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ORIGINAL PAPER

Identification and expression analysis of *APETALA1* homologues in poplar

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Abstract *APETALA1* plays a crucial role in floral transition from vegetative to reproductive phase and in flower development. In this study, a comprehensive analysis of *AP1* homologues in poplar was performed by describing the gene structure and chromosomal location. The phylogenetic relationship of the deduced amino acid sequences of *Arabidopsis AP1* and *AP1* homologues from *Populus*, to other AP1-like proteins was analyzed. The expression of *PtAP1-1* and *PtAP1-2* in *Populus tomentosa* was examined by RT-qPCR. Expression profiles were similar and both genes exhibited a high expression level in the reproductive phase. Seasonal expression profiles in floral buds indicated that the pattern of *PtAP1-1* and *PtAP1-2* expression in male and female floral buds was different. The trends of the *PtAP1-1* and *PtAP1-2* transcript levels in both sex floral buds were similar, but the peak of expression of the two genes in male buds was earlier than in female buds. This work would be of value to future functional analysis of *AP1* homologues in poplar.

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Introduction

In high plants, flowering is an important developmental process in response to endogenous and environmental signals. During the past two decades, many genes related to flower initiation and development have been isolated and their functions have been well studied (Wellmer and Riechmann 2010). Particularly, *APETALA1* (*AP1*) orchestrates floral initiation by integrating growth, patterning, and hormonal pathways (Kaufmann et al. 2010). In *Arabidopsis thaliana*, flowering cues gather in *FLOWERING LOCUS T* (*FT*), a flowering time integrator, and FT protein interacts physically with FLOWERING LOCUS D (FD) and activates the floral meristem identity genes *LEAFY* (*LFY*) and *AP1* to specify floral meristems on the flanks of the shoot apical meristem (Abe et al. 2005; Corbesier et al. 2007). These two genes play central roles in the transition from the inflorescence meristems into floral meristems. *LFY* activates *AP1* and they also form a positive feedback loop. *AP1* is first observed throughout emerging floral primordia (Mandel et al. 1992; Wagner et al. 1999). *TERMINAL FLOWER1* (*TFL1*) is a shoot identity gene and is referred to as a floral repressor. *TFL1* is necessary for indeterminate shoot fate, since *tfl1* mutants show early flowering and conversion of inflorescence meristems to floral meristems (Bradley et al. 1997; Ratcliffe et al. 1998). *LFY* and *AP1* antagonize the activity of *TFL1* to establish floral meristems. In turn, *TFL1* acts to repress *LFY* and *AP1* in inflorescence meristems (Ratcliffe et al. 1998). The balance between these three genes determines the rate of shoot apical phase transition and flowering time (Bradley et al.

1997; Parcy et al. 2002; Ratcliffe et al. 1999). Three MADS-box transcription factors, *SHORT VEGETATIVE PHASE* (*SVP*, a floral repressor), *AGAMOUS-LIKE24* (*AGL24*, a floral activator) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*, a floral activator), are identified early as flowering time genes. In floral meristems before late stage 2, class B and C homeotic genes are not expressed because *SEP3* is repressed by *SVP*, *SOC1* and *AGL24*. As floral meristems proceed to late stage 2, direct repression of *SVP*, *SOC1* and *AGL24* by *AP1* gradually derepresses *SEP3*. SEP3 interacts physically with LFY to activate downstream other floral organ identity genes in the apical region of early stage 3 floral meristem (Liu et al. 2009). *AP1* functions not only as floral meristem identity gene, but also determines floral organ formation, and in later stages of floral development, its expression is confined to the first and second whorls of floral buds, where AP1 is involved in the specification of sepals and petals (Mandel et al. 1992; Weigel and Meyerowitz 1994).

Poplar (*Populus* spp.) is a perennial woody plant that has been widely grown for landscaping, forestation, timber, pulp and biofuels. Since the release of the *Populus trichocarpa* draft genome (Tuskan et al. 2006) and subsequent updates in the phytozome database (Goodstein et al. 2012), poplar has emerged as a model species for molecular genetics research in trees. However, the long juvenile phase in poplars presents a substantial obstacle to research and breeding. Additionally, shed flowers, allergenic properties of poplar pollen, and catkin production represent sources of environmental pollution and a hazard to human health (An et al. 2011a, b; Strauss et al. 1995). The ability to control the transition from the juvenile phase to the reproductive phase and identification of the key genes involved in flowering in poplar would represent a significant accomplishment. Recently, the functions of *FT* (Böhlenius et al. 2006; Hsu et al. 2006, 2011; Shen et al. 2012; Zhang et al. 2010), *CO* (Hsu et al. 2012), and *CEN* (Mohamed et al. 2010) homologues in poplar have been well investigated. Although *AP1* plays key roles in flowering time and floral organ formation, the mechanisms of controlling flowering in poplar and the patterns of gene expression during poplar floral bud development have not been fully elucidated. In this study, we analyzed the gene structure, chromosomal location and phylogenetic relationship of *Populus AP1* homologues and characterized their expression patterns throughout the vegetative and reproductive developmental stages in Chinese white poplar (*Populus tomentosa* Carr.). This work may help to elucidate the biological functions of *AP1* homologues in poplar.

