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Transcriptome wide identification and characterization of NAC Transcription factors in response to abiotic stress in Lowland bamboo [Oxytenanthera abyssinica (A.rich) munro]

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Abstract

NAC (**N**AM, no apical meristem, **A**TAF and **C**UC) are among the biggest plant transcription factor (TF) having important role in plant developmental and biological processes. NAC members which are found only in plant kingdom have distinct structures and diverse functions. They have tremendous role in cell differentiation and expansion, and hormone signaling in response to various stresses. Despite this importance, genome-wide analysis and expression profiling of NAC gene family under abiotic stress remains elusive in Ethiopian lowland bamboo (Oxytenanthera abyssinica). In this study, a genome-wide identification of 220 O. abyssinica TFs having intact and complete NAC DNA binding domain (PF01849) have been made from abiotic stress induced transcriptome. The identification was followed by functional annotation, protein property, phylogenetic relationship and expression profiling. Accordingly, significant variations were observed regarding gene length, molecular weights and isoelectric points among NAC members. The expression level of randomly selected OaNACs were examined using qRT-PCR. The results were consistent with the RNA-Seq results, confirming the differential expression of selected genes. This informs the key role of NACs in regulating abiotic stress tolerances in O. abyssinica. In total, 142 of the 220 OaNACs are differentially expressed due to either drought or salinity stress. The biological involvement of 26 genes in stress response and the up-regulation 35 genes in expression analysis points the likely association of NACs in stress response and adaption in O. abyssinica. In this regard, this study lays the basis for further studies associated with NACs abiotic stress tolerance and adaptation in O. abyssinica and related plants.

1. Introduction

Bamboos are one of the most versatile, renewable resources among the plant kingdom. *They belong to the family Poaceae and subfamily Bambusoideae that hosts approximately 1670 species within 125 genera. They are found in Asian, American and African continents (Basak et al.2021) with wider* distribution in the tropical and subtropical regions of the world (Qiao et al. 2014). Bamboo grows one m per day, which makes it the fastest-growing plant on earth, (Tao et al.2018). Bamboo serves as an interesting model for addressing fundamental biological questions largely because of its unique and some novel species-specific characteristics. Those characteristics include, ability to grow faster than any other plants on earth (grows one m per day, according to Tao et al.2018), having a long and irregular flowering habit, having unique rhizome-dependent systems*and possessing expanded gene families resulted from polyploidization (Guo et al. 2019; Basak et al. 2021). Oxytenanthera abyssinica* is among the drought tolerant species of bamboos native to African continent. It grows well in savanna woodlands, semi-arid wooded and thicket agro-ecologies of Africa. It flowers after long periods of vegetative growth, irregularly sets seed and then dies back, sometimes synchronously across large area (Inada and Hall 2008).

Plant growth and development is affected by various environmental change related stresses come (Hu et al. 2006; Ahuja et al. 2010). However, several plant species have developed different biochemical and physiological mechanisms escape the consequences of environmental stresses. One of such

mechanisms include developing temporal and spatial expression pattern of their genes (Mao et al.2012). Large group of regulatory genes collectively known as transcription factors (TFs) are among those genes that govern the switching on and off of different pathways necessary to regulate the plant response to the environmental stresses and hence confer cross talk between the plant and environment. In this regard, transcription factors regulate the expression of stress associated genes that binds to the cognate *cis*-acting elements responsible in controlling all biological processes in plants including growth, development, and regulation of gene responses that arises due to environmental and developmental changes (Tran et al.2004). Molecular responses to abiotic stress include signal transduction, gene expression and ultimately metabolic changes in the plant thus conferring stress tolerance (Agarwal et al. 2006). The NAC transcription factors (TFs) family is believed to be among the plant TFs which plays significant roles in plant growth, development, stress and defense response (Olsen et al. 2005; Puranik et al. 2012; Huang et al.2022). and hence, offers the plant critical regulatory functions in plant to different stresses.

Other than directly conferring abiotic stress tolerances, NAC gene members functions as a transcriptional activator and play a role in mediating abiotic stress responses in plants. The over-expression of OsNAP, a NAC member in rice plants did not show growth retardation, but showed a significantly reduced rate of water loss, enhanced tolerance to high salinity, drought and low temperature at the vegetative stage, and improved yield under drought stress at the flowering stage. More ever OsNAP up-regulated the expression of non-related but stress-associated genes (Chen et al.2014) implying pivotal and diverse functions of NAC gene members.

NAC TFs are composed of **N**AM, **A**TAF, and **C**UC proteins that only exist in plants. The name of this family was originally derived from the names of three proteins: no apical meristem (NAM), ATAF1-2, and CUC2 (cup-shaped cotyledon) (Aida et al. 1997). NAC members are among the most structurally distinct and functionally diverse plant gene families having a conserved NAC domain (approximately 150 amino acids in the N-terminal region), mostly persists in five subdomains (A, B, C, D and E) (Olsen et al.2005). The NAC domain is known for its crucial responsibility including DNA binding and dimer formation, whereas different C-terminal regions participate in transcriptional regulation (Ooka et al.2003; Olsen et al.2005).

