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Evolution of NAC transcription factors from early land plants to domesticated crops

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Abstract

NAC [NO APICAL MERISTEM (NAM), ARABIDOPSIS TRANSCRIPTION ACTIVATOR FACTOR 1/2 (ATAF1/2), and CUP-SHAPED COTYLEDON (CUC2)] transcription factors are key regulators of plant growth, development, and stress responses but were also crucial players during land plant adaptation and crop domestication. Using representative members of green algae, bryophytes, lycophytes, gymnosperms, and angiosperms, we expanded the evolutionary history of NAC transcription factors to unveil the relationships among members of this gene family. We found a massive increase in the number of NAC transcription factors from green algae to lycophytes and an even larger increase in flowering plants. Many of the NAC clades arose later during evolution since we found eudicot- and monocot-specific clades. *Cis*-elements analysis in NAC promoters showed the presence of abiotic and biotic stress as well as hormonal response elements, which indicate the ancestral function of NAC transcription factor genes in response to environmental stimuli and in plant development. At the transcriptional level, the expression of NAC transcription factors was low or absent in male reproduction, particularly mature pollen, across the plant kingdom. We also identified NAC genes with conserved expression patterns in response to heat stress in *Marchantia polymorpha* and *Oryza sativa*. Our study provides further evidence that transcriptional mechanisms associated with stress responses and development emerged early during plant land adaptation and are still conserved in flowering plants and domesticated crops.

Keywords: NAC; evolution; heat stress; development; green algae; bryophytes; lycophytes; plant adaptation; crop domestication

Introduction

Plants aquatic to terrestrial transition and crop domestication played a fundamental influence on plant evolution. While increased UV protectant and water desiccation were essential acquisitions for land plant adaptation, morphological traits including enlarged grain seeds, determinate growth, loss of seed dispersal, and seed dormancy were key features selected in favor of agricultural productivity during crop domestication (Doebley et al. 2006, Konishi et al. 2006, Olsen and Wendel 2013). A large number of gene families including transcription factors have been proposed to significantly contribute to land plant adaptation and crop domestication (Meyer and Purugganan 2013, Maugarny-Calès et al. 2016, Bowman et al. 2017).

Within the well-described gene families involved in both land plant adaptation and crop domestication, NAC [NO APICAL MERISTEM (NAM), ARABIDOPSIS TRANSCRIPTION ACTIVATOR FACTOR 1/2 (ATAF1/2), and CUP-SHAPED COTYLEDON (CUC2)] genes have been extensively studied (Shao et al. 2015), with hundreds of genes functionally characterized (Nuruzzaman et al. 2013, Yuan et al. 2019, Singh et al. 2021). Some are classified as modulators of developmental programs such as plant vasculature (Zhong et al. 2006, Mitsuda et al. 2007), cotyledon separation (Aida et al. 1997), and leaf shape establishment (Nikovics et al. 2006, Hasson et al. 2011). For example, *Arabidopsis VASCULAR-RELATED NAC-DOMAIN* (*AtVND4*), *AtVND5*, *AtVND6*, and *AtVND7* are expressed in proto- and metaxylem. While overexpression of *AtVND4* and *AtVND5* results in activation of secondary wall biosynthesis genes in vessels (Zhou et al. 2014), overexpression of *AtVND6* and *AtVND7* induces transdifferentiation of cells into metaxylem- and protoxylem-like vessel elements (Kubo et al. 2005). Many NACs are also involved insenescence as

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well as in stress responses (Podzimska-Sroka et al. 2015, Shao et al. 2015, Chen et al. 2016, Chase et al. 2024). For instance, in *Pinus pinaster*, *PpNAC2* and *PpNAC3* are induced following salinity and mechanical wounding (Pascual et al. 2015). Furthermore, rice *OsNAC2* is involved in various plant developmental responses including shoot growth retardation, root development, flowering time, leaf senescence, and abiotic stress tolerance (Chen et al. 2015, Mao et al. 2017, 2020, Shen et al. 2017, Begcy et al. 2024).

Generally, the NAC protein structure is composed of major two components: a conserved N-terminus DNA-binding domain and a variable C-terminus transcriptional regulatory region (Puranik et al. 2012). The NAC transcription factor gene family underwent various expansion events after tracheophyte divergence with known genome duplication events occurring within angiosperms (Zhu et al. 2012, Maugarny-Calès et al. 2016, Jin et al. 2017). NAC proteins cluster into several subgroups based on concomitant phylogenetics (Ooka et al. 2003, Zhu et al. 2012, Maugarny-Calès et al. 2016). NAC gene family emergence occurred in streptophyte algae within Charales and Klebsormidiales (Maugarny-Calès et al. 2016). Although NAC genes have been extensively explored in angiosperms, few studies exist in gymnosperms (Pascual et al. 2015, 2018, Raherison et al. 2015). Nevertheless, the NAC function in green algae and bryophytes has not been characterized (Xu et al. 2014). Moreover, characterization of the evolution of NAC diversity requires the analysis of more diverse plants evolutionarily, including green algae, bryophytes, lycophytes, gymnosperms, and angiosperms.

Although phylogenetic analyses have revealed the evolutionary history of NAC transcription factors (Zhu et al. 2012, Fan et al. 2015, Maugarny-Calès et al. 2016), evidence of functional conservation in early land plants to domesticated crops is limited. In this study, using representative species of green algae, bryophytes, lycophytes, gymnosperms, and angiosperms including several domesticated crops, we identified clades that arose later during evolution. Transcriptionally, we show conservation of several NAC transcription factor genes in *Marchantia polymorpha* and rice orthologs in response to heat stress. Our results complement previous studies on the involvement of NAC transcription factors in stress responses and broaden the evolutionary scope of this gene family. Functional studies are now necessary to evaluate the specific role of the NAC transcription factors identified in this study and to elucidate their contribution during plant development and stress responses.

Results

Evolutionary history of the NAC transcription factors family in the plant kingdom

We searched for NAC protein-coding sequences in the complete genome or genome assemblies of representative members of green algae, bryophytes, lycophytes, gymnosperms, and angiosperms and included reported evidence of whole-genome duplication (WGD) or whole-genome triplication (WGT) of land plants (Clark and Donoghue 2018) to understand the deeper evolutionary history of the NAC transcription family (TF) (Fig. 1). As representative of green algae, we used the filamentous terrestrial green alga *Klebsormidium nitens* and identified three NAC TF genes in its genome. *Marchantia polymorpha* was selected as a basal lineage of land plants and identified nine NAC TF genes. As bryophyte representatives, we selected *Physcomitrium patens*, and 29 NAC TF genes were identified. Interestingly, the number of NAC TF increased three folds for *M. polymorpha* and nine folds for *P. patens* (Fig. 1) compared to the green algae *K. nitens*. It is important to note that *P. patens* underwent WGD (Rensing et al. 2007), and it could be the reason for the larger increase in NAC TF gene members within the bryophytes. For the lycophytes, we selected *Selaginella moellendorffii*, a member of an ancient vascular plant lineage, and identified 20 NAC TF genes. *Ceratopteris richardii* was selected as a fern representative and a total of 39 NAC TF were identified.

For gymnosperms, we selected the basal gymnosperms *Ginkgo biloba*, *Gnetum montanum*, and *Cycas panzhihuaensis*. We also included *Thuja plicata*, *Pinus taeda*, and *Picea abies* as representative plant species of the large evergreen coniferous trees in the order Pinales. Within the basal gymnosperms, we identified 32 NAC genes in *G. biloba*, 42 in *G. montanum*, and 43 in *C. panzhihuaensis*. Notably, the number of NAC TF genes in these plant species remained similar to the fern plant *C. richardii* (Fig. 1). For the coniferous species, 54, 78, and 90 NAC TF genes in *T. plicata*, *P. abies*, and *P. taeda* were identified, respectively.

Inside the angiosperms, we included *Amborella trichopoda*, a native plant species to New Caledonia important in evolutionary biology as it is the sole living species in the sister lineage to all other extant flowering plants, and identified 41 NAC TF (Fig. 1). Within the angiosperms, we selected monocot and eudicot representatives. Eudicot species included the model plants *Aquilegia coerulea*, *Arabidopsis thaliana*, and *Populus trichocarpa*, as well as the horticultural crops *Lactuca sativa*, *Solanum lycopersicum*, *Nicotiana benthamiana*, *Gossypium raimondii*, *Brassica rapa*, *Phaseolus vulgaris*, and *Fragaria vesca*. We identified a large number of NAC TF in the model angiosperm species being 79 in *A. coerulea*, 100 in *A. thaliana*, and 154 in *P. trichocarpa*. For the horticultural angiosperm species, 96 NAC TF genes were identified in *L. sativa*, 84 in *S. lycopersicum*, 208 in *N. benthamiana*, 152 in *G. raimondii*, 96 in *B. rapa*, 91 in *P. vulgaris*, and 123 in *F. vesca* (Fig. 1). Noteworthy, the massive number of NAC TF in *N. benthamiana*, which underwent two independent WGT and WGD events (Clark and Donoghue 2018).