Materials and methods

Plant materials and growth conditions

One-month-old tissue-cultured plants of Chinese white poplar (*P. tomentosa* female clone TC1521) were grown and synchronized (using vegetative stem segments containing an axillary bud) on 1/2 MS solid medium supplemented with 0.4 mg l^{-1} IBA (pH 5.8) in a growth chamber at 25 °C under a 16-h light/8-h dark photoperiod.

Sequence and phylogenetic analyses of *AtAP1* and *PtrAP1*

The amino acid sequences of *AP1* homologue genes were retrieved from Phytozome v9.1 (http://www.phytozome.net/) and aligned with ClustalX 1.81 and Bioedit software. Conserved and functional domains sequences were analyzed using Expert Protein Analysis System (ExPASy) software available on the website of the Swiss Institute of Bioinformatics (http://cn.expasy.org) (Gasteiger et al. 2003). The tertiary structure of the AP1 proteins was predicted using SWISS-MODEL (http://swissmodel.expasy.org/workspace/index.php?func=modelling_simple1) software, and viewed with RasMol 2.7.2.1. Genomic sequence data were used to analyze gene structure and determine chromosomal locations. A phylogenetic tree was constructed using the Neighbor-Joining (N-J) method available in MEGA 5.0 software (Tamura et al. 2011).

PtAP1-1 and *PtAP1-2* gene expression analysis in *P. tomentosa*

RT-qPCR was used to obtain a transcriptomic profile of *PtAP1-1* and *PtAP1-2* genes in *P. tomentosa*. Total RNA was extracted as previously described (Chen et al. 2013) from (a) roots, stems and leaves of one-month-old tissue-cultured poplar plants, and (b) mature leaves, vegetative buds, female catkins, and female and male floral buds obtained from female and male adult trees located in the Beijing Forestry University nursery. Samples from mature trees were collected from June, 2012 through February, 2013 monthly, to cover major stages of floral development (An et al. 2010). Total RNA was treated with RQ1 DNase I (Promega, Madison, WI, USA) to remove any contaminating genomic DNA. First-strand cDNA was synthesized using 1.0 µg of DNase-treated total RNA, Superscript III (Invitrogen, Carlsbad, CA, USA) and oligo $d(T)_{20}$ in a total volume of 20 µl. First-strand cDNA was diluted 1:10 with ddH_2O, and 2 µl of the diluted cDNA was used as a template for RT-qPCR analysis. The

RT-qPCR reaction was performed using SYBR® Premix Ex Taq™ (TaKaRa, Otsu, Japan) on a DNA Engine Opticon™ 2 system (MJ Research™). Specific primers used for RT-qPCR analysis are listed in Table S1. The PCR condition was 95 °C for 10 s, followed by 40 cycles of amplification (95 °C for 5 s, 60 °C for 20 s and 72 °C for 15 s), with a final extension of 7 min at 72 °C. The plates were read every 0.2 °C for 1 s from 70 to 95 °C, to generate the melting curves. The generated melting curve was employed as a significant parameter to check the specificity of the amplified fragment. All reactions were carried out in triplicate for technical and biological repetitions of the three individuals, respectively, and the generated real-time data were analyzed using the Opticon Monitor Analysis Software 3.1 tool. Data were presented as a mean ± SD. The efficiency of the primer sets was calculated by performing real-time PCR on several dilutions of first-strand cDNAs. Efficiencies of the different primer sets were similar. The PCR products were analyzed by agarose gel electrophoresis and sequencing to verify the presence of a gene-specific PCR product. Relative expressions of all transcripts were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The results obtained for the different tissues and stages we analyzed were standardized to the levels of a poplar *ACTIN* gene, which have stable expression in different tissues and in different developmental stages (An et al. 2011a, b).

Results

Sequence and phylogenetic analyses of *AtAP1* and *PtrAP1*

A comprehensive analysis of *AP1* homologues in *P. trichocarpa* is presented in Table 1. Bioinformatics information for each gene includes the length of genomic DNA, length of the transcript, length of the CDS, number of amino acids, theoretical Mw and p*I*, and the location of the functional domains. Sequence alignment of AtAP1 (AT1G69120.1), PtrAP1-1 (Potri.008G098500.1) and PtrAP1-2 (Potri.010G154100.1) by BLAST indicated a 79.81 % identity to the proteins coded by *AP1* homologous genes in *Vitis vinifera* (VvAP1, GSVIVG01012250001), *Glycine max* (GmAP1, Glyma16g13070.1) and *Malus* × *domestica* (MdAP1, MDP0000013331). The proteins all contain MADS-box, I, K-box, and C-terminal domains, which are typical for MIKC-type MADS-box proteins. The MADS-box domain was located at the N-terminus from 1 to 60 aa. A highly conserved K-box domain was located from 79 to 174 aa. An I domain was located between the MADS-box domain and K-box domain. All of the proteins contain a C-terminal conserved euAP1 motif (Litt and Irish 2003). MADS-box and K-box domains are highly conserved but sequences can be widely variable in the C-terminal domain (Fig. 1a). The euAP1 motif has two short conserved motifs, one LxLT/NLx (where “x” is an acidic residue) type of ERF-associated amphiphilic repression (EAR) motif, which is distinct for negative transcriptional regulation (Kagale et al. 2010), and one CFAA/T (farnesylation/prenylation motif) that terminates the protein (Litt and Irish 2003). The predicted tertiary structures of MADS-box domain in AtAP1 and PtrAP1 are similar, consisting of one α-helix and two β-sheets (Fig. 1b–d). The MADS-box domain is the most highly conserved of the four major MIKC-type MADS-box protein domains and has been widely studied across taxonomic kingdoms (Leseberg et al. 2006).

