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RESEARCH ARTICLE

Transcriptome-wide identification and expression analysis of the NAC gene family in lowland bamboo [*Oxytenanthera abyssinica* (A.Rich) Munro] under abiotic stresses

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Abstract

NAC (NAM, no apical meristem, ATAF, and CUC) is one of the largest gene families of the plant-specific transcription factors (TF). NAC TFs have immense involvement in plant growth and developmental processes and have particular importance in enhancing plant resistance to multiple abiotic stresses. NAC members have unique structural makeup and a range of biological activities. Despite their enormous roles in plants, comprehensive study on identification, characterization, and expression profiling of NACs under abiotic stress is lacking in Lowland bamboo [*Oxytenanthera abyssinica* (A.Rich) Munro]. Thus, this study aimed to identify NAC members, characterize their protein properties, construct their phylogenetic relationships, and more importantly, establish their expression profiling under abiotic stress. From this abiotic stress-induced transcriptome, 220 lowland bamboo TFs with intact and complete NAC DNA binding domains (PF01849) were identified. Following their identification, analysis of functional annotation, protein characterization, phylogenetic relationships, and expression profiling were conducted. The analysis presented up-regulation of 142 unigenes in response to abiotic stress, the association of 26 unigenes directly to stress response, and the involvement of 92 unigenes in genetic information processing and 29 in environmental information processing according to KEGG analysis. These results suggest the most likely involvement of NACs in lowland bamboo stress response and adaptation. As a species best survived in a moisture-stressed environment, this study has provided valuable information that could shed light on further functional analysis research efforts aiming to exploit NACs in developing stress-resilient bamboo and related plants.

Keywords

drought; Lowland bamboo; NAC transcription factor; salinity

Introduction

Bamboo is one of the plant kingdom's most versatile and renewable resources. It belongs to the family Poaceae and subfamily Bambusoideae, which hosts approximately 1670 species within 125 genera. It is found in Asian, American, and African continents (1) with a wider distribution in the tropical and sub-tropical regions of the world (2). Because of its distinctive

and some novel species-specific characteristics, bamboo makes an intriguing model for tackling basic biological questions. Those characteristics include; the ability to grow faster than any other plant on earth (grows one m per day, according to (3), having a long and irregular flowering habit, having unique rhizome-dependent systems, *and possessing expanded gene families resulting from polyploidization (1,4).* Lowland bamboo is among the drought-tolerant species of bamboo native to the African continent. It grows well in savanna woodlands, semi-arid wooded, and thicket agroecologies of Africa (5).

Plant growth and development could be affected by a variety of environmental change-related factors (6, 7). However, numerous plant species have developed alternative biochemical and physiological methods to avoid or minimize some of the impacts. One of these mechanisms is the evolution of their genes' temporal and spatial patterns of expression (8). A sizable group of regulatory genes collectively referred to as transcription factors (TFs) are among those genes that control the activation and inactivation of various pathways required to control the plant's response to environmental stresses and, as a result, confer cross-talk between the plant and the environment. In this regard, transcription factors control the expression of stress-related genes by binding to the cognate cis-acting elements in charge of regulating all biological processes in plants, such as growth and development, as well as the regulation of gene responses brought on by environmental and developmental changes (9).

Signal transduction, gene expression, and eventually metabolic changes in plants are molecular responses to abiotic stress that impart stress tolerance (10). The NAC transcription factors (TFs) family is thought to be one of the key players in plant development, defense, and response to stress (11, 12, 13), thereby providing the plant with essential regulatory functions to protect it from various stresses. Abiotic stress responses in plants are mediated by NAC gene members, who also serve as transcriptional activators in addition to directly conferring abiotic stress tolerance. For example; the over-expression of *OsNAP*, a NAC member, in rice plants did not cause growth retardation, but it did result in significantly lower water loss rates, improved tolerance to low temperatures, drought, and high salinity during the vegetative stage, and increased yield during the flowering stage under drought stress. Moreover, *OsNAP* increased the expression of unrelated genes that are linked to stress (14); this suggests that NAC gene members have a variety of importance and activities. NAM, ATAF, and CUC proteins, which are found only in plants, make up NAC TFs. No apical meristem (NAM), ATAF1-2, and CUC2 (cup-shaped cotyledon) are the names of three proteins from which this family's name was originally formed (15). With a conserved NAC domain (around 150 amino acids in the N-terminal region), NAC members are among the most structurally unique and functionally diversified plant gene families. The NAC domain is primarily preserved in five sub-domains (A, B, C, D, and E) (11). While other C-terminal domains engage in transcriptional control, the NAC domain is well known for its critical function, which includes DNA binding and dimer formation (11, 16).

