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Research Article
Characterization and Expression Analysis of an *AP2* Gene from *Ginkgo biloba*

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Abstract

Background and Objective: *APETALA 2* (*AP2*) gene is an important transcription factor in plant flower development and involved in signal transduction of plant growth, development and physiological and biochemical reactions. The aim of this study was to characterize a *AP2* gene from *Ginkgo biloba* (*G. biloba*). **Materials and Methods:** The specific primers were designed based on *AP2* unigene sequence of transcriptome data. The full-length cDNA of homologous genes were cloned from *G. biloba* by RT-PCR. Tissue expression analysis was estimated by quantitative RT-PCR methods. **Results:** Sequence analysis results showed that the full-length cDNA of GbAP2 was 2018 bp and contained a 1974 bp open reading frame, which encoded a 657 amino-acid protein. The predicted molecular weight and isoelectric point were 72.03 kDa and 5.91, respectively. Multiple alignments showed that the GbAP2 protein had high homology with the AP2 protein of other plants. The *GbAP2* contains two *AP2* domains and belongs to the *AP2* subfamily of the *AP2/ERF* family. Phylogenetic tree analysis revealed that *GbAP2* had closer genetic relationship with *Cycas revolute* from *Cycadaceae*. Tissue expression analysis showed that *GbAP2* gene was expressed in roots, stems, leaves, male strobili, female strobili and fruit of *G. biloba* and strongly expressed in the leaves and female strobili. **Conclusion:** In this study, a novel *AP2* gene (*GbAP2*) was cloned and characterized from *G. biloba* for the first time. The results of expression pattern of *GbAP2* in different tissues suggested that *GbAP2* might be involved in whole plant growth and development in *G. biloba*.

Key words: *Ginkgo biloba*, *APETALA2*, *GbAP2*, qRT-PCR, phylogenetic analysis, expression pattern

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ginkgo biloba is the oldest relict plant in existing seed plants. It sets food value, economic value, garden value, ecological value, ornamental and medicinal value in one, known as "Living fossil" and "Plant panda"[1,2]. However, *G. biloba* has a long juvenile phase, usually 15-20 years before blooming, which has brought serious difficulties in propagation and breeding[3]. Therefore, it is of great significance to study the flowering mechanism of *G. biloba* and shorten its juvenile phase.

The development of plant flowers affects the growth and reproduction of plants. In recent years, the genes related to flower development were cloned from *Arabidopsis thaliana*, *Antirrhinum*, *Oryza sativa* and other plants, such as *LEAFY* (*LFY*), *APETALA1* (*AP1*), *APETALA2* (*AP2*), *AGAMOUS* (*AG*), *FLOWERJNG LOCUSC* (*FLC*), *EMBRYONIC FLOWER* (*EMF*), etc[3,4]. *AP2/ERF* (*APETALA2*/ethylene-responsive factor) is one of the largest gene families in plants. It encodes a plant-specific transcription factor and contains an *AP2/ERF* domain consisting of 60-70 amino acids[5,6]. The *AP2/ERF* gene family can be divided into three categories according to their sequence similarity and number of domains, namely *AP2*, *ERF* and *RAV* subfamily[7]. The *AP2* family contains two *AP2/ERF* domains, which play a very important role in regulating plant growth and development[8-10]. The *ERF* family consists of one *AP2/ERF* domain, which can be divided into two large subfamilies, the *CBF/DREB* subfamily and the *ERF* subfamily[11]. *CBF/DREB* subfamily members can identify drought and cold induced response elements (*DRE/CRT*, *A/GCCGAC*) and play a significant role in resisting abiotic stress[12,13]. The *ERF* subfamily members can recognize *GCC-box* (AGCCGCC) and play a vital role in the process of resistance to biological stress[14]. The *RAV* family contains one *AP2/ERF* domain and one B3 domain, which plays a momentous role in the response of ethylene[15], brassinolide[16], biological and abiotic stress[17].

