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Proteomic analysis of PEG-simulated drought stress-responsive proteins of rice leaves using a pyramiding rice line at the seedling stage

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ABSTRACT. Drought is one of the most severe limitations on the productivity of rice. To investigate the response of rice to drought stress, changes in protein expression were analyzed using a proteomic approach in a drought tolerant quantitative trait locus (DT QTL) pyramiding rice line PD86 by way of PEG simulated drought stress. After drought stress for 8 days, 23 proteins increased in abundance and the level of five proteins decreased. Twelve of the drought-responsive proteins were identified by mass spectrometry. These proteins are involved in redox metabolism, photosynthesis, cytoskeleton stability, defense, protein metabolism, and signal transduction. Among the identified proteins, peroxiredoxin, ribonuclease and putative chitinase, coincided with three QTL regions carried by PD86; in addition, voltage-dependent anion-selective channel protein, ribonuclease and putative chitinase, co-localized with DT QTLs on the chromosome. The expression patterns of some of the corresponding genes were further analyzed at the mRNA level using real-time RT-PCR. The comprehensive results suggested that the differentially displayed proteins might play a role in redox metabolism, photosynthesis, protein degradation, cytoskeleton organization and programmed cell death in the DT mechanism of rice.

Keywords: Drought; DT QTL; Mass spectrometry; Proteomics; Rice; Two-dimensional gel electrophoresis.

INTRODUCTION

The advancement of physiology, molecular biology and genetics has greatly improved our understanding of the responsiveness of rice to stress. Numerous genes induced by osmotic stress have been identified in plants using different functional genomics strategies (Yamaguchi-Shinozaki et al., 2006). With the completion of the rice genome (International Rice Genome Sequencing Project, 2005), identification of rice genes by proteomics is becoming the major part of functional genomics. Lots of effort has been applied to proteomic analysis in rice, and systematic studies have been made on the functional identification of proteins present in tissues, organs and development (Khan and Komatsu, 2004; Komatsu, 2005).

Salekdeh et al. (2002) studied the responses of leaf proteins in three-week-old rice plants over 23 days of transpiration without watering. Mass spectrometry helped to identify 16 of the drought-responsive proteins, including an actin depolymerizing factor. Four novel drought-responsive processes were revealed: up-regulation of an S-like RNase homologue, actin depolymerizing factor, and rubisco activase, and down-regulation of an isoflavone reductase-like protein. Ali and Komatsu (2006) studied the responses of leaf proteins in the leaf sheaths of two-week-old rice seedlings, After drought stress for 2 to 6 days, ten proteins increased in abundance, and two declined. The functional categories of these proteins were identified as defense, energy, metabolism, cell structure, and signal transduction. Results suggest that actin depolymerizing factor is one of the target proteins induced by drought stress.

The key goal of rice proteomics to date is to determine what specific proteins respond to environmental changes like biotic and abiotic stresses (Ali and Komatsu, 2006). Information from previously identified proteins and proteome databases are the basis for further identification of genes and unknown proteins. Knowledge of protein alterations under biotic and abiotic stresses should help us understand the molecular mechanism of stress tolerance in rice at the translation instead of the transcription level.

We report here on the proteomic analysis of the reversibility of drought responsiveness in rice. Proteins were separated by two-dimensional gel electrophoresis (2-DE), quantified by digital analysis and analyzed by mass spectrometry (MS). In addition, we tried to analyze the functions of differentially expressed proteins in the DT method by comparison of the chromosomal locations of the identified proteins with DT QTLs.

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MATERIALS AND METHODS

Plant materials and stress treatment

A drought tolerant QTL pyramiding rice line, PD86, was used in this experiment. According to our genotypic data (unpublished data), PD86 has the same genetic background as IR64 but carries at least 7 DT QTLs on chromosomes 1, 2, 3, 7 and 8 from BR24, and 4 DT QTLs on chromosomes 5, 6, 8 and 12 from OM1723 (Figure 1). Three-week-old seedlings of PD86 were stressed for 8 d by using 20% PEG6000 in the nutrient solution (Yoshida et al., 1976). Leaves were harvested from the treated plants in each treatment, frozen in liquid nitrogen, and kept at -70°C before use.

