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Expression pattern of *CYC*-like genes relating to a dorsalized actinomorphic flower in *Tengia* (Gesneriaceae)

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Abstract *Tengia* has been called a "natural peloria" in the family Gesneriaceae because it exhibits an almost perfect actinomorphic flower from whorl one to whorl three. It would be especially interesting to know whether or how *CYC*-like gene activities are related to this type of perfect actinomorphic flower. To address this, we have isolated four *CYC*-like TCP genes and carried out an investigation on their expression patterns in *Tengia*. *TsCYC1C* and *TsCYC1D* have similar expression patterns with strong signals being detected in all five petals and stamens, whereas *TsCYC2A* and *TsCYC2B* are only transiently expressed in the very early floral meristem. Our results suggest that the expansion of the expressions of *TsCYC1C* and *TsCYC1D* from the dorsal to the ventral petals is likely responsible for the evolutionary formation of the fully dorsalized actinomorphic corolla, that is, an expanded functional domain of *CYC*-like gene dorsal identity in *Tengia* corolla. However, the expressions of *TsCYC1C* and *TsCYC1D* are not correlated with stamen abortion; therefore, *TsCYC* genes do not functionally repress the stamen development in *Tengia* flowers. This is probably due to changed *cis*-activities that result in the cell cycle-related genes uncoupling from the *TsCYC* regulatory pathway in *Tengia*.

Key words actinomorphy, *CYCLOIDEA*, dorsalized, Gesneriaceae, RNA *in situ* hybridization, *Tengia*.

Actinomorphic flowers are considered the ancestral state for angiosperms, and zygomorphic flowers have evolved several times independently from actinomorphic ancestors (Crepet, 1996; Donoghue et al., 1998). However, zygomorphy is frequently lost in the Asteridae, especially in Lamiales sensu lato that includes a major genetic model organism *Antirrhinum majus* (Donoghue et al., 1998; Endress, 1998, 2001).

In *A. majus*, *CYCLOIDEA* (*CYC*) together with its paralogue *DICHOTOMA* (*DICH*) plays a key role in the establishment of the floral dorsoventral asymmetry (zygomorphy) (Luo et al., 1996, 1999). *CYC* encodes a transcription factor of the teosinte branched 1, cycloidea, proliferating cell factors 1 and 2 (TCP) family (Cubas et al., 1999b) and function as specifying dorsal identity, that is, controlling the fate of the dorsal floral organs in the second and third whorls of flowers (Luo et al., 1996, 1999). In *Antirrhinum*, the *cyc*/*dich* double mutant has a fully ventralized peloric flower, whereas the *backpetals* mutant has a dorsalized actinomorphic flower due to the ectopic expression of *CYC* (Luo et al., 1996, 1999). In its close relative *Linaria vulgaris*, a ventralized peloric variant is caused by complete silencing of the *CYC* orthologue *LCYC* through extensive DNA methylation (Cubas et al., 1999a). In legumes, distantly related to *Antirrhinum*, the actinomorphic flowers of *Cadia* come from *LegCYC* expression in all five petals, similar to the *backpetals* mutant in *Antirrhinum* (Citerne et al., 2006). In Gesneriaceae, the basal-most family of Lamiales s.l., the downregulation of *CYC* orthologue *BlCYC1* after early floral development gives rise to a derived ventralized actinomorphic flower characteristic of *Bournea leiophylla* (Zhou et al., 2008). Recent study in *Plantago* (Plantaginaceae) shows that the single *CYC*-like gene *PlCYC* is not expressed in early floral meristems and has not apparently asymmetric expression during later flower development. Therefore, the evolution of actinomorphy in *Plantago* is correlated with loss of dorsal-specific *CYC*-like gene function (Reardon et al., 2009).

In contrast to the predominance of zygomorphic flowers in the major zygomorphic clades of angiosperms, the derived actinomorphy is relatively rare events occurring in convergence, scattered over different zygomorphic lineages, such as *Linaria*, *Plantago* (Plantaginaceae), and *Bournea* (Gesneriaceae) in Lamiales s.l. However, evo-devo studies to date have shown that the independent occurrence of derived actinomorphy is connected with diverse genetic pathways mainly controlled by *CYC*-like genes, as outlined above. As the basal-most group in Lamiales s.l. (Endress, 1998;

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Cubas, 2004; Wortley et al., 2005), the family Gesneriaceae with weak floral zygomorphy has the most diverse forms of derived actinomorphy and possesses the largest proportion of genera with actinomorphic flowers in Lamiales s.l. (Endress, 1998, 1999). The actinomorphies in Gesneriaceae have evolved based on a relatively weakly rooted genetic constitution for zygomorphy (Endress, 2001), their genetic mechanisms would be different from those of actinomorphies arisen from deeply fixed pattern of zygomorphy in other Lamiales s.l. For example, the derived actinomorphy in *Bournea* is achieved through an altered expression pattern of a *CYC*-like TCP gene during floral development, distinctive from the genetic pathways of other derived actinomorphies (Zhou et al., 2008).

