

Article

Comprehensive Identification and Expression Profiling of the NAC Family During Female Cone Development in *Torreya grandis*

Long Wang ¹, Chang Chen ¹, Meiyong Liu ¹, Wenfei Bi ¹, Su Li ¹, Xiong Zhang ^{2,*} and Tong Han ^{1,*}

¹ College of Advanced Agricultural Sciences, Weifang University, Weifang 261061, China; 20210013@wfu.edu.cn (L.W.); chen1064963455@163.com (C.C.); liumeiyong0919@163.com (M.L.); bwf1325533@163.com (W.B.); ls17386546434@163.com (S.L.)

² Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China

* Correspondence: zhangxiong@caas.cn (X.Z.); 20210031@wfu.edu.cn (T.H.)

Abstract

NAC transcription factors are key regulators involved in diverse cellular processes, stress responses, and developmental pathways in plants. However, their roles in female cone development of *Torreya grandis*, a representative gymnosperm species, remain largely unexplored. In this study, we performed a comprehensive identification and analysis of NAC transcription factors in *T. grandis* to investigate their potential functions in female cone development. A total of 82 TgNAC members containing conserved NAM domains were identified, distributed unevenly across 11 chromosomes. Phylogenetic analysis with *Arabidopsis* NACs classified them into 15 groups, with TgNACs represented in 10 groups and showing a notable enrichment in the TERN clade on chromosome 2. Promoter cis-element analysis revealed correlations between regulatory elements and expression patterns. Tissue-specific expression profiling indicated clear functional specialization, with some TgNACs showing no detectable expression in the examined tissues. During female cone development, several TgNACs were highly expressed in the early stages, whereas TgNAC72, TgNAC76 and TgNAC82 were upregulated during the latter stages. Among these, TgNAC72 exhibited the highest overall expression level. Subcellular localization confirmed TgNAC72 is localized in the nucleus. Dual-luciferase assays further demonstrated that TgNAC72 activates the *TgBGLU13* promoter, suggesting its role in starch and sucrose metabolism. Collectively, these findings provide novel insights into the regulatory involvement of TgNACs in reproductive organ development.

Keywords: transcription factors; cis-elements; plant reproductive organs



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1. Introduction

NAC proteins represent one of the largest families of plant-specific transcription factors, first identified in 1996 [1]. The name “NAC” is derived from three initially discovered members: the NAM gene in petunia, and ATAF1, ATAF2, and CUC2 in *Arabidopsis thaliana* [2]. Since then, the NAC family have been extensively characterized across numerous plant species, including 157 in maize and 152 in soybean [3–5]. The structural characteristics of NAC proteins is closely related to their diverse functions. The N-terminal region is highly conserved, comprising approximately 150 amino acids that form the NAC domain, which can be subdivided into five conserved subdomains (A–E) [5,6]. This domain

is responsible for DNA binding and for mediating homo- and heterodimerization, processes that may involve salt bridge formation. In addition, the positively charged surface of the NAC domain facilitates DNA–protein interactions. In contrast, the C-terminal region is highly variable and functions as the transcriptional regulatory domain. It is typically enriched in simple amino acid repeats and contains residues such as serine, threonine, proline, glutamine, or acidic amino acids, which contribute to the functional diversity of NAC [7–9].

Numerous studies have elucidated the diverse roles of NAC in plant development throughout the plant life cycle, such as embryogenesis, root development, shoot and leaf development, floral development and flowering, fruit ripening, and seed development [5,10]. For example, in *Arabidopsis*, the *nars1/nars2* double-mutant exhibits abnormal seed morphology and delayed integument degeneration, indicating that NARS1/NAC2 and NARS2/NAM are crucial for integument development [11]. NAC087 regulates programmed cell death to control lateral root cap size [12]. In banana, MaNAC1 and MaNAC2 regulate ethylene biosynthesis during the climacteric fruit ripening [13]. In maize, ZmNAC11 and ZmNAC29 promote nucellar tissue degeneration, thereby enhancing kernel size and weight [14]. In rice, OsNAC3 modulates seed germination by influencing multiple hormone pathways, including abscisic acid, and gibberellin [15]. In soybean, GmNAC81 participates in developmentally programmed leaf senescence [16]. Beyond development, NAC proteins also play critical roles in plant responses to abiotic stresses and biotic stresses [5,17]. For example, in maize, ZmNAC84 obviously enhances the drought and salt response by improving the antioxidant enzymes activities [18,19]. In *Arabidopsis*, NAC056 improves freezing tolerance by upregulating CBF-signaling-pathway-related genes [20], while in apple, MdNAC104 obviously enhances cold tolerance [21].

Torreya grandis, a member of the genus *Torreya* within the family Taxaceae of gymnosperms, is a rare gymnosperm producing dry fruits, and is endemic to southern China [22,23]. It has high nutritional, medicinal, and multi-purpose developmental value. Its nuts are rich in functional compounds, including lipids, and fatty acids, which confer both nutritional and pharmacological benefits [24,25]. *T. grandis* is a dioecious species, with male and female cones borne on separate individuals, and relies on wind for pollination. After pollination, female cones undergo an extended developmental phase in which the ovular integuments gradually form a fleshy aril, ultimately producing a drupe-like fruit. The developmental cycle includes a winter dormancy period lasting 5–6 months, followed by rapid fruit expansion from April to May of the following year [26]. However, this prolonged process results in more than 80% of female cones fail to mature properly each year, leading to a very low fruit set rate [27–29]. This severely restricts both natural population propagation and agricultural productivity. Therefore, elucidating the key genes involved in female cone development and their molecular regulatory networks is essential for optimizing reproduction, promoting vigorous growth, and enhancing yield in *T. grandis*.

