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In silico Comparative Analysis of Transcriptional Factor Binding Sites in Rice and Arabidopsis Calmodulin Binding Protein 60s Genes

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plants are usually subjected to one or more biotic stresses either alone or together which reduces agricultural output significantly, leading to huge losses. To cope with the various stimuli generated by diverse environmental stresses, plants have evolved with a complex mechanism of signal perception and transduction. Several phytohormones and secondary messengers are the main players in mediating cellular responses to various stimuli. Of the various secondary messengers in eukaryotes, the role of calcium ion (Ca^{2+}) has been most extensively studied. The Ca^{2+} is a well-known universal second messenger in plants regulating the responses of growth and development as well as different environmental stimuli in the plant. Changing cytosolic-free calcium concentration ($[Ca^{2+}]$cyt) is one of the earliest responses to biotic stresses. These changes in cellular Ca^{2+} level are being mediated by different Ca^{2+} binding proteins like calmodulin (CaM). CaM interacts with calmodulin binding protein (CBP) and activates downstream defence response. Among the several CBPs, CBP60 family of proteins is found to be involved in several environmental stresses in Arabidopsis. However, no rice *CBP60* (*OsCBP60*) has been identified in relation to pathogen infection. In this study, we have identified 15 *OsCBP60* genes using bioinformatics studies. The transcription of a gene is mainly regulated by the presence of transcriptional binding sites (BSs) that are specifically bound by regulatory proteins called transcription factors (TFs). *In silico* analysis using promoter scanning software is being widely used for identification of TFBSs. We carried out an *in silico* search for identification of TFBSs in *CBP60s* using Plant Promoter Analysis Navigator

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(*PlantPAN; http://PlantPAN2.itps.ncku.edu.tw*). Several TFBSs including WRKY, MYB, bZIP, bHLH, EIN3, CG-1, GATA, NAC/NAM were identified in our analysis. These TFs are responsible for modulation of several biotic stress-responsive genes.

Keywords: Rice CaM-binding proteins (OsCBP60s); transcription factor (TF); transcription factor binding protein (TFBSs); WRKY; MYB.

1. INTRODUCTION

Plants use complex recognition and response mechanisms to cope up with the invading pathogens. The successful resistance of plants against pathogens depends upon timely recognition of the invading pathogens and rapid activation of defence responses via a number of signal transduction pathways. Ca^{2+} is an important secondary messenger which involved in mediating the action of diverse signals including plant hormones, light, biotic and abiotic stresses and also symbiotic elicitors [1]. Changing cytosolic-free calcium concentration ($[Ca^{2+}]$cyt) is one of the earliest responses to biotic and abiotic stresses [2]. These changes in cellular Ca^{2+} level is being mediated by different Ca^{2+} binding proteins like calmodulin (CaM) and CaM-related proteins (CML), calcium-dependent protein kinases (CDPKs) and calcineurin-B-like proteins (CBL) by binding to EF-hand domain [3]; [4]. However, CaM, CML and CBL don't have any enzymatic activity of its own. Therefore, to further transmit Ca^{2+} signals, they interact with calmodulin binding proteins (CBPs) and regulate their gene expression [5]. Several families of CBPs have been identified and characterized in Arabidopsis genome. Among them Calmodulin-binding protein 60s (CBP60s) family of proteins are unique as it contains only CaM binding domain [6]. There are few reports which show the involvement of *AtCBP60* gene family in disease resistance [7]. Similarly, a large number of CaM and CaM-like (CML) proteins have been identified in rice genome [8,9]. However, there is no any published report of rice CBP60s (OsCBP60s). A plethora of literature are available regarding *in-silico* candidate-gene identification in rice using Arabidopsis gene/protein sequences as bait which relies on homology search [10,8]. Keeping in view that some members of *AtCBP60s* involved in disease resistance, we made an attempt to identify the rice homologue of CBP60s using *in silico* analysis. We have identified 15 *OsCBP60s* in rice (Unpublished data). *In silico* analysis using promoter scanning software is being widely used for identification of transcription factor binding sites (TFBSs). In the present investigation, an *in silico* search was performed for the identification of TFBSs in *CBP60s* using Plant Promoter Analysis Navigator (*PlantPAN; http://PlantPAN2.itps.ncku.edu.tw*).