Tengia has been called a "natural peloria" in the family Gesneriaceae (Donoghue et al., 1998) because it exhibits an almost perfect actinomorphic flower from whorl one to whorl three (Li & Wang, 2004) (Fig. 1). Unlike the actinomorphic flowers of *Bournea* with clear signs of residual zygomorphy (Zhou et al., 2008), the floral morphology of *Tengia* shows no vestigial traces of zygomorphy, especially in corolla and androecial structure, which is difficult to explain with the known genetic mechanisms revealed in the derived actinomorphies to date (Fig. 1). Therefore, it would be especially interesting to know whether or how *CYC*-like gene activities are related to this type of perfect actinomorphic flowers. To address this, we have isolated the *CYC* homologues in *Tengia* and carried out an investigation on the expression patterns of these genes. Our results would shed new light on exploring novel and diverse evolutionary pathways for derived actinomorphy in angiosperms, especially in Lamiales s.l.

1 Material and methods

1.1 Plant materials

The materials of *Tengia scopulorum* Chun used for this study, including gene isolation and RNA *in situ* hybridization, were collected from Xiuwen County, Guizhou province, China. For total RNA extraction, young flower buds were collected then immediately frozen in liquid nitrogen. For RNA *in situ* hybridization, floral meristems and floral buds at different developing stages were collected and fixed under vacuum in freshly prepared ice-cold formalin–acetic acid–alcohol. After an incubation of 16–18 h, they were completely dehydrated in an ethyl alcohol series, embedded in Paraplast

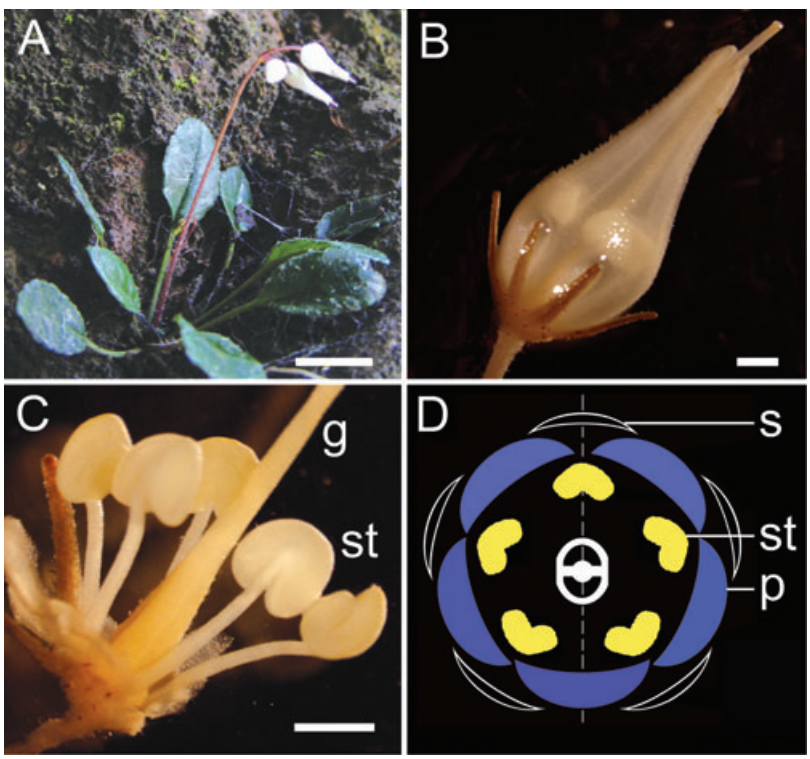

Fig. 1. Flower morphology of *Tengia*. **A,** The plant with inflorescence and flowers at anthesis, showing almost perfect actinomorphic corolla. **B,** The nearly closed corolla with a keyhole opening from which the stigma is exserted. All five stamens are completely included within the corolla. **C,** Five equal and fertile stamens. **D,** Floral diagram. g, gynoecium; p, petal (in blue); s, sepal; st, stamen (in yellow). Bars = 1 cm (A); 1 mm (B, C).

(Sigma, St. Louis, MO, USA), and stored at 4°C until use.

