Journal of Plant Biology, September 2000, 43(3) : 171-178

Expressed Sequence Tags of Young Floral Buds and Characterization of a Bud-Preferential Lectin-Like cDNA from *Pharbitis nil*

Soo-Jin Kim and Seong-Ryong Kim*
Department of Life Science, Sogang University, Seoul 121-742, Korea

To identify the genes involved in flower development, we analyzed 207 expressed sequence tags (ESTs) from a young floral bud cDNA library of *Pharbitis nil*. Of these, 87 clones (42%) showed significant homology to known protein sequences in the NCBI database. Four of these had not been reported previously in the plant kingdom, indicating that 1.9% of the ESTs were newly identified in plants. Functional categorization revealed that the genes involved in metabolic pathways, such as glycolysis and photosynthesis, were most abundant. Reverse-northern and northern analyses showed that one clone, *PnFP161*, was expressed preferentially in floral buds. DNA sequence analysis indicated that this clone contained 147 bp of 5'-UTR, 264 bp of 3'-UTR, and an open reading frame of 233 amino acids, thereby sharing 33% identity with a lectin from *Calystegia sepium*. The C-terminal region of *PnFP161* had well-conserved residue with that of the lectins. Southern blot analysis demonstrated that *PnFP161* exists as a multigene family.

Keywords: cDNA, EST, floral bud, lectin, *Pharbitis nil*

The initiation of flowering, i.e., the conversion of shoot apical meristems from vegetative to reproductive development, is a critical event in the life cycle of higher plants. Morning glory (*Pharbitis nil* Choisy cv Violet) is ideal for studying the early events in photoperiodic induction of flowering because young, light-grown seedlings can be induced to flower by exposing them to a single dark period of 16 h (Vinceprue and Gressel, 1985). RNA synthesis has been examined in the shoot apex following floral evocation by monitoring ^{3}H-uridine incorporation into the plumule RNA (Gressel et al., 1970, 1978). Although the molecular and physiological processes that occur at the meristem in response to the arrival of the floral stimulus are important, flower formation itself in morning glory is not well known.

During flower formation, new genes are expressed that are not active in the vegetative stage (Drews and Goldberg, 1998). Recently, *PnFP21*, encoding a non-photosynthetic ferredoxin, was isolated from apical buds of *P. nil* by differential screening under various photoperiodic conditions. The level of *PnFP21* transcript in apical buds was down-regulated under the short-day condition; therefore *PnFP21* is probably involved in the process of flower induction (Yoshizaki et al., 2000).

Expressed sequence tag (EST) analysis of emerging floral buds is used to identify the genes expressed during the early stage of flower formation. ESTs are short sequences, a few hundred base pairs long, that are derived by partial, single-pass sequencing of randomly selected cDNA clones. This type of analysis is an efficient way to gather information about an organisms genome. For example, as part of the human genome project, Adams et al. (1991) advocated that the method of sequencing random cDNA clones as being efficient in both its speed and cost. More than 111,745 ESTs from *Arabidopsis thaliana* and 60,237 ESTs from rice have been deposited in databases. About 40% of the *Arabidopsis* ESTs have homology to known genes in the database. The ESTs also can be used for comprehensive integration of expressed genes and for physical mapping of the genome (Rounsley et al., 1996).

In the present study, EST analysis was coupled with a differential screening by reverse-northern blot to characterize the preferential expression of a cDNA clone, *PnFP161*, in floral buds of *P. nil*.

MATERIALS AND METHODS

Plant Samples and Bacterial Strains

Seeds of *P. nil* Choisy cv Violet (purchased from Marutane Co., Kyoto, Japan) were soaked in concentrated sulfuric acid (H_2SO_4) for 30 min, then rinsed in running tap water for 20 h and sown for germination. Conditions in the plant growth chamber

*Corresponding author; fax +82-2-704-3601
e-mail sungkim@ccs.sogang.ac.kr

were set at 26 ± 1°C, with illumination by continuous fluorescent light (6.5 ~ 8 Watts m^{-2}). When the cotyledons had opened maximally (6 d after the H_2SO_4 treatment), the seedlings were subjected to photoperiods of 8 h light and 16 h dark for 3 days. Afterward, the seedlings were grown under continuous light for 8 days at 26°C. The emerging young floral buds (<2 mm long) that were produced in the fourth or fifth node were then harvested.

Escherichia coli strain XL-1 Blue MRF' [(F' Tn*10*, *proAB*, *lacI* ^{q}*Z* Δ*M15*) Δ(*mcrA*)183, Δ(*mcrCB-hsdSMR-mrr*) *recA1*, *endA1*, *gyrA96* (NalR), *thi-1*, *hsdR17* (rk^- mk^+), *supE44*, *relA1*, *lac*] served as a host for the molecular clonings. The ExAssist helper phage (M13) was used for in-vivo excision of the pBluescript plasmid vector from the lambda ZAP II phage (Stratagene, USA).

