Expression of α-Expansin and Expansin-Like Genes in Deepwater Rice[1]

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Previously, we have studied the expression and regulation of four α- and 14 β-expansin genes in deepwater rice (*Oryza sativa*). We now report on the structure, expression, and regulation of 22 additional α-expansin (*Os-EXP*) genes, four expansin-like (*Os-EXPL*) genes, and one expansin-related (*Os-EXPR*) gene, which have recently been identified in the expressed sequence tag and genomic databases of rice. α-Expansins are characterized by a series of conserved Cys residues in the N-terminal half of the protein, a histidine-phenylalanine-aspartate (HFD) motif in the central region, and a series of tryptophan residues near the carboxyl terminus. Of the 22 additional α-expansin genes, five are expressed in internodes and leaves, three in coleoptiles, and nine in roots, with high transcript levels in the growing regions of these organs. Transcripts of five α-expansin genes were found in roots only. Expression of five α-expansin genes was induced in the internode by treatment with gibberellin (GA) and by wounding. The wound response resulted from excising stem sections or from piercing pinholes into the stem of intact plants. EXPL proteins lack the HFD motif and have two additional Cys residues in their C- and N-terminal regions. The positions of conserved tryptophan residues at the C-terminal region are different from those of α- and β-expansins. Expression of the *Os-EXPL3* gene is correlated with elongation and slightly induced by applied GA. However, the expression of the *Os-EXPL1* and *Os-EXPL2* genes showed limited correlation with cell elongation and was not induced by GA. We found no expression of the *Os-EXPR1* gene in the organs examined.

Deepwater rice (*Oryza sativa*) is a subsistence crop in regions of Southeast Asia that are flooded during the monsoon season. To avoid drowning, deepwater rice has evolved the capacity to elongate very rapidly when it becomes submerged. The growth response of submerged deepwater rice plants is elicited by GA and is based on enhanced cell division activity in the intercalary meristem of the youngest internode and on increased elongation of the newly formed cells. Besides its importance as a crop plant, deepwater rice is also a well-suited model organism to study plant growth (for review, see Kende et al., 1998).

Expansins are proteins that mediate long-term extension of isolated cell walls. Originally, they were grouped into two related families, the α- and β-expansins (for review, see Cosgrove, 2000). Four α-expansins have been studied in deepwater rice (Cho and Kende, 1997a, 1997b). The expression of the corresponding genes is organ specific and correlated with growth and acid-inducible cell wall extensibility. Expression of two α-expansin genes, *Os-EXP2* and *Os-EXP4*, is induced by submergence and treatment with GA (Cho and Kende, 1997b). As the expressed sequence tag (EST) databases expanded and the rice genome was sequenced, it became clear that α- and β-expansin genes belong to a large superfamily of genes (Lee et al., 2001; Li et al., 2002). Genes encoding proteins with significant amino acid identities to α- and β-expansins but lacking some of their conserved motifs were also recognized in the databases, and phylogenetic analysis indicates that they belong to the expansin superfamily of genes (D.J. Cosgrove, Pennsylvania State University, University Park, http://www.bio.psu.edu/expansins; Lee et al., 2001; Li et al., 2002). The large number of expansins poses intriguing questions regarding the function of these proteins and the significance of their redundancy. Determining the pattern and control of expansin gene expression is the first step in elucidating the function of individual expansins.

Fourteen β-expansin genes have been identified in rice (Lee and Kende, 2001). Five of these are expressed in the internode, and their expression is induced by GA and wounding. Here, we report on the identification of 22 additional α-expansin (*EXP*) genes, four expansin-like (*EXPL*) genes, and one expansin-related (*EXPR*) gene of rice in the genomic and EST databases. We studied their expression patterns in various organs and tested the effect of GA and wounding on the level of their transcripts.

RESULTS

Sequence Analysis of α-Expansins of Rice

A search of the rice EST and genomic databases yielded 26 putative α-expansin genes (Lee et al.,

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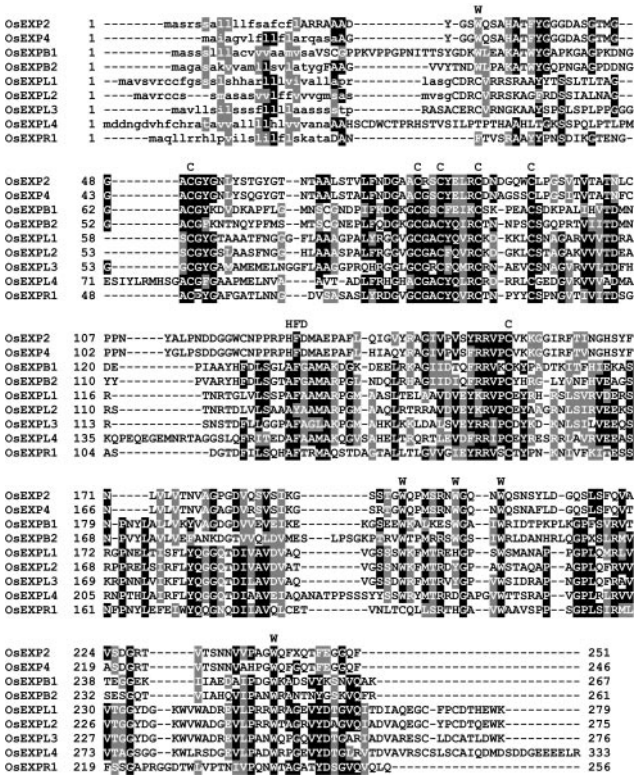

Figure 1. Alignment of the amino acid sequences of α-expansin, β-expansin, EXPL, and EXPR proteins. Positions of the highly conserved sequences, the conserved Cys (C) and Trp (W) residues, and the HFD motif are indicated by shading or by letters above the top sequence. In this alignment, the HFD motif of β-expansins is shifted by seven amino acids toward the amino terminus when compared with α-expansins. Signal peptide sequences are given as predicted by the PSORT program (Nakai and Kanehisa, 1992) and are represented in lowercase. The alignment was performed using the ClustalW program and was printed with the BOXSHADE program.

2001). Four of them (*Os-EXP1–Os-EXP4*) had already been studied extensively with respect to their sequence, expression, and regulation (Cho and Kende, 1997a, 1997b, 1998). Analysis with the PSORT program (Nakai and Kanehisa, 1992) predicts that α-expansins have a signal peptide for entry into the secretory pathway and secretion to the cell wall. The molecular masses of the mature α-expansin proteins range from 23.1 to 28.4 kD. They possess the characteristic expansin motifs, namely conserved Cys residues in the N-terminal region of the protein, a putative catalytic domain with the His-Phe-Asp (HFD) motif in the central portion of the protein, and conserved Trp residues in the putative cellulose-binding domain in the C-terminal region (Fig. 1). Rice α-expansins are somewhat less divergent from each other (55% average amino acid identity between mature proteins; Table I) than are rice β-expansins (51% average amino acid identity between mature proteins; Table I; Lee and Kende, 2001).

