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Analysis of transcripts that are differentially expressed in three sectors of the rice root system under water deficit

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Abstract Short periods of water deprivation can stimulate the growth of seminal and lateral roots in rice, and inhibit the emergence of adventitious roots. Identification of genes in the different tissues that respond to a water deficit may help us to understand the mechanism underlying root growth under conditions when water is scarce. cDNA-amplified fragment length polymorphism (AFLP) analysis was used to profile gene expression upon imposition of water deficit in three types of root tissue from the upland rice variety Azucena: seminal root tips, lateral root zones and adventitious root primordial zones. In all, 121 unique transcript-derived fragments (TDFs) were cloned, and Northern analysis was carried out for 30 TDFs to confirm their expression patterns. Sixty-six TDFs were differentially expressed in all three root samples. Four (AC2, D6, L22 and T23) were up-regulated by water deficit in seminal root tips and lateral root zones, and down-regulated in adventitious root primordial zones, an expression pattern which reflects the phenotypic changes observed in the different root sectors. In contrast, T17 and T37 showed the opposite expression pattern in Azucena: up-regulation in adventitious roots and repression in the other two zones. Functions could be assigned to five of these six TDFs on the basis of homology: they encode an expansin (T37), a fruit-ripening protein similar to ASR (T23), submergence-induced protein 2A (T17), a dehydrin (D6) and a 9- *cis* -epoxycarotenoid dioxygenase1 (L22), respectively. AC2 did not show a significant match to any known gene. Northern analysis showed that these six clones exhibited expression patterns that differed between the two cultivars tested (Azucena and the lowland variety IR1552) with respect to regulation by water limitation. Furthermore, T17, T37, D6 and T23 mapped within intervals known to contain QTLs (quantitative trait loci) for root growth in rice under water deficit. These genes may regulate or co-regulate the growth and development of the three root zones in a tissue-specific manner, and may play a role in the processes that underlie the early changes in root architecture under conditions of water deprivation.

Keywords *Oryza sativa* L. · Seminal root tips · Lateral root zones · Adventitious root primordial zones · Water deficit

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Introduction

Adaptation of root growth to soil factors is very important in rice, which is the most important food crop for human consumption (Price et al. 2002). The root system of rice consists of three types of roots: the seminal root, adventitious roots and lateral roots. In any given genotype different parts of the root system can exhibit differential growth responses to limited soil moisture (Yamauchi et al. 1996). Contradictory results may be expected in experiments where different parts of roots are treated as a single root type, or when the response of a single root type is attributed to the entire root system (Bushamuka and Zobel 1998). However, little attention is generally paid to such differences.

There is evidence that a water deficit can promote root growth in rice, and substantial genetic variation exists for this trait (Price et al. 2002). For example, upland rice, which is often exposed to drought stress, has a deeper and thicker root system than lowland rice (Yadav et al. 1997; Price et al. 2002; Sharp 2002). The genetic control of root growth is complex and polygenic, and

the underlying mechanisms have not been elucidated (Wu and Cosgrove 2000; Price et al. 2002; Sharp 2002). Comprehensive expression analysis is a valuable tool for isolating differentially expressed and functionally important stress-regulated genes, as well as for elucidating the genetic networks in which they participate (Breyne et al. 2003). Up to now, tissue-specific gene expression patterns in different parts of the root system under water limitation have not been reported. In this study, 121 TDFs (transcript-derived fragments) that respond differentially to drought stress in the three types of rice roots were identified using the cDNA-AFLP technique. Six selected TDFs were mapped, and their expression was analyzed in the three components of the root system in two contrasting cultivars. The possible functions of their products in root growth are also discussed.

Materials and methods

Plant material

Seeds were surface-sterilized in 3% NaClO for 20 min, rinsed six times, and left to germinate in darkness at 37°C for 3 days. Uniformly germinated seeds were transplanted to plastic pots (10 cm in diameter and 24 cm in height) containing sand and with holes at the bottom. Pots were placed in tanks filled with water and the seedlings (40 per pot) were allowed to grow under flooding conditions for 5 days. Water deficit was then imposed by allowing the excess water to drain through the holes in the bottom of the pots for 72 h. Control and stressed tissues were sampled at the same time. To assess the degree of drought, the mean relative water content (RWC) of six seedlings from each pot was measured at the time of sampling (Fig. 1). RNA was extracted from seedlings at the time of RWC measurement (Fig. 1). The experiment was conducted in a greenhouse under the conditions previously described by Yang et al. (2003a).

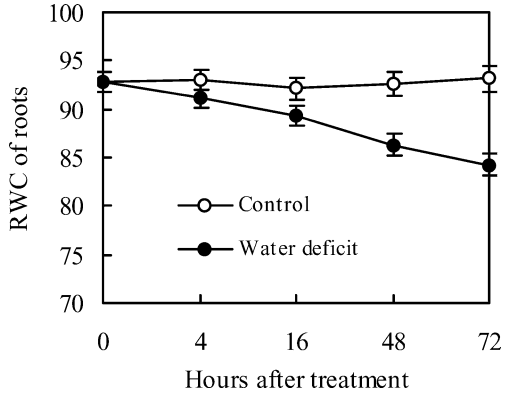


Fig. 1 Relative water content of roots at four time points. Data are mean values based on samples of six seedlings from each of three pots

Growth measurement of three parts of roots

Seminal root length was measured with a ruler and the number of adventitious roots was counted. The number and total length of lateral roots on seminal roots were measured using an image analysis system (WinRHIZO; Regent Instruments).