Construction of cDNA Library

Total RNAs were isolated by following the methods of Davis et al. (1986) and Wang and Vodkin (1994). To isolate poly $(A)^+$ RNA, an oligo dT-cellulose spun column (Pharmacia) was used. Approximately 5 μg of poly $(A)^+$ RNA served as a template, with oligo dT as the primer. After the cDNAs were synthesized, they were ligated into the Uni-ZAP XR vector (Stratagene), then in-vitro-packaged using the Gigapack II Gold packaging extracts (Stratagene).

Analysis of EST and DNA Sequence Analysis

For EST analysis, we sequenced the 5' ends of cDNA clones randomly selected from the bud cDNA library of *P. nil*. DNA preparation, sequencing, and computer analysis were performed, with a minor modification, according to Hong et al. (1998). Briefly, template DNAs were prepared via the alkaline lysis method, using the Wizard SV column (Promega). The inserts were sequenced with an *ABI PRISM™ BigDye™ Terminator Cycle Sequencing* kit (Perkim Elmer). Computer software, i.e., DNAsis, Prosis (Hitachi), ClustalX, ClustalW, and GeneDoc (Nicholas and Nicholas, 1997; Thompson et al., 1997), were used for sequence analysis. The Genbank, EMBL, and Swiss-Prot databases were then searched for amino acid sequence homology, using the BLASTX algorithm (Altschul et al., 1990).

Reverse-Northern Analysis

Slot-blots on a Hybond-N membrane (Amersham) were prepared in duplicate with 100 ng of plasmid DNA. Prehybridization was performed for 3 h at 65°C in 5X Denhardt's solution, 5X SSC, 0.5% SDS, and 4 mg mL^{-1} of denatured salmon sperm DNA. Hybridization was conducted for 16 h at 65°C. The hybridization probes were synthesized for 1 h at 37°C by reverse transcription of 5 μg of total RNA, using 50 μCi [α-^{32}P]dCTP (Amersham), 0.5 μg of oligo dT_{21} primer, 10 μM dNTP, 30 units of RNAsin (Promega), and 20 units of MMLV reverse transcriptase (Gibco-BRL).

Southern and Northern Blot Analysis

Genomic DNA was extracted from young leaves by the cethyltrimethylammonium bromide methods (Rogers and Bendich, 1988). Ten micrograms of DNA, digested with restriction enzymes (20 unit $μg^{-1}$ DNA) for 6 h at 37°C, were separated on a 0.8% agarose gel, and transferred to a Hybond-N membrane, using a vacuum transfer system (Hoefer). For northern blot analysis, 10 μg of total RNA were resolved on a 1.3% formaldehyde agarose gel and blotted onto a nylon membrane (Sambrook et al., 1989).

DNA and RNA blot analyses were performed using the radiolabeled *PnFP161* probe. The membrane was washed with 2X SSC, 0.1% SDS at RT for 15 min; then 1X SSC, 0.1% SDS at RT for 15 min; and 0.1X SSC, 0.1% SDS at RT for 15 min. Hybridization signals were detected with a BAS-1500 image analyzer (Fuji) and exposed on Agfa RP1 film. DNA fragments for hybridization were purified by electro-elution and radioactively labeled using [α-^{32}P] dCTP (3000 ci $mmole^{-1}$), following the random priming method described by Feinberg and Vogelstein (1983). Unincorporated nucleotides were removed through G-50 Sephadex column chromatography.

RESULTS

Construction of cDNA Library

A cDNA library was generated from floral buds emerging after photoinduction in order to isolate the genes involved in flower formation. The initial plaque forming unit (pfu) was 4.0×10^5. Insertion efficiency, analyzed by either X-gal selection or restriction analysis, was >98%. To determine the average size of cDNA inserts, the recombinant phages were converted *en masse* to pBluescript plasmids. Restriction analysis showed an average insert size of 0.8 kb (data not shown).

Characterization of Floral Bud ESTs

To identify genes expressed in the floral buds, 207 ESTs were generated by single-pass sequencing. All the sequences were automatically translated in the six open reading frames. They were compared with the protein sequence database in GenBank, using the subroutine BLASTX of Gapped Basic Local Alignment Search Tool (Gapped BLAST). Out of the 207 tags, 87 (42%) carried cDNAs with significant homology to

Table 1. Short-day induced young floral-bud ESTs of *P. nil* putatively identified by the database (DB) search.