With the development of molecular biology, it has been found that *AP2* gene has a major effect on plant flower development. However, so far, the study of *AP2* gene prevalent in plants is mainly focused on *Arabidopsis thaliana*[18], *Triticum aestivum*[19], *Oryza sativa*[20], *Zea mays*[21], etc and few articles related to *AP2* gene in *G .biloba*. Therefore, the aim of this study was to clone the *AP2* gene in *G. biloba* and obtain the *AP2* gene sequence and carry out biological information analysis and tissue expression analysis, to lay the foundation for the future study of *AP2* gene function, to clarify *GbAP2* involved in regulating *G. biloba* flowering and provide theoretical basis for the mechanism of growth.

MATERIALS AND METHODS

Materials: The materials were collected from 31 years old trees of the *G. biloba* cultivar "Jiafoshou", in the Ginkgo Science and Technology Garden, Yangtze University. The male and female strobili were sampled in early April. The roots, stems, leaves and young fruits were sampled in end of May. All these samples were quickly frozen by liquid nitrogen after collected and stored in -80°C ultra-low temperature refrigerator. The RNA extraction kit (MiniBest Plant RNA Extraction kit), reverse transcription kit (PrimeScript™ 1st Strand cDNA Synthesis Kit), gel recovery kit (Agarose Gel DNA purification Kit Ver.4.0) and PCR reagents for ampicillin (AMP), pMD19-T vector and *Escherichia coli* competent cell DH5α were purchased from TaKaRa, Dalian Bao Biotechnology Co. Sequencing and synthesis of the primers were performed by Shanghai Sangon Biological Engineering company.

Cloning of the *GbAP2* gene: The method of extracting total RNA reference TaKaRa Company of RNA extraction kit (MiniBest Plant RNA Extraction kit) specification. The purity and concentration of RNA were detected by nucleic acid quantification and 1% agarose gel electrophoresis and stored in a refrigerator at -80°C. The extracted RNA was subjected to reverse transcription and the procedure was carried out with reference to the instructions of the reverse transcription kit (PrimeScript™ 1st Strand cDNA Synthesis Kit). A pair of specific primers A1, A2 (Table 1) were designed according to the data of the previous *G. biloba* transcriptome. The PCR was carried out by reverse transcription of the cDNA. The reaction system was 25 μL. The amplification program was pre-denaturation at 94°C for 3 min; denaturation at 94°C for 30 sec, annealing at 37°C for 30 sec, extension at 72°C for 90 sec, Cycle, 72°C for 10 min. The PCR product was subjected to gel recovery by 1% agarose gel electrophoresis and the target fragment was ligated to the pMD19-T Vector and ligated overnight at 16°C. After the transformation was well transformed into *Escherichia coli* competent cells DH5α, single colonies were picked and the positive clones were confirmed by PCR. The positive bacteria were sent to Shanghai for sequencing.

Table 1: Primer sequences

Primers	Sequence 5'-3'
GbAP2 up A1	GAAACAGTTCAGATGACGGGC
GbAP2 down A2	GAGGGGCAAGTCTCATCGTC
qRT-PCR up R1	ATCTCGTCAATATACGCATCTGTCC
qRT-PCR down R2	GGGTGTTAGACATCGGAGACTTATC
GbGAPDH up H1	TTGGTCTCCCGTGCTAATGG
GbGAPDH down H2	CGAAGCGTCATCCTAAGACAACA

Bioinformatics analysis: Sequencing of the obtained gene sequence, the NCBI site using BLAST tool for nucleotide sequence and deduced amino acid sequence homology comparison, to obtain the highest degree of similar sequence. The open reading frame analysis was performed using Vector NTI 11.5 and the deduced amino acid sequence of *GbAP2* was compared with the known homology of the gene family. The deduced amino acid sequence was analyzed by CLUSTAL X 2 and MEGA6 software. The Neighbor-Joining (NJ) method was used to construct phylogenetic tree. The ExPASy online server ProtParam predicts the molecular weight of the protein and the theoretical isoelectric point.

Expression analysis of *GbAP2* gene: RNA was extracted from *Ginkgo* roots, stems, leaves, flowers and fruit samples and reverse transcribed into cDNA. The real-time quantitative primers R1 and R2 (Table 1) of the *GbAP2* gene fragment were designed according to the known sequence and *GbGAPDH* was selected as the internal reference gene[22,23] using reverse transcription cDNA as template and qRT-PCR amplification. The reaction system is 20 μL, the reaction program refer to PrimeScript™ RT reagent kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Dalian, China). Each sample was set up 3 times to repeat with ultrapure water as a negative control and *GbGAPDH* was an internal reference gene. Data processing using the relative quantitative method, with reference to the $2^{-\Delta\Delta Ct}$ method for the results of analysis[24].

