

Genome-wide identification and expression analysis of the PP2C gene family in *Apocynum venetum* and *Apocynum hendersonii*

Jiayi Chen

Hunan Provincial Key Laboratory of the Traditional Chinese Medicine Agricultural Biogenomics,
Changsha Medical University

Yue Wang

Hunan Provincial Key Laboratory of the Traditional Chinese Medicine Agricultural Biogenomics,
Changsha Medical University

Yongmei Wu

Hunan Provincial Key Laboratory of the Traditional Chinese Medicine Agricultural Biogenomics,
Changsha Medical University

Xiaoyu Huang

Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences

Xiaojun Qiu

Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences

Jikang Chen

Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences

Qian Lin

Hunan Provincial Key Laboratory of the Traditional Chinese Medicine Agricultural Biogenomics,
Changsha Medical University

Haohan Zhao

Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences

Fengming Chen

Hunan Provincial Key Laboratory of the Traditional Chinese Medicine Agricultural Biogenomics,
Changsha Medical University

Gang Gao

gaogang@caas.cn

Hunan Provincial Key Laboratory of the Traditional Chinese Medicine Agricultural Biogenomics,
Changsha Medical University

Keywords: Apocynum, Protein phosphatase 2C, Salt stress

Posted Date: April 24th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4268917/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

Protein phosphatase class 2C (PP2C) is the largest protein phosphatase family in plants, which plays an important role in plant growth and development and response to adversity stress. *Apocynum* is a perennial persistent herb, divided into *Apocynum venetum* and *Apocynum hendersonii*. It mainly grows in saline soil, desert and other harsh environments, and is widely used in saline soil improvement, ecological restoration, textile and medicine. It was found that *Apocynum hendersonii* is more tolerant of adverse conditions. The main purpose of this study was to investigate the PP2C gene family and its expression pattern under salt stress and to identify important candidate genes related to salt tolerance.

Results

In this study, 68 *AvPP2C* genes and 68 *AhPP2C* genes were identified from the genomes of *Apocynum venetum* and *Apocynum hendersonii*, respectively. They were classified into 13 subgroups based on their phylogenetic relationships and further analyzed for their subcellular locations, gene structures, conserved structural domains and cis-acting elements. The results of qRT-PCR analyses of seven *AvPP2C* genes and seven *AhPP2C* genes proved that they differed significantly in gene expression under salt stress. It has been observed that the PP2C genes in *Apocynum venetum* and *Apocynum hendersonii* exhibit different expression patterns. Specifically, *AvPP2C2, 6, 24, 27, 41* and *AhPP2C2, 6, 24, 27, 42* have shown significant differences in expression under salt stress. This indicates that these genes may play a crucial role in the salt tolerance mechanism of *Apocynum venetum* and *Apocynum hendersonii*.

Conclusions

In this study, we conducted a genome-wide analysis of the *AvPP2C* and *AhPP2C* gene families in *Apocynum*, which provided a reference for further understanding the functional characteristics of these genes.

Background

Plants are exposed to different environmental adversities such as drought, salinity, and high temperature during growth and development, which can affect the balance of ions, regulation of hormones, and the reception and transmission of signals in the plant body. These factors ultimately impact the growth and development of the plant and can significantly reduce crop yield and quality[1-3]. Plants have evolved an array of response mechanisms to thrive in challenging environments. Among these is protein reversible phosphorylation, a complex process that entails two opposing chemical reactions: protein phosphorylation and dephosphorylation. This modification process is facilitated by protein kinases (PKs) and protein phosphatases (PPs), and can promptly adjust the biological properties of proteins based on external stimuli. It plays a crucial role in plant growth and development, signal transduction, and in mitigating the effects of stress due to unfavorable conditions[4]. Phosphorylation occurs at specific sites

in proteins, including serine (Ser), threonine (Thr), and tyrosine (Tyr), and is regulated by protein kinases (PKs) and protein phosphatases (PPs). PPs are classified based on their substrate specificity, with Ser/Thr phosphatases (STPs), protein Tyr phosphatases (PTPs), and dual-specificity phosphatases (DSPTPs) being the main categories[5]. PTPs are further divided into two groups, phosphoprotein metal phosphatases (PPM) and phosphoprotein phosphatases (PPP), based on crystal structure, amino acid sequence, and response to inhibitors[6, 7]. The PPP family includes various types of protein phosphatases, such as PP1, PP2A, PP2B, PP4, PP5, PP6, and PP7, while type 2C protein phosphatase (PP2C) is the largest protein phosphatase family in plants. Although closely related to the PPP family, PP2C has no sequence homology and belongs to the PPM family[6-9]. PP2C has a conserved catalytic structure at the C-terminus and a functional extension region at the N-terminal that determines its various functions[9]. In *Arabidopsis*, distinct subgroups of *PP2C* genes exhibit specific functions. It is worth noting that these genes belong to the *PP2C* family, which plays a vital role in regulating plant growth and development. Group A comprises genes that participate in abscisic acid (ABA) signaling, such as *ABI1* and *ABI2*. These genes interact with *SnRK2*, binding and dephosphorylating it, which inhibits the ABA signaling pathway and stress response[10-13]. Group B is responsible for mitogen-activated protein kinase (MAPK) signaling, while Group C is mainly involved in flower development[14]. Members of Group D may have negative effects on cell elongation, but can also have positive effects on the ABA signaling pathway in response to saline stress[15-17]. Group E is responsible for regulating the opening and closing of plant stomata, while Group F induces stress responses in bacteria. The *PP2C* gene family has been extensively researched in various plants, such as rice[18, 19], maize[20, 21], and wheat[22], among others. Their roles in plant growth, development, and ability to combat negative stressors are increasingly being comprehended.

Apocynum is a perennial, persistent herb that grows mainly in harsh environments such as saline soils and deserts, and is widely used in saline land improvement, ecological restoration, textile and medicine[23-29]. *Apocynum* is divided into *Apocynum venetum* and *Apocynum hendersonii*, and these two species differ in morphological characteristics and physiological properties. The former is characterized by red stems and flowers, while the latter is more salt tolerant and sports white flowers, making it an ideal choice for soil improvement and restoration[30, 31]. At present, no systematic analyses of the *PP2C* gene family in *A. venetum* and *A. hendersonii*. It is important to explore the *PP2C* genes of *Apocynum* for salt tolerance in plants.

In this study, we performed genome-wide identification and analysis of *PP2C* gene family members in *Apocynum*. 68 *AvPP2C* genes and 68 *AhPP2C* genes were identified, and the physicochemical properties, gene structure, conserved structural domains, cis-acting elements, chromosomal location distribution, gene duplication, and phylogenetic relationships of *PP2C* gene family members were analyzed. And the expression patterns of *PP2C* genes in different tissues of *Apocynum* were analyzed based on the transcriptome data of our group. The response of the *PP2C* gene after NaCl stress was analyzed by qRT-PCR study. The results of this study laid the foundation for the study of *PP2C* gene function in *Apocynum* and provided a reference for the excavation and genetic improvement of *Apocynum* resistance genes.

Results

Identification, characteristics and phylogenetic analysis of the *PP2C* gene family in *Apocynum*.

In this study, we used BLASTp to identify 68 members of the *AvPP2C* gene family in *Apocynum venetum* genome and 68 members of the *AhPP2C* gene family in the *Apocynum hendersonii* genome, respectively, using the sequences of 80 *AtPP2C* proteins from *Arabidopsis thaliana* as a reference. We used TBtools to map the positional distribution of the *PP2C* genes on chromosomes of *Apocynum venetum* and *Apocynum hendersonii* respectively, which were named *AvPP2C1-AvPP2C68* and *AhPP2C1-AhPP2C68* according to their chromosomal positions (Fig. 1). Except for *AhPP2C68* which was not assembled, the remaining 68 *AvPP2C* genes and 67 *AhPP2C* genes were located on 11 chromosomes, respectively. There were fewer genes on chromosomes 4, 8 and 10.

