African Journal of Biotechnology Vol. 11(1), pp. 88-99, 3 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3474
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Identification and characterization of the Bcl-2-associated athanogene (BAG) protein family in rice

Rashid Mehmood Rana[1, 2], Shinan Dong[1], Zulfiqar Ali[3], Azeem Iqbal Khan[4] and Hong Sheng Zhang[1]*

[1]State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China.
[2]Department of Plant Breeding and Genetics, PMAS-Arid Agriculture University Rawalpindi, Pakistan.
[3]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad-38040, Pakistan.
[4]Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad-38040, Pakistan.

Accepted 9 December, 2011

The Bcl-2-associated athanogene (BAG) proteins are involved in the regulation of Hsp70/HSC70 in animals. There are six BAG genes in human that encode nine isoforms with different subcellular locations. *Arabidopsis thaliana* is reported to contain seven BAG proteins. We searched BAG proteins in *Oryza sativa* using profile-sequence (Pfam) and profile-profile (FFAS) algorithms and found six homologs. The BAG protein family in *O. sativa* can be grouped into two classes based on the presence of other conserved domains. Class I consists of four *OsBAG* genes (1 to 4) containing an additional ubiquitin-like domain, structurally similar to the human BAG1 proteins and might be BAG1 orthologs in plants. Class II consists of two *OsBAG* genes (5 and 6) containing calmodulin-binding domain. Multiple sequence alignment and structural models of *O. sativa* BAG proteins showed conservation of surface charge (except *OsBAG5*) and critical residues for the binding of BAG domain to Hsp70 nucleotide binding domain (NB). Meta analysis of microarray data showed that *OsBAG* genes are up or down regulated under different stresses (biotic and abiotic). Data obtained from real-time PCR of *OsBAG* genes under heat stress showed that maximum induction in the expression of all the genes occurred after one hour exposure to heat stress, while reduction in the expression was observed in the following time course and ultimately returned to the basal level at 24 h treatment. These results suggest that *OsBAG* genes might play important role at the onset of heat stress. A further detailed study may explore the exact function of the members of this gene family and help to make understanding of programmed cell death (PCD) mechanism in plants.

Key words: Rice, ubiquitin-like domain, nucleotide-binding domain, real-time PCR.

INTRODUCTION

Programmed cell death (PCD) plays a critical role in growth and development of plants and animals. The genes that control PCD showed conservation across wide evolutionary distances from *Caenorhabditis elegans* to humans. However, this conservation is still unclear in plants. Therefore the identification of homologous genes involved in plants cell death regulation is of great interest. Due to high sequence divergence, functional homology of animal apoptotic proteins in plant genomes cannot be revealed by tools such as BLAST and FASTA (Doukhanina et al., 2006; Yan et al., 2003). Identification and characterization of such divergent proteins with functional homology can be carried through profile-sequence (Pfam) and profile-profile (FFAS) algorithms (Doukhanina et al., 2006).

*Corresponding author. E-mail: hszhang@njau.edu.cn.

Abbreviations: BAG, Bcl-2-associated athanogene; **PCD,** programmed cell death; **NB,** nucleotide binding.

Bcl-2-associated athanogene (BAG), an evolutionarily conserved protein family, found to interact with ATPase binding domain of heat shock proteins 70 (Hsc70/Hsp70) and Bcl-2; and modulate their functions, either positively or negatively (Pascale et al., 2010). The BAG domain is an evolutionarily conserved region located at the C-terminus of the BAG-family proteins have been described (Sondermann et al., 2001) and/or proven in a variety of organisms including human (Sondermann et al., 2001), *Drosophila* (Coulson et al., 2005), *Bombyx mori* (silk worm) (Moribe et al., 2001) and Arabidopsis thaliana (Doukhanina et al., 2006). Human BAG-1 was the first member of this family discovered through a screen for Bcl-2 binding proteins (Takayama et al., 1995). All BAG proteins share a common Hsp70/Hsc70 interaction domain/binding domain (BD), but generally differ in the N-terminal region, which imparts specificity to particular proteins and pathways. Ubiquitin-like domain at the N terminus of human BAG proteins (BAG1 and BAG6) is probably functionally relevant and conserved in yeast, plants, and worms (Doukhanina et al., 2006). BAG proteins regulate diverse physiological processes in animals, including apoptosis, tumorigenesis, neuronal differentiation, stress responses, and the cell cycle.

BAG proteins are being extensively studied in animals. In plants, *A. thaliana* is reported to contain seven homologs of the BAG gene family. Domain organization of four AtBAGs was found similar to their animal homologs, while three members contain a calmodulin-binding domain near the BAG BD. The presence of calmodulin-binding domain is referred as a novel feature associated with plant BAG proteins reflecting possible divergent mechanisms involved with plant-specific PCD (Doukhanina et al., 2006).

In an attempt to study BAG proteins in plants, our searches of the *Oryza sativa* genome sequence revealed six homologs of the BAG family, including four with domain organization similar to their animal and *A. thaliana* homologs. The other two members contain a calmodulin-binding domain near the BD that showed similarity to their *A. thaliana* homologs. This study reports genome organization, phylogeny, and comparative genomics of rice BAG multigene family in rice that will provide a basis for further functional studies. It further reports the gene expression analysis of rice BAG gene family derived from publically available microarray data as well as through real-time PCR, representing their organ and stress specific expression pattern.

MATERIALS AND METHODS

Plant materials and growth conditions

Rice seeds (*Oryza sativa* L. subsp. japonica cv. Nipponbare) were surface sterilized with 0.1% $HgCl_2$, germinated and grown in an incubator at 28˚C using Yoshida nutrient solution as described previously (Yoshida et al., 1972; Zhou et al., 2006). Two week old seedlings were subjected to heat stress (42℃) and seedlings were sampled at 0, 1, 3, 6, 12 and 24 h after treatment and immediately stored at –80℃. For expression analysis in different tissues, plants were grown in the natural conditions and samples were collected at booting stage.

Identification of BAG family in rice

Seven BAG domain-containing proteins of the *A. thaliana* genome were individually used as query to BLAST in *Oryza sativa* genome. The resultant sequences were then screened for genome annotation as well as for Pfam, SMART, prosite and interpro BAG domain (accession numbers PF02179 and SM00264, PS51035 and IPR003103 respectively) created via hidden Markov model (HMM)-based data base searches. The resultant six members of BAG family in rice were processed for further study. Genomic and cDNA sequences of these proteins were retrieved from NCBI and gene structure was predicted by FGENESH+ (http://linux1.softberry.com/berry.phtml). The chromosomal location of each BAG gene in rice was determined from the rice physical map constructed by the International Rice Genome Sequencing Project (IRGSP) (http://rgp.dna.affrc.go.jp). *In silico* sub-cellular localization of *OsBAG* family proteins was predicted by WoLF PSORT (Horton et al., 2006).

Multiple sequence alignment and phylogenetic relationship

Alignment of rice or other plant/animal BAG protein sequences as well as rice BAG domain were performed with ClustalX v 1.83 (Thompson et al., 1994) and viewed by Jalview 2 software (Waterhouse et al., 2009). The phylogenetic tree was constructed with MEGA5 program (Tamura et al., 2011) using neighbor-joining (NJ) method. Bootstrap test of phylogeny was performed with 1,000 replicates using pair-wise deletion and p-distance model.