1.2 Isolation of *CYC*-like genes in *Tengia*

Total RNA was isolated from young flower buds of *Tengia* using an SV Total RNA Isolation System Kit (Promega, Madison, WI, USA), following the manufacturer's protocol. First-strand cDNA was synthesized by a RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany). Polymerase chain reaction (PCR) amplification was carried out using a forward primer F1 (5′-ATGTTTGGAAAGAGCCCATAC-3′) and a reverse primer R (5′-ATGAATTTGTGCTGATCCAAAATG-3′) (Gao et al., 2008; Song et al., 2009). The PCR products were purified then cloned into the pGEM-T Easy vector (Promega) and sequenced using universal vector primers T7 and SP6. These genes were independently cloned at least twice.

1.3 Sequence analysis of *CYC*-like genes in *Tengia*

We used DAMBE software (Xia & Xie, 2001) to translate the cDNA sequences of *CYC*-like genes in *Tengia*. The amino acid sequences were further compared with the CYC protein from *Antirrhinum* using DNAMAN 5.29 (Lynnon Biosoft, Los Angeles, CA, USA) and BioEdit version 7.0.4 (Hall, 1999). To identify the phylogenetic position of *TsCYC* genes in the CYC clade of the class II TCP genes, we selected several *CYC*-like genes for phylogenetic analysis. They were TB1 from *Zea mays* (AF415152), AtTCP1/12/18 from *Arabidopsis thaliana* (NM_001084312; NM_105554; NM_112741), LjCYC1/2/3 from *Lotus japonicus* (DQ202475; DQ202476; DQ202477), and AmCYC/AmDICH from *A. majus* (Y16313; AF199465). ClustalW was used for the multiple alignments of the amino acid sequences (Thompson et al., 1994), and the amino acid matrix was adjusted manually. This amino acid matrix was analysed by MEGA 3.1 (Kumar et al., 2004) to construct neighbor-joining (NJ) trees under default settings. The bootstrap values were calculated using 1000 replicates.

To clarify the phylogenetic position of *TsCYC* genes in *CYC*-like genes from Old World Gesneriaceae (*GCYC*; subfamily Cyrtandroideae), NJ analyses were carried out with nucleiotide sequences by PAUP* 4.0b10 (Swofford, 2002). *GCYC1A*/*1B* are from *Saintpaulia ionantha* (DQ064642, DQ064644) and *Streptocarpus primulifolius* (AF208340, AF208336). *GCYC1C*/*1D* (designated as *GYC1* in some taxa) are from *Chirita heterotricha* (Gao et al., 2008), *Bournea leiophylla* (EF486283), *Oreocharis benthami* (FJ710517), *Opithandra dinghushanensis* (FJ710518, FJ710519), *Jankaea heldreichii* (AF208332), *Haberlea ferdinandi-coburgii* (AF208322), *Conandron ramondioides* (AF208321), *Primulina tabacum* (AF208320), *Ramonda myconi* (AF208331), *Cyrtandra apiculata* (AY423160), *Didymocarpus citrinus* (AY423158), and *Loxostigma* (AY423161, AY423162). *GCYC2* (*2A*/*2B*) are from *Chirita heterotricha* (Gao et al., 2008), *Bournea leiophylla* (EF486284), *Oreocharis benthami* (FJ710516), *Opithandra dinghushanensis* (FJ710520, FJ710521), *Haberlea ferdinandi-coburgii* (AF208317), *Conandron ramondioides* (AF208316), *Ramonda myconi* (AF208318), and *Cyrtandra apiculata* (AY423147). *CYC* from *A.majus* and a *CYC*-like gene from *Linaria vulgaris* were used as outgroups. Phylogenetic analyses with the NJ method were carried out using PAUP* 4.0b10 (Swofford, 2002) and bootstrap values were estimated with 1000 resampling replicates.

1.4 RNA *in situ* hybridizations

The materials used for *in situ* hybridization were fixed, sectioned, and hybridized to digoxygenin-labeled probes of *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, and *TsCYC2B* according to described methods by Bradley et al. (1993). Four gene-specific fragments of *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, and *TsCYC2B* in the coding region were amplified, respectively, using primers of *TsCYC1C* (forward, 5′-ATGCTAGG TTTTGACAAGCC-3′; reverse, 5′-CAATGAAGAA TAGGCTGG-3′); *TsCYC1D* (forward, 5′-TTGTTAA CGAAATCAAAAGTAGCC-3′; reverse, 5′-GGGAC AATGAAGAATACTATTAG-3′); *TsCYC2A* (forward, 5′-AGTAAAACCCTTGAATGGCTGC-3′; reverse, 5′-GAAGGCGGTAATTTGCAAACTT-3′); and *TsCYC2B* (forward, 5′-CTTGCGGGACACTTTGTAGG-3′; reverse, 5′-AATCTGCGTCAAAGTAGTTCC-3′). Then the PCR products were purified and cloned into pGEM-T Easy vector. We prepared linearized templates using primer Yt7 (5′-CCCAGTCACGACGTTGTAAA-3′) and Ysp6 (5′-CACACAGGAAACAGCTATGAC-3′) from pGEM-T Easy plasmid. Digoxygenin-labeled probes were specific to *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, and *TsCYC2B*.