Numerous NAC/NAM TFs have been discovered in various plant species. To give a few examples, 125 NAC TFs have been found in Moso bamboo (17), 120 in Populus (6), 117 in *Arabidopsis thaliana* (18), 104 in *Solanum lycopersicum* (19), 147 in *Setaria italica* (20), 110 in *Solanum tuberosum* (21), 151 in *Oryza sativa* (22), 204 in *Brassica oleracea* (23), and 82 in *Cucumis* (24), 115 in sunflower (25). Despite the tremendous role of NACs in plant growth, development, and defense against stress, a comprehensive study is lacking in lowland bamboo. This study is poised to identify NAC members, characterize their protein properties, construct their phylogenetic relationships and investigate their expression profiling under abiotic stress. As a plant best survived under a moisture-stressed environment, the findings of this study could shed light on further functional studies aiming to exploit NACs in developing stress-tolerant bamboo and related plant species.

Materials and Methods

Plant materials and stress treatment

Lowland bamboo seeds obtained from the Ethiopian Biodiversity Institute were collected from the Asossa area of western Ethiopia. The seeds were germinated in a plastic pot under well-adjusted growth conditions such as varying temperatures during the day (26°C) and night (22° C), relative humidity of 75%, 16 h photoperiod, and 175 $\mu mol/m^2s^{-1}$ light intensity. One-month-old seedlings were transferred to uniform media for one week to create similar conditions before treatment. A total of 90 seedlings (30 seedlings for each treatment) were used for the three treatments, such as control, salt, and drought stress. Salt and drought stresses were induced using 200 mM NaCl and 25% PEG-6000 (Poly Ethylene glycol), respectively. Seedlings treated with 25% PEG-6000 get wilted and thus were not used for 48h RNA extraction. After successful treatment, 15 independent leave samples (six from control, six from salt, and three from drought treatment) were collected and directly frozen with liquid nitrogen and stored at -80^{o}C for further use.

RNA extraction, cDNA synthesis, and sequencing

Total RNA extraction was conducted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The quality and quantity of the extract were checked using a Nanodrop Lite spectrophotometer (Thermo Scientific Wilmington, Delaware, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with 1% agarose gel. The next-generation sequencing library was constructed according to the manufacturer's protocol (NEBNext Ultra™ RNA Library Prep Kit for Illumina). Poly(A) mRNA was isolated using NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB). The mRNA fragmentation was carried out using NEBNext First-Strand Synthesis Reaction Buffer, while priming was conducted using NEBNext Random Primers. First-strand cDNA was synthesized using

ProtoScript II Reverse Transcriptase and the second-strand cDNA was synthesized using Second Strand Synthesis Enzyme Mix. The purified double-stranded cDNA by AxyPrep Mag PCR Clean-up (Axygen, Union City, USA) was then treated with End Prep Enzyme Mix to repair both ends and add a dA-tailing in one reaction, followed by a T-A ligation to add adaptors to both ends. Size selection of Adaptor-ligated DNA was then performed using AxyPrep Mag PCR Clean-up (Axygen, Union City, USA), and fragments of ~360 bp (with the approximate insert size of 300 bp) were recovered. All samples were then PCR amplified for 11 cycles using P5 and P7 primers, with both primers carrying sequences that can anneal with flow cell to execute bridge PCR and P7 primer carrying a six-base index letting for multiplexing. The PCR products were purified using AxyPrep Mag PCR Clean-up (Axygen, Union City, USA), confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and quantified by Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Then libraries with different indices were multiplexed and loaded on an Illumina HiSeq instrument according to the manufacturer's protocol (Illumina, San Diego, CA, USA). Sequencing was carried out using a 2x150 bp Paired-End (PE) configuration; image analysis and base calling were conducted by the HiSeq Control Software (HCS) + OLB + GAPipeline-1.6 (Illumina) on the HiSeq instrument.

Data filtering and assembly

The quality of the raw paired-end reads was assessed using FastQC v0.11.2 (26). Pre-processing of raw reads was conducted using Cutadapt v.1.9.1 for adaptor trimming and quality filtering was conducted using Sickle v1.33 (27). The filtered reads were then assembled into full-length transcripts using Trinity v2.2.0 (28) which works by integrating three different software modules: Inchworm, Chrysalis, and Butterfly executing one after the other. Duplicate contigs were removed using CD-HIT v4.5.4 (29). Finally, the longest transcripts called unigenes were considered for functional annotation. The raw sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) under the SRP153816 accession number and the assembled transcripts were deposited in the Transcriptome Shotgun Assembly (TSA) Database, under the GGTK00000000 accession number.

