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In Silico Identification and Gene Expression Analysis of *SNAC* Subgroup of NAC Superfamily Members in *Zea mays*

Ashok Kumar K, Prashanth B, Anjana Priyadarshani K, Kavi Kishor PB, Prashant S*

Department of Genetics& Biotechnology, Osmania University, Hyderabad- 500 007 India

*Corresponding Author Email: prashantsingam@gmail.com

Abstract

Stress-related NAC (NAM, ATAF1-2 and CUC2) genes, known as SNAC sub-family have been identified in four different species such as *Arabidopsis thaliana*, *Oryza sativa*, *Sorghum bicolor* and *Zea mays* using the tools of bioinformatics. These genes have been characterized by finding out their introns, exons, *cis*-regulatory elements, sub-cellular localization, highly conserved motifs, and motif signatures. Phylogenetic tree has been constructed using protein sequences, calculated synonymous to non-synonymous substitution rates (Ka/Ks) of SNAC paralogs and generated 3-dimensional protein models. Predicted SNAC homologs in maize genome have been studied by analysing the synteny of *ZmSNAC* genes with *NAC* genes in *Arabidopsis thaliana*, *Oryza sativa* and *Sorghum bicolor*. miRNA binding sites have been predicted and analysis of *cis*-regulatory elements in the promoter regions of *SNAC* genes in the four plant species has been performed. Identification of SNAC transcription factor homologues in the four plant species and their comparative analysis may provide a basis for further characterization of SNAC transcription factors in various other plant species. Available data have been mined for gene expression analysis under different abiotic stress conditions which displayed up- and down-regulations indicating apparent involvement of *SNAC* genes during abiotic stress responses.

Keywords: *NAC* genes, *SNAC* sub-family genes, Abiotic stresses, *Zea mays*

Introduction

It is inarguable that numerous transcription factors (TFs) portray an indispensable role in improving plant tolerance to abiotic stresses (1,2).TF target genes form a regulon that is involved in the repression/activation of genes associated with biotic and abiotic stress responses. The NAC gene family is derived from three different TFs like no apical meristem (NAM), *Arabidopsis thaliana* activating factor (ATAF1-2), and cup-shaped cotyledon (CUC2). All the three TFs share N-terminal conserved NAC domain (~150 amino acid residues) for DNA-binding nuclear localization with transcriptional regulatory activity (3). The accessibility of many plant genome sequences made it possible to find out the number of NAC homologue genes in diverse plant species (4-9). TFs and *cis*-elements function in the promoter regions of different stress-related genes, and the overexpression of these genes can improve the plant's ability to tolerate abiotic stresses. Stress-related NAC (SNAC) super family TFs have pivotal roles to play in the control of growth, development, biotic and abiotic stress responses. NACs are associated with drought, salinity, and cold stress tolerance in diverse taxa (10-14). The members of NAC gene

family play crucial roles in the regulation of the transcriptional reprogramming associated with plant stress response. Their overexpression in plants has conferred stress tolerance. Single NAC gene often responds to several stress factors, and its protein products participate in the regulation of multiple processes as negative or positive regulators. The NAC proteins function via autoregulation or cross regulation. Overexpression of NACs has conferred stress tolerance in plants (70, 71). Overexpression of *TaNAC69* in transgenic wheat crop improved water stress tolerance and enhanced the expression levels of genes associated with stress tolerance (15). Another wheat NAC transcription factor *TaNAC29* was involved in response to drought, salt and abscisic acid (ABA) treatments (16). While Turnip Crinkle Virus-interacting protein (TIP) NACs have been known to be involved in biotic stresses, and plant development (17), other members of the NAC like NAM/CUC3 are associated with shoot apical meristem formation and abiotic stress responses (18). Stress-related NACs (SNACs) are implicated in virus infection, abiotic stress and cross talk between diverse signalling pathways (18). Many SNAC members from Arabidopsis (ANAC72), rice (TIP - SNAC members), sugarcane (SsNAC23), wheat (TtNAM-B1) and other species have been identified. These members are enmeshed with abiotic stress responses in plants (18-21). Overexpression of rice *SNAC1* gene improved drought and salt stress tolerance in transgenic cotton (22). Overexpression of *SNAC3* in rice showed an enhanced plant tolerance to high temperature and drought, whereas suppression of *SNAC3* by RNAi exhibited increased sensitivity to these stresses (23).

Maize is the third most important food crop after rice and wheat. India ranks sixth in global maize production. It is a staple food in many parts of the world. It is consumed directly by humans, and used as animal feed. Corn starch serves as a source for the production of biofuel. NAC transcription factors have been widely studied in various species such as Arabidopsis (24), rice (21), soybean (25), wheat (10), and also maize (5). However, *SNAC* genes have not been well characterized in *Z. mays*. Present study involves *in silico* characterization and gene expression of *SNAC* genes analysis in *Z. mays* with reference to that of other three plant species as mentioned above.

Material and Methods

Prediction of SNAC genes in Z. mays and other species, gene structure and conserved motif analysis

One of the characterized NAC proteins sequences from *Arabidopsis thaliana* were obtained from Phytozome database. It was used as a query sequence against the protein database of four plant species viz., *Z. mays, O. sativa, A. thaliana* and *S. bicolor* in Phytozome database (26) using BLASTP (Evalue>10) to find ZmSNAC, AtSNAC, OsSNAC and SbSNACs. Genes which show highest identity with AtNAC are selected for analysis. A total of 11 NAC encoding genes have been predicted from *Z. mays* and one gene from other three species. Subsequently, Pfam (http://pfam.xfam.org/) (27) and SMART (http://smart.embl.de/) (28,29) were used to authenticate the existence of the conserved NAM domain in putative NACs. The coding sequences of *SNAC* genes were downloaded from Phytozome database. The CDS of *SNAC* genes of four species were predicted by Gene Structure Display Server (GSDS) for identification of intron/exon distribution in *SNAC* genes. Similarly, all the protein sequences of *SNAC* genes were submitted to MEME online tool for prediction of conserved domains in SNAC proteins. The location of *SNAC* genes on chromosomes were mapped using online phenogram tool.

Sequences analysis, protein-protein interactions, 3D structure prediction and subcellular localization of NAC genes

The phylogenetic tree of ZmSNAC proteins was constructed with NAC proteins of closely related species by MEGA-X using

the Neighbour-Joining (NJ) method with1000 bootstraps (30). Multiple sequence alignment of SNAC proteins was aligned by using ClustalW. Protein parameters such as molecular weight, pI, GRAVY of all the NAC proteins were calculated by Protparam software. Sub-cellular localization of all the *SNAC* genes was predicted by using WOLFSPORT software. The protein-protein interactions of ZmSNAC1 and NAC of all the three species were analysed by STRING database with default parameters and the proteins which show interactions with NAC genes were explained in discussion. The 3D structures of all predicted ZmSNAC sequences along with NAC1 of other three species were modelled by using SWISS-MODEL server and their structures validated by SAVES server. Ramachandran plots of all the predicted SNAC proteins were analysed by PROCHECK server.

Ka/Ks and collinearity analysis of ZmSNAC gene homologs, Cis-regulatory elements, transcription factor analysis and miRNA predictions

Synonymous to non-synonymous substitution rates of *ZmSNAC* gene homologs were calculated by using TB tool, and the time of evolution of NAC genes were tabulated by TB tools software. The collinearity analysis of *ZmSNAC* homologs were analysed with *Arabidopsis*, *S.bicolor*, and *O.sativa* genomes by using MCS scanX and TB tools.The promoter sequences of all SNAC genes (upstream 1500 bp) were collected from Phytozomedata base. These promoter sequences were submitted to PlantCARE to predict *cis*-acting elements present in the promoter regions of SNAC genes. Also, the transcription factors which interact and regulate the expression of SNAC genes were predicted from Plant Transcription factor Database and their interaction network is visualized by Cytoscape. The miRNA binding sites in SNAC genes were predicted using Plant psRNA binding prediction tool.