For monocots, we selected the model species *Brachypodium distachyon* and the field crops *Oryza sativa*, *Sorghum bicolor*, and *Zea mays*. A massive increase in the total number of NAC TF were identified in monocots compared with green algae, bryophytes, and lycophytes. We identified 129 NAC TF genes for *B. distachyon*, 128 for *O. sativa*, 126 for *S. bicolor*, and 136 for *Z. mays*. Noteworthy, these monocot species underwent three independent rounds of WGD (Clark and Donoghue 2018). Our results show the presence of NAC TFs in all groups of the

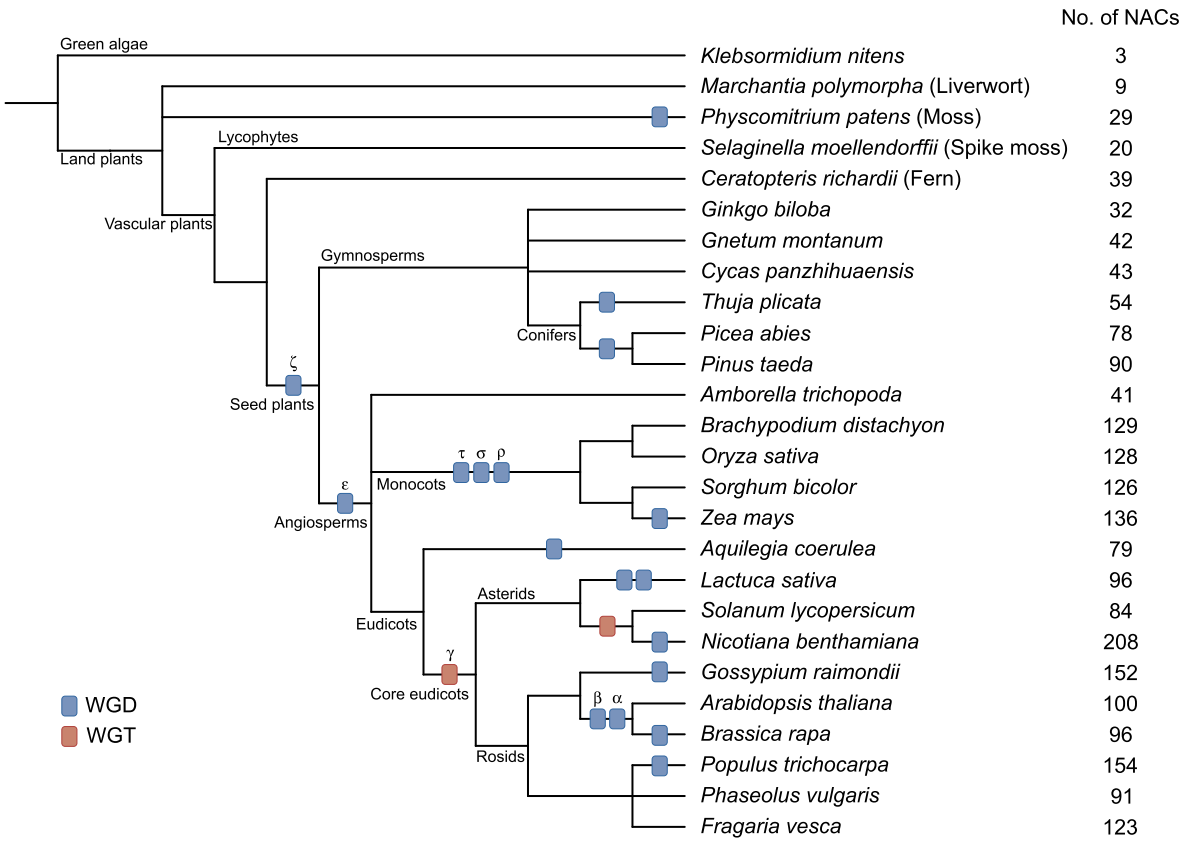

Figure 1. Evolutionary relationship of NAC transcription factors from green algae to domesticated crop. Representative plant species from green algae, bryophytes, lycophytes, gymnosperms, and angiosperms and the NAC gene number in their genomes are shown. Blue squares denote reported evidence of WGD events, and orange squares represent evidence of WGT events during their evolutionary history according to Clark and Donoghue (2018). The total count of identified NAC transcription factors is shown next to each plant species.

plant kingdom that expanded massively from green algae to modern cultivated crops. Multiple events of WGT and WGD could possibly explain the large increase of members of these gene families.

Novel nomenclature of the NAC transcription factor subfamilies

Aiming to understand the evolutionary relationships in the NAC transcription factor family, we generated orthogroups to compute phylogenetic trees using the NAC domain amino acid sequences from 26 plant species ranging from green algae, bryophytes, lycophytes, gymnosperms to angiosperms. A maximum likelihood analysis underpinned by the Q.Plant+R8 model (Ran et al. 2018) supports the existence, clade structure, branch length values, and topology of these clades (Fig. 2; Supplementary Fig. S1). A previously proposed NAC TF classification identified 21 different groups mainly derived from proteins discovered in nine plant species, most of them are within the flowering plants (Zhu et al. 2012). Even though this classification has been widely used to categorize the NAC TF family (Ooka et al. 2003, Fang et al. 2008, Nuruzzaman et al. 2010, Zhu et al. 2012), it has limitations, since a small number of plant species for the analyses was used. In our study, using 26 plant species covering all major groups of the plant kingdom, we propose an updated nomenclature that improves the current classification of the NAC subfamilies (Fig. 2). In general, our updated nomenclature is consistent with some of the subfamilies proposed by previous phylogenetic analyses (Ooka et al. 2003, Fang et al. 2008, Nuruzzaman et al. 2010, Zhu et al. 2012). However, major differences with previous studies are that (i) we defined a novel subfamily (Vc), (ii) split subfamilies VII and X into VIIa and VIIb, and Xa and Xb, respectively; and (iii) reclustered previously uncharacterized NAC into the subfamily VIII. We relabeled these subfamilies in simple Roman numerals, following previous nomenclatures (Zhu et al. 2012) (Supplementary Fig. S1).

In our updated nomenclature, 23 NAC subfamilies were identified, from which 10 subfamilies include only members from seed plants (Supplementary Fig. S2). Within these, one of them is a monocot-only family containing members. Another interesting finding is that subgroup VIa, previously described as angiosperm-specific (Zhu et al. 2012), also contains members from the fern *C. richardii* and *G. montanum*. Therefore, it is not as angiosperm-specific as previously postulated. Similarly, NAC subfamily VII was regarded as tree-specific (Zhu et al. 2012),

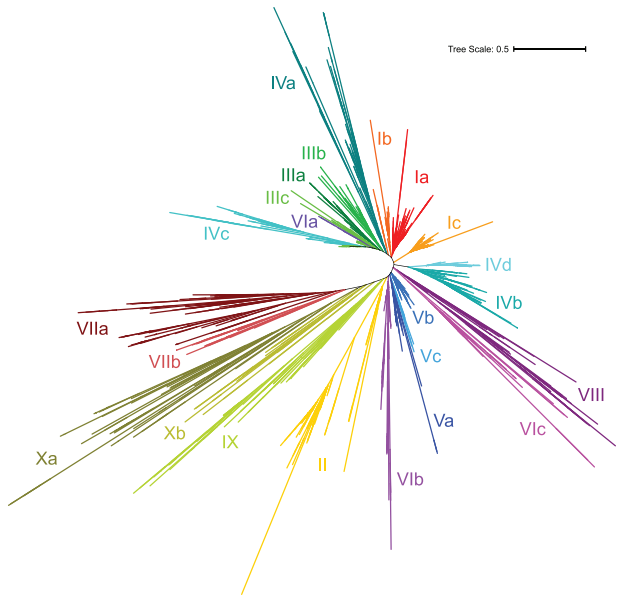

Figure 2. Evolutionary history of NAC transcription factors. Phylogenetic analysis of NAC transcription factors was inferred from 87 amino acids from the NAC domain. The maximum likelihood phylogenetic reconstruction and the *Q.Plant+R8* model using 2000 bootstrap replicates were used to support the estimation. The phylogenetic tree was visualized and edited using iTOL. Scale length indicates 0.5 substitutions per site.

since only contained members of *P. trichocarpa*. However, we identified members in the model plants *A. coerulea* and the horticultural crops *S. lycopersicum*, *N. benthamiana*, *G. raimondii*, *B. rapa*, and *F. vesca*. Our new classification including a large number of plant species indicates that (i) NAC TF factors are ubiquitously distributed across the plant kingdom and (ii) members of the monocot- and eudicot-specific subfamilies evolved separately and might have some unique developmental and stress response properties.