Here, we identified NAC genes in the *T. grandis* genome to explore their potential regulatory roles in female cone development. A comprehensive analysis of TgNAC genes was conducted, including their chromosomal localization and other features. The dynamic expression patterns of TgNAC genes at different developmental female cones were performed to unravel the key candidates potentially involved in cone development. In addition, expression correlation analyses and dual-luciferase reporter assays were conducted to validate potential target genes and to preliminarily construct the TgNAC72-mediated regulatory network involved in female cone development. Collectively, this study provides a theoretical framework for understanding the molecular mechanisms underlying female cone development in *T. grandis*.

2. Materials and Methods

2.1. Identification and Structural Analysis of NAC Family

To identify NAC genes in *Torreya grandis* genome, the hidden Markov model (HMM) profile of the NAM domain (PF02365) from PFAM and the protein dataset of *T. grandis* were used to search for candidate NAC family members with the HMMER search tool integrated in TBtools II. Candidate sequences were then filtered using a threshold set at $p\text{-value} < 10^{-5}$ and verified through domain analysis using NCBI Conserved Domain Database (NCBI-CDD), resulting in the identification of 82 putative NAC genes.

Conserved motifs and domains of these NAC proteins were subsequently conducted using MEME Suite (v5.5.8) and NCBI-CDD, respectively, with default parameters [30]. Gene structures and chromosomal locations on the 11 chromosomes of *T. grandis* were extracted from the genome GFF file, and all results were visualized with TBtools II (v2.330) [31].

2.2. Phylogenetic Analysis

First, the amino acid sequences of 105 NACs from *Arabidopsis thaliana* were obtained from the Plant Transcription Factor Database [32]. These sequences were combined with the 82 TgNAC protein sequences identified in *T. grandis*, and the combined sequence set was aligned using MAFFT (v7.526) [33]. A maximum likelihood phylogenetic tree for these NACs was constructed using IQ-TREE (v2.2.0) with default parameters and 1000 bootstrap replicates [34].

2.3. Expression Analysis of NAC Genes in *T. grandis*

To investigate the expression patterns of TgNAC genes, previously reported raw RNA-Seq data [29] were retrieved from the China National GeneBank DataBase. These data provided the basis for analyzing transcriptional levels of the identified TgNAC genes across different tissues and developmental stages. The FPKM values of these NAC genes in the transcriptome were normalized and then visualized using a heatmap.

2.4. Promoter Bioinformatics Analysis

To detect potential cis-acting regulatory elements, the 2000 bp promoter regions of these TgNAC genes were first extracted based on their chromosomal locations annotated in the GFF file of *T. grandis*. Subsequently, these retrieved promoter sequences were subjected to analysis using the Plant CARE (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>, accessed on 24 September 2025). Finally, the distribution and types of cis-elements identified in the promoter regions were visualized and presented using TBtools II [31].

2.5. Subcellular Localization

The CDS of TgNAC72 was cloned into the plant expression vector pCambia1300, generating a TgNAC72-GFP fusion construct. The TgNAC72-GFP vector was then transformed into *Agrobacterium* GV3101. Subsequently, the infiltration into tobacco leaves was performed following the method described by [35]. At 3 days post-infiltration, GFP fluorescence signals were examined in epidermal cells using a confocal laser scanning microscope (Leica TCS SP8, Wetzlar, Germany), with excitation at 488 nm and emission collected between 500 and 530 nm.

2.6. Luciferase (LUC) Assays in Tobacco

First, the coding sequence of TgNAC72 was constructed into pGreen II-62SK, while the 2000 bp promoter sequence upstream of the target gene was inserted into pGreen II-0800-LUC. Subsequently, *Agrobacterium* containing the two plasmids was injected into tobacco

leaves as described in Yan et al. [25]. After three days of post-infiltration, leaves from the injection sites were homogenized with plant cell lysis buffer. From the lysate, a 100 μ L aliquot was combined with 100 μ L of firefly luciferase substrate, and luminescence was recorded using a GloMax 20/20 detector (Promega, Fitchburg, WI, USA). The same sample was then supplemented with 100 μ L of Renilla luciferase substrate, and the luminescence was measured again. The ratio of firefly to Renilla signals was calculated.

2.7. Statistical Analysis

All experiments were conducted with three independent biological replicates. Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using SPSS Statistics 20.0. Different letters indicate a significant difference compared with the control as determined by one-way analysis of variance (ANOVA) at $p < 0.05$.

