

Gene expression analysis and data mining for the gene network analysis derived from drought stress treatments in rice

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Abstract

To elucidate global responses to drought stress in rice, a 60 mer oligomer microarray covering 22K unique genes based on the sequence of full-length cDNA clones was used to profile gene expression changes at the seedlings, using Apo and IR64 and in the peduncle at heading using IR64. As reported by Ji et al. (2005) Plant Mol Biol. 59:945-964, peduncle elongation in rice is inhibited by drought stress. Cluster analysis of genes up- and down-regulated by drought stress in these two different growth stages revealed the possible combination of the transcription factors and their down-streamers and stage-specific gene expression profiles. Gene expression analysis using the metabolic pathway data in Rice Cyc (<http://www.gramene.org/pathway/>) showed that genes encoding many enzymes of sugar metabolism, such as degradation of sucrose, glucose and galatose, are down-regulated, along with genes encoding enzymes of cell-wall biosynthetic, while genes encoding enzymes of some amino acid biosynthetic pathways are up-regulated. Drought-induced ABA is clearly involved in antagonizing GA-dependent events underlying peduncle elongation, but the biosynthetic genes related to these hormones are not clearly affected by the drought stress treatment. Among 613 differentially expressed transcription factor-related genes, the C3H, AP2-EREBP, bHLH, NAC, MYB and WRKY types of TF-related genes showed differential expression during drought stress treatment and re-watering treatment. Finally, the promoter regions (1kb upstream sequence) of the genes clustered after microarray experiments were examined using a newly developed cis-element analysis tool; the results of this analysis will also be discussed. Data mining using gene annotation data (ex. GO term), pathway data, and genome mapping data suggests the existence of transcription network of drought stress-responsive genes.

Method: Microarray system used in this paper

Agilent 22K rice Oligo Array

This array probes were designed from Rice (Nipponbare) FI-cDNA sequences, and 21495 rice probes were spotted on a array.

The annotation information of FL-cDNA clones were presented in KOME site (<http://cdna01.dna.affrc.go.jp/cDNA/>) and these clones are distributed from Rice Genome Resource Center (<http://www.rgrc.dna.affrc.go.jp/jp/index.html>)

Up-regulation								
	APO	IR64	Ped	PEG	Cold	salt	osmotic	Flood
APO	2466							
IR64	1340	1871						
Ped	922	745	2459					
PEG	1285	895	1128	2834				
Cold	1005	708	968	1320	3737			
salt	256	224	226	369	244	467		
osmotic	156	283	298	456	293	297	551	
Flood	798	553	737	1268	766	208	228	2628

Down-regulation								
	APO	IR64	Ped	PEG	Cold	salt	osmotic	Flood
APO	2707							
IR64	1551	1864						
Ped	692	451	2451					
PEG	1140	891	1272	2682				
Cold	1039	798	1015	1700	3285			
salt	174	140	284	421	417	542		
osmotic	156	148	247	415	397	288	492	
Flood	894	814	970	1676	1327	265	304	2581

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Specific Up-regulation								
	APO	IR64	Ped	PEG	Cold	salt	osmotic	Flood
	251	94	346	177	870	1	6	339

Specific Down-regulation								
	APO	IR64	Ped	PEG	Cold	salt	osmotic	Flood
	260	94	375	58	374	8	3	109