Identification of NACs

Abiotic stress (drought and salt) induced genome-wide analysis of Lowland bamboo was established by (30). Accordingly, the present study made use of the 406,181 de novo assembled unigenes of Lowland bamboo (Table 1) as a main source for the identification and characterization of NAC unigenes. Accordingly, to identify NACs, deduced protein sequences of Lowland bamboo were submitted to the plant transcription factor http://itak.feilab.net/cgi-bin/itak/online_itak.cgi (31)database and PFam analysis (https://pfam.xfam.org/).

Table 1: Summary of Illumina paired-end sequencing and *de novo* assembly of Lowland bamboo from the abiotic stress induced transcriptome.

Reads/ contigs/	Parameters	Values/numbers
Sequenced reads	Number of raw	809,219,680
	Number of clean reads	754,444,646
	Total read length (bp)	99,601,349,229
	GC content	53.72 %
	Q20 percentage	97.4 %
	Q30 percentage	93.09 %
Contigs	Total number	9,595,574
	Total length(bp)	1,101,480,760
	Mean length (bp)	374
	Contig N50 (bp)	566
Unigenes/ Transcripts	Total number	406,181
	Total length (bp)	240,231,095
	Mean Length(bp)	641
	Unigenes N50 (bp)	873
	Minimum length (bp)	201
	Maximum length	16651

Gene ontology and functional classification

Protein sequences of NAC unigenes were loaded into the CELLO2GO http://cello.life.nctu.edu.tw/ cello2go/ (32) to obtain the GO annotation against Eukaryotes. Unigenes were also categorized as those involved in biological processes and those enabling molecular function according to CELLO2GO GO functional classification. Metabolic pathway analysis was conducted using the Blast KOALA tool (33).

Expression analysis of NAC gene families

Expression patterns of Lowland bamboo NAC unigenes in response to drought and salt stress were investigated using previously developed RNA-Seq data (30). The differential gene expression analysis (RSEM and DESEq2) for NACs was extracted from already processed RNA-Seq data in (30).

Phylogenetic analysis of Lowland bamboo

Phylogenetic analysis was performed on 220 NAC proteins. ClustalX program was used to perform multiple sequences alignment of protein sequences. The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the number of differences method and are in the units of the number of amino acid differences per sequence. A phylogenetic tree was constructed using MEGA 11 (34). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

Verification of OaNACs DEGs using qRT-PCR

qRT-PCR analysis was conducted to validate the digital expression of randomly selected 28 unigenes. After avoiding the conserved regions from each gene using Expasy (https://www.expasy.org) from the protein sequences, the Primer quest tool was employed to design 28 gene-specific qRT-PCR primers from the full-length cDNA sequences of each gene. Total RNA was extracted using TaKaRa MiniVEST Plant RNA Extraction Kit (Takara, Dalian, China). The quality and quantity of total RNA were checked by 1% agarose gel electrophoresis and NanoDrop 2000c Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The qRT-PCR was performed on an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) using the SYBR Premix Ex Taq™ kit (Takara, Dalian, China), according to the manufacturer's instructions. The amplification protocol comprised a 5-min incubation at 94 °C then a cycle of 94 °C for 30 sec, 60 °C for the 30sec, 72 °C for 1 min, repeated for 35 cycles, 72 °C for 10 min, and a final 4 °C hold. Relative expression of qRT-PCR was calculated 2-ΔΔCT.NTBFTCTTGTTTGACACCGAAGAGGAG and NTB-R AATAGCTGTCCCT GGAGGAGTTT primers from *ERF76* gene that were confirmed to be used as references for qRT-PCR from Moso bamboo (35).

Results

Characterization of NAC unigenes in Lowland bamboo genome

NAC search from the iTAK database resulted in the identification of 304 putative NAC proteins. To confirm the presence of the conserved NAC binding domain (PF01849), the resulting protein sequences from the iTAK database were further subjected to PFam analysis (https://pfam.xfam.org/), and as a result, 265 proteins were confirmed to be under NAC binding domain. In the end, 220 proteins with intact NAC binding domains were identified for the entire analysis after 45 proteins with incomplete NAC subdomains were consciously eliminated. Finally, 220 proteins with intact NAC binding domains were identified and used for the entire analysis. Following the Sequence Manipulation Suite (http://www.detaibio.com/sms2/) analysis, the sequences lengths of the 220 OaNACs ranged from 68 to 1975 amino acids, with a molecular weight ranging from 8.49 to 214.13KDa and the isoelectric point ranged from 4.26 to 12.33. This suggests the presence of varied characteristics in NAC proteins. Sub-cellular localization prediction of the 220 NACs proteins showed the highest number (97) of NACs are localized in the nucleus and the lowest (5 NACs) in the chloroplast which is consistent with the characteristics of NAC as a transcription factor (Figure 1).

