Journal of Plant Biology, August 2007, 50(4) : 496-503

Gene Expression Profile for *Nicotiana tabacum* in the Early Phase of Flooding Stress

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Although flooding can often severely damage crop yields, few studies of this stress have been made at the genetic level. To identify the genes that probably function in plants at the onset of flooding stress, we constructed a cDNA library representing tobacco plants that experienced short-term stress, i.e., 2 to 4 h of submergence while under illumination. Differential screening of that library produced 73 cDNA clones that showed preferential hybridization with the probe prepared from these stressed plants. The cDNA inserts were isolated from the vector by restriction digest and subjected to reverse northern analysis, which confirmed preferential expression of 41 genes. The remainder either had no significant increase in expression under flooding stress or exhibited no identifiable signal. We then performed northern blot analyses for some selected genes to provide supporting evidence that strongly paralleled our results from the reverse-northern evaluation. Photosynthesis-related genes were the major group, followed by those for well-known glycolysis enzymes and fermentation enzymes. Other genes include those for hydrolytic enzymes and components of the ethylene synthesis pathway. Although many others also were induced, their functions could not be characterized here.

Keywords: cDNA clones, early timepoint of flooding stress, gene expression profile, tobacco

Flooding often causes severe damage to crops; this is especially important in about 16% of the production area worldwide (Boyer, 1962). Although this problem occurs mainly in the tropical rainforest, plants can also undergo such stress in cooler regions during much of the year when soil water remains saturated due to bad drainage and slow evaporation. Occasional heavy rainfalls also affect crops in temperate zones. In all of these scenarios, most of the damage results from a reduction in the amount of oxygen available to the plants (Dat et al., 2004).

The proteins specifically up-regulated when this type of stress occurs are referred to as anaerobic proteins (ANPs); most studied so far have comprised mainly metabolic pathway enzymes (Dennis et al., 2000; Dat et al., 2004). However, much more diverse genes are induced when plants become submerged. These include putative transcription factor genes, signal transduction pathway component genes, and some without predicted functions (Klok et al., 2002; Agarwal and Grover, 2005).

Plants differ significantly in their tolerance to flooding. Wetland species, such as rice, possess unique anatomical and physiological features that permit their survival in an aquatic environment or saturated soils. In contrast, plants that normally grow on dry land are easily damaged when waterlogged. When the roots of flooding-sensitive plants encounter anoxic conditions, their aerobic metabolism hardly proceeds, resulting in a reduced energy status in the cells. Most of these dry-land plants seldom develop specialized structures, e.g., aerenchyma tissue, for adapting to flooding stress (Drew et al., 2000). Such conditions can vary widely. For example, the depth and period of submersion can differ, as can the turbidity of the water that covers those plants, especially under sunlight (Pigliucci and Kolodynska, 2002; Mommer and Visser, 2005). Although flooding and anoxia intolerance of agricultural crops has long been studied, research at the genetic level has been limited, particularly with regard to the first stages of this stress. Therefore, to obtain a profile for transcripts early in the stress period, we constructed a cDNA library from tobacco plants that were submerged for a short time.

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MATERIALS AND METHODS

Plant Material and Stress Treatments

Following seed germination, *Nicotiana tabacum* W38 plants were cultured in pots for 6 weeks (to the 4- to 6-leaf stage) in a growth chamber (16-h photoperiod, 25°C, 60% relative humidity, and 200 μE m^2 s^{-1} from fluorescent lamps). Flooding stress was applied by fully immersing the pots in tap water for 2 or 4 h under light. Afterward, the young plants were quickly frozen in liquid nitrogen and stored at –80°C.

Total RNA Isolation

Total RNA was isolated from tobacco according to the methods of Sambrook et al. (1989), with minor modifications. Briefly, tissues were ground in liquid nitrogen with a mortar, and then combined with a mixture of homogenization buffer [50 mM LiCl, 25 mM Tris·Cl (pH 7.5), 35 mM EDTA, 35 mM EGTA, and 0.5% SDS] and phenol:chloroform:isoamylalcohol (25:24:1). After centrifugation, the aqueous phase was transferred to a new tube and extracted with a chloroform:isoamylalcohol (1:1) mixture. RNA was precipitated by adding an equal amount of 4 M LiCl to the aqueous phase, followed by centrifugation.

RNA Blot Hybridization

Twenty μg of total RNA was loaded in each lane and subjected to electrophoresis on a 1.2% formaldehyde gel. The RNA was then transferred onto a membrane (Hybond N; Amersham, USA). A probe for ADH (alcohol dehydrogenase, X81853) was synthesized by PCR amplification, using a forward primer of 5′-CATTCGTTGCAAAGCTGCG-3′ and a reverse primer of 5′-CAACATTGAGAGTTGCACC-3′. The membrane was pre-hybridized for 1 h, then hybridized for 16 to 22 h at 65°C in a solution of 0.5 M sodium phosphate (pH 7.2), 7% (w/v) SDS, and 1 mM EDTA (pH 7.0). Afterward, the membrane was washed in 2X SSPE and 0.1% (w/v) SDS at room temperature for 15 min, 1X SSPE and 0.1% (w/v) SDS at 65°C for 15 min, and 0.5X SSPE and 0.1% (w/v) SDS at room temperature for 15 min. It was then exposed to X-ray film with two intensifying screens (DuPont, USA) at −70°C.