3. Results

3.1. Identification and Structure of NAC Family in *T. grandis*

A total of 82 NAC family members were identified in *T. grandis* using an HMMER search with the NAM domain (PF02365) model against the *T. grandis* protein dataset, followed by filtering with a threshold of p -value $< 10^{-5}$ and validation via NCBI-CDD domain analysis (Table S1). Conserved motif analysis with MEME Suite revealed five conserved motifs in TgNAC proteins. Among them, motifs 1–3 and 5 were widely distributed across most members and were therefore inferred to represent components of the core NAC domain (Figure 1A). This inference was further supported by NCBI-CDD domain analysis, which confirmed that all 82 TgNAC proteins contained the characteristic NAM domain (PF02365) with high sequence conservation (Figure 1B).

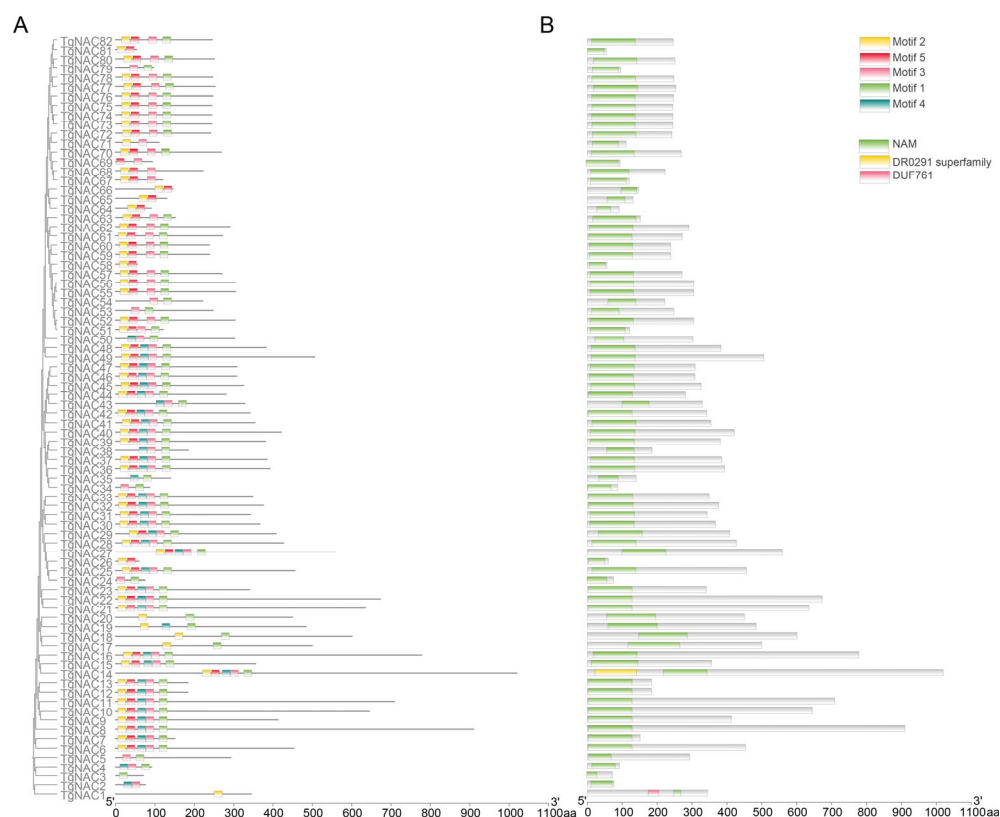


Figure 1. Protein motifs and gene structure and of NAC family in *T. grandis*. (A) The phylogenetic relationship (constructed using the maximum likelihood method) and motif distribution of the NAC family. (B) The conservative structural domains of NAC family.

Gene structure analysis showed that *TgNACs* varied in exon number, ranging from 1 to 8, with corresponding intron numbers ranging from 0 to 7 (Figure 1). The 82 *TgNACs* are distributed across all 11 chromosomes of *T. grandis*, but their distribution is highly uneven. For example, chromosome 7 (chr 7) and chromosome 4 (chr 4) contained only *TgNAC4* and *TgNAC33*, respectively, whereas chromosome 2 (chr 2) harbored 32 NAC members, which were clustered in distribution (Table S2) (Figure 2).

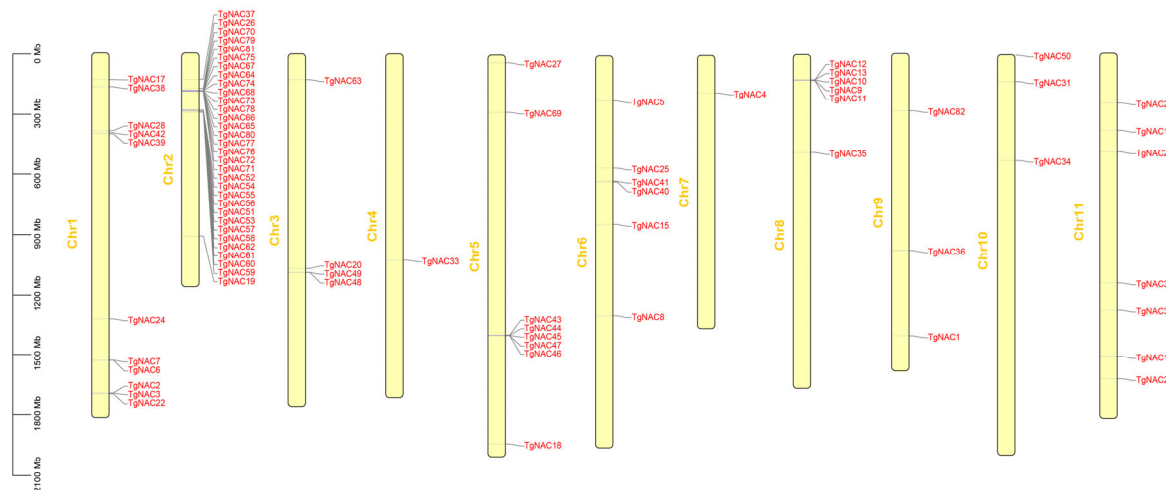


Figure 2. Chromosomal localization of NAC family in *T. grandis*. Each vertical bar represents a chromosome of *T. grandis*, labeled as chr1–chr11 (where “chr” is the abbreviation of “chromosome”).