2 Results

2.1 Character and sequence analyses of *TsCYC* genes

We isolated four *CYC*-like genes in *Tengia*, named *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, and *TsCYC2B* according to BLAST results. The open reading frames of *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, and *TsCYC2B* were 993, 1014, 987, and 996 bp, respectively. Furthermore,

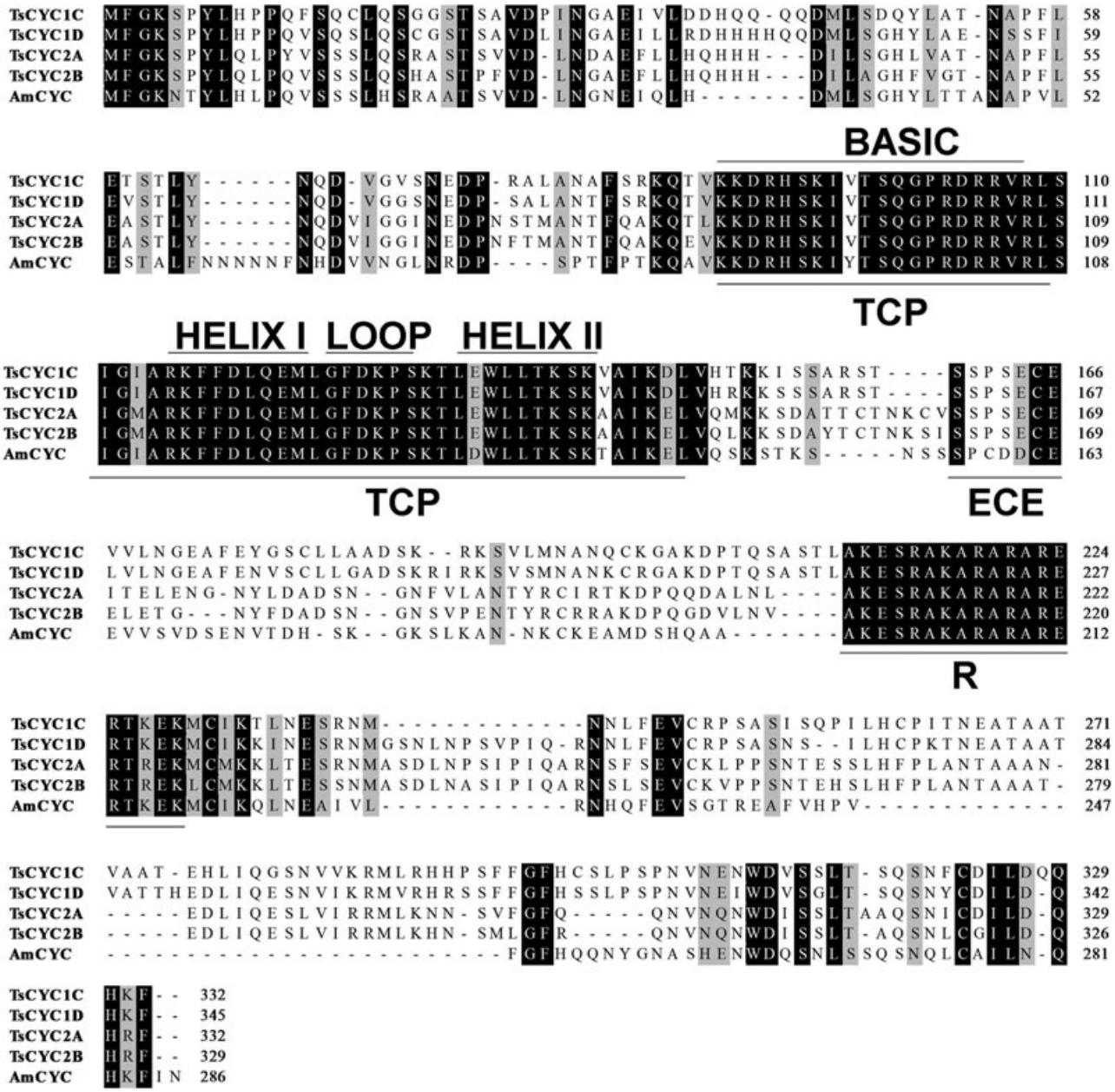

Fig. 2. Alignment of the predicted amino acid sequences of *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, *TsCYC2B*, and *AmCYC*. The TCP, ECE, and R domains are outlined and the identical amino acids are in black boxes. The TCP domain consists of the basic, helix I, loop, and helix II motifs.