Non-radioactive cDNA-AFLP analysis

An upland tropical *japonica* rice (*Oryza sativa* L.) cultivar was used for cDNA-AFLP analysis. Seminal root tips (1 cm) including elongation zones, the root-shoot junction (0.5 cm) corresponding to the adventitious root generative region, and lateral roots on a 2-cm section taken from the middle portion of 100 seedlings were harvested at 4, 16, 48 and 72 h after drainage. Poly(A)$^+$ RNA was prepared by pooling equal amounts of RNA from the same root segments at the four time points. Double-stranded cDNA synthesis, double digestion with the enzymes *Taq* I and *Ase* I, and AFLP reactions were performed according to published methods (Yang et al. 2003a). Silver staining was used to visualize the final fingerprint (Dubos and Plomion 2002). TDFs that showed differential expression in any one, two or all three segments of the root system were excised from the gel with a blade and soaked in water. DNA was purified by precipitation and reamplified as described by Dubos and Plomion (2002). Purified PCR products were ligated to the pUCm-T vector, and subsequently sequenced (Yang et al. 2003a).

Northern and Southern analyses

Aliquots (20 μg) of total RNA were denatured and fractionated by electrophoresis on 1.2% agarose formaldehyde gels. Total genomic DNA (5 μg/lane) was digested with each of eight restriction enzymes (*Apo*I, *Bam* HI, *Dra* I, *Eco* RI, *Eco* RV, *Hin* dIII and *Xba* I) and fractionated by electrophoresis on a 0.8% agarose gel. RNA or DNA was transferred to nylon membranes by standard capillary techniques. Probe labeling and hybridization were carried out as described by Yang et al. (2003a). Hybridized membranes were scanned using a Typhoon 8600 scanner (Molecular Dynamics).

Mapping of TDFs on the rice linkage map

Each mapping population consisted of 96 lines developed from a cross between IR1552 and Azucena, and a cross between IR64 and Azucena. The chromosomal location of the TDFs was determined using the Mapmaker program (Zheng et al. 2003).

In silico mapping

To identify the TDFs in the genomic DNA sequence of rice, we used BLASTN to align the sequences against

available BAC or PAC clone sequences. Only those matches that showed more than 95% identify were used. The locations of these BACs were inferred from the positions of their marker loci in the existing database (http://www.genome.arizona.edu/fpc/rice/WebAGCoL/WebFPC/) and the clones were anchored to the high-density linkage maps of rice (http://rgp.dna.affrc.go.jp/publicdata/geneticmap2000).

Results

Root growth under a water deficit

The growth of roots in Azucena was sensitive to water deficit, and changes in root phenotype were observed after 24 h of treatment. The rate of seminal root elongation was significantly stimulated ($P < 0.01$) during 72 h of water deficit, and peaked at 24 h (Yang et al. 2003a). Elongation and initiation of lateral roots both increased at similar rates ($P < 0.05$) with increasingly severe water deficit (Yang et al. 2003b). After 72 h of water deprivation, the lengths of seminal and lateral roots, and the number of lateral roots had increased significantly ($P < 0.01$) in Azucena. In contrast, the indices for the lowland rice cultivar IR1552 did not show significant changes upon water limitation (Table 1). However, the decrease in the number of adventitious roots in IR1552 ($P < 0.05$) was greater than that seen in Azucena (Table 1).

Identification of 121 differentially expressed TDFs in root tissues

To identify candidate genes involved in the changes in root phenotype, seminal root tips (1 cm) including elongation zones, the root-shoot junctions (0.5 cm) corresponding to the adventitious root generative region, and a 2-cm section of the seminal root where lateral roots were emerging and elongating were used for cDNA-AFLP analysis. Six templates were amplified by using 110 primer combinations, and the products thus generated were fractionated by gel electrophoresis and visualized by silver staining. The cDNA-AFLP fragments ranged in length from 50 to 1000 bp, and 50–70 bands were observed for each primer combination. Of the 6600 fragments inspected, most bands showed no change in intensity, but 156 TDFs were found to be differentially expressed. One hundred and thirty-eight of these were cloned and sequenced. Due to redundancy in the data set, this number corresponds to 121 unique transcripts. To evaluate the validity of the expression patterns revealed by cDNA-AFLP analysis, we performed Northern analysis for 30 randomly selected TDFs. While the nature of the variation (increase, decrease or invariance) revealed by cDNA-AFLP could be confirmed by Northern analysis, a slight difference was observed in the degree of variation in the cases of T95 and L15 (Fig. 2). Overall, however, the results obtained by cDNA-AFLP were in good agreement with those by RNA gel blot analysis. It is noteworthy that many Northern blots show double bands; these might results from differential splicing of a single gene or be derived from two homologous genes.

