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Genome-wide analyses of the NAC transcription factor gene family in *Acer palmatum* provide valuable insights into the natural process of leaf senescence

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Acer palmatum, a deciduous shrub or small tree, originated in Japan and China. It is a popular ornamental plant because of its beautiful leaves, which change colour in autumn. This study revealed 116 *ApNAC* genes within the genome of *A. palmatum*. These genes are unevenly distributed on the 13 chromosomes of *A. palmatum*. An analysis of the phylogenetic tree of *Arabidopsis thaliana* NAC family members revealed ApNAC proteins could be divided into 16 subgroups. A comparison of ApNAC proteins with *NAC* genes from other species suggested their potential involvement in evolutionary processes. Studies suggest that tandem and segmental duplications may be key drivers for the expansion of the *ApNAC* gene family. Analysis of the transcriptomic data and qRT–PCR revealed significant upregulation of most *ApNAC* genes during autumn leaf senescence compared with their expression levels in summer leaves. Coexpression network analysis revealed that the expression of 10 *ApNAC* genes was significantly correlated with that of 200 other genes, most of which are involved in plant senescence processes. In conclusion, this study elucidates the regulatory mechanisms of *NAC* genes in *A. palmatum* leaf senescence. These findings provide a valuable foundation for future genetic engineering efforts of woody ornamental plants.

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Abstract

Acer palmatum, a deciduous shrub or small tree, originated in Japan and China. It is a popular ornamental plant because of its beautiful leaves, which change colour in autumn. This study revealed 116 *ApNAC* genes within the genome of *A. palmatum*. These genes are unevenly distributed on the 13 chromosomes of *A. palmatum*. An analysis of the phylogenetic tree of *Arabidopsis thaliana* NAC family members revealed ApNAC proteins could be divided into 16 subgroups. A comparison of ApNAC proteins with *NAC* genes from other species suggested their potential involvement in evolutionary processes. Studies suggest that tandem and segmental duplications may be key drivers for the expansion of the *ApNAC* gene family. Analysis of the transcriptomic data and qRT–PCR, revealed significant upregulation of most *ApNAC* genes during autumn leaf senescence compared with their expression levels in summer leaves. Coexpression network analysis revealed that the expression of 10 *ApNAC* genes was significantly correlated with that of 200 other genes, most of which are involved in plant senescence processes. In conclusion, this study elucidates the regulatory mechanisms of *NAC* genes in *A. palmatum* leaf senescence. These findings provide a valuable foundation for future genetic engineering efforts of woody ornamental plants.

roduction

The genus *Acer,* which belongs to the Aceraceae family, is rich in deciduous and evergeen trees and shrubs and is distributed across Asia, Europe, and North America (o et al., 2020; Tao et al., 2020). China serves as the global centre for modern Acer diversity, with over 100 recognized species (Areces-Berazain et al., 2021). The diverse genus *Acer* (Aceraceae, Sapindales) includes the species *Acer palmatum*, cultivated primarily in China, Japan, and South Korea (Contreras & Shearer, 2018). Clinical studies suggest that extracts from *A. palmatum*, known for their anti-proliferative and antioxidant properties, hold promise for application in cancer treatment or prevention (Bi et al., 2016). In addition to its medicinal applications, the captivating beauty of *A. palmatum* makes it a popular ornamental plant (Cao et al., 2020; Xie et al., 2023). The widespread

distribution of Acer species has attracted significant interest in basic molecular and genetic research on these plants.

Leaf senescence is an active and highly regulated process governed by genes (Ali et al., 2018). Transcription factors (TFs) act as the mediators, regulating dramatic shifts in gene expression during leaf senescence process (Guo, 2013). TFs are proteins that bind to specific cis-elements in the promoter region or interact with other regulators to activate or inhibit target genes (Manna et al., 2021). The acronym NAC were named after three founding members (NAM, ATAF1/2, and CUC2), which were originally found to contain specific NAC domain (Nakashima et al., 2012). NAC proteins have a highly conserved N-terminal NAC domain for DNA binding and a variable C-terminal region that determines their activation or repression function (Shao et al., 2015). The NAC domain can be further subdivided into subdomains A-E (Ooka et al., 2003). Research suggests that subdomains C and D may play critical roles in DNA binding, whereas subdomain A may contribute to the composition of homodimers or heterodimers. Sub-domains B and E likely contribute to the functional diversity within NAC proteins (Olsen et al., 2005).

The NAC proteins, which is unique to plants, is essential for the growth and development of plants and represents, one of the largest families of TFs among plant regulators (Singh et al., 2021). *NACs* have emerged as crucial regulators of leaf senescence across various plant species (Gan et al., 2015; Mao et al., 2017). Research suggests that NAC TFs promote age-dependent senescence by activating genes associated with this process (Nagahage et al., 2020; Wang et al., 2021). Additionally, NAC proteins influence senescence by regulating genes involved in the production of gibberellin (GA), a hormone that impacts chlorophyll degradation (Fan et al., 2020; Tan et al., 2021). Recent studies have further elucidated the role of NAC TFs in dark-induced senescence, wherein they participate by influencing chlorophyll breakdown (Chou et al., 2018; Sun et al., 2024).

The leaves of *A. palmatum* exhibited a vibrant green hue during the summer months. However, as the autumn season approaches and temperatures begin to decline, these leaves undergo a transformation, turning a striking shade of yellow before gracefully falling to the ground (Xie et al., 2023). Leaf senescence, the final act in a leaf's life cycle, is essential for plant life cycle. While research has extensively explored how NAC proteins respond to abiotic stresses in various plants, their specific functions in *A. palmatum* remain elusive. In this study, we identified the *NAC* genes within the *A. palmatum* whole genome.Through RNA-Seq data and qRT–PCR, we explored the expression profile of the *ApNAC* gene in *A. palmatum*, and identified potential candidates that might regulate leaf senescence. These results elucidated the molecular response mechanism that might be involved in ApNAC-regulated leaf senescence in *A. palmatum* and open avenues for exploring the broader functions of *NAC* genes.

Materials & Methods

Plant Material and Treatment

A. palmatum seedlings cultivated in the experimental field of the Anhui Academy of Agricultural Sciences, Hefei city, Anhui Province, China, were used as study samples (31.86°N, 1117.27°E).

