Expression of Expansin Genes Is Correlated with Growth in Deepwater Rice

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Expansins are a family of proteins that catalyze long-term extension of isolated cell walls. Previously, two expansin proteins have been isolated from internodes of deepwater rice, and three rice expansin genes, Os-EXP1, Os-EXP2, and Os-EXP3, have been identified. We report here on the identification of a fourth rice expansin gene, Os-EXP4, and on the expression pattern of the rice expansin gene family in deepwater rice. Rice expansin genes show organ-specific differential expression in the coleoptile, root, leaf, and internode. In these organs, there is increased expression of Os-EXP1, Os-EXP2, and Os-EXP4 in developmental regions where elongation occurs. This pattern of gene expression is also correlated with acid-induced in vitro cell wall extensibility. Submergence and treatment with gibberellin, both of which promote rapid internodal elongation, induced accumulation of Os-EXP4 mRNA before the rate of growth started to increase. Our results indicate that the expression of expansin genes in deepwater rice is differentially regulated by developmental, hormonal, and environmental signals and is correlated with cell elongation.

INTRODUCTION

Because plants are sessile organisms, it is an adaptive advantage that their growth and development are subject to control by environmental conditions. Regulatory effects of the environment are often mediated by hormonal signals and can therefore be mimicked by application of the appropriate plant growth regulator(s). Greatly accelerated internodal elongation of deepwater rice in response to submergence exemplifies how an environmental stimulus induces growth via a series of hormonal interactions (Raskin and Kende, 1984a, 1984b; Hoffmann-Benning and Kende, 1992). Deepwater rice is a subsistence crop in large areas of Southeast Asia that are inundated by flood waters during the monsoon season. The livelihood of >100 million people depends on the successful cultivation of this rice (Mannan, 1988). The remarkably high growth rates of deepwater rice—up to 25 cm a day (Vergara et al., 1976)—permit this rice to emerge from the rising flood waters and to avoid drowning. The yield of deepwater rice is far below that of improved rice cultivars, which lack elongation capacity (Mannan, 1988). Understanding the molecular basis of the growth response of deepwater rice is necessary for the eventual introduction of increased elongation capacity into high-yielding modern rice cultivars.

The primary signal for enhanced elongation is the reduced partial pressure of O2 in the submerged tissue. Under low O2 tension, ethylene synthesis is enhanced; ethylene renders the internode more responsive to gibberellin (GA) by lowering the level of endogenous abscisic acid (Raskin and Kende, 1984a, 1984b; Hoffmann-Benning and Kende, 1992). GA is the immediate growth-promoting hormone and acts by enhancing cell elongation and cell division activity in the intercalary meristem (Sauter and Kende, 1992; Sauter et al., 1993). Hormonal and environmental stimuli that promote growth of plant cells act by inducing stress relaxation of the primary cell wall, which is thought to be principally a network of cellulose microfibrils interconnected by hemicelluloses (Carpita and Gibeaut, 1993). On the basis of this cell wall model, two wall-loosening processes can be envisioned: breakage of covalent bonds within cross-linking polymers or disruption of noncovalent bonds, such as hydrogen or ionic bonds, between wall polymers. It has been proposed that cleavage of covalent bonds is catalyzed by wall hydrolases or endotransglycosylases (Fry, 1989; Fry et al., 1992; Nishitani and Tominaga, 1992). Current evidence does not support the notion that these enzymes are able to cause long-term extension of isolated cell walls (McQueen-Mason et al., 1993; Cosgrove and Durachko, 1994). Recently, a new group of wall proteins, the expansins, has been identified as wall-loosening factors that can promote long-term extension of isolated cell walls (reviewed in McQueen-Mason, 1995; Cosgrove, 1996). Evidence indicates that expansins cause cell wall loosening by disrupting hydrogen bonds between cellulose microfibrils and matrix polymers (McQueen-Mason and Cosgrove, 1994).