We analyzed the physicochemical properties such as molecular weight (MW), theoretical isoelectric point (pI), and subcellular localization of the 68 *AvPP2C* proteins and 68 *AhPP2C* proteins (Table S1). The 68 *AvPP2C* genes encoded proteins were located at lengths ranging from 278-1086, theoretical isoelectric points ranging from 4.38-9.78, and molecular weights ranging from 30369.58-123024.02 Da. The 68 *AhPP2C* genes encoded proteins with lengths ranging from 138-1092, theoretical isoelectric points ranging from 4.62-9.8, and molecular weights of 15540.2-123582.74 Da. The results of subcellular localization show that *PP2C* genes have the ability to appear in various areas of the cell, including the nucleus, chloroplast, cell wall, cell membrane, cytoplasm, mitochondria, and peroxisome. Some specific *PP2C* genes, such as *AvPP2C9, 10, 16, 19, 20, 21, 29, 31, 35, 38, 47, 53, 62* and *AhPP2C9, 10, 21, 29, 31, 35, 37, 38, 39, 48, 53, 54, 58* are predicted to be present in multiple cellular structures.

To investigate the phylogenetic relationship between *Apocynum venetum*, *Apocynum hendersonii* and *Arabidopsis thaliana* *PP2C* genes, a phylogenetic tree was constructed using the neighbor-joining (NJ) method based on 80 *AtPP2C* genes of *Arabidopsis thaliana*, 68 *AvPP2C* genes of *Apocynum venetum* and 68 *AhPP2C* genes of *Apocynum hendersonii* (Fig. 2). Based on phylogenetic analysis, the *PP2C* genes in *Apocynum* were categorized into 13 subgroups (A-L). These subgroups contained 9, 4, 4, 8, 7, 6, 4, 6, 3, 2, 2, 7, 2 *AvPP2C* genes and 9, 4, 4, 9, 7, 6, 3, 6, 3, 2, 2, 7, 2 *AhPP2C* genes, with similar groupings to those found in *Arabidopsis*. Notably, *PP2C* proteins from both *Apocynum* and *Arabidopsis* were present in each subfamily, suggesting a common ancestor. Interestingly, *AvPP2C3, AvPP2C7, AvPP2C31, AvPP2C61, AhPP2C3, AhPP2C7, AhPP2C31, and AhPP2C60* of *Apocynum* did not cluster with any other group. The evolutionary tree also showed that genes in *Apocynum venetum* and *Apocynum hendersonii* were tightly clustered, indicating high homology and similar gene functions.

Gene structures and conserved motifs analyses of the *AvPP2C* genes and the *AhPP2C* genes

Based on their phylogenetic relationships, the structures and conserved domains of the *AvPP2C* and *AhPP2C* genes were studied. The gene structures include positional information such as coding sequence (CDS), untranslated regions (UTR), introns and *PP2C* structural domains (Fig. 3A and C). Genes within the same subgroup share similar gene structures, but there are variations in the length and arrangement of

their CDSs and UTRs. For instance, all *PP2C* genes in group C contained UTR and CDS regions, but their lengths varied. *AvPP2C42* had the longest UTR region, while 16 genes in *Apocynum venetum* had no UTR regions and 14 genes in *Apocynum hendersonii* had no UTR regions. *AvPP2C39* and *AhPP2C40* had the highest number of CDS regions, totaling 16. After examining the number of introns, it was found that three *AvPP2C* genes and four *AhPP2C* genes in *Apocynum* had no introns, while the remaining 129 *PP2C* genes contained introns.

In order to study the structure and function of PP2C proteins and their evolutionary relationships, the conserved domains of *AvPP2Cs* proteins and *AhPP2Cs* proteins were analyzed using the online MEME website. The analysis revealed 15 different conserved domains, which were given the names motif 1 to motif 15 (Fig. 3A and B, Table S2). The results demonstrated that PP2C proteins in the same subgroups possess a similar distribution that may be linked to their functions. Motif 1 (except *AvPP2C62*, *AhPP2C67*, *AhPP2C68*), motif 2 (except *AvPP2C31*, *AvPP2C44*, *AhPP2C45*, *AhPP2C31*), and motif 3 (except *AhPP2C68*, *AhPP2C19*, *AvPP2C19*, *AvPP2C48*) were present in all subgroups. Motifs 6, 7, 11, 13 and 15 were absent in subgroups C and D, while motif 5 was only present in subgroups C and D, except *AhPP2C68*. Motif 9 and 10 were present only in subgroup D, and motif 11 was present only in subgroup E, but not in other groups.

Cis-element analysis of the *PP2Cs* promoter in *Apocynum venetum* and *Apocynum hendersonii*

The cis-acting elements were predicted in the promoter regions of *Apocynum venetum* and *Apocynum hendersonii* using analysis through the PlantCARE online website pairs and visualized using TBtools software, both of which retrieved a total of 68 cis-acting elements. Among them, there were 32 light-responsive elements, 13 phytohormone-responsive elements, 12 stress-responsive elements, and 11 elements related to plant growth and development (Fig. 4, Table S3). It was found that all the promoters of *AvPP2Cs* and *AhPP2Cs* contained light-responsive elements, phytohormone-responsive elements, and stress-responsive elements. However, elements associated with plant growth and development were missing in *AvPP2C18*, *AvPP2C33*, *AvPP2C59*, *AvPP2C61*, *AvPP2C65*, *AhPP2C18*, *AhPP2C19*, *AhPP2C32*, *AhPP2C58*, *AhPP2C60*, and *AhPP2C66*. Box 4 was identified as the most abundant light-responsive element, with 263 in *Apocynum venetum* and 290 in *Apocynum hendersonii*. Among the phytohormone response elements, a large number of abscisic acid response elements (ABRE) and AAGAA-motifs were found, with *AvPP2Cs* having 148 ABREs and 132 AAGAA-motifs, and *AhPP2Cs* having 141 ABREs and 129 AAGAA-motifs. The ethylene response element (ERE) was also found in large numbers, with *AvPP2Cs* having 147 and *AhPP2Cs* having 156. The stress-responsive elements MYB and MYC were found to be the most abundant with 247 and 235 in *Apocynum venetum* and 242 and 238 in *Apocynum hendersonii*, respectively. These elements are involved in drought and ABA-induced responses. Another stress-responsive element, ARE, was found to be essential for anaerobic induction, with 133 in *Apocynum venetum* and 130 in *Apocynum hendersonii*. The large number of these elements suggests that most *PP2C* genes may respond to various stresses and are closely related to plant stress resistance.

Collinear analysis and evolutionary analysis of *AvPP2Cs* proteins and *AhPP2Cs* proteins

The expansion of gene families in the genome is predominantly caused by tandem and segmental duplications. To gain insight into the *PP2C* gene family, 68 *AvPP2C* genes and 68 *AhPP2C* genes were analyzed for duplication (Fig. 5A and B, Table S4). It was discovered that in *Apocynum venetum*, one tandem duplication pair was located on chromosome LG06 (39/40), whereas 17 pairs of segmental duplication genes were unevenly distributed on chromosomes other than LG08 and LG10. In *Apocynum hendersonii*, there were 2 tandem replication gene pairs located on chromosome LG06 (38/39, 40/41), and 17 fragment replication gene pairs were unevenly distributed on 9 chromosomes except for LG08 and LG10. These findings suggest that the evolution of *PP2Cs* in *Apocynum* was primarily driven by segmental duplication events, as the number of segmental duplication gene pairs was significantly greater than that of tandem duplications.