101 nmer and fall harvests of healthy leaves were performed. each season, three separate
102 biological duplicates, each with 20 leaves, were collected to guarantee data accuracy. Following
103 harvest, every leaf was instantly frozen at -80°C and stored in liquid nitrogen.
104
105 **Database Search and Sequence Retrieval**
106 The *A. palmatum* genome data were obtained from the *A. palmatum* genome databases (accession
107 numbers: PRJNA850663; https://www.ncbi.nlm.nih.gov/). The Pfam database
108 (http://pfam.xfam.org/) was searched for the HMM maps of the NAC domain (PF02365). The
109 conserved domains of the candidate gene sequences were verified via the CD-Search tool
110 (https://www.ncbi.nlm.nih.gov/cdd/) and BLASTP searches. ExPASy (https://www.expasy.org)
111 was used for analysis to predict the physicochemical characteristics of the ApNAC proteins. Using
112 the CELLO v2.5 website (http://cello.life.nctu.edu.tw/; Yu et al. 2006), subcellular localization
113 was ascertained.
114
115 **Phylogenetic Analysis of ApNAC Proteins**
116 The NAC protein sequences for *Arabidopsis thaliana*, were downloaded from the TAIR database
117 (https://www.arabidopsis.org/) for conducting comparative evolutionary analysis. Protein
118 sequences from *A. palmatum* and *A. thaliana* were used to generate an unrooted phylogenetic tree
119 via the neighbour-joining (NJ) method and 1000 iterations of bootstrap testing. This analysis
120 allowed us to classify all ApNAC proteins into distinct subgroups on the basis of the established
121 *Arabidopsis* NAC protein classification system.
122
123 **Conserved Motifs and Gene Structure Analysis**
124 We utilized Multiple EM for Motif Elicitation (MEME) service (http://meme-
125 suite.org/tools/meme) to search for conserved motifs in NAC proteins. To understand the intron-
126 exon organization of each *ApNAC* gene, their genomic sequences were aligned with the
127 corresponding gene structure annotation files. TBtools software facilitated the visualization of both
128 the conserved motifs and the intron-exon structures.
129
130 **Chromosomal Location, Duplication Events, and Homology**
131 Genome annotation files revealed the chromosomal positions of the ApNAC gene family, which
132 were then visualized via the MG2C website (http://mg2c.iask.in/mg2c_v2.1/). We examined the
133 genomic sequences and GFF files for *A. thaliana*, *Populus tomentosa*, *Citrus sinensis*, and *Vitis*
134 *vinifera*, which we obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). *ApNAC*
135 gene duplication events were analysed via the MCScanX program (Tang et al., 2012). *ApNAC*
136 gene homology relationships were examined via the Dual Synteny Plotter software (Chen et al.,
137 2020).
138 **Analysis of Promoter Cis-Elements**
139 To elucidate potential regulatory mechanisms governing *ApNAC* gene expression, we investigated
140 2 kilobase (kb) regions upstream of the transcription start sites for *ApNAC* gene. The PlantCARE
141 database (http://www.plantcare.co.uk/) was used to identify potential cis-acting regulatory

elements. This analysis aimed to predict the presence of crucial cis-elements involved in influence plant development and the stress and hormone response. Subsequently, TBtools software was used to visualize the 22 most common cis-elements found within the *ApNAC* gene promoters (Chen et al., 2020).

Analysis of *ApNAC* Gene Expression Patterns

Transcriptomic data were downloaded from the NCBI database (accession numbers: PRJNA850663). The relative gene expression values were expressed as fragments per kilobase million (FPKM) and all of the transcriptomic data were converted to log2(FPKM+1) values. For *ApNAC* genes that were differentially expressed in response to senescence stress, the selection criteria were log2 fold change values. The TBtools program was used to generate a heatmap of the transcript profiles of the *ApNAC* genes (Chen et al., 2020).

qRT–PCR validation of *ApNAC* Gene Expression Levels

Total RNA was isolated from the summer and autumn leaves of *A. palmatum* via a commercially available plant RNA extraction kit (ZD02001, Zoonbio, Nanjing, China). The quality and purity of the RNA were confirmed via a NanoDrop spectrophotometer from Thermo Fisher Scientific, USA. To verify the integrity of the RNA, gel electrophoresis was performed on a 1.2% agarose gel containing 18% formaldehyde. RT–PCR was then completed via Hifair® First Strand cDNA Synthesis Kit (11123ES10, Yeasen, Shanghai, China) and DNase I (ZD02001, Zoonbio, Nanjing, China).

e SYBR Premix Ex Taq (Tli RNaseH Plus; RR420A, TaKaRa, Dalian, China) on a Lightcycle K Real-Time PCR Detection System (BIOER, Hangzhou, China) for quantitative real-time PCR (qRT–PCR). The reactions were performed as follows: 40 cycles of 95°C for 5 s, 60°C for 30 s, and 72°C for 20 s. Primer Premier 5 software was used to design gene-specific primers (Table S1; Lalitha, 2000). Actin served as an internal control gene. In accordance with Livak and Schmittgen (2001), each of the three biological replicates is supported by three technical replicates, and the relative gene expression levels of specific ApNACs were evaluated via the $2^{-\Delta\Delta CT}$ method (Table S2).

***ApNAC* Genes Correlation Analysis**

To explore the various TFs that participate in leaf senescence associated with ApNAC genes, we employed R to calculate transcriptome correlations via Pearson correlation coefficient (PCC) analysis. The expression profiles of *ApNAC* genes and non-*ApNAC* genes were compared in this manner. This analysis aimed to identify genes whose expression patterns closely mirrored those of the *ApNAC* genes. We subsequently employed Cytoscape software (version 3.6.1) for coexpression network visualization (Shannon et al., 2003).

Results

Identification and Characterization of *ApNAC* Genes

By using the Hidden Markov Model HMM profiles of NAC (PF02365) domain as queries to scan the *A. palmatum* genome, 116 potential *ApNAC* genes were found in the genome of *A. palmatum*. On the basis of their chromosomal positions, these genes were designated ApNAC1 through ApNAC116 (Table S3). We also examined the lengths, MWs, pIs, and subcellular locations of the encoded proteins. The smallest ApNAC protein (ApNAC28) harboured only 139 amino acids with a corresponding MW of 15833.98 kDa, whereas the largest protein (ApNAC108) contained 871 amino acids and had a predicted MW of 98111.96 kDa. The pI values of these ApNAC proteins exhibited a broad range, from 4.22 (ApNAC114) to 10.02 (ApNAC50). Subcellular localization predictions revealed diverse localizations for the ApNAC proteins: ApNAC77 was predicted to reside in the extracellular space, 4 proteins (ApNAC42, 50, 114, and 115) were predicted to localize to chloroplasts, and 15 proteins (ApNAC32, 33, 34, 38, 40, 48, 56, 57, 60, 61, 64, 97, 98, 99, and 116) were predicted to reside in the cytoplasm, with the remaining ApNAC proteins predicted to be localized within the nucleus (Table S3).

Classification and Phylogenetic Analysis of ApNAC Proteins

To look at the evolutionary connections between ApNAC family members and other recognized plant NAC proteins, we generated a phylogenetic tree via sequence alignments of 116 ApNAC proteins and 105 *Arabidopsis* ANAC proteins (Fig. 1). Our analysis grouped the ApNAC proteins into 16 distinct subfamilies on the basis of homology with *Arabidopsis* NAC proteins. These subfamilies included ATAF, ANAC3, NAP, ONAC022, NAM, NAC1, OsNAC7, ANAC001, TIP, ANAC011, NAC2, ONAC003, ANAC063, SENU5, TERN, and OSNAC8 (detailed breakdown in Figure 1). The ANAC063 subfamily had the greatest number of ApNAC members (20), whereas the NAC1 subfamily contained only one. This diversity within the ApNAC family mirrored observations previously reported in *A. thaliana* (*Ooka et al., 2003*).

Gene Structure and Motifs Analysis of ApNAC Proteins

By revealing the functional domains of the ApNAC proteins, we identified 20 conserved motifs using the MEME program designated as motifs 1-20 (Fig. 2A). All the motifs resided within the well-conserved N-terminal NAC domain. Motif 7 was universally present across all ApNAC protein families, whereas motifs 1 and 8 were prevalent among most members. The number of motifs per protein varied, with ApNAC34 containing the fewest (1 motif) and ApNAC107 harbouring the most (10 motifs). Most of the closely related members of the phylogenetic tree shared similar motif compositions. For example, members of the AtNAC3, SENU5, and OsNAC8 subfamilies all possessed motif 7, suggesting that members grouped in the same clade on the basis of similar conserved motifs might share similar functions.

To explore structural diversity within the ApNAC gene family, we analysed intron–exon distribution patterns (Fig. 2C). Subfamilies generally exhibited concordance in intron–exon structures and gene length. The number of introns varied between 0 and 11, with two genes lacking introns entirely and 20 genes containing just one intron. Genes with two introns were the most abundant, whereas 10 genes (ApNAC36, 65, 69, 56, 57, 34, 78, 76, 77, and 79) presented more than six introns.

