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ORIGINAL PAPER

Comparison of gene expression between upland and lowland rice cultivars under water stress using cDNA microarray

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Abstract To elucidate the differences in the regulation of water stress tolerance between two genotypes of rice, upland-rice (UR, resistant to water stress) and lowland-rice (LR, susceptible to water stress), we constructed subtracted cDNA libraries from polyethyleneglycol (PEG)-treated and non-treated rice seedlings (IRAT109, an upland-rice variety) by suppression subtractive hybridization (SSH), from which about 2,000 recombinant colonies were picked and amplified. Then, a cDNA microarray containing these expressed sequence tags (ESTs) was used to analyze the gene expression profiles in UR and LR in response to PEG treatment. Microarray data revealed that the majority of genes expressed in UR and LR are almost identical and Student's *t* test showed that 13% of all the ESTs detected in leaves and 7% of that in roots expressed differentially in transcripts abundance between the two genotypes. After sequencing, it was found that 64 and 79 unique ESTs expressed at higher levels in UR and LR, respectively. Many of the ESTs that showed higher expression in UR upon PEG treatment represented genes for transcription factors, genes playing roles in detoxification or protection against oxidative stress, and genes that help in maintaining cell turgor. In contrast, some ESTs that showed higher expression in LR were genes functioning in the degradation of cellular components. Based on data from this study and previous reports, we suggest that overexpression of some genes that expressed at higher level in UR may improve water stress tolerance in LR and other plant species.

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Introduction

Water stress is one of the most important environmental factors limiting plant growth and crop productivity. To survive against the stress, plants have evolved a number of physiological and biochemical responses (Bray 1997). Studies on the mechanism that plants evoke to tolerate water stress will be of great benefit to the breeding of water stress resistant crops. Research on the biology and genetics of water stress resistance have, thus, become an important field of contemporary research in plant molecular biology.

Plant molecular response to water stress has been the subject of many studies in the past decade. Water stress tolerance is the result of a complex cascade of molecular events that include gene activation (Ramanjulu and Bartels 2002), and most studies focused on identifying these water stress induced genes by comparing gene expression profile in water-stressed materials to that in non-stressed materials (Ozturk et al. 2002; Reddy et al. 2002; Zheng et al. 2004). However, not every up-regulated gene has a role in water stress tolerance, the change in expression in some of them may simply be the result of damages caused by stress (Bray 1997; Chaves et al. 2003; Zhu 2000). It remains a challenge to sort out these water stress inducible genes and identify key genes contributing to water stress tolerance.

There are two different cultivation regimes for rice, the upland and lowland rice (UR and LR). The cultivars suitable for each regime are developed by a long period of natural and human selection under different water conditions.

LR is cultivated in paddy fields, and UR is grown under rain-fed, naturally well-drained soils without surface water accumulation, similar to wheat or maize. Compared to LR, UR cultivation can reduce the demand for irrigation water by 50–70% (Wang et al. 2002). These two types of cultivars are similar in most of the morphological and agronomic traits, but differ greatly in water stress resistance. While LR is very susceptible, UR is resistant. This provides an excellent system for studying the mechanisms of plant water stress tolerance. However, reported researches in this area only addressed biological characteristics, allele and genetic markers (Chang 1972; Ishikawa et al. 1992, 1997), and little is known about the difference in gene expression between UR and LR during water stress.

In this study, we used suppression subtractive hybridization (SSH) (Diatchenko et al. 1996) to obtain transcripts that respond to polyethyleneglycol (PEG) stress, and then performed cDNA microarray analysis to reveal the differences in gene expression between UR and LR under water stress, in an attempt to understand the difference in water stress tolerance mechanisms between UR and LR at the gene expression level.

Materials and methods

Plant growth and stress treatments

Seeds of three UR var. IRAT109, Haogelao, Han297, and three LR var. Zhongzuo93, Yuefu, Nipponbare, were germinated at 32°C for 2 days, and then grown under controlled conditions with 28 ± 2°C temperature, 200 μmol m^{-2} s^{-2} light intensity with 14-h-light/10-h-dark photoperiod and 80% relative humidity. Water deficit was induced by drought treatment and polyethylene glycol (PEG). For drought treatment, the germinated seeds were transferred onto plastic pots (9 cm in diameter and depth) filled with 1:1 sand:vermiculite and were watered with nutrient solution. The nutrient solution contained 1.43 mM NH_4NO_3, 0.27 mM $NaH_2PO_4{\cdot}2H_2O$, 0.51 mM K_2SO_4, 1.0 mM $CaCl_2$, 1.46 mM $MnSO_4{\cdot}7H_2O$, 0.19 mM Na_2SiO_3, 9.5 μM $MnCl_2{\cdot}4H_2O$, 7.5×10^{-2} μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 18.8 μM H_3BO_3, 0.15 μM $ZnSO_4{\cdot}7H_2O$, 0.16 μM $CuSO_4{\cdot}5H_2O$, 35.6 μM $FeCl_3{\cdot}6H_2O$, pH 5.5–6.0. Four weeks after transfer, the seedlings were subjected to progressive drought by withholding water for 1 week. On the seventh day of water stress treatment, leaf rolling index (LRI) was scored at 1300 hours using a 0 to 5 scale with 0 being no rolling, 1 being the first evidence of rolling and 5 being a closed cylinder according to O'Toole and Cruz's methods (1980). Leaf water potential (LWP) was also measured using a pressure chamber (Boyer 1976) (ZS-I plant water potential instrument, China Agriculture University). The soil water content was measured by drying method. For PEG treatment, the germinated seeds were transferred to hydroponic growth conditions and the solution was changed every 2 days. Four weeks after transfer, the seedlings were exposed to 15% (w/v) PEG (molecular weight, 6000) for 9 h, then leaves and root were cut off, frozen in liquid nitrogen, and stored at −80°C until use. The procedure was the same for the control but without PEG treatment.

RNA isolation

Total RNA was isolated from PEG-treated and control seedling samples with TRIZOL reagent (Dingguo, China), and poly $(A)^+$RNA was enriched using a PolyATract mRNA isolation kit (Promega Inc., USA).

Suppression subtractive hybridization (SSH) of the rice variety IRAT109

The suppression subtractive hybridization was carried out using a PCR-select cDNA subtractive kit (Clontech, USA) according to the manufacturer's protocol. Subtraction was performed using cDNA synthesized from PEG-treated plants as tester and that from control plants as driver to enrich genes that are induced by PEG stress. An Advantage PCR cloning kit (Clontech, USA) was used to selectively amplify the cDNA fragments preferentially present in the tester from the subtraction hybridization products.

Construction of subtracted cDNA library

PCR-amplified cDNA produced by SSH, was ligated into the pGEM-T Easy vector (Promega Inc., USA) and transformed into competent *Escherichia coli* (strain DH5a). Individual positive clones were picked (based on blue/white selection) and grown overnight in LB medium containing 15% glycerol.

