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AP2/EREBP transcription factor family genes are differentially expressed in rice seedlings during infections with different viruses

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Abstract

AP2/EREBP transcription factor family members are important regulators of plant growth, development, and biotic stress responses. Expression profiles of the 147 *AP2/EREBP* gene family members were studied in rice infected with *Rice transitory yellowing virus* (RTYV) and *Rice dwarf virus* (RDV strains, RDV-D84, RDV-O, and RDV-S. Microarray analysis showed that 30% of the *OsAP2/EREBP* genes were differentially regulated during virus infection compared with the control. Rice infected with either RDV-S showed the highest number of up-regulated *OsAP2/EREBP* genes, while RTYV infected plants had the lowest. These results correlate with the severity of the syndromes induced by the different viruses. Three of the five genes in the RAV subfamily and B5 subgroup were more highly expressed during RDV-S and RDV-O virus infections, while all of the genes in the DREB subfamily or A1 to A6, B3 and B4 subgroups were down-regulated during RTYV infection, suggesting roles in response to virus infection. The number of genes activated during RDV infections was greatest during infection with the S-strain, followed by the D84-strain and the O-strain, with three *OsAP2/EREBP* genes up-regulated during infection by all three strains. A common set of 3 genes (*Os04g46220*, *Os05g39590*, and *Os07g22730*) showed higher expression during infection by at least three different viruses, of which, *Os04g46220* showed up-regulation during infection with all viruses.

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Introduction

Transcription factors (TFs) are regulators of gene expression that have been shown to have a vital role in plant responses to diseases. The *AP2/EREBP* gene family includes a large number of plant specific transcription factors. The *AP2/EREBP* (for apetala2/ethylene-responsive element-binding protein) transcription factor gene family has been classified into four subfamilies: APETALA2 or Apetalous (AP2), Related to ABI3/VP1 (RAV), dehydration-responsive element binding protein (DREB) and ethylene-responsive element binding factor (ERF).

The ERF and DREB subfamilies 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). Proteins encoded by the *AP2/EREBP* gene family are defined by the AP2/ERF DNA-binding domain (Riechmann *et al*. 1998), which consists of 50 to 60 amino acids. These proteins are required for a variety of regulatory mechanisms during the plant life cycle.

Plant disease symptoms caused by different virus infections are very common in Southeast Asian countries (Ramirez 2008). However, relatively little is understood concerning the role of specific TF families in viral disease responses in plants. Transcriptional regulation of stress-responsive genes is mediated by changes in the activity of DNA-binding transcription factors such as Myb, Myc, bZIP, Zn finger and NAC (Nuruzzaman *et al*. 2013).

Several pathogenesis-related (*PR*) genes induced during pathogen infection are up-regulated by one or more signaling molecules, such as salicylic acid (SA), ethylene and jasmonic acid (JA) (Koo *et al*. 2007). A number of TFs have been shown to play significant roles in controlling the expression of *PR* genes; for example, ERF proteins activate *PR* genes by binding to the GCC box (GCCGCC) of their promoters, thus regulating the plant defense response to pathogen infection (Giri *et al*. 2014). These findings indicate that the induction of *PR* genes in plants is mediated by different ERF proteins or signaling molecules. However, the regulation of *PR* genes by the subfamily members AP2, RAV, ERF and DREB and in response to biotic stress remains unclear. AP2/EREBP proteins were shown to be integral to biotic and abiotic stress responses through their interaction with *cis*-acting elements, the GCC box, and/or CRT/DRE (for C-repeat/dehydration response element) (Sun *et al*. 2014; Catinot *et al*. 2015).

The members of the ERF subfamily, which include tobacco (*Nicotianatabacum*) ERF1 to -4, Arabidopsis ERF1 to -5, ORA59, tomato Pti4 to -6, tomato ERF1 to -4, and tomato stress-responsive factor, have been discoveredto act as transcriptional activators that bind to the GCC box in response to biotic stresses (Pre *et al*. 2008). Although AtERF4/7 also regulates genes by interacting with a GCC box, it is a transcriptional repressor and thus a negative regulator capable of modulating both biotic and abiotic stress responses (Quan *et al*. 2010; Maruyama *et al*. 2013; Dong *et al*. 2015). The DREB and ERF subfamilies are of special concern owing to their involvement in plant responses to various stresses (Agarwal *et al*. 2010; Jisha *et al*. 2015). TFs encoded by genes in the DREB subfamily play an important role in the resistance of plants to biotic stresses (such as tobacco streak virus infection) and to abiotic stresses (Gutha and Reddy 2008).

