Differential expression of a poplar SK2-type dehydrin gene in response to various stresses

Eun-Kyung Bae, Hyoshin Lee*, Jae-Soon Lee & Eun-Woon Noh
Biotechnology Division, Korea Forest Research Institute, Suwon, Korea

Dehydrins are group II, late embryogenesis abundant proteins that act putatively as chaperones in stressed plants. To elucidate the function of dehydrins in poplar, we isolated the SK2-type dehydrin gene Podhn from Populus alba × P. tremula var. glandulosa suspension cells and analyzed its expression following treatments of abiotic stress, wounding and plant growth regulator. Sequence homology and phylogenetic analyses indicate Podhn encodes an acidic dehydrin (pI 5.14, 277 amino acids, predicted size 25.6 kDa) containing two lysine-rich "K-segments" and a 7-serine residue "S-segment", both characteristic of SK2-type dehydrins. Southern blots show Podhn genes form a small gene family in poplar. Podhn was expressed in all tissues examined under unstressed conditions, but most strongly in cell suspensions (especially in the stationary phase). Drought, salt, cold and exogenous abscisic acid (ABA) treatments enhanced Podhn expression, while wounding and jasmonic acid caused its reduction. Therefore, Podhn might be involved in ABA or stress response. [BMB reports 2009; 42(7): 439-443]

INTRODUCTION

Trees are sessile organisms with long lifespans that expose many to wide diurnal, seasonal and stochastic fluctuations in environmental variables such as periods of drought and various climatic stresses. Therefore, survival and reproduction is dependent upon an array of tolerance mechanisms that have evolved to help plants to overcome and adjust to stressful conditions throughout their growth and development. Such mechanisms involve a wide range of proteins and other molecules including dehydrins, which respond to dehydration caused by both drought and cold stresses (1, 2).

Dehydrins are Group II (D-11 family), late embryogenesis abundant (LEA) proteins that accumulate in the later stages of embryogenesis when the water content in seeds declines (3). However, they are also induced in vegetative tissues in response to drought and cold stresses (4, 5) where they appear to function in various cellular compartments including the cytoplasm, nucleus and mitochondria (6). Dehydrin proteins contain several identifiable sequence motifs: Y-segments with the consensus motif (T/VDEYGNP) of various permutation and number located near the N-terminus, S-segments consisting of 5 to 7 amino acid residues and K-segments with an EKKGMD KIKEKLPG motif near the C-terminus (3). Accordingly, they are classified into five subclasses (YnSK2, Kn, SKn, Y2Kn, and KnS) depending on the number of Y- S- and K-segments they individually contain (1). Specifically, most of the SK2-type dehydrins are either SK2- or SK3-type and contribute to tolerance to drought and cold stresses (1, 7). Although SKn-type dehydrins from crops and herbaceous plants have been investigated in a number of studies (1, 3), little attention has been paid to their role in trees, which are often exposed to both cold and drought stresses at various times in their life cycles.

In this study we isolated and characterized an SK2-type dehydrin genomic sequence and its corresponding cDNA from a hybrid poplar (Populus alba × P. tremula var. glandulosa), followed by analyzing its expression in response to drought, salt, low temperature and plant hormones.

RESULTS AND DISCUSSION

Isolation and structural characterization of the Podhn gene

A full-length EST clone derived from a cDNA library prepared from suspension cells of Populus alba × P. tremula var. glandulosa was selected with more than 50% homology to previously described plant dehydrins. As shown in Fig. 1, the cDNA is 1,077 bp long and has a 681 bp open reading frame (ORF) with an 103 bp upstream sequence and a 279 bp downstream sequence. The cDNA ORF starts at nucleotide position 104 and ends at position 788, encoding a protein of 227 amino acid residues with a predicted molecular mass of ca. 25.7 kDa and a pI of 5.13. According to the predicted amino acid sequence of the protein the most abundant amino acid residues are glutamate, lysine and glutamine, constituting 28%, 27% and 21% of the total, respectively, while cysteine or tryptophan residues are absent. Almost half (48.5%) of the predicted amino acid residues are polar with hydrophilic character, as supported by a mean hydrophy value of -1.6. An
amino acid sequences of Eun-Kyung Bae, et al. indicated well-conserved Y-, S- and K-segments. tioned above) containing various permutations and numbers of dues, lacking both cysteine and tryptophan while (as men- dehydrins have low levels of hydrophobic amino acid resi- 
scribed by Close are consistent with the general characteristics of dehydrins as detected. Therefore, the characteristics of this poplar dehydrin dehydrin containing an S-segment located between residues 87 to 93 upstream of two K-segments (named K-1 and K-2, respectively, found in this study is higher than 80% when compared to con-
ponents was found near the C-terminus, yet no Y segment was 
S-segment consisting of seven serine residues and two K-seg-
ments was found in the untranslated region. A nucleotide cluster in bold. An intron is shown in lower-
line, the S-segment with a single line, the K-segment in italics 
ners on the right. The putative NLS is underlined with a double 
ment with the two probes gave the same pattern as shown in 
s strain intron between residues 91 and 92 within the S-segment 
of dehydrin is indicated in parenthesis. Tissue expression profile of 
The copy number of the Podhn gene was investigated by Southern blotting with a full-length Podhn cDNA probe. It was found digestion of genomic DNA with either EcoRI (E), HindIII (H) or XbaI (X) was fractionated by electrophoresis in an 1.0% agarose gel. The gel was blotted onto a nylon membrane and hy-
bridized with 32P-labeled full-length Podhn cDNA.