2. MATERIALS AND METHODS

The promoter sequences of all the *CBP60s* of *A. thaliana* and *O. sativa* were retrieved from the Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/) and Rice Genome Annotation Project (RGAP) (http://rice.plantbiology.msu.edu/), respectively. Upstream sequences (1000bp) of the gene were obtained from (http://rapdb.dna.affrc.go.jp/tools/dump)data base. The promoter analysis was performed using PlantPan2 database: (http://plantpan2.itps.ncku.edu.tw/) tool. The flowchart of all the steps was depicted in Fig. 1.

3. RESULTS

3.1 Identification TFBSs in Arabidopsis and Rice CBP60s

The upstream sequences (1000 bp) of *ACBP60s* and *OsCBP60s* genes were analysed using PlantPan VERSION. 2/. Different types of TFBS were scored in both Arabidopsis and rice *CBP60s* sequences. A total list of *AtCBP60s* and *OsCBP60s* TFBSs was shown in Tables 1 and 2.

3.2 Identification of Stress Responses TFBSs

All the identified TFBSs are analyzed for its role in stress responses using different database searches and previously published reports. Total number of stress-related TFs varied from 50 to 230 in different *CBP60s* genes (Fig. 2). The enriched promoter elements like WRKY (3 to 96) and Myb (2 to 43) were observed in most of the promoter sequences studied (Fig. 3). The highest number of WARKY TFs was observed in the promoter sequences of LOC_Os11g44680. But, no WRKY TFs in LOC_Os03g18960 and

LOC_Os02g08120 were observed. The highest number of Myb/SANT TFs was observed in LOC_Os04g36660. BESI transcription factor found in only in the promoter sequences of LOC_Os03g32160. LEA-5 transcription factor present in all rice *CBP60s* gene promoter sequences except LOC_Os12g36910, LOC_Os04g36660 and LOC_Os02g35470.CG-1transcription factor found in promoter sequence of gene LOC_Os03g56660, LOC_Os11g44680, LOC_Os11g44600, LOC_Os12g36920, LOC_Os12g36940impart role in calcium ion (Ca^{2+}) regulation. In promoter region *AtCBP60s*defense related TFs Myb/SANT;Myb;ARR-B, Myb/SANT; Myb – related, Myb/SANT, WRKY, Dehydrin, C2H2, GATA;tify, AP2;B3;RAV,AP2; ERF, bZIP, NAC;NAM, bHLH, CG-1; CAMATA, BES1, EIN3;EIL, Trihelix present and their numbers varies from 248 to 339 (Table 2, Fig. 4).The enriched promoter elements like WRKY (1 to 57) and Myb (2 to 38) were observed in most of the promoter sequences studied. The highest number of WARKY TFs was observed in the promoter sequences of At5g26920 and lowest in At5g57580 (Table 2). Similarly, highest Myb/SANT studied in At5g62570 and lowest in At4g31000 (Fig. 5). BES1 transcription factor characterized for brassinosteroid hormone regulation fond in At4g25800, At4g31000 and absent in other *AtCBP60s* genes.CG-1; CAMATATFs studied in promoter sequence ofAt4g25800, At4g31000, At5g26920, At5g62570.

Apart from WRKY and Myb, a large number of enriched TFBSs namely TBP, Homodomain-TALE, NF-YB, TCP and α-amylase which has role in developmental process were also found. However, a number of non-enriched TFBSs likeDof;GATA, MAD box;MICK, BES1, E2F, PSaH, MADF, ERF, LEA-5. TCR, Homodomain and Dof;GATA were also found in promoter sequences of *AtCBP60s* and *OsCBP60s* genes. Trihelix and Dehydrin transcription factor absent in *OsCBP60s* genes and LEA-5 absent in *AtCBP60s* genes. The consensus sequences of an important family of defence related TFBSs such as WRKY, bZIP, bHLH, NAC/NAM, CG-1, AP2, AP2; ERF, AP2:B3: RAV, EIN3, GATA, Myb/SANT, C2H2 and their consensus sequence were shown in Table 3.

