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Genome-Wide Identification and Transcriptional Analysis of Ap2/EREBP Transcription Factor Family in Rice Under Abiotic and Biotic Stress Conditions

Akhter, Most. Sharoni

University of Rajshahi

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GENOME-WIDE IDENTIFICATION AND TRANSCRIPTIONAL ANALYSIS OF AP2/EREBP TRANSCRIPTION FACTOR FAMILY IN RICE UNDER ABIOTIC AND BIOTIC STRESS CONDITIONS

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SUBMITTED

BY

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June, 2015

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DECLARATION

I do hereby declare that the whole work reported in this thesis entitled **"Genome-wide identification and transcriptional analysis of AP2/EREBP transcription factor family in rice under abiotic and biotic stress conditions"** for the degree of Doctor of Philosophy in the Department of Botany, University of Rajshahi, is the result of my won investigation and was carried out under the supervision of **Dr. Shoshi Kikuchi,** Professor, Plant Genome Research Unit, Division of Genome and Biodiversity Research, National Institute of Agrobiological Sciences (NIAS), Kan'non dai 2-1-2, Tsukuba Ibaraki 305-8602 Japan and **Dr. A.K.M. Rafiul Islam**, Professor, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh. I further declare that this work has not been submitted in the substance for any other degree.

Date:10-06-2015 (Most. Sharoni Akhter)

CERTIFICATE

This is to certify that **Most. Sharoni Akhter** has worked under our supervision. We are pleased to forward her thesis entitled "**Genome-wide identification and transcriptional analysis of AP2/EREBP transcription factor family in rice under abiotic and biotic stress conditions"** which is the record of bonafied research carried out at the Plant Genome Research Unit, Division of Genome and Biodiversity Research, National Institute of Agrobiological Sciences (NIAS), Kan'non dai 2-1-2, Tsukuba Ibaraki 305-8602 Japan. She has fulfilled all the requirements of the regulations relating to the nature and prescribed period of research for submission of the thesis. The data presented in the thesis are genuine and original. No part of this thesis has been submitted in the substance for any other degree.

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CONTENTS

TITLES	Page
LIST OF TABLES	I
LIST OF FIGURES	III
ABBREVIATIONS	VI
ABSTRACT	VII
CHAPTER – I	1
Gene Structures, Classification, and Expression Models of the AP2/EREBP Transcription Factor Family in Rice	1
1. Introduction	4
2. Materials and Methods	4
2.1. Compilation and classification of *OsAP2/EREBP* gene family members	4
2.2. Phylogenetic analysis and sequence alignment	4
2.3. Gene locations on chromosomes and duplications	4
2.4. Biotic and abiotic treatments	5
2.5. Data analysis (44K and 22K arrays)	5
2.6. Transcript-level analysis	6
3. Results	8
3.1. Detection of *OsAP2/EREBP* genes	8
3.2. Phylogenetic analysis and alignments	14
3.3. Chromosomal locations of *AP2/EREBP* genes	19
3.4. Comparison of expression profiles of duplicated *OsAP2/EREBP* genes	28
3.5. Gene expression profiles under biotic and abiotic treatments	30
4. Discussion	53
4.1. Domain/motif duplication and gene expansion	53
4.2. Gene function under biotic and abiotic stress conditions	55

CHAPTER – II 64

Comparative Transcriptome Analysis of *AP2/EREBP* Gene Family under Normal and Hormone Treatments, and under Two Drought Stresses in NILs Setup by Aday Selection and IR64 64

5. Introduction 64

6. Materials and methods 68

6.1. Expression profile analysis (Minghui 63) 68

6.2. Plant growth and drought-treatment conditions for rice 61

6.3. Rice oligo-microarray (44K) and data analysis 72

6.4. Transcript-level analysis 73

7. Results 74

7.1. Expression patterns of *OsAP2/EREBP* genes under normal growth conditions using Affymetrix arrays 74

7.2. Responses of *OsAP2/EREBP* genes to GA3, KT, NAA, SA, JA, and ABA treatments 87

7.3. Gene expression patterns in different rice genotypes under different stress conditions 88

7.4. *AP2/EREBP* gene expression in the root common to both NILs under severe and mild stress conditions 98

7.5. *AP2/EREBP* gene expression in the leaf common to both NILs under severe and mild stress conditions 106

7.6. *AP2/EREBP* panicle genes expression common in two NILs under severe and mild stress conditions 107

7.7. *AP2/EREBP* gene expression in both NILs under both DSCs in root vs. all tissues and in leaf vs. panicle and all tissues 109

7.8. Identification of putative drought responsive genes, subgroups, and subfamilies 110

7.9. Consensus cis-regulatory elements in tolerant NIL 116

7.10. Introgressed regions of the *OsAP2/EREBP* gene in the IR64 background 117

7.11. Gene enrichment analysis for differentially expressed genes in NILs 118

7.12. RT-PCR analysis for confirmation of the transcriptome data 119

8. Discussion 120

8.1. Expression patterns of *OsAP2/EREBP* genes under normal stress conditions 120

8.2. Responses of *OsAP2/EREBP* genes in different treatments 122

8.3. *AP2/EREBP* gene expression profiles under severe stress and mild stress conditions in NILs 123

8.4. Defense system of *AP2/EREBP* genes in rice 126

References 128

List of Publications 140

LIST OF TABLES

Tables	Title	Page
1	Gene primers used for RT-PCR analysis.	7
2	Detailed genomic information about each OsAP2/EREBP gene in rice	9
3	*AP2/EREBP* genes present on duplicated chromosomal segments of rice.	21
4	Tandemly duplicated *AP2/EREBP* genes of rice.	22
5	*OsAP2/EREBP* gene expression profile (log2-based ratio) under RSV infection (44K oligoarray data).	33
6	*OsAP2/EREBP* gene expression profile (log2-based ratio) under RTSV infection (44K oligoarray data).	37
7	*OsAP2/EREBP* gene expression profiles (log 2–based ratio) under RDV infection (three strains S, O and D84) (44K-oligoarray data).	42
8	Preferentially gene induced in specific subgroups under biotic and abiotic stresses conditions.	47
9	*OsAP2/EREBP* gene expression (up or down) profile under various stress treatments (22K oligoarray data).	49
10	The 13 genes expressed (upregulated) by at least three abiotic treatments.	52
11	Stress response of transgenic plants overexpressing DREBs and ERFs.	60
12	Sample vegetative and reproductive developmental stage analyses in Minghui 63.	70
13	Gene primers used for RT-PCR analysis.	74
14	Result of differential expression analysis used seed as reference. Differential expression genes have been taken with P value less than 0.05 and fold change >1 or <-1, which are described by "U" or "D". When fold change >1, regulation is up and when fold change <-1, regulation is down.	77
15	Results of differential expression analysis in 7 day-old seedlings subjected to three phytohormones (GA3, KT, and NAA) treatments in Minghui 63, and another three (SA, JA, and ABA) treated in seedlings of rice. Differential expression genes have been taken p value less than 0.05 and fold change >0.585 or <-0.585, which are described by "U" or "D". When fold change >0.585, regulation is up, and when fold change <-0.585, regulation is down. [CK= untreated sample].	90

16	*OsAP2/EREBP* gene expression (up or down) profile under drought stress at different tissues of plant (44K oligoarray data).	93
17	Differentiated expressed genes in IR77298-14-1-2-B-10 (NIL 10) versus IR77298-14-1-2-13 (NIL 13) and IR 64.	97
18	Overlapped and specific genes were detected in R77298-14-1-2-B-10 (NIL10) and IR77298-14-1-2-B-13 (NIL13) lines under severe and mild stress conditions in all tissues examined.	101
19	Specific up-regulated genes in the tolerant line IR77298-14-1-2-B-10 compared with the sister line IR77298-14-1-2-B-13 and control IR64.	112
20	Subgroup specific differentially expressed genes in all tissues under sever and mild stress conditions in three genotypes.	114
21	The number of differentially expressed gene patterns in Minghui 63 under non-stress conditions and severe stress conditions in the drought-tolerant IR77298-14-1-2-B-10.	115
22	Putative *cis*-elements enriched in promoters of rice *AP2/EREBP* genes.	116

LIST OF FIGURES

Figures	Title	Page
1	Evolutionary relationships among rice AP2/EREBP domain sequences. The unrooted tree was generated by the neighbor-joining method with the ClustalX program. Domains a and b were found in subfamilies RAV and AP2, for example, in *Os01g59780a* and *Os01g59780b*, respectively. The RAV subfamily showed motif duplication, and the AP2 subfamily showed domain duplication. (B) Structure of *AP2/EREBP* genes.	14
2	Relationships among rice AP2/EREBP proteins after alignment with ClustalW. OsAP2/EREBP proteins were allocated to two distinct subfamilies (DREB, subgroups A-1 to A-6; ERF, subgroups B-1 to B-7).	15
3	Alignment of the AP2/ERF domain in the *OsAP2/EREBP* gene family. Comparison of deduced amino acid sequences of the AP2/EREBP DNA-binding domains of the DREB/ERF proteins with those of other proteins with a single AP2/EREBP domain in rice. Amino acid sequences of the AP2/EREBP domains were aligned with the ClustalW multiple sequence alignment program. The different-colored vertical bars show conserved amino acid residues in each subgroup. The tan-colored horizontal bars show where the WLG motif was not conserved.	18
4	Locations of *AP2/EREBP* genes on the 12 rice chromosomes. Chromosome numbers are indicated at the top of each bar. The filled white circle on each chromosome (vertical bars) shows the putative position of the centromere. Genes with open reading frames with opposite orientations are marked on the chromosome (filled black circles represent downward and filled green circles indicate upward orientations). Straight lines connect the *OsAP2/EREBP* genes present on duplicated chromosomal segments, and tandemly duplicated gene clusters are marked by vertical black bars. The scale is in megabases (Mb).	21
5	Exons (black boxes), introns (lines), and untranslated regions (white boxes) of *OsAP2/EREBP*-encoding genes.	27
6	Examples of expression patterns of *OsAP2/EREBP* genes found in duplicated regions of the rice genome. (A) Expression patterns of two *OsAP2/EREBP* genes found in segmentally duplicated regions. (B)	29

Expression patterns of two pairs of tandemly duplicated *OsAP2/EREBP* genes. The different stresses are shown on the *X*-axis and the expression intensity on the *Y*-axis. Gene names are indicated at the top of each graph.

7 Number of differentially expressed genes at different time points: (A) under RSV infection at 3, 6, 9, and 12 days after inoculation (DAI); (B) under RTSV infection at 6, 9, 12, and 15 DAI. Different DAI are shown on the *X*-axis, and the number of differentially expressed genes on the *Y*-axis. (C) The differentially genes were expressed under RDV infection with three strains (S, O, and D84). Number of strains is shown on the *X*-axis, and the differentially expressed genes on the *Y*-axis. 32

8 (A) Histogram of microarray data for eight *OsAP2/EREBP* genes differentially expressed under RSV infection. (B) RT-PCR analysis of eight genes that showed higher expression than the control under RSV infection at 12 DAI. (C) Histogram of microarray data for eight genes downregulated under RSV infection. (D) RT-PCR analysis of eight genes that showed lower expression than the control under RSV infection at 12 DAI. *Actin* gene expression was used as the internal control; mk = mock (control); rsv = RSV- infected plants sampled 12 DAI. The differentially expressed genes are shown along the *X*-axis of the histograms, and the log2 ratios of expression values are shown on the *Y*-axis. 57

9 (A) Histogram of microarray data for seven *OsAP2/EREBP* genes differentially expressed under RTSV infection. (B) RT-PCR analysis of seven genes that showed higher expression than the control under RSV infection at 15 DAI. (C) Histogram of microarray data for five genes downregulated under RTSV infection. (D) RT-PCR analysis of eight genes that showed lower expression than the control under RSV infection at 15 DAI. *Actin* gene expression was used as the internal control; mk = mock (control); rtsv = RTSV- infected plants sampled 15 DAI. The differentially expressed genes are shown along the *X*-axis of the histograms, and the log2 ratios of expression values are shown on the *Y*-axis. 58

10 RT-PCR analysis of differential gene expression under various abiotic treatments. 61

11	Differential expression of *OsAP2/EREBP* genes under eight types of abiotic stress (log 10–ratio). The color bar at top shows levels of expression: red indicates upregulated genes and green indicates downregulated ones.	62
12	(A) Number of up- or down-regulated genes in different tissues under normal conditions in Minghui 63 are presented. (B) Tissue-specific up-regulated genes in Minghui 63:	86
13	Expression profiles of differentially expressed *OsAP2/EREBP* genes in 7-day old seedlings subjected to three phytohormones (GA3= gibberellic acid, KT= kinetin, and NAA= naphthalene acetic acid). *X-axis* represents differentially expressed genes. *Y-axis* represents log_2 expression values. Each treatment is indicated at the top of each diagram. CK= control.	88
14	Up-regulated overlapped and specific genes were detected in NIL10 and NIL13 lines under severe and mild stress conditions in the root, leaf, and panicle. 0.2= severe stress and 0.5 = mild stress.	100
15	Identification of the reference genes showing highest expression in the tolerant NIL compare to the counterparts under severe and mild stress in the root, leaf, and panicle tissues. Fold changes in log_2 values are shown on the *Y*-axis and the different genotypes are indicated on the *X*-axis. NIL10= IR77298-14-1-2-B-10and NIL13= IR77298-14-1-2-B-13.	113
16	The expression intensity of up-regulated genes was higher in NIL10 than in NIL13 or IR64 in all tissues except the leaf under severe stress conditions. The *X*-axis represents the selected tissues, and the *Y*-axis represents the number of up-regulated genes. The number of genes is provided at the top of each diagram.	116
17	Introgressed regions of four *AP2/EREBP* genes are presented in IR64 background.	118
18	RT-PCR analysis of differential gene expression (up- and down-regulated) in all tissues under both drought treatments in all genotypes**.** (A) In the root under severe conditions. (B) In the leaf under severe conditions. (C) In the panicle under severe conditions. *Actin* gene expression was used as the internal control.	120

ABBREVIATIONS

ABA	Abscisic acid
AP2	APETALA 2
DAI	Days after inoculation
DREB	Dehydration-responsive element binding protein
ERF	Ethylene-responsive factor
EREBP	Ethylene-responsive element binding protein
GA3	Gibberellic acid
JA	Jasmonic acid
KT	Kinetin
NAA	Naphthalene acetic acid
NILs	**N**ear-isogenic lines
QTL	Quantitative trait loci
RAV	Related to ABI3/VP1
RDV	*Rice dwarf virus*
RSV	*Rice stripe virus*
RTSV	*Rice tungro spherical virus*
RT-PCR	Reverse transcription – polymerase chain reaction
SA	Salicylic acid

ABSTRACT

The *AP2/EREBP* (Ethylene Responsive Element Binding Protein) genes play various roles in developmental processes and in stress-related responses in plants. We identified 163 *AP2/EREBP* genes in rice. We analyzed gene structures, phylogenies, domain duplication, genome localizations, and expression profiles. Conserved amino acid residues and phylogeny construction using the AP2/ERF conserved domain sequence suggest that in rice the *OsAP2/EREBP* gene family can be classified broadly into four subfamilies (AP2, RAV, DREB, and ERF). The chromosomal localizations of the *OsAP2/EREBP* genes indicated 20 segmental duplication events involving 40 genes; 58 redundant *OsAP2/EREBP* genes were involved in tandem duplication events. There were fewer introns after segmental duplication. We investigated expression profiles of this gene family under biotic stresses (infection with rice viruses such as *Rice stripe virus* [RSV], *Rice tungro spherical virus* [RTSV], and *Rice dwarf virus* [RDV, three virus strains S, O and D84]), and various abiotic stresses. Symptoms of virus infection were severe in RSV infection than by RTSV and RDV. Biotic stresses are novel work and enhance ability to identify the best candidate genes for further functional analysis. The genes of subgroup B-5 were not induced under abiotic treatments whereas activated by three RDV strains. None of the genes of subgroups A-3 were differentially expressed by all biotic stresses. Our 44K- and 22K-microarray results suggest that 53 and 52 non-redundant genes in this family were upregulated in response to biotic and abiotic stresses, respectively. We further examined the stress responsiveness of most genes by RT-PCR. The study results should be useful in selecting candidate genes from specific subgroup for functional analysis.

The *AP2/EREBP* genes play various roles in developmental processes and in stress-related responses in plants. Genome-wide microarrays based on the gene expression profiles of the *AP2/EREBP* family were analyzed under conditions of normal growth and drought stress. The preferential expression of fifteen genes was observed in specific tissues, suggesting that these genes may play important roles in vegetative and reproductive stages of growth. A large number of redundant genes were differentially expressed following phytohormone treatments (NAA, GA3, KT, SA, JA, and ABA). To investigate the gene expression responses in the root, leaf, and panicle of three rice genotypes, two drought stress conditions were applied using the fraction of transpirable soil water (FTSW) under severe (0.2 FTSW), mild (0.5 FTSW), and control (1.0 FTSW) conditions. Following treatment, transcriptomic analysis using a 44K oligoarray from Agilent was performed on all the tissue samples. We identified

common and specific genes in all tissues from two near-isogenic lines, IR77298-14-1-2-B-10 (drought-tolerant) and IR77298-14-1-2-B-13 (drought-susceptible), under drought stress conditions. The majority of the genes that were activated in the IR77298-14-1-2-B-10 line were members of the *AP2/EREBP* gene family. Non-redundant genes (sixteen) were found in the drought-tolerant line, and four genes were selected as candidate novel reference genes because of their higher expression levels in IR77298-14-1-2-B-10. Most of the genes in the AP2, B3, and B5 subgroups were involved in the panicle under severe stress conditions, but genes from the B1 and B2 subgroups were down-regulated in the root. Of the four subfamilies, RAV exhibited the highest number of up-regulated genes (80%) in the panicle under severe stress conditions in the drought-tolerant line compared to Minghui 63 under normal conditions, and the gene structures of the RAV subfamily may be involved in the response to drought stress in the flowering stage. These results provide a useful reference for the cloning of candidate genes from the specific subgroup for further functional analysis.

Chapter 1

Gene Structures, Classification, and Expression Models of the AP2/EREBP Transcription Factor Family in Rice

1. Introduction

Under different adverse environmental conditions such as drought, cold, high salinity, flood, submergence, and pathogen attack, genes show specific expression patterns in accordance with their biological and physiological functions. Transcription factors (TFs) are important for maintaining expression of functional protein genes in the genome. Proteins enhance or repress the TFs of candidate genes in response to biotic or abiotic stimuli and developmental processes. In the plant kingdom, *AP2/EREBP* is a large family of TF genes. TFs encoded by *AP2/EREBP* genes contain the highly conserved AP2/ERF DNA-binding domain (Riechmann et al. 1998). Jofuku et al. (1994) first reported this conserved DNA-binding domain in the homeotic gene APETALA2 (AP2), and Ohme-Takagi and Shinshi (1995) found ethylene-responsive element binding proteins (EREBPs) in tobacco. Three proteins (Pti-4, Pti-5, and Pti-6) were identified by Zhou et al. (1997) to interact with the tomato disease-resistance protein Pto in yeast two-hybrid assays. Each of these proteins has a conserved domain that is very similar to the domain identified by Ohme-Takagi and Shinshi (1995); this domain defines a subgroup of the AP2 family that was later designated as the ethylene-responsive factor (ERF, or ethylene-responsive element binding factor) subfamily. On the basis of the number of AP2/ERF domains encoded and gene function, the

AP2/EREBP gene family has been divided into four subfamilies: APETALA2 (AP2), RAV, dehydration-responsive element binding protein (DREB), and ERF (Sakuma et al. 2002).

Proteins encoded by the *AP2/EREBP* supergene family are defined by the AP2/ERF domain, which consists of 50 to 60 amino acids, and these proteins are involved in a variety of regulatory mechanisms throughout the plant life cycle. Both the DREB and ERF subfamilies are of particular interest owing to their involvement in plant responses to stresses. TFs encoded by genes in the DREB subfamily play an important role in the resistance of plants to abiotic stresses by recognizing the dehydration responsive element (DRE), which has a core motif of A/GCCGAC (Liu et al. 1998). ERF subfamily genes encode a large number of ERFs (Fujimoto et al. 2000), which have been shown to participate in the plant response to biotic stresses such as pathogens by recognizing the *cis*-acting element AGCCGCC, known as the GCC box (Hao et al. 1998). TFs encoded by some members of this gene family bind to both GCC and the DRE *cis*-element (Park et al. 2001, Sakuma et al. 2002). ERF- and DREB-subfamily transcription factors have been identified in various plant species, including rice (Cao et al. 2006), *Arabidopsis* (Liu et al. 1998), and cotton (Jin and Liu 2008). The roles of ERF and DREB proteins in the plant response to biotic and abiotic stress have also been extensively documented (Agarwal et al. 2006, 2010). In the genomes of *Arabidopsis* (Sakuma et al. 2002), grapevine (Jaillon et al. 2007), and poplar (Zhuang et al. 2008), 145, 132, and 200 AP2/ERF-related proteins, respectively, are encoded. Genetic and molecular approaches have been used in combination to characterize a series of regulatory genes of the *AP2/EREBP* gene family involved in many different pathways, including genes related to drought (Dubouzet et al. 2003), high salt concentration (Dubouzet et al. 2003), low temperature (Qin et al. 2007a), diseases (Gutterson and Reuber 2004, Agarwal et al. 2006), and the control of flowering (Elliott et al. 1996). Overexpression of DREB1A (CBF3) or CBF1 (DREB1B) in transgenic *Arabidopsis* has been shown to induce strong expression of target stress-inducible genes and result in improved tolerance to drought, high salt, and

freezing (Jaglo-Ottosen et al. 1998, Liu et al. 1998, Gilmour et al. 2000, Quan et al. 2010, Zhu et al. 2010). Likewise, overexpression of some ERF genes enhances resistance to biotic and abiotic stresses in tobacco (Park et al. 2001). Recently, members of the RAV subfamily have been shown to be involved in the ethylene response (Alonso et al. 2003), the brassinosteroid response (Hu et al. 2004), and biotic and abiotic stress responses in pepper (Sohn et al. 2006). In general, the *AP2/EREBP* gene family has been well studied in *Arabidopsis* (Sakuma et al. 2002).

Characterization of *AP2/EREBP*-family genes in rice can help us understand the molecular mechanisms of resistance to stress, and thus aid in the development of rice varieties, using transgenic technology, with greater tolerance to many adverse environments. Some *AP2/EREBP*-family genes have been isolated from rice (Dubouzet et al. 2003), but most of their functions remain to be determined. Completion of the high-quality sequencing of the rice genome (International Rice Genome Sequencing Project 2005) has provided an excellent opportunity for genome-wide analysis of genes belonging to specific gene families. Here, we identified 163 *OsAP2/EREBP* genes in rice by database searches and classified these genes according to their homology with known genes. In this study, we describe subfamilies more specifically, and we present novel information on their role in the plant response to biotic stresses. Some specific subgroups of this gene family were differentially expressed under biotic and abiotic stress conditions. *OsAP2/EREBP* genes play an important role in the crosstalk of different kinds of stresses signaling pathways. We analyzed the phylogenetic relationships of the *AP2/EREBP* genes in rice, and attempted the complete alignment of the ERF subfamily. In this study, we examined segmental and tandem expression of duplicated genes and exon and intron structures of *OsAP2/EREBP* genes. Furthermore, we studied the expression intensities of *OsAP2/EREBP* genes under different biotic and abiotic stresses using 44K-and 22K-microarray data. Taken together, our results should be helpful for determining the functions of each *OsAP2/EREBP* gene.

2. Materials and Methods

2.1. Compilation and classification of *OsAP2/EREBP* gene family members

We searched for AP2/EREBP TF genes in rice by searching various databases: the DRTF database (http://drtf. cbi.pku.cbi.pku.edu.cn; Gao et al. 2006), the MSU database (http://rice.plantbiology.msu.edu/), the Rice Transcription Factor Database (http://ricetfdb.bio.uni-potsdam. de/v3.0; Riano-Pachon et al. 2007), the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/), and the Knowledge-Based Oryza Molecular Biological Encyclopedia (http://cdna01.dna.affrc.go.jp/cDNA/). We then further conducted BLASTP and TBLASTN searches of MSU and NCBI databases using two search parameters as follows: maximum number of target sequences, 300, and expected value, less than 10. SMART (http://smart.embl-heidelberg.de/) and Pfam (http://pfam.sanger.ac.uk/) database searches were used to confirm and classify each predicted *OsAP2/EREBP* gene. Exon and intron structures of this gene family were investigated by NCPGR (http://gbrowse.ncpgr.cn/cgi-bin/gbrowse/japonica/) database.

2.2. Phylogenetic analysis and sequence alignment

A phylogenetic tree was constructed by using OsAP2/EREBP domain sequences, and an unrooted tree was generated by using ClustalX version 1.83 (Thompson et al. 1997) by the neighbor-joining method (Saitou and Nei 1987) and bootstrap analysis (1000 replicates). The tree was displayed by using MEGA software version 4 (Tamura et al. 2007), and sequence alignments were performed with ClustalW (Thompson et al. 1994).

2.3. Gene locations on chromosomes and duplications

OsAP2/EREBP genes were located on rice chromosomes according to the positions specified in the MSU rice database. To find large segmental duplications, we identified genome

duplications of rice in the MSU database with a maximum permitted distance between collinear gene pairs of 100 kb or 500 kb (http://rice.plantbiology.msu.edu/segmental_dup/index.shtml). We considered genes to be tandemly duplicated if two *OsAP2/EREBP* genes were separated by three or fewer gene loci according to the Rice Genome Annotation Release 6 of MSU.

2.4. Biotic and abiotic treatments

Seedlings (*japonica* cv.Nipponbare) were grown on absorbent tissue paper at 28 °C under a 16 h light: 8 h dark photoperiod for 10 days and were exposed to the various stress treatments: cold (24 h, 48 h, and 72 h, incubation at 10 °C); drought (1 h, 09 h, and 24 h, with the addition of 25% polyethylene glycol 6000 to the planter box); submergence (24 h, 48 h, and 72 h); laid-down submergence (6 h, 24 h, and 48 h); osmotic (addition of 260 mM mannitol to the planter box for 24 h); salt (addition of 150 mM sodium chloride to the planter box for 24 h); plant hormones abscisic acid (ABA), and gibberellic acid (GA) (30-day-old calli were treated with 50 µM ABA or 50 µM GA for 3 days). Control and treatment seedlings were harvested and stored at –80 °C until RNA extraction. Methods used to infect rice plants with biotic stresses were published by Satoh et al. (2010).

2.5. Data analysis (44K and 22K arrays)

For both biotic and abiotic stress treatments [*Rice stripe virus* (RSV, GSE12681); *Rice tungro spherical virus* (RTSV, GSE16141); *Rice dwarf virus* (RDV, GSE24937)], we used 44K-microarray data available at NCBI-GEO. Expression patterns of all samples (at least three biological repeats) were transformed into log 2–based numbers and normalized according to the quantile method for standardization of array data. Expression of a gene (up-or downregulated) was defined as a gene with a log 2–based ratio (RSV, RTSV and RDV-inoculated sample/mock-inoculated sample) higher than 0.585 or lower than –0.585; and a

significant difference in gene expression between the treated plants and the control indicated by $P \leq 0.05$ by paired *t*-test (permutations, all possible combinations; FDR correction, adjusted Bonferroni method). Data processing was done with MeV version 4.3. We identified 162 *OsAP2/EREBP* genes from 44K-microarray data collected under all biotic stress conditions.

We downloaded 22K-microarray data from NCBI-GEO (GSE7532, GSE2415, and GSE661) for eight abiotic stresses above. In all, 22K-microarray gene expression data corresponding to 100 *OsAP2/EREBP* genes were retrieved. Expression intensities on response to all abiotic treatments are log 10–ratio values, and then chose those genes with a threshold value higher than 0.176 or lower than –0.176 in both replications. The differentially expressed genes (DEGs) were either both up-or downregulated in the two repetitions.

2.6. Transcript-level analysis

Total RNA samples were extracted from plant materials with an RNA extraction kit (RNeasy Maxi Kit, Qiagen) in accordance with the manufacturer's instructions. Total RNA samples were extensively pretreated with RNase-free DNase I to eliminate any contaminating genomic DNA. The first-strand cDNA was synthesized from 1 µg of total RNA in a 20-µl reaction volume using Superscript II reverse transcriptase (Invitrogen), and 2 µl of the reaction mixture was subsequently used for RT-PCR runs in a 50-µl reaction volume. RT-PCR was performed using SuperScriptII reverse transcriptase (Invitrogen) in accordance with the manufacturer's instructions to synthesize first-strand cDNA from the DNase I-treated total RNA. The RNA samples for hybridization and RT-PCR were same. About 1/20 of the first-strand cDNA generated from 1 µg total RNA was used as a template for PCR in a reaction volume of 50 µL with rTaq DNA polymerase (Takara). The RT-PCR runs consisted of 25–38 cycles, depending on the linear range of PCR amplification for each gene expression. Each PCR was performed (repeated three times) in an ABI 9700 Thermocycler

(Applied Biosystems) consisted of incubation at 94 °C for 1 min, at 55 °C for 50 s, and at 72 °C for 1 min. Rice *actin* gene was used for RT-PCR as an internal control and the primers are listed in **Table 1**.

Table 1. Gene primers used for RT-PCR analysis.

Gene name	Forward primer	Reverse Primer
Os01g12440	CCACCAACTTCAACCAGTCC	GGGGAGGTTGGACAAGAAGT
Os01g21120	GGGAGCGGAAGAACCAGTA	TGCCGAGGAAGCTCATGTA
Os01g04750	CGACCGATTCAAAGCAAGA	CTCGTCGTCGTAGGTGTGC
Os01g66270	ACACGGCCGTCTACTTCCT	CAGGATCTCCATGTCATCCA
Os02g29550	CAAGGAAATCAAGCGCAGA	CGCCACCATTGATCATCTC
Os02g32140	GACGCTGTCGTCGGAATC	GATCAGGTCGGGGAACG
Os02g45420	GACGCGGTGCTCAATTTC	CGGTAGCTCCACAGGCTCT
Os03g08470	GCGGAGGAAGAAGACGAAG	GCCGTTGGTGAAGAAAGGT
Os03g09170	TGGTGGTGGATAGCAAGGA	AGCTCGGGGAAGTTGAGC
Os03g64260	CACCACCAACAGCTCATCC	GGTCCTCCAGCTCCATCAC
Os04g32620	ACCAAGGCCAAGCTCAACT	CGACGAATCCTTCTTCTTGC
Os04g34970	CGCGGTGACAAGGACAG	ATGCTCCACGAGTGCAAG
Os04g46250	GCCTATGTCGTGGCAAGAG	AGTTGAGGATGGCCTTGG
Os04g46400	GCATCTGGCTGGGTACCT	TACTCGGCCCACAAGAGTG
Os04g57340	ACTTCCCGCTCGTCTCCT	GGAGGTTGAGGTCGAACG
Os05g29810	GATCATCGCCGACTTCGT	AGGAGCCTGAGCTCTCCTG
Os05g39590	ATGGAGGTGGAGACGAAGG	GTGGTGGCAGGAGAACAGA
Os05g41760	AGGAGGCGGTGGAGTAGTG	CTCGCCGGGAAGTAGACAT
Os05g45954	GAAGAAGGGCAAGCAGGTC	GTTCATGGCAACATGAGCA
Os06g07030	GAGGAGGGAGGCAGTATCG	AGGTCGGGGTTCTTGGTC
Os06g10780	CGTACAAGGGGGTGAGGAT	GCGAGCAAGCTGTAGTCCA
Os06g43220	GGAGTGGAGGCAGACATCA	ACGTCAGGCTCGTCCTCT
Os08g31580	CACGGGTTCCACTACATGG	GACCTGGATGAGGCGTTCT
Os08g34360	CACGACACCGTCCTCAACT	CATCGTCTTCGATGGTGG
Os08g36920	GCAGAGGAGGAGGAGGAAGA	TCTGACGACTGTGGGAACC
Os08g42550	ACGGTGCTTCCAGCTCTCT	TGCTGCATCACTTGCTGTC
Os08g43200	CGGACCAAGTTCCAGGAGA	ATTGTCGTCGTTGGGTGAC
Os08g43210	GCACCAAGTTCCACGAGAC	GTCCAGCTCGAACGGTGAT
Os09g13940	GATTCAGGCAAAGCCACA	ACCCGTCCCAGTTGAGTTC
Os09g35030	GACCAAGTTCAGGGAGACGA	ACTCAGGACGTCCAGTTCG
Os10g38000	GGAAGCCGAGCAGGATATG	CAGCTGTGATCCCACAACC
Os11g06770	CCGTCTACTTCCCCTGCAT	CAAGAACCTGGCGTACTCG
Os11g13840	GTGGTGGTGATGCAGAACG	GGAGCAGCATCGTCGTCT
Os12g41060	ATGAGGAGGAGGAGGACGA	TGACATTGTCCCGGATCTC
Actin	CAATGAGCTTCGTGTTGCAC	GGCACCTGAACCTTTCTGAC

3. Results

3.1. Detection of *OsAP2/EREBP* genes

To identify *OsAP2/EREBP* genes in the rice genome, we searched several different public databases, (see Materials and Methods), and used non-redundant sequences of the same genes from the different databases. First, we identified 167 putative *OsAP2/EREBP* genes. We then confirmed all protein sequences of the 167 *OsAP2/EREBP* genes by SMART and Pfam searches for the presence of the AP2/ERF domain. Four of the 167 genes (LOC_Os03g06920, LOC_Os05g28800, LOC_Os12g40960, and LOC_Os12g41040) that had a very small domain were excluded from further analysis, because this domain could not be used to construct an acceptable phylogenesis. Therefore, we used 163 *OsAP2/EREBP* genes for phylogenetic and expression analysis, which had corresponding locus IDs in the Michigan State University Rice Genome Annotation (MSU, http://rice.plantbiology.msu.edu/) and Database of Rice Transcription Factors databases (DRTF, http://drtf.cbi.pku.edu.cn; Gao et al. 2006). Detailed information about the genes and the structures of representative *OsAP2/EREBP* genes is presented in **Table 2**. Hereafter, in this paper the ‘‘LOC_’’ prefix is omitted from the MSU locus IDs.

Table 2. Detailed genomic information about each *OsAP2/EREBP* gene in rice.

1	2	3	4	5	6	7	8	9	10	Protein			14	15
										11	12	13		
Os01g04020	1017	2	1	1	1746088 - 1747104	D	P0504H10	AP002526	AK119645	339	36529	4.5185	A2	DREB
Os01g04750	954	2	0	1	2153326 - 2154279	D	P0480E02	AP002913	AK065008	318	35296	7.6994	RAV	RAV
Os01g04800	1098	2	0	1	2201404 - 2202501	D	P0480E02	AP002913	AK065008	366	39280	10.3208	RAV	RAV
Os01g07120	846	5	2	1	3356377 - 3357222	D	P0701D05	AP003301	AK067313	282	31591	6.073	A4	DREB
Os01g10370	687	1	0	1	5454619 - 5453933	U	B1015E06	AP003197	AK108873	229	24558	6.419	B1	ERF
Os01g12440	1143	2	0	1	6813070 - 6814212	D	P0417G05	AP002835	AK101054	381	40507	4.5781	B2	ERF
Os01g21120	624	1	1	1	11783014 - 11783719	D	B1011A07	AP003722	AK111775	208	22302	7.1866	B1	ERF
Os01g46870	879	1	1	1	26734081 - 26733182	U	P0417G05	AP002835	AK101054	293	30953	4.2163	B3	ERF
Os01g49830	1182	2	0	1	28619938 - 28618757	U	P0034C09	AP003450	AK241984	394	41181	9.3407	RAV	RAV
Os01g54890	714	1	1	1	31566847 - 31567697	D	OJ1003_B06	AP004676	AK105940	238	24000	10.9079	B1	ERF
Os01g58420	708	1	0	1	33766030 - 33766737	D	P0699H05	AP003299	AK061380	236	24270	9.9084	B4	ERF
Os01g59780	1290	3	5	1	34573789 - 34576560	D	OSJNBa0056E15	AC123525	AK112088	430	46708	8.7023	AP2	AP2
Os01g64790	1056	3	1	1	37591403 - 37588991	U	OSJNBb0013K10	AP008245	AK106361	352	36317	6.3474	A5	DREB
Os01g66270	570	1	0	1	38488557 - 38489126	D	N/F	N/F	N/F	190	19825	7.9777	A1	DREB
Os01g67410	2088	4	7	1	39144199 - 39140524	U	P0035F12	AP003313	AK240892	696	71890	7.067	AP2	AP2
Os01g73770	660	1	0	1	42726451 - 42727110	D	OSJNBb0076O03	AP005866	AK287692	220	23818	6.1627	B1	ERF
Os02g06330	612	1	0	2	3165032 - 3164421	U	B1103G11	AP004843	AK287559	204	20557	5.6365	B1	ERF
Os02g09650	600	1	0	2	4959523 - 4958924	U	P0665C04	AP004464	AK064252	200	21093	8.2356	A3	DREB
Os02g10760	618	1	1	2	5688851 - 5689589	D	P0453G09	AP004873	AK110871	206	22444	9.3301	A4	DREB
Os02g13710	744	1	0	2	7365310 - 7366053	D	OSJNBb0034G17	AL663000	AK108473	248	25408	4.9562	B7	ERF
Os02g29550	669	7	6	2	17571123 - 17576025	D	OSJNBb0021C10	AP005630	AK067156	223	25330	9.3184	B1	ERF
Os02g32040	720	1	0	2	18918472 - 18917753	U	P0625E02	AP004016	AK105365	240	25158	4.5898	B4	ERF
Os02g32140	783	1	1	2	18982637 - 18981231	U	OSJNBb0003H22	AP005842	AK109226	261	26409	10.3516	B4	ERF
Os02g34260	654	1	1	2	20486000 - 20485245	U	OSJNBa0069D13	AP004122	AK107852	218	22448	11.1567	B4	ERF
Os02g34270	651	1	1	2	20494008 - 20493260	U	OSJNBa0069D13	AP004122	AK107852	217	22518	11.5575	A4	DREB
Os02g35240	513	1	0	2	21178778 - 21178266	U	N/F	N/F	N/F	171	18048	5.3043	B1	ERF
Os02g38090	1038	1	0	2	23018928 - 23019965	D	OSJNBa0054L03	AP005164	AK241762	346	35946	7.5738	A6	DREB
Os02g40070	2103	5	7	2	24255944 - 24260108	D	OJ2055_H10	AP005300	AK112070	701	73183	6.8005	AP2	AP2

Continue

Os02g42585	1422	2	0	2	25591508 - 25590087	D	OSJNBa0014E22	AP005513	AK242986	474	50587	7.7256	B3	ERF
Os02g43790	912	1	0	2	26417556 - 26416645	U	OJ1003_B06	AP004676	AK105940	304	31632	6.3008	B3	ERF
Os02g43820	1035	1	0	2	26450712 - 26451746	D	P0519E06	AP005006	AK241246	345	36330	5.7499	A4	DREB
Os02g43940	864	1	0	2	26518794 - 26519657	D	OSJNBb0034G17	AL663000	AK107119	288	29450	4.7425	A4	DREB
Os02g43970	678	1	0	2	26545232 - 26545909	D	OJ1112_F09	AP005289	AK108830	226	23144	5.0812	A5	DREB
Os02g45420	786	1	0	2	27615619 - 27616404	D	P0135D07	AP006060	AK243146	262	26966	5.5747	A1	DREB
Os02g45450	675	1	0	2	27647066 - 27648337	D	P0135D07	AP006060	AK106041	225	23946	4.9599	A6	DREB
Os02g51300	1104	4	7	2	31407752 - 31404472	U	OSJNBa0078N11	AP005848	AK242387	368	40145	6.5312	AP2	AP2
Os02g51670	1017	2	0	2	31643162 - 31644178	D	OJ1288_G09	AP004119	AK105991	339	36300	8.098	B4	ERF
Os02g52670	729	1	0	2	32199378 - 32198650	U	P0539D10	AP004817	AK107146	243	25789	6.9863	A5	DREB
Os02g54050	636	1	0	2	33101876 - 33102544	U	N/F	N/F	N/F	212	22783	6.6764	B2	ERF
Os02g54160	1098	3	2	2	33193938 - 33196812	U	OJ1311_D08	AP004849	AK103783	366	40141	4.64	A3	DREB
Os02g55380	519	1	1	2	33915036 - 33914404	U	OSJNBb0019L07	AP007257	AK108355	173	18705	6.9454	B3	ERF
Os03g05590	426	1	0	3	2799334 - 2799759	D	B1234D02	BX842602	AK107745	142	15269	6.7995	A2	DREB
Os03g07830	957	1	0	3	3983896 - 3984852	D	OJ1288_G09	AP004119	AK242121	319	33456	6.3088	B3	ERF
Os03g07940	1650	4	6	3	4060310 - 4056163	U	P0035F12	AP003313	AK240892	550	58450	7.6076	AP2	AP2
Os03g08460	981	2	2	3	4344664 - 4343122	U	OSJNBa0032G08	AC079633	AK073133	327	35853	6.1932	B2	ERF
Os03g08470	1005	2	2	3	4349439 - 4348326	U	OSJNBa0032G08	AC079633	AK101949	335	35760	6.0764	B3	ERF
Os03g08490	966	1	0	3	4367330 - 4366365	U	OSJNBa0032G08	AC079633	AK106987	322	33806	4.7462	B2	ERF
Os03g08500	999	1	0	3	4372399 - 4371228	U	OSJNBa0032G08	AC079633	AK119553	333	38018	11.7413	A6	DREB
Os03g09170	894	1	0	3	4759361 - 4760672	U	OSJNBb0019D17	AC116604	AK287694	298	31793	6.5073	A5	DREB
Os03g12950	1929	4	8	3	6971992 - 6976294	U	OJ1017C11	AC103891	AK106306	643	68662	6.7756	AP2	AP2
Os03g15660	777	1	0	3	8635982 - 8635206	U	OJ1041F02	AC135206	AK068934	259	26070	5.3077	B2	ERF
Os03g19900	1473	5	8	3	11188024 - 11183225	U	OSJNBb0011G21	AC118340	AK109839	491	52869	7.3239	AP2	AP2
Os03g22170	732	1	1	3	12712189 - 12713011	D	OSJNBb0050D18	AC121491	AK111414	244	26170	6.5475	B1	ERF
Os03g56050	2280	5	#	3	31861000 - 31866063	D	OSJNBa0072F13	AC133450	AK241712	760	79716	7.4601	AP2	AP2
Os03g60120	726	1	0	3	34186328 - 34185603	U	OSJNBb0042K11	AC133007	AK068252	242	25487	10.8382	B3	ERF
Os03g60430	1305	4	#	3	34351637 - 34354948	D	P0407H12	AP004303	AK111874	435	47277	7.9709	AP2	AP2
Os03g64260	678	1	0	3	36295184 - 36294507	U	OSJNBa0033P04	AC092263	AK109390	226	24165	6.5094	B3	ERF
Os04g18650	387	1	0	4	10308101 - 10307715	U	B1234D02	BX842602	AK107745	129	14194	5.957	B4	ERF
Os04g32620	807	2	1	4	19464268 - 19466627	D	OSJNBa0039C07	AL731591	AK101501	269	27806	10.2157	B1	ERF
Os04g32790	879	1	0	4	19618461 - 19619339	D	B1103G11	AP004843	AK287559	293	29764	4.3061	B4	ERF
Os04g34970	735	1	0	4	21077651 - 21076917	U	OSJNBa0041K23	AL713902	AK107125	245	24655	7.6742	A4	DREB
Os04g36640	555	1	0	4	21912920 - 21913474	U	B1015E06	AP003197	AK108873	185	19798	5.3535	A6	DREB
Os04g42570	1869	4	6	4	24963397 - 24967270	D	OSJNBa0014K14	AL606604	AK287726	623	63143	5.9724	AP2	AP2

Continue

Os04g44670	1401	1	0	4	26255318 - 26253918	U	OSJNBa0074L08	AL662976	AK107680	467	49861	8.9509	B3	ERF
Os04g46220	957	2	0	4	27152100 - 27153757	U	OSJNBa0079A21	AL607006	AK243188	319	33141	4.7654	B5	ERF
Os04g46240	957	1	0	4	27213167 - 27214123	D	OSJNBa0065H10	AL731613	AK109141	319	33820	4.8782	B5	ERF
Os04g46250	987	1	0	4	27223986 - 27224972	D	OSJNBa0065H10	AL731613	AK109141	329	34817	5.2234	A4	DREB
Os04g46400	855	1	0	4	27332130 - 27332984	D	OSJNBb0034G17	AL663000	AK107119	285	29618	5.0673	A4	DREB
Os04g46410	744	1	0	4	27338261 - 27339004	D	OSJNBb0034G17	AL663000	AK108473	248	26202	4.5625	A4	DREB
Os04g46440	654	1	0	4	27354784 - 27355437	D	OSJNBb0034G17	AL663000	AK108473	218	23015	5.2825	A1	DREB
Os04g48350	660	1	0	4	28601755 - 28602414	D	P0135D07	AP006060	AK106041	220	23900	5.36	B1	ERF
Os04g52090	669	1	0	4	30754173 - 30754841	D	OJ1118_C04	AC108523	AK111755	223	22798	10.2507	A5	DREB
Os04g55520	702	1	0	4	32846598 - 32845897	U	OSJNBa0010D21	AL606635	AK099333	234	25434	10.0932	A3	DREB
Os04g55560	1383	4	8	4	32886859 - 32889487	D	OSJNBa0010D21	AL606635	AK121346	461	48499	7.0458	AP2	AP2
Os04g55970	1488	5	8	4	33156978 - 33160864	D	OSJNBb0022F16	AL606446	AK111891	496	53556	6.0537	AP2	AP2
Os04g56150	420	1	1	4	33252646 - 33252077	U	OSJNBb0019L07	AP007257	AK108355	140	14869	5.1887	B1	ERF
Os04g57340	573	1	0	4	33949333 - 33948761	U	OSJNBa0043A12	AL606619	AK067373	191	19890	9.3677	B1	ERF
Os05g03040	1539	4	7	5	1159687 - 1156284	U	OSJNBa0010D21	AL606635	AK099991	513	55608	7.2189	AP2	AP2
Os05g25260	846	2	0	5	14605766 - 14606611	D	OSJNBa0065C11	AC124141	AK110613	282	30331	10.3509	A2	DREB
Os05g27930	1122	3	1	5	16242725 - 16239854	U	B1164G01	AC134928	AK071850	374	40473	4.4881	A2	DREB
Os05g28350	810	1	0	5	16544295 - 16543486	U	OSJNBa0074L08	AL662976	AK107680	270	27627	7.8566	B2	ERF
Os05g29810	597	1	1	5	17186672 - 17187467	D	P0692D12	AC135929	AK100184	199	20772	7.7097	B7	ERF
Os05g32270	1047	4	7	5	18749964 - 18754112	D	OSJNBa0073E05	AC136219	AK059324	349	39108	8.689	A5	DREB
Os05g34730	594	1	0	5	20539295 - 20538702	U	N/F	N/F	N/F	198	20067	9.8746	B4	ERF
Os05g36100	1095	4	1	5	21342132 - 21346473	D	OSJNBb0050D18	AC121491	AK111414	365	37064	4.9708	B3	ERF
Os05g37640	1188	1	0	5	21966665 - 21968486	D	B1122D01	AC130602	AK063748	396	41158	6.4712	A2	DREB
Os05g39590	786	2	1	5	23185369 - 23187225	D	P0486C01	AC135924	AK102559	262	27689	4.8025	B5	ERF
Os05g41760	570	1	0	5	24366103 - 24365534	U	OJ1118_C04	AC108523	AK242393	190	19789	10.1626	B1	ERF
Os05g41780	711	1	0	5	24382500 - 24381790	U	OJ1118_C04	AC108523	AK111755	237	24113	9.3484	B3	ERF
Os05g45954	1296	1	6	5	26571116 - 26569248	D	P0708B04	AP004764	AK106769	432	47683	8.2816	AP2	AP2
Os05g47650	1185	2	0	5	27240341 - 27241525	D	P0554F08	AC136492	AK243363	395	41381	10.055	RAV	RAV
Os05g49010	849	1	0	5	28047643 - 28046795	U	OJ1003_B06	AP004676	AK105940	283	30274	8.2477	A6	DREB
Os05g49700	822	8	0	5	28437830 - 28437009	U	OSJNBa0095C07	AC077693	AK287696	274	29706	10.119	A1	DREB
Os06g03670	645	1	0	6	1433798 - 1434442	D	OSJNBb0076O03	AP005866	AK287692	215	23110	4.7872	B3	ERF
Os06g05340	1200	3	5	6	2401591 - 2398692	U	P0708B04	AP004764	AK106769	400	43093	7.2791	AP2	AP2
Os06g06540	1029	1	0	6	3066710 - 3067738	D	P0417G05	AP002835	AK101054	343	36458	4.6277	A1	DREB
Os06g06970	762	1	0	6	3309944 - 3310705	D	OSJNBb0076O03	AP005866	AK287692	254	27668	10.7473	A5	DREB
Os06g07030	555	1	0	6	3336163 - 3336717	D	OSJNBa0015I14	AP002536	AK107775	185	19678	11.1922	A3	DREB

Continue

Os06g08340	537	1	1	6	4039909 - 4040552	D	OSJNBb0019L07	AP007257	AK108355	179	19168	6.0005	B2	ERF
Os06g09390	1089	3	2	6	4730451 - 4732320	D	P0648E08	AP004280	AK069833	363	39764	4.5227	A5	DREB
Os06g09717	624	1	0	6	4959148 - 4959771	D	N/F	N/F	N/F	208	22187	4.5266	A5	DREB
Os06g09760	375	1	1	6	4971238 - 4971774	D	P0453D01	AP004691	AK068312	125	13393	9.7514	A5	DREB
Os06g09790	630	1	1	6	4990220 - 4991150	D	N/F	N/F	N/F	210	23023	4.6622	A5	DREB
Os06g09810	624	1	0	6	4997065 - 4997688	D	N/F	N/F	N/F	208	21805	4.6033	A5	DREB
Os06g10780	834	2	1	6	5628417 - 5630683	D	OJ1041F02	AC135206	AK068934	278	29197	4.9661	A6	DREB
Os06g11860	1083	2	0	6	6305028 - 6303946	U	OJ1288_G09	AP004119	AK242121	361	37817	7.1939	A5	DREB
Os06g11940	672	1	1	6	6357047 - 6357856	D	P0453D01	AP004691	AK068312	224	24191	4.6858	A4	DREB
Os06g36000	837	1	1	6	21039439 - 21040540	D	OJ1112_F09	AP005289	AK108830	279	28752	4.5211	A3	DREB
Os06g40150	732	2	1	6	23898590 - 23897627	U	P0486H12	AP003615	AK061163	244	25396	10.0322	B3	ERF
Os06g42990	1179	1	0	6	25825906 - 25827084	D	P0417G05	AP002835	AK101054	393	40886	9.4028	B1	ERF
Os06g43220	1005	4	9	6	25979076 - 25976399	U	P0523F01	AP003573	AK242957	335	36104	6.9249	AP2	AP2
Os06g44750	1410	4	8	6	27024607 - 27027968	D	B1047G05	AP004803	AK101959	470	50263	6.9301	AP2	AP2
Os06g47590	567	1	0	6	28817331 - 28817897	D	B1103G11	AP004843	AK287559	189	19923	6.5253	B7	ERF
Os07g03250	945	5	6	7	1298912 - 1302863	D	OSJNBa0060M02	AP005309	AK109848	315	33838	7.2309	B6	ERF
Os07g10410	801	1	0	7	5592911 - 5593711	D	OJ1260_B01	AL772427	AK109606	267	29029	8.395	B1	ERF
Os07g12510	849	1	0	7	7139780 - 7138932	U	P0665C04	AP004464	AK064252	283	30603	10.8225	B3	ERF
Os07g13170	1311	4	9	7	7544758 - 7548436	D	P0407H12	AP004303	AK111874	437	47439	7.3059	AP2	AP2
Os07g22730	666	1	0	7	12826220 - 12825555	U	P0710F09	AP005325	AK108503	222	22888	8.9855	B3	ERF
Os07g22770	720	1	0	7	12847360 - 12846641	U	P0710F09	AP005325	AK062612	240	25357	9.8115	B6	ERF
Os07g38750	948	1	0	7	23251291 - 23250344	U	OJ1260_B01	AL772427	AK109606	316	33367	7.4217	B2	ERF
Os07g42510	1029	2	1	7	25441474 - 25440125	U	B1056G08	AP004988	AK067060	343	37168	4.4297	B1	ERF
Os07g47330	957	1	0	7	28299982 - 28299026	U	P0625E02	AP004016	AK105365	319	33541	7.0289	B2	ERF
Os07g47790	651	1	1	7	28540687 - 28539943	U	P0037D09	AP006268	AK288040	217	23655	6.7903	B1	ERF
Os08g07440	837	3	6	8	4177451 - 4175101	U	OJ1590_E05	AP005606	AK105232	279	30030	6.6916	AP2	AP2
Os08g07700	528	1	0	8	4303635 - 4303108	U	OSJNBa0054L03	AP005164	AK241762	176	19447	5.5007	B7	ERF
Os08g27220	939	1	0	8	16618395 - 16617457	U	N/F	N/F	N/F	313	33359	9.7012	A6	DREB
Os08g31580	843	2	0	8	19414486 - 19416168	D	P0453D01	AP004691	AK105725	281	29525	7.7621	A5	DREB
Os08g34360	1260	4	7	8	21561639 - 21565428	D	P0708B04	AP004764	AK106769	420	45602	5.3283	AP2	AP2
Os08g35240	732	1	0	8	22233806 - 22234537	D	N/F	N/F	N/F	244	25967	4.5395	B4	ERF
Os08g36920	849	1	0	8	23351203 - 23352051	D	P0451G12	AP004399	AK062882	283	30076	6.0545	B7	ERF
Os08g41030	570	1	0	8	25947602 - 25948171	D	OJ1003_A09	AP005509	AK108208	190	20296	9.1806	B4	ERF
Os08g42550	1626	8	8	8	26885294 - 26892449	D	P0665C04	AP004464	AK119425	542	58748	9.3749	RAV	RAV
Os08g43200	711	1	0	8	27187835 - 27188545	U	OSJNBb0076O03	AP005866	AK287692	237	24470	5.6332	A1	DREB

Continue

Os08g43210	756	1	0	8	27322565 - 27321810	U	OSJNBb0076O03	AP005866	AK287692	252	26812	4.3989	A1	DREB
Os08g44960	411	0	0	8	28221606 - 28222016	D	B1234D02	BX842602	AK107745	137	14994	8.441	B3	ERF
Os08g45110	726	1	0	8	28319655 - 28318930	U	OSJNBa0044E16	AP005411	AK108143	242	26280	6.4938	A2	DREB
Os09g11460	699	2	1	9	6388653 - 6387955	U	P0663H05	AC090056	AK065344	233	26039	8.4969	B2	ERF
Os09g11480	768	2	1	9	6404950 - 6404183	U	P0663H05	AP006758	AK068688	256	27818	4.6319	B2	ERF
Os09g13940	957	1	0	9	8200282 - 8199326	U	OSJNBa0019B22	AP005687	AK100575	319	35680	5.1888	B7	ERF
Os09g20350	NF	2	0	9	20398856-20399777	D	OJ1759_F09	AP005580	AK058899	247	25491	5.1038	A6	DREB
Os09g25600	1140	4	7	9	15363890 - 15368113	D	P0418B08	AP005420	AK111617	380	41020	8.203	AP2	AP2
Os09g26420	1191	3	2	9	15958541 - 15961244	D	OJ1081_G10	AP005551	AK111690	397	42626	4.626	B2	ERF
Os09g28440	825	1	0	9	17305162 - 17305986	D	B1045B05	AP005891	AK067195	275	29190	6.2795	B4	ERF
Os09g35010	657	1	0	9	20395679 - 20395023	U	OSJNOa273B05	AP006859	AK071519	219	23238	4.9735	A1	DREB
Os09g35020	741	1	0	9	20399717 - 20398977	U	OSJNOa273B05	AP006859	AK071519	247	25491	5.1038	A1	DREB
Os09g35030	717	1	0	9	20403772 - 20403056	U	N/F	N/F	N/F	239	25390	4.8433	A1	DREB
Os09g39810	396	0	0	9	22821495 - 22821100	U	OSJNBa0065H10	AL731613	AK109141	132	14686	8.0015	B3	ERF
Os09g39850	651	1	0	9	22846805 - 22847455	D	B1331F11	AP006162	AK241281	217	23449	9.1838	B3	ERF
Os10g22600	966	1	0	10	11400208-11401173	D	OJ1288_G09	AP004119	AK242121	322	33784	8.7234	A6	DREB
Os10g25170	969	1	1	10	12920864 - 12921935	D	OSJNBb0061I18	AC079936	AK110269	323	34187	5.2297	B1	ERF
Os10g26590	684	1	0	10	13791722 - 13792405	D	N/F	N/F	N/F	228	24478	7.2963	B7	ERF
Os10g30840	375	0	0	10	15992996 - 15992622	U	B1234D02	BX842602	AK107745	125	13307	9.41	B3	ERF
Os10g38000	582	1	0	10	20283556 - 20284137	D	OSJNBa0056G13	AC134236	AK108930	194	20435	6.8039	A5	DREB
Os10g41130	870	1	0	10	22028979 - 22028110	D	B1015E06	AP003197	AK108873	290	29729	4.4779	A4	DREB
Os10g41330	822	2	2	10	22141682 - 22142922	D	OSJNBa0065H10	AL731613	AK109141	274	29769	7.2154	B5	ERF
Os11g03540	1350	5	6	11	1368690 - 1363704	U	OSJNBa0056E15	AC123525	AK112088	450	49880	4.5427	AP2	AP2
Os11g06770	957	2	1	11	3305797 - 3303585	U	P0665C04	AP004464	AK064252	319	32431	5.417	B4	ERF
Os11g13840	606	1	0	11	7624018 - 7623413	U	B1015E06	AP003197	AK108873	202	21647	4.6476	A4	DREB
Os11g19060	1680	5	7	11	10857728 - 10862590	D	OSJNBb0044M15	AC136501	AK287621	560	59975	6.5132	AP2	AP2
Os12g03290	1332	5	5	12	1273766 - 1268804	U	OSJNBa0056E15	AC123525	AK112088	444	49305	4.5454	AP2	AP2
Os12g39330	1209	1	0	12	24169201 - 24170409	D	OJ1260_B01	AL772427	AK109606	403	42370	4.6106	B6	ERF
Os12g41030	423	0	0	12	25351984 - 25351562	U	OJ1118_C04	AC108523	AK111755	141	15751	7.7982	B7	ERF
Os12g41060	972	4	1	12	25381958 - 25385125	U	OSJNBb0062H20	AL837528	AK287544	324	34249	4.3968	B7	ERF

Note: 1=MSU locus ID; 2=ORF length; 3=Gene Length kbp; 4=NO. of Introns in ORF; 5=Chrom. No.; 6=TIGR v6 Pseudomolecule position (5'-3'); 7=Gene Orientation; 8=BAC Name; 9=BAC Accession No.; 10=FL-cDNA; 11= Length (aa); 12=Molecular Weight (KDa); 13=PI; 14=Phy.Subgroup; 15=Phy. Subfamily; D= Downward; U=Upward; N/F= Not found.