Construction of cDNA Library and Screening

Poly(A)$^+$ RNA was purified from total RNA with the Poly(A) Tract mRNA Isolation System (Promega, USA). A unidirectional *Eco*RI-*Xho*I cDNA library was constructed using a ZAP-cDNA synthesis kit and ZAP-cDNA GigapackIII gold packing extracts (Stratagene, USA) according to the manufacturer's instructions. The cDNA library had a complexity of 2.5×10^6 pfu ml^{-1}, and >95% of the phages contained cDNA inserts (data not shown). Differential screening of the cDNA library was done as described by Sambrook et al. (1989), with some modifications. Briefly, after amplification and estimation of titer, the library was plated at a density of ~50,000 pfu per 150-mm plate, for a total of 20 plates. The duplicated plaques were then lifted onto membranes (Hybond-N). These plaque lifts were denatured, neutralized, rinsed, and fixed by UV cross-linking. The membranes were pre-hybridized, then hybridized with one of two probes for differential screening. Probes were synthesized using poly(A)$^+$ RNA isolated from either flooding-stressed or normally grown tobacco plants, both of which were labeled with ^{32}P using reverse transcriptase. The hybridized membranes were washed and exposed to X-ray film. From this differential screening, we obtained 102 plaques that showed a highly increased signal from the stressed plants. Isolated cDNA clones were *in vivo*-excised from the UniZAP-XR vector and sub-cloned into pBluescript SK(-) using the Exassit helper phage (M13; Stratagene).

Partial Nucleotide Sequencing

Plasmid DNA was purified with a plasmid DNA purification kit (Intron, Korea). The T7 primer of 5′-TAATACGACTCACTATAGG-3′ was used for partial sequencing. Sequencing reactions were run on an automated sequencer (Model 3100; Applied Biosystems, USA). All ESTs were annotated on the basis of the existing annotation for non-redundant databases at the NCBI (http://www.ncbi.nlm. nih.gov), using BLASTX (Altschul et al., 1997). Homologies that showed an e-value <1e-08 with more than 102 nucleotides (34 amino acids) were considered significant.

Reverse Northern Blot Analysis

To further confirm and characterize the cDNA clones selected from this plaque hybridization, we performed "reverse northern" analysis (Zegzouti et al., 1997). Plasmid DNA from each selected clone was isolated, and the DNA inserts were released from the pBluescript SK(-) plasmid in the Uni-ZAP XR vector via double digestion with *Eco*RI and *Xho*I. The digested DNAs were run in duplicate on the agarose gel, and after transfer to the membrane in duplicate, one membrane was hybridized to ^{32}P-labeled first-strand cDNAs from the flooding-stressed tobacco while the other was used with the cDNA probe from the untreated tobacco. The membranes were washed, then exposed to X-ray film as described above. Clones that showed different hybridization intensities between the two probes were selected and analyzed.

Phylogenetic Analysis

cDNA clones coding chlorophyll a/b-binding proteins and ribulose-1,5-bisphosphate carboxylases were subjected to phylogenetic analysis. The deduced amino acid sequences for these were used for multiple alignment. Highly overlapped sequences (from 390 b to 790 b of X52744 in the case of chlorophyll a/b-binding protein, and from 170 b to 380 b of X01722 for ribulose-1,5-bisphosphate carboxylase) from the first analysis were chosen for phylogenetic grouping of the genes with the CLUSTALW program (www.ebi.ac.uk/clustalw).

RESULTS AND DISCUSSION

Alcohol Dehydrogenase Expression in Tobacco under Flooding Stress

Alcohol dehydrogenase (ADH) is a well-known enzyme that is induced under anaerobic conditions, especially at transcription of the gene. Examples of this are found in soybean (Brzezinski et al., 1986), maize (Subbaiah et al., 1996), wheat (Albrecht and Mustroph, 2003) and *Arabidopsis* (Ismond et al., 2003). RNA blot hybridization of our flood-stressed tobacco plants showed that *ADH* was rapidly induced. After 2 h, its northern band was markedly thickened compared with the signal from the non-stressed plants. The intensity of this band became stronger as the stress period lengthened (Fig. 1). This fast induction of *ADH* expression probably reflected the sensitivity of tobacco to flooding.

