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ORIGINAL ARTICLE

Genome-wide identification of major transcription factor superfamilies in rice identifies key candidates involved in abiotic stress dynamism

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Abstract

Transcription factors (TFs) are pivotal players in plant stress signaling and signal transduction pathways. Among the key TFs, NAC, ZF-HD, AP2-EREBP, WRKY and bHLH proteins play crucial roles in the regulation of reprogramming the transcriptome and associated responses in stress. Considering this, genome-wide identification of NAC, ZF-HD, AP2-EREBP, WRKY and bHLH TF families were performed in the C3 model plant, *Oryza sativa*. The computational study identified 144 NAC, 15 ZF-HD, 164 AP2-EREBP, 103 WRKY, and 135 bHLH proteins and their physicochemical properties and, expression profiling by computational analysis. Genome-wide in silico expression analysis of NAC, ZF-HD, AP2-EREBP, WRKY, bHLH genes showed their differential expression profiling in different tissues. Expression patterns, gene structure, subcellular localization, gene ontology of 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH key genes suggested the putative novel variants in stress and signal transduction. These key players are needed to be studied in order to categorize and outline their functional roles in AbS signaling network.

Keywords Abiotic stress · AP2-EREBP · bHLH TF families · Expression profiling · NAC · *Oryza sativa* · WRKY · ZF-HD

Abbreviations

AbS	Abiotic stress
AbSR	Abiotic stress responsive
GO	Gene ontology
TFs	Transcription factors

Introduction

A large number of the world's population depend on rice for future food requirement. Unlike animals, plants are exposed to diverse environmental stresses. Especially abiotic stresses (AbS's) are predominant, which negatively affects their growth and development of plants thereby reduction in productivity. Drought, cold, salinity, flood, submergence are the major stressors responsible for substantial yield loss. At the molecular and physiological level, expression and signalling of several genes are affected by these stresses, which known as abiotic stress responsive (AbSR) genes (Hirayama and Shinozaki 2010; Sharoni et al. 2011; Hadiarto and Tran 2011; Lata et al. 2015). Among many AbSR genes, transcription factors (TFs) are the crucial targets to understand the molecular cross-talks of AbS responses and to conducting overexpression studies in plants for AbS tolerance, they can act as key regulators overdriving the expression of several target genes (Nakashima et al. 2009; Urano et al. 2010; Yang et al. 2010; Golldack et al. 2011; Mickelbart et al. 2015). Plant genome contains around 7% TFs (Udvardi et al.

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2007), which are categorized into 58 TF families (Jin et al. 2014). Among these, NAC, ZF-HD, AP2-EREBP, WRKY, bHLH are the largest TF families, excluding ZF-HD TFs (http://grassius.org/grasstfdb.php) (Singh et al. 2002; Gong et al. 2004, Xiong et al. 2005).

Various investigation and available literatures reveal the regulatory role of NAC, ZF-HD, AP2-EREBP, WRKY and bHLH TF families in signal transduction and inflection of several physiological and molecular processes including somatic embryogenesis (Toledo-Ortiz et al. 2003; Albrecht et al. 2005; Sonnenfeld et al. 2005; Mittler et al. 2006; Xu et al. 2008), plant development (Shigyo and Ito 2004), pollen development and function (Guan et al. 2014), internode elongation (Hattori et al. 2009), biosynthesis of secondary metabolites (Ma et al. 2009; Suttipanta et al. 2011), seed development (Johnson et al. 2002; Luo et al. 2005), seed dormancy (Rushton et al. 2010; Ding et al. 2014), biomass (Yu et al. 2013), flowering period and plant height (Cai et al. 2014), leaf senescence (Miao et al. 2004; Guo and Gan 2006), anther and ovule development (Zhao et al. 2008) and hormone signaling (Rashotte and Goertzen 2010; Feller et al. 2011; Hu et al. 2013). Significantly NAC, ZF-HD, AP2-EREBP, WRKY, bHLH TF families are activated in response to diverse biotic stress (Shinozaki et al. 2003; Cao et al. 2006; Singh et al. 2010; Xia et al. 2010; Muthamilarasan and Prasad 2013) including fungal invasion (Zheng et al. 2006; Marchive et al. 2007), virus attack (Huh et al. 2012), bacterial infection (Du and Chen 2000; Deslandes et al. 2002; Kim et al. 2008), disease resistance (Gutterson and Reuber 2004; Oh et al. 2005; Nakashima et al. 2007) and as well as AbS (Shinozaki et al. 2003; Cao et al. 2006; Singh et al. 2010; Xia et al. 2010; Tang et al. 2013) like drought and heat (Rizhsky et al. 2002; Kiribuchi et al. 2004, 2005; Sakuma et al. 2006; Wu et al. 2009; Zheng et al. 2009; Qiu and Yu 2009; Ren et al. 2010), salinity (Mukhopadhyay et al. 2004; Gutha and Reddy 2008; Qiu and Yu 2009; Zheng et al. 2009), cold (Pnueli et al. 2002; Wang et al. 2003; Mukhopadhyay et al. 2004; Kume et al. 2005; Qiu and Yu 2009), desiccation, submergence, heavy metals and wounding (Mukhopadhyay et al. 2004; Kiribuchi et al. 2004, 2005), osmotic stress (Gutha and Reddy 2008), low temperatures (Zheng et al. 2009; Sharoni et al. 2011) and phosphate starvation (Yi et al. 2005).

Therefore, the significant role of NAC, ZF-HD, AP2-EREBP, WRKY, bHLH TF families in several biological, molecular and physiological process were studied in assorted crop plants (Ledent and Vervoort 2001; Toledo-Ortiz et al. 2003; Ulker and Somssich 2004; Zheng et al. 2009; Rushton et al. 2010; Sharoni et al. 2011; Figueiredo et al. 2012; Chen et al. 2016). However, only minimum reports are available in the C3 model plant, *O. sativa*. For that reason, it is important to expedite the functional genomic approaches in the panicoideae family, especially C3 photosynthesis and AbS tolerance.

Based on the above addressed issues, the present study aimed at, in silico approaches to identify the potential AbSR encoding genes from rice NAC, ZF-HD, AP2-EREBP, WRKY, bHLH TF families. This is the first wide range investigation on these TFs *O. sativa*. The current study provides better insights about the functional aspects of these AbSR TFs, and unravels key genes for further validation toward describing their functional role in AbS dynamism.

Materials and methods

Database search for the identification of transcription factor family in *O. sativa* (L.)

Rice transcription factors (TF) like NAC, ZF-HD, AP2-EREBP, WRKY, bHLH and their protein sequences retrieved from the GRASSIUS Grass Regulatory Information Server (http://grassius.org/grasstfdb.html; Yilmaz et al. 2009).

Mining and meta-analysis of rice transcription factors

TF family members and their RAP-DB ID/Gene locus ID were collected. It was exposed to Rice Oligonucleotide Array Database (ROAD) meta-analysis search tool (Cao et al. 2012) for analyzing the tissues specific expression profile in different tissues in rice. The AbSR TF genes (Gene ID's) were used to retrieve the corresponding genomic transcript, coding sequences with their chromosomal localization and protein sequence from RiceSRTFDB (Priya and Jain 2013).

Gene structure prediction

The genomic sequences and coding sequences of potential AbSR TFs like 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH proteins were analyzed by GSDS web server v2.0 (Hu et al. 2015) to identify the position of exons and introns.

Physicochemical properties of identified proteins and phylogenetic analysis

The physicochemical properties including amino acid length, molecular weight, isoelectric point (pI), the number of positive/negatively charged residues, the instability index, and aliphatic index were predicted using the protparam tool of ExPASy (http://web.expasy.org/protparam/; Gasteiger et al. 2005). The 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH protein sequences were imported into Phylogeny.fr (http://www.phylogeny.fr/; Dereeper et al. 2008) to construct a phylogenetic tree by maximum likelihood method.