Statistical analysis: Data were analyzed with one-way ANOVA using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). The means were compared with Duncan's multiple range tests. $p<0.05$ was considered to be statistically significant.

RESULTS

***GbAP2* cDNA full-length cloning:** A pair of specific primers (Table 1) were designed and synthesized according to the sequencing results of the previous transcripts. The cDNA was amplified by PCR using reverse transcription cDNA as template. The *GbAP2* gene was 2018 bp in length. The results of Vector NTI 11.5 showed that the *GbAP2* gene contained a 1974 bp ORF encoding 657 amino acids (Fig. 1) named *GbAP2* (GenBank accession number MF422072). The online analysis of ExPASy-ProtParam showed that the molecular weight of the protein was 72.03 kDa and the isoelectric point was 5.91. The nucleotide sequence of the *GbAP2* gene is highly homologous to the *AP2* gene sequence of other plants (Table 2).

Table 2: Nucleotide sequence of *GbAP2* similarity to the *AP2* gene of other plant species

Species	Accession number	Identify (%)	E-value
Cicer arietinum	XP_0045119041	70	5e-102
Populus trichocarpa	XP_002310715.1	68	4e-100
Vigna angularis	XP_017408128.1	67	6e-101
Cajanus cajan	XP_020237263.1	66	4e-103
Betula platyphylla	AEL29576.1	64	1e-101
Populus euphratica	XP_011006856.1	63	9e-101
Nelumbo nucifera	XP_010270518.1	63	4e-101
Lupinus angustifolius	XP_019434830.1	60	3e-103

Table 3: Sequence accession numbers of genes

Gene	Species	Accession number
GaAP2	*Gossypium arboretum*	XP_017618998.1
CaAP2	*Cicer arietinum*	XP_004511904.1
VaAP2	*Vigna angularis*	XP_017408136.1
CcAP2	*Cajanus cajan*	XP_020237263.1
EgAP2	*Elaeis guineensis*	XP_010928347.1
PsAP2	*Paeonia suffruticosa*	ALI57167.1
GhAP2	*Gossypium hirsutum*	XP_016692286.1
PlAP2	*Paeonia lactiflora*	AGI61068.1
CrAP2	*Cycas revolute*	BAE48512.1
AdAP2	*Arachis duranensis*	XP_015963905.1
PdAP2	*Phoenix dactylifera*	XP_008799004.1
CsAP2	*Camelina sativa*	XP_010437250.1
RsAP2	*Raphanus sativus*	XP_018477329.1
BjAP2	*Brassica juncea*	ANV82484.1
NtAP2	*Nicotiana tabacum*	XP_016491203.1
SpAP2	*Solanum pennellii*	XP_015063599.1
BnAP2	*Brassica napus*	XP_013643407.1
GSap2	*Glycine soja*	KHN08340.1

Analysis of GbAP2 protein: The *GbAP2* gene coding sequence of amino acids in NCBI BLAST-protein on the site, the results showed that the GbAP2 protein sequence had high homology with other AP2 protein sequences. The sequence similarities of GbAP2 protein and other AP2 proteins were as follows: *Cicer arietinum*, *Populus trichocarpa*, *Vigna angularis*, *Cajanus cajan*, *Betula platyphylla*, *Populus euphratica*, *Nelumbo nucifera* and *Lupinus angustifolius* were 70, 68, 67, 66, 64, 63, 63 and 60%, respectively. Using AlignX software to compared *GbAP2* amino acid sequence with other *AP2* homologous proteins in multiple comparison (Fig. 2), the results showed that the GbAP2 protein contained two typical *AP2* domains, which were similar to the *AP2* conserved domain of *AP2/ERF* family transcription factors in other plants, indicating that *GbAP2* belongs to the *AP2* subfamily.