Homology analysis
Podhn has its highest sequence homology (95%) to the SK2-type dehydrin Peudhn1, which belongs to another Populus species, P. euramericana, located in a different section of the genus (10). Podhn also shows 59% and 50% sequence homol-
gy to two SK3-type dehydrins, DHN24 of Solanum sogardiaum (7) and Cadhn of Capsicum annuum (11), respectively. Phylogenetic analysis of YSK2-type and SK2-type dehydrins indicate Podhn clusters together with SK2-type dehydrins, especially with SK2-types (Fig. 1B). Thus, the results of both the homology and phylogenetic analyses suggest Podhn is an SK2-type dehydrin.

Genomic organization of the Podhn gene
The copy number of the Podhn gene was investigated by Southern blotting with a full-length Podhn cDNA probe. It was found digestion of genomic DNA with either EcoRI or HindIII yielded four bands that hybridized with the probe, while digestion with XbaI yielded only three bands (Fig. 2). We speculated this difference was potentially due to the presence of a restriction site in an intron in the S-segment, since many dehydrin genes contain such an intron (12-14). Therefore, to detect possible EcoRI, HindIII or XbaI restriction sites we determined the nucleotide sequences of the PCR-amplified DNA fragments of Podhn. The results confirmed both the presence of an 112 nt intron between residues 91 and 92 within the S-segment (residues 87 to 93) as well as the absence of any EcoRI, HindIII or XbaI restriction sites (Fig. 1A). After detection of a Ncol site in the intron, this enzyme was used to cut the Podhn into two DNA fragments that were later used as probes. Southern blotting with the two probes gave the same pattern as shown in Fig. 2 (data not shown). Therefore, 2 to 4 copies of the Podhn gene appear to be present in the poplar genome since an HindIII cutting site was detected in the untranslated region.

Tissue expression profile of Podhn
To characterize the tissue-specific expression of Podhn, total...
RNA was extracted from leaves, stems, roots and floral buds of whole poplar plants, and from suspension cells 14 days after subculturing for northern analysis. The results revealed that while Podhn was expressed in all the tissues examined, it was expressed most strongly in suspension cells and most weakly in stems and floral buds (Fig. 3A). Therefore, this SKn-type dehydrin gene seems to lack any tissue-specific expression pattern, which coincides with previous studies indicating these dehydrins are expressed differentially depending on the growth conditions and plant species rather than tissue. For instance OsDhn1, an SK3-type dehydrin in rice, is expressed in callus, seeds, leaves and shoots (15); pseudhn1, an SK2-type dehydrin from P. eurameriana, is reportedly expressed in both leaves and (less strongly) roots (10); and PgDhn1, an SK2-type dehydrin from white spruce (Picea glauca), is expressed in various tissues, including the petiole, stem, roots and buds (5).

Expression of Podhn during cell growth
Cultured plant cells can have high degrees of genetic and physiological homogeneity and thus have potential in the large-scale commercial production of various secondary metabolites (16). Cell culture systems are also useful in examining responses of cells to changes in culture conditions, cell division and growth. To examine changes in Podhn expression during the growth cycle of suspension cells, both the total RNA content and fresh weight of the suspension cells were monitored after sub-culturing in fresh medium. The observed changes in fresh weight are shown in Fig. 3B. Cells remained in an initial lag phase until entering an exponential growth phase from day 8 to day 16. Cells then appeared to grow very slowly until day 22, whereupon their growth completely ceased. During this growth cycle, cellular levels of Podhn mRNA changed substantially, rising rapidly upon entering the exponential growth phase at day 8 until day 24. The mRNA level in the late stationary phase (day 24) was 2.8-fold higher than in the lag phase (day 4) (Fig. 3C). The reason why the Podhn level was so high in the late stationary phase remains unknown, but may be related to nutrient deficiency in the culture medium and osmotic stress as the cells enter the apoptotic cell death phase (16). Nevertheless, more detailed analysis is needed to explain why Podhn mRNA increases steadily from the early exponential phase until the late stationary phase of the cell culture cycle.