Gene ontology and KEGG analysis

Gene ontology analysis is widely used to identify those genes that are involved in biological processes and molecular functioning and those that are part of a cellular component. In this regard, 99.09% (218 of the total 220 unigenes), were detected to carry out molecular functioning which tells that nearly all of NAC unigenes enable molecular function. Likewise, 97.72% (215 of the total 220 unigenes) were identified as cellular components. Biological process analysis has covered 98.18 % (216 of the total 220 unigenes) and the remaining 1.82 % did not show any significant functional similarity to the explored database.

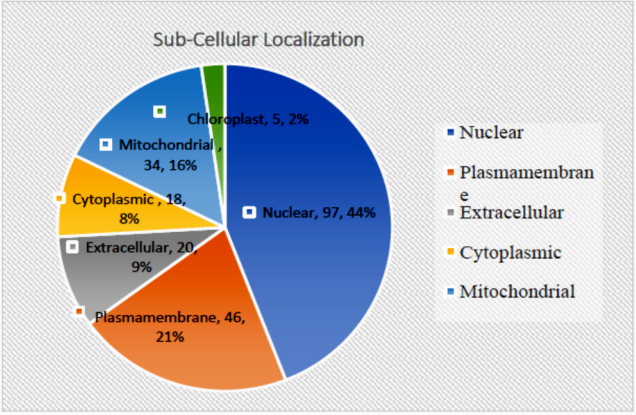

Fig.1. Sub-cellular localization of the 220 NAC gene family shows how NACs in the Lowland bamboo localized across various cellular compartments.

All parts of the cellular component (nucleus, organelle and cytoplasm) were represented by the proximate number of unigenes(Figure 2). Those ontologies with a significant role in enabling molecular function were identified to be DNA binding and nucleic acid binding transcription factor activity. A greater number of ontologies with diverse functions were involved in biological processes (11 ontologies) as compared to molecular functioning (three ontologies). The most represented ontologies in the biological process were found to be involved in the biosynthetic process, cellular nitrogen compound metabolic process, response to stress, and anatomical structure development. Most importantly, response to stress and signal transduction were represented by 26 and 18 unigenes, respectively, implying that NAC transcription factors in Lowland bamboo are highly responsive to abiotic stress.

Metabolic pathway analysis revealed that genetic information and environmental information processing pathway categories were highly represented. This result uncovers that NAC transcript factor families are actively involved in stress regulation (Figure 3).

Expression profiling analysis of NAC members

Among the identified 220 expressed unigenes under the NAC binding domain, 204 were differentially expressed genes (DEGs), 142 were up-regulated, 62 were down-regulated and 16 unigenes were not differentially expressed, implying their level of expression was not affected by both drought and salt stress. The most represented functional annotations of NACs in response to drought and salt stress in Lowland bamboo were stressed-induced transcription factor and NAC transcription factor (Table 2).