Preparation of cDNA microarrays

cDNA microarrays were fabricated in Beijing National Biochip Research and Engineering Center. Inserts of cDNA clones were amplified by PCR using nested primers of SSH. The yield and amplification quality of PCR products were confirmed by separating one aliquot of each finished reaction on 1% (w/v) agarose gel. PCR products were precipitated in isopropanol and re-suspended in 50% (v/v) dimethyl sulphoxide (DMSO). The purified PCR fragments were arrayed on poly-lysine-coated micro slide glass (Amersham Pharmacia Biotech). Each amplified sample was spotted three times on each glass slide. In addition, six yeast (*Saccharomyces cerevisiae*) genes that lacked

cross-hybridization to rice genes were used as external controls, and 50% (v/v) DMSO was used as a negative control for subtracting the background. Hex is positive control for nucleic acid fixation. Rice *alpha-tubulin* gene, *beta-tubulin* gene and *actin* gene were used as internal controls. After printing, the spotted cDNA was cross-linked to the slide surface by UV irradiation.

Experimental design, probe labeling, hybridization and data acquisition

The RNA of test sample (four leaf samples and four root samples) for hybridizations was isolated from PEG-treated seedlings of the four varieties. cRNA was generated by in vitro transcription using T7 RNA polymerase on each total RNA. A reference for each tissue type was created by pooling an equal amount of cRNA from every test sample of the same tissue type (leaf or root). Each test sample was Cy5-labeled and the reference samples Cy3 labeled. A test sample was co-hybridized with a reference to the cDNA microarray. The microarray experiment of the sample of Yuefu leaves was performed twice, and more, a 'dye-swap' experiment was carried out. The processes of labeling, hybridization, washing, and scanning were carried out at Beijing National Biochip Research and Engineering Center. Data acquisition and analysis were performed on a GenePix 4000B scanner with GENEPIX 4.0 software (Axon Instruments).

Data analysis and sequencing

We used GProcesser 1.0 (http://keck.med.yale.edu/biostats/software.htm) to normalize (Lowess methods) the signal intensity. Independent clones from the same genes that were spotted at different locations on the microarray were used to verify reliability of the hybridization data. The values of the negative controls were considered as background. The genes that showed expression value equal to or below their neighboring background in at least two independent hybridizations were considered as non-expressing. In the differential expression analysis, cDNAs showing fluorescent intensity levels less than 2 SD over average local background in either the Cy3 or the Cy5 channels of any RNA sample were not considered. The signal mean ratio of Cy5/Cy3 (test/reference) was generated on the basis of normalized signals and used as a relative measurement to determine the relative level of gene expression. Differentially expressed genes were identified by $P < 0.05$ in Students' *t* test.

The ESTs identified to be differentially expressed between the two genotypes were all single-pass sequenced using T7 reverse universal primer (Aoke, China). Unique ESTs were selected and annotated using the NCBI non-redundant databases by BLASTN and BLASTX with an e-value threshold of 1e-08. Functional classification of the ESTs was carried out according to previous function studies of the related genes and functional categories of Arabidopsis proteins (http://mips.gsf.de/proj/thal/db/index.html).

Real-time PCR

RT-PCR reactions were performed using a DNA Engine Opticon™ (Bio-Rad, USA), and the same total RNA samples were used for the microarrays. First stand cDNA was synthesized using AMV Reverse Transcriptase (Promega Inc., USA). SYBR Green I was used as fluorescence label. The EST-specific primers were designed by Primer 5.0 and synthesized by Sangon Company (Shanghai, China). Rice *α-tubulin* gene was used as endogenous reference for data normalization. Transcript concentration was calculated by $2^{-\Delta c(t)}$. Primer sequences are listed in Table 1.

Results

UR is extremely water stress tolerant

To examine the water stress tolerance of UR, four-week-old seedlings were subjected to soil drought. After 1 week without watering, the soil water content was 7%, the seedlings of two UR, IRAT109 and Haogelao, were only slightly affected by the drought and their leaves showed a small degree of leaf rolling (Fig. 1, Table 2). Leaf water potential (LWP) of these 2 UR varieties were −1.9 MPa and −2.2 MPa, respectively, a slight decrease from well-watered conditions. In contrast, severe leaf rolling was observed in all the LR seedlings (Fig. 1), and the leaf rolling index (LRI) of them was 5 or nearly 5 (Table 2). The leaves in these LR varieties were so dry that LWP could not be detected in our experiment (Table 2). Han297, regarded as a UR, did not show strong drought tolerance as the other two UR varieties. The reason may be that Han297 is not a typical UR variety, but was developed from crossbreeding between a UR variety and a LR variety. The result of LRI shows that Zhongzuo93, one of the three LR varieties, was a little more tolerant to leaf rolling than the other two LR varieties (Table 2). We also observed that the UR varieties had much larger root mass; the data is not present here because previous publications already documented this phenotype (Ge 1992; Ling et al. 2002). Based on these results, IRAT109 and Haogelao were selected as water stress resistant UR and Yuefu and Nipponbare as water stress susceptible LR in subsequent experiments.

Table 1 Primers used in RT-PCR to verify the expression pattern of differentially expressed genes from the microarray experiment

Clone ID	Forward primer sequence	Reverse primer sequence
L1F11[a]	5′-GACAAAGAATACAAGCCAAATC-3′	5′-CCGACAGCACCAGAAAGA-3′
L2D8[a]	5′-TAGTTGGTGGAGCGATTTGTC-3′	5′-CCCAGAACATCTAAGGGCATC-3′
L8F8[a]	5′-GGTTATGGAGTTGGTGACGT-3′	5′-AGAATTGTGAGCACCGGATA-3′
L5B5[b]	5′-AAAAGGGAATGGAATGGG-3′	5′-GCAACCTGCGACCTAAAA-3′
L14G10[b]	5′-GCATATTGGCCCCATTTTAT-3′	5′-TGCAACTGGGTTCATCACTG-3′
R2B4[c]	5′-AACAATCACCCAGCTCCAG-3′	5′-AACGCATAAACACCCAGAC-3′
R1G3[c]	5′-GGCTGATGTCCAAAGTGTG-3′	5′-TGTCGAGATGCTGAAGAGG-3′
L8D7[d]	5′-CACTGTGACCCTGACTACCT-3′	5′-GACCCTCCTAATATCCAAAA-3′
L11F3[d]	5′-AGAAGTGCCTCAGGGATG-3′	5′-AGCACCATAAGCAACAGC-3′
L13C11[e]	5′-CTGCTTCTCCTGGAATGGT-3′	5′-TTGGTGGTGTCAAGTAGTTTATC-3′
a-Tubulin	5′-TCAGATGCCCAGTGACAGGA-3′	5′-TTGGTGATCTCGGCAACAGA-3′

[a] EST highly expressed in LR in leaf microarray analysis

[b] EST highly expressed in UR in leaf microarray analysis

[c] EST highly expressed in UR in both leaf and root microarray analysis

[d] EST highly expressed in LR in root microarray analysis

[e] EST highly expressed in UR in root microarray analysis

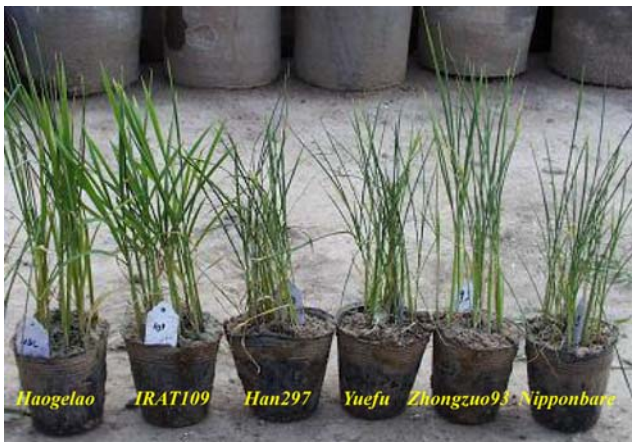

Fig. 1 Phenotypic response of UR and LR to drought stress. Water was withheld from 4-week old seedlings and the soil water content decreased to 7%. The 2 UR varieties on the *left* showed slight sign of stress while the 3 LR on the *right* were severely stressed. Han297 is a UR but showed signs of water stress

The demonstration of extreme water stress tolerance of UR implies the existence of important mechanisms for water stress tolerance in the whole seedling, including leaves and roots; therefore, it was necessary to collect both leaf and root materials for molecular analysis. However, it is difficult to harvest high quality root samples from soil-grown seedlings. We adopted a hydroponic culture system. This method enabled us to collect the roots easily.