Highly conserved NAC TF physicochemical properties across land plants

To further characterize NAC transcription factors in land plants, we collected their physiochemical features including mapping positions, molecular weight (MW), theoretical isoelectric point (pI), instability index, aliphatic index, hydropathicity, and predicted subcellular localization (Supplementary Table S1). NAC TF members of *M. polymorpha* were on average 50 kDa. However, their MWs ranged from ∼38 to 93 kDa, similar to the MWs found in *P. Patens* and the green algae *K. nitens*. Noteworthy, 80% of the *C. richardii* NAC proteins had <20 kDa of MW. NAC proteins of *S. moellendorffii* showed a small variation in MWs with an average of ∼20 kDa. Within the gymnosperms, *G. montanum* and *G. biloba* have an average MW of ∼40 kDa and 50 kDa, respectively. For angiosperms and particularly major crops, an average of 41–42 kDa was found in *O. sativa*, *S. bicolor*, and *Z. mays*.

We then explored the NAC TF subcellular localization (Fig. 3a). Consistent with their putativefunction as transcription factors, most of the NAC across all the plant species analyzed were predicted to be nuclear localized. Some of the members of subgroups IIIa, IIIb, IIIc, IVa, IVc, and VIa were also to be predicted in the cytoplasm and endoplasmic reticulum (Fig. 3a). We further tested the accuracy of the subcellular DeepLoc 2.0 (Thumuluri et al. 2022) prediction tool by selecting *ONAC005* (LOC_Os08g42400) and *OsNAC10* (LOC_Os11g03300) for subcellular localization analysis. As predicted both *ONAC005* and *OsNAC10* were nuclear localized (Fig. 3b).

To explore the possibility that the large expansion of NAC genes from green algae to domesticated crops gives rise to novel gene structures and motif features, we compared all the NAC genes in green algae, bryophytes, lycophytes, gymnosperms, and angiosperms. On average, the exon number was consistent in all plant species analyzed with an average of three to four exons. However, the variation in eudicots and monocots was more pronounced than that in any other group of plants (Fig. 3c). We then grouped their motifs by NAC subgroups and displayed them when the percentage of genes within each motif was >20% (Fig. 3d), as well as their conserved motifs identified using the Multiple Em for Motif Elicitation (MEME) analysis (Supplementary Fig. S4; Supplementary Tables S3 and S4). We found a high conservation in all subgroups. However, it was predominantly found in the N-terminal regions. The C-terminal ends contained fewer motifs and were less conserved, thereby corroborating previous studies suggesting that N-terminal NAC domains are conserved, whereas the C-terminus is highly divergent (Ooka et al. 2003). In general, each subgroup shared similar motifs (Fig. 3d). The presence of highly conserved motifs among NAC proteins of the same subfamily suggests that they are essential for the function of the NAC proteins within subfamilies and further supports the evolutionary phylogenetic relationships we have inferred (Fig. 2).

Cis-element analysis of NAC transcription factors

To understand NAC TF regulation, we performed a *cis*-regulatory element analysis using the 2-kb proximal upstream region of the annotated start codon of all genes identified from each plant species analyzed (Fig. 4; Supplementary Fig. S5; Supplementary Table S5). We focused on *cis*-regulatory regions related to core elements, stress, hormone and light responses, cell cycle, tissue specificity, metabolism, and circadian rhythm. The large group of *cis*-regulatory elements shared by green algae, bryophytes, lycophytes, gymnosperms, and angiosperms is related to stress and hormone responses (Fig. 4). Remarkably, we identified a unique *cis*-element (Pc-CMA2b) in *A. coerulea* and *N. benthamiana* related to light responses. We also identified a unique TGGCA *cis*-element related to light in *S. bicolor* (Fig. 4). Interestingly, the dehydration-responsive element core, MYB binding site, myeloblastosis (MYB) recognition site, and myelocytomatosis oncogene (MYC) *cis*-elements were highly abundant in all green algae, bryophytes, lycophytes, gymnosperms, and angiosperms plant species. The defense- and stress-responsive thymine and cytosine rich (TC-rich) repeat *cis*-element was absent in *P. patens*, *P. abies*, and *T. plicata* but

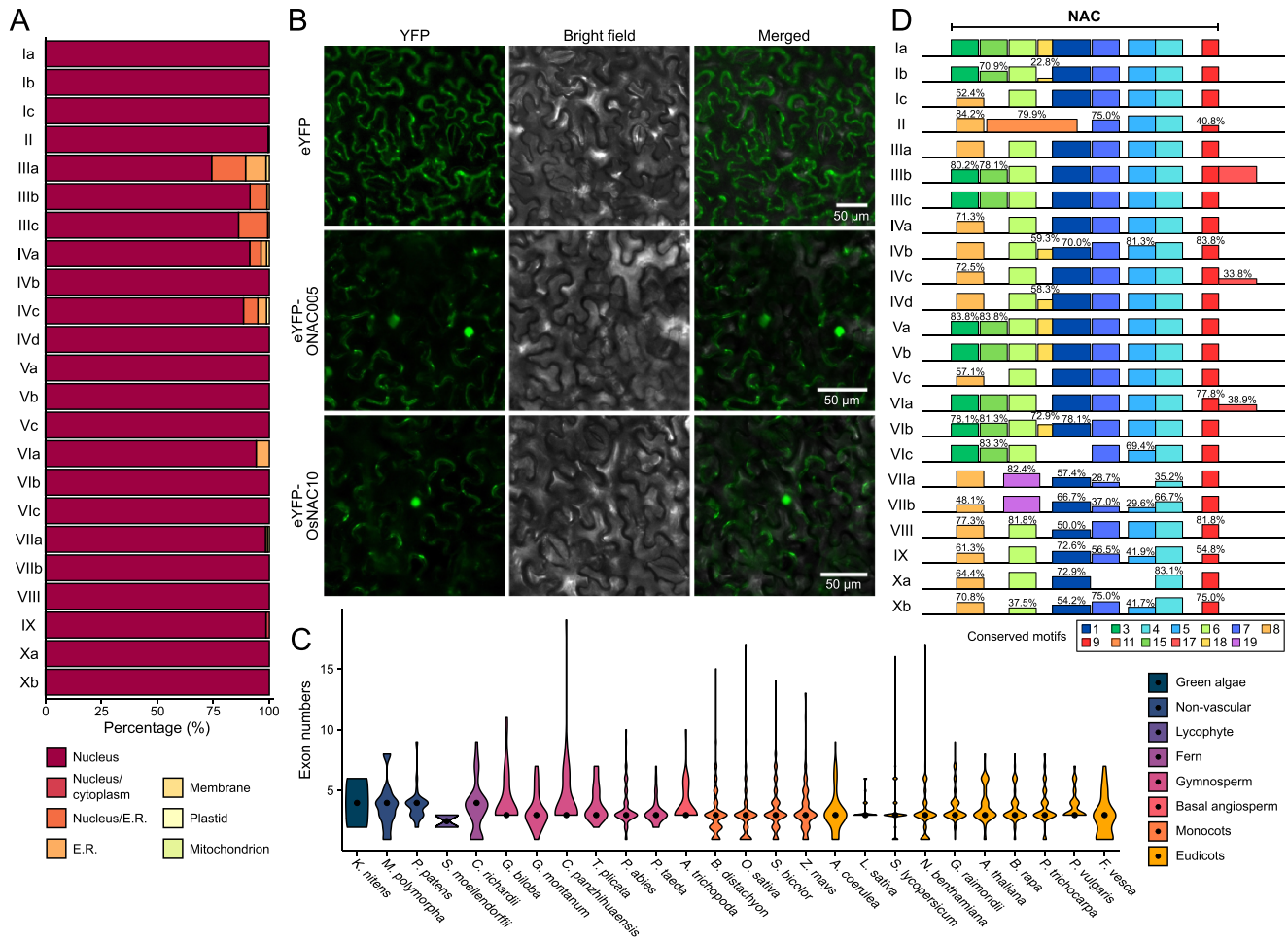

Figure 3. Subcellular localization, exon number, and motif analysis of NAC proteins. (A) Subcellular localization prediction of NAC proteins. (B) Subcellular localization analysis of *ONAC005* (LOC_Os08g42400) and *OsNAC10* (LOC_Os11g03300) in *N. benthamiana* leaves. *35S::eYFP* was used as a control. Scale bar = 50 μm. Golden Gate construct maps are illustrated in Supplementary Fig. S3. (C) Distribution of exon numbers in NAC transcription factor genes. (D) Conserved motifs displayed by phylogenetic groups. Motifs are shown when the percentage of genes within each motif is >20%. Percentages are not shown if the percentage is >85%.

present in at least 20% of all green algae, bryophyte, lycophyte, gymnosperm, and angiosperm NAC genes (Supplementary Table S5). The hormone response category was divided into auxin, abscisic acid (ABA), gibberellic acid, ethylene, jasmonic acid (JA), and salicylic acid *cis*-elements (Supplementary Table S5). The ethylene-responsive element was absent in *K. nitens*, only identified in a single *M. polymorpha* NAC gene, whereas it was present in at least 24% of angiosperms and 65% of gymnosperms. ABA- and JA-related *cis*-elements were ubiquitously present in most upstream regulatory regions of NAC transcription factors analyzed (Supplementary Table S5). The light response category was overrepresented in all upstream regions of NAC TF, suggesting their responsiveness to light. All the other remaining *cis*-regulatory element categories, including cell cycle, tissue specificity, metabolism, and circadian rhythm, showed *cis*-regulatory elements highly abundant in each category (Supplementary Table S5).