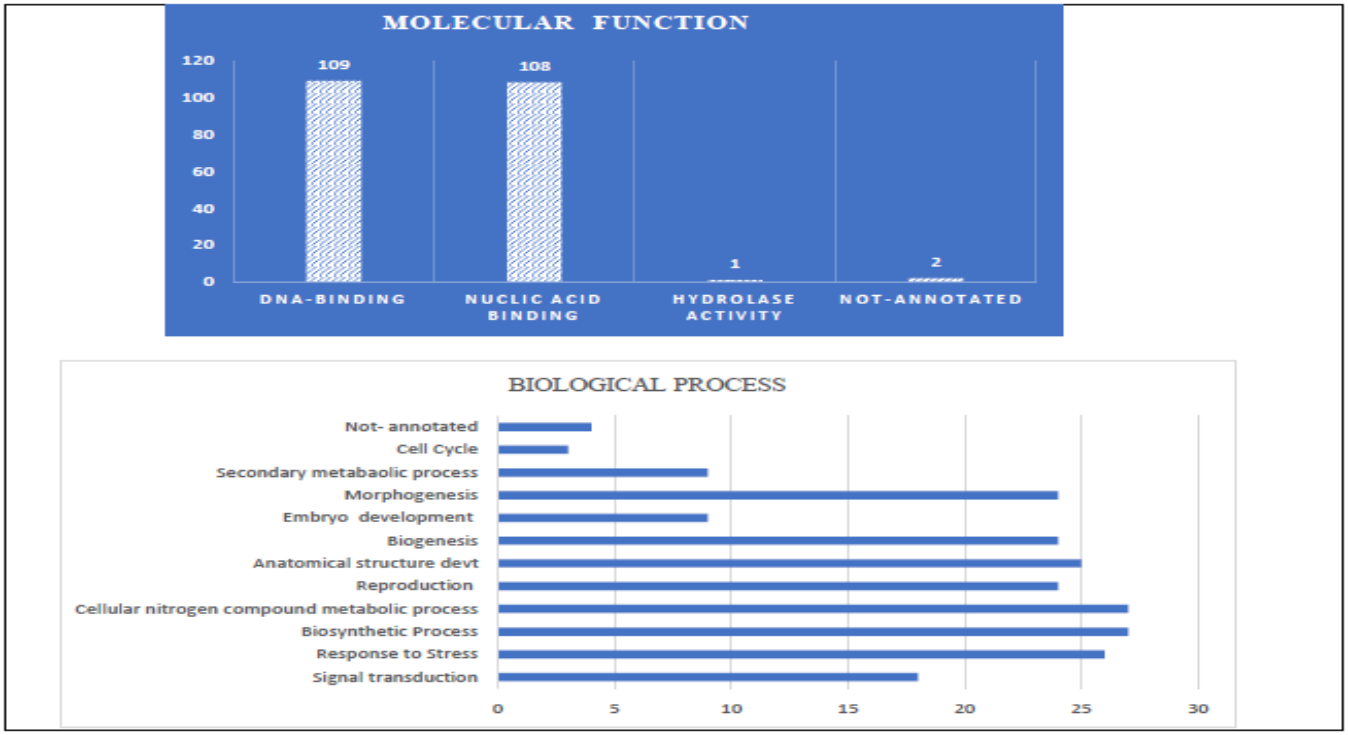

Fig. 2. Gene ontology classification of lowland bamboo NAC transcript factors dissecting the role of 220 NACs in the molecular function and biological process.

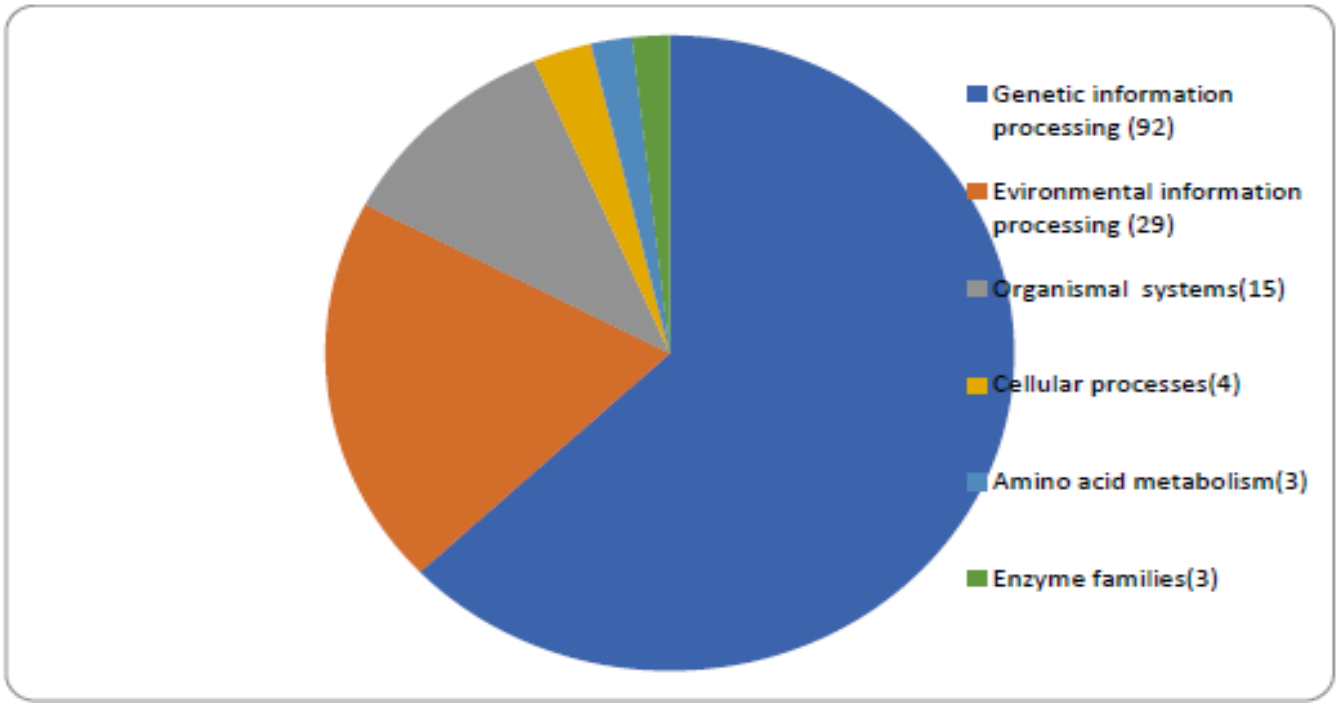

Fig.3. The KEGG analysis of NACs using the Blast KOALA tool shows the major metabolic categories where lowland bamboo NACs are involved.