Chromosomal Localization, Repeat Events, and Synteny Analysis of *ApNAC* Genes

To visualize the chromosomal locations of the *ApNAC* genes, we generated a distribution map using MG2C software (Fig. 3). The 116 *ApNACs* displayed an uneven distribution across the 13 chromosomes, with no apparent correlation between the number of genes on each chromosome and its length. Chromosome 3 harboured the most *ApNAC* genes (18), followed by chromosome 12 (13), whereas chromosome 1 contained the fewest (3).

To determine repeat events in *ApNAC* genes, we performed homology analysis via the MCScanX software. Segmental duplications were identified among the 116 ApNAC members. Chromosome 5 presented the highest abundance of segmental duplication gene pairs (3), followed by chromosomes 4 and 2 (2 pairs each) (Fig. 4 and Table S4). Additionally, we identified 21 pairs of tandem duplications, which were located on chromosomes 2, 3, 4, 6, 7, 9, 11, and 13 (Figure 3 and Table S5). These findings suggest that segmental and tandem duplications are likely the primary causes driving ApNAC gene family expansion.

To gain deeper insights into *ApNAC* gene evolution, we constructed a comparative homology map using *NAC* genes from *A. thaliana*, *P. trichocarpa*, *C. sinensis*, and *V. vinifera*. This analysis revealed collinearity between *ApNAC* genes and *NAC* genes from these plant species: *P. trichocarpa* (134 homologous gene pairs), *A. thaliana* (69 pairs), *C. sinensis* (90 pairs), and *V. vinifera* (81 pairs) (Fig. 5).

Cis-Acting Regulatory Element (CARE) Analysis of *ApNAC* Genes

To investigate the possible cis-elements of *ApNAC* gene expression, we extracted 2 kilobase sequences upstream of the promoter region for all *ApNAC* genes. Putative cis-elements within the promoter region sequences were identified using the PlantCARE database (Fig. 6 and Table S6). This analysis revealed a diverse array of cis-elements associated with various biological processes, including developmental stages, plant hormone signalling, and abiotic stress responses. The cis-elements associated with plant development were predominantly responsive to light and specific to meristematic tissues. Additionally, cis-elements responsive to hormones such as ABA, GA, MeJA, and ethylene were identified. These findings suggest that *ApNAC* genes, potentially under hormonal regulation, might be essential for the successful growth, development, and stress tolerance of *A. palmatum*.

Expression Pattern of *ApNAC* Genes During Leaf Senescence

To explore the potential involvement of *ApNAC* genes in the regulation of leaf senescence, we analysed their expression patterns via analysis of transcriptomic data. This analysis revealed significant expression changes in the expression of 68 *ApNAC* genes in leaves between autumn (SUS) and summer (SUS) (Fig. 7 and Table S7). Especially ApNAC02, 04, 05, 06, 41, 48, 51, 83, 91, and 100, increased during autumn senescence. Conversely, the expression of ApNAC71 and ApNAC93 decreased.

To further explore the potential regulators of leaf senescence in *A. palmatum*, we selected 10 *ApNAC* genes and analysed their relative transcript abundance via qRT–PCR. This analysis

confirmed the upregulation of ApNAC02, 04, 05, 06, 41, 48, 51, 83, 91, and 100 (Fig. 8) during autumn senescence compared with summer leaves.

Construction of a Coexpression Network for *ApNAC* Genes

To elucidate the regulatory networks of *ApNAC* gene function during leaf senescence, we employed R language to analyse the expression levels of the TFs within the *A. palmatum* leaf transcriptomic data. This analysis aimed to identify coexpressed TFs of *ApNAC* genes. 200 significantly expressed TFs exhibited strong correlations with 10 *ApNAC* genes ($|PCC| > 0.95$) (Fig. 9 and Table S8). The top five coexpressed TF families were WRKY (58), AP2/ERF (51), bZIP (41), MYB-related (37), and C2H2 zinc finger (11). These findings suggest that *ApNAC* genes may interact with these TF families to establish regulatory networks, potentially acting cooperatively to promote leaf senescence.

Discussion

NAC genes act as critical players in the fundamental development processes and in the response to environmental stresses (Nuruzzaman et al., 2013). *NAC* genes have been identified in *Arabidopsis*, rice, and barley (Cai et al., 2019). However, whether NAC TFs play various roles in regulating the senescence of *A. palmatum* leaves remains unclear. This study we delved into the characterization of *ApNAC* genes in the *A. palmatum* genome. We explored the potential roles of these genes in regulating leaf senescence at the whole-genome level. Our primary aim was to establish a foundation for understanding how *ApNAC* genes influence leaf senescence in *A. palmatum*, paving the way for future investigations into their functional and molecular mechanisms.

To elucidate the potential functional relationships among *ApNAC* genes, we employed the neighbour-joining (NJ) method to generate an unrooted phylogenetic tree on the basis of protein sequences. This analysis classified the 116 *ApNAC* genes into 16 subgroups on the basis of their homology to *Arabidopsis* NAC proteins (Ooka et al., 2003). This marked conservation of intron–exon structures and motif compositions within each subgroup. Early studies suggests that members of the same subgroup likely share similar biological functions (Hu et al., 2015; Wei et al., 2016). Two subgroups, ATAF and NAP, contained the most ApNAC members in both *A. palmatum* and *Arabidopsis* which have been linked to leaf senescence regulation in other plant species (Garapati et al., 2015; Guo and Gan, 2006), suggesting a potential role for ATAF and NAP subfamily members in *A. palmatum* leaf senescence. Analysis of RNA sequencing (RNA-Seq) data revealed that ApNAC genes present significant transcriptional responses throughout *A. palmatum* leaf senescence. To validate these findings, we further investigated the expression profiles of 10 *ApNAC* genes from various subgroups via qRT–PCR analysis. In accordance with the RNA-Seq analysis results, all 10 genes presented increased expression during senescence .

The increase in ApNAC genes was caused mostly by tandem and segmental duplication. Tandem duplications have been reported to play a significant role in the expansion of the NAC gene family in *Oryza sativa* (Manimekalai et al., 2010) and *Eucalyptus grandis* (Hussey et al.,

2015). Segmental duplications may also contribute to NAC gene family expansion, as observed in *Panicum miliaceum* (Shan et al., 2020) and *Vigna radiate* (Tariq et al., 2022). 116 ApNAC genes have 21 pairs of tandemly duplicated genes and 11 pairs of segmentally duplicated genes. These duplication events were likely driving forces in the diversification and expansion of the NAC gene family within *A. palmatum*. The *A. palmatum* genome experienced an ancient whole-genome duplication event (γ-genome-wide replication) (Han et al., 2020) but evidence of recent independent whole-genome duplication events is lacking (Lu et al., 2023).

NAC TFs are one of the largest groups of plant regulatory proteins, that can either activate or repress gene expression, affecting how plants react to biotic and abiotic stressors (Nuruzzaman et al., 2013). In this study, promoter analysis revealed ABREs within the promoter regions of 8 *ApNAC* genes (ApNAC07, 22, 59, 64, 72, 96, 103, and 114). In *Arabidopsis*, ABREs have been linked to ABA-responsive stress signalling (Jensen et al., 2010), and NAC-TFs are known to respond to ABA (Nuruzzaman et al., 2013). These findings suggest a potential role for ABA signalling in regulating ApNAC gene expression during leaf senescence in *A. palmatum*.