The 121 differentially expressed TDFs identified can be divided into seven groups: 66 TDFs were differentially expressed in response to water deficit in all three sectors of the root system: 32 in both lateral root zones and seminal root tips, 9 in both seminal root tips and adventitious root primordial zones, 3 in both lateral root zones and adventitious root primordial zones, 9 only in seminal root tips, and 1 each only in lateral root zones and in adventitious root primordial zones (Fig. 3). However, none of the genes identified was specifically expressed (presence/absence) in a single tissue under unstressed and water-stressed conditions. Among the 121 TDFs, 116 were differentially expressed in seminal root tips and 79 were differentially expressed in adventitious root primordial zones. These results suggest that the seminal root tip may be the root tissue that is most sensitive to drought, while adventitious root primordial zones are the least sensitive to water deficit at the young seedling stage. Some TDFs show similar expression patterns under both conditions in the three root sectors, whereas many show different behaviors in the different root tissues. Notably, four genes (AC2, L22, T23 and D6) were up-regulated by water deficit in both seminal root tips and lateral root zones, but were down-regulated in adventitious root primordial zones (Table 2). Thus, they exhibited an expression pattern that reflects the growth responses of the different root tissues in Azucena (Table 1). In contrast, T17 and T37 showed the opposite response: these transcripts were down-regulated in both seminal root tips and lateral root zones, and up-regulated in adventitious root primordial zones (Table 2).

Table 1 Length and number of roots grown after exposure of seedlings to flooding conditions or water deficit for 72 h

Growth conditions	Length of seminal roots (cm)		Number of adventitious roots		Length of lateral roots (cm)		Number of lateral roots	
	Azucena	IR1552	Azucena	IR1552	Azucena	IR1552	Azucena	IR1552
Flooding	9.6 ± 0.43	9.2 ± 0.51	5	6	151.6 ± 14.4	169.3 ± 21.1	69 ± 11	62 ± 15
Water deficit	13.5 ± 0.58	9.8 ± 0.49	4	4	192.8 ± 23.6	180.7 ± 24.2	102 ± 16	84 ± 19

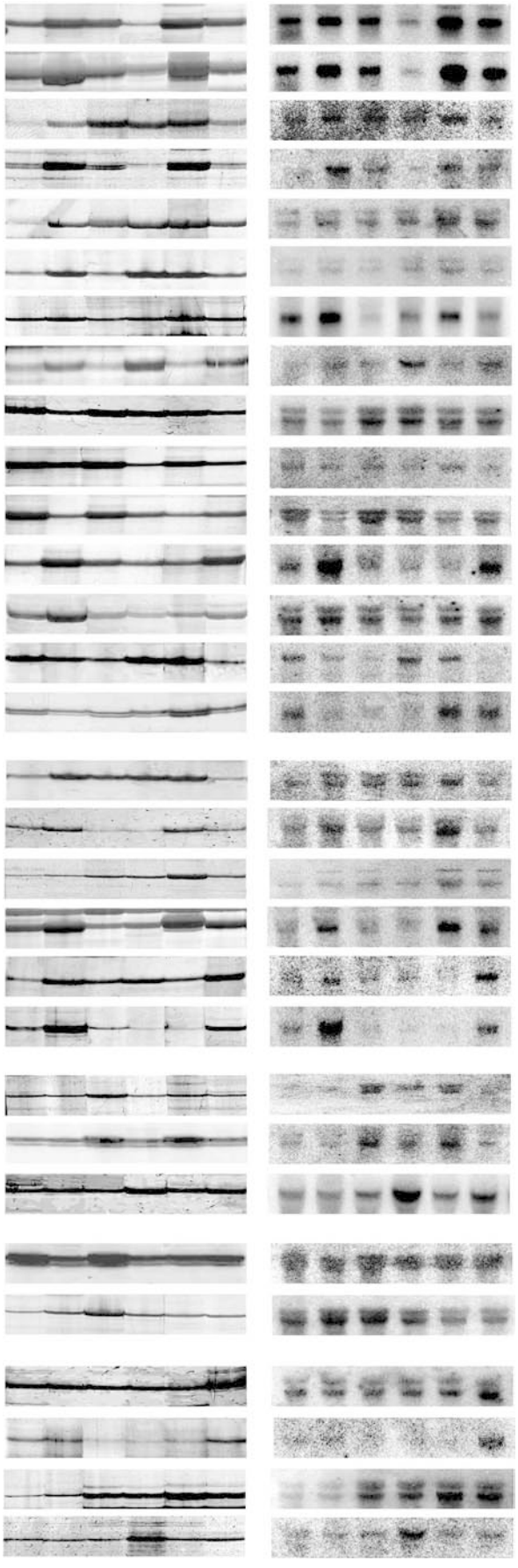


Fig. 2 Expression analysis of TDFs by cDNA-AFLP analysis (*left*) and verification by Northern hybridization analysis (*right*). Lanes 1–6 are templates derived from a mixture of RNA samples isolated from control (-) or treated (+) root tissues: L, lateral root zones; A, adventitious root primordial zones; T, seminal root tips

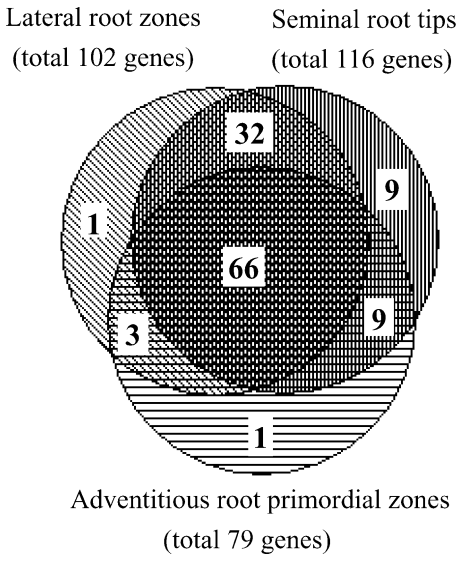


Fig. 3 Classification of TDFs on the basis of their differential expression in the three root sectors