Analysis of *GbAP2* gene evolutionary tree: The AP2 protein sequences of other species were downloaded from GenBank (Table 3). To understand *GbAP2* AP2 protein molecular evolution of relations with other plants, using CLUSTALX2 and MEGA6 software, the GbAP2 protein sequence was homologous to the AP2 protein of other species on GenBank and phylogenetic tree was constructed by NJ method.

```
1       GAAACAGTTCAGATGACGGGCCAGATAGTGCACTTTTCAAGCATTGAGGCGACTGGTGTGACAGCCAGGAGCCCA
1                     M  T  G  Q  I  V  H  F  S  S  I  E  A  T  G  V  T  A  R  S  P
76      GACGACCCCGAGTCGGTATGCGATGAAGCAGCCAGCAACAATGATAAAGTTCCTCCTGGATCATTAATCGAGCCG
26       D  D  P  E  S  V  C  D  E  A  A  S  N  N  D  K  V  P  P  G  S  L  I  E  P
151     GTGTTGGTTTTCGACGAGTCTGGAACCTCCGACTCCTCTGTTGTAAATGCCATTGCGATTGAAAACGTTAATTCC
51       V  L  V  F  D  E  S  G  T  S  D  S  S  V  V  N  A  I  A  I  E  N  V  N  S
226     CTTGAATGCGAAGCCTCCAGCAAGGCAGTGGAAGGAGATATCTCGTCAATATACGCATCTGTCCGTCAGTCTGGA
76       L  E  C  E  A  S  S  K  A  V  E  G  D  I  S  S  I  Y  A  S  V  R  Q  S  G
301     GACGGTAATGATAAAATCTTTGGGTTTTCTTTCAGTATTCTCAGAGATAAGTCTCCGATGTCTAACACCCCCAAC
101      D  G  N  D  K  I  F  G  F  S  F  S  I  L  R  D  K  S  P  M  S  N  T  P  N
376     GAGAAATTGATGACCTGTGGGACAGAAAACGATGCACCCAGTGGAGTCTGTGTTACACGGCAGTTTTTTCCGTCA
126      E  K  L  M  T  C  G  T  E  N  D  A  P  S  G  V  C  V  T  R  Q  F  F  P  S
451     CGCAATGTGGAGTCTTCTGGAATGCTGATAGAAAGTCGTCGTGCAGATAAGTTTACAGGAGCTCATTGGGCGGGC
151      R  N  V  E  S  S  G  M  L  I  E  S  R  R  A  D  K  F  T  G  A  H  W  A  G
526     TCAGGTTTCTGTCAGTCAGAAGACGCAGCCGGACTTTCAAAGCAAATAGAAAACACTCAGCGGGGTAAGAAAAGT
176      S  G  F  C  Q  S  E  D  A  A  G  L  S  K  Q  I  E  N  T  Q  R  G  K  K  S
601     AGACGAGGGCCAAGATCGAGAAGCTCCCAATATCGTGGGGTGACATTCTACCGACGTACTGGTCGATGGGAATCC
201      R  R  G  P  R  S  R  S  S  Q  Y  R  G  V  T  F  Y  R  R  T  G  R  W  E  S
676     CACATATGGGATTGTGGAAAACAAGTCTATCTGGGTGGATTTGACACCGCTGATGCAGCTGCCAGAGCGTACGAT
226      H  I  W  D  C  G  K  Q  V  Y  L  G  G  F  D  T  A  D  A  A  A  R  A  Y  D
751     AGGGCGGCCATCAAGTTTAGAGGAATTGAAGCTGACATCAATTTTACTCTCAGTGACTATGAAGAAGATGTGAAC
251      R  A  A  I  K  F  R  G  I  E  A  D  I  N  F  T  L  S  D  Y  E  E  D  V  N
826     CAGATGAACAATCTCTCGAAGGAAGAATTTGTGCATATCCTTCGTCGTCAAAGCACTGGTTTCTCTCGAGGGAGT
276      Q  M  N  N  L  S  K  E  E  F  V  H  I  L  R  R  Q  S  T  G  F  S  R  G  S