Table 1 Potential abiotic stress responsive TF genes and their details

S. no	Gene name	Gene ID	Gene position	Exons	Introns	CDS
NAC family						
1	OsNAC4	LOC_Os01g15640	8791646–8796363 (−)	4	3	1470
2	OsNAC11	LOC_Os01g66120	38397474–38400490 (−)	3	2	744
3	OsNAC13	LOC_Os01g70110	40571776–40572977 (+)	2	1	687
4	OsNAC24	LOC_Os02g57650	35297324–35301411 (+)	5	4	1899
5	OsNAC29	LOC_Os03g12120	6357095–6364595 (−)	17	16	2661
6	OsNAC30	LOC_Os03g21030	11936154–11938191 (−)	3	2	1077
7	OsNAC37	LOC_Os03g60080	34158969–34160416 (+)	2	1	951
8	OsNAC47	LOC_Os05g10620	5784079–5789498 (−)	5	4	1047
9	OsNAC51	LOC_Os05g34830	20615442–20617784 (+)	2	1	774
10	OsNAC52	LOC_Os05g35170	20817763–20821884 (−)	4	3	909
11	OsNAC59	LOC_Os06g15690	8879072–8883884 (+)	6	5	1257
12	OsNAC69	LOC_Os07g12340	6967004–6968316 (+)	1	0	831
13	OsNAC76	LOC_Os07g48550	29039441–29041305 (−)	3	2	906
14	OsNAC80	LOC_Os08g06140	3388020–3392453 (−)	7	6	2178
15	OsNAC86	LOC_Os08g42400	26764102–26767631 (+)	2	1	495
16	OsNAC87	LOC_Os08g44820	28141867–28146558 (−)	4	3	1533
17	OsNAC112	LOC_Os11g08210	4295051–4297685 (−)	3	2	990
ZF-HD family						
1	OsZHD10	LOC_Os09g24820	14811098–14815388 (−)	1	0	1026
2	OsZHD12	LOC_Os11g03420	1297251–1297994 (+)	1	0	318
3	OsZHD14	LOC_Os12g03110	1179358–1180099 (+)	1	0	318
AP2-EREBP family						
1	OsEREB1	LOC_Os01g04020	1745416–1747549 (+)	1	0	1017
2	OsEREB9	LOC_Os01g49830	28618439–28620073 (−)	1	0	1182
3	OsEREB10	LOC_Os01g54890	31566847–31567697 (+)	2	1	714
4	OsEREB11	LOC_Os01g58420	33765935–33767041 (+)	1	0	708
5	OsEREB36	LOC_Os02g51670	31642599–31644669 (+)	1	0	1017
6	OsEREB39	LOC_Os02g54160	33193935–33196815 (−)	2	1	1098
7	OsEREB49	LOC_Os03g08500	4371117–4372462 (−)	2	1	999
8	OsEREB75	LOC_Os04g52090	30754057–30755155 (+)	1	0	669
9	OsEREB87	LOC_Os05g29810	17186605–17187906 (+)	2	1	597
10	OsEREB104	LOC_Os06g09390	4730331–4732912 (+)	2	1	1089
11	OsEREB132	LOC_Os08g31580	19544608–19546313 (+)	1	0	849
12	OsEREB145	LOC_Os09g20350	12215431– 12217345 (+)	1	0	876
13	OsEREB147	LOC_Os09g26420	15958323–15961974 (+)	1	0	1191
WRKY family						
1	OsWRKY4	LOC_Os01g14440	8083372–8086044 (+)	5	4	1752
2	OsWRKY20	LOC_Os01g60640	35061691–35063897 (+)	2	1	843
3	OsWRKY25	LOC_Os02g08440	4542759–4544980 (+)	4	3	651
4	OsWRKY31	LOC_Os03g20550	11649542–11650862 (+)	3	2	444
5	OsWRKY53	LOC_Os05g27730	16092750–16095231 (+)	5	4	1464
6	OsWRKY68	LOC_Os06g44010	26508922–26510702 (−)	3	2	1191
7	OsWRKY76	LOC_Os08g09900	5712948–5718163 (−)	4	3	2565
8	OsWRKY79	LOC_Os08g29660	18217328–18219695 (+)	2	1	960
9	OsWRKY90	LOC_Os11g02480	758588–762335 (−)	3	2	675
10	OsWRKY103	LOC_Os12g32250	19445198–19450076 (+)	6	5	1623
11	OsWRKY104	LOC_Os12g40570	25066933–25070629 (−)	4	3	1092
bHLH family						
1	OsbHLH26	LOC_Os02g02480	874691–878615 (−)	5	4	711

Table 1 (continued)

S. no	Gene name	Gene ID	Gene position	Exons	Introns	CDS
2	OsbHLH87	LOC_Os05g38140	22308646–22311010 (+)	5	4	732
3	OsbHLH94	LOC_Os06g09370	4708744–4714553 (−)	9	8	1206
4	OsbHLH105	LOC_Os07g35870	21465743–21468864 (−)	5	4	645
5	OsbHLH110	LOC_Os08g04390	2147786–2150534 (−)	5	4	762
6	OsbHLH116	LOC_Os08g38210	24219692–24226032 (+)	7	6	876
7	OsbHLH127	LOC_Os09g29930	18206612–18214107 (+)	9	8	432
8	OsbHLH129	LOC_Os09g32510	19405779–19407978 (−)	7	6	789

CDS: coding sequence; (+) leading strand; (−) lagging strand

Analysis of subcellular localization in TF families

The subcellular localization of TF family proteins of *O. sativa* was predicted using CELLO2GO (http://cello.life.nctu.edu.tw/cello2go/; Yu et al. 2014), Wolf Psort 2 (http://wolfpsort.org/; Horton et al. 2007), Bacello (http://gpcr.biocomp.unibo.it/bacello/; Pierleoni et al. 2006), ESLPred2 (http://www.imtech.res.in/raghava/eslpred2/; Garg and Raghava 2008), SubLoc (http://www.bioinfo.tsinghua.edu.cn/SubLoc/; Hua and Sun 2001).

Gene ontology analysis

Identified potential TF family members (Table 1) were loaded into CELLO2GO (http://cello.life.nctu.edu.tw/cello2go/; Yu et al. 2014) to find the Gene Ontology (GO) annotation against eukaryote. TF genes were characterized as per biological process, molecular function and cellular components according to CELLO2GO GO annotation.

Results

Identification of potent transcription factors (TF) in rice

A total of 144 NAC, 15 ZF-HD, 164 AP2-EREBP, 103 WRKY and 135 bHLH TFs of rice with their gene ID's were analyzed computationally for tissues specific (TS) gene expression studies. Seventeen NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH were potential abiotic stress responsive (AbSR) TF genes (Table 1), which have been involved in TS gene expression in all the 22 different tissues (Fig. 1a–e). This higher expression heatmap profiling was also evidenced among 5 different TF families, thereby reasonably delineating their function in tissue-specific manner.

Structure of AbSR TF genes

Positions of exons and introns within the 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY and 8 bHLH genes were predicted. Gene structure determination showed the numbers and arrangements of exons and introns (Fig. 2a–e and Table 1). The majority of AbSR TF genes (11; 21.153%) were found to contain a single intron, while 7 genes (13.46%) have two introns. Six AbSR TF genes (11.538%) have three introns; whereas 8 genes (15.38%) have four introns. Three AbSR TF genes (5.769%) were found to contain six introns; while 4 genes (7.692%) contained five and 8 introns, respectively. One AbSR TF gene (1.92%) has 16 introns. Among these, 12 AbSR TF genes (23.07%) were found intronless (Fig. 2a–e and Table 1).

Gene ontology annotation

Gene characteristic features of TF families were analyzed by protein sequence using CELLO2GO and showed the putative involvement of these proteins in different molecular function, and biological process (Table 2). A large number of proteins were predicted to be involved in stress response, embryo development, anatomical structure development, metabolic and biosynthetic process, and signal transduction (Fig. 3a–e). The molecular functions of these proteins paralleled to transcriptional regulator activity, protein binding and DNA binding activity (Fig. 4a–e).

Protein properties of potentially expressed TFs

All five different TF families and their well-expressed protein properties were analyzed. Smallest and biggest amino acids with respect to molecular weight, pI ranging, stability index, aliphatic index and grand average of hydropathicity (GRAVY) of potential TF genes were determined (Table 3).