we used BioEdit version 7.0.4 (Hall, 1999) to compare protein sequences of *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, and *TsCYC2B* with *AmCYC*; they share 46.5–48.0% similarity to AmCYC. When only comparing the TCP and R domain, they have 93.8–95.0% similarity to AmCYC, suggesting they are functionally related. In the three conserved domains (TCP domain, R domain, and ECE motif) there are several amino acid differences of TsCYC relative to AmCYC. One difference is located in the basic region (Y–V) and one is in the helix II region (D–E) of TCP domain (Fig. 2). In addition, we found two amino acid substitutions in TCP domain among TsCYC and AmCYC. One is between the basic and helix I (I–M) regions, and the other is after the helix II (T–V/A) region. The R domain is conserved between TsCYC and AmCYC. ECE motif of TsCYC and AmCYC is clearly divergent (Fig. 2). The amino acid sequences are remarkably divergent in the

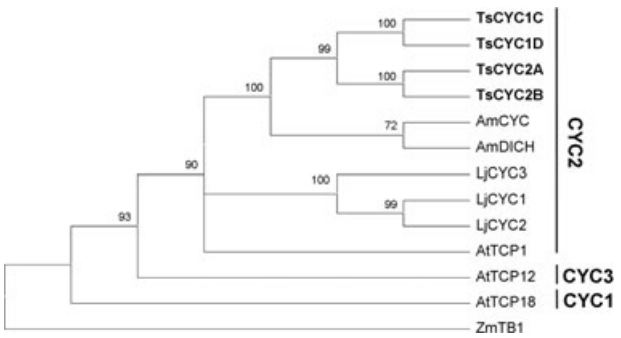

Fig. 3. Neighbor joining tree of proteins encoded by the ECE lineage genes in the CYC/TB1 subfamily, showing that TsCYC1C/TsCYC1D and TsCYC2A/TsCYC2B form a branch that is sister to AmCYC/AmDICH from *Antirrhinum majus*, which belong to the CYC2 clade in the ECE lineage. Bootstrap values with support >50% are shown.

non-conserved regions between TsCYC and AmCYC (Fig. 2).

Our phylogenetic analyses showed that *TsCYC* genes have a close relationship with *AmCYC* and *AmDICH* (100% bootstrap) (Fig. 3), and all of them, together with *AtTCP1* and *LjCYC*1/2/3, belong to the ECE-CYC2 clade in the ECE lineage (CYC/TB1 subfamily) of the TCP gene family (Howarth & Donoghue, 2006). The results show that they are homologues of *CYC*. The *CYC*-like genes in Gesneriaceae (*GCYC*) constitute a small gene family that is considered to be derived from gene duplication (Citerne et al., 2000; Gao et al., 2008; Song et al., 2009). Four *CYC* homologues isolated from *Tengia* belong to different lineages of *GCYC* genes in Gesneriaceae, in which *TsCYC* is closely related to *GCYC* from *Chirita heterotricha* (Fig. 4.).

2.2 Tissue-specific expressions of *TsCYC*

To assess the potential role of *CYC*-like genes in floral development, we used RNA *in situ* hybridization to reveal spatial and temporal expression patterns of *TsCYC* genes in *Tengia.* Transcripts of *TsCYC1C* were first detected in the whole floral primordium (Fig. 5: A). After sepal initiation, *TsCYC1C* has strong mRNA signals in sepals and the floral apex inside sepals where five petal and stamen primordia were to emerge (Fig. 5: B). *TsCYC1C* transcripts were distributed in all five petals and stamens with strong mRNA signals when they became visible (Fig. 5: C–E). As stamens enlarged, *TsCYC1C* mRNA signals became gradually weak in stamens, but had strong expression in all sepals and petals (Fig. 5: F, G). Then, *TsCYC1C* transcripts were only observed in pollen sacs when anthers began to develop (Fig. 5: H, I). Although the expression domains of *TsCYC1D* and *TsCYC1C* were largely over-