3.2. Phylogenetic analysis and alignments

It was reported that at least 139 ERF members with a conserved AP2/ERF domain exist in the *japonica* rice genome (Oh et al. 2009). A total of 163 *AP2/EREBP* genes were identified in rice from the different database searches. These were divided into four subfamilies based on their sequence similarities and the number of AP2/ERF domains in the encoded protein. Among them, (i) 24 genes were predicted to encode two complete AP2/ERF domains and assigned to the AP2 subfamily, and (ii) five genes predicted to encode a single AP2/ERF domain and a single B3 domain were assigned to the RAV subfamily. Thus, *AP2/EREBP* genes having a double domain (domain a and domain b) were divided into subfamilies AP2 and RAV (**Fig. 1**).

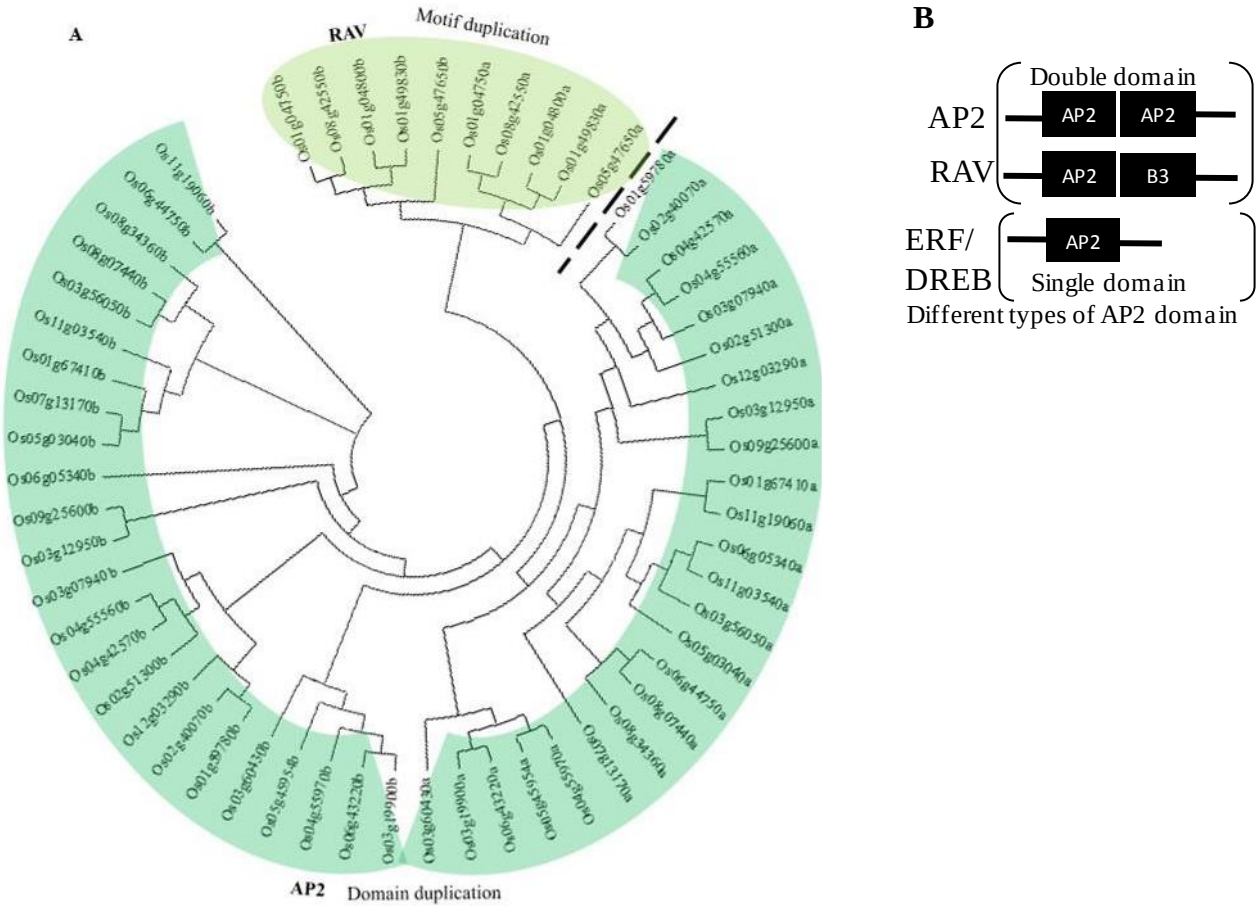
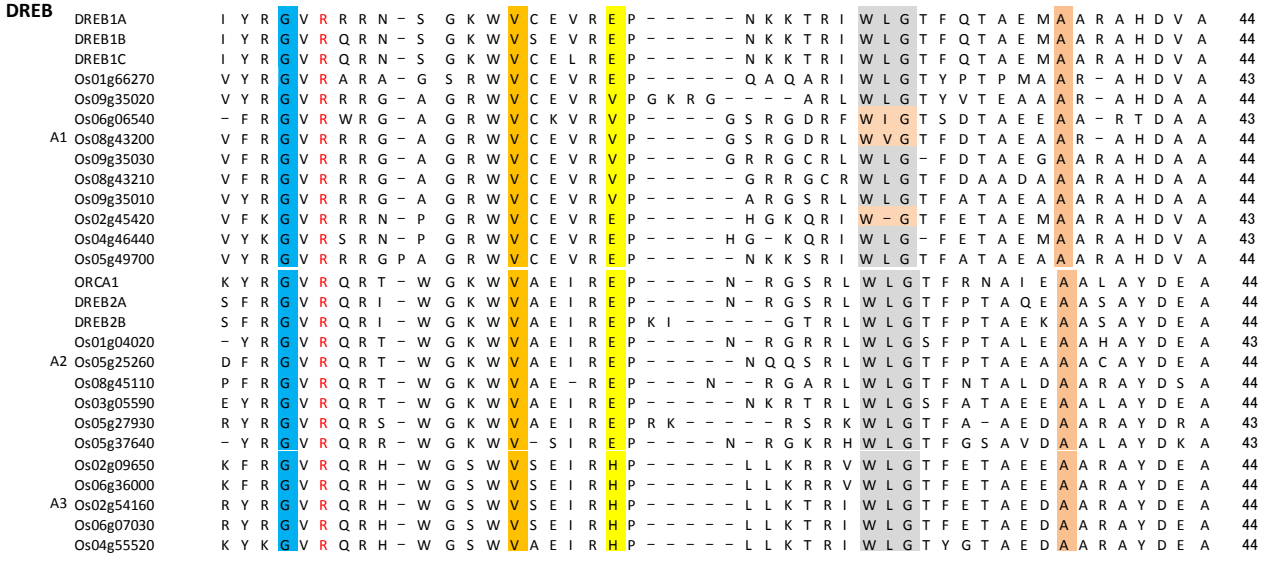


Fig. 1 (A) Evolutionary relationships among rice AP2/EREBP domain sequences. The unrooted tree was generated by the neighbor-joining method with the ClustalX program. Domains a and b were found in subfamilies RAV and AP2, for example, in *Os01g59780a* and *Os01g59780b*, respectively. The RAV subfamily showed motif duplication, and the AP2 subfamily showed domain duplication. (B) Structure of *AP2/EREBP* genes.

The phylogenetic relationships of the genes are the same in each subfamily; for example, in the AP2 subfamily, domain all of the proteins duplicated before the gene duplication, whereas the RAV subfamily proteins showed domain duplication but no gene duplication. This is not surprising that *AP2/EREBP* genes are old family and functionally diverse. This is a common phenomenon in old gene families (Nuruzzaman et al. 2008). (iii) A large number of *AP2/EREBP* genes (134) encoding a single AP2/ERF domain were assigned to the DREB and ERF subfamilies. To clarify the phylogenetic relationships among ERF subfamily genes and to infer the evolutionary history of this gene family, a combined phylogenetic tree was constructed by alignment of the 27 published AP2/ERF domain sequences (**Fig. 2**).

Fig. 2 Relationships among rice AP2/EREBP proteins after alignment with ClustalW. OsAP2/EREBP proteins were allocated to two distinct subfamilies (DREB, subgroups A-1 to A-6; ERF, subgroups B-1 to B-7).

The 134 genes were further classified into two subfamilies on the basis of similarity of the amino acid sequence of the AP2/ERF domains: 57 of them encode DREB/CBF-like proteins (subfamily DREB; Figs. 2, 3), and 77 encode ERF-like proteins (subfamily ERF; Figs. 2, 3). Of these, 127 genes contain a conserved WLG motif of the AP2/ERF domain (excepting seven genes: *Os02g45420*, *Os06g06540*, *Os06g47590*, *Os07g22730*, *Os08g43200*, *Os09g26420*, and *Os10g26590*) (**Fig. 3**). All of the proteins encoded by genes of subfamily DREB contain the position 14 is normally valine (important), that position 19 is glutamic acid (not important) in the DREB/CBF DNA-binding domain, respectively (Sakuma et al. 2002). Most of the genes of subfamily B have alanine in the A14 position and aspartic acid in the D19 position in the ERF-DNA binding domain. We found all of the genes of subfamilies DREB and ERF contain alanine in the A37 position, α-helix in A37 position have been demonstrated to be essential for binding with the DRE and GCC box *cis*-elements (Liu et al. 2006) and Allen et al. (1998) have been described the three dimensional structure (β-sheet and α-helix) of ERF domain. The proteins of subfamily DREB/CBF were divided into six subgroups. Among these subgroups A-4 and A-5 contained the greatest numbers of genes. Likewise, the proteins of subfamily ERF were divided into seven subgroups and largest numbers of genes were assigned to subgroups B-1 and B-3 (**Fig. 2**).



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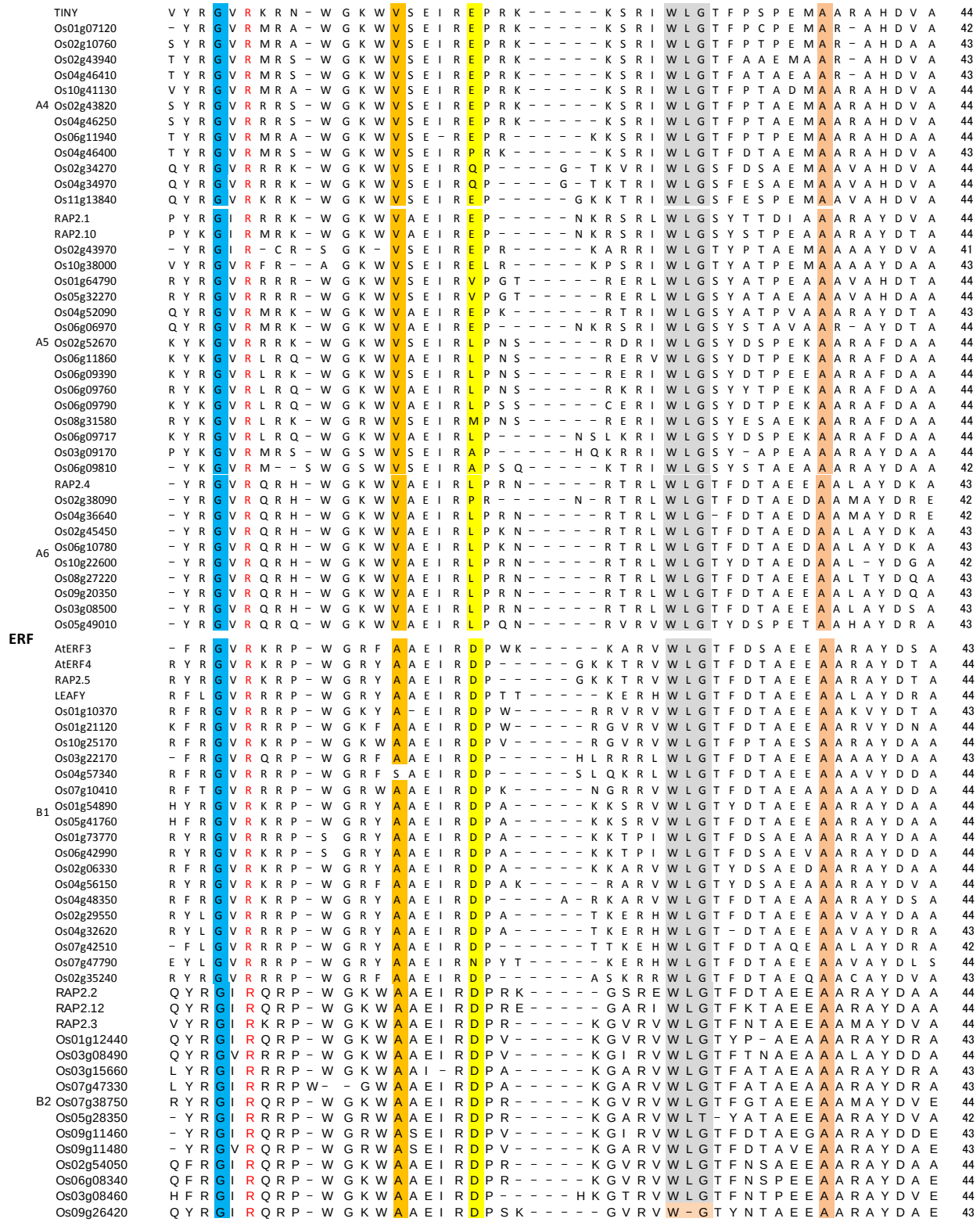

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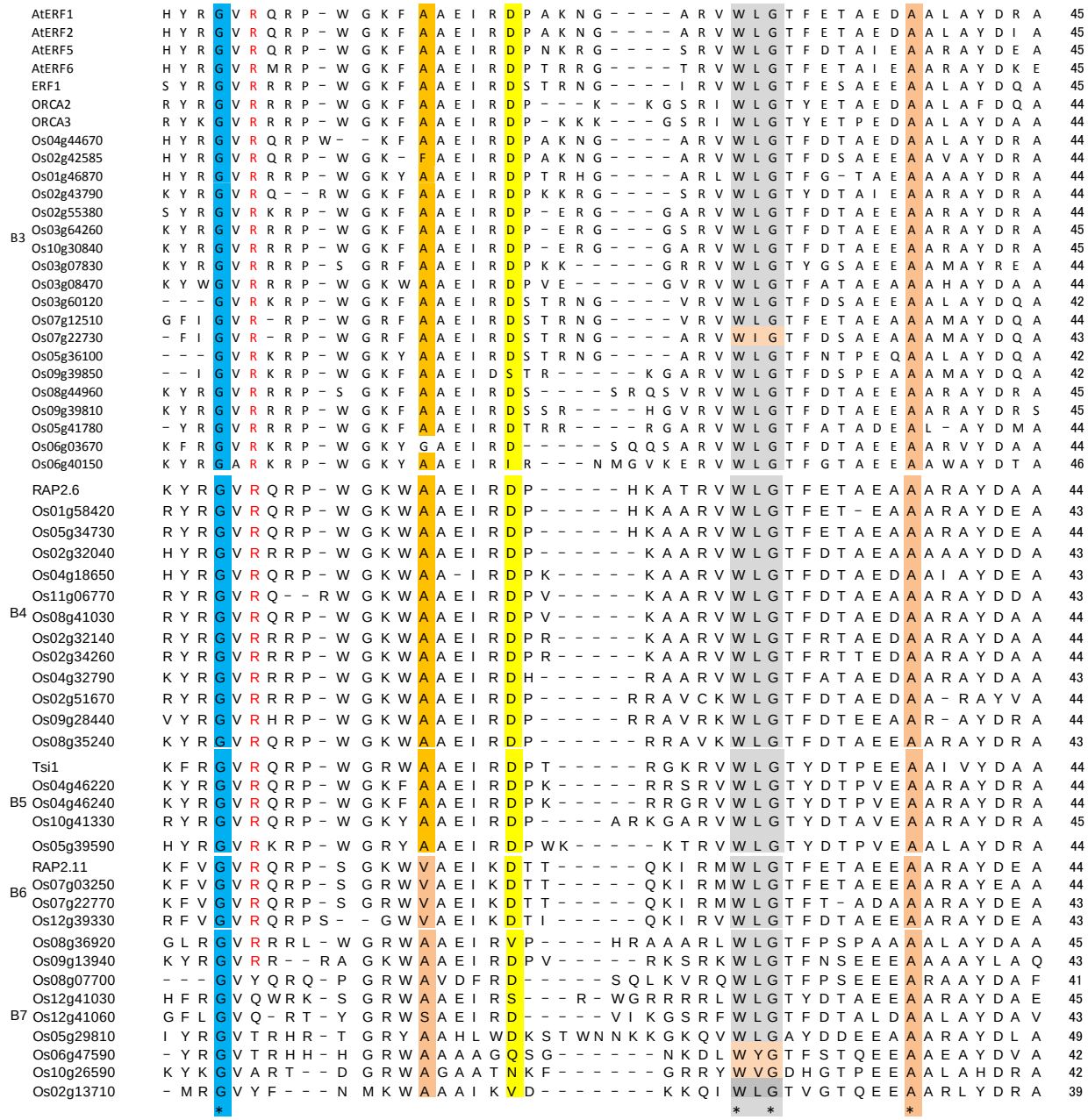
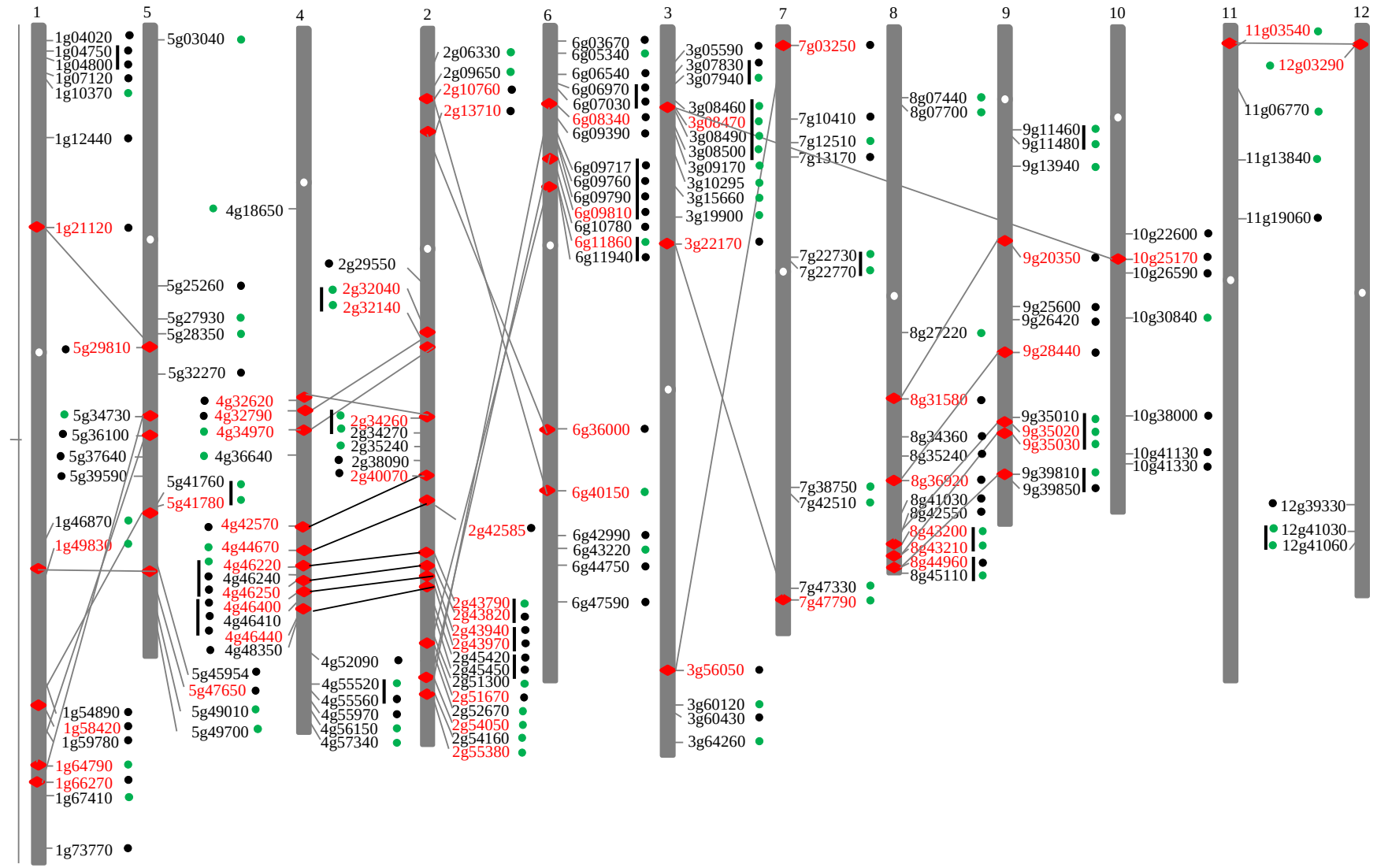


Fig. 3 Alignment of the AP2/ERF domain in the *OsAP2/EREBP* gene family. Comparison of deduced amino acid sequences of the AP2/EREBP DNA-binding domains of the DREB/ERF proteins with those of other proteins (from *Arabidopsis thaliana*, *Brassica napus*, *Zea mays*, *Catharanthus roseus*, *Nicotiana tabacum*, and *Lycopersicon esculentum*) with a single AP2/EREBP domain in rice. Amino acid sequences of the AP2/EREBP domains were aligned with the ClustalW multiple sequence alignment program. The different-colored vertical bars show conserved amino acid residues in each subgroup. The tan-colored horizontal bars show where the WLG motif was not conserved.

3.3. Chromosomal locations of *AP2/EREBP* genes

To examine the genomic distribution of *AP2/EREBP* genes on rice chromosomes, we identified their positions by MSU database search. A total of 163 rice *AP2/EREBP* genes could be localized on the 12 chromosomes with an obviously uneven distribution. *OsAP2/EREBP* genes were present in all regions on a single chromosome (i.e., at the telomeric ends, near the centromere, and in between) and could be distributed individually or in clusters (**Fig. 4**). Chromosome 2 had the largest number (25) of *OsAP2/EREBP* genes, and chromosomes 4 and 6 had 20 each. Only four *OsAP2/EREBP* genes were identified on each of chromosomes 11 and 12. Interestingly, all four *OsAP2/EREBP* genes on chromosome 11 were found on the short arm, whereas three *OsAP2/EREBP* genes of the four on chromosome 12 were on the long arm. Two *OsAP2/EREBP* genes (*Os03g22770* and *Os07g22730*) encoding proteins having only one AP2/ERF domain were positioned around the centromere on chromosome 7 and were arranged as a tandem duplication. Additionally, only one gene (*Os08g45110*) was located near a telomeric region. There were fewer than 10 *OsAP2/EREBP* genes on each of chromosomes 10, 11, and 12.

In plants, gene numbers are expanded by segmental and tandem duplication in gene families (Cannon et al. 2004). To elucidate the potential mechanism of evolution of the *OsAP2/EREBP* gene family, we analyzed segmental and tandem duplication events, identifying 20 segmental duplication events in rice (**Fig. 4, Table 3**). One segmental duplication was found in the RAV subfamily, and 19 segmental duplications were found in DREB and ERF subfamilies. Neither segment was duplicated in subfamily AP2 in rice. We also found 29 tandemly duplicated genes in rice (**Fig. 4, Table 4**). In rice, 58 *OsAP2/EREBP* genes were involved in tandem duplications, consisting of 29 pairs or 22 clusters in rice (**Fig. 4, Table 4**). The number of *OsAP2/EREBP* genes arranged in the tandem repeats varied from two to four, and these sets of genes showed the same orientation on the chromosomes, with the exception of seven pairs (*Os02g43970* and *Os02g43820*; *Os03g07830* and *Os03g07940*; *Os04g46220* and *Os04g46240*; *Os04g55520* and *Os04g55560*; *Os06g11860* and *Os06g11940*; *Os08g44960* and *Os08g45110*; *Os09g39810* and *Os09g39850*). The degree of homology in the protein sequences of these genes is shown in **Tables 3 and 4**.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5g39590
1g46870
1g49830
1g54890
1g58420
1g59780
1g64790
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1g67410
1g73770
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3g10295
3g15660
3g19900
3g22170
3g56050
3g60120
3g60430
3g64260
7g03250
7g10410
7g12510
7g13170
7g22730
7g22770
7g38750
7g42510
7g47330
7g47790
8g07440
8g07700
8g27220
8g31580
8g34360
8g35240
8g36920
8g41030
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8g43200
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8g44960
8g45110
9g11460
9g11480
9g13940
9g20350
9g25600
9g26420
9g28440
9g35010
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10g38000
10g41130
10g41330
11g03540
12g03290
11g06770
11g13840
11g19060
12g39330
12g41030
12g41060
44 Mb

Fig. 4 Locations of *AP2/EREBP* genes on the 12 rice chromosomes. Chromosome numbers are indicated at the top of each bar. The filled white circle on each chromosome (vertical bars) shows the putative position of the centromere. Genes with open reading frames with opposite orientations are marked on the chromosome (filled black circles represent downward and filled green circles indicate upward orientations). Straight lines connect the *OsAP2/EREBP* genes present on duplicated chromosomal segments, and tandemly duplicated gene clusters are marked by vertical black bars. The scale is in megabases (Mb).

Table 3. AP2/EREBPgenes present on duplicated chromosomal segments of rice.

Gene 1			Gene 2			BlastP (e-value)	%Homology[3]
Gene name	Chr	Subfamily[1]	Gene name	Chr	Subfamily[2]		
Os01g49830	1	RAV	Os05g47650	5	RAV	6.30E-131	57%
Os01g58420	1	ERF	Os05g41780	5	ERF	2.20E-80	42%
Os01g66270	1	DREB	Os05g34730	5	ERF	2.60E-45	40%
Os02g32040	2	ERF	Os04g32790	4	ERF	2.20E-48	35%
Os02g32140	2	ERF	Os04g32620	4	ERF	5.80E-41	40%
Os02g42585	2	ERF	Os04g44670	4	ERF	7.00E-116	42%
Os02g43790	2	ERF	Os04g46220	4	ERF	3.30E-86	45%
Os02g43820	2	DREB	Os04g46250	4	DREB	5.00E-74	44%
Os02g43940	2	DREB	Os04g46400	4	DREB	5.10E-65	48%
Os02g43970	2	DREB	Os04g46440	4	DREB	1.80E-71	42%
Os02g10760	2	DREB	Os06g40150	6	ERF	1.00E-66	57%
Os02g51670	2	ERF	Os06g11860	6	DREB	1.20E-102	40%
Os02g54050	2	ERF	Os06g09810	6	DREB	8.80E-38	51%
Os02g55380	2	ERF	Os06g08340	6	ERF	3.80E-60	48%
Os03g22170	3	ERF	Os07g47790	7	ERF	3.00E-37	45%
Os08g31580	8	DREB	Os09g20350	9	DREB	4.70E-78	49%
Os08g43200	8	DREB	Os09g35020	9	DREB	3.50E-50	35%
Os08g43210	8	DREB	Os09g35030	9	DREB	1.40E-64	43%
Os08g44960	8	ERF	Os09g39810	9	ERF	6.80E-31	50%
Os10g25170	10	ERF	Os03g08470	3	ERF	9.10E-36	79%

[1]-subfamily of the first gene

[2]-subfamily of the segmentally duplicated gene

[3]-Homology between proteins encoded by segmentally duplicated genes

Table 4. Tandemly duplicated *AP2/EREBP* genes of rice.

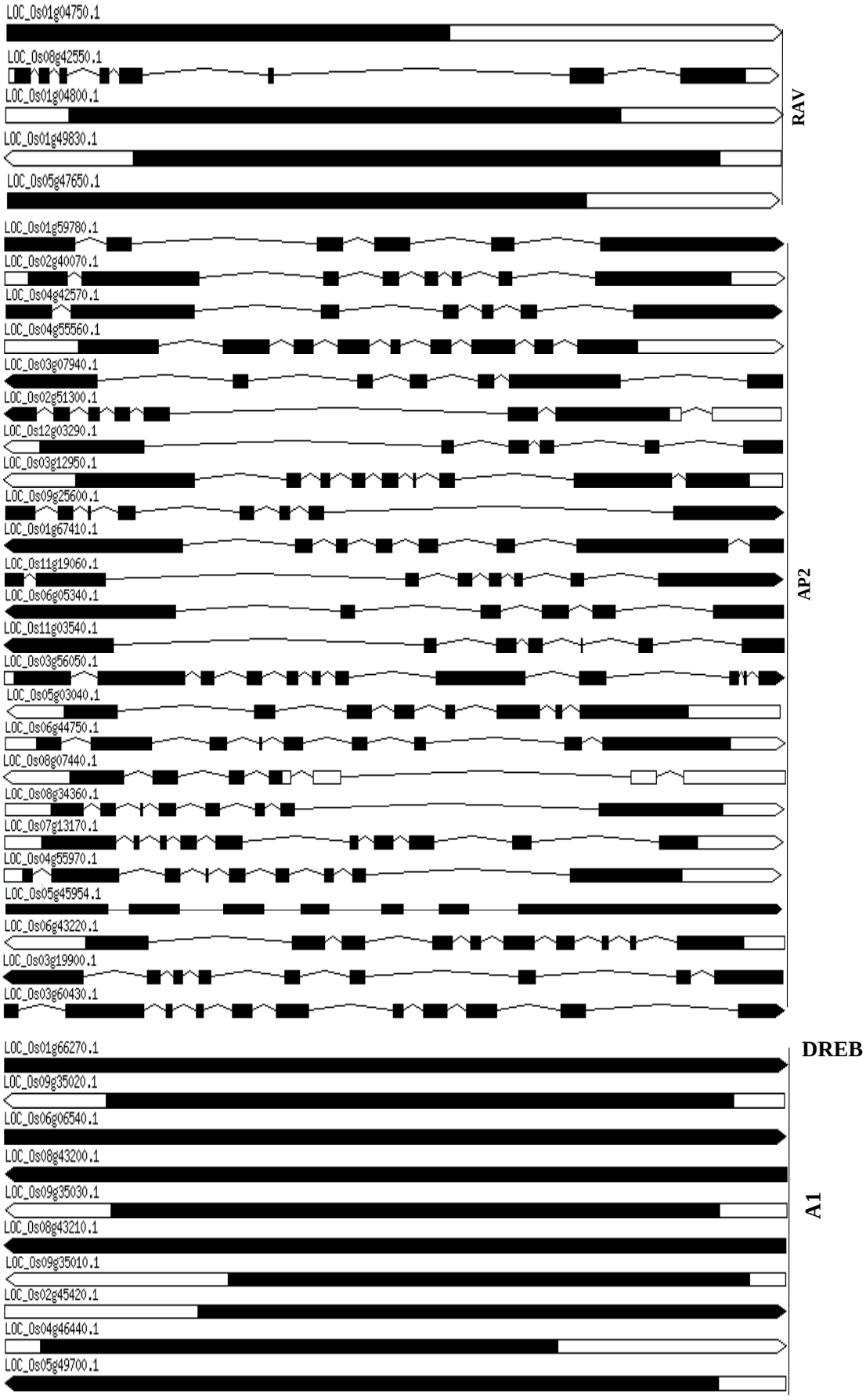
Cluster	Pair	Gene 1	Subfamily[1]	Gene 2	Subfamily[2]	%Homology[3]
1	1	Os01g04750	RAV	Os01g04800	RAV	70%
2	2	Os02g32040	ERF	Os02g32140	ERF	71%
3	3	Os02g34260	ERF	Os02g34270	DREB	54%
4	4	Os02g43790	ERF	Os02g43820	DREB	55%
5	5	Os02g43940	DREB	Os02g43970	DREB	84%
6	6	Os02g45420	DREB	Os02g45450	DREB	37%
7	7	Os03g07830	ERF	Os03g07940	AP2	36%
	8	Os03g08460	ERF	Os03g08470	ERF	34%
	9	Os03g08470	ERF	Os03g08490	ERF	39%
8	10	Os03g08490	ERF	Os03g08500	DREB	31%
	11	Os04g46220	ERF	Os04g46240	ERF	55%
9	12	Os04g46240	ERF	Os04g46250	DREB	50%
	13	Os04g46400	DREB	Os04g46410	DREB	80%
10	14	Os04g46410	DREB	Os04g46440	DREB	47%
11	15	Os04g55520	DREB	Os04g55560	AP2	42%
12	16	Os05g41760	ERF	Os05g41780	ERF	63%
13	17	Os06g06970	DREB	Os06g07030	DREB	29%
	18	Os06g09717	DREB	Os06g09760	DREB	64%
	19	Os06g09760	DREB	Os06g09790	DREB	77%
14	20	Os06g09790	DREB	Os06g09810	DREB	45%
15	21	Os06g11860	DREB	Os06g11940	DREB	54%
16	22	Os07g22730	ERF	Os07g22770	ERF	31%
17	23	Os08g43200	DREB	Os08g43210	DREB	37%
18	24	Os08g44960	ERF	Os08g45110	DREB	39%
19	25	Os09g11460	ERF	Os09g11480	ERF	35%
	26	Os09g35010	DREB	Os09g35020	DREB	32%
20	27	Os09g35020	DREB	Os09g35030	DREB	38%
21	28	Os09g39810	ERF	Os09g39850	ERF	75%
22	29	Os12g41030	ERF	Os12g41060	ERF	35%

[1]-subfamily of the first gene

[2]-subfamily of the tandemly duplicated gene

[3]-Homology between proteins encoded by tandemly duplicated genes

We analyzed the intron and exon structures of *OsAP2/EREBP* genes belonging to the four subfamilies based on the phylogenetic tree. We found no obvious differences among the different subgroups of the DREB and ERF subfamilies, except for subgroups A-5, B-1, and B-6 (**Fig. 5**). Both the locations and numbers of introns and exons of the *OsAP2/EREBP* gene family varied among genes. The highest numbers of introns and exons were identified in subfamily AP2. In subfamily AP2, most coding sequences of *OsAP2/EREBP* genes were disrupted by introns, with the number of introns varying from 5 to 11 (**Fig. 5**). Most of the genes in subfamilies RAV and ERF had no introns. In rice, some researchers have reported that the rate of intron loss is faster than the rate of intron gain after segmental duplication (Lin et al. 2006, Nuruzzaman et al. 2008). Therefore, we speculate that the genes in subfamilies RAV and ERF may be young members of this gene family; in each subfamily, those genes with more introns may be the original genes in that subfamily, or they may have diversified from subfamily AP2 (**Fig. 5**). The mechanisms of intron gain and loss are not yet clear, however (Lin et al. 2006).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LOC_Os03g19900.1
LOC_Os03g60430.1
AP2
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DREB
A1

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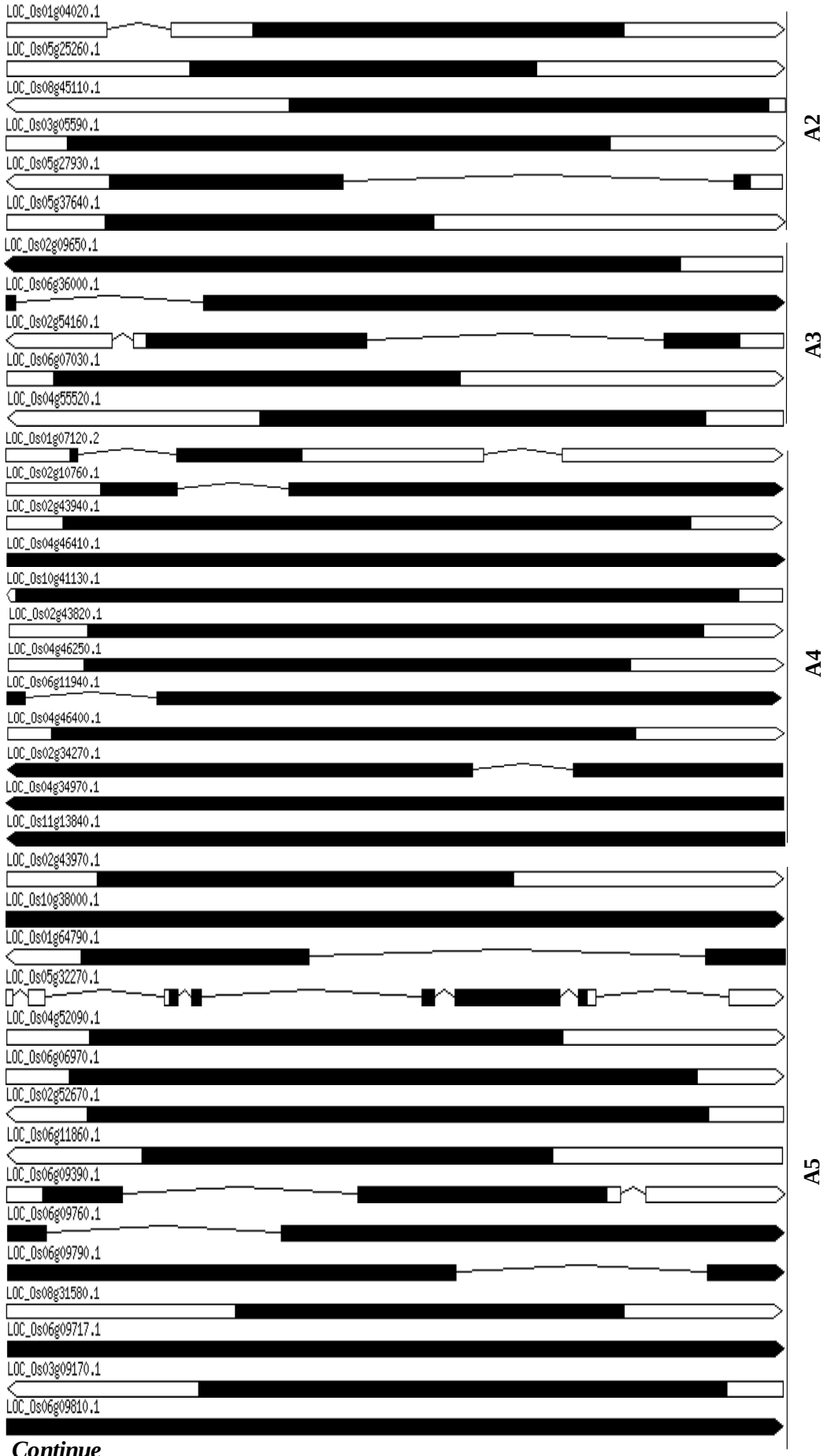
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A5

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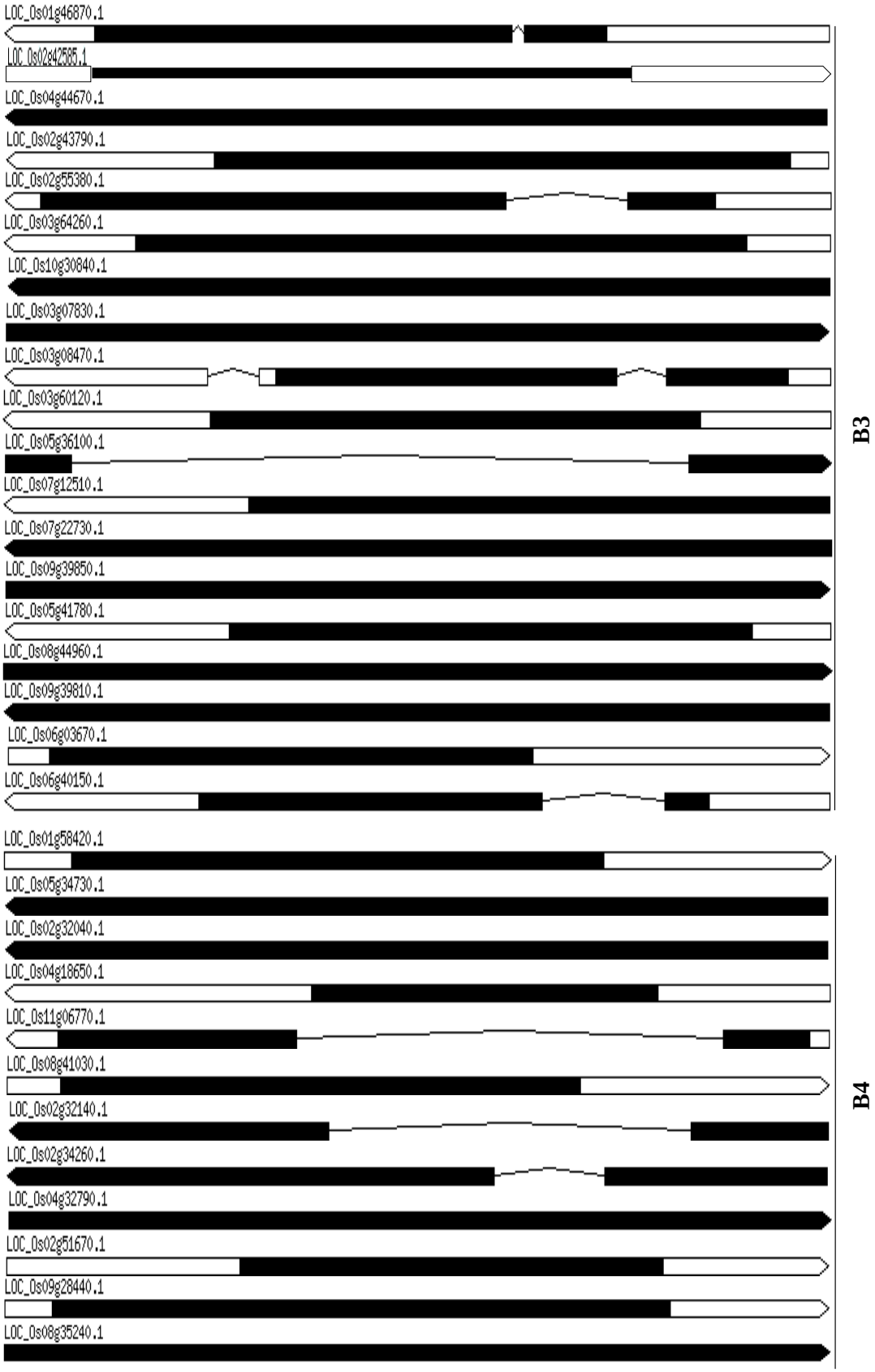
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B3
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B4

Continue

Fig. 5 Exons (black boxes), introns (lines), and untranslated regions (white boxes) of *OsAP2/EREBP*-encoding genes.

3.4. Comparison of expression profiles of duplicated *OsAP2/EREBP* genes

In the course of evolution, there are three possible novel functions of gene duplication: nonfunctionalization, neofunctionalization, and subfunctionalization (Lynch and Conery 2000). Divergence of gene expression plays a very important role in the preservation of duplicated genes. In this study, we examined the expression patterns of segmentally and tandemly duplicated genes under different stress conditions. Probes were matched to 11 of the 20 genes located in segmentally duplicated regions. Nine pairs of genes showed highly similar expression patterns under most of the tested stresses, indicating subfunctionalization after the duplication events (**Fig. 6A**). Likewise, we identified 22 clusters of tandemly duplicated *OsAP2/EREBP* genes (**Table 4**). Among them, only eight clusters of the gene probe set were found in our 22K-microarray data. Five clusters of genes showed highly similar expression intensities, which may indicate subfunctionalization. The expression patterns of four clusters of tandemly duplicated genes (such as *Os02g32040* and *Os02g32140*) were dissimilar, which may indicate neofunctionalization (**Fig. 6B**). Neofunctionalization occurs when a neofunctionalized allele is fixed in one of duplicated genes and it asserts that after duplication one daughter gene retains the ancestral function while the other acquires new functions. *Os02g32040* gene showed downregulation under cold, drought and submergence while *Os02g32140* gene upregulated under all abiotic treatments. On the basis of various roles, we may predict that these duplicated genes have diverse functions under stress conditions.

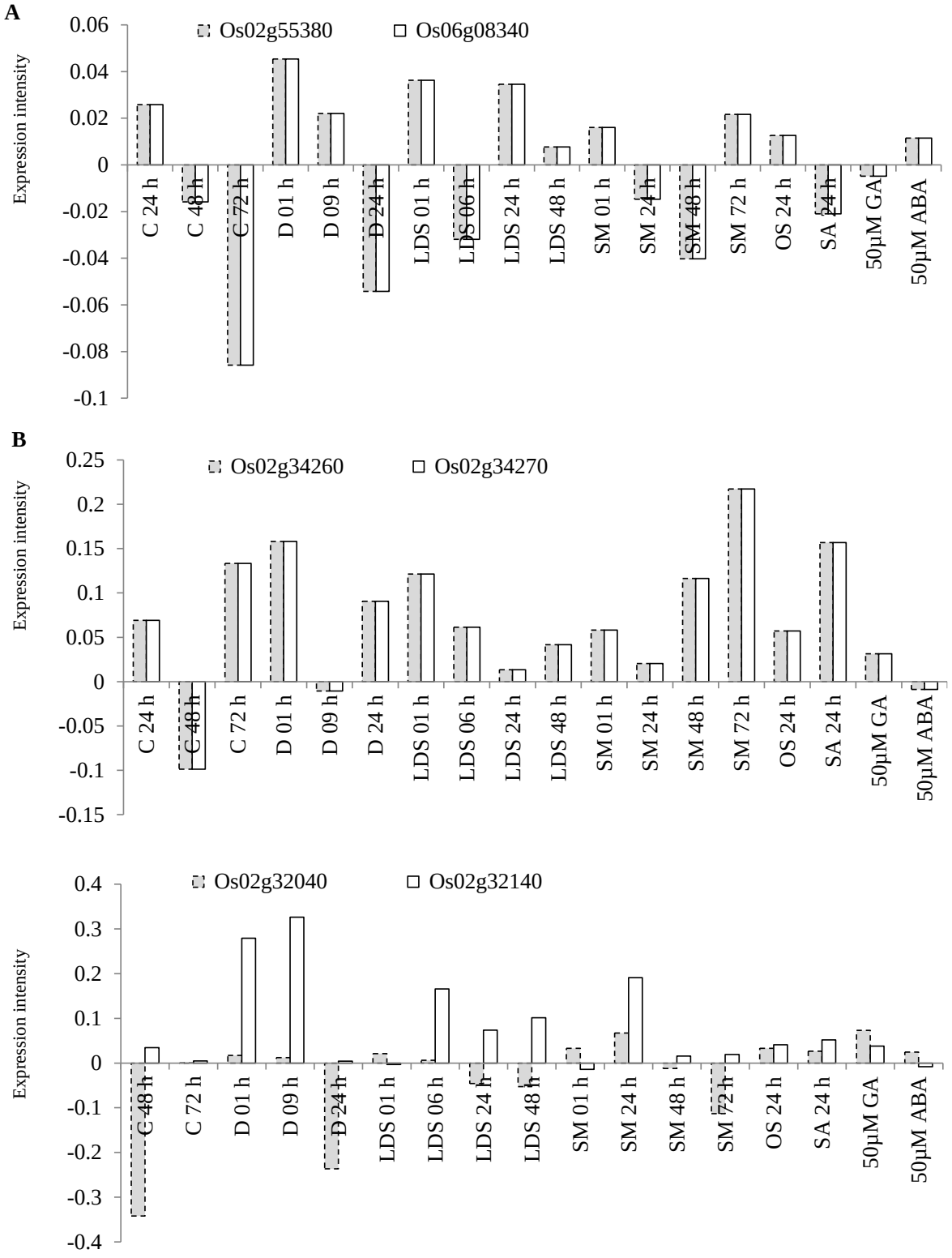

Fig. 6 Examples of expression patterns of *OsAP2/EREBP* genes found in duplicated regions of the rice genome. (A) Expression patterns of two *OsAP2/EREBP* genes found in segmentally duplicated regions. (B) Expression patterns of two pairs of tandemly duplicated *OsAP2/EREBP* genes. The different stresses are shown on the *X*-axis and the expression intensity on the *Y*-axis. Gene names are indicated at the top of each graph.

3.5. Gene expression profiles under biotic and abiotic treatments

We used microarray analyses to investigate the response of the *OsAP2/EREBP* genes to both biotic and abiotic stresses. We investigated the expression profiles of *OsAP2/EREBP* genes in rice seedlings infected with RSV, RTSV and RDV (three strains S, O and D84), and expressed the results as fold changes with respect to the controls. Multiplication and motion of viruses might be inhibited by plant defense system (Kitanaga et al. 2006). We found 37 non-redundant genes differentially expressed under RSV infection at 3, 6, 9, and 12 days after inoculation (DAI) (**Table 5**). Among these, 13 were upregulated and 24 were downregulated. The number of genes upregulated was highest at 12 DAI, followed by 3, 6, and 9 DAI (listed in decreasing order) under RSV infection, although may genes involved in gene silencing were activated at 12 DAI (**Fig. 7A**). It may suggest that the gene-silencing system in the host was not activated in timely manner to suppress RSV replication. Recent researches have shown that the p3 (*NS3*) gene of RSV encodes a gene-silencing suppressor that inhibits local and systemic gene silencing (Levy et al. 2008, Xiong et al. 2008, 2009). We also found 46 non-redundant differentially expressed genes (DEGs) under RTSV infection at 6, 9, 12, and 15 DAI (**Fig. 7B, Table 6**). Among these, 28 were upregulated and 18 were downregulated. Only one gene (*Os09g35030*) was found to be upregulated at all time points. The number of upregulated genes was largest at 9 DAI and smaller at 12, 6, and 15 DAI (in that order) under RTSV infection (**Fig. 7B**). Out of 10 genes of subgroup A-1, 8 were differentially expressed under RTSV. Six genes of subgroup B-1 were up-or downregulated under RTSV infection. Interestingly, only two genes (*Os01g12440* and *Os09g35020*) were very commonly upregulated under both RSV and RTSV infection. Furthermore, we observed 54 non-redundant DEGs under RDV infection with three strains S, O and D84. Number of genes activated was highest in RDV S-strain and then decreased in the order of strain D84 and strain O (**Fig. 7C, Table 7**). In case of RSV, up < down, while in the case of RTSV and RDV, up > down. Defense systems are activated in RTSV and RDV but in RSV host was

nearly death. In the phylogenetic tree, we found that expression of 46 non-redundant genes of subfamilies DREB and ERF was induced by all biotic stresses, whereas four genes from subfamily RAV were upregulated under both stresses (RSV and RTSV) and three genes of this subfamily were induced by RDV S-strain. Only two genes from the AP2 subfamily were upregulated under RSV infection and all of the genes under this subfamily were not differentially expressed by RDV treatment except Os04g42570. Out of 12 genes of subgroup B-4, Os02g32140 was activated by RSV and RDV S-strain, respectively. Most of the genes of subgroups A-2, A-3 and B-6 were not differentially expressed by all biotic treatments. Interestingly, 7 of 10 genes in subgroup A-1 were upregulated under RTSV infection, whereas only one gene of subgroup A-1 showed high expression under RSV infection. Likewise, subgroups B-1 and B-5 of ERF subfamily genes were preferentially induced under RTSV, RSV and RDV, respectively (**Table 8**). Comparatively speaking, the gene responses of the *OsAP2/EREBP* family were higher under RDV than under RTSV and RSV infections. Some transcription factors are known to act specifically on transcriptional regulation of genes responding to biotic and abiotic stresses (Shimono et al. 2007). The result of global gene expression analysis for plants infected with three viruses suggested inter-correlations among the numbers of DEGs, the degrees of gene response, the symptom severity, and the accumulation of virus in plants.

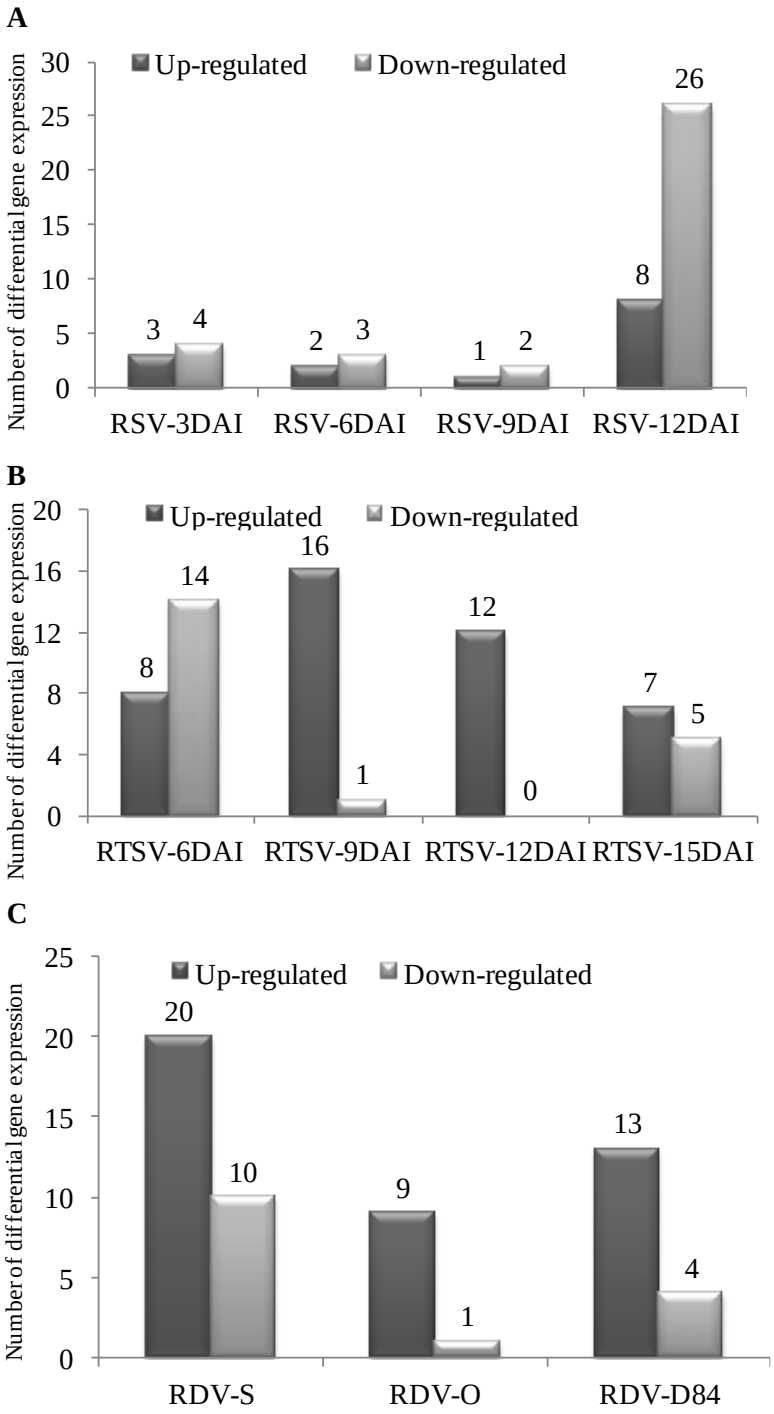

Fig. 7 Number of differentially expressed genes at different time points: (A) under RSV infection at 3, 6, 9, and 12 days after inoculation (DAI); (B) under RTSV infection at 6, 9, 12, and 15 DAI. Different DAI are shown on the *X*-axis, and the number of differentially expressed genes on the *Y*-axis. (C) The differentially genes were expressed under RDV infection with three strains (S, O, and D84). Number of strains is shown on the *X*-axis, and the differentially expressed genes on the *Y*-axis.

Table 5. *OsAP2/EREBP* gene expression profile (log2-based ratio) under RSV infection (44K oligoarray data).

