Plant Science 231 (2015) 40–51

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Ectopic expression of a *Catalpa bungei* (Bignoniaceae) *PISTILLATA* homologue rescues the petal and stamen identities in *Arabidopsis pi-1* mutant

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ARTICLE INFO

Article history:
Received 17 August 2014
Received in revised form 2 November 2014
Accepted 17 November 2014
Available online 25 November 2014

Keywords:
Catalpa bungei
B-class MADS box gene
PISTILLATA
Ectopic expression
Flower development
Functional conservation

ABSTRACT

PISTILLATA (*PI*) plays crucial roles in *Arabidopsis* flower development by specifying petal and stamen identities. To investigate the molecular mechanisms underlying organ development of woody angiosperm in *Catalpa*, we isolated and identified a *PI* homologue, referred to as *CabuPI* (*C. bungei PISTILLATA*), from two genetically cognate *C. bungei* (Bignoniaceae) bearing single and double flowers. Sequence and phylogenetic analyses revealed that the gene is closest related to the eudicot *PI* homologues. Moreover, a highly conserved PI-motif is found in the C-terminal regions of CabuPI. Semi-quantitative and quantitative real time PCR analyses showed that the expression of *CabuPI* was restricted to petals and stamens. However, *CabuPI* expression in the petals and stamens persisted throughout all floral development stages, but the expression levels were different. In 35S::*CabuPI* transgenic homozygous *pi-1* mutant *Arabidopsis*, the second and the third whorl floral organs produced normal petals and a different number of stamens, respectively. Furthermore, ectopic expression of the *CabuPI* in transgenic wild-type or heterozygote *pi-1* mutant *Arabidopsis* caused the first whorl sepal partially converted into a petal-like structure. These results clearly reveal the functional conservation of *PI* homologues between *C. bungei* and *Arabidopsis*.

1. Introduction

The well-established ABCE-model predict how a few genes act together to specify any flower organ that make up a perfect flower in *Arabidopsis* [1–7]. According to this model, the class A and E genes together specify sepal formation, the class A, B, and E genes combine to regulate petal formation, the class B, C, and E genes together determine stamen identity, the class C and E genes combine to regulate carpel identity. Furthermore, most ABCE-genes encode MADS box proteins which contain four conserved domains, the MADS-, Intervening-, Keratin-like, and C-terminal (MIKC) domains [2,7–11].

The B class MADS-box genes, members of *PISTILLATA* (*PI*) and *APETALA3* (*AP3*) gene lineages, have been shown to play a major role in specifying petal and stamen development [12–18]. In *Arabidopsis*, mutations in *PI* or *AP3* cause homeotic changes, in which the second whorl floral part (i.e., petal) is converted into a sepal structure and the third whorl floral part (i.e., stamen) is converted into a carpel structure [13]. Moreover, molecular genetics and protein-protein interaction assays suggest that PI-like and AP3-like proteins of eudicot species are thought to act as obligate heterodimers and the presence of both gene products is required to cause an ectopic phenotype [12,19]. Phylogenetic analysis revealed that the two major lineages of B class genes have arisen from a duplication that probably occurred in the common ancestor of angiosperms after it has diverged from the gymnosperms, and generated the *PI* and paleo*AP3* lineages [9,15,20–22]. The *PI* lineage is composed of *PI* homologues that encode a highly conserved region in the C-terminal domain known as the PI-motif [14]. The paleo*AP3* lineage is composed of *AP3* homologues identified in magnolid dicots, lower eudicots, and basal angiosperms [14]. A second duplication from the basal paleo*AP3* lineage is thought to have generated two classes of *AP3*-like genes during evolution, replace paleo*AP3* by the eu*AP3* and *TM6* gene lineages, which is close to the base of core eudicots [14]. However, proteins of the eu*AP3* and paleo*AP3* lineages are not able to form homodimers, and have to interact with PI to form heterodimers to specify petal

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http://dx.doi.org/10.1016/j.plantsci.2014.11.004
0168-9452/

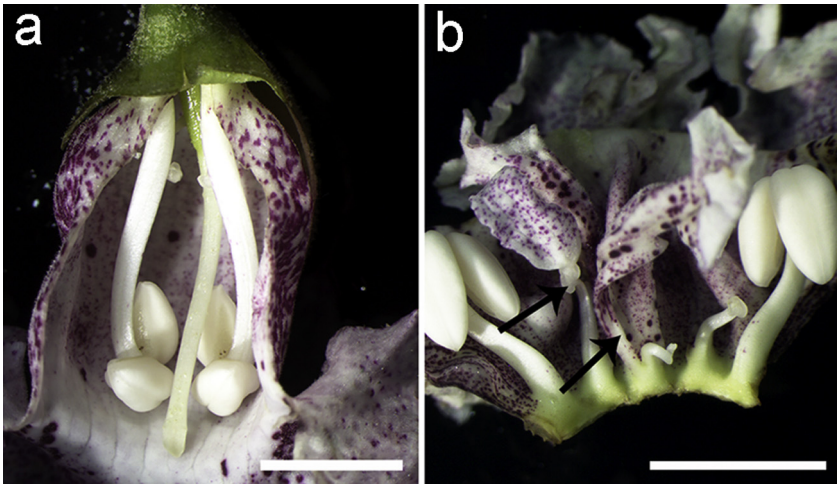

Fig. 1. The morphological observation on the single and double flowers of *C. bungei*. (a) The petals, stamens and a carpel from the single flower of *C. bungei*; (b) The double flower of *C. bungei*, showing homeotic conversional petals from stamens (black arrows). Bars = 2 mm.

and stamen identities [23–26]. Besides these two major gene duplications, a number of small scale duplications have also been identified in various clades within the *PI* lineage [14,22]. It has been suggested that *PI* homologues shaped floral diversification of some asterids in a dramatic way by modularizing the floral perianth [25,27]. However, the crucial roles of *PI* homologues in *Catalpa* flower development are sorely lacking.