Several NAC/NAM TFs have been reported from different plant species. For instances,125 NAC TFs have been identified from Moso bamboo (Li et al.2015), 120 from *Populus* (Hu et al.2010), 117 from *Arabidopsis thaliana* (Tran et al. 2011), 104 from *Solanum lycopersicum* (Yang et al.2011), 147 from *Setaria italica* (Puranik et al. 2013), 74 from *Vitis vinifera* (Wang et al. 2013a,) 110 from *Solanum tuberosum* (Singh et al. 2013), 151 from *Oryza sativa* (Nuruzzman et al. 2010), 204 from *Brassica oleracea* (Liu et al. 2014) and 82 from *Cucumis melon* (Wei et al.2016), 150 from Sunflower (Li et al.2021).

However, genome-wide identification and characterization of NACs gene families remains unavailable in Ethiopian lowland bamboo (*O. abyssinica)*. Thus, the present study was initiated to carryout

comprehensive identification of NAC member, their protein property, phylogenetic relationship and more importantly to establish their expression profiling in response to abiotic stress The findings of this study could serve as a baseline for furthers studies of NACs in *O. abyssinica* and related species.

2. Materials And Methods

2.1 Plant materials and stress treatment

O. abyssinica seeds collected from Asossa area, western Ethiopia and deposited at the Ethiopian Biodiversity Institute (EBI). The seeds were allowed to germinate in plastic pot under well-adjusted growth conditions such as varying temperatures during day (26°C) and night (22°C) times, relative humidity of 75%, 16 hr photoperiods and 175 µmol/ (m^2s^{-1}) light intensity. One month old seedlings were transferred to water media for one week to create uniform condition before treatment. A total of 90 seedlings (30 seedlings for each treatment) were considered for the three treatments such as control, salt and drought stress. Salt and drought stresses were induced using 200 nm NaCl and 25% PEG-6000 (Poly Ethylene glycol), respectively. Seedlings treated with 25% PEG-6000 wilted and thus not used for 48hr RNA extraction. After successful treatment, 15 independent leave samples (six from control, six from salt and three from drought treated) were collected and directly frozen with liquid nitrogen and stored at -80^oC until use.

2.2 RNA Extraction, cDNA Synthesis, and Sequencing

Total RNA extraction was conducted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Quality and quantity of the extract was checked using Nanodrop Lite spectrophotometer (Thermo Scientific Wilmington, Delaware, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with 1% agarose gel. 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 which 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

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.

2.3 Data filtering and assembly

Quality of the raw paired-end reads was assessed using FastQC v0.11.2 (Andrews, 2010). 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 (Joshi and Fass 2011). The filtered reads were then assembled into full length transcript using Trinity v2.2.0 (Grabherr et al. 2011) that 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 (Fu et al. 2012). 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 SRP153816 accession number and the assembled transcripts were deposited in the Transcriptome Shotgun Assembly (TSA) Database, under GGTK00000000 accession number.

2.4 Gene ontology and functional classification

Plant materials, stress treatments and employed methods are described in Adem et al. 2019. Protein sequences of NAC genes were loaded into the CELLO2GO http://cello.life.nctu.edu.tw/ cello2go/ (Yu et al., 2014) to obtain the GO annotation against Eukaryote. Genes were also categorized as those involving in biological process and those enabling molecular function according to CELLO2GO GO functional classification. Metabolic pathway analysis was conducted using the Blast KOALA tool (Kanehisa et al. 2015).

2.5 Expression analysis of NAC gene families

Expression patterns of *O. abyssinica* NAC genes in response to drought and salt stress were investigated using previously developed RNA-Seq data (Adem et a1.2019). The differential gene expression analysis (RSEM and DESEq2) for NACs was extracted from already processed RNA-Seq data in Adem et al. 2019. Accordingly, genes with a fold-change ≥ 1 and a false discovery rate (FDR) < 0.01 were treated as DEGs.

2.6 Phylogenetic analysis of *O. abyssinica*

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. Phylogenetic tree was constructed using MEGA 11 (Tamura et al. 2021). All ambiguous positions were removed for each sequence pair (pairwise deletion option).

2.7 Verification of *OaNACs* DEGs using qRT-PCR

Quantitative Reverse Transcription–Polymerase Chain Reaction(qRT-PCR) analysis was conducted to validate the digital expression of randomly selected 28 genes. After avoiding the conserved regions from

each gene using Expasy (https://www.expasy.org) from the protein sequences, Primer quest tool was employed to design 28 gene specific qPCR 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-minute incubation at 94°C then a cycle of 94°C for 30 s, 60°C for the 30s, 72°C for 1 min, repeat 35 times, 72°C for 10 min, and a final 4°C hold. Relative expression of qRT-PCR was calculated 2-ΔΔCT.NTBFTCTTGTTTGA CACCGAAGAGGAG and NTB-R AATAGCTGTCCCT GGAGGAGTTT primers from ERF76 gene that was confirmed to be used as references for qRT-PCR from Moso bamboo (Fan et al.2013).

3. Results

3.1 Identification and Characterization of NAC genes in *O. abyssinica* genome

Abiotic stress (drought and salt) induced genome-wide transcriptome analysis of *O. abyssinica* was established by (Adem et al. 2019). Accordingly, the present study made use of the 406,181 *de novo* assembled unigenes of *O. abyssinica* (Table 1) as a main source for identification and characterization of NAC genes.

Table 1
Summary of Illumina paired-end sequencing and de novo assembly of *O. abyssinica*.