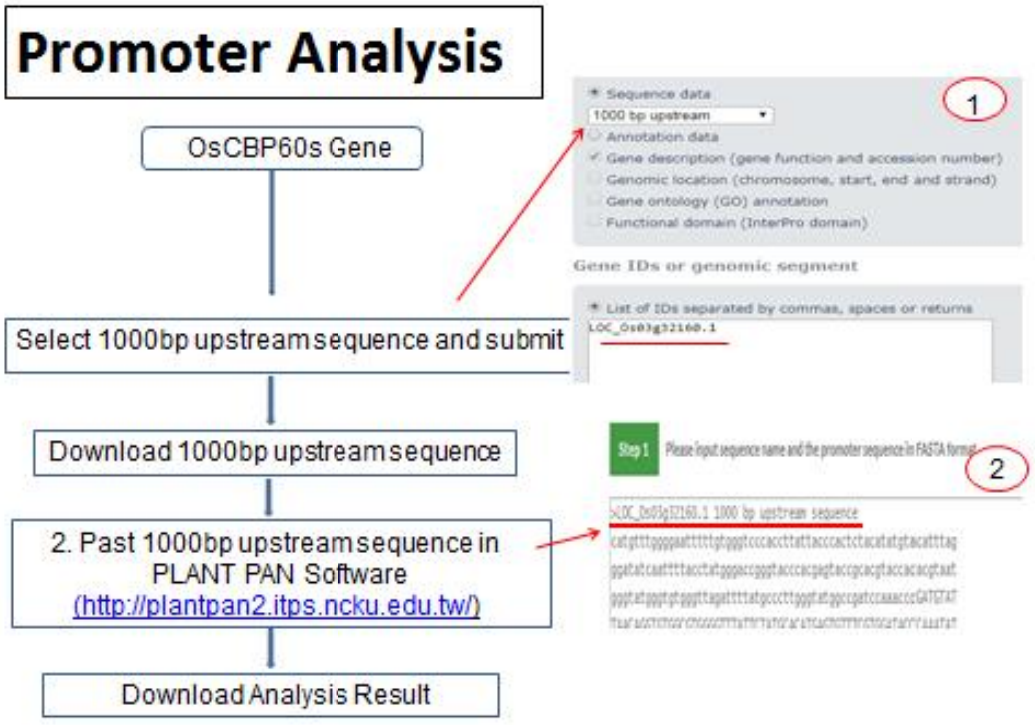

Fig. 1. Promoter analysis by PlantPAN2.0 software. Upstream sequences of 1000bp were used for promoter analysis