Functional classification of differentially expressed genes

Among the 121 differentially expressed TDFs, 80 are similar to genes with known functions. Their expression patterns and homology to known proteins are summarized in Table 2. We classified these 121 sequences into 11 groups based on their putative function. The major group (23.9%) is involved in cellular organization and cell-wall biogenesis—which reflects remodeling of the cell wall. A fairly high proportion (10.7%) is involved in transport facilitation, metabolism and energy, and these provide the material and energy needed for the growth of seminal and lateral roots. Stress- and defense-related proteins also account for 10.7% of the total TDFs, and comprised nutrition-, cold- or drought-stress-induced, as well as members of disease-resistance gene families. These results indicate cross-talk between different stress-signaling processes. In addition, some 14% of the TDFs are involved in signal transduction, gene expression and regulation. Furthermore, 23.9% matched a gene of unknown function, whereas 1.6% showed homology to rice ESTs. The remaining 8.2% either showed homology to unannotated genomic sequences or did not match any sequences in the nucleotide database. Overall, 9.8% of the TDFs may represent either previously uncharacterized genes or AFLP fragments that are too short to reveal any significant homology.

Table 2 Summary of the expression behavior of 80 TDFs that are similar to genes encoding proteins of known function

TDF[a]	Expression[b]			Homology	TDF[a]	Expression[b]			Homology
	L	A	T			L	A	T	
Gene expression and regulation: 9 (7.4%)									
T49	–	+	+	Gibberellin action negative regulator	L16	–	+		Similar to SART-1 protein
T44	–	–	+	AP2 domain-containing protein	L7	+	+		Transformer-2-like protein
T82	–	–	+	bHLH protein	AC7		–	–	DEAD/DEAH RNA helicase
T9	+	+	+	DNA-binding protein	AR18			–	knotted1-type homeobox protein
L10	+	+	+	Co-repressor protein					
Signal transduction: 8 (6.6%)									
L13	+	+	+	Contains protein kinase domain	L14	+		+	GTPase
T32	+	+	+	Altered Response to Gravity protein	T50	+		+	Jasmonic acid regulatory protein
T6	+		+	Receptor protein kinase precursor	T83		+	+	Calmodulin (CaM)
T28	+		+	Inositol 1,3,4-trisphosphate kinase	T16			+	MAP3 K beta 1 protein kinase
Transport facilitation: 13 (10.7%)									
T7	–	+	+	Stomatin-like protein	T17*	–	+	–	Submergence induced protein 2A
T47	–	+	+	Vacuolar protein sorting protein	AC1		–	+	Permease 1
L9	–	+	+	Autophagocytosis protein—like	T51		–		GTP-binding protein rab11b
T21	–	+	+	Integral membrane protein	T73	+		+	ABC transporter-like protein
T72	–	+	+	Cadmium/zinc-transporting ATPase	T59	–		+	CLIP-associating protein 2
T65	+		+	Guanine nucleotide exchange protein	T79	–		+	Aquaporin (PIP2a)
T18		+	+	Guanine nucleotide exchange protein 2					
Cellular organization and cell wall biogenesis: 18 (14.8%)									
T37*	–	+	–	alpha-expansin (OsEXP2)	T77	–	–	+	Glycine-rich protein
T61	+	+	+	alpha-expansin	T95	–	–	+	Caffeoyl-CoA O-methyltransferase
T40	+	+	+	Xyloglucan endotransferase	AR9	–	–	–	Xylose isomerase
T71	+	+	+	Endo-glucanase	T92		+	–	Cellulose synthase-like protein
T94	+	+	+	Endoxyloglucan glycosyltransferase	AR5		–	–	Phenylalanine ammonia-lyase
T11	+	+	+	Myosin-like protein	T81	+		+	H1 histone
T93	+	+	+	GDP-mannose pyrophosphorylase	AR8	–		+	*p* -coumarate 3-hydroxylase
AR4	+	+	–	4-coumarate:CoA ligase isoform	T62	+			Actin-depolymerizing factor 1
T56	–	+	+	Alpha-mannosidase	AR6			–	Cinnamyl-alcohol dehydrogenase
Cell growth and division: 3 (2.4%)									
L22*	+	–	+	Nine-cis-epoxycarotenoid dioxygenase1	T63	+	+	+	Similar to mRNA for KIAA0039
T84		+	+	Regulator of chromosome condensation					
Transposable elements: 3 (2.4%)									
L5	+	+	+	Retroelement	T52	–		+	Reverse transcriptase
T41	+	+	+	Polyprotein from transposon TNT					
Metabolism and energy: 13 (10.7%)									
D4	+	+	+	Cytochrome c oxidase subunit 5c	A2	–		+	Pyruvate dehydrogenase kinase
T75	+	+	+	Deoxyguanosine kinase	T24	+		+	Aspartate-tRNA synthetase
T31	+	+	+	Enolase	T27	+		+	N methyltransferase
T74	+	+	+	FtsH-metalloproteinase	T78		+	+	Glutamyl-tRNA amidotransferase
AC5	–	–	–	Homoserine dehydrogenase-like protein	T57			+	Xaa-Pro aminopeptidase 1
A3	–	–	–	Adenine phosphoribosyltransferase	T36			+	Lipase
T1	–		+	Cysteine synthase 1					
Stress- and defense-related: 13 (10.7%)									
D6*	+	–	+	Dehydrin COR410	L15		+	+	SR1 sucrose-regulated mRNA
T23*	+	–	+	Fruit-ripening protein similar to ASR	T70		+	+	Pathogenesis-related protein 1
T90	+	+	+	Drought-induced protein Di19	L1	+		+	Disease resistance protein I2
T34	+	+	+	NBS-LRR type resistance gene	T54	+		+	NBS-LRR type resistance protein
D2	+	+	–	Cytochrome P450 monooxygenase	T26	–		+	Peroxidase
AC3	–	–	–	Protein expressed under carbonate stress	T38	–		+	hin1 protein
A1	+	+		Late embryogenesis abundant protein					