Genetic and molecular approaches have been used in combination to characterize *AP2/EREBP* gene family members involved in various biotic stress response pathways, including genes related to tomato bacterial speck disease (Gutterson and Reuber 2004), Arabidopsis growth promoting rhizobacteria, Arabidopsis plant defense against biotrophic and hemibiotrophic pathogens such as *At4g13040* (Giri *et al*. 2014) and enhanced resistance to *Phytophthorasojae* in soybean (Dong *et al*. 2015).

Previously, we discovered 163 *OsAP2/EREBP* genes in rice gene databases of which only a small number of the genes had known functions Sharoni *et al*. (2011). Our current study extends information on this gene family by comparative analysis of data on the transcription of AP2/EREBP genes during infection

of rice with four different viruses which were *Rice transitory yellowing virus* (RTYV) and three different strains of *Rice dwarf virus* (RDV strains RDV-D84, RDV-O, and RDV-S). RTYV and RDV are single-stranded RNA (ssRNA) viruses. Thus the aim of this study was to determine genes that are commonly expressed in all virus infections, to identify genes which show the highest expression levels in one or more virus infections.

Materials and methods

Plant materials

Nipponbare rice seeds (*Oryza sativa* L. ssp. *japonica* cv.) were supplied by Drs. M. Yano and T. Matsumoto of the National Institute of Agrobiological Sciences (NIAS), Japan.

Inoculation of different viruses

RTYV and RDV were obtained from Dr. Toshihiro Omura, Research Team for Vector-Borne Plant Pathogens, National Agricultural Research Center, Tsukuba, Ibaraki, 305-8666, Japan. Experiments for the above virus infections were tested at the National Institute of Agrobiological Sciences, Japan. RDV three strains (RDV-D84, -O, and –S) were independently inoculated into rice seedlings via viruliferous green leafhopper (GLH: *Nephotettixcincticeps*). Inoculated plants were maintained in a temperature-controlled greenhouse (28 ± 3 ºC, natural sunlight). Samples were collected from RDV-infected plants at 21 days post-inoculation (dpi); and RTYV-infected plants at 24 dpi. For each sample, the shoots of the inoculated plants were cut 3 to 5 cm above the soil surface. The plant samples/tissues were frozen in liquid nitrogen and stored at –80 ºC until extraction of RNA.

Estimations of virus accumulation

For samples for infection with each of the viruses, shoots from ten individual plants were collected and used to make extracts which were diluted 1 in 10 (w/v) in phosphate-buffered saline. Virus accumulation in plants was estimated by enzyme-linked immunosorbent assay (ELISA) (Shibata *et al*. 2007). All experiments were conducted in triplicate (3 biological replications).

Microarray experiments

Total mRNA samples from rice infected with RTYV, and RDV were extracted using a fluorescent linear amplification kit (Agilent Technologies, USA) in accordance with the manufacturer's instructions.Cyanine-3 (Cy3)- and cyanine-5 (Cy5)-labeled target complementary RNA (cRNA) samples were prepared from 850 ng total mRNA. Transcriptome profiles specific to infected plants were examined by direct comparison of transcription activities between infected and uninfected plants on the same oligo array. Hybridization solution containing 825 ng of each of the Cy3- and Cy5-labeled cRNA preparations was prepared using an in situ Hybridization Kit Plus (Agilent Technologies, USA). The fragmented cRNAs were added to the hybridization buffer, applied to the microarray, and hybridized for 17 h at 60°C.

The scanned microarray images were analyzed using Feature Extraction 6.1.1 software (Agilent Technologies, USA), and the dye-normalized, background-subtracted intensity and ratio data were exported to a text file. In cases where the software flagged corrupted spots or detected a lack of differences between sample spots and the background, these data were not included in further analysis. All arrays were performed in triplicate with independent samples.

Microarray-based gene expression data analysis

Gene expression data from all virus infections RTYV (GSE34266: www.ncbi.nlm.nih.gov/gds/?term=GSE34266 and RDV (GSE24937: www.ncbi.nlm.nih.gov/gds/?term= GSE24937) are available at NCBI. In addition, we applied our 44k array data virus- infected rice plants above, which are available at NCBI Gene Expression Omnibus GEO (platform number GPL7252, www.ncbi.nlm.nih.gov/geo; or www.ncbi.nlm.nih.gov/gds; (Barrett *et al*. 2009). The Cy3 and Cy5 signal intensities were normalized applying rank-consistency filtering and the LOWESS method and worked by Feature Extraction version 9.5 (Agilent Technologies, USA). Expression profiles of samples were transformed into $\log_2$-based numbers

and normalized by EXPANDER version 5.0 (Shamir *et al*. 2005) according to the quantile method for the standardization of array slides. Up- or down-regulation was defined as a gene with a $\log_2$–based ratio (infected samples/control) ≥0.585 or ≤–0.585. A significant difference in gene expression patterns between the treated plants and the control was indicated by P≤0.05 by paired t-test (permutations, all possible combinations; FDR collection, adjusted Bonferroni method). Data processing was performed through MeV version 4.4 (Saeed *et al*. 2006). We disclosed 147 *OsAP2/EREBP* genes out of 163 Sharoni *et al*. (2011) from 44K microarray data collected during different virus infections.