Two expansin proteins have been isolated from deepwater rice internodes (Cho and Kende, 1997a), and three expansin
genes have been identified from rice (Shcherban et al., 1995; Cho and Kende, 1997a). Expansin gene families have also been found in cucumber and Arabidopsis (Shcherban et al., 1995). The redundancy of expansin genes indicates that expression of individual expansin genes may be differentially regulated at various developmental stages and by diverse environmental stimuli. This would permit the plant to fine-tune its response to developmental signals and changes in the environment. We report here on the identification of a fourth rice expansin gene, Os-EXP4, whose expression is highly correlated with growth, and show tissue-specific expression of expansin genes and their developmental, hormonal, and environmental regulation. Our results support the hypothesis that expansins may at least in part determine the growth rate of plant organs. Therefore, increasing the expression of the proper expansin genes may raise the growth potential of high-yielding rice cultivars.

RESULTS

Cloning of Os-EXP4 and Sequence Analysis of the Rice Expansin Gene Family

We have previously isolated two expansin proteins, SP20 and SP29, from rice internodes and have sequenced their N-terminal amino acids (Cho and Kende, 1997a). The N-terminal amino acid sequence of SP20 is identical to that encoded by Os-EXP1. Because six of eight N-terminal amino acids of SP29 match an amino acid sequence deduced from the nucleotide sequence of Os-EXP2, we concluded tentatively that SP29 is encoded by a rice expansin gene that is different from yet very similar to Os-EXP2. To isolate the cDNA coding for SP29, we screened a cDNA library prepared from deepwater rice internodes with a probe from the conserved coding region of Os-EXP2. Positive clones were further screened with gene-specific probes of Os-EXP1 and Os-EXP2. One cDNA clone that did not hybridize with the gene-specific probes was sequenced and shown to represent the new rice expansin gene Os-EXP4 (GenBank accession number U85246).

Os-EXP4 consists of 1229 bp, with an open reading frame of 738 bp, 54 bp of the 5' flanking sequence, and 437 bp of the 3' flanking sequence. The open reading frame encodes a protein of 246 amino acids, with a signal peptide predicted by the PSORT program (Nakai and Kanehisa, 1992) (Figure 1A). The mature protein of 228 amino acids has a calculated molecular mass of 24 kD, which agrees well with the molecular mass of SP29 as determined by SDS-PAGE (Cho and
### Figure 2. Gene-Specific Rice Expansin cDNA Probes.

(A) The percentage of nucleotide identity of the gene-specific rice expansin cDNA probes.

(B) DNA gel blot analysis showing the specificity of the gene-specific probes. Plasmid DNAs given above the blots were digested with restriction enzymes, subjected to electrophoresis, blotted, and hybridized under stringent conditions with the gene-specific probes indicated at left.

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Figure 3. DNA Gel Blot Analysis of the Expansin Gene Family in the Rice Genome.

DNA gel blot analysis was used to estimate the complexity of the expansin gene family in the rice genome (Figure 3). Each gene-specific probe detected a single band, indicating that the four known rice expansin genes exist as single copies (Figures 3A to 3D). Because some probes contained short stretches from the coding region and because relatively low-stringency conditions were used (55°C washing temperature), some blots showed additional faint bands whose sizes corresponded to known expansin bands. Os-EXP3 has a BamHI site in the middle of the coding region. Therefore, a band (~1 kb) shown in lane B+H in Figure 3C corresponds to the 3' half of the gene. A probe prepared from the 5' flanking and coding region of Os-EXP3 detected the 5' half (~1.3 kb) of the gene (data not shown). The DNA gel blot analysis performed with a probe from the coding region of Os-EXP1 showed the four expansin genes and several unidentified bands (Figure 3E, lane E). The bands of ~10.3 and 6.3 kb (Figure 3E, lane E) could be detected in other gel blot analyses that were performed with probes from the coding regions of other expansin cDNAs (results not shown). These additional bands may represent other expansin genes or related genes, such as those encoding pollen