The genomic sequence of *AtAP1* is 3984 bp and consists of eight exons of 185, 79, 65, 100, 42, 42, 155 and 103 bp, encoding a putative protein of 256 amino acids. The genomic sequence length of *PtrAP1-1* is 5341 bp and consists of eight exons of 185, 79, 65, 100, 42, 42, 116 and 97 bp, encoding a putative protein of 241 amino acids. The genomic sequence of *PtrAP1-2* is 5867 bp and consists of eight exons of 185, 79, 65, 100, 42, 42, 134 and 100 bp, encoding a putative protein of 248 amino acids (Fig. S1a). The lengths of the first six exons in these three genes are the same while the other two are variable. The coding sequence of *PtrAP1-1* cDNA exhibited 88.80 % identity to *PtrAP1-2* cDNA at the nucleotide level, and the variation is mainly in the seventh and eighth exons (92.43, 92.41, 96.92, 90.00, 92.86, 92.86, 77.61, and 77.67 % for the exons 1–8, respectively). *PtrAP1-1* is located on chromosome Scaffold 8, while *PtrAP1-2* on Scaffold 10 (Fig. S1b).

In order to clarify the relationship among the homologous AP1/FUL proteins, a phylogenetic tree, based on the deduced amino acid sequences, was constructed using the Neighbor-Joining (N-J) method (Fig. 2). The

Table 1 Characteristics of *AP1*-like genes in poplar

Gene name	Transcript name	gDNA size (nts)	Transcript size (nts)	CDS size (nts)	Protein size		p*I*	Functional domains (start–end)	
					Peptide residues	Mw (kDa)		MADS-box domain	K-box region
PtrAP1-1	Potri.008G098500.1	5341	1313	726	241	28.13	8.19	1–60	79–174
PtrAP1-2	Potri.010G154100.1	5867	1258	747	248	28.5	8.91	1–60	79–174