In silico expression analysis of ZmSNAC genes

The expression data of *Z. mays* was downloaded from maize genetics and genomic database. The FKPM values of identified *ZmSNAC* genes from different parts of maize plant (B73 cultivar) were extracted and under drought, salt, cold, heat and salinity stress conditions also extracted. All the FKPM values were visualized by TB tools software.

Results and Discussion

Identification of SNAC gene homologs in Z. mays and other species

Eleven highly identical *SNAC* gene homologs have been identified in *Z. mays* genome based on *A. thaliana* characterized NAC genes and one each from *Oryza*, *Sorghum* and *Arabidopsis* from Phytozome database. All the above predicted *SNAC* genes were screened for conserved NAM domain. Genes which contain conserved NAM domain were considered as candidate genes for further analysis, and rest were discarded. All the predicted *Z. mays NAC* genes were designated as *ZmSNAC1* to *ZmSNAC11*and remaining species as *AtNAC1*, *OsNAC1* and *SbNAC1*.

Characterization of SNAC genes

Intron/exon distribution of *SNAC* genes were identified in all four species. *ZmSNAC*7,8,9,10 and 11 comprise three exons and two introns, rest of the *ZmSNAC* gene homologs displayed two exons and one intron (Fig.1a). In *AtSNAC*, three exons and two introns, in *OsSNAC* and *SbSNAC* two exons and one intron respectively have been identified (Fig. 1c). All the *ZmSNAC* genes have been mapped on the chromosomes. *ZmSNAC11*showed the longest coding sequence of 1197 bp and *ZmSNAC5*,*6* the shortest coding sequence of 846 bp respectively. *AtNAC1* displayed the coding sequence of 852 bp, *SbNAC1* 960 bp, whil-e*OsNAC1* 951 bp. On the other hand, *ZmSNAC* homologs in the same group displayed equal number of intron and exon distribution (Table:1).

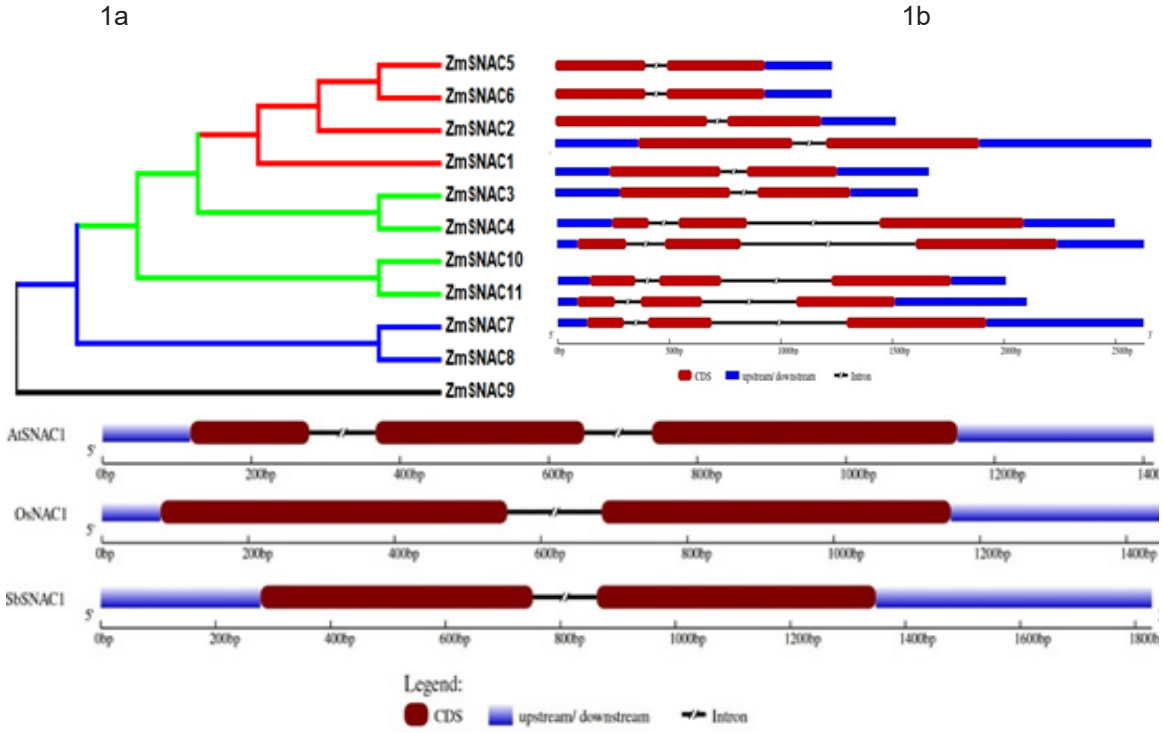


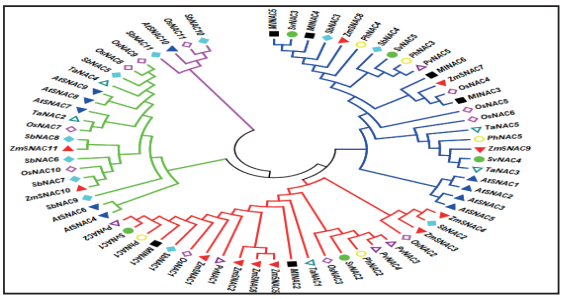
Fig. 1. Phylogenetic analysis(a), intron/exon distribution of *ZmSNAC* genes (b) and Intron/exon distribution of *AtSNAC1*, *OsSNAC1* and *SbSNAC1* genes (c).

Table 1. Characteristics of *SNAC* genes

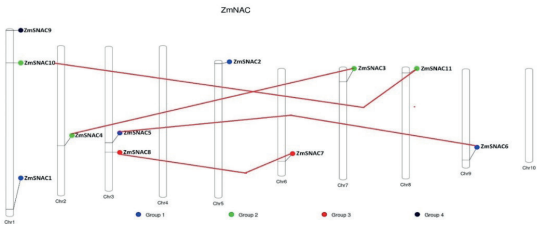
Transcript ID	Gene name	Chromosome	Strand	CDS (bp)	No. ofintrons/ exons
Zm00001d034601_P001	*ZmSNAC1*	1	forward	939	01:02
Zm00001d013003_P001	*ZmSNAC2*	5	reverse	1104	01:02
Zm00001d019207_P001	*ZmSANC3*	7	forward	903	01:02
Zm00001d005208_P001	*ZmSNAC4*	2	reverse	912	01:02
Zm00001d042246_P001	*ZmSNAC5*	3	forward	846	01:02
Zm00001d048044_P001	*ZmSNAC6*	9	reverse	846	01:02
Zm00001d038221_P001	*ZmSNAC7*	6	forward	1020	02:03
Zm00001d042609_P001	*ZmSNAC8*	3	forward	888	02:03
Zm00001d000112_P001	*ZmSNAC9*	1	reverse	1080	02:03
Zm00001d028999_P001	*ZmSNAC10*	1	reverse	1122	02:03
Zm00001d008399_P001	*ZmSNAC11*	8	forward	1197	02:03
Sobic.001G040200	*SbNAC1*	1	reverse	960	01:02
AT5G08790	*AtSNAC1*	5	reverse	852	02:03
LOC_Os03g6080080	*OsNAC1*	3	forward	951	01:02