Table 2: Differentially expressed genes and functional analysis of selected up regulated NAC transcript factors that could be prioritized for further research.

Gene ID	Log2FoldChange	Go_ID	KO_Definition
TRINITY_DN148808_c14_g7_i1	-	GO:0006355	Stress-induced transcription factor
TRINITY_DN141689_c2_g10_i2	8.5727262	GO:0006355	NAC transcription factor 1
TRINITY_DN149849_c1_g1_i2	7.482913019	GO:0016310	Putative receptor kinase
TRINITY_DN142740_c6_g1_i2	7.341014366	GO:0006355	NAC domain transcription factor
TRINITY_DN148808_c14_g20_i1	6.033535534	GO:0006355	Stress-induced transcription factor
TRINITY_DN207123_c0_g1_i1	5.633097149	GO:0009651	NaCl stress protein1
TRINITY_DN147541_c3_g2_i1	1.045496411	GO:0006355	TPA: NAC transcription factor
TRINITY_DN148808_c14_g20_i1	7.553369158	GO:0006355	Stress-induced transcription factor

Phylogenetics analysis

Phylogenetic analysis was conducted to understand the evolutionary implication of NACs in Lowland bamboo. The tree was constructed using 220 OaNAC proteins by employing the maximum parsimony method and bootstrap values over 1000 replicates. The phylogenetic analysis grouped NAC proteins into six distinct groups: from clusters I to VI. The number of NAC proteins in clusters ranges from (07) in cluster II to 70 in cluster VI (Figure 4).

qRT-PCR

To validate the expression levels measured by RNA-Seq, the ratio of expression of selected genes as measured by qRT-PCR was compared to the ratio of expression under drought and salt stress conditions from RNA-Seq. Randomly selected 28 unigenes were used and confirmed the reliability of the results. qRT-PCR was made for the entire transcriptome, but from the total 28 genes used, 7 genes were found to be under NAC binding domain. The expression results of qRT-PCR and RNA-Seq for genes were highly correlated (R^2 ⍰ 0.90).

Discussion

A detailed investigation into the molecular and genetic analysis of Lowland bamboo is absent. Until recently, only a few attempts have been made to reveal its global transcriptome profiling in response to abiotic stress (30). Furthermore, thorough genetic characterization of the species has been significantly hampered by the absence of its entire genome sequencing data. Despite the discovery of 220 NAC TF proteins, the absence of a reference genome for the species has cast a shadow over our research and forced us to forgo some analyses, including those of motif, gene structures, exons, and untranslated regions (UTR). Therefore, the comprehensive transcriptome sequence data obtained from Lowland bamboo using the Illumina Seq platform played a crucial role in conducting functional analysis and could be further used in gene cloning, expression analysis, and the development of EST-SSR markers. This allowed for the compromise of the limitation and the completion of some detailed analysis.

The identified NAC proteins were effectively characterized and predicted into different sub-cellular localizations. In this regard, the result obtained (97