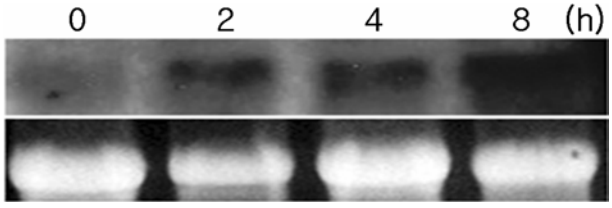

Figure 1. Accumulation of ADH transcripts in flooding-stressed tobacco. Total RNAs isolated from treated and normally grown plants were electrophoresed and hybridized to ^{32}P-labeled *ADH*. Number on figure indicates length of flooding treatment. rRNA bands were stained with ethidium bromide to serve as equal-loading controls.

Table 1. Annotation of 73 ESTs from flooding-stressed tobacco. (FSI, Flooding-Stress Induced)

EST ID	Description of best match	Score (Blastx)	E-value
FSI 1	Avr9/Cf-9 rapidly elicited protein 140	231	6e-59
FSI 2	*Nicotiana tabacum* voucher Et6 mRNA	468	2e-128
FSI 3	chlorophyll a/b-binding protein (Cab-C) gene	486	1e-147
FSI 4	putative oligopeptidase A	204	3e-51
FSI 5	ribulose-1,5-bisphosphate carboxylase small subunit	353	1e-95
FSI 6	ATP-binding / argininosuccinate synthase AT4G24830 mRNA	54	8e-04
FSI 7	unknown [*Arabidopsis thaliana*]	246	2e-63
FSI 8	light harvesting chlorophyll a/b-binding protein	525	1e-147
FSI 9	glyceraldehyde-3-phosphate dehydrogenase A-subunit precursor	154	8e-36
FSI 10	oligopeptidase A-like protein	80	1e-13
FSI 11	nucleoid DNA-binding-like protein	192	2e-47
FSI 12	hypothetical protein	259	2e-67
FSI 13	mRNA capping enzyme-like protein	52	1e-05
FSI 14	copia-like retroelement pol polyprotein	57	8e-07
FSI 15	putative ATP-dependent RNA helicase	289	1e-76
FSI 16	putative RNase H domain containing protein	93	8.1
FSI 17	hypothetical protein XP_613463	43	1.5
FSI 18	S-adenosylmethionine decarboxylase	409	0
FSI 19	inositol-tetrakisphosphate 1-kinase 2	233	1e-59
FSI 20	glycine-rich RNA-binding protein mRNA	289	5e-75
FSI 21	TRIP-1 (TGF-beta receptor interaction protein)	161	2e-36
FSI 22	nucleotide-binding AT2G46280 (TRIP-1) transcript variant	192	6e-46
FSI 23	putative ribulose bisphosphate carboxylase small subunit protein	353	6e-96
FSI 24	chlorophyll a/b-binding protein CP29	126	2e-28
FSI 25	putative ribulose bisphosphate carboxylase small subunit protein	57	2e-07
FSI 26	chlorophyll a/b-binding protein Type III precursor	415	1e-114
FSI 27	chlorophyll a/b-binding protein Type III precursor	273	3e-116
FSI 28	Photosystem I light-harvesting chlorophyll a/b-binding protein	478	2e-133
FSI 29	glycine decarboxylase complex H-protein	269	4e-71
FSI 30	light-harvesting chlorophyll a/b-binding protein	267	1e-94
FSI 31	light-harvesting chlorophyll a/b-binding protein	413	3e-114
FSI 32	ribulose bisphosphate carboxylase small chain S41	332	2e-89
FSI 33	light-harvesting chlorophyll a/b-binding protein	324	0
FSI 34	light-harvesting chlorophyll a/b-binding protein	501	0
FSI 35	chlorophyll a/b-binding protein	177	2e-11
FSI 36	chlorophyll a/b-binding protein	535	5e-151
FSI 37	ribosomal protein L11	77	1e-12
FSI 38	light-harvesting chlorophyll a/b-binding protein	524	2e-147
FSI 39	ribulose bisphosphate carboxylase small subunit protein	752	3e-12
FSI 40	ribulose bisphosphate carboxylase small subunit protein	315	3e-12
FSI 41	light-harvesting chlorophyll a/b-binding protein	506	7e-142
FSI 42	putative nodulin-like protein	150	7e-35
FSI 43	ACC oxidase	448	2e-124
FSI 44	light-harvesting chlorophyll a/b-binding protein	149	2e-34
FSI 45	unknown protein	187	6e-46
FSI 46	putative ribulose bisphosphate carboxylase small subunit protein	353	6e-96
FSI 47	putative ribulose bisphosphate carboxylase small subunit protein	341	4e-92
FSI 48	putative ribulose bisphosphate carboxylase small subunit protein	353	6e-96
FSI 49	light-harvesting chlorophyll a/b-binding protein	207	6e-52
FSI 50	chloroplast Photosystem II 22 kDa component	259	1e-67
FSI 51	putative ribulose bisphosphate carboxylase small subunit protein	288	3e-76

Table 1. Continued.