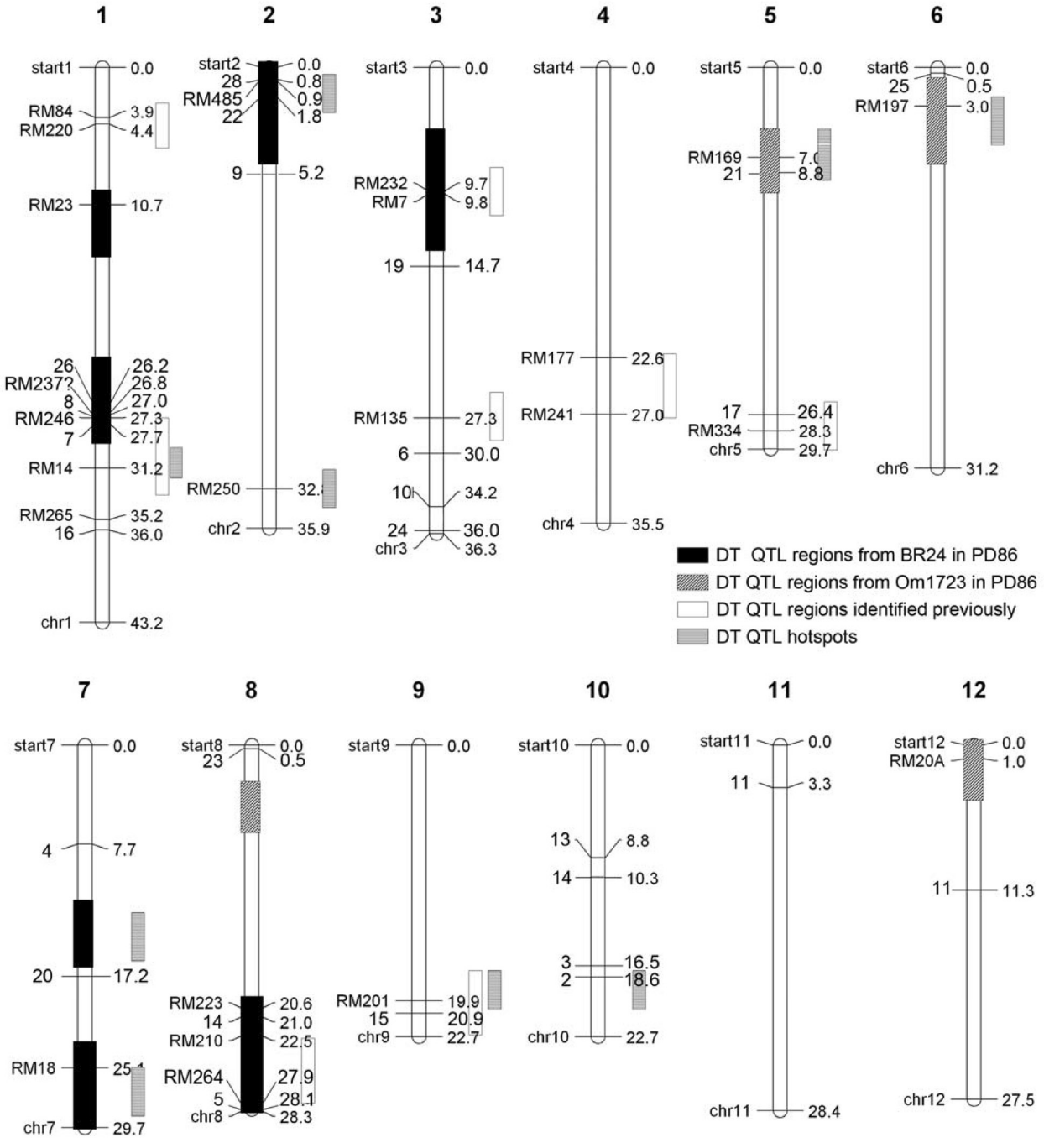

Figure 1. Genomic locations of the overlapped genes of the differentially displayed proteins with previously identified QTLs for DT and related traits. Chromosome numbers are indicated above each of the chromosomes. The numbers on the right side of each chromosome indicate the physical locations (Mb) of the corresponding markers and the overlapped genes. RM on the left side of each chromosome represents the anchor SSR markers, and 1-28 represent the differentially displayed proteins. ▭ and ▤ represent QTL clusters identified in previous studies.