Large overlap of NAC syntenic clusters among bryophytes, lycophytes, gymnosperms, and angiosperms

To investigate the syntenic relationships among NAC genes, we performed 676 pairwise all-against-all comparisons of protein sequences using the Diamond tool (Buchfink et al. 2021), followed by collinearity detection on MCScanX (Wang et al. 2012). We conducted a syntenic network analysis to identify conserved or lineage-specific syntenic clusters. We identified 138 syntenic clusters across the bryophytes, lycophytes, gymnosperms, and angiosperms studied. We quantified gene copy numbers in each plant species and categorized the identified clusters into five distinct groups based on the presence of NAC genes (Fig. 5a; Supplementary Table S6): *P. patens*-specific (6 clusters), Gymnosperm-specific (15 clusters), monocot-specific (45 clusters), eudicot-specific (29 clusters), and angiosperm-wide (14 clusters).

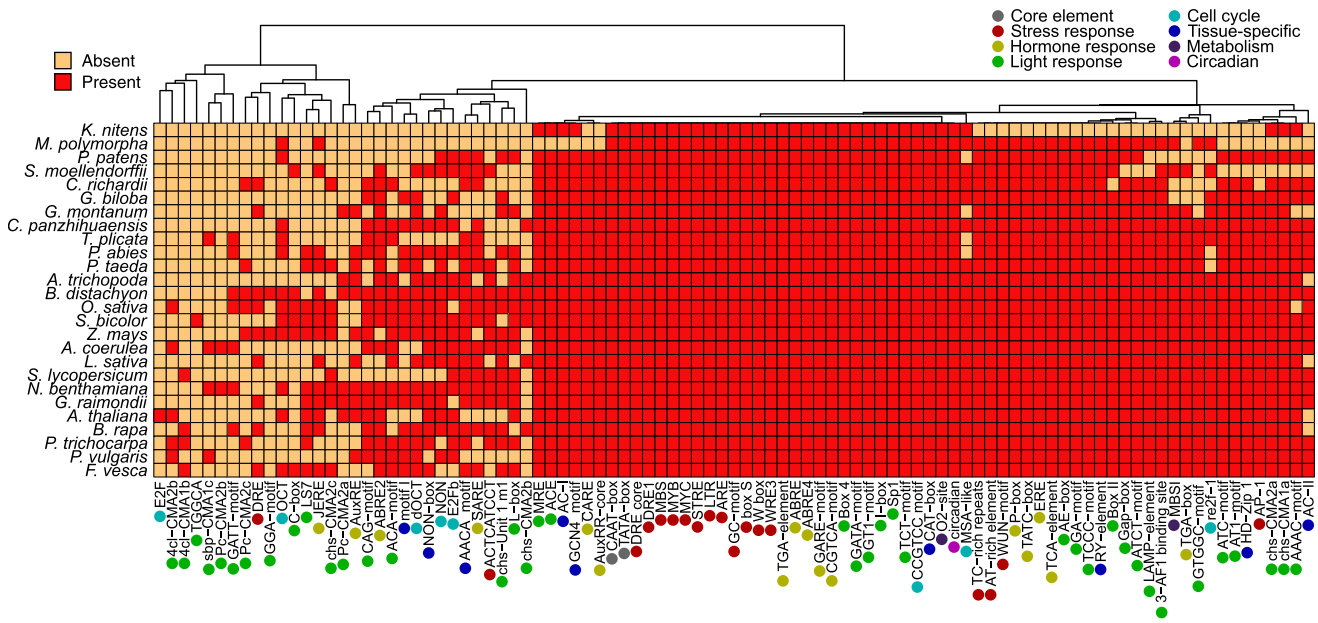

Figure 4. Shared and unique *cis*-regulatory elements in NAC transcription factor promoters. From each plant species, 2-kb upstream regions of each NAC gene identified were used to analyze *cis*-regulatory elements. Orange color represents the absence and red represents the presence of a particular *cis*-regulatory element in each species. The identity of *cis*-regulatory elements is indicated in colored circles as follows: gray for core elements; maroon for stress responses; olive for hormone responses; emerald for light responses; turquoise for cell cycle; dark blue for tissue-specific elements; indigo for metabolism; and magenta for the circadian clock.

Interestingly, *P. patens*-specific clusters contain multiple syntenic pairs of NAC genes in groups Ia, Ic, IIIc, and Va. In these specific groups, *P. patens* showed a significantly higher gene count compared to *M. polymorpha*, despite both bryophyte species having an identical gene count in groups II and IVb (Supplementary Fig. S2). This indicates that the WGD event, as shown in Fig. 1, likely contributed to the expansion of gene numbers within these groups for *P. patens*. Furthermore, the majority of angiosperm-wide syntenic clusters contain *A. trichopoda* NAC genes, along with an increased copy number of NAC genes in monocot and eudicot species. This implies that the NAC genes within these clusters appeared early in evolutionary history and experienced amplification in gene copies during the diversification of monocot and eudicot lineages.

To further estimate the selection pressure on NAC genes within syntenic relationships, we calculated the ratio of nonsynonymous to synonymous substitutions (dN/dS) for the identified syntenic gene pairs. The dN/dS ratio is used as an indicator of gene selection pressure, with dN/dS > 1 meaning positive selection, dN/dS = 1 denoting neutral selection, and dN/dS < 1 representing negative selection (Kryazhimskiy et al. 2008). Our results revealed that most analyzed NAC genes exhibit dN/dS ratios <1 except for *N. benthamiana* (Fig. 5b; Supplementary Table S7), indicating that while the vast majority of NAC genes were subjected to negative selection pressure, *N. benthamiana* NAC genes were subjected to positive selection. These results show the importance of NAC genes for plants in general, since the negative selection has allowed to maintain long-term stability of NAC gene function by removing deleterious mutations.

Developmental expression of NAC transcription factors in bryophytes, lycophytes, gymnosperms, and angiosperms

We explored whether a conserved developmental expression was present in NAC transcription factor genes from bryophytes to angiosperms (Supplementary Fig. S7). We divided our analysis into reproductive and vegetative development. NAC TF transcriptional data in *M. polymorpha* showed strong expression in thallus and rhizoid. However, the gemmaling and male reproductive organs (sperm and antheridium) were substantially low, although *MpNAC6* (Mp5g01530) clustered in clade IVd has a strong gemmaling expression. Contrarily, all nine *M. polymorpha* NAC genes have a high expression in female archegoniophores, especially *MpNAC8* (Mp6g02670) (Supplementary Fig. S7).

In *P. patens*, we identified members in clusters Ia, IIIc, and Vb as preferentially expressed in spores. Noteworthy, *PpNAC025* (Pp3c16_23260) clustered in subgroup IVb showed strong female and male expression (Supplementary Fig. S7). For *S. moellendorffii*, low expression was detected across all NAC genes, but the only high transcriptional expression was detected in roots (Supplementary Fig. S7). Whitin gymnosperms, while NAC genes in *G. biloba* showed strong expression in roots and ovules, in *P. abies* higher expression was found in female buds than male buds (Supplementary Fig. S7).