3.2. Phylogenetic Analysis of NAC Family

To clarify the evolutionary relationships within the NAC family, NAC proteins of *A. thaliana*, which have been well classified in previous studies, were incorporated as reference sequences for phylogenetic tree analysis. The full-length amino acid sequences of 82 NACs from *T. grandis* and 105 NACs from *A. thaliana* were aligned using MAFFT. As shown in Figure 3, these NAC sequences were grouped into 15 clusters in the phylogenetic tree. *TgNACs* were distributed across 10 clusters, with the exception of the TIP, ANAC001, SENU5, ANAC063, ANAC011, and NAC1 clusters. Notably, a large number of *TgNACs* were grouped within the TERN cluster, which contained only two *AtNACs*. Interestingly, most of these *TgNACs* were located on chromosome 2.

3.3. Tissue-Specific Expression Analysis of NAC Family in *T. grandis*

To investigate the potential biological functions of *TgNACs*, their expression patterns were analyzed across five tissues of *T. grandis*, including roots, stems, leaves, seeds, and arils, using RNA sequencing data. As shown in Figure 4A, 18 *TgNACs*, including *TgNAC78*, *TgNAC79*, and *TgNAC81* were not expressed in any of the examined tissues. Several members, such as *TgNAC7*, *TgNAC9*, *TgNAC33*, and *TgNAC19*, exhibited high expression levels in roots, whereas *TgNAC40*, *TgNAC63*, *TgNAC74*, and *TgNAC71* showed preferential expression in arils. Distinct sets of *TgNACs* also displayed elevated expression in stems, leaves, and seeds, respectively.

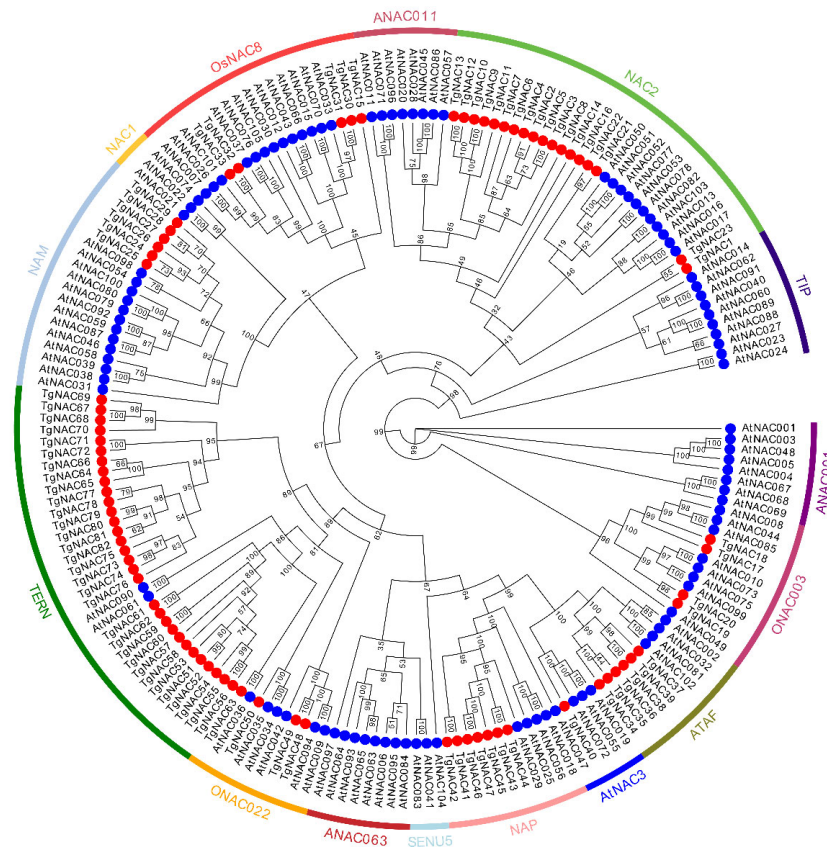


Figure 3. Phylogenetic relationships among NAC members in *T. grandis* and *A. thaliana*. In the tree, red circles represent NAC proteins derived from *T. grandis*, while blue circles indicate those originating from *A. thaliana*.