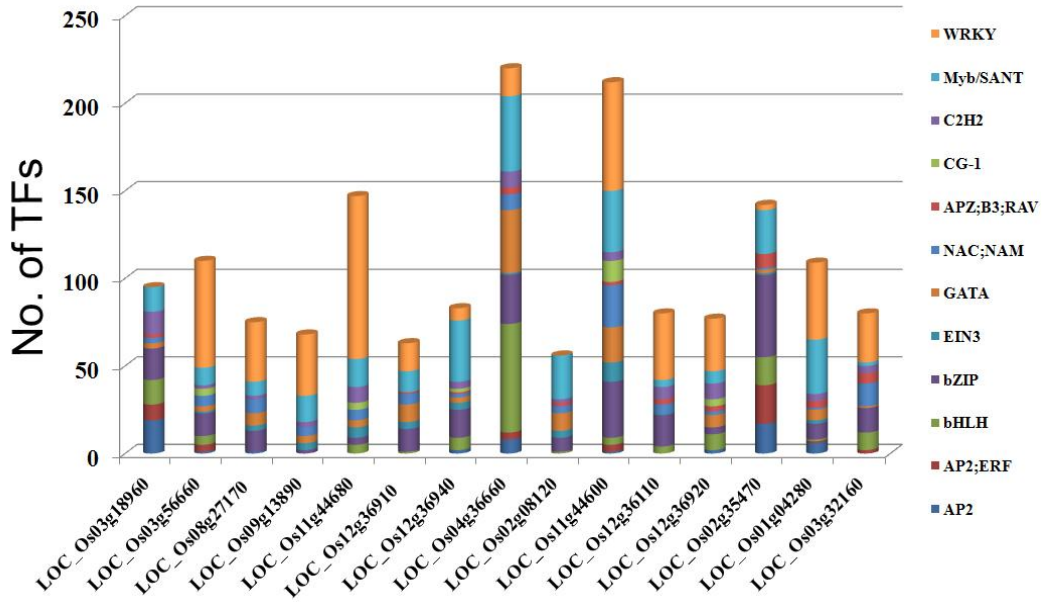

Fig. 2. Putative cis-acting regulatory elements identified in the promoter of *OsCBP60s* genes. Stress responsive transcription factor WRKY, NAC/NAM, bZIP, bHLH, C2H2, Myb/SANT, GATA, EIN3, AP2;ERF, CG-1 impart crucial role in defense signaling in rice*OsCBP60s*genes

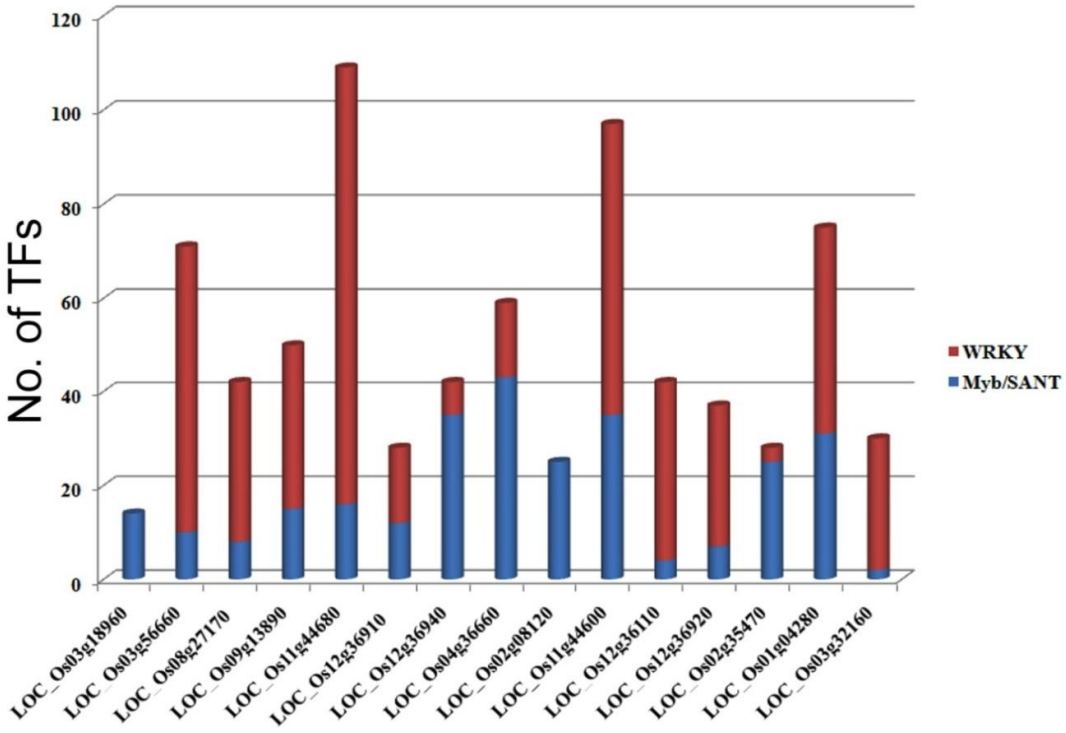

Fig. 3. Putative cis-acting WRKY and Myb/SANT TFs identified in the promoter of *OsCBP60s*. WRKY and Myb/SANT TFs have predominantly role in defense signaling in rice *OsCBP60s* genes