Sequence Analysis of *EXPL* and *EXPR* Genes of Rice

We identified five rice genes that show significant amino acid identity to α- and β-expansins but lack some of the conserved features of expansins (Fig. 1; Table I). The derived amino acid sequences of these five rice genes are similar to those of four Arabidopsis genes. Phylogenetic analysis indicates that the Arabidopsis genes belong to two new subfamilies of the expansin superfamily. They were named *EXPL* (*EXPANSIN-LIKE*) and *EXPR* (*EXPANSIN-RELATED*; D.J. Cosgrove, http://www.bio.psu.edu/expansins; Lee et al., 2001). In analogy to the Arabidopsis genes, we designated four of the rice genes as expansin like (*Os-EXPL1–Os-EXPL4*) and one as expansin related (*Os-EXPR1*). The deduced Os-EXPL proteins show 50% average amino acid identity to each other (Table I). They contain conserved Cys residues in the N-terminal and conserved Trp residues in the C-terminal regions, and an ambiguous signal peptide. In addition, all EXPL proteins have from one to three NXT/S motifs, which may be N-linked glycosylation sites. They lack, however, the HFD motif in the putative catalytic domain of the protein; one otherwise conserved Trp is missing, and the positions of the other Trp residues are different from those in α- and β-expansins. EXPL proteins have additional Trp residue in the C-terminal and two additional Cys residues in the N- and C-terminal regions (Fig. 1).

The *EXPR* gene in the rice genome shows 40% amino acid identity to the only *EXPR* gene of Arabidopsis (protein accession no. CAB80974.1). The deduced Os-EXPR protein has the conserved Cys residues in the N-terminal and the conserved Trp residues in the C-terminal region, three NXT/S motifs, and a signal peptide for secretion to the cell wall (Fig. 1). It lacks the HFD motif in the center of the protein, and two otherwise conserved Trp are missing in the C-terminal region. The major difference between EXPL and EXPR proteins is in the C- and N-terminal regions. EXPR of both Arabidopsis and rice lack the amino acid extensions that are present at the C and N terminus of EXPL proteins and that contain two conserved Cys residues each (Fig. 1).

Table I. *Average percent identity of amino acid sequences between members of each expansin family of rice*

Values were calculated using the amino acid sequences without the signal peptides. Nos. of the proteins are 26, 14, 4, and 1 for Os-EXP, Os-EXPB (Lee and Kende, 2001), Os-EXPL, and Os-EXPR, respectively. NA, Not available because there is only one EXPR gene.

Family	Os-EXP	Os-EXPB	Os-EXPL	Os-EXPR
Os-EXP	55	–	–	–
Os-EXPB	21	51	–	–
Os-EXPL	15	20	50	–
Os-EXPR	17	23	28	NA

Organ-Specific Expression of α-Expansin and *EXPL* Genes in Deepwater Rice

To determine which of the recently identified α-expansin, *EXPL*, and *EXPR* genes are expressed in deepwater rice, we prepared gene-specific probes consisting mainly of the 3′-untranslated regions (UTRs) of the respective genes (Table II). When no cDNA was available, the gene-specific regions were amplified by RT-PCR from mRNA or by PCR from genomic DNA. DNA gel-blot analyses performed under the same stringency conditions as used for the RNA gel blots showed that, with one exception, the probes were specific and that no detectable cross hybridization to other genes occurred (Fig. 2). A probe corresponding to the 3′-UTR region of *Os-EXPL4* (accession no. AP003624) cross-reacted with multiple bands on a DNA gel blot (results not shown). Because of this lack of specificity, no expression studies for *Os-EXPL4* were performed. However, the presence of two ESTs in the database of *Indica* rice indicates that the *Os-EXPL4* gene is expressed (Yu et al., 2002; http://210.83.138.53/rice). The transcript levels of the recently identified 22 α-expansin, three *EXPL*, and one *EXPR* genes were examined in the basal 1-cm region of the uppermost internode, which contains the intercalary meristem and the elongation zone; in the basal 1-cm region of GA-treated and control internodes; in the basal 5-cm region of elongating leaves; in the apical 1-cm region of coleoptiles from 3-d-old seedlings; and in the apical 0.5-cm region of roots from 3-d-old seedlings (Fig. 3).

Of the 22 recently identified α-expansin genes, 10 (*Os-EXP5*, *Os-EXP6*, *Os-EXP7*, *Os-EXP10*, *Os-EXP11*, *Os-EXP12*, *Os-EXP13*, *Os-EXP15*, *Os-EXP16*, and *Os-EXPB17*) are expressed in deepwater rice. *Os-EXP5* and *Os-EXP10* are expressed in all organs tested; *Os-EXP6* is expressed in all organs tested except in roots; *Os-EXP7* and *Os-EXP16* are expressed in all organs tested except in coleoptiles; and *Os-EXP11*, *Os-EXP12*, *Os-EXP13*, *Os-EXP15*, and *Os-EXP17* are expressed in the roots only. *Os-EXPL1* is highly expressed in the root and coleoptile, *Os-EXPL2* in the internode, and *Os-EXPL3* in the internode, leaf, coleoptile, and root. We could not find any transcripts of the *Os-EXPR1* gene, and no *Os-EXPR1* cDNA exists in the database.

Expression of α-Expansin and *EXPL* Genes in Different Developmental Regions of Internodes

In rice, as in other grasses, stem elongation occurs at the base of the highest internode, just above the second highest node. Five of the recently identified α-expansin genes—*Os-EXP5*, *Os-EXP6*, *Os-EXP7*, *Os-EXP10*, and *Os-EXP16*—are expressed in the highest internode and in the subtending node of intact plants (Fig. 4). The transcript level of three α-expansins—*Os-EXP6*, *Os-EXP7*, and *Os-EXP16*—is highest in the basal region of the internode, 0 to 1 cm above the second highest node. This region contains the intercalary meristem and the elongation zone (Kende et al., 1998). *Os-EXP5* is expressed at the highest level in the second highest node. Except for *Os-EXP10*, α-expansin mRNA was not detected beyond the 1-cm zone above the node. *Os-EXP10* is evenly expressed in all regions of the internode. *Os-EXPL1* showed highest expression in the nongrowing regions of the internode, whereas expression of *Os-EXPL2* and *Os-EXPL3* was most pronounced in the second highest node and decreased with distance from the node (Fig. 4).

Expression of α-Expansin and EXPL Genes and Elongation in Different Developmental Regions of Leaves

In grasses, such as rice, only the youngest leaves grow, and elongation occurs mainly at the base of the leaf, just above the leaf collar. Seven α-expansin genes—*Os-EXP1*, *Os-EXP2*, *Os-EXP5*, *Os-EXP6*, *Os-EXP7*, *Os-EXP10*, and *Os-EXP16*—were expressed in the youngest leaf of intact plants (Fig. 5A). Except for *Os-EXP7*, all α-expansins showed highest expressions in the 2- to 7-cm region above the collar. The expression of *Os-EXP7* was highest in the 0- to 2-cm region above the collar. The expression level of α-expansin was very low or undetectable in the nonelongating region of the leaf. The expression pattern of *Os-EXPL3* was similar to that of α-expansins. In contrast, the transcript level of *Os-EXPL1* was highest in the nonelongating regions and that of *Os-EXPL2* remained high in the nonelongating regions.