Phylogenetic analysis, chromosome location and Ka/Ks analysis of ZmSNAC genes

Evolutionary tree of ZmSNAC protein sequences were constructed with SNAC homologs in *Arabidopsis, Oryza, Sorghum, Miscanthus sinensis, Panicum virgatum, Panicum hallii, Triticum aestivum, Setaria viridis* with NJ method, and 1000 bootstrap values using MEGA.7(Fig 2 a). According to evolutionary relationship, NAC genes are divided into four groups (I, II, III, IV). *ZmSNAC* homologs (*ZmSNAC11*, *ZmSNAC10*, *ZmSNAC4*, *SbNAC8*, *SbNAC7*, and *SbNAC2*) showed three ortholog pairs with *S. bicolor* NAC homologs. Based on the evolutionary studies, four *ZmSNAC* paralog pairs (*ZmSNAC5-6*, *ZmSNAC3-4*, *ZmSNAC10-11*and *ZmSNAC7-8*) have been identified. Synonymous to non-synonymous substitution rates (Ka/Ks) of ZmSNAC homologs have been calculated (Table2). While *ZmSNAC11-ZmSNAC10* shows the highest Ka/Ks ratio, *ZmSNAC4-ZmSNAC3* the least Ka/Ks ratio. All the discovered *ZmSNAC* paralog pairs show Ka/Ks <1. Based on this, it has been found that *ZmSNAC* paralog pairs experienced purifying selection during evolution. Evolutionary time of *ZmSNAC* paralog pairs have been predicted which appears few million years. *ZmSNAC* homologs have been mapped on *Zea mays* chromosomes. While chromosome1 consists of *ZmSNAC1*,*9*, *10*, third chromosome localizes*ZmSNAC5* and *8*. *ZmSNAC* homologs distributed on all chromosomes except chromosome 4and 10. Sorghum *SbSNAC1* appears to be localized on chromosome 1, *AtSNAC1* on chromosome 5 and*OsSNAC1* on chromosome 3 (Fig. 2b).



2a



2b

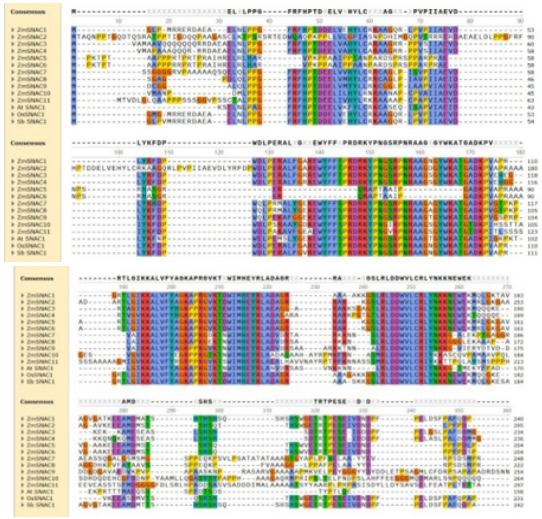
Fig.2. Evolutionary analysis of ZmSNAC orthologs in different species (a) and Physical mapping of *ZmSNAC* homologs (b)

Table 2.Ka/Ks analysis of *ZmSNAC*paralogs

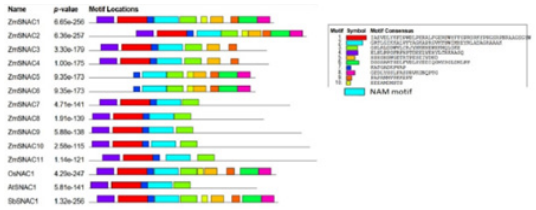
Gene pairs	Ka	Ks	Ka/Ks ratio	T(MYA)
ZmSNAC1-ZmSNAC2	0.1301	0.15525	0.838003221	9.916158537
ZmSNAC6-ZmSNAC5	0.00258081	0.00794802	0.32471106	0.196708079
ZmSNAC9-ZmSNAC7	0.18315	0.33925	0.539867354	13.95960366
ZmSNAC4-ZmSNAC3	0.029552825	0.094005805	0.314372341	2.252501905
ZmSNAC11-ZmSNAC10	0.2337	0.2772	0.843073593	17.8125

Multiple sequence alignment and conserved motif analysis

Multiple sequence analysis of ZmSNAC1 proteins was performed with AtSNAC1, OsNAC1 and SbNAC1 proteins using Clustal W algorithm (Fig. 3a). Highly conserved residues have been identified in all NAC genes, which maybe involve in regulatory functions and constitute conserved domain such as NAM domain. Conserved motifs present in the NAC genes have also predicted by MEME tool with default parameters. All NAC genes investigated in the present study displayed conserved NAM motif. ZmSNAC7 to 11 displayed only 5 motifs out of 10 including conserved NAM motif and the same motif pattern has been observed in AtSNAC1. SbNAC1 and OsNAC1 showed the same number of motifs and similar motif distribution (Fig. 3b).



3a

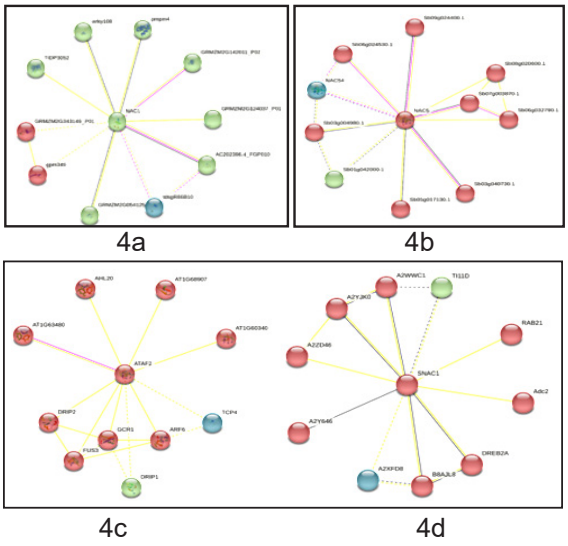


3b

Fig.3. Multiple sequence alignment of ZmSNAC proteins with Arabidopsis, Sorghum and Rice NAC1 proteins respectively (a) and Motif pattern of NAC genes (b)

Protein parameters, sub-cellular localization, protein-protein interaction and 3D structure prediction

In all the predicted ZmSNAC homologs, ZmSNAC11 is the longest protein which consists of 399 amino acids; ZmSNAC5 and ZmSNAC6 are the shortest with 282 amino acids. ZmSNAC11 has the highest molecular weight (42481.38 D), followed by ZmSNAC2 (40706.86 D), and ZmSNAC6 has been found to be the smallest protein with a molecular weight of 30578.82 Daltons. ZmSNAC6 has a pI value of 9.99, and ZmSNAC9 has 5.25. While most of the ZmSNAC homologs have been found localized in the nucleus including AtSNAC1, OsSNAC1 and SbSNAC1, ZmSNAC5 and 7 in the chloroplast, and ZmSNAC8 have been seen in the mitochondria (Table3). The string protein-protein analysis of ZmSNAC1(Fig: 4a) shows 11 nodes with several interacting partners. The interacting partners include putative regulator of chromosome condensation (RCC1) family protein (TIDP3052), putative WRKY DNA-binding domain superfamily protein (GRMZM2G054125_P01), BZIP transcription factor (gpm34), DnaJ heat shock N-terminal domain-containing protein (GRMZM2G343149_P01), peroxidase family protein (GRMZM2G142011_P02), DREB-like protein (GRMZM2G124037_P01), and dehydration-responsive element-binding protein 1B (tdsgR86B10). AtNAC1, OsNAC1, and SbNAC1 have also been analysed by STRING (Fig:4. b, c &d). The 3D structures of all ZmSNAC homologs and SNAC genes from *Arabidopsis*, *Oryza* and *Sorghum* have been predicted using SWISS-MODEL server. All the SNAC genes showed significant similarity with respective templates. Template identities (IDs), identity percentage are tabulated. The 3D structures are validated by SAVES server and Ramachandran plots have been predicted. Most of the amino acids of SNAC proteins are localized in favourable regions of Ramachandran plot.