Reads/contigs/unigenes	Parameters	Values/numbers
Sequenced reads	Number of raw reads	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(bp)	16651

To identify NAC genes, deduced protein sequences of *O. abyssinica* were submitted to plant transcription factor database, http://itak.feilab.net/cgi-bin/itak/online_itak.cgi (Zheng et al., 2016), these results in to the identification of 304 putative NAC proteins. To confirm the presences of the conserved NAC binding domain (PF01849), the resulting protein sequences from iTAK database were further subjected to PFam analysis (https://pfam.xfam.org/), and then 265 proteins were confirmed to be under NAC binding domain. But 45 proteins were deliberately removed since they were found to be with incomplete NAC subdomains. Finally, 220 proteins with intact NAC binding domain were identified and used for the entire analysis.

Following the Sequence Manipulation Suite (http:// www. detai bio. com/ sms2/) analysis, the sequences lengths of the 220 OaNACs ranged from 68 to 1975 amino acids, with a molecular weight ranged from 8.49 to 214.13KDa and the isoelectric point ranged from 4.26 to 12.33. This suggests the presences of varied characteristics in NAC proteins.

As presented in Fig. 1, sub-cellular localization prediction of the 220 NACs proteins showed that 97 of are localized in the nucleus which is consistent with the characteristics of NAC as a transcription factor. Among the NAC unigenes, two unigenes are not classified to any sub cellular localization.

3.2 Gene ontology and KEGG analysis

Gene ontology analysis is widely used to identify those genes that are largely involved in biological process, molecular functioning and those that are part of cellular component.in this regard, 99.09% (218 of the total 220 unigenes), were detected to carryout molecular functioning which tells that nearly all of NAC unigenes enables molecular function. Likewise, 97.72% (215 of the total 220 unigenes) identified as cellular component.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.

As it's displayed in Fig. 2, all parts of the cellular component (nucleus, organelle, cytoplasm, and intracellular) were represented by proximate number of unigenes. Those ontologies with significant role in enabling molecular function were identified to be DNA binding and nucleic acid binding transcription factor activity. Greater number of ontologies with diverse functions were involved in biological process (11 ontologies) as compared molecular functioning (three ontologies). The most represented ontologies in biological process were found to be involved in 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 *O. abyssinica* are highly responsive to abiotic stress.

As it's shown in Fig. 3, 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.

3.3 Expression profiling analysis of NAC members

Among the identified 220 expressed genes under the NAC binding domain, 204 were differentially expressed genes (DEGs), 142 were up-regulated, 62 were down-regulated and 16 genes 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 *O. abyssinica* were stressed induced transcription factor and NAC transcription factor (Table 2).

Table 2
DEGs and functional analysis of selected up regulated NAC transcript factors

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

3.4 Phylogenetics analysis

Phylogenetic analysis was conducted to understand the evolutionary implication of NACs in *O. abyssinica*. The tree was constructed using 220 OaNAC proteins by employing maximum parsimony method and bootstrap values over 1000 replicates. The phylogenetic analysis grouped NACs 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. Cluster VI holds the highest number of NAC proteins (70 NAC members), implying significant variation in the number of NAC proteins between clusters (Fig. 4).

3.5 Quantitative Reverse-Transcription PCR (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 genes 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 ($R^2 \geq 0.90$).

4. Discussions

Except limited reports on the global transcriptome profiling of *O. abyssinica* in response to abiotic stress (Adem et al. 2019), detailed research work regarding the molecular and genetic properties of the plant remained untouched. In addition, the lack of its whole genome sequences data has significantly

hampered detailed genetic characterization of the species. Such lack of refences genome for the species casted a shadow on our work and left various analysis including, motif, gene structures, exons and untranslated regions (UTR) aside despite the identified 220 NAC TFs proteins. Thus, to compromise the limitation and to carry out some detailed analysis, the comprehensive transcriptome sequences data we obtained from *O. abyssinica* using the Illumina Seq platform played a crucial role in conducting functional analysis and could be further utilized in gene cloning, expression analysis, and EST-SSR markers development.

The identified NAC proteins were effectively characterized and predicted in to different sub-cellular localizations. In this regard, the result obtained (97 localized in the nucleus), in the molecular function (109 enabled DNA binding and 108 enabled nucleic acid binding) was consistent with the previous reports revealing NAC as a potential transcription factor (Chen et al.2014; Suchithra et al.2018; Li et al.2021) and in addition, the result confirms NACs potential of having distinct structure and diverse function within a given or in different plant species (Olsen et al.2005). Likewise, gene ontology analysis of the plant revealed that several genes (26 genes) played a key biological role to stress response.

KEGG analysis fully annotated larger number of the identified unigenes (146 or 66.36%) confirming that they had a homologues sequence in the KEGG database. On the other hand, considerable number of the unigenes left un annotated hence lack their corresponding homologues in the KEGG database which is a common phenomenon in different species (Berardini et al. 2004). To this end, regardless of their significant diverse role, one third of the identified NACs in *O. abyssinica* didn't get their functional match in the KEGG database. This could be attributed to lack of references genome of *O. abyssinica*, the highly divergent nature of the unigenes or genes being novel and perform species specific functions. In addition, the transcriptome being un-translated regions, inclusion of new *O. abyssinica* specific transcripts to the data set could be reasonable explanations (Muller et al. 2017).