Clone	Putative identification	Species	L C[a]	% id[b]	Acc. No.[c]	DB[d]
Metabolism						
PnFP17	Triseophosphate isomerase	*Petunia x hybrida*	133	54	P48495	SP
PnFP21	Vacuolar ATP synthase subunit	*Arabidopsis thaliana*	92	93	P11574	SP
PnFP51	Indole-3-glycerol phosphate synthase	*Arabidopsis thaliana*	129	82	AC006951	GB
PnFP82	PSII protein psbK precursor	*Spinacia oleracea*	88	81	I410212	PRF
PnFP84	endo-1,4-β-D-glucanase	*Lycopersicon esculentum*	94	76	Y11268	EMBL
PnFP85	Glyceraldehyde-3-phosphate dehydrogenase	*Nicotiana tabacum*	130	78	P09094	SP
PnFP95	Ferredoxin precursor	*Citrus sinensis*	88	55	S62722	PIR
PnFP98	Chloroplast aminopeptidase precursor	*Solanum tuberosum*	64	82	CAA48038	EMBL
PnFP111	LLR protein	*Lycopersicon esculentum*	179	73	X95269	EMBL
PnFP112	Bundle sheath defective protein 2	*Zea mays*	67	58	AF126742	GB
PnFP118	Cystatin	*Castanea sativa*	91	63	AJ224331	EMBL
PnFP119	Thioredoxin-like protein	*Arabidopsis thaliana*	99	63	AC007658	GB
PnFP123	Uridine monophosphate synthase	*Nicotiana tabacum*	94	82	Q42942	SP
PnFP126	Neutral peroxidase	*Ipomoea batatas*	109	91	Z84473	EMBL
PnFP129	Oxygen evolving protein of PS II	*Solanum tuberosum*	120	84	CAA67696	EMBL
PnFP137	Adenosyl-methionine-sterol-methyltransferase	*Nicotiana tabacum*	102	66	U71107	GB
PnFP140	Water-oxidizing complex of PSII	*Nicotiana tabacum*	132	85	X64349	EMBL
PnFP170	Aminopeptidase-like protein	*Arabidopsis thaliana*	92	73	CAB16823	EMBL
PnFP171	PSI subunit III precursor	*Arabidopsis thaliana*	162	44	CAB52747	EMBL
PnFP172	Allene oxide synthase precursor	*Linum usitatissimum*	97	41	P48417	SP
PnFP184	S-adenosylmethionine synthetase	*Lycopersicon esculentum*	106	94	P43282	SP
PnFP186	LHC a/b-binding protein	*Nicotiana sylvestris*	134	91	BAA25392	DDBJ
PnFP190	NADH-ubiquinone-oxidoreductase precursor	*Solanum tuberosum*	113	66	Q43844	SP
PnFP200	Ubiquitin/ribosomal protein CEP52	*Nicotiana sylvestris*	102	84	P42027	SP
PnFP201	14-3-3-like protein 16R	*Solanum tuberosum*	135	96	P93785	SP
PnFP202	Cyclophilin	*Solanum tuberosum*	121	78	AAD22975	GB
Transcription/Translation						
PnFP4*	Translational inhibitor protein P14.5	*Homo sapiens*	122	52	P52758	SP
PnFP5	Ribosomal protein S27	*Arabidopsis thaliana*	86	82	AF111029	GB
PnFP7	40S ribosomal protein S18	*Arabidopsis thaliana*	144	91	P34788	SP
PnFP10	Initiation factor 5A-1	*Nicotiana tabacum*	88	98	P24921	SP
PnFP11	mRNA for sigma-like factor	*Arabidopsis thaliana*	64	98	Y18550	EMBL
PnFP12	Ribosomal protein S26	*Pisum sativum*	73	72	AF112440	GB
PnFP16	Ribosomal protein L12	*Arabidopsis thaliana*	76	93	AC006260	GB
PnFP32	40S ribosomal protein S16	*Gossypium hirsutum*	129	81	P46293	GB
PnFP34	DNA-directed RNA polymerase	*Arabidopsis thaliana*	396	34	AL049481	EMBL
PnFP38	30S ribosomal protein S1(CS1)	*Spinacia oleracea*	111	83	P29344	SP
PnFP42	60S ribosomal protein L27A	*Arabidopsis thaliana*	68	79	P49637	SP
PnFP48*	snRNP D3 polypeptide	*Homo sapiens*	103	60	P94333	SP
PnFP50	50S ribosomal protein L27	*Nicotiana tabacum*	106	81	M75731	GB
PnFP61	Release factor 1	*Arabidopsis thaliana*	118	78	P35614	SP
PnFP70	30S ribosomal protein S17	*Arabidopsis thaliana*	90	55	P16180	SP
PnFP87	Transcription factor	*Nicotiana tabaum*	94	80	Y09109	EMBL
PnFP106	40S ribosomal protein S15	*Arabidopsis thaliana*	100	54	Q08112	SP
PnFP110	40S ribosomal protein S17	*Lycopersicon esculentum*	120	85	AC007018	GB
PnFP143	40S ribosomal protein S7	*Secale cereale*	114	80	AF118149	GB
PnFP151	Putative DNA binding protein	*Arabidopsis thaliana*	131	60	CAB36546	EMBL
PnFP176*	Cleavage polyadenylation specific subunit	*Xenopus lavis*	102	40	AAD33061	GB
PnFP196	Chloroplast ribosomal protein L10	*Nicotiana tabacum*	69	79	BAA31511	DDBJ