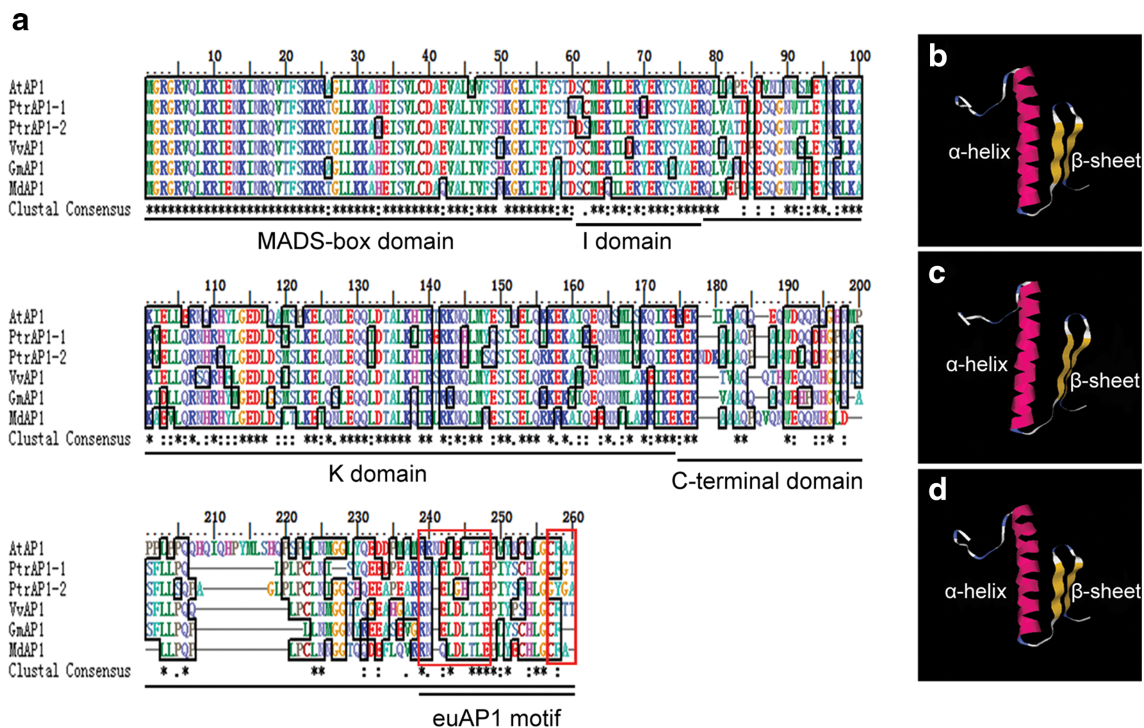

Fig. 1 **a** Alignment of amino acid sequences of AP1 protein homologues from *Arabidopsis*, poplar, grapevine, pea and apple. The phytozome v9.1 accession number of each protein is as follows: AtAP1 (*A. thaliana*, AT1G69120.1), PtrAP1-1 and PtrAP1-2 (*P. trichocarpa*, Potri.008G098500.1 and Potri.010G154100.1, respectively), VvAP1 (*V. vinifera*, GSVIVG01012250001), GmAP1 (*G. max*, Glyma16g 13070.1) and MdAP1 (*M.* × *domestica*, MDP0000013331). The gaps are attributed to the lack of amino acids. Conserved region, including a MADS-box domain, I domain, K domain, and C-terminal domain, is underlined. The euAP1 motif is double underlined. The first red box indicates an EAR motif within the euAP1 motif. The second red box indicates a prenylation motif. **b** The predicted tertiary structure of MADS-box domain in AP1 from *A. thaliana* contains an α-helix (*pink*) and two β-sheets (*yellow*). **c** The predicted tertiary structure of MADS-box domain in PtrAP1-1 from *P. tomentosa* contains an α-helix (*pink*) and two β-sheets (*yellow*). **d** The predicted tertiary structure of MADS-box domain in PtrAP1-2 from *P. tomentosa* contains an α-helix (*pink*) and two β-sheets (*yellow*)

resulting phylogenetic tree consisted of three major clades, represented by eudicots AP1, eudicots FUL, and monocots AP1/FUL-like. PtrAP1-1 and PtrAP1-2, together with AtAP1, were classified in the eudicots AP1 clade. They were more closely related to AP1-like members in *Capsella rubella*, *Linum usitatissimum*, *Manihot esculenta*, *Vitis vinifera* and *Carica papaya*. AP1/FUL-like proteins from the monocots *Brachypodium distachyon*, *Setaria italic*, *Zea mays*, and *Oryza sativa* were in another clade in the phylogenetic tree.

Expression patterns of *PtAP1-1* and *PtAP1-2* in *P. tomentosa*

Expression profiles of *PtAP1-1* and *PtAP1-2* in various tissues of poplar in the juvenile phase and reproductive (adult) phase were obtained using RT-qPCR, with gene-specific primers (Table S1). Overall, the level of *PtAP1-1* transcript was relatively higher than that of *PtAP1-2*. *PtAP1-1* transcript accumulated mainly during the reproductive phase in tissues or organs of poplar, such as mature leaves, vegetative buds, male floral buds, female floral buds and female catkins (Fig. 3a). In contrast, the level of *PtAP1-1* transcript was low in tissues of one-month-old poplar seedlings (juvenile phase), including roots and leaves, with little detection in stems (Fig. 3a). *PtAP1-2* transcript was also mainly detected in the reproductive phase in various tissues and organs, except for mature leaves (Fig. 3b). Similar to *PtAP1-1*, expression of *PtAP1-2* was very low or not detected in tissues, including roots, stems, and leaves, of one-month-old poplar seedlings (Fig. 3b).

As indicated in Fig. 3c, d, the trend of *PtAP1-1* and *PtAP1-2* seasonal expression pattern in male or female floral buds was similar, however, the timing of expression was different. In developing male floral buds, the level of *PtAP1-1* transcript was relatively higher than *PtAP1-2* during most of the sampling period. The expression of *PtAP1-1* and *PtAP1-2* was upregulated rapidly during the summer. Expression of both genes peaked in August and decreased thereafter (Fig. 3c). On the other hand, the transcript level of *PtAP1-1* was higher than *PtAP1-2* in developing female floral buds prior to November, but the pattern subsequently differed from that observed in male floral buds (Fig. 3d). Expressions of *PtAP1-1* and *PtAP1-2*