Effects of abiotic stress, wounding and plant growth regulators on Podhn expression
To examine the effects of various stresses and abscisic acid

Fig. 3. Podhn expression in various tissues of poplar cells during normal growth in suspension culture. (A) Northern analysis of total RNA extracted from mature leaves (L), stems (S), roots (R), flowers (F) and cell suspension culture (SC). (B) Growth kinetics of poplar cells in suspension culture. After sub-culturing, fresh cellular mass was determined every 2 days for 26 days. (C) Northern analysis of total RNA extracted from the cells at the indicated times. Full-length Podhn cDNA was used as the probe and ethidium-bromide stained ribosomal RNA served as a loading control. Podhn expression levels represented as relative values when compared with those of 4-day cultured cells were determined by computer-based densitometry, as described in Materials and Methods.

Fig. 4. Podhn expression under various treatment conditions. (A) Podhn expression in suspension cells in response to mannitol treatment (Man, 250 mM), NaCl (150 mM), ABA (25 μM) and cold (2°C) for 2 and 10 h. Untreated control cells (Con) were incubated for the same time periods. (B) Podhn expression in leaf tissues in response to wounding. Ca. 1 cm cuts were made with sterile scissors along the edges of leaves from 1-year-old poplar plants. Total RNA was extracted from the trimmed leaves at the indicated times. (C) Podhn expression in suspension cells in response to treatment with plant growth regulators including ABA (20 μM), SA (20 μM), JA (10 μM) and GA3 (20 μM) for 0.5 and 6 h. Podhn expression levels represented as relative values when compared to those of untreated controls were determined by computer-based densitometry, as described in Materials and Methods.
(ABA) on Podhn expression, poplar suspension cells were treated separately with 250 mM mannitol, 150 mM NaCl, 25 μM ABA and low temperature (2°C). Northern analysis of RNA sampled from the cells revealed that all treatments increased Podhn expression (Fig. 4A). Changes in gene expression induced by each of the treatments over time were also compared. While ABA treatment or low temperature caused 2-3 fold increases in Podhn mRNA levels within 2 hrs, mannitol and NaCl induced increases in mRNA levels more slowly (after 10 hrs) and of greater magnitude (ca. 4 to 6 fold). The increases induced by treatment with mannitol (which is known to induce osmotic stress by decreasing water availability in plant cells; 17, 18) and NaCl are consistent with the previously reported effects of salt and PEG6000 on Peudhn1 expression (10), which indicated the level of SK4-type dehydrins in woody species increases in response to increased cellular osmotic pressure. ABA treatment and low temperatures also cause increases in the level of an SK4-type dehydrin (BpuDhn2) in birch (13). Our results indicate SK4-type dehydrins in woody species are likely upregulated via an ABA-dependent signaling pathway in response to low temperature, drought or salt stresses (19, 20). However, whether the ABA-mediated pathway or other stress-specific pathways are solely responsible for increases in Podhn expression in response to low temperature, drought or salt remains to be elucidated. In contrast, the expression level of Podhn began to decrease as early as 30 min after wounding and did not recover to pre-wounding levels for up to 24 h (Fig. 4B).

The effects of applying various hormones-ABA, jasmonic acid (JA), salicylic acid (SA) and gibberellic acid (GA3)-for either 30 min or 6 h on the expression level of Podhn were also examined. As shown in Fig. 4C, Northern blot analysis revealed Podhn transcript levels increased only in response to ABA while decreasing in response to JA (Fig. 4C). The latter finding is interesting since JA regulates the expression of numerous defense genes, with cells accumulating JA upon becoming wounded or infected by pathogens (19). While the SK4-type dehydrin PgDhn1 has been shown to be up-regulated by either wounding or JA treatment (5), the present study shows SK2-type Podhn was downregulated by the same treatments. Therefore, SK2 and SK4-type dehydrins appeared to respond differently to wounding and pathogen infection. Further, given that PgDhn1 in Picea glauca is also reportedly up-regulated by drought and low temperature (5), we speculate all SK2-type dehydrins are induced in response to drought and low temperature stresses yet differ in their response to wounding and pathogen infection stresses.