[a]TDFs marked with *asterisks* showed an expression pattern that either reflected or was the converse of the pattern of phenotypic change in root growth (see text for details). AC2 is also included in this group

[b]Up-regulated (+) or down-regulated (–) in lateral root zones, adventitious root primordial zones and seminal root tips

Analysis of promoter sequences associated with genes that are differentially expressed under a water deficit

Several *cis* -acting elements involved in drought-regulated gene expression, such as the dehydration-responsive element (DRE), the ABA-responsive element (ABRE), recognition sites for MYB and MYC proteins, and the W-box-binding motif, have been reported (Seki et al. 2001, 2002; Abe et al. 2003, Brown et al. 2003; Rabbani et al. 2003). Sequence data for the clones identified in the present study were compared with those of rice full-length cDNA clones (http://cdna01.dna.affrc.go.jp/cDNA) (Rice Full-Length cDNA Consortium 2003). The *cis* -acting elements in promoter regions (1 kb

Table 3 Analysis of drought-related *cis*elements in the promoter regions of 101 differentially expressed genes identified by cDNA-AFLP

Motif name	Motif sequence	Number of promoters with motif	Corresponding transcription factor	Reference
Myb	(A/T)AACCA	71	MYB (Myb box binding factor)	Abe et al. (2003)
	TAACTG	33		Abe et al. (2003)
	CACCTG	36		Seki et al. (2002)
Myc	CACATG	54	MYC (Myc box binding factor)	Seki et al. (2002)
	CATGTG	53		Abe et al. (2003)
DRE box	CCGAC	60	CBF (DRE box binding factor)	Seki et al. (2002)
W box	TTGAC(C/T)	44	WRKY (W box binding protein)	Seki et al. (2002)
GCC box	GCCGCC	33	ERF (ethylene response factor)	Brown et al. (2003)
ERE	AWTTCAAA	24	ERF (ethylene response factor)	Montgomery et al. (1993)
ABRE	ACGTGKC	22	ABF (ABA response factor)	Rabbani et al. (2003)

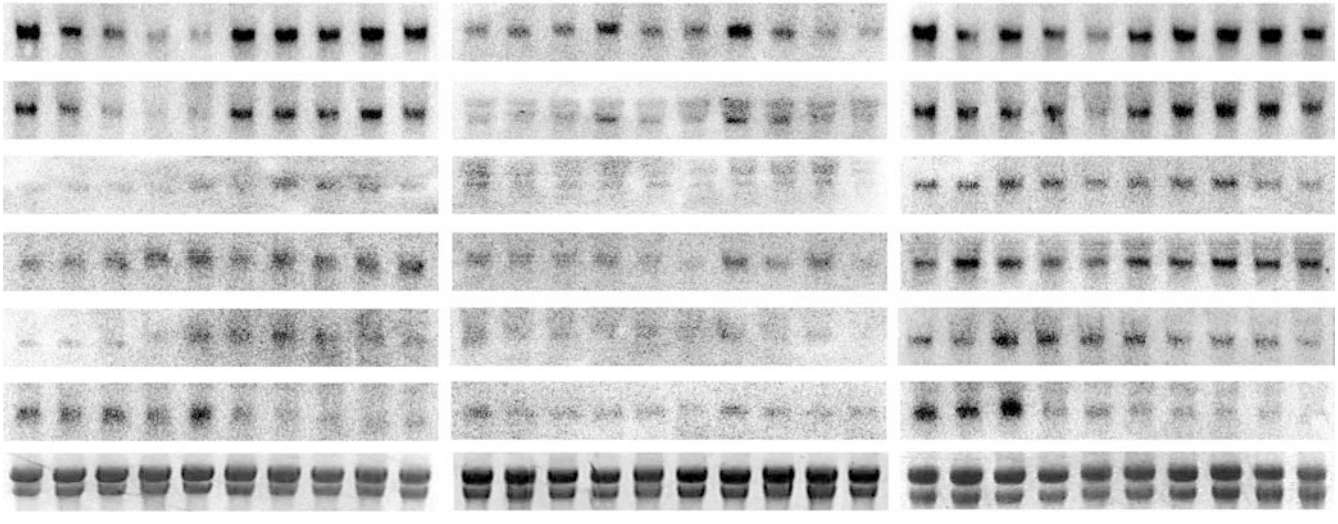
upstream) of 87 matched genes were obtained from the PLACE database (http://ricegaas.dna.affrc.go.jp/usr/) (Higo et al. 1999), and sequences that showed no matches were analyzed further. First, they were assigned to specific BAC or PAC clones, and the possible *cis* -acting elements of 11 genes were analyzed using the Rice Genome Annotation Database (http://ricegaas.dna.affrc.go.jp/rgadb/). Table 3 lists ten motifs observed in the 101 genes that are differentially expressed under water limitation and were identified by cDNA-AFLP analysis. Seventy-one genes contain (A/T)AACCA in their promoters, suggesting that these genes are regulated by MYB transcription factors. Sixty genes contained the DRE-related CCGAC core motif in their promoters, suggesting that they are regulated by the transcription factors DREB1 and/or DREB2. The results presented here also suggest that some members of the MYC, WRKY, ERF and ABF families of transcription factors can spatially and temporally regulate gene expression in response to water deficit.