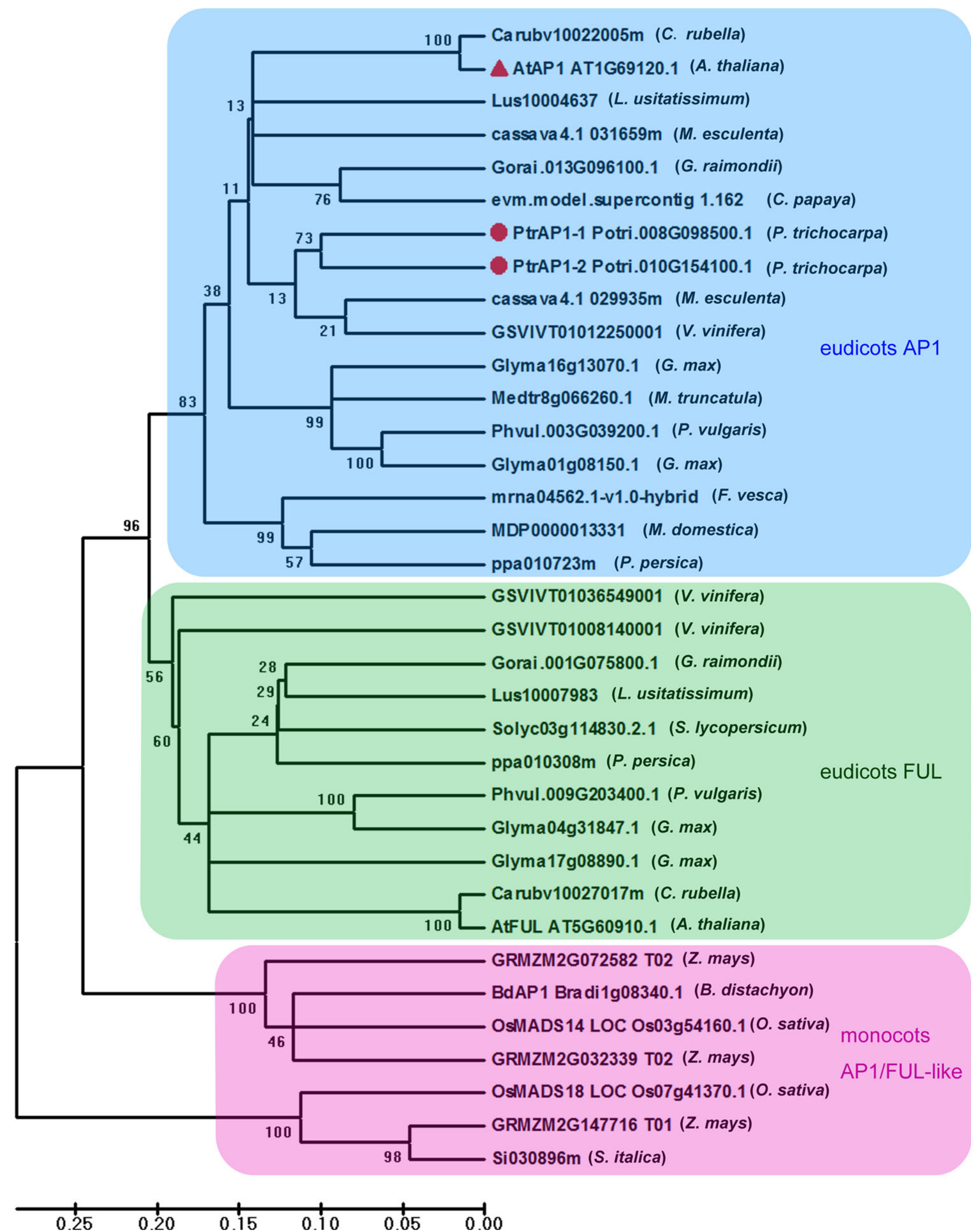

Fig. 2 Phylogenetic analysis of AP1/FUL-like proteins. The tree was constructed using the Neighbor-Joining (N-J) method for the deduced amino acid sequence of members of the *AP1/FUL* from *Arabidopsis thaliana*, *Populus trichocarpa*, *Malus* × *domestica*, *Capsella rubella*, *Linum usitatissimum*, *Manihot esculenta*, *Vitis vinifera*, *Carica papaya*, *Brachypodium distachyon*, *Setaria italic*, *Zea mays*, *Oryza sativa*, *Fragaria vesca*, *Gossypium raimondii*, *Medicago truncatula*, *Phaseolus vulgaris*, *Prunus persica* and *Solanum lycopersicum*. The protein sequence data were obtained from Phytozome v9.1 database. *Numbers* on each branch indicate bootstrap values for 1000 replicates

increased (more slowly than in male floral buds) during the summer and fall and both peaked in November and thereafter decreased. These results indicate that *PtAP1-1* and *PtAP1-2* are closely associated with the sexual differentiation of *P. tomentosa* floral organs.

Discussion

Sequence and phylogenetic analyses of *AtAP1* and *PtrAP1*

MADS-box transcription factors play important roles in several aspects of plant growth and development, including the control of flowering time, meristem identity, floral organ identity, and the development of vegetative organs (Arora et al. 2007). AtAP1, PtrAP1-1 and PtrAP1-2 belong to the MADS-box family of transcription factors. The amino acid sequences of *AtAP1*, *PtrAP1-1* and *PtrAP1-2* share 81.10 % identity with each other and also have a high degree of identity with AP1 orthologues in *Vitis vinifera*, *Glycine max* and *Malus* × *domestica*, especially in the MADS-box domain. The MADS-box is a DNA-binding domain and is also involved in dimerization and the functional operation of transcription factors (Riechmann et al. 1996). The K domain is involved in protein–protein interaction and appears to be plant-specific (Davies et al. 1996). The I domain, located between the MADS-box and