EST ID	Description of best match	Score (Blastx)	E-value
FSI 52	membrane protein COV-like	168	2e-40
FSI 53	no significant similarity found		
FSI 54	chlorophyll a/b-binding protein CP29	379	6e-104
FSI 55	putative Photosystem I subunit III precursor	197	4e-49
FSI 56	chlorophyll a/b-binding protein CP29	189	9e-45
FSI 57	serine/threonine protein phosphatase 2A	40	0.079
FSI 58	unknown protein	149	8e-35
FSI 59	isocitrate dehydrogenase (NADP+)	161	4e-38
FSI 60	unnamed protein product	161	1e-38
FSI 61	cytochrome P450 monooxygenase CYP72B	308	2e-82
FSI 62	ribosomal protein L38-like	139	2e-31
FSI 63	putative callose synthase catalytic subunit	215	2e-54
FSI 64	putative reverse transcriptase	747	7e-12
FSI 65	putative ribosomal protein	427	4e-56
FSI 66	soluble starch synthase 1, chloroplast precursor	258	4e-67
FSI 67	*Nicotiana tabacum* Avr9/Cf-9 induced kinase 1 (ACIK1) mRNA	48.1	0.025
FSI 68	putative elongation factor 2	324	2e-87
FSI 69	putative bHLH transcription factor	106	8e-22
FSI 70	putative ribulose bisphosphate carboxylase small subunit protein	300	4e-80
FSI 71	light-harvesting chlorophyll a/b-binding protein	424	2e-117
FSI 72	putative GTP-binding protein	54	5e-06
FSI 73	ribulose bisphosphate carboxylase activase	280	5e-74

Highly Induced cDNA Clones from Tobacco in the Early Phase of Flooding Stress

It is important to understand, at the molecular level, the changes that occur in flooding-sensitive plants. Here, we constructed a cDNA library representing mRNAs from tobacco plants exposed to short-term flooding. After differential screening of $\sim 1 \times 10^6$ plaques from the library, we isolated 102 clones with apparently increased expression (data not shown). On them we performed single-path nucleotide sequencing that gave sequences for about 500 nucleotides for each gene. After we eliminated identical genes and those without reliable sequences, this resulted in 73 cDNA clones. Their putative identities were searched for through databases (Table 1).

Reverse northern blot analysis was used to screen again the isolated clones for their induction under flooding stress. Their digested DNAs were run in duplicate on an agarose gel (Fig. 2A). When the hybridization patterns corresponding to flooding-treated and untreated plants were compared, 41 cDNA clones showed greater expression under stress while the rest had similar or reduced signal intensities as a result of treatment (Fig. 2B). Considering the condition of the plaque hybridization during the differential screening of the cDNA library, we had expected a large portion of the cDNA clones to present false-positive signals.

To confirm our results from the reverse northern blot analysis, we randomly selected several cDNA clones for RNA blot analysis -- FSI (Flooding Stress Induced) 3, 13, 20, and 43. In the reverse northerns, *FSI3* had shown about equal signal intensity from both probes, one for stressed, the other for unstressed. RNA blot analysis of *FSI3* essentially paralleled that earlier result, i.e., no significant induction of expression was found upon flooding stress. Likewise, *FSI13* and *FSI20* proved to be flooding stress-inducible genes in both reverse northern and RNA blot analysis. *FSI43* did not exhibit enhanced intensity from the flooding stress-related probe in the reverse northern, a result identical to the RNA blot analysis at the 4-h time point. However, this RNA blot analysis did indicate significant induction of *FSI43* expression after 24 h of flooding, suggesting that *FSI43* is a late gene (Fig. 3). Thus, our typical examples of RNA blot analyses supported the results from our reverse northern blots.

Based on these analyses, we propose that the following are early genes in the flooding stress response by tobacco: putative oligopeptidase, ATP binding/argininosuccinate synthase, several members of the light-harvesting chlorophyll a/b-binding proteins, several members of ribulose bisphosphate carboxylase small subunits, glyceraldehyde-3-phosphate dehydrogenase A-subunit precursor, nucleoid DNA binding-like protein, mRNA capping enzyme-like protein, copia-like retroelement pol polyprotein, putative RNase H domain-containing protein, S-adenosylmethionine decarboxylase, inositol-tetrakisphosphate 1-kinase, glycine-rich RNA-binding protein, TGF-beta receptor interaction protein (TRIP-1), nucleotide-binding TRIP-1 variant, glycine decarboxylase complex H-protein, ribosomal proteins L11 and L38, isocitrate dehydrogenase, putative callose synthase catalytic subunit, putative reverse transcriptase, Avr9/Cf-9 induced kinase 1, putative elongation factor 2, and several unknown or hypothetical proteins.