Table 1. Continued

Clone	Putative identification	Species	L C[a]	% id[b]	Acc. No.[c]	DB[d]
Stress/Resistance						
PnFP13	Disease-resistance response protein	*Arabidopsis thaliana*	86	44	AC006264	GB
PnFP19	Ascorbate peroxidase	*Cucumis sativus*	124	83	D88649	DDBJ
PnFP20	FK506-binding protein	*Arabidopsis thaliana*	114	83	U57838	PIR
PnFP43	Proline-rich APG-like protein	*Arabidopsis thaliana*	55	69	CAA22974	EMBL
PnFP46	Major latex protein homolog	*Mesembryanthemum crystallinum*	108	37	AAC14179	GB
Signal Transduction						
PnFP6	AHP1	*Arabidopsis thaliana*	145	71	BAA36335	DDBJ
PnFP35	Receptor protein kinase	*Arabidopsis thaliana*	69	52	AL030978	EMBL
PnFP49	Calmodulin-related protein	*Petunia hybrida*	106	100	M80831	GB
PnFP64	Casein kinase, alpha chain II	*Zea mays*	144	70	X61387	EMBL
PnFP67	Receptor-like Ser/Thr kinase	*Sorghum bicolor*	52	71	Y14600	EMBL
PnFP99	RAB1-like small GTP-binding protein	*Lotus japonicus*	118	98	X97853	EMBL
PnFP115	Phytochrome-associated protein 1	*Arabidopsis thaliana*	154	52	AF088281	GB
Others						
PnFP3	Ccr protein	*Citrus x paradisi*	127	55	S52663	PIR
PnFP9	Phosphate-induced gene-1	*Nicotiana tabacum*	177	81	BAA33810	DDBJ
PnFP15	Auxin-repressed protein	*Fragaria x ananassa*	51	64	X52429	EMBL
PnFP27	Actin	*Solanum tuberosum*	138	70	P30173	SP
PnFP33	Histone H2B	*Capsicum annuum*	84	95	AC007184	GB
PnFP60	RNase LX	*Lycopersicon esculentum*	107	71	X79338	EMBL
PnFP62	Dormancy-associated protein	*Arabidopsis thaliana*	106	51	AF053746	GB
PnFP66	ERECTA	*Arabidopsis thaliana*	135	77	D83257	DDBJ
PnFP68	Aquaporin	*Oryza sativa*	61	87	AJ224327	EMBL
PnFP72	Cop1	*Lycoperscon esculentum*	88	95	AF02984	GB
PnFP76	Hydroxyproline-rich glycoprotein	*Arabidopsis thaliana*	92	43	AJ224327	EMBL
PnFP79*	Ring-box protein 1	*Homo sapiens*	90	94	AC006284	GB
PnFP96	Tubulin	*Oryza sativa*	81	97	X91806	EMBL
PnFP109	Histone H2A	*Lycopersicon esculentum*	96	73	P25469	SP
PnFP121	Patatin-like protein	*Arabidopsis thaliana*	124	36	AC004697	GB
PnFP149	RNA helicase	*Arabidopsis thaliana*	106	65	CAA09199	EMBL
PnFP180	α-tubulin	*Hordeum vulgare*	105	90	CAA10663	EMBL
PnFP182	TOM20	*Solanum tuberosum*	107	71	CAA63223	EMBL
PnFP207	Translocon-associated protein	*Arabidopsis thaliana*	83	49	P45434	SP

*Indicates non-plant matched EST clones. **a,** the number of amino acid residues between a query and its matched protein sequence. **b,** percentage identity at the peptide level. **c,** accession number of the matched sequences. **d,** databases: **SP,** SwissProt. **PIR,** Protein Identification Resource Data Bank. **GB**, GenBank.

previously identified genes in databases. Among these cDNAs, four tags had not previously been identified in the plant kingdom. This indicated that 1.9% of the ESTs were new in plants. Of the remaining ESTs, 27 (13%) shared homology with genes from the *Solanaceae* family, 27 (13%) with *Brassicaceae*, and 5 (2.4%) with *Poaceae*. The eight ESTs having >90% identity over a 50-nucleotide stretch were considered to be redundant clones. Table 1 presents 79 ESTs, grouped by expected function. Genes involved in metabolic pathways such as glycolysis and photosynthesis were most abundant; genes for transcription and translation ranked next in abundance. Six resistance- or stress-related genes, such as for ascorbate peroxidase, also were identified.