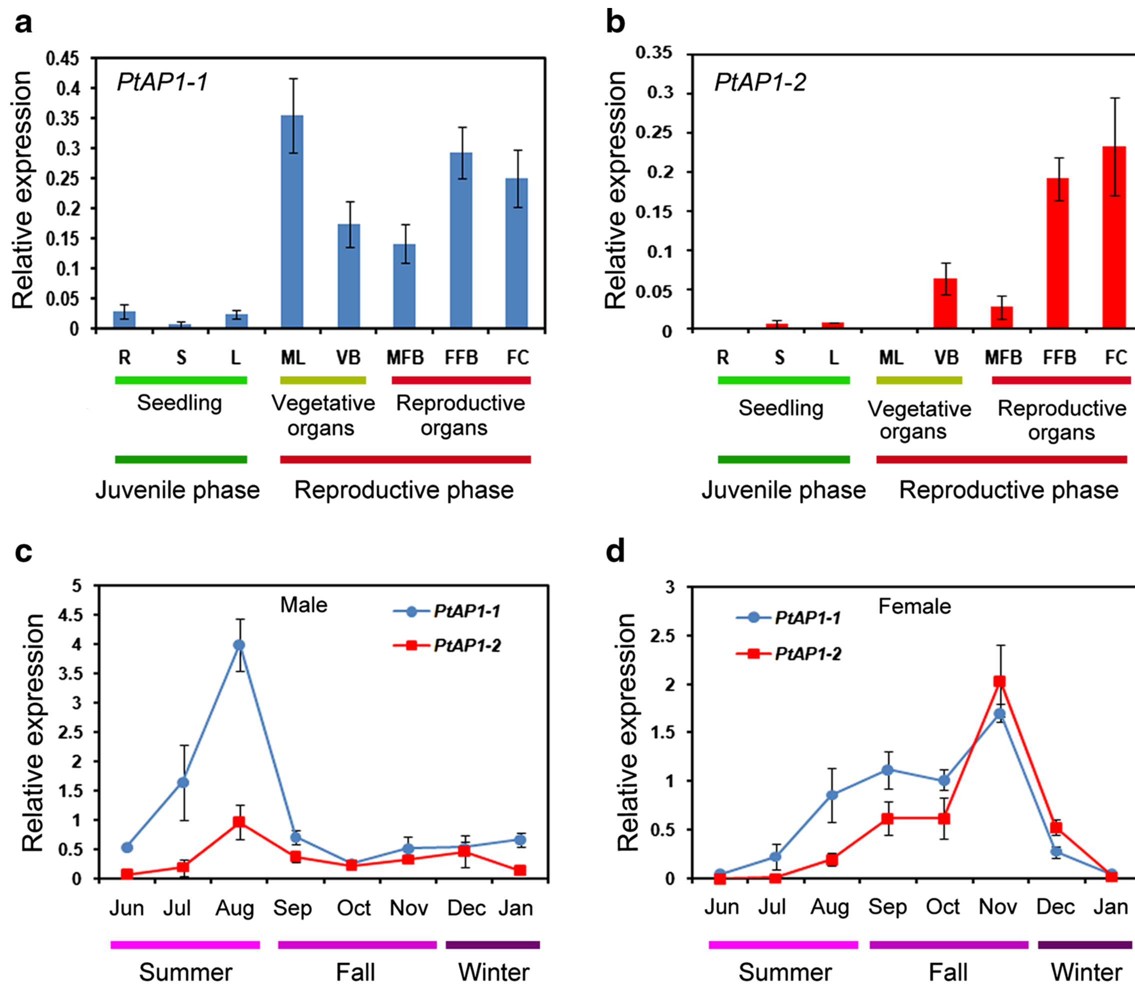

Fig. 3 Expression profiles of *PtAP1-1* (**a**) and *PtAP1-2* (**b**) in various tissues, as well as the seasonal expression patterns of *PtAP1-1* and *PtAP1-2* in developing male (**c**) and female (**d**) floral buds as determined by RT-qPCR. Samples for (**a**) and (**b**) from left to right are as follows: roots (R), stems (S) and leaves (L) from one-month-old tissue-cultured plantlets; mature leaves (ML), vegetative buds (VB), male floral buds (MFB), female floral buds (FFB) and female catkins (FC) from adult trees. Samples for male (**c**) and female (**d**) floral buds collected from late June through late January. A native poplar *ACTIN* gene was used as a reference gene for normalization. Data presented represent the mean ± SD