Catalpa bungei, a Chinese originated ornamental tree, belongs to the Bignoniaceae family in Asterids, and is cultivated broadly. The single flowers of *C. bungei* have four whorls of normal floral organs, including sepals in whorl 1, petals in whorl 2, stamens in whorl 3, and one carpel in whorl 4 (Fig. 1a), respectively. However, the double-flower *C. bungei*, a recently discovered natural variation, only found in the Northwest of Hubei Province, having four whorls of floral organs, including sepals in whorl 1, petals in whorl 2, stamens and homeotic conversional petals from stamens in whorl 3 (Fig. 1b), and one carpel in whorl 4. In order to uncover the possible roles of *PI* homologue in regulating flower development of *C. bungei*, we isolated a *PI* homologue, *CabuPI*, from two genetically cognate *C. bungei* bearing single and double flowers, respectively. Sequence and phylogenetic analyses revealed that *CabuPI* fall into the clades of eudicot *PI* lineages. Moreover, semi-quantitative PCR and quantitative real time RT-PCR analyses suggested that the expression pattern of *CabuPI* was consistent with other classic core eudicot petal and stamen identity program. However, the expression levels were different during the flower development in single and double floral buds of *C. bungei*. Furthermore, ectopic expression of the *CabuPI* revealed that it could substitute endodgenous *PI* to rescue the petals and stamens development in homozygous *pi-1* mutant *Arabidopsis*. Moreover, the 35S::*CabuPI* transgenic *Arabidopsis* in wild-type or heterozygote *pi-1* mutant background could produce flowers with partially petaloid speals. These observations suggested that the function of *PI* homologues between *C. bungei* and *Arabidopsis* was conservative in specifying petal and stamen identities.

2. Materials and methods

2.1. Plant material

Flower buds at different development stages were collected from the single and double flowers of *C. bungei* growing in a natural, secondary forest in Xiangyang, in the Northwest of Hubei Province. Before anthesis, the root, stem, juvenile leaves, sepals, petals, stamens and carpels were sampled, frozen immediately in liquid nitrogen and stored at −80 °C until used.

2.2. Morphological analysis the flowers of C. bungei

The floral buds from the single and double flowers of *C. bungei* were collected in series of development stages and fixed in the FAA under vacuum. Samples were dehydrated by a graded aqueous ethanol series 70, 85, 95 and 100% ethanol, then decolorized through dimethylbenzene: ethanol (50:50, by vol.) and 100% dimethylbenzene. Samples then were gone through three changes of 100% paraffin at 63 °C and finally embedded. Paraffin-embedded materials were cut to a thickness of 6 μm and stained with safranine-fast green solution after dewaxed. Morphology was examined and photographed using Nikon D3000, Leica M205 FA, and Leica DM 6000B fully automated upright microscope (Leica Microsystems GmbH, Wetzlar, Germany).

2.3. Isolation of PI homologue in C. bungei

Total RNA was extracted from floral buds of the single and double flowers at different developmental stages using the modified CTAB method [28]. First-strand cDNA was synthesized from 2 μg of the DNase I-treated RNA using oligo(dT)-18 adaptor primer and M-MLV reverse transcriptase (TaKaRa, Japan). To isolate the *PI* homologue from *C. bungei*, a 393-bp fragment was amplified from the cDNA using the forward primer PIF and the reverse primer PIR. Comparison with sequences in the NCBI databases revealed that the fragment is an internal coding region of a *PI* homologue. Isolation of the 3′ end of the *PI* homologue from *C. bungei* was carried out through a 3′ RACE using the 3′-full RACE Core Set Version 2.0 kit (TaKaRa, Japan), according to instructions from the manufacturer and the gene-specific primer GSPPI1 and 3′ RACE Outer Primer. A second PCR was performed using the nested gene specific primer GSPPI2 and 3′ RACE Inner Primer. The 5′ partial cDNA of *PI* homologue from *C. bungei* was isolated by using the 5′-Full RACE Kit (TaKaRa, Japan) for Rapid Amplification of cDNA Ends, following the protocol from the manufacturer. First strand cDNA was synthesized from 1 μg of total RNA using the Random 9 mers and 5′ RACE Adaptor. Amplification by PCR was accomplished with LA Taq DNA polymerase (TaKaRa, Japan), a gene specific primer PIGSP1 and 5′ RACE Outer Primer. A second PCR was performed using the nested gene specific primer PIGSP2 and 5′ RACE Inner Primer. To verify the integrity of the cDNA sequences of the *PI* homologue from *C.*