Protein and RNA extraction

RNA and proteins were extracted using a modified protocol according to Chomczynski (Chomczynski et al., 1993). Five-hundred-milligram samples were ground into fine powder in liquid nitrogen, and RNAs were extracted with 5 mL of TRIZOL® Reagent (Invitrogen) following manufacturer's protocols. For real-time RT-PCR, RNA was resuspended in nuclease-free water and quantified with a spectrophotometer at 260 nm. Then mRNA was isolated from total RNA using the Polytract RNA-mRNA Isolation System (Promega) according to the manufacturer's protocol. RNA was treated with RQ1 DNase (Promega) to remove genomic DNA contamination. Proteins were isolated from the phenol-ethanol supernatant obtained after precipitation of DNA with ethanol. Precipitated proteins with 7.5 mL of isopropyl alcohol. Stored samples for 10 min at 15 to 30°C, and sedimented the protein precipitate at 12,000 × g for 10 min at 4°C. Washed the protein pellet 3 times in 10 mL of solution containing 0.3 M guanidine hydrochloride in 95% ethanol. After the final wash, we vortexed the protein pellet in 2 ml of ethanol and centrifuged it at 7,500 × g for 5 min at 4°C; we vacuum dried the protein pellet for 5 min and then dissolved it in 2 ml of 1% SDS by pipetting and incubating the sample at 50°C; we sedimented any insoluble material by centrifugation at 10,000 × g for 10 min at 4°C and transferred the supernatant to a fresh tube. Proteins were precipitated with 2 ml acetone then washed in 2 ml ethanol and vacuum dried the protein pellet for 10 min. The RNA and protein was stored at -70°C until use.

2-DE analysis

The protein samples were dissolved in 300 µl of rehydration buffer (8 M urea, 65 mM DTT, 4% w/v CHAPS, 0.2% w/v Bio-Lyte pH 3-10, and 0.001% w/v bromophenol blue). IPG strips (17 cm, pH 3-10. BioRad ReadyStrip, BioRad) were rehydrated for 16 h. Proteins were focused using a Protean IEF Cell (BioRad, Hercules,CA, USA) at 17°C, applying 250V (30 min), 1000V (60 min), 10000V (5 h) and 10000V for a total of 60KVh. After focusing, proteins were reduced by incubating the IPGstrips with 1% w/v DTT for 15 min and alkylated with 2.5% w/v iodoacetamide in 10 mL of equilibration buffer (6M urea, 2% SDS, 375 mM Tris-HCl [pH 8.8], and 20% v/v glycerol) for 10 min. Electrophoresis in the second dimension was performed with 12% polyacrylamide gels using the Protean xi Cell system (Bio-Rad). The gels were run at 15 mA per gel for the first 30 min followed by 30 mA per gel. Each sample was run in triplicate. Three replications were conducted using independently isolated total protein from stressed and unstressed plants. 2-DE gels were stained with colloidal coomassie brilliant blue (CBB) G-250 and scanned using a GS-800 calibrated densitometer (Bio-Rad). Image analysis was performed with the PDQuest 8.0 software program (Bio-Rad). One gel image was selected as a reference followed by automated spot matching among the gels. The unmatched spots of the member gels were added to the reference gel. The amount of protein spot was expressed as the volume of that spot which was defined as the sum of the intensities of all the pixels that made up that spot. To correct the variability due to CBB staining, and to reflect the quantitative variations in intensity of protein spots, the spot volumes were normalized as the percentage of the total volume in all of the spots present in the gel. The standard error was calculated from spots of the gels from three independent experiments and used as error bars.

Protein identification

Spots from 2-DE were excised from the gel and digested with trypsin (Trypsin Gold, Promega, Madison, WI, USA) according to manufacturer's protocols. The protein spots were excised from gels, washed with 25% methanol and 7% acetic acid for 12 h, and destained with 50 mM NH_4HCO_3 in 50% methanol for 1 h at 40°C. Proteins were reduced with 10 mM DTT in 100 mM NH_4HCO_3 for 1 h at 60°C and incubated with 40 mM iodoacetamide in 100 mM NH_4HCO_3 for 30 min. The gel pieces were minced and allowed to dry and then rehydrated in 100 mM NH_4HCO_3 with 1 pM trypsin at 37°C overnight. The digested peptides were extracted from the gel pieces with 0.1% TFA in 50% ACN/water for 3 times. The peptide solution thus obtained was dried and reconcentrated with 30 ml of 0.1% TFA in 5% ACN/water and then desalted with NuTip C-18 pipette tips (Glygen, USA). The above peptide solution was mixed with CHCA. A matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) was performed using an Ultra flex TOF/TOF tandem mass spectrometer (Bruker, Germany). Mascot software was used to carry out PMF searching in the NCBI rice database. To determine the confidence level of the identification results, we used the MOWSE score and sequence coverage as criteria. Usually, results with a score above 60 are credible. Only the best matches with high confidence levels were selected.