Probe ID	MSU locus	RSV3D	mock3D	DEGs	RSV6D	mock6D	DEGs	RSV9D	mock9D	DEGs	RSV12D	mock12D	DEGs	Phg. Subfamily	Subgroup
R5_c01_00261_F	Os01g04020	10.69	10.52	0	10.60	10.45	0	10.73	10.66	0	10.29	10.42	0	DREB	A2
R5_c01_00331_F	Os01g04750	7.48	7.69	0	7.50	7.32	0	7.51	7.84	0	7.56	8.24	0	RAV	RAV
R5_c01_00335_F	Os01g04800	12.13	12.72	0	12.14	12.06	0	12.60	12.59	0	13.30	13.29	0	RAV	RAV
R5_c01_00531_F	Os01g07120	8.21	8.53	0	8.50	8.51	0	9.05	8.92	0	9.65	9.26	0	DREB	A4
R5_c01_00811_R	Os01g10370	5.98	6.30	0	6.13	6.32	0	5.96	6.21	0	5.68	5.90	0	ERF	B1
R5_c01_00982_F	Os01g12440	7.93	7.48	0	7.94	7.75	0	8.54	8.22	0	9.21	7.98	1	ERF	B2
R5_c01_01593_F	Os01g21120	7.89	7.86	0	9.72	9.33	0	10.21	10.24	0	10.80	11.89	0	ERF	B1
R5_c01_03200_R	Os01g46870	15.89	16.08	0	15.60	15.83	0	15.32	15.64	0	15.01	15.50	0	ERF	B3
R5_c01_03456_R	Os01g49830	11.97	12.49	0	13.43	12.37	0	13.49	13.26	0	14.43	14.62	0	RAV	RAV
R5_c01_03895_F	Os01g54890	12.18	12.26	0	11.91	12.45	0	11.67	12.52	-1	12.44	12.76	0	ERF	B1
R5_c01_04215_F	Os01g58420	14.20	14.68	0	13.78	14.57	-1	14.50	15.06	0	14.50	15.01	0	ERF	B4
R5_c01_04330_F	Os01g59780	3.00	2.97	0	2.79	2.49	0	2.52	2.74	0	2.07	2.07	0	AP2	AP2
R5_c01_04750_R	Os01g64790	7.77	8.42	0	8.50	7.97	0	9.07	9.01	0	9.13	9.07	0	DREB	A5
R5_c01_04894_F	Os01g66270	7.93	8.22	0	8.63	8.41	0	8.91	9.86	0	9.23	10.51	-1	DREB	A1
R5_c01_04997_R	Os01g67410	2.34	2.17	0	2.32	2.26	0	2.25	2.23	0	2.26	2.23	0	AP2	AP2
R5_c01_05517_F	Os01g73770	5.22	5.59	0	6.54	5.43	0	6.68	7.63	0	6.92	8.29	0	ERF	B1
R5_c02_06063_R	Os02g06330	11.33	10.77	1	10.27	10.30	0	10.12	10.30	0	9.74	9.67	0	ERF	B1
R5_c02_06322_R	Os02g09650	16.91	16.84	0	16.79	16.72	0	16.55	16.54	0	16.26	16.39	0	DREB	A3
R5_c02_06426_F	Os02g10760	5.19	5.34	0	8.27	8.57	0	8.01	8.25	0	8.34	9.34	-1	DREB	A4
R5_c02_06658_F	Os02g13710	8.96	8.67	0	8.97	8.50	0	8.69	8.68	0	7.93	8.41	0	ERF	B7
R5_c02_07701_F	Os02g29550	14.37	14.69	0	14.08	14.53	0	13.63	14.21	0	13.19	14.28	0	ERF	B1
R5_c02_07845_R	Os02g32040	11.81	11.80	0	11.43	11.65	0	11.31	11.54	0	11.26	11.69	0	ERF	B4
R5_c02_07852_R	Os02g32140	8.98	9.11	0	8.42	8.65	0	8.46	8.43	0	8.45	7.75	1	ERF	B4
R5_c02_08009_R	Os02g34260	3.99	4.23	0	3.22	3.53	0	2.76	2.42	0	2.96	3.91	0	ERF	B4
R5_c02_08011_R	Os02g34270	4.57	4.44	0	4.04	4.08	0	3.12	3.14	0	3.63	4.42	0	DREB	A4
R5_c02_08098_R	Os02g35240	4.90	5.02	0	2.23	2.18	0	2.18	2.47	0	2.21	2.35	0	ERF	B1
R5_c02_08335_F	Os02g38090	5.19	4.63	0	5.10	4.77	0	4.94	4.43	0	4.73	4.79	0	DREB	A6
R5_c02_08502_F	Os02g40070	2.09	2.41	0	2.13	2.29	0	2.09	2.24	0	2.46	2.32	0	AP2	AP2
R5_c02_08784_R	Os02g43790	9.92	10.30	0	11.07	10.18	0	11.03	10.91	0	11.66	11.99	0	ERF	B3
R5_c02_08786_F	Os02g43820	5.96	6.87	-1	7.23	7.12	0	7.44	7.60	0	7.57	8.15	0	DREB	A4
R5_c02_08800_F	Os02g43940	10.17	9.66	0	9.55	9.98	0	9.37	9.88	0	8.82	10.00	-1	DREB	A4
R5_c02_08802_F	Os02g43970	11.75	11.41	0	11.16	11.46	0	11.18	11.50	0	10.44	11.68	-1	DREB	A5
R5_c02_08926_F	Os02g45420	5.63	5.86	0	6.92	6.14	0	7.38	7.30	0	7.02	7.44	0	DREB	A1
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R5_c02_09449_R	Os02g51300	9.06	9.17	0	9.27	8.80	0	9.35	9.19	0	9.35	8.98	0	AP2	AP2
R5_c02_09486_F	Os02g51670	16.55	16.55	0	16.21	16.47	0	15.88	16.33	0	15.75	15.99	0	ERF	B4
R5_c02_09570_R	Os02g52670	8.24	8.10	0	8.74	8.76	0	9.45	9.46	0	9.82	9.83	0	DREB	A5
R5_c02_09691_R	Os02g54050	3.85	4.40	0	6.15	4.87	0	5.88	6.24	0	6.50	7.30	0	ERF	B2

Continue

R5_c02_09702_R	Os02g54160	13.12	13.05	0	13.28	13.29	0	13.41	13.34	0	13.80	13.44	0	DREB	A3
R5_c02_09817_R	Os02g55380	4.96	5.49	0	5.16	5.05	0	4.72	5.40	0	5.47	5.34	0	ERF	B3
R5_c03_10544_F	Os03g05590	4.54	3.90	0	2.91	2.77	0	2.12	2.07	0	2.12	2.07	0	DREB	A2
R5_c03_10733_F	Os03g07830	4.53	4.81	0	4.59	4.73	0	4.59	4.83	0	5.01	5.18	0	ERF	B3
R5_c03_10742_R	Os03g07940	2.74	2.35	0	2.70	2.50	0	3.59	2.63	0	2.54	2.55	0	AP2	AP2
R5_c03_10795_R	Os03g08460	9.96	10.14	0	10.24	10.51	0	10.96	11.29	0	11.22	12.34	0	ERF	B2
R5_c03_10797_R	Os03g08470	11.14	11.23	0	11.04	11.28	0	11.04	11.36	0	10.56	11.49	0	ERF	B3
R5_c03_10799_R	Os03g08490	13.33	14.01	0	14.10	13.69	0	14.79	14.55	0	14.68	14.74	0	ERF	B2
R5_c03_10800_R	Os03g08500	16.74	17.04	0	17.75	17.11	0	17.57	17.56	0	17.63	17.76	0	DREB	A6
R5_c03_10865_R	Os03g09170	11.09	11.27	0	10.91	10.73	0	10.27	10.80	0	9.21	10.58	0	DREB	A5
R5_c03_11174_R	Os03g12950	4.03	4.51	0	4.70	4.73	0	4.23	4.40	0	3.48	4.13	0	AP2	AP2
R5_c03_11390_R	Os03g15660	3.66	3.74	0	4.62	5.05	0	5.16	5.15	0	5.52	5.35	0	ERF	B2
R5_c03_11776_R	Os03g19900	2.68	3.60	0	2.81	3.75	0	4.28	4.57	0	2.47	3.50	0	AP2	AP2
R5_c03_11968_F	Os03g22170	7.01	6.93	0	6.60	7.20	0	6.34	6.79	0	5.96	6.18	0	ERF	B1
R5_c03_14178_F	Os03g56050	5.16	5.47	0	5.41	5.51	0	5.08	5.38	0	4.53	5.36	0	AP2	AP2
R5_c03_14544_R	Os03g60120	12.72	12.69	0	12.59	12.91	0	12.69	13.08	0	13.41	14.37	0	ERF	B3
R5_c03_14570_F	Os03g60430	10.97	11.12	0	10.98	11.09	0	10.89	11.12	0	10.67	11.05	0	AP2	AP2
R5_c03_14900_R	Os03g64260	6.07	6.34	0	5.62	6.31	0	6.40	6.83	0	9.01	8.11	0	ERF	B3
R5_c04_15784_R	Os04g18650	4.86	4.28	0	4.51	4.17	0	3.06	3.62	0	3.46	3.82	0	ERF	B4
R5_c04_16592_F	Os04g32620	7.53	7.88	0	7.95	8.00	0	8.00	8.13	0	8.19	8.17	0	ERF	B1
R5_c04_16607_F	Os04g32790	7.56	7.58	0	7.62	7.73	0	7.68	7.96	0	7.71	7.89	0	ERF	B4
R5_c04_16797_R	Os04g34970	6.23	6.68	0	6.27	6.87	0	6.70	6.67	0	7.41	6.68	1	DREB	A4
R5_c04_16906_R	Os04g36640	3.22	4.30	0	5.73	5.31	0	5.72	5.67	0	5.88	6.97	0	DREB	A6
R5_c04_17379_F	Os04g42570	3.20	2.85	0	2.24	2.31	0	2.38	2.20	0	2.96	2.33	0	AP2	AP2
R5_c04_17558_R	Os04g44670	8.97	8.76	0	7.95	8.78	-1	7.94	8.72	-1	6.90	7.55	0	ERF	B3
R5_c04_17698_R	Os04g46220	14.26	14.63	0	15.25	14.47	0	15.16	15.11	0	15.72	15.91	0	ERF	B5
R5_c04_17699_F	Os04g46240	8.08	8.02	0	7.79	8.06	0	7.63	7.78	0	7.40	7.70	0	ERF	B5
R5_c04_17700_F	Os04g46250	3.04	3.52	0	3.70	3.69	0	3.07	3.80	0	3.13	3.34	0	DREB	A4
R5_c04_17712_F	Os04g46400	11.51	10.87	0	11.44	11.77	0	11.52	12.08	0	10.67	12.29	-1	DREB	A4
R5_c04_17713_F	Os04g46410	5.57	5.61	0	5.11	5.73	0	5.44	5.72	0	5.07	5.32	0	DREB	A4
R5_c04_17715_F	Os04g46440	11.64	11.18	0	11.18	11.12	0	11.15	11.08	0	11.25	10.93	0	DREB	A1
R5_c04_17876_F	Os04g48350	11.31	10.06	1	11.13	10.61	0	10.60	10.91	0	9.09	9.90	-1	ERF	B1
R5_c04_18159_F	Os04g52090	13.20	13.82	0	13.09	14.07	0	13.84	14.39	0	14.44	14.35	0	DREB	A5
R5_c04_18473_R	Os04g55520	11.04	11.65	0	11.84	11.33	0	12.30	12.34	0	12.13	12.44	0	DREB	A3
R5_c04_18476_F	Os04g55560	8.46	8.85	0	8.56	8.89	0	8.39	8.84	0	8.19	8.58	0	AP2	AP2
R5_c04_18509_F	Os04g55970	7.33	7.57	0	7.04	7.17	0	6.87	7.20	0	7.02	7.24	0	AP2	AP2
R5_c04_18526_R	Os04g56150	2.90	3.45	0	2.75	3.20	0	3.08	2.74	0	2.52	2.67	0	ERF	B1
R5_c04_18636_R	Os04g57340	15.25	15.27	0	15.35	15.34	0	15.52	15.42	0	15.91	15.41	0	ERF	B1
R5_c05_19028_R	Os05g03040	12.71	13.12	0	13.23	13.02	0	13.14	13.24	0	12.36	13.00	0	AP2	AP2
R5_c05_20302_F	Os05g25260	12.48	12.72	0	13.09	12.56	1	13.22	12.89	0	12.97	12.82	0	DREB	A2
R5_c05_20463_R	Os05g27930	13.82	13.88	0	14.16	14.04	0	14.40	14.16	0	14.35	13.97	0	DREB	A2
R5_c05_20495_R	Os05g28350	3.23	2.98	0	3.19	2.83	0	3.11	3.09	0	3.07	2.99	0	ERF	B2
R5_c05_20570_F	Os05g29810	5.24	5.86	0	6.79	7.22	0	7.54	7.87	0	8.36	8.36	0	ERF	B7
R5_c05_20760_F	Os05g32270	10.18	10.14	0	10.27	10.40	0	9.85	10.18	0	9.02	10.08	-1	DREB	A5
R5_c05_20969_R	Os05g34730	4.65	5.05	0	4.75	4.99	0	5.34	6.24	0	4.60	5.27	0	ERF	B4
R5_c05_21085_F	Os05g36100	2.52	2.24	0	2.27	2.11	0	2.23	2.13	0	2.23	2.14	0	ERF	B3

Continue

R5_c05_21164_F	Os05g37640	12.04	12.12	0	12.22	12.00	0	12.24	12.15	0	12.25	12.16	0	DREB	A2
R5_c05_21330_F	Os05g39590	4.02	3.98	0	4.49	3.71	0	6.04	4.19	1	8.42	4.66	1	ERF	B5
R5_c05_21511_R	Os05g41760	12.25	12.50	0	12.28	12.47	0	12.59	13.16	0	11.78	13.41	-1	ERF	B1
R5_c05_21512_R	Os05g41780	14.05	14.43	0	14.23	14.34	0	14.66	14.96	0	14.75	15.02	0	ERF	B3
R5_c05_21809_R	Os05g45954	7.92	7.45	0	7.05	7.47	0	6.51	6.89	0	7.61	6.97	1	AP2	AP2
R5_c05_21922_F	Os05g47650	10.23	10.15	0	10.47	10.16	0	10.57	10.33	0	10.36	10.26	0	RAV	RAV
R5_c05_22040_R	Os05g49010	4.84	4.92	0	4.79	4.71	0	5.16	5.22	0	4.72	4.88	0	DREB	A6
R5_c05_22104_R	Os05g49700	9.85	9.89	0	9.75	9.60	0	9.68	10.10	0	9.06	9.46	0	DREB	A1
R5_c06_22500_F	Os06g03670	6.49	6.88	0	7.37	7.27	0	7.54	7.84	0	7.31	7.52	0	ERF	B3
R5_c06_22659_R	Os06g05340	4.69	4.74	0	4.40	4.28	0	4.02	4.64	0	3.88	4.42	0	AP2	AP2
R5_c06_22775_F	Os06g06540	9.61	9.64	0	9.09	9.15	0	8.66	8.83	0	8.83	9.59	0	DREB	A1
R5_c06_22818_F	Os06g06970	4.82	4.95	0	3.90	4.71	0	4.62	5.05	0	4.04	4.28	0	DREB	A5
R5_c06_22824_F	Os06g07030	11.50	12.34	0	12.53	12.32	0	13.09	13.16	0	13.72	13.67	0	DREB	A3
R5_c06_22929_F	Os06g08340	2.48	3.55	0	3.01	3.63	0	2.38	3.01	0	3.09	4.26	0	ERF	B2
R5_c06_23022_F	Os06g09390	9.90	9.48	0	9.95	9.70	0	9.76	9.88	0	9.14	9.85	-1	DREB	A5
R5_c06_23044_F	Os06g09717	5.24	5.12	0	4.78	4.99	0	5.11	5.34	0	5.06	5.92	0	DREB	A5
R5_c06_23047_F	Os06g09760	5.84	5.83	0	6.62	6.42	0	5.21	5.43	0	5.16	5.25	0	DREB	A5
R5_c06_23049_F	Os06g09790	2.29	2.20	0	2.28	2.20	0	2.28	2.16	0	2.57	2.16	0	DREB	A5
R5_c06_23051_F	Os06g09810	4.15	4.08	0	4.31	3.87	0	4.05	3.48	0	4.32	4.30	0	DREB	A5
R5_c06_23134_F	Os06g10780	4.21	4.09	0	4.69	4.17	0	6.45	4.97	0	6.11	5.47	0	DREB	A6
R5_c06_23229_R	Os06g11860	11.84	12.24	0	12.18	11.99	0	12.30	12.33	0	12.79	12.76	0	DREB	A5
R5_c06_23234_F	Os06g11940	2.90	3.02	0	3.13	3.66	0	2.94	3.18	0	3.25	3.16	0	DREB	A4
R5_c06_24648_F	Os06g36000	4.44	4.33	0	4.73	4.58	0	4.27	4.90	0	4.06	4.57	0	DREB	A3
R5_c06_24943_R	Os06g40150	7.22	7.37	0	10.68	11.13	0	10.35	10.56	0	11.01	11.99	-1	ERF	B3
R5_c06_25143_F	Os06g42990	5.33	5.29	0	5.54	5.73	0	4.85	5.16	0	4.36	4.96	0	ERF	B1
R5_c06_25165_R	Os06g43220	6.90	5.80	1	6.05	6.14	0	5.48	5.61	0	4.85	6.39	-1	AP2	AP2
R5_c06_25294_F	Os06g44750	4.12	3.25	0	3.41	3.38	0	2.86	3.29	0	2.34	3.27	0	AP2	AP2
R5_c06_25550_F	Os06g47590	12.43	12.58	0	12.58	12.56	0	12.77	12.59	0	13.00	12.58	0	ERF	B7
R5_c07_26062_F	Os07g03250	8.15	8.63	0	7.73	7.91	0	7.71	7.91	0	6.77	7.83	-1	ERF	B6
R5_c07_26640_F	Os07g10410	4.88	5.00	0	4.62	4.50	0	4.40	4.52	0	4.26	4.48	0	ERF	B1
R5_c07_26784_R	Os07g12510	12.95	12.79	0	12.81	12.71	0	12.69	12.57	0	13.06	12.88	0	ERF	B3
R5_c07_26830_F	Os07g13170	7.36	7.60	0	7.78	7.56	0	7.72	7.57	0	7.48	7.16	0	AP2	AP2
R5_c07_27279_R	Os07g22730	4.76	6.09	-1	5.50	5.63	0	6.01	6.21	0	7.30	6.78	0	ERF	B3
R5_c07_27282_R	Os07g22770	3.80	3.38	0	3.87	3.08	0	2.34	2.67	0	3.18	3.43	0	ERF	B6
R5_c07_28366_R	Os07g38750	5.62	5.49	0	5.57	5.21	0	5.14	5.16	0	5.01	5.00	0	ERF	B2
R5_c07_28680_R	Os07g42510	6.85	7.29	0	7.29	7.29	0	7.81	7.69	0	8.11	7.70	0	ERF	B1
R5_c07_29085_R	Os07g47330	2.96	3.08	0	2.88	3.63	0	3.47	2.61	0	3.97	3.69	0	ERF	B2
R5_c07_29128_R	Os07g47790	3.04	3.62	0	4.20	4.87	0	5.92	5.75	0	7.43	6.99	0	ERF	B1
R5_c08_29813_R	Os08g07440	12.77	12.63	0	12.83	12.70	0	12.88	12.57	0	12.90	12.49	0	AP2	AP2
R5_c08_29833_R	Os08g07700	3.36	3.94	0	3.68	4.26	0	2.97	4.00	0	4.09	3.87	0	ERF	B7
R5_c08_30932_R	Os08g27220	8.40	8.16	0	8.08	7.85	0	7.88	7.73	0	7.70	7.57	0	DREB	A6
R5_c08_31221_F	Os08g31580	12.95	13.67	0	13.27	13.88	0	13.50	13.99	0	13.84	14.01	0	DREB	A5
R5_c08_31442_F	Os08g34360	10.07	10.17	0	10.03	10.02	0	10.03	10.16	0	9.35	10.10	-1	AP2	AP2
R5_c08_31516_F	Os08g35240	2.76	2.63	0	2.72	2.98	0	2.60	3.27	0	3.06	2.66	0	ERF	B4
R5_c08_31636_F	Os08g36920	5.38	5.65	0	10.09	7.66	0	8.78	9.41	0	9.15	11.71	-1	ERF	B7
R5_c08_31995_F	Os08g41030	2.15	2.17	0	2.14	2.15	0	2.13	2.25	0	2.66	2.11	0	ERF	B4

Continue

R5_c08_32118_F	Os08g42550	8.81	8.79	0	9.25	8.86	0	9.62	9.17	0	11.29	9.56	1	RAV	RAV
R5_c08_32176_R	Os08g43200	4.46	4.21	0	3.25	4.33	0	3.45	4.65	0	3.88	5.04	0	DREB	A1
R5_c08_32177_R	Os08g43210	10.10	9.75	0	8.72	9.36	0	7.77	8.55	0	7.00	8.26	-1	DREB	A1
R5_c08_32329_F	Os08g44960	5.82	5.69	0	5.99	5.53	0	5.87	5.53	0	5.73	5.33	0	ERF	B3
R5_c08_32342_R	Os08g45110	2.38	2.96	0	2.72	2.38	0	2.40	3.40	0	2.43	3.57	0	DREB	A2
R5_c09_32888_R	Os09g11460	5.77	5.43	0	5.95	6.08	0	5.97	6.33	0	6.33	5.97	0	ERF	B2
R5_c09_32890_R	Os09g11480	9.76	10.04	0	10.72	10.29	0	11.26	10.87	0	10.81	10.52	0	ERF	B2
R5_c09_33027_R	Os09g13940	11.90	11.78	0	11.81	11.94	0	12.05	11.80	0	12.54	11.81	1	ERF	B7
R5_c09_33424_F	Os09g20350	15.98	16.16	0	16.19	16.38	0	16.39	16.49	0	16.39	16.67	0	DREB	A6
R5_c09_33800_F	Os09g25600	11.54	11.40	0	11.77	11.89	0	11.32	11.70	0	11.44	12.10	-1	AP2	AP2
R5_c09_33870_F	Os09g26420	16.50	16.65	0	16.49	16.63	0	16.54	16.74	0	16.56	17.03	0	ERF	B2
R5_c09_34047_F	Os09g28440	7.07	6.37	0	8.49	7.69	0	8.08	8.59	0	8.04	9.36	0	ERF	B4
R5_c09_34510_R	Os09g35010	11.60	11.08	0	12.71	11.53	0	11.94	12.50	0	11.06	12.00	-1	DREB	A1
R5_c09_34511_R	Os09g35020	9.04	8.67	0	10.35	9.20	1	9.38	9.82	0	8.45	9.66	-1	DREB	A1
R5_c09_34512_R	Os09g35030	13.01	13.38	0	15.15	13.69	0	14.36	14.56	0	14.51	15.71	0	DREB	A1
R5_c09_34919_R	Os09g39810	6.70	6.87	0	6.77	6.62	0	6.36	6.76	0	5.44	6.54	0	ERF	B3
R5_c09_34921_F	Os09g39850	5.83	6.08	0	5.63	5.97	0	5.24	5.68	0	5.52	5.86	0	ERF	B3
R5_c10_36036_F	Os10g22600	9.30	9.33	0	9.24	9.78	0	8.77	9.20	0	8.35	9.27	-1	DREB	A6
R5_c10_36164_F	Os10g25170	6.15	6.24	0	6.42	6.58	0	7.13	7.49	0	7.13	7.89	0	ERF	B1
R5_c10_36265_F	Os10g26590	6.22	6.21	0	5.96	5.94	0	5.94	6.01	0	5.48	5.87	0	ERF	B7
R5_c10_36541_R	Os10g30840	9.17	8.86	0	8.69	8.58	0	8.21	8.32	0	7.29	7.78	0	ERF	B3
R5_c10_37098_F	Os10g38000	15.94	16.14	0	16.11	16.11	0	16.66	16.25	0	17.34	16.57	1	DREB	A5
R5_c10_37379_R	Os10g41130	10.12	9.77	0	9.92	10.04	0	9.86	9.85	0	9.68	10.21	0	DREB	A4
R5_c10_37398_F	Os10g41330	9.94	11.14	-1	10.31	10.12	0	10.98	10.60	0	10.64	10.41	0	ERF	B5
R5_c11_37752_R	Os11g03540	4.56	4.50	0	5.35	6.23	0	4.94	5.80	0	5.86	5.99	0	AP2	AP2
R5_c11_38041_R	Os11g06770	5.73	6.34	0	6.75	6.68	0	7.77	8.11	0	9.41	9.12	0	ERF	B4
R5_c11_38534_R	Os11g13840	5.65	5.17	0	5.56	4.57	0	5.53	5.18	0	5.12	4.68	0	DREB	A4
R5_c11_38831_F	Os11g19060	3.20	2.49	0	3.32	2.42	0	3.12	2.46	0	2.86	2.49	0	AP2	AP2
R5_c12_40849_R	Os12g03290	8.66	8.77	0	9.00	9.32	0	8.81	9.13	0	9.02	9.56	0	AP2	AP2
R5_c12_43079_F	Os12g39330	3.40	3.24	0	3.05	4.25	0	3.33	3.50	0	4.16	3.66	0	ERF	B6
R5_c12_43200_R	Os12g41030	8.16	8.37	0	7.99	7.97	0	7.99	8.19	0	7.67	7.88	0	ERF	B7
R5_c12_43202_R	Os12g41060	6.80	6.63	0	6.84	6.96	0	6.50	6.83	0	5.60	6.99	-1	ERF	B7

Footnote: Fold change gene expression data of 162 *OsAP2/EREBP* genes probe on the 44K rice oligoarray under RSV infection are listed. More than 0.585 fold upregulated genes were assigned 1, less than –0.585 fold downregulated genes were assigned –1 , others are assigned as 0. DEGs = differentially expressed genes. Phg = Phylogentic subgroups or subfamilies. RSV = Rice stripe virus.

Table 6. *OsAP2/EREBP* gene expression profile (log2-based ratio) under RTSV infection (44K oligoarray data).

ID	MSU locus	RTSV6D	mock6D	DEGs	RTSV9D	mock9D	DEGs	RTSV12D	mock12D	DEGs	RTSV15D	mock15D	DEGs	Phg. Subfamily	Subgroup
R5_c01_00261_F	Os01g04020	10.674667	10.655	0	10.808666	10.68	0	10.931	10.803333	0	11.18767	10.99867	0	DREB	A2
R5_c01_00331_F	Os01g04750	10.301	9.542999	1	10.618667	10.04733	0	9.974334	9.895667	0	10.25167	10.06233	0	RAV	RAV
R5_c01_00335_F	Os01g04800	15.666667	14.80867	1	15.650666	15.65667	0	15.271	15.383667	0	15.34467	14.73933	0	RAV	RAV
R5_c01_00531_F	Os01g07120	9.998334	10.248	0	10.614333	10.22233	0	11.477666	11.179333	0	11.09167	10.597	0	DREB	A4
R5_c01_00811_R	Os01g10370	6.0780005	6.245333	0	6.044333	5.873334	0	6.8040004	7.1056666	0	7.285334	7.314667	0	ERF	B1
R5_c01_00982_F	Os01g12440	12.572666	12.297	0	12.431667	11.968	0	12.345	12.312	0	13.10833	12.181	1	ERF	B2
R5_c01_01593_F	Os01g21120	14.498	13.196	1	14.098334	13.30067	1	13.740001	13.301	0	14.75167	13.807	1	ERF	B1
R5_c01_03200_R	Os01g46870	12.13	12.27567	0	12.509	12.18433	0	13.394	13.525333	0	11.584	11.83067	0	ERF	B3
R5_c01_03456_R	Os01g49830	14.988	14.40967	1	15.053	14.645	0	14.922666	14.945999	0	14.93133	14.71233	0	RAV	RAV
R5_c01_03895_F	Os01g54890	12.429334	12.51467	0	12.457334	11.86767	0	13.481667	13.156001	0	12.976	12.817	0	ERF	B1
R5_c01_04215_F	Os01g58420	14.742	15.347	0	15.113999	14.82733	0	15.747001	15.700665	0	15.53133	15.91433	0	ERF	B4
R5_c01_04330_F	Os01g59780	3.342	3.089334	0	3.7670002	3.819667	0	3.5909998	3.4733334	0	3.765667	3.159333	0	AP2	AP2
R5_c01_04750_R	Os01g64790	11.839333	11.64267	0	11.967334	11.37867	0	12.768333	12.554	0	12.54067	12.06267	0	DREB	A5
R5_c01_04894_F	Os01g66270	10.773666	9.479	0	10.333667	8.862	1	10.515334	9.552333	1	10.577	9.161333	1	DREB	A1
R5_c01_04997_R	Os01g67410	2.1816666	2.167667	0	2.1853335	2.181	0	2.173	2.220333	0	3.277667	3.352667	0	AP2	AP2
R5_c01_05517_F	Os01g73770	9.894334	8.625	0	9.916333	9.141666	0	10.520667	9.227	1	10.05033	8.782333	0	ERF	B1
R5_c02_06063_R	Os02g06330	9.9053335	9.835	0	9.917	9.357667	0	10.354	9.887	0	10.15967	10.00633	0	ERF	B1
R5_c02_06322_R	Os02g09650	15.902333	15.72267	0	15.838333	15.62267	0	15.796666	15.824668	0	15.66367	15.49667	0	DREB	A3
R5_c02_06426_F	Os02g10760	2.3546667	2.191334	0	2.2903333	2.231334	0	2.8066666	2.3886669	0	2.726333	4.305666	0	DREB	A4
R5_c02_06658_F	Os02g13710	10.157001	9.892667	0	9.525333	9.444667	0	9.553	9.024333	0	9.134334	8.839667	0	ERF	B7
R5_c02_07701_F	Os02g29550	11.960667	12.47867	0	11.918334	12.085	0	12.604333	12.995667	0	11.75433	12.401	-1	ERF	B1
R5_c02_07845_R	Os02g32040	10.817333	10.91	0	11.043666	10.863	0	11.389	11.375	0	11.12767	11.09233	0	ERF	B4
R5_c02_07852_R	Os02g32140	11.162334	12.00267	-1	12.107666	11.80167	0	12.677667	12.917666	0	12.52333	12.30833	0	ERF	B4
R5_c02_08009_R	Os02g34260	3.9529998	5.61	0	5.5926666	5.946	0	6.4706664	7.2310004	0	5.278	5.745667	0	ERF	B4
R5_c02_08011_R	Os02g34270	4.7409997	5.939333	0	6.113333	6.089667	0	6.8870006	7.315	0	5.822	6.085667	0	DREB	A4
R5_c02_08098_R	Os02g35240	6.523333	7.194334	0	7.5646667	6.865667	1	8.399333	9.139	0	7.498	8.044667	0	ERF	B1
R5_c02_08335_F	Os02g38090	5.087333	4.453333	0	3.4069998	3.455667	0	3.2686665	3.1456668	0	4.082667	4.182667	0	DREB	A6
R5_c02_08502_F	Os02g40070	2.066	2.190667	0	2.2	2.240333	0	2.9626667	3.2903335	0	2.262	2.048	0	AP2	AP2
R5_c02_08784_R	Os02g43790	10.874667	10.78733	0	11.336667	10.60367	1	11.866	11.066667	1	11.496	11.59567	0	ERF	B3
R5_c02_08786_F	Os02g43820	8.064	9.231999	-1	9.070334	9.15	0	10.163667	10.434667	0	10.049	10.06233	0	DREB	A4

Continue

R5_c02_08800_F	Os02g43940	10.222667	9.888001	0	10.564667	10.57833	0	10.709332	10.522666	0	10.423	10.842	0	DREB	A4
R5_c02_08802_F	Os02g43970	8.6206665	9.733666	-1	9.302667	9.261333	0	10.444667	9.508666	1	9.322333	9.794666	0	DREB	A5
R5_c02_08926_F	Os02g45420	12.574	11.866	1	12.263667	11.84467	0	12.678668	12.301	0	13.23867	12.13067	1	DREB	A1
R5_c02_08928_F	Os02g45450	10.212667	9.352333	0	10.274333	9.956	0	11.817666	10.888667	0	11.623	11.38167	0	DREB	A6
R5_c02_09449_R	Os02g51300	9.211667	9.162334	0	9.174333	9.105667	0	9.711333	9.365001	0	9.846667	9.545001	0	AP2	AP2
R5_c02_09486_F	Os02g51670	15.554	15.581	0	15.483001	15.57167	0	15.423	15.618668	0	15.61733	15.75433	0	ERF	B4
R5_c02_09570_R	Os02g52670	13.510666	13.02133	0	13.414333	12.338	1	14.513333	13.545667	1	14.50667	13.77433	0	DREB	A5
R5_c02_09691_R	Os02g54050	8.05	7.533	0	8.269	7.807667	0	8.773666	7.6193333	0	8.977333	8.430667	0	ERF	B2
R5_c02_09702_R	Os02g54160	14.429333	14.37533	0	14.504334	14.544	0	14.426	14.500333	0	14.93933	14.77967	0	DREB	A3
R5_c02_09817_R	Os02g55380	6.5136666	6.102999	0	7.2479997	6.655	1	6.027	6.068333	0	6.828	6.626	0	ERF	B3
R5_c03_10544_F	Os03g05590	2.1363335	2.089334	0	2.1379998	2.082333	0	2.127	2.0719998	0	2.118333	2.485	0	DREB	A2
R5_c03_10733_F	Os03g07830	4.3356667	4.625333	0	4.678	4.373333	0	4.6703334	4.773333	0	4.702667	4.449	0	ERF	B3
R5_c03_10742_R	Os03g07940	2.8926666	2.562667	0	3.2619998	2.381666	0	3.3553333	2.4466667	0	2.356	2.445	0	AP2	AP2
R5_c03_10795_R	Os03g08460	9.460334	9.358334	0	10.377	10.75067	0	10.973666	11.234333	0	10.167	9.932667	0	ERF	B2
R5_c03_10797_R	Os03g08470	13.647667	13.53133	0	13.534333	14.316	-1	12.928666	13.196332	0	13.98233	13.842	0	ERF	B3
R5_c03_10799_R	Os03g08490	16.289999	15.79667	0	16.674667	16.45667	0	15.630333	15.969666	0	16.55133	16.05967	0	ERF	B2
R5_c03_10800_R	Os03g08500	18.066332	17.95933	0	17.824333	18.169	0	17.882	18.005	0	18.15	18.07533	0	DREB	A6
R5_c03_10865_R	Os03g09170	13.017333	13.82767	0	13.644333	13.42133	0	14.489334	14.764333	0	13.86933	14.45867	0	DREB	A5
R5_c03_11174_R	Os03g12950	3.8913333	3.782	0	3.7246666	3.977	0	2.6673334	3.038	0	3.43	3.371333	0	AP2	AP2
R5_c03_11390_R	Os03g15660	5.2963333	5.375	0	5.424	5.504	0	6.0200005	5.636667	0	6.469667	6.305666	0	ERF	B2
R5_c03_11776_R	Os03g19900	4.492334	5.665333	0	5.1123333	5.473333	0	5.5429997	6.1003337	0	4.959333	5.654333	0	AP2	AP2
R5_c03_11968_F	Os03g22170	6.903333	6.530333	0	6.8519998	6.947666	0	6.792	7.154667	0	7.439333	6.951	0	ERF	B1
R5_c03_14178_F	Os03g56050	3.183667	3.339	0	2.7266667	3.257	0	3.2630002	3.255	0	3.048333	3.765	0	AP2	AP2
R5_c03_14544_R	Os03g60120	13.097333	13.12533	0	12.978333	13.47833	0	12.88	13.068001	0	13.242	13.341	0	ERF	B3
R5_c03_14570_F	Os03g60430	10.647666	10.47433	0	10.141334	10.327	0	9.949667	9.886001	0	10.53567	10.284	0	AP2	AP2
R5_c03_14900_R	Os03g64260	7.9990005	8.395	0	9.644333	9.434667	0	9.821	10.014333	0	9.607333	10.237	-1	ERF	B3
R5_c04_15784_R	Os04g18650	3.9726667	3.976667	0	3.8533332	3.112333	0	4.0406666	3.806	0	4.465666	4.379667	0	ERF	B4
R5_c04_16592_F	Os04g32620	10.694667	10.83833	0	11.224	11.303	0	11.396667	11.662	0	11.67367	11.40333	0	ERF	B1
R5_c04_16607_F	Os04g32790	9.117667	10.22767	-1	9.556334	9.663667	0	11.698667	11.630333	0	10.419	10.87367	0	ERF	B4
R5_c04_16797_R	Os04g34970	6.345333	7.682333	-1	8.415333	7.833334	0	9.312667	9.429334	0	9.548333	9.004001	0	DREB	A4
R5_c04_16906_R	Os04g36640	7.5166664	8.052334	0	8.2543335	7.689667	1	8.866333	9.272667	0	8.776999	9.202667	0	DREB	A6
R5_c04_17379_F	Os04g42570	2.8876667	2.562	0	2.9143333	2.122	0	2.2509997	2.3903334	0	2.754	2.297333	0	AP2	AP2
R5_c04_17558_R	Os04g44670	8.514333	9.321334	0	9.036666	9.152333	0	10.931	10.622001	0	9.635667	9.530666	0	ERF	B3
R5_c04_17698_R	Os04g46220	15.720666	15.323	0	15.703	15.90867	0	15.568001	15.763667	0	15.38867	15.85767	0	ERF	B5
R5_c04_17699_F	Os04g46240	7.5126667	7.417667	0	7.3606668	7.206667	0	7.9143333	8.026999	0	7.560999	7.242334	0	ERF	B5

Continue

R5_c04_17700_F	Os04g46250	3.1066666	4.287667	-1	4.955333	5.104666	0	6.049	6.6913333	0	5.905667	6.800334	-1	DREB	A4
R5_c04_17712_F	Os04g46400	10.521333	10.36833	0	10.383334	10.372	0	10.531667	10.356999	0	10.476	10.55867	0	DREB	A4
R5_c04_17713_F	Os04g46410	4.3103333	4.223667	0	3.8516667	4.001667	0	4.5263333	4.668	0	4.571333	5.148333	0	DREB	A4
R5_c04_17715_F	Os04g46440	12.425	11.899	0	11.655334	10.79033	1	12.127	11.126334	1	12.18467	11.58267	0	DREB	A1
R5_c04_17876_F	Os04g48350	11.186666	10.40967	0	11.124333	9.713666	0	11.293667	9.429334	0	11.84267	11.48267	0	ERF	B1
R5_c04_18159_F	Os04g52090	14.656334	15.247	0	15.155	14.623	0	15.879334	15.416667	0	15.19367	15.93033	0	DREB	A5
R5_c04_18473_R	Os04g55520	13.467999	13.31433	0	13.555333	13.00734	0	13.597333	13.746333	0	13.658	13.28067	0	DREB	A3
R5_c04_18476_F	Os04g55560	8.320666	8.498334	0	8.065333	8.405	0	8.279334	8.652667	0	8.227666	8.461667	0	AP2	AP2
R5_c04_18509_F	Os04g55970	7.5690002	7.476667	0	7.6716666	7.527667	0	7.4900002	7.626	0	7.713666	7.473	0	AP2	AP2
R5_c04_18526_R	Os04g56150	4.1809998	5.21	0	5.522	4.279	0	6.228667	6.448667	0	5.371334	5.576666	0	ERF	B1
R5_c04_18636_R	Os04g57340	15.429334	15.661	0	15.887	15.41767	0	16.366333	15.806333	0	15.98033	16.175	0	ERF	B1
R5_c05_19028_R	Os05g03040	13.518333	13.74267	0	13.471334	13.81733	0	13.961333	14.051999	0	13.48833	13.76467	0	AP2	AP2
R5_c05_20302_F	Os05g25260	14.422	13.951	0	14.203334	14.32133	0	13.869	14.148999	0	13.99733	13.99533	0	DREB	A2
R5_c05_20463_R	Os05g27930	13.811666	14.04833	0	14.001332	14.05133	0	14.094334	14.083	0	14.13367	14.2	0	DREB	A2
R5_c05_20495_R	Os05g28350	3.3283336	3.144334	0	2.8076668	2.451333	0	4.5736666	4.2436666	0	2.703334	2.777	0	ERF	B2
R5_c05_20570_F	Os05g29810	8.783334	9.282001	0	9.947	9.361667	0	10.866	11.056667	0	10.52267	11.167	-1	ERF	B7
R5_c05_20760_F	Os05g32270	9.408999	9.994	0	10.188666	10.56333	0	10.678666	10.507	0	9.898	10.60033	0	DREB	A5
R5_c05_20969_R	Os05g34730	8.993333	8.607333	0	8.830667	7.885667	1	9.741333	8.838333	1	9.047334	9.096666	0	ERF	B4
R5_c05_21085_F	Os05g36100	2.5410001	2.404667	0	2.2513332	2.156667	0	2.2183332	2.1353333	0	2.257667	2.798333	0	ERF	B3
R5_c05_21164_F	Os05g37640	8.271	8.574667	0	8.507667	8.363334	0	8.145667	8.408667	0	8.419666	8.374333	0	DREB	A2
R5_c05_21330_F	Os05g39590	5.273667	4.566667	0	5.3156667	5.661334	0	4.4876666	3.6553333	0	5.648333	5.151667	0	ERF	B5
R5_c05_21511_R	Os05g41760	13.127	13.453	0	13.255333	12.59867	1	13.647334	13.135667	0	13.819	13.97367	0	ERF	B1
R5_c05_21512_R	Os05g41780	15.254334	15.38867	0	15.274667	14.99133	0	15.653333	15.273666	0	15.45533	15.65567	0	ERF	B3
R5_c05_21809_R	Os05g45954	2.4209998	2.411	0	3.2543333	2.696667	0	2.1913333	2.5863333	0	2.567	2.248333	0	AP2	AP2
R5_c05_21922_F	Os05g47650	12.280334	12.139	0	12.796001	12.40233	0	12.602666	12.941667	0	13.118	12.81467	0	RAV	RAV
R5_c05_22040_R	Os05g49010	5.9933333	4.788667	0	5.9636664	5.360667	0	6.021667	5.3093333	0	5.484666	5.078	0	DREB	A6
R5_c05_22104_R	Os05g49700	8.297	9.388333	-1	8.902333	8.506333	0	10.252	9.885333	0	9.816667	9.815	0	DREB	A1
R5_c06_22500_F	Os06g03670	9.8203335	10.60667	0	10.704667	9.806	0	12.139999	11.455001	0	11.87333	11.47933	0	ERF	B3
R5_c06_22659_R	Os06g05340	3.6780002	3.940667	0	3.190667	3.206	0	3.3093336	2.894	0	2.865667	3.015667	0	AP2	AP2
R5_c06_22775_F	Os06g06540	10.339667	10.51267	0	10.167	9.908334	0	10.276	10.592	0	10.161	10.28433	0	DREB	A1
R5_c06_22818_F	Os06g06970	4.077	4.100334	0	2.7363331	2.991	0	3.4723332	3.3803337	0	2.900667	2.821	0	DREB	A5
R5_c06_22824_F	Os06g07030	14.227333	15.21933	-1	15.311333	15.65667	0	15.297333	15.915	0	15.04967	15.355	0	DREB	A3
R5_c06_22929_F	Os06g08340	3.2796667	3.558	0	3.9566667	3.743333	0	5.252667	4.5103335	0	3.493333	4.423334	0	ERF	B2
R5_c06_23022_F	Os06g09390	10.345667	10.23833	0	10.148334	9.952	0	9.982333	9.642333	0	10.71467	10.60867	0	DREB	A5
R5_c06_23044_F	Os06g09717	4.944	5.309	0	4.9273334	4.866667	0	6.1493335	5.2706666	0	5.935333	5.806333	0	DREB	A5

Continue

R5_c06_23047_F	Os06g09760	6.004667	5.715334	0	6.207333	5.824667	0	6.951	6.4440002	0	6.842001	6.905333	0	DREB	A5
R5_c06_23049_F	Os06g09790	2.2443333	2.329667	0	2.832	2.482333	0	2.9426668	2.743333	0	3.06	3.009333	0	DREB	A5
R5_c06_23051_F	Os06g09810	3.2673333	3.421667	0	3.6933334	3.554667	0	4.376667	3.7896664	0	4.649667	4.409667	0	DREB	A5
R5_c06_23134_F	Os06g10780	10.858666	9.109	1	9.841667	9.061	0	10.157333	9.014	1	11.10933	9.803666	1	DREB	A6
R5_c06_23229_R	Os06g11860	12.527	12.811	0	12.780334	13.19167	0	12.793332	12.971334	0	13.68533	12.983	0	DREB	A5
R5_c06_23234_F	Os06g11940	4.081	4.443667	0	3.6350002	3.167666	0	4.8973336	3.8076668	0	4.822	3.871667	0	DREB	A4
R5_c06_24648_F	Os06g36000	2.7156668	3.784334	0	3.0240002	3.923333	0	4.372333	4.4173336	0	3.952667	4.079	0	DREB	A3
R5_c06_24943_R	Os06g40150	2.846	2.093667	0	2.6490002	2.818	0	4.9266663	4.166	0	4.006667	6.646667	0	ERF	B3
R5_c06_25143_F	Os06g42990	5.220667	5.412333	0	5.291	5.175334	0	5.083	5.141333	0	4.795	4.933	0	ERF	B1
R5_c06_25165_R	Os06g43220	3.3359997	3.763333	0	4.058	3.321	0	3.7296665	3.4233334	0	3.430667	3.335	0	AP2	AP2
R5_c06_25294_F	Os06g44750	2.2666667	2.631333	0	2.253	3.043	0	2.24	2.5713332	0	2.239	2.210667	0	AP2	AP2
R5_c06_25550_F	Os06g47590	13.103665	12.93	0	13.349334	13.12467	0	13.421666	13.342667	0	13.45767	13.04067	0	ERF	B7
R5_c07_26062_F	Os07g03250	6.945667	6.947667	0	6.761667	7.003666	0	6.570333	6.803	0	6.710667	6.792667	0	ERF	B6
R5_c07_26640_F	Os07g10410	2.374	2.510333	0	2.7006664	2.804	0	2.306	2.4186666	0	2.670667	2.437667	0	ERF	B1
R5_c07_26784_R	Os07g12510	12.135333	12.47467	0	12.424335	12.40933	0	13.026	12.932667	0	12.52533	12.69733	0	ERF	B3
R5_c07_26830_F	Os07g13170	8.356667	8.327	0	8.110333	7.979666	0	8.579666	8.571667	0	8.301333	8.160666	0	AP2	AP2
R5_c07_27279_R	Os07g22730	7.59	7.790333	0	7.7350006	6.866	0	9.130334	9.054667	0	9.085667	8.483334	0	ERF	B3
R5_c07_27282_R	Os07g22770	2.2893333	2.38	0	2.2793334	2.419	0	2.25	2.2043333	0	2.761667	2.302	0	ERF	B6
R5_c07_28366_R	Os07g38750	3.7553332	3.646	0	4.1666665	3.804667	0	3.0733335	3.7676666	0	3.128333	3.793	0	ERF	B2
R5_c07_28680_R	Os07g42510	10.153667	9.809334	0	10.351333	10.35167	0	9.815666	9.908668	0	10.18533	10.292	0	ERF	B1
R5_c07_29085_R	Os07g47330	2.1829998	2.146667	0	2.1603334	2.130333	0	2.1533334	2.111	0	2.194333	2.533334	0	ERF	B2
R5_c07_29128_R	Os07g47790	7.7206664	7.798667	0	8.351333	7.604	0	9.856334	8.728666	1	9.234333	9.087334	0	ERF	B1
R5_c08_29813_R	Os08g07440	13.079	12.86067	0	13.038667	13.04233	0	12.926666	12.875667	0	12.99	12.81567	0	AP2	AP2
R5_c08_29833_R	Os08g07700	2.331	2.469667	0	2.3426666	3.413667	0	4.316	5.1543336	0	3.014667	4.036	0	ERF	B7
R5_c08_30932_R	Os08g27220	7.7756667	7.528667	0	7.8266664	7.303667	0	7.7466664	7.771667	0	7.527667	7.257	0	DREB	A6
R5_c08_31221_F	Os08g31580	12.436	14.138	-1	12.984333	13.352	0	14.144666	14.111333	0	13.25433	14.64267	-1	DREB	A5
R5_c08_31442_F	Os08g34360	9.516	10.11233	0	10.573	10.872	0	10.610333	10.827333	0	9.942334	10.16133	0	AP2	AP2
R5_c08_31516_F	Os08g35240	2.4493334	2.445334	0	2.3806667	2.473667	0	2.7986667	2.608	0	2.418333	3.053667	0	ERF	B4
R5_c08_31636_F	Os08g36920	12.32	11.69833	0	11.891334	10.585	1	12.098666	11.419666	0	12.46233	12.305	0	ERF	B7
R5_c08_31995_F	Os08g41030	3.8243332	5.762667	-1	4.688	4.657333	0	6.5519996	6.2599998	0	7.392666	7.192333	0	ERF	B4
R5_c08_32118_F	Os08g42550	9.520667	9.833333	0	10.453333	10.43633	0	10.143333	10.309001	0	10.36167	10.244	0	RAV	RAV
R5_c08_32176_R	Os08g43200	6.921	6.643667	0	7.2843337	6.805334	0	8.887667	8.454999	0	8.343667	7.454667	1	DREB	A1
R5_c08_32177_R	Os08g43210	3.2540004	2.906667	0	3.6069996	2.958333	0	5.297667	4.636	0	4.200333	4.258	0	DREB	A1
R5_c08_32329_F	Os08g44960	4.199333	4.157333	0	4.3110003	4.036333	0	3.9246666	4.089	0	3.887	3.93	0	ERF	B3
R5_c08_32342_R	Os08g45110	2.1133335	2.098333	0	3.4016666	3.415	0	2.686	2.6850002	0	3.059333	2.861333	0	DREB	A2

Continue

R5_c09_32888_R	Os09g11460	10.216333	9.122334	1	9.980667	9.78	0	9.269334	9.391	0	9.519	9.116333	0	ERF	B2
R5_c09_32890_R	Os09g11480	16.052332	15.314	0	15.543332	15.93734	0	14.424333	14.655332	0	15.078	14.93133	0	ERF	B2
R5_c09_33027_R	Os09g13940	10.957001	11.045	0	10.898	11.18867	0	11.25	11.499	0	11.237	11.33967	0	ERF	B7
R5_c09_33424_F	Os09g20350	15.869667	15.90567	0	15.598667	15.93367	0	15.47	15.456	0	16.09733	16.082	0	DREB	A6
R5_c09_33800_F	Os09g25600	9.496667	9.766666	0	9.718333	9.348666	0	9.928666	9.915666	0	9.511	9.632	0	AP2	AP2
R5_c09_33870_F	Os09g26420	16.504667	16.374	0	16.023333	16.43833	0	15.907666	16.178999	0	16.06	16.24367	0	ERF	B2
R5_c09_34047_F	Os09g28440	11.889667	11.35867	0	11.664666	9.586	1	11.771	10.786667	1	12.47433	12.41067	0	ERF	B4
R5_c09_34510_R	Os09g35010	13.211667	12.47867	0	12.662666	11.46267	1	12.935	12.019666	0	13.66167	13.38667	0	DREB	A1
R5_c09_34511_R	Os09g35020	9.575333	9.105	0	8.800667	7.304667	1	9.4053335	8.635334	0	9.934001	9.700334	0	DREB	A1
R5_c09_34512_R	Os09g35030	16.73	15.342	1	16.466667	15.41833	1	15.935666	15.272667	1	16.98667	16.10967	1	DREB	A1
R5_c09_34919_R	Os09g39810	4.6353335	4.604333	0	4.506	4.603667	0	5.0620003	4.641333	0	4.438334	4.566667	0	ERF	B3
R5_c09_34921_F	Os09g39850	3.4423332	4.473	0	4.3093333	5.002333	0	4.3276668	4.617	0	4.093333	4.884333	0	ERF	B3
R5_c10_36036_F	Os10g22600	7.0769997	7.836	-1	7.232	7.769	0	8.134	8.158333	0	7.220333	7.935999	0	DREB	A6
R5_c10_36164_F	Os10g25170	4.090667	4.711	0	5.0883336	5.170667	0	5.1743336	5.132	0	5.944334	6.310999	0	ERF	B1
R5_c10_36265_F	Os10g26590	6.4133334	6.299	0	6.4653335	5.908	0	5.633667	5.6516666	0	5.625333	5.475667	0	ERF	B7
R5_c10_36541_R	Os10g30840	7.0206666	7.061333	0	6.1349998	5.969	0	6.531	6.706	0	5.776333	5.858334	0	ERF	B3
R5_c10_37098_F	Os10g38000	16.697	16.52367	0	16.519999	16.65134	0	15.872001	15.999001	0	16.69833	16.434	0	DREB	A5
R5_c10_37379_R	Os10g41130	9.3	9.188666	0	9.066333	9.071667	0	9.824333	9.660333	0	9.308333	9.273	0	DREB	A4
R5_c10_37398_F	Os10g41330	11.464665	12.06467	0	12.082	11.81533	0	12.167333	11.305333	1	11.52067	12.72433	0	ERF	B5
R5_c11_37752_R	Os11g03540	6.243667	7.414	-1	7.348	7.798667	0	8.833667	8.744332	0	8.393333	8.972	0	AP2	AP2
R5_c11_38041_R	Os11g06770	13.389667	14.18233	-1	14.329333	14.15467	0	15.255	15.530666	0	14.614	14.842	0	ERF	B4
R5_c11_38534_R	Os11g13840	6.994	8.653	-1	9.042666	7.895334	1	10.728667	10.585667	0	10.91633	10.40733	0	DREB	A4
R5_c11_38831_F	Os11g19060	2.912	2.435	0	2.7133334	2.476	0	2.6406667	2.42	0	2.682	2.473667	0	AP2	AP2
R5_c12_40849_R	Os12g03290	9.006333	9.595	0	9.906333	9.843667	0	10.579667	10.587667	0	10.408	10.67367	0	AP2	AP2
R5_c12_43079_F	Os12g39330	2.5536668	3.179	0	2.3406668	2.679333	0	2.8920002	2.8316667	0	2.781667	3.159	0	ERF	B6
R5_c12_43200_R	Os12g41030	7.347	7.287666	0	7.6723332	7.544	0	7.337667	7.573667	0	7.318667	7.261334	0	ERF	B7
R5_c12_43202_R	Os12g41060	4.8336663	4.409333	0	4.1996665	3.859667	0	3.7666671	4.0246663	0	4.568667	4.632	0	ERF	B7

Footnote: Fold change gene expression data of 162 *OsAP2/EREBP* genes probe on the 44K rice oligoarray under RTSV infection are listed. More than 0.585 fold upregulated genes were assigned 1, less than –0.585 fold downregulated genes were assigned –1, others are assigned as 0. DEGs = differentially expressed genes. Phg = Phylogentic subgroups or subfamilies. RTSV = Rice tungro spherical virus.

Table 7. *OsAP2/EREBP* gene expression profiles (log 2–based ratio) under RDV infection (three strains S, O, and D84) (44K-oligoarray data).