Protein structure model

The homology models for rice BAG proteins were generated through SWISS-MODEL web server. Each model was generated based on automated identification of template model. The models were processed by the deepview/Swiss-PdbViewer program (Guex and Peitsch, 1997). All BAG proteins were analyzed for the presence of the additional domains in target sequences using the prosite programs.

‘‘Digital northern’’ analysis of BAG gene expression in *Oryza sativa*

Rice BAG gene expression evidence based on EST data was obtained using the PASA program based server (http://rice.plantbiology.msu.edu/expression_anatomy.shtml; Campbell et al., 2006) which utilizes a number of alignment programs to maximally align transcripts to the genome. MSU rice locus ID was used as identifier against available database.

Expression analysis of *OsBAG* family in rice based on microarray data

Condition (abiotic and biotic) specific expression of *OsBAG* genes was estimated using publicly available web-based tool “genevestigator” for meta-analysis of gene expression based array platforms (https://www.genevestigator.com) (Hruz et al., 2008). Affymetrix OS_51K data were used to draw heat map of four *OsBAG* genes (no Affymetrix probes detected for *OsBAG2* and *OsBAG6*). Tissue specific expression of *OsBAG* genes was illustrated using another web-based tool with reclassified Affymetrix data (www.ricearray.org)

Table 1. List of the primers used in the study.

Name	Transcript identifier	Primer sequence (5' to 3')	
OsBAG1	LOC_Os09g35630.1	GCGTCAAGGTCAAGTTCAACGG	Forward
		CTTCTTCAGCTCACCGAAGGAC	Reverse
OsBAG2	LOC_Os08g43270.1	AGAAGCTCCTGTCGGAGAAGAC	Forward
		TCTCCTTGTCCCTGTACACCAC	Reverse
OsBAG3	LOC_Os06g03640.1	ATGGTGGTGCTTGGTTGGCAAG	Forward
		TCATCTCATCAGGGACAGGACACC	Reverse
OsBAG4	LOC_Os01g61500.1	ACAAAGGAAGGCTGAGGTACGC	Forward
		TGCCTTCAGCTTATCCAACGTCTC	Reverse
OsBAG5	LOC_Os11g31060.1	ACAAGAAGCTCAAGAAGGAAGCC	Forward
		ATCGCTGTAGCAGCTTCGGATG	Reverse
OsBAG6	LOC_Os02g48780.1	ATTGGCAAAGCACAGGTTTGGG	Forward
		AAGAAGTGGGAGGTGGAGGTGTAG	Reverse
18S rRNA	LOC_Os09g00999.1	AACTAGCTATGCGGAGCCATCC	Forward
		TCAAACTTCCGTGGCCTAAACGG	Reverse

Table 2. Genomic organization and chromosomal localization of rice BAG genes.

Protein	Nucleotide accession number	Chromosome	Number of exons	Affmatrix probe ID
OsBAG1	NM_001070251.1	9	4	Os.51065.1.S1_at
OsBAG2	NM _001068954.2	8	6	N/A
OsBAG3	NM_001063194.1	6	4	Os.10179.1.S1_at
OsBAG4	NM_001051235.1	1	4	Os.20681.1.S1_x_at
OsBAG5	NM_001074483.2	11	1	OsAffx.19095.1.S1_at
OsBAG6	NM_001054480.1	2	1	N/A

(Jung et al., 2008).

Expression of *OsBAG* gene family in rice by real-time RT-PCR

Real-time PCR was performed in ABI-7500 fast real-time PCR (Applied Biosystems, USA) using SYBR green real-time PCR master mix (Toyobo, Japan) with two-step PCR according to manufacturer's protocol. Quantitative gene expression was analyzed by comparative CT (ΔΔCT) method (Livak and Schmittgen, 2001), using 18S-rRNA as an internal control (Jain et al., 2006). Primers used in the study were designed using quantprime primer designing tool (Arvidsson et al., 2008). All the primers are listed in Table 1.

RESULTS

Identification of BAG family in rice

Low sequence identities (8.1 to 47.3%) and similarities (16.2 to 66.2%) of rice BAG proteins to animal and *Arabidopsis* BAG proteins requires more sensitive methods such as HMM-based protein search tools and profile-profile alignment algorithms to be used (Bateman and Haft, 2002). To identify rice BD-containing proteins, we used HMM-based protein search tools (Pfam and SMART) and found six BD-containing proteins in the rice genome. The database annotation of putative rice BAG proteins was described as "unknown" or "hypothetical". This gene family was named as BAG gene family in current study and nomenclature was adopted for rice BAG genes (Table 2).

Genomic organization, chromosomal distribution and subcellular localization

Comparisons of the full-length cDNA sequences with corresponding genomic DNA sequences showed that

Table 3. Properties of predicted BAG proteins in rice.

Protein	MSU locus ID	Protein accession number	Subcellular localization	Protein properties		
				Amino acid	MW (Da)	PI
OsBAG1	LOC_Os09g35630	NP_001063716.1	chloroplast	334	35963	10.35
OsBAG2	LOC_Os08g43270	NP_001062419.2	cytoplasm	501	55069.29	8.46
OsBAG3	LOC_Os06g03640	NP_001056659.2	Nucleus	339	36431.7	10.44
OsBAG4	LOC_Os01g61500	NP_001044700.1	cytoplasm	262	28781.5	5.53
OsBAG5	LOC_Os11g31060	NP_001067951.2	cytoplasm	455	49699.9	4.39
OsBAG6	LOC_Os02g48780	NP_001047945.1	cytoplasm	213	23082.8	6.36