901     TCCAAGTTCAGGGGCGTTACTTTGCACAAATGTGGCCGGTGGGAAGCCAGGATGGGGCAATTCCTAGGGAAAAAG
301      S  K  F  R  G  V  T  L  H  K  C  G  R  W  E  A  R  M  G  Q  F  L  G  K  K
976     TATATATATTTGGGATTATTTGACACGGAAGAGGAAGCTGCAAGGGCATATGACAGAGCAGCCATCAGATGCAAT
326      Y  I  Y  L  G  L  F  D  T  E  E  E  A  A  R  A  Y  D  R  A  A  I  R  C  N
1051    GGTAAAGAGGCAGTAACGAACTTTGATCCTAGTGTATATGAGAAAGACATGCTCGAGGAAGGAGGAGAGGGACAT
351      G  K  E  A  V  T  N  F  D  P  S  V  Y  E  K  D  M  L  E  E  G  G  E  G  H
1126    GCAAGCACAGGTTGCAAGCAGAATCTGGACCTTTGTCTTGGCATTTCGGCTCCAGTGGAGGGTACCACCTTGGTA
376      A  S  T  G  C  K  Q  N  L  D  L  C  L  G  I  S  A  P  V  E  G  T  T  L  V
1201    TCAGAGGACTGCAAGATTGTAGGATACAAATCAAAATTCAATCCGTCCACCTCAACTGAAATAGATTGGAAGAAA
401      S  E  D  C  K  I  V  G  Y  K  S  K  F  N  P  S  T  S  T  E  I  D  W  K  K
1276    GCTCATCTTTTTAAACCTCTGGTTGAACAAGAGACTCAGCAGAACCACATGGGAGTGCTCTATTCCGGCCAGTTT
426      A  H  L  F  K  P  L  V  E  Q  E  T  Q  Q  N  H  M  G  V  L  Y  S  G  Q  F
1351    AATTTACCCTCTAAATTAAAGGATGCAAGCATTTTGTCATATCAAAGGAGAAATCCTGGAAGTGGTAACTTTGAA
451      N  L  P  S  K  L  K  D  A  S  I  L  S  Y  Q  R  R  N  P  G  S  G  N  F  E
1426    AAGGATAGGCGGTTCCAGTTCATGTCACAAGGTCAGAATGAGAGTTCAAATTCACAAAACCTGCAAGTTCATGAC
476      K  D  R  R  F  Q  F  M  S  Q  G  Q  N  E  S  S  N  S  Q  N  L  Q  V  H  D
1501    ATGGTCATGAAACACTATCCATTTAGGACTTCACCTCTAATTATGGATGATCGTGTCTGTGTCTCATCGGAGTCA
501      M  V  M  K  H  Y  P  F  R  T  S  P  L  I  M  D  D  R  V  C  V  S  S  E  S
1576    GTATCAGAACACAGCCAGATCCCTTCTGAATCCTTCAGCAGCTGTAAGGCACCAATCCTCCTGGAGAGTTCCAAG
526      V  S  E  H  S  Q  I  P  S  E  S  F  S  S  C  K  A  P  I  L  L  E  S  S  K
1651    GAGCAAAGAGGTGGAATTTTGTCAACTATATCCTCTTTGCCAATGGAACAACATTCTGGATGGGCTTGGCAACTG
551      E  Q  R  G  G  I  L  S  T  I  S  S  L  P  M  E  Q  H  S  G  W  A  W  Q  L
1726    CACGTCACTGGGCCGGCTGCTCCAGTATTTGCAAACGCATCCTCATCAGGATTCTCACCCCAAATTGTACCCACT
576      H  V  T  G  P  A  A  P  V  F  A  N  A  S  S  S  G  F  S  P  Q  I  V  P  T
1801    ACTATTTCTTCTTCAGATTGGCTGCAGAAAACAGGCACACATTCCCAGGCGCATTCTACCAATCCTGCGTTTCAG
601      T  I  S  S  S  D  W  L  Q  K  T  G  T  H  S  Q  A  H  S  T  N  P  A  F  Q
1876    ACACAAAGTTTGCATTTAGCAACTCCTCCGGGCACTGGTACTGCTGCCGAATCGGGCGAACATTTTCTGGGTTTG
626      T  Q  S  L  H  L  A  T  P  P  G  T  G  T  A  A  E  S  G  E  H  F  L  G  L
1951    ACTCTGGACTTTCCAACGAAGTCGTCTAACACGTAAATCCACTTCTAAGACGATGAGACTTGCCCCTC
651      T  L  D  F  P  T  K  S  S  N  T  *
```