Furthermore, the study investigated the homology between *Apocynum venetum*, *Apocynum hendersonii*, and *Arabidopsis thaliana* to understand the evolutionary relationship of *PP2C* genes (Fig. 5C, Table S5). The results indicated that there were 84 homologous gene pairs between *Apocynum venetum* and *Arabidopsis thaliana*, and 82 homologous gene pairs between *Apocynum hendersonii* and *Arabidopsis thaliana*. Among these pairs, *Apocynum venetum* and *Apocynum hendersonii* had the highest number of homologous gene pairs on chromosome LG06, with 13 pairs each. These findings suggest that *Apocynum venetum* and *Apocynum hendersonii* may have a high degree of homology with *Arabidopsis*.

Tissue-specific expression of *AvPP2C* genes and *AhPP2C* genes in different tissues

To gain a better understanding of the function of *PP2C* genes in the growth and development of *Apocynum venetum* and *Apocynum hendersonii*, we analyzed the expression patterns of *PP2C* genes using RNA-Seq data of roots, stems, and leaves of both species (Fig. 6). Most of the *PP2C* genes were expressed in all tissues, except for four genes (*AvPP2C19*, *AvPP2C38*, *AhPP2C39*, and *AhPP2C68*), which were barely expressed. In *Apocynum venetum*, *AvPP2C47* was highly expressed in roots, stems, and leaves. *AvPP2C9*, *AvPP2C24*, *AvPP2C49*, *AvPP2C60*, and *AvPP2C68* were moderately expressed in roots; *AvPP2C13* and *AvPP2C60* were highly expressed in stems; *AvPP2C13* was highly expressed in leaves, and *AvPP2C7* and *AvPP2C60* were moderately expressed in leaves. In *Apocynum hendersonii*, *AhPP2C29* and *AhPP2C67* were highly expressed in roots, while *AhPP2C24*, *AhPP2C13*, *AhPP2C49*, *AhPP2C48*, and *AhPP2C59* were moderately expressed in roots. *AhPP2C9*, *AhPP2C13*, *AhPP2C59*, and *AhPP2C48* were highly expressed in stems, and *AhPP2C13*, *AhPP2C48*, and *AhPP2C59* were highly expressed in leaves.

Relative expression changes of *AvPP2C* genes and *AhPP2C* genes under NaCl treatment

In order to study the expression of *AvPP2C* genes and *AhPP2C* genes under salt stress conditions and to identify important *PP2C* genes related to salt tolerance. Based on phylogenetic analysis and homology of known *PP2C* genes in *Arabidopsis thaliana*, five genes in subgroup D and two genes in subgroup G were selected in *Apocynum hendersonii* and their expression patterns in different tissues of *Apocynum venetum* under salt stress were investigated by qRT-PCR (Fig. 7).

The results showed that the *AvPP2C1* gene was down-regulated in roots after NaCl stress, with the lowest expression at 50 mM concentration. It was up-regulated in leaves and phloem, with the highest expression at 200 mM and 100 mM concentrations, respectively, reaching 2.14-fold and 1.40-fold of CK. *AhPP2C1* gene was up-regulated in leaves and increased with increasing NaCl concentration; it showed down-regulation in bast and decreased with increasing NaCl concentration. *AvPP2C2* gene showed up-regulation under NaCl stress, in which the expression of the gene in leaves varied the most with NaCl concentration, which was 10.44 times that of CK. The expression trend of *AvPP2C2* in root, xylem and phloem was the same, and all of them reached the maximum expression at 100 mM concentration. While the *AhPP2C2* gene was down-regulated in roots and up-regulated in leaves after NaCl stress, there was little change in the xylem and phloem, but the expression reached the maximum at 50 mM concentration in all. *AvPP2C6* gene did not change significantly in roots, but showed up-regulation in leaves and xylem phloem, and it is noteworthy that its expression in leaves increased with the increase of NaCl concentration and reached the maximum at 200 mM, which was 13.55 times that of CK. And *AhPP2C6* was different from *AvPP2C6* in that it showed down-regulation in roots. The expression of *AvPP2C14* was different in roots, leaves, xylem and phloem, with the highest in leaves and the lowest in roots; after NaCl stress, the *AvPP2C14* gene showed a tendency of increasing and then decreasing with the increase of NaCl concentration, and its expression reached the maximum in all tissues at the concentration of 50 mM. While the expression of *AhPP2C14* did not change significantly under NaCl treatment, it only showed up-regulation in leaves, and the expression of the gene was the highest at 50 mM concentration, and its expression gradually declined with the increase of concentration. *AvPP2C24* gene had the highest expression in leaves and showed up-regulation in all tissues after NaCl stress. *AhPP2C24* gene was the same as *AvPP2C24* gene in that the expression of *AhPP2C24* gene was up-regulated in all tissues after NaCl stress, except in phloem at 50 mM concentration. The difference was that the expression of *AhPP2C24* gene changed more significantly in leaves after NaCl treatment. Both *AvPP2C27* and *AhPP2C27* genes showed up-regulation after NaCl treatment, but the expression of *AvPP2C27* gene in roots and leaves increased with increasing NaCl concentration, while the expression of *AhPP2C27* gene in leaves reached a maximum at 50 mM concentration and then decreased with increasing concentration. The expression of *AvPP2C41* and *AhPP2C42* genes in roots increased with increasing NaCl concentration, *AvPP2C41* gene showed down-regulation in leaves at 100 mM and up-regulation at 50 mM and 200 mM concentrations. While *AhPP2C42* gene showed up-regulation after NaCl stress, the expression was maximum in leaves at 50 mM concentration, which was 8.20 times of CK, and then decreased with the increase of NaCl concentration.

Discussion

PP2C genes are closely related to abscisic acid ABA signaling pathway, plant adversity stress, and stomatal opening and closing, etc. The *PP2C* gene families of *Arabidopsis thaliana*, rice, maize, and wheat have been investigated by previous authors. In this study, we comprehensively analyzed the *AvPP2C* genes of *Apocynum venetum* and the *AhPP2C* genes of *Apocynum hendersonii*, including the identification of the gene families, phylogenetic relationships, and chromosomal locations, collinear