Extensive profiling has been done for flooding stress-

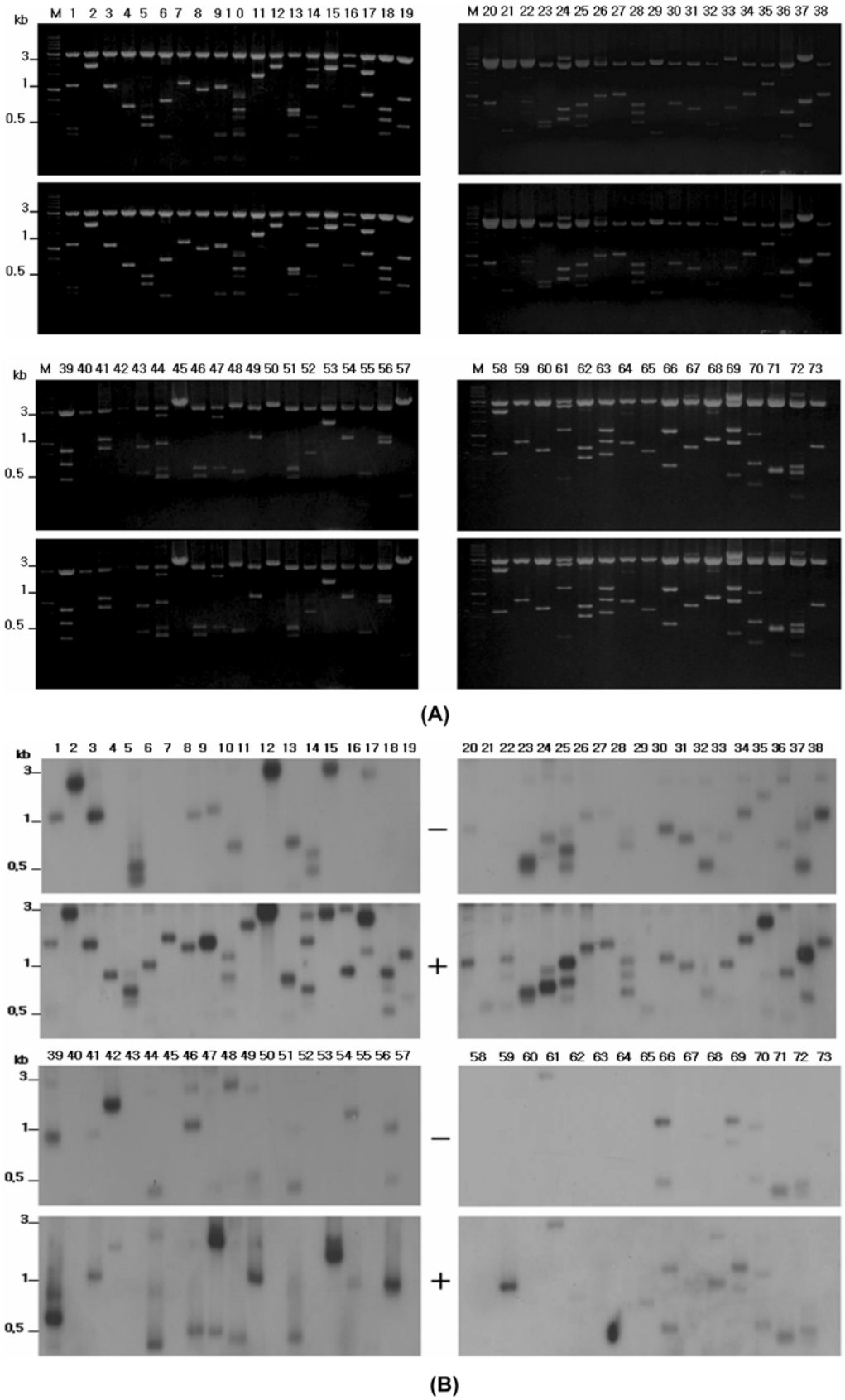

Figure 2. Results from differential screening of clones were confirmed by reverse northern blot hybridization. (**A**) Equal amounts of digested DNAs were placed on agarose gel in duplicate. DNA bands were visualized by ethidium bromide staining. (**B**) Results from reverse northern blot hybridization of DNA fragments in (**A**). Digested DNA fragments were transferred onto membrane in duplicate, and one set was hybridized to ^{32}P-labeled 1st cDNAs from flooding-treated (+) tobacco plants; the other, to ^{32}P-labeled cDNAs from untreated tobacco (–).