Kende, 1997a). The deduced amino acid sequence of Os-EXP4 indicates that it indeed encodes SP29 (Figure 1A). Eight N-terminal amino acids of SP29 match those encoded by Os-EXP4. At position 8, amino acid sequencing yielded both T and S residues; the amino acid deduced from Os-EXP4 is serine. The cleavage sites of the signal peptides of Os-EXP1 and Os-EXP4, predicted by the PSORT program, are consistent with the N-terminal amino acid sequences of SP20 and SP29. The deduced amino acid sequences of the rice expansin family show that Os-EXP2 and Os-EXP4 are most closely related to each other, with an amino acid identity of 85.8%; Os-EXP3 is least related to the other rice expansin genes and also to all other known plant expansin genes (Figure 1B).

To investigate the differential expression of each rice expansin gene in various rice tissues, gene-specific probes were prepared consisting mainly of the 3' untranslated regions of the respective cDNAs. These regions have 21.4 to 26.4% nucleotide identity to each other (Figure 2A). DNA gel blot analyses performed under the same conditions as used for RNA gel blotting (65°C washing temperature) showed that all four probes were indeed gene specific (Figure 2B).

Genomic Analysis

DNA gel blot analysis was used to estimate the complexity of the expansin gene family in the rice genome (Figure 3). Each gene-specific probe detected a single band, indicating that the four known rice expansin genes exist as single copies (Figures 3A to 3D). Because some probes contained short stretches from the coding region and because relatively low-stringency conditions were used (55°C washing temperature), some blots showed additional faint bands whose sizes corresponded to known expansin bands. Os-EXP3 has a BamHI site in the middle of the coding region. Therefore, a band (~1 kb) shown in lane B+H in Figure 3C corresponds to the 3' half of the gene. A probe prepared from the 5' flanking and coding region of Os-EXP3 detected the 5' half (~1.3 kb) of the gene (data not shown). The DNA gel blot analysis performed with a probe from the coding region of Os-EXP1 showed the four expansin genes and several unidentified bands (Figure 3E, lane E). The bands of ~10.3 and 6.3 kb (Figure 3E, lane E) could be detected in other gel blot analyses that were performed with probes from the coding regions of other expansin cDNAs (results not shown). These additional bands may represent other expansin genes or related genes, such as those encoding pollen...
Organ-Specific Expression of Expansin Genes

The transcript levels of the four expansin genes were examined in the expanding region of young leaves from 11- to 13-week-old air-grown plants; in the basal 1-cm region of the uppermost internode, which contains the intercalary meristem and elongation zone from the same adult plants; and in the apical region of coleoptiles and roots from 3-day-old seedlings (Figure 4). Air-grown mature plants grow slowly, whereas the coleoptile and root of seedlings elongate at a fast rate. In the coleoptile and internode, Os-EXP1, Os-EXP2, and Os-EXP4 were expressed, but no detectable Os-EXP3 signal was found in either tissue. In the root, all expansin genes were expressed. This was the only tissue in which Os-EXP3 mRNA was found. Only Os-EXP2 transcript could be detected at low abundance in the growing zone of leaves. Expansin mRNAs accumulated to higher levels in the apical regions of coleoptiles and roots than in the more slowly expanding tissues of internodes and leaves. The transcript level of E37, which encodes a major 37-kD protein of the inner plastid envelope (Teyssier et al., 1996), served as control for the quantity of total RNA loaded because its expression remains constant in rice internodes induced to grow rapidly by GA (Van der Knaap and Kende, 1995); it is also of similar magnitude in root and coleoptile apices. In leaves, which contain more plastids than do other organs, the abundance of E37 mRNA was increased. Therefore, 17S rRNA was used as an additional internal loading control.