Probe name	MSU locus	RDV-S	mock RDV-S	DEGs	RDV-O	mock RDV-O	DEGs	RDV-D84	mock RDV-D84	DEGs	Phg. Subfamily	Subgroup
R5_c01_00261_F	Os01g04020	11.163	10.980884	0	10.5384	10.362469	0	10.75246	10.950129	0	DREB	A2
R5_c01_00331_F	Os01g04750	5.8986	5.811695	0	5.59601	5.7634	0	5.737957	6.0541706	0	RAV	RAV
R5_c01_00335_F	Os01g04800	12.761	11.4205	1	10.7019	11.021422	0	11.45333	11.268677	0	RAV	RAV
R5_c01_00531_F	Os01g07120	10.979	9.229093	0	8.82374	8.71016	0	10.08142	9.0287285	1	DREB	A4
R5_c01_00811_R	Os01g10370	6.1118	5.776237	0	5.50988	5.8751407	0	5.273167	6.187985	0	ERF	B1
R5_c01_00982_F	Os01g12440	9.3137	9.228337	0	8.91176	8.566508	0	9.18082	9.163092	0	ERF	B2
R5_c01_01593_F	Os01g21120	12.075	9.977259	0	9.8133	9.247656	0	10.60025	9.921872	0	ERF	B1
R5_c01_03200_R	Os01g46870	8.6647	9.124392	0	8.6969	8.700806	0	8.945331	9.021412	0	ERF	B3
R5_c01_03456_R	Os01g49830	15.009	13.837941	1	13.6732	13.272112	0	14.475	13.593165	0	RAV	RAV
R5_c01_03895_F	Os01g54890	13.646	12.698906	0	12.6465	12.237601	0	13.27172	12.624104	0	ERF	B1
R5_c01_04215_F	Os01g58420	14.984	15.909831	0	15.3328	15.28578	0	14.82529	15.644516	0	ERF	B4
R5_c01_04330_F	Os01g59780	1.9959	3.0216553	0	3.15342	2.06053	0	2.210441	2.6067677	0	AP2	AP2
R5_c01_04750_R	Os01g64790	10.719	7.367089	1	7.60273	7.0018587	0	9.044999	7.2593617	1	DREB	A5
R5_c01_04894_F	Os01g66270	14.769	11.496715	0	11.7417	10.930849	0	14.16022	11.311533	0	DREB	A1
R5_c01_04997_R	Os01g67410	1.8369	2.0605056	0	1.28909	1.7485471	0	1.878966	1.7515152	0	AP2	AP2
R5_c01_05517_F	Os01g73770	9.0903	5.6036777	0	5.20902	5.4409833	0	9.64813	5.478961	1	ERF	B1
R5_c02_06063_R	Os02g06330	9.0244	9.688809	0	9.26486	9.143054	0	9.71137	9.57198	0	ERF	B1
R5_c02_06322_R	Os02g09650	13.656	13.954677	0	13.1739	13.312374	0	13.28225	13.728299	0	DREB	A3
R5_c02_06426_F	Os02g10760	4.8667	7.921659	-1	5.64003	7.378635	-1	5.781932	7.8222637	-1	DREB	A4
R5_c02_06658_F	Os02g13710	7.5419	8.809592	-1	8.43343	8.574733	0	8.527508	9.073228	0	ERF	B7
R5_c02_07701_F	Os02g29550	13.44	14.134137	-1	13.5131	13.926623	0	13.69837	14.1648855	0	ERF	B1
R5_c02_07845_R	Os02g32040	12.296	12.256731	0	11.793	11.670291	0	12.21357	12.38479	0	ERF	B4
R5_c02_07852_R	Os02g32140	9.9629	8.113794	1	7.52378	7.614241	0	8.524657	7.994753	0	ERF	B4
R5_c02_08009_R	Os02g34260	5.786	4.4751883	0	2.22285	3.5365577	0	4.368618	4.908523	0	ERF	B4
R5_c02_08011_R	Os02g34270	6.0356	4.003947	1	2.90644	3.5270314	0	4.496422	4.0359645	0	DREB	A4
R5_c02_08098_R	Os02g35240	1.7969	2.2875922	0	2.08047	1.6443561	0	1.833592	1.7139052	0	ERF	B1
R5_c02_08335_F	Os02g38090	2.7029	2.9395642	0	2.77951	2.5054681	0	1.880116	3.0402577	0	DREB	A6
R5_c02_08502_F	Os02g40070	2.7029	2.1005673	0	1.10385	1.8801491	0	1.806572	1.648291	0	AP2	AP2
R5_c02_08784_R	Os02g43790	13.168	12.315904	0	13.3515	11.589167	0	13.44262	12.208239	0	ERF	B3
R5_c02_08786_F	Os02g43820	9.4682	8.497771	0	8.18362	7.758795	0	8.743385	8.392126	0	DREB	A4
R5_c02_08800_F	Os02g43940	9.1013	10.115473	0	9.9344	9.567813	0	9.843276	10.227817	0	DREB	A4
R5_c02_08802_F	Os02g43970	10.408	12.979394	-1	12.6989	12.554759	0	11.86751	12.929764	0	DREB	A5
R5_c02_08926_F	Os02g45420	8.6063	4.3074145	0	5.16932	4.069795	0	8.793424	4.371066	0	DREB	A1

Continue

R5_c02_08928_F	Os02g45450	10.317	7.161295	0	8.17783	6.700183	0	10.56692	7.393895	0	DREB	A6
R5_c02_09449_R	Os02g51300	9.515	9.434227	0	9.31128	9.0432625	0	9.343315	9.550994	0	AP2	AP2
R5_c02_09486_F	Os02g51670	15.895	16.164875	0	16.0219	16.054434	0	15.86302	16.519018	0	ERF	B4
R5_c02_09570_R	Os02g52670	11.869	10.293354	1	10.2238	9.759136	0	12.25207	10.418573	1	DREB	A5
R5_c02_09691_R	Os02g54050	9.0725	7.0263	0	6.74496	5.830954	0	8.779275	6.653546	1	ERF	B2
R5_c02_09702_R	Os02g54160	13.558	13.081182	0	12.79	12.796628	0	13.25083	13.176049	0	DREB	A3
R5_c02_09817_R	Os02g55380	6.3447	5.8773155	0	4.71027	4.6485386	0	5.60614	4.795493	0	ERF	B3
R5_c03_10544_F	Os03g05590	1.7031	2.0208602	0	1.06508	1.7041779	0	1.811399	1.6521951	0	DREB	A2
R5_c03_10733_F	Os03g07830	2.5717	2.3579752	0	2.42649	2.6248782	0	3.276099	2.6332746	0	ERF	B3
R5_c03_10742_R	Os03g07940	1.9373	2.2115178	0	1.36637	1.958182	0	2.027403	1.9446418	0	AP2	AP2
R5_c03_10795_R	Os03g08460	12.701	11.668816	0	11.0599	11.312423	0	12.5463	11.749802	0	ERF	B2
R5_c03_10797_R	Os03g08470	12.836	10.690633	0	10.1517	10.515156	0	11.64797	10.835318	0	ERF	B3
R5_c03_10799_R	Os03g08490	15.582	14.622437	0	14.7884	14.361506	0	15.10668	14.552823	0	ERF	B2
R5_c03_10800_R	Os03g08500	17.569	17.627861	0	17.5836	17.201097	0	17.3973	17.499561	0	DREB	A6
R5_c03_10865_R	Os03g09170	11.987	10.69218	0	10.4826	10.510662	0	11.42838	10.800673	0	DREB	A5
R5_c03_11174_R	Os03g12950	2.5015	2.348075	0	2.12112	1.7293831	0	2.375231	2.3976595	0	AP2	AP2
R5_c03_11390_R	Os03g15660	6.9647	5.368713	1	6.59043	4.827181	0	6.433989	5.367289	0	ERF	B2
R5_c03_11776_R	Os03g19900	3.6106	2.864181	0	1.77025	3.9751194	0	2.200528	3.6829777	0	AP2	AP2
R5_c03_11968_F	Os03g22170	5.6213	5.8213415	0	4.84225	5.4662843	0	5.53108	5.8801184	0	ERF	B1
R5_c03_14178_F	Os03g56050	2.7941	2.4739313	0	1.39956	3.491793	0	3.687179	3.5999463	0	AP2	AP2
R5_c03_14544_R	Os03g60120	14.361	14.708447	0	14.5285	14.351744	0	14.58138	14.550351	0	ERF	B3
R5_c03_14570_F	Os03g60430	10.472	11.383172	0	10.7004	11.010113	0	10.9884	11.38114	0	AP2	AP2
R5_c03_14900_R	Os03g64260	9.1415	8.150419	0	6.85362	7.3638	0	8.260234	7.839719	0	ERF	B3
R5_c04_15784_R	Os04g18650	1.9754	2.5722797	0	2.41935	1.89906	0	2.018153	1.9422176	0	ERF	B4
R5_c04_16592_F	Os04g32620	10.391	9.134718	1	8.39265	8.321431	0	8.894318	8.591709	0	ERF	B1
R5_c04_16607_F	Os04g32790	4.0728	4.740995	0	4.22463	4.492899	0	4.915194	4.7563663	0	ERF	B4
R5_c04_16797_R	Os04g34970	8.5594	7.5710464	1	8.50737	7.3250194	1	8.652676	7.677761	0	DREB	A4
R5_c04_16906_R	Os04g36640	3.2845	4.495458	-1	2.96779	4.6948533	0	5.682706	5.1818595	0	DREB	A6
R5_c04_17379_F	Os04g42570	3.1509	2.5404935	1	2.79249	1.7758521	1	2.827827	2.4337595	0	AP2	AP2
R5_c04_17558_R	Os04g44670	7.4427	8.001918	0	7.25088	7.49948	0	6.572434	8.093305	0	ERF	B3
R5_c04_17698_R	Os04g46220	16.31	15.088549	1	15.3493	14.400996	1	15.95524	15.038901	1	ERF	B5
R5_c04_17699_F	Os04g46240	7.0472	7.2137127	0	7.46129	7.018822	0	7.423328	7.5327153	0	ERF	B5
R5_c04_17700_F	Os04g46250	4.0536	2.100502	1	1.57695	1.7132168	0	1.756948	2.3791234	0	DREB	A4
R5_c04_17712_F	Os04g46400	10.433	12.589254	-1	11.7386	12.220393	0	11.61324	12.710452	-1	DREB	A4
R5_c04_17713_F	Os04g46410	2.6128	3.346791	0	2.67217	3.8687856	0	2.867171	2.4668183	0	DREB	A4
R5_c04_17715_F	Os04g46440	11.748	12.30817	0	12.5687	11.887412	0	11.96199	12.412195	0	DREB	A1
R5_c04_17876_F	Os04g48350	12.582	11.771568	0	12.1761	11.038134	0	13.51217	11.800066	0	ERF	B1
R5_c04_18159_F	Os04g52090	15.18	16.605259	0	16.5326	15.900404	0	15.38736	16.40853	0	DREB	A5
R5_c04_18473_R	Os04g55520	12.44	11.381604	0	10.6257	10.790391	0	11.83576	11.1826	0	DREB	A3
R5_c04_18476_F	Os04g55560	7.5562	8.533199	0	7.8155	8.14548	0	8.055271	8.450425	0	AP2	AP2

Continue

R5_c04_18509_F	Os04g55970	4.314	3.6676185	0	3.49877	3.921166	0	3.910717	4.629567	-1	AP2	AP2
R5_c04_18526_R	Os04g56150	1.742	2.0449631	0	1.1149	1.8397396	0	2.357477	2.4824324	0	ERF	B1
R5_c04_18636_R	Os04g57340	15.825	16.303003	0	16.1411	15.665791	0	15.81189	16.122007	0	ERF	B1
R5_c05_19028_R	Os05g03040	11.114	12.18066	0	11.2495	11.571055	0	11.49965	12.124772	0	AP2	AP2
R5_c05_20302_F	Os05g25260	13.909	13.53083	0	12.9045	12.742237	0	13.43555	13.394768	0	DREB	A2
R5_c05_20463_R	Os05g27930	13.974	13.836683	0	13.5054	13.390131	0	13.87814	13.912195	0	DREB	A2
R5_c05_20495_R	Os05g28350	3.8094	3.1037364	0	2.77415	3.0977633	0	3.385642	3.3899384	0	ERF	B2
R5_c05_20570_F	Os05g29810	9.0775	7.7037783	0	7.10231	7.34694	0	8.517164	7.913097	1	ERF	B7
R5_c05_20760_F	Os05g32270	9.5872	10.029617	0	9.56818	9.583058	0	9.514339	9.825575	0	DREB	A5
R5_c05_20969_R	Os05g34730	9.2475	6.3551564	0	6.62628	5.829629	0	8.051376	6.463315	0	ERF	B4
R5_c05_21085_F	Os05g36100	1.7657	2.081926	0	1.13302	1.7392861	0	1.847472	1.7092395	0	ERF	B3
R5_c05_21164_F	Os05g37640	11.951	11.946488	0	11.4206	11.518869	0	12.11484	11.964856	0	DREB	A2
R5_c05_21330_F	Os05g39590	8.0212	4.5275416	1	7.52635	4.584383	1	8.077802	5.048247	1	ERF	B5
R5_c05_21511_R	Os05g41760	13.659	14.861269	-1	14.4132	14.477178	0	14.08196	14.698279	0	ERF	B1
R5_c05_21512_R	Os05g41780	15.53	15.755988	0	16.208	15.076159	0	15.95615	15.510682	0	ERF	B3
R5_c05_21809_R	Os05g45954	6.5504	7.0877194	0	5.82606	6.575006	0	6.491392	6.7716823	0	AP2	AP2
R5_c05_21922_F	Os05g47650	10.531	9.592537	0	9.01251	9.02657	0	10.11089	9.459401	0	RAV	RAV
R5_c05_22040_R	Os05g49010	3.4156	3.040706	0	2.73065	2.5143023	0	4.178425	3.6178415	0	DREB	A6
R5_c05_22104_R	Os05g49700	10.591	9.231805	1	8.99707	8.89217	0	10.3883	9.087201	0	DREB	A1
R5_c06_22500_F	Os06g03670	10.212	7.306829	1	7.69969	6.5459943	0	9.31078	6.989802	1	ERF	B3
R5_c06_22659_R	Os06g05340	2.0631	2.313774	0	1.47039	2.0571723	0	2.178896	2.2704222	0	AP2	AP2
R5_c06_22775_F	Os06g06540	6.1801	6.4182777	0	6.44207	6.6099834	0	7.022007	7.5833144	0	DREB	A1
R5_c06_22818_F	Os06g06970	2.4949	2.2970185	0	1.05167	1.5690608	0	2.147453	1.8163625	0	DREB	A5
R5_c06_22824_F	Os06g07030	13.986	12.52521	0	12.1553	11.892306	0	13.23634	12.30683	0	DREB	A3
R5_c06_22929_F	Os06g08340	2.6401	2.4345393	0	2.3914	2.72669	0	2.319814	2.7315416	0	ERF	B2
R5_c06_23022_F	Os06g09390	9.0688	10.296287	-1	9.49651	9.756839	0	9.508147	10.254201	0	DREB	A5
R5_c06_23044_F	Os06g09717	5.7375	3.7720652	1	3.2116	2.815379	0	4.500301	3.675888	0	DREB	A5
R5_c06_23047_F	Os06g09760	3.9486	3.9306464	0	3.1222	3.884723	0	3.802516	3.8568692	0	DREB	A5
R5_c06_23049_F	Os06g09790	2.6555	2.5752013	0	2.40401	1.9071149	0	2.352481	2.5217063	0	DREB	A5
R5_c06_23051_F	Os06g09810	3.0111	2.4591186	0	2.34447	1.8459215	0	4.58131	2.5822592	0	DREB	A5
R5_c06_23134_F	Os06g10780	7.6271	5.389081	1	5.98281	5.684426	0	7.504623	5.903976	1	DREB	A6
R5_c06_23229_R	Os06g11860	13.988	13.054767	0	12.8624	12.885559	0	13.5418	13.150952	0	DREB	A5
R5_c06_23234_F	Os06g11940	2.9487	2.2197435	0	2.3656	1.8921096	0	2.331643	1.8186723	0	DREB	A4
R5_c06_24648_F	Os06g36000	2.8821	2.7160237	0	2.40504	2.5806613	0	3.808833	3.8430452	0	DREB	A3
R5_c06_24943_R	Os06g40150	4.9196	11.154316	-1	9.18916	10.522064	0	8.550446	11.003506	0	ERF	B3
R5_c06_25143_F	Os06g42990	4.1582	4.2965703	0	4.0328	4.0780616	0	4.509135	4.8098783	0	ERF	B1
R5_c06_25165_R	Os06g43220	4.8121	4.4306774	0	4.31092	4.236125	0	4.519737	4.157408	0	AP2	AP2
R5_c06_25294_F	Os06g44750	2.361	2.436372	0	2.35074	1.8725774	0	3.337724	2.5125067	0	AP2	AP2
R5_c06_25550_F	Os06g47590	12.756	12.774928	0	12.583	12.60228	0	12.97292	12.927485	0	ERF	B7
R5_c07_26062_F	Os07g03250	4.2016	4.587444	0	3.96014	4.394858	0	4.126943	4.7999344	0	ERF	B6

Continue

R5_c07_26640_F	Os07g10410	2.0103	2.5871758	0	2.39801	1.902781	0	2.251142	2.042486	0	ERF	B1
R5_c07_26784_R	Os07g12510	12.411	13.316633	-1	12.6101	12.876545	0	12.46854	13.129	-1	ERF	B3
R5_c07_26830_F	Os07g13170	6.6352	6.9051666	0	6.45424	6.4970303	0	6.802284	6.918123	0	AP2	AP2
R5_c07_27279_R	Os07g22730	8.4765	6.537742	1	6.98081	6.211359	1	8.295916	6.6334906	1	ERF	B3
R5_c07_27282_R	Os07g22770	2.7801	2.129356	0	2.17592	1.9321886	0	2.380006	1.8330625	0	ERF	B6
R5_c07_28366_R	Os07g38750	1.8727	3.1497056	0	2.35239	2.4731338	0	1.965966	2.592391	0	ERF	B2
R5_c07_28680_R	Os07g42510	7.3777	6.949946	0	6.13698	6.353845	0	6.609146	6.810881	0	ERF	B1
R5_c07_29085_R	Os07g47330	1.729	2.0384383	0	1.11259	1.7927475	0	1.797325	1.6515436	0	ERF	B2
R5_c07_29128_R	Os07g47790	7.7781	5.9834776	0	6.83241	5.363863	1	7.650062	5.9851084	0	ERF	B1
R5_c08_29813_R	Os08g07440	13.31	13.030958	0	12.8716	12.785539	0	13.29561	13.107072	0	AP2	AP2
R5_c08_29833_R	Os08g07700	1.7946	2.0851812	0	1.32946	1.6931492	0	1.860522	1.7230657	0	ERF	B7
R5_c08_30932_R	Os08g27220	5.1451	5.3110037	0	4.89989	5.2347503	0	5.007811	5.732813	0	DREB	A6
R5_c08_31221_F	Os08g31580	14.484	15.142495	0	14.8619	14.497135	0	14.66502	15.032661	0	DREB	A5
R5_c08_31442_F	Os08g34360	10.634	11.026309	0	10.7955	10.742708	0	10.75643	11.251065	0	AP2	AP2
R5_c08_31516_F	Os08g35240	1.9749	2.2270386	0	1.40555	1.9776968	0	2.05613	1.9778928	0	ERF	B4
R5_c08_31636_F	Os08g36920	13.512	8.2832985	0	11.4744	7.019435	0	13.65907	7.6875463	1	ERF	B7
R5_c08_31995_F	Os08g41030	2.5312	2.0509934	0	1.10251	1.6842581	0	1.89249	1.6740077	0	ERF	B4
R5_c08_32118_F	Os08g42550	11.932	10.273841	1	10.5851	9.623489	0	11.36927	10.224053	1	RAV	RAV
R5_c08_32176_R	Os08g43200	6.2038	2.1580195	0	2.69355	1.725478	0	4.360004	2.5765803	0	DREB	A1
R5_c08_32177_R	Os08g43210	6.9414	7.6094418	0	5.63201	7.0034842	0	5.777145	7.609173	0	DREB	A1
R5_c08_32329_F	Os08g44960	4.2478	2.6449375	0	3.96607	2.2618034	0	2.8709	3.6126685	0	ERF	B3
R5_c08_32342_R	Os08g45110	2.6081	3.215497	0	2.24616	2.766122	0	2.23352	3.1530018	0	DREB	A2
R5_c09_32888_R	Os09g11460	6.5368	6.92786	0	6.47062	6.52774	0	6.158955	7.001246	0	ERF	B2
R5_c09_32890_R	Os09g11480	12.822	12.490712	0	11.9859	12.09578	0	12.09405	12.327159	0	ERF	B2
R5_c09_33027_R	Os09g13940	12.384	13.119514	0	12.6795	12.445346	0	12.5129	13.005921	0	ERF	B7
R5_c09_33424_F	Os09g20350	16.574	17.282288	0	16.5607	16.693384	0	16.34363	17.042368	0	DREB	A6
R5_c09_33800_F	Os09g25600	11.751	12.001287	0	11.1778	11.68627	0	11.65649	12.187086	0	AP2	AP2
R5_c09_33870_F	Os09g26420	16.679	16.981674	0	16.4513	16.51025	0	16.70689	16.907822	0	ERF	B2
R5_c09_34047_F	Os09g28440	11.079	10.232696	0	12.1207	9.901435	0	13.40332	10.425256	0	ERF	B4
R5_c09_34510_R	Os09g35010	13.422	12.381052	0	13.6058	11.732451	0	14.77385	12.345105	0	DREB	A1
R5_c09_34511_R	Os09g35020	9.5297	9.552239	0	10.4146	8.923912	1	11.66592	9.481013	0	DREB	A1
R5_c09_34512_R	Os09g35030	15.954	14.061434	0	15.0717	13.363675	1	16.44329	13.923292	0	DREB	A1
R5_c09_34919_R	Os09g39810	3.1205	4.433894	0	3.58617	4.1782837	0	3.159603	4.7372584	0	ERF	B3
R5_c09_34921_F	Os09g39850	2.0064	2.1450047	0	1.29471	1.994114	0	2.452628	2.581848	0	ERF	B3
R5_c10_36036_F	Os10g22600	8.6838	8.866413	0	8.31142	8.485049	0	9.013309	8.779008	0	DREB	A6
R5_c10_36164_F	Os10g25170	8.0693	6.5259132	0	6.16051	6.267733	0	6.603316	6.5663543	0	ERF	B1
R5_c10_36265_F	Os10g26590	2.9123	2.4224029	0	1.56778	2.1190946	0	2.34331	3.176877	0	ERF	B7
R5_c10_36541_R	Os10g30840	3.392	3.9073715	0	3.14844	3.4870284	0	3.281843	3.899136	0	ERF	B3
R5_c10_37098_F	Os10g38000	16.17	16.184465	0	15.121	15.273051	0	16.00238	15.844246	0	DREB	A5
R5_c10_37379_R	Os10g41130	9.9252	10.950436	0	10.8191	10.727695	0	10.86929	11.15412	0	DREB	A4

Continue

R5_c10_37398_F	Os10g41330	11.364	11.237111	0	12.3	10.592808	1	11.78733	11.047922	0	ERF	B5
R5_c11_37752_R	Os11g03540	8.1285	7.0894217	0	6.54567	6.573103	0	7.015597	7.0640216	0	AP2	AP2
R5_c11_38041_R	Os11g06770	9.0725	8.920619	0	6.59983	7.8693657	0	7.248645	8.550306	0	ERF	B4
R5_c11_38534_R	Os11g13840	6.5193	4.9270597	0	5.08224	4.558143	0	5.747954	4.316903	0	DREB	A4
R5_c11_38831_F	Os11g19060	2.0417	2.2966018	0	1.44343	2.0350065	0	2.15617	2.034471	0	AP2	AP2
R5_c12_40849_R	Os12g03290	10.685	9.907672	0	9.48097	9.493508	0	9.677241	9.807309	0	AP2	AP2
R5_c12_43079_F	Os12g39330	3.3319	2.4043982	0	2.43189	1.9001786	0	3.652298	1.8427203	0	ERF	B6
R5_c12_43200_R	Os12g41030	5.5773	4.926096	1	5.10095	4.7982817	0	5.006499	5.3577013	0	ERF	B7
R5_c12_43202_R	Os12g41060	3.1491	3.0716386	0	3.74879	4.08474	0	3.663958	4.3385444	0	ERF	B7

Footnote: Fold change gene expression data of 162 *OsAP2/EREBP* genes probe on the 44K rice oligoarray under RDV infection are listed. More than 0.585 fold upregulated genes were assigned 1, less than –0.585 fold downregulated genes were assigned –1, others are assigned as 0. DEGs = differentially expressed genes. Phg = Phylogentic subgroups or subfamilies. RDV = Rice dwarf virus (three virus strains S, O and D84).

Table 8. Preferentially gene induced in specific subgroups under biotic and abiotic stresses conditions.

Number in bold of specific subgroups indicates up-or downregulated under different stress conditions.

Subgroup	No. of genes	RTSV		RSV		RDV					
						S		O		D84	
		Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
A-1	**10**	**7**	**1**	1	4	1	0	2	0	0	0
A-2	**6**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
A-3	**5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
A-4	12	1	4	1	4	3	2	1	1	1	2
A-5	15	2	2	1	3	3	2	0	0	2	0
A-6	9	1	1	0	1	1	1	0	0	1	0
B-1	**18**	**5**	**1**	**2**	**3**	1	2	1	0	1	0
B-2	12	2	0	1	3	1	0	0	0	1	0
B-3	19	2	2	0	0	2	2	1	0	2	1
B-4	12	2	4	1	1	1	0	0	0	0	0
B-5	**4**	1	0	1	1	**2**	**0**	**3**	**0**	**2**	**0**
B-6	**3**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
B-7	9	1	1	0	2	1	1	0	0	2	0
AP2	24	0	1	2	3	1	0	1	0	0	1
RAV	**5**	**3**	**0**	1	0	**3**	**0**	**0**	**0**	**1**	**0**

Subgroup	Induced genes	Treatments	Total	Subgroup	Induced genes	Treatments	Total
A-1	2/10	C, LDS, SM	3	B-1	4/18	C, D, LDS, SM	4
A-2	3/6	LDS, SM	2	B-2	4/12	LDS, SM,	2
A-3	**4/5**	**C, D, LDS, SM,**	**5**	**B-3**	**7/19**	**C, D, LDS, SM**	**4**
A-4	4/12	C, D, LDS, SM	4	B-4	5/12	C, D, LDS, SM,	4
A-5	3/15	C, D, LDS, SM, GA	5	**B-5**	**0/4**	**0**	**0**
A-6	1/9	LDS, SM,	2	B-6	2/3	SA	1
AP2	8/24	C, LDS, SM, GA	4	**B-7**	**3/9**	**C, LDS, SM, OS**	**3**
RAV	2/5	LDS, SM,	2				

RTSV = Rice tungro spherical virus; RSV = Rice stripe virus; RDV = Rice dwarf virus; C = Cold; D = Drought; LDS = Laid-down submergence; SM = submergence; GA = Gibberellic acid; SA = Salt.

The expression levels of 70 non-redundant *OsAP2/EREBP* genes were up- or downregulated under different abiotic stresses (cold, drought, laid-down submergence, submergence, osmotic stress, salt, and hormone stress) compared with their expression in control seedlings (**Table 9**). Under the different stress conditions, more than 60% of the genes were upregulated. The greatest number of genes (33) was upregulated under laid-down submergence stress, and the lowest number (1) was upregulated under osmotic or salt stress. Under the ABA (hormone) treatment, none of the genes were up- or downregulated. Thirteen genes were upregulated by at least three abiotic treatments and comparatively subgroups A-3 and A-4 of subfamily DREB were preferentially induced by more than three abiotic treatments (**Table 10**). Five genes showed both up- and downregulation at different time points of laid-down submergence stress, among which two genes (*Os04g57340* and *Os09g35010*) had identical patterns of up-and downregulation under both submergence stresses (**Table 9**). Altogether, expression of 10 genes of subfamilies AP2 and RAV was influenced by one or more abiotic treatments, and 6 of the 10 genes were affected by both laid-down submergence and submergence. A small number of genes of the different A and B subgroups were upregulated under different abiotic stresses, whereas a number of genes belonging to the A-3 and B-3 subgroups were upregulated similarly in response to various abiotic stresses (**Table 9**).

Table 9. *OsAP2/EREBP* gene expression (up or down) profile under various stress treatments (22K oligoarray data).

MSU loc ID	C24h	C48h	C72h	D01h	D09h	D24h	LDS01h	LDS06h	LDS24h	LDS48h	SM01h	SM24h	SM48h	SM72h	OS24h	SA24h	50µMGA	50µMABA	Phy. Subfamily/subgroup
Os01g04750		-0.66	-0.66					0.4281		0.4355			0.437				-0.21251		RAV
Os01g04800		-0.66	-0.66					0.4281		0.4355			0.437				-0.21251		RAV
Os01g07120		0.332		0.366	0.424					0.3482				0.32					A4
Os01g10370		-0.29	-0.4		-0.26			-0.308		-0.3607			-0.235	-0.229					B1
Os01g59780		0.403																	AP2
Os02g09650		-0.22			0.462			0.3531		0.6017			0.475	0.502					A3
Os02g10760									-0.517										A4
Os02g13710		0.296											-0.266						B7
Os02g29550								-0.3		-0.2977			-0.319	-0.3					B1
Os02g32140				0.279	0.326							0.191							B4
Os02g40070																	-0.20459		AP2
Os02g43940				-0.3	-0.35			-0.397		-0.7613			-0.292	-0.348					A4
Os02g43970							0.391	-0.228			0.392	0.316					0.24617		A5
Os02g45450		-0.67	-1.09	-0.56	-0.53					0.2067				0.375					A6
Os02g54160		0.273			0.32														A3
Os03g05590		0.27																	A2
Os03g08460													0.37	0.381			-0.24248		B2
Os03g08470		-0.46	-0.65		0.708			0.7164		0.802			0.607	0.53			-0.34041		B3
Os03g08490								0.3357											B2
Os03g12950																	0.20037		AP2
Os03g22170		-0.26						0.3014									-0.47336		B1
Os03g64260		-0.39	-0.47	0.317	0.417					0.3785									B3
Os04g18650		0.27																	B4

Continue

Os04g32620	0.493	0.543	0.662	0.922		0.3174		0.6025							B1
Os04g34970	0.617		1.004	0.966		0.3204									A4
Os04g36640	-0.29	-0.4		-0.26		-0.308		-0.3607			-0.235	-0.229			A6
Os04g46240							-0.2285		-0.184						B5
Os04g46250							-0.2285		-0.184						A4
Os04g46400			-0.3	-0.35		-0.397		-0.7613			-0.292	-0.348			A1
Os04g46410	0.296										-0.266				A4
Os04g46440	0.296										-0.266				A1
Os04g48350	-0.67	-1.09	-0.56	-0.53				0.2067				0.375			B1
Os04g52090												0.344			A5
Os04g57340					-0.2975	0.2943		0.2115	-0.357			0.269			B1
Os05g25260						0.288		0.396			0.351	0.312			A2
Os05g27930						0.3138									A2
Os05g36100	-0.26					0.3014								-0.47336	B3
Os05g37640		-0.29													A2
Os05g41780												0.344			B3
Os05g45954	-0.26	-0.39		-0.4				0.35			0.321	0.424			AP2
Os06g05340	-0.26	-0.39		-0.4				0.35			0.321	0.424			AP2
Os06g07030	-0.3	-0.28	0.661	0.613				0.3607			0.295			0.19421	A3
Os06g09760	0.502		0.789	0.691		0.2839						0.739		-0.25721	A5
Os06g11940	0.502		0.789	0.691		0.2839						0.739		-0.25721	A4
Os06g36000					0.391	-0.228			0.392	0.316				0.24617	A3
Os06g40150			-0.43	-0.44				-0.2735							B2
Os07g03250								-0.5335					0.193		B6
Os07g12510	-0.22			0.462		0.3531		0.6017			0.475	0.502			B3
Os07g22730		-0.24						-0.2424							B3
Os07g22770					0.2285				0.221						B6

Continue

Os08g34360	-0.26	-0.39		-0.4					0.35			0.321	0.424				AP2
Os08g36920	-1.12	-1.26	-0.5	-0.38								-0.486					B7
Os08g41030						0.298				0.348							B4
Os08g44960	0.27																B3
Os08g45110					-0.41										-0.23		A2
Os09g11460							0.7115		0.4765								B2
Os09g13940	0.841					0.307				0.319				0.186			B7
Os09g25600										0.556							AP2
Os09g26420							0.4671	-0.2215									B2
Os09g28440		-0.33							0.2636				0.181				B4
Os09g35010	-1.09	-1.36	-0.67	-0.28		-0.232	0.2406	-0.29				-0.482	0.404			-0.37748	A1
Os09g39810								-0.2285		-0.184							B3
Os10g30840	0.27																B3
Os10g41130	-0.29	-0.4		-0.26			-0.308		-0.3607			-0.235	-0.229				A4
Os10g41330								-0.2285		-0.184							B5
Os11g03540	0.403																AP2
Os11g06770	-0.22			0.462			0.3531		0.6017			0.475	0.502				B4
Os11g13840	-0.29	-0.4		-0.26			-0.308		-0.3607			-0.235	-0.229				A4
Os12g03290	0.403																AP2
Os12g41030													0.344				B7

Footnote: Fold change gene expression data of 70 *OsAP2/EREBP* genes probe on the 22K rice oligoarray under different abiotic stress treatments are listed. Genes upregulated more than 1.5-fold were assigned a log 10–ratio value of > 0.176, downregulated less than 1.5-fold were assigned a log 10–ratio value of < –0.176, and others were assigned blank. For those stress experiments in which analyses were made at one or more time points, genes upregulated at one or more time points were considered to be always upregulated, and those downregulated at one or more time points were considered to be always downregulated by that stress treatment. C = Cold; D = Drought; LDS = Laid-down submergence; SM = submergence; GA = Gibberellic acid; SA = Salt.

Table 10. The 13 genes expressed (upregulated) by at least three abiotic treatments.

Upregulated gene	Cold	Drought	Laid-down Submergence	Submergence	Osmotic	Salt	50µM GA	50µM ABA	Subgroup
Os01g07120	1	1	1	1					**A4**
Os02g09650		1	1	1					**A3**
Os02g43970			1	1			1		A5
Os03g08470		1	1	1					B3
Os04g32620	1	1	1						B1
Os04g34970	1	1	1						**A4**
Os06g07030		1	1	1			1		**A3**
Os06g09760	1	1	1	1					A5
Os06g11940	1	1	1	1					**A4**
Os06g36000			1	1			1		**A3**
Os07g12510		1	1	1					B3
Os09g13940	1		1	1	1				B7
Os11g06770		1	1	1					B4

Genes upregulated more than ≥ 1.5-fold were assigned a value of 1. Number in bold of specific subgroups indicates highest numbers of genes induced by different abiotic stresses.

The *Os01g04750* and *Os01g04800* genes of the RAV subfamily, *Os05g45954* of the AP2 subfamily, *Os05g25260* of the A-2 subgroup, *Os04g34970* of the A-4 subgroup, *Os02g43970* of the A-5 subgroup, *Os04g48350* of the B-1 subgroup, *Os09g11460* of the B-2 subgroup, and *Os09g28440* of the B-4 subgroup were upregulated under different abiotic and biotic stresses. All the genes of subgroup B-5 were not induced under abiotic treatments, while only one gene was upregulated by RSV and RTSV infections but most of the genes were activated by three RDV strains. On the other hand, subgroup A-1 of the DREB subfamily comprises several genes that regulate disease-resistance pathways, as inferred from the increased resistance to pathogens conferred by their overexpression under the control of a constitutive promoter (Shin et al. 2002). All most all the genes of subgroup A-3 of DREB subfamily were upregulated under five different abiotic treatments while several genes of subgroup B-3 of ERF subfamily showed upregulation under four abiotic treatments. Out of the 3 genes, two showed preferential expression under SA treatment, which is assigned to B-6

subgroup of ERF subfamily. On a broad scale, our findings might improve our knowledge of gene responses in research on different biotic and abiotic stresses. Some researchers have reported that overexpression of some transcription factor genes from different families such as NAC (Jeong et al. 2010), DREB (Haake et al. 2002), bZIP (Uno et al. 2000), and zinc finger (Mukhopadhyay et al. 2004) improved tolerance to various abiotic stresses. AP2/EREBP transcription factors have a very wide range of functions in specific plant species. Taken together, these results increase our knowledge of the involvement of OsAP2/EREBP TFs in plant resistance and show that certain members or subgroups of this gene family are involved in resistance to abiotic and biotic stresses.

4. Discussion

4.1. Domain/motif duplication and gene expansion

We have presented a detailed, specific phylogenetic analysis of AP2/EREBP proteins based on their DNA-binding domains. The subgroups obtained by our analysis fit well with known AP2/EREBP function classes. Proteins with similar domains may have the same or similar biological functions (Lin et al. 2007). For example, the different subgroups of ERF or DREB (**Fig. 2**) represent five well-known functions of AP2/EREBP proteins: regulation of seed development, cell division and expansion, organ formation or initiation, response to biotic or abiotic stress, and crosstalk between different signaling pathways. Our goals in this study were to (i) annotate the *OsAP2/EREBP* gene family, (ii) predict domain and motif duplication of subfamilies AP2 and RAV, (iii) provide a new avenue to predict the group-based classification in plants, (iv) determine the expression patterns of duplicated genes, (v) assess the number of genes responding to different stresses (responses to biotic stresses, especially, are a novel contribution), and (vi) select the best candidate genes for further functional analysis. There are some inconsistencies in *OsAP2/EREBP* gene classification between our

results and previous findings. *OsAP2/EREBP* genes play vital roles in various developmental processes, including signaling, stress responses, and plant defenses. In combination, the processes of gene duplication, nucleotide substitution, domain duplication, and intron/exon shuffling can generate a complex set of related genes that may differ substantially in their expression patterns and functions. Gene duplication is one of the major evolutionary mechanisms leading to functional diversification and speciation (Lynch and Conery 2000). Likewise, portions of genes, such as specific exons or those encoding specific domains, may be duplicated within a gene, further complicating the history of gene evolution. Just as we compare gene trees with species trees to co-infer patterns of organismal and genetic evolution, we can compare gene trees to "domain trees" to investigate patterns of domain evolution.

We found 163 *OsAP2/EREBP* genes in the rice genome that could be classified into different subfamilies distributed on all 12 chromosomes and Nakano et al. (2006) have been reported that 147 *AtAP2/EREBP* genes distributed on the *Arabidopsis* chromosomes. Gene duplication is a primary driving force of new gene functions in the evolution of genetic systems and genomes (Moore and Purugganan 2003). Cannon et al. (2004) reported that segmental duplications occur commonly in the slowly evolving *MYB* gene family. Tandem duplications in local genomic clusters with low levels of retention of segmental duplications are common in the large NBS-LRR disease-resistance gene family (Cannon et al. 2004). The present study results revealed more tandemly duplicated genes than duplicated chromosomal segments in the *AP2/EREBP* gene family in rice. Therefore, we consider the number of *OsAP2/EREBP* family genes to have increased rapidly during the course of evolution, and the tandem duplications of chromosomal regions to have played a key role in the expansion of this family. This phenomenon has also been found in the F-box family of rice genes (Jain et al. 2007). The number of segmental duplication genes in *Arabidopsis* was around twice the number in rice, whereas there were about 2.5 times the number of tandem duplication genes

in rice as in *Arabidopsis* (Nakano et al. 2006). Gene duplication of this gene family is thus species-specific.

4.2. Gene function under biotic and abiotic stress conditions

OsAP2/EREBP genes play crucial roles in various developmental processes, including signaling, stress responses, and plant defenses. ERF and DREB proteins constitute subfamilies of the AP2/EREBP TFs that are distinctive to plants, and they play significant roles in plant resistance to biotic and abiotic stresses. In the *AP2/EREBP* gene family, 53 non-redundant genes were upregulated under all tested biotic stresses (RSV, RTSV and RDV); of these 53 genes, the ERF and DREB subfamilies included 46 non-redundant genes. Comparison of symptoms induced by the virus infections (RSV, RTSV and RDV) and changes of gene expression of *AP2/EREBP* genes, it suggested that members of this family are heavily contributed to the defense mechanism of virus infection. Global gene response and defense system were described by RSV infection (Satoh et al. 2010). Significantly, several ERF TFs that confer enhanced disease resistance when overexpressed, such as *ERF1*, *Pti4*, and *AtERF1*, are transcriptionally regulated by pathogens and by the plant hormones ethylene and jasmonic acid (Fujimoto et al. 2000, Gutterson and Reuber 2004). Posttranscriptional regulation of *ERF* genes by phosphorylation may be a significant form of regulation. The *OsEREBP1* gene of rice has been shown to be phosphorylated (Fujimoto et al. 2000) by pathogen-induced mitogen-activated protein kinase. The basic-domain leucine-zipper (bZIP), MYC, MYB, NAC, and WRKY-binding (WRKY) ERF TFs are important families of stress-responsive transcription factors. WRKY proteins, which are unique to plants, contain either one or two WRKY domains and play a key role in regulating pathogen-induced defense responses (Dong et al. 2003). Interestingly, all of the AP2/EREBP sequences that are known to play a role in disease-resistance responses are in one AP2/EREBP subfamily. The AP2/EREBP TF family has undergone extensive expansion through gene

duplication events. Like that within other TF families (e.g., the MYB) family (Dias et al. 2003), the rate of evolution within the *AP2/EREBP* gene family differs significantly between the conserved domains and other regions of the proteins. Evidence from both the MYB family and the basic helix–loop–helix family (Atchley et al. 1994) suggests that this differential sequence conservation reflects differing structural constraints on the functions of the conserved domain and other domains. We found that the expression levels in our results were very similar to the intensities of the microarray data, and the transcription of many genes encoding transcription factors was induced at 12 and 9 DAI under the RSV and RTSV biotic stresses, respectively. Similar expression models have been published for genes encoding proteins containing protein kinase, leucine-rich, NB-ARC, and EF-hand domains, which might function in signal transduction for defense systems (Tameling and Baulcombe 2007, Li et al. 2009). Expression of the genes tested in this study was induced in the middle and late phases of infection, when plants showed obvious symptoms (**Fig. 7**). Some host defense systems have been associated with genes for transcription factors in the WRKY family (*OsWRKY45*, Shimono et al. 2007). We studied large numbers of differentially expressed genes by reverse transcription RT-PCR analysis to determine mRNA expression levels from our 44K-microarray data (Figs. 8, 9). We found that the expression levels in our results were very similar to the intensities of the microarray data, and the transcription of many genes encoding transcription factors was affected at different days of DAI under both biotic stresses. The inactivation of defense systems in rice plants in the early infection stage probably promotes the propagation of RSV and RTSV.

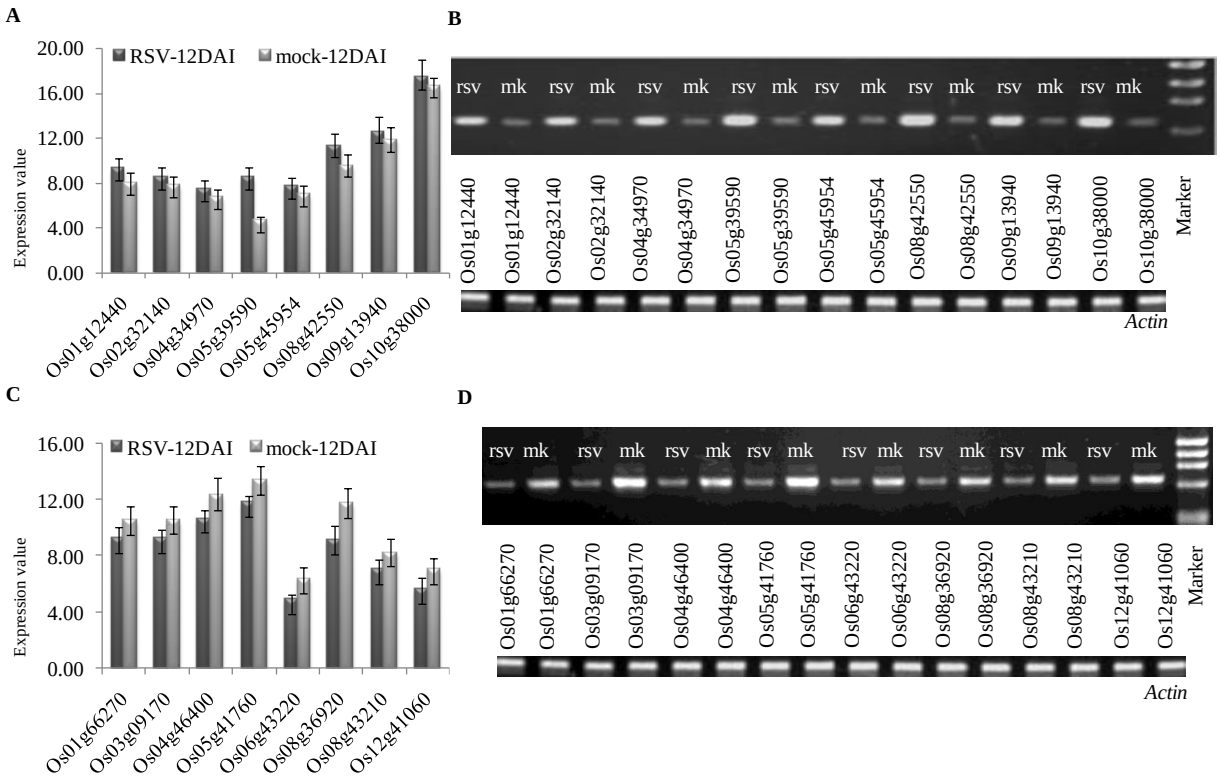

Fig. 8 (A) Histogram of microarray data for eight *OsAP2/EREBP* genes differentially expressed under RSV infection. (B) RT-PCR analysis of eight genes that showed higher expression than the control under RSV infection at 12 DAI. (C) Histogram of microarray data for eight genes downregulated under RSV infection. (D) RT-PCR analysis of eight genes that showed lower expression than the control under RSV infection at 12 DAI. *Actin* gene expression was used as the internal control; mk = mock (control); rsv = RSV- infected plants sampled 12 DAI. The differentially expressed genes are shown along the *X*-axis of the histograms, and the log2 ratios of expression values are shown on the *Y*-axis.

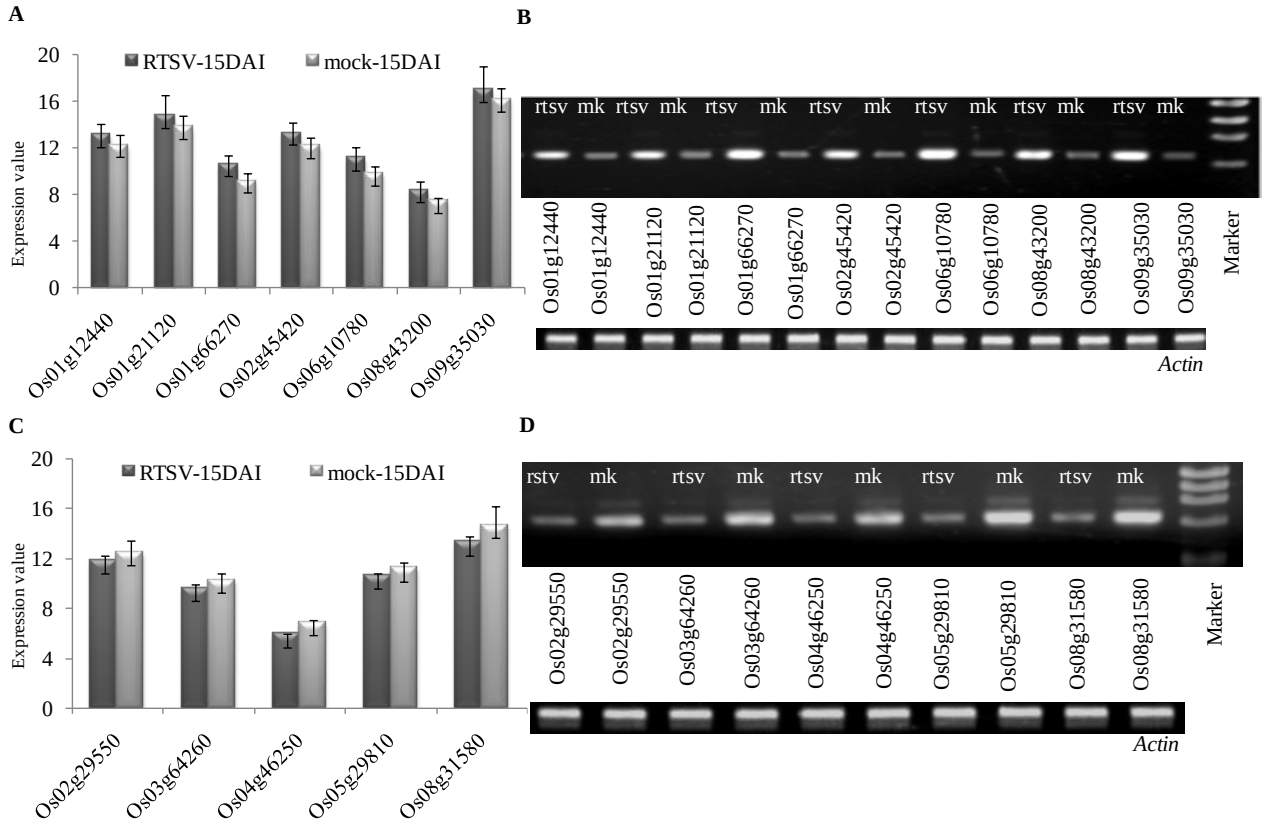

Fig. 9 (A) Histogram of microarray data for seven *OsAP2/EREBP* genes differentially expressed under RTSV infection. (B) RT-PCR analysis of seven genes that showed higher expression than the control under RSV infection at 15 DAI. (C) Histogram of microarray data for five genes downregulated under RTSV infection. (D) RT-PCR analysis of eight genes that showed lower expression than the control under RSV infection at 15 DAI. *Actin* gene expression was used as the internal control; mk = mock (control); rtsv = RTSV- infected plants sampled 15 DAI. The differentially expressed genes are shown along the *X*-axis of the histograms, and the log2 ratios of expression values are shown on the *Y*-axis.

Subfamily DREB has been demonstrated to play a key role in the resistance of plants to abiotic stresses such as cold, high salinity, and drought (Shinozaki et al. 2000). Also some DREBs, such as DBF1, DBF2 (Kizis and Pages 2002), and CBF4 (Haake et al. 2002), are responsive to ABA. DREB/ERF proteins share a conserved 50–60 amino acid domain (the AP2/ERF domain) that binds to two *cis*-elements: the GCC box, found in many pathogen-related gene promoters conferring ethylene responsiveness (Gu et al. 2000), and the C-repeat

CRT/DRE element motif, involved in the expression of cold- and dehydration-responsive genes. *OsDREB1A–1D* genes are induced under different abiotic stresses in rice (Dubouzet et al. 2003). Similarly, in many studies, overexpression of stress-inducible DREB transcription factors activates the expression of many target genes having DRE elements in their promoters, and the resulting transgenic plants show improved stress tolerance (**Table 11**). The present study showed in detail that expression of rice *AP2/EREBP* genes is induced by abiotic as well as biotic stresses. From the various studies summarized in **Table 11**, it is clear that DREB proteins are important transcription factors regulating abiotic stress-related genes and that they play a critical role in imparting stress endurance to plants. To obtain an overview of the expression pattern changes in the rice *AP2/EREBP* gene family under different stress conditions, we examined responses in 10-day-old seedlings and 30-day-old calli to cold, drought, laid-down submergence, submergence, osmotic, salt, and hormone stresses. The expression patterns of the *OsAP2/EREBP* genes may provide clues to determine the function of each gene under both biotic and abiotic conditions. A total of 70 genes were differentially expressed under at least one of these abiotic stress conditions, among which 52 were upregulated by at least one time point under the one or more of the treatments and some subgroups (i.e. A-1, A-3 and B-5) were discriminatory induced by abiotic or biotic stress. For confirmation, we carried out an RT-PCR analysis to determine mRNA expression levels; all genes had strikingly higher or lower expression profiles in seedlings than in controls at one or more time points (**Fig. 9A–D**). Similarly, MeV cluster analysis based on log 10–ratio values showed that *OsAP2/EREBP* genes had very diverse expression patterns (**Fig. 10, Table 9**). In order to gather more details on the expression profiles under different stresses, we included three biotic and eight abiotic stresses not considered before.

Table 11. Stress response of transgenic plants overexpressing DREBs and ERFs.

Gene	Transgenic plants	Performance of transgenic plants	Reference
AtDREB2A	*Arabidopsis*	Dehydration tolerance	Sakuma et al. 2006
BNCBF5, BNCBF17	Canola	Freezing tolerance	Savitch et al. 2005
AtDREB1A	Tobacco	Freezing and dehydration tolerance	Kasuga et al. 2004
OsDREB1A	*Arabidopsis*	Freezing, dehydration and salt tolerance	Dubouzet et al. 2003
AtCBF4	*Arabidopsis*	Freezing and dehydration tolerance	Haake et al. 2002
AtCBF1	Potato	Freezing tolerance	Pino et al. 2008
Tsil1	Tobacco	Biotic, osmotic stress tolerance	Park et al. 2001
AtCBF3	*Arabidopsis*	Freezing tolerance	Gilmour et al. 2000
AtCBF1	*Arabidopsis*	Freezing tolerance	Jaglo-Ottosen et al. 1998
AtDREB1A	*Arabidopsis*	Freezing and dehydration tolerance	Liu et al. 1998, Oh et al. 2005
TERF1	Rice	Drought and high-salinity tolerance	Gao et al. 2008
TSRF1	Rice	drought tolerance	Quan et al. 2010
PgDREB2A	Tobacco	Salinity and drought tolerance	Agarwal et al. 2010
RAP2.2	*Arabidopsis*	Low oxygen response	Hinz et al. 2010
RAP2.6	*Arabidopsis*	ABA, salt and osmotic tolerant	Zhu et al. 2010

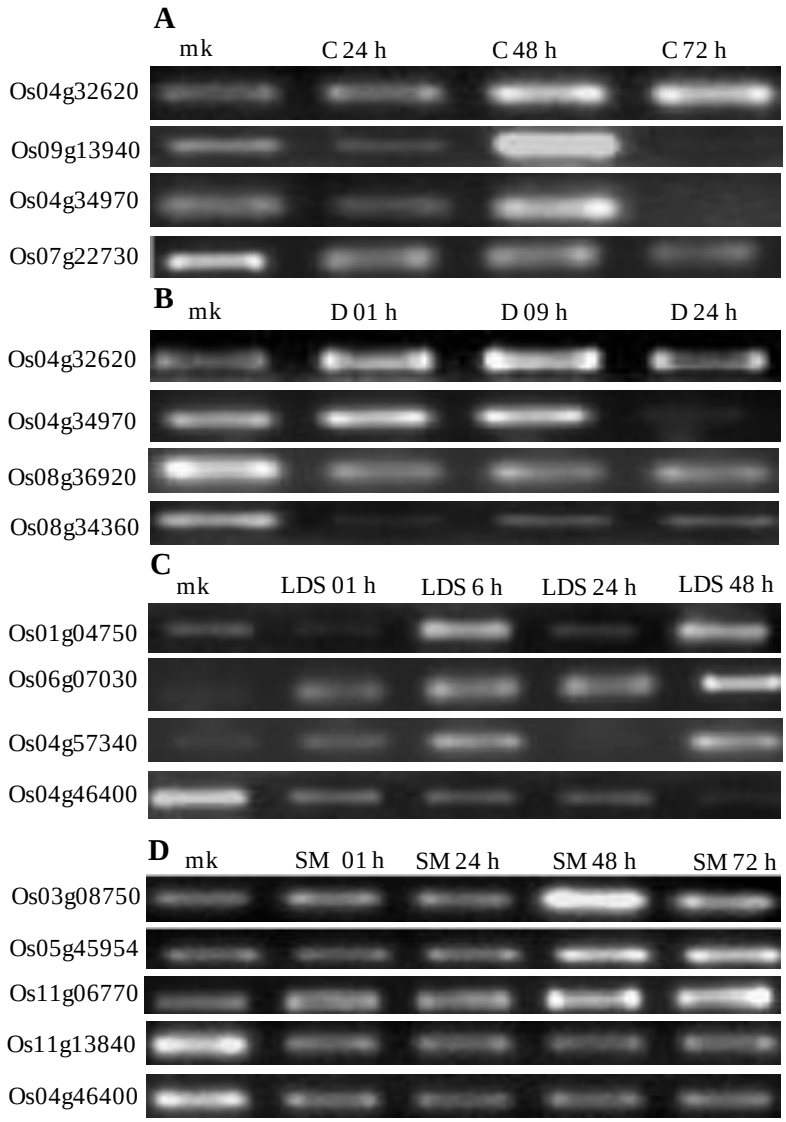

Fig. 10 RT-PCR analysis of differential gene expression under various abiotic treatments. (A) Three genes (*Os04g32620*, *Os09g13940*, and *Os04g34970*) were upregulated and one (*Os07g22730*) was downregulated under cold stress (mk = mock/control; C 24 h, C 48 h, and C 72 h = cold for 24, 48, and 72 h, respectively). (B) Of four genes, three (*Os04g32620*, *Os04g34970*, and *Os08g36920*) were upregulated to high levels and one was downregulated (*Os08g34360*) to low levels under drought stress (mk = mock/control; D 01 h, D 09 h, and D 24 h = drought for 1, 9, and 24 h, respectively). (C) Three genes showed higher expression (*Os01g04750*, *Os06g07030*, and *Os04g57340*) and one gene showed lower expression (*Os04g46400*) than the control under laid-down submergence stress (mk = mock/control; LDS 01 h, LDS 06 h, LDS 24 h, and LDS 48 h = laid-down submergence for 1, 6, 24, and 48 h, respectively). (D) Among five genes, three genes were upregulated (*Os03g08470*, *Os05g45954*, and *Os11g06770*), and two genes were downregulated (*Os11g13840* and *Os04g46400*) under submergence stress (mk = mock/control; SM 24 h, SM 48 h, and SM 72 h = submergence for 24, 48, and 72 h, respectively).