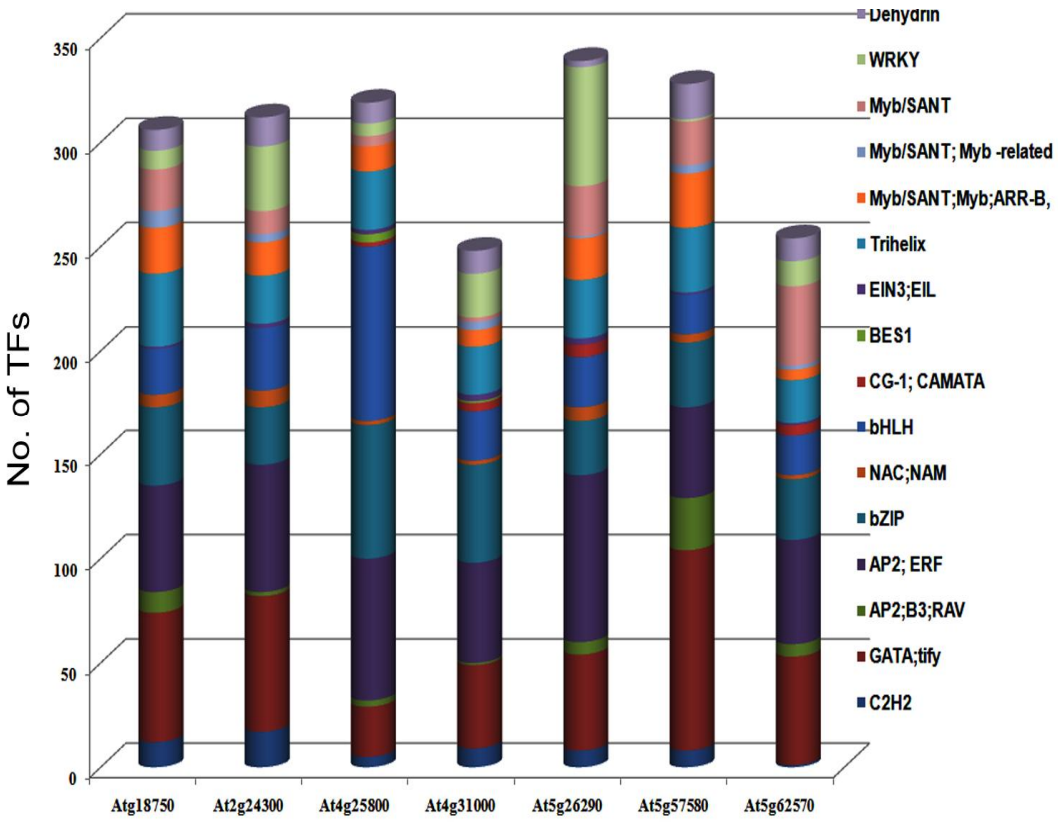

Fig. 4. Putative cis-acting regulatory elements identified in the promoter of *AtCBP60s* genes. Stress responsive transcription factor WRKY, NAC/NAM, bZIP, bHLH, C2H2, Myb/SANT, GATA, EIN3;EIL, AP2;ERF, CG-1; CAMATA, BES1, Dehydrin, Trihelix impart crucial role in defense signaling in Arabidopsis *AtCBP60s*