3.4. Expression Analysis of NAC Family During Female Cone Development in *T. grandis*

T. grandis is a dioecious plant, and the development of male cones before pollination is critical for successful pollination. Transcriptome data from five developmental stages of female cone development were used for the analysis, which were categorized into three key phases: the initial stages (CH1–CH2), the middle stages (CH3–CH4), and the late stage (CH5). As shown in Figure 4B, several *TgNACs*, including *TgNAC54*, *TgNAC55*, *TgNAC51*, and *TgNAC3*, were not expressed at any stage of female cone development. In contrast, *TgNAC45*, *TgNAC39*, and *TgNAC48*, showed elevated expression during the initial stages of female cone development (CH1–CH2), whereas *TgNAC72*, *TgNAC76*, *TgNAC82*, and *TgNAC66* showed high expression at the late stage of female cone development (CH5). To further identify *TgNACs* potentially involved in late-stage development, we examined the expression levels of the four CH5-enriched members (*TgNAC72*, *TgNAC76*, *TgNAC82*, and *TgNAC66*). Among these, *TgNAC72*, *TgNAC82*, and *TgNAC66* showed significant upregulation: their expression levels at CH5 were more than 2-fold higher than those at the preceding CH4 stage, showing fold changes of 2.37, 2.98, and 2.17, respectively. Notably, *TgNAC72* had the highest overall expression level among these upregulated members, suggesting it acts as a potential key regulator in the late-stage development of *T. grandis* female cones.

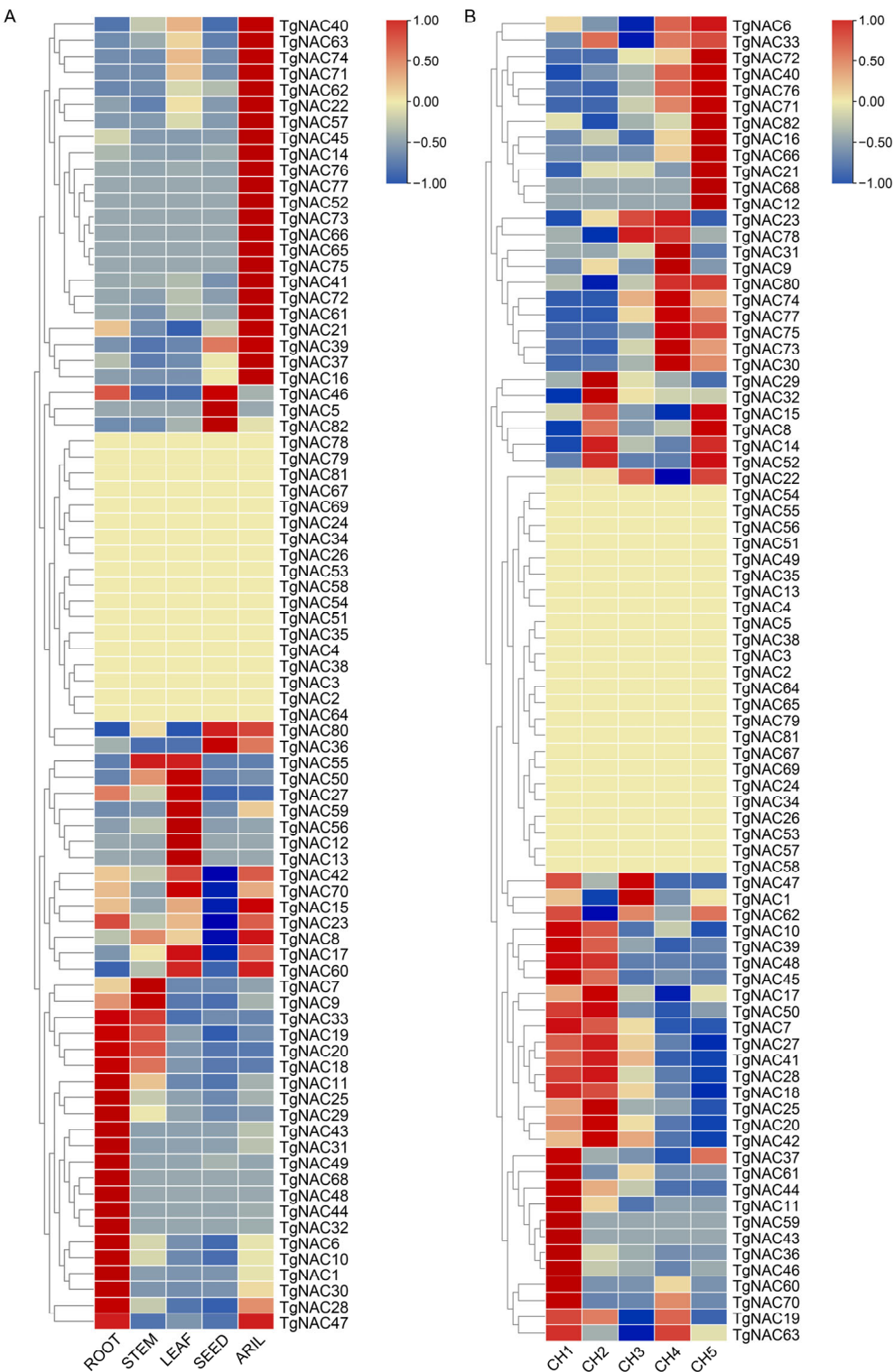


Figure 4. Expression patterns of TgNAC members in *T. grandis*. (A) Heatmap showing the expression levels of TgNAC genes in different tissues of *T. grandis*, including root, stem, leaf, seed, and aril. (B) Heatmap illustrating the expression levels of TgNAC genes in different developmental stages of female cones of *T. grandis*. CH: Cone development.