Fig. 1: Nucleotide sequence and deduced amino acid sequence of *GbAP2*. The start codon and termination codon are indicated by the box, the primers are indicated by the underline

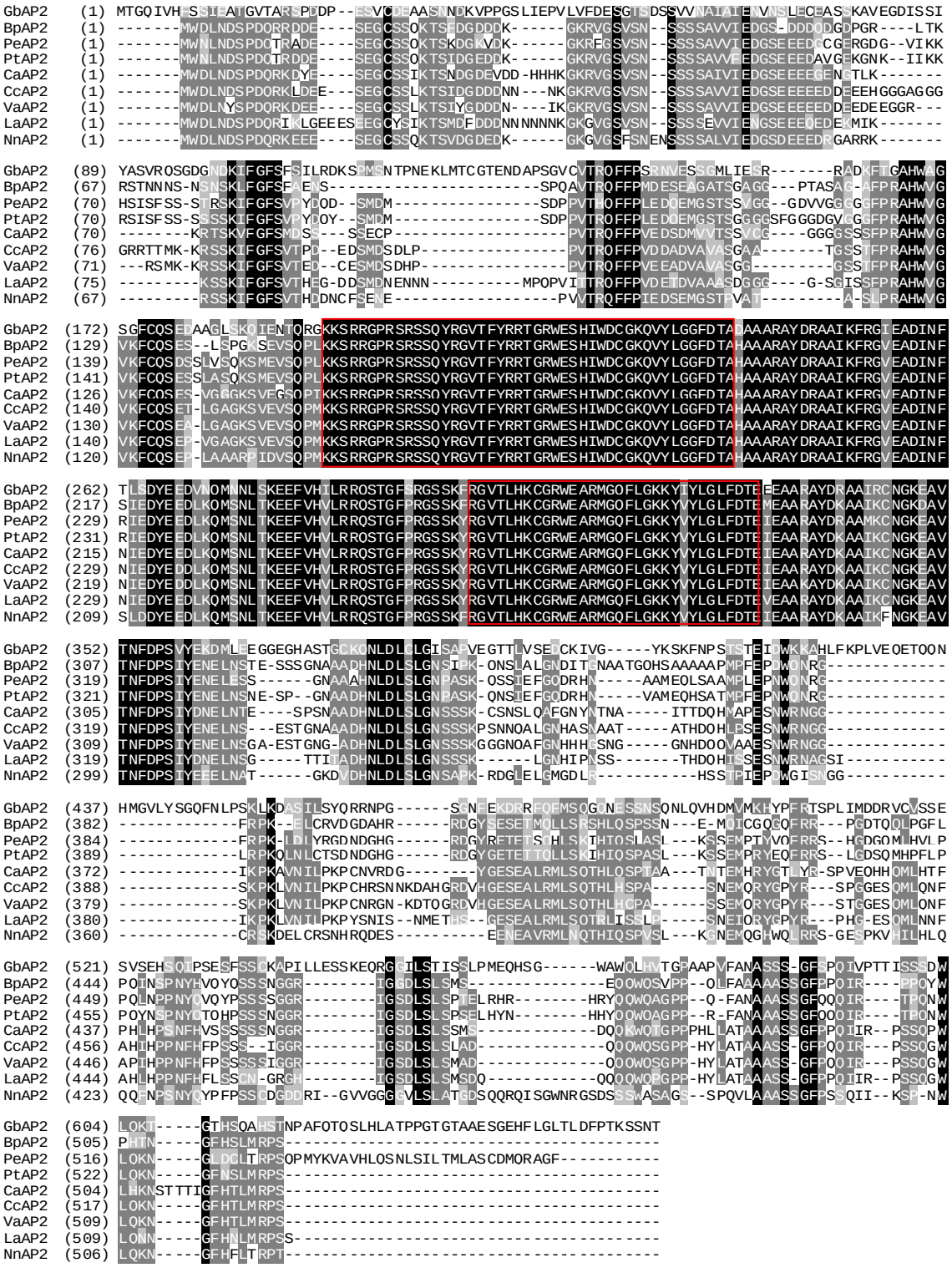

Fig. 2: Similarity analysis of *GbAP2* coding protein and other known proteins, red box represent the AP2 domain. *GbAP2* (*Ginkgo biloba*), *BpAP2* (*Betula platyphylla* AEL29576.1), *PeAP2* (*Populus euphratica* XP_011006856.1), *PtAP2* (*Populus trichocarpa* XP_002310715.1), *NnAP2* (*Nelumbo nucifera* XP_010270518.1), *CaAP2* (*Cicer arietinum* XP_004511904.1), *CcAP2* (*Cajanus cajan* XP_020237263.1), *VaAP2* (*Vigna angularis* XP_017408128.1), *LaAP2* (*Lupinus angustifolius* XP_019434830.1)