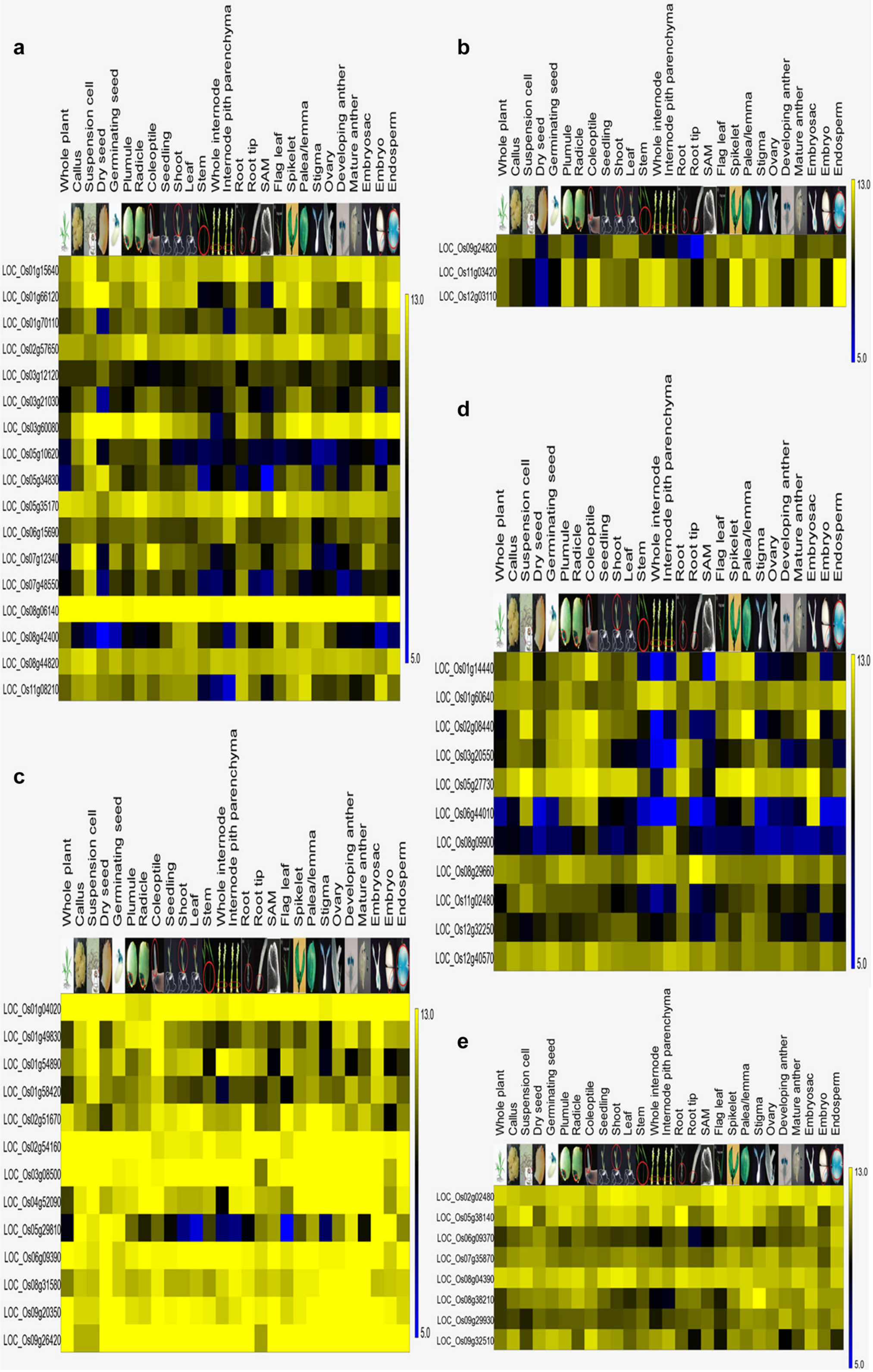

Fig. 1 Differential expression patterns of rice TF family genes. Heat map representing expression profiling of **a** NAC, **b** ZF-HD, **c** AP2-EREBP, **d** WRKY, **e** bHLH potent AbSR TF genes with respect to specific tissues. Yellow color indicates up regulation; Blue color indicates down regulation; Black color indicates unchanged expression level of AbSR TF genes. The colored scale bar at right side denotes relative expression value, where 5.0 and 13.0 represent down regulation and up regulation respectively (color figure online)

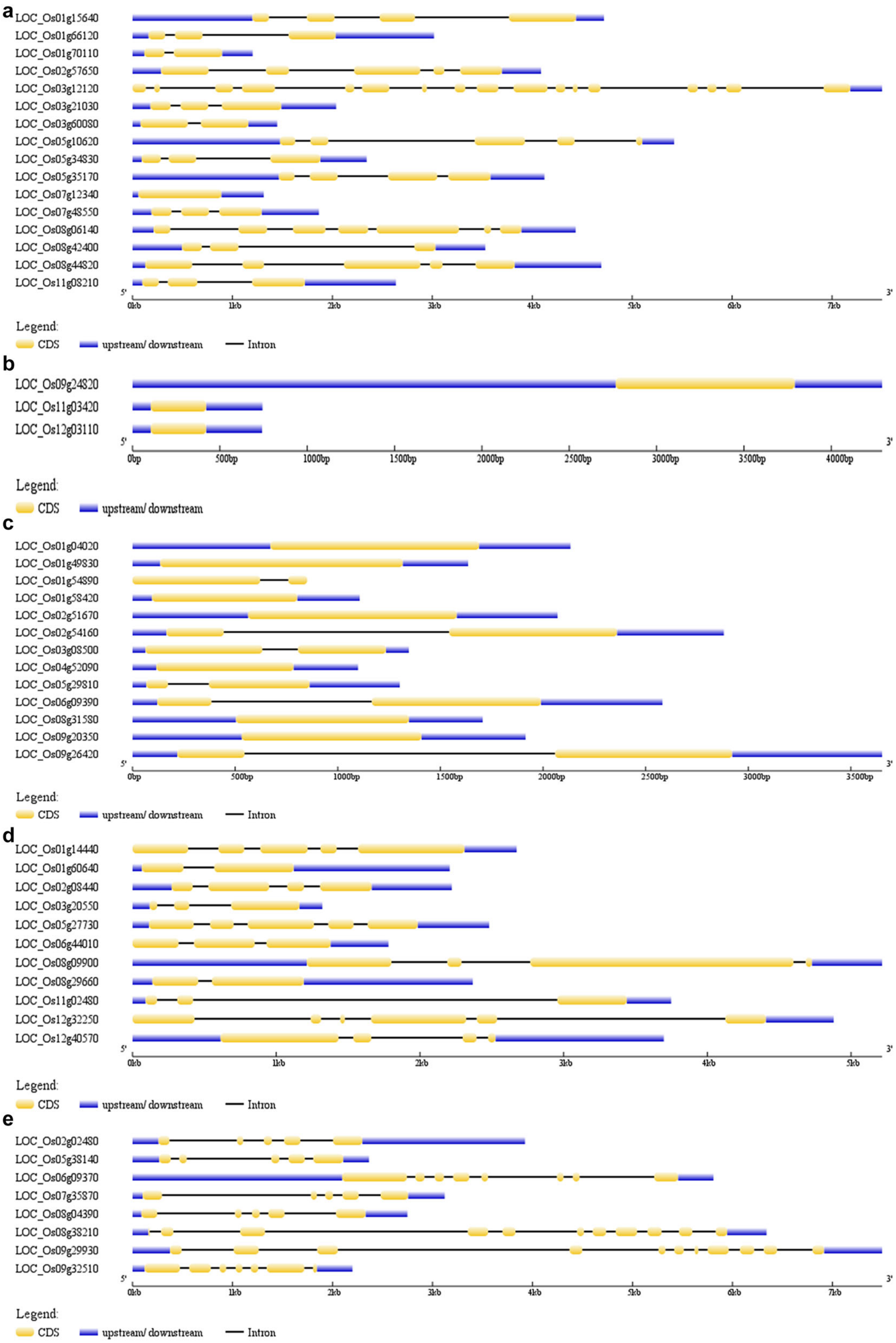

Fig. 2 Gene organization of major TF family genes. **a** NAC, **b** ZF-HD, **c** AP2-EREBP, **d** WRKY, and **e** bHLH TF key players. The blue lines indicate the UTR regions, orange box indicate the exons and black lines shows the introns (color figure online)

Table 2 Gene ontology of key AbSR TF genes

S. no	Gene Name	Gene ID	Molecular function	Biological process
NAC family				
1	OsNAC4	LOC_Os01g15640	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development;anatomical structure formation involved in morphogenesis; biogenesis
2	OsNAC11	LOC_Os01g66120	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
3	OsNAC13	LOC_Os01g70110	Transcription regulator activity; DNA binding	Response to stress; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
4	OsNAC24	LOC_Os02g57650	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
5	OsNAC29	LOC_Os03g12120	Transcription regulator activity; DNA binding	Response to stress; biosynthetic process; metabolic process; anatomical structure development; biogenesis
6	OsNAC30	LOC_Os03g21030	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development;anatomical structure formation involved in morphogenesis; biogenesis
7	OsNAC37	LOC_Os03g60080	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
8	OsNAC47	LOC_Os05g10620	Transcription regulator activity; DNA binding	Response to stress; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
9	OsNAC51	LOC_Os05g34830	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
10	OsNAC52	LOC_Os05g35170	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
11	OsNAC59	LOC_Os06g15690	Transcription regulator activity; DNA binding	Response to stress; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
12	OsNAC69	LOC_Os07g12340	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
13	OsNAC76	LOC_Os07g48550	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
14	OsNAC80	LOC_Os08g06140	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
15	OsNAC86	LOC_Os08g42400	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis

Table 2 (continued)

S. no	Gene Name	Gene ID	Molecular function	Biological process
16	OsNAC87	LOC_Os08g44820	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
17	OsNAC112	LOC_Os11g08210	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development; anatomical structure formation involved in morphogenesis; biogenesis
ZF-HD family				
1	OsZHD10	LOC_Os09g24820	Transcription regulator activity; DNA binding	Biosynthetic process; metabolic process; embryo development; anatomical structure development; Response to stress
2	OsZHD12	LOC_Os11g03420	Transcription regulator activity; DNA binding	Biosynthetic process; metabolic process; embryo development; anatomical structure development; Response to stress
3	OsZHD14	LOC_Os12g03110	Transcription regulator activity; DNA binding	Biosynthetic process; metabolic process; embryo development; anatomical structure development; Response to stress
AP2-EREBP family				
1	OsEREB1	LOC_Os01g04020	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
2	OsEREB9	LOC_Os01g49830	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
3	OsEREB10	LOC_Os01g54890	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
4	OsEREB11	LOC_Os01g58420	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
5	OsEREB36	LOC_Os02g51670	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
6	OsEREB39	LOC_Os02g54160	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
7	OsEREB49	LOC_Os03g08500	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
8	OsEREB75	LOC_Os04g52090	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
9	OsEREB87	LOC_Os05g29810	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
10	OsEREB104	LOC_Os06g09390	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
11	OsEREB132	LOC_Os08g31580	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
12	OsEREB145	LOC_Os09g20350	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
13	OsEREB147	LOC_Os09g26420	Transcription regulator activity; DNA binding	Response to stress; signal transduction; biosynthetic process; metabolic process; anatomical structure development
WRKY family				
1	OsWRKY4	LOC_Os01g14440	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transduction; anatomical structure formation involved in morphogenesis; anatomical structure development
2	OsWRKY20	LOC_Os01g60640	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transduction; anatomical structure formation involved in morphogenesis; anatomical structure development
3	OsWRKY25	LOC_Os02g08440	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development

Table 2 (continued)