Construction of subtracted cDNA library

Since UR is water stress resistant, genes highly expressed in UR may be important for discovering functional genes in water stress tolerance. Thus, we select IRAT109, a UR variety, to construct the subtractive cDNA library, and then use the ESTs from this library to perform cDNA microarray analysis. The method ensured the enrichment of genes that are UR-specific or highly expressed in UR. To identify water stress-inducible genes in IRAT109, we treated plants with PEG (6000) at 15% (w/v) for 9 h. The reasons for using this condition are as follows: (1) PEG (6000)-stress is often used to simulate water stress (Kaufmann and Eckard 1971; Zheng et al. 2004); (2) Li et al. (2001) compared the effect of different concentration of PEG (6000) to the growth of UR and LR seedlings, and concluded that 15% PEG (6000) could distinguish between the resistant genotype and the susceptible genotype; (3) leaf chlorosis is not observed within 9 h in the susceptible LR; (4) genes induced in early stage are more likely to be involved in the signaling responses to water stress than in damaging responses.

Two SSH experiments were performed: subtraction of PEG-treated IRAT109 leaves cDNA (tester) from non-treated IRAT109 leaves cDNA (driver) and that of the same for PEG-treated and non-treated roots. The subtracted DNA fragments were then cloned into T-easy vector, prior to transferring into *E. coli*. A total of 1,344 white colonies derived from leaf materials, and 768 white colonies derived from root materials. The length of insert fragments in these clones ranged from ~200 to ~700 bp (Fig. 2). After discarding clones whose PCR products were showing several DNA fragments according to the result of agarose gel electrophoresis, 1,991 clones were finally selected for microarray analysis.

Table 2 LWP (Mpa) and LRI of rice seedlings under drought stress and well watered conditions

Varieties	Well water		Drought stress	
	LWP (Mpa)	LRI	LWP (Mpa)	LRI
UR				
Haogelao	−1.27	0	−1.9	1.5
IRAT109	−1.39	0	−2.2	1.8
Han297	−1.60	0	–	5.0
LR				
Yuefu	−1.67	0	–	5.0
Zhongzuo93	−1.45	0	–	4.5
Nipponbare	−1.53	0	–	5.0

LWP leaf water potential, *LRI* leaf rolling index

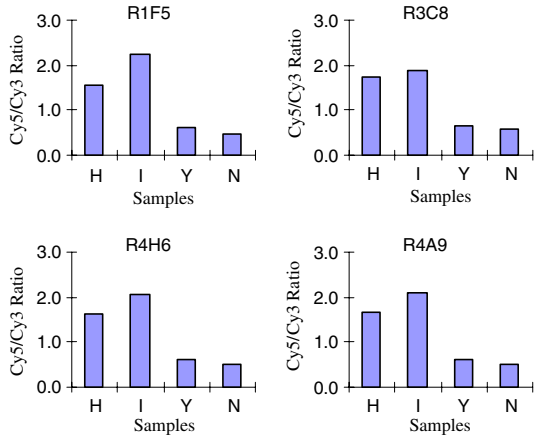

Fig. 3 Comparison of microarray expression pattern of 4 ESTs coding for a putative 1,4-benzoquinone reductase. ID of ESTs is on the *top* of each panel. *Y*-axis represents the Cy5 to Cy3 ratio in hybridization. *X*-axis represents root samples from Haogelao, IRAT109, Yuefu and Nipponbare, respectively

The reliability of cDNA microarray in profiling gene expression during PEG stress

We have used two ways to examine the quality of the microarray data. In the first method, we checked the expression pattern of several ESTs that were arrayed at different locations in the microarray but shared similar sequence (ESTs R1F5, R3C8, R4H6) or coded the same gene (R4A9, putative 1,4-benzoquinone reductase). As shown in Fig. 3, these ESTs had similar expression pattern as detected by the microarray hybridization. In the second method, ten ESTs that showed significant differential expression between UR and LR were selected and their expression was examined by RT-PCR. *α-tubulin* gene was used as endogenous reference for data normalization. *α-tubulin* was selected not only because it is widely used as a housekeeping gene, but also because it did not show differential expression between the four rice cultivars based on our microarray analysis. Except for a few small expression level differences, the expression patterns of all ten ESTs were generally similar between microarray and RT-PCR experiments with a correlation coefficient (r) of 0.9444 (Fig. 4). Furthermore, we performed a dye-exchange replicate of the microarray for the sample of Yuefu leaves to check the consistence between the two dye systems, and the results of the two hybridization experiments showed a good correlation ($r = 0.84$; data not shown).

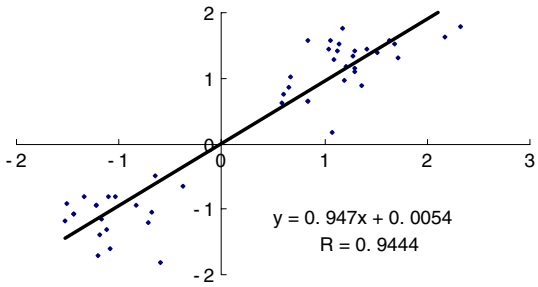

Fig. 4 Correlation analysis of the ratio of differentially expression level from microarray experiment to that from RT-PCR

Expression of PEG-stress induced genes in UR and LR

To compare gene expression between samples subjected to PEG, we used a pooled reference design in which individual experimental RNA samples were hybridized with a common reference. There were three spots for each EST clone on an array. The genes that showed expression value equal to or below their neighboring background in at least two independent hybridizations were considered as non-expressing. When an EST was not expressed in either UR, its expression was not detected in LR, and vice versa. In

Fig. 2 PCR analysis of partial clones from the subtracted library. *M* marker; bands from *bottom* to *top* represent 100, 300, 500, 700, 900, and 1,200 bp, respectively. *Lane 1* to *lane 33*, PCR products from different clones

other words, no EST was found expressed only in UR or LR. The genes that showed signal value more than 2 SD over average local background in both channels (sample and ref) in at least two spots were used in further analyses. By this criterion, expression data from 1,959 cDNA clones could be used in leaf differential expression analysis, and expression data from 1,980 cDNA clones could be used in root differential expression analysis.