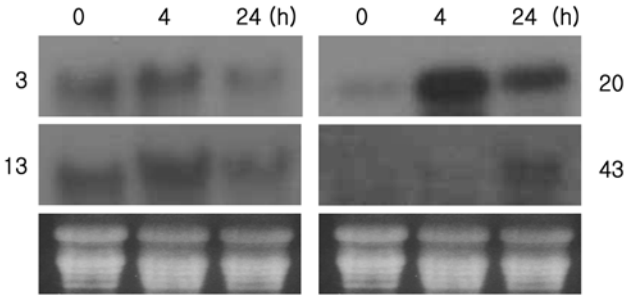

Figure 3. Northern blot analyses for cDNA clones from differential screening of cDNA library. Total RNA was isolated from tobacco plants that had been subjected to flooding stress for 4 or 24 h, under normal growing conditions. Each lane was loaded with 20 μg of total RNA. Numbers on side of figure indicate clone identifications. rRNAs were visualized by staining with ethidium bromide to serve as equal-loading controls.

inducible genes or proteins in plants. Dennis et al. (2000) have reviewed previous studies that found sucrose synthase, α-amylase, glucose-6-phosphate isomerase, pyrophosphate-dependent phosphofructokinase, hexokinase, fructose-1,6-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, enolase, alcohol dehydrogenase, pyruvate decarboxylase, lactate dehydrogenase, alanine aminotransferase, glutamine synthase, nitrate reductase, nitrite reductase, formate dehydrogenase, xyloglucan endotransglycosylase, 1-aminocyclopropane-1-carboxylic acid synthase, haemoglobin, vacuolar H^+-translocating pyrophosphatase, cytosolic pyruvate orthophosphate dikinase, AtMYB2, Myb7 from rice, G-box binding factor, and calcium-dependent protein kinase. Chang et al. (2000) have identified 46 proteins induced from the root tips of maize seedlings under short-term hypoxia. These include glucose transporter, F1-ATPase subunit, UDP-glucose pyrophosphorylase, phosphoglycerate mutase, glucose dehydrogenase, malate dehydrogenase, aconitase, aspartate aminotransferase, methionine synthase, S-adenosylmethionine synthetase, heat shock protein 70, elongation factor 2, translation initiation factor 4A, mitochondrial chaperonin 60, se-wap41, elongation factor Tu, and aldehyde dehydrogenase. Klok et al. (2002) have conducted EST analysis for *Arabidopsis* roots cultured under hypoxic conditions, and have reported on lectin, RING-H2 zinc finger-containing protein, TIP2, RNA-binding protein, adenosylhomocysteinase, *O*-methyltransferase, RING-H2 finger protein, protein kinase, brassinosteroid receptor kinase, respiratory burst oxidase, peroxidase, glutathione *S*-transferase, metallothionein 1b, ABC transporter, chloroplast superoxide dismutase, epoxide hydrolase, acyl-carrier protein, pathogen-inducible protein CXc, ubiquitin-specific protease 8, SF3-transcription factor, several leucine zipper-containing proteins, several MYB transcription factors, nucleotide sugar epimerase, glucosyltransferase, nodulin-like protein, glutamate synthase, pectate lyase, peroxidase, 4-