Metabolic pathway analysis of NAC gene members revealed their active involvement in the six different pathway categories. In this regard, the larger number of NACs are involved in environmental and genetic information processing pathways which are perceived to be the most likely categories associated with abiotic stress (Hou et al.2020). This unveils the key role of NACs in plant stress response and adaptation. The transgenesis experiments on the substantial role of plant NAC transcription factors in conferring abiotic stress tolerances and adaptation has been conducted on Arabidopsis and rice (Xie et al.2020; Hou et al.2020; Li et al.2021). During the experiment, the Moso bamboo originated gene *PheNAC3* overexpression has conferred significant drought and salt tolerance to Arabidopsis. Under salt stress conditions overexpression of PheNAC3 induced faster seed germination, better seedling growth and a higher survival rate than the wild-type (Xie et al.2020). Another bamboo originated NAC members, PeSANC-*1* overexpressed plants exhibited enhanced tolerance to drought and salt stress. The transgenic rice performed far better than its wild counterpart as demonstrated in the analysis of physiological indexes such as activities of superoxide dismutase, peroxidase and catalase and contents of malondialdehyde, H_2O_2 and proline. Moreover, protein interaction analysis revealed that PeSNAC − 1 is likely to interact with stress responsive PeSNAC-2/4 and PeNAP-1/4/5 in both yeast and plant cells,

attesting the synergistic effect of those proteins in regulating the moso bamboo stress response (Hou et al. 2020). A NAC gene identified from Tartary buckwheat, FtNAC70, overexpressed in Arabidopsis has showed significant tolerance to salt and drought stress (Huang et al.2022)

In differential expression genes (DEG) analysis, larger number (204 genes) of the identified genes showed differential result among which 142 were up-regulated and 42 were down-regulated. Majority of NAC members were expressed at larger differential levels under polyethylene glycol (PEG) treated samples implying their more responsiveness to drought stress, which is consistent to the study of NAC expression profiling under abiotic stress in peanut (Li et al.2021). The expression pattern analysis of NAC gene members in Miscanthus sinensis in response to high salinity, PEG, and heavy metals, presented nearly similar results (Nie et al.2021).

5. Conclusions

From the abiotic stress induced transcriptome, this study has identified 220 OaNAC gene members with intact and complete DNA binding domains. Despite lack of references genome impaired comprehensive analysis, characterization of NACs including their functional annotation, protein property, phylogenetic relationship and expression profiling analysis was performed and helped to understand NAC transcription factors in *O. abyssinica* particularly their response associated to abiotic stress. Gene ontology analysis showed the involvement of 35 NAC members in the biological process towards stress response. Metabolic pathway analysis also predicted that large numbers NACs involved in environmental and genetic information pathways that are highly associated to stress response. Differential expression genes analysis revealed the stress responsiveness of NAC genes, as the level of expression of 142 genes was increased in response to drought and salt stress. Those OaNACs genes with the highest level of expression could serve as the basis for further functional characterization, elucidating their specific regulatory mechanisms and eventually could be applied for genetic improvement programs aiming to promote plant growth and development in the face of abiotic stress.

Declarations

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Authors contribution: MA conceived the study and general data, FA edited the manuscript KZ performed the qRT-PCR, DB supervised the work.

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Ethical approval: the article does not contain studies performed on Human or animals.

References

1. Jiang T (2019) De novo assembly and transcriptome profiling of Ethiopian lowland bamboo [Oxytenanthera abyssinica (A.Rich) Munro] under drought and salt stresses. Open Biotechnol J 13:6–17Feyissa T*)*
2. Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:1263–1274
3. Ahuja I, de Vos RC, Bones AM, Hall RD (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15:664–674
4. Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997) Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. Plant Cell 9:841–857 PMID: 9212461
5. Andrews S (2010) FastQC: A quality control tool for high throughput sequence data
6. Chen X, Wang Y, Lv B, Li J, Luo L, Lu S, Zhang X, Ma H, Ming F (2014) The NAC Family Transcription Factor OsNAP Confers AbioticStress Response through the ABA Pathway Plant Cell Physiol. 55: 604–619 (2014) doi:10.1093/pcp/pct204
7. Fan C, Ma J, Guo Q, Li X, Wang H, Lu M (2013) Selection of Reference Genes for Quantitative Real-Time PCR in Bamboo (*Phyllostachys edulis*). PLoS ONE 8(2):e56573
8. Grabherr MG, Haas BJ, Yassour M (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 2011; 29(7): 644 – 52
9. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci USA 103:12987–12992
10. Fu L, Niu B, Zhu Z, Wu S, Li W (2012) CD-HIT: Accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152
11. Hou D, Qiutao H, Naresh V, Wei Z, Xinchun L (2020) Zhongyu Z, Qiutao H, Ling L, Naresh V, Juan Z, Wei Z, Aimin W, Xinchun L *() PeSNAC-1 a NAC transcription factor from moso bamboo (Phyllostachys edulis) confers tolerance to salinity and drought stress in transgenic rice*
12. Huang J, Ren R, Rong Y, Tang B, Deng J, Chen Q, Shi T (2022) Identification, Expression, and functional Study of Seven NAC Transcription Factor Genes Involved in Stress Response in Tartary Buckwheat (Fagopyrum tataricum (L.) Gaertn.). Agronomy 12:849. https://doi.org/10.3390/
13. Hu R, Qi G, Kong Y, Kong D, Gao Q, Zhou G (2010) Comprehensive analysis of NAC domain transcription factor gene family in populus trichocarpa. BMC Plant Biol 10:145
14. Inada T, Hall JB (2008) *Oxytenanthera abyssinica* (A. Rich). Munro. Record from Protabase
15. Kanehisa M, Sato Y, Morishima K (2015) BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. Databases