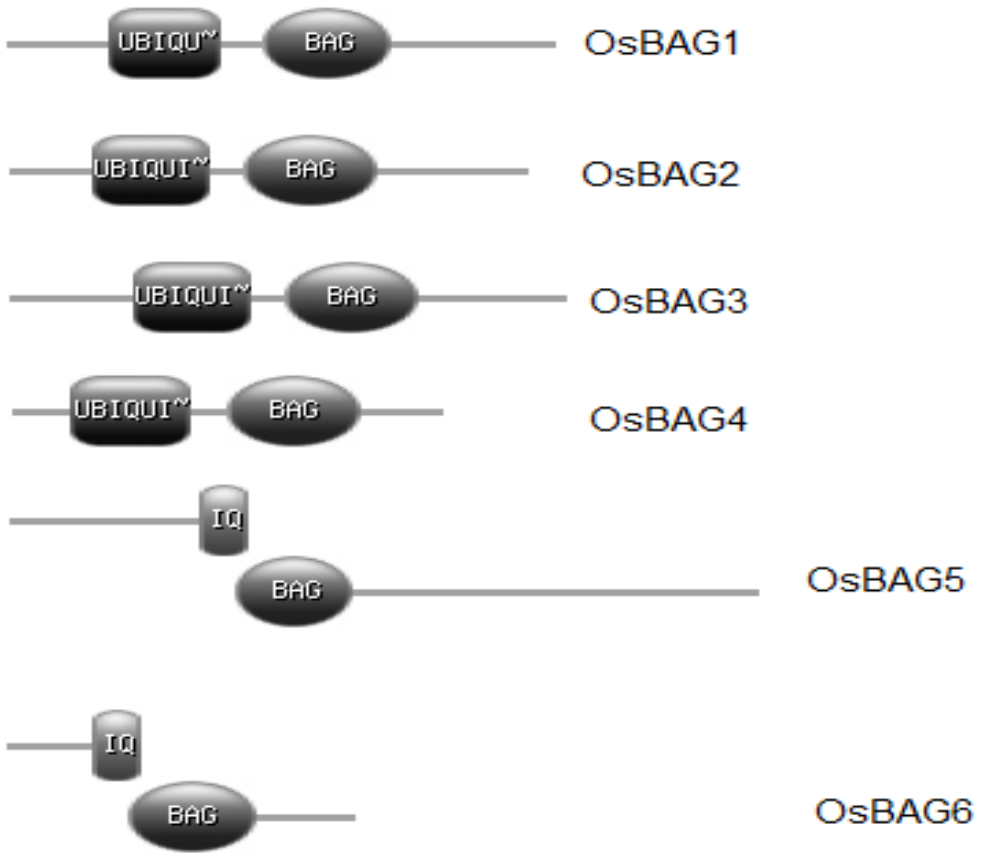

Figure 1. Domain organization of rice BAG proteins; UBIQUI represent Ubiquitin domain; BAG represent BAG domain, IQ represent IQ or calmodulin domain.

coding region of four *OsBAG* genes (*OsBAG1* to *OsBAG4*) was disrupted by introns. Chromosomal location of each BAG gene in rice showed that *OsBAG1*, *OsBAG2*, *OsBAG3*, *OsBAG4*, *OsBAG5* and *OsBAG6* were mapped on chromosome 9, 8, 6, 1 11 and 2 respectively (Table 2). The *in silico* subcellular locations depicted that four proteins (*OsBAG2*, *OsBAG4*, *OsBAG5* and *OsBAG6*) were localized in cytoplasm, one (*OsBAG3*) in nucleus and one (*OsBAG1*) in chloroplast (Table 3). Properties of *OsBAG* proteins, number of amino acids and their accession numbers are listed in Table 3. Domain organization of rice BAG proteins showed the presence of additional domain in N-terminus. However, four *OsBAG* proteins (*OsBAG1* to *OsBAG4*) contained ubiquitin-like domains, while *OsBAG5* and *OsBAG6* contained calmodulin-binding motif. Figure 1 represents the domain organization of rice BAG proteins.

Alignment and phylogenetic relationship

The alignment of predicted protein sequences of six rice

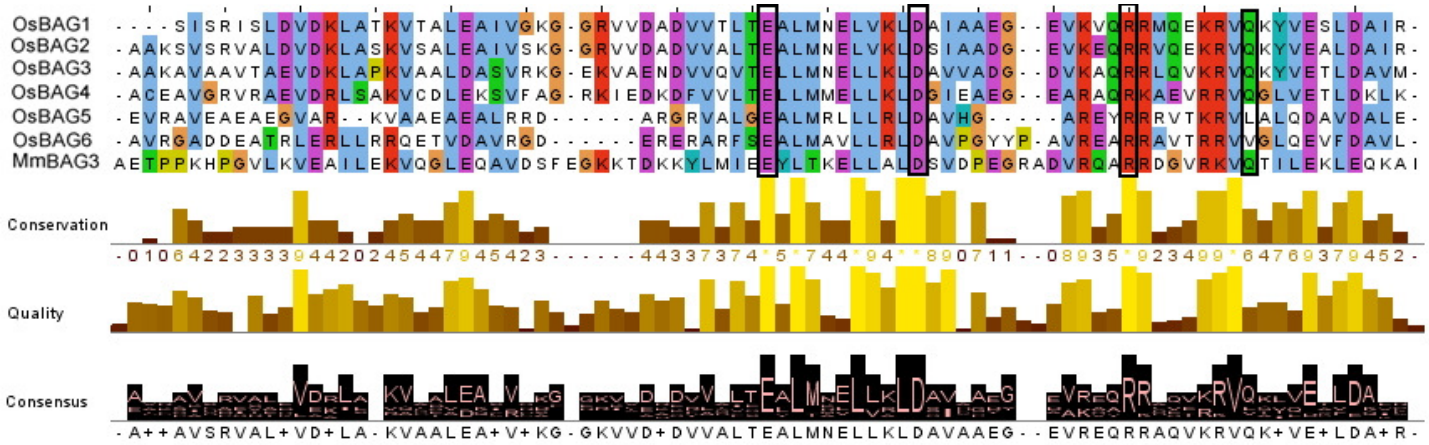

Figure 2. Alignment of amino acid sequences from six *OsBAGs* and *MmBAG3*. Clustalx v1.8 was used for multiple sequence alignment and Jalview 2 was used to visualize the alignment. Box represents the residues critical for BAG/HSP70 interaction.

BAG genes using ClustalX and Jalview 2 showed that residues responsible for electrostatic interaction of BAG domain with nucleotide binding domain (NBD) of HSP70/HSC70 were highly conserved among all rice BAG proteins (Figure 2). Pair-wise comparisons of rice BAG protein sequences showed that the similarities among full length proteins were relatively low (81.4 to 25.3%) as compared to their BAG domain region (92.7 to 45.6%).

Multiple sequence alignment of full-length rice BAG protein sequences were used to determine their phylogenetic relationship. Rice BAG proteins cluster into two groups (Figure 3 A). Group-I consisted of *OsBAG1* to *OsBAG4* proteins which contained ubiquitin-like domains located in the N terminus as in some mammalian BAG proteins like *HsBAG1*. Therefore, *OsBAG* proteins of Group-I are similar to animal counterparts. Group II consisted of *OsBAG5* and *OsBAG6* which contained calmodulin-binding motif as AtBAG5 to AtBAG7.

Phylogenetic relationship among the BAG domain containing proteins from different species was also determined. A phylogenetic tree was constructed for BAG proteins from plants, animals and yeasts. The rice BAG proteins, *OsBAG1* to *OsBAG4* were grouped with those of *Arabidopsis* and *Cicer arietinum* BAG proteins. The *OsBAG5* and *OsBAG6* grouped with AtBAG5 (Figure 3 B).

Comparison of the rice BAG domains with template model

Multiple sequence alignment of BD sequences from *OsBAG*s and *MmBAG3* revealed that most of the residues required for interaction of BAG domain with nucleotide-binding domain (NBD) of Hsp70 are conserved in *O. sativa* BAG proteins (Figure 2). The structure of the BAG5-BD5/NBD-HSP70 complex reveals that Asp^{410}, Arg^{424}, and Gln^{432} are critical for BAG/NBD interaction (Arakawa et al., 2010). These interaction residues are also found conserved in *OsBAGs* except that of Gln^{432}, which was not found in the BDs of *OsBAG5* and *OsBAG6* (Figure 4). However, the overall residual similarity of rice BAG domains to mammalian BAG domain reveals that rice BAG proteins may also bind to NBD-HSP70.

The three-dimensional structure of the *Mus musculus* BAG3 BD (PDB ID: 1uk5A) was chosen as a template based on maximum identity (~23 to 30%) to the *OsBAG* BDs. Three-dimensional structure of six OsBAG proteins was predicted through SWISS-model server. We compared the surface of the reported BD solution NMR structure of *M. musculus* BAG3 with a three-dimensional model of the BD of *OsBAG1* to *OsBAG6*. *M. musculus* BAG3 protein showed high similarities with respect to charged residue distributions in α2 and α3 helix surface residues of the *OsBAG1* to *OsBAG4* and *OsBAG6*, while less similarity to *OsBAG5*. It was observed that surface of *OsBAG5* was found more positively charged, with an acidic cluster in the left bottom corner.