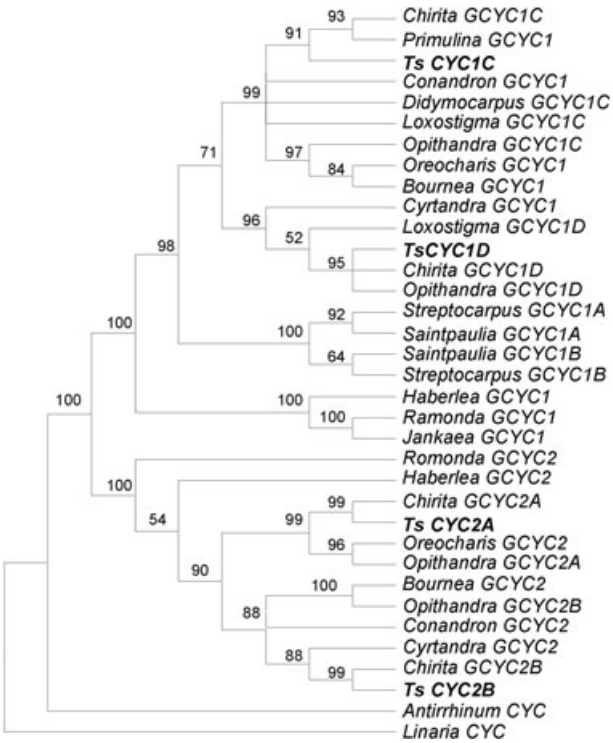

Fig. 4. Phylogram of *GCYC*, showing the phylogentic relations of *TsCYC* genes with other *GCYC* in Gesneriaceae. Phylogenetic analyses were carried out using PAUP* 4.0b10, and bootstrap values with support >50% (1000 replicates) are indicated for each branch.

lapping, suggestive of functional redundancy, *TsCYC1D* appeared to have increasing expression level and expression duration relative to *TsCYC1C* in the developing flowers. *TsCYC1D* expression signals were observed in sepals and the whole domain of the floral apex inside sepals (Fig. 5: J, K). When petal and stamen primordia emerged, *TsCYC1D* mRNA intensively accumulated in all floral organs including sepals, petals, and stamens (Fig. 5: L–N). As anthers began to develop, *TsCYC1D* expression signals were only observed in pollen sacs (Fig. 5: O). Transcripts of *TsCYC2* (*TsCYC2A* and *TsCYC2B*) were only transiently expressed in the very early florescence meristem (Fig. 5: P), then disappeared quickly (Fig. 5: Q–T).

3 Discussion

In *Tengia*, five equal and short teeth emerge from the top of the highly fused corolla tube. The urceolate corolla is nearly closed with only a keyhole opening from which the stigma is far exserted while all five stamens are completely included within the corolla, making the stigma and anthers completely separated