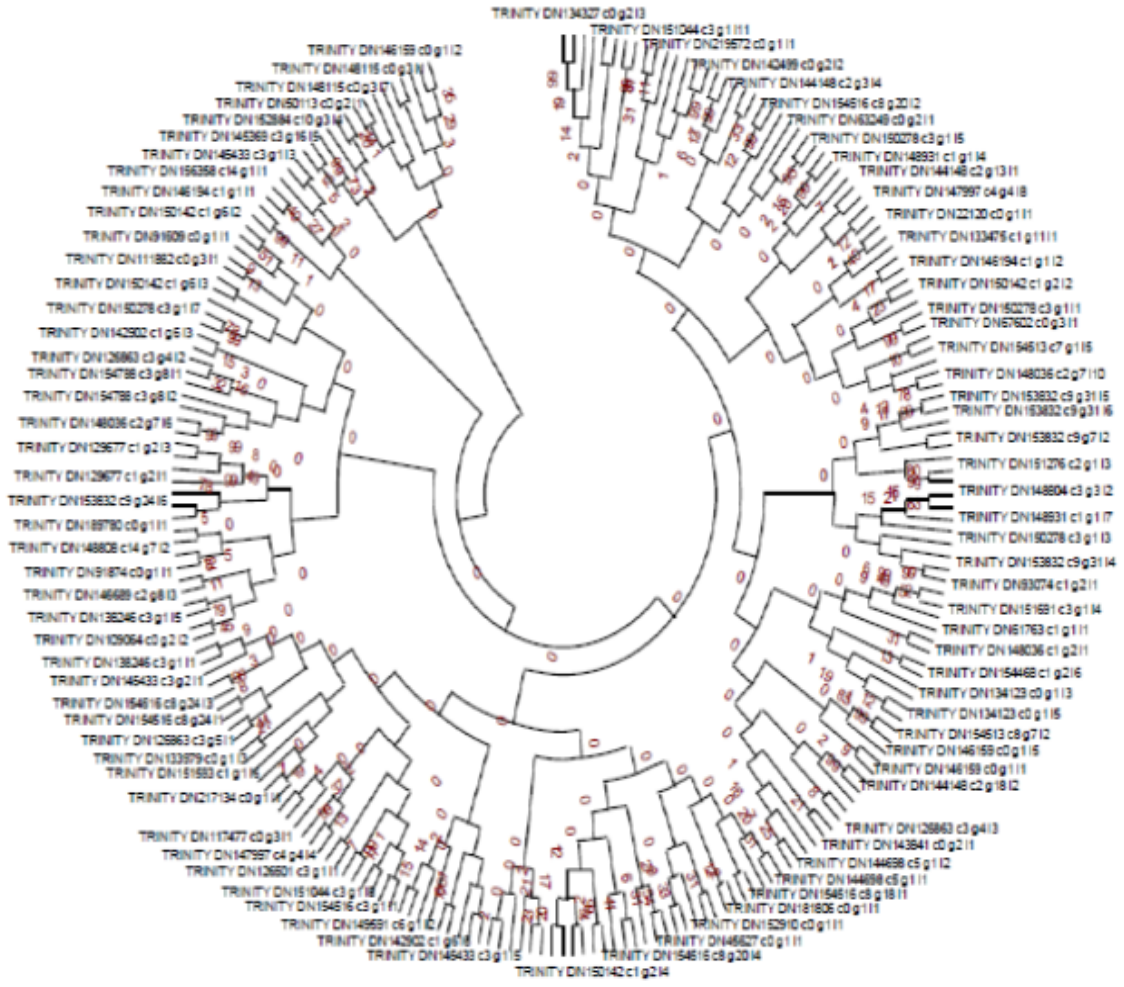

Fig.4. Phylogenetic tree of 220 aligned NAC proteins constructed using MEGA 11. The evolutionary history was inferred using the Neighbor-Joining method.

localized in the nucleus; 109 enabled DNA binding and 108 enabled nucleic acid binding) was consistent with the previous reports revealing NAC as a potential transcription factor (14; 25; 36). In addition, the result approves NACs' potential to have a distinct structure and diverse functions in plant species (11). Likewise, gene ontology analysis of the plant revealed a larger number of genes (26 unigenes) as being involved in biological functioning associated with the stress response. A larger percentage of the detected unigenes (146 or 66.36 %) were annotated by KEGG analysis, indicating that they possessed a homologous sequence in the KEGG database. However, a sizable portion of unigenes, remained unannotated, partly because of a lack of reference genome and this is a common occurrence in various species, including Lowland bamboo. As a result, one-third of the discovered NACs failed to find their functional match in the KEGG database regardless of their significantly diverse role. This could be explained by the lack of references, the highly diverse characters of the novel, species-specific unigenes, or both. In addition to the lack of references for the Lowland bamboo genome, the transcriptome's untranslated sections and the insertion of new Lowland bamboo-specific transcripts into the data set could be another potential cause (37). The phylogenetic analysis clustered OaNACs into 6 groups. Cluster VI holds the highest number of NAC proteins (70 NAC members), implying significant variation in the number of NAC proteins between clusters.

NAC TFs are active players in plant growth and development, especially in conferring plants the ability to resist multiple abiotic stresses such as drought, salinity, and cold (18, 38). For instance, the overexpression of three NAC genes, *ANAC019*, *ANAC055,* and *ANAC072*, significantly improved the drought resistance of plants. These genes are also in control of the expression of *ERD1* and its downstream genes by binding to the CATGTG core region of the promoter of the ERD1 gene, which is also involved in response to drought stress (18). In support of these claims, this study showed that 26 unigenes were directly attached to stress response in the biological process and the involvement of 92 unigenes in the genetic information processing and 29 unigenes in the environmental information processing pathways clarifies the claims, since these pathways are thought to be the most likely categories linked to abiotic stress (39). This reveals that NACs play crucial roles in helping plants deal with stress. Arabidopsis and rice have been used in transgenesis experiments to examine the important functions that plant NAC transcription factors play in facilitating abiotic stress tolerance and adaptability (25; 39;40). The over-expression of the *PheNAC3* gene from Moso bamboo significantly increased Arabidopsis' resistance to salt and drought stress. When exposed to salt stress, *PheNAC3* overexpression led to faster seed germination, greater seedling growth, and a higher rate of survival than the wild -type (40). Another NAC member originating from bamboo, *PeSANC-1* overexpressed plants showed improved resistance to salt and drought stress. The study of physiological indices, such as the activities of superoxide dismutase, peroxidase, and catalase, and the levels of malondialdehyde, H_2O_2, and proline, showed that the transgenic rice performed significantly better than its wild counterpart. Additionally, a study of protein interactions found that *PeSNAC-1* is likely to interact with the stress-response proteins of *PeSNAC-2/4* and *PeNAP-1/4/5* in yeast and plant cells, demonstrating the synergistic role those proteins play in regulating the Moso bamboo stress response (39). The stress response of NACs has been investigated in many species. The Tartary buckwheat NAC gene, *FtNAC70*, overexpressed Arabidopsis displayed notable resistance to salt and drought stress (13).