S. no	Gene Name	Gene ID	Molecular function	Biological process
4	OsWRKY31	LOC_Os03g20550	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development
5	OsWRKY53	LOC_Os05g27730	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development
6	OsWRKY68	LOC_Os06g44010	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development
7	OsWRKY76	LOC_Os08g09900	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development
8	OsWRKY79	LOC_Os08g29660	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development
9	OsWRKY90	LOC_Os11g02480	Transcription regulator activity; ion binding protein binding; DNA binding	Biosynthetic process; metabolic process; signal transduction; anatomical structure formation involved in morphogenesis; anatomical structure development
10	OsWRKY103	LOC_Os12g32250	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development
11	OsWRKY104	LOC_Os12g40570	Transcription regulator activity; ion binding; protein binding; DNA binding	Biosynthetic process; metabolic process; signal transductions; anatomical structure formation involved in morphogenesis; anatomical structure development
bHLH family				
1	OsbHLH26	LOC_Os02g02480	Transcription regulator activity; DNA binding	Biosynthetic process; metabolic process; response to stress
2	OsbHLH87	LOC_Os05g38140	Transcription regulator activity; DNA binding	Biosynthetic process; metabolic process; response to stress; cell morphogenesis; cell differentiation; anatomical structure development
3	OsbHLH94	LOC_Os06g09370	Transcription regulator activity; DNA binding; protein binding	Biosynthetic process; metabolic process; response to stress; cell differentiation; anatomical structure development; signal transduction
4	OsbHLH105	LOC_Os07g35870	Transcription regulator activity; DNA binding; protein binding	Biosynthetic process; metabolic process; response to stress; homeostatic process; cell morphogenesis; cell differentiation; anatomical structure development; signal transduction
5	OsbHLH110	LOC_Os08g04390	Transcription regulator activity; DNA binding	Biosynthetic process; metabolic process; response to stress
6	OsbHLH116	LOC_Os08g38210	Transcription regulator activity; DNA binding; protein binding	Biosynthetic process; metabolic process; response to stress; cell differentiation; anatomical structure development; signal transduction
7	OsbHLH127	LOC_Os09g29930	Transcription regulator activity; DNA binding; protein binding	Biosynthetic process; metabolic process; response to stress; cell differentiation; anatomical structure development; signal transduction
8	OsbHLH129	LOC_Os09g32510	Transcription regulator activity; DNA binding; protein binding	Biosynthetic process; metabolic process; cell differentiation; anatomical structure development; signal transduction

Phylogenetic analysis of TFs

To study the phylogenetic organization of the 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY and 8 bHLH family, the imputed protein sequences were used to generate an unrooted phylogenetic tree (Fig. 5). The unrooted tree divided the potentially expressed TF family genes into 5 major groups (groups I–V) based on the conserved NAC,

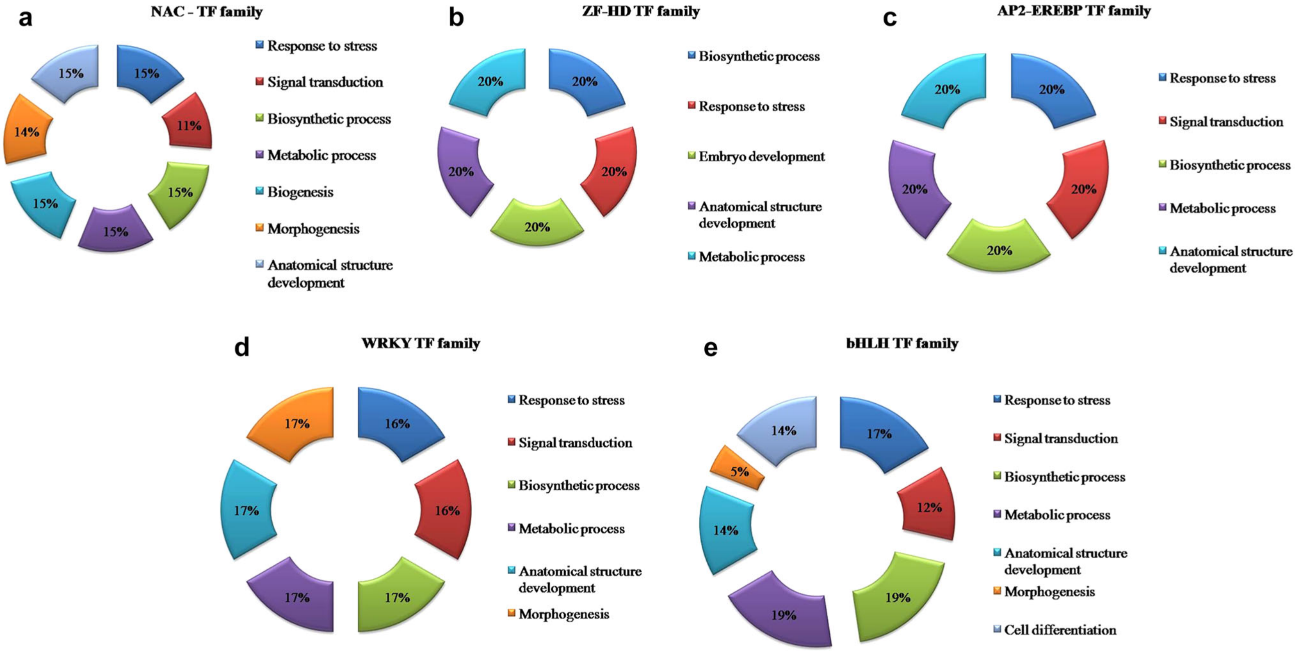

Fig. 3 Classification of AbSR TF genes based on their biological process. GO biological process based categorization for **a** NAC, **b** ZF-HD, **c** AP2-EREBP, **d** WRKY, and **e** bHLH family genes to AbS

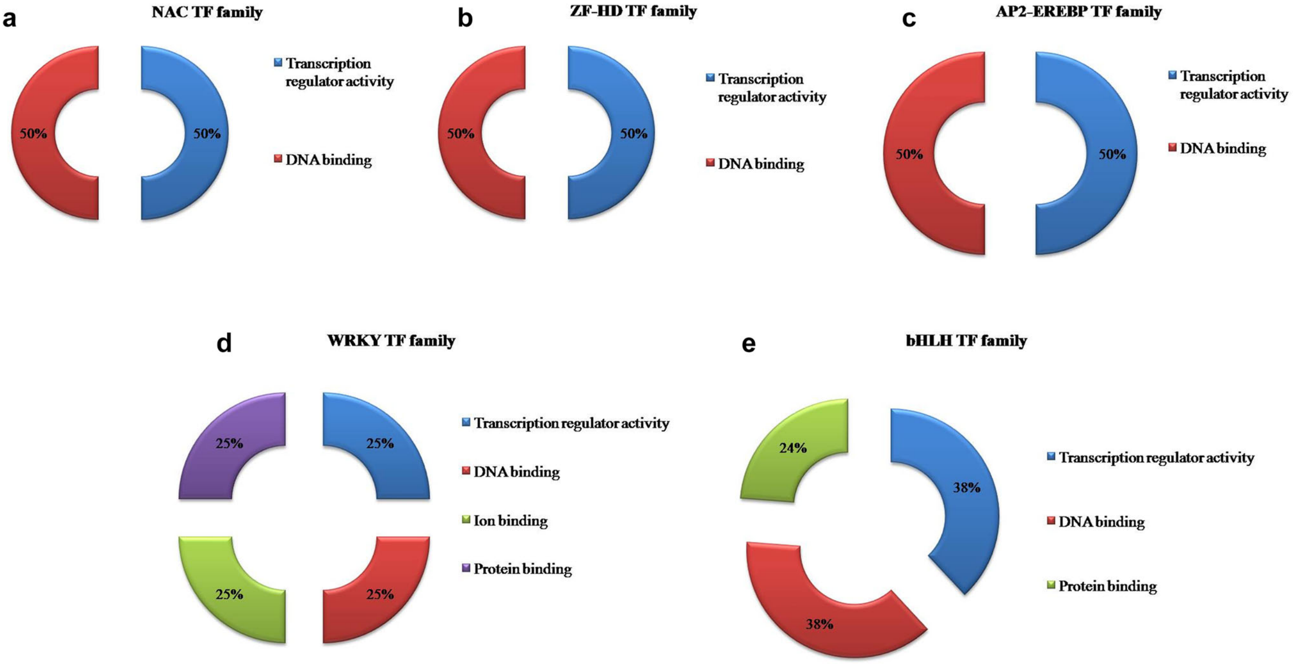

Fig. 4 Classification of TF genes according to their molecular function. GO molecular function based categorization for **a** NAC, **b** ZF-HD, **c** AP2-EREBP, **d** WRKY, and **e** bHLH family genes to AbS

ZF-HD, AP2-EREBP, WRKY, bHLH domains and homology of TF family gene sequences. Twenty proteins belongs to group—I (9 NAC; 1 AP2 –EREBP; 5 WRKY; 5 bHLH), 8 to group—II (2 NAC; 1 WRKY; 2 AP2-EREBP; 3 ZF-HD), 5 to group—III (1 NAC; 4 AP2-EREBP), 4 to group—IV (4 NAC); 15 to group—V (1 NAC; 6 AP2-EREBP; 5 WRKY; 3 bHLH) (Fig. 5).