the K domain, is less conserved (Riechmann et al. 1996). Sequence differences occur mainly in the C-terminus region which is involved in transcriptional activation and ternary complex formation. The information provided in the current study supports the premise that the C-terminus domain of plant MADS-box proteins is the most variable (Egea-Cortines et al. 1999). While the euAP1-like motif in AP1-like proteins is well characterized, neither PtrAP1-1 or PtrAP1-2 contains the farnesylation motif "CFAA" in the C-terminus domain that was found to play an important role in the determining the function and specificity of AtAP1 in *Arabidopsis* (Litt and Irish 2003). PtrAP1-2 has a C-terminal amino acid motif "GYGA" instead of the farnesylation motif "CFAA" found in AtAP1 and a few other AP1 homologues. The motif "GYGA" is also found in SAP1-1 and SAP1-2 from *Salix discolor* (Fernando and Zhang 2006). *Populus* and *Salix* are both dioecious woody perennials with flowers that lack sepals and petals. The absence of the farnesylation motif "CFAA" is common to many AP1 homologues in several species, such as VvAP1 (*Vitis vinifera*), MdAP1 (*Malus* × *domestica*), GmAP1 (*Glycine max*), PEAM4 (*Pisum sativum*), and NtMADS11 (*Nicotiana tobacum*). These observations support the hypothesis that this kind of modification is not an essential factor in the function of AP1 (Berbel et al. 2001; Chi et al. 2011; Jang et al. 2002).

Comparison of the exon–intron structure of *AtAP1* with *PtrAP1-1* and *PtrAP1-2* indicates that it contains the same number of exons and introns. The length of first six exons of these three genes is the same, indicating that exon length is highly conserved. The length of the gDNAs is largely different due to the length of introns which indicates that

intron mutations may distinguish different *AP1*-like genes in different species. The phylogenetic tree analysis conducted in the present study characterized the relationship between *AP1/FUL* homologues from several eudicots and monocots. The analysis identified three different clades, designated as eudicots AP1, eudicots FUL, and monocots AP1/FUL-like proteins. AtAP1 from *A. thaliana* and PtrAP1-1 and PtrAP1-2 from *P. trichocarpa* are all placed in the eudicots AP1 clade. AtAP1 is most closely associated to the AP1 homologue from *Capsella rubella* which supports the premise that *A. thaliana* and *C. rubella* are closely related species. Additionally, the AP1 homologues from eudicots are well separated from those of the monocots. Our results are consistent with previous study that eudicots AP1 and eudicots FUL belong to core eudicot and relative distance is close to each other (Litt and Irish 2003). In the present phylogenetic tree, non-core eudicot FUL-like is not included and monocot clade is separated from core eudicot gene clade.

Populus trichocarpa is the first sequenced perennial woody plant. Since its public release (Tuskan et al. 2006) and subsequent updates in the phytozome (Goodstein et al. 2012), the availability of the *Populus* genome has spawned researches in plant molecular biology, morphology, ecology, comparative and functional genomics (Wullschleger et al. 2012). During the past decade, tree physiologists have used this resource in identifying candidate genes that underlie physiological and morphological traits of interest, and the structure, chromosomal location, phylogeny and function of these candidate genes in *P. trichocarpa* were well and deeply studied. In order to make a comprehensive analysis of *AP1*-like gene in poplar more concisely, we choose the sequence of *AP1*-like gene from *P. trichocarpa*. *P. trichocarpa* is mainly distributed in western North America and difficult to obtain in China. Previous study showed that all *Populus* species are closely related, and the sequence identity between homologous genes in different *Populus* species is often 99 % (Ingvarsson 2005). So, in the gene expression experiment, we choose plant samples from an indigenous tree species (*P. tomentosa*).

PtAP1-1 and *PtAP1-2* expression profiles in *P. tomentosa*

In *Arabidopsis*, *AP1* is initially expressed specifically in young floral primordia but not in inflorescence meristems, and later becomes localized to sepals and petals (Mandel et al. 1992). In the herbaceous plant, *Dendranthema grandiflorum*, the *AP1* homologue, *CDM111*, is expressed in inflorescence meristems and developing bracts (Shchennikova et al. 2004). In the woody plant, *Betula pendula*, the *AP1* homologue, *BpMADS3*, is expressed in both male and female inflorescence meristems (Elo et al. 2001). *VAP1*, an *AP1* homologue in *Vitis vinifera*, is also expressed in inflorescence meristems (Calonje et al. 2004). In apple (*Malus* × *domestica*), the *AP1* homologue, *MdAP1*, is expressed in leaf primordia, the upper cell layers of the shoot apex, inflorescence primordia, both young floral primordia and the inner cell layer of sepal primordia. It is also expressed in the outer cell layer of receptacle primordia, the floral axis, and in developing floral organ primordia (Kotoda et al. 2000; Mimida et al. 2011). In the present study, the expression patterns of *PtAP1-1* and *PtAP1-2* in *P. tomentosa* were similar in that they were highly expressed during the reproductive phase and low transcript levels were observed during the vegetative phase (Fig. 3).