Research has demonstrated that some NAC TFs, such as JUNGBRUNNEN1 (JUB1) and VND-INTERACTING2 (VNI2), function as negative regulators of leaf senescence (Yang et al., 2011; Wu et al., 2012). VNI2 inhibits ABA-induced leaf senescence through the modulation of specific genes associated with the cold response (COR) and dehydration stress (RD) (Yang et al., 2011; Matsuda et al., 2023). Previous studies on functional prediction of TFs family members on the basis of phylogenetic analysis (Yuan et al., 2020). VNI2 belongs to the NAP and SENU subfamilies, whereas JUB1 belongs to the ONAC022 and TREN subfamilies (Li et al., 2018). Our analysis revealed that two ApNAC genes, ApNAC103 and ApNAC62, closely clustered with the negative senescence regulator VNI2 on the phylogenetic tree. Similarly, ApNAC93 and ApNAC71 groups together with JUB1. These findings suggest that ApNAC62, 71, 93, and 103 could act as negative regulators of leaf senescence in *A. palmatum*. Compared with senescence-promoting *NAC* genes, senescence-suppressing *NAC* genes may present a more intricate evolutionary history.

Numerous families of TFs, including WRKY, AP2/ERF, bZIP, and NAC TFs, play an essential role in leaf senescence (Song et al., 2012; Mizoi et al., 2012; Puranik et al., 2012; Rushton et al., 2012; Licausi et al., 2013; Wang et al., 2018). In *A. palmatum*, our analysis using the Pearson correlation coefficient (PCC) identified potential coregulators of *NAC* genes during leaf senescence. These included 58 WRKY, 51 AP2/ERF, 41 bZIP, 37 MYB-related, and 11 C2H2 zinc finger TFs that exhibited strong positive correlations with NAC expression. These findings suggest the existence of a complex regulatory network involving multiple interacting TFs families in regulating leaf senescence. For example, OsWRKY5 in rice has been shown to accelerates chlorophyll degradation, promoting leaf senescence of rice (*Oryza sativa*) by indirectly upregulating the expression of *NAC* genes related to leaf senescence such as OsNAP and OsNAC2 (Kim et al., 2019). In *Arabidopsis*, MYB108 directly regulates genes associated with senescence by binding to the ANAC003 promoter (Chou et al., 2018). The findings presented here underscore the importance of exploring the interplay of TFs in regulatory mechanisms of *A. palmatum* leaf senescence.

Conclusions

In *A. palmatum*, 116 *NAC* genes were identified in this study. Using an *Arabidopsis* NAC protein-based phylogenetic tree, we classified these TFs into 16 distinct subfamilies. Analysis of gene distribution across chromosomes and sequence homology suggested that segmental and tandem duplications were the major contributors to *ApNAC* gene family expansion. Analysis of the RNA-seq data revealed distinct expression profiles for the *NAC* genes. Most *ApNAC* genes displayed significant upregulation during leaf senescence. These *ApNACs* with prominent expression changes are promising candidates for further investigation into their regulatory roles in *A. palmatum* leaf senescence. Overall, this research provides a foundational understanding of the regulatory role of *ApNAC* genes, offering valuable insights for future investigations into the biological functions in *A. palmatum*.

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Competing Interests

The authors declare that they have no conflict of interests.

Data Availability

The following information was supplied regarding data availability:
Data supporting the findings of this study are available from the corresponding author (Jie Ren) upon request. The *A. palmatum* genome has been deposited under BioProject Accession number: PRJNA850663.

Supplementary Information

Table S1 Primer used for qRT-PCR analysis of *ApNACs*
Table S2 qRT-PCR quantitative data

Table S3 Annotation of *A. palmatum* NAC transcription factors
Table S4 Details of gene segmental-duplication of *ApNACs*
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Table S7 Expression patterns of 68 *ApNACs* during *A. palmatum* leaf senescense
Table S8 Coexpression network analysis between 10 *ApNACs* and other genes

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Figure 1

Building a phylogenetic tree with NAC proteins from *Arabidopsis* and *A. palmatum*

The neighbor-joining (NJ) phylogenetic trees of the NAC proteins constructed from *Arabidopsis* and *A. palmatum*. MEGA 7.0 was used for multiple sequence alignment, and 1000 bootstrap repetitions, pairwise deletion, and p-distance were used to build the phylogenetic tree. 16 subfamilies were created from the tree, and each subfamilies was given a unique colour and label.

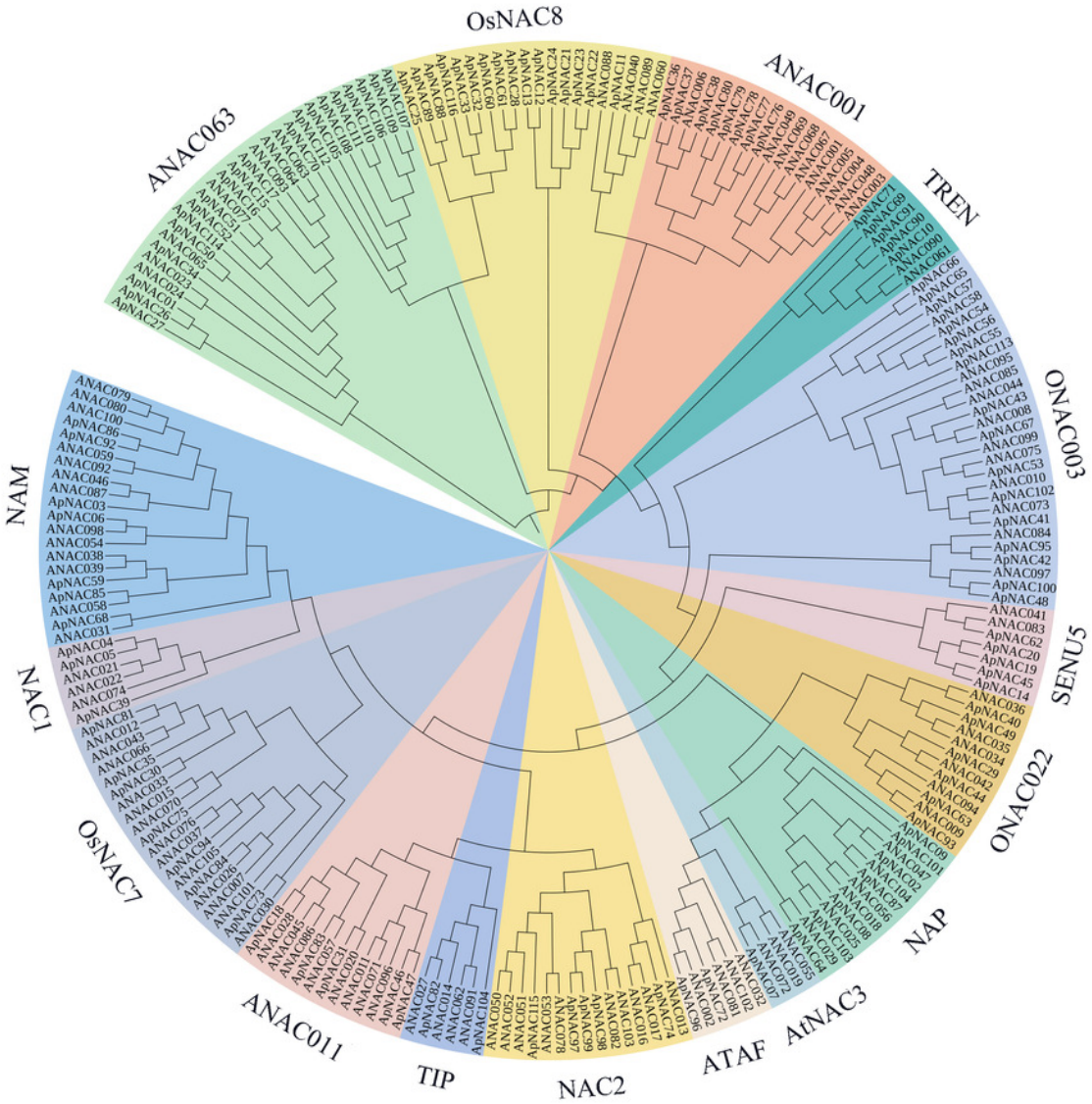
OsNAC8
ANAC001
TREN
ONAC003
SENU5
ONAC022
NAP
AtNAC3
ATAF
NAC2
TIP
ANAC011
OsNAC7
NAC1
NAM
ANAC063

Figure 2

Phylogenetic tree, conserved domains, motifs, and intron-exon gene structure of the *A. palmatum NAC* genes

(A) The MEME motifs were shown as different coloured modules at the N-terminal indicating the NAC domain region. (B) Conserved domains of ApNAC proteins. (C) Exons are indicated by green boxes, and introns are indicated by black lines.