16. Joshi NA, Fass JN (2011) Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ fles 201h1t.tp://www.citeulike.org /user/mvermaat/article/13260426
17. Li B, Hu SL, Cao Y, Gang X (2015) Bioinformatics analysis of NAC gene family in moso bamboo. Genomics and Applied Biology 34(8):1769–1777. Doi 10.13417/j.gab.034.001769
18. Li P, Peng Z, Xu P, Tang G, Ma C, Zhu J, Shan L, Wan S (2021) Genome-Wide dentification of NAC Transcription Factors and Their Functional Prediction of Abiotic Stress Response in Peanut. Front Genet 12:630292. doi: 10.3389/fgene.2021.630292
19. Liu T, Song X, Duan W, Huang Z, Liu G, Li Y, Hou X (2014) Genome-wide analysis and expression patterns of NAC transcription factor family under different developmental stages and abiotic stresses in Chinese cabbage. Plant Mol Biology Report 32:1041–1056
20. Li W, Zeng Y, Fangliu Yin F, Wei R, Xiaofei Mao X (2021) Genome–wide identification and comprehensive analysis of the NAC transcription factor family in sunflower during salt and drought stress. Sci Rep 11:19865. https://doi.org/10.1038/s41598-021-98107-4
21. Mao X, Zhang H, Qian X, Li A, Zhao G, Jing R (2012) TaNAC2, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in *Arabidopsis*. J Exp Bot 63:2933–2946
22. Muller M, Seifert S, LuÈbbe T, Leuschner C, Finkeldey R (2017) *De novo* transcriptome assembly and analysis of differential gene expression in response to drought in European beech. PLoS ONE 12:e0184167. https://doi.org/ 10.1371/journal.pone.0184167
23. Nie G, Yang Z, He J, Liu A, Chen J, Wang S, Wang X, Feng G, Li D, Peng Y, Huang L, Zhang X (2021) Genome-Wide Investigation of the NAC Transcription Factor Family in Miscanthus sinensis and Expression Analysis Under Various Abiotic Stresses. Front. Plant Sci 12:766550. doi: 10.3389/fpls.2021.766550
24. Nuruzzaman M, Manimekalai R, Sharoni AM, Satoh K, Kondoh H, Ooka H, Kikuchi S (2010) Genome-wide analysis of NAC transcription factor family in rice. Gene 465:30–44
25. Olsen AN, Ernst HA, Leggio LL, Skriver K (2005) NAC transcription factors: structurally distinct, functionally diverse. Trends Plant Sci 10:79–87
26. Ooka H et al (2003) Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. DNA Res 10:239–224
27. Puranik S, Sahu PP, Mandal SN, Parida SK, Prasad M (2013) Comprehensive genome-wide survey, genomic constitution and expression profiling of the NAC transcription factor family in foxtail millet (Setaria italica L. PLoS ONE 8:e64594
28. Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: regulation and role in stress tolerance. Trends Plant Sci 17:369–381
29. Singh AK, Sharma V, Pal AK, Acharya V, Ahuja PS (2013) Genome-wide organization and expression profiling of the NAC transcription factor family in potato *(Solanum tuberosum L.)* DNA. Research 20:403–423
30. Babu RN (2018) *Genome Wide Analysis of NAC Transcription Factors and their Expression Pattern During High Temperature and Drought Stress in Groundnut.* African Crop. Sci J 26:327–348Devaraj

VR*)*

31. Tamura K, Stecher G, Kumar S (2021) MEGA 11: Molecular Evolutionary Genetics Analysis. Version 11. Molecular Biology and Evolution
32. Tran L-SP, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of Arabidopsis stress inducible NAC transcription factors that bind to a drought-responsive cis-element in the early responsive to dehydration stress 1 promoter. Plant Cell 16:2481–2498
33. Tran LSP, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K (2004) Isolation and functional analysis of Arabidopsis stress-inducible NAC transcription factors that bind to a drought-responsive cis-element in the early responsive to dehydration stress1 promoter. Plant Cell 16:2481–2498
34. Wang N, Zheng Y, Xin H, Fang L, Li S (2013a) Comprehensive analysis of NAC domain transcription factor gene family in *Vitis vinifera*.. Plant Cell Rep 32:61–75
35. Wei S, Gao L, Zhang Y, Zhang F, Yang X, Huang D (2016) Genome-wide investigation of the NAC transcription factor family in melon (*Cucumis melo L.)* and their expression analysis under salt stress. Plant Cell Rep 35:1827–1839
36. Xie L, CaiM, Li X, Zheng H, Xie Y, Cheng Z, Bai Y, Li J, Mu S, Gao J (2020) Overexpression of PheNAC3 from moso bamboo promotes leaf senescence and enhances abiotic stress tolerance in Arabidopsis. PeerJ 8:e8716. DOI 10.7717/peerj.8716
37. Yang R, Deng C, Ouyang B, Ye Z (2011) Molecular analysis of two salt-responsive NAC-family genes and their expression analysis in tomato. Mol Biol Rep 38:857–863
38. Lu Ch (2014) CELLO2GO: A Web Server for Protein Sub-Cellular Localization Prediction with Functional Gene Ontology Annotation. Plos ONE 9(6):E99368Hwang JChang Kc, Huang S*)*
39. Zheng Y, Jia C, Sun H (2016) **iTAK**: a program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein Kinases. Mol Plant 9:1667–1670