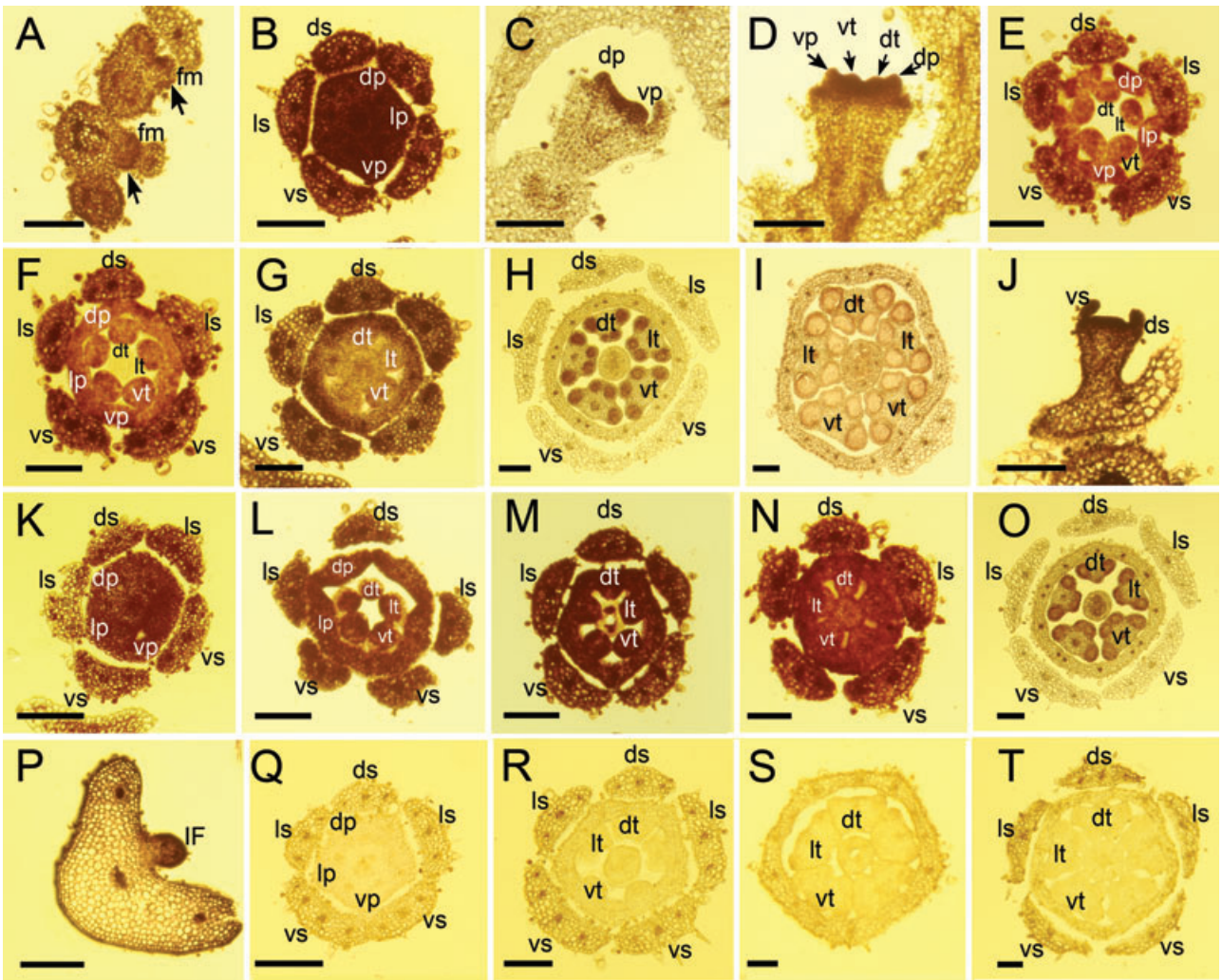

Fig. 5. Tissue-specific expression of *TsCYC1* and *TsCYC2* during floral development in *Tengia*. **A–I,** RNA *in situ* hybridizations with antisense probe of *TsCYC1C*. **A,** mRNA is first observed in the whole floral primordium. **B,** mRNA is detected in sepals, and all five petal and stamen primordia after sepal initiation. **C–E,** mRNA is distributed in all petals and stamens when they become visible. **F, G,** mRNA becomes gradually weak in stamens, but has strong signals in sepals and petals when stamens begin to enlarge. **H, I,** mRNA only exists in pollen sacs weakly when anthers begin to develop. **J–O,** RNA *in situ* hybridizations with antisense probe of *TsCYC1D*. **J, K,** mRNA is observed in sepals and the whole domain of the floral apex where all five petal and stamen primordia are to emerge. **L–N,** mRNA has strong signals in petals and stamens when stamens begin to enlarge. **O,** mRNA has only a weak signal in pollen sacs when anthers begin to develop. **P–T,** RNA *in situ* hybridizations with antisense probe of *TsCYC2A* (*TsCYC2B* not shown). **P,** mRNA expressed in whole inflorescence meristem. **Q–T,** mRNA disappears in all tissues. Contrast and color balance were adjusted using Adobe Photoshop 7.0. dp, dorsal petal; ds, dorsal sepal; dt, dorsal stamen; fm, floral primordium; IF, inflorescence; lp, lateral petal; ls, lateral sepal; lt, lateral stamen; vp, ventral petal; vs, ventral sepal; vt, ventral stamen. Bar = 100 μm.

spatially (Fig. 1). This combination of characters in *Tengia* might be related to new pollinators, such as small-sized insects, for cross-pollination in the moist and shady habitats that plants of *Tengia* prefer (Wang et al., 2010). We isolated four *CYC*-like genes from *Tengia*, *TsCYC1C*, *TsCYC1D*, *TsCYC2A*, and *TsCYC2B*. Sequence and phylogenetic analyses show that they are closely related to the *CYC* gene from the model plant *Antirrhinum*, indicating that they are the orthologues of *CYC*. Correlative with the morphological actinomorphy of corolla, strong expression signals of both *TsCYC1C* and *TsCYC1D* are almost equally distributed in the corolla tube and five petals (including two dorsal, two lateral, and one ventral) whereas no expression signal of *TsCYC2* (*2A*/*2B*) is detected in the floral organs of *Tengia*.

In the *backpetals* mutant of *Antirrhinum majus*, the ectopic expression of *CYC* in the lateral and ventral petals results in a dorsalized actinomorphic corolla (Luo et al., 1999). A mimic or reminiscence of the