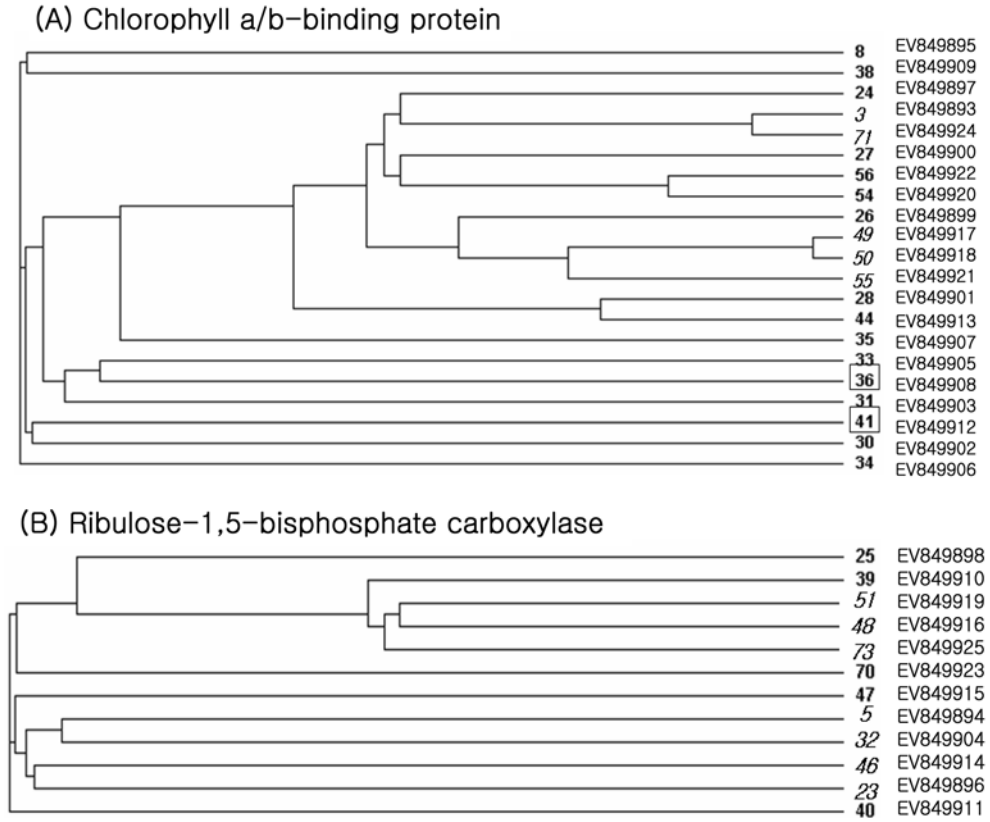

Figure 4. Phylogenetic tree for chlorophyll a/b-binding protein (**A**) and ribulose-1,5-bisphosphate carboxylase (**B**). Letters in italics represent uninduced genes in reverse northern analysis. Clones in square boxes have longer sequences than those closest in proximity on phylogenetic tree, and have identical overlapping sequences. Database accession number for each gene is given at the rightmost. Tree was constructed based on those overlapped amino acid sequences. CLUSTALW was used.

coumarate-CoA ligase, peroxidase ATP 14a, feebly-like protein, jasmonate inducible protein, and over 40 unknown proteins. These three summaries list a large number of identical genes induced under flooding stress as well as differential expression of genes for individual cases. Finally, Agarwal and Grover (2005) have done transcription profiling for a rice genotype tolerant of flooding stress, which provides an expression profile quite different from these others. There, many hexose transporters, ion channel proteins, and RNA-binding proteins are heavily represented.

Obviously, our current work names genes that overlap genes and proteins reported previously. For example, glyceraldehyde-3-phosphate dehydrogenase, which is involved in glycolysis, is a commonly occurring anaerobic protein (Sivalinganna and Sachs, 1997). Nevertheless, a significant portion of the genes identified here are unique to flooding-stressed tobacco plants.

Photosynthesis-Related Genes at the Early Phase of Flooding Stress

Among the analyzed cDNA clones, the largest groups contained genes for photosynthesis, light-harvesting chlorophyll a/b-binding protein and ribulose-1,5-bisphosphate carboxylase. Maintenance of photosynthesis is very important when plants are adapting to and recovering from stress. However, this aspect of plant responses to flooding is poorly investigated (Briggina et al., 2002; Yordanova et al., 2003).

Among the 73 cDNA clones selected by differential screening of the cDNA library for stressed tobacco, 23 chlorophyll a/b-binding protein genes and 12 ribulose-1,5-bisphosphate carboxylase genes were involved. The next round of screening via reverse northern blots demonstrated that, of those 23 chlorophyll a/b-binding protein genes, 12 showed increased levels of transcript under early flooding stress while the rest displayed no significant changes (Fig. 2B; Table 1). Furthermore, among the 12 ribulose-1,5-bisphosphate carboxylase genes, 5 had increased transcript levels as a result of our flooding treatment (Fig. 2B; Table 1). Phylogenetic analyses of the 23 chlorophyll a/b-binding protein genes and the 12 ribulose-1,5-bisphosphate carboxylase genes presented several groupings for them (Fig. 4). Those with flooding stress-inducible expression in the reverse northern analyses could be separated in the tree from the ones with no significant changes. This split could be an important indicator of flooding stress-inducible genes among chlorophyll a/b-binding protein family members and those of the ribulose-1,5-bisphosphate carboxylase family. As plants become immersed in water, the environment for photosynthesis is dramatically altered. Shortage of O_2 supply during the flooding has been repeatedly reported and emphasized. (Dat et al., 2004; Huynh et al., 2005; Dong and Xu, 2006). However, shortage of light energy and atmospheric CO_2 also can be damaging (Mommer and Visser, 2005). Indications of flooding stress-induced chlorophyll a/b-binding protein genes and ribulose-1,5-bisphosphate carboxylase genes probably suggest the existence of one adaptation mechanism for tobacco plants in the early phases of flooding.

In conclusion, we emphasize that, depending on the degree of flooding stress as well as the plant species involved, the particular genes that are induced may vary significantly. Abiotic stresses are often interconnected in signal transduction and response in plants (Chen et al., 2002; Cho et al., 2003; Ashrafuzzaman et al., 2005; Shinozaki and Yamaguchi-Shinozaki, 2007). Genes that were revealed in the current study as being induced by flooding stress are often detected in association with other types of stress as well. This might indicate a strong linkage between the flooding-stress response and the complex network of abiotic stress signaling and response pathways. The genes newly reported here could be a good addition in this effort.