3.5. Cis-Element Analysis of NAC Family in *T. grandis*

Cis-acting elements in the promoter regions of *TgNAC* genes were identified using PlantCARE to investigate the regulatory mechanisms underlying *TgNAC*s expression. As shown in Figure 5, multiple functional cis-elements were detected in the promoter of *T. grandis* *TgNAC* genes, with considerable variation in both type and number among members. Many light-responsive cis-elements were distributed in the promoters of these *TgNAC*s. Specifically, *TgNAC7*, *TgNAC20*, and *TgNAC25*, previously characterized as root-highly expressed members, contained abscisic acid-responsive and salicylic acid-responsive, in their promoters (Figure 5). In members highly expressed in arils, such as *TgNAC40*, *TgNAC62*, and *TgNAC63*, the promoters harbored MeJA- and gibberellin-responsive elements (Figure 5). By contrast, *TgNAC38*, *TgNAC3*, and *TgNAC26*, which showed no expression in roots, stems, leaves, seeds, or arils, contained relatively few cis-elements, with only a small number of light-responsive motifs. Collectively, these results indicate a correlation between the cis-element composition in *TgNAC* promoters and the expression patterns of *TgNAC*s.

3.6. Functional Characterization of *TgNAC72* During Female Cone Development

Given that NAC proteins act as transcription factors in plants, we first examined the subcellular localization of *TgNAC72*. A fusion construct was generated by linking the CDS of *TgNAC72* to *GFP*, and this construct was co-expressed with a nuclear marker in tobacco leaf epidermal cells via *Agrobacterium*-mediated transformation. As shown in Figure 6, confocal microscopy revealed that the fluorescent signal of the *TgNAC72*-GFP fusion protein was strongly concentrated in the nucleus and completely overlapped with the nuclear marker. This co-localization pattern confirms that *TgNAC72* is localized to the nucleus in tobacco leaf cells.

To further identify potential downstream target genes of *TgNAC72* during female cone development, the cluster analysis on the expression patterns of all genes involved in female cone development was analyzed using the mfuzz method. As shown in Figure 7A, the genes in Cluster 1, to which *TgNAC72* belongs, exhibited a consistent upregulation trend during female cone development. KEGG enrichment analysis of this cluster revealed significant representation in several biological pathways, including brassinosteroid biosynthesis (ko00905), circadian rhythm (ko04712), starch and sucrose metabolism (ko00500), and fatty acid elongation (ko00062) (Figure 7B). Because carbohydrate metabolism is closely associated with reproductive organ maturation [27], we focused on genes within the starch and sucrose metabolism pathway. Notably, two β -glucosidase genes (*TgBGLU13* and *TgBGLU15*), both belonging to Cluster 1, were progressively upregulated during cone development. Given the known roles of β -glucosidases in carbohydrate hydrolysis and reproductive development, these genes were considered putative targets of *TgNAC72*. To test this possibility, we performed a dual-luciferase assay. The results demonstrated that *TgNAC72* significantly enhanced luciferase activity driven by the *TgBGLU13* promoter in tobacco leaves (Figure 8), indicating that *TgNAC72* may directly regulate β -glucosidase expression during cone maturation.



Figure 5. Distribution of cis-acting elements in the promoters of *TgNAC* members in *T. grandis*. Promoters were defined as the 2000 bp upstream of the transcription start site. Different colors represent different types of responsive elements.

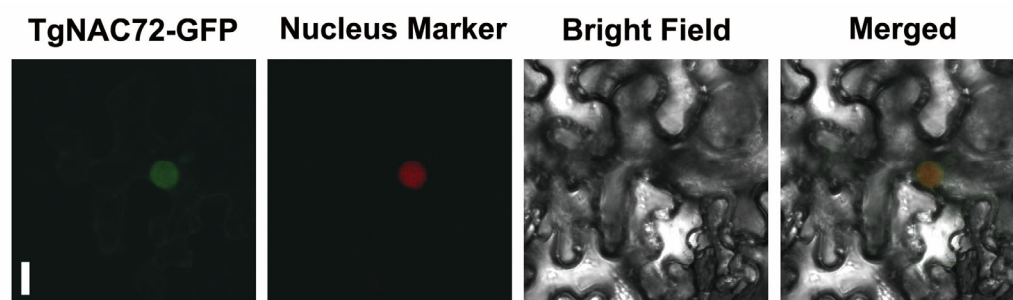


Figure 6. Subcellular localization of TgNAC72 protein in tobacco cells. Tobacco cells were co-transfected with GFP-TgNAC72 and a nuclear marker vector. Scale bar = 10 μ m.