We also measured the elongation of various regions of rice leaves (Fig. 5B). Most elongation occurred in the region 0 to 1 cm above the collar and gradually decreased with distance from the collar. No growth was recorded 7 cm above the collar.

Expression of α-Expansin and *EXPL* Genes in Response to GA Treatment

We tested the effect of GA on the expression of five α-expansin genes (*Os-EXP5*, *Os-EXP6*, *Os-EXP7*, *Os-EXP10*, and *Os-EXP16*) and three *EXPL* genes (*Os-EXPL1*, *Os-EXPL2*, and *Os-EXPL3*) in the internode (Fig. 6, A and B). The time course and magnitude of induction by GA varied according to genes. Transcripts of the above α-expansin genes, except those of *Os-EXP10*, accumulated gradually during the 24 h of incubation. The level of *Os-EXP10* mRNA increased rapidly during the first 12 h of incubation and leveled off thereafter. The expression level of *Os-EXPL3* increased slightly as a result of treatment with GA, but, interestingly, the expression levels of *Os-EXPL1* and *Os-EXPL2* decreased after treatment with GA. These results confirm those of an independently performed experiment shown in Figure 3. The effect of GA on the expression of individual α-expansin and *EXPL*

Table II. *Primer sets used for the PCR amplifications of gene-specific probes of α-expansins, EXPL, and EXPR genes of rice*

Gene Name	Primer Sequence (5′ to 3′)	Template	Product Size	Accession No.	
				cDNA	Genomic DNA
			bp		
Os-EXP1	CTCGACCTCACAGCAGTTCTCTTA TCATCGATTGGCAAGCACCTC	S5074[a]	291	AF261270	AF394543
Os-EXP2	GGCTAATTCCGTTTTTCAGTT TGCTCCCCCACAATCTCC	R1518[a]	369	U30477	AF394544
Os-EXP3	GTCGCCCCGTCCAACTGGTTC AATTGGTGGGCAAAACATTCA	R0448[a]	268	U30479	CL006919.165[b]
Os-EXP4	CCAGTTCTAGCCGCCACCGACATC ATTCCGTTGCAAGGCCATCACTCC	cDNA clone	388	U85246	AF394545
Os-EXP5	TTCCGCTCAATTTTACTCG TTTTTTTTTTTTTTTTTTTTTTA	E31402[a]	505	AF247162	AF394546
Os-EXP6	TAGATTAGTTTAGCCAAGAGG TTTTTTTTTTTTTTTTTTTTTTA	E60552[a]	429	AF247163	CL014794.24[b]
Os-EXP7	AAAACTGCAATATACCCTCTTA TTTTTTTTTTTTTTTTTTTTTTC	E31204[a]	335	AF247164	AF394547
Os-EXP8	CATTAATTGCAAGCCTATCTCA GAGAGGGAGTATGCCGAATGT	Reverse transcriptase (RT) product of mRNA	320	NA[c]	AC007789
Os-EXP9	CAAATTGGGCTTAGTTCAGGT CTCTGAAAACCGTTGCAAATC	RT product of mRNA	198	NA	AC007789
Os-EXP10	GAGAGGAAAGGGTACCAATAGCA TTTTTTTTTTTTTTTTTTTTTTA	C61881[a]	332	AF247165	AL662987
Os-EXP11	GCCAGTTCTAGCCGTTCC CATTGCGCTCGTCACCACT	RT product of mRNA	276	NA	AP000837
Os-EXP12	ACCAGCGGCGTGCAGTTCTAC AGGCATGGCGGGCTATCG	Genomic DNA	296	AY046929	AF394548
Os-EXP13	TTCGCGAGCAACATACAG TACATCGCACATACATACAAAAG	Genomic DNA	254	NA	AF394549
Os-EXP14	CAAGCTGCAATTCAAGTAA GATAGTTCGGGAGGGTAA	Genomic DNA	376	NA	AF394550
Os-EXP15	ATAAGGCTGCAGATTGAAGAAG CACGGTAGAAATGACTGGTAGC	Genomic DNA	303	NA	AF394551
Os-EXP16	AAGGCAAGCAGTTCGTC AGTTTATGCACCTCTATTATGTC	Genomic DNA	334	NA	AF394552
Os-EXP17	CCAGGGATCCAACAACTTCTACTA CGGATCGGATATACATAAACCAAT	Genomic DNA	498	NA	AP000616
Os-EXP18	TTCGGGCAAACCTTCAGC TTGCAACTTAATTTACATCCATCA	Genomic DNA	233	NA	AF394553
Os-EXP19	CGGCCAAACATTCAGCACCTACCA CAAACGGGGCCGAAGAAACCACTA	Genomic DNA	297	NA	AF394554
Os-EXP20	CGGCCAAACCTTCAGCACCTACCA GCCTTTAATTGCCACTCTGCTTCC	Genomic DNA	365	NA	AF394555
Os-EXP21	CGCGCCTAGAACGATGATGTATGT GGTGGTTGGGGTAAAATGGAAAGA	Genomic DNA	404	NA	AF394556
Os-EXP22	TTGGGTTTCTACTGCCTGACTGAT CGCGTTGCAACTGTTTTTCTTAT	Genomic DNA	493	NA	AF394557
Os-EXP23	TGATACCGGAGCGTTTCTTTTG CCTGCGTGGTGTGGACTATGA	Genomic DNA	316	NA	AF394558
Os-EXP24	TTCTACGGCCTGCGACTGATT TGTTCCCCTTTTATTTATGATTTG	Genomic DNA	280	NA	AF394559
Os-EXP25	GACATTCACCAGCAACCAG GTACGTAACAGCAGTCCTCTCCT	Genomic DNA	337	NA	AF394560
Os-EXP26	CTACCAGGCCAAGAAGAA TTAGTAAATACGCAGAGGAT	Genomic DNA	171	AY100692	AF394561
Os-EXPL1	ACGCACGAGTGGAAGTAGAAGC GAAGAAGAAGAAACGGAGGAGGAA	Genomic DNA	188	AY100693	AY039022
Os-EXPL2	ACGCAGGAGTGGAAGTGACA CACCCCGAGTCGAGTAAACAACAC	Genomic DNA	214	NA	AC051633
Os-EXPL3	CTGGAAGTGAAAGGTGTGCTA TAGCAATTAGTATGGGGGTAGC	Genomic DNA	344	AY100694	AP004315
Os-EXPR1	CTTGGCTTGTTTCACCTCTCCTAT CTTTATGCATTTCCGAGTTTA	Genomic DNA	317	NA	AP003956

[a] Expressed sequence tag clone no. [b] Contig no. of genomic DNA from Torrey Mesa Research Institute database (Goff et al., 2002; http://portal.tmri.org/rice/). [c] NA, Not available.