Fig. 4 Northern analysis of six TDFs in three root sectors in Azucena and IR1552 after exposure to water deficit. T, seminal root tips; A, adventitious root primordial zones; L, lateral root zones. About 20 μg of total RNA was loaded in each lane. Equality of loading was confirmed using a rice ribosomal DNA as a probe on the same blot

Expression patterns of six selected clones in different cultivars after a water deficit

The predicted products of D6, L22 and T37 are most similar to a dehydrin (wcor410), 9- *cis* -epoxycarotenoid dioxygenase1 (NCED1) and Os-EXP2, respectively. T17 is 95% identical to the submergence-induced protein 2A (SIP2A), while the extended sequence of T23 is homologous to an abscisic acid (ABA)-, stress- and ripening-inducible protein (ASR). Another TDF (AC2) showed no significant match to any known gene or EST sequence in the databases searched.

The lowland rice variety IR1552 and the upland rice variety Azucena were chosen for Northern analysis using the six TDFs described above as probes. Their expression profiles in the three parts of the root system in response to a water deficit are shown in Fig. 4. In both seminal root tips and lateral root zones, the transcripts of T17 and T37 were negatively regulated by water deficit in Azucena, but were not affected in IR1552. In adventitious root primordial zones, T17 and T37 were induced in Azucena, but repressed in IR1552, upon exposure to water deficit. In seminal root tips, the expression of AC2 was induced only after a 48-h water deficit in Azucena, but the gene was transiently induced within 48 h in IR1552. In lateral root zones, AC2 was transiently induced in both genotypes under water lim-



itation, but a slight difference in the expression pattern was observed. In adventitious root primordial zones, the level of the AC2 transcript was found to decrease in Azucena and transiently increase in IR1552. Although D6 was induced in seminal root tips, there was no difference in expression levels between Azucena and IR1552. In lateral root zones, D6 was transiently induced at different time points in Azucena and IR1552. In adventitious root primordial zones, D6 was repressed at 72 h in Azucena, whereas it was rapidly induced in IR1552, where transcript levels remained high for 48 h and then decreased. In seminal root tips and lateral root zones, T23 was induced, with maximal expression at 16 h and 48 h, in Azucena, but was present constitutively in IR1552. In adventitious root primordial zones, T23 was repressed in Azucena, but was transiently induced at 4 h in IR1552. The transcripts corresponding to L22 behaved similarly to those of T23 in the three root sectors in both cultivars.

Mapping candidate genes

Based on in silico mapping, six TDFs were mapped to chromosomes 1, 2, 3, 4 and 9, respectively (Table 4): *OsEXP2* (T37) and *SIP2A* (T17) were genetically mapped on chromosomes 1 and 3 (Table 4), respectively (Zheng et al. 2003), while the dehydrin sequence (D6) was localized on chromosome 2 (Fig. 5)

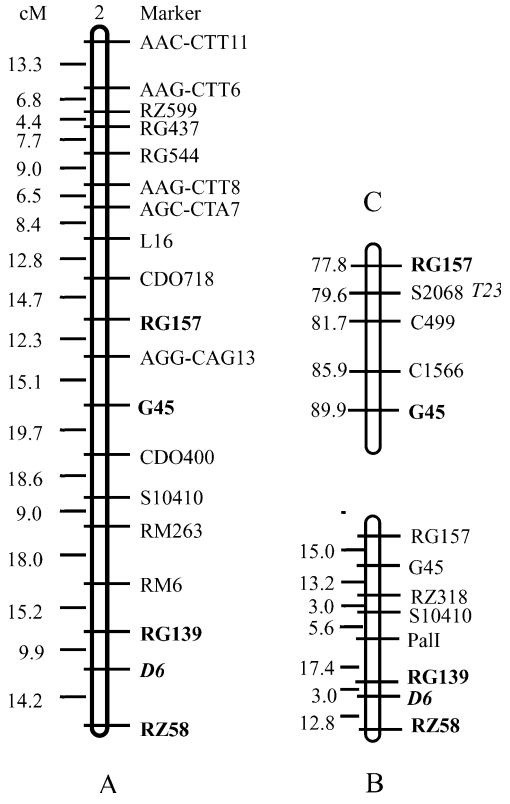


Fig. 5A–C Mapping of two genes that are regulated by water deficit. *D6* was mapped to chromosome 2 in the populations IR1552×Azucena (**A**) and IR64×Azucena (**B**). *T23* was mapped by in silico analysis, and was localized on an integrated map of QTL intervals for root growth by alignment of RFLP markers with an RGP physical map (**C**)

Discussion

A plant's root system is its primary site of drought perception. Changes in gene expression induced by water deficit that lead to the synthesis and activation of novel proteins have been well documented in maize (Riccardi et al. 1998) and in the model organism *Arabidopsis thaliana* (Seki et al. 2001). However, very little is known about the molecular mechanisms involved in drought avoidance in rice, especially with respect to the responses of different parts of its roots.