Signal ratio of a sample over reference was used in Student's *t* test; each of the two UR samples was compared to each of the two LR (four comparisons for each cDNA) samples. Only ESTs that passed *t* test at $P = 0.05$ for each of all the four comparisons were considered to have a significant difference in expression between UR and LR. Using these statistical criteria, 254 (13%) and 136 (7%) ESTs were identified to be differentially expressed between UR and LR in leaves and roots, respectively.

After removing redundant sequences, 39 and 31 unique ESTs remained as highly expressed in UR leaves and roots, respectively (Table 5); and six of these ESTs were expressed in both leaves and roots. Similarly, 65 and 20 unique ESTs were highly expressed in LR leaves and roots, respectively, with six overlapping in both tissues. The percentage of differentially expressed gene overlapping both tissue types were only 9% (UR, 6/64) and 8% (LR, 6/79), and the majority of the differentially expressed ESTs were found either in leaves or roots. This tissue specificity suggested that the stress response mechanisms in rice leaves might be different from roots.

Upon PEG treatment, a larger number of genes showed higher expression in the water stress susceptible LR than in the water stress resistant UR (Table 5). Genes from certain functional categories showed a high expression in the LR, but not in the UR. These genes are more likely to be involved in degradation of cell components and cell death (Tables 3 and 4). In contrast, UR had larger proportion of the root genes that showed higher expression compared to that in LR. Many genes that showed higher expression in LR in response to PEG treatment had a much larger magnitude of difference compared to those genes that were differentially expressed in UR (Tables 3 and 4).

The sources of GenBank EST hits of each of the sequences that showed differential expression in the microarray were profiled and included in Tables 3 and 4. Many of the profiles showed EST origin from tissues treated by a variety of stresses, supportive of our microarray data.

There were five genes in the category of general protection that were expressed highly in the UR, and only two in the LR (Table 4). Of the five that showed a higher expression in the UR, four were pathogen defense related genes (Table 3).

Although similar number of cell wall related genes were expressed highly in UR and LR, the genes that showed a higher expression level in UR were generally involved in the synthesis of cell wall components and those with higher expression level in LR were involved in the hydrolysis of cell wall components.

In the sugar/osmotant category, a raffinose synthase and an *s*-adenosylmethionine synthetase transcript showed a higher level of expression in the UR. The products from these enzymes are well known for providing osmotic adjustment to protect water stressed cells.

Functional categories of genes differentially expressed between UR and LR

All the sequenced ESTs were searched in the GenBank database using BLAST programs to find annotation information. All the top GenBank hits of each EST were carefully studied and most of the publications associated with the hits were examined to extract the most likely annotation and relevant information for the EST. The ESTs were then classified into various function categories based on the above information (Tables 3 and 4). In some cases, the EST sequence from the microarray was too short to search for reliable annotation information. In such instances, the annotation was based on the GenBank unigene that matched the short EST. A summary of these two tables is presented in Table 5.

The largest function category that showed differential expression between UR and LR is signal transduction (Table 5). This may be due to the short time (9 h) of PEG treatment, but a large number of signaling processes were observed in both water stress resistant and water stress susceptible responses of the two different rice genotypes. A closer look at the gene members in this category indicated that there were more transcription factors expressed highly in UR while more protein kinases in LR (compare Tables 3 and 4), suggesting that the water stress resistant response involved more transcriptional control in gene expression while water stress susceptible response involved more enzyme activities.

There were far more metabolism related genes expressed highly in LR than in UR. Most of these genes in LR seemed to be involved in catabolism, while those in UR were related to biosynthesis. Also the expression of all of these genes in LR were highly expressed in the leaves, indicating that the leaves were affected more than the roots by water stress and the destructive process may have started well before leaf rolling could be visually observed in the LR.

The trend in expression of genes related to protein synthesis and turnover was very similar to those related to metabolism. The majority of the UR expressed genes in this function category play roles in the translation initiation of protein synthesis. In contrast, the LR expressed genes in this category were related to the construction of the ribo-

Table 3 ESTs preferentially expressed in UR in response to PEG treatment

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
1 Metabolism					
R6E2	NM192800	CTP synthase	Many EST from a variety of tissues	1.4	4.0
R1E1	XM464909	Uracil phosphoribosyltransferase, increased 2–10-fold in response to P starvation in shoots (Hewitt et al. 2005)	Callus, panicles, root, shoot, germinating seeds	1.8	
L15B1	XM474263	Phospholipid/glycerol acyltransferase, involved in phospholipid biosynthesis	Most EST from roots of salt or benzylaminopurine (BAP) treated seedlings		1.5
L4C12	NM185498	Putative cytochrome P-450	EST hits from a variety of tissues	1.5	
2 Development regulators					
L14G10	XM493738	Putative early flowering 3 protein, expression slightly affected by salt stress (Boxall et al. 2005)	Many EST hits from pathogen infected rice leaves (Jantasuriyarat et al. 2005)	2.4	
R5C10	XM479432	Putative sex determination protein tasselseed 2	EST hits from a variety of tissues	1.5	
L6E1	AY754864	Maize INDETERMINATE gene, flower time regulation	Panicles, root, shoot, mature leaf	1.5	
3 Protein synthesis/turnover					
R2G3	AK105387	Proteinase inhibitor. Protease inhibitors may perform a defensive role against the proteases	Many EST hits from a variety of stress treatments		1.8
L8F2	XM475493	Protein translation initiation factor Sui1	EST hits from a variety of tissues	1.6	
L11B8	XM467617	Putative nucellin-like aspartic protease	Many EST hits from cold treated calli. An aspartic proteinase was constitutive in drought tolerant cowpea and up-regulated by drought in drought susceptible bean (Cruz de Carvalho et al. 2001)	1.5	
R2B1	AF094774	Translation initiation factor	EST hits from a variety of tissues		1.5
L14G12	XM469841	Translation initiation factor 5A	EST hits from a variety of tissues	1.4	
L6B10	AK100869	Translation initiation factor 2, phosphorylation of this gene promotes a cytoprotective gene expression program known as the integrated stress response (Jousse et al. 2003)	EST hits from a variety of tissues	1.3	
R5D8	AB037153	26S proteasome regulatory particle non-ATPase subunit12	Large number of EST from a variety of tissues		1.2
R5D11	NM197590	Ubiquitin protein	Panicles, shoots, stress or hormone treated calli, drought stressed panicles	1.4	
4 Cell wall					
L10G7	XM481677	Hydroxyproline-rich glycoprotein, cell wall protein	Hydroxyproline-rich glycoprotein has been found to be upregulated in salt-stressed roots (Gu et al. 2004)	2.6	
L3B7	NM183638	Class III peroxidase, involved in cell elongation, wall construction and differentiation, and in the defense against pathogens (Passardi et al. 2004).	ESTs mostly found in roots and shoots	2.6	
L3B6	XM479034	Proline-rich protein, cell-type-specific wall structure during plant development and contributing to defense reactions against physical damage and pathogen infection (Fowler et al. 1999)	Most EST hits from etiolated seedlings or salt-treated roots	1.9	
L8F7	XM470040	Cellulose synthase catalytic subunit	EST hits from a variety of tissues		1.4