Expression of Expansin Genes in Different Developmental Regions of Coleoptiles, Roots, and Internodes

Os-EXP1 and Os-EXP4 were expressed at higher levels in the apical 1-cm region of the coleoptile, where growth occurs, than in the adjacent basal region; the transcript level of Os-EXP2 was similar in both regions (Figures 5B and 5C). In the root, expression of Os-EXP1, Os-EXP3, and Os-EXP4

![Figure 4. Tissue-Specific Expression Pattern of Rice Expansin Genes.](image)

Each lane contains 20 µg of total RNA isolated from the basal 1-cm region of uppermost internodes, expanding leaves of mature plants, and the apical region of coleoptiles and roots. The transcript levels of E37 encoding a plastid inner envelop protein and 17S rRNA served as internal loading controls.

![Figure 5. Differential Expression of Rice Expansin Genes in the Apical and Basal Regions of the Coleoptile.](image)

(A) The apical and basal regions of the coleoptile from dark-grown 3-day-old rice seedings. (B) RNA gel blot analysis. Each lane contains 20 µg of total RNA isolated from each region. E37 was used as the internal loading control. (C) Quantification of mRNA levels of each expansin gene. Blots shown in (B) were quantified with a PhosphorImager, and the relative mRNA levels were calculated by setting the value for the apical region to 100.
transcripts was confined to the apical 5-mm region, which corresponds to the growing zone (Figures 6B and 6C). Os-EXP2 mRNA also accumulated mainly in the apical region but was expressed in the root hair zone as well. The level of E37 mRNA was lower in the root hair zone than in the apical region, but the abundance of 17S rRNA remained constant along the root, confirming equal loading of total RNA. In air-grown internodes, Os-EXP1 and Os-EXP4 were expressed at the highest levels in the basal growing zone, with transcript levels declining in the nongrowing upper regions (Figures 7B and 7C). The mRNA abundance of Os-EXP2 showed the opposite tendency; it was much lower in the basal growing zone than in the upper nongrowing regions.

Differential Cell Wall Extensibility along the Developmental Regions of the Coleoptile, Root, and Internode

To correlate expansin action and expression of expansin genes, we examined acid-induced extensibility of isolated cell walls from the same developmental regions that were
used in the gene expression studies. Extensibility of the cell wall from the apical region of the coleoptile was approximately twofold higher than that from the basal region (Figure 8A). In the root, wall extensibility was entirely confined to the apical 5-mm region (Figure 8B). In internodes, cell wall extensibility also declined sharply from the growing to the nongrowing region (Figure 8C).

**Accumulation of Expansin Transcripts in Response to GA and Submergence**

We compared the levels of the three internodal expansin transcripts in air-grown (control), submerged, and GA-treated internodes from 11- to 13-week-old plants (Figures 9A and 9B). Os-EXP1 mRNA levels decreased at the beginning of incubation, probably as a result of excising the stem sections that contain the growing internode. Between 24 and 48 hr of incubation, the transcript level of Os-EXP1 returned to its initial value in GA-treated internodes; in submerged internodes, it returned to approximately half of its original value. Os-EXP2 mRNA accumulated gradually during 48 hr of treatment with GA but rapidly between 3 and 6 hr of submergence. In contrast, the abundance of Os-EXP4 transcript increased rapidly within 6 hr of incubation with GA and within 3 hr of submergence.

Because the level of Os-EXP4 transcript increased earlier than that of Os-EXP2 in response to both treatment with GA and submergence, we determined the time course of Os-EXP4 mRNA accumulation during the first 6 hr of submergence and incubation in GA (Figure 10). Under both experimental conditions, the transcript level of Os-EXP4 increased within 30 min and reached a maximum after 3 hr.

**DISCUSSION**

**Conservation of Expansins during Evolution**

The rice expansin gene family does not represent a separate monocotyledonous branch in the phylogenetic tree of known
Expansins (Figure 1B). The closely related rice expansins Os-EXP2 and Os-EXP4 form a subfamily with a Pinus taeda expansin (Pt-EXP1), a cucumber expansin (Cs-EXP1), and an Arabidopsis expansin (At-EXP2). The high similarity of expansins from a gymnosperm and from monocotyledonous and dicotyledonous angiosperms is evidence for the conservation of this family of proteins during the evolution of seed plants. Os-EXP1 and another cucumber expansin, Cs-EXP2, are closely related, whereas Os-EXP3 showed least similarity to other expansins.