relationship, gene structure, conserved motifs, and expression patterns. A total of 136 *PP2C* genes, including 68 *AvPP2C* genes and 68 *AhPP2C* genes, were identified in *Apocynum*, and the physicochemical properties and subcellular localization of the genes were predicted, and it was found that the vast majority of *PP2C* genes were located in the nucleus and chloroplasts, and a few genes might be present in the mitochondria, cell wall, cytoplasm, peroxisomes, and cell membrane. Therefore, it is hypothesized that they may be related to photosynthesis, cell growth and development, and respiration. According to phylogenetic and evolutionary relationship analysis, *Apocynum hendersonii* and *Apocynum venetum* are closely related to each other, and a total of 136 *PP2C* genes in *Apocynum hendersonii* and *Apocynum venetum* were divided into 13 subgroups (A-L), which is consistent with the grouping of *Arabidopsis thaliana*. Previous studies found that genes in the A subgroup of *PP2C* genes in *Arabidopsis* mainly inhibit ABA receptor activity and negatively regulate ABA signaling[32]; the B subgroup is involved in the mitogen-activated protein kinase signaling pathway and participates in the process of MAPK phosphorylation, and is capable of dephosphorylating and inactivating MAPK[33]; Subgroup C gene family members are involved in stem cell maintenance and differentiation[34]; Members of subgroup D negatively regulate small auxin up RNA (SAUR)-mediated cell elongation, and PP2C.D1, PP2C.D2, and PP2C.D5 in *Arabidopsis* act during leaf greening, and PP2C.D1 regulates hook formation by affecting ethylene accumulation[15, 35, 36]. Moreover, members of this group respond to saline and alkaline stress, which may be related to stomatal movement and function, by inhibiting plasma membrane H-ATPase activity. It has been shown that *Arabidopsis* PP2C.D negatively regulates aluminium resistance by modulating malate secretion[16, 37]; Members of the E subgroup gene family may be associated with inflorescence stem growth, regulation of stomatal signaling, and so on[38-40]. Based on the phylogenetic relationship between *Apocynum* and *Arabidopsis*, it is hypothesized that the functions of gene family members located in the same subgroup in *Apocynum* may be similar to those in *Arabidopsis*. The prediction of the subcellular localization of *PP2C* gene family members in *Apocynum* showed that most of the *PP2C* gene family in *Apocynum venetum* and *Apocynum hendersonii* were predicted to be located in the nucleus and chloroplasts, in addition to which some of the genes might be located in the cell wall, cytoplasm, cell membrane, mitochondria, and peroxisomes. The predictions of *PP2C* gene family in *Apocynum venetum* and *Apocynum hendersonii* were partially different. For example, *AvPP2C9* was predicted to be located in mitochondria and the nucleus, whereas *AhPP2C9* was predicted to be located in chloroplasts, mitochondria, and the nucleus. *AvPP2C20* was located in the nucleus and chloroplasts, whereas *AhPP2C20* was predicted to be located in chloroplasts only. This may imply differences in the location and function of some *PP2C* genes in *Apocynum hendersonii* and *Apocynum venetum*.

The diversity of gene structures, conserved structural domains, and cis-acting elements is also closely related to the functions of gene families. Genes in the same subgroup are similar in structure, which may imply that they have the same function[41]. The gene structures of *Apocynum hendersonii* and *Apocynum venetum* genes from the same evolutionary tree branching were similar, except for the *AhPP2C68* gene. However, the gene structures of individual *PP2C* genes were different again, for example, *AvPP2C18* and *AhPP2C18* in subgroup L had significantly more CDS regions compared with *AvPP2C33* and *AhPP2C33*, which might be related to the increase of introns during the evolutionary

process. The *PP2C* gene family is evolutionarily conserved, and most *PP2Cs* have a highly conserved structural domain with a unique non-catalytic N-terminal extension region. The specificity of the function of each sub-group of *PP2C* genes may be related to an N-terminal substrate docking domain[42]. A total of 15 conserved motifs were identified in the amino acid sequences of *AvPP2C* and *AhPP2C* genes, and the distribution of conserved motifs was similar for genes in the same subgroup, which may be closely related to the function of *PP2C* proteins. Cis-acting elements are involved in the regulation of gene expression and are closely related to plant growth and development, hormone responses and responses to various stresses. Among *AvPP2C* and *AhPP2C* genes, Box4 elements were the most abundant (*AvPP2Cs*: 263; *AhPP2Cs*: 290), followed by MYB elements (*AvPP2Cs*: 247; *AhPP2Cs*: 242). Among all the response elements, light responsive and stress responsive were the most numerous elements, with a high number of 1,318 stress responsive in *Apocynum hendersonii*. This may be related to the characteristics of *Apocynum hendersonii* high-stress tolerance.

In this study, chromosomal position analysis and covariance analysis of *PP2C* gene family of *Apocynum venetum* and *Apocynum hendersonii* were carried out, which is important to understand the amplification mechanism of the genes. It was found that both *AvPP2Cs* and *AhPP2Cs* were distributed on 11 chromosomes. Seventeen pairs of segmental duplications and one pair of tandem duplications were identified in *Apocynum venetum*; 17 pairs of segmental duplications and two pairs of tandem duplications were identified in *Apocynum hendersonii*. Gene duplication is the main driver of gene family amplification, which mainly includes segmental duplication, tandem duplication, and genomic duplication, and segmental duplication is more conducive to maintaining gene function than tandem duplication[43]. Therefore, we hypothesize that segmental duplication is the main force driving the evolution and expansion of the *PP2C* gene family, which is similar to the results found for *PP2C* in *Arabidopsis*[44], woodland and pineapple strawberry[45], and cucumber[4].

AvPP2C genes and *AhPP2C* genes showed specific expression in different tissues, and most of the *PP2C* genes were lowly expressed in roots, stems and leaves. However, among all the *PP2C* genes, *AvPP2C13*, *AvPP2C47*, *AvPP2C60*, *AhPP2C48*, and *AhPP2C59* showed higher expression in all three tissues: roots, stems, and leaves, whereas *AhPP2C29*, and *AhPP2C67* showed high expression in roots and lower expression in stems and leaves. In cucumber, most of the *CsPP2C* genes were highly expressed in fertilized ovaries, male, female, and leaf (except *CsPP2C11*, 41, 5, 33, 50), and low in other tissues [4]. In maize, most of the *ZmPP2C* genes were expressed in all tissues, with *ZmPP2C42* and *ZmPP2C47* being the most highly expressed in mature pollen, and *ZmPP2C59* being expressed in primary roots and root cortex[46]. This suggests that *PP2C* genes in different plants may play different roles at different stages of plant growth, and some of these *PP2C* genes may have special functions in specific tissues.

Studies on *Arabidopsis* have shown that members of subgroup D respond to saline stress, and *AtPP2C.D6* and *AtPP2C.D7* interact with *SCaBP8* of the SOS pathway, whose phosphatase activity is inhibited under salt stress. *AtPP2C.G1* in group G is dependent on ABA to positively regulate salt tolerance. Our study showed that the expression of some *AvPP2C* genes differed significantly from that of *AhPP2C* genes after NaCl stress, in which the expression of *AvPP2C2* gene was significantly up-

regulated after salt stress, and the expression was significantly higher in roots, xylem, phloem, and leaves. It indicated that *AvPP2C2* gene might play an important role in these four tissues when *Apocynum venetum* resisted salt stress. The expression of *AhPP2C2* gene was down-regulated in roots and up-regulated in leaves after salt stress, while it showed a similar expression pattern in xylem and phloem, both of which peaked at a concentration of 50 mM. *AvPP2C2* and *AhPP2C2* showed different expression patterns in *Apocynum venetum* and *Apocynum hendersonii* under salt stress, which was hypothesized to be related to their differences in salt tolerance. The expression of *AvPP2C6* and *AhPP2C6* was up-regulated in leaves, xylem and phloem, and the change of *AvPP2C6* was not obvious in roots, while *AhPP2C6* showed down-regulation in roots. *AvPP2C24*, 27 and 41 in roots showed similar trends after salt stress. In summary, *PP2C* genes located in the same subgroup responded differently in the face of salt stress, while there may be different expression patterns in *Apocynum venetum* and *Apocynum hendersonii*, where *AvPP2C2*, 6, 24, 27, 41 and *AhPP2C2*, 6, 24, 27, 42 showed significant differences in expression under salt stress, suggesting that these genes may play important roles in *Apocynum venetum* and *Apocynum hendersonii* when facing salt stress.