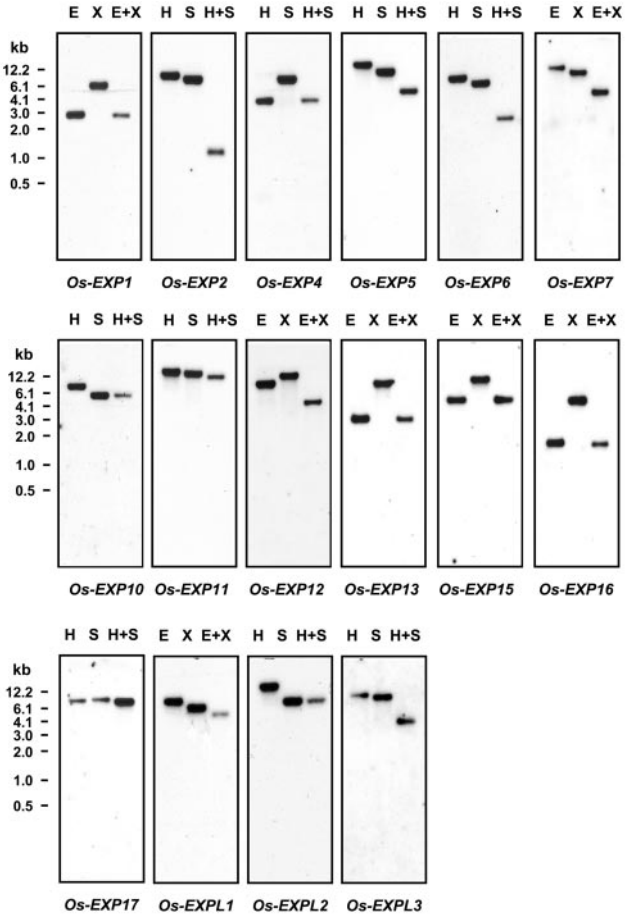

Figure 2. DNA gel-blot analyses showing the specificity of the gene-specific probes. Genomic DNA was digested with *Eco*RI (E), *Xba*I (X), *Hin*dIII (H), *Sac*I (S), *Eco*RI and *Xba*I (E+X), or with *Hin*dIII and *Sac*I (H+S). The digested DNA was separated by gel electrophoresis, blotted onto a Hybond N^+ membrane, and hybridized to the gene-specific probes indicated under each blot under the same conditions as described for RNA gel-blot analysis. We are only showing the DNA gel-blot analysis of those genes whose expression was detected in deepwater rice.

genes was confirmed in two to four independent experiments.

The Time Course of α-Expansin Gene Expression in Internodes during Incubation of Stem Sections in Water

The expression of α-expansin genes increased in internodes after excision of stem sections. We determined the time course of this increase during incubation in water and found that the expression pattern differed among the eight genes that are expressed in the internode after isolation of the sections (Fig. 7, A and B). The stem sections used to investigate the effect of GA on α-expansin and *EXPL* transcript accumulation were first incubated in water for 8 h to dissipate the effect of excision before GA was applied. The effect of excising stem sections on the

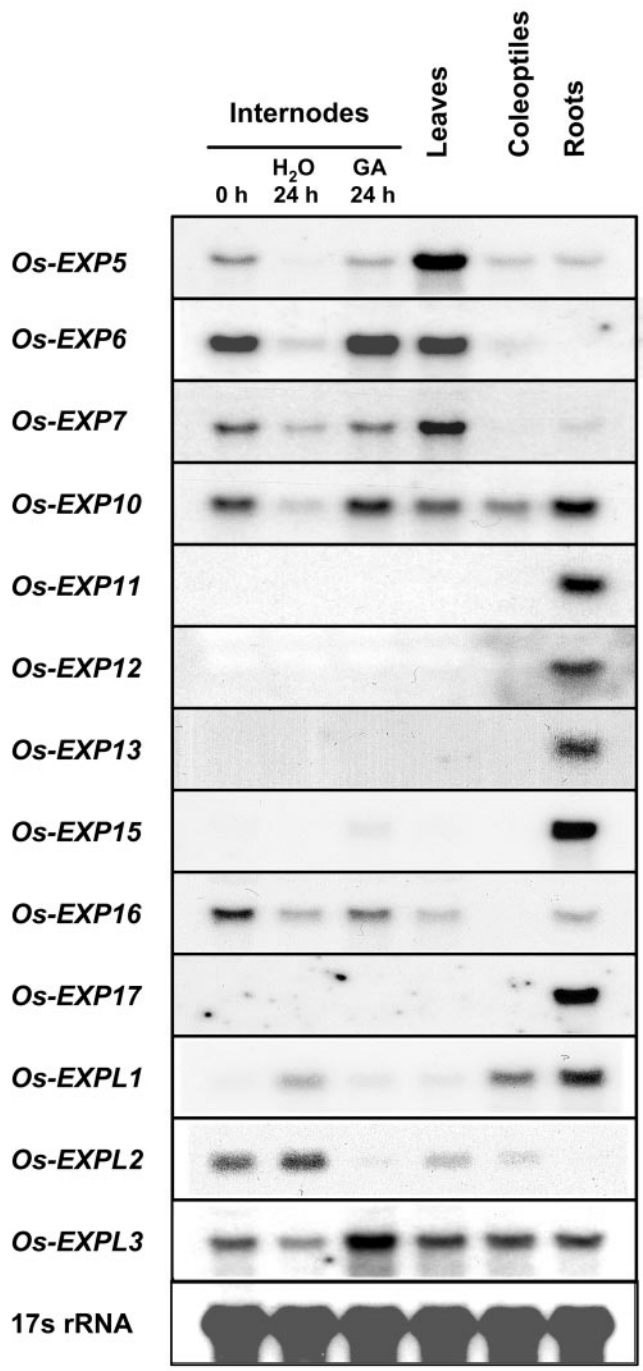

Figure 3. RNA gel-blot analysis of α-expansin and *EXPL* gene expression in internodes, leaves, coleoptiles, and roots of deepwater rice. Each lane contained 20 μg of total RNA isolated from the organs indicated above the lanes. Internodes, 0 to 1 cm above the second highest node of the youngest, growing internode of adult plants; to test the effect of GA, excised 20-cm-long stem sections were incubated with or without 50 μM GA_3 for 24 h; leaves, 0 to 5 cm above the basal collar of growing leaves of adult plants; coleoptiles, 0 to 1 cm below the tip of growing coleoptiles of 3-d-old seedlings; roots, 0 to 0.5 cm above the tip of growing roots of 3-d-old seedlings. 17s rRNA was used as internal loading control.