Gene expression analysis by RT-PCR

RT-PCR was performed to confirm the differential expression of representative *OsAP2/EREBP* genes identified by microarray data analysis using gene-specific primers (Table 1).

The complementary DNA (cDNA) fragments for transcripts of selected *OsAP2/EREBP* genes for the RDV and RTYV viruses' genome were synthesized using 1,000 ng of the corresponding RNA with 50 ng/µl of random hexamer by Super Script III reverse transcriptase (Invitrogen, USA). The resultant reaction mixtures containing cDNA were diluted four times. Four µl of diluted mixture was used for this test. Each PCR was done in triplicate using an ABI 9700 Thermocycler (Applied Biosystems, USA) with incubation at 94 °C for 1 min, 55 °C for 50 s, and 72 °C for 1 min.

The RT-PCR tests consisted of 30–40 cycles, depending on the linear range of PCR amplification for each gene. The "LOC_" prefix has been excluded from the Michigan State University (Osa1) locus IDs in this study.

Results

Disease symptoms for different virus infections

In this study, we infected rice with each of the viruses then sampled the RDV-infected plants at 21 dpi and RTYV-infected plants at 24 dpi (Figure 1). Rice plants infected with RDV showed disease symptoms such as stunted growth, chlorotic specks on leaves (Figure 1). The rice plants infected with RTYV and RDV were much shorter and had more leaf yellowing than mock-infected or control (uninfected) plants (Figure 1).

OsAP2/EREBP gene expression patterns during virus infections

To gain insight into the comparative functions of the *OsAP2/EREBP* gene family members in response to infections caused by RTYV and RDV microarray analysis was used to determine expression profiles of infected rice seedlings. In the RAV subfamily and B5 subgroup, 3 of the 5 genes were more highly expressed during RDV-O and RDV-S virus infections than RTYV virus (Table 2). While all of the genes in the DREB subfamily or subgroups A1 to A6, B3 and B4 were down-regulated during RTYV infection (Table 2). We compared common and specific up-regulated and down-regulated genes among all virus infections. *Os04g46220* gene was commonly up-regulated in all virus infections (Table 2). We discovered two *OsAP2/EREBP* genes (*Os05g39590* and *Os07g22730*) were up-regulated at least three viruses (Table 2).

Identification of common infection-responsive candidate genes

Out of 163 *OsAP2/EREBP* genes, we identified 147 *OsAP2/EREBP* genes in our 44K array data. By using a threshold of 0.585–fold (up or down) difference between infected and control samples for each virus, 45 (30%) of the 147 *OsAP2/EREBP* genes were differentially expressed in at least one of the four virus infections (Table 2). The number of genes up-regulated (20) was highest at 21 dpi during RDV-S infection, followed by RDV-84, RDV-O, and 24 dpi (listed in decreasing order) during RTYV infections (Figure 2). Among the three strains of RDV tested, the degree of gene response to RDV-O infection was generally lower and the degree of gene response to RDV-S infection was generally higher, with all infectious responses showing a greater number of up- than down-regulated *OsAP2/EREBP* genes (Figure 2). To discover candidate genes that may be responsible for virus infection responses in the rice seedlings, this study focused on genes that exhibited the largest changes in expression in infected plants compared to control plants, using a threshold of ≥1.5-fold or ≥0.585 $\log_2$–based ratio (Table 1).

Table 1. Selected genes and corresponding primer sequences used for RT-PCR.

Gene	Forward primer	Reverse primer
Os05g47650	CAGAGCTACGTGCTCACCAA	TGTGCTTGTGACTCAGCCAT
Os10g41330	GGGTACTGGGCGTGTCAG	GCCATGATCAGCCTCTCCTT
Os06g10780	CAGAAGACGCGGATATGGCT	GGAGACTGGAAGAACGCCTC
Os02g43790	TCCTACGACGGGTCCTCCAT	ATGAAGGGGAAGAAGAGCCG
Os08g43210	CATCATCGTCCGTGTTCCCA	GCTCCACAGAGACACGTCAG
Os03g05590	GGGAAATCGTCGTCGTCGTA	CACTCCAGCTCCACCTTGTC
Os02g29550	CCAGGCAAGGAAATCAAGCG	TCACTCTGTCCTACCAGCGA
Os11g06390 Actin	AGTGCTCCTCGTCGTCGT	GAGCTGCAGGAGAAGCTCAT

For convenience, the "LOC_" prefix has been omitted from the Michigan State University (Osa1) locus IDs in this manuscript.