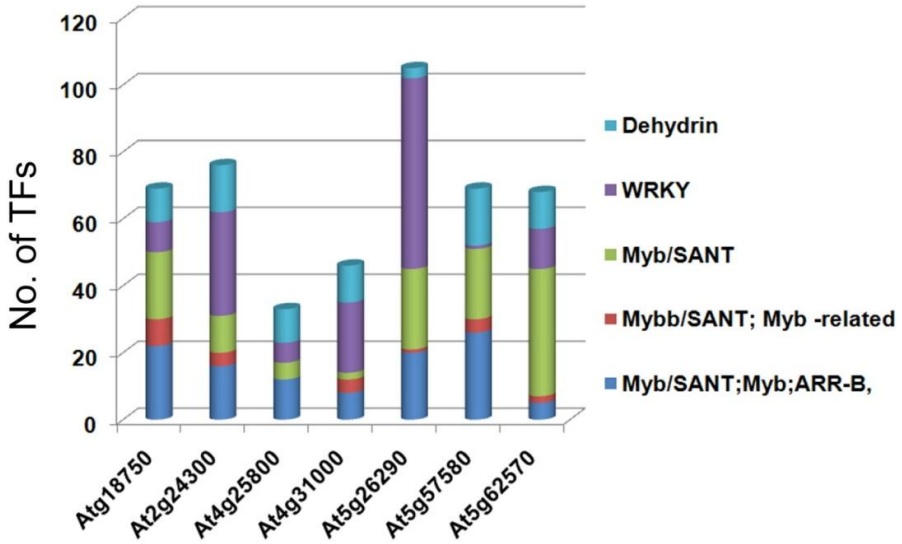

Fig. 5. Putative cis-acting defense related TFs identified in the promoter of *AtCBP60s* genes. WRKY and Myb/SANT TFs have predominantly role in defense signaling in Arabidopsis *AtCBP60s* genes

Table 1. List of defense related TFs in rice OsCBP60s

GENE ID	AP2	AP2; ERF	bHLH	bZIP	EIN3	GATA	NAC; NAM	WRKY	C2H2	Myb/ SANT	LEA-5	CG-1
LOC_Os03g18960	19	9	14	18	0	3	3	0	13	14	2	0
LOC_Os03g56660	1	4	5	13	1	3	6	61	2	10	1	4
LOC_Os08g27170	1	0	0	12	3	7	8	34	2	8	1	0
LOC_Os09g13890	0	0	0	2	4	4	5	35	3	15	1	0
LOC_Os11g44680	0	0	5	4	6	10	6	93	9	16	2	4
LOC_Os12g36910	0	0	1	13	4	3	6	16	0	12	0	0
LOC_Os12g36940	2	0	7	16	4	36	2	7	4	35	2	2
LOC_Os04g36660	8	4	62	28	1	10	9	16	9	43	0	0
LOC_Os02g08120	0	0	1	8	4	20	4	0	2	25	7	0
LOC_Os11g44600	1	0	4	32	11	0	24	62	5	35	5	12
LOC_Os12g36110	2	0	4	18	1	7	5	38	7	4	2	0
LOC_Os12g36920	2	0	9	4	0	2	2	30	9	7	2	4
LOC_Os02g35470	17	22	16	47	1	6	1	3	0	25	0	0
LOC_Os01g04280	6	1	1	9	2	1	1	44	4	31	1	0
LOC_Os03g32160	0	2	10	14	0	1	13	28	4	2	4	0

Table 2. Putative list of cis-acting regulatory elements identified in the promoter of *AtCBP60s* genes

TFs	Atg18750	At2g2430	At4g25800	At4g31000	At5g26920	At5g57580	At5g62570
Myb/SANT;Myb; ARR-B	22	16	12	8	20	26	5
Myb/SANT; Myb -related	8	4	0	4	1	4	2
Myb/SANT	20	11	5	2	24	21	38
WRKY	9	31	6	21	57	1	12
Dehydrin	10	14	10	11	3	17	11
C2H2	12	17	5	9	8	8	1
GATA;tify	62	65	24	40	46	96	52
AP2;B3;RAV	10	2	3	1	6	25	6
AP2; ERF	51	61	68	48	80	44	50
bZIP	38	28	64	47	26	31	29
NAC;NAM	6	8	2	2	7	4	2
bHLH	22	30	84	24	24	19	19
CG-1; CAMATA	0	0	2	4	6	0	5
BES1	0	0	4	1	0	0	0
EIN3;EIL	1	2	2	3	3	1	1
Trihelix	35	3	28	23	28	31	21