Table 3 continued

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
5 Transportation					
R6C3	XM550409	Small basic membrane integral protein (MIP), water transport (Chaumont et al. 2001). Many MIP function as water channels and regulate water transport or cell turgor. Enhanced aquaporin expression in drought-stressed plants (Mariaux et al. 1998)	Many EST from a variety of tissues		2.3
L11F4	AJ535082	ATP-binding cassette (ABC) transporter, a multidrug resistance-associated protein and plays a role in cellular detoxification by transporting toxic compounds from the cytosol into the vacuole (Klein et al. 2003)	Green shoot, etiolated shoot	1.9	
L9E2	XM482515	Myosin-like protein, plays an important role in various developmental processes in plants (Jiang and Ramachandran 2004). Arabidopsis myosin XI mutant is defective in organelle movement and polar auxin transport (Holweg and Nick 2004)	Endosperm, callus, flower, drought stressed or 1-aminocyclopropane-1-carboxylic acid (ACC) treated tissues		1.6
L4D7	XM479449	Potassium transporter	EST hits from a variety of tissues		1.4
L12D1	XM470637	Putative endosomal protein, cross membrane transport	Many EST from BAP or salt treated roots, elicitor treated callus, ACC treated leaves, cold or Cu treated seedlings	1.3	
6 Signaling					
L11F6	AK073264 XM450304	Ubiquitin-protein ligase, protein degradation	Many EST hits from drought or other stress treatments	1.9	
R1F8	AF254558	NAC6, TF, a role in adaptation to abiotic stresses (Ohnishi et al. 2005; Fujita et al. 2004). Over-expression (OE) increased drought tolerance and the expression of drought-inducible genes (Tran et al. 2004)	Etiolated shoot, immature panicle, callus	1.9	
L1F12	AK070914	ER6 protein, ethylene response	EST hits from a variety of tissues	1.8	
R2H7	XM467394	Putative ethylene-responsive protein	Many stress-treated cell cultures, calli, and other tissues		1.8
L2B3	XM472679	Protein phosphatase type 2C	EST hits from a variety of tissues	1.7	
L5C3	XM472655	Putative microsomal signal peptidase	EST hits from a variety of tissues	1.7	
L14D8	AF190770	Ethylene-responsive element-binding proteins, involved in plant responses to stresses (Feng et al. 2005). OE enhanced disease resistance and salt tolerance (Guo et al. 2004; Shen et al. 2003b)	Besides panicles, roots and etiolated shoots, also found in heat, cold, ABA or α-naphthylacetic acid (NAA) treated callus		1.6
R3H6	U49113	Protein phosphatase 2A	EST hits from a variety of tissues	1.6	
L2G3	AK073324	Tetratricopeptide repeat protein, signal transduction and cell communication	Cold and Cu treated callus, salicylic acid (SA) treated leaves or BAP treated roots	1.5	
R2G12	AK101949 XM470558	Putative AP2 domain containing protein, OE of some AP2 containing genes increased stress tolerance (Yi et al. 2004; Tang et al. 2005)	EST hits from a variety of tissues		1.5
L15B6	AB246780	Cytokinin response regulator, involved in cytokinin signaling (Asakura et al. 2003)	NAA or ABA treated calli, roots of irradiated seedlings	1.4	
L13H8	XM478310	Protein phosphatase type 2C, important members of signal transduction	Many EST from gamma-irradiated, UVB iradiated, H2O2, SA or BAP treated tissues		1.3
7 General protection					
L8D12	D64038	EL2, involved in pathogen response (Minami et al. 1996)	Elicitor and irradiation treated tissues		1.7
L12D3	XM479143	Putative RSH, disease resistance-related protein	Many EST hits from rice panicle and 4 nuclei stage pistil	1.7	

Table 3 continued

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
L7D5	AK073894	Putative Avr9/Cf-9 rapidly elicited protein 141, signaling components and play pivotal roles in the initial development of the defense response (Rowland et al. 2005)	Elicitor or Cd treated callus, drought stressed panicle or gamma-irradiated leaves		1.5
R5F8	D55708 AK065866	Putative chitinase, many are pathogenesis-related genes (Van Loon and Van Strien 1999). Expression enhanced by stresses (Wubben et al. 1996; Kastner et al. 1998)	A variety of stress treated tissues	1.5	
L7B3	AK064904	DnaJ domain containing protein, molecular chaperones	EST hits from a variety of tissues		1.4
8 Sugars and osmotant					
R3H4	XM476598	UDP-galactose/glucose 4-epimerase. Epimerisation of UDP-glucose to UDP-galactose	Many EST from a variety of tissues		2.0
L4H11	XM477103 AK120944	Raffinose synthase or seed imbibition protein	Many EST hits from UVC irradiated seedlings, and from heavy metal or drought stressed tissues	1.9	
L7E2	AK102158	Sucrose synthase	EST hits from a variety of tissues		1.8
L2D7	AJ296743	*S*-adenosylmethionine synthetase, provides a methyl group to many metabolites including important compounds under high salinity conditions, such as glycinebetaine, methylated polyols and polyamines	Many EST hits from heat, cold, BAP, ABA, NAA or Cd treated calli	1.5	
9 Oxidation protection					
R1H4	NM191522 AK104580	Quinone oxidoreductase, may have a role in stress defence (Maruyama et al. 2003)	Many EST hits are from ABA-treated histone deacetylase OE lines (Jang et al. 2003) and jasmonic acid (JA) carboxyl methyltransferase OE lines (Seo et al. 2001)	1.5	3.6
L11E5	NM197178	Phytochelatin synthetase, heavy metal tolerance; COBRA protein precursor, cell expansion (Schindelman et al. 2001). Phytochelatin accumulation is related to oxidative stress (Tsuji et al. 2003)	EST hits mostly from desiccated leaves of a ABA-responsive element binding transcription factor 3 OE line, and from cold, heat, Cd or H_2O_2-treated rice seedlings (Kikuchi et al. 2003)	3.0	1.7
L15G12	AF251065	4-hydroxyphenylpyruvate dioxygenase, invloved in tocopherol production (Garcia et al. 1999), which respond to stress (Collakova and DellaPenna 2003) and is believed to protect chloroplast membranes (Fryer 1992; Munne-Bosch 2005)	EST abundant in a variety of rice tissues (Kikuchi et al. 2003)	2.7	
R6B9	AK103977 NM125047	Myo-inositol oxygenase, synthesis of cell-wall matrix polysaccharides (Kanter et al. 2005); plays a role in ascorbate biosynthesis (Lorence et al. 2004)	Large number of EST hits, many from pathogen or other stress treated tissues	1.6	2.7
L13C11	NM188571	Hypothetical protein, some homology to oxidoreductase	Large number of EST hits, most from pathogen (Jantasuriyarat et al. 2005) or stress treated tissues		2.1
L7A2	AF323586	Aldehyde dehydrogenase, catalyze the oxidation of aldehydes to the corresponding carboxylic acid (Skibbe et al. 2002). Removal of aldehydes is essential for cellular survival	Cold, Cd, elicitor, Zn, ABA or NAA treated callus	1.7	
R5E3	AC145324	Oxidoreductase, zinc-binding dehydrogenase family	Roots and panicles	1.7	
R2H8	XM463870	Catalase, ubiquitous H_2O_2-detoxifying enzymes central to the cellular antioxidant response (Kawasaki et al. 1997) and protect cells from different reactive oxygen species (Noventa-Jordao et al. 1999)	EST hits from a variety of tissues	1.6	