In other species, expression profiles of *AP1* homologues are somewhat variable. For example in *Glycine max*, the *AP1* homologue, *GmAP1*, is specifically expressed in the flower, especially in sepals and petals, but not in other organs. RNA in situ hybridization analysis revealed that *GmAP1* transcript can be detected in both the outer cell layers of both apical inflorescence meristems and lateral floral meristems of soybean (Chi et al. 2011). In contrast, we observed low levels of *AP1* expressions in vegetative tissues or organs of *P. tomentosa* during the vegetative phase of growth (Fig. 3). Sepals and petals are absent in flowers of both *Populus* and *Salix*. In *S. discolor*, the expression profile of *SAP1* (*AP1* homologue) in various parts of male reproductive buds indicates that this gene is expressed in inflorescence meristems, bracts, and floral meristems (Fernando and Zhang 2006). In *Prunus avium*, *PaAP1* (*AP1* homologue) is highly expressed in petals, sepals, styles, and flower buds (Wang et al. 2013). However, *AP1* homologue is expressed in all four whorls of floral organs in *Prunus serrulata*, *Magnolia grandiflora* and *Persea americana* while in all floral organs excluding stamens in *P. persica* (Kim et al. 2005; Zhang et al. 2008). These data indicate that *AP1* homologues in different species may vary considerably in their expression and regulatory function.

RT-qPCR analysis revealed that *PtAP1-1* and *PtAP1-2* mRNAs were detected in both male and female floral buds at different developmental stages (Fig. 3). Similar observations for *AP1* homologues have been reported in other species (Chi et al. 2011; Kotoda et al. 2010). Seasonal expression profiles of *PtAP1-1* and *PtAP1-2* in male and female floral buds exhibited a similar trend with initial increased expression levels and subsequent reduction. *PtAP1-1* exhibited continuous differential expression in male and female floral buds. This indicated that it may play some roles during entire development phase of male and female floral buds. The relative level of *PtAP1-1* and *PtAP1-2* transcript in developing male floral buds was higher than that in developing female floral buds during

June to August. In poplar, more stamen primordia exist in male floral buds than gynoecia primordia in female floral buds. The differences in the level of expression in the male and female floral buds may indicate that higher level of *PtAP1* transcript is necessary for male floral buds development and stamen morphogenesis in *P. tomentosa* from primordial formation stage to enlargement stage. From September to November, the *PtAP1-1* and *PtAP1-2* mRNA were higher in female floral buds suggesting that a higher level of *PtAP1-1* and *PtAP1-2* mRNA is necessary to archespore formation in female flowers. A previous study reported that the level of expression of *PtLFY* (another floral meristem identity gene in *P. tomentosa*) in male floral buds was higher than that in female floral buds at different developmental stages from floral bud initiation to maturity (An et al. 2011a, b). *PtAP1-1* and *PtAP1-2* reached a peak in expression three months before in male floral buds than in female floral buds. An anatomical analysis of male and female floral buds in *P. tomentosa* revealed that initiation and differentiation of male floral buds occur earlier than female floral buds (An et al. 2010). Therefore, the differential expression of *PtAP1-1* and *PtAP1-2* in male and female floral buds is likely associated with the earlier development of male vs. female floral buds.

Flower induction in *P. tomentosa* in Beijing, China occurs in June (An et al. 2010). Expression of endogenous *PtAP1-1* and *PtAP1-2* was low during the period of floral induction in mature trees of *P. tomentosa*. These data indicate that *PtAP1-1* and *PtAP1-2* may have little effect on floral initiation. This premise is consistent with previous studies where overexpression of the *Populus AP1* orthologue, *PTAP1-1* did not induce early flowering in *Populus* (Strauss et al. 2004). Previous study showed that *Like-AP1* (a tree ortholog of *Arabidopsis AP1*) mediates in photoperiodic control of seasonal growth cessation downstream of the CO/FT module in hybrid aspen and ectopic expression of *Like-AP1* fails to induce early flowering in hybrid aspen trees (Azeez et al. 2014). Expressions of both *PtAP1-1* and *PtAP1-2* increase, however, during floral bud development, indicating that their expression is at least associated with floral bud development. Studies on *Citrus* plants indicated that the citrus *AP1* homologue, *CsAP1*, has little effect on seasonal flowering of Satsuma mandarin (*Citrus unshiu*) but may affect floral bud development in trifoliate orange (*Poncirus trifoliata*) and kumquat (*Fortunella crassifolia*) (Nishikawa et al. 2007, 2009, 2011). In sweet orange, *CsAP1* transcript increased at the end of the floral induction period which suggested that it was involved in floral organ development rather than floral induction (Pillitteri et al. 2004). In addition, seasonal patterns of *AP1* homologue expression have been reported in other species such as apple and also in soybean (Chi et al. 2011; Kotoda et al. 2010).

In summary, we analyzed the gene structure, chromosomal location and phylogenetic relationship of *Populus AP1* homologues and characterized their temporal and spatial expression profiles. Further studies to investigate the functions of these genes by overexpression, RNAi or using CRISPR-Cas system in transgenic *P. tomentosa* plants might provide more details about their roles in floral transition and development. To elucidate the molecular mechanism of flowering in poplar would be helpful for shortening the poplar breeding cycle and laying foundation to breed sterility poplar cultivars.

Author contribution statement Xinmin An and Zhong Chen designed the experiment. Zhong Chen drafted the manuscript. Zhong Chen and Pian Rao performed the experiments. Zhong Chen, Xiong Yang and Xiaoxing Su analyzed the data. Kai Gao and Bingqi Lei helped improve the manuscript. All authors read and approved the final manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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