4a 4b

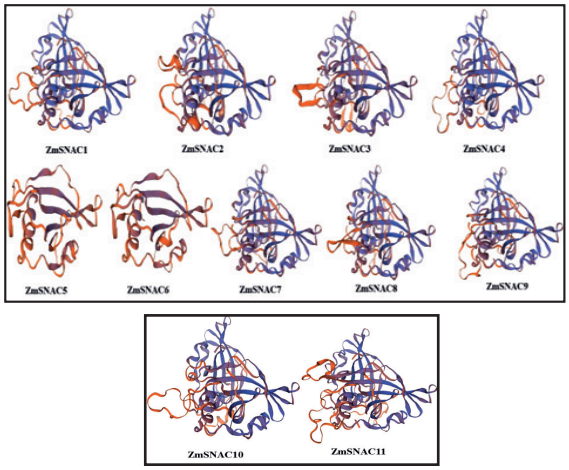
4c 4d

Fig. 4. PPI analysis of ZmSNAC1 (a) AtSNAC1 (b), OsSNAC1 (c) and SbSNAC1 (d)

Table 3. ZmSNAC proteins length, molecular weight, pI, sub-cellular localization and GRAVY

Gene name	Protein length	Mol.wt(Da)	pI	Sub-cellular localization	GRAVY
ZmSNAC1	313	34841.31	6.27	Nucleus	-0.622
ZmSNAC2	368	40706.86	6.63	Nucleus	-0.689
ZmSANC3	301	33628.77	5.31	Nucleus	-0.704
ZmSNAC4	304	33893.14	5.74	Nucleus	-0.691
ZmSNAC5	282	30579.85	9.91	Chloroplast	-0.702
ZmSNAC6	282	30578.82	9.99	Nucleus	-0.723
ZmSNAC7	340	35805.54	9.15	Chloroplast	-0.401
ZmSNAC8	296	32544.2	8.46	Mitochondria	-0.452
ZmSNAC9	360	38842.42	5.25	Nucleus	-0.48
ZmSNAC10	374	40588.62	8.7	Nucleus	-0.573
ZmSNAC11	399	42481.38	6.18	Nucleus	-0.344
SbNAC1	320	35422.92	6.1	Nucleus	-0.604
AtSNAC1	284	32225.23	5.55	Nucleus	-0.686
OsNAC1	317	35177.8	6.62	Nucleus	-0.611

5a



5b

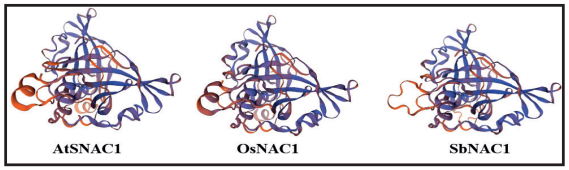
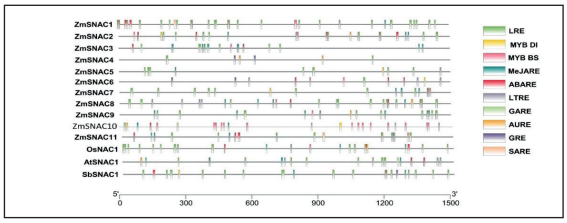


Fig. 5. 3D structures of ZmSNAC (a) and *Arabidopsis*, *Oryza* and *Sorghum* (b)

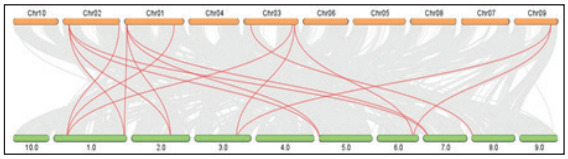
Cis-regulatory elements prediction and collinearity analysis of *SNAC* genes

Cis-elements which are present in the promoter regions of *ZmSNAC*, *AtSNAC*, *OsSNAC* and *SbSNAC* have been analysed using PlantCARE software tool (Fig.6.a). *Cis*-acting elements that interact with NAC genes such as light-responsive, low-temperature-responsive, MYB-drought-inducible and MYB-binding elements have been identified. Further, abscisic acid-responsive, gibberellic acid-responsive, auxin-responsive, salicylic acid-responsive and methyl jasmonic acid-responsive elements have been identified. Collinearity analysis of ZmSNAC genes were performed with *Arabidopsis*, *Sorghum* and *Oryza* genomes using MCScanX and visualized by TB tools (Fig. 6.b, c and d). While maize chromosome 1 shows 2 homologs each on *S. bicolor* chromosomes 2 and 1, chromosome 2 displays one homolog each on sorghum chromosome 1, and 2. Similarly, chromosome 3 shows one homolog each on sorghum chromosome 3 and 9, and chromosome 5 displays two homologs on sorghum

and 5 and chromosome 7 displays 2 homologs each one on rice chromosome 3 and 7. There is no significant similarity of collinearity of maize *SNAC* genes with Arabidopsis genome.



6a



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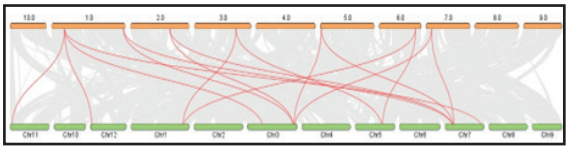
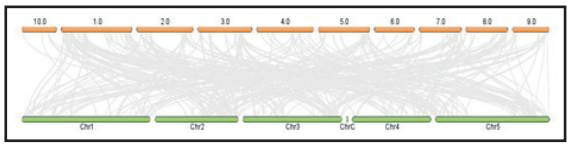


6c

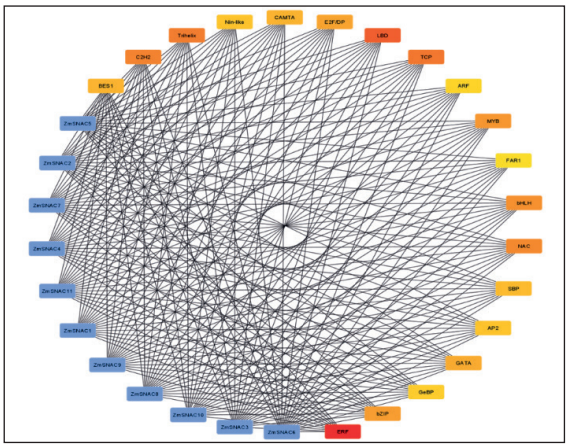


6d

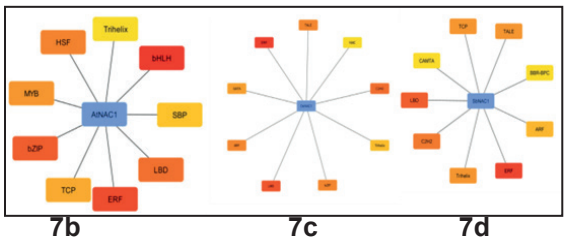
Fig 6: *Cis*-regulatory analysis of SNAC transcription factors (a), Collinearity analysis of *ZmSNAC* homologs with *S. bicolor* genome (b), Collinearity analysis of *ZmSNAC* homologs with *O. sativa* genome(c), Collinearity analysis of *ZmSNAC* homologs with *A. thaliana* genome (d).

Transcription factor prediction and miRNA binding analysis

In the present study, TFs which interact and regulate the expression of *SNAC* genes have been identified in *ZmSNAC (*Fig.7.a*)*, *AtSNAC1*, *SbSNAC1*, and *OsNAC1*genes (Fig.7.b, c &d). Interaction of TFs with C2H2-type zinc fingers, Trihelix, calmodulin binding transcription activators(CAMTA), TFs necessary for the transactivation of the adenoviral E2 promoter and the expression of viral DNA replication proteins(E2F) and dimerization partner (DP) (together known as E2F/DP), LATERAL ORGAN BOUNDARIES DOMAIN (LBD), auxin-response factors (ARF), MYB, SQUAMOSA promoter binding protein (SBP),APETALA2 TFs (AP2), zinc finger proteins that bind the consensus DNA sequence (T/A)GATA(A/G) (GATA), basic leucine zipper (bZIP), ethylene-response factor (ERF) and other NAC transcription factors. The miRNA binding sites have also been predicted. While*ZmSNAC*10 shows two miRNA binding sites, *AtSNAC1* displays 4 miRNA binding sites. All the predicted miRNAs exhibit cleavage inhibition.