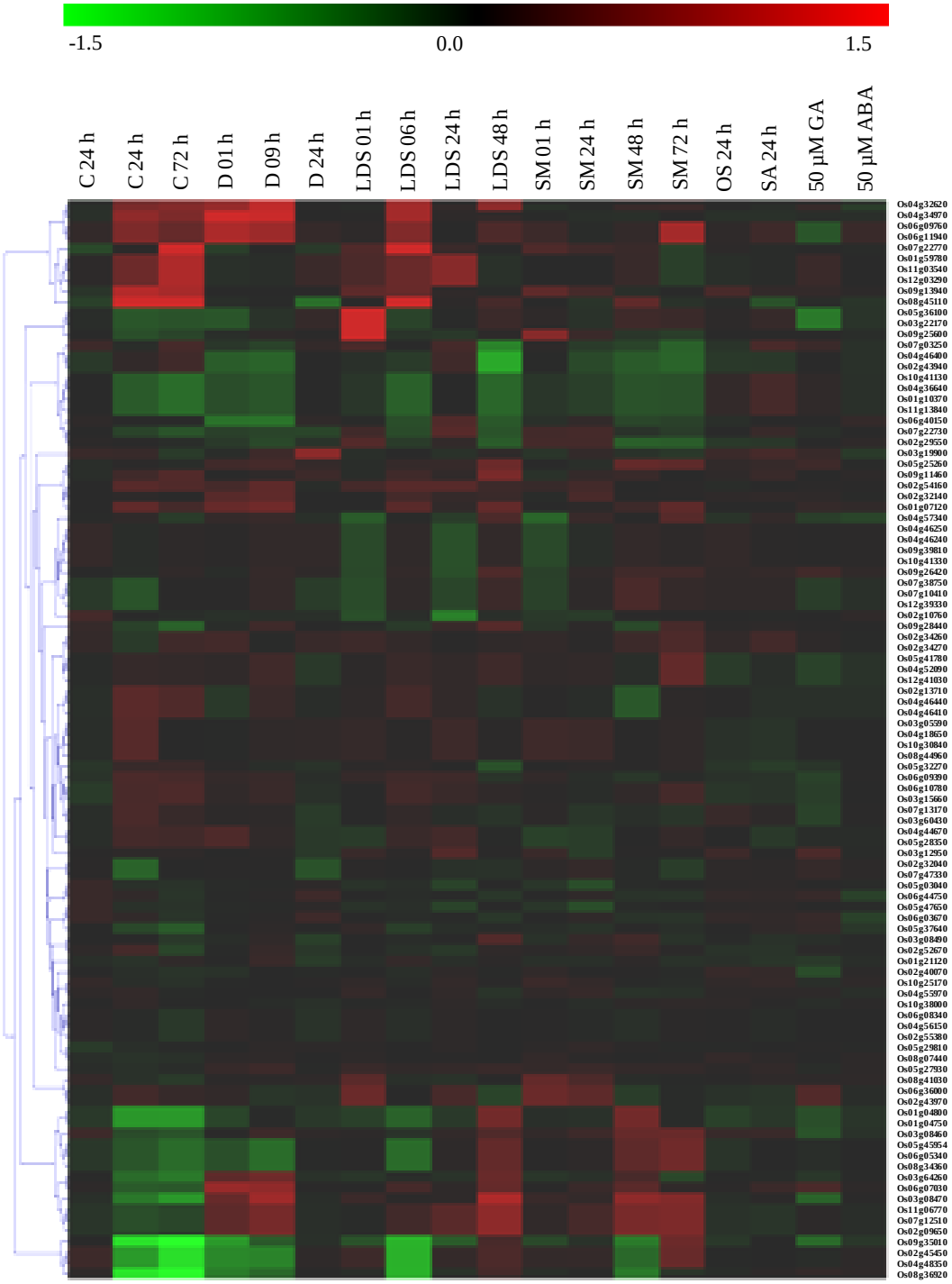

Fig. 11 Differential expression of *OsAP2/EREBP* genes under eight types of abiotic stress (log 10–ratio). The color bar at top shows levels of expression: red indicates upregulated genes and green indicates downregulated ones. Stresses: C 24 h, C 48 h, and C 72 h = cold for 24, 48, and 72 h, respectively; D 01 h, D 09 h, and D 24 h = drought for 1, 9, and 24 h, respectively; LDS 01 h, LDS 06 h LDS 24 h, and LDS 48 h = laid-down submergence for 1, 6, 24, and 48 h, respectively; SM 24 h, SM 48 h, SM 72 h = submergence for 24, 48, and 72 h, respectively; OS = osmotic 24 h; SA = salt 24 h; 50µM GA = 50 µM gibberellic acid; 50µM ABA = 50 µM abscisic acid.

Functional and regulatory proteins are involved in gene expression in response to stress conditions. Functional proteins include membrane proteins that control water movement through membranes, proteins (such as LEA proteins, osmotin, and mRNA-binding proteins). LEA proteins, for example, improve the drought or high-salinity tolerance of plants. Regulatory proteins (e.g., bZIP, MYC, MYB, and DREB), protein kinases (e.g., MAP kinase, CDP kinase, and receptor protein kinase), and proteinases are involved in the regulation of signal transduction and gene expression. Transcription factors, together with *cis*-elements, function in the promoter region of different stress-related genes and overexpression of these genes may improve tolerance to abiotic or biotic stress. A microarray analysis in *Arabidopsis* has shown that there are several pathways that independently respond to abiotic stress, and one such important pathway involves the DREB regulon (Fowler and Thomashow 2002). By a transcriptome analysis, genes induced by different stresses can be identified from the functions of their products.

In conclusion, taken together, our data showed gene and domain/motif duplication in subfamilies AP2 and RAV. Segmental and tandem duplications might have been the main contributors to the expansion of this family in rice. Some subgroups showed a high level of expression profile in abiotic stress and biotic stress, suggesting that they might have in functional divergence. Interestingly, two genes were common and upregulated under two biotic stresses (RSV and RTSV), whereas three genes were induced by RDV infection (all strains). Thirteen genes were activated by at least three abiotic stress conditions. *OsAP2/EREBP* genes showed temporal and spatial patterns of expression under different kinds of stress conditions. Specific subgroup of this gene family provides a new avenue for determining the best candidate genes for functional analysis. Overexpression, knockdown or mutagenesis, and promoter analyses of selected members of this gene family are underway in our laboratory so that we can accurately determine molecular pathways in the *OsAP2/EREBP* gene family.

Chapter 2

Comparative Transcriptome Analysis of *AP2/EREBP* Gene Family under Normal and Hormone Treatments, and under Two Drought Stresses in NILs Setup by Aday Selection and IR64

5. Introduction

Under different adverse environmental conditions, genes show specific expression patterns in accordance with their biological and physiological functions. Hence, an important goal in developmental biology is to detect the expression of unique genes or groups of genes and to determine their expression patterns to identify the exact functional network of that gene(s) and whether it is linked to specific or multiple tissues. Transcription factors (TFs) are important for maintaining the expression of genes that encode functional proteins in the genome. In plants, the *AP2/EREBP* gene family encodes a large number of TF genes. TFs that are encoded by *AP2/EREBP* genes contain the highly conserved AP2/ERF DNA-binding domain (Riechmann et al. 1998). Jofuku et al. (1994) first described this conserved DNA-binding domain in the homeotic gene APETALA2 (AP2) and in ethylene-responsive element binding proteins (EREBPs) in tobacco (Ohme-Takagi and Shinshi, 1995). Based on the number of AP2/ERF domains and the gene structure, the *AP2/EREBP* gene family has been divided into four subfamilies, AP2, RAV, dehydration-responsive element binding protein (DREB), and ERF (ethylene-responsive element binding factor) (Sakuma et al. 2002; Sharoni et al. 2011). The RAV, DREB, and ERF subfamilies are of particular interest due to their involvement in plant responses to stress. The C-terminal domain of RAV subfamily members is similar to that of the ABI3 and VP1 transcription factors, which have two core motifs

CAACA and CACCTG (Kagaya et al. 1999). TFs encoded by genes in the DREB subfamily bind to both GCC and the DRE (dehydration responsive element) *cis*-elements (Park et al. 2001; Sakuma et al. 2002). The ERF subfamily of genes encode a large number of ERFs (Fujimoto et al. 2000) that participate in plant responses to biotic stresses, such as pathogens, by recognizing the *cis*-acting element AGCCGCC, known as the GCC box (Hao et al. 1998). The ERF- and DREB-subfamily transcription factors have been discovered in several plant species including rice (Quan et al. 2010), *Arabidopsis* (Sakuma et al. 2006) and tobacco (Agarwal et al. 2010). Furthermore, the functions of the ERF and DREB transcription factors in the plant response to biotic and abiotic stress have been extensively documented (Agarwal et al. 2006, 2010). A combination of genetic engineering and molecular approaches have been used to characterize a series of regulatory genes of the *AP2/EREBP* gene family that are involved in various pathways, including genes related to drought and high salinity (Dubouzet et al. 2003), diseases (Gutterson and Reuber, 2004; Agarwal et al. 2006), cold (Qin et al. 2007a), and the control of flowering (Elliott et al. 1996). The overexpression of DREB1A (CBF3) or DREB1B (CBF1) in transgenic *Arabidopsis* induces the strong expression of target stress-inducible genes and results in increased tolerance to drought and high salt (Quan et al. 2010; Zhu et al. 2010). Similarly, the overexpression of a number of ERF genes enhances the resistance to biotic and abiotic stresses in tobacco (Park et al. 2001). In addition, TERF1 and TSRF1 exhibit drought and salinity tolerance in rice (Gao et al. 2008; Quan et al. 2010). Members of the RAV subfamily are involved in the ethylene response (Alonso et al. 2003), the brassinosteroid response (Hu et al. 2004), and biotic and abiotic stress responses in pepper (Sohn et al. 2006). A microarray analysis in *Arabidopsis* has shown that there are different pathways that independently respond to abiotic stress, and one such important pathway involves the DREB regulon (Fowler and Thomashow, 2002).

Drought is a serious abiotic stress that imposes a constraint on rice production and severely reduces the grain yield potential of rice (Serraj et al. 2009). Among cereal crops, rice

is very sensitive to soil water deficits and evaporative demands, and the greatest sensitivity is found in the lowland-adapted genotypes (Parent et al. 2010). Different mechanisms of the drought response can be measured at many different levels, from the whole plant down to the molecular level. In plants, knowledge of the molecular aspects of drought control or drought tolerance is limited. Therefore, a promising approach is to examine near-isogenic lines (NILs) that have a common genetic background but contrasting levels of tolerance to water deficits in a long-term drought stress situation that is similar to field conditions, using a dry down method. Isogenic lines and NILs are invaluable tools for testing hypotheses in physiological and genetic studies because there is no interference from variations in other traits (Lafitte et al. 2006). Therefore, the use of NILs reduces the noise of gene expression data by comparing it with the IR64 recurrent parent. Through selection in the drought-breeding program of IRRI (International Rice Research Institute), a series of advanced backcrossed rice plant lines was developed by backcrossing Aday Selection (Aday Sel), a drought-tolerant traditional variety, to IR64 (Khush et al. 2004). IR64 is resistant to blast, bacterial leaf blight, and brown planthopper, but it is susceptible to tungro (Khush et al. 2004) and drought stress (Guan et al. 2011). IR64 is the most widely grown high-yielding rice variety in the tropics and has important agronomic traits. Using the IR64 as a recurrent parent, the following pair of NILs with contrasting drought tolerances (severe and mild) was selected (Venuprasad et al. 2007) from the IR77298-14-1-2-B family: IR77298-14-1-2-B-10 (highly drought-tolerant) and IR77298-14-1-2-B-13 (susceptible to drought). These advanced backcrossed lines are considered pre-NILs because they are sister lines that were derived from a single family that was segregated for drought-tolerance phenotypes. Venuprasad et al. (2011) reported that the two NILs of the IR77298-14-1-2-B family are 96.5% genetically similar using 491 SSR markers. The genome-wide identification of genes that are regulated by drought treatments has manifold significance (Degenkolbe et al. 2009). The identification of these genes enables a more detailed understanding of the transcriptional response to stress and provides a clue for

further elucidating the role of single genes in the stress response, which will have a significant impact on crop engineering. The rice genome is thought to contain 163 *OsAP2/EREBP* genes (Sharoni et al. 2011); however, only a few of these genes have been characterized in this species. The characterization of genes from the *OsAP2/EREBP* family in rice facilitate the understanding of the molecular mechanisms of resistance to stress and thus, will aid in the development of drought-resistant rice varieties using transgenic technology. In this study, we used Affymetrix microarray studies to systematically characterize the expression of the entire *OsAP2/EREBP* gene family in the rice Minghui 63 genotype to gain a better understanding of the role each family/subfamily member or subgroup plays in several developmental stages under normal conditions and following phytohormone treatments. Furthermore, we used the Agilent 44K oligoarray system to profile the transcriptomes of root, leaf, and panicle tissues from three rice genotypes (IR77298-14-1-2-B-10, IR77298-14-1-2-B-13, and IR64) grown under different drought stress conditions to identify the putative drought responsive genes in tolerant NIL by examining their different gene expression patterns and comparing them with the control. Initially, global gene expression analyses in root tissue from these NILs grown under drought stress conditions were performed, and the gene expression data were analyzed using gene ontology profiling (Moumeni et al. 2011, personal communication). Although there were many more genes in this array, this study focuses only on *OsAP2/EREBP* gene family members to select the best candidate genes for further functional analysis. A number of specific subfamily genes or subgroup family genes were differentially expressed, and the data revealed novel information about their roles in the plant response to different stress conditions. The conserved *cis*-elements in the 2-kb upstream sequence of the promoter region of differentially expressed genes (DEGs) under different stress conditions were revealed. Introgressed regions were examined and Gene Ontology (GO) enrichment tests were performed. We used RT-PCR to confirm the expression of selected *OsAP2/EREBP* genes under stressed and non-stressed conditions. To our knowledge,

this is the first report that focuses on *OsAP2/EREBP* genes to identify family-level expression patterns, and these results provide a solid basis for future functional genomic research of the *OsAP2/EREBP* gene family.

6. Materials and methods

6.1. Expression profile analysis (Minghui 63)

The expression profile data for *OsAP2/EREBP* genes in Minghui 63 and in different hormone-treated seedlings were extracted from the NCBI-GEO database (GSE 19024, GSE7567, GSE32634, and GSM26043; http://www.ncbi.nlm.nih.gov/). This database contains Affymetrix rice microarray data from 26 tissues that cover the entire life cycle of rice. As shown in **Table 12**, vegetative and reproductive tissues at different developmental stages covering the whole rice plant body were used for the *OsAP2/EREBP* gene expression profile analyses used in this study. After normalization, the average signal value of two biological replicates of each sample was used for analysis. To identify preferentially expressed genes, Student's *t*-test was performed. A gene in a given tissue was defined as being differentially expressed only if the expression value of the gene in this tissue was more than 2-fold ($P \leq 0.05$) greater that in the control (CK). In the phytohormone-treated samples, genes that were up- or down-regulated more than 1.5-fold ($P \leq 0.05$) compared with the control or an untreated sample were considered differentially expressed. Trefoil-stage seedlings were exposed to 100 mM concentrations of gibberellic acid (GA3), kinetin (KT), and naphthalene acetic acid (NAA) for 15, 30, and 60 min, and the samples were then mixed (Nuruzzaman et al. 2008). The different hormone treatments were performed in seedlings growing under normal agricultural conditions of temperature, light, and humidity. Benzothiadiazole (BTH) is a functional analog of salicylic acid (SA) and protects plants from disease by activating the SA signaling pathway. The single-color method was used to

compare 4 biological replicates of BTH-treated leaves with mock-treated leaves. Leaf disks (6 mm diameter) from seedlings were floated on jasmonic acid (JA, 500μM) and incubated under light at 25 ºC. The samples were harvested at 6, 12, 24, and 48 h after the treatment. The *japonica* rice variety Nipponbare were grown for 3 weeks at 28 ºC in the dark and were treated with abscisic acid (ABA, 100 μM) for 5 h. Plants treated with H_2O for 5 h were used as controls. Whole plants were used for RNA extractions (Yazaki et al. 2003). The $\log_2$ ratio values of the means were normalized, and up- or down-regulated genes with values > 0.585 or < – 0.585 were selected for the JA and ABA treatments.

Table 12. Sample vegetative and reproductive developmental stage analyses in Minghui 63.

Sample	Developmental Tissues
CK	Seed: germination (72 h after imbibition)
1	Calli 1: 15 days after subculture
2	Calli 2: screening stage
3	Calli 3: 5 days after regeneration
4	Seedling 1: 3 days after sowing
5	Seedling 2: Root and leaf at the three leaf stage
6	Shoot: Seedling with 2 tillers
7	Root : Seedling with 2 tillers
8	Stem 1: 5 days before heading
9	Stem 2: heading stage
10	Flag leaf 1: 5 days before heading
11	Flag leaf 2: 14 days after heading
12	Leaf 1: young panicle at stage 3
13	Leaf 2: 4-5 cm young panicle
14	Sheath 1: young panicle at stage 3
15	Sheath 2: 4-5 cm young panicle
16	Panicle 1: young panicle at stage 3
17	Panicle 2: young panicle at stage 4
18	Panicle 3: young panicle at stage 5
19	Panicle 4: 4-5 cm young panicle
20	Panicle 5: heading stage
21	Stamen: just before heading
22	Spikelet: 3 days after pollination
23	Endosperm 1: 7 days after pollination
24	Endosperm 2: 14 days after pollination
25	Endosperm 3: 21 days after pollination

6.2. Plant growth and drought-treatment conditions for rice

The two NILs and the IR64 plants used for this study were chosen because of their different rice yields under drought stress conditions (DSCs). The two NILs plants were derived from the IR77298-14-1-2 family and developed at IRRI. Under stress, IR77298-14-1-2-B-10 is a high-yielding (highly drought-tolerant) plant, whereas IR77298-14-1-2-B-13 is a low-yielding (drought-susceptible) plant. Both IR77298-14-1-2-B-10 and IR77298-14-1-2-B-13 NILs plants exhibit similar yield potentials under normal growth conditions (Venuprasad et al. 2011). Plants were grown in a PVC pipe filled with 10 kg of a soil and sand mixture (2:1) in which all the soil nutrients were controlled. The test was initially conducted in a green house, and the plants were moved outside to natural conditions before imposing drought stress. Before planting, the saturated soil in the pots was covered with white or transparent plastic covers with an opening in the center to facilitate planting. Feeder pipes were fixed to the pots to facilitate watering. In one pot, five germinated seeds were transplanted, and the plants were subsequently separated to one plant per pot when they reached the three-leaf stage. This test was carried out on eight replicate samples using a randomized complete block design. All of the pots were watered twice daily to keep the soil saturated. The day before beginning the progressive soil drying process, the soil in each pot was saturated with water. Stress was imposed by initiating a soil dry-down protocol starting 35 days after sowing. The dry-down process continued until the pot reached the target fraction of transpirable soil water (FTSW) (Sinclair and Ludlow 1986). The weight of each pot was determined every day during the dry-down process to estimate the transpiration of soil water. The DSCs were as follows: a control (1.0 FTSW) consisting of well-watered plants with soil that was kept saturated throughout the experiment, and two drought stresses, one severe (0.2 FTSW) and one mild (0.5 FTSW). No water was applied to the soil during the dry-down period. The target FTSW was maintained in all of the pots until the plants were harvested. The water-use efficiency in the different rice genotypes was calculated using the lysimeter system.

6.3. Rice oligo-microarray (44K) and data analysis

Cyanine-3 (Cy3) and cyanine-5 (Cy5)-labeled target complementary RNA (cRNA) samples were prepared from 850 ng total mRNA using a fluorescent linear amplification kit (Agilent Technologies) in accordance with the manufacturer's instructions. Transcriptome profiles specific to stressed plants were examined by the direct comparison of transcription activities between stressed and non-stressed plants on the same oligoarray. We hybridized 1 µg of fluorescent linear-amplified, Cy3- and Cy5-labeled cRNA (825 ng of each cRNA) to a customized rice oligo microarray. The fragmented cRNAs were added to the hybridization buffer, applied to the microarray, and hybridized for 17 h at 60 °C. The slides were washed with SSC (10 min in 6× SSC and 0.005% Triton X-102 at room temperature, followed by 5 min in 0.1× SSC and 0.005% Triton X-102 at 4 °C). The slides were dried and scanned using an Agilent Technologies G2565BA microarray scanner system. The scanned microarray images were analyzed using Feature Extraction 6.1.1 software (Agilent Technologies), and the dye-normalized, background-subtracted intensity and ratio data were exported to a text file. This software flags corrupted spots and detects a lack of differences between sample spots and the background. The slide images were scanned using a DNA microarray scanner (G2505B, Agilent Technologies).

The image files of the slides were processed using Feature Extraction version 9.5 (Agilent Technologies). The Cy3 and Cy5 signal intensities were normalized using rank-consistency filtering and the LOWESS method. The signal intensities of all the samples were transformed into $\log_2$-based numbers and normalized according to the quantile method for standardization among the array data using Expander version 5.0 (Shamir et al. 2005). A significantly DEG was defined as an expressed gene with a $\log_2$-based ratio (stressed sample and control sample) > 1 or < –1, and significance in changes in gene expression between two plants was defined as $P \leq 0.05$ using a paired *t*-test (permutations, all possible combinations, FDR correction, adjusted Bonferroni method). Data processing was performed using MeV

version 4.5 (Saeed et al. 2003). The Michigan State University (Osa1, Ouyang et al. 2007) database contained at least 163 *AP2/EREBP* genes in the rice genome and contained corresponding locus IDs. We identified 140 *OsAP2/EREBP* genes from our 44K microarray data collected under DSCs. The probe arrangement of the array data (platform number GPL7252) is available at NCBI-GEO (series number GSE30464, Barrett et al. 2007).

6.4. Transcript-level analysis

Total RNA was extracted from the root, leaf, and panicle of all of the rice genotypes using an RNeasy Maxi Kit (Qiagen) in accordance with the manufacturer's instructions. The concentration and quality of the RNA samples were determined using a spectrophotometer (Nanodrop ND-1000, Nanodrop Technologies) and a model 2100 bioanalyzer (Agilent Technologies). The RNA samples were pretreated with RNase-free DNase I (Invitrogen) to eliminate any contaminating genomic DNA. The first-strand cDNA was synthesized from 1 µg of total RNA in a 20-µl reaction volume using SuperScript II Reverse Transcriptase (Invitrogen), and 2 µl of the reaction mixture was subsequently used for RT-PCR in a 50-µl reaction volume. The RT-PCR was performed using SuperScriptII Reverse Transcriptase in accordance with the manufacturer's instructions to synthesize first-strand cDNA from the DNase I–treated total RNA. The same RNA samples were used for hybridization and RT-PCR experiments. Approximately 1/20 of the first-strand cDNA that was generated from 1 µg total RNA was used as a template for PCR (reaction volume 50 µl) using rTaq DNA polymerase (Takara). The RT-PCR procedures consisted of 25–35 cycles, depending on the linear range of the PCR amplification for each gene. Each PCR was performed (in triplicate) in an ABI 9700 thermocycler (Applied Biosystems). The program entailed incubation at 94 °C for 45 s, at 55 °C for 50 s, and at 72 °C for 1 min. The rice *actin* gene (LOC_Os05g36290) was used as an internal control (Nuruzzaman et al. 2010; Sharoni et al.

2011), and the primers are listed in **Table 13**. For convenience, all the "LOC_" prefixes in the Osa1 (Ouyang et al. 2007) locus ID are omitted in the remainder of the manuscript.

Table 13. Gene primers used for RT-PCR analysis.

Gene name	Forward primer	Reverse primer
Os01g66270	ACACGGCCGTCTACTTCCT	CAGGATCTCCATGTCATCCA
Os03g12950	CACCACCAGCTAGTCCCCTA	ACTTGCCTTCCCTTCCTTGT
Os03g64260	ACCACCAACAGCTCATCCTC	AGGTGATATCGATGGGCTGA
Os04g46400	GCATCTGGCTGGGTACCT	TACTCGGCCCACAAGAGTG
Os05g49700	CGTCTACTACCGCCAGAGC	TGGCTCTTCTTGCTCTCCT
Os07g22730	AGCCGTCTGGCTACGACTAC	GCGTGGAGTCCCTGATCTC
Os10g22600	AGATCCTCCAAGCCACCAC	GAAGACCCTGTGGACGAGAC
Actin	CAATGAGCTTCGTGTTGCAC	GGCACCTGAACCTTTCTGAC

7. Results

7.1. Expression patterns of *OsAP2/EREBP* genes under normal growth conditions using Affymetrix arrays

The rice tissues from the vegetative and reproductive stages that were selected for microarray analysis cover the complete life cycle of the rice plant. Detailed information on the selected tissues is presented in **Table 12**. The expression pattern of the microarray data was confirmed to reveal the expression profiles of the various genes (Nuruzzaman et al. 2008; Ye et al. 2009). Probes for 116 of the 163 *OsAP2/EREBP* genes that were identified in the microarray and demonstrated differential expression during various stages of development when compared with their expression in the seed are summarized in **Table 14**, and the *P* values and the fold change values are included in this table. The *AP2/EREBP* genes were classified into 4 subfamilies and two subfamilies of those (ERF and DREB), and these were further divided into different subgroups, e.g., DREB-A1 to subgroup DREB-A6 and ERF-B1 to subgroup ERF-B7 (Sharoni et al. 2011). A number of up-regulated genes were found in the calli, shoot, leaf-2, and spikelet stages. In contrast, a higher number of down-regulated genes were observed in Minghui 63 in most of the stages of development (**Fig. 12A**). During the vegetative stages, 26 *OsAP2/EREBP* genes were up-regulated and 25 genes were down-regulated by two-fold or more in Minghui 63. These data show that the differentially regulated *OsAP2/EREBP* genes did not exhibit high variations, implying that their expression may be somewhat conservative during vegetative development. In contrast, of the 52 genes those were differentially regulated in reproductive development, 22 *OsAP2/EREBP* genes were up-regulated more than two-fold and 30 were down-regulated. This observation suggests that the expression pattern of *OsAP2/EREBP* genes in reproductive development is complex and implies the activation of different genes at different developmental stages. A biphasic expression pattern was also observed for *OsAP2/EREBP* genes during reproductive

development, 9 genes (e.g., *Os06g11860*, *Os09g11480*, and *Os06g07030*) were up-regulated during several stages but were down-regulated during the other stages. Specific genes were up-regulated or down-regulated during vegetative and reproductive development, probably because of the prevalence of similar processes in these stages. *OsAP2/EREBP* genes, 9 in total (e.g., *Os02g43940*, *Os03g09170*, and *Os08g31580*, subfamily DREB), exhibited up-regulated expression during both vegetative and reproductive development, whereas five genes were down-regulated (e.g., *Os02g34260*, *Os01g12440*, and *Os04g48350*, subfamily ERF). Overall, the DREB subfamily exhibited the highest number of up-regulated genes, followed by ERF, AP2, and RAV, whereas the AP2 subfamily demonstrated a higher number of down-regulated genes, followed by RAV, ERF, and DREB, respectively (**Table 14**). Moreover, we found 15 up-regulated genes in specific tissues of plants grown under normal growth conditions (**Fig. 12B, Table 14**). For example, three genes (*Os03g12950*, *Os05g25260*, and *Os08g36920*) were specifically expressed in the panicle-2 stage under non-stressed conditions (**Fig. 12B**). The number of differentially expressed genes under normal conditions was compared with the number of differentially expressed genes under different drought stresses, as described later. Taken together, these results provide a global overview of the *OsAP2/EREBP* transcriptome in relation to rice tissues and the developmental stages of the plant life cycle.

Table 14. Result of differential expression analysis used seed as reference. Differential expression genes have been taken with P value less than 0.05 and fold change >1 or <-1, which are described by "U" or "D". When fold change >1, regulation is up and when fold change <-1, regulation is down.

Gene name	Phg	Probe Set ID Os.	Calli 1 Vs S			Calli 2 Vs S			Calli 3 Vs S			Sling 1 Vs S			Sling 2 Vs S			Shoot Vs S			Root Vs S			Stem 1 Vs S			Stem 2 Vs S		
			PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R
Os01g04020	A2	4663.1.S2_at	0.05	-1.62		0.05	-1.40		0.10	-1.56		0.01	-1.75	D	0.05	-1.97		0.09	-1.40		0.19	-1.34		0.33	-1.11		0.08	-1.72	
Os01g04750	RAV	27299.1.A1_at	0.10	-0.87		0.23	-0.81		0.07	-0.79		0.46	-1.65		0.00	-2.13	D	0.28	-0.31		0.46	0.10		0.02	-2.93	D	0.06	-4.81	
Os01g04800	RAV	36672.1.S1_at	0.88	0.11		0.96	-0.03		0.64	-0.33		0.96	0.02		0.72	-0.07		0.96	0.03		0.05	4.81		0.68	0.04		0.83	-0.13	
Os01g07120	A4	21563.1.S2_at	0.09	-0.56		0.04	-0.78		0.13	-1.45		0.14	-1.43		0.07	-2.00		0.04	-0.74		0.22	0.41		0.11	-1.08		0.16	-0.30	
Os01g10370	B1	55837.1.S1_at	0.14	-3.07		0.15	-3.24		0.12	-3.04		0.10	-3.48		0.09	-3.40		0.10	-2.57		0.15	-1.14		0.25	-2.07		0.23	-2.46	
Os01g12440	B2	31908.1.S1_at	0.22	0.62		0.86	-0.04		0.20	-0.60		0.02	-2.38	D	0.01	-2.78	D	0.12	-2.37		0.18	-2.00		0.02	-1.09	D	0.01	-2.14	D
Os01g21120	B1	8031.1.S1_at	0.04	-0.94		0.66	-0.06		0.40	-0.44		0.19	-3.98		0.01	-7.78	D	0.10	-3.63		0.24	-2.74		0.01	-3.19	D	0.07	-3.09	
Os01g46870	B3	51970.1.S1_at	0.17	3.21		0.19	2.83		0.08	2.40		0.79	-0.26		0.65	-0.60		0.87	0.17		0.71	0.34		0.73	0.47		0.99	-0.02	
Os01g49830	RAV	56351.1.S1_at	0.04	-1.59	D	0.58	-0.07		0.03	-0.32		0.06	-1.11		0.10	-0.96		0.05	-0.78		0.20	-0.86		0.04	-0.70		0.00	-0.82	
Os01g54890	B1	53660.1.S1_at	0.05	3.20	U	0.22	2.26		0.22	1.67		0.04	2.54	U	0.46	0.78		0.45	2.14		0.08	4.40		0.08	2.72		0.71	0.80	
Os01g58420	B4	3766.1.S1_at	0.39	1.36		0.26	1.47		0.15	1.38		0.31	1.68		0.60	0.31		0.48	0.64		0.20	1.78		0.10	1.63		0.59	0.71	
Os01g59780	AP2	16683.1.S1_at	0.06	-1.67		0.06	-1.26		0.11	-1.04		0.04	-1.11	D	0.01	-1.70	D	0.20	-1.34		0.05	-0.31		0.19	-1.61		0.10	-0.92	
Os01g67410	AP2	14775.1.S1_at	0.62	0.15		0.29	-0.55		0.30	-0.32		0.17	-1.16		0.06	-2.14		0.05	-2.27	D	0.05	-1.81		0.20	-1.39		0.16	-0.97	
Os01g73770	B1	55365.1.S1_at	0.58	0.11		0.10	-0.30		0.05	-0.63		0.85	0.05		0.36	0.40		0.02	-0.63		0.17	-0.43		0.21	-0.51		0.52	-0.29	
Os02g10760	A4	50235.2.S1_at	0.68	0.09		0.11	0.03		0.45	-0.15		0.13	-0.58		0.06	-0.44		0.09	-0.37		0.12	-0.18		0.22	0.34		0.17	-0.34	
Os02g13710	B7	55643.1.S1_at	0.45	0.06		0.45	0.12		0.51	0.27		0.23	-1.14		0.00	-1.56	D	0.09	-1.85		0.01	-1.66	D	0.47	0.25		0.37	-0.09	
Os02g29550	B1	39004.1.S1_at	0.18	-0.42		0.30	-0.12		0.26	0.25		0.03	1.32	U	0.06	1.48		0.01	2.74	U	0.16	-0.50		0.76	0.03		0.01	0.63	
Os02g32040	B4	32291.1.S1_at	0.06	3.70		0.05	3.83	U	0.29	1.21		0.49	-0.64		0.57	-0.56		0.30	-0.73		0.83	0.08		0.19	-0.77		0.28	-0.91	
Os02g32140	B4	12318.1.S1_at	0.59	-0.08		0.38	-0.28		0.78	-0.11		0.84	0.02		0.84	-0.06		0.38	0.06		0.02	0.41		0.73	0.05		0.72	0.12	
Os02g34260	B4	55316.1.S1_at	0.03	-1.16	D	0.96	0.08		0.01	-1.43	D	0.01	-1.67	D	0.01	-1.48	D	0.09	-1.27		0.02	-1.13	D	0.06	-1.37		0.06	-1.35	
Os02g34270	A4	6079.1.S1_at	0.23	-0.54		0.74	0.14		0.10	-0.33		0.05	-2.33	D	0.04	-1.74	D	0.05	-1.50		0.03	-1.26	D	0.40	-0.41		0.22	-1.38	
Os02g40070	AP2	49859.1.S1_at	0.01	3.02	U	0.30	1.36		0.14	0.84		0.01	-3.98	D	0.01	-3.72	D	0.01	-4.15	D	0.01	-1.14	D	0.01	-4.42	D	0.02	-4.28	D
Os02g42585	B3	54384.1.S1_at	0.19	1.08		0.12	1.14		0.06	0.93		0.00	-1.24	D	0.15	-1.04		0.58	-0.11		0.44	-0.33		0.78	0.22		0.42	-0.79	
Os02g43790	B3	10904.1.S1_at	0.27	0.59		0.94	-0.02		0.56	0.35		0.51	0.07		0.62	-0.23		0.51	0.47		0.13	1.27		0.47	0.38		0.21	1.25	
Os02g43820	A4	52763.1.S1_at	0.23	0.91		0.04	0.61		0.26	0.84		0.51	-0.35		0.49	-0.44		0.61	-0.32		0.62	0.25		0.35	0.64		0.61	0.17	
Os02g43940	A4	54929.1.S1_at	0.01	3.60	U	0.01	2.75	U	0.06	1.28		0.03	2.20	U	0.17	1.69		0.02	1.09	U	0.06	2.61		0.01	4.78	U	0.03	4.10	U
Os02g43970	A5	28045.1.S1_at	0.44	-0.14		0.76	0.08		0.26	-0.57		0.02	-0.57		0.09	-0.66		0.90	0.12		0.95	-0.01		0.00	-2.96	D	0.07	-2.41	
Os02g45420	A1	9146.1.S1_at	0.48	0.21		0.87	-0.04		0.85	-0.07		0.67	0.13		0.98	0.01		0.63	0.23		0.98	0.01		0.68	-0.19		0.43	0.36	
Os02g45450	A6	51078.1.S1_at	0.31	-2.88		0.60	0.57		0.29	-2.01		0.53	2.95		0.35	-0.53		0.18	1.40		0.91	-0.29		0.08	3.91		0.28	1.63	
Os02g51300	AP2	47948.1.S1_at	0.16	-1.75		0.14	-1.94		0.16	-1.84		0.21	1.57		0.28	0.68		0.28	1.05		0.21	-0.50		0.02	1.68	U	0.12	3.48	
Os02g51670	B4	6076.1.S1_at	0.24	2.01		0.18	1.43		0.26	1.45		0.31	1.06		0.60	0.56		0.36	0.61		0.21	2.24		0.44	1.00		0.76	0.33	
Os02g52670	A5	54944.1.S1_at	0.00	-3.20	D	0.40	-1.26		0.01	-3.08	D	0.96	-0.13		0.06	-3.33		0.84	0.25		0.26	2.31		0.05	-1.78		0.61	-1.04	
Os02g54050	B2	53362.2.S1_at	0.84	-0.05		0.51	0.13		0.66	-0.13		0.77	0.06		0.98	0.00		0.62	-0.15		0.59	-0.14		0.46	-0.10		0.35	-0.07	
Os02g54160	A3	14181.1.S1_at	0.33	0.54		0.07	-0.77		0.30	-0.64		0.07	-0.97		0.16	-1.15		0.11	-0.90		0.24	-1.20		0.51	-0.34		0.17	-1.99	

Continue

Os02g55380	B3	55579.1.S1_at	0.05	-3.23		0.09	-1.45		0.07	-0.64		0.04	-4.80	D	0.09	-4.34		0.06	-4.30		0.15	-4.78		0.06	-5.00		0.01	-4.97	D
Os03g05590	A2	55258.1.S1_at	0.47	-0.48		0.23	-0.40		0.22	-0.57		0.60	-0.07		0.76	-0.11		0.84	-0.17		0.28	-0.11		0.17	-0.52		0.55	-0.29	
Os03g07830	B3	32557.1.S1_at	0.10	0.52		0.13	1.02		0.13	0.74		0.07	-0.82		0.29	-0.48		0.18	0.50		0.49	-0.06		0.34	0.78		0.66	-0.26	
Os03g07940	AP2	8271.1.S1_at	0.07	0.99		0.67	-0.32		0.69	0.16		0.46	-0.20		0.61	0.06		0.81	-0.04		0.02	1.50	U	0.24	0.49		0.11	0.60	
Os03g08460	B2	13876.2.S1_x_at	0.37	0.57		0.37	0.71		0.32	0.65		0.02	0.27		0.44	0.26		0.81	0.13		0.93	0.05		0.22	0.63		0.79	0.14	
Os03g08470	B3	6009.3.S1_a_at	0.18	1.78		0.16	2.87		0.40	2.51		0.19	2.29		0.14	2.99		0.55	1.17		0.08	4.87		0.03	2.33	U	0.49	0.42	
Os03g08490	B2	23028.1.S1_s_at	0.08	0.96		0.15	1.70		0.06	2.32		0.19	3.21		0.00	2.36	U	0.06	2.58		0.07	4.75		0.05	0.53		0.29	-0.18	
Os03g08500	A6	12889.2.S1_at	0.09	-0.37		0.94	-0.03		0.33	-0.42		0.64	-0.11		0.36	0.40		0.30	-0.22		0.23	-0.18		0.39	-0.14		0.31	-0.27	
Os03g09170	A5	38849.1.S1_at	0.04	4.07	U	0.01	2.80	U	0.08	3.11		0.10	3.88		0.05	3.77	U	0.12	3.21		0.06	3.88		0.37	0.94		0.06	4.53	
Os03g12950	AP2	54629.1.S1_at	0.11	1.75		0.22	2.15		0.08	2.49		0.25	-1.41		0.10	-1.72		0.03	-1.08	D	0.12	-1.45		0.18	-1.80		0.14	-1.97	
Os03g15660	B2	35111.1.S1_at	0.49	-0.51		0.00	2.39	U	0.14	0.94		0.18	-1.43		0.04	-2.14	D	0.12	-2.03		0.25	-1.52		0.04	-1.87	D	0.89	-0.13	
Os03g19900	AP2	56314.1.S1_at	0.18	-0.18		0.13	-0.13		0.70	-0.52		0.05	-2.44		0.02	-2.44	D	0.10	-2.26		0.04	-2.06	D	0.03	-2.20	D	0.03	-2.31	D
Os03g22170	B1	57381.1.S1_at	0.30	1.49		0.55	0.97		0.51	-1.00		0.09	-3.01		0.18	-3.28		0.07	-2.60		0.23	0.81		0.61	-0.58		0.35	-1.91	
Os03g60120	B3	8951.1.S1_x_at	0.08	-0.33		0.17	-0.86		0.17	-0.98		0.01	-1.73	D	0.00	-1.64	D	0.10	-1.55		0.76	-0.32		0.16	-0.58		0.01	-1.00	D
Os03g60430	AP2	27471.1.S1_at	0.14	2.12		0.27	1.36		0.23	1.65		0.44	-0.76		0.12	-0.80		0.16	-0.78		0.36	0.96		0.30	-1.13		0.38	-0.90	
Os03g64260	B3	12966.1.S1_at	0.13	0.28		0.76	0.17		0.69	0.16		0.10	1.34		0.12	1.27		0.14	1.17		0.15	0.65		0.54	0.24		0.42	0.37	
Os04g32620	B1	9523.1.S1_at	0.13	0.86		0.06	1.25		0.48	0.10		0.03	-2.70	D	0.00	-2.63	D	0.08	-2.52		0.18	-0.97		0.03	-3.07	D	0.03	-3.00	D
Os04g32790	B4	38106.1.S1_at	0.09	0.35		0.98	-0.01		0.10	0.48		0.84	-0.06		0.85	-0.12		0.28	0.44		0.05	0.12		0.46	0.28		0.34	0.42	
Os04g34970	A4	54932.1.S1_at	0.38	-0.36		0.28	0.42		0.10	-0.81		0.56	-0.28		0.01	-0.59		0.40	0.50		0.03	4.23	U	0.14	-0.84		0.09	-0.81	
Os04g42570	AP2	55217.1.S1_at	0.66	0.19		0.90	-0.05		0.37	0.56		0.37	-0.73		0.07	-1.13		0.38	-1.27		0.31	-0.55		0.19	0.43		0.24	0.85	
Os04g44670	B3	6539.1.S1_at	0.00	-1.75	D	0.20	-0.35		0.06	-1.71		0.38	0.31		0.47	0.58		0.08	-0.63		0.04	3.23	U	0.15	1.12		0.56	0.44	
Os04g46240	B5	5646.1.S1_at	0.05	-0.42		0.17	-0.46		0.19	-0.35		0.07	0.90		0.39	0.46		0.36	0.41		0.18	-0.16		0.19	0.23		0.67	-0.10	
Os04g48350	B1	10507.1.S1_at	0.05	-0.90		0.01	-1.52	D	0.07	-1.03		0.15	-1.18		0.02	-1.17	D	0.02	-0.60		0.03	-2.35	D	0.10	-2.39		0.07	0.29	
Os04g52090	A5	23103.1.S1_at	0.66	0.25		0.49	0.51		0.45	0.38		0.92	0.07		0.08	-1.07		0.53	-0.15		0.25	0.68		0.03	0.58		0.73	-0.21	
Os04g55520	A3	51638.1.S1_s_at	0.01	-0.92		0.48	-0.50		0.01	-0.53		0.23	0.99		0.14	-0.74		0.24	-0.63		0.03	1.27	U	0.77	-0.13		0.16	-0.66	
Os04g55560	AP2	9874.1.S1_at	0.41	0.28		0.37	0.41		0.01	0.26		0.09	1.88		0.25	0.60		0.14	1.17		0.07	2.10		0.41	0.42		0.76	-0.06	
Os04g57340	B1	28406.1.S1_at	0.00	4.48	U	0.14	3.90		0.40	3.35		0.46	0.90		0.01	2.67	U	0.24	3.03		0.25	2.63		0.46	1.51		0.04	-0.26	
Os05g03040	AP2	6461.1.A1_at	0.85	-0.11		0.61	0.35		0.87	0.06		0.88	0.10		0.20	-0.40		0.89	-0.15		0.21	0.76		0.18	-0.81		0.27	-1.06	
Os05g25260	A2	56944.1.S1_at	0.18	0.92		0.42	0.56		0.17	0.58		0.01	-1.00	D	0.18	-0.69		0.15	-0.58		0.10	-0.38		0.60	-0.23		0.19	-1.49	
Os05g27930	A2	7718.1.S1_at	0.13	0.31		0.06	1.17		0.88	-0.08		0.77	-0.11		0.15	-0.36		0.76	-0.14		0.59	-0.38		0.32	0.58		0.90	-0.03	
Os05g29810	B7	4893.1.S1_at	0.02	1.25	U	0.05	0.31		0.08	0.60		0.08	-3.68		0.13	-3.63		0.12	-2.01		0.33	-1.32		0.04	-0.80		0.15	-2.46	
Os05g32270	A5	49193.1.S1_at	0.08	2.90		0.03	1.64	U	0.07	2.35		0.13	2.41		0.01	2.37	U	0.11	2.84		0.08	2.62		0.02	3.82	U	0.06	3.07	
Os05g37640	A2	51802.1.S1_at	0.75	0.20		0.91	-0.06		0.49	-0.33		0.67	-0.43		0.17	-0.56		0.48	-0.37		0.97	-0.02		0.73	0.06		0.59	-0.33	
Os05g39590	B5	10500.1.S1_at	0.74	0.16		0.16	0.67		0.00	0.61		0.77	0.45		0.26	-0.53		0.09	0.56		0.19	0.97		0.14	0.49		0.07	0.78	
Os05g41760	B1	51998.1.S1_at	0.19	-0.52		0.17	-0.81		0.21	-0.78		0.26	-0.68		0.27	-0.80		0.65	-0.21		0.25	-0.75		0.34	-0.73		0.18	-0.85	
Os05g41780	B3	5532.1.S1_at	0.01	2.50	U	0.10	2.19		0.04	2.21	U	0.12	2.13		0.06	1.79		0.12	2.24		0.03	3.48	U	0.01	1.75	U	0.04	3.26	U
Os05g45954	AP2	54786.1.S1_at	0.60	0.35		0.48	0.60		0.80	-0.16		0.17	0.49		0.97	0.02		0.83	-0.14		0.85	-0.06		0.65	-0.18		0.63	-0.25	
Os05g47650	RAV	31989.1.S1_at	0.14	-0.71		0.05	0.39		0.01	0.79		0.46	0.07		0.07	-1.14		0.74	0.08		0.15	1.58		0.00	-1.54	D	0.17	-0.73	
Os05g49700	A1	57303.1.S1_x_at	0.05	-1.34		0.08	-0.62		0.15	0.48		0.22	0.14		0.67	-0.13		0.87	-0.08		0.04	0.68		0.48	-0.41		0.70	0.11	
Os06g03670	B3	23123.1.S2_at	0.96	-0.01		0.70	0.06		0.82	-0.06		0.16	-0.85		0.03	-0.84		0.05	-0.58		0.02	-0.38		0.44	0.17		0.53	-0.11	
Os06g05340	AP2	52397.1.S1_at	0.94	-0.02		0.16	0.14		0.37	0.16		0.15	1.21		0.04	1.41	U	0.04	1.48	U	0.12	0.08		0.75	0.09		0.17	1.52	
Os06g06540	A1	31624.1.S1_at	0.19	-0.15		0.41	-0.18		0.57	-0.17		0.09	-0.35		0.77	-0.10		0.90	-0.04		0.53	0.05		0.04	-0.27		0.82	-0.07	
Os06g06970	A5	6789.1.S1_at	0.17	1.20		0.10	0.66		0.01	1.18	U	0.21	1.65		0.02	1.57	U	0.07	1.21		0.20	1.47		0.14	-1.69		0.11	1.48	
Os06g07030	A3	4804.1.S1_at	0.69	-0.04		0.09	-0.39		0.00	-1.71	D	0.32	-1.18		0.08	-2.33		0.42	-1.27		0.49	0.49		0.02	-2.05	D	0.00	-4.24	D

Continue

Os06g08340	B2	15722.1.S1_s_at	0.16	-0.33		0.15	0.61		0.68	0.10		0.44	0.65		0.11	0.92		0.25	-0.17		0.80	-0.04		0.57	-0.13		0.30	-0.89	
Os06g09390	A5	14664.1.A1_at	0.28	-0.89		0.49	0.55		0.86	0.13		0.55	-0.33		0.39	-0.54		0.67	-0.83		0.41	-0.62		0.30	0.45		0.45	0.64	
Os06g09760	A5	5599.1.S1_at	0.62	-0.05		0.37	-0.71		0.11	-0.89		0.50	-0.86		0.34	-0.72		0.05	-2.11		0.73	0.12		0.87	-0.06		0.08	-0.75	
Os06g11860	A5	10845.1.S1_s_at	0.06	2.03		0.72	-0.14		0.09	0.24		0.06	-0.77		0.30	-0.26		0.01	0.08		0.08	1.69		0.02	2.55	U	0.07	0.32	
Os06g36000	A3	12997.1.S1_at	0.54	0.39		0.56	0.54		0.74	0.21		0.05	-0.78		0.52	-0.48		0.97	0.00		0.45	-0.36		0.95	-0.06		0.48	-0.70	
Os06g40150	B3	51150.1.S1_at	0.16	-1.69		0.27	-1.25		0.29	-1.03		0.36	0.68		0.84	0.14		0.22	0.97		0.17	-1.18		0.11	-1.27		0.23	0.93	
Os06g43220	AP2	50002.1.S1_at	0.09	0.53		0.05	1.25		0.01	2.16	U	0.09	1.52		0.09	1.77		0.07	1.74		0.01	3.04	U	0.07	2.05		0.02	2.56	U
Os06g44750	AP2	21891.1.S1_at	0.09	4.35		0.12	3.51		0.02	3.08	U	0.26	-1.52		0.11	-1.78		0.18	-2.28		0.30	1.25		0.21	-2.70		0.13	-3.00	
Os06g47590	B7	30258.1.S1_at	0.26	0.18		0.28	0.48		0.15	0.46		0.21	0.42		0.39	0.08		0.23	0.13		0.30	0.23		0.17	-0.11		0.19	-0.27	
Os07g03250	B6	56321.1.S1_at	0.17	-0.84		0.36	-0.47		0.23	-0.39		0.23	-0.57		0.14	-0.61		0.29	-0.33		0.13	-0.90		0.25	-0.63		0.19	-0.76	
Os07g10410	B1	56201.1.S1_at	0.68	0.11		0.03	0.54		0.11	-0.17		0.14	-0.45		0.15	0.54		0.29	-0.46		0.27	0.18		0.81	-0.09		0.11	-0.19	
Os07g13170	AP2	31647.1.S1_at	0.66	0.14		0.89	0.03		0.47	0.40		0.35	0.19		0.32	-0.12		0.52	-0.13		0.12	0.06		0.84	0.04		0.05	0.40	
Os07g22730	B3	30670.1.S1_at	0.20	1.89		0.21	2.42		0.38	1.38		0.36	2.38		0.02	1.17	U	0.03	1.74	U	0.11	2.86		0.35	1.50		0.05	-0.18	
Os07g22770	B6	36817.1.S1_at	0.05	0.37		0.68	0.08		0.18	0.06		0.18	0.23		0.05	0.57		0.24	-0.10		0.12	1.02		0.52	0.53		0.12	-0.53	
Os07g42510	B1	37967.1.S1_at	0.55	0.60		0.52	-0.43		0.45	0.95		0.88	-0.21		0.71	0.25		0.49	0.87		0.83	0.27		0.92	0.12		0.41	0.98	
Os07g47330	B2	51144.1.S1_at	0.42	0.48		0.34	1.50		0.15	1.62		0.06	4.33		0.03	4.27	U	0.01	3.66	U	0.13	4.39		0.02	3.38	U	0.00	6.37	U
Os07g47790	B1	48988.1.S1_at	0.02	0.32		0.17	0.33		0.46	0.15		0.16	-0.02		0.75	0.05		0.10	-0.20		0.41	-0.07		0.70	0.05		0.17	-0.44	
Os08g07440	AP2	10323.1.S1_at	0.14	0.46		0.19	0.44		0.48	0.14		0.84	-0.03		0.46	0.16		0.97	0.00		0.68	-0.11		0.47	0.42		0.84	0.05	
Os08g07700	B7	31056.1.S1_at	0.57	0.11		0.01	-1.09	D	0.03	-0.89		0.08	-1.74		0.17	-1.49		0.03	-2.16	D	0.02	-1.55	D	0.07	-2.00		0.05	-1.97	
Os08g27220	A6	20018.2.S1_x_at	0.13	0.52		0.92	-0.03		0.03	-0.32		0.38	-0.22		0.44	-0.29		0.81	0.18		0.73	0.17		0.68	0.25		0.88	-0.07	
Os08g31580	A5	6824.1.S1_at	0.01	1.95	U	0.12	0.83		0.04	2.37	U	0.01	2.64	U	0.10	3.11		0.03	3.11	U	0.02	1.54	U	0.04	0.79		0.06	1.98	
Os08g34360	AP2	49821.3.A1_at	0.18	-0.37		0.68	-0.06		0.68	0.19		0.13	-1.24		0.09	-1.29		0.23	-0.91		0.06	-0.56		0.24	-0.58		0.17	-0.46	
Os08g36920	B7	15849.1.S1_s_at	0.28	-1.13		0.91	-0.02		0.15	-0.68		0.41	2.75		0.13	-1.38		0.35	0.99		0.08	1.90		0.20	2.16		0.42	1.79	
Os08g41030	B4	55500.1.S1_at	0.03	-0.47		0.18	-0.44		0.16	-0.33		0.30	-0.13		0.26	-0.24		0.16	-0.38		0.56	-0.09		0.18	-0.28		0.20	-0.36	
Os08g42550	RAV	49952.1.S1_at	0.86	-0.10		0.07	1.55		0.05	0.71		0.77	-0.08		0.93	-0.02		0.16	0.34		0.13	2.05		0.86	0.07		0.75	0.17	
Os08g43200	A1	25479.1.S1_at	0.15	1.42		0.32	0.66		0.12	0.99		0.49	-0.27		0.15	-1.05		0.65	0.13		0.94	-0.04		0.31	0.88		0.67	0.24	
Os08g45110	A1	49350.1.S1_at	0.12	1.62		0.20	0.66		0.24	1.13		0.47	-0.45		0.80	-0.27		0.85	0.18		0.28	0.78		0.15	1.83		0.18	1.32	
Os09g11460	B3	5237.1.S1_at	0.04	0.47		0.28	-0.10		0.57	-0.08		0.13	-0.41		0.09	-0.60		0.09	-0.62		0.03	0.20		0.81	-0.05		0.22	-0.20	
Os09g11480	B2	19325.1.S1_at	0.95	-0.03		0.61	0.27		0.65	0.33		0.09	0.93		0.18	0.82		0.04	2.09	U	0.25	-1.07		0.17	0.90		0.24	0.44	
Os09g13940	B7	11725.1.S1_at	0.49	0.19		0.14	1.92		0.03	0.77		0.04	-1.59	D	0.24	-1.31		0.19	-0.55		0.18	-0.95		0.39	0.78		0.55	-0.50	
Os09g20350	A6	11944.1.S2_s_at	0.11	-0.60		0.28	-0.88		0.01	-0.88		0.25	-0.07		0.95	-0.02		0.32	-0.72		0.19	-0.63		0.81	-0.14		0.20	0.61	
Os09g25600	AP2	20663.1.S1_at	0.10	0.15		0.22	-0.57		0.39	-0.52		0.10	0.40		0.45	0.49		0.00	0.78		0.19	0.12		0.13	2.47		0.85	0.08	
Os09g26420	B2	51487.1.S1_at	0.60	-0.35		0.67	-0.34		0.53	-0.32		0.89	-0.06		0.64	0.29		0.45	-0.33		0.91	-0.08		0.68	-0.31		0.88	-0.11	
Os09g28440	B4	52451.1.A1_at	0.62	-0.80		0.23	2.07		0.06	-0.42		0.57	0.72		0.24	-2.18		0.56	-0.85		0.68	0.57		0.01	2.86	U	0.97	0.01	
Os09g39850	B3	54835.1.S1_s_at	0.38	-0.53		0.63	-0.23		0.66	0.15		0.26	0.37		0.22	0.56		0.21	0.67		0.19	0.23		0.84	-0.07		0.16	1.58	
Os10g25170	B1	56665.1.A1_at	0.00	3.13	U	0.03	1.94	U	0.16	1.33		0.21	1.50		0.26	1.23		0.14	-0.26		0.10	4.84		0.46	0.36		0.56	0.13	
Os10g38000	A5	55877.1.S1_at	0.32	1.07		0.08	1.35		0.00	0.49		0.57	0.37		0.32	-0.19		0.00	0.80		0.48	0.74		0.08	-0.15		0.04	0.46	
Os11g19060	AP2	51993.1.S1_at	0.24	-0.21		0.55	-0.14		0.29	-0.31		0.29	0.36		0.83	-0.02		0.13	0.14		0.87	-0.04		0.63	0.21		0.59	-0.05	