Quantitative real-time PCR (qRT-PCR) analysis

Real-time RT-PCR was performed using an ABI PRISM 7000 (Applied Biosystems) machine and the SYBR Premix ExTaq (TaKaRa, Japan) kit according to the manufacturers' recommendations. After optimizing each of the primer pairs, samples were assayed in 25 ul of the RT-PCR reaction mixture. Real-time amplified RT-PCR products were sequenced, and only those showing 100% homology to the target sequences were used for further analyses. The results were shown as ratios of the concentration of the PCR products from mRNAs divided by the dose of the Actin mRNA. The cycling conditions were 10 s polymerase activation at 95°C and 40 cycles at 95°C for 5 s and 60°C for 31 s. Each assay included (in duplicate) a standard curve of six serial dilution points for the internal control gene or for the target gene (ranging from 50 ng to 640 fg). A non-template control was used for each primer set and each test cDNA. All assays were performed in

duplicate. All experiments were repeated 3 times, and the average ratios of the treated samples vs untreated control were presented. ANOVA was used to analyze the data and to determine the levels of the expression ratios.

Electronic mapping of the subtracted cDNAs onto rice chromosomes

All the mRNA sequences of the identified proteins sequences were aligned against the TIGR japonica rice assembly through the BLASTN algorithm. The chromosomal locations of the mRNA sequence across the twelve rice chromosomes were determined by using MapChart software. Information of QTLs related to DT or osmotic adjustment was aligned onto individual rice chromosomes by using cMap on the Gramene website (http://www.gramene.org), based on physically anchored SSR markers flanking the mapped QTL(s) and cDNAs.

RESULTS

Protein expression in rice leaves under stress

To assess the initial drought response of rice leaves, changes in protein accumulation were examined after increasingly longer drought periods. Comparing 2-DE maps of the control and treatment materials, 28 proteins were found to be changed in response to drought stress (Figure 2). Among them, 23 were up-regulated and five were down-regulated (nos. 2, 3, 4, 5, 18 Figure 2). A digital image analysis was performed, and comparison between the control and treatment groups with respect to the proteins is shown in Figure 3 ($p<0.05$).

Protein identification

In accordance with the criteria, 12 of the 28 differentially displayed protein spots were identified with high probability (Table 1).

All protein sequences identified in this study were searched against databases and sorted into functional categories (Schoof et al., 2002). The results revealed that these proteins were involved in redox metabolism (Prx and putative thioredoxin peroxidase), photosynthesis (rbcS and rbcL), cytoskeleton stability (ABP), defense (putative chitinase), protein metabolism (ribonuclease) and signal transduction (VDAC and osmotin-like protein).

QRT-PCR analysis

Twelve proteins identified by MALDI-TOF were examined at the mRNA level using quantitative RT-PCR (Table 2). Among them, under drought stress, the unknown protein, putative chitinase and rbcL were up-regulated 9.32, 8.11 and 6.93 fold respectively; VDAC, rbcS, ABP and Peroxiredoxin were up-regulated 3.85, 3.41, 3.16 and 2.43 folds; hypothetical protein (spot 4), ribonuclease (spot 14) and ABA/WDS induced protein (spot 11) were slightly up-regulated no more than 1.5 fold; the expressions of osmotin-like protein (spot 16), putative thioredoxin peroxidase and hypothetical protein genes at the mRNA level were not in line with their expression at the protein level. Both peroxiredoxin (Prx) and the unknown protein were strongly up-regulated under the abiotic stress, but down-regulated under ABA, ETH and GA_3.

Table 1. Proteins identified by MALDI-TOF mass spectrometry.

Spot no.	Mr (kDa)/pI		Score	Coverage (%)	Description	Accession no.	Previous study
	Experimental	Theoretical					
↑ 1	15.81/5.94	19.71/8.87	131	64%	rbcS	ABG21973	Yan et al. (2006)
↑ 7	17.61/5.65	17.28/ 5.58	62	26%	Peroxiredoxin	NP916886	Hajheidari et al. (2005)
↑ 9	17.62/4.44	23.28/6.15	125	52%	Putative thioredoxin peroxidase	XP464429	Lee et al. (2007)
↑ 10	19.8/4.9	16.28/4.9	67	46%	Putative actin-binding protein	XP470137	Yan et al. (2005)
↑ 11	20.57/6.34	15.46/6.2	68	40%	ABA/WDS induced protein	ABA91705	Riccardi et al. (1998)
↑ 13	23.8/4.7	16.51/ 4.7	62	41%	Unknown protein	NP920733	(-)
↑ 14	24.2/4.8	24.07/ 4.95	126	58%	Ribonuclease	BAB19805	(-)
↑ 16	26.3/8.7	27.14/7.87	66	34%	Osmotin-like protein	NP915414	Hajheidari et al. (2005)
↑ 17	28.29/8.83	29.58/8.56	62	28%	Voltage-dependent anion-selective channel protein	XP475771	Tanaka et al. (2005)
↑ 21	28.3/5.8	32.76/6.08	98	34%	Putative chitinase	AAV43981	Lee et al. (2006)
↑ 27	38.4/6.7	53.33/6.23	71	23%	rbcL	BAF19909	Yan et al. (2006)
↓ 4	18.9/9.1	10.52/8.77	61	51%	Hypothetical protein	NP911624	(-)

