with the developmental transition to flowering, perhaps due to programmed decline in APX activity (Ye *et al.* 2000).

As aforementioned, the peroxidases are both referred to ascorbate peroxidase, which is belonged to class I peroxidase. But according to the cDNA, *OPOX1*, cloned from pseudobulb by using a 3b02 cDNA as probe, the deduced amino acid sequence showed a sequence homology (E-value of $1e-15$) with the conserved domain of ascorbate peroxidase.

The higher expression of 1f11, 1g06 and 3b02 ESTs, which are homologous to glutathione peroxidase, secretory peroxidase, and peroxidase, respectively, in the initial inflorescence stage may indicate higher oxidative stresses in this stage. Besides oxidative stresses, the function of 3b02 EST may also be involved in flowering time. Another similar experiment, conducted in our laboratory (Tan *et al.* 2005), also identified genes homologous to plant peroxidases. By subtracting

*Rsa*I-digested cDNAs of leaf from those of pseudobulbs, 1,080 subtractive ESTs were obtained, including 126 ESTs homologous to peroxidases (class III peroxidases), and five ESTs homologous to secretory peroxidase. It is worthy to discover why pseudobulbs require so many peroxidase in this stage and whether these enzymes do involved in the flowering time.

Metallothioneins (MTs) are the low-molecular-weight, cystein-rich proteins with the capacity to coordinate metal atoms and are widely distributed throughout the animal and plant kingdom. The up-regulation of metallothionein genes during fruit or seed development has been reported for many plants, such as bananas (Clendennen and May 1997), apples (Reid and Ross 1997), and rice (Zhou *et al.* 2005). Metallothionein genes mRNA expression is especially abundant in developing pineapples (50%) (Moyle *et al.* 2005) and mature citrus fruits (21%) (Moriguchi *et al.* 1998). In *Quercus suber*, the expression of *QsMT* gene is mainly observed under conditions related to oxidative stress, for example, in response to H₂O₂ or paraquat treatments. One link between the reported up-regulation of metallothionein during the development of fruit, and their abundance in developing pineapples and mature citrus fruit may be oxidative stress (Moyle *et al.* 2005). Based on these results, it hinted that the higher expression of metallothionein in the pseudobulb of initial inflorescence may be involved in oxidative stress.

The dicarboxylate transporter DCAT1 can carry dicarboxylatic acids and phosphate. In alder (*Alnus glutinosa*), AgDCAT1 is a nodule-specific dicarboxylate transporter, which can transport malate, succinate, fumarate, and oxaloacetate. AgDCAT1 has been suggested to supply intracellular bacteria with dicarboxylates as carbon source (Jeeyon *et al.* 2004). The higher expression of the dicarboxylate transporter in the pseudobulb with initial inflorescence may be related to greater carbon input.

Heat shock proteins (HSPs) are molecular chaperones that participate in various normal cellular processes. The most studied of the HSPs have been concentrated Hsp70 family, which has several members, *i.e.*, hsc70 and hsp70. The hsc70 genes are expressed in the absence of heat stress

(Lindquist and Craig 1988) and participate in protein folding, assembly and disassembly, as well as transport and signal transduction (Hartl 1996). In the mung bean, the amount of hsc70 species declines while new hsc70s species appear after germination, suggesting that the expression of hsc70 is governed by developmental regulation (Wang and Lin 1993).

Hsp70 genes are heat-inducible and include DnaK and DnaJ. DnaK, the Hsp70 molecular chaperon system in *Escherichia coli*, is induced by various environmental stresses and believed to be involved in the protection and recovery of cells from the ill effects of stress, non-native protein binding, and refolding of these denature proteins. It was found that DnaJ can stimulate the chaperon activity of DnaK (Johnson *et al.* 1989).

Profilins (PRF) are small proteins, can bind to the actin monomer, and they are ubiquitous in eukaryotic cells. Profilins can cause actin polymerization (Hopmann and Miller 2003) and depolymerization (Bubb *et al.* 2003). In *Arabidopsis*, PRF1 transcripts are strongly expressed in all vegetative tissues during various stages of development (Muthugapatti *et al.* 2002). A transgenic *Arabidopsis*, underexpressing profilin PFN-1, exhibited an overall dwarf phenotype and flowered early. It strongly indicated that profiling did affect the flowering time in *Arabidopsis* (Ramachandran *et al.* 2000).

ADP-ribosylation factors, a family of small GTP-binding proteins, are involved in intracellular trafficking, the maintenance of endoplasmic reticulum morphology (Lee *et al.* 2002), and epidermal cell polarity (Jian and Ben 2005).

Allantoinase catalyzes the hydrolysis of allantoin to allantoic acid in ureide metabolism. The activity of allantoinase increases in the cotyledon of legumes and soybean during development (Tonin and Sodek 1990). The higher expression of allantoinase in the pseudobulb during the initial inflorescence may indicate that allantoinase was involved in the use of nitrogen from other sources.

In animals, the GTP-binding protein Rac participates in the signal transduction pathway that regulates the organization of the actin cytoskeleton. In cotton, the gene *Rac13* exhibited enhanced expression in developing cotton fibers,

with maximal expression at the time of transition between primary and secondary wall syntheses, at which time the cytoskeleton is reorganized (Delmer *et al.* 1995). The higher expression of GTP-binding protein Rac in the pseudobulb with initial inflorescence may reveal that it is involved in the signal transduction pathway that controls cytoskeletal organization.

Ubiquitin/26S proteasome contributes significantly to development by participating in a wide range of processes, including embryogenesis, hormone signaling, and senescence. In *Arabidopsis thaliana*, over 1,400 genes (ca. 5% of the proteome) encode components of the ubiquitin /26S proteasome pathway (Jennifer *et al.* 2004).

Latex is thick milky or clear juice or emulsion of diverse composition that is found in the plants such as rubber trees and spurge. It is a stress-related protein and shares the anthelmintic activity.

Plant lectins are found in many flowering plants and defined as proteins, contain at least one of non-catalytic domains that bind reversibly with a specific mono- or oligosaccharide (Peumans and Van Damme 1995). One of these, monocot mannose-binding lectins are preferentially bind to 1-3- or 1-6- linked D-mannoses with the highest affinity (Van Damme *et al.* 1988), and are described as an anti-fungal stress protein. For instance, gastrodianin, a mannose-binding lectin from orchid *Gastrodia elata*, exhibits strong anti-fungal activity toward various fungi (Xu *et al.* 1998). *Dendrobium officinale* agglutinin 2 (DOA2), a mannose-binding lectin from orchid *Dendrobium officinale*, exhibits anti-fungal activities toward *Gibberella zeae* (Chen *et al.* 2005).

Microtubule arrays are always constructed by the polymers of α - and β -tubulin heterodimers. The eukaryotic microtubule cytoskeleton participates in various processes, such as cell division, intracellular transport, cell shape maintenance, and cell mobility.

The *rd22* gene responds to the dehydration and is effectively mediated by ABA. It may have physiological and molecular importance in the processes that underlies the memory functions of plants in response to ABA and light pulses (Goh *et al.* 2003).

This is the first report about the identification of differentially expressed genes during the initial

inflorescence in *Oncidium* pseudobulb. Northern blot analysis and transgenic manipulation should provide a more complete picture of the genes that participate in the initial inflorescence development and their interaction.

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