Acid-induced cell elongation occurs in algae, mosses, ferns, gymnosperms, and angiosperms (Taiz, 1984; Cosgrove, 1996). Because expansins mediate extension of isolated plant cell walls under acid conditions, it is conceivable that they play a role in cell enlargement of both lower and higher plants. Even though phylogenetically separate organisms have cell walls of different composition, expansins may act by a common mechanism suggested for higher plant cell walls, namely, by disrupting hydrogen bonds between load-bearing wall polymers (McQueen-Mason and Cosgrove, 1994).

Differential Expression Pattern of Expansin Genes

Expansin genes are differentially expressed in the major vegetative parts of rice plants, and their mRNAs are most

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**Figure 9.** Accumulation of Rice Expansin Transcripts in GA-Treated and Submerged Internodes.

(A) RNA gel blot analysis. Each lane contains 20 μg of total RNA isolated from the basal 2-cm region of the uppermost internodes that had been treated with 50 μM GA3, submerged, or kept in air for 0 to 48 hr. E37 served as internal control.

(B) Quantification of the mRNA levels of each gene. Blots shown in (A) were quantified with a PhosphorImager.
Os-EXP2 and the other expansin genes leads us to suggest a distinct role for the Os-EXP2 protein. The nongrowing regions of the internode and the root are both differentiating tissues. Therefore, Os-EXP2 may play a role in the differentiation of the vascular system and perhaps in the differentiation and growth of root hairs. In maize roots, cell wall proteins extracted from the basal region showed higher extension activity than did cell wall proteins from the apical region (Wu et al., 1996). It is possible that an expansin with a function(s) similar to that of Os-EXP2 is expressed at an elevated level in the basal region of the primary root of maize as well.

We have previously reported on the highly localized occurrence of expansin protein along the inner epidermal layer and around immature vascular bundles of rice internodes (Cho and Kende, 1997b). We do not know the role of expansins in those tissues, but the high concentration of expansins in specific cells further supports the notion that certain expansins, for example, those associated with the developing vasculature, may have physiological functions other than promoting cell elongation. McQueen-Mason and Cosgrove (1995) showed that the two cucumber expansins have slightly different pH dependence and cell wall rheological effects, which may indicate differences in functions, and Rose et al. (1997) have identified a family of expansins that may play a role in cell wall disassembly of ripening fruits. Thus, there is mounting evidence that besides mediating cell wall extension, expansins may also function in other processes involving cell wall modifications. However, the biochemical mechanism of expansin action, namely, disruption of hydrogen bonds between cell wall polymers, may be common to all expansin-mediated reactions.

Expansin Gene Expression Is Correlated with Rapid Internodal Growth of Deepwater Rice Induced by Submergence and GA

The response of deepwater rice internodes to submergence illustrates how a physiological process, in this case rapid internodal elongation, is controlled by the interaction between plant hormones (Raskin and Kende, 1984b; Hoffmann-Benning and Kende, 1992). The initial environmental signal, submergence, and the immediate growth-promoting hormone, GA, greatly increased the mRNA abundance of Os-EXP2 and Os-EXP4 (Figure 9). Submergence and GA promoted expression of Os-EXP4 within 30 min (Figure 10), which is within the observed lag time of 40 min for GA-induced internodal growth (Sauter and Kende, 1992) and 3 hr and 20 min for submergence-induced elongation (Rose-John and Kende, 1985). The accumulation of Os-EXP2 mRNA was considerably slower than that of Os-EXP4 in both GA-treated and submerged internodes. These results support the hypothesis that Os-EXP4 plays a role in submergence- and GA-enhanced growth of deepwater rice.

Figure 10. Accumulation of the Os-EXP4 Transcript in GA-Treated and Submerged Internodes.