MATERIALS AND METHODS

Plant materials and growth conditions

Poplar suspension cells were maintained by sub-culturing when they reached the stationary phase by transferring 0.4 g fresh cellular mass to 100 ml of liquid MS medium (21) containing 1 mg l⁻¹ 2,4-dichlorophenoxyacetic acid, 0.1 mg l⁻¹ 1-naphthalene acetic acid and 0.01 mg l⁻¹ 6-benzylaminopurine (22). The suspensions were maintained at 120 rpm on a gyratory shaker placed inside a culture room at 22 ± 1°C under dim (20 μmol m⁻² s⁻¹) cool-white fluorescent light. To analyze expression during normal growth cycles, cells were harvested every two days after sub-culturing for 26 days, followed by vacuum filtration through 3MM filter paper, weighing, liquid nitrogen freezing, and storage at 70°C for RNA isolation. For tissue-specific expression analysis, leaves, stems and roots were harvested from 1-year-old poplar plants growing in a nursery while flowers were harvested from approximately 25-year-old plants.

Construction of cDNA library and isolation of the dehydrin gene

Total RNA was extracted from a cell suspension 8 days after sub-culturing by the guanidine thiocyanate method (23), followed by purification of poly (A)+ RNA from the total RNA by oligo (dT) column chromatography. A cDNA library was constructed using a ZAP-cDNA Gigapack III Gold Cloning kit (Stratagene, La Jolla, CA) according to manufacturer’s instructions. Using an ExAssist helper phage, the cDNA library was randomly excised in vivo, the plasmid DNAs were isolated and the 3′-single pass sequences were determined. Public databases were then searched using BLASTX to select clones homologous to known plant dehydrins. The selected cDNA clones were then sequenced and analyzed by Vector NTI advance 9.0 (Invitrogen, USA) and shown to encode an SK2-type dehydrin.

PCR amplification was performed with genomic DNA using primers specific for the Podhn gene. Genomic DNA was extracted from the leaves of 1-year-old poplar plants growing in a nursery using a MagExtractor-Plant Genome kit (Toyobo, Osaka, Japan). The primers used were Podhn-s (5′-AAGTGTATTGT GATTCGATCAG-3′) and Podhn-as (5′-CGTTCTATAGAAC CATAATTACT-3′). PCR amplification consisted of 35 cycles of 40 s denaturation at 94°C, 30 s annealing at 60°C and a 120 s extension at 72°C. The PCR product was cloned into the pGEM-T Easy vector (Promega, Madison, WI) and sequenced using 7T and SP6 primers. Sequences of the PCR product were compared with those of Podhn.

Southern blot analysis

Ten μg of genomic DNA were digested with EcoRI, HindIII or Xbal restriction enzymes overnight. The DNA was then electrophoretically separated on an 1% agarose gel, transferred to a Hybond-XL nylon membrane (Amersham-Pharmacia, Piscataway, NJ) according to the capillary transfer method (24) and probed using labeled full-length Podhn cDNA. Labeling of the probe, hybridization and membrane washing were done as described by Lee et al. (25).

Stress treatments and northern blot analysis

To mimic drought-induced osmotic stress, four-day-old suspension cultures were supplemented with mannitol or NaCl. To assess their response to the drought-associated hormone ABA,
replicate suspensions were treated with the hormone. To examine the effects of cold stress, suspension cells were incubated in Erlenmeyer flasks at ca. 2°C in ice on a gyratory shaker. In addition, the effects of other plant growth regulators were tested by supplementing cells with SA, ABA, JA or GA3. Replicate flasks (n = 3) were harvested for each case. Amounts and duration of the treatments are indicated in the figure legends. A wounded treatment was also applied to the leaves of 1-year-old poplar plants by making ca. 1 cm cuts along their edges with sterile scissors followed by leaf collection. All treated cells and leaves were immediately frozen in liquid nitrogen and stored at 70°C until analyzed. Total RNA was isolated from the samples using TRI Reagent (Molecular Research Center, Cincinnati, Ohio), followed by electrophoretically separating 10 μg portions of the RNA on 1.2% formaldehyde agarose gels and transfer to a Hybond-XL nylon membrane. Labeling of the probe, hybridization and membrane washing were done as described by Lee et al. (25). Signals were quantified by densitometric calculations using ImageJ 1.4 software (http://rsb.info.nih.gov/ij).

REFERENCES