Amborella trichopoda showed transcriptionally active expression of all its members in flowers, fruits, shoots, leaves, and roots. However, expression in mature pollen and sperm cells was practically absent, while female reproduction was mainly attributed to NAC membersclustered in subgroups II, IIIa, IIIb,

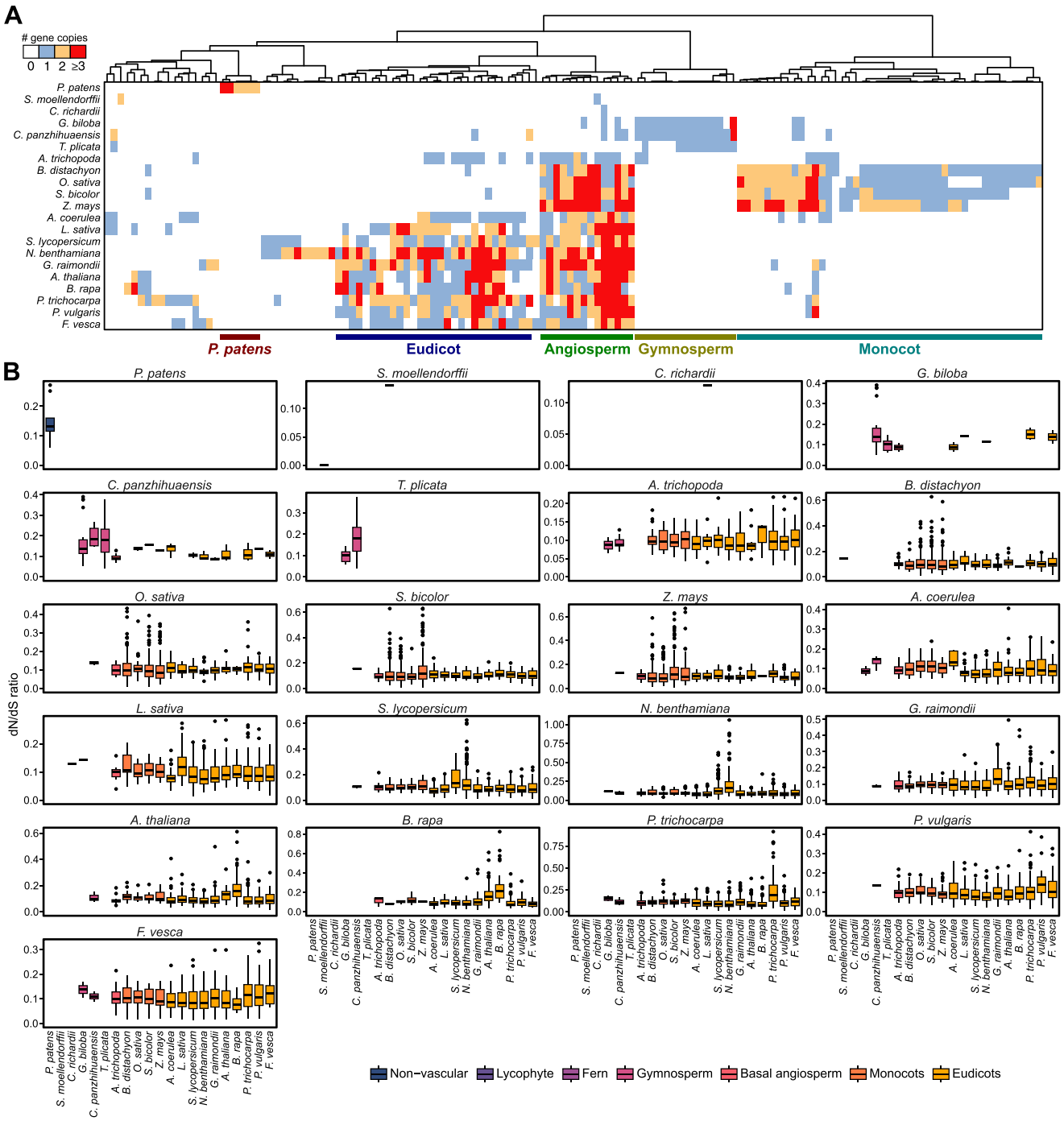

Figure 5. Syntenic analysis of NAC genes. (A) Phylogenomic profiling of syntenic clusters. NAC gene counts for each syntenic cluster of each species analyzed are displayed. Detailed information on syntenic clusters is shown in Supplementary Table S6. (B) dN/dS ratios of syntenic gene pairs. Species (*K. nitens*, *M. polymorpha*, *G. montanum*, *P. abies*, and *P. taeda*) that did not show collinearity with the other plant species analyzed were excluded. The dN/dS ratio between each gene pair is detailed in Supplementary Table S7.

and Vb and one single member of subgroup IX (Supplementary Fig. S7).

In the monocots, *B. distachyon*, *O. sativa*, *S. bicolor*, and *Z. mays* a clear root-specific expression was identified in most of the clades. Interestingly, clades VIII–Xb did not show strong NAC expression in root tissues in rice. Another interesting expression pattern in monocots is the low transcriptional expression in mature pollen. For eudicots, *A. thaliana*, *S. lycopersicum*, *B. rapa*, and *L. sativa* showed similar patterns of expression mostly in roots with a clear reduced mature pollen expression (Supplementary Fig. S7). Our results show that developmentally NAC TFs are expressed in most plant tissues. However, a low or absent expression in mature pollen was observed commonly across all the plant species analyzed. This

is intriguing based on their known role in plant development (Supplementary Fig. S7).

Transcriptional responses of NAC TF under heat stress in *M. polymorpha* and rice

NAC transcription factors have been shown to be master regulators of stress responses (Chen et al. 2015, Mao et al. 2017, 2020, Shen et al. 2017, Begcy et al. 2024). Therefore, we tested whether their responses were evolutionary conserved. We selected heat stress due to its relevance to the current climate change scenario (Folsom et al. 2014, Chen et al. 2016, Begcy et al. 2018a, 2018b, 2019, Li et al. 2024). First, to determine whether heat stress responsive clusters arose during evolution, we used transcriptional data generated from moderate (35°C) and severe (39°C) heat stress on rice plants at 24 h post-fertilization and maintained for 48 and 72 h (Chen et al. 2016). We focused on the NAC TF responses during seed development (Supplementary Fig. S8). First, we clustered the RNA-sequencing (RNA-seq) data based on our phylogenetic group classification (Fig. 2). In general, we observed that some NAC transcription factors from clades Ia, IVd, VIII, IX, and Xa and Xb are not expressed during early seed development (Supplementary Fig. S8). Interestingly, at least one member of all rice NAC genes containing clades were induced by heat stress. However, the induction was more pronounced in clades Ia, II, VIII, and IX at 72 h (Supplementary Fig. S8). Our results point to a clade-specific heat stress response most likely occurring during early seed development.

We then selected *M. polymorpha* and *O. sativa* as representative plant species to explore the transcriptional responses under heat stress conditions. *Marchantia polymorpha* has a low paralog number (Berrie 1960), simplifying the genetic analysis needed to unravel the molecular stress response machinery in orthologs species. Within domesticated crops, rice is the most important crop in the world from a global food security perspective and it is an attractive system to study mechanisms of plant response to environmental stresses (Folsom et al. 2014, Chen et al. 2016, Begcy et al. 2018a). Rice has a high-quality sequenced genome and a suite of genomic resources that make it a suitable model to study gene family evolution (Sasaki 2005, Jackson 2016). We imposed heat stress (40°C/35°C day/night temperatures) for 48 h on both plant species and quantified their transcriptional responses. Control plants were maintained at 28°C/25°C day/night temperature. We tested all nine *M. polymorpha* NAC transcription factor genes and their close rice orthologs (Fig. 6). We did not observe any morphological changes resulting from the increased temperatures in *M. polymorpha* and rice (Supplementary Fig. S9).

MpNAC1 (Mp2g07720) and *MpNAC2* (Mp6g02590) paralogs clustered in group Ia and showed a stiff decline after 24 h of heat stress and under control conditions. However, both *MpNAC1* and *MpNAC2* had a sharp increase at 48 h, while control conditions remained unchanged (Fig. 6). We selected *OsNAC2* (LOC_Os04g38720) and *ONAC107* (LOC_Os08g44030) as representatives of group Ia for rice. Both rice genes and corresponding *M. polymorpha* orthologs showed a similar decrease of expression at 24 h of heat stress (Fig. 6). However, after 48 h of heat stress only *OsNAC2* (LOC_Os04g38720) resembled *M. polymorpha* group Ia transcriptional expression. *ONAC107* (LOC_Os08g44030) remained downregulated during the heat stress period (Fig. 6). In group Ic, *MpNAC5* (Mp6g20920) showed a decrease in expression in both control and heat stress conditions after 24 h followed by a statistically significant upregulation at 48 h compared to control conditions. *ONAC084* (LOC_Os04g59470) and *OsSWN1* (LOC_Os06g04090) were not responsive to high temperatures. *MpNAC9* (Mp5g08410), belonging to group II, showed a similar expression pattern to that of *MpNAC5*, which was clustered in group Ic (Fig. 6). Both rice genes *ONAC050* (LOC_Os02g38130) and *ONAC066* (LOC_Os01g09550) did not respond to heat stress as they were transcriptionally similar in both conditions (Fig. 6).