ACKNOWLEDGEMENTS

This work was supported by research grants from the Korea Science and Engineering Foundation, and for the Priority Research Institute from the Korean Research Foundation (grant no. 2005-005-J16002) to CBH.

Received July 7, 2007; accepted August 14, 2007.

LITERATURE CITED

Agarwal S, Grover A (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

Albrecht G, Mustroph A (2003) Localization of sucrose synthase in wheat roots: Increased *in situ* activity of sucrose synthase correlates with cell wall thickening by cellulose deposition under hypoxia. Planta 217: 252-260

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucl Acids Res 25: 3389-3402

Ashrafuzzaman M, June Oh S, Hong CB (2005) Low expression profiles of heat stress-related genes in *Capsicum annuum*. J Plant Biol 48: 85-95

Boyer (1962) Should we regularly use city water coming from river? Rev Prat 12: 3559-3563

Briggina TV, Drozdova IS, Ponomareva YV, Alekhin VI, Grineva GM (2002) Photosynthesis, respiration, and transpiration in maize seedling under hypoxia induced by complete flooding. Dokl Biol Sci 384: 840-843

Brzezinski R, Talbot BG, Brown D, Klimuszko D, Blakeley SD, Thirion JP (1986) Characterization of alcohol dehydrogenase in young soybean seedlings. Biochem Genet 24: 643-656

Chang WW, Huang L, Shen M, Webster C, Burlingame AL, Roberts JK (2000) Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment and identification of proteins by mass spectrometry. Plant Physiol 122: 295-318

Chen W, Provart NJ, Glazebrook J, Katagiri F, Eulgem T, Mauch F, Luan S, Zou G, Whitham SA, Budworth PR, Tao Y, Xie Z, Chen X, Lam S, Kreps JA, Harper JF, Si-Ammour A, Mauch-Mani B, Heinlein M, Kobaya K, Hohn T, Dang JL, Wang X, Zhu T (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 14: 559-574

Cho YB, Jeon HJ, Hong CB (2003) Cloning and functional annotation of rare mRNA species from drought-stressed hot pepper. J Plant Biol 46: 83-89

Dat JF, Capelli N, Folzer H, Bourgeade P, Badot PM (2004) Sensing and signaling during plant flooding. Plant Physiol Biochem 42: 273-282

Dennis ES, Dolferus R, Ellis M, Rahman M, Wu Y, Hoeren FU, Grover A, Ismond KP, Good AG, Peacock WJ (2000) Molecular strategies for improving waterlogging tolerance in plants. J Exp Bot 51: 89-97

Dong D, Xu Y (2006) Immediate oxidative burst onto the leaves after cutting the stem or flooding the roots of *Nicotiana benthamiana*. Plant Physiol Biochem 44: 158-160

Drew MC, He CJ, Morgan PW (2000) Programmed cell death and aerenchyma formation in roots. Trends Plant Sci 5: 123-127

Huynh LN, VanToai T, Streeter J, Banowetz G (2005) Regulation of flooding tolerance of *SAG12:ipt Arabidopsis* plants by cytokinin. J Exp Bot 56: 1397-1407

Ismond KP, Dolferus R, De Pauw M, Dennis ES, Good AG (2003) Enhanced low oxygen survival in *Arabidopsis* through increased metabolic flux in the fermentative pathway. Plant Physiol 132: 1292-1302

Klok EJ, Wilson IW, Wilson D, Chapman SC, Ewing RM, Somerville SC, Peacock WJ, Dolferus R, Dennis ES (2002) Expression profile analysis of the low-oxygen response in *Arabidopsis* root cultures. Plant Cell 14: 2481-2494

Mommer L, Visser EJW (2005) Underwater photosynthesis in flooded terrestrial plants: A matter of leaf plasticity. Ann Bot 96: 581-589

Pigliucci M, Kolodynska A (2002) Phenotypic plasticity and integration in response to flooded conditions in natural accessions of *Arabidopsis thaliana* (L.) Heynh (Brassicaceae). Ann Bot 90: 199-207

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

Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. J Exp Bot 58: 221-227

Sivalinganna M, Sachs MM (1997) Molecular characterization and promoter analysis of the maize cytosolic glyceraldehyde 3-phosphate dehydrogenase gene family and its expression during anoxia. Plant Mol Biol 33: 97-112

Subbaiah CC, Saab IN, Sachs MM (1996) Anaerobic gene expression and flooding tolerance in maize. J Exp Bot 47: 1-15

Yordanova RY, Alexieve VS, Popova LP (2003) Influence of root oxygen deficiency on photosynthesis and antioxidant status in barley plants. Russ J Plant Phys 50: 163-167

Zegzouti H, Marty CL, Jones B, Bouquin T, Latch A, Pech J C, Bouzayen M (1997) Improved screening of cDNA generated by mRNA Differential Display enables the selection of true positives and the isolation of weakly expressed messages. Plant Mol Biol Rep 15: 236-245