Table 3 continued

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
R5A11	AF001396	Metallothionein-like protein, a drought-induced type-2 metallothionein of drought tolerant wild watermelon have potent hydroxyl radical-scavenging activity (Akashi et al. 2004)	EST hits from a variety of tissues		1.5
R1E7	NM191522	1,4-benzoquinone reductase, roles in the detoxification of quinone and protecting against oxidative stress (Laskowski et al. 2002)	EST hits from a variety of tissues		4.1
10 Function unknown					
L5G2	AK067108	Hypothetical protein	Many EST hits from different stress treated tissues		3.1
L5B5	AK065438	Function unknown	EST most abundant in UVB irradiated leaves (Kikuchi et al. 2003)	3.0	
R1G3	AK120906	Rice gene with no known function	Many EST hits Cd treated rice roots.	2.3	2.9
R2B4	XM468449	Unknown protein	EST hits from a variety of tissues.	1.8	2.0
L15H1	AF001395	salT gene, induced by salt, PEG, drought and other stress (Claes et al. 1990)	Green shoots, etiolated shoots; Cu treated calli or gamma-irradiated irradiated seedlings	1.4	
R5E12	XM465274	Nuclear protein with unknown function	EST hits from a variety of tissues		1.4
R3C9	NM196722	Unknown protein	EST hits from a variety of tissues		1.3
L4H6	XM468725	Expressed protein	Many EST from gamma-irradiated, UVB irradiated tissues		1.3
L14D7	XM468654	Unknown protein	EST hits from a variety of tissues		1.2

Numbers in the 'Leaf' and 'Root' columns are the average fold in expression in the 2 UR over the expression in the 2 LR varieties

some. Similarly, several RNA splicing factors were upregulated in LR but not in UR.

One of the most contrasting differences in gene expression between UR and LR was that there were many genes expressed highly in UR that directly or indirectly offer cellular protection against oxidative stress. Ten of these genes showed a higher expression in UR, while only one showed a higher expression in LR.

The expression of several genes involved in cellular transportation increased in UR and LR upon PEG treatment. The majority of these transporters highly expressed in LR were involved in the transportation of inorganic ions and other small molecules, while most of those showing higher expression in UR were associated with membrane mediated movement.

Discussion

The uniqueness of this study

Large-scale gene expression related to water stress has been studied in rice comparing stressed-treated and non-treated plants (Rabbani et al. 2003; Gorantla et al. 2005; Lan et al. 2005). Upland and lowland rice cultivars were also compared (Kathiresan et al. 2006), but the samples were from plants under long-term water stress; and the difference in gene expression could be from morphological and structural changes. Lian et al. (2006) also studied PEG stress responses in upland and lowland rice varieties, but only expression of aquaporins was compared.

The data set from our study is unique and valuable considering the following points. The two UR used in the experiment were proven to be significantly more water stress-tolerant than the two LR cultivars (Fig. 1, Table 2 and Li et al. 2005a). Difference in transcript expression between them under the same stress treatment is useful in associating the genes with the difference in stress response. Changes of gene expression due to a 9 h PEG treatment is more likely to reflect the difference between the UL and LR types of rice in response to water stress, rather than the effects of long-term adaptation. Analysis of both leaves and roots to detect changes in gene expression in this study provided additional insights into tissue specific response to stress. Detailed analyses of the sequences of genes that showed differential expression in this study allowed us to see interesting and informative patterns.

Transcription factors that may be used to engineer water stress tolerance in rice

Many sequences expressed highly in UR by PEG treatment were transcription factors (signaling group in Table 3). Some of these genes were previously reported to be upregulated under different stress treatments; for example, NAC6 (clone R1F8) was induced by dehydration in Arabidopsis (Fujita et al. 2004) and by various stresses in rice (Ohnishi et al. 2005). Even more interesting was clones L14D8

Table 4 ESTs preferentially expressed in LR in response to PEG treatment

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
1 Metabolism					
R5A6	AP004877 AU031599	Serine decarboxylase, catalyzes the conversion of serine to ethanolamine in plants. Expression increased by heavy metal (Ni^{2+} and Mn^{2+}) treatment in Arabidopsis (Fujimori and Ohta 2003)	Various tissue types	6.1	
L12A2	D21287	NADP dependent malic enzyme	Most abundant in leaves	5.1	
L11B3	XM479895	Glyceraldehyde 3-phosphate dehydrogenase	Most abundant in panicles and leaves	4.3	
L14F5	Y07766	*S*-adenosylmethionine decarboxylase, participates in polyamines biosynthesis. Polyamines are reported to maintain ion balance, protect chromatin and decrease the generation of active oxygen species (Bohnert and Jensen 1996)	Many EST are from etiolated leaves, pathogen treated leaves	4.1	
R1B4	AK060371	Glycolate oxidase, activity results in the production of H_2O_2 (Recalcati et al. 2003)	A variety of tissue types	3.9	
L4A5	XM480480	Glyoxalase I, up-regulated by salt stress (Espartero et al. 1995), OE in increased salt tolerance (Veena Reddy and Sopory 1999)	A variety of tissue types	3.9	
L8F8	NM189189	ATP citrate lyase (ACL)	A variety of tissue types	3.9	
L1F11	AK120755	Citrate synthase, glyoxysomal precursor Citrate synthase involved in TCA cycle	A variety of tissue types	3.8	
R1E6	AY335488	Enolase, an enzyme in glycolysis	A variety of tissue types	3.7	
L9F2	NM188089	Similar to isocitrate dehydrogenase.	Mostly in inflorescence	3.7	
R5G7	AF364304	Succinate dehydrogenase subunit 4	EST from a variety of tissues	3.4	
L7G11	XM550301	Glutaredoxin-related protein.	Most abundant in calli, roots and shoots	3.4	
L1B12	XM473160	Glycoside hydrolase	Most abundant in leaves and shoots	3.3	
2 Development regulators					
L3D8	AK120175	Senescence-associated protein	Most abundant in leaves and shoots	4.7	
L9D9	AC145366	SAM dependent carboxyl methyltransferase, enzyme that act on a variety of substrates including SA, JA and 7-methylxanthine	Drought stress panicles	3.7	4.3
R3G7	XM479736	Putative shoot gravitropism 2	Abundant in rice 4 nuclei stage pistil.	4.0	
L11G12	AK066696	Auxin-independent growth promoter	Most abundant in calli and shoots		2.9
3 RNA synthesis					
L10H4	XM463929	Arginine/serine-rich splicing factor RSp41	Many EST from calli, leaves and shoots.	5.9	
L1D9	AY663851	SYD chromatin remodeling ATPase, transcriptional control via chromatin remodeling	Most EST from leaves	4.0	
L6E4	AK064782	DEAD-box helicases	A variety of tissue types		3.9
R2C6	XM468503	Putative splicing factor 3B subunit 2	BAP or NAA treated calli; roots of UVB, NAA and gamma-ray treated seedlings	3.8	
4 Protein synthesis/turnover					
R5C2	AF069218	17S ribosomal RNA gene	EST from a variety of tissues	6.1	
R4G5	M11585	25S ribosomal RNA gene	Etiolated shoot, root, NAA or BAP treated callus, water-stressed sorghum	5.9	
R2A2	AF030517	Elongation factor 1 alpha	Many ESTs from ABA or ABA related treatments	5.2	
L5E11	XM450544	Eukaryotic translation initiation factor 5	A variety of tissue types	4.5	
L8H7	XM470417	Translational elongation factor Tu	Mostly in seeds, meristems and inflorescences	4.3	
L8G7	XM464995	Putative ribosomal protein S3a	A variety of tissue types	4.1	
L2D8	AK059783	Rice gene for 17S ribosomal RNA		4.1	
L15C4	AB026567	Beta 5 subunit of 20S proteasome	Most abundant in panicles	3.4	