Common Up-regulation		
		33
Common Down-regulation		
		17

Table 2. Genes respond to specific stress and respond commonly

Family	22K whole	Dr_up	Ratio Dr_up	Dr_Down	Ratio Dr_Down	Comment
AP2-EREBP	20	15	33.3%	14	17.5%	
bHLH	70	11	15.7%	25	34.7%	
NAC	64	20	31.3%	16	25.0%	
MYB	40	15	25.0%	16	20.0%	
SB	58	16	27.6%	10	17.2%	
ZIP	54	15	27.8%	11	20.4%	
C3H	54	20	37.0%	5	9.3%	
GRAS	26	5	19.2%	6	23.1%	
WRKY	52	9	17.3%	6	11.5%	
MYB-related	47	14	29.8%	10	19.2%	
PHD	43	16	37.2%	0	0.0%	Dr_up_specific
AUX/IAA	27	7	25.9%	12	44.4%	Dr_up_specific
GRAS	27	8	29.6%	0	0.0%	
GRAS	26	5	19.2%	6	23.1%	
GRAS	26	4	15.4%	10	38.5%	
ARF	23	1	4.3%	3	13.0%	
WRKY	23	2	8.7%	4	17.4%	
WRKY	21	5	23.8%	6	28.6%	
C3C2-GATA	18	2	11.1%	4	22.2%	
HSF	16	9	56.3%	2	12.5%	Dr_down_specific
TUB	15	5	33.3%	2	13.3%	
ZIM	15	3	20.0%	6	40.0%	
SRP	14	5	35.7%	3	21.4%	
C3C2-Def	14	4	28.6%	1	7.1%	
WRKY	11	0	0.0%	3	27.3%	Dr_down_specific
WRKY	11	0	0.0%	6	45.5%	
WRKY	10	3	30.0%	3	30.0%	
C3C2-HAP2	10	2	20.0%	3	30.0%	
C3C2-CC-1	9	2	22.2%	2	22.2%	
WRKY	9	3	33.3%	1	11.1%	
C3C2-HAP2	8	3	37.5%	2	25.0%	
HMG	8	3	37.5%	3	37.5%	
ARID	7	1	14.3%	1	14.3%	
ELL	7	2	28.6%	1	14.3%	
LSD	7	2	28.6%	1	14.3%	

Table 5. List of drought stress responsive TFs classified by gene families

EXP_Pat.	GeneName	RAP Description
Up	AK088555	DnaJ protein homolog AN1
Up	AK060423	Alkylglyoxylate aminotransferase-like protein (Fragment)
Up	AK060757	Adipolytic dihydrogenase family 7 member A1 (EC 1.2.1.3) (Astrigalin 1) (Matured fruit 60 kD protein) (MF-60)
Up	AK061438	Ribonuclease T2 family protein
Up	AK062784	Conserved hypothetical protein
Up	AK092333	Short highly repeated, interspersed DNA (Fragment)
Up	AK063833	Twin-sigrane translocation pathway signal domain containing protein
Up	AK063896	Hypothetical protein
Up	AK063923	no-annotation
Up	AK065206	Phyrtene precursor (EC 3.4.23.40) (Aspartic proteinase)
Up	AK066091	Hypothetical protein
Up	AK068233	Importin alpha 2
Up	AK068727	ERD1 protein, chloroplast precursor
Up	AK069748	Amino acid/lysine transporter II family protein
Up	AK070268	Obolerin regulated protein family protein
Up	AK070556	no-annotation
Up	AK070872	Low-temperature induced protein b101.2
Up	AK070914	Universal stress protein (Usp) family protein
Up	AK070973	Hypothetical protein
Up	AK071108	Temperature stress-induced lipocalin
Up	AK071203	ChcC-like protein family protein
Up	AK072280	Universal stress protein (Usp) family protein
Up	AK099336	Importin alpha 2
Up	AK099418	Dromacyclon-associated family protein
Up	AK100465	Cys/Met metabolism pyridoxal-phosphate-dependent enzymes family protein
Up	AK101209	Myb, DNA-binding domain containing protein
Up	AK101609	Single-stranded nucleic acid binding RCH domain containing protein
Up	AK101837	Thaumatin-like protein
Up	AK102352	Conserved hypothetical protein
Up	AK103194	A-ribonuclease A-like domain (EC 2.3.1.5)
Up	AK105096	Electron transfer flavoprotein alpha subunit, mitochondrial precursor (Alpha-ETF)
Up	AK111578	YT21-B-like protein family protein