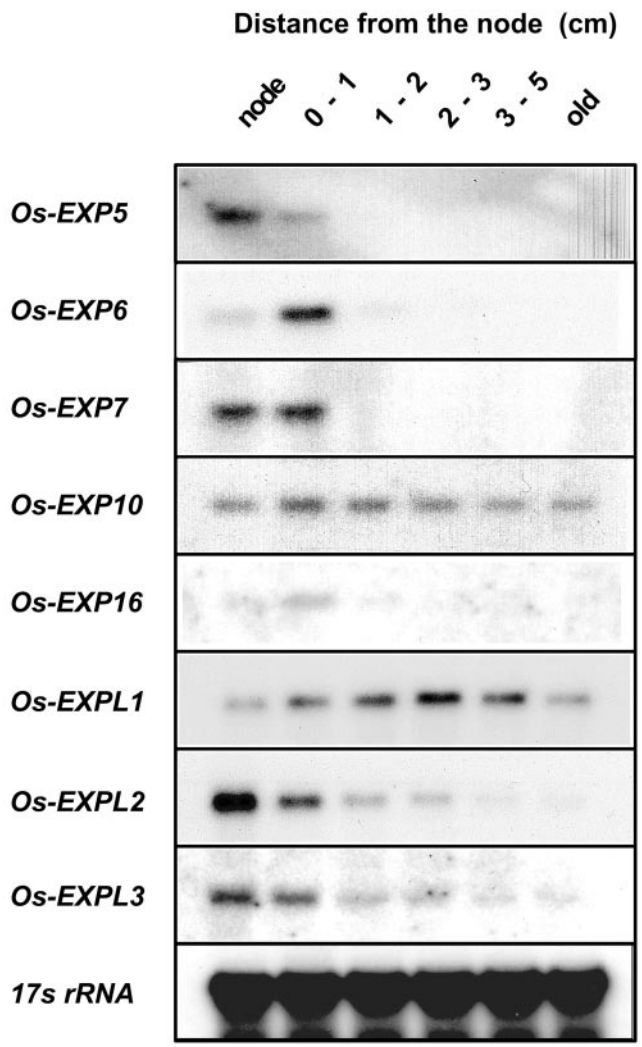

Figure 4. RNA gel-blot analysis of α-expansin and *EXPL* gene expression in the second highest node and in different regions of the uppermost internode. Each lane contained 20 μg of total RNA isolated from the node and the internodal regions indicated above the lanes. Node, Second highest node; 0 to 1 cm, internodal region containing the intercalary meristem and most of the elongation zone; 1 to 2 cm, internodal region containing the upper part of the elongation zone and the differentiation zone; 2 to 3 cm and 3 to 5 cm, internodal regions containing the differentiation zone; old, oldest part of the internode 5 cm above the node. 17s rRNA was used as internal loading control.

expression of individual α-expansin genes was confirmed in two to three independent experiments.

The Time Course of α-Expansin Expression in Internodes of Whole Plants in Response to Wounding

To examine whether the enhanced expression of α-expansin genes in excised stem sections was the result of wounding, we determined the level of *Os-EXP* transcripts in internodes of whole plants that had been wounded by piercing six pinholes into the stem 2 cm below the second highest node where the stem section would have been excised. Transcripts of all eight *Os-EXP* genes accumulated as a result of wounding, but again with varying time courses (Fig. 8, A and B). The expression of the *Os-EXP* genes declined after 9 h but at a slower rate than in stem sections. The effect of wounding on the expression of individual α-expansin genes was confirmed in two independent experiments.

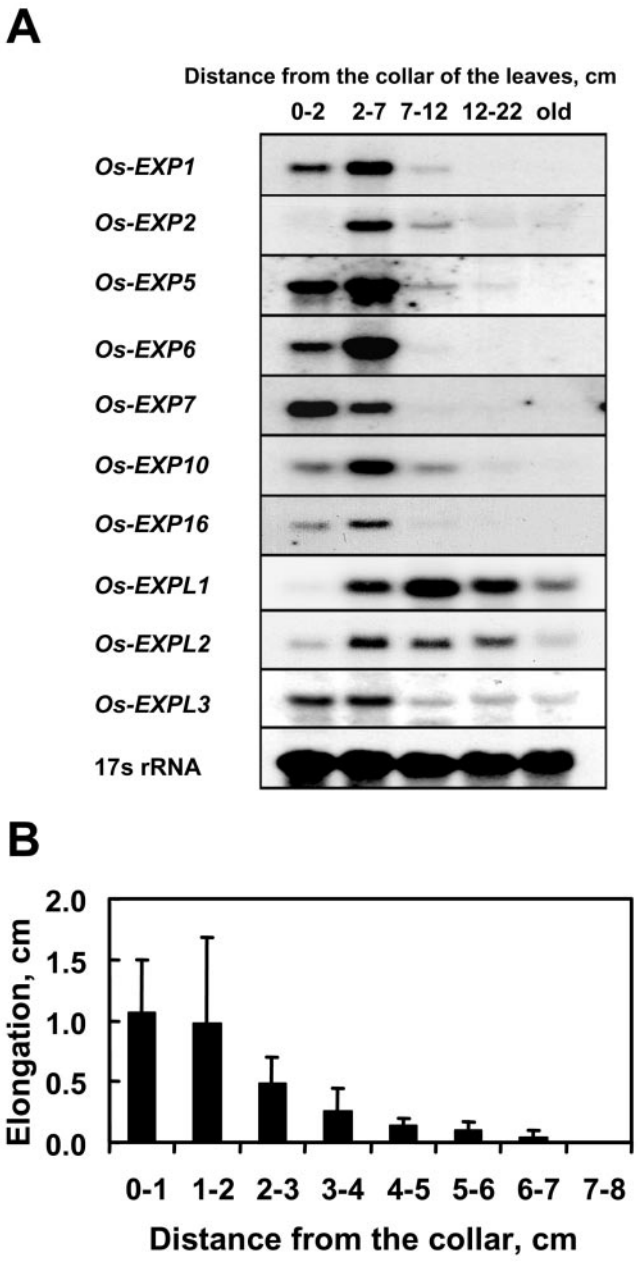

Figure 5. RNA gel-blot analysis of α-expansin and *EXPL* gene expression in the leaves and growth of leaves. A, RNA gel-blot analysis of α-expansin and *EXPL* gene expression in growing rice leaves. Each lane contained 20 μg of total RNA isolated from the regions indicated above the lanes. Old, Oldest part of the leaves 22 cm above the collar. 17s rRNA was used as internal loading control. B, Elongation of each region of the leaves during a 48-h period.