In the rice seedlings, several genes such as *Os04g46220* (B5), and *Os08g36920* (B7) exhibited higher expression levels at 21 and 24 dpi during RTYV and RDV virus infections (Table 2). *Os05g39590* (B5) gene was highly activated in the seedlings infected with all RDV strains (RDV-84, -O, and -S) (Table 1). In addition, in the seedlings under RDV- O and RDV-S strains of RDV virus infections, we noted that genes assigned to the RAV subfamily and B5 (or ERF subfamily) were highly expressed when compared with the control (Table S2).

Table 2. Differentially expressed genes under different virus infections, > 0.585 =Up-regulated and < -0.585=Down-regulated.

MSU locus/Gene	P. group	RTYV	RDV-D84	RDV-O	RDV- S
Os01g66270	A1				
Os02g45420	A1				
Os04g46440	A1				
Os05g49700	A1				1.36
Os06g06540	A1				
Os08g43200	A1				
Os08g43210	A1				
Os09g35010	A1				
Os09g35020	A1			1.49	
Os09g35030	A1			1.71	
Os01g04020	A2				
Os03g05590	A2				
Os05g25260	A2				
Os05g27930	A2				
Os05g37640	A2				
Os08g45110	A2				
Os02g09650	A3				
Os02g54160	A3				
Os04g55520	A3				
Os06g07030	A3				
Os06g36000	A3				
Os01g07120	A4		1.05		
Os02g10760	A4		-2	-1.7	-3.1
Os02g34270	A4				2.03

Os02g43820	A4			
Os02g43940	A4			
Os04g34970	A4		1.18	0.99
Os04g46250	A4			1.95
Os04g46400	A4	-1.1		-2.2
Os04g46410	A4			
Os06g11940	A4			
Os10g41130	A4			
Os11g13840	A4			
Os01g64790	A5	1.79		3.35
Os02g43970	A5			-2.6
Os02g52670	A5	1.83		1.58
Os03g09170	A5			
Os04g52090	A5			
Os05g32270	A5			
Os06g06970	A5			
Os06g09390	A5			-1.2
Os06g09717	A5			1.97
Os06g09760	A5			
Os06g09790	A5			
Os06g09810	A5			
Os06g11860	A5			
Os08g31580	A5			
Os10g38000	A5			
Os02g45450	A6			
Os03g08500	A6			
Os04g36640	A6			-1.2
Os05g49010	A6			
Os06g10780	A6	1.6		2.24
Os08g27220	A6			
Os09g20350	A6			
Os10g22600	A6			
Os01g10370	B1			
Os01g21120	B1			
Os01g54890	B1			
Os01g73770	B1	4.17		
Os02g29550	B1			-0.7
Os02g35240	B1			
Os03g22170	B1			
Os04g32620	B1			1.26
Os04g48350	B1			
Os04g57340	B1			
Os05g41760	B1			-1.2
Os06g42990	B1			
Os07g10410	B1			
Os07g42510	B1			
Os07g47790	B1		1.47	
Os10g25170	B1			
Os01g12440	B2			

Os02g54050	B2		2.13		
Os03g08460	B2				
Os03g08490	B2				
Os03g15660	B2				1.6
Os05g28350	B2				
Os06g08340	B2				
Os07g47330	B2				
Os09g11460	B2	0.68			
Os09g11480	B2				
Os01g46870	B3				
Os02g43790	B3				
Os02g55380	B3				
Os03g08470	B3				
Os03g60120	B3				
Os03g64260	B3				
Os04g44670	B3				
Os05g36100	B3				
Os05g41780	B3				
Os06g03670	B3		2.32		2.91
Os06g40150	B3				-6.2
Os07g12510	B3		-0.7		-0.9
Os07g22730	B3		1.66	0.77	1.94
Os08g44960	B3				
Os09g39810	B3				
Os09g39850	B3				
Os10g30840	B3				
Os01g58420	B4				
Os02g32140	B4				1.85
Os02g34260	B4				
Os04g18650	B4				
Os05g34730	B4				
Os08g35240	B4				
Os08g41030	B4				
Os09g28440	B4				
Os11g06770	B4				
Os04g46220	B5	0.66	0.92	0.95	1.22
Os05g39590	B5		3.03	2.94	3.49
Os10g41330	B5			1.71	
Os07g03250	B6				
Os07g22770	B6				
Os12g39330	B6				
Os02g13710	B7				-1.3
Os05g29810	B7		0.6		
Os06g47590	B7				
Os08g07700	B7				
Os08g36920	B7		5.97		
Os10g26590	B7				
Os12g41030	B7				0.65
Os12g41060	B7				