Identification and Sequence Analysis of a Floral Bud-Preferential Lectin Gene, *PnFP161*

Reverse-northern analysis for 94 randomly chosen ESTs was performed to identify those that were floral bud-preferential. Among those hybridized with either floral-bud mRNA or leaf mRNA, *PnFP161* showed a differential hybridization pattern (data not shown). DNA sequence analysis of both strands of *PnFP161* was conducted (Fig. 1). The *PnFP161* cDNA was 1113 bp long and contained an open reading frame of 233 amino acids. This clone had 147 bp of 5′-UTR and 264 bp of 3′-UTR, with a poly(A) tail. The calculated molecular mass of deduced PnFP161 was 25.1 kDa, with a predicted pI value of 8.40.

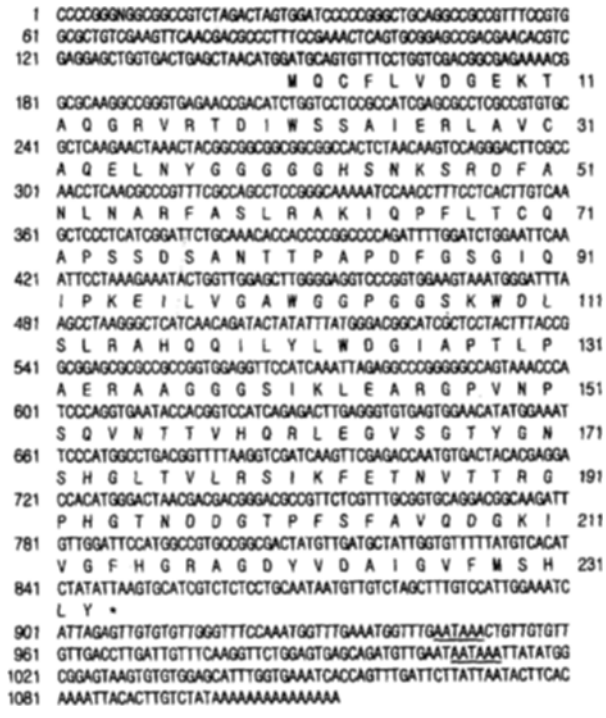

Figure 1. Nucleotide and deduced amino-acid sequence of *PnFP161*. The position of nucleotides is shown on the left and the position of amino acids on the right. Two putative polyadenylation sites are underlined.

Although the *PnFP161* EST clone did not show any significant sequence homology in the databases, the full-length PnFP161 ORF shared homology with lectin proteins, as it existed in the C-terminal region. Figure 2 shows the alignment of the PnFP161 sequence with lectins or putative lectins. PnFP161 shared an amino acid identity of 33% with the sequence of Calsepa, a mitogenic mannose/maltose-specific lectin protein from *Calystegia sepium* (van Damme et al., 1996). PnFP161 also had >30% homology with both jacalin from *Artocarpus integrifolia* (Yang and Czapla, 1993) and MPA from *Maclura pomifera* (Young et al., 1991). Overall homology between the different lectins was about 30%, and was significant in the C-terminal regions. As with Calsepa, no signal peptide could be traced in the deduced sequence of PnFP161.

Northern Blot Analysis of *PnFP161*

RNA blot analysis was conducted to study the expression pattern of *PnFP161* (Fig. 3). *PnFP161* was highly expressed in young floral buds, as had been expected from the reverse-northern analysis. Transcript size was about 1.1 kb, which corresponded to the size of the *PnFP161* cDNA. The mRNA was also