Conclusions

Genome-wide identification and analysis of *PP2C* family members in *Apocynum venetum* genomes and *Apocynum hendersonii* genomes. In this study, a total of 68 *AvPP2C* genes and 68 *AhPP2C* genes were identified and classified into 13 subgroups, which were further analyzed for subcellular localization, gene structure, conserved structural domains, and cis-acting elements. By analyzing the expression patterns of *PP2C* genes in *Apocynum venetum* and *Apocynum hendersonii* under salt stress, it was found that the *PP2C* genes in subgroup D and subgroup G showed significant differences under salt stress, which might be related to the differences in salt tolerance between *Apocynum venetum* and *Apocynum hendersonii*. This study provides a reference for the subsequent research on the function of *PP2C* gene.

Materials and methods

Identification of *PP2C* genes in *Apocynum*

The protein sequences of 80 *AtPP2C* genes of *Arabidopsis* were downloaded from Tair (<https://www.arabidopsis.org/>). Protein sequences of *Apocynum venetum* and *Apocynum hendersonii* were obtained from the whole genome data sequenced in our laboratory. The *PP2C* genes were identified by two methods. First, the putative *PP2C* protein present in the genome was identified using BLASTp. Then, the *PP2C* structural domain (PF00481) of the *PP2C* protein was downloaded from Pfam (<http://pfam.xfam.org/>) and searched against the local protein database using HMMER 3.0[47, 48]. Meanwhile, the SMART (<https://smart.embl.de/>) was used to predict its structural domain with E-value <e-5[49]. The results obtained by both methods were intersected and the protein sequences were extracted from the local protein database using TBtools and the gene IDs identified by screening.

Sequence analysis and basic information of the *Apocynum PP2C* gene family

Physicochemical property analysis of PP2C proteins including number of amino acid, molecular weight, theoretical pI, instability index, aliphatic index, grand average of hydropathicity were analyzed by Protein Paramter Calc in TBtools. Prediction of the subcellular location of PP2C protein by Plant-mPloc (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>) of Cell-Ploc 2.0.

Analysis of chromosome location and collinearity analysis

Chromosome position information was obtained from the annotation file of the *Apocynum* genome and the chromosome position distribution was mapped using TBtools. Homology and collinearity within the *Apocynum venetum* and *Apocynum hendersonii* genomes and between them and *Arabidopsis thaliana* were analyzed using the MCScanX software and visualized using the Advanced Circos and Multiple Synteny Plot functions in TBtools[50, 51].

Construction of phylogenetic tree

The phylogenetic tree of the *PP2C* gene family of *Arabidopsis* and *Apocynum* was constructed using MEGA-X with the Neighbor-Joining method, Bootstrap value set to 1000 and other default parameters[52, 53]. Finally, the phylogenetic tree was embellished with iTOL(<https://itol.embl.de/>)[54].

Analysis of gene structures and protein conserved motifs

The gene structure of *Apocynum PP2C* was analyzed using the Batch CD-search (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>) on the NCBI website. The MEME website (<https://meme-suite.org/meme/tools/meme>) was used to predict the conserved structural domains of the *PP2C* genes in *Apocynum*, setting the motif number to 15 and the rest to default values[55]. Gene Structure View in TBtools was used to generate the final overall map of the phylogenetic tree, gene structure and conserved structural domains of *PP2Cs*.

Analysis of cis-acting elements in *PP2C* gene promoters

The sequences of 2.0 kb upstream of *AvPP2C* genes and *AhPP2C* genes were extracted using TBtools and then the results were submitted to PlantCARE website (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) for cis-acting element prediction[55].

Expression Analysis of *PP2C* Genes

From the transcriptome data of roots, stems and leaves of *Apocynum venetum* and *Apocynum hendersonii*, the FPKM (Fragments Per Kilobase of exon model per Million mapped fragments) values of *AvPP2Cs* and *AhPP2Cs* were extracted and heat maps were generated using TBtools[51].

Plant material treatment methods

Seedlings grown under hydroponic conditions for 6-8 weeks were divided into four groups and treated with different concentrations (0, 50, 100, and 200 mM) of NaCl stress, and after 14 days of treatment,

respectively, the roots, leaves, xylem and phloem were sampled and stored in a refrigerator at -80°C.

RNA Isolation and qRT-PCR

Total RNA was extracted using the *SteadyPure* Plant RNA Extraction Kit (Accurate Biotechnology (Changsha, China) Co., Ltd.), and the RNA was reverse transcribed into cDNA using the *Evo M-MLV* One Step RT-PCR Kit (Accurate Biotechnology (Changsha, China) Co., Ltd.)[56]. Specific primers were designed for qRT-PCR using Primer5(Table S6). ACT gene was used as an internal reference gene. qRT-PCR was performed on a CFX96 Touch Deep Well Real-Time Quantitative PCR System (Bio-Rad) using SYBR® Green Premix *Pro Taq* HS qPCR Kit II (Accurate Biotechnology (Changsha, China) Co., Ltd.)[56]. The relative expression of *PP2C* genes was calculated using the $2^{-\Delta\Delta CT}$ method and histograms were plotted using GraphPad Prism 8.

List of Abbreviations

PP2C: Protein phosphatase class 2C

qRT-PCR: Quantitative Real-time PCR

MAPK: Mitogen-activated protein kinase

MW: Molecular weight

pI: Isoelectric point

CDS: Coding sequence

UTR: Untranslated regions

CK: Control check

SAUR: Small auxin up RNA

Declarations

Ethics approval and consent to participate

The plant material used in this study was *Apocynum venetum* and *Apocynum hendersonii*, grown in the laboratory of the Institute of bast fiber crops, Chinese Academy of Agricultural Sciences (CAAS) and no permits were required to collect plant samples. In the present study, all methods were carried out following relevant guidelines and regulations. Ethical approval or consent was not required for this study because no endangered or protected species were involved.

Consent for publication

Not applicable.

Availability of data and materials

The sequence information of *Arabidopsis PP2C* family genes were collected from The *Arabidopsis* Information Resource (<https://www.arabidopsis.org/>). The *PP2C* family expression data were generated by qRT-PCR. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by Hunan Provincial Department of Education general project (No.22C0674) ESI Discipline Special Project of Changsha Medical University (No.2022CYY023 and No.2022CYY006) Science Research Project of Hunan Provincial Department of Education (No.23A0662) National Undergraduate Innovation and Entrepreneurship Training Program Project under Grant (No.202210823006).

Contributions

Jiayi Chen: Methodology, writing-review.

Yue Wang: Conceived, designed the experiments and writing, Methodology.

Xiaoyu Huang, Xiaojun Qiu: Methodology, writing-review & editing.

Jikang Chen, Qian Lin: Methodology.

Haohan Zhao, Yongmei Wu: Edit improves the manuscript.

Gang Gao, Fengming Chen: Concept of study, Supervision and revised the manuscript.

Acknowledgements

The authors thank laboratory colleagues for their technical assistance. The authors thank the editors and reviewers for their rigorous evaluation of the manuscript.

References

1. Yang S, Gong Z, Guo Y, Gong J, Zheng S, Lin R, Yang H, Mao L, Qin F, Luo L *et al*: **Studies on plant responses to environmental change in China:the past and the future**. *Scientia Sinica Vitae* 2019, **49**(11):1457-1478.