Table 3 Protein properties of key TF genes

S. no	Gene name	Gene ID	Amino acid	M.Wt	pI	II	AI	GRAVY	S/US
NAC family									
1	OsNAC4	LOC_Os01g15640	489	54693.92	5.19	52.43	68.83	− 0.703	US
2	OsNAC11	LOC_Os01g66120	303	32906.68	8.95	55.68	71.55	− 0.366	US
3	OsNAC13	LOC_Os01g70110	228	24504.68	9.17	72.58	61.71	− 0.510	US
4	OsNAC24	LOC_Os02g57650	632	69559.58	4.54	39.72	63.91	− 0.607	S
5	OsNAC29	LOC_Os03g12120	886	99173.84	5.23	52.59	76.84	− 0.621	US
6	OsNAC30	LOC_Os03g21030	358	39199.34	5.52	42.47	69.02	− 0.348	US
7	OsNAC37	LOC_Os03g60080	316	35177.80	6.62	35.24	59.11	− 0.611	S
8	OsNAC47	LOC_Os05g10620	357	39112.06	9.25	47.38	62.58	− 0.691	US
9	OsNAC51	LOC_Os05g34830	322	34595.21	8.54	54.70	65.59	− 0.439	US
10	OsNAC52	LOC_Os05g35170	449	49739.57	4.85	42.93	69.04	− 0.445	US
11	OsNAC59	LOC_Os06g15690	418	46875.30	5.12	42.15	71.12	− 0.702	US
12	OsNAC69	LOC_Os07g12340	276	30860.93	6.02	47.25	68.12	− 0.591	US
13	OsNAC76	LOC_Os07g48550	301	34121.60	5.55	48.94	64.55	− 0.539	US
14	OsNAC80	LOC_Os08g06140	730	80654.77	4.66	55.79	65.26	− 0.747	US
15	OsNAC86	LOC_Os08g42400	232	24,691.18	9.76	75.51	68.97	− 0.281	US
16	OsNAC87	LOC_Os08g44820	656	71,937.63	4.59	43.84	71.08	− 0.459	US
17	OsNAC112	LOC_Os11g08210	329	35,522.79	5.47	47.20	60.03	− 0.470	US
ZF-HD family									
1	OsZHD10	LOC_Os09g24820	341	35,323.62	7.71	75.52	54.31	− 0.564	US
2	OsZHD12	LOC_Os11g03420	105	10,859.95	8.56	51.07	44.10	− 0.571	US
3	OsZHD14	LOC_Os12g03110	105	10,817.86	8.22	50.26	44.10	− 0.562	US
AP2-EREBP family									
1	OsEREB1	LOC_Os01g04020	338	36,528.59	4.75	58.83	61.66	− 0.526	US
2	OsEREB9	LOC_Os01g49830	393	41,181.26	9.09	43.51	70.20	− 0.290	US
3	OsEREB10	LOC_Os01g54890	237	24,000.22	10.46	53.27	80.55	0.015	US
4	OsEREB11	LOC_Os01g58420	235	24,270.46	9.55	48.91	64.98	− 0.175	US
5	OsEREB36	LOC_Os02g51670	338	36,300.52	7.77	67.52	61.18	− 0.475	US
6	OsEREB39	LOC_Os02g54160	365	40,141.53	4.89	48.42	60.49	− 0.659	US
7	OsEREB49	LOC_Os03g08500	329	35,304.76	4.56	45.25	62.61	− 0.496	US
8	OsEREB75	LOC_Os04g52090	222	22,798.00	9.62	56.09	81.08	− 0.003	US
9	OsEREB87	LOC_Os05g29810	198	20,772.19	7.07	60.56	61.06	− 0.533	US
10	OsEREB104	LOC_Os06g09390	362	39,764.69	4.78	57.83	61.02	− 0.617	US
11	OsEREB132	LOC_Os08g31580	280	29,524.91	7.03	51.93	62.57	− 0.441	US
12	OsEREB145	LOC_Os09g20350	291	30,752.19	6.22	58.76	65.43	− 0.375	US
13	OsEREB147	LOC_Os09g26420	396	42,625.83	4.88	42.83	63.76	− 0.507	US
WRKY family									
1	OsWRKY4	LOC_Os01g14440	583	60,502.43	8.87	55.13	59.69	− 0.493	US
2	OsWRKY20	LOC_Os01g60640	280	29,553.03	5.58	58.95	68.18	− 0.223	US
3	OsWRKY25	LOC_Os02g08440	348	37,227.08	7.68	58.95	64.60	− 0.584	US
4	OsWRKY31	LOC_Os03g20550	210	23,642.19	8.38	66.24	49.38	− 0.888	US
5	OsWRKY53	LOC_Os05g27730	487	50,980.26	8.05	54.14	55.01	− 0.648	US
6	OsWRKY68	LOC_Os06g44010	396	42,643.21	9.4	67.9	66.94	− 0.489	US
7	OsWRKY76	LOC_Os08g09900	854	95,172.91	6.31	45.74	79.03	− 0.321	US
8	OsWRKY79	LOC_Os08g29660	319	33,550.28	6.18	41.48	67.40	− 0.316	US
9	OsWRKY90	LOC_Os11g02480	224	25,187.68	8.54	52.33	42.72	− 0.891	US
10	OsWRKY103	LOC_Os12g32250	540	57,760.18	6.90	54.84	57.72	− 0.710	US
11	OsWRKY104	LOC_Os12g40570	365	38,551.71	9.73	57.09	61.78	− 0.479	US

Table 3 (continued)

S. no	Gene name	Gene ID	Amino acid	M.Wt	pI	II	AI	GRAVY	S/US
bHLH family									
1	OsbHLH26	LOC_Os02g02480	236	25,592.97	5.15	41.38	76.53	− 0.565	US
2	OsbHLH87	LOC_Os05g38140	243	26,197.61	7.73	59.05	72.06	− 0.511	US
3	OsbHLH94	LOC_Os06g09370	478	50,725.77	5.17	49.62	78.68	− 0.339	US
4	OsbHLH105	LOC_Os07g35870	256	27,873.70	5.88	65.33	61.13	− 0.655	US
5	OsbHLH110	LOC_Os08g04390	253	27,226.84	5.75	60.22	61.38	− 0.609	US
6	OsbHLH116	LOC_Os08g38210	508	55,082.95	7.31	59.20	60.06	− 0.796	US
7	OsbHLH127	LOC_Os09g29930	504	54,188.80	6.84	53.57	62.22	− 0.671	US
8	OsbHLH129	LOC_Os09g32510	398	42,178.08	4.95	46.10	69.70	− 0.353	US

M.Wt: molecular weight; pI: isoelectric point; II: instability index; AI: aliphatic index; GRAVY: grand average hydropathicity; S/US: stable/unstable

Subcellular localization of rice TFs

The 4 programes were classed into two based on their resolution as high resolution and low-resolution predictions. The prediction principles and the competences of the four programs described in literature (Horton et al. 2007; Garg and Raghava 2008; Pierleoni et al. 2006; Hua and Sun 2001). The prediction results for the key TF family genes among the five different TF families and summarized in Table 4. It revealed the localization of these key players and their products in nucleus.

Discussion

NAC, ZF-HD, AP2-EREBP, WRKY, and bHLH type of TF's have been reported to play vital roles in regulating various plant processes and physiological responses like plant development, normal growth, response to environmental stimuli and involved in plant defenses and so on (Ledent and Vervoort 2001; Toledo-Ortiz et al. 2003; Ulker and Somssich 2004; Zheng et al. 2009; Rushton et al. 2010; Sharoni et al. 2011; Figueiredo et al. 2012; Chen et al. 2016). This class of TFs are one of the well-studied proteins whose mode of action, cross regulation in signaling, auto regulation and evolution have been reported (Singh et al. 2002; Shimono et al. 2007; Puranik et al. 2012; Bakshi and Oelmüller 2014; Chen et al. 2016). These TFs play a crucial role in conferring tolerance to various AbS's that includes cold, salinity, drought (Hu et al. 2006; Nakashima et al. 2007; Hu et al. 2008; Zheng et al. 2009), low temperature (Zheng et al. 2009) in NAC; cold, drought, salinity, metal, submergence (Kreps et al. 2002; Mukhopadhyay et al. 2004; Vij and Tyagi 2006; Kilian et al. 2007; Figueiredo et al. 2012; Giri et al. 2013) in ZF-HD; salinity, drought (Sharoni et al. 2010; Hsieh et al. 2013), low temperature (Sharoni et al. 2010), submergence, flooding (Sharoni et al. 2010), osmotic stress (Hsieh et al. 2013) in AP2—EREBP; salinity, drought, heat (Li et al. 2009, 2011), cold (Zou et al. 2010), H_2O_2 (Song et al. 2009),ozone oxidative stress, UV radiation (Jiang and Deyholos 2009), sugar starvation (Song et al. 2010), phosphate deprivation (Chen et al. 2009) in WRKY; cold, drought (Shinozaki et al. 2003) in bHLH TF families.

Expression of NAC, ZF-HD, AP2-EREBP, WRKY, bHLH TFs in response to diverse AbS decodes their putative involvement in the regulation of signaling mechanisms associated with transcriptional reprogramming during physiological stress. In silico identification of NAC, ZF-HD, AP2-EREBP, WRKY, bHLH TFs has been analyzed in various crop plants and their expression profiling in response to many AbS's have been well studied. To the best of our knowledge, so far no such study has been reported in rice which is a panicoid C3 model crop with potential tolerance to diverse AbS.