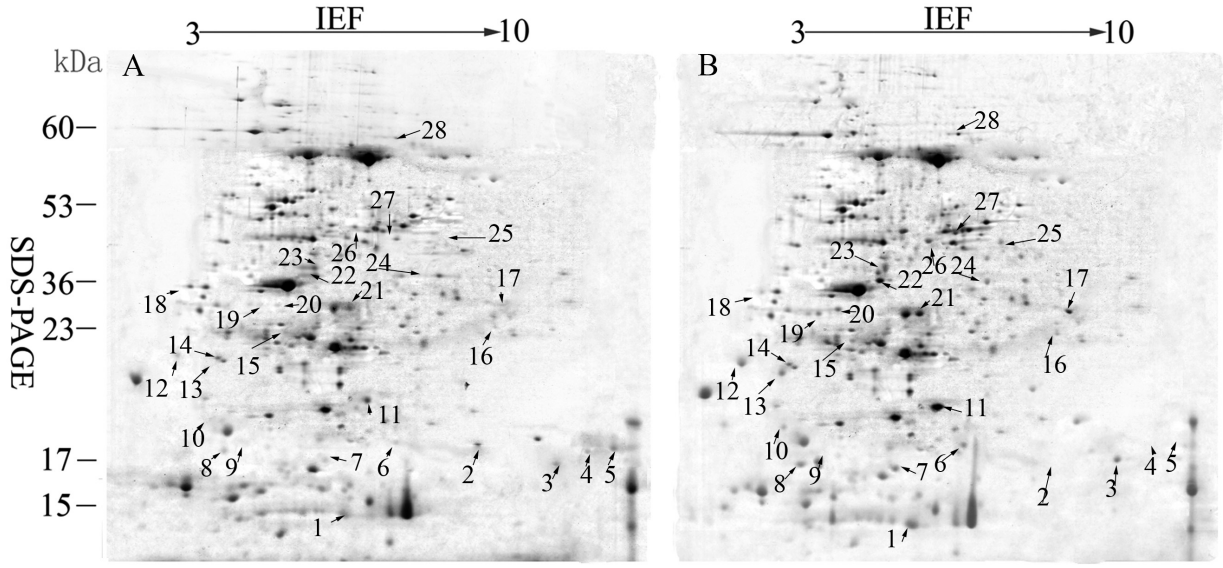

Figure 2. Protein patterns changed by drought stress in rice leaf. Proteins were extracted from leaves, separated by 2D-PAGE and stained by CBB. Following scanning, the gel patterns were analyzed using the PDQuest 8.0 software. Arrows indicate proteins that responded differently to stress. Three-week-old seedlings cultured under the normal nutrient solution were used as control (A) or treated with drought by 20% PEG6000 in nutrient solution for 8 days (B).