Digital northern analysis

Digital northern analysis using the PASA based program categorized *OsBAG* genes with respect to the EST numbers (Figure 5). The rice genes *OsBAG2* and *OsBAG6* have ten and six ESTs respectively. *OsBAG1* and *OsBAG5* have 21 and 25 ESTs respectively, which represent a higher expression level. *OsBAG3* and *OsBAG4* have 37 and 52 ESTs, which displays that *OsBAG4* has the highest level of gene expression is among all *OsBAG* genes in rice.

Expression analysis of *OsBAG* gene family in rice based on microarray data

The expression profiles of BAG genes in various rice

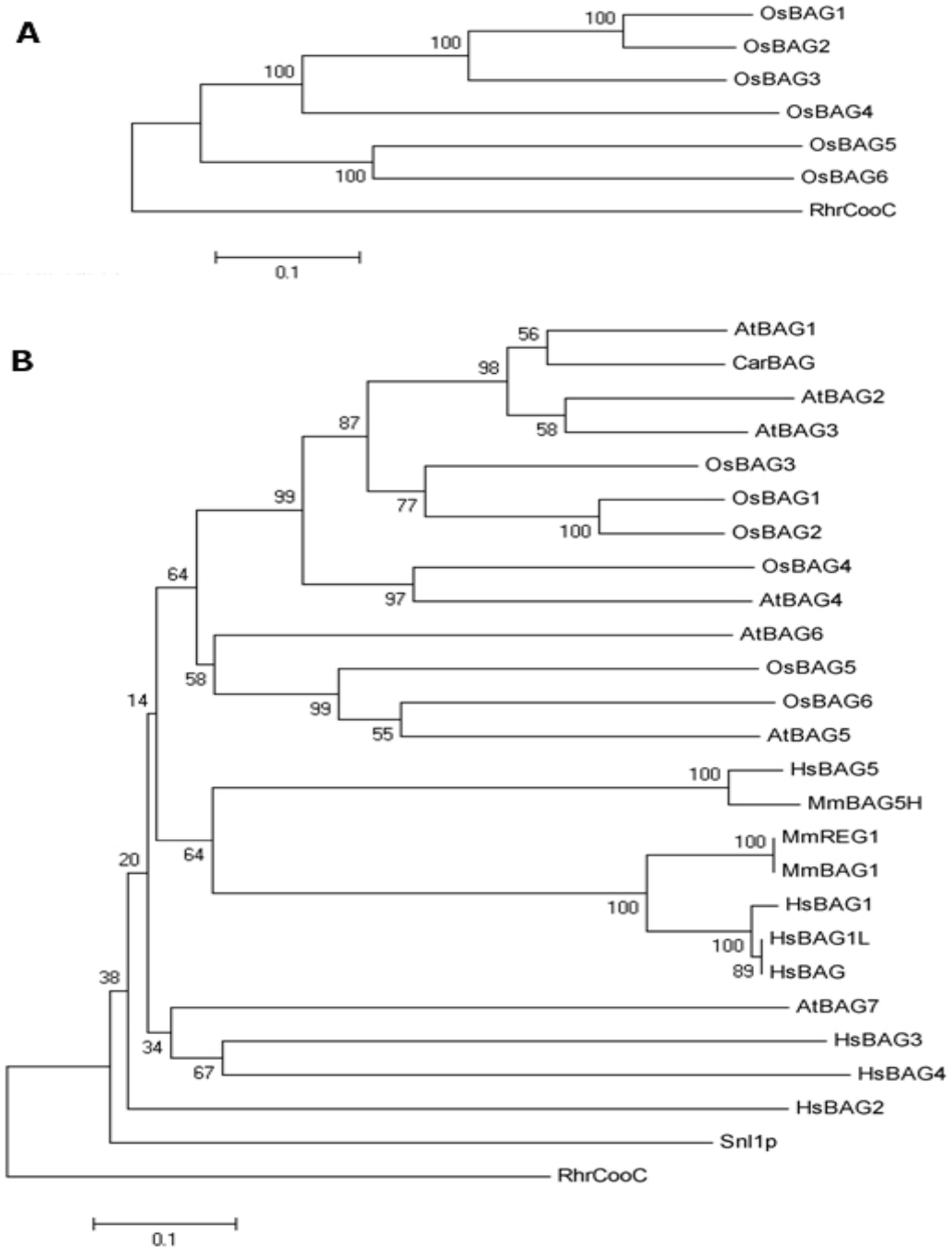

Figure 3. Phylogenetic relationship of BAG proteins. The tree was generated using MEGA5 program by neighbor-joining method. The bootstrap values from 1000 replicates are indicated at each branch. (A) Phylogenetic tree of *Oryza sativa* BAG proteins (full length) using RhrCooC as out-group. (B) Phylogenetic tree of BAG proteins from various taxonomic groups; GI numbers of sequences used in the study are as follows: *O. sativa OsBAG1* (GI: 115480245), *OsBAG2* (GI: 297608934) *OsBAG3* (GI: 297605098), *OsBAG4* (GI: 115440841) *OsBAG5* (GI: 297611880), *OsBAG6* (GI: 115448331) *Arabidopsis thaliana* proteins AtBAG1 (GI:18423349), AtBAG2 (GI:10176928), AtBAG3 (GI:21537107), AtBAG4 (GI:3068705), AtBAG5 (GI:3157923), AtBAG6 (GI:3702325), and AtBAG7 (GI:15241803); *CarBAG,* the *Cicer arietinum* (chickpea) BAG protein (GI:10334497); the *Saccharomyces cerevisiae* (yeast) *Snl1p* protein (GI:6322173); *Homo sapiens* (Hs) proteins *HsBAG* (GI:17384436), *HsBAG1* (GI:4204712), *HsBAG1L* (GI:3523107), *HsBAG2* (GI:4757834), *HsBAG3* (GI:5868898), *HsBAG4* (GI:6631075), and *HsBAG5* (GI:6631077); *Mus musculus* proteins *MmREG1* (GI:5915764), *MmBAG5H* (GI:12838157), *MmBAG1* (GI:12851044), and the *Rhodospirillum rubrum* protein CooC (AAC45124; out-group).