Table 3. 33 commonly up-regulated genes among eight treatments

Specific	Family	GeneName	RAP locus description	Description
Dr_comm_U	AP2-EREBP	AK051349	Pathogen-related transcription factor and ERP domain containing protein	
Dr_comm_U	AP2-EREBP	AK060543	Ethylene responsive element binding factor 1 (AREP1)	
Dr_comm_U	AP2-EREBP	AK060552	AP2 domain containing protein PAP2.1 (Fragment)	
Dr_comm_U	AP2-EREBP	AK060670	Ethylene responsive element binding factor 5 (AREP5)	
Dr_comm_U	AP2-EREBP	AK060713	DRE binding protein 2	
Dr_comm_U	AP2-EREBP	AK060775	Drydown responsive element binding protein 1D (DREB1D protein) (C-repeat binding factor 4) (C-repeat/ethylene responsive element binding factor 4) (CRTDRE binding factor 4)	
Dr_comm_U	AUX/IAA	AK073365	AUX/IAA protein family protein	
Dr_comm_U	ZIP	AK063889	Non-protein-coding transcript, untranslatable transcript	
Dr_comm_U	ZIP	AK067919	OSE2-like protein (Fragment)	
Dr_comm_U	ZIP	AK071142	Caserep hypothetical protein	
Dr_comm_U	C3C2-GATA	AK060931	Zn finger, GATA type domain containing protein	
Dr_comm_U	C3H	AK063896	Hypothetical protein	
Dr_comm_U	C3H	AK070857	Zn finger, C-to-C-to-C-to-H type domain containing protein	
Dr_comm_U	C3H	AK060392	Zn finger, C-to-C-to-C-to-H type domain containing protein	
Dr_comm_U	CPP	AK108664	Terminator/TSO-like, CXC domain containing protein	
Dr_comm_U	HD	AK060885	Short highly repeated, interspersed DNA (Fragment)	
Dr_comm_U	HMG	AK103092	HMG protein	
Dr_comm_U	HSF	AK106488	Heat shock transcription factor 29 (Fragment)	
Dr_comm_U	HSF	AK106455	Heat shock factor protein 3 (HSF 3) (Heat shock transcription factor 3) (HSF 3)	
Dr_comm_U	MYB-related	AK065594	Transcription factor MYB52	
Dr_comm_U	MYB-related	AK101209	Myb, DNA-binding domain containing protein	
Dr_comm_U	NAC	AK108083	No special consensus (NAM) protein domain containing protein	
Dr_comm_U	SBP	AK062581	SBP domain containing protein	
Dr_comm_U	SNF2	AK060590	Chromatin remodeling factor (CHR) (CHROMOSPICKLE)	
Dr_comm_U	TbHLH	AK090666	Myb, DNA-binding domain containing protein	
Dr_comm_U	Atf1-like	AK109447	Zn finger-like, PHD finger domain containing protein	
Dr_comm_U	AUX/IAA	AK060938	AUX/IAA protein family protein	
Dr_comm_U	AUX/IAA	AK060618	AUX/IAA protein family protein	
Dr_comm_U	BES1	AK106748	Plant protein of indoleamine synthase DUF922 family protein	
Dr_comm_U	bHLH	AK064946	Transcription factor bHLH1 (Subclass of bHLH expression 1) (Basic helix-loop-helix protein 116) (bHLH116) (bHLH116)	
Dr_comm_U	bHLH	AK101863	TAL1 protein (Fragment)	
Dr_comm_U	K2ZF	AK071639	Eukaryotic transcription factor, DNA-binding domain containing protein	
Dr_comm_U	MYB	AK111803	MYB transcription factor	
Dr_comm_U	NAC	AK070667	OHNAC3 protein	
Dr_comm_U	WRKY	AK066255	WRKY transcription factor 45	
Dr_comm_U	ZIM	AK065170	ZIM domain containing protein	