Figures

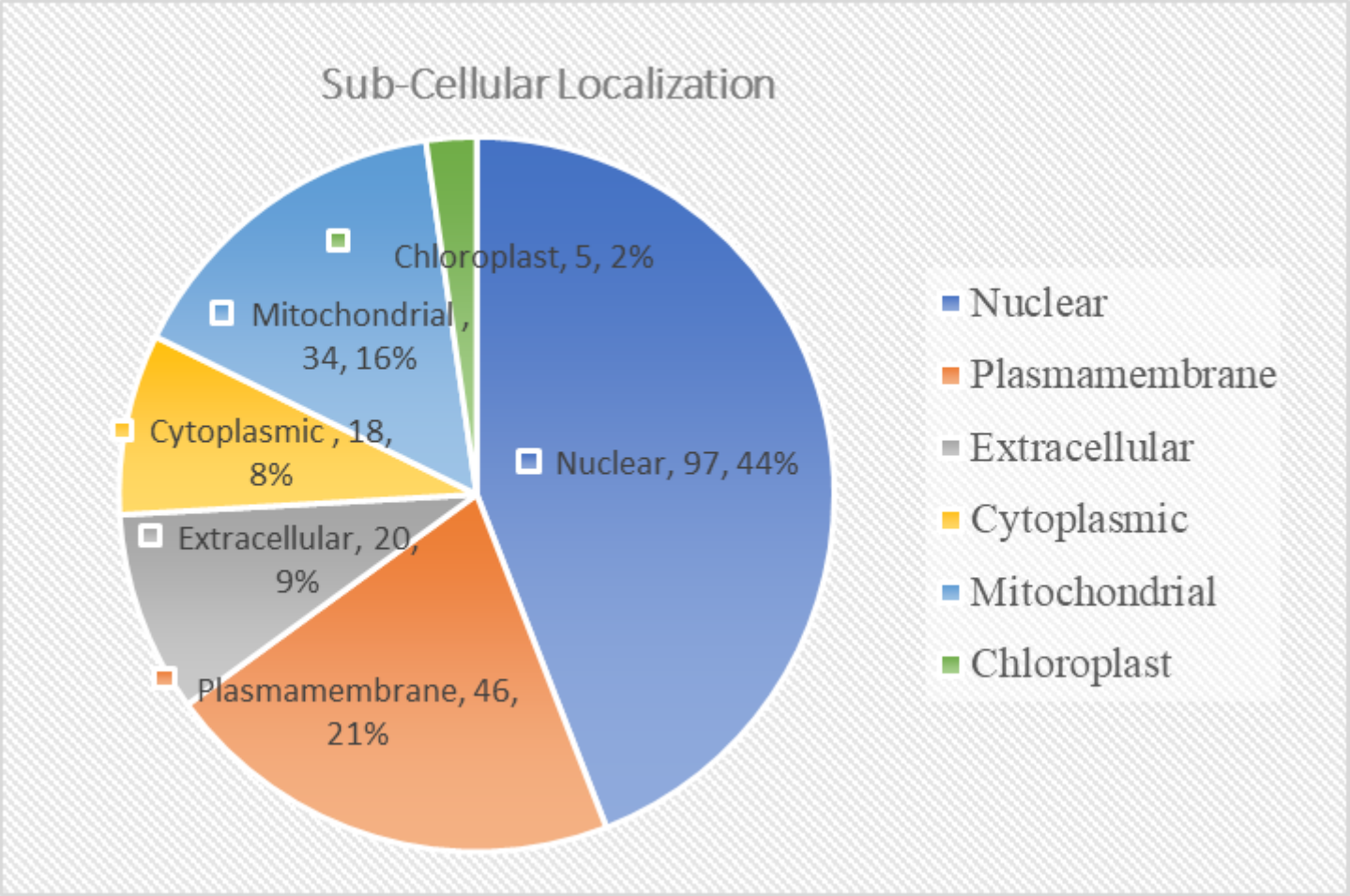


Figure 1

Sub-cellular localization of 220 proteins of NAC transcription factor family.