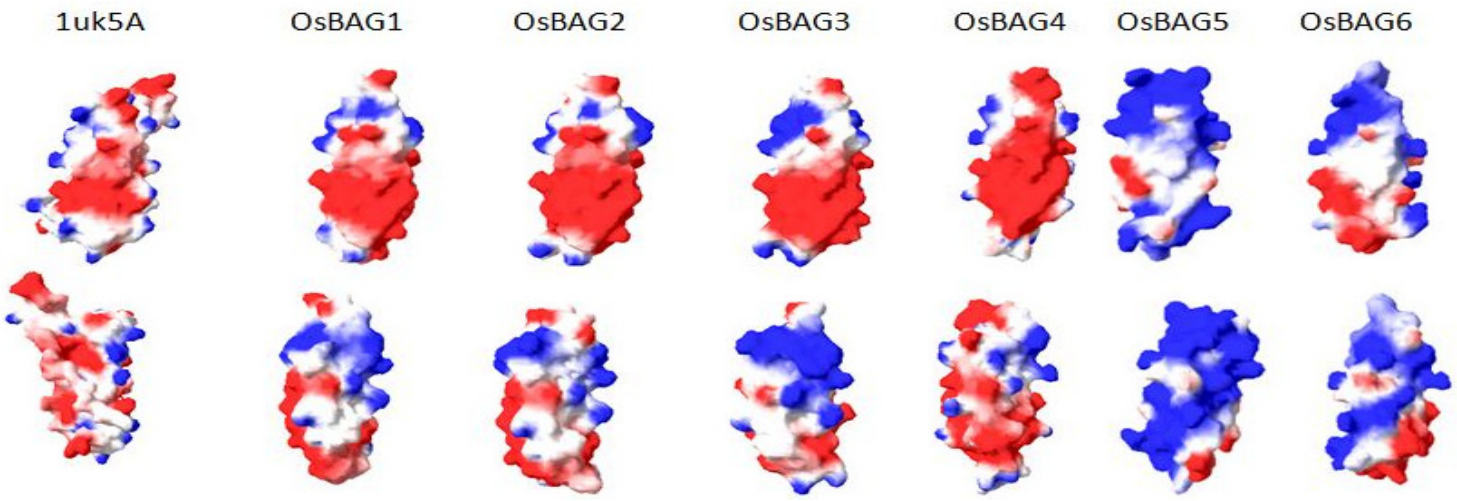

Figure 4. Homology models of six *Oryza sativa* BAG BDs as compared to the mouse BAG3 BD (PDB ID: 1uk5A). Positively charged regions are shown in blue, and negatively charged regions in red, upper row showing front while bottom row showing back view of the 3D model of each protein. The structures was predicted by using SWISS-MODEL web server (http://swissmodel.expasy.org/) and processed by Swiss-Pdb viewer program.

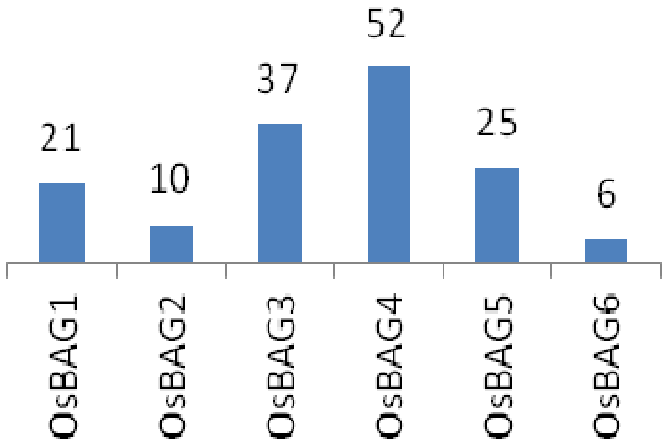

Figure 5. EST based "Digital northern" analysis. PASA based server (http://rice.plantbiology.msu.edu/expression_anatomy.shtml) was used to analyze EST profile of *OsBAG* genes.

tissues were investigated based on microarray database. The expression level of *OsBAG1* and *OsBAG3* was higher in radicle, stem and internode. For *OsBAG4*, it showed higher expression level in stem and internode. The expression of *OsBAG5* shows lowest expression in all tissues. The rest of tissues analyzed showed much less or no expression (Figure 6). Affymetrix probes for *OsBAG2* and *OsBAG6* were not available in database. Based on microarray data, we analyzed expression of rice BAG genes using genevestigator. Heat map generated by genevestigator showed three BAG genes significantly induced by different stresses (Figure 7). *OsBAG5* showed maximum up-regulation under anoxia, salt, heat and rice blast (*Magnaporthe grisea*) while *OsBAG1* showed maximum up-regulation rice blast. *OsBAG3* and *OsBAG4* showed maximum expression under trans-zeatin application.

Expression pattern of six *OsBAG* genes in rice tissues

Quantitative real time RT-PCR (qRT-PCR) was performed to analyze the expression pattern of *OsBAG* genes in different tissues. *OsBAG1*, *OsBAG4* and *OsBAG6* genes showed maximum expression in culm; *OsBAG2* expressed at very low level with maximum expression in panicle; *OsBAG3* expression was observed at maximum level in node and panicle, while *OsBAG5* expressed in leaf (Figure 8).

Expression analysis of six OsBAG genes in rice seedlings under heat stress

Expression analysis of *OsBAG* genes under heat stress with different time course was evaluated through qRT-PCR. Data show that maximum induction in the expression of all the genes occurred after one hour exposure to heat stress, while reduction in the expression was observed in the following time course and ultimately returned to the basal level at 24 h treatment (Figure 9).

DISCUSSION

BAG family proteins have been reported to participate in several cellular mechanisms, including apoptosis, proliferation, differentiation, and stress signaling in animals (Arakawa et al., 2010; Coulson et al., 2005; Moribe et al.,

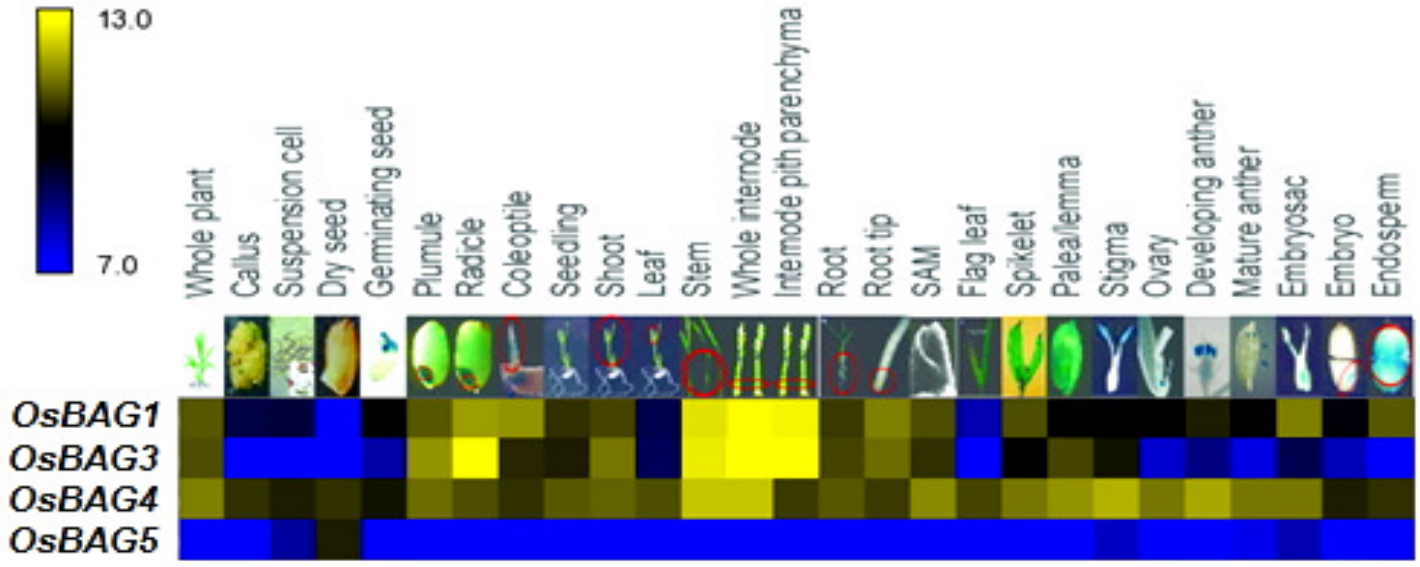

Figure 6. Tissue specific expression of *OsBAG* genes in rice based on affymetrix data using web-based tool with reclassified Affymetrix data (www.ricearray.org).