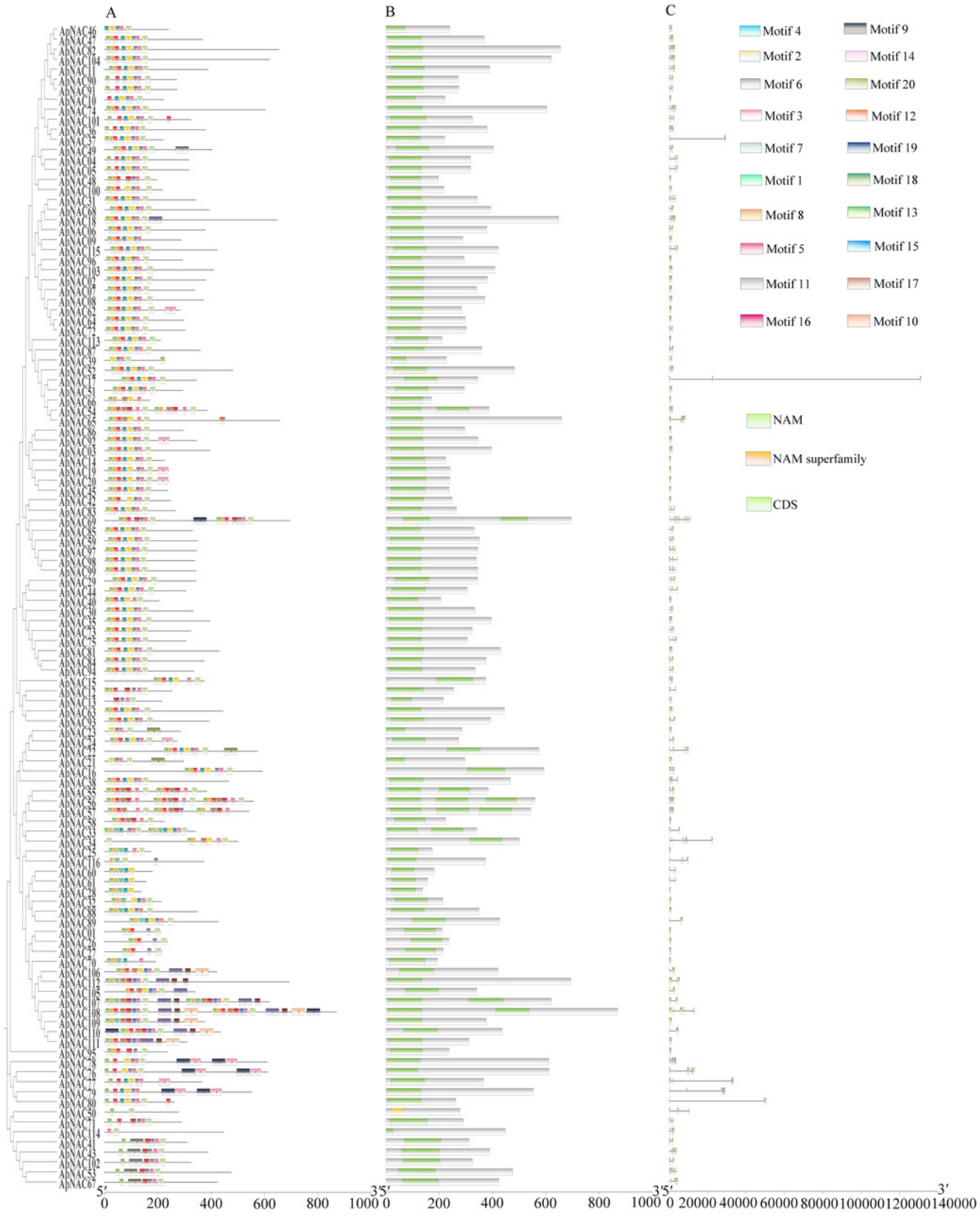
A
B
C
Motif 4
Motif 9
Motif 2
Motif 14
Motif 6
Motif 20
Motif 3
Motif 12
Motif 7
Motif 19
Motif 1
Motif 18
Motif 8
Motif 13
Motif 5
Motif 15
Motif 11
Motif 17
Motif 16
Motif 10
NAM
NAM superfamily
CDS
5′
3′
0
200
400
600
800
1000
20000
40000
60000
80000
100000
120000
140000

Figure 3

Chromosomal locations of ApNAC transcription factors

116 *ApNAC* genes are distributed throughout 13 chromosomes. The chromosomes of *A. palmatum* are represented by bars. Each chromosome has its number on the left side. The chromosome length is indicated by the scale on the left.