Marchantia polymorpha genes of group IIIc showed an opposite gene expression. While *MpNAC3* (Mp4g11910) was upregulated, *MpNAC4* (Mp4g22890) was downregulated in response to heat stress (Fig. 6). *ONAC054* (LOC_Os03g02800) and *ONAC121* (LOC_Os10g42130) did not show transcriptional differences when compared to heat stress and control conditions. *MpNAC8* (Mp6g02670), *ONAC022* (LOC_Os03g04070), and *ONAC095* (LOC_Os06g51070) were representative genes selected for group IVb (Fig. 6). *MpNAC8* was not significantly expressed in response to heat stress (Fig. 6). Both *ONAC022* and *ONAC095* were only upregulated at 36 h of heat stress, but no transcriptional differences were observed at 48 h (Fig. 6).

In group IVd, *MpNAC6* (Mp5g01530) was upregulated at 24 and 48 h of heat stress (Fig. 6). *OsNAC4* (LOC_Os07g04560) behaved similarly as *MpNAC6* as both genes were upregulated in all time points. However, *ONAC063* (LOC_Os08g33910) did not change its gene expression during heat stress, but at 36 h control, 5 folds increased in expression was detected (Fig. 6). In group Vb, *MpNAC7* (Mp6g02620) did not change at 24 h, but it was upregulated at 48 h of heat stress (Fig. 6). Rice selected genes, *OsNAC3* (LOC_Os07g12340) and *OsNAC9* (LOC_Os03g60080), were upregulated throughout the heat stress conditions (Fig. 6). In summary, our results showed that although not all tested rice NAC genes behaved similarly to *M. polymorpha* NAC genes, some clusters showed conserved transcriptional responses between both species under heat stress conditions suggesting a common function of those genes in bryophytes and monocots.

Discussion

During plant evolution and domestication, key gene families and genetic signatures have allowed plants to adequately respond to a variety of biotic and abiotic stresses. Therefore, they have developed versatile systems for accurate stress signal perception and transduction as well as during development. One instance is the vast plant-specific NAC transcription factor family, known to play a role in multiple abiotic stress responses (Puranik et al. 2012). However, functional evidence on early land plants is limited since the large abundance of NAC transcription factors has beendocumented in crop species (Nuruzzaman et al.

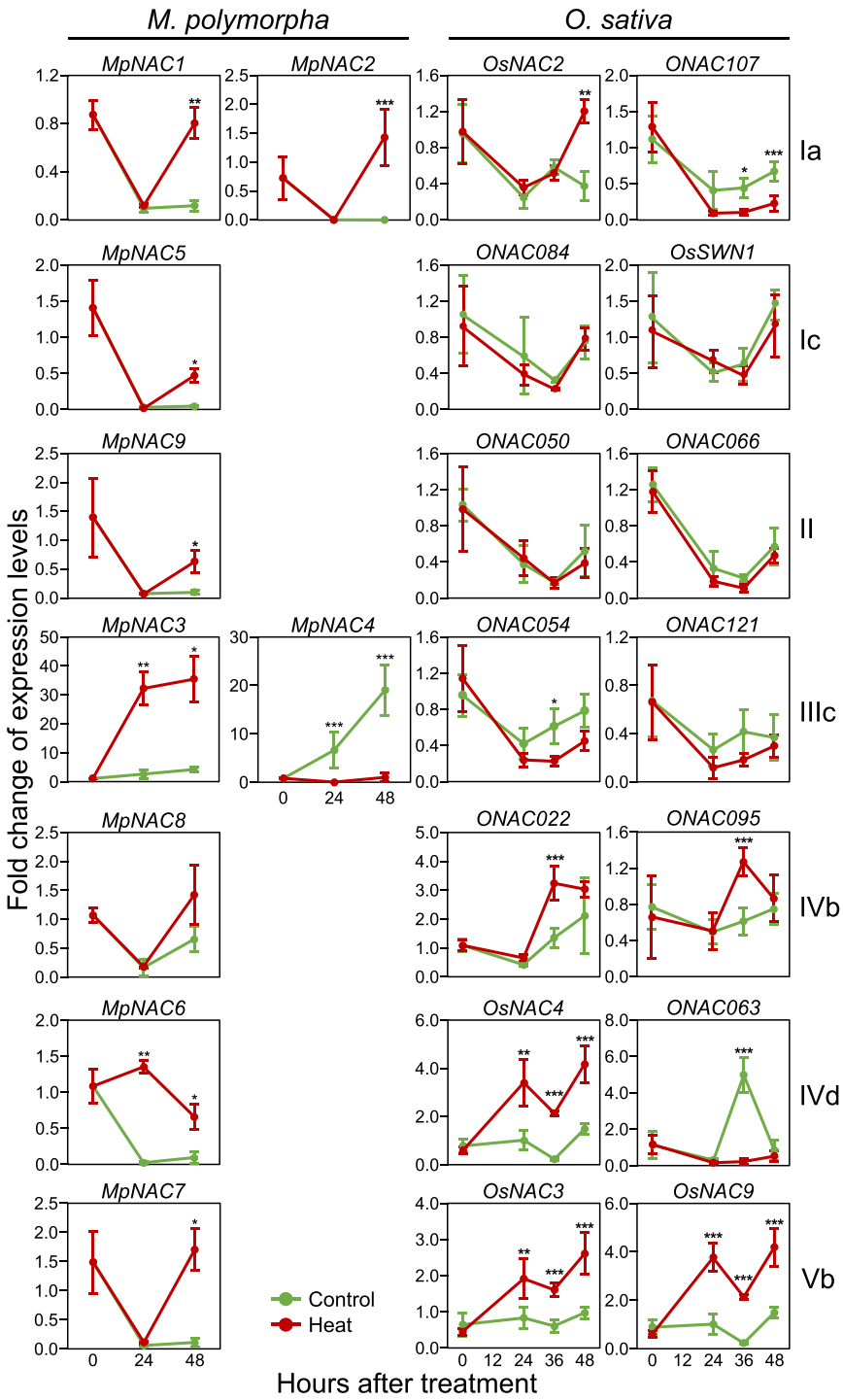

Figure 6. Conserved transcriptional expression patterns in response to heat stress in *M. polymorpha* and *O. sativa*. Gene expression levels of *MpNACs* and *OsNACs* in *MpNAC*-containing clades were determined by qPCR analysis. Expression levels of *MpNAC* and *OsNAC* genes were normalized to those of *MpACTIN7* (Mp6g11010) and *OseEF-1a* (LOC_Os03g08020), respectively. The mean of three biological replicates and the standard deviation are presented. Heat stress treatment was applied using 40°C/35°C day/night temperatures for 2 days. Control plants were maintained at 28°C/25°C day/night temperature. Asterisks denote statistically significant differences between heat stress and control conditions determined by Student's *t*-test (*$P < .05$, **$P < .01$, and ***$P < .001$).

2010, Zhu et al. 2012). We used representative plant species from diverse taxa including green algae, bryophytes, lycophytes, gymnosperms, and angiosperms to explore the evolution of NAC TF across the plant kingdom. Previous studies suggest that NAC members in clades Ia and Ic are developmental regulators. For instance, *AtVND7* (AT1G71930) is associated with

flowering time alteration (Fujiwara and Mitsuda 2016). *AtVND4* (AT1G12260) and *AtVND5* (AT1G62700) activate secondary wall biosynthesis genes in vessels, and *AtVND6* (AT5G62380) and *AtVND7* (AT1G71930) induce transdifferentiation of cells into metaxylem- and protoxylem-like vessel elements (Kubo et al. 2005, Zhou et al. 2014). *ANAC094* (AT5G39820) has been described as a regulator of ER-programmed cell death (Yang et al. 2014). *MpNAC1* (Mp2g07720) has been previously shown to have enriched expression in sporeling tissue at a 3-day time frame (Flores-Sandoval et al. 2018). *MpNAC1* (Mp2g07720) is in the same clade as *CUC3* (AT1G76420), a member of the Cup-Shaped Cotyledon involved in both separation of cotyledons and reproductive organs (Aida et al. 1997) as well as leaf serration degree (Nikovics et al. 2006, Hasson et al. 2011). Within the *CUC1/2/3* paralogs, *CUC1* (AT3G15170) and *CUC2* (AT5G53950) are regulated by miR164. Post-transcriptional regulation of *CUC* is also an ancient mechanism that is present in *A. trichocarpa*. Genome duplication of the *CUC3* (AT1G76420) lineage gave rise to an miRNA-mediated regulation of *CUC1* (AT3G15170) and *CUC2* paralogs (Vialette-Guiraud et al. 2011). Given the vegetative growth from the apical notch in *M. polymorpha* and shoot apical meristem in *Arabidopsis*, it is unknown whether *MpNAC1* and *MpNAC2* function in boundary separation as in the functionally redundant *AtCUC1* (AT3G15170), *AtCUC2* (AT5G53950), and *AtCUC3* (AT1G76420) or a different role altogether. Based on our results, the increased expression of *MpNAC1* in vegetative thallus (Supplementary Fig. S7) and findings from previous research (Romani et al. 2024) indicate that *MpNAC1* (Mp2g07720) regulates vegetative growth and development. Mutant studies are now required to support this assumption.