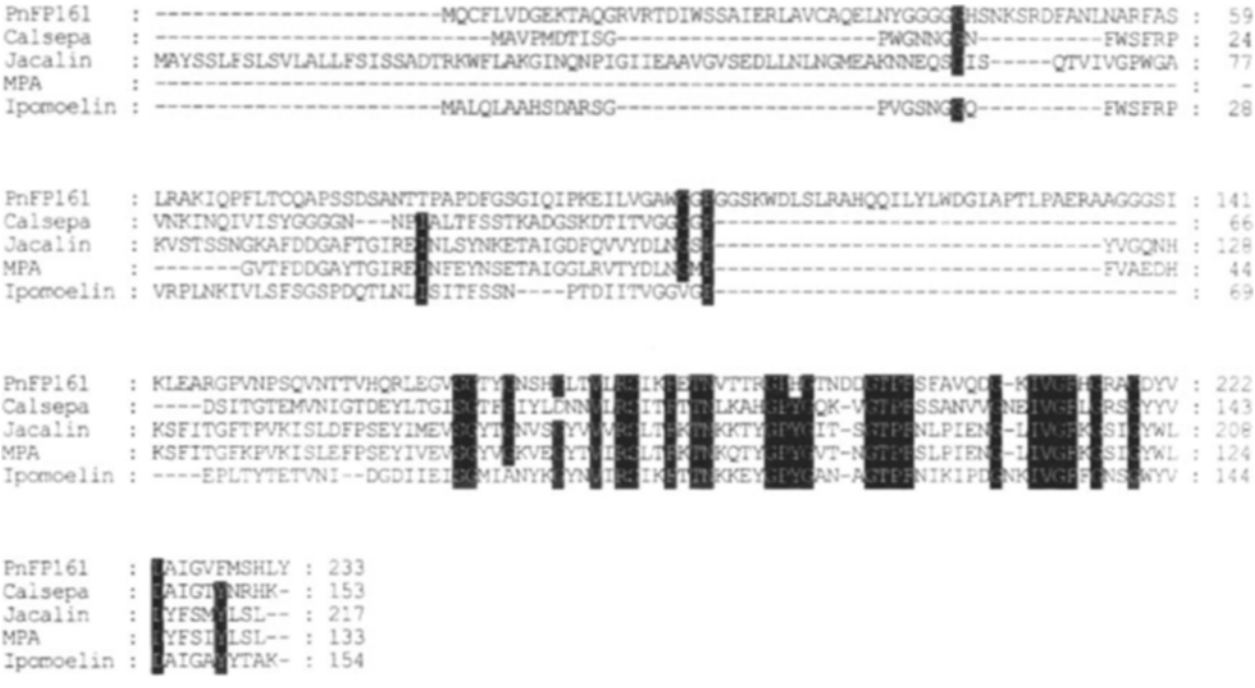

Figure 2. Comparison of the PnFP161 protein with Calsepa (van Damme et al., 1996), jacalin (Yang and Czapla, 1993), MPA (Young et al., 1991), and ipomoelin (Imanishi et al., 1997) lectins. The alignment was made using ClustalW (Thompson et al., 1997) and displayed using GeneDoc (Nicholas and Nicholas, 1997). White letters in a black box indicate 4 out of 5 matches or better.

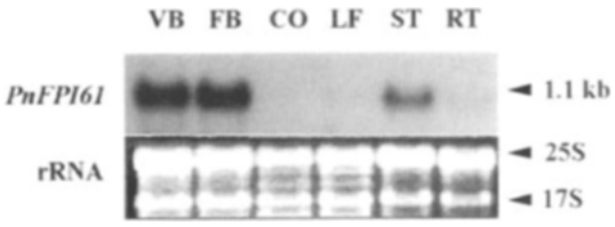

Figure 3. Northern blot analysis of *PnFP161*. Ten micrograms of total RNA from vegetative buds (VB), young floral buds (FB), cotyledons (CO), stems (ST), leaves (LF), and roots (RT) were separated on an agarose gel, blotted onto a nylon membrane, and hybridized with the radiolabeled *PnFP161* probe. EtBr-stained rRNA bands indicate an equal amount of loading for total RNA.

highly present in vegetative buds formed under continuous light. mRNA was less abundant in stems, minimal in leaves and roots, and absent in cotyledons. This indicated that *PnFP161* was a bud-preferential gene, which had not been demonstrated in the other lectin genes.

Southern Blot Analysis of *PnFP161*

DNA gel blot analysis was performed to determine the number of lectin genes present in the genome of *P. nil*. Ten micrograms of genomic DNA were digested with EcoRI, BamHI, and HindIII, then hybridized with the radiolabeled *PnFP161* probe. More than five bands hybridized to the probe (Fig. 4). Because the restriction enzymes used in this study had no target sites within the *PnFP161* cDNA, the results of the Southern analysis indicated that the morning glory genome contained several copies of genes encoding the lectin proteins or related proteins. This result was similar to that found in other plant species.

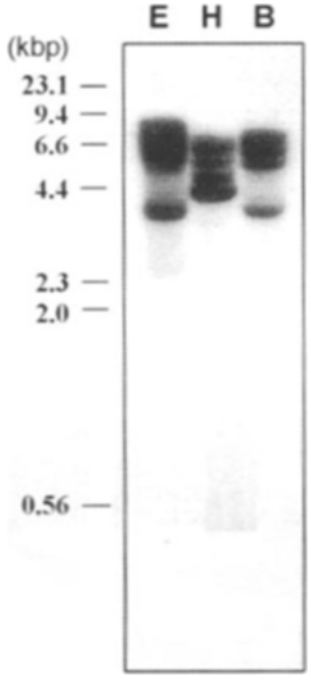

Figure 4. Southern blot analysis of *PnFP161*. Ten micrograms of total genomic DNA cut with EcoRI, HindIII, and BamHI were electrophoresed, blotted onto a nylon membrane, and hybridized with the radiolabeled *PnFP161* probe. Positions and sizes in kbp of HindIII-digested lambda DNA fragments are indicated.