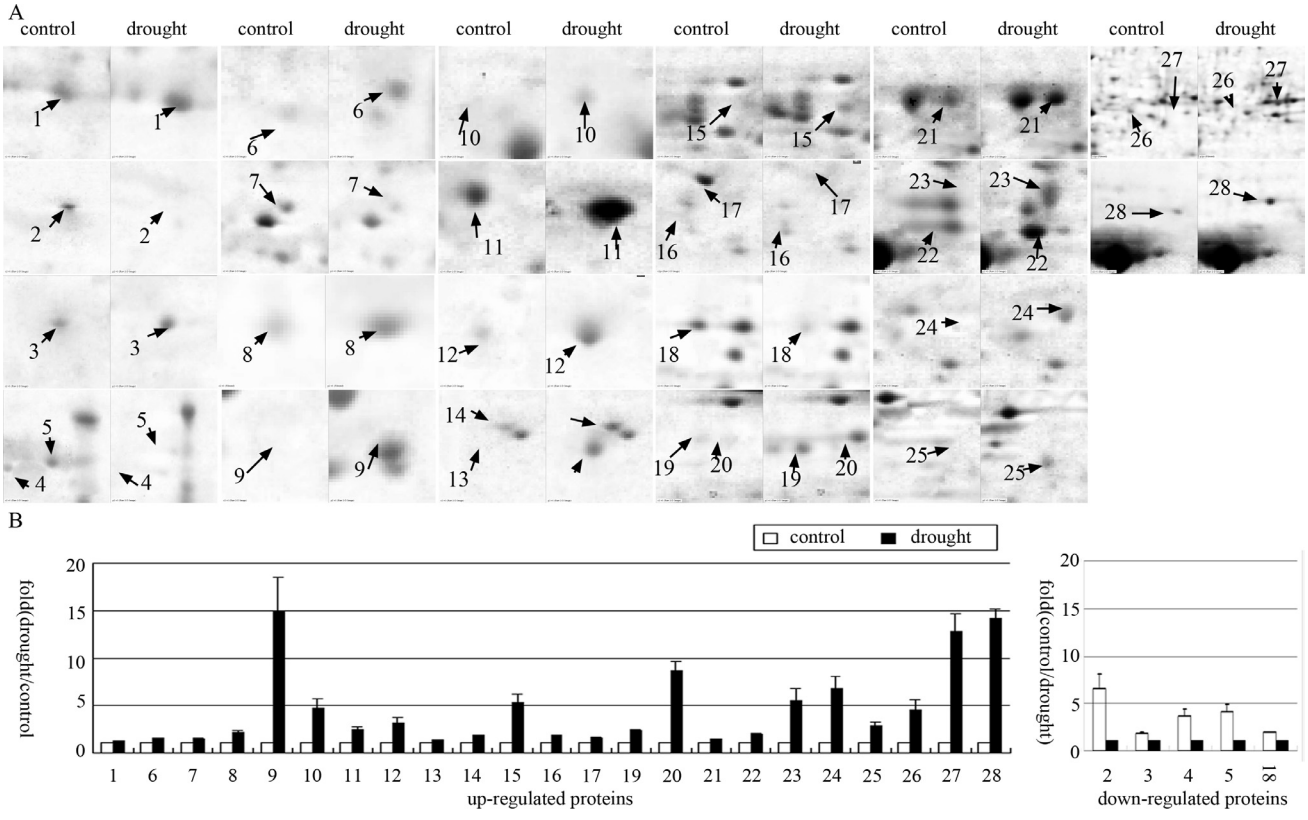

Figure 3. Comparison of the control and treatment groups with respect to the proteins. The changed protein spots in Figure 1 were displayed (A). Following scanning, the gel patterns were analyzed using the PDQuest 8.0 software, and the relative abundance ratio of proteins was analyzed (B). The X-axis denotes the concentration at which rice seedlings were treated, and Y-axis denotes the relative levels of protein expression. X-axis denotes the spot number, and the Y-axis denotes the relative levels of protein expression. The bars in the graphs represent an average volume ± SD of three replicates of the experiment. The white bar shows control and the black bar drought treatment.

Table 2. Genes from the identified proteins showing differential expression detected by quantitative real time RT-PCR under different stresses.

Gene Code	Gene Function Homology	DT[a]/Control	ST[b]/Control	CD[c]/Control	AT[d]/Control	ET[e]/Control	FD[f]/Control	GD[g]/Control
1	RuBisCO small subunit (rbcS)	3.41	11.29	6.53	6.00	3.76	0.82	1.66
7	Peroxiredoxin (Prx)	2.43[h]	2.53	1.31	0.66	0.90	0.86	0.51
9	Putative thioredoxin peroxidase	0.81	1.01	5.50	0.03	0.98	1.09	0.40
10	Putative actin-binding protein (ABP)	3.16	7.37	5.10	4.06	14.01	3.37	2.55
11	ABA/WDS induced protein	1.13	0.74	2.19	NA[j]	NA	NA	NA
13	Unknown protein	9.32	8.28	32.00	0.59	0.24	0.54	0.24
14	Ribonuclease	1.15	9.44	5.21	3.97	3.82	1.75	2.10
16	Osmotin-like protein	0.86	0.09	0.11	0.30	0.14	11.39	0.17
17	Voltage-dependent anion-selective channel protein(VDAC)	3.85	13.86	11.27	15.32	5.32	7.76	3.86
21	Putative chitinase	8.11	9.55	0.76	15.62	26.17	13.64	5.66
27	RuBisCO subunit (rbcL)	6.93	15.64	9.92	17.43	10.55	4.47	8.03
4	Hypothetical protein	1.05	4.73	3.33	6.71	7.49	0.64	2.62