Continue

Gene name	Phg	Probe Set ID Os.	Flag Leaf 1 Vs S			Flag Leaf 2 Vs S			Leaf 1 Vs S			Leaf 2 Vs S			Sheath 1 Vs S			Sheath 2 Vs S			Panicle 1 Vs S			Panicle 2 Vs S		
			PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R
Os01g04020	A2	4663.1.S2_at	0.15	-1.57		0.11	-1.19		0.47	-0.11		0.80	0.10		0.23	-0.22		0.24	-0.41		0.12	-1.56		0.05	-1.56	D
Os01g04750	RAV	27299.1.A1_at	0.03	-3.61	D	0.11	-2.96		0.09	-1.09		0.54	-0.69		0.56	-1.26		0.09	-1.84		0.11	-4.49		0.06	-4.08	
Os01g04800	RAV	36672.1.S1_at	0.98	0.01		0.74	-0.23		0.82	-0.15		0.84	-0.11		0.66	-0.19		0.41	-0.51		0.83	0.04		0.85	0.07	
Os01g07120	A4	21563.1.S2_at	0.10	-1.12		0.54	1.18		0.66	0.53		0.16	0.94		0.09	-0.82		0.22	-0.20		0.18	-1.53		0.13	-1.27	
Os01g10370	B1	55837.1.S1_at	0.23	-2.27		0.08	-2.61		0.10	-3.22		0.15	-2.90		0.12	-3.25		0.10	-3.30		0.13	-3.30		0.06	-2.48	
Os01g12440	B2	31908.1.S1_at	0.05	-3.48		0.04	-2.56	D	0.40	-2.48		0.55	-0.78		0.00	-1.94	D	0.45	-1.47		0.18	-0.92		0.37	-1.01	
Os01g21120	B1	8031.1.S1_at	0.05	-7.05	D	0.03	-4.96	D	0.09	-5.58		0.00	-4.95	D	0.08	-6.33		0.08	-5.57		0.06	-4.00		0.18	-0.64	
Os01g46870	B3	51970.1.S1_at	0.54	-0.72		0.58	-0.61		0.46	-0.87		0.53	-0.53		0.53	-0.75		0.64	-0.63		0.13	3.76		0.14	3.51	
Os01g49830	RAV	56351.1.S1_at	0.06	-1.05		0.18	-0.93		0.01	-0.93		0.32	-0.61		0.11	-1.08		0.06	-1.22		0.11	-0.61		0.13	-0.47	
Os01g54890	B1	53660.1.S1_at	0.08	1.73		0.04	5.91	U	0.07	3.58		0.03	3.54	U	0.22	2.81		0.20	3.12		0.98	0.02		0.28	0.89	
Os01g58420	B4	3766.1.S1_at	0.29	-1.05		0.94	-0.16		0.22	1.83		0.12	2.34		0.15	2.03		0.15	1.91		0.27	1.47		0.12	3.07	
Os01g59780	AP2	16683.1.S1_at	0.06	-2.61		0.06	-2.13		0.04	-1.99	D	0.13	-1.42		0.46	-0.34		0.83	-0.01		0.08	-1.27		0.01	-1.27	D
Os01g67410	AP2	14775.1.S1_at	0.08	-3.34		0.08	-3.33		0.02	-1.88	D	0.09	-2.18		0.02	-2.14	D	0.05	-1.63	D	0.11	-0.55		0.43	-0.40	
Os01g73770	B1	55365.1.S1_at	0.21	-0.07		0.19	-0.41		0.12	-0.45		0.01	-0.23		0.91	-0.02		0.63	0.09		0.53	0.17		0.15	0.54	
Os02g10760	A4	50235.2.S1_at	0.54	-0.35		0.28	-0.29		0.76	0.07		0.98	0.00		0.89	0.02		0.18	-0.37		0.09	-0.17		0.04	-0.25	
Os02g13710	B7	55643.1.S1_at	0.00	-1.59	D	0.04	-3.28	D	0.07	-1.92		0.08	-1.86		0.01	-2.13	D	0.01	-1.34	D	0.29	-0.64		0.27	0.38	
Os02g29550	B1	39004.1.S1_at	0.03	3.16	U	0.09	2.20		0.53	0.63		0.25	1.16		0.06	1.13		0.13	1.28		0.07	-0.16		0.74	0.21	
Os02g32040	B4	32291.1.S1_at	0.32	-0.83		0.24	-0.68		0.24	-0.92		0.80	-0.29		0.30	-0.89		0.29	-0.96		0.20	1.71		0.46	0.90	
Os02g32140	B4	12318.1.S1_at	0.43	0.29		0.49	0.31		0.49	0.31		0.48	0.32		0.09	0.15		0.57	0.08		0.73	-0.05		0.15	-0.34	
Os02g34260	B4	55316.1.S1_at	0.02	-1.32	D	0.02	-1.28	D	0.08	-1.42		0.10	-1.35		0.07	-1.46		0.06	-1.39		0.07	-1.50		0.15	-0.56	
Os02g34270	A4	6079.1.S1_at	0.37	-0.97		0.24	-0.71		0.45	-1.03		0.52	-0.09		0.63	-0.10		0.25	-0.28		0.15	-0.36		0.10	-0.70	
Os02g40070	AP2	49859.1.S1_at	0.02	-4.55	D	0.03	-4.33	D	0.01	-4.37	D	0.04	-4.45	D	0.01	-4.29	D	0.04	-4.32	D	0.05	-4.01	D	0.02	-4.33	D
Os02g42585	B3	54384.1.S1_at	0.68	0.40		0.85	-0.07		0.90	0.12		0.24	0.60		0.32	0.62		0.23	0.81		0.28	0.95		0.35	0.78	
Os02g43790	B3	10904.1.S1_at	0.74	-0.14		0.85	-0.12		0.56	0.26		0.38	0.20		0.92	0.03		0.83	-0.06		0.79	0.16		0.68	0.15	
Os02g43820	A4	52763.1.S1_at	0.09	0.32		0.38	0.63		0.21	1.01		0.30	0.78		0.40	0.49		0.28	0.40		0.44	1.08		0.40	1.03	
Os02g43940	A4	54929.1.S1_at	0.10	0.57		0.13	-1.98		0.97	0.06		0.29	0.30		0.90	0.21		0.45	0.84		0.26	0.26		0.19	0.55	
Os02g43970	A5	28045.1.S1_at	0.31	0.44		0.68	-0.38		0.26	-0.26		0.30	-0.29		0.11	-1.55		0.12	-0.84		0.11	-1.26		0.02	-0.84	
Os02g45420	A1	9146.1.S1_at	0.79	-0.13		0.70	0.16		0.87	0.03		0.43	0.34		0.18	0.50		0.57	0.26		0.21	-0.78		0.15	-1.03	
Os02g45450	A6	51078.1.S1_at	0.71	0.46		0.19	5.38		0.42	3.02		0.22	4.23		0.59	0.35		0.09	3.01		0.57	-1.25		0.80	0.67	
Os02g51300	AP2	47948.1.S1_at	0.12	-1.15		0.14	-1.74		0.19	-1.02		0.29	-1.30		0.15	-1.02		0.21	-0.34		0.23	1.06		0.27	0.89	
Os02g51670	B4	6076.1.S1_at	0.40	1.09		0.30	2.20		0.19	0.99		0.29	0.97		0.34	1.29		0.08	1.15		0.28	1.57		0.31	1.58	
Os02g52670	A5	54944.1.S1_at	0.08	-2.21		0.39	0.29		0.99	0.00		0.22	1.41		0.44	-0.54		0.45	0.50		0.18	-2.29		0.04	-0.80	
Os02g54050	B2	53362.2.S1_at	0.12	-0.31		0.77	-0.04		0.87	-0.06		0.99	0.00		0.39	-0.20		0.89	-0.08		0.08	0.62		0.03	0.34	
Os02g54160	A3	14181.1.S1_at	0.86	-0.25		0.15	-0.35		0.43	-0.09		0.69	-0.09		0.24	-0.92		0.37	-0.36		0.57	-0.37		0.36	-0.41	
Os02g55380	B3	55579.1.S1_at	0.03	-5.97	D	0.07	-5.85		0.06	-5.63		0.00	-5.41	D	0.09	-4.80		0.00	-5.09	D	0.09	-3.18		0.04	-4.48	D
Os03g05590	A2	55258.1.S1_at	0.47	-0.29		0.71	-0.06		0.52	-0.21		0.33	-0.45		0.23	-0.38		0.16	-0.37		0.23	-0.53		0.23	-0.27	
Os03g07830	B3	32557.1.S1_at	0.64	0.37		0.35	0.15		0.43	0.73		0.08	0.86		0.38	0.45		0.18	0.80		0.35	0.41		0.07	0.16	
Os03g07940	AP2	8271.1.S1_at	0.17	-0.91		0.46	-0.98		0.26	-0.50		0.59	-0.14		0.18	0.49		0.18	0.57		0.07	1.49		0.01	1.70	U
Os03g08460	B2	13876.2.S1_x_at	0.20	0.78		0.70	0.04		0.29	0.54		0.65	0.24		0.20	0.79		0.33	0.31		0.19	1.45		0.23	1.13	

Continue

Os03g08470	B3	6009.3.S1_a_at	0.20	-1.30		0.40	0.72		0.24	0.89		0.26	1.39		0.00	3.74	U	0.07	1.81		0.06	-3.63		0.15	-2.94	
Os03g08490	B2	23028.1.S1_s_at	0.26	1.20		0.01	2.63	U	0.02	4.59	U	0.01	4.81	U	0.04	4.73	U	0.04	4.39	U	0.38	-0.45		0.40	-0.27	
Os03g08500	A6	12889.2.S1_at	0.02	-0.41		0.11	-0.26		0.03	-0.24		0.91	-0.03		0.46	0.13		0.76	0.04		0.57	-0.11		0.52	0.20	
Os03g09170	A5	38849.1.S1_at	0.28	-1.26		0.25	-1.89		0.15	-1.11		0.19	-1.41		0.40	-0.88		0.93	0.18		0.05	1.98	U	0.17	1.35	
Os03g12950	AP2	54629.1.S1_at	0.06	-2.11		0.08	-1.96		0.02	-2.09	D	0.19	-1.43		0.02	-1.93	D	0.10	-1.76		0.06	4.97		0.05	4.40	U
Os03g15660	B2	35111.1.S1_at	0.15	-2.36		0.08	-2.03		0.06	-2.58		0.15	-2.49		0.13	-2.06		0.07	-2.12		0.13	-1.37		0.26	-1.10	
Os03g19900	AP2	56314.1.S1_at	0.02	-2.41	D	0.01	-2.08	D	0.10	-2.12		0.03	-2.32	D	0.03	-2.17	D	0.04	-2.30	D	0.70	-0.18		0.12	-1.48	
Os03g22170	B1	57381.1.S1_at	0.09	-4.32		0.07	-2.96		0.14	-3.17		0.15	-3.44		0.22	-2.64		0.12	-3.30		0.06	-4.03		0.13	-3.64	
Os03g60120	B3	8951.1.S1_x_at	0.03	-1.68	D	0.09	-1.38		0.21	-0.96		0.05	-0.68		0.05	-1.15	D	0.01	-1.12	D	0.03	-1.42	D	0.00	-1.38	D
Os03g60430	AP2	27471.1.S1_at	0.29	-1.00		0.10	-1.46		0.57	-0.11		0.03	0.42		0.32	-1.28		0.20	-0.43		0.89	-0.13		0.50	-0.39	
Os03g64260	B3	12966.1.S1_at	0.18	0.16		0.64	0.30		0.02	0.35		0.07	0.27		0.47	0.75		0.36	0.68		0.45	0.39		0.62	0.10	
Os04g32620	B1	9523.1.S1_at	0.02	-2.71	D	0.07	-2.25		0.53	-1.09		0.53	-0.55		0.04	-1.41	D	0.22	-1.81		0.00	-2.71	D	0.01	-2.79	D
Os04g32790	B4	38106.1.S1_at	0.19	0.68		0.53	0.16		0.08	0.27		0.47	0.45		0.22	0.47		0.16	0.43		0.40	0.43		0.14	0.29	
Os04g34970	A4	54932.1.S1_at	0.18	-0.32		0.58	-0.21		0.07	4.02		0.03	4.26	U	0.03	4.94	U	0.03	4.15	U	0.05	-1.02		0.03	-0.60	
Os04g42570	AP2	55217.1.S1_at	0.14	-0.35		0.19	-0.47		0.38	-0.09		0.92	-0.05		0.97	-0.02		0.73	-0.11		0.05	1.14		0.22	0.87	
Os04g44670	B3	6539.1.S1_at	0.82	-0.19		0.87	-0.02		0.17	-1.36		0.14	-1.17		0.03	-1.17	D	0.23	-1.61		0.85	-0.26		0.55	0.46	
Os04g46240	B5	5646.1.S1_at	0.99	0.01		0.44	-0.05		0.25	-0.16		0.49	-0.27		0.33	-0.19		0.63	0.16		0.85	0.04		0.89	0.03	
Os04g48350	B1	10507.1.S1_at	0.10	-3.10		0.01	-4.17	D	0.06	-4.52		0.11	-4.14		0.14	-4.05		0.50	-2.36		0.04	-0.73		0.03	-0.85	
Os04g52090	A5	23103.1.S1_at	0.00	-1.76	D	0.30	1.06		0.78	0.26		0.09	0.57		0.36	0.22		0.30	0.25		0.38	0.27		0.16	1.42	
Os04g55520	A3	51638.1.S1_s_at	0.14	-1.41		0.13	-1.20		0.01	-1.40	D	0.12	-1.17		0.09	-1.10		0.64	-0.52		0.13	-2.32		0.03	-1.81	D
Os04g55560	AP2	9874.1.S1_at	0.14	0.88		0.42	0.15		0.19	1.88		0.06	1.92		0.38	1.24		0.10	2.15		0.63	0.20		0.53	0.48	
Os04g57340	B1	28406.1.S1_at	0.06	5.60		0.14	4.74		0.04	2.69	U	0.14	2.54		0.37	2.57		0.04	1.93	U	0.07	0.10		0.19	0.14	
Os05g03040	AP2	46461.1.A1_x_at	0.05	-0.92		0.23	-1.25		0.91	-0.10		0.57	-0.24		0.96	-0.05		0.51	-0.21		0.41	-1.05		0.29	-1.31	
Os05g25260	A2	56944.1.S1_at	0.50	-0.36		0.22	-1.12		0.39	0.44		0.22	0.21		0.60	-0.14		0.54	-0.35		0.12	1.03		0.04	1.14	U
Os05g27930	A2	7718.1.S1_at	0.07	0.84		0.04	0.89		0.29	0.80		0.06	0.79		0.82	-0.11		0.02	0.19		0.49	-0.09		0.56	0.24	
Os05g29810	B7	4893.1.S1_at	0.03	-7.07	D	0.14	-5.68		0.02	-2.88	D	0.07	-1.79		0.15	-0.83		0.01	-0.76		0.07	0.90		0.01	1.30	U
Os05g32270	A5	49193.1.S1_at	0.12	0.91		0.09	-0.58		0.16	0.75		0.40	0.58		0.08	2.47		0.10	2.15		0.02	3.77	U	0.06	3.50	
Os05g37640	A2	51802.1.S1_at	0.75	0.08		0.62	-0.25		0.96	0.02		0.81	-0.12		0.80	0.02		0.76	-0.24		0.96	0.05		0.28	-0.41	
Os05g39590	B5	10500.1.S1_at	0.43	-0.36		0.17	1.80		0.13	1.28		0.12	2.11		0.50	0.54		0.07	1.35		0.19	0.71		0.12	1.10	
Os05g41760	B1	51998.1.S1_at	0.50	-0.57		0.12	-0.64		0.08	-0.45		0.11	0.16		0.14	-0.73		0.18	-0.53		0.20	-0.67		0.29	-0.40	
Os05g41780	B3	5532.1.S1_at	0.88	0.05		0.06	1.98		0.49	0.66		0.01	1.50	U	0.02	1.10	U	0.17	1.45		0.05	2.08		0.01	1.73	U
Os05g45954	AP2	54786.1.S1_at	0.76	-0.18		0.30	-0.57		0.69	0.22		0.82	0.11		0.37	0.88		0.59	0.37		0.30	0.76		0.31	0.71	
Os05g47650	RAV	31989.1.S1_at	0.47	-0.52		0.16	-0.86		0.23	-0.64		0.39	-0.44		0.14	-0.57		0.17	-0.87		0.04	-0.74		0.07	-0.38	
Os05g49700	A1	57303.1.S1_x_at	0.40	-0.73		0.35	-1.13		0.09	-1.96		0.08	-0.42		0.64	-0.18		0.06	-1.04		0.11	0.39		0.49	0.54	
Os06g03670	B3	23123.1.S2_at	0.13	-0.43		0.87	-0.09		0.65	-0.04		0.21	0.22		0.32	-0.28		0.12	-0.30		0.97	0.00		0.03	-0.07	
Os06g05340	AP2	52397.1.S1_at	0.30	0.24		0.47	0.22		0.10	0.43		0.79	0.10		0.15	0.57		0.52	0.46		0.27	0.09		0.68	0.14	
Os06g06540	A1	31624.1.S1_at	0.13	0.02		0.61	-0.14		0.25	0.12		0.85	0.03		0.23	0.27		0.04	0.13		0.89	0.04		0.39	-0.48	
Os06g06970	A5	6789.1.S1_at	0.10	-2.03		0.42	-0.78		0.97	-0.02		0.13	0.46		0.37	-0.81		0.56	-0.28		0.13	1.71		0.16	2.13	
Os06g07030	A3	4804.1.S1_at	0.05	-2.88	D	0.15	-0.89		0.02	1.28	U	0.05	1.82	U	0.12	1.06		0.10	1.37		0.18	-2.03		0.02	-1.12	D
Os06g08340	B2	15722.1.S1_s_at	0.12	0.46		0.21	0.11		0.33	0.44		0.43	0.21		0.40	-0.32		0.14	-0.12		0.37	0.31		0.50	0.63	
Os06g09390	A5	14664.1.A1_at	0.25	0.38		0.99	-0.01		0.94	0.11		0.32	-0.92		0.57	0.21		0.20	-1.03		1.00	0.00		0.60	-0.64	
Os06g09760	A5	5599.1.S1_at	0.37	-0.85		0.18	-0.52		0.47	-0.46		0.50	-0.77		0.24	-0.17		0.53	-0.87		0.11	1.19		0.01	2.47	U
Os06g11860	A5	10845.1.S1_s_at	0.03	-0.11		0.27	1.04		0.07	2.30		0.03	2.63	U	0.01	2.78	U	0.18	3.05		0.05	2.33	U	0.04	2.55	U
Os06g36000	A3	12997.1.S1_at	0.78	0.38		0.30	-0.18		0.51	0.34		0.91	-0.07		0.65	-0.26		0.26	-0.65		0.95	-0.03		0.46	-0.43	

Continue

Os06g40150	B3	51150.1.S1_at	0.16	-2.29		0.00	-2.30	D	0.04	-2.27	D	0.08	-2.73		0.16	-1.90		0.10	-3.42		0.27	-0.13		0.25	0.44									
Os06g43220	AP2	50002.1.S1_at	0.02	3.20	U	0.01	2.39	U	0.05	3.62	U	0.02	4.02	U	0.00	3.01	U	0.03	3.33	U	0.06	0.83		0.02	0.81									
Os06g44750	AP2	21891.1.S1_at	0.17	-2.87		0.21	-2.50		0.14	-3.03		0.12	-2.77		0.15	-2.99		0.14	-2.86		0.18	-2.54		0.06	-2.07									
Os06g47590	B7	30258.1.S1_at	0.31	-0.31		0.03	0.37		0.53	-0.02		0.82	-0.05		0.86	0.05		0.61	-0.24		0.83	0.04		0.16	-0.07									
Os07g03250	B6	56321.1.S1_at	0.07	-0.94		0.27	-1.06		0.06	-0.92		0.18	-0.88		0.13	-0.79		0.39	-0.62		0.07	3.40		0.07	2.08									
Os07g10410	B1	56201.1.S1_at	0.08	-0.65		0.23	-0.28		0.60	-0.05		0.51	-0.04		0.31	-0.26		0.35	-0.36		0.37	-0.13		0.04	0.76									
Os07g13170	AP2	31647.1.S1_at	0.17	0.13		0.30	0.32		0.16	-0.03		0.06	-0.24		0.14	-0.30		0.45	-0.10		0.19	0.05		0.24	-0.29									
Os07g22730	B3	30670.1.S1_at	0.34	-1.26		0.12	0.34		0.36	-0.84		0.58	-0.70		0.27	1.22		0.07	0.59		0.12	-0.94		0.20	-1.30									
Os07g22770	B6	36817.1.S1_at	0.14	0.61		0.18	2.04		0.88	0.05		0.48	0.67		0.45	0.84		0.33	0.25		0.20	0.64		0.98	0.01									
Os07g42510	B1	37967.1.S1_at	0.60	-0.60		0.13	-3.52		0.06	-3.12		0.15	-2.68		0.48	-0.34		0.43	-0.84		0.20	2.38		0.17	2.61									
Os07g47330	B2	51144.1.S1_at	0.22	0.52		0.09	0.66		0.59	0.15		0.27	0.20		0.17	0.52		0.92	-0.02		0.22	-0.24		0.91	-0.05									
Os07g47790	B1	48988.1.S1_at	0.36	-0.37		0.21	-0.41		0.74	-0.13		0.67	-0.03		0.07	0.16		0.89	0.04		0.13	0.30		0.25	0.18									
Os08g07440	AP2	10323.1.S1_at	0.13	0.76		0.09	0.45		0.05	0.77		0.17	0.61		0.09	0.51		0.21	0.53		0.79	0.08		0.93	0.01									
Os08g07700	B7	31056.1.S1_at	0.01	-2.03	D	0.04	-1.89	D	0.01	-2.24	D	0.03	-2.16	D	0.02	-2.10	D	0.01	-2.18	D	0.01	-1.34	D	0.12	-0.90									
Os08g27220	A6	20018.2.S1_x_at	0.48	0.69		0.48	0.28		0.14	0.57		0.12	0.30		0.93	0.04		0.58	0.38		0.95	-0.03		0.56	0.10									
Os08g31580	A5	6824.1.S1_at	0.01	4.03	U	0.09	5.26		0.02	5.27	U	0.02	4.87	U	0.06	4.61		0.05	4.35		0.37	0.67		0.24	0.49									
Os08g34360	AP2	49821.3.A1_at	0.06	-1.13		0.14	-1.07		0.10	-0.99		0.05	-0.96		0.12	-0.68		0.07	-1.25		0.41	0.55		0.67	0.16									
Os08g36920	B7	15849.1.S1_s_at	0.54	-0.79		0.07	2.89		0.65	1.51		0.30	1.71		0.42	-0.59		0.98	-0.02		0.08	1.27		0.03	4.43	U								
Os08g41030	B4	55500.1.S1_at	0.22	-0.30		0.33	-0.35		0.16	-0.52		0.11	-0.53		0.09	-0.39		0.22	-0.46		0.40	-0.37		0.35	-0.20									
Os08g42550	RAV	49952.1.S1_at	0.02	0.43		0.19	0.66		0.16	1.60		0.03	1.99	U	0.09	1.01		0.22	2.52		0.94	0.02		0.39	0.18									
Os08g43200	A1	25479.1.S1_at	0.68	0.22		0.14	-0.42		0.68	-0.06		0.39	0.53		0.20	-0.37		0.92	0.04		0.13	2.06		0.11	1.95									
Os08g45110	A1	49350.1.S1_at	0.02	1.18	U	0.10	0.80		0.21	1.49		0.07	1.30		0.40	0.60		0.21	0.87		0.64	0.57		0.59	0.57									
Os09g11460	B3	5237.1.S1_at	0.14	0.40		0.23	0.93		0.29	0.53		0.92	0.01		0.33	-0.09		0.24	-0.31		0.46	-0.25		0.13	-0.26									
Os09g11480	B2	19325.1.S1_at	0.04	3.08	U	0.28	1.58		0.04	0.58		0.67	0.42		0.04	1.23	U	0.42	0.96		0.92	-0.04		0.98	-0.01									
Os09g13940	B7	11725.1.S1_at	0.20	1.09		0.33	0.21		0.04	1.21	U	0.01	0.99		0.22	1.05		0.20	1.07		0.03	-0.22		0.37	-0.91									
Os09g20350	A6	11944.1.S2_s_at	0.59	-0.49		0.78	0.13		0.15	0.30		0.76	0.14		0.19	0.21		0.73	-0.20		0.14	1.40		0.09	1.40									
Os09g25600	AP2	20663.1.S1_at	0.26	0.53		0.58	0.61		0.60	0.81		0.28	0.45		0.75	0.08		0.68	0.06		0.47	0.48		0.78	0.09									
Os09g26420	B2	51487.1.S1_at	0.77	0.10		0.79	-0.11		0.82	-0.11		0.97	0.01		0.79	0.16		0.87	-0.06		0.99	-0.01		0.83	-0.20									
Os09g28440	B4	52451.1.A1_at	0.39	-0.80		0.07	2.73		0.93	0.35		0.74	0.29		0.31	-1.07		0.27	-0.86		0.25	0.41		0.06	3.95									
Os09g39850	B3	54835.1.S1_s_at	0.44	-0.41		0.41	-0.26		0.31	-0.25		0.42	-0.21		0.51	0.07		0.29	-0.56		0.02	-0.53		0.49	-0.47									
Os10g25170	B1	56665.1.A1_at	0.33	-0.62		0.26	-0.72		0.08	-0.44		0.47	-0.36		0.11	-0.33		0.64	-0.25		0.21	-0.63		0.12	-0.44									
Os10g38000	A5	55877.1.S1_at	0.12	0.81		0.70	-0.13		0.12	-0.61		0.26	-0.59		0.35	-0.10		0.33	0.87		0.36	0.42		0.10	0.26									
Os11g19060	AP2	51993.1.S1_at	0.46	0.16		0.31	-0.30		0.71	-0.06		0.86	0.04		0.74	-0.06		0.69	0.07		0.17	0.25		0.34	0.26									

Continue

Gene name	Phg	Probe Set ID Os.	Panicle 3 Vs			Panicle 4 Vs S			Panicle 5 Vs S			Stamen Vs S			Spikelete Vs S			Endosperm 1 Vs			Endosperm 2 Vs			Endosperm 3 Vs		
				FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R	PV	FC	R
Os01g04020	A2	4663.1.S2_at	0	-1.26		0.10	-1.61		0.15	-1.24		0.11	-3.14		0.21	-0.96		0.07	-0.85		0.19	-1.14		0.19	-0.47	
Os01g04750	RAV	27299.1.A1_at	0	-4.92	D	0.02	-4.38	D	0.07	-4.77		0.05	-5.21		0.07	-4.68		0.05	-4.01		0.08	-5.06		0.03	-5.15	D
Os01g04800	RAV	36672.1.S1_at	0	-0.17		0.56	-0.28		0.79	-0.19		0.91	0.05		0.59	-0.21		0.96	-0.03		0.70	-0.25		0.79	-0.16	
Os01g07120	A4	21563.1.S2_at	0	-1.34		0.05	-1.17		0.07	-2.01		0.19	-1.26		0.25	-0.40		0.42	0.11		0.10	0.93		0.33	1.55	
Os01g10370	B1	55837.1.S1_at	0	-1.40	D	0.19	-1.99		0.45	-0.74		0.12	-3.59		0.18	-2.81		0.10	-3.01		0.09	-2.63		0.15	-2.05	
Os01g12440	B2	31908.1.S1_at	0	-0.38		0.11	-1.66		0.02	-2.96	D	0.01	-4.30	D	0.13	-2.01		0.15	-3.48		0.08	-3.35		0.08	-1.95	
Os01g21120	B1	8031.1.S1_at	0	-1.01	D	0.10	-6.00		0.04	-6.38	D	0.03	-8.84	D	0.13	-3.84		0.12	-1.13		0.19	-1.33		0.02	-2.48	D
Os01g46870	B3	51970.1.S1_at	0	3.48		0.15	3.27		0.38	1.12		0.53	-0.76		0.36	0.98		0.69	0.66		0.75	-0.46		0.54	-0.72	
Os01g49830	RAV	56351.1.S1_at	0	0.11		0.10	0.14		0.14	-0.48		0.02	-0.71		0.00	-1.01	D	0.11	-1.05		0.20	-1.07		0.09	-1.17	
Os01g54890	B1	53660.1.S1_at	0	0.08		0.73	-0.56		0.29	1.85		0.02	-1.69	D	0.05	3.61		0.23	1.66		0.38	0.66		0.47	-0.33	
Os01g58420	B4	3766.1.S1_at	0	3.38		0.31	1.26		0.70	0.34		0.41	0.15		0.28	1.19		0.32	0.73		0.23	1.61		0.36	1.08	
Os01g59780	AP2	16683.1.S1_at	0	-1.40		0.04	-0.85		0.10	-0.47		0.53	0.58		0.48	0.57		0.19	1.14		0.82	0.08		0.33	0.38	
Os01g67410	AP2	14775.1.S1_at	0	-0.94		0.08	-0.93		0.05	-2.55	D	0.04	-4.89	D	0.04	-2.06	D	0.12	-2.78		0.26	-1.35		0.14	-0.54	
Os01g73770	B1	55365.1.S1_at	0	-0.02		0.28	-0.29		0.40	-0.32		0.79	0.09		0.30	-0.16		0.21	0.45		0.25	0.15		0.82	0.14	
Os02g10760	A4	50235.2.S1_at	0	-0.13		0.74	0.07		0.67	-0.10		0.78	-0.13		0.21	-0.46		0.53	-0.02		0.59	0.23		0.07	0.41	
Os02g13710	B7	55643.1.S1_at	0	0.88		0.06	-1.48		0.05	-1.68	D	0.02	-3.48	D	0.10	-0.77		0.42	0.59		0.89	0.08		0.10	-0.75	
Os02g29550	B1	39004.1.S1_at	0	0.32		0.18	0.50		0.44	0.36		0.08	-0.71		0.03	0.33		0.15	1.41		0.81	0.11		0.69	0.05	
Os02g32040	B4	32291.1.S1_at	0	-0.20		0.75	-0.27		0.37	-0.74		0.99	-0.01		0.05	-0.70		0.15	-0.84		0.49	-0.55		0.67	-0.41	
Os02g32140	B4	12318.1.S1_at	0	-0.13		0.92	0.04		0.01	5.19	U	0.04	5.67	U	0.82	0.09		0.64	0.18		0.61	0.27		0.60	0.11	
Os02g34260	B4	55316.1.S1_at	0	-1.23	D	0.03	-1.31	D	0.06	-1.27		0.53	0.51		0.11	-1.52		0.26	-1.06		0.07	-1.21		0.37	0.44	
Os02g34270	A4	6079.1.S1_at	0	-0.61		0.36	-0.37		0.22	-0.60		0.02	-3.49	D	0.15	-0.15		0.06	-0.42		0.32	-0.55		0.12	-0.96	
Os02g40070	AP2	49859.1.S1_at	0	-4.20	D	0.04	-4.32	D	0.04	-4.11	D	0.03	-4.30	D	0.00	-4.32	D	0.02	-4.37	D	0.02	-4.21	D	0.01	-4.41	D
Os02g42585	B3	54384.1.S1_at	0	0.57		0.20	1.32		0.88	0.08		0.05	-3.18		0.74	-0.23		0.27	-1.32		0.21	-0.68		0.30	-0.50	
Os02g43790	B3	10904.1.S1_at	0	0.07		0.50	0.37		0.13	1.74		0.09	1.99		0.18	0.59		0.48	-0.48		0.37	-0.40		0.77	0.21	
Os02g43820	A4	52763.1.S1_at	0	0.74		0.36	0.95		0.82	0.13		0.77	-0.23		0.81	0.17		0.46	-0.43		0.85	-0.06		0.35	0.41	
Os02g43940	A4	54929.1.S1_at	0	0.55		0.18	0.18		0.58	0.08		0.29	-0.55		0.25	-1.05		0.25	-1.74		0.41	-1.25		0.06	-1.45	
Os02g43970	A5	28045.1.S1_at	0	-1.21	D	0.01	-0.68		0.05	-1.21		0.09	-1.44		0.12	-1.73		0.06	-2.19		0.43	-0.71		0.01	-1.01	D
Os02g45420	A1	9146.1.S1_at	0	-0.56		0.59	-0.18		0.30	0.66		0.22	0.82		0.97	-0.02		0.27	-0.87		0.29	-0.13		0.56	-0.36	
Os02g45450	A6	51078.1.S1_at	0	1.56		0.31	-2.40		0.01	1.19	U	0.57	-1.13		0.22	4.20		0.14	-1.07		0.64	-1.10		0.56	-1.68	
Os02g51300	AP2	47948.1.S1_at	0	1.70		0.07	2.08		0.60	-0.45		0.18	-1.98		0.31	1.10		0.41	-0.95		0.12	-2.01		0.13	-1.97	
Os02g51670	B4	6076.1.S1_at	0	0.92		0.36	1.20		0.46	0.98		0.33	-1.04		0.61	0.51		0.32	-1.06		0.85	0.13		0.57	0.44	
Os02g52670	A5	54944.1.S1_at	0	-0.79		0.08	-3.22		0.20	-2.03		0.19	-2.55		0.87	-0.16		0.02	-3.35	D	0.08	-2.50		0.08	-2.20	
Os02g54050	B2	53362.2.S1_at	0	0.12		0.00	-0.29		0.31	0.19		0.02	0.03		0.18	-0.23		0.26	-0.15		0.80	0.09		0.32	-0.31	
Os02g54160	A3	14181.1.S1_at	0	-0.67		0.29	-1.15		0.06	-2.65		0.21	-3.73		0.16	-2.88		0.02	-2.18	D	0.46	-0.52		0.18	0.91	
Os02g55380	B3	55579.1.S1_at	0	-4.52	D	0.07	-3.01		0.03	-5.72	D	0.07	-5.48		0.02	-5.56	D	0.06	-5.87		0.11	-5.59		0.13	-4.67	
Os03g05590	A2	55258.1.S1_at	0	-0.25		0.37	-0.55		0.48	-0.54		0.01	0.11		0.33	-0.25		0.52	-0.30		0.56	-0.26		0.93	-0.06	
Os03g07830	B3	32557.1.S1_at	0	0.33		0.03	0.60		0.88	0.06		0.09	-2.06		0.12	0.22		0.09	-0.44		0.13	0.25		0.05	1.35	
Os03g07940	AP2	8271.1.S1_at	0	1.43		0.05	2.02		0.18	0.85		0.08	-0.65		0.05	0.54		0.35	0.11		0.03	-1.78	D	0.25	-1.42	

Continue

Os03g08460	B2	3876.2.S1_x_at	0	1.33		0.24	1.28		0.44	0.61		0.55	-0.28		0.52	0.39		0.37	-0.76		0.93	-0.03		0.56	0.24	
Os03g08470	B3	6009.3.S1_a_at	0	-2.46		0.20	-2.14		0.66	0.44		0.06	3.27		0.05	3.94	U	0.10	4.10		0.15	3.10		0.75	-0.35	
Os03g08490	B2	23028.1.S1_s_at	0	-0.28		0.38	-0.82		0.05	2.63	U	0.05	2.49	U	0.05	2.26	U	0.61	-0.08		0.03	2.14	U	0.03	2.71	U
Os03g08500	A6	12889.2.S1_at	0	0.31		0.60	-0.18		0.80	-0.09		0.95	0.03		0.78	-0.04		0.56	0.20		0.77	-0.20		0.48	0.66	
Os03g09170	A5	38849.1.S1_at	0	2.04	U	0.08	3.86		0.05	4.51		0.50	0.50		0.10	5.14		0.12	2.68		0.22	1.86		0.89	0.07	
Os03g12950	AP2	54629.1.S1_at	0	3.17		0.12	2.94		0.29	-1.46		0.13	-1.59		0.10	-2.11		0.00	-1.80	D	0.12	-1.20		0.19	-1.26	
Os03g15660	B2	35111.1.S1_at	0	-0.99		0.09	-1.49		0.27	-1.54		0.15	-1.60		0.09	-1.70		0.14	-1.91		0.16	-1.47		0.11	-1.72	
Os03g19900	AP2	56314.1.S1_at	0	-1.82	D	0.04	-1.84	D	0.03	-2.18	D	0.03	-2.12	D	0.34	1.06		0.85	0.10		0.80	-0.04		0.18	0.90	
Os03g22170	B1	57381.1.S1_at	0	-3.76		0.10	-3.76		0.12	-3.66		0.00	-3.32	D	0.13	-3.36		0.72	0.89		0.34	1.22		0.93	0.09	
Os03g60120	B3	8951.1.S1_x_at	0	-1.36	D	0.07	-1.67		0.07	-1.06		0.10	-0.46		0.08	-0.96		0.09	-0.98		0.08	-1.22		0.17	-0.76	
Os03g60430	AP2	27471.1.S1_at	0	-0.14		0.50	0.60		0.77	0.27		0.21	-1.94		0.96	-0.03		0.81	-0.20		0.07	-0.75		0.57	0.59	
Os03g64260	B3	12966.1.S1_at	0	0.32		0.47	0.29		0.01	0.47		0.12	0.22		0.74	0.07		0.21	-0.11		0.37	0.14		0.90	-0.03	
Os04g32620	B1	9523.1.S1_at	0	-3.01	D	0.03	-3.11	D	0.01	-2.93	D	0.04	-2.86	D	0.15	-1.46		0.01	-1.39	D	0.15	-1.45		0.25	-1.61	
Os04g32790	B4	38106.1.S1_at	0	-0.25		0.22	0.89		0.09	1.79		0.05	2.86		0.26	0.58		0.68	0.08		0.88	0.06		0.86	0.06	
Os04g34970	A4	54932.1.S1_at	0	-1.00		0.26	-0.72		0.16	-0.80		0.08	-0.76		0.14	-0.82		0.14	-0.95		0.64	-0.15		0.49	0.97	
Os04g42570	AP2	55217.1.S1_at	0	1.12		0.14	1.59		0.18	-0.73		0.23	-0.87		0.21	-0.56		0.11	-1.95		0.03	-2.04	D	0.12	-2.15	
Os04g44670	B3	6539.1.S1_at	0	-0.10		0.25	1.04		0.93	-0.06		0.93	0.09		0.66	0.10		0.04	-0.40		0.21	1.01		0.05	-1.65	
Os04g46240	B5	5646.1.S1_at	0	-0.14		0.23	-0.54		0.04	0.28		0.15	-0.38		0.87	-0.02		0.35	-0.17		0.08	-0.62		0.03	-0.36	
Os04g48350	B1	10507.1.S1_at	0	-0.47		0.12	0.48		0.05	-2.14	D	0.05	-5.11		0.05	-2.91		0.06	-4.63		0.02	-5.54	D	0.01	-5.18	D
Os04g52090	A5	23103.1.S1_at	0	1.55		0.45	-0.52		0.25	-0.66		0.06	-1.02		0.41	0.57		0.48	-0.43		0.42	-0.53		0.59	-0.53	
Os04g55520	A3	51638.1.S1_s_at	0	-2.13	D	0.01	-1.75	D	0.03	2.23	U	0.78	-0.16		0.13	1.80		0.26	-0.28		0.02	-0.77		0.21	-1.16	
Os04g55560	AP2	9874.1.S1_at	0	0.60		0.71	0.17		0.28	1.49		0.06	0.78		0.22	0.87		0.39	0.66		0.64	0.20		0.37	0.22	
Os04g57340	B1	28406.1.S1_at	0	-0.10		0.82	0.13		0.12	0.96		0.37	1.37		0.39	1.62		0.02	2.97	U	0.02	2.04	U	0.52	0.76	
Os05g03040	AP2	46461.1.A1_at	0	-0.73		0.74	-0.21		0.99	0.01		0.24	-1.40		0.74	0.24		0.01	1.64	U	0.90	0.12		0.27	-0.70	
Os05g25260	A2	56944.1.S1_at	0	1.41		0.23	0.73		0.34	-0.65		0.07	-2.21		0.09	-0.27		0.13	-1.47		0.21	0.31		0.45	0.66	
Os05g27930	A2	7718.1.S1_at	0	-0.09		0.33	0.47		0.96	0.01		0.14	-1.06		0.17	-0.40		0.88	0.05		0.33	0.95		0.11	0.40	
Os05g29810	B7	4893.1.S1_at	0	1.27	U	0.26	-0.11		0.01	-3.22	D	0.00	-6.75	D	0.09	-2.28		0.05	-1.54		0.10	-0.42		0.44	-0.60	
Os05g32270	A5	49193.1.S1_at	0	3.64	U	0.06	3.73		0.13	1.38		0.96	-0.01		0.20	1.65		0.40	-0.80		0.65	0.11		0.06	1.39	
Os05g37640	A2	51802.1.S1_at	0	-0.47		0.64	-0.22		0.70	-0.35		0.11	2.08		0.72	-0.20		0.72	0.27		0.18	1.08		0.21	0.63	
Os05g39590	B5	10500.1.S1_at	0	1.34		0.71	-0.17		0.20	-0.60		0.11	-1.44		0.48	0.28		0.47	0.16		0.28	0.59		0.72	-0.41	
Os05g41760	B1	51998.1.S1_at	0	-0.50		0.29	-0.32		0.48	-0.34		0.24	-0.82		0.20	-0.90		0.11	-0.83		0.79	0.14		0.21	0.94	
Os05g41780	B3	5532.1.S1_at	0	1.65		0.03	2.51	U	0.08	1.29		0.36	0.83		0.04	2.34	U	0.29	1.14		0.07	-1.51		0.12	-1.31	
Os05g45954	AP2	54786.1.S1_at	0	0.88		0.99	0.00		0.71	-0.19		0.95	-0.03		0.55	-0.25		0.84	-0.10		0.98	0.01		0.63	-0.27	
Os05g47650	RAV	31989.1.S1_at	0	-0.54		0.09	-0.74		0.01	-0.83		0.15	0.72		0.07	-1.06		0.11	-1.54		0.13	-1.67		0.01	-1.72	D
Os05g49700	A1	57303.1.S_at	0	0.89		0.15	1.89		0.16	-0.48		0.98	0.05		0.57	-0.44		0.38	-0.74		0.07	-1.34		0.01	-1.28	D
Os06g03670	B3	23123.1.S2_at	0	-0.16		0.98	-0.01		0.37	-0.34		0.01	-0.63		0.21	-0.39		0.52	-0.35		0.32	-0.36		0.63	-0.26	
Os06g05340	AP2	52397.1.S1_at	0	0.31		0.09	1.11		0.29	0.09		0.01	0.63		0.08	0.38		0.22	0.74		0.11	0.79		0.00	0.81	
Os06g06540	A1	31624.1.S1_at	0	-0.18		0.16	0.08		0.69	-0.15		0.05	0.80		0.61	-0.08		0.57	0.17		0.43	0.20		0.65	-0.09	
Os06g06970	A5	6789.1.S1_at	0	2.56		0.06	2.25		0.17	0.85		0.05	-0.76		0.19	0.97		0.33	0.80		0.20	0.44		0.51	0.23	
Os06g07030	A3	4804.1.S1_at	0	-2.73		0.02	-1.46	D	0.03	-2.78	D	0.37	-1.53		0.37	-0.92		0.34	0.40		0.06	1.78		0.01	2.64	U
Os06g08340	B2	15722.1.S1_s_at	0	0.80		0.36	0.31		0.85	-0.10		0.08	1.16		0.35	0.20		0.54	0.46		0.18	-0.59		0.34	-0.88	
Os06g09390	A5	14664.1.A1_at	0	0.05		0.89	-0.09		0.69	-0.35		0.15	1.57		0.87	-0.07		0.57	0.27		0.66	-0.47		0.40	-0.43	
Os06g09760	A5	5599.1.S1_at	0	2.57	U	0.70	0.08		0.03	-1.51	D	0.56	-0.58		0.13	0.62		0.42	-0.50		0.12	0.52		0.01	0.82	

Continue

Os06g11860	A5	10845.1.S1_s_at	0	2.07	U	0.09	1.54		0.08	0.78		0.36	1.01		0.07	0.54		0.21	-0.34		0.00	-2.74	D	0.07	-2.64	
Os06g36000	A3	12997.1.S1_at	0	-0.33		0.72	-0.17		0.79	0.08		0.98	0.00		0.59	-0.49		0.29	-1.30		0.39	-0.70		0.95	-0.05	
Os06g40150	B3	51150.1.S1_at	0	0.36		0.49	0.54		0.87	-0.09		0.13	-1.13		0.23	1.30		0.09	1.12		0.19	-1.00		0.51	-1.15	
Os06g43220	AP2	50002.1.S1_at	0	0.63		0.03	1.82	U	0.04	2.11	U	0.06	0.87		0.03	1.80	U	0.11	2.15		0.10	1.12		0.00	0.25	
Os06g44750	AP2	21891.1.S1_at	0	-2.35		0.15	-2.87		0.11	-3.16		0.09	-2.49		0.10	-2.52		0.20	-2.54		0.13	-2.47		0.05	-2.15	
Os06g47590	B7	30258.1.S1_at	0	0.09		0.91	-0.01		0.64	-0.10		0.22	0.10		0.25	-0.10		0.04	-0.09		0.36	0.17		0.95	-0.02	
Os07g03250	B6	56321.1.S1_at	0	0.86		0.61	0.45		0.13	-0.78		0.20	-0.61		0.18	-0.82		0.12	-0.75		0.29	-0.69		0.19	-0.78	
Os07g10410	B1	56201.1.S1_at	0	-0.09		0.42	-0.23		0.30	-0.17		0.17	-0.15		0.32	-0.31		0.35	-0.11		0.23	0.19		0.06	-0.24	
Os07g13170	AP2	31647.1.S1_at	0	0.22		0.79	-0.01		0.04	2.20	U	0.02	4.50	U	0.63	-0.08		0.18	-0.15		0.05	-0.16		0.17	-0.15	
Os07g22730	B3	30670.1.S1_at	0	-0.99		0.98	0.00		0.54	0.61		0.02	-1.14	D	0.22	2.33		0.33	-0.72		0.23	-0.78		0.73	0.81	
Os07g22770	B6	36817.1.S1_at	0	0.08		0.09	0.68		0.02	3.75	U	0.53	0.22		0.03	1.65	U	0.47	-0.12		0.28	-0.38		0.82	0.08	
Os07g42510	B1	37967.1.S1_at	0	2.18		0.21	2.34		0.24	2.71		0.16	-2.45		0.27	1.47		0.53	1.08		0.35	-2.36		0.03	-2.26	D
Os07g47330	B2	51144.1.S1_at	0	-0.04		0.06	-0.19		0.57	-0.13		0.66	0.37		0.43	0.37		0.27	1.18		0.02	3.53	U	0.68	0.66	
Os07g47790	B1	48988.1.S1_at	0	0.30		0.12	0.19		0.02	-0.46		0.04	-2.13	D	0.12	-0.24		0.21	-0.50		0.06	-0.90		0.01	-0.22	
Os08g07440	AP2	10323.1.S1_at	0	0.06		0.07	0.55		0.14	0.09		0.23	-0.38		0.32	0.28		0.12	0.58		0.05	0.39		0.08	1.06	
Os08g07700	B7	31056.1.S1_at	0	-1.17		0.10	-0.84		0.05	-1.58		0.07	-1.53		0.08	-1.10		0.00	-1.32	D	0.09	-1.74		0.01	-2.02	D
Os08g27220	A6	20018.2.S1_at	0	0.09		0.48	0.49		0.03	3.54	U	0.01	7.32	U	0.50	-0.16		0.75	0.11		0.57	0.53		0.00	1.36	U
Os08g31580	A5	6824.1.S1_at	0	0.24		0.06	1.10		0.05	2.16		0.06	1.74		0.07	1.58		0.14	0.87		0.34	0.61		0.65	0.22	
Os08g34360	AP2	49821.3.A1_at	0	0.03		0.43	0.15		0.08	-0.64		0.44	-0.24		0.11	-0.73		0.28	-0.64		0.15	-0.72		0.51	-0.54	
Os08g36920	B7	15849.1.S1_s_at	0	4.46		0.89	-0.11		0.76	-0.41		0.14	-1.02		0.91	0.09		0.09	-0.66		0.65	0.39		0.07	-0.93	
Os08g41030	B4	55500.1.S1_at	0	-0.20		0.04	-0.60		0.19	0.59		0.32	0.20		0.19	5.80		0.01	8.63	U	0.17	4.67		0.17	2.20	
Os08g42550	RAV	49952.1.S1_at	0	-0.14		0.73	0.30		0.95	-0.04		0.10	0.51		0.10	-0.36		0.74	-0.27		0.30	-0.06		0.41	-0.44	
Os08g43200	A1	25479.1.S1_at	0	1.51		0.13	2.10		0.44	0.57		0.95	-0.04		0.38	0.41		0.79	0.21		0.21	-1.26		0.36	-0.30	
Os08g45110	A1	49350.1.S1_at	0	0.39		0.07	1.97		0.42	0.55		0.08	2.64		0.75	0.14		0.60	0.40		0.33	0.74		0.44	1.03	
Os09g11460	B3	5237.1.S1_at	0	-0.44		0.15	-0.58		0.78	-0.09		0.03	1.16	U	0.03	-0.57		0.16	-0.43		0.07	-0.58		0.23	-0.36	
Os09g11480	B2	19325.1.S1_at	0	-0.30		0.19	0.66		0.11	1.19		0.02	-1.15	D	0.18	0.56		0.64	0.29		0.95	-0.01		0.83	-0.11	
Os09g13940	B7	11725.1.S1_at	0	-1.42		0.45	0.35		0.24	1.38		0.36	0.67		0.08	-0.42		0.58	-0.37		0.38	0.16		0.00	0.77	
Os09g20350	A6	11944.1.S2_s_at	0	1.87		0.33	0.23		0.06	-0.80		0.51	-0.38		0.05	0.58		0.65	0.10		0.08	-0.58		0.35	-0.32	
Os09g25600	AP2	20663.1.S1_at	0	0.08		0.74	0.05		0.47	0.28		0.62	0.17		0.70	0.17		0.21	-0.23		0.51	-0.18		0.63	0.39	
Os09g26420	B2	51487.1.S1_at	0	0.27		0.05	-0.09		0.65	-0.40		0.38	0.38		0.84	-0.08		0.67	-0.33		0.72	-0.09		0.95	0.04	
Os09g28440	B4	52451.1.A1_at	0	3.85		0.10	-2.20		0.13	-1.20		0.15	-1.71		0.08	-1.09		0.04	-2.22	D	0.12	-2.17		0.04	-2.19	D
Os09g39850	B3	54835.1.S1_s_at	0	-0.38		0.14	-0.39		0.39	-0.34		0.47	-0.04		0.04	-0.26		0.95	-0.02		0.01	0.50		0.88	-0.04	
Os10g25170	B1	56665.1.A1_at	0	-0.35		0.34	-0.59		0.76	0.09		0.11	-0.75		0.36	0.42		0.50	0.68		0.06	0.31		0.12	-0.31	
Os10g38000	A5	55877.1.S1_at	0	0.37		0.08	1.18		0.30	-0.11		0.32	0.08		0.33	0.82		0.28	0.80		0.71	-0.16		0.40	0.33	
Os11g19060	AP2	51993.1.S1_at	0	0.25		0.51	-0.15		0.89	0.00		0.81	-0.07		0.89	-0.02		0.48	-0.16		0.69	-0.08		0.95	0.02	

R = Regulation; U = Up-regulated; D = Down-regulated; S =Seed (CK); PV = P value; FC = Fold change; Phg = Phylogentic subgroups or subfamilies.

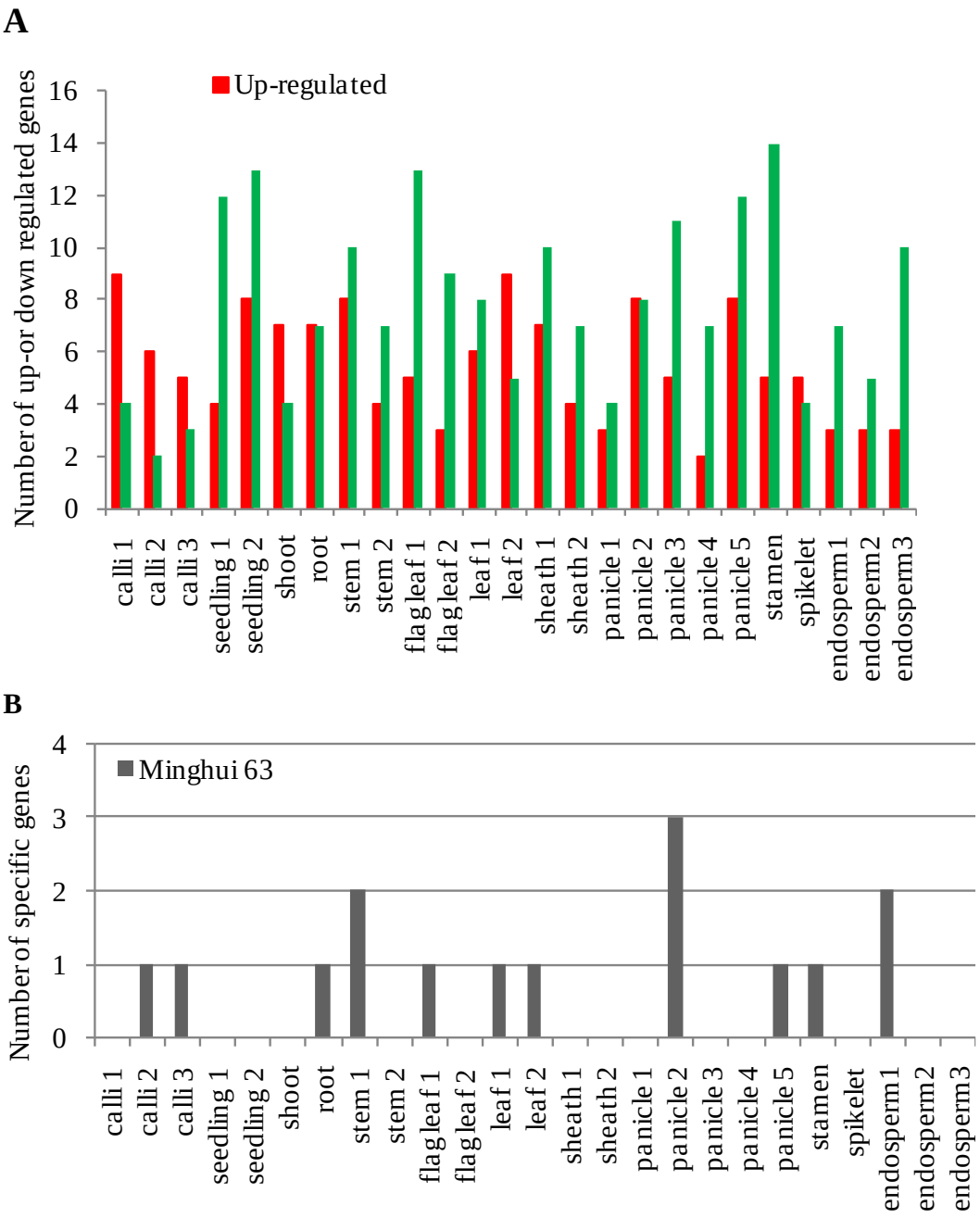

Fig. 12 (A) Number of up- or down-regulated genes in different tissues under normal conditions in Minghui 63 are presented. (B) Tissue-specific up-regulated genes in Minghui 63: calli 2, *Os02g32040*; calli 3, *Os06g44750*; root, *Os04g44670*; stem 1, *Os02g51300* and *Os09g28440*; flag leaf 1, *Os08g45110*; leaf 1, *Os09g13940*; leaf 2, *Os08g42550*; panicle 2, *Os03g12950*, *Os05g25260*, and *Os08g36920*; panicle 5, *Os02g45450*; stamen, *Os09g11460*, and endosperm 1, *Os05g03040* and *Os08g41030*. The number of genes is shown on the *Y*-axis, and the different developmental stages are indicated on the *X*-axis. Tissue-specific up-regulated genes are shown in **Table 14**.

7.2. Responses of *OsAP2/EREBP* genes to GA3, KT, NAA, SA, JA, and ABA treatments

Phytohormones play a critical role in plant growth and development. To investigate the response of *OsAP2/EREBP* genes to phytohormone treatment, microarray analysis was performed. To understand the expression patterns of the *OsAP2/EREBP* gene family, data were extracted from the NCBI database as described previously. The Minghui 63 rice cultivar was used for microarray expression analysis following three different hormonal treatments of plants at the seedling stage. We identified a total of 21 redundant *OsAP2/EREBP* genes out of 116 from Gene Chip that were differentially expressed following treatments with one or more of the phytohormone GA3, KT, and NAA in the Minghui 63 genotypes compared with the control (**Fig. 13**). The fold-change values of differentially expressed genes in the seedling stage with respect to the control (CK, untreated sample) are shown in **Table 15**. Of these 116 genes, 18 genes were up-regulated, whereas three genes were down-regulated following different hormone treatments. Interestingly, two genes (*Os02g51300* and *Os05g03040*, subfamily AP2) were up-regulated following treatment with all three phytohormones. For instance, the *Os03g60430* gene of the AP2 subfamily was up-regulated specifically by NAA treatment of Minghui 63, and the *Os06g40150* gene of the B3 subgroup of the ERF subfamily was up-regulated by GA3 and NAA treatments in seedlings (**Table 15**). Furthermore, 12 DEGs were found between the SA and ABA treatments, whereas 41, 54, 63, and 57 redundant DEGs were observed following JA treatment at four different time points (**Table 15**). We observed 24 up- or down-regulated genes that were common to all the time points in JA-treated plants. The gene expression profiles of up- or down-regulated genes following treatment of Minghui 63 with different phytohormones were compared in the root, leaf, and panicle of the NILs under severe and mild drought stress conditions.

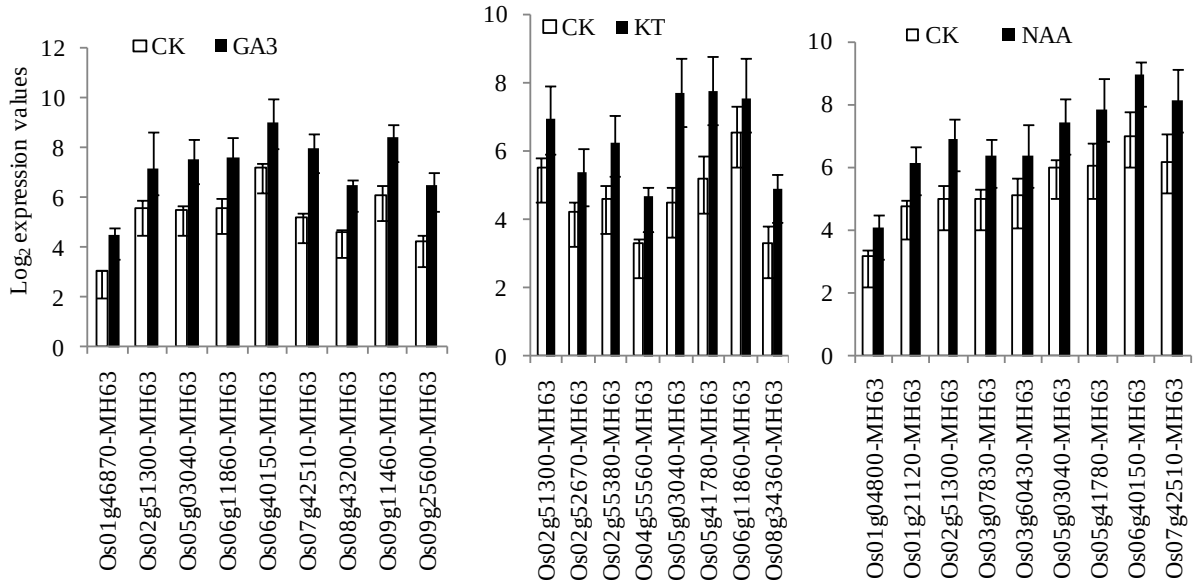

Fig. 13 Expression profiles of differentially expressed *OsAP2/EREBP* genes in 7-day old seedlings subjected to three phytohormones (GA3= gibberellic acid, KT= kinetin, and NAA= naphthalene acetic acid). *X-axis* represents differentially expressed genes. *Y-axis* represents log_2 expression values. Each treatment is indicated at the top of each diagram. CK= control.

7.3. Gene expression patterns in different rice genotypes under different stress conditions

IR64 and two NILs, IR77298-14-1-2-B-10 and IR77298-14-1-2-B-13, were used in this study. In these NILs, gene identification and the determination of gene expression patterns are important to understand the molecular basis of plant responses and to improve drought stress tolerance in the progeny from a common genetic background using transgenic techniques. The three water regimes used were severe stress (0.2 FTSW), mild stress (0.5 FTSW), and control (1.0 FTSW), and gene expression in the root, leaf, and panicle tissues of the two NILs and IR64 at the reproductive stage were examined. Therefore, the goals of this study are to identify DEGs in IR77298-14-1-2-B-10 versus IR77298-14-1-2-B-13 and IR64, to determine the number of DEGs that are specifically induced in each NIL, to determine the

number of DEGs that are common to both NILs under both DSCs in multiple tissues, and to identify putative drought responsive genes in the drought-tolerant line for further functional analysis. Altogether, the results of the expression patterns and analyses indicate the involvement of this large gene family in a number of signaling pathways that are triggered by the drought response.