Table 4 continued

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
L14D9	XM464508	26S proteasome regulatory particle triple-A ATPase subunit 4	A variety of tissue types	3.4	
L11B8	XM467617	Putative nucellin-like aspartic protease	A variety of tissue types		3.3
R6A10	AY120865	Small subunit ribosomal RNA	Many EST from a variety of tissues	3.3	
L1F7	AF475098	40S ribosomal protein S9	A variety of tissue types	3.1	
5 Cell wall					
R6E1	NM197139	Alpha-galactosidase, hydrolysis of cell wall components (Feurtado et al. 2001) and storage reserves (Overbeeke et al. 1989). OE impaired and antisense increased freezing tolerance (Pennycooke et al. 2003). It is also involved in disease resistance (Evers et al. 2006) and desiccation response (Pukacka and Wojkiewicz 2002)	A variety of tissue types	8.8	
L6E12	AK106980	Polygalacturonase, cell-wall-modifying enzymes with precise temporal and organ-specific expression (Mahalingam et al. 1999). Water loss in harvested cucumber fruits leads to its expression (Kubo et al. 2000). Drought stress caused an increase in its activity in tomato (Huberman et al. 1993)	Leaves and roots treated by a variety of stresses	3.3	
R1G10	XM480452	Germin, constitute a large and highly diverse family of ubiquitous plant cell wall proteins (Mathieu et al. 2003)	Most abundant in seedlings	3.5	3.3
6 Chromosome organization					
R1B12	XM470806	Histone H3.2 protein	A variety of tissue types		3.6
R2D3	AF093632	High mobility group protein, chromatin-associated and act as architectural factors in various nucleoprotein structures, which regulate transcription and recombination (Grasser et al. 2004)	Various tissue types	3.3	
7 Transportation					
R6G1	AK067286 XM473769	Vacuolar H+-ATPase, creating proton gradients and maintenance of pH homeostasis in membrane compartment (Rouquie et al. 1998)	EST in a variety of tissues and treatments	8.5	
L12H6	XM480477	Microtubial binding protein, intracellular membrane trafficking and autophagy	A variety of tissue types	4.4	
R5B9	AY266290	Zinc transporter	Most abundant in etiolated shoot	3.4	4.3
L10C11	AK072976	N-ethylmaleimide sensitive fusion protein, an ATPase involved in intracellular membrane transport	Most abundant in calli	4.1	
L8G8	AK102621	Glutamate transporter	A variety of tissue types	3.7	
R2B2	XM466794	Putative ammonium transporter	A variety of tissue types		3.6
R6A7	XM478702	Monosaccharide transporter, up-regulated by wounding, elicitors, and pathogens (Truernit et al. 1996)	Drought-stressed root and NAA treated tissues	3.5	
L13D5	XM481225	Plant syntaxin, involved in vesicle sorting, docking and fusion in the secretory pathway	A variety of tissue types	3.2	
8 Signaling					
R4H5	AY569615	MYB transcription factor. AtMYB48 and AtMYB59 are induced by stress and strongly induced by salicylic acid (Chen et al. 2006)	More than half of the EST hits are from leaves	9.5	
L7A9	AK104775	Zinc finger (C3HC4-type RING finger) protein, protein-protein interaction	Most abundant in flowers	7.7	
L11E3	AK070809	Zinc finger protein ZFP-like	Most abundant in leaves and calli	5.6	
L13G7	D86925	C-type cyclin, cell cycle regulation	Most EST from leaves	5.1	

Table 4 continued

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
R4F11	XM469882	Zinc finger transcription factor ZF1	EST from a variety of tissues and different stress and hormone treatments	4.5	
L14H8	NM191692	Putative protein kinase	Most abundant in panicles and leaves	4.3	4.4
R3F6	XM469008	Putative protein kinase	Mostly in leaves	4.4	
L9B6	AK065500	PHD finger protein, involved in chromatin-mediated transcriptional regulation	A variety of tissue types	3.6	
L11A9	XM477251	Calreticulin, a major Ca^{2+}-sequestering protein implicated in Ca^{2+} storage, signaling and chaperone activity. OE in rice inhibited callus regeneration and seedling growth (Shen et al. 2003a)	Many EST from a variety of tissues		4.1
L9E10	XM469511	Phosphatidylinositol kinase, plays a vital role in cellular signaling processes in both animals and plants	A variety of tissue types	4.1	
L11G11	NM197752	Unknown protein, belong to dual specificity protein phosphatase family protein	A variety of tissue types	3.8	
R5E8	AY574990	Zinc finger protein	Leaf, shoot, and ABA treated callus	3.8	
R2E10	AK073725	Shaggy-related protein kinase, a key component of the wingless signaling pathway and is required for the establishment of tissue patterning and cell fate (Dornelas et al. 2000)	More than 80% EST found in meristem	3.4	
L11H3	AK121767 NM192781	Ubiquitin activating enzyme	Most abundant in leaves		3.2
L10D12	XM470055	ADP-ribosylation factor, a family of myristoylated small GTP-binding proteins. OE induced many pathogen-related genes, reduced susceptibility to a fungal pathogen, and caused accumulation of SA (Lee et al. 2003)	Most abundant in flowers	3.1	
R6B12	XM469674	WRKY, super family of transcription factors found only in plants	Most abundant in roots		2.9
9 General protection					
L11F3	X67711	Heat shock protein 70, molecular chaperon protecting enzymes, protein complexes, and membranes (Maestri et al. 2002)	A variety of tissues from many stress treatments	3.5	4.9
R4E7	AK073583	24 kDa seed maturation protein, likely belong to LEA proteins and functions in the maintenance of membrane and protein structure (Shao et al. 2005)	Various tissue types	3.1	
10 Sugars and osmotant					
R1G7	Z15028	Sucrose synthase	Many EST from a variety of tissues	5.6	
R2F5	NM194797	Alpha-mannosidase	Mostly in leaves	4.5	
11 Oxidation protection					
L14F3	XM477983	Gamma-glutamylcysteine synthetase, involved in biosynthesis of glutathione, considered a key antioxidant in plants	Abundant in calli, leaves and shoots	4.1	
12 Cell death					
L12D2	XM467920	Zinc metalloproteinase, play a role in plant extracellular cell matrix degradation and may be involved in programmed cell death in cucumber (Delorme et al. 2000)	Most abundant in leaves		3.5
13 Function unknown					
R6E10	XM476999	Unknown protein	Many EST from a variety of tissues		5.2
R1F1	AK120970	Expressed protein	Mostly in pistil	4.9	
R2C12	AK103846	Unknown protein	A variety of tissue types	4.9	4.6
R1H5	CI274161	Unknown protein	A variety of tissue types		4.8
L12F2	AK106513	Expressed protein	Most EST from BAP treated calli	3.9	
L14B9	AK106100	Dormancy/auxin associated protein	Most EST from leaves	3.8	