Many other NAC genes are involved in stress responses. For instance, in rice, *ONAC063* (LOC_Os08g33910) expression is upregulated in response to dehydration and salt stress (García-Morales et al. 2014). In *Arabidopsis*, *ANAC009/FEZ* (AT1G26870) is expressed in the root cap to regulate proper cell division and it is in a regulatory feedback loop with *ANAC033/SMB/URP7* (Willemsen et al. 2008). Expression of *M. polymorpha AUXIN RESPONSE FACTOR3* results in decreased expression of *MpNAC6* (Mp5g01530) (Flores-Sandoval et al. 2018), ortholog of *ANAC009/FEZ*, suggesting that auxins may integrate MpNAC transcription factors during development. Functional studies are now required to validate this hypothesis. Therefore, some common NAC functions among green algae, bryophytes, lycophytes, gymnosperms, and angiosperms have been maintained, whereas others have evolved to adapt to terrestrial landscapes (Maugarny-Calès et al. 2016).

To examine whether NAC gene stress responses were present in *M. polymorpha*, we explored their transcriptional expression in response to heat stress and compared it with domesticated crop rice. The only NAC *M. polymorpha* gene that did not show statistically significant differences against control conditions was *MpNAC8* (Mp6g02670). All the other *M. polymorpha* NAC genes were differentially expressed at both or one of the time points analyzed (Fig. 6). For instance, *MpNAC1* and *MpNAC2* were significantly upregulated after 48 h of heat stress (Fig. 6). Both genes cluster together in group Ia (Fig. 2; Supplementary Fig. 1). Thus, it is tempting to speculate that *MpNAC1* (Mp2g07720) and *MpNAC2* (Mp6g02590) may be functionally redundant in the heat stress response. However, further studies are needed to corroborate our findings. In the case of flowering and domesticated crops, members of clade Vb appear to have a role in stress responses as *OsNAC6* (LOC_Os01g66120) is regulated by drought, salt, wounding, and JA (Ohnishi et al. 2005). Furthermore, other members of subgroup Vb, such as *OsNAC3* (LOC_Os07g12340) (Zhang et al. 2021), *OsNAC5* (LOC_Os11g08210) (Jeong et al. 2013), *OsNAC9* (LOC_Os03g60080) (Redillas et al. 2012), and *OsNAC52* (LOC_Os05g34830) (Gao et al. 2010), have been reported to regulate drought and salt stress responses. Consistently, we found that *MpNAC7* (Mp6g02620) cladded in subgroup Vb significantly upregulated after 48 h of heat stress treatment (Fig. 6), suggesting that several genes from these two clades might function in similar mechanisms in response to heat stress across different developmental stages. Functional studies are now needed to support this hypothesis.

The analysis of *cis*-elements in the promoter regions of NAC transcription factors provides evidence to further understand the regulatory control of the stress-inducible expression of this gene family (Fig. 4). We found a large number of the stress-related *cis*-elements in all plant species analyzed. *Cis*-regulatory elements vary based on genetic and evolutionary properties. For instance, the high level of conservation of *cis*- and *trans*-regulatory elements in the chicken genome was attributed to the relatively short evolutionary distance between the breed lines analyzed (Wang et al. 2019). Therefore, according to our analyses, the high variability of many *cis*-regulatory elements in green algae, bryophytes, lycophytes, gymnosperms, and angiosperms could be attributed to millions of years of divergence these species have gone through. Further investigations are now necessary to understand the function of these *cis*-regulatory elements in the NAC transcription factor family. In summary, we showed that NAC transcription factors are an ancient family spanning from green algae, bryophytes, lycophytes, gymnosperms to angiosperms including several subgroups sharing unique characteristics. The massive expansion experienced by NAC transcription factors might help to explain the complex adaptative mechanisms shown by plants during development and in response to environmental stresses.

Materials and Methods

Genome resource databases

Genomic information and primary transcript sequences from 26 plant species were retrieved from different databases: JGI PhycoCosm (https://phycocosm.jgi.doe.gov/phycocosm/home) for *K. nitens* (v1.1) (Hori et al. 2014); MarpolBase (https://marchantia.info) for *M. polymorpha* (v6.1) (Montgomery et al. 2020); GinkgoDB (https://ginkgo.zju.edu.cn) for *G. biloba* (v2019) (Gu et al. 2022); Cycas genome database (https://db.cngb.org/codeplot/datasets/PwRftGHfPs5qG3gE) for *C. panzhihuaensis* (Liu et al. 2022); TreeGenes (https://treegenesdb.org) (Wegrzyn et al. 2019) for *G. montanum* (v1.0), *P. abies* (v1.0b),

and *P. taeda* (v2.01); NbenBase (https://nbenthamiana.jp) for *N. benthamiana* (v1.1.2) (Bombarely et al. 2012); and Phytozome (https://phytozome-next.jgi.doe.gov) for *P. patens* (v3.3) (Lang et al. 2018), *S. moellendorffii* (v1.0) (Banks et al. 2011), *C. richardii* (v2.1) (Marchant et al. 2019), *T. plicata* (v3.1) (Shalev et al. 2022), *A. trichopoda* (v1.0) (Albert et al. 2013), *A. coerulea* (v3.1) (Filiault et al. 2018), *A. thaliana* (Araport11) (Cheng et al. 2017), *S. lycopersicum* (ITAG4.0) (Hosmani et al. 2019), *L. sativa* (v8) (Reyes-Chin-Wo et al. 2017), *B. distachyon* (v3.1) (The International Brachypodium Initiative 2010), *O. sativa* (v7.0) (Kawahara et al. 2013), *S. bicolor* (v3.1.1) (McCormick et al. 2018), *Z. mays* (Zm-B73-REFERENCE-NAM-5.0.55) (Hufford et al. 2021), *F. vesca* (v4.0.a2) (Li et al. 2019), *P. vulgaris* (v2.1) (Schmutz et al. 2014), *P. trichocarpa* (v4.1) (Tuskan et al. 2006), *B. rapa* (FPsc v1.3), and *G. raimondii* (v2.1) (Paterson et al. 2012).

Genome-wide identification and phylogenetic analyses of NAC transcription factors InterProScan (Jones et al. 2014) and National Center for Biotechnology Information - Conserved Domains Database (NCBI-CDD) (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Wang et al. 2023) were used to confirm the presence and the integrity of the NAC domain (IPR003441). Proteins with incomplete NAC domains were subsequently excluded from further analyses. A total of 2182 NAC transcription factor protein sequences from all the species analyzed were submitted to the Mafft software V7.525 (Katoh and Standley 2013) for alignment using default parameters and a 1000 maxiterate. TrimAL software version 2.rev0 (Capella-Gutiérrez et al. 2009) was used to trim gaps in the multiple alignments with a gap threshold set at 0.8 and a similarity score at 0.1%. Eighty-seven amino acids from the NAC domain were used to identify the best-fit model inference using IQTree v2.1.2 (Minh et al. 2020). The Q.Plant+R8 model (Ran et al. 2018) was selected and assigned for the maximum likelihood phylogenetic reconstruction using 2000 bootstrap replicates to support the estimation. The resulted phylogenetic tree was visualized and edited using iTOL v6.8.1 (https://itol.embl.de). NAC groups' nomenclature was initially assigned as previously described (Zhu et al. 2012), and then novel groups were designated based on their subgroup location and bootstrap values.

Physicochemical properties and subcellular localization of NAC transcription factors

Physical and chemical properties including MW, isoelectric point, instability index, aliphatic index, and hydropathicity of NAC transcription factors were estimated using the ProtParam tool from the ExPASy server (Gasteiger et al. 2005). Localization by subcellular compartment predictions of identified NAC transcription factor protein sequences was performed using DeepLoc-2.0 (Thumuluri et al. 2022) with the top probability score after running both fast and accurate methods. To confirm the DeepLoc-2.0 prediction, total RNA was extracted from rice leaves using TRIzol (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. First-strand cDNA synthesis was performed using the iScript cDNA synthesis kit using oligo(dT) primers (Bio-Rad, Hercules, CA, USA). Plasmids for subcellular localization were constructed using 35S promoter, eYFP, and the NOS terminator fragments amplified from pN_35S/mCitrine/P_UBQ10/Derlin1-mOrange2 (Addgene plasmid no. 118000) using the Golden Gate system (Weber et al. 2011). *ONAC005* (LOC_Os08g42400) and *OsNAC10* (LOC_Os11g03300) were amplified from rice leaf cDNA using gene-specific primers (Supplementary Table S2). To generate *35S::eYFP*, *35S::eYFP-ONAC005*, and *35S::eYFP-OsNAC10* constructs, a Golden Gate cloning reaction was performed to assemble each fragment into pICH47732 plasmid (Marillonnet and Grützner 2020) using 20 U BsaI-HFv2 (New England Biolabs Inc., Ipswich, MA, USA) and 2.5 U T4 DNA ligase (Thermo Scientific, Waltham, MA, USA).