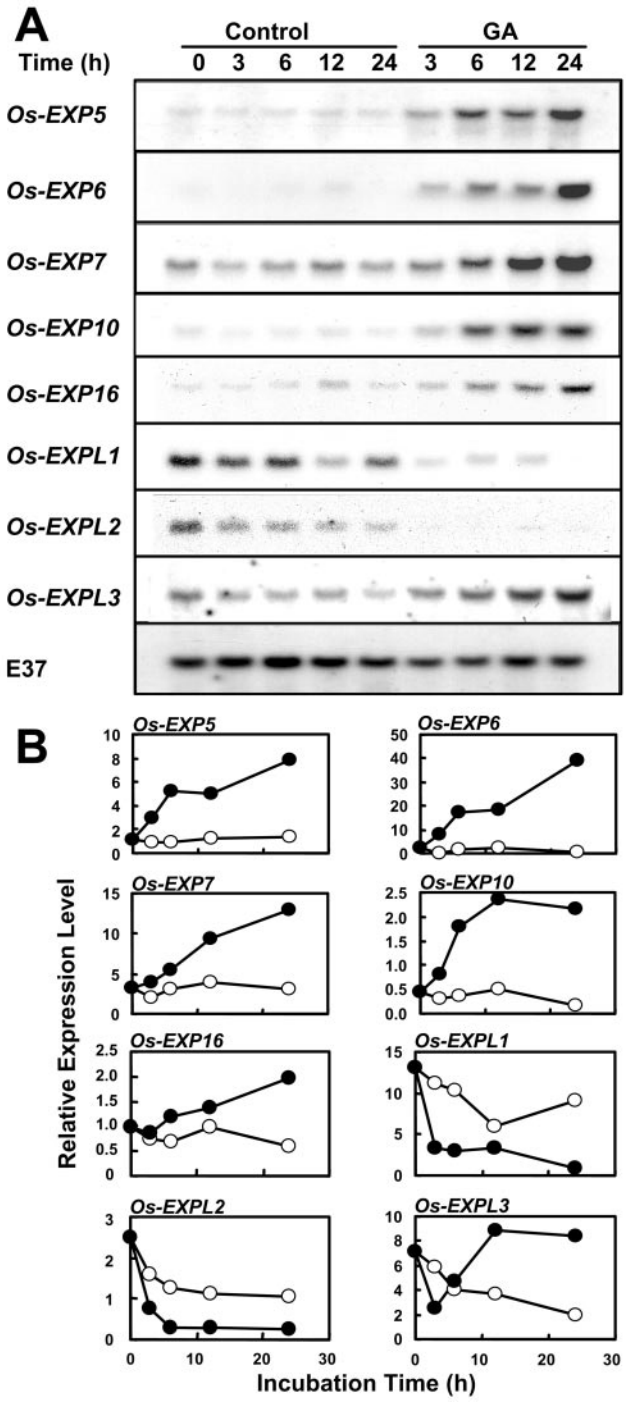

Figure 6. Expression of α-expansin and *EXPL* genes in GA-treated stem sections. Stem sections were first incubated for 8 h in distilled water to dissipate the wound effect and then transferred to 50 μM GA_3 or distilled water (control) for the times indicated above the lanes. A, RNA gel-blot analysis. Each lane contained 20 μg of total RNA isolated from the 0- to 2-cm region above the second highest node, which includes the intercalary meristem, the elongation zone, and the lower part of the differentiation zone. *E37* was used as internal loading control. B, Quantification of transcript levels of each gene. Expression levels are shown in PhosphorImager values × 10^{-3} after 24 h of exposure. ○, Control; ●, GA treatment.

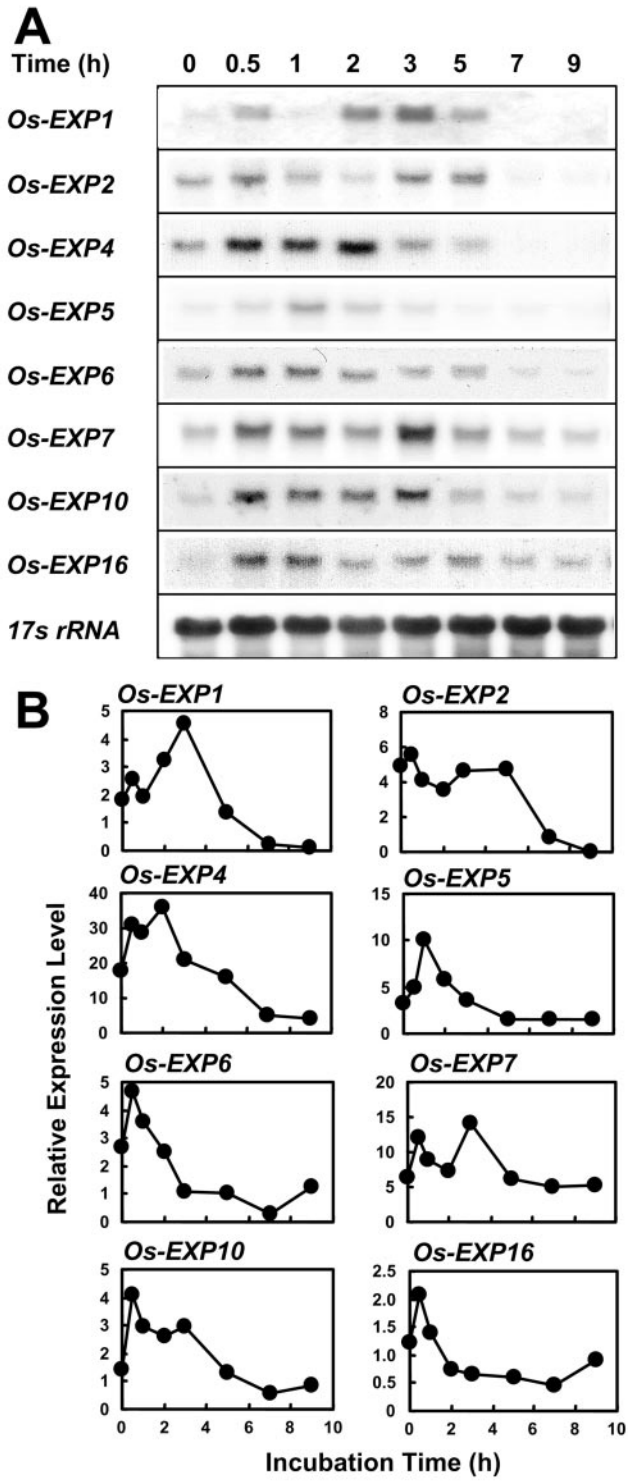

Figure 7. Expression of α-expansin genes in stem sections. Stem sections were excised and incubated in water for the times indicated above the lanes. A, RNA gel-blot analysis. Each lane contained 20 μg of total RNA isolated from the 0- to 2-cm region above the second highest node, which includes the intercalary meristem, the elongation zone, and the lower part of the differentiation zone. 17s rRNA was used as loading control. B, Quantification of the mRNA level of each gene. Expression levels are shown in PhosphorImager values × 10^{-3} after 24 h of exposure.