Up to now, data on drought-responsive genes in rice roots have been limited. Using the cDNA-AFLP technique, we identified and cloned 121 transcripts that were differentially expressed in response to drought in seminal root tips, lateral root zones or adventitious root primordial zones. We compared the identified genes with those reported by Rabbani et al. (2003), who constructed cDNA libraries from both root and leaf tissues. Table 2 shows that the expression of many classes of signal transduction components is affected by water stress, perhaps reflecting the fact that roots are the first organs to perceive drought stress and respond to it by signal transduction. Our sample contains a higher proportion of genes (15%) involved in cellular organization and cell-wall biogenesis than that described by Rabbani et al. (2003) (3%); these genes may have contributed to the rapid changes in root phenotype observed under a

Table 4 Mapping of six candidate genes

Chr.	Gene	In silico mapping			Mapping by Southern analysis[b]
		BAC or PAC	Marker[a]	Map position (cM)	Region
1	T37 (*OsEXP2*)	AP003348	(RZ730)-G370	140.5	C86-RG109B
2	T23 (*ASR*)	AP004019	(RG157)-S2068	79.6	-
2	D6 (*COR410*)	AP005055	(RG139)-L737	111.2–112.6	RG139-RZ58
3	T17 (*SIP2A*)	AC105729	E31168-(RG409)	16.8	RG409-CDO1395
4	AC2 (No hit)	OSJNBa0039C07	RM417-(C975)	52.6	-
9	L22 (*NCED1*)	AP005684	RM3855-(C1454)	26.7–30.6	-

[a]The marker in *parentheses* was the neighboring one
[b]Mapped in the progeny population obtained from the cross IR1552×Azucena

mild water deficit. Some TDFs, such as T83, T79, T37, T61, T40, T71 and T94, have previously been reported to be regulated by water limitation (Wu and Cosgrove 2000; Seki et al. 2001), which corroborates our results based on cDNA-AFLP analysis.

Interestingly, many clones showed different behaviors in the different parts of the rice root system upon the imposition of water deficit. Sixty-six TDFs were differentially expressed in all three root sectors. Four genes were up-regulated in both seminal root tips and lateral root zones, and down-regulated in adventitious root primordial zones. This mirrors the growth response of the different sectors of the root system upon exposure to water stress. Two TDFs showed precisely the opposite expression pattern (Table 2).

Possible roles of six differentially expressed candidate genes in root growth

Roots that are adapted to conditions of water limitation continue to grow by increasing the extensibility of the cell walls in their apical sectors. This increase in wall extensibility results, at least in part, from the accumulation of expansin transcripts (Wu and Cosgrove 2000; Lee et al. 2001). In most instances, the highest levels of expansin transcripts have been found in the most rapidly growing regions of tissues and organs of rice (Wu and Cosgrove2000; Lee and Kende 2002). However, *Os-EXP2* mRNA (Accession No. U30382) is also present in non-growing regions of roots, internodes and leaves, and shows highest expression in regions that are still elongating but at a much slower rate (Cho and Kende 1997; Lee and Kende 2002). In rice root-hair zones, where growth of the primary root has ceased, *Os-EXP2* continues to be expressed (Cho and Kende 1997). In this study, expression of *Os-EXP2* (T37) was repressed in the seminal root tips and lateral root zones, where growth is accelerated under water deficit (Table 1 and Fig. 4). The distinctive behavior of *Os-EXP2* indicates that Os-EXP2 may be linked to primary growth, and may play a role in the differentiation of the vascular system (Cho and Kende 1997). Os-EXP2 may act by disrupting hydrogen bonds between cell wall polymers (Cho and Kende 1997; Lee et al. 2001).

wcor410 (D6), a cold-regulated wheat gene coding for an acidic dehydrin, is also up-regulated by severe water stress and by exposure to ABA (Danyluk et al. 1998). In the experiments described here, it was up-regulated in both seminal root tips and lateral root zones, and down-regulated in adventitious root primordial zones in response to water deficit (Fig. 4). WCOR410 proteins are largely hydrophilic (only 17% of residues are hydrophobic amino acids), which makes them well suited to retaining water and stabilizing membranes through polar interactions. On the other hand, they may help to counteract the otherwise irreversible damage caused by the increased ion concentrations that result from dehydration. Immunoblot analysis showed that WCOR410 proteins accumulate to very high levels in the vicinity of the plasma membrane of cold-acclimated cells in the vascular transition area. The properties, abundance and localization of these proteins suggest that they are involved in cryoprotection of the plasma membrane against dehydration stress (Danyluk et al. 1998).

Asr (T23) genes have been identified in several species. Vaidyanathan and co-workers (1999) isolated a clone, *OsAsr1* , from a cDNA library constructed from salt-stressed rice tissues. The *OsAsr1* sequence encodes a hydrophilic protein with a molecular mass of 15.4 kDa, and is up-regulated by exogenous application of ABA and by osmotic stress caused by mannitol and salt. OsASR1, like all other ASR proteins, is a potential kinase substrate, and is predicted to have a predominantly alpha-helical structure. A potential myristoylation site in its sequence suggests that myristoylation may play an important role in the function of this protein (Vaidyanathan et al. 1999). However, the functions of ASR proteins in the response to stress are unknown.

AC2 may represent a novel gene, but this result has to be considered with caution since the TDF obtained by cDNA-AFLP is very short, which may in turn limit the accuracy of homology searches.