Os01g59780	AP2					
Os01g67410	AP2					
Os02g51300	AP2					
Os03g07940	AP2					
Os03g12950	AP2	0.63				
Os03g19900	AP2	-2.1				
Os03g56050	AP2					
Os03g60430	AP2					
Os04g42570	AP2				1.02	0.61
Os04g55560	AP2					
Os04g55970	AP2		-0.7			
Os05g03040	AP2					
Os05g45954	AP2	0.94				
Os06g43220	AP2					
Os07g13170	AP2					
Os08g34360	AP2					
Os09g25600	AP2					
Os11g03540	AP2					
Os11g19060	AP2					
Os12g03290	AP2					
Os01g04750	RAV					
Os01g04800	RAV					1.34
Os01g49830	RAV					1.17
Os05g47650	RAV	0.65				
Os08g42550	RAV		1.15			1.66

Footnote: Not differentially expressed genes are indicated as blank space. 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). RTYV =*Rice transitory yellowing virus* and RDV =*Rice dwarf virus*.

Gene expression analysis by RT-PCR

To assess the accuracy of the microarray data, we selected five non-redundant genes *Os06g10780, Os07g22730*, *Os05g39590*, *Os04g46220*, and *Os07g12510* that were differentially expressed in response to viral infection and examined the similarity between gene responses observed by microarray and by RT-PCR (Figure 3). Rice *actin* gene (*LOC_Os11g06390*) was used as an internal control, and its expression remained nearly constant under all experimental conditions (Figure 3). We observed that microarray and RT-PCR data, which were calculated based on the median of three repeated measurements, showed good correlation for RDV-84 and RDV-S virus infections compared with the mock/control (Figure 3). The oligoarray data of our lab have also been confirmed or published previously from our lab Satoh *et al*. 2011, 2013).

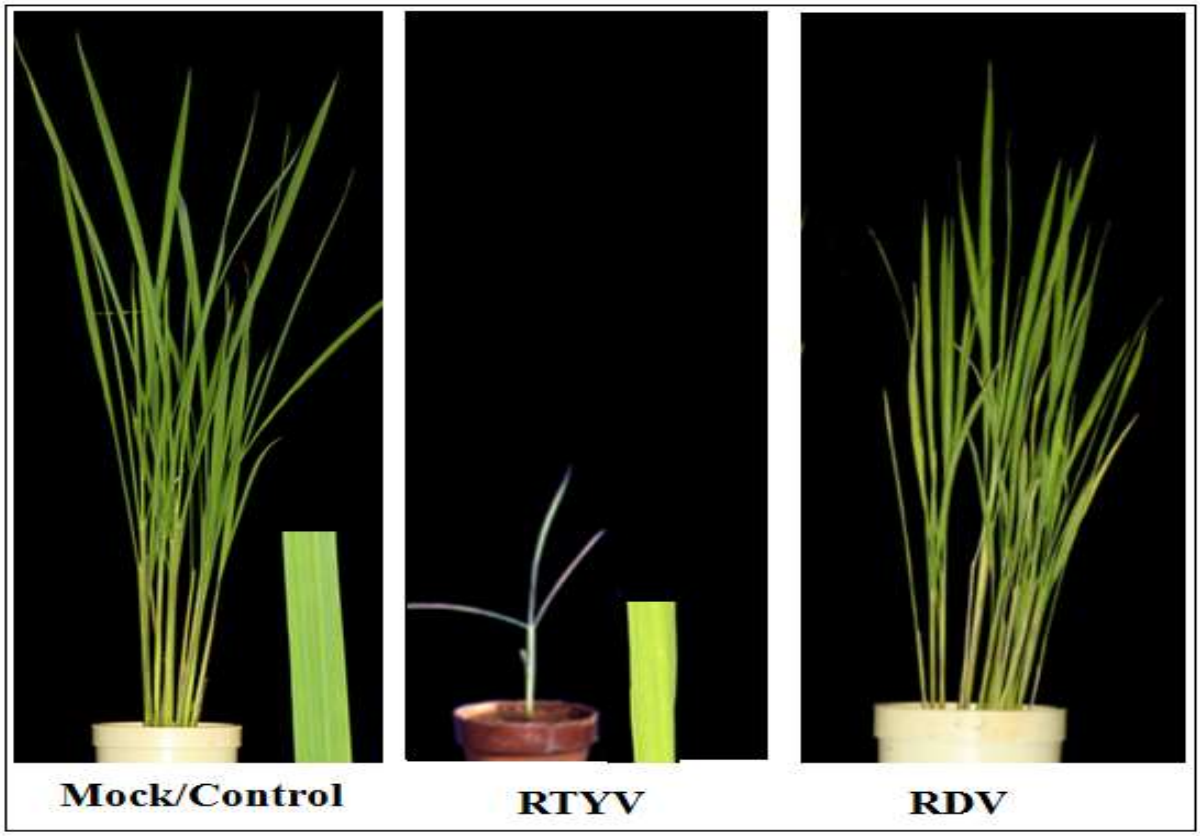

Fig. 1. Disease phenotype and virus accumulation in Nipponbare cultivar rice after infection with RDV (21 dpi) and RTYV (24 dpi). On the right hand side of each panel is a representative leaf showing the symptoms of each respective viral infection. Leaf yellowing of RTYV-infected plants at 24 days after inoculation. RDV infected plants showed stunted growth and chlorotic specks on leaves at 21 days after inoculation. The rice plants infected with RTYV were much shorter and had more leaf yellowing than mock-infected or control.