The identified unigenes showed divergent results in the differential expression gene (DEG) study, of which 142 were up-regulated and 62 were down-regulated. Similarly, 173 differentially expressed *VrNAC* genes were identified in mung beans (41). DEG analysis of NAC expression profiling under abiotic stress in peanuts showed that the majority of NAC members were highly upregulated in response to drought stress, suggesting their greater responsiveness to drought stress (42). In *Nelumbo nucifera,* the encoding genes for NAC016, NAC025, CCC, and NAC070 proteins were significantly upregulated in response to salt and ABA stress. Further analysis demonstrated that the three NAC proteins undergo transcription activation roles and they form complexes with other proteins to execute integrated functions (43). The results of the examination of the expression patterns of the NAC gene members in *Miscanthus sinensis* in response to high salinity, PEG, and heavy metals have also shown remarkably comparable results (44). Expression analysis with RNA-seq revealed that most *Hibiscus hamabo* NACs were expressed in response to drought and salt stress. qRT-PCR suggested that *HhNAC54* was significantly upregulated under multiple abiotic stresses. Overexpression of *HhNAC54* in *Arabidopsis thaliana* significantly increased its tolerance to salt (45). When exposed to drought stress, dozens of NAC genes, such as *ItbNAC110*, *ItbNAC114*, *ItfNAC15*, *ItfNAC28*, and *ItfNAC62* from hexaploid cultivated sweet potato also showed significant up-regulation (46). Using transgenic tobacco, yeast, and Arabidopsis plants as model systems, the most recent investigation with the *PeNAC-19* gene, a passion fruit NAC family member, revealed that the gene has been highly differentially regulated in response to multiple stresses such as drought, salt, cold, and high temperature (47). In response to drought and salt stresses, the *AvNACs, AvNAC58,* and *AvNAC69* genes of *Apocynum venetum* exhibited significant differential expression (48).

Although better adapted to moisture-stressed environments, like any other plants, lowland bamboo faces multiple abiotic stresses. Mining lowland bamboo stress-tolerant genes, functional characterization of such genes and consequently devising molecular breeding strategies are the most important for developing stress-resilient bamboo and related plant species. Comprehensive studies of the NAC gene family are very important as one of the most crucial gene families with diverse functions in the growth, development, and stress response (49, 50). The results of this study provided important details about the discovered OaNAC gene family

and should be functionally confirmed by employing various biotechnological methods in the future.

Conclusion

The study has discovered 220 OaNAC gene members with intact and complete DNA binding domains from the abiotic stress-induced transcriptome. The characterization of the NACs, including; functional annotation, protein properties, phylogenetic relationships, and expression profiling analysis. This helped to understand NAC transcription factors in Lowland bamboo, particularly their response to abiotic stress. A gene ontology analysis revealed that 26 NAC members were involved in the biological process leading to the stress response. Large numbers of NACs were anticipated to be engaged in genetic and environmental information pathways that are strongly related to the stress response. DEG analysis showed the expression level of 142 unigenes was upregulated in response to salt and drought stress, demonstrating the stress responsiveness of NAC genes. The findings of the study offered valuable information about the identified OaNAC gene family members' response to abiotic stress that could stimulate further research aiming to develop stress-resilient bamboo and related species. We strongly recommend that further functional confirmation studies should focus particularly on the OaNACs unigenes exhibiting the highest level of expression, which may also help elucidate the molecular regulatory mechanisms and ultimately, apply for successful genetic improvement programs.

Supplementary data: OaNAC amino acid sequences

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Authors contributions

MA conducted the experiment, data analysis and wrote the manuscript, FA edited the manuscript, KZ performed the RT-qPCR, DB supervised the work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest :Authors declare that they have no conflict of interest.

Ethical issues : None

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