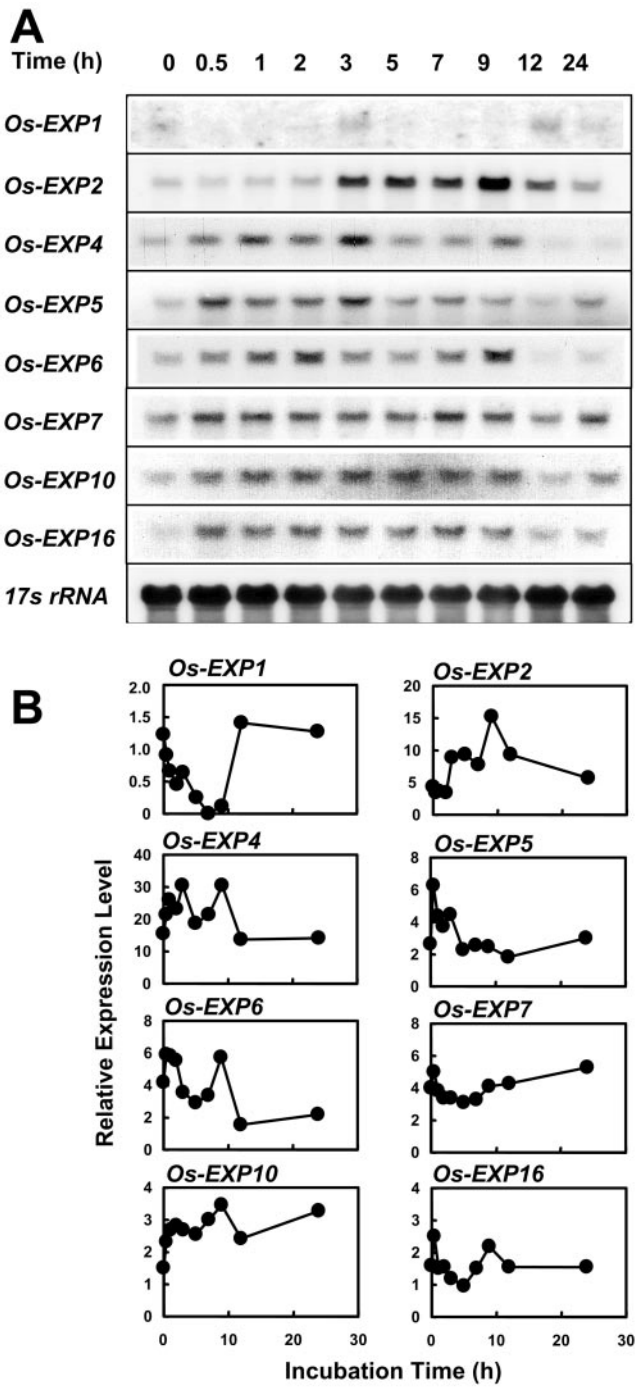

Figure 8. Expression of α-expansin genes in the internodes of wounded plants. The internodes were wounded by piercing pinholes around a circle into the stems 2 cm below the second highest node. The tissue samples were collected at the times indicated above the lanes. A, RNA gel-blot analysis. Each lane contained 20 μg of total RNA isolated from the 0- to 2-cm region above the second highest node, which includes the intercalary meristem, the elongation zone and the lower part of the differentiation zone. 17s rRNA was used as loading control. B, Quantification of the mRNA levels of each gene. Expression levels are shown in PhosphorImager values $\times 10^{-3}$ after 24 h of exposure.

DISCUSSION

In earlier work, we studied the expression pattern and regulation of four α- and 14 β-expansin genes in deepwater rice (Cho and Kende, 1997a, 1997b; Lee and Kende, 2001). Recently, we found 22 new α-expansins in the rice databases (Lee et al., 2001), as well as five genes, which, in analogy to the nomenclature in Arabidopsis (D.J. Cosgrove, http://www.bio.psu.edu/expansins; Lee et al., 2001), we named expansin like (*Os-EXPL*) and expansin related (*Os-EXPR*). These genes are grouped on two separate branches of the phylogenetic tree representing the expansin superfamily (D.J. Cosgrove, http://www.bio.psu.edu/expansins; Lee et al., 2001; Li et al., 2002). Li et al. (2002) proposed a new nomenclature for the expansins and assigned Arabidopsis EXPL and EXPR to the β-expansins. Although purified α- and β-expansins have been shown to possess wall-loosening activity (McQueen-Mason et al., 1992; Cosgrove et al., 1997), no expansin function has been demonstrated for EXPL and EXPR proteins. Because of their distinct amino acid sequences, their separate position on the phylogenetic tree, and because we do not know whether they have wall-loosening activity, we agree with the nomenclature of D.J. Cosgrove (http://www.bio.psu.edu/expansins), as also used by Lee et al. (2001), and refer to these proteins as expansin like and expansin related.

Eight expansin genes are expressed in the root only (Table III). It is interesting to note that there are no internode-, leaf-, or coleoptile-specific expansin transcripts. In our previous study, no expansin transcripts were detected in rice leaves (Cho and Kende, 1997b). In the experiments reported here, we found that seven α-expansin genes are expressed in leaves, including two (*Os-EXP1* and *Os-EXP2*) for which no transcripts had been found in leaves before. The discrepancy between our earlier and present results is because of the differences in the leaf regions examined. In both instances, young, expanding leaves were analyzed. However, Cho and Kende (1997b) determined expansin mRNA levels in the tip zone of the leaf; in the present study, expansin mRNA levels were found to be highest at the base of the leaf.

In most instances, the highest levels of expansin transcripts were found in the most rapidly growing regions of tissues and organs of rice (Cho and Kende, 1997a, 1997b, 1998; Huang et al., 2000; Lee and Kende, 2001). A few exceptions to this correlation have been noted, however. For example, *Os-EXP2* mRNA is also present in nongrowing regions of roots and internodes (Cho and Kende, 1997b). With the exception of *Os-EXP7*, whose transcript level in the leaf is highest 0 to 2 cm above the leaf collar, all other α-expansin genes showed highest expression in the region 2 to 7 cm above the collar (Fig. 5A). This region is still elongating but at a much slower rate than the region 0 to 2 cm above the collar (Fig. 5B). In leaves of the grass *Festuca pratensis*, expression of one

Table III. *Expression of expansin superfamily genes in various organs of rice*

Family	Organs			
	Internodes	Leaves	Coleoptiles	Roots
α-Expansins	Os-EXP1[a]	Os-EXP1	Os-EXP1[a]	Os-EXP1[a]
	Os-EXP2[a]	Os-EXP2	Os-EXP2[a]	Os-EXP2[a]
	Os-EXP4[a]	Os-EXP5	Os-EXP4[a]	Os-EXP3[a,b]
	Os-EXP5	Os-EXP6	Os-EXP5	Os-EXP4[a]
	Os-EXP6	Os-EXP7	Os-EXP6	Os-EXP5
	Os-EXP7	Os-EXP10	Os-EXP10	Os-EXP7
	Os-EXP10	Os-EXP16		Os-EXP10
	Os-EXP16			Os-EXP11[b]
				Os-EXP12[b]
				Os-EXP13[b]
				Os-EXP15[b]
				Os-EXP16
				Os-EXP17[b]
β-Expansins	Os-EXPB3[c]	Os-EXPB4[d]	Os-EXPB3[d]	Os-EXPB2[b,d]
	Os-EXPB4[c]	Os-EXPB12[d]	Os-EXPB4[d]	Os-EXPB3[d]
	Os-EXPB6[c]		Os-EXPB6[d]	Os-EXPB4[d]
	Os-EXPB11[c]		Os-EXPB11[d]	Os-EXPB6[d]
	Os-EXPB12[c]			Os-EXPB8[b,d]
				Os-EXPB11[d]
EXPL	Os-EXPL1	Os-EXPL1	Os-EXPL1	Os-EXPL1
	Os-EXPL2	Os-EXPL2	Os-EXPL2	Os-EXPL3
	Os-EXPL3	Os-EXPL3	Os-EXPL3	

[a] Cho and Kende (1997b). [b] Of the four organs tested, expressed only in roots. [c] Lee and Kende (2001). [d] Y. Lee and H. Kende (unpublished data).