The physical appearance of the plants, such as plant height, transpiration, flowering, the total biomass (root, shoot, and panicle) at harvest, the spikelet fertility score and the grain yield were recorded for the IR77298-14-1-2-B family (Moumeni et al. 2011; Venuprasad et al. 2011). The drought-tolerant NIL in the IR77298-14-1-2-B family exhibited a significantly higher grain yield compared with the drought-susceptible NIL under drought-stress but not under non-stress conditions (Venuprasad et al. 2011). We found 162 out of a possible 163 *OsAP2/EREBP* genes in our 44K array data. Among these genes, 140 were differentially expressed under both DSCs in different experimental tissues. A gene was defined as preferentially responsive if the signal values of the gene in this tissue increased more than 2-fold and had a *P* value less than 0.05 compared with the control (**Table 16**). We compared gene expression profiles of three rice genotypes under two DSCs. **Table 17** shows the number of DEGs obtained from microarray experiments using tissues from the different rice genotypes under both DSCs.

Table 15. Results of differential expression analysis in 7 day-old seedlings subjected to three phytohormones (GA3, KT, and NAA) treatments in Minghui 63, and another three (SA, JA, and ABA) treated in seedlings of rice. Differential expression genes have been taken p value less than 0.05 and fold change >0.585 or <-0.585, which are described by "U" or "D". When fold change >0.585, regulation is up, and when fold change <-0.585, regulation is down. [CK= untreated sample].

Gene Name	Phg	GA3 Vs Seedling			KT Vs Seedling			NAA Vs Seedling			SA			JA								ABA	
		PV	FC	DEG	PV	FC	DEG	PV	FC	DEG	PV	FC	DEG	6h	DEG	12h	DEG	24h	DEG	48h	DEG		DEG
Os01g04020	A2	0.579	0.201		0.42	0.195		0.99	0.003		0.863	0.374		-0.51		**-0.88**	**D**	**-1.41**	**D**	**-1.61**	**D**	**0.768**	**U**
Os01g04750	RAV	0.394	0.818		0.35	0.772		0.466	0.744					**1.058**	**U**	-0.06		-0.11		-0.4			
Os01g04800	RAV	0.155	0.522		0.02	0.46		**0.042**	**0.644**	U	0.479	-0.05		-0.56		-0.38		0		0			
Os01g07120	A4	0.092	0.716		0.24	0.785		0.135	0.281		0.673	0.042		**0.934**	**U**	**1.583**	**U**	**1.231**	**U**	**0.976**	**U**		
Os01g10370	B1	0.461	0.226		0.53	0.529		0.392	0.452		0.408	0.246		**1.23**	**U**	**1.373**	**U**	**1.613**	**U**	0.33			
Os01g12440	B2	0.29	0.157		0.34	0.132		0.181	0.089		0.909	0.08		-0.38		**1.184**	**U**	**-1.53**	**D**	0			
Os01g21120	B1	0.153	1.524		0.35	1.017		**0.012**	**1.414**	U	0.277	0.723		**1.325**	**U**	**1.748**	**U**	**2.926**	**U**	**5.131**	**U**	0.3	
Os01g46870	B3	**0.011**	**0.894**	U	0.35	0.621		0.056	0.847					**-1.16**	**D**	**-0.9**	**D**	**-0.79**	**D**	**-1.02**	**D**		
Os01g49830	RAV	0.028	0.193		0.61	0.23		0.21	0.038		0.488	0.351		**0.996**	**U**	0.281		**0.972**	**U**	**1.535**	**U**		
Os01g54890	B1	0.645	0.368		0.49	0.739		0.817	0.145					**0.96**	**U**	**0.908**	**U**	**-1.2**	**D**	-0.37			
Os01g58420	B4	0.127	-0.54		0.05	-0.5		**0.045**	**-0.86**	D				0.404		-0.04		**0.956**	**U**	**-1.26**	**D**	0.511	
Os01g59780	AP2	0.049	0.455		0.14	0.583		0.304	0.546		0.669	-0.08		**0.859**	**U**	**2.86**	**U**	**1.505**	**U**	**1.779**	**U**		
Os01g67410	AP2	0.17	0.376		0.41	0.066		0.806	0.118					**1.39**	**U**	**3.07**	**U**	**2.467**	**U**	**2.443**	**U**		
Os01g73770	B1	0.101	-0.77		0.01	-0.4		0.08	-0.41		0.431	-0.17		**-0.8**	**D**	**-0.83**	**D**	**-1.19**	**D**	**-0.68**	**D**		
Os02g10760	A4	0.708	0.092		0.16	0.349		0.53	0.286		0.191	-0.63		**0.89**	**U**	-0.16		0.117		-0.11			
Os02g13710	B7	0.642	-0.22		0.42	-0.23		0.268	-0.53		**0.008**	**-0.96**	**D**	-0.01		0.444		**-0.89**	**D**	**0.743**	**U**		
Os02g29550	B1	0.263	0.221		0.43	-0.15		0.534	0.219		0.607	-0.06		**-1.66**	**D**	**-2.29**	**D**	**-1.72**	**D**	**-2.25**	**D**	**-0.67**	**D**
Os02g32040	B4	0.211	0.189		0.25	0.509		0.943	-0.01		0.679	0.094		0.121		0		0		0			
Os02g32140	B4	0.574	-0.12		0.64	-0.07		0.494	-0.34		0.728	0.111		0		0		0		0		-0.55	
Os02g34260	B4	0.74	-0.05		0.03	0.456		0.942	0.008		0.269	-0.23		**0.604**	**U**	-0.56		-0.47		**-2.07**	**D**		
Os02g34270	A4	0.354	0.228		0.9	-0.02		0.761	0.042		0.765	-0.02		-0.02		0.301		-0.13		-0.02		**1.047**	**U**
Os02g40070	AP2	0.07	0.572		0.3	0.482		0.198	0.599		0.728	0.082		-0.27		0		0.314		0.521			
Os02g42585	B3	0.356	0.515		0.47	0.34		0.395	0.443		0.284	-0.28		-0.08		-0.23		-0.56		-0.46			
Os02g43790	B3	0.093	0.834		0.15	0.849		0.079	0.702					0.335		**1.023**	**U**	**0.629**	**U**	0.108			
Os02g43820	A4	0.765	0.139		0.41	0.421		0.551	0.351					0.528		0.424		0.094		0.284			
Os02g43940	A4	0.582	-0.52		0.56	-0.49		0.409	-0.6		0.059	-2.77		**1.523**	**U**	**4.281**	**U**	**3.622**	**U**	**3.241**	**U**		
Os02g43970	A5	0.415	0.089		0.8	0.032		0.403	-0.08		0.423	-0.06		0.246		**-0.68**	**D**	-0.35		-0.32			
Os02g45420	A1	0.037	0.381		0.11	0.426		0.115	0.404		0.158	-0.21		0.042		0.182		**0.899**	**U**	0.313			
Os02g45450	A6	0.558	0.251		0.28	2.152		0.492	0.545		0.064	2.358		0		0		**2.466**	**U**	**0.625**	**U**		
Os02g51300	AP2	**0.044**	**1.639**	U	**0.04**	**1.419**	U	**0.023**	**1.389**	U				0.032		0		0		0		-0.13	
Os02g51670	B4	0.809	-0.03		0.87	0.076		0.776	-0.05					**1.081**	**U**	**1.06**	**U**	**1.699**	**U**	**1.639**	**U**		
Os02g52670	A5	0.305	-0.07		**0.04**	**1.192**	U	0.681	-0.25		0.954	-0.02		**2.068**	**U**	**2.188**	**U**	**2.036**	**U**	**1.528**	**U**		

Continue

Os02g54050	B2	0.118	-0.46		0.43	-0.29		0.141	-0.3		0.238	0.17		0		0		-0.37		0						
Os02g54160	A3	0.647	0.222		0.25	0.43		0.491	0.26					0.409		0.014		-0.02		-0.38						
Os02g55380	B3	0.407	0.335		**0.01**	**0.668**	U	0.028	0.438		0.402	0.09		-0.11		**-0.72**	**D**	0		**-0.86**	**D**	-0.05				
Os03g05590	A2	0.306	-0.2		0.92	-0.01		0.932	0.009		0.439	0.171		0		0.549		**0.798**	**U**	**1.407**	**U**					
Os03g07830	B3	0.104	0.58		0.15	0.787		**0.038**	**0.701**	U				-0.01		0.327		-0.3		-0.19						
Os03g07940	AP2	0.485	0.277		0.47	0.272		0.102	0.155		0.185	-0.47		-0.45		0.121		0.195		0.028						
Os03g08460	B2	0.183	0.287		0.15	0.3		0.232	0.314					-0.08		-0.15		-0.4		0.02						
Os03g08470	B3	0.453	-0.09		0.41	0.075		0.585	0.114		**0.01**	**0.588**	**U**	**1.125**	**U**	**3.181**	**U**	**4.596**	**U**	**4.389**	**U**					
Os03g08490	B2	0.495	0.386		0.5	0.349		0.927	0.06		**0.002**	**1.753**	**U**	**1.563**	**U**	-0.17		**-0.77**	**D**	**-1.5**	**D**					
Os03g08500	A6	0.174	-0.66		0.62	-0.34		0.108	-0.52		0.316	0.588		0.411		-0.02		0.236		0.552						
Os03g09170	A5	0.557	0.122		0.67	0.18		0.773	0.064		0.136	0.381		-0.17		**0.745**	**U**	0		**-0.65**	**D**					
Os03g12950	AP2	0.32	0.808		0.43	0.784		0.34	0.715		0.469	0.072		0.165		0		0		0						
Os03g15660	B2	0.322	0.323		0.06	0.309		0.106	0.279					-0.26		-0.31		**1.005**	**U**	0.186						
Os03g19900	AP2	0.834	-0.04		0.59	0.051		0.989	-0		0.72	0.069		**1.505**	**U**	**0.863**	**U**	0		0.034						
Os03g22170	B1	0.193	2.233		0.05	2.15		0.051	2.355		0.224	1.468		**2.796**	**U**	**2.659**	**U**	**3.07**	**U**	**4.013**	**U**	0.549				
Os03g60120	B3	0.95	-0.01		0.04	0.116		0.815	0.068					0.115		0.387		**0.661**	**U**	0.4						
Os03g60430	AP2	0.082	0.819		0.17	0.943		**0.009**	**0.718**	U	0.16	-0.33		-0.16		0.535		0.24		-0.2		-0.39				
Os03g64260	B3	0.604	0.356		0.69	-0.11		0.395	0.491					0.361		**-1.98**	**D**	**-0.65**	**D**	**-3.67**	**D**					
Os04g32620	B1	0.047	-0.11		0.98	-0		0.287	-0.22		**0.043**	**1.044**	**U**	**1.005**	**U**	**1.559**	**U**	**1.688**	**U**	**2.162**	**U**	0.132				
Os04g32790	B4	0.342	0.176		0.08	0.083		0.266	0.476		0.262	-0.5		**-1.01**	**D**	0.474		-0.39		0.018						
Os04g34970	A4	0.5	0.539		0.16	0.34		0.958	-0.02		0.457	0.04		**4.255**	**U**	**2.606**	**U**	**3.127**	**U**	**2.484**	**U**					
Os04g42570	AP2	0.129	1.211		0.17	1.233		0.134	1.199		0.174	0.384		-0.24		-0.01		-0.48		-0.35						
Os04g44670	B3	0.9	0.1		0.95	-0.05		0.5	0.415		**0.005**	**-1.47**	**D**	**0.615**	**U**	**-1.63**	**D**	0.473		**2.344**	**U**					
Os04g46240	B5	0.152	-0.52		0.14	-0.57		0.144	-0.31		0.079	-0.81		**1.425**	**U**	**2.591**	**U**	**1.068**	**U**	**1.732**	**U**					
Os04g48350	B1	0.143	0.425		0.04	0.442		0.307	0.225					**-1.06**	**D**	**-0.79**	**D**	**-3.3**	**D**	**-4.76**	**D**	-0.14				
Os04g52090	A5	0.424	0.103		0.95	0.035		0.36	-0.32		**0.005**	**1.939**	**U**	0.342		**1.243**	**U**	**1.236**	**U**	**0.615**	**U**	0.242				
Os04g55520	A3	0.658	0.207		0.64	0.322		0.262	0.476		0.216	-0.98		**1.978**	**U**	**4.802**	**U**	**1.574**	**U**	**2.464**	**U**					
Os04g55560	AP2	0.103	1.207		**0.01**	**1.019**	U	0.089	1.032		0.376	-0		-0.56		-0.38		**-2.16**	**D**	-0.43		0.293				
Os04g57340	B1	0.512	0.531		0.74	0.299		0.421	0.887		0.005	-0.45		0.13		**-1.32**	**D**	**-2.22**	**D**	**-3.21**	**D**					
Os05g03040	AP2	**0.012**	**1.092**	U	**0.04**	**1.235**	U	**0.003**	**0.958**	U	0.297	-0.4		-0.53		**-0.94**	**D**	**-1.91**	**D**	**-0.97**	**D**					
Os05g25260	A2	0.199	-0.66		0.16	-0.33		**0.026**	**-0.8**	D	0.685	0.726		-0.21		0.162		**-1.04**	**D**	-0.53						
Os05g27930	A2	0.822	0.057		0.61	0.253		0.046	0.124					0.086		-0.53		-0.35		-0.07		0.357				
Os05g29810	B7	0.034	0.377		0.81	0.092		0.6	-0.22		0.609	-0.26		**2.036**	**U**	**3.906**	**U**	**5.183**	**U**	**3.845**	**U**					
Os05g32270	A5	0.129	0.481		0.03	0.398		0.023	0.254		0.391	-0.3		**-1.63**	**D**	**-2.87**	**D**	**-1.86**	**D**	**-2.02**	**D**					
Os05g37640	A2	0.735	-0.08		0.75	-0.08		0.958	0.024		0.896	-0.09		-0.54		**-0.62**	**D**	**-0.95**	**D**	**-1.37**	**D**					
Os05g39590	B5	0.528	0.186		0.13	0.411		0.427	0.311		**0.049**	**0.93**	**U**	0.191		0.33		**0.653**	**U**	**0.661**	**U**					
Os05g41760	B1	0.366	-0.17		0.04	-0.07		0.582	-0.08					0.321		**1.444**	**U**	0.175		0.106						
Os05g41780	B3	0.073	0.463		**0.04**	**0.601**	U	**0.009**	**0.638**	U				-0.01		**1.095**	**U**	**2.335**	**U**	**2.874**	**U**	0.294				
Os05g45954	AP2	0.824	0.135		0.71	0.166		0.86	0.126		0.008	0.162		-0.44		-0.55		**-1.94**	**D**	**-4.71**	**D**					
Os05g47650	RAV	0.39	0.488		0.52	0.525		0.245	0.449		0.421	-0.01		**0.721**	**U**	**1.125**	**U**	**1.053**	**U**	**1.319**	**U**					
Os05g49700	A1	0.439	0.218		0.46	0.246		0.445	0.157		0.157	-0.63		**-0.85**	**D**	**0.586**	**U**	0.527		-0.12						
Os06g03670	B3	0.261	0.297		0.16	0.342		0.124	0.236		0.62	-0.01		0.209		0.539		0.285		0.398		0.351				
Os06g05340	AP2	0.452	-0.34		0.86	0.091		0.53	-0.32					0.089		0		0		0						
Os06g06540	A1	0.549	0.162		0.02	0.416		0.24	0.356		0.737	0.153		0.058		0		-0.12		**0.98**	**U**					

Continue

Os06g06970	A5	0.303	0.093		0.99	-0		0.484	0.12		0.025	0.531		-0.13		0.441		-0.54		**-0.97**	**D**	0.203
Os06g07030	A3	0.817	-0.08		0.57	-0.33		0.772	-0.27		0.107	1.081		**0.588**	**U**	0.396		-0.56		-0.11		0.011
Os06g08340	B2	0.446	-0.17		0.07	-0.35		0.537	-0.12		0.11	-0.48		**-0.7**	**D**	-0.15		**-0.95**	**D**	**-0.68**	**D**	
Os06g09390	A5	0.05	0.499		0.23	0.637		0.066	0.3					-0.01		-0.18		-0.27		-0.21		
Os06g09760	A5	0.022	-0.47		0.14	-0.62		0.31	-0.58					-0.12		0.51		-0.44		**-0.67**	**D**	
Os06g11860	A5	**0.015**	**1.073**	U	**0.04**	**0.994**	U	0.146	1.076		**0.004**	**0.915**	U	-0.18		**0.637**	**U**	**0.877**	**U**	**0.588**	**U**	
Os06g36000	A3	0.927	-0.01		0.9	0.06		0.686	0.1					0.037		0.038		-0.35		**-1**	**D**	
Os06g40150	B3	**0.005**	**1.133**	U	0.08	1.174		**0.005**	**1.06**	U	0.887	-0		0.459		0		0		0		
Os06g43220	AP2	0.021	-0.23		0.82	0.015		0.59	-0.03		0.793	0.101		**0.644**	**U**	**-1.72**	**D**	**-1.26**	**D**	**-1.61**	**D**	
Os06g44750	AP2	0.156	0.531		0.49	0.23		0.748	0.136		0.693	0.108		-0.12		-0.18		0		0		
Os06g47590	B7	0.616	-0.12		0.8	0.054		0.525	-0.16		0.356	-0.06		-0.52		**-1.68**	**D**	**-0.66**	**D**	**-0.81**	**D**	
Os07g03250	B6	0.038	0.525		0.12	0.782		0.009	0.505		0.031	-0.58		-0.36		**-0.82**	**D**	-0.41		-0.54		
Os07g10410	B1	0.067	0.057		0.51	0.407		0.6	0.088		**0.039**	**0.83**	**U**	0.06		0		-0.02		0		
Os07g13170	AP2	0.869	0.034		0.38	0.39		0.5	0.264		0.076	-1.05		0.358		-0.53		**-0.71**	**D**	**-1.94**	**D**	
Os07g22730	B3	0.149	-1.09		**0.04**	**-1.24**	D	0.237	-0.95		0.055	1.555		**2.59**	**U**	**1.457**	**U**	**-1.02**	**D**	-0.26		
Os07g22770	B6	0.264	-0.4		0.39	-0.21		0.395	-0.51		0.225	-0.75		**1.224**	**U**	0		0.336		-0.35		
Os07g42510	B1	**0.021**	**0.803**	U	0.09	0.962		**0.009**	**0.921**	U				**-1.72**	**D**	**-2.49**	**D**	**-2.4**	**D**	**-3.77**	**D**	0.149
Os07g47330	B2	0.134	-0.39		0.32	-0.38		0.271	-0.46		0.175	1.122		-0.16		0.011		**-2.37**	**D**	**-5.04**	**D**	
Os07g47790	B1	0.157	-0.14		0.95	0.01		0.229	-0.07		0.599	0.052		-0.06		0.378		-0.36		**-1.41**	**D**	
Os08g07440	AP2	0.32	-0.28		0.69	-0.13		0.372	-0.12		0.41	-0.07		-0.15		-0.17		**-0.65**	**D**	-0.24		
Os08g07700	B7	0.374	-0.34		0.97	0.021		0.629	-0.29		0.179	-0.74		0.423		**1.403**	**U**	**2.23**	**U**	0.573		
Os08g27220	A6	0.105	0.564		0.35	0.383		0.216	0.657					0.052		-0.01		-0.32		-0.5		
Os08g31580	A5	0.202	-0.4		0.33	-0.44		0.199	-0.37		0.16	-0.42		0.064		-0		**0.867**	**U**	0.463		
Os08g34360	AP2	0.15	0.42		**0.03**	**0.975**	U	0.158	0.792		0.874	0.079		0		**-0.75**	**D**	0		0		
Os08g36920	B7	0.463	0.111		0.49	1.219		0.872	-0.06		0.296	1.86		0.481		**2.06**	**U**	**5.69**	**U**	**3.259**	**U**	
Os08g41030	B4	0.158	-0.23		0.99	0.003		0.68	-0.1		0.361	-0.32		**0.659**	**U**	0.149		**0.794**	**U**	0		
Os08g42550	RAV	0.168	0.449		0.48	0.223		0.379	0.309		0.719	0.17		0.185		**0.845**	**U**	0.536		0.061		
Os08g43200	A1	**0.002**	**0.895**	U	0.17	0.857		0.084	0.92		0.11	-0.16		-0.36		-0.46		**-0.61**	**D**	-0.41		
Os08g45110	A1	0.02	0.552		0.05	0.701		0.16	0.638		0.162	-0.59		0.129		0.124		0.108		-0.04		
Os09g11460	B3	**0.009**	**0.586**	U	0.28	0.507		0.115	0.588					0.352		0.377		0.463		**0.682**	**U**	0.562
Os09g11480	B2	0.038	0.16		0.21	0.437		0.025	0.284		0.36	-0.02		**-0.8**	**D**	**-1.95**	**D**	**-1.14**	**D**	**-1.29**	**D**	
Os09g13940	B7	0.7	0.273		0.9	0.148		0.726	0.313		0.221	-0.2		-0.19		0.276		0.25		**0.621**	**U**	-0.16
Os09g20350	A6	0.403	0.128		0.09	0.25		0.624	-0.17		0.659	0.062		-0.32		0.533		-0.1		0.019		
Os09g25600	AP2	**0.03**	**1.259**	U	0.3	0.645		0.178	0.518		0.194	-0.16		0.514		**0.743**	**U**	-0.24		-0.22		
Os09g26420	B2	0.264	-0.59		0.48	-0.44		0.092	-0.6		**0.005**	**-0.87**	**D**	-0.55		**-0.63**	**D**	0.123		-0.37		
Os09g28440	B4	0.004	-0.54		0.41	-0.34		0.079	-0.74					**0.771**	**U**	**2.939**	**U**	**2.766**	**U**	**1.973**	**U**	
Os09g39850	B3	0.257	0.311		0.51	-0.13		0.174	0.209		0.47	0.424		0.079		0.392		0.433		-0.15		
Os10g25170	B1	0.613	0.239		0.49	0.122		0.823	0.223		0.605	0.069		**0.665**	**U**	**1.99**	**U**	**1.025**	**U**	**1.798**	**U**	
Os10g38000	A5	0.174	0.635		0.04	0.527		0.073	0.727		0.151	-0.31		-0.23		**1.481**	**U**	**1.096**	**U**	0.354		
Os11g19060	AP2	0.511	-0.15		0.44	-0.17		0.681	-0.04					0.507		-0.05		**-1.4**	**D**	-0.12		

PV = P value; FC= Fold change; DEG= Differential expression genes; Control (CK) = Seedling; U = Up-regulated; D = Down-regulated

Table 16. *OsAP2/EREBP* gene expression (up or down) profile under drought stress at different tissues of plant (44K oligoarray data).

MSU locus ID	Root severe			Root mild			Leaf severe			Leaf mild			Panicle severe			Panicle mild			Phg
	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	
Os01g66270	-3.01	-3.65	-3.89	-1.39	-1.13	-1.78	-1.77	-2.22	-3.22			-1.67				4.43	1.98	1.96	A1
Os02g45420							4.18	4.79	3.68					1.76	2.72				A1
Os04g46440		1.4	2.44	2.38	2.14	2.58		-1.02	-1.11				1.29	1.22	1.27				A1
Os05g49700	8.11	6	7.33	8.34	7.67	7.53	2.41	2.57	3.01	2.67	2.8	2.18	1.74	1.26	3.51	5.35	3.87	2.99	A1
Os06g06540														-1.14	-1.02				A1
Os08g43200																			A1
Os08g43210	-2.96	-6.52	-3.72	-4.56	-6.44	-4.97													A1
Os09g35010	-3.24	-2.83	-2.29		-1.2					1.14	1.19	-2.45	1.93	1.08	1.07	1.27	1.11		A1
Os09g35020	-3.21	-2.57	-2.57							1.34	1.62	-2.83							A1
Os09g35030	-1.91	-1.42										-2.11	2.35	1.99	1.37				A1
Os01g04020	2.05	1.86	1.69										1.36	1.52	1.55				A2
Os03g05590	-2.26	-1.35	-1.17	-1.52	-1.68	-2.61													A2
Os05g25260				1.29	1.1					1.27	1.22		2.13	1.85	2.23	2.25	1.5		A2
Os05g27930						1.02							1.07		1.03				A2
Os05g37640													-2.9		1.13				A2
Os08g45110	4.98	5	5.14	6.44	6.3	3.82					1.13	2.74	3.56	2.96	2.09	4.23	2.86	2.45	A2
Os02g54160	1.25	1.32	1.13		1.27		1.38	1.23	1.06	1.15	1.08								A3
Os04g55520		-1.35					-1.6	-1.57	-1.7							1.26			A3
Os06g07030							1.53	1.58	1.76		1.26		1.9	1.82	2.26	2.45	2.39	1.29	A3
Os06g36000						-1.12					-1.71		-1.85	-2.23	-2.04				A3
Os01g07120	1.61	1.52	1.06	1.12	1.39		2.74	3.67	2.8	1.54	2.24		2.05	2.43	1.44	2.07	1.88		A4
Os02g34270													3.34	3.97	3.6				A4
Os02g43820							2.5	2.36	2.91				2.21	2.62	2.66				A4
Os02g43940	-3.46	-2.61	-2.66	-2.38	-1.62	-2.69	-1.73	-2.05	-2.67	1.62			-2.2	-2.82	-1.73	-1.64	-2.73		A4
Os04g34970	2.89	2.36	2.46	2.71	2.93	2.3	2.98	3.07	3.26		1.09					2.37	2	2	A4
Os04g46250													3.4	2.31	3.36				A4
Os04g46400							3.38	1.87	2.46					-1.02					A4
Os04g46410													-1.01	-2.56	-2.43				A4
Os10g41130	-1.08	-1.49	-1.56	-1.23		-1.58							-1.85			-1.81	-1.79		A4
Os11g13840	-2.46	-1.88	-1.55		-1.1	-1.78	-1.45	-2.02		2.13	3.54					4.15	3.93	2.06	A4
Os01g64790	2.44	2.6	2.11	2.62	1.1	1.22		1.41					3.51	2.7	2.97	2.23	2.27	1.4	A5

Continue

Os02g43970	-1.18						-1.69	-4.21	-1.64				-1.49	-1.86	-1.12	-1.19	-2.51		A5
Os02g52670	1.45	1.2	1.58	1.99		1.56	4.18	4.04	4.09	3.01	2.77		4.13	4.06	4.13	3.97	4.12	2.85	A5
Os03g09170													-3.13	-3.23	-2.04				A5
Os04g52090							1.38	1.29	1.15										A5
Os05g32270	1.31	2.22			1.19								2.98	2.04	1.32				A5
Os06g09390		1.26		2.01	1.9	1.25					1.47		-1.6	-1.44					A5
Os06g09717				-1.14		-1.01					-2.55	-1.51							A5
Os06g09810	-4.55	-3.72	-4.38	-2.75	-2.08	-1.27									-1.09				A5
Os06g11860	-1	-1.3	-1.08	-1.46	-1.7	-1.05													A5
Os08g31580							1.01		1.38	1.12	1.34		1.86	1.47	1.92	2.19	1.9	1.27	A5
Os10g38000							1.11						1.28	1.13	1.03				A5
Os02g38090							-1.38						-2.29	-1.93	-1.61				A6
Os03g08500				1.52		1.25	1.01						1.42			1.04			A6
Os04g36640													-2.42	-2.47					A6
Os05g49010	-1.67	-2.08	-2.22	-4	-2.56	-2.5		-1.04					-2.48	-2.19	-1.32	1.36			A6
Os06g10780	-2.54	-3.1	-1.44	-2.38	-1.98	-1.39					2.42		-2.47	-2.87					A6
Os08g27220					-1.39	-1.13		-1.03	-2.07		-1.24								A6
Os10g22600	-2.72	-2.75	-3.34			-2.87	-2.24	-1.68	-2.2				-6.6	-6.19	-3.18	-1.09	-1.43	-1.17	A6
Os01g10370							1.53		1.35						-1.08				B1
Os01g21120	-7.04	-6.13	-6.22	-6.14	-5.86	-4.35							5.49	5.62	4.29	1.27			B1
Os01g54890													2.91	2.75	1.17				B1
Os01g73770		-1.4	1.02	-3.61	-1.54	-2.69				-1.46						3.47	1.75	3.16	B1
Os02g06330							-1.21	-1.52											B1
Os02g29550							-1.6	-1.48	-1.7										B1
Os02g35240													-2.43	-2.26					B1
Os03g22170	-9.07	-8.34	-7.79	-7.15	-6.69	-6.3	-1.17		-1.93	-1.4	-2.38	-2.1					1.07		B1
Os04g32620					-1.1	1.31	1.98	2.04	2.04				2	1.77	2.2	3.38	2.55	1.34	B1
Os04g48350	-3.73	-3.7	-1.48	-2.15	-2.28		-1.54	-3.49	-3.71	1.14	2.53	-1.37	-2.2	-1.73	-1.28				B1
Os04g56150														-1.41	-1.47				B1
Os04g57340								1.17											B1
Os05g41760							-1.11	-1.18											B1
Os06g42990	1.29			-1.27			-1.24	-1.67			-1.35	-1.06	-1.44	-1.96	-1.13				B1
Os07g10410																			B1
Os07g42510	-1.39	-2.01	-1.82		-1.92					-1.63		-1.63	2.76	2.51	2.52	2.59	2.01	1.62	B1
Os07g47790	-6.92	-6.56	-4.63	-3.7	-5.32	-3.88				1.43	2.71	1.61	1.12	1.96	1.29	1.62	1.47	2.6	B1
Os10g25170	-5.55	-4.49	-6.14	-5.67	-3.71	-4.09						1.24				1.02			B1
Os01g12440							2.35	2.65	1.86				2.43	2.53	2.03				B2
Os02g54050	-1.74	-2.14	-2.29	-1.92	-2.54	-3.17					1.39	-1.62						1.15	B2

Continue

Os03g08460	-6.25	-5.64	-5.86	-7.32	-6.23	-5.8											-1.52	B2
Os03g08490							-1.54	-2.45	-2.09				-3.16	-2.46	-2.01			B2
Os03g15660	-1.96	-1.4	-1.17		-1.31													B2
Os06g08340													1.75					B2
Os07g47330	-1.72		-1.4	-2.28		1.09												B2
Os09g11460	-2.19	-1.95	-2.39	-2.34	-2.66	-1.81	1.99	2.17	2.26	1.53								B2
Os09g11480	-4.66	-3.81	-4.12	-5.88	-4.86	-3.18	3.95	3.55	4.13				3.71	3.03	3.95	2.99	3.45	1.21	B2
Os09g26420	-4.86	-3.7	-4.22	-5.51	-3.95	-2.13		-1.31					-1.37	-1.71					B2
Os01g46870													1.28						B3
Os02g43790							-2.16		-2.37				2.81	2.5	1.33				B3
Os02g55380	-2.36	-2.54	-3.93	-3.54		-1.14	-1.2	-3.27	-2.82							2.1	1.77	1.76	B3
Os03g07830													2.78	3					B3
Os03g08470	-5.62	-4.51	-4.44	-2.3	-2.49	-1.44	5.81	5.01	5.5	1.04	1.33	1.18	1.35	1.42	1.86	1.35	1.3		B3
Os03g60120	2.56	2.49	2.59	1.68	2		2.38	1.82	2.44	1.33	1.37		1.68	1.39	1.68				B3
Os03g64260	-2.38	-3.59	-3.89	-1.78	-2.07	-2.23				-1.45		-1.68	3.67	1.03	2.35	1.81	2.09	2.21	B3
Os04g44670	-5.04	-3.74	-4.23	-3.48	-3.39	-3.25				1.27						1.44	1.14		B3
Os05g41780							1.04	1.14					1.04	1.35	1.21				B3
Os06g03670							5.04	5.46	5.46	4.47	4.59		1.66	1.8	4.06	3.46	3.45	2.47	B3
Os06g40150				-2.1								1.26				-2.04	-3.3		B3
Os07g22730	-1.62	-3.6	-2.43	-1.42	-1.31		-1.92	-1.14	-2.43	-2.62			3.18	1.84	1.11		1.15		B3
Os08g44960		-2.53	-1.38		-1.59	-1.16							2.06	1.33	1.17				B3
Os09g39850				1.53									1.74	2.03	1.47				B3
Os10g30840					-1.08		-1.15		-2.75		-1.29			-1.41	-1.25				B3
Os01g58420										1.66	1.12				1.02		1.21		B4
Os02g32040													1.77	1.63	1.29				B4
Os02g32140				1.73		1.11	4.15	3.57	4.05	1.62	1.58		2.7	2.46	2.6	4.32	3.99	2.97	B4
Os02g34260													2.69	4.14	3.66				B4
Os02g51670	-1.01	-1.09		-1.58	-1.37														B4
Os04g32790													1.94	1.95	1.35				B4
Os05g34730	-2.52	-3.22	-2.62	-1.06	-1.39	-1.55	-1.2	-1.05	-1.99			-1.5		-1.14		1.38	1.41		B4
Os08g35240	-5.31	-5.16	-5.44	-3.14	-3.36	-3.08													B4
Os08g41030													-2.17	-2.08	-1.25				B4
Os09g28440	-4.38	-4.03	-2.77		-1.76					1.8	3.01	-1.14			2.07	1.23			B4
Os11g06770	1.33	-1.24		2.44	1.63	2.06	1.18	2.7	1.5	-1.08	-1.17	-2.44	1.87		3.04	3.08	2.22	3.74	B4
Os04g46220	-1.64	-1.54	-1.26	-1.21	-1.25								2.25	2.1	1.92	1.4	1.39		B5
Os04g46240	-1.07	-1.47	-1.01	-1.32	-1.54														B5
Os05g39590								-1.81	-2.12				1.04	1.52					B5
Os10g41330	-5.71	-5.27	-6.01	-3.39	-2.45	-2.1	-2.62	-1.27	-2.03			-1.78	1.98	1.77		1.44	2		B5

Continue

Os07g03250										-1.18	-2.85					-1.25	-1.06		B6
Os07g22770							1.09		1.34				1.24						B6
Os12g39330	-3.58	-4.96	-3.46	-3.47	-2.94	-2.55							1		1.25				B6
Os02g13710	-3.56	-2.58	-2.48	-2.62	-2.38	-2.21										-1.73	-1.41		B7
Os05g29810							2.72	1.47	2.49				1.7	1.68	2.74				B7
Os08g36920													1.12	1.89	3.43				B7
Os09g13940	1.08	1.05																	B7
Os10g26590	-1.29	-1.27			-1.55		-1.45	-1.26	-1.17		-1.38		-1.27	-1.63	-1.01				B7
Os12g41030																			B7
Os12g41060													1.08	1.71					B7
Os01g59780	2.4	3.15	2.73	2.95	1.39	1.75	3.27	3.82	4.06	2.69	3.88								AP2
Os01g67410	-4.33		-2.84	-1.85	1.98														AP2
Os02g40070	-1.05	1.37	-2.16	-1.42															AP2
Os02g51300	1.01	1.36		1.83	1.87	1.39				1.11			-1.13	-1.02					AP2
Os03g12950													3.98	1.99	-1.06				AP2
Os03g19900	1.14	1.54	1.32	3.05		1.56				-2.3	-2.37		2.07	1.29					AP2
Os03g56050													1.97	1.56					AP2
Os03g60430			-1.15																AP2
Os04g42570	-2.33		-1.36	-2.12	1.21														AP2
Os04g55560							-2.07	-1.71	-1.96		-1.43		-1.39	-1.24	-1.75	-1.94	-1.04		AP2
Os04g55970		1.69		2.18	1.28	2.17							3.24	1.92					AP2
Os05g03040	-1.54																		AP2
Os06g05340				1.12			-1.17	-1.67					1.3						AP2
Os06g43220	-2.43		-1.61	-1.76	-1.61						-1.25	1.61	-2	-2.18	-3.21	-2.68	-2.29		AP2
Os08g34360							-1.65	-1.47	-1.36				-2.56	-1.98	-2.59				AP2
Os09g25600													1.37	1.21					AP2
Os11g03540								2.18											AP2
Os12g03290										-1.1	-1.37								AP2
Os01g04750	-4.64	-4.13	-5.23	-4.5	-4.27	-5.33													RAV
Os01g04800	-8.28	-7.69	-6.32	-5.49	-5.95	-5.18	-1.55	-2.43	-2.28	-2.46		-1.79	2.67	1.63	1.29				RAV
Os01g49830													2.41	2.17	1.85				RAV
Os05g47650	-1.76	-2.41					-1.17	-1.32	-1.5				1.62	1.51	2.65	2.99	3.72	2.41	RAV
Os08g42550							3.56	3.46	4.27	1.58	2.88	1.26	2.94	2.57	2.72	2.17	2.15	1.02	RAV

Note: NIL10= IR77298-14-1-2-B-10, NIL13= IR77298-14-1-2-B-13; Phg = Phylogentic subgroups or subfamilies.

The differentiation of the expression patterns in the three tissues of the rice genotypes indicated that a higher number of DEGs was observed under severe stress compared with mild stress conditions. We observed that the tolerant line IR77298-14-1-2-B-10 exhibited the highest number of up-regulated genes, followed by IR77298-14-1-2-B-13 and IR64, in all tissues under both stresses, with the exception of the root and the leaf under severe and mild stress conditions. In response to severe stress, this tolerant line demonstrated the highest number of up-regulated genes (61) in the panicle. Therefore, despite their common genetic background as backcrossed progeny from Aday Sel x IR64, the two NILs showed distinctive differences in their gene expression patterns in response to drought. We further analyzed the DEGs in two NILs of the IR77298-14-1-2-B family and compared their expression profiles under normal conditions and after the hormone treatment of seedlings, as outlined below.

Table 17. Differentiated expressed genes in IR77298-14-1-2-B-10 (NIL 10) versus IR77298-14-1-2-13 (NIL 13) and IR 64.

Root		Severe			
DEG		NIL 10 VS	NIL 13	IR64	Common
Activated	> 2	16	18	13	11
	> 4	7	7	7	6
Suppressed	> 2	51	48	47	40
	> 4	35	34	32	26

Root		Mild			
DEG		NIL 10 VS	NIL 13	IR64	Common
Activated	> 2	19	17	17	10
	> 4	10	5	6	4
Suppressed	> 2	43	22	36	21
	> 4	27	22	22	16

Leaf		Severe			
DEG		NIL 10 VS	NIL 13	IR64	Common
Activated	> 2	28	26	25	22
	> 4	16	17	17	12
Suppressed	> 2	26	23	27	17
	> 4	4	0	13	0

Leaf		Mild			
DEG		NIL 10 VS	NIL 13	IR64	Common
Activated	> 2	13	25	8	4
	> 4	5	11	2	1
Suppressed	> 2	10	13	16	2
	> 4	3	4	5	0

Panicle		Severe			
DEG		NIL 10 VS	NIL 13	IR64	Common
Activated	> 2	61	54	51	44
	> 4	29	21	25	13
Suppressed	> 2	24	27	22	16
	> 4	13	12	7	3

Panicle		Mild			
DEG		NIL 10 VS	NIL 13	IR64	Common
Activated	> 2	35	32	22	21
	> 4	21	16	11	8
Suppressed	> 2	9	9	2	1
	> 4	2	4	0	0

7.4. *AP2/EREBP* gene expression in the root common to both NILs under severe and mild stress conditions

In this study, gene expression profiles were compared between the drought-tolerant and drought-susceptible lines. It is believed that genotypes responding differentially to drought stress exhibit variations in gene expression and that these differences are strongly related to drought avoidance or to drought response. Spollen et al. (1993) reported that mild osmotic stress rapidly inhibits the growth of the leaves and stems, whereas the roots continue to elongate, and the root architecture is a key trait for dissecting genotypic differences in rice responses to DSCs. Thus, the analysis of the genes observed entirely in two NILs i.e. up-regulated genes in both NILs and in individual is of interest to identify the most promising candidate genes among three tissues at procreative stage under both DSCs, which will be provide a starting point for further elucidating role of single gene in stress response. In the root under severe stress, fourteen genes were shared common in IR77298-14-1-2-B-10 and in IR77298-14-1-2-B-13 (**Fig. 14, Table 18**), while most of them were also up- or down-regulated in different tissues under both DSCs in two NILs and assigned to DREB subfamily (**Table 16**). We considered a gene specifically expressed in one NIL if that gene was not differentially expressed and exhibited no change in expression levels or a low-level of expression compared with susceptible NIL under severe or mild stress in a certain tissue. In IR77298-14-1-2-B-10, we identified 2 specific genes (*Os06g42990* and *Os11g06770,* DREB subfamily) that were expressed in the root under severe stress but were down-regulated or highly sensitive in the root under severe stress in IR77298-14-1-2-B-13. Similarly, in IR77298-14-1-2-B-13, 4 genes (*Os02g40070*, *Os04g46440*, *Os04g55970*, and *Os06g09390*, ERF and AP2 subfamilies) were specifically induced in the root under severe stress, and all of the genes were down-regulated, their expression levels did not change or these genes were highly sensitive in IR77298-14-1-2-B-10 under conditions of severe stress (**Table 18**). *Os02g40070* was down-regulated in the root under non-stressed conditions. This study

assumed that these genes are line- and tissue-specific under conditions of severe stress. In the root under mild stress conditions, 13 up-regulated genes were common to both NILs (**Fig. 14, Table 18**). Out of these 13 genes, eight genes belong to the DREB subfamily. A total of six genes (*Os02g52670*, *Os03g08500*, *Os09g39850*, *Os02g32140*, *Os03g19900*, and *Os06g05340*) assigned to subfamilies DRED, ERF, and AP2 were specifically up-regulated in IR77298-14-1-2-B-10 under mild stress conditions, but they were down-regulated or exhibited no change in expression in IR77298-14-1-2-B-13 in the root. These genes were not up- or down-regulated following treatment with the different plant hormones, whereas *Os03g19900* of the AP2 subfamily was expressed with a 6-fold increase in the drought-tolerant line compared with the drought-susceptible line in the root, and its expression was down-regulated in root tissue under normal growth conditions. In IR77298-14-1-2-B-13, four genes (*Os02g54160*, *Os05g32270*, *Os01g67410*, and *Os04g42570*, DREB and AP2 subfamilies) were up-regulated under mild stress conditions. *Os01g67410* and *Os05g32270* expression was induced in leaf-1 and panicle-1, respectively, under normal conditions, whereas *Os01g67410* was up-regulated and *Os05g32270* was down-regulated at all time points of the JA treatment. Interestingly, two genes of the AP2 subfamily were severely down-regulated in IR77298-14-1-2-B-10 under mild stress conditions. In the root, under both DSCs, the two NILs shared 8 common genes (**Fig. 14, Table 18**). Of these 8 genes, *Os02g51300* is from the AP2 subfamily and was up-regulated following treatment of seedlings with GA3, KT, and NAA. Under both DSCs in the root, three genes were up-regulated specifically in IR77298-14-1-2-B-10. Among these three genes, *Os11g06770* (subgroup B4, ERF subfamily) was down-regulated under severe stress conditions, and its expression was induced in the root under mild stress conditions in IR77298-14-1-2-B-13. *Os02g52670* (A5 subgroup, DREB subfamily) and *Os03g19900* (AP2 subfamily) exhibited a low level of expression in the root in IR77298-14-1-2-B-13 under mild stress conditions, whereas *Os03g19900* was down-regulated under non-stress conditions in the same tissue.

Similarly, 5 genes were commonly found in IR77298-14-1-2-B-13 under both stress conditions, although the majority of these genes exhibited high expression levels under either DSC in the drought-tolerant line compared with the drought-susceptible line. We speculate that expression responses of these genes are root tissue- and NILs-specific under both DSCs.

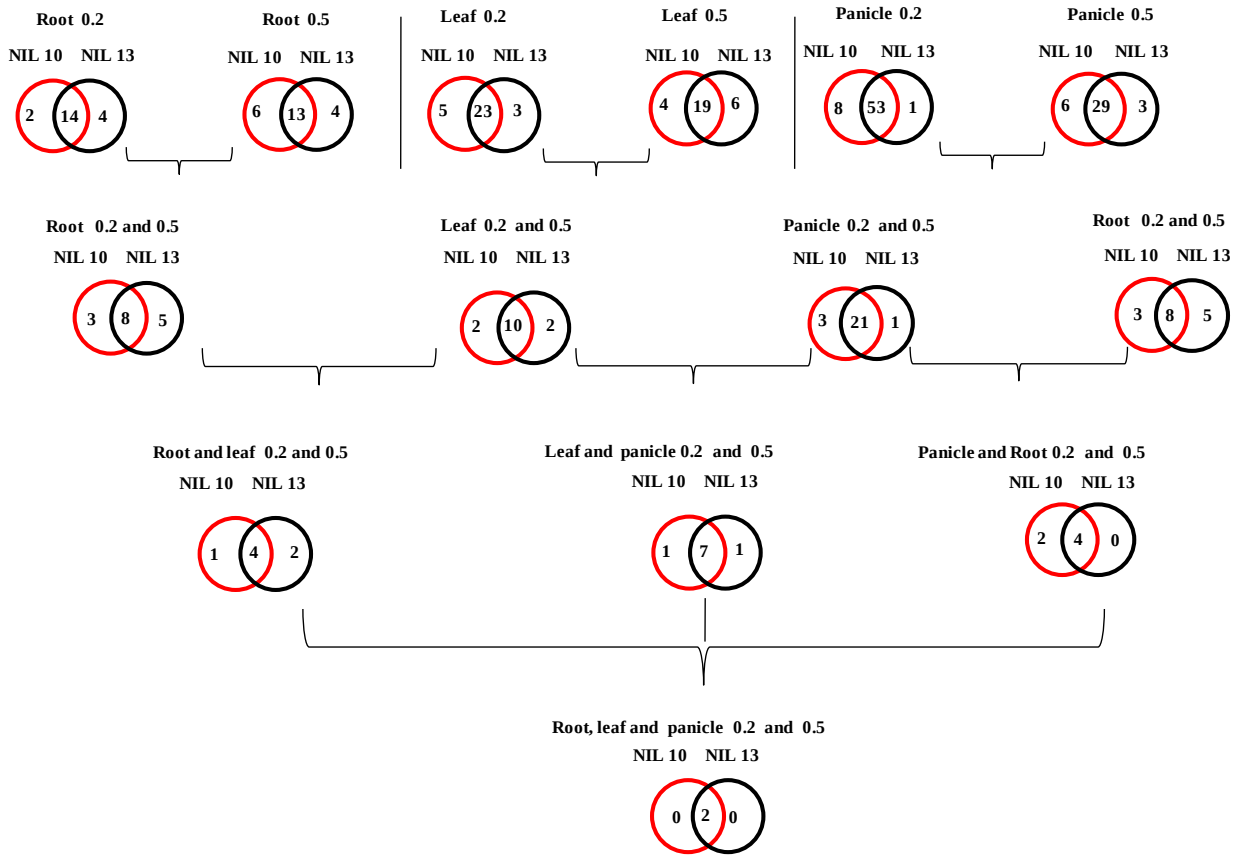

Fig. 14 Up-regulated overlapped and specific genes were detected in NIL10 and NIL13 lines under severe and mild stress conditions in the root, leaf, and panicle. NIL10= R77298-14-1-2-B-10 and NIL13= IR77298-14-1-2-B-13, 0.2= severe stress and 0.5 = mild stress.

Table 18. Overlapped and specific genes were detected in NIL10 and NIL13 lines under severe and mild stress conditions in all tissues examined.

		Root severe	
Common	Phg	NIL10	NIL13
Os05g49700	A1	8.112	6
Os01g04020	A2	2.053	1.863
Os08g45110	A2	4.983	5.004
Os02g54160	A3	1.248	1.321
Os01g07120	A4	1.61	1.523
Os04g34970	A4	2.886	2.361
Os01g64790	A5	2.444	2.601
Os02g52670	A5	1.446	1.197
Os05g32270	A5	1.314	2.215
Os03g60120	B3	2.556	2.489
Os09g13940	B7	1.082	1.054
Os01g59780	AP2	2.402	3.15
Os02g51300	AP2	1.013	1.364
Os03g19900	AP2	1.137	1.542

		Root severe	
NIL 10 specific	Phg	NIL10	NIL13
Os06g42990	B1	1.293	
Os11g06770	B4	1.325	-1.238

		Root severe	
NIL 13 specific	Phg	NIL10	NIL13
Os04g46440	A1		1.397
Os06g09390	A5		1.263
Os02g40070	AP2	-3.052	1.366
Os04g55970	AP2		1.69

		Root mild	
Common	Phg	NIL10	NIL1
Os04g46440	A1	2.384	2.137
Os05g49700	A1	8.336	7.666
Os05g25260	A2	1.288	1.097
Os08g45110	A2	6.44	6.297
Os01g07120	A4	1.118	1.393
Os04g34970	A4	2.71	2.925
Os01g64790	A5	2.624	1.099
Os06g09390	A5	2.005	1.902
Os03g60120	B3	1.677	2.004
Os11g06770	B4	2.441	1.626
Os01g59780	AP2	2.948	1.392
Os02g51300	AP2	1.832	1.87
Os04g55970	AP2	2.175	1.277

		Root mild	
NIL 10 specific	Phg	NIL10	NIL13
Os02g52670	A5	1.99	
Os03g08500	A6	1.52	
Os09g39850	B3	1.528	
Os02g32140	B4	1.726	
Os03g19900	AP2	3.047	
Os06g05340	AP2	1.12	

		Root mild	
NIL 13 specific	Phg	NIL10	NIL13
Os02g54160	A3		1.266
Os05g32270	A5		1.188
Os01g67410	AP2	-1.851	1.978
Os04g42570	AP2	-2.121	1.211

		Root severe		Root mild	
Common	Phg	NIL10	NIL13	NIL10	NIL13
Os05g49700	A1	8.112	6	8.336	7.666
Os08g45110	A2	4.983	5.004	6.44	6.297
Os01g07120	A4	1.61	1.523	1.118	1.393
Os04g34970	A4	2.886	2.361	2.71	2.925
Os01g64790	A5	2.444	2.601	2.624	1.099
Os03g60120	B3	2.556	2.489	1.677	2.004
Os01g59780	AP2	2.402	3.15	2.948	1.392
Os02g51300	AP2	1.013	1.364	1.832	1.87

		Root severe		Root mild	
NIL 10 specific	Phg	NIL10	NIL13	NIL10	NIL13
Os02g52670	A5	1.446	1.197	1.99	
Os11g06770	B4	1.325	-1.238	2.441	1.626
Os03g19900	AP2	1.137	1.542	3.047	

		Root severe		Root mild	
NIL 13 specific	Phg	NIL10	NIL13	NIL10	NIL13
Os04g46440	A1		1.397	2.384	2.137
Os02g54160	A3	1.248	1.321		1.266
Os05g32270	A5	1.314	2.215		1.188
Os06g09390	A5		1.263	2.005	1.902
Os04g55970	AP2		1.69	2.175	1.277

Continue

		Leaf severe	
Common	Phg	NIL10	NIL13
Os02g45420	A1	4.184	4.793
Os05g49700	A1	2.407	2.574
Os02g54160	A3	1.379	1.234
Os06g07030	A3	1.532	1.583
Os01g07120	A4	2.736	3.673
Os02g43820	A4	2.504	2.359
Os04g34970	A4	2.983	3.07
Os04g46400	A4	3.382	1.866
Os02g52670	A5	4.182	4.04
Os04g52090	A5	1.376	1.289
Os04g32620	B1	1.982	2.038
Os01g12440	B2	2.348	2.651
Os09g11460	B2	1.989	2.168
Os09g11480	B2	3.95	3.546
Os03g08470	B3	5.805	5.007
Os03g60120	B3	2.379	1.815
Os05g41780	B3	1.039	1.14
Os06g03670	B3	5.035	5.457
Os02g32140	B4	4.146	3.565
Os11g06770	B4	1.182	2.702
Os05g29810	B7	2.715	1.468
Os01g59780	AP2	3.268	3.816
Os08g42550	RAV	3.56	3.46

		Leaf severe	
NIL 10 specific	Phg	NIL10	NIL13
Os08g31580	A5	1.007	
Os10g38000	A5	1.11	
Os03g08500	A6	1.007	
Os01g10370	B1	1.53	
Os07g22770	B6	1.094	

		Leaf severe	
NIL 13 specific	Phg	NIL10	NIL13
Os01g64790	A5		1.413
Os04g57340	B1		1.17
Os11g03540	AP2		2.177

		Leaf mild	
Common	Phg	NIL10	NIL13
Os05g49700	A1	2.669	2.797
Os09g35010	A1	1.143	1.187
Os09g35020	A1	1.339	1.621
Os05g25260	A2	1.273	1.215
Os02g54160	A3	1.145	1.078
Os01g07120	A4	1.539	2.239
Os11g13840	A4	2.132	3.535
Os02g52670	A5	3.007	2.774
Os08g31580	A5	1.123	1.343
Os04g48350	B1	1.144	2.531
Os07g47790	B1	1.429	2.709
Os03g08470	B3	1.039	1.326
Os03g60120	B3	1.333	1.374
Os06g03670	B3	4.471	4.589
Os01g58420	B4	1.663	1.115
Os02g32140	B4	1.617	1.576
Os09g28440	B4	1.804	3.006
Os01g59780	AP2	2.69	3.876
Os08g42550	RAV	1.579	2.877

		Leaf mild	
NIL 10 specific	Phg	NIL10	NIL13
Os02g43940	A4	1.619	
Os09g11460	B2	1.528	
Os04g44670	B3	1.271	
Os02g51300	AP2	1.106	

		Leaf mild	
NIL 13 specific	Phg	NIL10	NIL13
Os08g45110	A2		1.131
Os06g07030	A3		1.26
Os04g34970	A4		1.086
Os06g09390	A5		1.465
Os06g10780	A6		2.419
Os02g54050	B2		1.391

Continue

		Leaf severe		Leaf mild	
Common	Phg	NIL10	NIL13	NIL10	NIL13
Os05g49700	A1	2.407	2.574	2.669	2.797
Os02g54160	A3	1.379	1.234	1.145	1.078
Os01g07120	A4	2.736	3.673	1.539	2.239
Os02g52670	A5	4.182	4.04	3.007	2.774
Os03g08470	B3	5.805	5.007	1.039	1.326
Os03g60120	B3	2.379	1.815	1.333	1.374
Os06g03670	B3	5.035	5.457	4.471	4.589
Os02g32140	B4	4.146	3.565	1.617	1.576
Os01g59780	AP2	3.268	3.816	2.69	3.876
Os08g42550	RAV	3.56	3.46	1.579	2.877

		Leaf severe		Leaf mild	
NIL 10 specific	Phg	NIL10	NIL13	NIL10	NIL13
Os08g31580	A5	1.007		1.123	1.343
Os09g11460	B2	1.989	2.168	1.528	

		Leaf severe		Leaf mild	
NIL 13 specific	Phg	NIL10	NIL13	NIL10	NIL13
Os06g07030	A3	1.532	1.583		1.26
Os04g34970	A4	2.983	3.07		1.086

		Panicle severe	
Common	Phg	NIL10	NIL13
Os04g46440	A1	1.286	1.219
Os05g49700	A1	1.736	1.261
Os09g35010	A1	1.925	1.082
Os09g35030	A1	2.353	1.986
Os01g04020	A2	1.36	1.52
Os05g25260	A2	2.128	1.852
Os08g45110	A2	3.559	2.956
Os06g07030	A3	1.901	1.821
Os01g07120	A4	2.054	2.43
Os02g34270	A4	3.337	3.965
Os02g43820	A4	2.21	2.616
Os04g46250	A4	3.398	2.313
Os01g64790	A5	3.513	2.699
Os02g52670	A5	4.13	4.06
Os05g32270	A5	2.983	2.035
Os08g31580	A5	1.86	1.471
Os10g38000	A5	1.282	1.129
Os01g21120	B1	5.485	5.619
Os01g54890	B1	2.911	2.745
Os04g32620	B1	2	1.768
Os07g42510	B1	2.757	2.512
Os07g47790	B1	1.124	1.958
Os01g12440	B2	2.43	2.528

Continue

		Panicle severe	
Common	Phg	NIL10	NIL13
Os09g11480	B2	3.714	3.031
Os02g43790	B3	2.812	2.499
Os03g07830	B3	2.781	3.002
Os03g08470	B3	1.35	1.42
Os03g60120	B3	1.678	1.39
Os03g64260	B3	3.667	1.026
Os05g41780	B3	1.043	1.345
Os06g03670	B3	1.655	1.797
Os07g22730	B3	3.184	1.835
Os08g44960	B3	2.056	1.333
Os09g39850	B3	1.742	2.033
Os02g32040	B4	1.766	1.633
Os02g32140	B4	2.701	2.457
Os02g34260	B4	2.688	4.137
Os04g32790	B4	1.939	1.947
Os04g46220	B5	2.252	2.099
Os05g39590	B5	1.043	1.522
Os10g41330	B5	1.98	1.769
Os05g29810	B7	1.701	1.682
Os08g36920	B7	1.12	1.89
Os12g41060	B7	1.076	1.711
Os03g12950	AP2	3.978	1.989
Os03g19900	AP2	2.065	1.29
Os03g56050	AP2	1.965	1.558
Os04g55970	AP2	3.242	1.923
Os09g25600	AP2	1.371	1.206
Os01g04800	RAV	2.67	1.633
Os01g49830	RAV	2.412	2.174
Os05g47650	RAV	1.623	1.512
Os08g42550	RAV	2.943	2.565

		Panicle severe	
NIL 10 specific	Phg	NIL10	NIL13
Os05g27930	A2	1.073	
Os03g08500	A6	1.424	
Os06g08340	B2	1.745	
Os01g46870	B3	1.283	
Os11g06770	B4	1.868	
Os07g22770	B6	1.238	
Os12g39330	B6	1.002	
Os06g05340	AP2	1.297	

		Panicle severe	
NIL 13 specific	Phg	NIL10	NIL13
Os02g45420	A1		1.759

		Panicle mild	
Common	Phg	NIL10	NIL13
Os01g66270	A1	4.43	1.982
Os05g49700	A1	5.347	3.865
Os09g35010	A1	1.269	1.105
Os05g25260	A2	2.252	1.502
Os08g45110	A2	4.234	2.859
Os06g07030	A3	2.448	2.391
Os01g07120	A4	2.072	1.881
Os04g34970	A4	2.371	1.996
Os11g13840	A4	4.15	3.926
Os01g64790	A5	2.234	2.265
Os02g52670	A5	3.967	4.115
Os08g31580	A5	2.189	1.901
Os01g73770	B1	3.465	1.753
Os04g32620	B1	3.378	2.553
Os07g42510	B1	2.594	2.006
Os07g47790	B1	1.62	1.47
Os09g11480	B2	2.991	3.447
Os02g55380	B3	2.096	1.774
Os03g08470	B3	1.345	1.299
Os03g64260	B3	1.806	2.089
Os04g44670	B3	1.444	1.144
Os06g03670	B3	3.462	3.452
Os02g32140	B4	4.321	3.987
Os05g34730	B4	1.376	1.409
Os11g06770	B4	3.078	2.223
Os04g46220	B5	1.399	1.393
Os10g41330	B5	1.443	2.004
Os05g47650	RAV	2.985	3.722
Os08g42550	RAV	2.173	2.146

		Panicle mild	
NIL 10 specific	Phg	NIL10	NIL13
Os04g55520	A3	1.257	
Os03g08500	A6	1.036	
Os05g49010	A6	1.358	
Os01g21120	B1	1.274	
Os10g25170	B1	1.015	
Os09g28440	B4	1.229	

		Panicle mild	
NIL 13 specific	Phg	NIL10	NIL13
Os03g22170	B1		1.071
Os07g22730	B3		1.146
Os01g58420	B4		1.21

		Panicle severe		Panicle mild	
Common	Phg	NIL10	NIL13	NIL10	NIL13
Os05g49700	A1	1.736	1.261	5.347	3.865
Os09g35010	A1	1.925	1.082	1.269	1.105
Os05g25260	A2	2.128	1.852	2.252	1.502
Os08g45110	A2	3.559	2.956	4.234	2.859
Os06g07030	A3	1.901	1.821	2.448	2.391
Os01g07120	A4	2.054	2.43	2.072	1.881
Os01g64790	A5	3.513	2.699	2.234	2.265
Os02g52670	A5	4.13	4.06	3.967	4.115
Os08g31580	A5	1.86	1.471	2.189	1.901
Os04g32620	B1	2	1.768	3.378	2.553
Os07g42510	B1	2.757	2.512	2.594	2.006
Os07g47790	B1	1.124	1.958	1.62	1.47
Os09g11480	B2	3.714	3.031	2.991	3.447
Os03g08470	B3	1.35	1.42	1.345	1.299
Os03g64260	B3	3.667	1.026	1.806	2.089
Os06g03670	B3	1.655	1.797	3.462	3.452
Os02g32140	B4	2.701	2.457	4.321	3.987
Os04g46220	B5	2.252	2.099	1.399	1.393
Os10g41330	B5	1.98	1.769	1.443	2.004
Os05g47650	RAV	1.623	1.512	2.985	3.722
Os08g42550	RAV	2.943	2.565	2.173	2.146

		Panicle severe		Panicle mild	
NIL 10 specific	Phg	NIL10	NIL13	NIL10	NIL13
Os03g08500	A6	1.424		1.036	
Os01g21120	B1	5.485	5.619	1.274	
Os11g06770	B4	1.868		3.078	2.223

		Panicle severe		Panicle mild	
NIL 13 specific	Phg	NIL10	NIL13	NIL10	NIL13
Os07g22730	B3	3.184	1.835		1.146

Continue

		Root severe		Root mild		Leaf severe		Leaf mild	
Common	Phg	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13
Os05g49700	A1	8.112	6	8.336	7.666	2.407	2.574	2.669	2.797
Os01g07120	A4	1.61	1.523	1.118	1.393	2.736	3.673	1.539	2.239
Os03g60120	B3	2.556	2.489	1.677	2.004	2.379	1.815	1.333	1.374
Os01g59780	AP2	2.402	3.15	2.948	1.392	3.268	3.816	2.69	3.876

		Root severe		Root mild		Leaf severe		Leaf mild	
NIL 10 specific	Phg	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13
Os02g52670	A5	1.446	1.197	1.99		4.182	4.04	3.007	2.774

		Root severe		Root mild		Leaf severe		Leaf mild	
NIL 13 specific	Phg	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13
Os02g54160	A3	1.248	1.321		1.266	1.379	1.234	1.145	1.078
Os04g34970	A4	2.886	2.361	2.71	2.925	2.983	3.07		1.086

		Leaf severe		Leaf mild		Panicle severe		Panicle mild	
Common	Phg	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13
Os05g49700	A1	2.407	2.574	2.669	2.797	1.736	1.261	5.347	3.865
Os01g07120	A4	2.736	3.673	1.539	2.239	2.054	2.43	2.072	1.881
Os02g52670	A5	4.182	4.04	3.007	2.774	4.13	4.06	3.967	4.115
Os03g08470	B3	5.805	5.007	1.039	1.326	1.35	1.42	1.345	1.299
Os06g03670	B3	5.035	5.457	4.471	4.589	1.655	1.797	3.462	3.452
Os02g32140	B4	4.146	3.565	1.617	1.576	2.701	2.457	4.321	3.987
Os08g42550	RAV	3.56	3.46	1.579	2.877	2.943	2.565	2.173	2.146

		Leaf severe		Leaf mild		Panicle severe		Panicle mild	
NIL 10 specific	Phg	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13
Os08g31580	A5	1.007		1.123	1.343	1.86	1.471	2.189	1.901

		Panicle severe		Panicle mild		Root severe		Root mild	
NIL 10 specific	Phg	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13
Os02g52670	A5	4.13	4.06	3.967	4.115	1.446	1.197	1.99	
Os11g06770	B4	1.868		3.078	2.223	1.325	-1.24	2.441	1.626

		Root severe		Root mild		Leaf severe		Leaf mild		Panicle severe		Panicle mild	
Common	Phg	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13	NIL10	NIL13
Os05g49700	A1	8.11	6.00	8.34	7.67	2.41	2.57	2.67	2.80	1.74	1.26	5.35	3.87
Os01g07120	A4	1.61	1.52	1.12	1.39	2.74	3.67	1.54	2.24	2.05	2.43	2.07	1.88

Note: Blank indicates not differentially expressed genes; Phg = Phylogentic subgroups or subfamilies.