[a]20% PEG treatment; [b]1% salt treatment; [c]4°C cold treatment; [d]0.003 mmol/L ABA sprayed on the leaves under 20% PEG treatment; [e]0.005 mg/L Ethephon sprayed on the leaves; [f]0.003 mmol/L Flouridon sprayed on the leaves under 20% PEG treatment; [g]0.005 mmol/L GA_3 sprayed on the leaves; [h]qRT-PCR ratio values of the stress over control, in which the significance levels at $P < 0.05$; [j]no data.

Genomic distribution of the identified proteins and DT QTLs with comparison of the identified proteins

Using the available bioinformatics tools, we were able to localize the mRNA sequences of the twelve identified proteins onto the rice sequence physical map based on their sequence similarities to genome sequences (Figure 1). Three of the identified proteins coincided with 3 QTL regions carried by PD86 (spot 7 and 14 in DT QTL regions from BR24; spot 21 in DT QTL regions from OM1723). Three proteins (spots 14, 17 and 21) co-localized with 3 DT QTLs identified previously, which are hotspots on chromosomes 5 and 8. All the regions were chromosome regions harboring clusters of the subtracted cDNAs and DT QTLs from our previous study (Fu et al., 2007).

DISCUSSION

We studied changes in the protein expression of rice in response to PEG-simulated drought stress. Twelve drought-responsive proteins were identified by MALDI-TOF. Our research combined QTL, RT-PCR, and proteomic analysis to find the important proteins in the DT mechanism.

Redox metabolism

Two out of twelve identified proteins, Prx (spot 7) and thioredoxin peroxidase (spot 9), which are known to contribute to redox metabolism, were up-regulated under drought stress. In addition, Prx coincided with 1 QTL region carried by PD86. Prx was detected as a highly accumulated protein, and its mRNA was up-regulated under PEG, salt, and cold stresses in this experiment. Prx may play a role in the activation or inhibition of a signal transduction pathway of oxidative stress (Feussner and Wasternack, 2002). Prx, which has a high affinity for peroxide, may also act as a kinetic peroxide and redox sensor (Dietz, 2003). Considering all these specific redox interactions, it may be reasonable to propose that Prx is a knot point in redox metabolism.

Protein degradation

Two proteins, rbcS (spot 1) and rbcL (spot 27), increased under drought stress, but the molecular weights observed in the gels fell short of their theoretical molecular weight. Other studies have yielded similar results and confirmed them as a phenomenon of protein degradation (Yan et al., 2006). Reactive oxygen species and the disorder in physiological metabolism caused by

stress could also lead to incorrect folding or assembly of proteins, so the activity of protein degradation appeared to be particularly important (Luo et al., 2002). The 20S proteasome subunit plays an important role in protein degradation and processing during growth and development (Sullivan et al., 2003). Our results suggest that the protein degradation system may be activated during PEG-simulated drought stress.

Photosynthesis

The two proteins rbcS (spot 1) and rbcL (spot 27) are also involved in photosynthesis: they were detected as increasing under drought stress. It has been reported that rbcS decreased under drought stress in leaf sheath (Yan et al., 2005). In sugar beet, proteome analysis of leaves under drought stress showed that four fragments of the rbcL were up-regulated in drought treated plants of both genotypes (Hajheidari et al., 2005). One of the reasons for the different results might be different tissues as material; another reason might be the different drought treatments and treatment times. It was reported that photosynthesis was repressed under drought stress (Wingler et al., 2000). We suggest one reason for this may be that oxidation stress damaged rbcS and rbcL as discussed above. At the transcription level, the rbcS genes were up-regulated by various external factors such as salicylic acid (SA), salt stress, and drought stress, and rbcL was also up-regulated under drought stress (Lilley et al., 1996; Fu et al., 2007). From these results, we conclude that the expression of rbcS and rbcL of rice seedlings was up-regulated to compensate for the loss of these two proteins to reactive oxygen species. It is proposed that this would maintain photosynthesis and prevent damage in the stressed rice plants.