7a



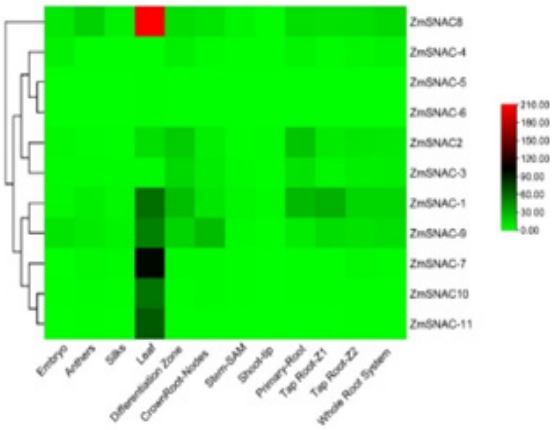
7b 7c 7d

Fig. 7. Transcription factor network of *ZmSNAC* homologs (a) Arabidopsis (b), sorghum (c), and rice (d) SNAC1 homologs

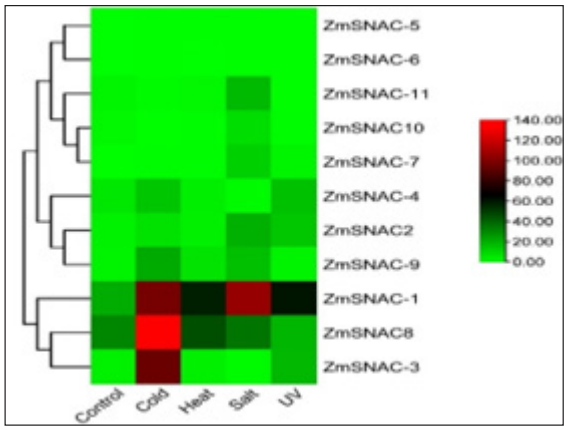
Expression analysis of ZmSNAC genes

To explore the expression patterns of

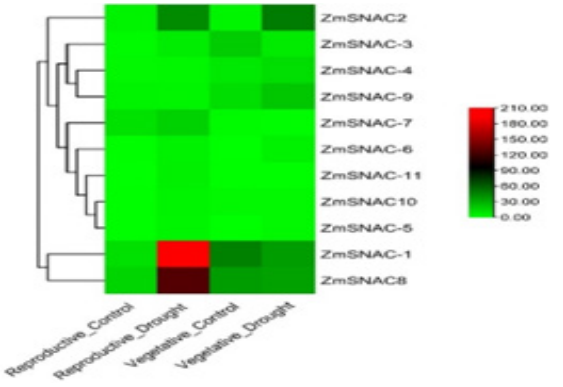
maize *ZmSNAC* genes, maize microarray data that presents a complete atlas of global transcription profiles across diverse developmental stages have been utilized (31). The maize microarray data provides transcription patterns in 12 different tissues representing 8 major organs of inbred lineB73. Data reveal high expression of *ZmSNAC8* in leaf tissues followed by *ZmSNAC7* and *ZmSNAC1*, *11*, *9*,*10*(Fig. 8.a). No significant expression was noticed for *ZmSNAC* genes in the shoot tip. *ZmSNAC5*,*6*,*7*,*10*, and *11* show meagre expression levels in all the tissues except leaf. Rest of the *ZmSNAC* genes exhibit moderate to low expression pattern in all the examined tissues except in shoot-tips. Expression patterns of *ZmSNAC* genes in maize seedlings under cold, heat, salt and ultraviolet light (UV) stress conditions were assessed (Fig. 8.b). Results revealed upregulation of *ZmSNAC1* in cold and salt stress, but moderate to low expression patterns under UV and heat tress conditions respectively. *ZmSNAC8* also displayed enhanced expression under cold stress, but low expression patterns in salt and heat stress conditions. *ZmSNAC3* was up-regulated under cold stress, but no significant expression was noticed in other stress conditions. *ZmSNAC5* and *6* displayed insignificant expression patterns in all the studied stress responses. Remaining *ZmSNAC* genes showed low expression patterns under the stress conditions examined. Expression patterns of all *ZmSNAC* genes were examined at reproductive and vegetative stages of development under drought stress. *ZmSNAC1* gene showed the highest expression levels at reproductive stage under drought conditions followed by *ZmSNAC8*. But these genes showed low expression patterns in vegetative stage under drought conditions (Fig. 8.c). *ZmSNAC2* exhibited moderate expression in both reproductive and vegetative stages under drought stress responses. Rest of the *ZmSNAC* genes displayed no significant similarity at any developmental stage under drought stress conditions. Expression patterns of *ZmSNAC* homologs were examined in the leaf tissues under drought and salt stress conditions after 10-daytreatment (T0) followed by re-watering (T7). Interestingly, expression of *ZmSNAC1* gene was highly upregulated under all stress conditions (Fig. 8.d).



8a

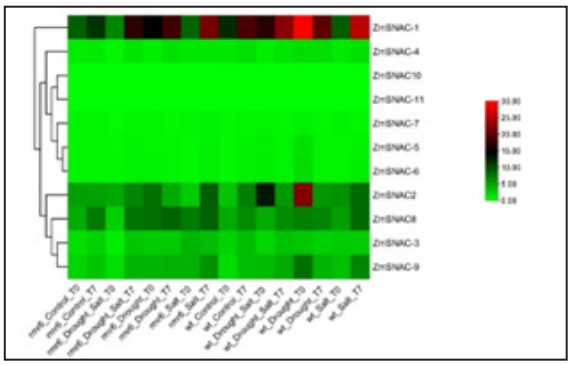


8b



8c

In silico identification and gene expression analysis of SNAC subgroup of NAC superfamily members in Zea mays



8d

Fig. 8. Heat maps showing expression patterns of *ZmSNAC* homologs in various organs of *Z. mays* (a), expression patterns of *ZmSNAC* homologs in seedlings under cold, heat, salt and UV stress conditions and control (b), expression patterns of *ZmSNAC* homologs at reproductive and vegetative stages under drought conditions (c) and expression patterns of *ZmSNAC* homologs under drought and salt stress conditions in leaf tissue of rmr6 cultivar and control samples (d).

Discussion

NAC is a large group of TFs and play central roles in plant developmental processes, diverse abiotic stress responses, signalling pathways, and numerous plant defence mechanisms. NAC TFs have been identified in Arabidopsis (32), *Oryza sativa* (19), *Zea mays* (5), *Musa acuminata* (33), *Solanum lycopersicum* (34), *Asparagus officinalis* (35), and *Medicago sativa* (36). SNAC subgroup is a part of this family, and has not been studied in detail. The identified *ZmSNAC* gene homologs have been divided into four groups (I, II, III, IV), and are closely related to *SbSNAC*, *AtSNAC* and *OsSNAC* genes. SNAC proteins comprise conserved NAM domain, and the conserved motif pattern and number of motifs have been found similar in each group. Phylogenetic analysis of the *SNAC* gene family in maize revealed that the genes in the same subdivisions or subclades generally contained the same intron/exon pattern, with the location of the intron(s) existence fully conserved. NAC proteins undoubtedly act as TFs, and modulate scores of physiological and biochemical functions by interacting with *cis*-elements in the promoter regions (1). Interestingly, NAC/SNAC TFs have been found to regulate senescence, biotic and abiotic stresses and plant hormone homeostasis (37, 38, 39). ABRE is the most frequently found *cis*-regulatory element found in all studied *SNAC* genes. In fact, stress-mediated *cis*-element TF interactions are known in plants (40). These findings propound the involvement of *SNAC* genes in drought and abiotic stress responses (41,42). Presence of auxin, and gibberellic acid-responsive *cis*-elements in the promoter regions of *SNAC* genes suggest that these genes may involve in growth and developmental activities of plants (43). Identification of *cis*-elements related to methyl jasmonic acid and salicylic acid also provides evidence for the implication of *SNAC* genes in plant defence responses. Low temperature-responsive elements found in this study indicate the involvement of *SNAC* genes in cold stress-response (44). Likewise, light-responsive elements noticed in this investigation suggest that *SNAC* genes may be regulated by light and circadian clocks, alongside their involvement in nutrient uptake and utilization (45). The finding of MYB binding and MYB-drought inducibility-responsive elements indicate the role of *SNAC* genes in drought-induced stress responses.