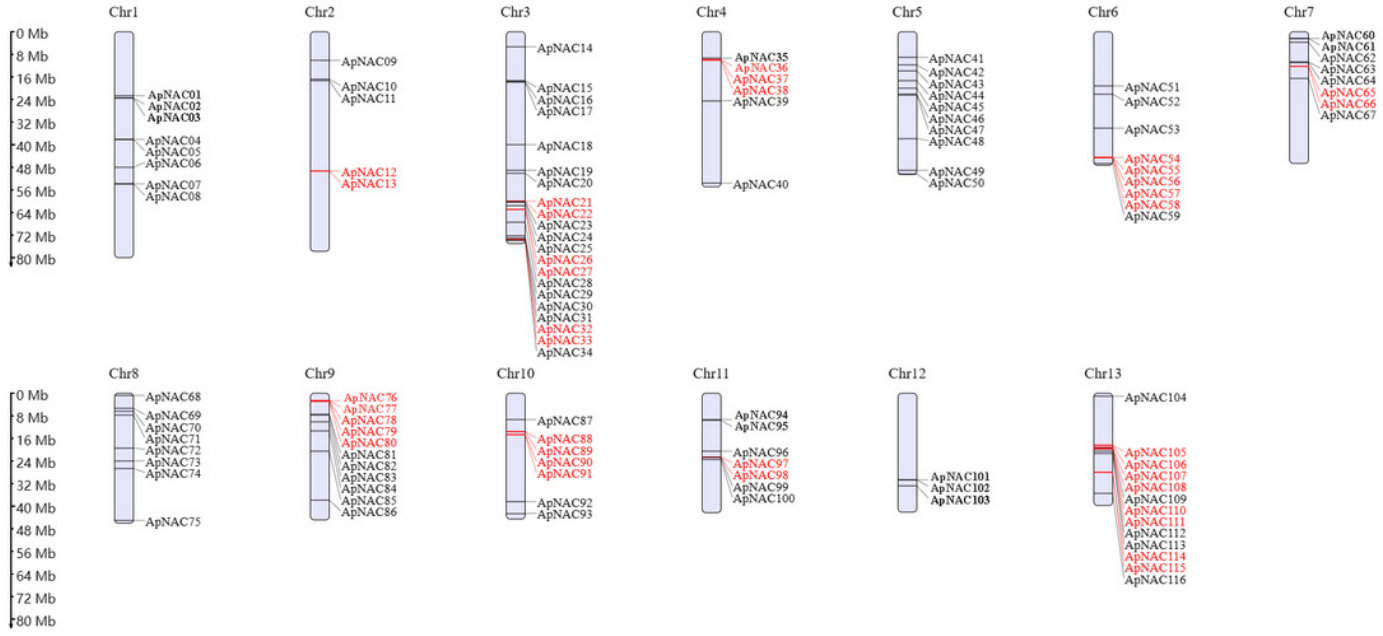

Figure 4

Chromosomal relationships of *ApNAC* genes shown schematic

Pairs of *ApNAC* genes with segmental duplications are shown by coloured lines. Gene density information is represented by the heatmap and line graph, where red denotes high gene density and yellow denotes low gene density.

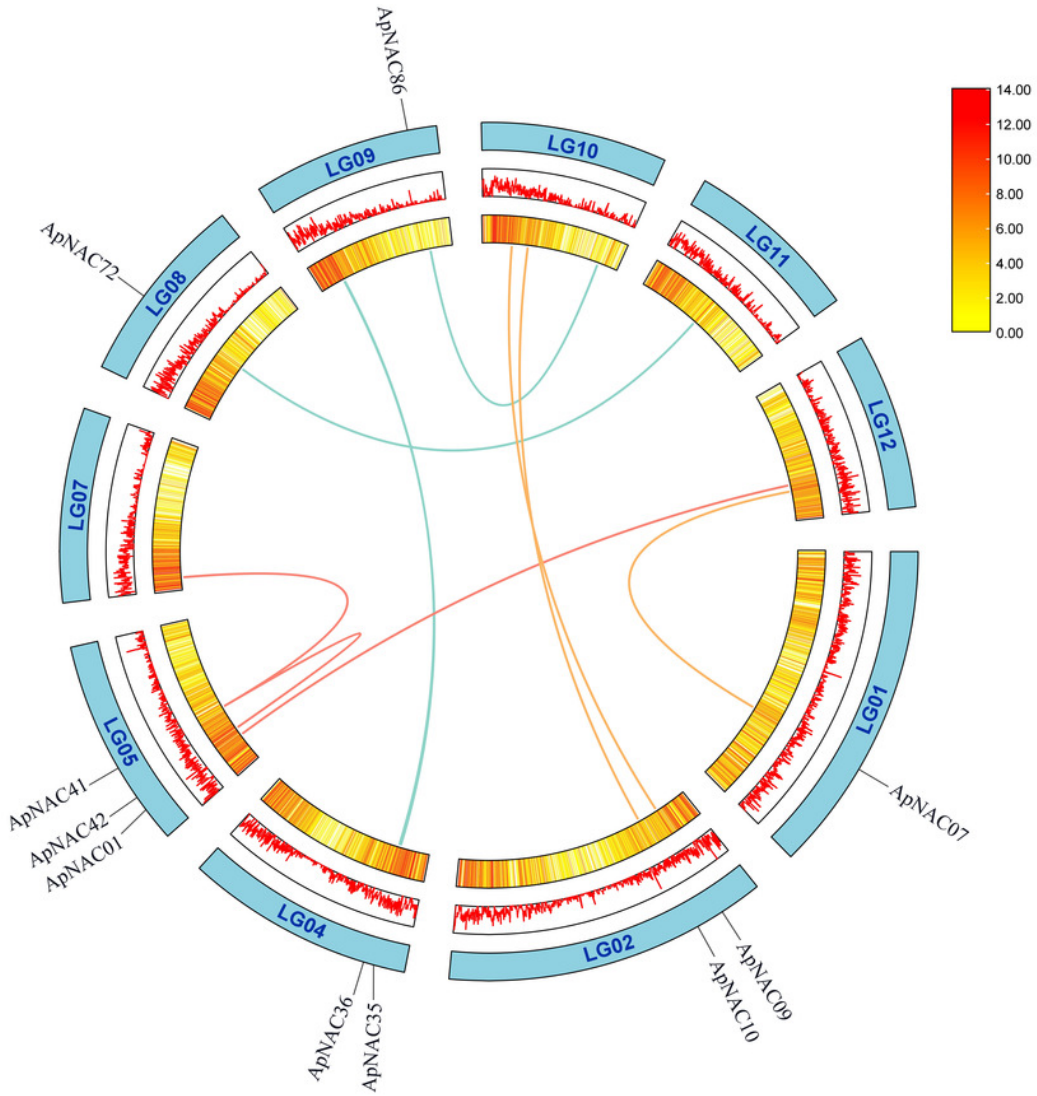
ApNAC86
LG09
LG10
LG11
LG12
LG01
ApNAC07
ApNAC09
ApNAC10
LG02
LG04
ApNAC36
ApNAC35
LG05
ApNAC41
ApNAC42
ApNAC01
LG07
LG08
ApNAC72
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00

Figure 5

NAC genes synteny analyses between four representative plants and *A. palmatum*

Gray lines in the background represent collinear blocks between the genomes of *A. palmatum* and other plant species, while red lines represent pairs of *NAC* genes with segmental duplications.

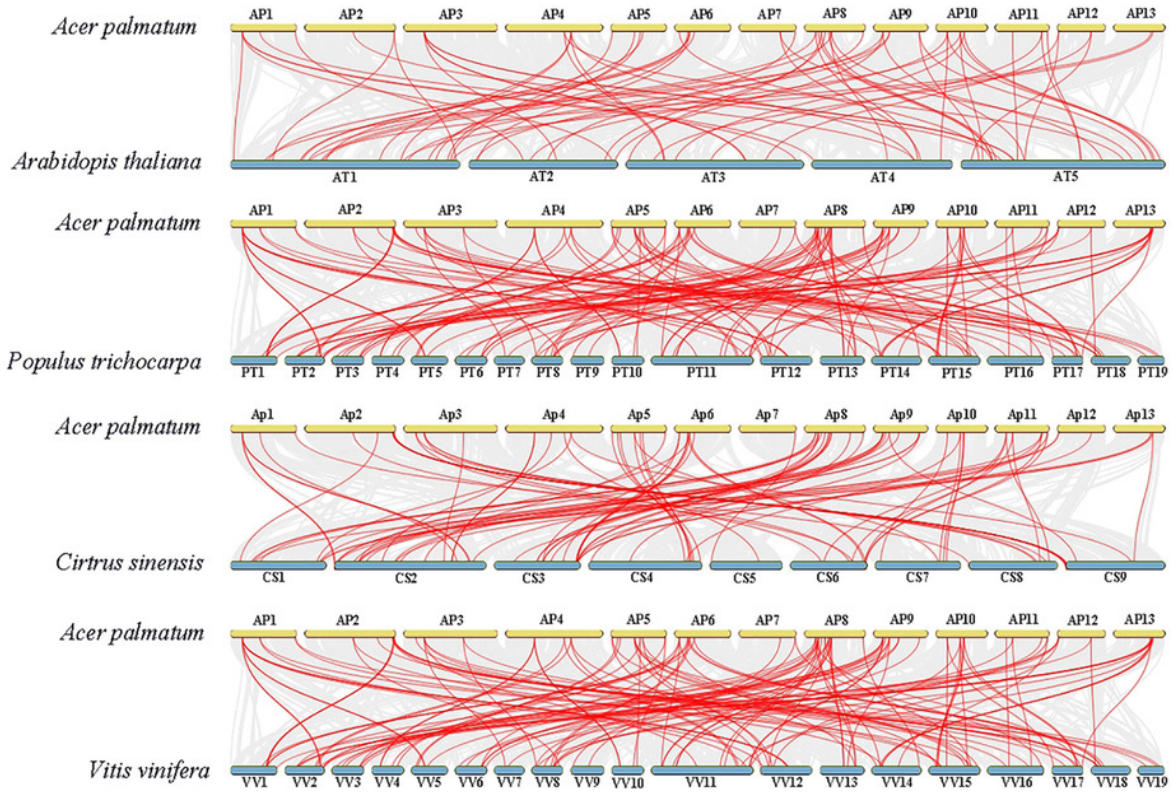

Figure 6

Analysis of ApNAC gene promoter cis-elements statistically

The colour and number of the grid indicate the number of cis-elements in the promoter region of *ApNAC* gene.