(A) RNA gel blot analysis. Each lane contains 20 µg of total RNA isolated from the basal 2-cm region of the uppermost internodes that had been treated with 50 µM GA₃ or submerged for 0 to 6 hr.

(B) Quantification of Os-EXP4 mRNA levels. Blots shown in (A) were quantified with a PhosphorImager.
Expansin Gene Expression and Cell Wall Extensibility

Acid-induced extensibility of native cell walls is indicative for the expansin content of the wall and for the susceptibility of the cell wall to expansin action (Cosgrove, 1996). These two parameters may at least in part also determine the growth rate of the tissue. In oat coleoptiles (Cosgrove and Li, 1993) and cucumber hypocotyls (McQueen-Mason, 1995), susceptibility to expansins rather than expansin content may be a determining factor in establishing the rate of growth. In contrast, elongation of maize roots under water stress (Wu et al., 1996) and growth of deepwater rice internodes as a function of the developmental stage or as a result of submergence are correlated with both expansin content and susceptibility of the cell wall to expansin (Cho and Kende, 1997b). The pattern of acid-induced wall extensibility of different developmental regions of the coleoptile, root, and internode (Figure 6) is similar to the expression pattern of the Os-EXP1, Os-EXP3, and Os-EXP4 genes (Figures 5 to 7). This is further evidence that growth of these three vegetative organs is determined at least in part by the level of expansin.

Conclusions

In this study, we have shown that (1) four expansin genes are differentially expressed in the coleoptile, root, and internode of deepwater rice; (2) developmental, hormonal, and environmental signals affect expression of expansin genes differentially; and (3) the expression pattern of some of the expansin genes is highly correlated with wall extensibility and cell elongation. We propose that individual expansins play different roles in cell expansion and differentiation and that expansin action contributes to the growth of the major vegetative organs of rice and ultimately to survival of deepwater rice under submergence.

METHODS

Plant Material

Rice (Oryza sativa cv Pin Gaew 56) seeds were germinated on two sheets of moist Whatman No. 1 filter paper in a Petri dish in darkness at 30°C for 3 days. Seedlings with coleoptiles 2- to 2.5-cm long and roots 4- to 6-cm long were selected for RNA isolation and cell wall extension measurements. For experiments with internodes, rice plants were grown as described previously (Stünzi and Kende, 1989). Twenty-centimeter-long stem sections containing the uppermost internode were excised from 11- to 13-week-old plants, according to the method of Raskin and Kende (1984a). For treatment in air and gibberellic acid (GA3), 15 to 20 sections were incubated in distilled water or in 50 µM GA3 such that the water level remained below the basal node. For submergence, the sections, with a weight attached, were completely immersed in 2.5-liter, 60-cm-deep plexiglass cylinders containing distilled water (Kutschera and Kende, 1988). They were incubated for up to 48 hr at 27°C under continuous light (cool-white fluorescent tubes, 53 µmol m-2 sec-1; General Electric, Cleveland, OH).

cDNA Cloning and DNA Sequencing

The rice expansin Os-EXP4 cDNA was cloned from a rice internode cDNA library. A 750-bp probe containing the coding region was generated from Os-EXP2 by digestion using Sall and Eagl. Plaque-forming units (100,000) of the cDNA library were screened with this probe. Single positive plaques were screened with Os-EXP1- and Os-EXP2-specific probes in the fifth screening. Hybridization of the plaque blots on nitrocellulose (Protran; Schleicher & Schuell) was performed in 6 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.5% SDS, and 5 × Denhardt’s solution (1 × Denhardt’s solution is 0.01% Ficoll, 0.01% PVP, 0.01% BSA) at 62°C for 16 hr. The nitrocellulose membranes were washed with 0.2 × SSC and 0.1% SDS at 62°C. A clone that hybridized with the probe from the Os-EXP2 coding region but not with Os-EXP1- and Os-EXP2-specific 3' probes was isolated. The insert from the phage DNA was isolated and cloned into pBluescript SK– phagemid (Stratagene, La Jolla, CA).