Table 4 continued

Clone	Hit to NR	Annotation	EST profile	Leaf	Root
R5H12	AK109082	Unknown protein	Roots of gamma-irradiated or H_2O_2 treated seedlings		3.8
L4E11	AK066751	Unknown protein	Most abundant in leaves and shoots		3.5
L8D7	AK061357	Unknown protein	A variety of tissue types		3.6
L6A4	AK073324	Tetratricopeptide repeat protein	A variety of tissue types	3.5	
11 Other					
L11E9	AY187678	Selenium binding protein, participates in intra-Golgi protein transport and in controlling the oxidation/reduction status of target proteins. OE in rice enhanced tolerance to different pathogens (Sawada et al. 2004)	Most abundant in leaves and panicles	3.7	

Numbers in the 'Leaf' and 'Root' columns are the average fold in expression in the 2 LR over the expression in the 2 UR varieties

Table 5 Number of ESTs in different function categories and expressed differentially in UR, LR, leaves and roots

Gene function category	UR > LR			LR > UR		
	Leaf	Root	Total	Leaf	Root	Total
Metabolism	3	2	4	13		13
Development regulators	3		3	3	2	4
RNA synthesis				3	1	4
Protein synthesis/turnover	5	3	8	11	1	12
Cell Wall	3	1	4	3	1	3
Chromosome organization				1	1	2
Transportation	2	3	5	7	2	8
Signaling	8	4	12	13	4	16
General protection	2	3	5	2	1	2
Sugars and osmotant	2	2	4	2		2
Oxidation protection	7	6	10	1	0	1
Cell death					1	1
Function unknown	4	7	9	5	6	10
Other				1		1
Total	39	31	64	65	20	79

The information in this table was extracted from Tables 3 and 4

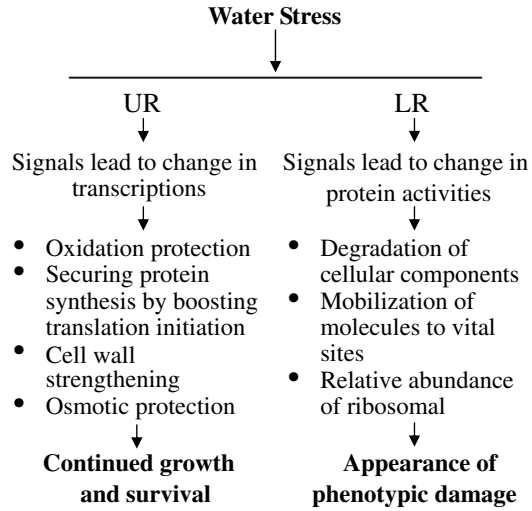

Fig. 5 Summary of transcript response to water (PEG) stress in UR and LR

(a EREBP) and R2G12 (AP2 domain containing protein). They belong to a large AP2 family of transcription factors (Feng et al. 2005; Shigyo et al. 2006). The expression of some members in this family was water stress inducible (Feng et al. 2005; Gorantla et al. 2005; Lan et al. 2005; Li et al. 2005b; Pandey et al. 2005). There have been many instances that overexpression of some AP2 domain containing genes enhanced stress tolerance, including water stress tolerance (Wang et al. 2004; Yi et al. 2004; Li et al. 2005a; Tang et al. 2005; Gao et al. 2005). These early studies support the results of our gene expression study. Taking all these together, it would be interesting to test if overexpression of the genes represented by clones L14D8 or R2G12 in LR can improve water stress tolerance in LR.

Importance of anti-oxidative genes in water stress tolerance

How the transcription factors affect downstream genes that confer water stress tolerance in UR could not be inferred from this gene expression study; however, an increased expression of genes related to combating oxidative stress was observed (Table 3). Water stress is known to increase oxidative stress in rice as measured by an increase in the concentration of superoxide anion (Sharma and Dubey 2005). How the plant can deal with oxidative stress may have an effect on water stress tolerance phenotype. Oxidative stress response was also reported in *Populus przewalskii*, where activity of two enzymes capable of removing oxidative molecules, guaiacol peroxidase and glutathione reductase, was found to be significantly higher in the water

stress tolerant cultivar compared to cultivar that usually grows in wet environment (Lei et al. 2006). In our study, we observed a higher expression of ten (16%) genes involved in oxidation protection in UR cultivars. In contrast, there was only one gene in this category that showed higher expression in the LR cultivars. These results support the notion that water stress causes oxidative stress in plants and water stress tolerance includes a detoxification mechanism that limits the accumulation of reactive oxygen species and oxidative stress.

The conclusion above is also supported by the results of overexpression studies. An aldehyde dehydrogenase (represented by L7A2 in our experiment, Table 3) was overexpressed in Arabidopsis (Sunkar et al. 2003). The transgenic lines showed improved tolerance when exposed to dehydration and the tolerance was correlated with decreased accumulation of lipid peroxidation-derived reactive aldehydes. Transgenic tomato overexpressing a catalase (R2H8 in Table 3) offered protection against photo-oxidation caused by application of herbicide paraquat, water stress and chilling (Mohamed et al. 2003).

Why UR is water stress resistant while LR is susceptible

Based on the microarray data and the annotation information of the differentially expressed sequences, we have proposed a model to explain the different responses of UR and LR to water stress, as shown in Fig. 5. In the water stress tolerant UR, PEG treatment stimulated or maintained the expression of many transcription factors, which then presumably upregulated the expression of genes that help prevent the accumulation of oxidative stress, thus maintaining the integrity of cellular components. Some UR abundant gene transcripts seemed to strengthen cell wall and boost the levels of osmotants so that turgor can be maintained. In contrast, the gene expression in the LR upon PEG treatment reflected a path of degradation. The lack of an effective mechanism to limit oxidative stress may have caused damage to vital molecular and cellular structures. Genes of protein kinases and of enzymes with catabolic activities may have been induced causing further degradation of cellular components. The abundance of transporters indicated that at least some of the resources from degradation might be mobilized to critical tissues such as meristem.

LR is likely to have all the UR genes

Lowland rice typically grows in water and is not considered water stress tolerant. This raises the question whether LR posses any genes that can enhance its ability to deal with water stress. A cDNA microarray study by Rabbani et al. (2003) identified many rice genes that showed high expression upon water stress treatment in Nipponbare (a LR variety). However, it is conceivable that the expression of all those genes was a passive response to water stress and it was still not clear if any of those genes could confer water stress tolerance in lowland rice. In our study, the UR cultivars were significantly more tolerant to water stress than the LR cultivars; therefore, we can assume that there are 'water stress tolerant genes' in UR. At least some of the genes found to be highly expressed in the UR upon PEG treatment in our experiment might be one of those 'water stress tolerant genes'. However, all those genes showing higher expression in UR do exist in LR, although they may not be expressed at a significant level. These results suggested that water stress susceptible LR have the genes that enable UR to be water stress tolerant, but the suppression of the expression of these genes rendered LR susceptible to water stress.

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