Studies have revealed that segmental duplication was largely responsible for the expansion of maize gene family, such as CCCH, HD-Zip and HS (46,47,48). In this study, the number of *ZmSNAC* genes involved in the segmental duplication is almost the same as that of tandem duplication. It has been described that the maize genome has experienced several rounds of whole genome duplication, including an earliest duplication prior to the divergence of grasses (~50-70 million years), and further whole genome duplication (~5 million years), which discriminate maize from sorghum (49,50). The Ka/Ks analysis of *ZmSNAC* genes

revealed that all of them experienced purifying selection during divergence. Collinearity analysis of *ZmSNAC* gene homologs mapped on *O. sativa*, *S. bicolor* and *A. thaliana* genomes displayed different number of homologs on different genomes. The SNAC protein structures predicted in this study may help to understand their interaction with other protein networks. The protein-protein interactions of *ZmSNAC1*, *SbNAC1*, *AtSNAC1* and *OsNAC1* and their interacting partners have been identified. ZmSNAC1 protein displayed interaction with WRKY108 protein. Zhang et al. (51) reported that WRKY21 and WRKY108 transcription factors in *Oryza* function redundantly to promote Pi uptake by activating *OsPHT1;1* expression under Pi-replete conditions.ZmSNAC1 showed interaction with dehydration-responsive element-binding protein DREB1B. DREBs play crucial regulatory roles in abiotic stress responses in plants (52).*DREB1A* displayed drought and freezing tolerance in transgenic Arabidopsis and chickpea plants alongside yield gain (53,54,55).*ZmSNAC1*has been found interacting with CBF3-like protein; CRT/DRE binding factor. In Arabidopsis, a family of transcription factors that bind to the CRT/DRE promoter region has been identified and designated as CBF or DRE genes (56,57,58). These genes can activate the expression of cold-response (COR) genes and thus are vital components of the cold acclimatization pathway and may function as “Master Switches” that activate a signal transduction pathway leading to improved freezing tolerance (59). Overexpression of Arabidopsis core binding factor1 (CBF1) and CBF3 result in constituent expression of *COR* genes and enhanced freezing tolerance in Arabidopsis (57,60). Similar results have been noticed in protein-protein interaction analysis of AtSNAC1, OsSNAC1 and SbSNAC1 proteins.

C2H2 zinc finger (ZF)sequences (CX2-4CX3FX5LX2HX3-5H) (C2H2 proteins) comprise two cysteines and two histidines that coordinate a zinc atom, forming a dense nucleic acid-binding domain. Majority of such proteins characterized to date are DNA-binding transcription factors, and many have been shown to play crucial roles in the development of plants (61). miRNA have been detected in this study. They are known to play pivotal roles during plant growth, development besides abiotic stress tolerance (62). Such a role for the detected miRNAs in this study cannot be ruled out, but needs further investigations. Expression analysis under abiotic stress conditions indicated a clear role for *SNAC* genes. Further, by attaching the target mRNA, NACs are known to modulate post-transcriptional activities (63). Overexpression of micro-RNA-targeted NAC ameliorated salt stress via ABA-mediated pathway (64). Stress-responsive NAC TFs (SNACs) are involved in plant abiotic stress responses (65). While overexpression of SNAC1 enhanced the water stress tolerance by increasing root development and reducing transpiration rate in transgenic cotton (22). Stress-responsive NAC TF gene ONAC022 improved the drought and salt stress tolerance in rice (66). Such transgenics displayed lower levels of Na^+ in roots and higher concentrations of proline and soluble carbohydrates (66). NAC transcription factor JUNGBRUNNEN1 enhances drought tolerance in *Solanum lycopersicum* (67). Overexpression of ZmNAC33 in *A. thaliana* improved the seed germination percentage under the influence of ABA as well as osmotic stress (68). In *Malus-baccata*, overexpression of MbNAC25 gene in Arabidopsis enhances tolerance to cold and salt stresses (63). Similarly, overexpression of cotton GhNAC072 increased the drought and salt stress tolerance in transgenic Arabidopsis (69). The expression patterns of *ZmSNAC* genes in diverse abiotic conditions (drought, cold, heat, salt and UV stress) in different organs at different stages of development inferred the involvement of SNACs in stress. While *ZmSNAC1*, *ZmSNAC8* displayed the highest expression patterns, *ZmSNAC2* showed moderate expression under the abiotic stress conditions studied. This study helps to understand SNAC gene structure and functions in detail. Further, the study helps to design and develop crop plants with superior abiotic stress tolerance. Breeding plants with NAC TFs help us to develop crops

that are resilient to biotic and abiotic stresses.

Conclusions

In the present study, 11 *ZmSNAC* genes in *Z. mays* genome have been predicted based on *A. thaliana* characterized *SNAC1* sequences. Motif analysis of *SNAC* genes showed the presence NAM domain, which is highly conserved in the NACs of *Z. mays*, *A. thaliana*, *O. sativa*, and *S. bicolor*. Evolutionary studies of *ZmSNAC* genes revealed that these genes are closely related to *SbSNAC* genes in comparison with *O. sativa*. All the *ZmSNAC* genes have been divided into four groups based on distribution, indicating the evidence of four ancestor genes in NAC gene family in *Z. mays*. The Ka/Ks analysis of *ZmSNAC* genes revealed that these genes experienced purifying selection during the course of evolution. Identification of *cis*-acting elements, transcription factor and miRNA predictions and expression analysis provide insights into the regulation of *ZmSNAC* genes. Further, incorporating such TFs via breeding program or through genetic engineering methods would lead to tolerance to diverse abiotic stress without any yield penalty.

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References:

1. Puranik, S, Sahu, P.P, Srivastava, P.S, Prasad, M. (2012). NAC proteins: regulation and role in stress tolerance. Trends in Plant Science 17; 369-381.
2. Wang Z, Fenny D (2013). NAC (NAM/ATAF/CUC) transcription factors in different stresses and their signaling pathway. Acta Physiologiae Plantarum 35; 1397-1408.
3. Aida M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997). Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. Plant Cell 9; 841-857.
4. Nuruzzaman, M., Sharoni, A. M., Satoh, K., Moumeni, A., Venuprasad, R., Serraj, R., et al. (2012b). Comprehensive gene expression analysis of the NAC gene family under normal growth conditions, hormone treatment, and drought stress conditions in rice using near-isogenic lines (NILs) generated from crossing a day selection (drought tolerant) and IR64. Mol. Genet. Genomics 287; 389-410.
5. Peng X, Zhao Y, Li X, Wu M, Chai W, Sheng L, Wang Y, Dong Q, Jiang H, Cheng B (2015). Genome-wide identification, classification and analysis of NAC type gene family in maize. J. Genet. 94; 377-390.
6. Sun, M.M., Liu, X., Huang, X.J., Yang, J. J., Qin, P.T., Zhou, H., & Liao, H.Z. (2022). Genome-Wide Identification and Expression Analysis of the NAC Gene Family in Kandeliaobovata, a Typical Mangrove Plant. Current Issues in Molecular Biology 44; 5622-5637.
7. Le, D.T., Nishiyama, R.I.E., Watanabe, Y.A.S.U.K.O., Mochida, K.E.I.I.C.H.I., Yamaguchi-Shinozaki, K., Shinozaki, K., Tran, L. S. P. (2011). Genome-wide survey and expression analysis of the plant-specific NAC transcription factor family in soybean during development and dehydration stress. DNA Research, 18; 263-276.
8. 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.
9. Xu, W., Chen, C., Gou, N., Huang, M., Wuyun, T., Zhu, G., Wang, L. (2021). Genome-wide identification and expression

analysis of NAC transcription factor family genes during fruit and kernel development in Siberian Apricot. J. American Soc. Horticult. Sci. 146; 276-285.