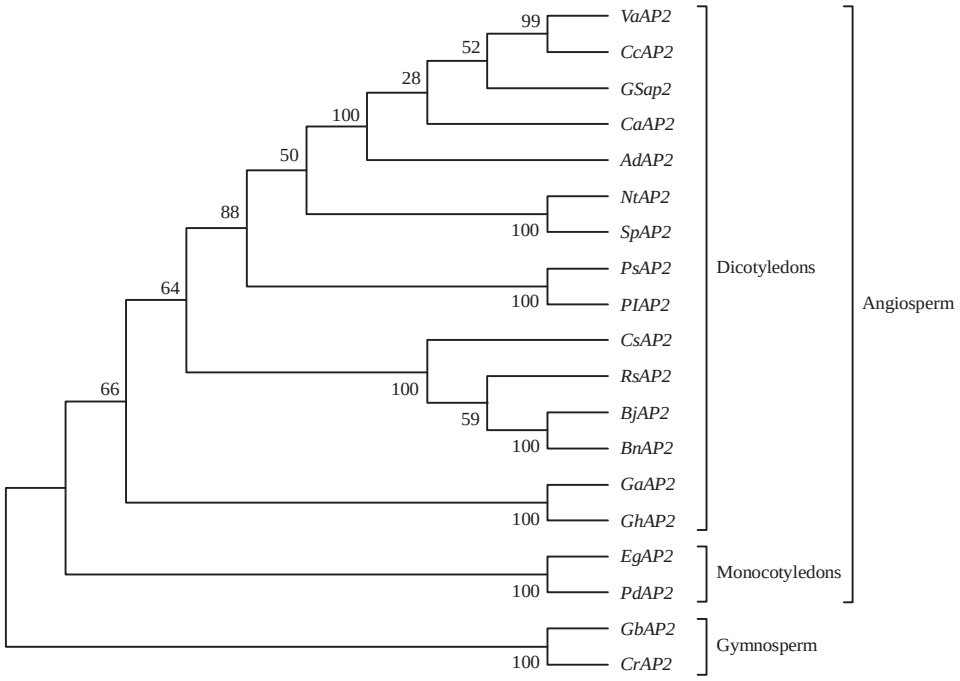

Fig. 3: Phylogenetic tree of genes in *GbAP2* gene family

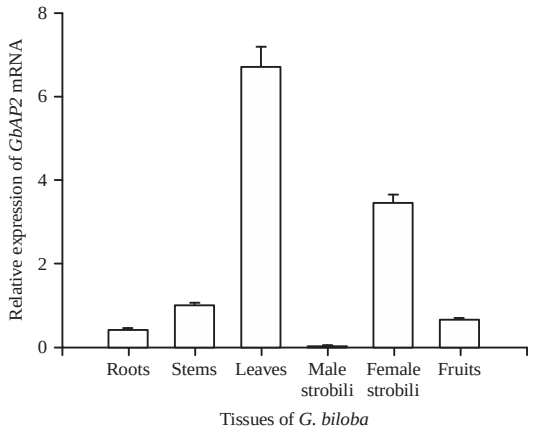

Fig. 4: *GbAP2* gene expression in different tissues of *G. biloba*
Data from qRT-PCR were shown as the Mean±SD (standard deviation) of three biological replicated assays. Means with different letters were significantly different by p<0.05 by Duncan's multiple range tests

Phylogenetic tree is divided into two as shown in Fig. 3, one for the gymnosperm, the other for the angiosperm, the angiosperm are divided into monocotyledons and dicotyledons. Phylogenetic tree shows that *AP2*gene is widely found in plants and has some conservatism in evolution. The *GbAP2* had closer genetic relationship with *Cycas revolute* from *Cycadaceae*, indicating that *GbAP2*was the most closely related to *AP2* gene of gymnosperm, followed by monocotyledons, but had the greatest affinity for dicotyledons.