Discussion

AP2/EREBP transcription factor family genes are key regulators of development and stress responses. Several members of this family have been associated with plant responses to viral infections (Sharoni *et al.* 2011). In the current study, we aimed to determine the role of AP2/EREBP in rice seedlings responding to infection by various viruses through examining common and differentially expressed *AP2/EREBP* transcription factor family genes. Finally, we selected some putative candidate genes for further functional analysis.

OsAP2/EREBP genes have distinct responses to different viral infections in rice

The various symptoms associated with virus infection in plants are a consequence of altered patterns of gene expression, often mediated by transcription factors.

A link between biotic stress and ERF transcription factor function was first established via a screen for binding partners of a bZIPTF functioning in pathogen defense in Arabidopsis (Büttner and Singh 1997) with later similar reports for EFR in soybean (Dong *et al.* 2015) and rice (Jisha *et al.* 2015).

In the current study, we observed that together with different physical changes due to infection, the *OsAP2/EREBP* gene expression profiles were distinct for each of the different viruses with only a small proportion of these genes responding to one or more of the viruses. Despite this, when looking at gene expression data for infection across all four viruses, (RTYV and RDV-3 strain; Table 1; Figure 2), 30% redundant *OsAP2/EREBP* genes were up-regulated during four tested virus infections.

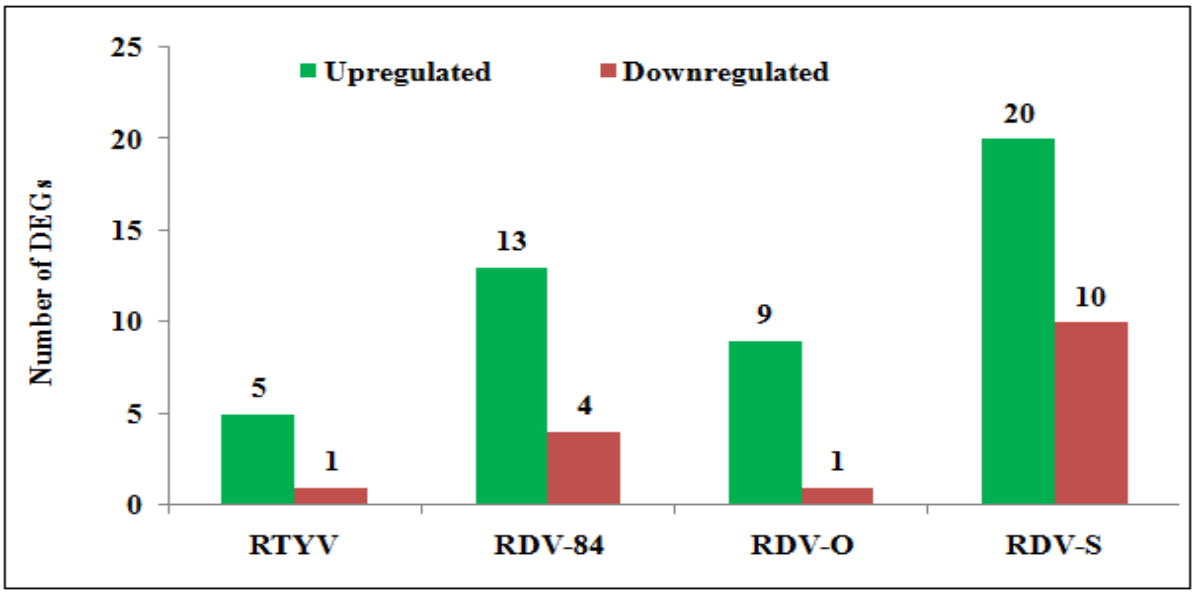

Fig. 2. Number of differentially expressed genes (DEGs) in rice seedlings in Nipponbare cultivar. Plants were infected with RTYV and RDV. The *Y*-axis represents the number of DEGs, and four treatments are indicated on the *X*-axis.