10. Borrill, P., Harrington, S. A., Uauy, C. (2017). Genome-wide sequence and expression analysis of the NAC transcription factor family in polyploid wheat. G3: Genes, Genomes, Genetics 7; 3019-3029.

11. Kadier, Y., Zu, Y. Y., Dai, Q. M., Song, G., Lin, S. W., Sun, Q. P., Lu, M. (2017). Genome-wide identification, classification and expression analysis of NAC family of genes in sorghum [*Sorghum bicolor (*L.) Moench]. Plant Growth Reg. 83; 301-312.

12. Kou X, Wang S, Wu M, Guo R, Xue Z, Meng N, Tao X, Chen M, Zhang Y (2014). Molecular characterization and expression analysis of NAC family transcription factors in tomato. Plant Mol. Biol Rep. 32; 501-516.

13. Saidi, M. N., Mergby, D., Brini, F. (2017). Identification and expression analysis of the NAC transcription factor family in durum wheat (*Triticum turgidum* L. ssp. durum). Plant Physiol. Biochem. 112; 117-128.

14. Sun, H., Hu, M., Li, J., Chen, L., Li, M., Zhang, S., Yang, X. (2018). Comprehensive analysis of NAC transcription factors uncovers their roles during fiber development and stress response in cotton. BMC Plant Biology 18; 1-15.

15. Xue GP, Way HM, Richardson T, Drenth J, Joyce PA, McIntyre CL (2011) Overexpression of TaNAC69 leads to enhanced transcript levels of stress up-regulated genes and dehydration tolerance in bread wheat. Mol. Plant 4; 697-712.

16. Xu Z, et al. (2015). Wheat NAC transcription factor TaNAC29 is involved in response to salt stress. Plant Physiol. Biochem. 96; 356-363.

17. Yoshii, M., Shimizu, T., Yamazaki, M., Higashi, T., Miyao, A., Hirochika, H., Omura, T. (2009). Disruption of a novel gene for a NAC-domain protein in rice confers resistance to rice dwarf virus. Plant J. 57; 615-625.

18. Nuruzzaman, M., Sharoni, A. M., Kikuchi, S. (2013). Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. Front. Microbiol. 4; 248.

19. Fang, Y., You, J., Xie, K., Xie, W., Xiong, L. (2008). Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice. Mol. Genet. Genom. 280; 547-563.

20. Nuruzzaman, M., Sharoni, A. M., Satoh, K., Moumeni, A., Venuprasad, R., Serraj, R., et al. (2012b). Comprehensive gene expression analysis of the NAC gene family under normal growth conditions, hormone treatment, and drought stress conditions in rice using near-isogenic lines (NILs) generated from crossing a day selection (drought tolerant) and IR64. Mol. Genet. Genom. 287; 389-410.

21. Nuruzzaman M, Sharoni AM, Satoh K, Karim MR, Harikrishna JA, Shimizu T, Sasaya T, Omura T, Haque MA, Hasan SMZ, Ahmad A, Kikuchi S (2015). NAC transcription factor family genes are differentially expressed in rice during infections with rice dwarf virus, rice black-streaked dwarf virus, rice grassy stunt virus, rice ragged stunt virus, and rice transitory yellowing virus. Front. Plant Sci. 6; 676.

22. Liu, G., Li, X., Jin, S., Liu, X., Zhu, L., Nie, Y., Zhang, X. (2014). Overexpression of rice NAC gene SNAC1 improves drought and salt tolerance by enhancing root development and reducing transpiration rate in transgenic cotton. PLoS One, 9; e86895.

23. Fang, Y., Liao, K., Du, H., Xu, Y., Song, H., Li, X., Xiong, L. (2015). A stress-responsive NAC transcription factor SNAC3 confers heat and drought tolerance through modulation of reactive oxygen species in rice. J. Exp. Bot. 66; 6803-6817.

24. Capella M., Ré, D.A., Arce A. L., Chan R.L. (2014). Plant homeodomain-leucine zipper I transcription factors exhibit different functional AHA motifs that selectively interact with TBP or/and TFIIB. Plant Cell Reports 33; 955-967.

25. Hao Y.J., Wei W., Song Q.X., Chen H.W., Zhang Y. Q., Wang, F., Chen, S.Y. (2011). Soybean NAC transcription factors promote abiotic stress tolerance and lateral root formation in transgenic plants. Plant Journal 68; 302-313.

26. Goodstein D.M., Shu S., Howson R., Neupane, R., Hayes R.D., Fazo J., Rokhsar D.S. (2012). Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res. 40(D1); D1178-D1186.

27. Mistry J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G.A., Sonnhammer, E.L., Bateman, A. (2021). Pfam: The protein families database in 2021. Nucleic Acids Res. 49(D1), D412-D419.

28. Schultz J., Milpetz, F., Bork P., Ponting, C.P. (1998). SMART, a simple modular architecture research tool: identification of signaling domains. Proc. Nat. Acad. Sciences, 95; 5857-5864.

29. Letunic I., Khedkar, S., Bork, P. (2021). SMART: recent updates, new developments and status in 2020. Nucleic Acids Res. 49(D1); D458-D460.

30. Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35; 1547.

31. Sekhon, R.S., Lin, H., Childs, K. L., Hansey, C.N., Buell, C. R., De Leon, N., Kaeppler, S. M. (2011). Genome-wide atlas of transcription during maize development. Plant J. 66; 553-563.

32. Ooka, H., Satoh, K., Doi, K., Nagata, T., Otomo, Y., Murakami, K., Kikuchi, S. (2003). Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. DNA Res. 10; 239-247.

33. Li, B., Fan, R., Yang, Q., Hu, C., Sheng, O., Deng, G., Yi, G. (2020). Genome-wide identification and characterization of the NAC transcription factor family in Musa acuminata and expression analysis during fruit ripening. Internat. J. Mol. Sci. 21; 634.

34. Jin, J.F., Wang, Z.Q., He, Q.Y., Wang, J.Y., Li, P.F., Xu, J.M., Yang, J.L. (2020). Genome-wide identification and expression analysis of the NAC transcription factor family in tomato (*Solanum lycopersicum*) during aluminum stress. BMC Genomics, 21; 1-14.

35. Li, C., Zhang, J., Zhang, Q., Dong, A., Wu, Q., Zhu, X., Zhu, X. (2022). Genome-wide identification and analysis of the NAC transcription factor gene family in garden *Asparagus* (*Asparagus officinalis*). Genes 13; 976.

36. He, F., Zhang, L., Zhao, G., Kang, J., Long, R., Li, M., Chen, L. (2022). Genome-wide identification and expression analysis of the NAC gene family in Alfalfa revealed its potential roles in response to multiple abiotic stresses. Internat. J. Mol. Sci. 23; 10015.

37. Kosová, K., Vítámvás, P., Urban, M. O., Klíma, M., Roy, A., Prášil, I.T. (2015). Biological networks underlying abiotic stress tolerance in temperate crops-a proteomic perspective. Int. J. Mol. Sci. 16; 20913-20942.