The current study, 144 NAC, 15 ZF-HD, 164 AP2-EREBP, 103 WRKY, 135 bHLH from *O. sativa* TF family members were identified (Yilmaz et al. 2009). According to the heat map data, well expressed 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH potential abiotic stress responsive (AbSR) TF genes were retrieved and subjected to ROAD. These AbSR TFs genes were chosen for expression profiling under AbS. Publically available microarray hybridization of ROAD expression values showed tissue-specific expression patterns in the 27 tissues of all the identified (17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH) potential AbSR TF genes. High level expression of these TFs expressed genes from multiple tissues and their expression during the individual AbS's, delineates their multiple roles in diverse biological process

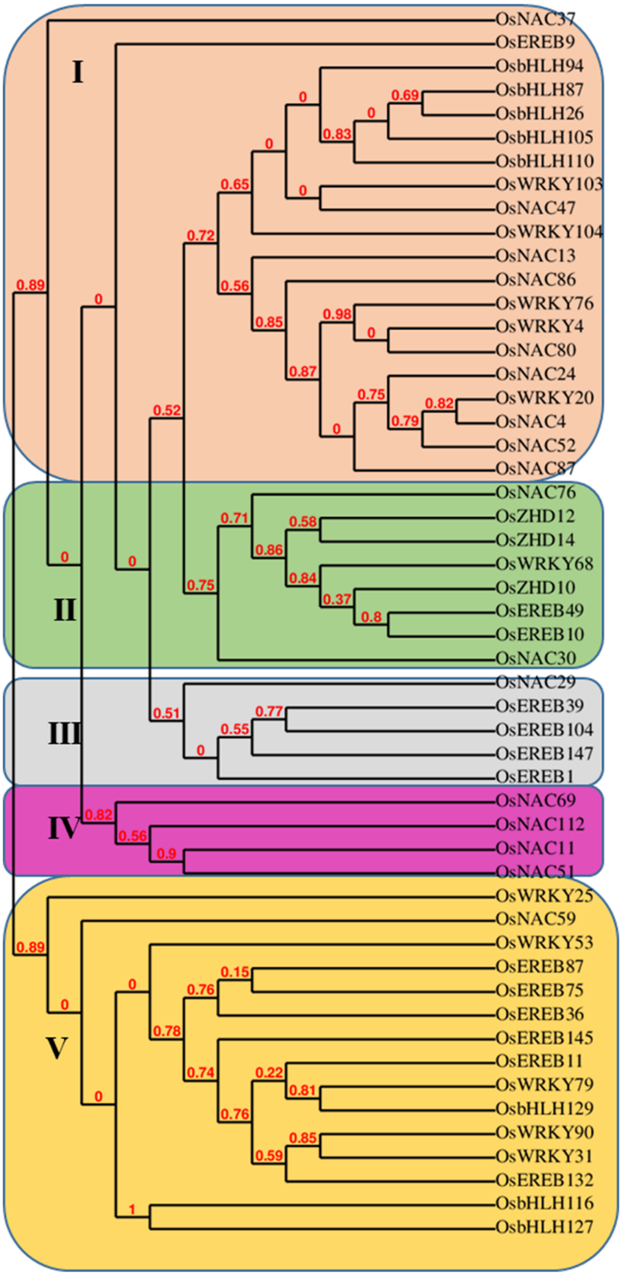

Fig. 5 Unrooted maximum likelihood tree constructed with NAC, ZF-HD, AP2-EREBP, WRKY, and bHLH proteins of rice. Groups are differentiated with different colors (color figure online)

and molecular crosstalks. This data could be further exploited for selecting key genes showing distinct expression pattern for explaining their functional roles. It highlighted the genome-wide analysis of genes expressed in different tissues. We outlined information on co-regulation among TF genes under abiotic stress conditions (Cao et al. 2012; Muthuramalingam et al. 2017). Identified each AbSR TF genes involved in the molecular crosstalks of the plant stress response was predicted. Further, this heatmap data could pave the way for conducting over-expression studies of key genes in different plant tissues in order to develop the AbSR protein content in rice.

Phylogenetic analysis of the potentially expressed TFs genes in rice showed the genes are present in the same subclades/subgroups. In addition, a joined evolutionary tree was constructed from expressed rice 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH proteins involved in diverse aspects of plant growth, development and involved in multiple abiotic stress tolerance mechanisms. The potent TF genes with the same functions revealed a tendency to cluster into one subgroup, which provided an vital resource to explore the functions of the TF genes. It implied that these potent TF genes may be involved in the responses to unique and combined AbS, and this hypothesis was also supported using computational tissues specific expression and gene ontology annotation.

These AbSR TFs encoding proteins have huge variations in length of amino acid, isoelectric point, molecular weight, aliphatic index, instability index and GRAVY values of these proteins. Additionally, subcellular localization of these proteins at independent organelles can be attributed to the presence of putative novel variants, which are prerequisite for further validation.

Emerging advancement of ultra-high- throughput omics tools and approaches, including molecular physiology and computational strategies, the pivotal role of NAC, ZF-HD, AP2-EREBP, WRKY, bHLH TFs in gene regulation and signal transduction mechanisms has been studied well in all major crops and tree species. Though, no such reports on these TFs have been conducted in *O. sativa*, considered as model systems for scrutinizing C3 photosynthesis and AbS tolerance mechanisms. Considering the significance of this crop and NAC, ZF-HD, AP2-EREBP, WRKY, bHLH TFs, the current study used a wide range of computational approaches to categorize and characterize these TF gene family members. The identified TF genes were used for gene ontology annotation, gene structure prediction, physicochemical properties and subcellular localization prediction analysis. In toting, in silico expression profiling of 17 NAC, 3 ZF-HD, 13 AP2-EREBP, 11 WRKY, 8 bHLH genes were used to understand the different tissues specific differential expression profiling. As a result

Table 4 Subcellular localization of potential TF genes

S. no	Gene name	Gene ID	Bacello	WolfPsort	ESL Pred	CELLO2GO
NAC family						
1	OsNAC4	LOC_Os01g15640	N	Cyto	N	Cyto
2	OsNAC11	LOC_Os01g66120	N	N	N	Ct
3	OsNAC13	LOC_Os01g70110	N	N	Ct	N
4	OsNAC24	LOC_Os02g57650	N	Ct	Ct	N
5	OsNAC29	LOC_Os03g12120	N	N	N	N
6	OsNAC30	LOC_Os03g21030	N	N	N	N
7	OsNAC37	LOC_Os03g60080	N	N	N	Cyto
8	OsNAC47	LOC_Os05g10620	N	N	N	N
9	OsNAC51	LOC_Os05g34830	N	N	Ct	N
10	OsNAC52	LOC_Os05g35170	N	N	N	Cyto
11	OsNAC59	LOC_Os06g15690	N	N	Mt	Cyto
12	OsNAC69	LOC_Os07g12340	N	N	Ct	Cyto
13	OsNAC76	LOC_Os07g48550	N	Cyto	N	Cyto
14	OsNAC80	LOC_Os08g06140	N	N	N	N
15	OsNAC86	LOC_Os08g42400	N	Ct	Ct	Ct
16	OsNAC87	LOC_Os08g44820	N	Ct	Ct	N
17	OsNAC112	LOC_Os11g08210	N	N	N	Ct
ZF-HD family						
1	OsZHD10	LOC_Os09g24820	Seceretory	N	N	N
2	OsZHD12	LOC_Os11g03420	N	Ct	ct	N
3	OsZHD14	LOC_Os12g03110	N	Ct	Ct	N
AP2-EREBP family						
1	OsEREB1	LOC_Os01g04020	N	Ct	Ct	Ct
2	OsEREB9	LOC_Os01g49830	N	N	Ct	Ct
3	OsEREB10	LOC_Os01g54890	Ct	Ct	Ct	Ct
4	OsEREB11	LOC_Os01g58420	Secretory	Ct	Ct	Ct
5	OsEREB36	LOC_Os02g51670	N	N	Ct	N
6	OsEREB39	LOC_Os02g54160	N	N	Ct	Ct
7	OsEREB49	LOC_Os03g08500	N	N	Ct	Cyto
8	OsEREB75	LOC_Os04g52090	Ct	Cyto	Ct	PM
9	OsEREB87	LOC_Os05g29810	N	N	Ct	N
10	OsEREB104	LOC_Os06g09390	N	N	Ct	N
11	OsEREB132	LOC_Os08g31580	N	N	Ct	N
12	OsEREB145	LOC_Os09g20350	N	N	Cyto	N
13	OsEREB147	LOC_Os09g26420	N	Ct	Ct	Ct
WRKY family						
1	OsWRKY4	LOC_Os01g14440	N	N	N	N
2	OsWRKY20	LOC_Os01g60640	N	N	Ct	EC
3	OsWRKY25	LOC_Os02g08440	N	N	Ct	N
4	OsWRKY31	LOC_Os03g20550	N	N	N	N
5	OsWRKY53	LOC_Os05g27730	N	N	N	N
6	OsWRKY68	LOC_Os06g44010	N	N	N	N
7	OsWRKY76	LOC_Os08g09900	N	N	N	N
8	OsWRKY79	LOC_Os08g29660	N	N	Cyto	EC
9	OsWRKY90	LOC_Os11g02480	N	N	N	N
10	OsWRKY103	LOC_Os12g32250	N	N	Ct	N
11	OsWRKY104	LOC_Os12g40570	N	N	N	N
bHLH family						
1	OsbHLH26	LOC_Os02g02480	N	Cyto	N	Cyto

Table 4 (continued)

S. no	Gene name	Gene ID	Bacello	WolfPsort	ESL Pred	CELLO2GO
2	OsbHLH87	LOC_Os05g38140	N	N	N	N
3	OsbHLH94	LOC_Os06g09370	N	N	N	N
4	OsbHLH105	LOC_Os07g35870	N	N	N	N
5	OsbHLH110	LOC_Os08g04390	N	N	EC	N
6	OsbHLH116	LOC_Os08g38210	N	N	EC	N
7	OsbHLH127	LOC_Os09g29930	N	N	EC	N
8	OsbHLH129	LOC_Os09g32510	Secretory	N	EC	N