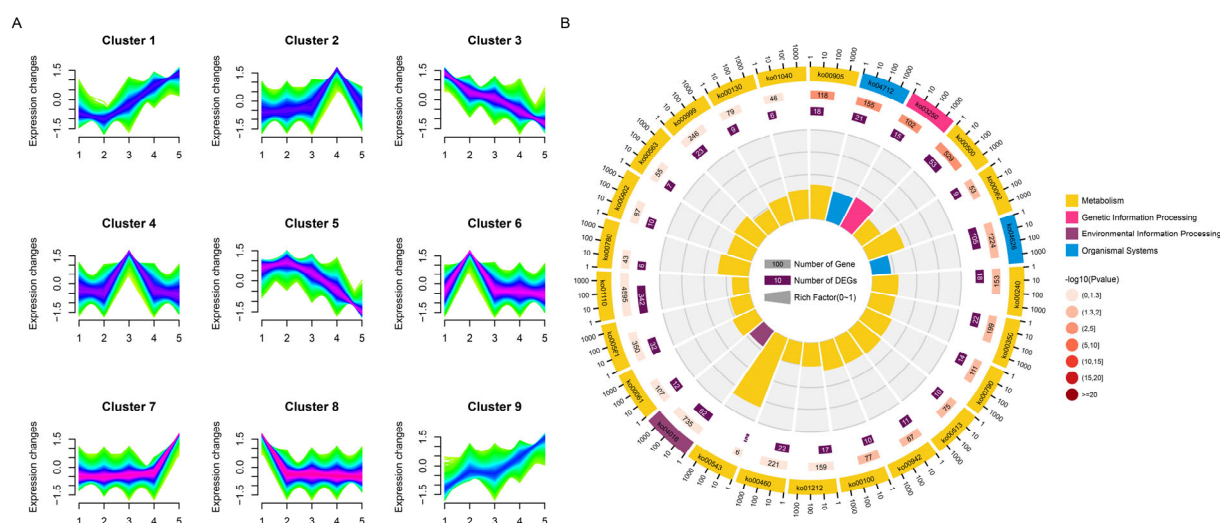


Figure 7. Co-expression gene analysis of TgNAC72 during female cone development of *T. grandis*. (A) All gene expression analyses in this co-expression study were performed using mfuzz. (B) KEGG pathway enrichment analysis was performed on genes in Cluster 1, where TgNAC72 is located. This cluster was generated using the mfuzz, as the genes within it exhibit similar expression trends to TgNAC72 and therefore were clustered together.

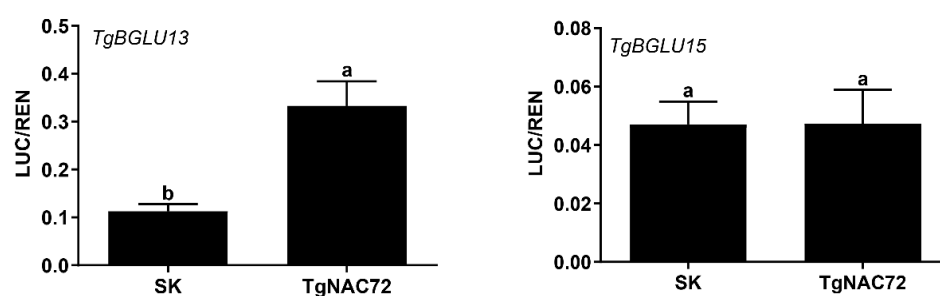


Figure 8. Screening of potential downstream target genes of TgNAC72 in *T. grandis*. Data are presented as mean $\pm$ standard deviation (SD) based on three biological replicates ($n = 3$). Different letters indicate a significant difference compared with the control, as determined by one-way analysis of variance (ANOVA) at $p < 0.05$.

4. Discussion

NAC transcription factors are key regulators of diverse biological processes underlying plant growth and development [8,9]. Genome-wide analyses of the NAC family have been performed in many plant species, but most studies have focused on angiosperms [5,36]. In contrast, systematic investigations on gymnosperms remain scarce, particularly regarding family size and structural characteristics.

In this study, we identified 82 TgNAC genes, all containing conserved NAM domains and core motifs (Figure 1). Gene structure and chromosomal distribution analysis revealed that these genes not only retain the conservation typical of the NAC family, but also exhibit species-specific expansion, as reflected by the clustered aggregation of many members on chromosome 2. Phylogenetic comparison with *Arabidopsis* further showed that the *T. grandis* NACs are grouped into 15 clades, covering 10 of them, with marked enrichment in the TERN clade, but lacking several clades present in *Arabidopsis* (Figure 3). This pattern suggests that the NAC family in *T. grandis* has undergone adaptive evolution, acquiring both conserved functions and species-specific features. Notably, the strong concentration of TgNACs on chr 2 implies that this region may have experienced unique evolutionary events that facilitated the expansion of TERN-clade members. Alternatively, it may indicate that TERN-related functions are particularly important for the adaptive evolution of *T. grandis*, leading to their preferential retention on chromosome 2. Taken together, these findings provide strong evidence for lineage-specific diversification of NACs in *T. grandis*, highlighting that this family is not only widely present, but is also likely to exhibit considerable functional diversity, thereby laying a solid foundation for subsequent functional studies.