38. Singh, S., Grover, A., Nasim, M. (2016).

Biofuel potential of plants transformed genetically with NAC family genes. Front. Plant Sci. 7; 22.

39. Baillo, E.H., Kimotho, R.N., Zhang, Z., Xu, P. (2019). Transcription factors associated with abiotic and biotic stress tolerance and their potential for crops improvement. Genes 10; 771.

40. Sheshadri S.A., Nishanth M.J., Simon B. (2016). Stress-mediated cis-element transcription factor interactions interconnecting primary and specialized metabolism in planta. Frontiers in plant science, 7, 1725.

41. Tuteja N. (2007). Abscisic acid and abiotic stress signalling. Plant Signal Behav. 2; 135-138.

42. Cheng M.C., Liao P.M., Kuo W.W., Lin, T.P. (2013). The Arabidopsis ETHYLENE RESPONSE FACTOR1 regulates abiotic stress-responsive gene expression by binding to different cis-acting elements in response to different stress signals. Plant Physiology, 162; 1566-1582.

43. Castro-Camba R., Sánchez, C., Vidal, N., Vielba, J.M. (2022). Interactions of gibberellins with phytohormones and their role in stress responses. Horticulturae, 8; 241.

44. Iqbal, A., Bocian, J., Hameed, A., Orczyk, W., Nadolska-Orczyk, A. (2022). *Cis*-regulation by NACs: A promising frontier in wheat crop improvement. Int. J. Mol. Sci. 23; 15431.

45. Xu, J., Guo, Z., Jiang, X., Ahammed, G. J., Zhou, Y. (2021). Light regulation of horticultural crop nutrient uptake and utilization. Horticult. Plant J. 7; 367-379.

46. Lin Y. X., Jiang H. Y., Chu Z. X., Tang X. L., Zhu S. W. and Cheng B. J. 2011 Genome-wide identification, classification and analysis of heat shock transcription factor family in maize. BMC Genomics 12; 76

47. Zhao, Y., Zhou, Y., Jiang, H., Li, X., Gan, D., Peng, X., Cheng, B. (2011). Systematic analysis of sequences and expression patterns of drought-responsive members of the HD-zip gene family in maize. PLoS One 6; e28488.

48. Peng, X., Zhao, Y., Cao, J., Zhang, W., Jiang, H., Li, X., Cheng, B. (2012). CCCH-type zinc finger family in maize: genome-wide identification, classification and expression profiling under abscisic acid and drought treatments. PLoS One 7; e40120.

49. Gaut B.S. (2002). Evolutionary dynamics of grass genomes. New Phytol. 154; 15-28.

50. Salse J., Bolot, S., Throude, M., Jouffe, V., Piegu, B., Quraishi, U. M., ...& Feuillet, C. (2008). Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. Plant Cell 20; 11-24.

51. Zhang, J., Gu, M., Liang, R., Shi, X., Chen, L., Hu, X., Xu, G. (2021). OsWRKY21 and OsWRKY108 function redundantly to promote phosphate accumulation through maintaining the constitutive expression of OsPHT1; 1 under phosphate-replete conditions. New Phytol. 229, 1598-1614.

52. Haake, V., Cook, D., Riechmann, J., Pineda, O., Thomashow, M.F., Zhang, J. Z. (2002). Transcription factor CBF4 is a regulator of drought adaptation in Arabidopsis. Plant Physiol. 130; 639-648.

53. Dubouzet J.G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E. G., Miura, S., Yamaguchi-Shinozaki K. (2003). OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt-and cold-responsive gene expression. Plant J. 33; 751-763.

54. Anbazhagan K, Bhatnagar-Mathur P, Vadez V, Reddy DS, Kavi Kishor PB, Sharma KK (2015a). DREB1A overexpression

in transgenic chickpea alters key traits influencing plant water budget across water regimes. Plant Cell Rep. 34; 199-210.

55. Anbazhagan K, Bhatnagar-Mathur P, Sharma KK, Baddam R, Kavi Kishor PB, Vadez V (2015b). Changes in timing of water uptake and phenology favors yield gain in terminal water stressed chickpea *AtDREB1A*transgenics. Funct. Plant Biol. 42: 84-94.

56. Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki K. (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10; 1391-1406.

57. Kasuga M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K. (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotechnol. 17, 287-291.

58. Medina, J., Bargues, M., Terol, J., Pérez--Alonso, M., Salinas, J. (1999). The Arabidopsis CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. Plant Physiol. 119; 463-470.

59. Thomashow M.F. (2001). So, what's new in the field of plant cold acclimation? Lots! Plant Physiol. 125; 89-93.

60. Gilmour, S.J., Sebolt, A.M., Salazar, M.P., Everard, J.D., Thomashow, M.F. (2000). Overexpression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol. 124; 1854-1865.

61. Chrispeels, H.E., Oettinger, H., Janvier, N., Tague, B.W. (2000). AtZFP1, encoding *Arabidopsis thaliana* C2H2 zinc-finger protein 1, is expressed downstream of photomorphogenic activation. Plant Mol, Boil. 42; 279-290.

62. Zhang B., Wang Q. (2015). MicroRNA-based biotechnology for plant improvement. J. Cell. Physiol. 230; 1-15.

63. Han, D., Du, M., Zhou, Z., Wang, S., Li, T., Han, J., Yang, G. (2020). Overexpression of a *Malus baccata* NAC transcription factor gene MbNAC25 increases cold and salinity tolerance in Arabidopsis. Int. J. Mol. Sci. 21; 1198.

64. Jiang, D., Zhou, L., Chen, W., Ye, N., Xia, J., Zhuang, C. (2019). Overexpression of a microRNA-targeted NAC transcription factor improves drought and salt tolerance in Rice via ABA-mediated pathways. Rice 12; 1-11.

65. Nakashima, K., Takasaki, H., Mizoi, J., Shinozaki, K., Yamaguchi-Shinozaki, K. (2012). NAC transcription factors in plant abiotic stress responses. Biochim. et Biophys. Acta (BBA)-Gene Reg. Mech. 1819; 97-103

66. Hong, Y., Zhang, H., Huang, L., Li, D., Song, F. (2016). Overexpression of a stress-responsive NAC transcription factor gene ONAC022 improves drought and salt tolerance in rice. Front. Plant Sci. 7; 4.

67. Thirumalaikumar, V.P., Devkar, V., Mehterov, N., Ali, S., Ozgur, R., Turkan, I., Balazadeh, S. (2018). NAC transcription factor JUNGBRUNNEN 1 enhances drought tolerance in tomato. Plant Biotechnology Journal, 16(2), 354-366.

68. Liu, W., Zhao, B. G., Chao, Q., Wang, B., Zhang, Q., Zhang, C., Li, X. (2019). Function analysis of ZmNAC33, a positive regulator in drought stress response in Arabidopsis. Plant Physiol. Biochem. 145;

174-183.

69. Mehari, T. G., Hou, Y., Xu, Y., Umer, M. J., Shiraku, M. L., Wang, Y., Liu, F. (2022). Overexpression of cotton GhNAC072 gene enhances drought and salt stress tolerance in transgenic Arabidopsis. BMC Genom. 23; 1-17.

70. Jin, H., Huang, F., Cheng, H., Song, H., Yu, D. (2013). Overexpression of the GmNAC2 gene, an NAC transcription factor, reduces abiotic stress tolerance in tobacco. Plant Mol. Biol. Rep. 31; 435-442.

71. Yang, X., Kim, M.Y., Ha, J., Lee, S.H. (2019). Overexpression of the soybean NAC gene GmNAC109 increases lateral root formation and abiotic stress tolerance in transgenic Arabidopsis plants. Front. Plant Sci. 10; 1036.