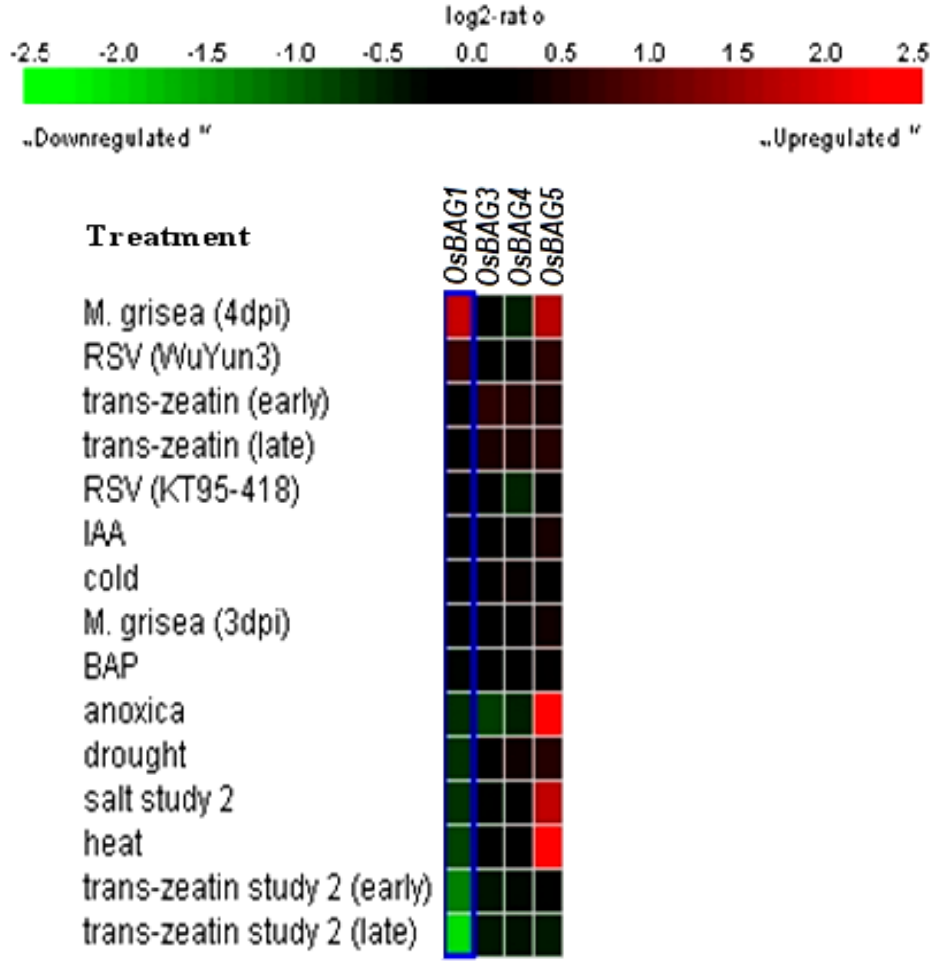

Figure 7. Expression of OsBAG genes under different stresses in rice based on Affymetrix data using web-based tool (www.genevestigator.com).

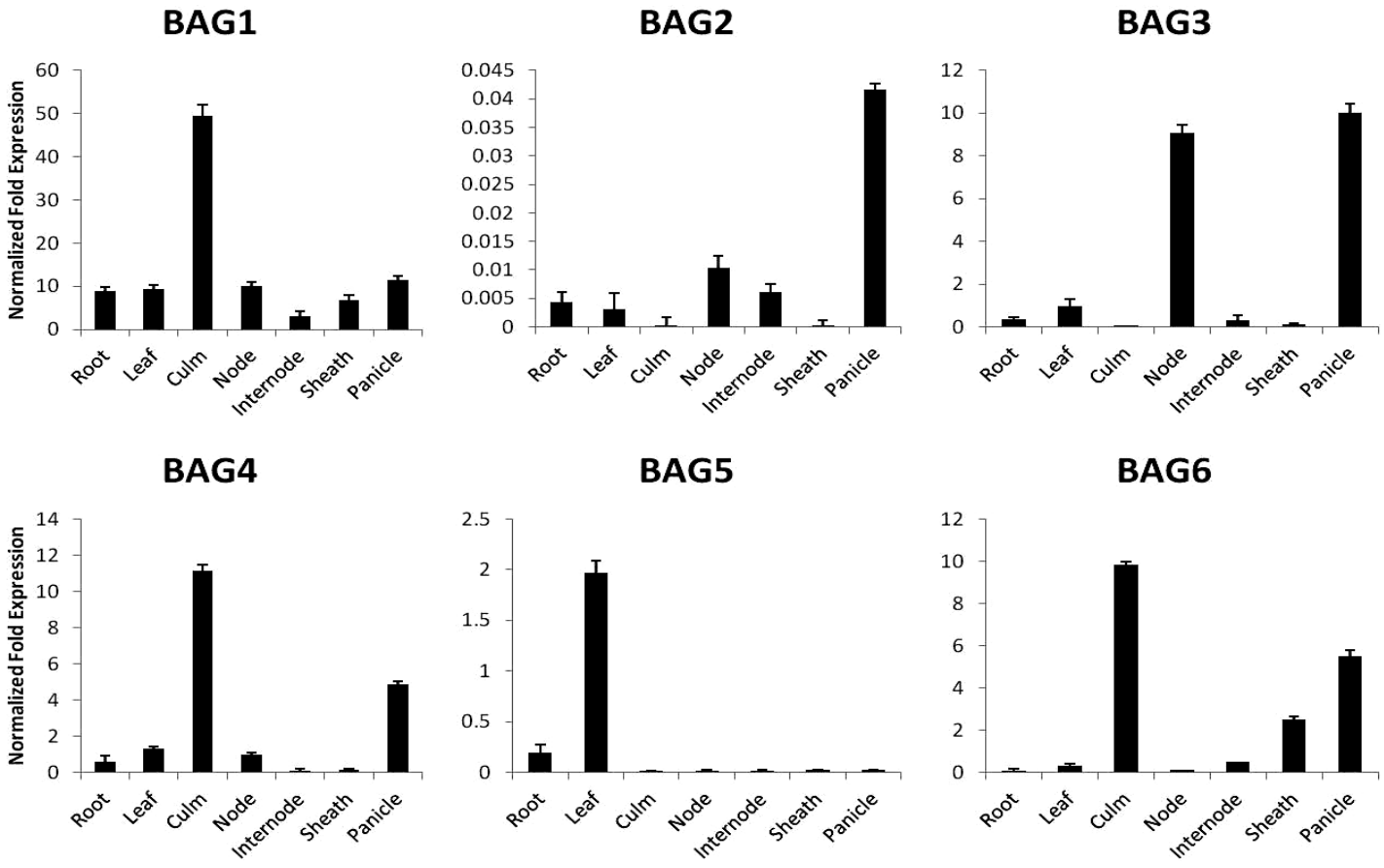

Figure 8. Expressions of six *OsBAG* genes in different rice tissues. The expression patterns of *OsBAG genes* were analyzed by quantitative real-time RT-PCR in different tissues at reproductive stage. Plants were grown under normal conditions. Bar represents standard deviation, n=3.