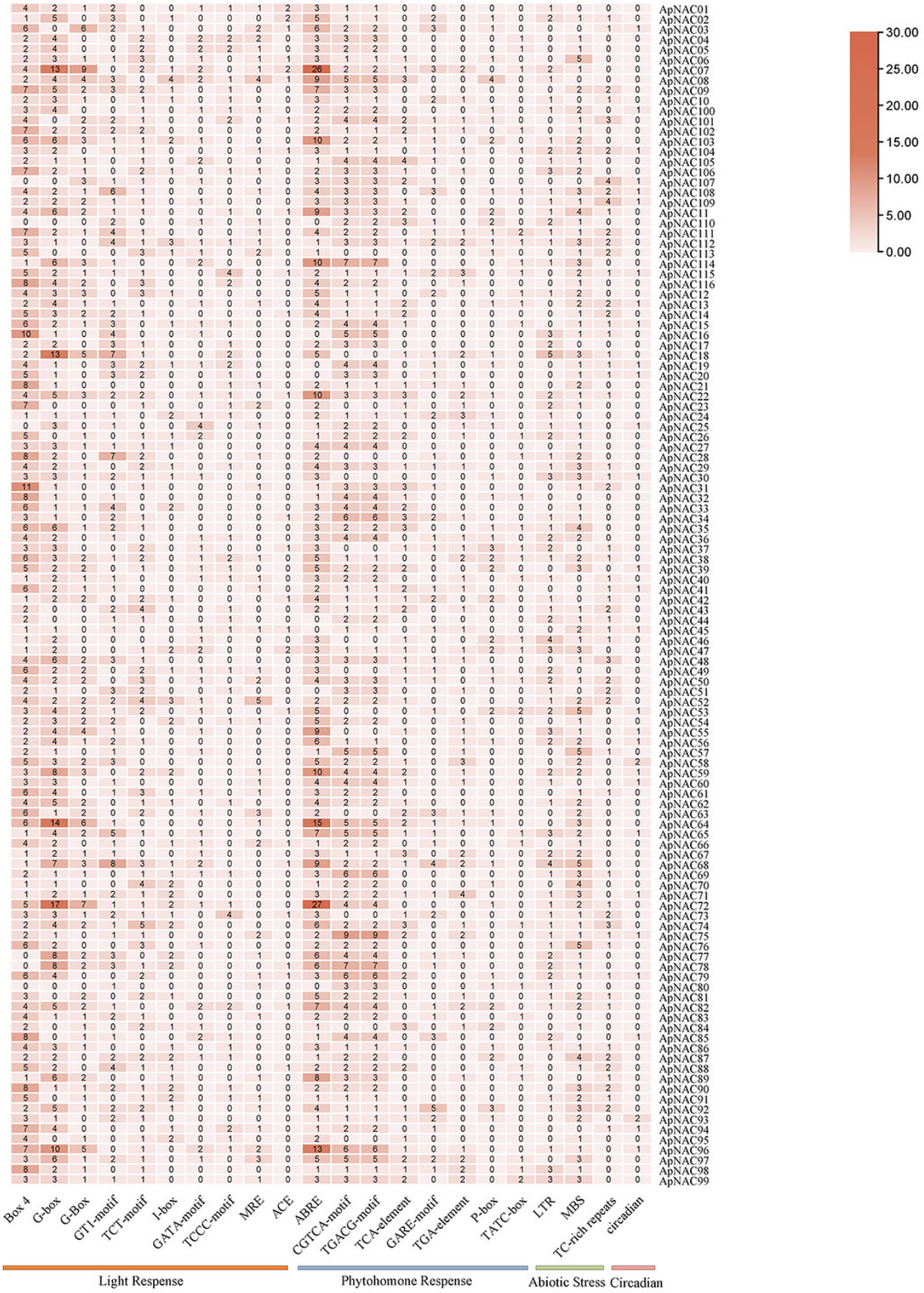
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Box 4
G-box
G-Box
GT1-motif
TCT-motif
I-box
GATA-motif
TCCC-motif
MRE
ACE
ABRE
CGTCA-motif
TGACG-motif
TCA-element
GARE-motif
TGA-element
P-box
TATC-box
LTR
MBS
TC-rich repeats
circadian
Light Respense
Phytohomone Respense
Abiotic Stress
Circadian

Figure 7

The *ApNAC* genes implicated in autumn leaf senescence are represented in a hierarchical clustering heatmap

Analysing the RNA-Seq data resulted in the creation of a heatmap on the basis of the log2 fold change values in summer and autumn. A colour gradient in the upper right corner depicts the shift in expression levels from blue (downregulated) to red (upregulated).