Table 6. List of common drought responsive TFs

Table 4. 17 commonly down-regulated genes

SIGNAL_NAME	SIGNAL_ID	SIGNAL_SEQ	whole	Dr_up	APO	IR64	PED	PEG	Common	TF
ABRELATERD1	3000414		31.6%	35.8%	33.6%	33.0%	28.3%	37.4%	43.2%	AP2/EREBP (DREB)
SEF3MOTIFGM	3000115	AAACCA	15.2%	15.8%	18.5%	20.9%	14.4%	15.1%	16.2%	-
BOJ1MPTATB	3000296	ATAGAA	15.0%	15.0%	14.6%	20.9%	15.5%	15.0%	12.9%	-
DRB1TCOREAT	3000418	AGCCAA	15.0%	16.7%	17.0%	13.2%	16.2%	15.6%	20.2%	AP2/EREBP (DREB)
CANB9NAPA	3000148	CNAACAC	13.3%	14.2%	13.2%	17.0%	10.2%	14.3%	18.4%	AP2/EREBP (DREB)
CACOTMOTIF	3000042	CACCTO	13.3%	16.3%	13.1%	13.7%	12.8%	15.2%	24.7%	AP2/EREBP (DREB)
TATOCACRAMY	3000083	TATCCA	13.2%	13.8%	13.9%	18.1%	13.4%	16.1%	13.2%	Myb
CAREBSEFP1	3000421	CAACTC	12.6%	12.9%	11.3%	18.7%	15.0%	12.7%	12.2%	Myb
RAT1BAT	3000015	CACCTO	10.8%	11.9%	13.2%	11.2%	9.6%	10.3%	15.1%	AP2/EREBP (DREB)
IBOX	3000134	GTATAG	10.1%	11.0%	9.4%	15.4%	12.9%	9.7%	10.9%	-
HEX2MOTIFATB34	3000033	ACATCA	9.7%	11.3%	12.9%	8.2%	11.2%	10.3%	13.3%	Myb
TATABOX	3000110	TATTAAT	9.3%	9.6%	9.4%	11.0%	8.2%	11.3%	12.9%	-
SIB2OCREMBAB7	3000023	ATATTA	9.2%	9.8%	10.6%	12.1%	11.1%	6.9%	9.2%	TbHLH (DT-Parv)
GT1OCE	3000125	GTITAA	8.9%	9.0%	7.7%	13.7%	8.7%	8.6%	8.2%	Myb
ACOT4ARBMOTIFAZO8EM	3000294	ACOTRC	7.8%	10.8%	7.2%	6.0%	6.6%	10.3%	17.6%	AP2/EREBP (DREB)
DREOCREMBAB7	3000402	ACATCA	7.9%	8.2%	9.1%	4.6%	8.3%	8.3%	10.4%	AP2/EREBP (DREB)
TATOCAYMOTIFORAMYD	3000236	TATOCAY	7.8%	7.5%	7.2%	9.3%	8.2%	7.6%	7.8%	Myb
TOACOTFMAMY	3000377	TOACOT	7.0%	7.1%	9.6%	6.6%	4.5%	7.0%	8.9%	-
ABREBSEFP1	3000012	ACOTSSC	6.2%	8.0%	6.2%	6.6%	6.1%	10.1%	13.2%	AP2/EREBP (DREB)
CDT1REBHC2	3000411	GTTCAC	5.9%	6.0%	4.6%	9.3%	5.4%	6.7%	4.7%	AP2/EREBP (DREB)
IBOXOREBNT	3000404	GATAGC	5.7%	6.2%	5.3%	6.0%	6.2%	4.8%	6.4%	-
SPYACOREB9HAN	3000123	GTTCAC	5.5%	5.5%	4.6%	4.6%	7.3%	4.2%	7.1%	-
REBETALH1R21	3000363	CGATTA	5.3%	6.4%	6.7%	4.4%	3.8%	7.7%	7.1%	-
SPERFSEFP1	3000184									