The DNA insert was sequenced using Taq FS DNA polymerase and fluorescent-dideoxy terminators in a cycle-sequencing reaction. The resultant DNA fragments were separated by electrophoresis and analyzed using an automated Applied Biosystems (Foster City, CA) 373A Stretch DNA sequencer at the W.M. Keck facility of Yale University (New Haven, CT). This new rice expansin cDNA clone was named Os-EXP4.

Preparation of the Probes

Gene-specific probes were prepared mainly from the 3' untranslated regions of the respective transcripts. A 440-bp fragment between the HincII and Eagl restriction sites (including 142 bp from the coding region) was used for the Os-EXP1–specific probe, and a 580-bp fragment between two Eagl restriction sites (including 72 bp from the coding region) was used for the Os-EXP2–specific probe. Probes were generated by polymerase chain reaction with oligonucleotide primers corresponding to 3' gene-specific regions of Os-EXP3 (5'-GTGCACCCTCACCAGTTCTAGCCGCCACCGAC-3') and Os-EXP4 (5'-CCAGTTCTAGCCGCCACCGAC-3'). The length of the polymerase chain reaction probes was 270 bp for Os-EXP3 (including 35 bp from the coding region) and 457 bp for Os-EXP4. A probe (656 bp) used for genomic DNA gel blot analysis was prepared from the coding region of Os-EXP1 by HincII restriction digestion.

RNA and DNA Gel Blot Analyses

Total RNA was extracted according to the method of Puissant and Houdeline (1990). Twenty micrograms of total RNA was separated on formaldehyde gels and stained with ethidium bromide to ensure equal loading of RNA. The RNA was then capillary transferred to nylon membranes (Hybond N; Amersham) with 20 × SSC for 16 hr. Radiolabeled DNA probes were prepared using a random priming DNA labeling kit (Boehringer Mannheim). The membranes were prehybridized at 42°C in 5 × SSC, 10 × Denhardt’s solution, 0.1% SDS, 0.1 M potassium phosphate, pH 6.8, and 0.2 mg mL-1 salmon sperm DNA and hybridized at 42°C in 5 × SSC, 30% formamide, 10% dextran.
Measurement of Cell Wall Extension

Cell wall extension (creep) was measured according to the method described in Cosgrove (1989), as modified for rice tissue (Cho and Kende, 1997a). Coleoptiles, roots, and the uppermost internodes were frozen at −80°C, abraded with carborundum (300 mesh; Fisher, Fair Lawn, NJ) slurry, and thawed. One-centimeter-long sections of the coleoptiles, 5-mm-long sections of the roots, and 1.5-cm-long sections of the internodes were cut from each region (Figures 5A, 6A, and 7A) and insertions were inserted between two clamps spaced 5 mm apart for the 5-mm-long sections of the roots, and 1.5-cm-long sections of the internodes. For the 17s rRNA probe and the deepwater rice cDNA library. This work was supported by Grant No. IBN 9407763 from the National Science Foundation and Grant No. DE-FG02-91ER20021 from the U.S. Department of Energy.

ACKNOWLEDGMENTS

We thank Drs. Yoshiaki Nagamura and Takuji Sasaki (Rice Genome Research Program, Tsukuba, Japan) for the generous gift of rice expressed sequence tag clones; Dr. Athanasios Theologis (U.S. Department of Agriculture Plant Gene Expression Center, Albany, CA) for the 17s rRNA probe; and Esther Van der Knaap (Michigan State University—Department of Energy Plant Research Laboratory) for the E37 cDNA probe and the deepwater rice cDNA library. This work was supported by Grant No. IBN 9407763 from the National Science Foundation and Grant No. DE-FG02-91ER20021 from the U.S. Department of Energy.

Received April 29, 1997; accepted July 11, 1997.

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*Plant Cell* 1997;9:1661-1671
DOI 10.1105/tpc.9.9.1661

This information is current as of October 27, 2017