2. Zhu JK: **Abiotic stress signaling and responses in plants.** *Cell* 2016, **167**(2):313-324.
3. Waadt R, Seller CA, Hsu PK, Takahashi Y, Munemasa S, Schroeder J: **Plant hormone regulation of abiotic stress responses.** *Nature Reviews Molecular Cell Biology* 2022, **23**(10):680-694.
4. Zhang G, Zhang Z, Luo S, Li X, Lyu J, Liu Z, Wan Z, Yu J: **Genome-wide identification and expression analysis of the cucumber *PP2C* gene family.** *Bmc Genomics* 2022, **23**(1).
5. Cao J, Jiang M, Li P, Chu Z: **Genome-wide identification and evolutionary analyses of the *PP2C* gene family with their expression profiling in response to multiple stresses in *Brachypodium distachyon*.** *Bmc Genomics* 2016, **17**.
6. Luan S: **Protein phosphatases in plants.** *Annual Review of Plant Biology* 2003, **54**:63-92.
7. Shazadee H, Khan N, Wang J, Wang C, Zeng J, Huang Z, Wang X: **Identification and expression profiling of protein phosphatases (*PP2C*) gene family in *Gossypium hirsutum* L.** *International Journal of Molecular Sciences* 2019, **20**(6).
8. Schweighofer A, Hirt H, Meskiene L: **Plant *PP2C* phosphatases: emerging functions in stress signaling.** *Trends in Plant Science* 2004, **9**(5):236-243.
9. Zhang J, Tao N: **Research progress of plant *PP2C*-type protein phosphatase in ABA signal transduction and adversity stress regulation mechanism.** *Guangxi Zhiwu / Guihaia* 2015, **35**(6):935-941.
10. Peirats-Llobet M, Han SK, Gonzalez-Guzman M, Jeong CW, Rodriguez L, Belda-Palazon B, Wagner D, Rodriguez PL: **A direct link between abscisic acid sensing and the chromatin-remodeling ATPase *BRAHMA* via Core ABA signaling pathway components.** *Molecular Plant* 2016, **9**(1):136-147.
11. Hsu PK, Dubeaux G, Takahashi Y, Schroeder JI: **Signaling mechanisms in abscisic acid-mediated stomatal closure.** *Plant Journal* 2021, **105**(2):307-321.
12. Li ZX, Waadt R, Schroeder JI: **Release of GTP exchange factor mediated down-regulation of abscisic acid signal transduction through ABA-induced rapid degradation of *RopGEFs*.** *Plos Biology* 2016, **14**(5).
13. Shi Y, Liu XN, Zhao SS, Guo Y: **The *PYR-PP2C-CKL2* module regulates ABA-mediated actin reorganization during stomatal closure.** *New Phytologist* 2022, **233**(5):2168-2184.
14. Wu P, Wang W, Li Y, Hou X: **Divergent evolutionary patterns of the MAPK cascade genes in *Brassica rapa* and plant phylogenetics.** *Horticulture Research* 2017, **4**.
15. Rovira A, Sentandreu M, Nagatani A, Leivar P, Monte E: **The sequential action of *MIDA9/PP2C.D1*, *PP2C.D2*, and *PP2C.D5* is necessary to form and maintain the hook after germination in the dark.** *Frontiers in Plant Science* 2021, **12**.
16. Chen C, Yu Y, Ding X, Liu B, Duanmu H, Zhu D, Sun X, Cao L, Zaib un N, Li Q *et al*: **Genome-wide analysis and expression profiling of *PP2C* clade D under saline and alkali stresses in wild soybean and *Arabidopsis*.** *Protoplasma* 2018, **255**(2):643-654.
17. Xue TT, Wang D, Zhang SZ, Ehltng J, Ni F, Jakab S, Zheng CC, Zhong Y: **Genome-wide and expression analysis of protein phosphatase 2C in rice and *Arabidopsis*.** *Bmc Genomics* 2008, **9**.

18. Singh A, Jha SK, Bagri J, Pandey GK: **ABA inducible rice protein phosphatase 2C confers ABA insensitivity and abiotic stress tolerance in *Arabidopsis*.** *Plos One* 2015, **10**(4).
19. Xie W, Liu S, Gao H, Wu J, Liu D, Kinoshita T, Huang C-F: **PP2C.D phosphatase SAL1 positively regulates aluminum resistance via restriction of aluminum uptake in rice.** *Plant Physiology* 2023, **192**(2):1498-1516.
20. He Z, Wu J, Sun X, Dai M: **The maize clade a PP2C phosphatases play critical roles in multiple abiotic stress responses.** *International Journal of Molecular Sciences* 2019, **20**(14).
21. Guo YZ, Shi YB, Wang YL, Liu F, Li Z, Qi JS, Wang Y, Zhang JB, Yang SH, Wang Y *et al*: **The clade F PP2C phosphatase *ZmPP84* negatively regulates drought tolerance by repressing stomatal closure in maize.** *New Phytologist* 2023, **237**(5):1728-1744.
22. Wang JY, Li CA, Li L, Gao LF, Hu G, Zhang YF, Reynolds MP, Zhang XY, Jia JZ, Mao XG *et al*: ***DIW1* encoding a clade I PP2C phosphatase negatively regulates drought tolerance by de-phosphorylating *TaSnRK1.1* in wheat.** *Journal of Integrative Plant Biology* 2023.
23. Guo X, Chai W, Bai J, Ma Z: **Cloning and bioinformatics analysis of *AvFLS* gene from *Apocynum venetum*.** *Molecular Plant Breeding* 2019, **17**(15):4978-4985.
24. Huang X, Qiu X, Wang Y, Abubakar AS, Chen P, Chen J, Chen K, Yu C, Wang X, Gao G *et al*: **Genome-wide investigation of the NAC transcription factor family in *Apocynum venetum* revealed their synergistic roles in abiotic stress response and trehalose metabolism.** *International Journal of Molecular Sciences* 2023, **24**(5).
25. Zhang M, Lu X, Ren T, Marowa P, Meng C, Wang J, Yang H, Li C, Zhang L, Xu Z: **Heterologous overexpression of *Apocynum venetum* flavonoids synthetase genes improves *Arabidopsis thaliana* salt tolerance by activating the IAA and JA biosynthesis pathways.** *Frontiers in Plant Science* 2023, **14**.
26. Cao C, Lin DF, Zhou YJ, Li N, Wang YW, Gong WB, Zhu ZH, Liu CW, Yan L, Hu ZX *et al*: **Solid-state fermentation of *Apocynum venetum* L. by *Aspergillus niger*: Effect on phenolic compounds, antioxidant activities and metabolic syndrome-associated enzymes.** *Frontiers in Nutrition* 2023, **10**.
27. Li X, Li JJ, Su HY, Sun P, Zhang Z, Li MF, Xing H: **Physiological and transcriptional responses of *Apocynum venetum* to salt stress at the seed germination stage.** *International Journal of Molecular Sciences* 2023, **24**(4).
28. Zhang Y, Liu S, Ma JL, Chen C, Huang P, Ji JH, Wu D, Ren LQ: ***Apocynum venetum* leaf extract alleviated doxorubicin-induced cardiotoxicity through the AKT/Bcl-2 signaling pathway.** *Phytomedicine* 2022, **94**.
29. Jiang L, Wang L, Tian CY: **High lithium tolerance of *Apocynum venetum* seeds during germination.** *Environmental Science and Pollution Research* 2018, **25**(5):5040-5046.
30. Gao G, Abubakar AS, Chen J, Wang Y, Chen P, Chen K, Yu C, Wang X, Qiu X, Huang X *et al*: **Comparative genome and metabolome analyses uncover the evolution and flavonoid biosynthesis between *Apocynum venetum* and *Apocynum hendersonii*.** *Iscience* 2023, **26**(5).