2001) and shown functional conservation in the model plant, *A. thaliana* (Cheng, 2006; Kang et al., 2005). Using HHMs based methods as previously used in *Arabidopsis* (Doukhanina et al., 2006), we identi-fied six BAG genes in *Oryza sativa.* Out of six identified rice BAG proteins, two (*OsBAG5* and *OsBAG6*) have calmodulin-binding motif, present near the BAG-BD. The presence of calmodulin-binding motif reveals that calmo-dulin and Ca^{2+} may be involved in the regulation of these proteins and conserved in plant specific BAG proteins (Doukhanina et al., 2006).

The structure of the BAG5-BD5/NBD-HSP70 complex revealed that Asp^{410}, Arg^{424}, and Gln^{432} are critical for BAG/NBD interaction. Mutation in these residues caused reduction in the binding affinities of BAG domain to NBD the complex (Arakawa et al., 2010). Therefore we examined *O. sativa* BAG-BDs for these critical residues. *OsBAG* proteins showed conservation in these residues except Gln^{432} that was not found in the BDs of *OsBAG5* and *OsBAG6*. Similarly, the absence of Gln^{245} in BDs of AtBAG2, AtBAG5, and AtBAG6 has been reported when compared to human BAG4BD (Doukhanina et al., 2006).

This suggests the conserved pattern of plant specific BAG proteins.

Furthermore, a highly conserved sequence motif of 8 amino acid residues (EVRPGGML) was observed near the N-terminus of four rice BAG proteins (*OsBAG1* to *OsBAG4*) but missing in *OsBAG5* and *OsBAG6*. Presence of extra motif is also evident in *Arabidopsis* (EXRPGGML/VVQXR) and human BAG1 isoforms (TRSEEX) (Yan et al., 2003). Although, the function of this motif (EXRPGGML) is unknown, high conservation is suggesting that it may play some important role. Mouse BAG3 proteins showed high similarities with respect to charged residues distributions in α2 and α3 helix surface residues of the *OsBAG1* to *OsBAG4* and *OsBAG6*, while less similarity to *OsBAG5* proteins. It was observed that surface of *OsBAG5* was found more positively charged, with an acidic cluster in the left bottom corner, similar observations are reported for *AtBAG5* (Doukhanina et al., 2006).

The tissues specific expression profiling of BAG genes in various rice tissues showed high expression of *OsBAG1*, *OsBAG3* and *OsBAG4* in radicle, stem and internode when analyzed through microarray data. Similar results were observed when we analyzed the expression of *OsBAG*

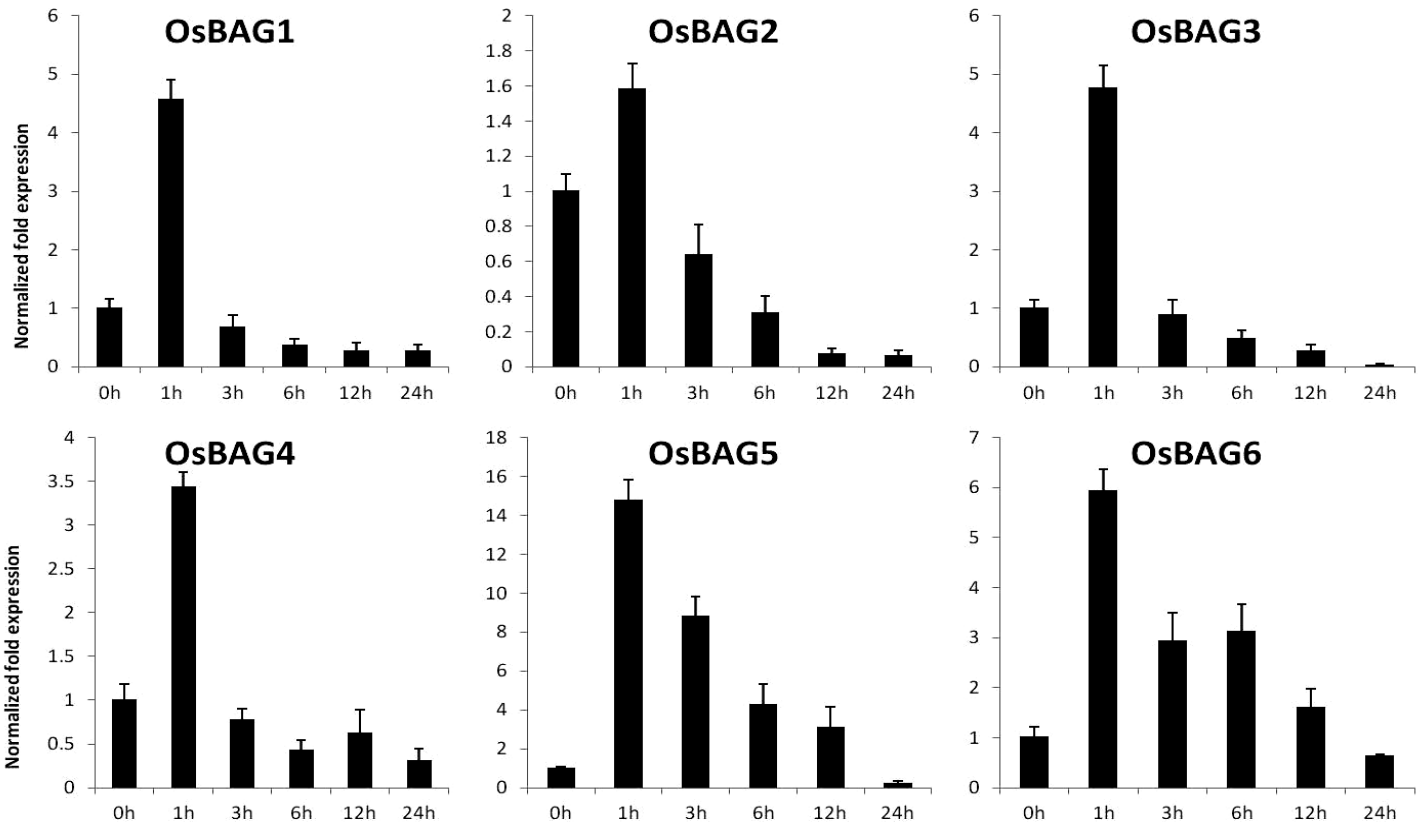

Figure 9. Expressions of six *OsBAG* genes in response to heat stress in rice seedlings. The expression patterns of *OsBAG genes* were analyzed by real-time quantitative PCR. Two week old rice seedlings were treated with heat shock at 42 ℃ from 1h to 24 h, 0 h represents untreated control. Bar represents standard deviation, n=3.

genes through qRT-PCR showing maximum expression of *OsBAG1*, *OsBAG3*, *OsBAG4* and *OsBAG6* genes in stem (culm or node). The higher expression in these tissues suggested involvement in cellular elongation/ proliferation, a conserved feature of BAG domain (Kabbage and Dickman, 2008).