Cytoskeleton organization

One possible cause of reduced growth rate under stress is a direct inhibition of cell division and expansion. Slower growth is an adaptive feature for plant survival under stress because it allows plants to rely on multiple resources (e.g. building blocks and energy) to combat stress (Zhu, 2001). ABP plays an important role in regulation of the cytoskeleton (Ouellet et al., 2001). Actin depolymerizing factor is one kind of ABP. Ali and Komatsu (2006) studied the responses of leaf proteins in leaf sheaths of two-week-old rice seedlings, and the result indicates that osmotic stress caused by drought may subsequently induce ABP. It also showed that ABP increases in expression under other stresses such as salt, drought, and cold stresses in rice (Yan et al., 2005; Salekdeh et al., 2002). The signal molecules, PI4 or 5P2, might have combined with ABP, which controlled their activity and then acted in tandem to reorganize the skeleton (Fukami et al., 1992). Alternatively, osmotin-like protein might have been involved in cytoskeleton regulation. Osmotin-like protein and chitinase were identified as actin-binding proteins in suspension-cultured potato cells (Takemoto et al., 1997), and osmotin induces cold protection by affecting cytoskeleton organization (Angeli et al., 2007). The results presented here suggest that ABP (spot 10) and osmotin-like protein (spot 16) might play an important role in osmotic adjustment in the plant through cytoskeleton organization.

Programmed cell death

Biochemical and electrophysical studies have shown that the proapoptotic proteins, Bax and Bak, enhance VDAC activity so that cytochrome *c* passes through the channel (Shimizu et al., 2001). In fact, VDAC controls mitochondria-mediated cell death by acting as main components of the permeability transition pore (PTP) or interacting with the Bcl-family protein (Newmeyer et al., 2003; Shimizu et al., 2000).

Another protein involved in programmed cell death (PCD) was osmotin-like protein described above (Angeli et al., 2007). However at the mRNA level, osmotin-like protein was down-regulated under drought, salt, and cold stresses. One cause of this might be that osmotin gene expression was post-transcriptionally regulated (Larosa et al., 1992).

Alternatively, we found that rice seedlings exhibited PCD in response to the osmotic and salt stresses, and two ecotypes (IRAT109 and IR20) appeared to adapt to the two types of stresses through different timing and tissue specificity of cell death (Liu et al., 2007). In this study, VDAC and osmotin-like protein increased under drought stress. VDAC co-localized with a major QTL affecting osmotic adjustment on chromosome 5 (Figure 1) (Nguyen et al., 2004). The results indicated that the increase of VDAC (spot 17) and osmotin-like protein (spot 16) under drought stress might play an important role the tolerance of rice to different abiotic stresses by promoting PCD.

Proteomic approaches are providing unprecedented insights into plant biology, microbiology, and human disease. We report here a systematic proteomic analysis of the leaf proteins in rice under PEG-simulated drought stress conditions. The expression patterns in protein and mRNA levels were analyzed in twelve of these genes under drought conditions. The disaccord of some genes between protein and mRNA levels suggested a post-transcriptional effect or more profound phenomenon might occur. In addition, our research combined QTL, RT-PCR, and proteomic analysis to find the important proteins in the DT mechanism. This global approach will provide a starting platform for further investigation of their functions using genetic and other approaches. The proteins analyzed in this work represent only a small part of the rice proteome. Many other drought-responsive proteins still need to be identified. Deeper proteomic analysis may help us better understand the drought response in rice.

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水稻導入系幼苗葉片在 PEG 模擬乾旱脅迫條件下的蛋白組學分析

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乾旱是制約水稻產量的一個關鍵引物。為了調查水稻對乾旱脅迫的反應，我們以抗旱 QTL 導入系 PD86 為材料通過 PEG 模擬乾旱脅迫，運用雙向電泳技術研究其蛋白水準上的表達變化。經過 8 天的脅迫處理，我們發現有 28 個蛋白的表達發生了變化，其中 23 個蛋白表現上調，5 個下調。通過質譜鑒定出其中的 12 個蛋白，這些蛋白參與抗氧化代謝，光合成，細胞骨架，防禦，蛋白代謝和信號傳導等功能路徑。鑒定的蛋白中，peroxiredoxin，ribonuclease 和 putative chitinase 這 3 個蛋白基因與導入系導入的親本抗旱 QTL 重疊，另外，voltage-dependent anion-selective channel protein, ribonuclease 和 putative chitinase 與已知的主效抗旱 QTL 位元點重合，顯示這些蛋白基因在抗旱機制中發揮重要作用。結合轉錄組水準的螢光定量分析的結果揭示了抗旱途徑在可能存在抗氧化代謝，光合成，蛋白降解，細胞骨架組織和細胞凋亡等機制。

關鍵詞：乾旱；抗旱；雙向電泳；蛋白組；水稻；質譜。