Plasmid constructs (*35S::eYFP*, *35S::eYFP-ONAC005*, or *35S::eYFP-OsNAC10*) were transformed into *Agrobacterium tumefaciens* strain EHA105 using the freeze–thaw method. Transient expression using Agroinfiltration was performed on leaves of *N. benthamiana* plants grown in a greenhouse for 4 weeks. Three days after infiltration, leaves were harvested, mounted in a microscope slide, and observed for fluorescent signals (Schmitz et al. 2015) using a confocal laser scanning microscope (IX81-DSU, Olympus, Tokyo, Japan) (Kim et al. 2022).

Conserved motif, Cis-regulatory element analysis, and collinearity of NAC transcription factors Motif analyses were conducted using the MEME Suite program (v5.5.5) (Bailey et al. 2009). Conserved motifs among NAC proteins were determined using the following setting: maximum motif numbers = 20; site distributions = any number of repetitions; motif width = 6–50. Motifs with motif similarity >0.6 were considered redundant, thereby being omitted from further analysis (Supplementary Table S3). NAC transcription factor *cis*-regulatory elements were identified using 2000-bp upstream regions using the PlantCARE database (Lescot 2002). For collinearity analysis, comprehensive proteome comparisons were conducted by performing all-against-all comparisons within and between species using Diamond (Buchfink et al. 2021) with default parameters as previously described (Kim et al. 2021, 2024). Subsequently, pairwise collinear blocks were identified through MCScanX (Wang et al. 2012) using default settings. Collinearity among the plant genomes analyzed was depicted using Circos (Krzywinski et al. 2009). All syntenic blocks were integrated, and NAC-containing regions were filtered using a custom Python script available in a GitHub repository (https://github.com/taehoonkim7/Kim_PCP_2024). Syntenic network clusters were identified and visualized following the SynNet-Pipeline (https://github.com/zhaotao1987/SynNet-Pipeline) (Zhao et al. 2017).

Non-synonymous (dN) to synonymous substitution (dS) ratio (dN/dS) analysis

Pairwise protein sequence alignments of all syntenic gene pairs were performed using EMBOSS Water pairwise alignment (Smith and Waterman 1981). Corresponding coding sequence alignments were then processed with the PAL2NAL tool (Suyama et al. 2006). Subsequently, ratios of non-synonymous mutations (dN) to synonymous mutations (dS) were computed by using the KaKs_calculator 2.0 (Wang et al. 2010), employing the γ-MYN (gamma - Modified version of Yang-Nielsen) method (Wang et al. 2009) as previously described (Kim et al. 2021, 2024).

RNA-seq analysis RNA-seq data from moderate and severe rice heat-stressed plants were retrieved from a previous study (Chen et al. 2016). Briefly, 1 day after pollination (24 HAP), moderate and severe heat stress conditions were imposed on rice plants at the reproductive stage. For moderate stress, the day/night photoperiod was set at 35°C/30°C, while for severe stress, rice plants were exposed to 39°C/35°C day/night photoperiod. A parallel set of plants were maintained at 28°C/25°C diurnal temperature regime corresponding to control plants. Reanalysis of the RNA-seq data was performed using a pipeline previously reported (Antosz et al. 2020). Raw reads were trimmed using the tool Trimmomatic with a minimum average quality score of >30 and a minimum length of 70 bp. Processed reads were mapped to the rice reference transcriptome using the software TopHat v2.1.1 (Kim et al. 2013) and BowTie v2.3.4 (Langmead and Salzberg 2012) allowing up to two base mismatches per read. Reads mapped to multiple locations were discarded. Genes with transcript per million (TPM) $\geq$5 were considered transcriptionally active.

Expression profile analysis To identify transcriptional expression of NAC genes in different tissue types, transcriptome data were retrieved from public databases: EvoRepro (https://evorepro.sbs.ntu.edu.sg) (Ruprecht et al. 2017), EMBL-EBI Expression Atlas (https://www.ebi.ac.uk/gxa/home) (Moreno et al. 2022), LettuceGDB (https://lettucegdb.com) (Guo et al. 2023), and PvGEA (https://www.zhaolab.org/PvGEA/) (O'Rourke et al. 2014) (Supplementary Table S8). TPM values were normalized to $\log_2(\text{TPM}+1)$, scaled, and subsequently visualized using heatmaps.

Growth conditions and heat stress treatment *Marchantia polymorpha* was obtained from the Carolina Biologicals (NC, USA). Thallus propagules

were separated and maintained on potting media (Southern Agricultural Insecticides, FL, USA) at 22°C. For heat stress treatment, plants were subjected to 40°C, 80% RH, with continuous light for 2 days. Samples were collected at 0, 24, and 48 h after heat stress treatment.

Rice seeds (*O. sativa* cv. Nipponbare) were germinated in water under dark conditions at 28°C ± 2°C and 80% humidity. Seedlings were transplanted to soil and grown under controlled conditions (28°C ± 2°C, 14-h-light/10-h-dark photoperiod) in the greenhouse until plants reached the 3-leaf stage. Then, plants were transferred to growth chambers with similar conditions to the greenhouse (14-h-day and 10-h-night settings, 28°C/25°C day/night temperature, and 80% humidity) several days before starting the heat stress treatment. To apply heat stress treatment, plants were exposed to 40°C/35°C day/night temperatures for 2 days. A parallel set of control plants were maintained under a 28°C/25°C day/night temperature regime. Samples were collected at 0, 24, and 48 h after the initiation of the heat stress treatment. We selected 40°C as heat stress treatment because it is a temperature commonly observed in tropical and subtropical rice-growing regions.

RNA extraction and reverse transcription–quantitative PCR Total RNA was isolated from leaves of rice plants at the 3-leaf stage and thalli of *M. polymorpha* plants under control conditions and heat stress using TRIzol reagent (Ambion, Life Technologies, Austin, TX, USA) according to the manufacturer's protocol. Total RNA was treated with DNase I (Thermo Scientific, Waltham, MA, USA) to remove DNA contamination. cDNA synthesis was performed from 1 μg of DNase I-treated RNA using an iScript cDNA synthesis kit using oligo(dT) primers (Bio-Rad, Hercules, CA, USA). Real-time PCR [quantitative PCR (qPCR)] reactions were conducted using AzuraView GreenFast qPCR Blue Mix LR (Azura Genomics, Raynham, MA, USA) in the QuantStudio 3 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of genes (heat stress/control) using a sample that was not subjected to stress as a control. *MpACTIN7* (Mp6g11010) was used as an internal reference for *M. polymorpha* and *OseEF-1a* (LOC_Os03g08020) for rice (Folsom et al. 2014). Reverse transcription–qPCR (RT-qPCR) reactions were performed in triplicate for each RNA sample on three biological replicates of each condition. Primers used for RT-qPCR reactions are listed in Supplementary Table S2.

Statistical analysis R software/environment was used for the statistical analyses of the RT-qPCR data. Three independent experiments in which each experiment had three biological replicates were used. Student's *t*-test was used to compare gene expression between heat stress and nonstressed conditions. Differences in means were considered significant at $P < .05$.

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Author contributions

T.K. and J.C.A. performed the bioinformatic analysis. T.K. performed the *rice* growth chamber experiments. D.R. performed the cloning and subcellular localization experiments. J.P. performed the *M. polymorpha* growth chamber experiments. T.L. supervised the *M. polymorpha* experiments. T.K. prepared the figures. T.K. and K.B. analyzed the data. K.B. conceived the experiments and wrote the manuscript with input from all authors. All authors read and approved the manuscript.

Supplementary Data

Supplementary data is available at *PCP* online.

Disclosures

None declared.

Conflict of interest. None declared.

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Data availability

The datasets were derived from sources in the public domain: JGI PhycoCosm (https://phycocosm.jgi.doe.gov/phycocosm/home); MarpolBase (https://marchantia.info); GinkgoDB (https://ginkgo.zju.edu.cn); Cycas genome database (https://db.cngb.org/codeplot/datasets/PwRftGHfPs5qG3gE); TreeGenes (https://treegenesdb.org); NbenBase (https://nbenthamiana.jp); and Phytozome (https://phytozome-next.jgi.doe.gov).

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