DISCUSSION

We characterized 207 ESTs from the floral buds of *P. nil*. To our knowledge, this was the first EST analysis performed in this species, thereby providing information on the floral development of this short-day plant. The genes involved in metabolic pathways were most abundant in the floral buds; genes involved in transcription and translation ranked next in abundance. This pattern had also been observed with the floral buds of Chinese cabbage (Lim et al., 1996).

Several redundant clones appeared twice, including the thioredoxin-like protein (PnFP119), water-oxidizing complex of Photosystem II (PnFP140), light-harvesting complex a/b-binding protein (PnFP186), cyclophilin (PnFP202), ribosomal protein S26 (PnFP12), DNA-directed RNA polymerase (PnFP34), 40S ribosomal protein S7 (PnFP143), and proline-rich APG-like protein (PnFP124). Various ribosomal protein genes were especially abundant in the floral buds, suggesting that these bud cells were metabolically active. This observation is consistent with previous EST data from Arabidopsis (Höfte et al., 1993) and Chinese cabbage (Lim et al., 1996). Many of the database-matched ESTs were similar to known housekeeping genes, including the thioredoxin-like protein, adenosyl-methionin-sterol-methyltransferase, actin, tubulin, and histone H2 protein.

Five defense- or stress-related genes also were identified, indicating that floral buds respond actively to various environmental stresses such as moisture, temperature, and pathogens. PnFP66, with 77% identity to ERECTA over a 104 amino acid overlap, should have an important role in flower development because ERECTA confers inflorescence development (Yokoyama et al., 1998). Another EST, *PnFP3*, showed 55% identity with the citrus *Ccr* transcripts that display circadian rhythm (Abied and Holland, 1994).

Further characterization is needed to determine whether *PnFP3* is also involved in the circadian rhythm of morning glory and has a role in flower development.

Reverse-northern analysis of 94 ESTs used mRNAs from young floral buds and leaves. This technique has been advocated as an efficient and timesaving methodology for obtaining differentially expressed genes (Zegzouti et al., 1997). Northern analysis showed that the floral bud-preferential clone, *PnFP161*, was also highly abundant in vegetative buds, thereby indicating that this clone is preferentially expressed in both vegetative and reproductive buds.

The deduced amino acid sequence of PnFP161 has shown more than 30% sequence homology with lectins from a *Convolvulaceae* species *C. sepium* (hedge bindweed), and two *Moraceae* species, *A. integrifolia* (jackfruit) and *M. pomifera* (Osage orange). PnFP161 also has sequence homology with ipomoelin, a putative sweet potato (*Ipomoea batatas*) lectin protein (Imanishi et al., 1997). Ipomoelin, which is induced by wounding, can have insecticidal activity. It would be interesting to examine whether PnFP161 has a similar property because both *P. nil* and *I. batatas* belong to the same family, *Convolvulaceae*.

Lectins are carbohydrate-specific, cell-agglutinating proteins found widely in plants, animals, and microorganisms (Etzler, 1985). Because their carbohydrate specificities are so varied, they have been useful reagents for studying carbohydrate moieties of glycoproteins on cell surfaces (Lis and Sharon, 1986). Tyr, Trp, and His residues are involved in the binding of sugars (Manhanta et al., 1992). Whether these residues in PnFP161 have similar sugar-binding activities awaits further characterization. Because no signal peptide was present in PnFP161, the putative lectin in *P. nil* may be a cytoplasmic protein, as is seen in *C. sepium* (van Damme et al., 1996). Carbohydrate specificity of PnFP161 is unknown. The role of lectin has not been documented in bud development, so further characterization would be necessary.

ACKNOWLDGEMENTS

We thank Mi-Yeon Lee for helping with the EST analysis and Ju-Seon Maeng for helpful discussions. This work was supported by Korea Research Foundation Grant (KRF-98-019-D00068).

Received September 5, 2000; accepted September 19, 2000.