N: nucleus; Ct: chloroplast; Mt: mitochondria; EC: extracellular membrane, Cyto: cytoplasm; PM: plasma membrane

proving an important indication of their controlling and regulatory functions in AbS conditions.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

References

Albrecht C, Russinova E, Hecht V, Baaijens E, de Vries S (2005) The Arabidopsis thaliana somatic embryogenesis receptor like kinases1 and 2 control male sporogenesis. Plant Cell 17:3337–3349

Bakshi M, Oelmüller R (2014) WRKY transcription factors: Jack of many trades in plants. Plant Signal. Behav 9:e27700

CaiY Chen X, Xie K, Xing Q, WuY Li J, Du C, Sun Z, Guo Z (2014) Dlf1, a WRKY transcription factor, is involved in the control of flowering time and plant height in rice. PLoS One 9(7):e102529

Cao Y, Song F, Goodmand RM, Zheng Z (2006) Molecular characterization of four rice genes encoding ethylene-responsive transcriptional factors and their expressions in response to biotic and abiotic stress. J Plant Physiol 163:1167–1178

Cao P, Jung KH, Choi D, Hwang D, Zhu J, Ronald PC (2012) The rice oligonucleotide array database: an atlas of rice gene expression. Rice. 5:17

Chen YF, Li L, Xu Q, Kong YH, Wang H, Wu WH (2009) The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in Arabidopsis. Plant Cell 21:3554–3566

Chen L, Han J, Deng X, Tan S, Li L, Li L, Zhou J, Peng H, Yang G, He G, Zhang W (2016) Expansion and stress responses of AP2/EREBP superfamily in *Brachypodium Distachyon*. Scientific reports 6:21623

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36:W465–W469

Deslandes L, Olivier J, Theulieres F, Hirsch J, Feng DX, Bittner-Eddy P, Beynon J, Marco Y (2002) Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes. Proc Natl Acad Sci USA 99:2404–2409

Ding ZJ, Yan JY, Li GX, Wu ZC, Zhang SQ, Zheng SJ (2014) WRKY41 controls Arabidopsis seed dormancy via direct regulation Of ABI3 transcript levels not downstream of ABA. Plant J 79:810–823

Du L, Chen Z (2000) Identification of genes encoding receptor-like protein kinases as possible targets of pathogen-and salicylic acid- induced WRKY DNA-binding proteins in Arabidopsis. Plant J 24:837–847

Feller A, Machemer K, Braun EL, Grotewold E (2011) Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. Plant J 66:94–116

Figueiredo DD, Barros PM, Cordeiro AM, Serra TS, Lourenc T, Chander S, Oliveira MM, Saibo NJ (2012) Seven zinc-finger transcription factors are novel regulators of the stress responsive gene OsDREB1B. J Exp Bot 63(10):3643–3656

Garg A, Raghava GP (2008) ESLpred2: improved method for predicting subcellular localization of eukaryotic proteins. BMC Bioinform 9:503

Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A (2005) Protein identification and analysis tools on the ExPASy server. In: Walker JM (ed) The proteomics protocols handbook. Springer, NewYork, pp 571–607

Giri J, Dansana PK, Kothari KS, Sharma G, Vij S, Tyagi AK (2013) SAPs as novel regulators of abiotic stress response in plants. BioEssays 35:639–648

Golldack D, Lu'king I, Yang O (2011) Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network. Plant Cell Rep 30:1383–1391

Gong W, Shen YP, Ma LG, Pan Y, Du YL, Wang DH, Yang JY, Hu LD, Liu XF, Dong CX, Ma L, Chen YH, Yang XY, Gao Y, Zhu D, Tan X, Mu JY, Zhang DB, Liu YL, DineshKumar SP, Li Y, Wang XP, Gu HY, Qu LJ, Bai SN, Lu YT, Li JY, Zhao JD, Zuo J, Huang H, Deng XW, Zhu YX (2004) Genome-wide ORFeome cloning and analysis of Arabidopsis transcription factor genes. Plant Physiol 135:773–782

GuanY Meng X, Khanna R, LaMontagne E, Liu Y, Zhang S (2014) Phosphorylation of a WRKY transcription factor by MAPKs is required for pollen development and function in Arabidopsis. PLoS Genet 10:e1004384

Guo Y, Gan S (2006) AtNAP, a NAC family transcription factor, has an important role in leaf senescence. Plant J 46:601–612

Gutha LR, Reddy AR (2008) Rice DREB1B promoter shows distinct stress-specific responses, and the overexpression of cDNA in tobacco confers improved abiotic and biotic stress tolerance. Plant Mol Biol 68:533–555

Gutterson N, Reuber TL (2004) Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr Opin Plant Biol 7:465–471

Hadiarto T, Tran LS (2011) Progress studies of drought-responsive genes in rice. Plant Cell Rep 30:297–310

Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. Nature 460:1026–1030

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J. 61:1041–1052

Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, Nakai K (2007) WoLF PSORT: protein localization predictor. Nucl Acids Res 35:W585–W587

Hsieh EJ, Cheng MC, Lin TP (2013) Functional characterization of an abiotic stress-inducible transcription factor AtERF53 in *Arabidopsis thaliana*. Plant Mol Biol 82:223–237

Hu H, Dai M, Yao J, Xiao B, Xianghua L, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci USA 103:12987–12992

Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L (2008) Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. Plant Mol Biol 67:169–181

Hu Y, Jiang L, Wang F, Yu D (2013) Jasmonate regulates the inducer of cbf expression-C-repeat binding factor/DRE binding factor1 cascade and freezing tolerance in Arabidopsis. Plant Cell 25:2907–2924

Hu B, Jin J, Guo A-Y, Zhang H, Luo J, Gao G (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31(8):1296–1297

Hua S, Sun Z (2001) Support vector machine approach for protein subcellular localization prediction. Bioinformatics 17:721–728

Huh SU, Choi LM, Lee GJ, Kim YJ, Paek KH (2012) *Capsicum annuum* WRKY transcription factor (CaWRKYd) regulates hypersensitive response and defense response upon Tobacco mosaic virus infection. Plant Sci 197:50–58

Jiang Y, Deyholos MK (2009) Functional characterization of Arabidopsis NaCl-inducible WRKY25 and WRKY33 transcription factors in abiotic stresses. Plant Mol Biol 69:91–105

Jin J, Zhang H, Kong L, Gao G, Luo J (2014) PlantTFDB3.0: a portal for the functional and evolutionary study of plant transcription factors. Nucl Acids Res 42:D1182–D1187

Johnson CS, Kolevski B, Smyth DR (2002) Transparent testa glabra2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. Plant Cell 14:1359–1375

Kilian J, Whitehead D, Horak J, Wanke D, Weinl S, Batistic O, D'Angelo C, Bornberg-Bauer E, Kudla J, Harter K (2007) The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. Plant J 50:347–363

Kim KC, Lai Z, Fan B, Chen Z (2008) Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. Plant Cell 20:2357–2371

Kiribuchi K, Sugimori M, Takeda M, Otani T, Okada K, Onodera H, Ugaki M, Tanaka Y, Tomiyama-Akimoto C, Yamaguchi T, Minami E, Shibuya N, Omori T, Nishiyama M, Nojiri H, Yamane H (2004) RERJ1, a jasmonic acid-responsive gene from rice, encodes a basic helixloop—helix protein. Biochem Biophys Res Commun 325:857–863

Kiribuchi K, Jikumaru Y, Kaku H, Minami E, Hasegawa M, Kodama O, Seto H, Okada K, Nojiri H, Yamane H (2005) Involvement of the basic helix-loop-helix transcription factor RERJ1 in wounding and drought stress responses in rice plants. Biosci Biotechnol Biochem 69:1042–1044

Kreps JA, Wu Y, Chang HS, Zhu T, Wang X, Harper JF (2002) Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol 130:2129–2141

Kume S, Kobayashi F, Ishibashi M, Ohno R, Nakamura C, Takumi S (2005) Differential and coordinated expression of Cbf and Cor/Lea genes during long-term cold acclimation in two wheat cultivars showing distinct levels of freezing tolerance. Genes Genet Syst 80:185–197

Lata C, Muthamilarasan M, Prasad M (2015) Drought stress responses and signal transduction in plants. In: Pandey GK (ed) Elucidation of abiotic stress signaling in plants. Springer, New York, pp 195–225

Ledent V, Vervoort M (2001) The basic helix-loop-helix protein family: comparative genomics and phylogenetic analysis. Genome Res 11(5):754–770

Li S, Fu Q, Huang W, Yu D (2009) Functional analysis of an Arabidopsis transcription factor WRKY25 in heat stress. Plant Cell Rep 28:683–693

Li S, Fu Q, Chen L, Huang W, Yu D (2011) Arabidopsis thaliana WRKY25, WRKY26, and WRKY33 coordinate induction of plant thermotolerance. Planta 233:1237–1252

Luo M, Dennis ES, Berger F, Peacock WJ, Chaudhury A (2005) Miniseed3 (MINI3), a WRKY family gene, and HAIKU2 (IKU2), a leucine- rich repeat (LRR) Kinase gene, are regulators of seed size in Arabidopsis. Proc Natl Acad Sci USA 102:17531–17536

Ma D, Pu G, Lei C, Ma L, Wang H, GuoY Chen J, Du Z, Wang H, Li G, Ye H, Liu B (2009) Isolation and characterization of AaWRKY1, an *Artemisia annua* transcription factor that regulates the amorpha- 4,11-diene synthase gene, a key gene of artemisin in biosynthesis. Plant Cell Physiol 50:2146–2161