Organizational expression analysis: The *GbGAPDH* gene was used as the internal reference to analyze the specificity of *GbAP2* gene expression in different tissues of *G. biloba*. The *GbAP2* gene was expressed in the roots, stems, leaves, male strobili and female strobili of *G. biloba* as shown in Fig. 4. Among them, the expression level in the leaves was the highest, the female strobili was the second and the expression level in the roots, stems and fruits was close and the expression level was the lowest in the male strobili.

DISCUSSION

The *AP2* gene as class A of flower organ, is a kind of important transcription factors in the development of plant flowers, involved in plant growth, development and a variety of physiological and biochemical reaction of signal transduction[25,26]. In this study, *GbAP2*, a homologous gene of *AP2*, was cloned from *G. biloba* by RT-PCR. The full-length cDNA of *GbAP2* was 2018 bp and contained a 1974 bp open reading frame, which encoded a encodes 657 amino-acid protein. The predicted molecular weight and isoelectric point were 72.03 kDa and 5.91, respectively. Its coding protein has the characteristics of typical *AP2*family, specifically comprising two highly conserved repeats, the *AP2* domain, which identify and bind the cis-acting elements of the DNA[27]. In addition, there is a serine-rich transcriptional activation region between its N-terminal 16-60 amino acids and a basic region between

194-203 amino acids, containing the nuclear localization signal KKSR[28]. The above results indicate that the amino acid sequence difference between *GbAP2* and other *AP2* homologous genes is large except for the nuclear localization signal sequence and *AP2* domain, suggesting that there may be some differences in the function of *AP2* homologous genes between species, especially among species with different flowering characteristics.

In addition, the *GbAP2* gene in *G. biloba* was expressed in roots, stems, leaves, male strobili, female strobili and fruits and the highest expression was found in leaves. Zhou *et al*.[29] isolated the *MAP2A* gene of *Malus* and found that *MAP2A* was expressed in many tissues of *Malus*, such as flower bud, sepals, petals, male and female, ovary and leaf, but *MAP2A* gene was not detected in young fruit, suggesting that the *MAP2A* gene not only regulated the development of floral organ, but also participated in the regulation of vegetative tissue. Wang *et al*.[30] isolated the *Vv-AP2* gene from the *Vitis vinifera*. The results showed that the expression of *Vv-AP2* in the inflorescence and flower was significantly higher than that in the leaves and stems, indicating that *Vv-AP2* had a different effect on the nutrition of the *Vitis vinifera* and the development of the reproductive organs. Chen *et al*.[31] isolated the *PeAP2* gene from *Phyllostachys edulis* and found that *PeAP2* was expressed in the roots, stems, leaves. Among them, the expression level in the leaves was the highest, followed by the sheath and the expression level in the roots, stems and sections was close. These results indicated that *AP2* gene was expressed in different tissues, suggesting that the gene was involved in the development of each tissue, but its expression mechanism and regulation and development pattern were different in different tissues. Therefore, in the study of *AP2* gene in the role of flower development, should also pay attention to the expression of these genes and control of phenotypic changes.

As a key gene for the development of plant flower, the biological function of *AP2* gene needs to be studied in a more systematic and in-depth way, which will provide the theoretical basis for revealing the function and regulation mechanism of this gene. This will be the focus of our future attention and exploration.

CONCLUSION

An *AP2* gene from *G. biloba* was cloned and its coding protein has the characteristics of typical *AP2* family, specifically comprising two highly conserved repeats, the *AP2* domain. It is strongly expressed in leaves and female strobili. This finding lays the foundation for shortening the juvenile phase of *G. biloba* and other woody plants.

SIGNIFICANCE STATEMENTS

The *GbAP2* gene is an important transcription factor in plant flower development, which is useful for shortening the long juvenile phase of *G. biloba*. This study cloned and characterized an *AP2* gene from *G. biloba* for the first time. Tissue expression pattern analysis shows that *GbAP2* is strongly expressed in leaves and female strobili, suggesting *GbAP2* might be involved in the development of leaves and female strobili. These findings will provide the theoretical basis for revealing the function and regulation mechanism of *GbAP2* gene.

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