Expression of *OsBAG* genes was illustrated in different abiotic and biotic condition using “genevestigator” (Hruz et al., 2008). Affymetrix OS_51K data were used to draw heat map of four *OsBAG* genes (Affymetrix probes were not detected for *OsBAG2* and *OsBAG6*). The heat map generated by genevestigator revealed that *OsBAG5* was found up-regulated under abiotic (arsenate, anaerobic, heat and salt) and biotic (*Magnaporthe grisea*) stress conditions. In contrast *OsBAG3* showed down regulation during biotic (*M. grisea*) stress and plant hormone treatment (trans-zeatin). It revealed the conserved role of *OsBAG3* in cell proliferation and differentiation as trans-zeatin (cytokinin) is reported to play important role in such processes and some other metabolic processes. Furthermore, the involvement of *OsBAG* genes in heat stress response was illustrated through qRT-RCR analysis, as all of the *OsBAG* genes were induced by heat stress. This result is in the consent of previous reports about BAG, as they have been demonstrated to interact with HSP70 and found expressed under heat stress (Liao et al., 2001; Song et al., 2001; Williams et al., 2010). Therefore the proposed function of BAG genes in rice might be their involvement in stress response, which highlights the importance of this gene family.

In conclusion, BAG protein family has proved to play key role in animals as well as in plants. Here we reported BAG domain containing proteins and briefly described their structural and functional homology. The four rice BAG genes were similar to animal BAGs, as they contained ubiquitin domain in their N-terminus; while two rice BAG genes contained plant specific calmodulin-binding motif. Most of the identified rice BAG genes might be involved in cell elongation, differentiation, response to stresses and cell death process. Further investigations of this gene family in rice may elaborate the process of PCD in plants that is not well understood yet.

ACKNOWLEDGEMENTS

The authors thank PMAS-Arid Agriculture University (Pakistan) for financial assistance to Rashid Mehmood

Rana during his Ph.D. studies. This work was supported by Natural Science Foundation of China (30971758, 31071387) and the Ph.D. programs foundation of Ministry of Education, China (200803070036).

REFERENCES

Arakawa A, Handa N, Ohsawa N, Shida M , Kigawa T, Hayashi F, Shirouzu M, Yokoyama S (2010). The C-terminal BAG domain of BAG5 induces conformational changes of the Hsp70 nucleotide-binding domain for ADP-ATP exchange. Structure, 18: 309-319.

Arvidsson S, Kwasniewski M, Riaño-Pachón D, Mueller-Roeber B (2008). QuantPrime–a flexible tool for reliable high-throughput primer design for quantitative PCR. BMC Bioinformatics, 9: p. 465.

Bateman A, Haft DH (2002). HMM-based databases in InterPro. Briefings in Bioinformatics, 3: 236.

Campbell M, Haas B, Hamilton J, Mount S, Buell CR (2006). Comprehensive analysis of alternative splicing in rice and comparative analyses with Arabidopsis. BMC Genomics, 7: p. 3 27.

Cheng K (2006). The Role of AtBAG-4 in phytochrome signaling, Graduate Institude of Life Science, Tzu Chi University, Taiwan. p. 73.

Coulson M, Robert S, Saint R (2005). *Drosophila starvin* encodes a tissue-specific BAG-domain protein required for larval food uptake. Genetics, 171: 1799.

Doukhanina EV, Chen S, Van Der Zalm E, Godzik A, Reed J, Dickman MB (2006). Identification and Functional Characterization of the BAG Protein Family in *Arabidopsis thaliana*. J. Bio. Chem. 281: 18793-18801.

Guex N, Peitsch MC (1997). SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis, 18: 2714-23.

Horton P, Park KJ, Obayashi T, Nakai K (2006). Protein subcellular localization prediction with WoLF PSORT, Citeseer. p. 48.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P (2008). Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformatics, pp. 420747.

Jain M, Nijhawan A, Tyagi AK, Khurana JP (2006). Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. Biochem. Biophys. Res. Commun. 345: 646-651.

Jung KH, Dardick C, Bartley LE, Cao P, Phetsom J, Canlas P, Seo YS, Shultz M, Ouyang S, Yuan Q (2008). Refinement of light-responsive transcript lists using rice oligonucleotide arrays: evaluation of gene-redundancy. PLoS One, 3: p. 3337.

Kabbage M, Dickman M (2008). The BAG proteins: a ubiquitous family of chaperone regulators. Cell. Mol. Life Sci. 65: 1390-1402.

Kang C, Jung W, Kang Y, Kim J, Kim D, Jeong J, Baek D, Jin J, Lee J, Kim M (2005). AtBAG6, a novel calmodulin-binding protein, induces programmed cell death in yeast and plants. Cell Death and Differentiation, 13: 84-95.

Liao Q, Ozawa F, Friess H, Zimmermann A, Takayama S, Reed JC, Kleeff J, Buchler MW (2001). The anti-apoptotic protein BAG-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines. FEBS Lett, 503: 151-157.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-[Delta][Delta] CT method. Methods, 25: 402-408.

Moribe Y, Niimi T, Yamashita O, Yaginuma T (2001). Samui, a novel cold-inducible gene, encoding a protein with a BAG domain similar to silencer of death domains (SODD/BAG-4), isolated from Bombyx diapause eggs. Eur. J. Biochem. 268: 3432-3442.

Pascale M, Rosati A, Festa M, Basile A, d'Avenia M, Falco A, Torino G, Turco MC (2010). BAG3 Protein: Role in Some Neoplastic Cell Types and Identification as a Candidate Target for Therapy. Apoptosome, pp. 137-146.

Sondermann H, Scheufler C, Schneider C, Höhfeld J, Hartl FU, Moarefi I (2001). Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science, 291:1553.

Song J, Takeda M, Morimoto RI (2001). Bag1-Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. Nat. Cell Biol. 3: 276-282.

Takayama S, Sato T, Krajewski S, Kochel K, Irie S, Millan JA, Reed JC (1995). Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. Cell, 80: 279-84.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28: 2731-2739.

Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: p. 4673.

Waterhouse AM, Procter JB, Martin D, Clamp M, Barton GJ (2009). Jalview Version 2-a multiple sequence alignment editor and analysis workbench. Bioinformatics, 25: p. 1189.

Williams B, Kabbage M, Britt R, Dickman MB (2010). AtBAG7, an Arabidopsis Bcl-2-associated athanogene, resides in the endoplasmic reticulum and is involved in the unfolded protein response. Proc. Natl. Acad. Sci. USA, 107: 6088-6093.

Yan J, He C, Zhang H (2003). The BAG-family proteins in *Arabidopsis thaliana*. Plant Sci. 165: 1-7.

Yoshida S, Forno DA, Cock JH, Gomez K (1972). In: Laboratory Manual for Physiological Studies of Rice. 2nd ed. The International Rice Research Institute, Los Baños, Philippines. pp. 53-57.

Zhou GA, Jiang Y, Yang Q, Wang JF, Huang J, Zhang HS (2006). Isolation and characterization of a new Na+/H+ antiporter gene OsNHA1 from rice (Oryza sativa L.). DNA Seq, 17: 24-30.