LITERATURE CITED

Abied MA, Holland D (1994) Two newly isolated genes from citrus exhibit a different pattern of diurnal expression and light response. Plant Mol Biol 26: 165-173

Adams MD, Kelly JM, Gocayne JD, Dubnick M, Polymeropoilos MH, Xiao H, Merril CR, Wu A, Olde B, Kerlavage AR, McCombieand WR, Venter JC (1991) Complementary DNA sequencing: Expressed sequence tags and human genome project. Science 252: 1651-1656

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403-411

Davis LG, Dibner MD, Batty JF (1986) Basic Methods in Molecular Biology. Elsevier, New York

Drews GN, Goldberg RB (1998) Genetic control of flower development. Trends Genet 5: 256-261

Etzler ME (1985) Plant lectins: Molecular and biological aspects. Annu Rev Plant Physiol 36: 209-334

Feinberg A, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6-13

Gressel J, Silberstein A, Arzee T (1970) Bursts of incorporation into RNA and ribonuclease activities associated with induction of morphogenesis in *Pharbitis*. Develop Biology 22: 31-42

Gressel J, Zilberstein A, Straubauch L, Arzee T (1978) Photoinduction of Pharbitis flowering: Relationship of RNA synthesis and other metabolic events. Photochem Photobiol. 27: 237-240

Höfte H, Desprez T, Amselem J, Chiapello H, Caboche M, Moisan A, Jourjon MF, Charpenteau JL, Berthomjeu P, Guerrier D, Giraudat J, Quigley F, Thomas F, Yu DY, Mache R, Ranynal M, Cooke R, Grellet F, Delsenu M, Parmentier Y, Matcjillac GD, Gigot C, Fleck J, Philipps G, Axelos M, Bardet C, Tremousaygue D, Lescure B (1993) An inventory of expressed sequence tags obtained by partial sequencing of cDNAs from *Arabidopsis thaliana*. Plant J 4:1051-1061

Hong ST, Chung JE, An G, Kim SR (1998) Analysis of 176 expressed sequence tags generated from cDNA clones of hot pepper by single-pass sequencing. J Plant Biol 41: 116-124

Imanishi S, Nakamura KK, Matsuoka K, Morikami A, Nakamura K (1997) A major jasmonate-inducible protein of sweet potato, ipomoelin, is an ABA-independent wound-inducible protein. Plant Cell Physiol 38: 643-652

Lim CO, Kim HY, Kim MG, Lee SI, Chung WS, Park SH, Hwang I, Cho MJ (1996) Expressed sequence tags of Chinese cabbage flower bud cDNA. Plant Physiol 111: 577-588

Lis H, Sharon N (1986) Biosynthesis and action of jasmonates in plants. Annu Rev Biochem. 55: 35-67

Manhanta SK, Sanker S, Rao NVSAVP, Swamy MJ, Surolia A (1992) Primary structure of Thomsen-Friedenreich-antigen-specific lectin, jacalin [*Artocarpus integrifolia*

(jack fruit) agglutinin]. Biochem J 284: 95-101

Nicholas KB, Nicholas HB (1997) GeneDoc: Analysis and visualization of genetic variation. Http://www.cris.com/~Ketchup/genedoc.html

Rogers SO, Bendich AJ (1988) Extraction of DNA from plant tissues, *In* SB Gilson, RA Schilperoort, eds, Plant Molecular Biology Manual, Kluwer Academic Publishers, Dordrecht, A6: 1-10

Rounsley SD, Glodek A, Sutton G, Adams MD, Somerville CR, Venter JC, Kerlavage AR (1996) The construction of *Arabidopsis* expressed sequence tag assemblies. Plant Physiol 112: 1177-1181

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-4882

van Damme EJM, Barre A, Verhaert P, Rouge P, Peumans WJ (1996) Molecular cloning of the mitogenic mannose/maltose-specific rhizome lectin from *Calystegia sepium*. FEBS Lett 18: 352-356

Vince-prue D, Gressel J (1985) *Pharbitis nil*, *In* AH Halevy, ed, Handbook of Flowering, Vol IV. CRC Press, Boca Raton, FL, 47-88

Wang CS, Vodkin LO (1994) Extraction of RNA from tissues containing high levels of procyanidins that bind RNA. Plant Mol Biol 12: 132-145

Yang H, Czapla TH (1993) Isolation and characterization of cDNA clones encoding jacalin isolectins. J Biol Chem 268: 5905-5910

Yokoyama R, Takahashi T, Kato A, Torii KU, Komeda Y (1998) The Arabidopsis *ERECTA* gene is expressed in the shoot apical meristem and organ primordia. Plant J 15: 301-310

Yoshizaki M, Furumoto T, Hata S, Shinozaki M, Izui K (2000) cDNA cloning and expression analysis of a non-photosynthetic ferredoxin gene in morning glory (*Pharbitis nil*). Biochim Biophys Acta 1491: 273-278

Young NM, Johnston RAZ, Watson DC (1991) The amino acid sequences of jacalin and the *Maclura pomifera* agglutinin. FEBS Lett 282: 382-384

Zegzouti H, Marty C, Jones B, Bouquin T, Latche A, Pech JC, Bouzayen M (1997) Improved screening of cDNAs generated by mRNA differential display enables the selection of true positives and the isolation of weakly expressed messages. Plant Mol Biol Reporter 15: 236-245