Table 3. Defense related transcription factor consensus sequences

Transcription factor	Consensus
AP2	C(C/G)CCGA/C
AP2;ERF	CCCGAC
EIN3	TACAT
CG-1	A/CGCGT
WRKY	T/G(T)G/C(A)C/A
MYB/SANT	NATTC
bZIP	T/C(G/T/A)A/T/G(C/G/T)N
bHLH	G/C(C/A/G)NNNN(C/G/A)G/A
GATA	A/T/GATC/A/G/T
NAC/NAM	A/C/G/T(C/G/T/A)NNNN(A/C/G/T)
AP2;B3;RAV	CANNNNC/G/T(A/T/G)

4. DISCUSSION

Transcription factors (TFs) regulate gene expression through binding to cis-regulatory specific sequences in the promoters of their target genes [11]. The advancement in the determination of TFBSs using *in silico* searches has helped researchers to decipher expression of genes in different conditions including environmental stresses [12,13]. Expression of a large number of defense-related plant genes is regulated at the transcriptional level in response to pathogen infection [14]. Timely transcriptional regulation of defence-related genes is crucial for effective responses to pathogens [15]. In present investigation, the enrichment of WRKY and Myb TFBSs in *OsCBP60s* demonstrate their role in biotic stress. WRKY and Myb family are the most important transcription factors for the regulation of plant defence response pathways in plant [16,17,18,19]. Many WRKY genes are the key factors in controlling plant response to disease resistance especially pathogen infections that can trigger SA-dependent defence signalling [20]. In Arabidopsis, WRKY70 was identified as an important node of SA signalling during plant defense responses [21]. Recent studies on rice have strongly confirmed the importance of WRKY TFs in plant defence signalling [22]. The rice genome contains more than 100 WRKY genes [23,24]. The majority of these genes respond to biotic stresses and various phytohormones [25,24]. The overexpression of *OsWRKY13* enhances resistance to the bacterial blight *Xanthomonas oryzae* pv. *oryzae* (Xoo) and the fungal blast *Magnaportha* oryzae [22]. It exerts its function by activating SA-biosynthesis and SA-response genes while suppressing JA signaling [26,27]. The recent release (TAIR10) of the Arabidopsis genome annotationhas 27,416 protein coding genes (Among them, >2000 proteins (>7% of the total proteome) are identified as putative DNA binding TFs that are classified into 58 families according to their DNA binding domain and other conserved motifs [28] .About half of them belong to plant-specific families [29]. Calmodulin binding transcription activators (CAMTAs; also referred to as signal-responsive proteins or ethylene-induced CaM-binding proteins), screened for CaM binding proteins [30]. CAMTAs are characterized by a CG-1 DNA binding domain at the N terminus, a TIG domain (an immunoglobulin-like fold found in some TFs) involved in nonspecific DNA binding, several ankyrin repeats that are implicated in protein–protein interaction, a Ca^{2+}-dependent CaMbinding domain, and Ca^{2+}-independent CaM binding domains called the IQ motif [31]. Functional stress-responsive genes have been identified Arabidopsisand rice encoding important enzymes and metabolic proteins (functional proteins) such as late embryogenesis abundant (LEA) protein, which directly functions to protect cells from stresses [32]. Members of the LEA genes family have been associated with plant responses to many different stresses including drought, salt, cold, heat, and wounding [33]. Transgenic expression of an LEA protein from barley demonstrated increased tolerance to water and salt stress in rice [34]. The presence of a large number of defence related TFBSs in both *ACBP60s* and *OsCBP60s* indicates their role in disease resistance.

5. CONCLUSION

The study investigate to carried out an *in silico* search for identification of TFBSs in *CBP60s* using Plant Promoter Analysis Navigator. The transcription of a gene is mainly regulated by the presence of transcriptional binding sites (BSs) that are specifically bound by regulatory proteins called transcription factors (TFs). The presence of a large number of defense related TFBSs in both *ACBP60s* and *OsCBP60s* indicates their role in disease resistance. Several TFBSs including WRKY, MYB, bZIP, bHLH, EIN3, CG-1, GATA, NAC/NAM were identified in our analysis. These TFs are responsible for modulation of several biotic stress-responsive genes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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