The number of *OsAP2/EREBP* genes with up-regulated expression was higher in infected compared with control plants, which indicates that defense systems were activated in RTYV and RDV infections (Figure 2). Differences in the genome structure (such as dsRNA or ssRNA) may affect the viral genome replication. dsRNA is a target for the host gene silencing system, so dsRNA viruses enclose the parental and replicating RNA to protect against gene silencing, which may involve host AP2/EREPB TF factors. According to our previous results based on microarray assay of gene expression in rice plants infected with RDV virus (Shimizu *et al.* 2007), there were significant decreases in expression of genes that are involved in the formation of cell walls, reflecting the stunted growth of diseased plants. The expression of plastid-related genes also was suppressed, as predicted from the white chlorotic appearance of infected leaves. Interestingly, *Os04g46220* exhibited preferential expression in all studied virus infections. The degree of suppression in plants infected with RDV-D84 was lower compared to RDV-O and RDV-S. Many differentially expressed genes were related to defense, stress response, and development and morphogenesis processes activated by RDV infection (Satoh *et al.* 2011).

Nicotiana plants infected with RNA viruses (i.e., *Cymbidium ringspot virus* and *Turnip crinkle virus*) exhibited suppression of host genes that was associated with the development of serious symptoms (Havelda and Várallyay 2008). Some transcription factor genes, for example those encoding the homeobox-like HD-Zip genes, MADS-box, SNAC, WRKY, and bZIP transcription factors are suppressed during virus infection (Satoh *et al.* 2013b). The responses of *AP2/EREBP* genes to viral infection seem to be dependent on the encoded domain types, which may be related to distinctive gene functions (Huang *et al.* 2015; Zhang *et al.* 2015). Several of the genes that have similar expression patterns in rice after infection with different viruses encode signaling components, including transcription factors and protein kinases, that have been previously reported to be associated with various environmental, biotic and abiotic stress responses (Satoh *et al.* 2013a,b; Sharoni *et al.* 2011). Changes in gene expression profiles in large numbers of *OsAP2/EREBP* genes concur with symptoms induced by infection of rice with the different viruses examined and deserve special consideration in further functional analysis.

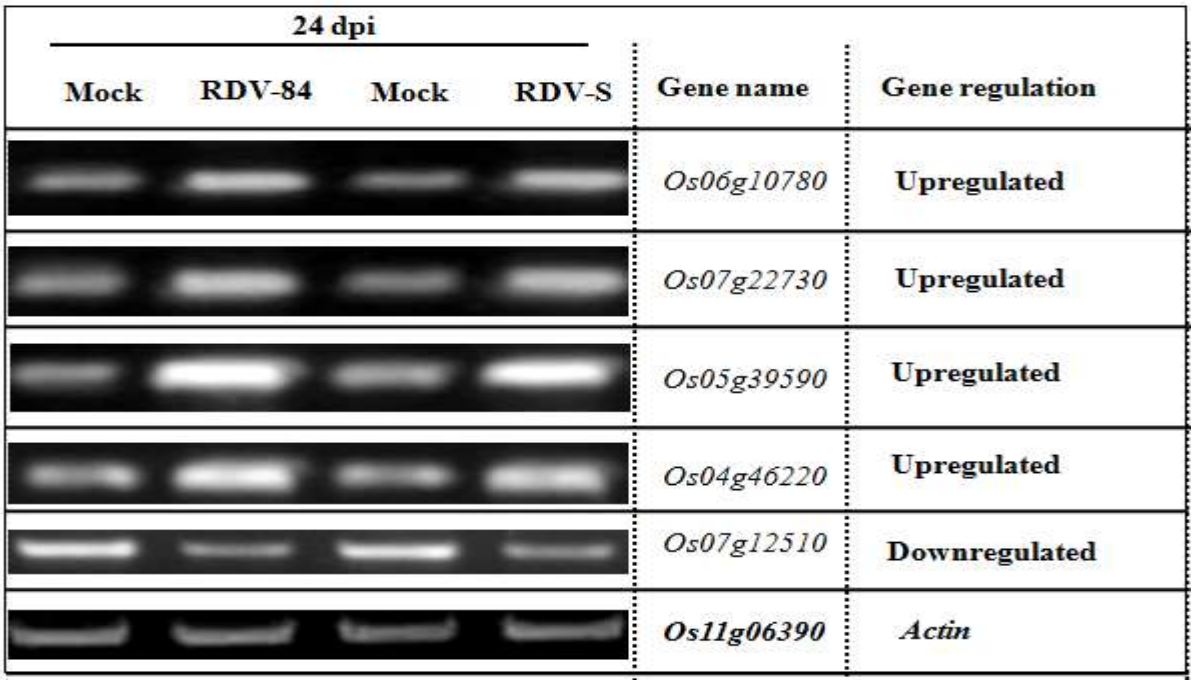

Fig. 3. Evaluation for the expression levels of selected DEGs by RT-PCR under RDV-84 and RDV-S virus infections.