31. Yuan N, Li MM, Jia CL: **De novo transcriptome assembly and population genetic analyses of an important coastal shrub, *Apocynum venetum* L.** *Bmc Plant Biology* 2020, **20**(1).
32. Ma Y: **Regulators of PP2C phosphatase activity function as abscisic acid sensors (vol 324, pg 1064, 2009).** *Science* 2009, **324**(5932):1266-1266.
33. Umbrasaite J, Schweighofer A, Meskiene I: **Substrate analysis of *Arabidopsis* PP2C-type protein phosphatases.** In: *Plant Kinases: Methods and Protocols*. Edited by Dissmeyer N, Schnittger A, vol. 779; 2011: 149-161.
34. Yu LP, Miller AK, Clark SE: **POLTERGEIST encodes a protein phosphatase 2C that regulates CLAVATA pathways controlling stem cell identity at *Arabidopsis* shoot and flower meristems.** *Current Biology* 2003, **13**(3):179-188.
35. Sentandreu M, Martín G, González-Schain N, Leivar P, Soy J, Tepperman JM, Quail PH, Monte E: **Functional profiling identifies genes involved in organ-specific branches of the PIF3 regulatory network in *Arabidopsis*.** *Plant Cell* 2011, **23**(11):3974-3991.
36. Spartz AK, Ren H, Park MY, Grandt KN, Lee SH, Murphy AS, Sussman MR, Overvoorde PJ, Gray WM: **SAUR inhibition of PP2C-D phosphatases activates plasma membrane H⁺-ATPases to promote cell expansion in *Arabidopsis*.** *Plant Cell* 2014, **26**(5):2129-2142.
37. Fu H, Yu X, Jiang Y, Wang Y, Yang Y, Chen S, Chen Q, Guo Y: **SALT OVERLY SENSITIVE 1 is inhibited by clade D Protein phosphatase 2C D6 and D7 in *Arabidopsis thaliana*.** *Plant Cell* 2023, **35**(1):279-297.
38. Mishra G, Zhang WH, Deng F, Zhao J, Wang XM: **A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in *Arabidopsis*.** *Science* 2006, **312**(5771):264-266.
39. Sugimoto H, Kondo S, Tanaka T, Imamura C, Muramoto N, Hattori E, Ogawa Ki, Mitsukawa N, Ohto C: **Overexpression of a novel *Arabidopsis* PP2C isoform, *AtPP2CF1*, enhances plant biomass production by increasing inflorescence stem growth.** *Journal of Experimental Botany* 2014, **65**(18):5385-5400.
40. Wong JH, Klejchová M, Snipes SA, Nagpal P, Bak G, Wang B, Dunlap S, Park MY, Kunkel EN, Trinidad B *et al*: **SAUR proteins and PP2C.D phosphatases regulate H⁺-ATPases and K⁺ channels to control stomatal movements.** *Plant Physiology* 2021, **185**(1):256-273.
41. Yang J, Chen R, Hu W, Wu Q, Tong X, Li X: **Identification and expression analysis of PP2C gene family in *Poncirus trifoliata*.** *Journal of Fruit Science* 2022, **39**(4):532-547.
42. Meskiene I, Baudouin E, Schweighofer A, Liwosz A, Jonak C, Rodriguez PL, Jelinek H, Hirt H: **Stress-induced protein phosphatase 2C is a negative regulator of a mitogen-activated protein kinase.** *Journal of Biological Chemistry* 2003, **278**(21):18945-18952.
43. Lynch M, Conery JS: **The evolutionary fate and consequences of duplicate genes.** *Science* 2000, **290**(5494):1151-1155.
44. Cannon SB, Mitra A, Baumgarten A, Young ND, May G: **The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*.** *BMC plant biology* 2004, **4**:10-10.

45. Guo L, Lu S, Liu T, Nai G, Ren J, Gou H, Chen B, Mao J: **Genome-wide identification and abiotic stress response analysis of *PP2C* gene family in *Woodland* and *Pineapple Strawberries*.** *International Journal of Molecular Sciences* 2023, **24**(4).
46. Wu H, Zhu L, Cai G, Lv C, Yang H, Ren X, Hu B, Zhou X, Jiang T, Xiang Y *et al*: **Genome-wide identification and characterization of the *PP2C* family from *Zea mays* and its role in long-distance signaling.** *Plants-Basel* 2023, **12**(17).
47. Chen CJ, Chen H, Zhang Y, Thomas HR, Frank MH, He YH, Xia R: **TBtools: An integrative toolkit developed for interactive analyses of big biological data.** *Molecular Plant* 2020, **13**(8):1194-1202.
48. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, Tosatto SCE, Paladin L, Raj S, Richardson LJ *et al*: **Pfam: The protein families database in 2021.** *Nucleic Acids Res* 2021, **49**(D1):D412-D419.
49. Ren M, Wang Q, Zhang F-h, Wang Y-l, Wang Y-y, Li W, Qi K-j, Xie Z-h, Zhang S-l, Tao S-t: **Genome-wide identification of the *GAox* gene family and functional characterization of *PbGA3ox4* during stone cell formation in Chinese white pear.** *Scientia Horticulturae* 2024, **330**.
50. Wang YP, Tang HB, DeBarry JD, Tan X, Li JP, Wang XY, Lee TH, Jin HZ, Marler B, Guo H *et al*: **MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity.** *Nucleic Acids Res* 2012, **40**(7).
51. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R: **TBtools: An integrative toolkit developed for interactive analyses of big biological data.** *Molecular Plant* 2020, **13**(8):1194-1202.
52. Kumar S, Stecher G, Li M, Knyaz C, Tamura K: **MEGA X: Molecular Evolutionary genetics analysis across computing platforms.** *Molecular Biology and Evolution* 2018, **35**(6):1547-1549.
53. Saitou N, Nei M: **The neighbor-joining method: a new method for reconstructing phylogenetic trees.** *Molecular biology and evolution* 1987, **4**(4):406-425.
54. Letunic I, Bork P: **Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation.** *Nucleic Acids Res* 2021, **49**(W1):W293-W296.
55. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren JY, Li WW, Noble WS: **MEME SUITE: tools for motif discovery and searching.** *Nucleic Acids Res* 2009, **37**:W202-W208.
56. Qiu X, Zhao H, Abubakar AS, Shao D, Chen J, Chen P, Yu C, Wang X, Chen K, Zhu A: **Genome-wide analysis of *AP2/ERF* gene superfamily in Ramie (*Boehmeria nivea* L.) revealed their synergistic roles in regulating abiotic stress resistance and ramet development.** *International Journal of Molecular Sciences* 2022, **23**(23).