NCED (L22) cleaves 9′- *cis* -neoxanthin to xanthoxin, a precursor of ABA. Several studies have indicated that this cleavage reaction is a key step in the pathway that controls water-stress-induced ABA synthesis. In other species the induction of *NCED* by water stress is correlated with stress-induced ABA synthesis. Mutations in *NCED* genes reduce ABA production, while overexpression of *NCED1* results in increased ABA accumulation. The localized expression of *NCED* in root tips and cortex cells at the base of lateral roots suggests that ABA regulates root growth and development under water deficit (Sharp 2002; Tan et al. 2003). The fact that the *NCED1* expression pattern matched the phenotypic changes in the three root sectors indicates that an increased concentration of endogenous ABA may function to promote rice root growth under water limitation. ABA has also been implicated in the regulation of lateral root initiation and growth (Tan et al. 2003). A recent study of ABA-deficient maize seedlings showed that endogenous ABA plays an important role in maintaining root growth under conditions of water limitation by preventing the production of excess ethylene (Sharp 2002).

The Sip2A (T17) transcript detected in this study is orthologous to IDI1 of barley. Hypoxia, which mimics submerged conditions, did not induce *IDI1* expression in barley roots. Thus, signals other than hypoxia that are activated by submergence must induce the expression of SIP proteins in rice. SIP2A proteins are quite similar to the E-2 protein, an enzyme that catalyzes the oxidation of 1,2-dihydroxy-3-keto-5-methylthiopentene anion to formate and 2-keto-4-methylthiobutylic acid in the methionine salvage pathway. In our experiments water deficit also suppressed adenine phosphoribosyltransferase (APRT, A3) (Table 2), which catalyzes the conver-

Table 5 Positions of known functional motifs in the 5' regions of five candidate genes

Gene	Motif		
	ABRE [PyACGTG(G/T)C] or CE3 [CGCGTG(T/G)C]	ERE (GCCGCC) (AWTTCAAA)	DRE (A/GCCGACNN)
D6 (Dehydrin COR410)	tACGTGGCt (−949 to −957)	tGCCGCCc (207–214) cGCCGCCc (544–551) cGCCGCCg (580–587) cGCCGCCg (756–763) cGCCGCCg (884–891) cGCCGCCg (887–894) cGCCGCCc (890–897)	aACCGACat (−185 to –193) aACCGACat (198 to 206) tGCCGACtc (−327 to –334) gGCCGACgg (−417 to –426) cGCCGACga (−720 to –728) aGCCGACgt (−964 to –972)
L22 (9-*cis* -epoxycarotenoid dioxygenase 1, *NCED1*)	cCACGTGGCg (−552 to −561) cCACGTGGCg (554−563)		
T17 (Submergence induced protein 2A, *SIP2A*)	tCGCGTGGCc (641−650)	tGCCGCCt (371–378) gGCCGCCg (682–689) gGCCGCCg (689–696) gGCCGCCa (696–703) cGCCGCCg (−724 to −731) tGCCGCCg (−727 to –734)	
T23 (Fruit-ripening protein similar to ASR, *ASR*)		aATTTCAAAc (242−251)	
T37 (alpha-expansin, *OsEXP2*)		cGCCGCCt (881−888)	tACCGACca (−564 to −572)

sion of adenine from the methionine salvage pathway and provides the ATP required for the formation of S-adenosylmethionine (Yamaguchi et al. 2000). Ethylene biosynthesis depends on the methionine salvage pathway to maintain a constant supply of methionine and ATP (Wang et al. 2002). The inhibition of both *Sip2A* and *APRT*, together with the induction of *NCED1*, is thus likely to reduce ethylene production in vivo. This is compatible with the notion that ABA maintains the growth of seminal roots and lateral roots under a water deficit by restricting ethylene production (Sharp 2002). Adventitious root formation in rice has been shown to be induced by ethylene (Zhou et al. 2002). In addition, ABA and ethylene induce the expression of hundreds of genes. *Cis* -acting elements such as ABRE and CE3 or ERE were observed in the promoters of all five genes (Table 5) (the sequence of the AC2 promoter was not obtained), suggesting that they may be ABA-inducible or ethylene-inducible (Montgomery et al. 1993; Seki et al. 2002; Brown et al. 2003).

Coincidence between expression candidate genes and root trait QTLs

OsEXP2 and *Sip2A* were found to be linked to a QTL for seminal root length and lateral root length under a water deficit, respectively (Zheng et al. 2003). *Dehydrin* was mapped at the locus on chromosome 2 (Fig. 5) where QTLs for maximum root length and root thickness have been detected (Yadav et al. 1997). *Asr* was mapped in silico within the interval containing a QTL for seminal root length and root thickness on chromosome 2 (Zheng et al. 2003). The expression patterns of all four of these genes differed between Azucena and IR1552 (Fig. 4). This suggests that they could be candidate genes for root growth, although the precise functions and roles of their gene products are still unclear.

The elongation of seminal roots and lateral roots, and the initiation of lateral roots, are stimulated by water deficit, maximizing water capture and allowing access to water at depth (Price et al. 2002). Cellular responses to a water deficit probably involve the coordinated positive and negative regulation of several genes, as has been suggested previously (Blomstedt et al. 1998). These four genes may positively or negatively regulate the growth and development of the three components of the rice root system in a tissue-specific manner, and may be involved in the processes that underlie the early changes in root architecture in upland rice upon exposure to drought.

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