Subfamilies of the OsAP2/EREBP genes show functional redundancy

OsAP2/EREBP genes play vital roles in various developmental processes, including signaling, stress responses and plant defenses. We found that there is functional redundancy among the subgroups of the *OsAP2/EREBP* gene family. Overall, three (60%) *OsAP2/EREBP* genes belong to RAV and to the B5 subgroups were up-regulated during at least one of the four virus infections (Table 2), indicating that these related genes can be triggered during infection by different viruses. Array data showed that rice *AP2/EREBP* genes that were up-regulated during virus infection included those previously reported to be induced by abiotic stresses (SA, JA, and ABA) such as *Os05g39590* (Sharoni *et al.* 2011). ERF-dependent regulation has been linked to biotic stress responses such as pathogen attack (*At4g13040*; Giri *et al.* 2014), application of ethylene and methyl jasmonate (Catinot *et al.* 2015). The DREB transcription factor TINY connects abiotic stress signaling via DRE-dependent regulation to biotic stress signaling via ERE. The RAV subfamily includes the known *PR* genes protein ABI3/VP1 (RAV) while other members in that subgroup share homology with Arabidopsis CBF1 (AtCBF1) and with tomato (SlRAV2) RAV2 proteins (Li *et al.* 2011). Three genes (60%) from the RAV subfamily were induced by RDV-O infection and three genes from the B5 subgroup were induced by RDV-S infection (Table 2). Some genes such as *Os01g73770* (B1), *Os05g39590* (B5), and *Os08g36920* (B7) exhibited higher expression profiles (≥ 2 fold) during RTYV and RDV infections in the rice seedlings (Table 2). Sharoni *et al.* (2011) reported phylogenetic analysis of this gene family showing homology and tight clustering of members in each subfamily or subgroup. Although phylogenetic analysis delivers vital support for candidate gene selection, it is only indicative of gene function. Thus, our tissue expression analysis at the level of mRNA transcription and the preserved subfamily or subgroup-specific residues of the *AP2/EREBP* domain defined in this study lead us to suggest that functional redundancy among*AP2/EREBP* subfamily members responding to viral infections, can narrow the selection of candidates for further functional validation and potential application in selecting and/or developing virus-resistant rice lines.

OsAP2/EREBP genes respond to numerous treatments

Several *OsAP2/EREBP* genes exhibited relatively high or low expression in rice seedlings during infection by four viruses (Figure 2; Table 1). Some of the virus-responsive *OsAP2/EREBP* genes were amongst those we previously reported to be triggered by at least one of the treatments with SA, ABA, JA or abiotic stress treatments (cold, drought, and submergence) of rice seedlings (Sharoni *et al.* 2011). *AP2/EREBP* genes (e.g., *CRL5*, *crownrootless5* and *OsAP239/Os04g52090*) in plants are affected by auxin, cytokinin, ABA and GA3 (Yaish *et al.* 2010; Kitomi *et al.* 2011). ABA plays a vital role in plant adaptive responsesby stimulating root growth and increasing the ability of the plant to uptake water from the soil during drought stress. In the current study, the *Os07g22730, Os04g46220* and *Os05g39590* genes were up-regulated specifically in the seedlings during infections with all RDV strains (Table 2). *Os05g39590* and *Os07g22730* genes showed up-regulation under different hormonal (SA, JA, and ABA) treatments in rice seedlings (Sharoni *et al.* 2011). Thus, *OsAP2/EREBP* genes, including *Os07g22730, Os04g46220,* and *Os05g39590* might have a role in ABA signaling pathways related to defense responsesduring viral infections. Many of these genes that have similar expression levels in Arabidopsis after infection with different viruses, encode signaling molecules, including transcription factors (e.g., BdDREB2) and protein kinases (Sun *et al.* 2014).While different gene family members respond to the different viral infections, it seems likely that commonly expressed *OsAP2/EREBP* genes play functional roles in rice seedlings during different virus infections, and that these roles include signal transduction through growth factor pathways.

Conclusion

In conclusion, using a comprehensive 44K oligo array platform we determined gene expression profiles of rice seedlings infected with each of four different viruses. Comparison of the gene expression profiles for *AP2/EREBP* family members in seedlings under virus-infected and uninfected conditions enabled the identification of *AP2/EREBP* family transcription factors that were differentially expressed including some genes that were commonly expressed during infections with each of the four viruses. This could be particularly viable for determining the functions of the *OsAP2/EREBP* family of genes in rice during seedling growth and development in addition to response to virus infection. Some subgroups showed a high level of expression during different virus infections, suggesting that they might have undergone functional divergence. Genes belonging to the RAV subfamily and B5 subgroup were activated in the seedlings infected with at least 3 of the 4 viruses and thus are attractive candidate genes for further functional analysis. Present research to characterise a number of these genes through over-expression and knockdown/mutant analyses is underway in our laboratory towards the optimization of molecular breeding schemes for the *OsAP2/EREBP* gene family in rice.

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