Figures

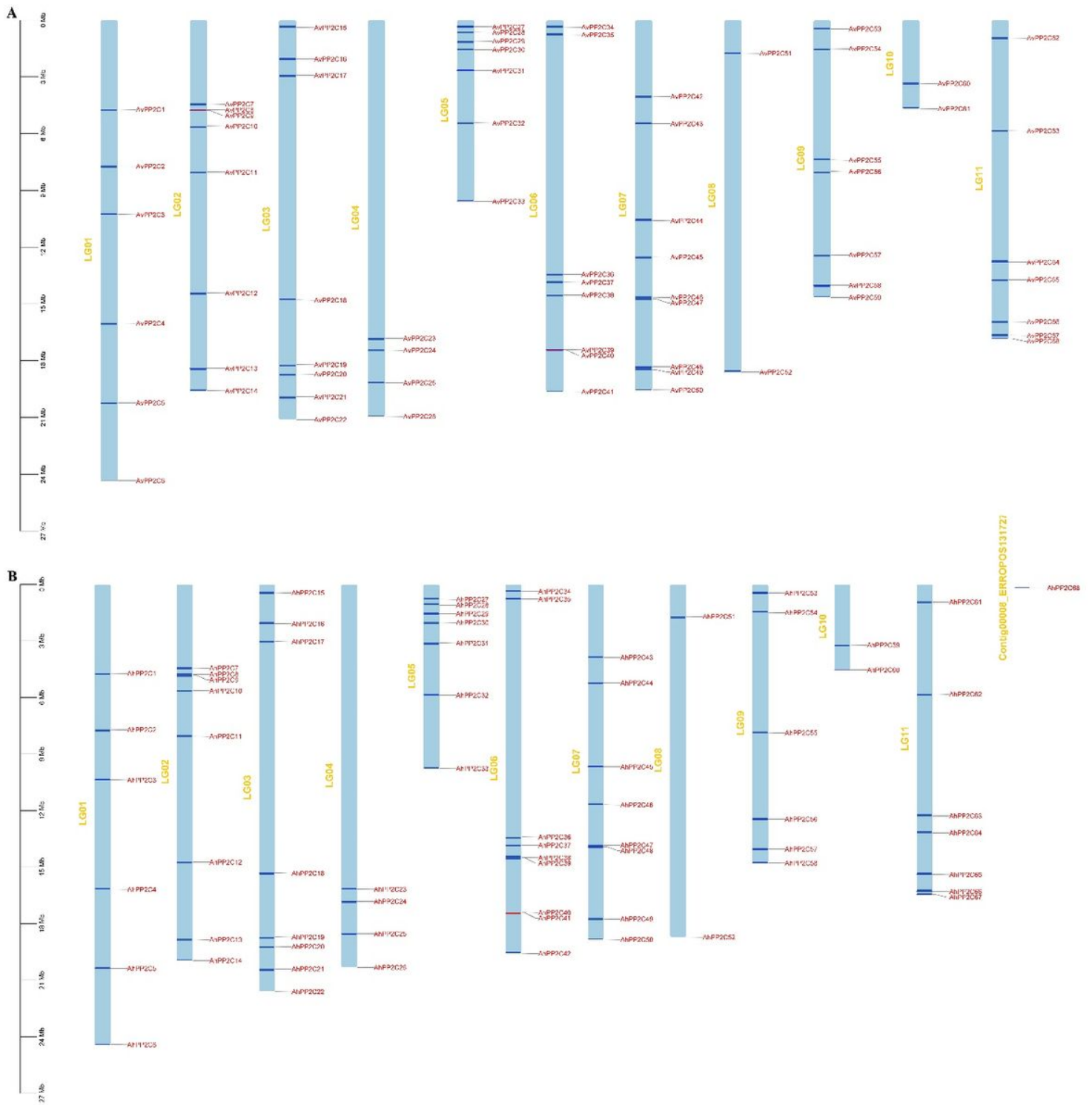


Figure 1

Chromosomal distribution and localization of the *PP2C* gene family in *Apocynum venetum* and *Apocynum hendersonii*. The blue rectangular bars represent chromosomes of *Apocynum*, and chromosome names are shown on the left side of each chromosome. The 0-27 Mb scale bar on the left represents chromosome length. (A) Chromosome distribution and localization of *AvPP2Cs*. (B) Chromosome distribution and localization of *AhPP2Cs*.

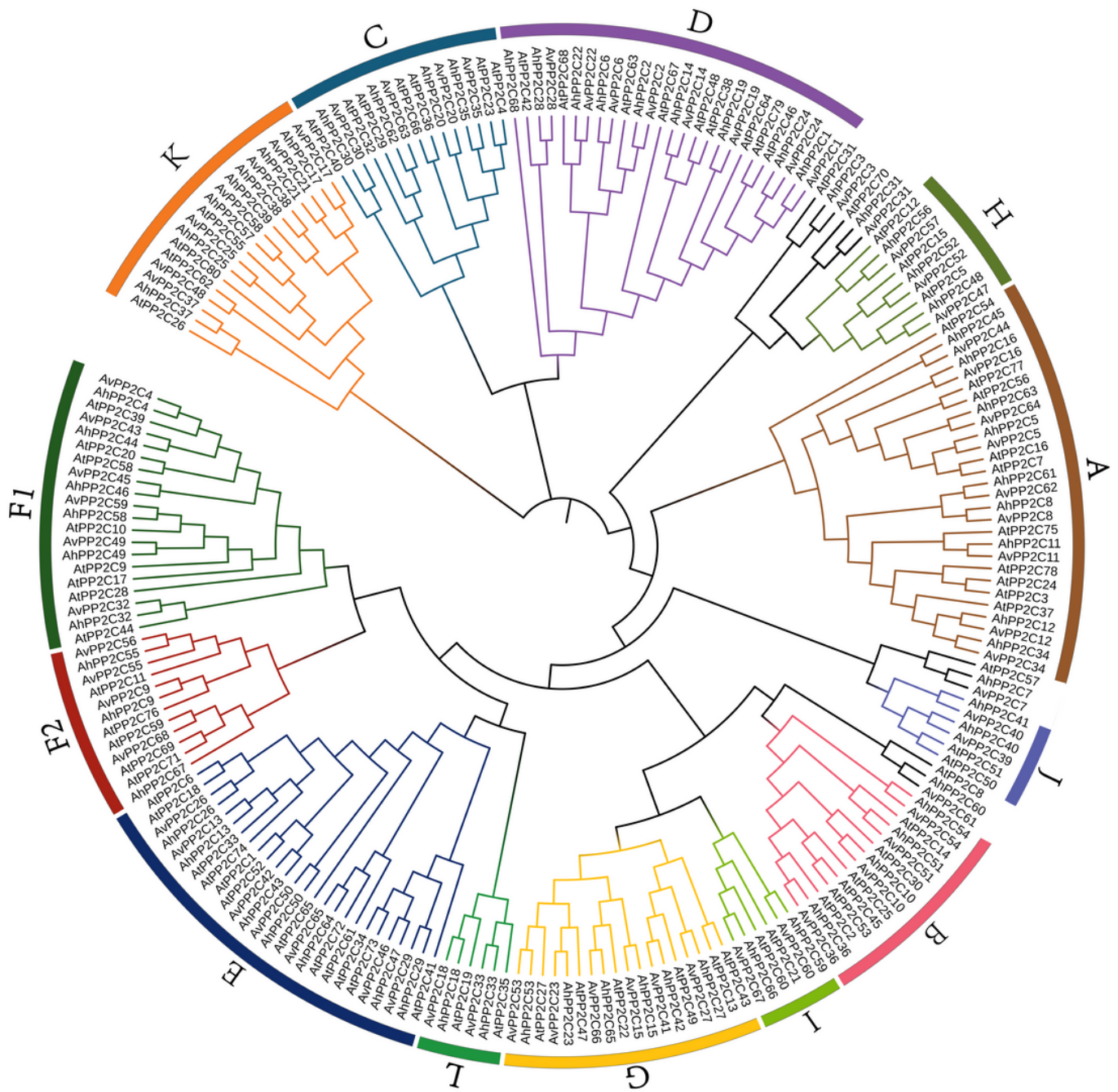


Figure 2

Phylogenetic analysis of PP2C family between *Apocynum venetum*, *Apocynum hendersonii* with *Arabidopsis*. The phylogenetic tree was constructed by MEGA 7 using the neighbor-joining method. The subgroups (A-L) are represented by different colors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xlsx](#)
- [TableS2.xlsx](#)
- [TableS3.xlsx](#)
- [TableS4.xlsx](#)
- [TableS5.xlsx](#)
- [TableS6.xlsx](#)