The NAC family is broadly associated with structural features and functional diversity [17]. Functional divergence of NAC genes is closely linked to their tissue-specific expression during plant development [5]. In this study, tissue expression profiling of the NAC family in *T. grandis* revealed distinct expression patterns across roots, stems, leaves, seeds, and arils. For instance, TgNAC7 and TgNAC9 were predominantly expressed in roots, whereas TgNAC40 and TgNAC63 were highly expressed in arils. These results are consistent with finding in *Arabidopsis* and maize, where NAC genes regulate tissue-specific processes such as root and crown development and grain size determination [12,37]. These results indicate that TgNACs have evolved tissue-specific expression patterns, suggesting their functional specialization in regulating different biological processes associated with specific tissues in *T. grandis*. Promoter cis-element analysis showed that the promoters of root-expressed genes were enriched in abscisic acid- and salicylic acid-responsive elements, as well as stress-related motifs, whereas those highly expressed in arils contained methyl jasmonate- and gibberellin-responsive elements. This correlation between cis-element composition and tissue expression provides insights into the regulatory mechanisms underlying TgNAC expression.

During female cone development in *T. grandis*, dynamic expression patterns of TgNACs indicate stage-specific roles in reproductive regulation. Among them, TgNAC72 was significantly upregulated during maturation and localized to the nucleus (Figures 4B and 6). Dual-luciferase assays further demonstrated that TgNAC72 activates the expression of the β -glucosidase gene (*TgBGLU13*) (Figure 8). Together with the enrichment of starch and sucrose metabolism pathways in KEGG analysis, these findings suggest that TgNAC72 promotes female cone maturation by regulating carbohydrate metabolism. This parallels the roles of OsNAC3 in rice seed germination [15], highlighting the conserved function of NAC proteins in reproductive development. The tissue- and stage-specific expression of the *T. grandis* NAC family, combined with promoter cis-element characterization and preliminary functional validation of TgNAC72, expands our understanding of NAC functional diversity and identifies key candidate genes for reproductive development. Future studies should apply gene editing to validate in vivo functions of TgNAC72 and related members, as well as chromatin immunoprecipitation (ChIP) to uncover downstream regulatory networks and elucidate NAC-mediated pathways in *T. grandis* reproduction.

In plants, β -glucosidase (BGLU, EC 3.2.1.21), a member of the glycoside hydrolase family 1 (GH1), participates in diverse developmental processes and stress responses by

hydrolyzing β -glycosidic bonds in carbohydrates [38]. GH1-type BGLUs specifically act on the non-reducing ends of their substrates and can bind not only to glucose, but also to various aglycones, thereby contributing to their functional diversity [39,40]. β -Glucosidases are essential across multiple organisms, regulating lignin biosynthesis, hormone metabolism, and the activation of defense compounds in plants to counter pathogens and herbivores [41]. For example, in *Arabidopsis*, AtBGLU18 and AtBGLU33 hydrolyze abscisic acid glucose ester (ABA-GE) to release active abscisic acid (ABA), thereby mediating drought stress responses [42]. In rice, Os4BGlu10, Os6BGlu24, and Os9BGlu33 are involved in abiotic stress responses and seed germination [43]. In reproductive processes, GH1 family members also play essential roles; suppression of AtBGLU20 in *Arabidopsis* disrupts pollen development, and TaBGLU81 in wheat is associated with fertility conversion [44,45]. Our results extend these findings to gymnosperms, showing that the expression of TgBGLU13 and TgBGLU15 progressively increased during the late stages of female cone development in *T. grandis*. Suo et al. (2024) found that sucrose played a vital role in modulating the female cone development in *T. grandis* [27]. Thus, we speculate that this temporal pattern suggests that BGLU activity may be particularly important for cone maturation, potentially by modulating carbohydrate reserves or mobilizing signaling molecules required for seed development. The positive regulation of TgBGLU13 by TgNAC72, which is strongly expressed during cone maturation, further supports the hypothesis that a TgNAC72–TgBGLU13 module contributes to reproductive regulation. Given that BGLUs can influence both primary metabolism (e.g., starch and sucrose hydrolysis) and hormone homeostasis (e.g., ABA metabolism), their upregulation at late developmental stages may ensure the metabolic flexibility and signaling control necessary for successful cone maturation and seed viability.

5. Conclusions

We identified 82 NAC transcription factors in *Torreya grandis*, which show both conserved features and lineage-specific diversification, including a notable expansion of TERN-clade members on chromosome 2. Tissue-specific and developmental expression profiling highlighted functional specialization, and TgNAC72 was found to be a key regulator during female cone maturation. Subcellular localization and dual-luciferase assays confirmed its nuclear activity and activation of TgBGLU13, suggesting a TgNAC72–TgBGLU13 module that may regulate carbohydrate metabolism and reproductive development. These findings contribute to our understanding of the molecular basis of reproductive regulation in gymnosperms and identify candidate genes for further functional exploration. However, this study is limited by its reliance on transcriptome data and transient assays. Future work will focus on functional validation of the TgNAC72–TgBGLU13 module and its regulatory network during female cone development and deepen insights into cone development and adaptive evolution in *T. grandis*.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11101229/s1>, Table S1: The list of NAC family; Table S2: The distribution of coding sequences in NAC family; Table S3: Primers used in this study.

Author Contributions: Conceptualization, X.Z. and T.H.; project administration, X.Z. and T.H.; funding acquisition, X.Z. and T.H.; supervision, X.Z. and T.H.; methodology, L.W., C.C., M.L., W.B. and S.L.; formal analysis, L.W., C.C., M.L., W.B. and S.L.; writing—review and editing, L.W., X.Z. and T.H. All authors have read and agreed to the published version of the manuscript.

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