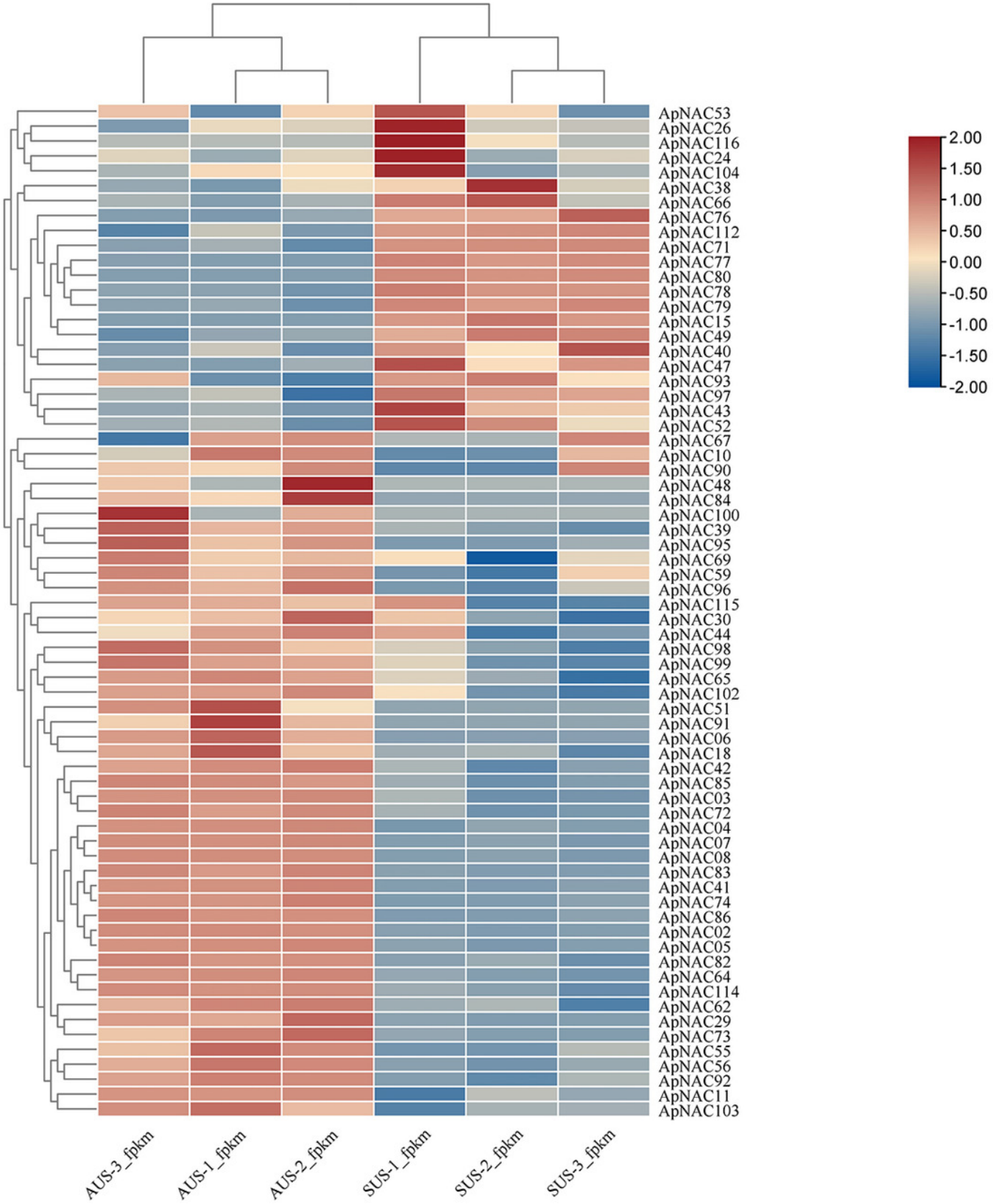
ApNAC53
ApNAC26
ApNAC116
ApNAC24
ApNAC104
ApNAC38
ApNAC66
ApNAC76
ApNAC112
ApNAC71
ApNAC77
ApNAC80
ApNAC78
ApNAC79
ApNAC15
ApNAC49
ApNAC40
ApNAC47
ApNAC93
ApNAC97
ApNAC43
ApNAC52
ApNAC67
ApNAC10
ApNAC90
ApNAC48
ApNAC84
ApNAC100
ApNAC39
ApNAC95
ApNAC69
ApNAC59
ApNAC96
ApNAC115
ApNAC30
ApNAC44
ApNAC98
ApNAC99
ApNAC65
ApNAC102
ApNAC51
ApNAC91
ApNAC06
ApNAC18
ApNAC42
ApNAC85
ApNAC03
ApNAC72
ApNAC04
ApNAC07
ApNAC08
ApNAC83
ApNAC41
ApNAC74
ApNAC86
ApNAC02
ApNAC05
ApNAC82
ApNAC64
ApNAC114
ApNAC62
ApNAC29
ApNAC73
ApNAC55
ApNAC56
ApNAC92
ApNAC11
ApNAC103
AUS-3_fpkm
AUS-1_fpkm
AUS-2_fpkm
SUS-1_fpkm
SUS-2_fpkm
SUS-3_fpkm
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00

Figure 8

The expression profiles of ten representative *ApNAC* genes were analysed using qRT-PCR

The seasons are represented by the x-axis, while the relative expression is shown by the y-axis. The *Actin* gene was utilized to standardize the qRT-PCR data. The standard deviation (SD) of the three biological replicates is represented by the bar chart.

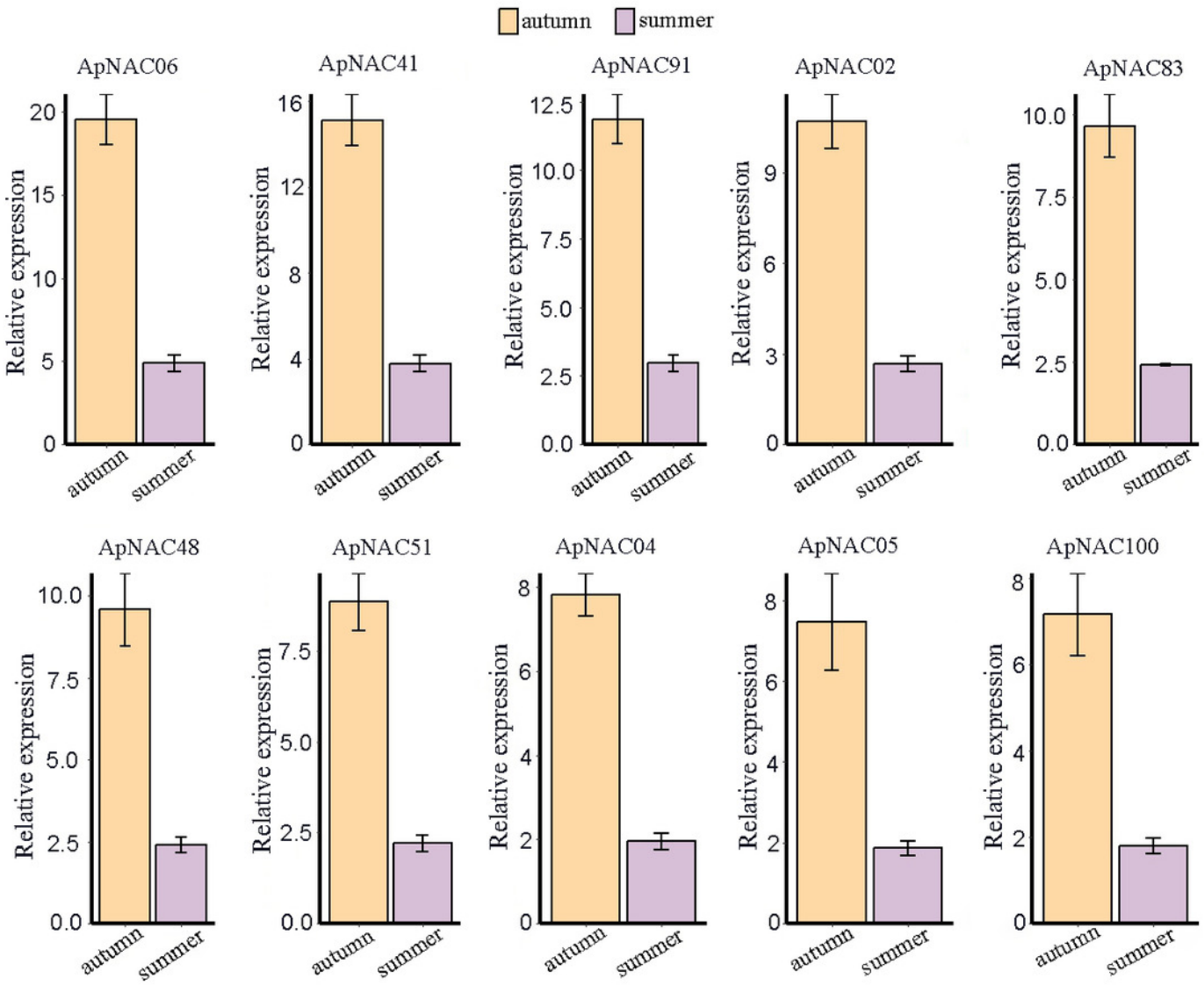

Figure 9

ApNAC gene coexpression network built with transcriptomic data

The number of related genes is represented by the hue, with lighter purple denoting fewer related genes and darker purple denoting more related genes.