α- and two β-expansin genes was also highest in the zone whose growth rate had diminished (Reidy et al., 2001). It appears likely that occurrence of expansin transcripts in slowly growing or nongrowing tissues reflects expansin functions related to cellular differentiation.

The expression of α-expansin genes in the growing internode of deepwater rice is enhanced by GA (Fig. 6, A and B), by excision of stem sections (Fig. 7, A and B), and by wounding of whole plants (Fig. 8, A and B). The accumulation of α- and β-expansin mRNA in GA-treated tissue is consistent with the notion that expansins are involved in mediating GA-induced rapid internodal elongation in deepwater rice (Cho and Kende, 1997b; Lee and Kende, 2001). Wound-induced expression of β-expansin genes was reported previously (Lee and Kende, 2001). The relative induction of α-expansin gene expression by wounding was not as pronounced as that of β-expansins. The significance of expansin mRNA accumulation in wounded deepwater rice internodes is not known.

The DNA-derived amino acid sequences of *EXPL* and *EXPR* genes show significant amino acid identity to α- and β-expansins, although some of the characteristic motifs of the expansins are missing (Table I; Fig. 1). We could not find any expression of the *EXPR* gene in the rice organs tested, and the expression patterns of the *Os-EXPL1* and *Os-EXPL2* genes are quite different from those of the α- and β-expansins. Expression of both expansin-like genes in rice internodes was down-regulated by GA and was high in the nonelongating region of leaves. These results indicate that the expansin-like proteins do not act in GA-regulated stem elongation of rice and that their function may be different from that of α- and β-expansins.

Our combined results indicate that at least eight α- and five β-expansin genes are expressed in deepwater rice internodes (Table III). With the exception of *Os-EXP1* (Cho and Kende, 1997b), GA enhances their expression, and most, if not all, may be involved in mediating internodal elongation. It is not yet known whether each of the α- and β-expansins has a specific function in growth or whether their functions are largely overlapping. Individual expansins could have different substrate specificities, there may be differences in their biochemical modes of action, and they could act in different cell types of the internode. The significance of expansin gene activation by wounding and the role of the EXPL and EXPR proteins are entirely unknown.

MATERIALS AND METHODS

Growth and Treatment of Plants

Seeds of deepwater rice (*Oryza sativa* L. cv Pin Gaew 56) were obtained from the International Rice Research Institute (Los Baños, Philippines). Plants were grown as described by Stünzi and Kende (1989). The plant material was collected as described previously (Lee and Kende, 2001). Treatment of stem sections with GA_3 and wounding of internodal tissue were performed as described by Lee and Kende (2001). To measure the elongation of leaves, pinholes were pierced through the leaf sheath into the underlying young leaf with a 26-gauge needle starting at the collar and

moving up the leaf sheath at 1-cm intervals. The distance between the pinholes was measured 48 h later.

Isolation of Nucleic Acids

Genomic DNA was isolated according to Dellaporta et al. (1983), and total RNA according to Verwoerd et al. (1989). The PolyATtract kit (Promega, Madison, WI) was used to enrich poly(A^+) RNA; the enriched product is referred to as poly(A^+) RNA.

Preparation of Probes

For RT-PCR, total RNA was isolated from mature plants, and poly(A^+) RNA was purified. One hundred nanograms of poly(A^+) RNA was subjected to RT-PCR using Superscript II (Life Technologies, Rockville, MD) and 1 μL of oligo(dT)$_{18}$ (500 μg mL^{-1}) as a reverse primer. After incubation at 42°C for 50 min and inactivation of the reverse transcriptase for 15 min at 70°C, the reactions were subjected to 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min, in the presence of the gene-specific primer pairs (Table II). PCR was performed with *Taq* DNA polymerase (Promega) according to the manufacturer's instructions in a PTC200 thermal cycler (MJ Research, Watertown, MA). For PCR amplification from plasmid or plant genomic DNA, gene-specific primer pairs (Table II) were used to amplify the fragments containing the putative 3′-UTRs under the conditions given above. The PCR products were purified by gel electrophoresis and cloned into the pGEM-T Easy vector (Promega) for sequencing.

DNA fragments containing the inserts of gene-specific regions of α-expansin, *EXPL*, and *EXPR* genes, of *E37*, and of 17S rDNA were excised from the cloning vectors with restriction enzymes and isolated from agarose gels with a DNA purification system (Wizard PCR Preps, Promega). *E37* is a truncated cDNA encoding parts of a chloroplast inner membrane protein; the *E37* transcript is constitutively expressed in deepwater rice internodes (Van der Knaap and Kende, 1995). 17S rRNA (Zarembinski and Theologis, 1993) and *E37* served as loading controls.

RNA Gel-Blot Analysis

Twenty micrograms of total RNA was separated electrophoretically in a 1.2% (w/v) formaldehyde-agarose gel (Ausubel et al., 1987) and transferred to Hybond N$^+$ membrane (Amersham Pharmacia, Piscataway, NJ). Blots were prehybridized and hybridized as described previously (Lee and Kende, 2001). The radioactivity on blots was visualized by autoradiography using Hyperfilm MP (Amersham Pharmacia). The exposure time was adjusted to the intensity of the signals. The radioactivity associated with the transcripts was quantified by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA) after 24 h of exposure.

DNA Gel-Blot Analysis

Four micrograms of genomic DNA was digested with *Eco*RI, *Hin*dIII, *Sac*I, *Xba*I, *Eco*RI, and *Xba*I, or *Hin*dIII and *Sac*I, separated in an agarose gel (0.8% [w/v]), and transferred to a Hybond N$^+$ membrane. Preparation of the probes and hybridizations were performed as described previously (Lee and Kende, 2001). The radioactivity on blots was visualized by autoradiography using Hyperfilm MP (Amersham Pharmacia). The exposure time was adjusted to the intensity of the signals.

DNA and Amino Acid Sequence Analysis

The nucleotide and deduced amino acid sequences were analyzed with the DNASTAR program (DNASTAR, Madison, WI). The protein localization site was predicted using the PSORT program (Nakai and Kanehisa, 1992). Multiple sequence alignments were performed using the ClustalW (version 1.8) Multiple Sequence Alignment program (http://searchlauncher.bcm.tmc.edu) and printed using BOXSHADE 3.20 (http://www.ch.embnet.org).

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