Marchive C, Mzid R, Deluc L, Barrieu F, Pirrello J, Gauthier A, Corio-Costet MF, Regad F, Cailleteau B, Hamdi S, Lauvergeat V (2007) Isolation and characterization of a *Vitis vinifera* transcription factor, *VvWRKY1*, and its effect on responses to fungal pathogens in transgenic tobacco plants. J Exp Bot 58:1999–2010

MiaoY Laun T, Zimmermann P, Zentgraf U (2004) Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis. Plant Mol Biol 55:853–867

Mickelbart MV, Hasegaw PM, Bailey-Serres J (2015) Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat Rev Genet 16:237–251

Mittler R, Kim Y, Song LH, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Stevenson B, Zhu JK (2006) Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to ablotic stress. FEBS Lett 580:6537–6542

Mukhopadhyay A, Vij S, Tyagi AK (2004) Overexpression of a zinc finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proc Natl Acad Sci USA 101:6309–6314

Muthamilarasan M, Prasad M (2013) Plant innate immunity: an updated insight into defense mechanism. J Bio Sci 38:433–449

Muthuramalingam P, Krishnan SR, Pothiraj R, Ramesh M (2017) Global transcriptome analysis of combined abiotic stress signaling genes unravels key players in *Oryza sativa* L.: an in silico approach. Front. Plant Sci 8:759

Nakashima K, Tran LS, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress responsive gene expression in rice. Plant J 51:617–630

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. Plant Physiol 149:88–95

Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK (2005) Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol 138:341–351

Pierleoni A, Martelli PL, Fariselli P, Casadio R (2006) BaCelLo: a balanced subcellular localization predictor. Bioinformatics 22:e408–e416

Pnueli L, Hallak-Her E, Rozenberg M, Cohen M, Goloubinoff P, Kaplan A, Mittler R (2002) Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume *Retama raetam*. Plant J. 31:319–330

Priya P, Jain M (2013) RiceSRTFDB: a database of rice transcription factors containing comprehensive expression, cis-regulatory element and mutant information to facilitate gene function analysis. Database bat027

Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: regulation and role in stress tolerance. Trends Plant Sci 17:369–381

Qiu Y, Yu D (2009) Over-expression of the stress-induced OsWRKY45 enhances disease resistance and drought tolerance in Arabidopsis. Env. Exp Bot 65:35–47

Rashotte AM, Goertzen LR (2010) The CRF domain defines cytokinin response factor proteins in plants. BMC Plant Biol 10:74

Ren X, Chen Z, Liu Y, Zhang H, Zhang M, Liu Q, Hong X, Zhu JK, Gong Z (2010) ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in Arabidopsis. Plant J. 63:417–429

Rizhsky L, Liang H, Mittler R (2002) The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol 130:1143–1151

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci 15:247–258

Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, Yamaguchi-Shinozaki K (2006) Dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. Proc Natl Acad Sci USA 103:18822–18827

Sharoni AM, Nuruzzaman M, Satoh K, Shimizu T, Kondoh H, Sasaya T, Choi IR, Omura T, Kikuchi S (2010) Gene structures, classification and expression models of the AP2/EREBP transcription factor family in rice. Plant and cell physiol 52:344–360

Sharoni AM, Nuruzzaman M, Satoh K, Shimizu T, Kondoh H, Sasaya T, Choi IR, Omura T, Kikuchi S (2011) Gene structures, classification and expression models of the AP2/EREBP transcription factor family in rice. Plant Cell Physiol 52(2):344–360

Shigyo M, Ito M (2004) Analysis of gymnosperm two-AP2-domain-containing genes. Dev Genes Evol 214:105–114

Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, Takatsuji H (2007) Rice WRKY45 plays a crucial role in benzothiadiazole inducible BLAST resistance. Plant Cell 19:2064–2076

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6(5):410–417

Singh KB, Foley RC, Oñate-Sánchez L (2002) Transcription factors in plant defense and stress responses. Curr Opin Plant Biol 5(5):430–436

Singh AK, Sopory SK, Wu R, Singla-Pareek SL (2010) Transgenic approaches. In: Pareek A, Sopory SK, Bohnert HJ, Govindjee (eds.) Abiotic stress adaptation in plants: physiological, molecular and genomic foundation, Springer, Berlin, Germany, pp 417–450

Song Y, Jing SJ, Yu DQ (2009) Overexpression of the stress induced OsWRKY08 improves the osmotic stress tolerance in Arabidopsis. Chin Sci Bull 54:4671–4678

Song Y, Chen LG, Zhang LP, Yu DQ (2010) Overexpression of OsWRKY72 gene interferes in the ABA signal and auxin transport pathway of Arabidopsis. J Biosci 35:459–471

Sonnenfeld MJ, Delvecchio C, Sun X (2005) Analysis of the transcriptional activation domain of the Drosophila tango bHLH-PAS transcription factor. Dev Genes Evol 215:221–229

Suttipanta N, Pattanaik S, Kulshrestha M, Patra B, Singh SK, Yuan L (2011) The transcription factor CrWRKY1 positively regulates the terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*. Plant Physiol 157:2081–2093

Tang J, Wang F, Wang Z, Huang Z, Xiong A, Hou X (2013) Characterization and co-expression analysis of WRKY orthologs involved in responses to multiple abiotic stresses in Pak-choi (*Brassicacampestris* ssp. *chinensis*). BMC Plant Biol 13:188

Toledo-Ortiz G, Huq E, Quail PH (2003) The Arabidopsis basic/helixloop- helix transcription factor family. Plant Cell 15:1749–1770

Udvardi MK, Kakar K, Wandrey M, Montanari O, Murray J, Andriankaja A, Zhang JY, Benedito V, Hofer JMI, Chueng F, Town CD (2007) Legume transcription factors: global regulators of plant Development and response to the environment. Plant Physiol 144:538–549

Ulker B, Somssich IE (2004) WRKY transcription factors: from DNA binding towards biological function. Curr Opin Plant Biol 7:491–498

Urano K, Kurihara Y, Seki M, Shinozaki K (2010) 'Omics' analyses of regulatory networks in plant abiotic stress responses. Curr Opin Plant Biol 13:1–7

Vij S, Tyagi AK (2006) Genome-wide analysis of the stress associated protein (SAP) gene family containing A20/AN1 zinc-finger(s) in rice and their phylogenetic relationship with Arabidopsis. Mol Genet Genomics 276:565–575

Wang YJ, Zhang ZG, He XJ, Zhou HL, Wen YX, Dai JX, Zhang JS, Chen SY (2003) A rice transcription factor OsbHLH1 is involved in cold stress response. Theor Appl Genet 107:1402–1409

Wu X, Shiroto Y, Kishitani S, Ito Y, Toriyama K (2009) Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing *OsWRKY11* under the control of HSP101 promoter. Plant Cell Rep 28:21–30

Xia N, Zhang G, Liu XY, Deng L, Cai GL, Zhang Y, Wang XJ, Zhao J, Huang LL, Kang ZS (2010) Characterization of a novel wheat NAC transcription factor gene involved in defense response against stripe rust pathogen infection and abiotic stresses. Mol Biol Rep 37:3703–3712

Xiong Y, Liu T, Tian C, Sun S, Li J, Chen M (2005) Transcription factors in rice: a genome wide comparative analysis between monocots and eudicots. Plant Mol Biol 59:191–203

Xu DQ, Huang J, Guo SQ, Yang X, Bao YM, Tang HJ, Zhang HS (2008) Overexpression of a TFIIIA-type zinc finger protein gene ZFP252 enhances drought and salt tolerance in rice (*Oryza sativa* L.). FEBS Lett 582:1037–1043

Yang S, Vanderbeld B, Wan J, Huang Y (2010) Narrowing down the targets: towards successful genetic engineering of drought-tolerant crops. Mol Plant 3:469–490

Yi K, Wu Z, Zhou J, Du L, Guo L, Wu Y, Wu P (2005) OsPTF1, a novel transcription factor involved in tolerance to phosphate starvation in rice. Plant Physiol 138:2087–2096

Yilmaz A, Nishiyama MY, Fuentes BG, Souza GM, Janies D, Gray J, Grotewold E (2009) GRASSIUS: a platform for comparative regulatory genomics across the grasses. Plant Physiol 149:171–180

Yu Y, Hu R, Wang H, Cao Y, He G, Fu C, Zhou G (2013) MlWRKY12, a novel Miscanthus transcription factor, participates in pith secondary cellwall formation and promotes flowering. Plant Sci 212:1–9

Yu CS, Cheng CW, Su WC, Chang KC, Huang SW, Hwang JK, Lu CH (2014) CELLO2GO: a web server for protein subcellular localization prediction with functional gene ontology annotation. PLoS One 9:e99368

Zhao X, de Palma J, Oane R, Gamuyao R, Luo M, Chaudhury A, Hervé P, Xue Q, Bennett J (2008) OsTDL1A binds to the LRR domain of rice receptor kinase MSP1, and is required to limit sporocyte numbers. Plant J 54:375–387

Zheng Z, Qamar SA, Chen Z, Mengiste T (2006) Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. Plant J 48:592–605

Zheng X, Chen B, Lu G, Han B (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Biochem Biophys Res Commun 379:985–989

Zou CS, Jiang WB, Yu DQ (2010) Male gametophyte-specific WRKY34 transcription factor negatively mediates cold stress tolerance of mature pollen in Arabidopsis. J Exp Bot 14:3901–3914