7.5. *AP2/EREBP* gene expression in the leaf common to both NILs under severe and mild stress conditions

We found that 23 up-regulated genes in the leaf were common to both lines under severe stress conditions (**Fig. 14, Table 18**). Of these 23 genes, 14 were not differentially expressed in the root under both DSCs (**Table 16**). Under severe stress conditions, five genes (*Os08g31580*, *Os10g38000*, *Os03g08500*, *Os01g10370*, and *Os07g22770*, subfamilies DREB and ERF) were up-regulated in the leaf, specifically in IR77298-14-1-2-B-10. With the exception of *Os03g08500*, these genes were up-regulated at least one JA treatment time point. All of these genes were expressed in the leaf at a low-level or was highly sensitive in IR77298-14-1-2-B-13 under severe stress conditions (**Table 18**). In IR77298-14-1-2-B-13, three genes (*Os01g64790*, *Os04g57340*, and *Os11g03540*; AP2, DREB, and ERF subfamilies) were highly activated in the leaf under severe stress conditions and down-regulated or expressed at a low-level in that tissue in IR77298-14-1-2-B-10 under severe stress conditions. These genes are specific to NILs and to leaf tissue under severe stress. Of the genes expressed at high levels in the leaf under mild stress, 19 were common both NILs (**Fig. 14, Table 18**). In IR77298-14-1-2-B-10, 4 genes (*Os02g43940*, *Os09g11460*, *Os04g44670*, and *Os02g51300*, subfamilies AP2, DREB, and ERF) were highly responsive, specifically in IR77298-14-1-2-B-10, under mild conditions in the leaf and not differentially expressed or highly responsive in IR77298-14-1-2-B-13. Of these four genes, two (*Os09g11460* and *Os02g43940*) were up-regulated by JA treatment at the seedling stage (**Table 15**), whereas *Os04g44670* was highly induced specifically in the root under normal conditions and was down- and up-regulated in seedlings following SA and JA treatments, respectively (**Fig. 12B, Tables 14 and 15**). Similarly, six genes (*Os08g45110*, *Os06g07030*, *Os04g34970*, *Os06g09390*, *Os06g10780*, and *Os02g54050*, ERF and DREB subfamilies) were highly activated in the leaf, specifically in IR77298-14-1-2-B-13 under mild stress condition, but were not differentially expressed and did not exhibit lower intensities in

IR77298-14-1-2-B-10. *Os04g34970* was activated in root tissue under normal conditions. During both stress conditions, 10 genes were up-regulated in the leaf in both NILs (**Fig. 14, Table 18**). All of these genes were expressed in the different experimental tissues under both DSCs (**Table 16**). *Os08g31580* (DREB-A5) and *Os09g11460* (ERF-B2) were up-regulated in the leaf under both stress conditions in IR77298-14-1-2-B-10, but one of them (*Os09g11460*) was more highly expressed in IR77298-14-1-2-B-13. *Os09g11460* exhibited up-regulation specifically in the stamen under normal conditions and following GA3 and JA treatments (**Fig. 12B, Tables 14 and 15**). On the other hand, *Os06g07030* and *Os04g34970* genes (subgroups A3 and A4 of the DREB subfamily) were expressed at high levels in IR77298-14-1-2-B-13 under both DSCs, whereas both genes were down-regulated in drought-tolerant NILs under mild stress conditions (**Fig. 14, Table 18**). These *Os06g07030* and *Os04g34970* genes were up-regulated in the root and the leaf-1 tissue under normal conditions in Minghui 63, respectively.

7.6. *AP2/EREBP* panicle genes expression common in two NILs under severe and mild stress conditions

Plants are very susceptible to drought during the reproductive stage of development. Under severe stress conditions, a greater number up-regulated genes (53) common to both NILs were found in the panicle compared with the root and leaf (**Fig. 14, Table 18**). Of these 53 genes, thirteen genes (e.g., *Os02g34270* and *Os04g46250*) were up-regulated in the panicle in both NILs under severe stress conditions, but they were not up-regulated in any other tissue (**Table 16**). *Os02g34270* exhibited down-regulation in the root under non-stressed conditions. Interestingly, eight genes (*Os05g27930*, *Os03g08500*, *Os06g08340*, *Os01g46870*, *Os11g06770*, *Os07g22770*, *Os12g39330*, and *Os06g05340*) belonged to the subfamilies AP2, DREB, and ERF and were up-regulated specifically in IR77298-14-1-2-B-10 in the panicle; however; they were not differentially expressed or only expressed at a low level in IR77298-

14-1-2-B-13 under severe stress. Out of these 8 genes, five belong to the ERF subfamily, and one gene (*Os01g46870*, ERF-B3) was up-regulated in the 3-leaf stage following treatment with GA3 (**Table 15**). Similarly, *Os02g45420* (subfamily DREB-A1) was up-regulated specifically in the IR77298-14-1-2-B-13 panicle under severe stress (**Fig. 14, Table 18**). This study detected 29 common genes in the panicles of IR77298-14-1-2-B-10 and IR77298-14-1-2-B-13 under mild stress conditions (**Fig. 14, Table 18**). All of these genes were differentially expressed in at least one tissue in both NILs (**Table 16**). In IR77298-14-1-2-B-10 under mild stress conditions, six genes (*Os04g55520*, *Os03g08500*, *Os05g49010*, *Os01g21120*, *Os10g25170*, and *Os09g28440*; subfamilies DREB and ERF) were up-regulated specifically in the panicle but were not differentially expressed or highly sensitive in IR77298-14-1-2-B-13. Of these six genes, four (*Os04g55520, Os01g21120, Os10g25170,* and *Os09g28440*) were up-regulated following JA treatment at all the time points, whereas *Os01g21120* was up-regulated following NAA treatment. In leaf-1 tissue, *Os04g55520* gene was down-regulated under normal conditions. Similarly, three genes (*Os03g22170*, *Os07g22730*, and *Os01g58420* from ERF) were activated specifically in the IR77298-14-1-2-B-13 panicle under mild stress conditions but exhibited low expression levels in IR77298-14-1-2-B-10 (**Table 18**). In the panicle under both DSCs, both NILs shared 21 common genes, and these genes were expressed in at least one of the stress conditions in the root or leaf, as indicated above. The *Os03g08500* gene (subgroup A6, DREB subfamily) and the *Os01g21120* and *Os11g06770* (subgroups B1 and B4, ERF subfamily) genes were highly induced in the panicle under both DSCs in IR77298-14-1-2-B-10, whereas *Os03g08500* in the panicle was highly sensitive in IR77298-14-1-2-B-13 under both DSCs. In the drought-tolerant line, the expression intensity of the *Os01g21120* gene under severe stress conditions was higher than under mild stress. Similarly, the *Os07g22730* (subgroup B3, ERF subfamily) was activated in the panicle in IR77298-14-1-2-B-13 under both DSCs but up-regulated approximately 9-fold in IR77298-14-1-2-B-10 under severe stress conditions. The rapid

change in the expression level of this gene under the drought stress conditions in the drought-tolerant line suggests the recruitment of genes that are normally expressed at lower level for emergencies. In contrast, some genes exhibit ubiquitous expression under normal conditions and during phytohormone and drought stress treatments. A number of genes were up-regulated in the drought-tolerant line, and these genes may be required to maintain grain yield in response to severe drought. Functional analyses are needed to further explore these possibilities.

7.7. *AP2/EREBP* genes expression in both NILs under both DSCs in root vs. all tissues and in leaf vs. panicle and all tissues

The up-regulation of four genes was observed in both lines under the two DSCs in the root and the leaf (**Fig. 14, Table 18**). *Os02g52670* (subfamily DREB-A5) was expressed in the root and in the leaf in both NILs under both stress conditions but was highly sensitive in the root in IR77298-14-1-2-B-13 under mild stress. Similarly, *Os02g54160* and *Os04g34970* (DREB subfamily) were activated in the root and leaf under severe and mild stress conditions in both lines but were not differentially expressed in the root and leaf under mild stress in IR77298-14-1-2-B-10. We observed that seven overlapping genes were up-regulated in both the leaf and panicle in both NILs under both DSCs (**Fig. 14, Table 18**). The *Os08g31580* (DREB-A5) gene exhibited low expression levels in the leaf under severe stress in IR77298-14-1-2-B-13. *Os06g07030* (DREB-A3) was down-regulated in the leaf under mild stress in IR77298-14-1-2-B-10. The expression responses of both genes were higher in IR77298-14-1-2-B-10 than in IR77298-14-1-2-B-13 in all tissues (**Fig. 14, Table 18**). The induction of four genes in both the panicle and the root were observed in the tolerant and susceptible lines under both DSCs (**Fig. 14, Table 18**). The *Os05g49700* and *Os01g07120* (BREB subfamily) genes were uniformly expressed in all tissues in both lines, suggesting that these are housekeeping genes. The *Os05g49700* gene response was higher in the root and panicle

under both DSCs in IR77298-14-1-2-B-10 when compared with IR77298-14-1-2-B-13 (**Fig. 14, Table 18**). On a broad scale, our findings contribute to the understanding of gene responses to different abiotic stresses.

7.8. Identification of putative drought responsive genes, subgroups, and subfamilies

The specific *AP2/EREBP* genes that were activated during the drought stress response in the drought-tolerant line are of particular interest. In total, sixteen non-redundant genes were up-regulated in IR77298-14-1-2-B-10, and these genes were expressed at a low-level or were not differentially expressed in the drought-susceptible IR77298-14-1-2-B-13 line under both DSCs in all tissues (**Table 19**). To identify putative genes that are responsible for drought avoidance in the tolerant NIL, this study focused on DEGs with high levels of expression in response to two DSCs in the tolerant line versus the susceptible line and IR64. In the tolerant line, two (*Os05g49700* and *Os06g42990*) out of four genes demonstrated higher expression levels in the root than in the leaf and panicle under severe stress and two of these genes (*Os01g66270* and *Os03g12950*) demonstrated higher expression levels in the panicle compared with the root and leaf under severe and mild stress (**Fig. 15**). *Os03g12950* is induced in the panicle and *Os05g49700* is down-regulated in the endosperm under normal conditions, whereas *Os01g66270* and *Os06g42990* are absent in the Affymetrix Gene Chip. Therefore, we suspected that these genes might play functional roles in the specific tissue under both stress conditions. However, further work is required to distinguish these possibilities. We found that most of the genes belonging to subgroups B3 and B5 and to the RAV subfamily were responsive in the panicle under severe stress, but the genes belonging to subgroups B and B2 of the ERF subfamily were down-regulated in the root. The genes belonging to subgroup A2 of the DREB subfamily were involved in the root and panicle, whereas other stress-responsive genes are randomly distributed in the other phylogentic subgroups or subfamilies (**Table 20**). Among the four subfamilies, we compared the DEGs of

two rice genotypes, Minghui 63 and IR77298-14-1-2-B-10, in the root, leaf, and panicle tissues under normal and severe drought stress conditions. **Table 21** shows that none of the RAV subfamily genes were differentially expressed in any tissue under normal conditions in Minghui 63, but this subfamily exhibited a large number of up-regulated genes (80%) in the panicle under severe drought stress condition in IR77298-14-1-2-B-10, followed by the ERF, DREB, and AP2 subfamilies. Our data suggest that the up-regulation of RAV subfamily genes may be tissue specific during severe drought stress conditions. Furthermore, the expression intensities of a number of up-regulated genes in IR77298-14-1-2-B-10 were higher than or equal to their expression intensities in IR77298-14-1-2-B-13 or IR64 in all examined tissues (**Fig. 16**). Taken together, these results enhance our knowledge of the involvement of *OsAP2/EREBP* TFs in plant resistance to drought and show that members of certain subgroups or subfamilies of the *OsAP2/EREBP* gene family are activated in response to drought stress.

Table 19. Specific up-regulated genes in the tolerant line IR77298-14-1-2-B-10 compared with the sister line IR77298-14-1-2-B-13 and control IR64.

Root severe			
NIL10 specific	IR77298-14-1-2-B-10	IR77298-14-1-2-B-13	IR64
Os06g42990	1.293	0	0
Root mild			
NIL10 specific	IR77298-14-1-2-B-10	IR77298-14-1-2-B-13	IR64
Os09g39850	1.528	0	0
Os06g05340	1.12	0	0
Leaf severe			
NIL10 specific	IR77298-14-1-2-B-10	IR77298-14-1-2-B-13	IR64
Os10g38000	1.11	0	0
Os03g08500	1.007	0	0
Leaf mild			
NIL10 specific	IR77298-14-1-2-B-10	IR77298-14-1-2-B-13	IR64
Os02g43940	1.619	0	0
Os04g44670	1.271	0	0
Os02g51300	1.106	0	0
Panicle severe			
NIL10 specific	IR77298-14-1-2-B-10	IR77298-14-1-2-B-13	IR64
Os03g08500	1.424	0	0
Os06g08340	1.745	0	0
Os01g46870	1.283	0	0
Os07g22770	1.238	0	0
Os06g05340	1.297	0	0
Panicle mild			
NIL10 specific	IR77298-14-1-2-B-10	IR77298-14-1-2-B-13	IR64
Os04g55520	1.257	0	0
Os03g08500	1.036	0	0
Os05g49010	1.358	0	0
Os01g21120	1.274	0	0
Os10g25170	1.015	0	0
Os09g28440	1.229	0	0

Note: 0 = Genes not differentially expressed in IR77298-14-1-2-B-13 and IR64

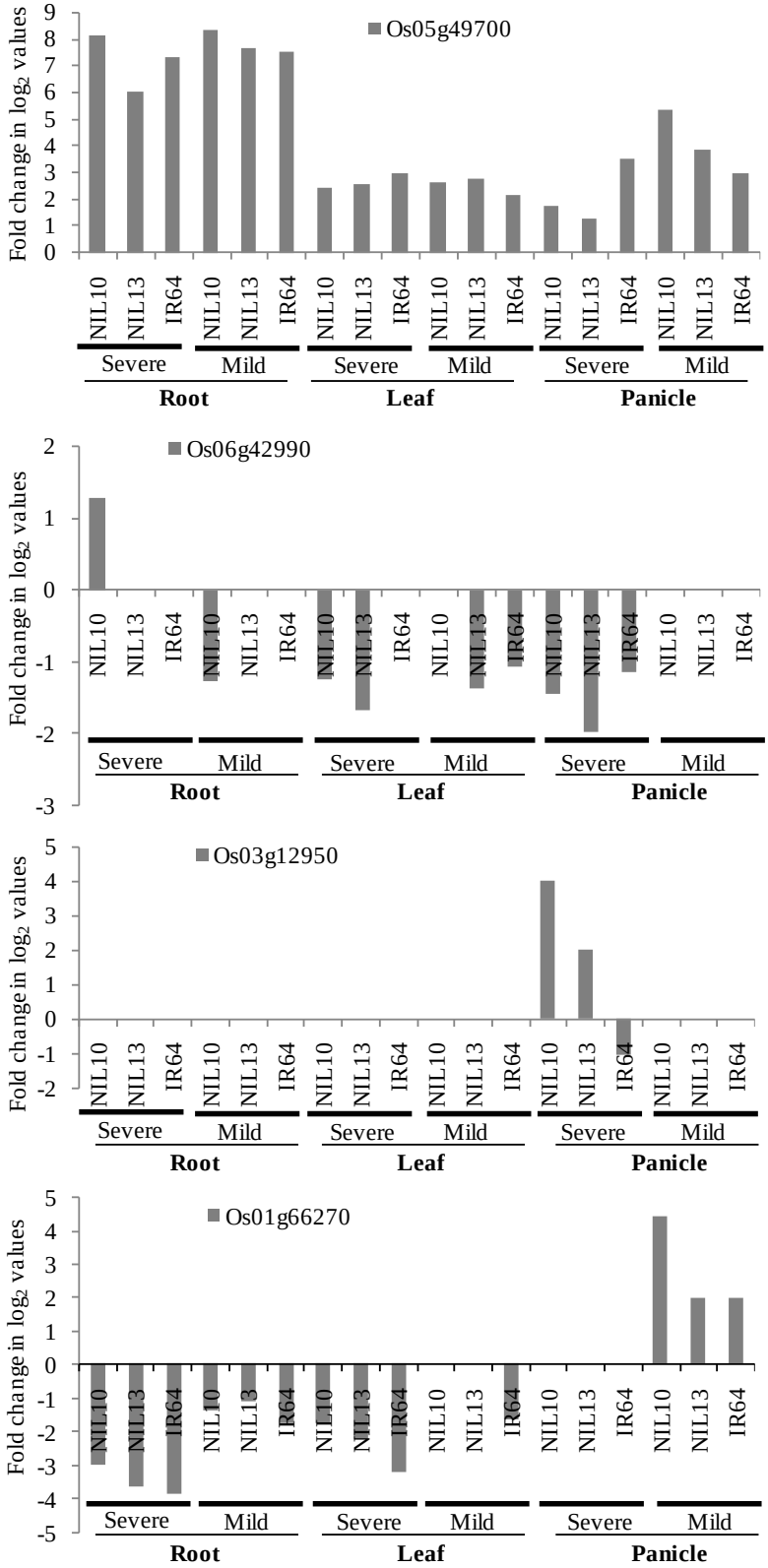

Fig. 15 Identification of the reference genes showing highest expression in the tolerant NIL compare to the counterparts under severe and mild stress in the root, leaf, and panicle tissues. Fold changes in log_2 values are shown on the *Y*-axis and the different genotypes are indicated on the *X*-axis. NIL10= IR77298-14-1-2-B-10and NIL13= IR77298-14-1-2-B-13.

Table 20. Subgroup specific differentially expressed genes in all tissues under sever and mild stress conditions in three genotypes.

Up-regulated

Subfamily/	No. of	Root severe			Root mild			Leaf severe			Leaf mild			Panicle severe			Panicle mild		
Subgroup	genes	NIL1	NIL1	IR6	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64
DREB-A1	10 (10)	1	2	2	2	2	2	2	2	2	3	3	1	4	5	5	3	3	3
DREB-A2	**6 (6)**	**2**	**2**	**2**	**2**	**2**	**2**	**0**	**0**	**0**	**1**	**2**	**1**	**4**	**3**	**5**	**2**	**2**	**1**
DREB-A3	4 (5)	1	1	1	0	1	0	2	2	2	1	2	0	1	1	1	2	1	1
DREB-A4	10 (12)	2	2	2	1	2	1	4	4	4	3	3	0	4	4	4	3	3	2
DREB-A5	12 (15)	3	4	2	3	3	3	4	3	3	2	3	0	5	5	5	3	3	3
DREB-A6	**7 (9)**	**0**	**0**	**0**	1	0	1	1	0	0	0	1	0	1	0	0	2	0	0
ERF-B1	18 (18)	1	0	0	0	0	1	2	2	2	2	2	2	5	5	5	6	5	4
ERF-B2	10 (12)	0	0	0	0	0	1	3	3	3	1	1	0	3	2	2	1	1	2
ERF-B3	15 (19)	1	1	1	2	1	0	4	4	3	4	3	2	**11**	**10**	**10**	**5**	**6**	**3**
ERF-B4	11 (12)	0	0	0	2	1	2	2	2	2	3	3	0	5	4	7	4	4	2
ERF-B5	4 (4)	0	0	0	0	0	0	0	0	0	0	0	0	**3**	**3**	**1**	**2**	**2**	**0**
ERF-B6	3 (3)	0	0	0	0	0	0	1	0	1	0	0	0	**2**	0	1	0	0	0
ERF-B7	7 (9)	1	1	0	0	0	0	1	1	1	0	0	0	3	3	2	0	0	0
AP2	18 (2)4	3	5	2	5	5	4	1	2	1	2	1	1	6	5	0	0	0	0
RAV	5 (5)	0	0	0	0	0	0	1	1	1	1	1	1	**4**	**4**	**4**	**2**	**2**	**2**

Down-regulated

Subfamily/	No. of	Root severe			Root mild			Leaf severe			Leaf mild			Panicle severe			Panicle mild		
Subgroup	genes	NIL1	NIL1	IR6	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64	NIL10	NIL13	IR64
DREB-A1	10 (10)	5	5	4	2	3	2	1	2	2	0	0	5	0	1	1	0	0	0
DREB-A2	**6 (6)**	1	1	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0
DREB-A3	4 (5)	0	1	0	0	0	1	1	1	1	0	1	0	1	1	1	0	0	0
DREB-A4	10 (12)	3	3	3	2	2	3	2	2	1	0	0	0	3	3	3	2	2	0
DREB-A5	12 (15)	3	2	2	3	2	3	1	1	1	0	1	1	3	3	3	1	1	0
DREB-A6	**7 (9)**	3	3	3	2	3	4	2	3	2	0	1	0	5	5	3	1	1	1
ERF-B1	18 (18)	6	7	6	7	8	5	6	5	3	2	2	4	3	4	4	0	0	0
ERF-B2	10 (12)	**7**	**6**	**7**	**6**	**6**	**5**	1	2	1	0	0	1	2	1	1	0	0	1
ERF-B3	15 (19)	5	6	6	6	6	5	4	2	4	2	1	1	0	1	1	1	1	0
ERF-B4	11 (12)	5	5	3	3	4	2	1	1	1	1	1	3	1	2	1	0	0	0
ERF-B5	4 (4)	**3**	**3**	**3**	**3**	**3**	**1**	**1**	**2**	**2**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
ERF-B6	3 (3)	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	1	1	0
ERF-B7	7 (9)	2	2	1	1	2	1	1	1	1	0	1	0	1	1	1	1	1	0
AP2	18 (2)4	5	0	5	4	1	0	3	3	2	2	4	0	3	4	3	2	2	0
RAV	5 (5)	**3**	**3**	**2**	2	2	2	2	2	2	1	0	1	**0**	**0**	**0**	**0**	**0**	**0**

Note; NIL10= IR77298-14-1-2-B-10, NIL13= IR77298-14-1-2-B-13; () = The number of genes in each subgroup and subfamily is indicated in parenthesis, classification by Sharoni et al. (2011).

Table 21. The number of differentially expressed gene patterns in Minghui 63 under non-stress conditions and severe stress conditions in the drought-tolerant IR77298-14-1-2-B-10.

Subfamily	No. of gene	Root		Leaf		Panicle	
		Up	Down	Up	Down	Up	Down
Minghui 63 under normal conditions							
AP2	20 (24)	2 (10%)	2	1	2	0	1
RAV	**5 (5)**	**0**	**0**	**0**	**0**	**0**	**0**
DREB	35 (57)	**3 (8.5%)**	**1**	**2 (5.7%)**	**2**	**3 (8.5%)**	**0**
ERF	56 (77)	2 (3.5%)	4	2 (3.5%)	8	**0**	3
	Total	7	7	5	12	3	4
IR77298-14-1-2-B-10 under stress conditions							
AP2	18 (24)	3 (16.6%)	5	1	3	**6 (33%)**	4
RAV	**5 (5)**	**0**	**3**	**1**	**2**	**4 (80%)**	**0**
DREB	49 (57)	**9 (18.3%)**	15 (30.5%)	13 (26.5%)	7 (14.2%)	**19 (38.7%)**	13 (26.5%)
ERF	68 (77)	4 (5.8%)	28 (41.1%)	13 (19.1%)	14 (20.5%)	**32 (47.1%)**	7 (10.2%)
	Total	16	31	28	26	**61**	24

Note: () = The number of genes in each subfamily is indicated in parentheses, classification by Sharoni et al. (2011). Selected tissues of Minghui 63, Root= seedling with 2 tillers, Leaf 1= young panicle at stage 3, Panicle 1= young panicle at stage 3, Up= up-regulated genes, Down= down-regulated genes. Bold values indicate greater difference between normal and stressed conditions.

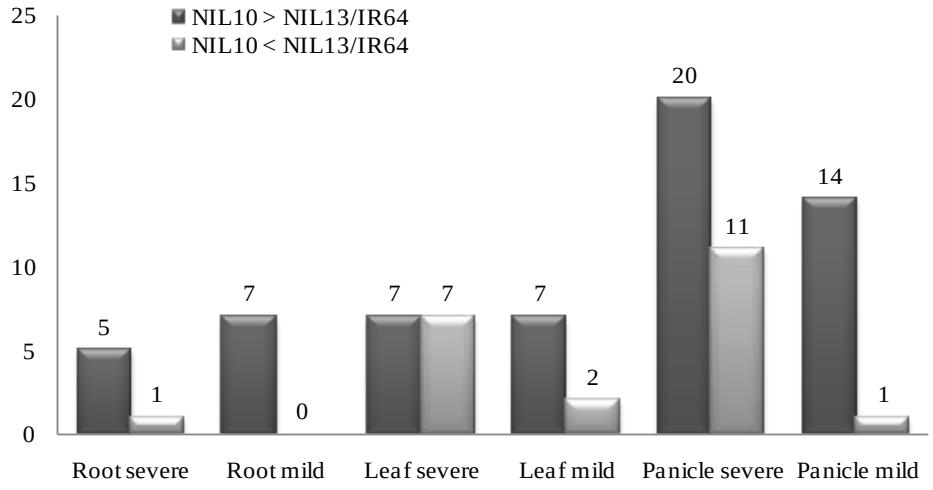

Fig. 16 The expression intensity of up-regulated genes was higher in NIL10 than in NIL13 or IR64 in all tissues except the leaf under severe stress conditions. The *X*-axis represents the selected tissues, and the *Y*-axis represents the number of up-regulated genes. The number of genes is provided at the top of each diagram. NIL10= IR77298-14-1-2-B-10 and NIL13= IR77298-14-1-2-B-13.

7.9. Consensus *cis*-regulatory elements in tolerant NIL

The interaction between TFs and *cis*-regulatory DNA sequences controls gene expression and constitute the essential functional linkage of gene regulatory networks. We examined the *cis*-elements in the promoter sequences (2-kb upstream region) of genes that were specifically up-regulated in tolerant NIL using a Rice *Cis*-Element Searching tool (RiCES, Doi et al. 2008). We determined that in the tolerant NIL under both DSCs, the most important *cis*-elements contained *cis*-motifs matching the published ARF, ASF-1/2, Dof1, GT-1, RAV1, WRKY, W-box, and MYB/C motifs. The different types of *cis*-elements that are involved in the responses to abiotic and biotic stresses are listed in **Table 22**. The majority of the *OsAP2/EREBP* up-regulated genes with tissue-specific expression patterns contained at least two of these *cis*-elements (**Table 22**), whereas uncommon *cis*-elements were found in genes in the susceptible line (data not shown). With the help of bioinformational analysis, we predict that further analysis of a number of these TFs will contribute to a better understanding of gene regulatory networks in rice under different kinds of drought stress conditions.

Table 22. Putative *cis*-elements enriched in promoters of rice *AP2/EREBP* genes.

Cis-element name	Sequence	TF	Stimulus/tissue
SURECOREATSULTR11	GAGAC	ARF	Auxin
CBFHV	RYCGAC		Dehydration, GA3
GATABOX	GATA	ASF-2	Seed
GT1GMSCAM4	GRWAAW	GT-1	Salt, Pathogen
SORLIP1AT	GCCAC	GT-1	Light
DOFCOREZM	AAAG	Dof1	carbon metabolism
OSE2ROOTNODULE	AAAGAT		
DRECRTCOREAT	RCCGAC	DRE/CRT	Drought
DRE2COREZMRAB17	ACCGAC	DRE	Drought response
WBOXATNPR1	TTGCA	W-box	SA
WRKY71OS	TGCA	WRKY	GA3
LTRECOREATCOR15	TGGCCGAC		Light
POLLEN1LELAT52	AGAAA		Pollen-specific
PRECONSCRHSP70A	SCGAY		
RAV1AAT	CAACA	RAV1	Seed maturation
RAV1AAT	CACCTG	RAV1	Seed maturation
MYBCORE	CNGTTR	MYB	Dehydration
MYBPLANT	MACCWAMC	MYB	Drought
MYCCONSENSUSAT	CANNTG	MYC	Drought
CAATBOX1	CAAT		Seed
ASF1MOTIFCAMV	TGACG	ASF-1	Auxin, SA, Biotic

7.10. Introgressed regions of the *OsAP2/EREBP* gene in the IR64 background

Advanced backcross quantitative trait loci (QTLs) analysis facilitates simultaneous identification and introgression of identified QTLs into a recipient genotype. We observed introgressed lines in our present study that can be used as an important genetic material to decipher the physiological and molecular basis of tolerance in individual genomic regions. In different rice genotypes, this study identified four *OsAP2/EREBP* genes that were introgressed (McNally et al. 2009) on chromosomes 3, 5, 7, and 10 in the IR64 background (**Fig. 17**). From this genome-wide comparison, four regions showed differential hybridization; these regions were further genotyped using SSR markers to confirm polymorphism between the parental lines. The four regions mapped for QTL identification were at 6.8-7.3 Mb, 6.7-7.2, 14.6-16.5 and 18.6-19.3 Mb on chromosomes 2, 4, 9 and 10, respectively (personal communication, Arvind Kumar). Of these 4 genes, one (*Os07g23710*) was not differentially expressed under both DSCs in the tissues that were examined. The *Os03g08460* gene was down-regulated in the root under both DSCs conditions in all genotypes, whereas the *Os09g30840* gene was down-regulated in the root and leaf under mild stress and in the panicle under severe stress in the drought susceptible line. Only *Os05g29810* was up-regulated in the leaf and panicle under severe stress in all genotypes (**Table 16**). The data show that the effect of the introgressed QTL of the *OsAP2/EREBP* genes on grain yield under drought conditions is unclear. The *Os09g25070*, *Os09g24490*, and *Os06g48950* genes are from WRKY, MYC, and ARF families, which are placed in the QTL region (personal communication, Arvind Kumar). We observed that the *cis*-element motif of *OsAP2/EREBP* genes is found in the different transcription factors mentioned above. *AP2/EREBP* may regulate target genes directly or indirectly thorough intermediary WRKY, MYB/C, and ARF transcription factors in tolerant NILs. Functional analysis is required to substantiate this suggestion. Bernier et al. (2007) have suggested that marker-assisted mapping and the introgression of major-effect QTLs for grain yield under drought conditions may provide a

fast-track approach for developing drought tolerant varieties, and Venuprasad et al. (2009) have reported several QTLs for yield and yield-related traits on different rice chromosomes under drought stress conditions.

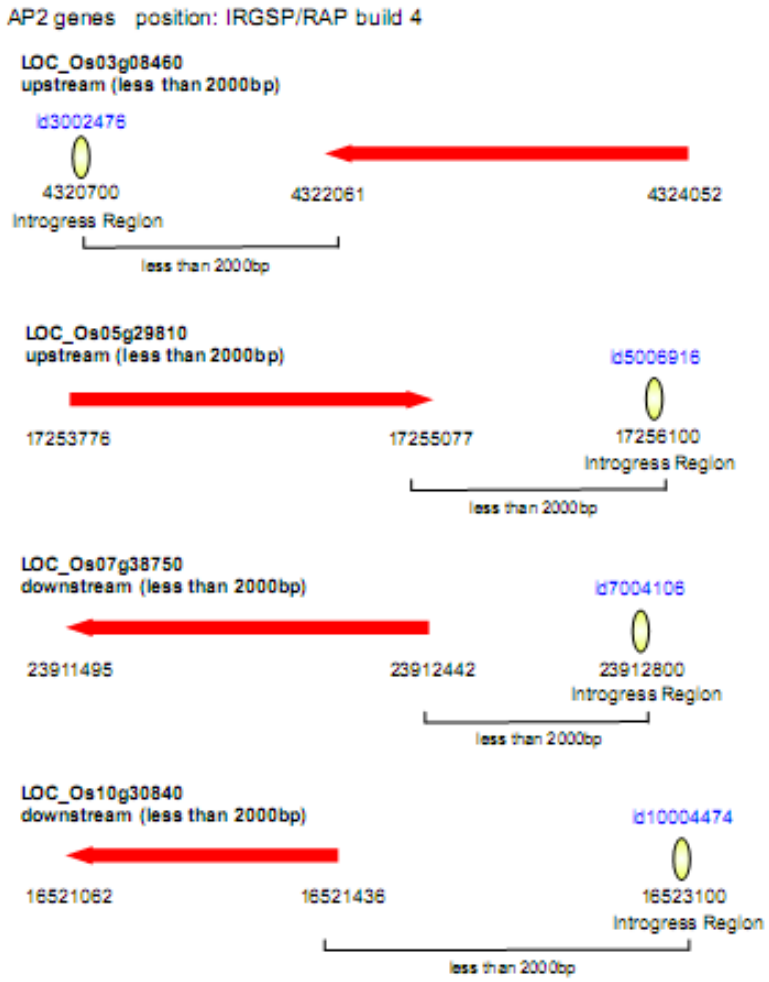

Fig. 17 Introgressed regions of four *AP2/EREBP* genes are presented in IR64 background.

7.11. Gene enrichment analysis for differentially expressed genes in NILs

The specifically expressed genes in tolerant NILs versus susceptible sister NILs during two drought stress treatments were examined for Gene Ontology (GO, Du et al. 2010) category enrichment using agriGO and KEGG orthology (KO, Kanehisa et al. 2008) analyses. The terms KEGG orthology (KO) and Gene Ontology (GO) are widely applied to understand the biological significance of microarray differential gene expression data (Du et al. 2010).

Overall, a global view of the gene expression analysis demonstrated that approximately 85% of genes were differentially expressed in all rice tissues in response to drought stress treatment. The GO and KO enrichment tests and biological pathway analysis indicate that the up-regulated *OsAP2/EREBP* genes in the drought-tolerant NIL IR77298-14-1-2-B-10 are not involved in other pathways, such as secondary metabolism, amino acid metabolism, response to stimuli, defense response, transcription, photosynthesis, cell wall growth, and signal transduction, and the down-regulated genes were not involved in any biological pathway. An assumption of this analysis is that the *OsAP2/EREBP* genes that demonstrated up-regulated expression in tolerant NIL contribute indirectly to these categories of gene function; however, further research is required to support this hypothesis. Information such as this would contribute to a deeper understanding of gene expression linked to regulatory networks under drought stress.

7.12. RT-PCR analysis for confirmation of the transcriptome data

To validate the accuracy of our microarray data, we selected 8 up- or down-regulated genes from the different tissues of all genotypes and both DSCs. We found that in most cases, the DEGs that were identified using the microarray were also detected using RT-PCR analysis (**Fig. 18**). The PCR results suggest that the DEGs observed through microarray analysis represent real differences between drought-stressed and non-stressed rice genotypes. Furthermore, Moumeni et al. (2011) confirmed this microarray data using qRT-PCR analysis on 10 root tissue DEGs selected from different functional pathways.

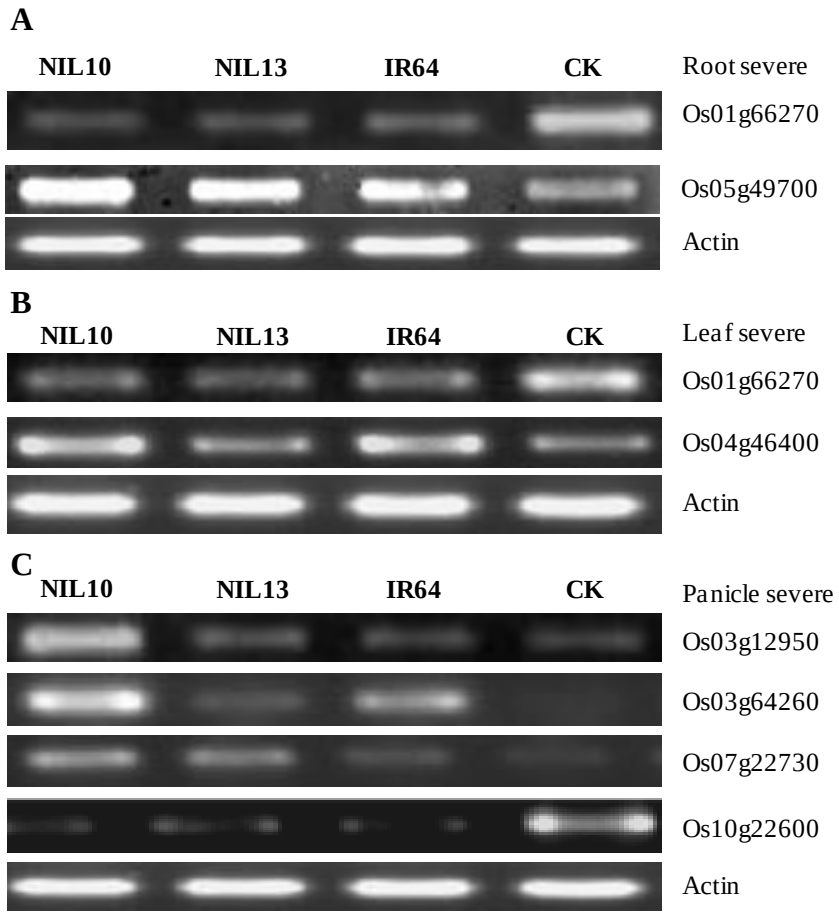

Fig. 18 RT-PCR analysis of differential gene expression (up- and down-regulated) in all tissues under both drought treatments in all genotypes**.** (A) In the root under severe conditions. (B) In the leaf under severe conditions. (C) In the panicle under severe conditions. *Actin* gene expression was used as the internal control.

8. Discussion

8.1. Expression patterns of *OsAP2/EREBP* genes under normal stress conditions

The objectives of this study were (i) to determine the expression patterns of members of this gene family under normal, hormone, and different drought stress conditions, (ii) to assess the number of genes responding to normal conditions or different stress treatments (responses in the drought tolerant line in particular is a novel contribution), (iii) to identify the important subfamily based on gene expression profiles in specific tissues during normal or stress

treatments, (iv) to identify the drought response *cis*-element, which may assist the understanding of the function of these novel genes in network systems, and (v) to select the most appropriate candidate genes for further functional analysis. Our studies present genomic information on the *OsAP2/EREBP* gene family in rice. These results indicate that, to varying extents, normal treatment (no drought stress) induced different expression levels of a number of *OsAP2/EREBP* genes. The similarity of the expression patterns among the *OsAP2/EREBP* genes may reflect shared induction mechanisms. However, differences in the accumulation levels of transcripts during normal treatment indicate that *OsAP2/EREBP* genes may be specifically controlled by specific factors. For example, *Os09g11460* was highly expressed in the stamen before flowering, and we can speculate that it plays an important role in pollen tube growth at this stage. In addition, *Os03g12950*, *Os05g25260*, and *Os08g36920* are mainly transcribed in panicles, suggesting that they participate in the development of young panicles (**Fig. 12B**). Consequently, pollen is the major site of variations in the expression levels for many genes (Becker et al. 2003; Czechowski et al. 2005). Moreover, *Os08g41030* is preliminarily expressed in the endosperm, suggesting that it might be needed for endosperm development (**Fig. 12B**). *Os03g08460/OsEBP-89* and *Os02g43970/AP59* exhibited a low expression or down-regulation in Minghui 63, and these plants exhibited a lower grain yield under normal and drought stress conditions (Yang et al. 2002; Oh et al. 2009). A significant percentage of *OsAP2/EREBP* genes were identified as showing higher expression levels in the reproductive stage compared with the vegetative stage. The expression pattern of a gene can reflect its functional relevance, e.g., the wide expression patterns of some *OsAP2/EREBP* members suggest that these genes may play regulatory roles at multiple development stages, whereas the unique expression patterns of other genes may indicate that these genes participate in specific biological processes.

8.2. Responses of *OsAP2/EREBP* genes in different treatments

Hormones regulate plant growth and development through a complex set of interactions. *OsAP2/EREBP* gene expression under hormone treatment requires extensive cross talk between the response pathways, and there are likely substantial physiological connections between AP2/EREBP protein production and hormone treatments. A higher expression pattern of a particular gene under these circumstances suggests that the genes play an important protective role against stressful hormone treatments (**Fig. 13**). *OsAP2/EREBP* genes (e.g., *CRL5*, *crownrootless5* and *OsAP239/Os04g52090*) in plants are affected by auxin and cytokinin (Kitomi et al. 2011), ABA and GA3 (Yaish et al. 2010). Shen et al. (2002) reported that the levels of different types of proteins increase or decrease following GA3 treatment in rice leaf sheaths. Under adverse conditions, abscisic acid (ABA) plays an important role in adapting the plant's response and can stimulate root growth and increase the ability of the plant to uptake water from the soil during drought stress. In this study, a number of genes (e.g., *Os05g41780*, *Os05g51300*, and *Os09g11460*) were induced in the root, leaf, and panicle under both hormone and drought stresses. Therefore, these genes may be involved in the uptake of water when the soil is dehydrated. The expression of *CRL5* (*crownrootless5*), which encodes a member of the AP2/ERF transcriptional factor family, was observed in the stem region where crown root initiation occurs (Kitomi et al. 2011). ABA triggers the closing of the stomata when soil water is insufficient to keep up with transpiration. Drought-stressed leaves produce large amounts of ABA, which triggers potassium ions to be transported out of the guard cells, causes the stomata to close, and water is held in the leaf. On basis of our results, we speculate that *OsAP2/EREBP* genes function in ABA signaling pathways and in the defensive response against water deficit. The expression of *OsAP239/Os04g52090*, which was up-regulated in the leaf in both NILs under severe stress but was higher in IR77298-14-1-2-B-10, was shown to control the ABA/GA3 balance in rice to allow seed production (Yaish et al. 2010). Based on the expression data, it is

difficult to configure the complex regulatory network of this gene family, but the data may provide valuable insights for understanding the responses to different hormones. A number of *OsAP2/EREBP* genes demonstrated a high or low expression under different hormone treatments at the seedling stage (**Fig. 13**).

8.3. *AP2/EREBP* gene expression profiles under severe stress and mild stress conditions in NILs

During drought stress, plants adapt to the new environment by changing their gene expression profiles after perceiving stress signals (Hazen et al. 2005). Analyses of *AP2/EREBP* gene family have revealed a wealth of information about the gene expression in this family of genes with fundamental implications for understanding the biological processes underlying growth and development of the plant. Knowing the gene expression profile of *OsAP2/EREBP* genes may provide clues for selecting the most appropriate candidate genes for functional analyses. Our study focused on the identification and expression profiles of drought stress–responsive genes from rice genotypes at different developmental stages under different stresses, with the aim of identifying important components of the drought-stress response. The analysis of the *OsAP2/EREBP* genes showed high water-usage efficiency in the drought-tolerant genotype. In the whole rice genome, approximately 7% of the genes are induced by drought or salt stresses (Zhou et al. 2007), whereas in the *AP2/EREBP* family, at least 85% (140 of 163 rice genes) of the genes were differentially expressed (up- or down-regulated) in one or more tissues with two folds under both DSCs. We considered the DEGs in a tolerant line compared with the sister (susceptible) line and the control and focused on those genes that were exclusively up-regulated in IR77298-14-1-2-B-10. Although the common genetic background of rice NILs, which are the backcross progeny from Aday Se. x IR64, is approximately 97% for the IR77298-14-1-2-B family (Venuprasad et al. 2011), the tolerant NIL showed distinctive differences in its gene expression responses to different drought

stress levels. Comparative gene expression profiling is an alternate and efficient way to identify pathways and genes regulating the stress response (Udupa et al. 1999).

Sharoni et al. (2011) identified 13 rice *AP2/EREBP* genes that were drought-inducible in the seedling stage at different time points; however, our study revealed some common and unique up-regulated genes in different tissues in both NILs under drought stress (**Fig. 14**) and during hormone treatments (**Figs. 12, 13**). Complex genetic networks function during the development of every organ in plants, and substantial gene-expression overlaps exist between the developmental pathways and the stress-response pathways (Cooper et al. 2003; Shinozaki et al. 2003). More DEGs and higher gene expression intensities were observed in IR77298-14-1-2-B-10 compared with IR77298-14-1-2-B-13 and IR64, and 16 genes were up-regulated in the IR77298-14-1-2-B-10 tolerant line specifically that were not up-regulated in IR77298-14-1-2-B-13 or IR64 (**Table 19**). For example, *Os09g39850*, *Os02g43940*, and *Os06g08340* were up-regulated in IR77298-14-1-2-B-10 specifically in the root, leaf, and panicle with higher expression under both DSCs than in IR77298-14-1-2-B-13/IR64. *OsDRED2A/Os01g07120*, *OsDREB1A/Os09g35030*, and *AP37/Os01g58420* expression intensities were higher in the root, leaf, and panicle in IR77298-14-1-2-B-10 compared with IR77298-14-1-2-B-13 and the control under severe or mild stress, and these genes are tolerant to drought, high salt, and cold stress in the transgenic rice plant (Dubouzet et al. 2003; Oh et al. 2009). TSRF1 improves the osmotic and drought tolerance of rice seedlings without growth retardation and increases the expression of MYB, MYC, and photosynthesis-related genes, probably by binding to dehydration responsive element and GCC boxes in the promoter regions of the target genes (Quan et al. 2010). A number of genes that are common or overlapped among tissues, for example, *Os05g49700* and *Os01g07120* (DRED-A1 and DREB-A4 subgroups) were highly expressed in all three tissues under severe and mild DSCs in both NILs. A large fraction of the genes that are regulated by dehydration are also up-regulated by pollination (Lan et al. 2005). In our study, the expression of several

OsAP2/EREBP genes (e.g., *Os03g08500* and *Os05g49010*) was highly induced at the pollination stage; thus, these genes may be involved in the drought response in IR77298-14-1-2-B-10 (**Table 19**), although further research is required to establish this possibility. Similar conclusions have been drawn from the analysis of drought-inducible *OsDREB2A/Os01g07120*, *AP37/Os01g58420*, and *OsDREB1A/Os09g35030* genes, which regulate plant tolerance in transgenic rice during conditions of drought stress (Dubouzet et al. 2003; Oh et al. 2009). Conversely, *Os03g08460/OsEBP-89* and *Os02g43970/AP59* genes are expressed at a low-level in the root, leaf, panicle, and endosperm, and a decrease in the grain yield by 23% to 43% was observed under normal and drought stress conditions (Yang et al. 2002; Oh et al. 2009). A number of novel genes are candidate reference genes because of their specific/higher expression patterns (**Fig. 15, Table 19**). Differential gene expression is thought to reflect the drought response. Among the ERF subgroups, the expression of a number of genes from subgroups ERF-B3 and ERF-5 was induced in the panicle under both DSCs, whereas almost all the genes in subgroup DREB-A6 exhibited very little up-regulation under both DSCs in all tissues that were analyzed (**Table 20**). The genes of the RAV subfamily are highly induced in panicle tissue under conditions of severe drought stress compared with normal growth conditions. The N-terminal regions of the RAV subfamily genes are homologous to the AP2 DNA- binding domain but their C-terminal regions are homologous to ABI3 and VP1 transcription factors and the AP-2 and B3-like domains of RAV bind autonomously to CAACA and CACCTG motifs (Kagaya et al. 1999). The protein product of the genus *Phaseolus vulgaris* ABI3-like factor (*PvAlf*) is similar to the ABI3 and VP1 proteins and functions to develop seed maturation and seed abscission (Bobb et al. 1995). CAACA and CACCTG motifs were found in the promoter region of RAV subfamily gene members and the genes were induced in the panicle under severe stress conditions in the drought-tolerant NIL, which indicates that these genes may be involved in endosperm development (**Table 21**).

8.4. Defense system of *AP2/EREBP* genes in rice

We predicted that some gene-specific *cis*-elements might be important in the regulation of the target gene by other factors and that these *cis*-elements may influence the introgressed regions of drought QTLs. Transcription factors and *cis*-elements function in the promoter region of different stress-related genes, and overexpression of these genes may improve the plant's tolerance to abiotic or biotic stress. In response to drought, the AP2/EREBP TF regulates many target genes by binding to the *cis*-element (A/GCCGAC) in the promoter region of candidate genes, and this transcriptional regulatory system is known as a regulon. ABA is produced under drought stress conditions and plays a crucial role in plant tolerance to drought (Shinozaki et al. 2003). AP2 and other regulons, such as *OsDREB2*, respond to dehydration in rice (Dubouzet et al. 2003), and the dehydration-responsive element binding protein (DREB1 and DREB2)/C-repeat binding factor (CBF) regulons function in ABA-independent gene expression. Meanwhile, the ABA-responsive element (ABRE) binding protein (AREB)/ABRE binding factor (ABF) or the ABRE and coupling element (CE) regulon functions in ABA-dependent gene expression. The DREB1/2 homologous genes (OsDERB1A and OsREB2A) were isolated from grasses, such as rice and maize (Dubouzet et al. 2003; Qin et al. 2007b). ABA-activated OSRK1 protein kinases phosphorylate and activate AREB/ABF-type proteins in rice (Chae et al. 2007). ABA-independent and ABA-dependent signal transduction pathways convert the initial stress signal into cellular responses. The members of TF families that are involved in both ABA-independent (AP2/ERF, bHLH, and NAC) and ABA-dependent (MYB, bZIP, and MYC) pathways are up-regulated in rice. TFs belonging to these families interact with specific *cis*-elements and/or proteins, and their overexpression confers stress tolerance in heterologous systems (Kizis and Pages 2002; Zhu et al. 2003; Lee et al. 2004). It has been shown that *OsAP2-39/Os04g52090* is overexpressed in transgenic rice and up-regulates the ABA biosynthetic gene *OsNCED-1* (Yaish et al. 2010). Microarray analyses have shown that up-regulated genes in rice plants overexpressing *AP37/Os01g58420* were drought stress tolerant

and exhibited increased seed setting (16%–57% more than controls) in the field (Oh et al,. 2009). The homologous genes, wheat *TaDREB1*, wheat *WDREB2*, and maize *ZmDREB2A* are also responsive to cold stress (Shen et al. 2003; Qin et al. 2007b). These results indicate that the AP2 regulon may have additional functions in rice. Therefore, comparative analysis of gene expression profiles under both DSCs to determine the functional role of these genes in the growth of the plant and its response to stress, and the identification of target genes for TFs involved in stress responses are vital. In this study, the regulation of *OsAP2/EREBP* genes was precise in terms of the spatial and temporal distribution.

This study provides an overview of gene expression patterns of the *OsAP2/EREBP* gene family under normal and stress conditions. The application of a new comprehensive 44K oligoarray platform together with rice NILs enabled us to determine gene expression patterns in different tissues of two NILs with contrasting yield performances under drought stress at the reproductive stage of growth. By comparing the gene expression patterns of NILs under drought stress and normal conditions, we identified several putative genes that may be responsible for the drought response in the drought-tolerant line. These genes should be considered novel reference genes, and further research is needed to prove their functions. A number of putative *cis*-elements were identified, which may be help understand the function of these key genes. These data provide a useful reference and establish a starting point for determining the functions of the *OsAP2/EREBP* family of genes in rice at the reproductive stage of growth. The genes belonging to the ERF-B3 and ERF-B5 subgroups and the RAV subfamily gene members were activated in leaf and panicle tissues but not in the root. The specific subfamily (RAV) or subgroups of this gene family provide a new avenue for investigating appropriate candidate genes for functional analysis under different treatments. Overexpression and knockdown analyses of selected members of this gene family are underway in our laboratory to investigate optimal molecular breeding schemes for the *OsAP2/EREBP* gene family.

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