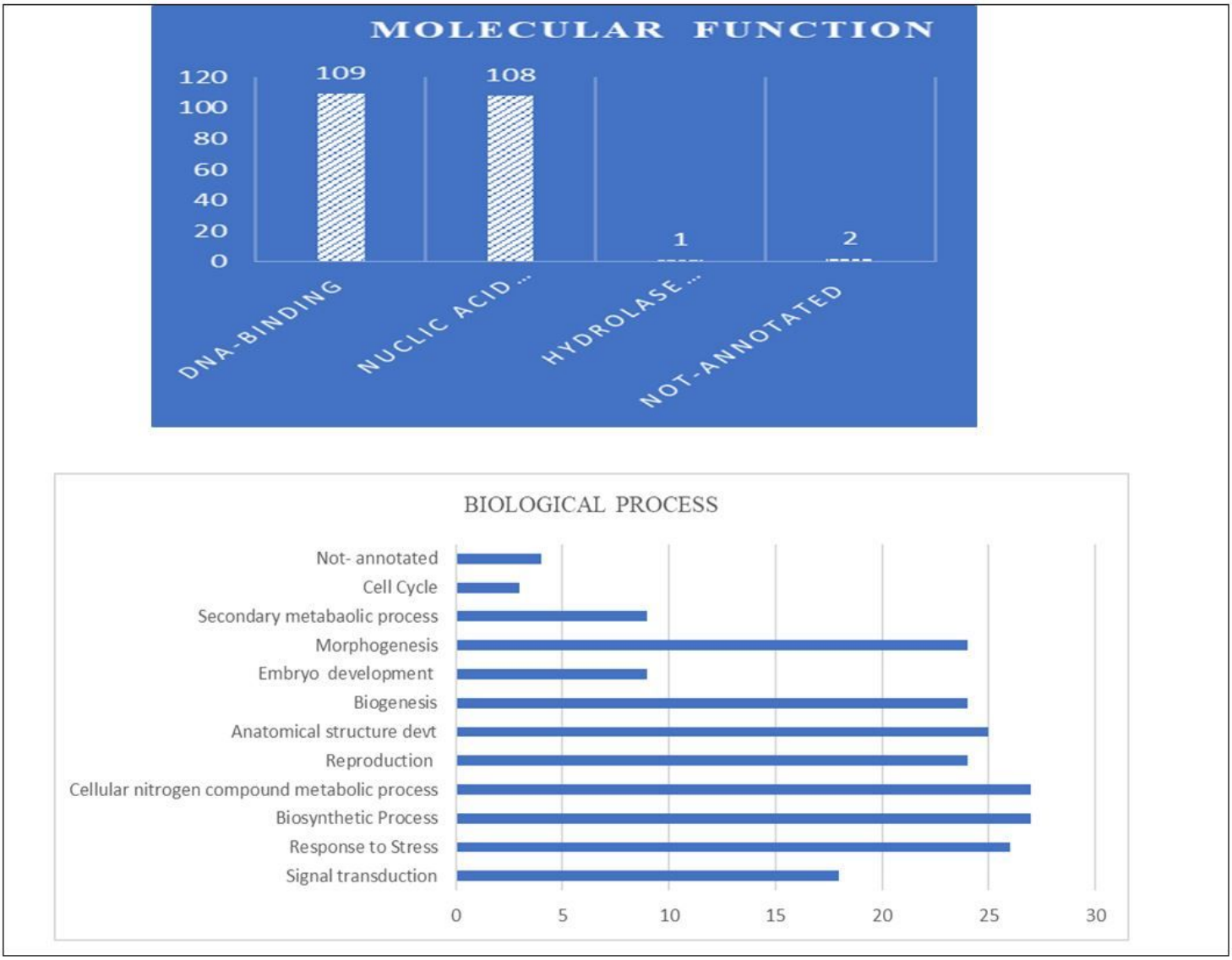


Figure 2

Gene ontology classification of 220 NAC transcript factors

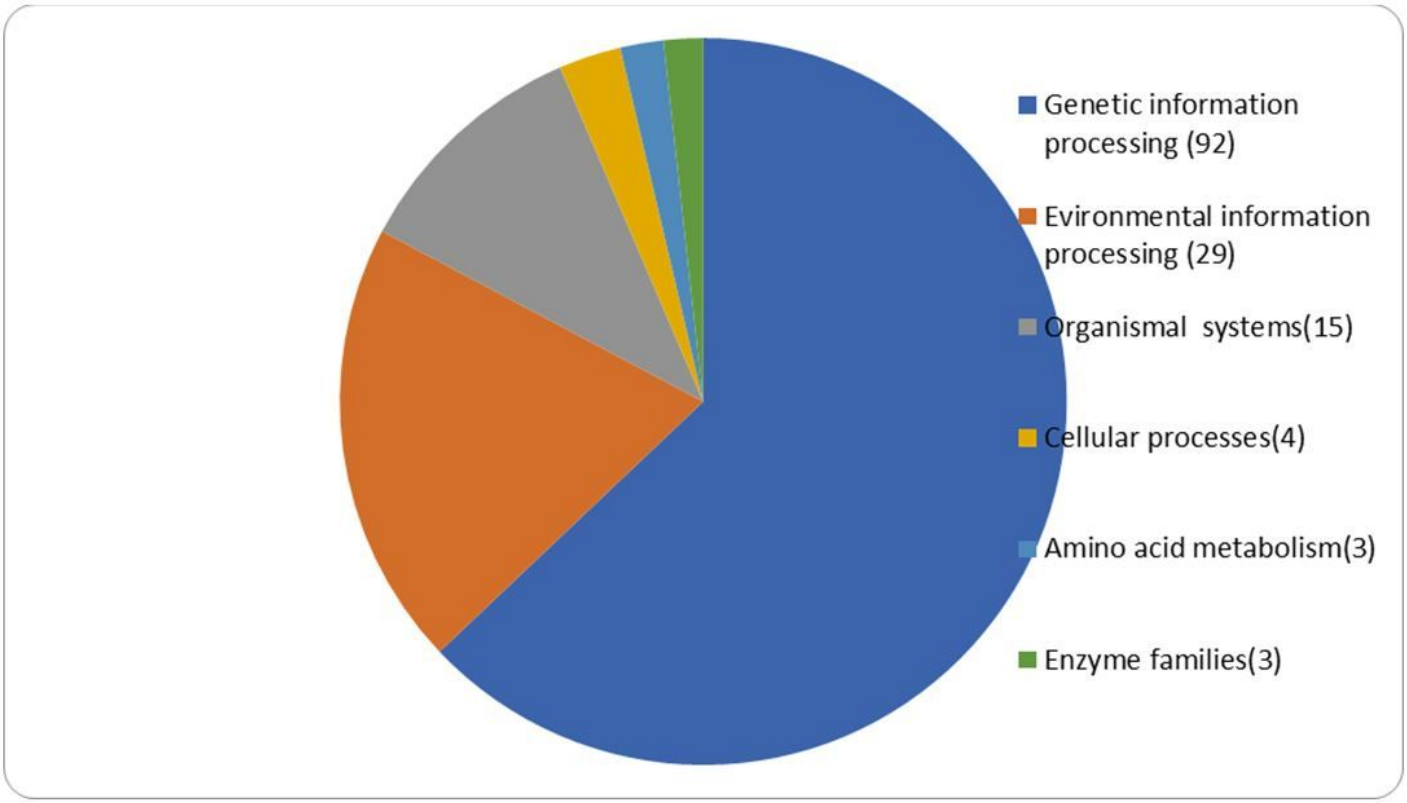


Figure 3

KEGG analysis using the Blast KOALA tool, among the 220 unigenes only 146 (66.36 %) were annotated.

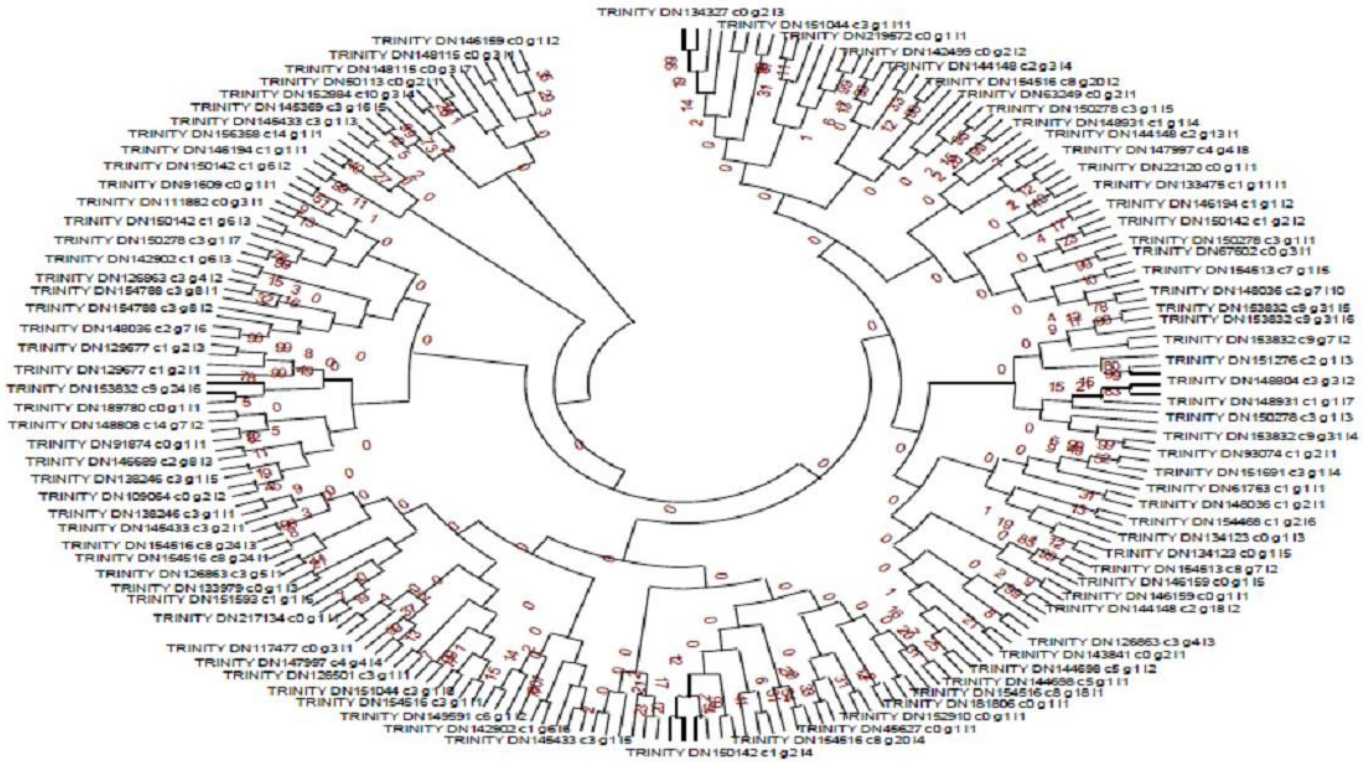


Figure 4

Phylogenetic tree of 220 aligned NAC gene family proteins in *O. abyssinica* constructed using MEGA 11. The evolutionary history was inferred using the Neighbor-Joining method.

Supplementary Files

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- OaNACaminoacidsequences.txt