backpetals mutation in the legume *Cadia* come about through a similar mechanism as in *Antirrhinum*, that is, *LegCYC1B* gene expression expansion from the dorsal petals to all five petals (Citerne et al., 2006). A recent study of actinomorphic flowers in *Bergia texana* (Elatinaceae) indicates that the *CYC*-like genes are expressed across all floral organs (Zhang et al., 2010). Even though *Tengia* is characteristic of a perfect actinomorphic flower, it is deeply nested within the core zygomorphic groups with diandrous flowers in the family Gesneriaceae, in which it is sister to *Petrocodon*, and further constitutes a monophyletic group with the monotypic genus *Calcareoboea*, and *Chirita* sect. *Gibbosaccus* (Wang et al., 2010). The phylogenetic relationship between *Tengia* and these zygomorphic groups is also reflected in the close relation between *TsCYC* and G*CYC* from *Chirita heterotricha* (Fig. 4). *Calcareoboea* is featured with a specialized bilabiate corolla with upper (dorsal) lip of four short teeth and lower (ventral) lip of a tongue-like single patent lobe (Li & Wang, 2004; Weber, 2004; Wang et al., 2010). *Petrocodon* further exhibits a morphologically transitional form between *Tengia* and *Calcareoboea*, in which its corolla is urceolate and almost actinomorphic with five equal and short teeth emerging from the top of the highly fused corolla tube, similar to that of *Tengia*, whereas its androecium consists of only two fertile stamens at the ventral position, as that of *Calcareoboea* (Wang et al., 2010). The short teeth of corolla lobes emerging from the top of the highly fused corolla tube is the synapomorphy shared among the three genera *Tengia*, *Petrocodon*, and *Calcareoboea* (Wang et al., 2010). Apparently, *Tengia* is characterized by a dorsalized actinomorphic corolla evolved from the dorsal petals (highly fused tube part with tooth-like lobes) of the *Calcareoboea* plants. Putting the *TsCYC1* expression pattern and the morphological and phylogenetic evidence together, we suggest that the expansion of the expressions of *TsCYC1C* and *TsCYC1D* from the dorsal to the ventral petals is likely responsible for the evolutionary formation of the fully dorsalized actinomorphic corolla, that is, an expanded functional domain of *CYC*-like gene dorsal identity in *Tengia* corolla.

CYC-like genes have been widely known in patterning floral dorsoventral asymmetry (zygomorphy) in angiosperms, especially in core eudicots, in which their expressions in the second and third whorls of floral organs usually repress stamen development and promote or retard petal growth according to the trait concerned (Luo et al., 1996, 1999; Hileman et al., 2003; Costa et al., 2005; Citerne et al., 2006; Feng et al., 2006; Busch & Zachgo, 2007; Gao et al., 2008; Wang et al., 2008; Song et al., 2009; Zhang et al., 2010). However, in the *backpetals* mutant of *A. majus*, the stamen growth is not affected by the ectopic expression of *CYC* because the two lateral and two ventral stamens are still fertile (Luo et al., 1999; Kalisz et al., 2006). In the actinomorphic flower of legume *Cadia*, the *LegCYC1B* expression is not related to the androecial development (Citerne et al., 2006). In *Veronica* and *Gratiola* (Veronicaceae), the *CYC*-like gene expression does not positively correlate with the ventral stamen abortion (Preston et al., 2009). In rice, the *CYC* homologue *REP1* expression has no apparent effect on stamen growth or fertility (Yuan et al., 2009), as well as *PlCYC* in *Plantago* (Reardon et al., 2009) and *BtCYC* genes in *Bergia texana* (Zhang et al., 2010). Similarily, even though the expressions of *TsCYC1C* and *TsCYC1D* are distributed in all five stamens, with *TsCYC1D* slightly stronger and longer than *TsCYC1C* in expression level and prolonged time, the five stamens are all fertile and almost equal in size. This phenomenon suggests that the expressions of *TsCYC1C* and *TsCYC1D* are not correlated with stamen abortion, that is, *TsCYC* genes do not functionally repress the stamen development in *Tengia* flowers. It is suggested that *CYC* activity in establishing floral dorsal identity is mediated by the cell cycle-related genes and an MYB family gene *RAD*, in which *CYC* directly or indirectly suppresses *cyclinD3b* activity in the stamen whorl and activates *RAD* expression in the petal whorl (Luo et al., 1996; Gaudin et al., 2000; Corley et al., 2005). The reasonable explanation for the androecial development unaffected by *CYC*-like gene expression herein is that the cell cycle-related genes might have not been co-opted into the *CYC*-like gene regulatory network in the *Tengia*-related lineage in the Gesneriaceae, or uncoupled from the *TsCYC* regulatory pathway in *Tengia*. In the *backpetals* mutant of *A.majus*, the ectopic expression of *CYC* in later floral development is due to a transposon insertion in the promoter region of *CYC*, 4.2 kb upstream from the transcription start site, which is believed to affect a *cis*-acting region that normally suppresses *CYC* transcription during the later stages of development (Luo et al., 1999). Functional *cis*-regulatory elements located in non-coding DNA regions of a gene at varying distances from the transcription start site usually control where, when, and at what level a gene is expressed though interacting with *trans*-acting factors (Wittkopp et al., 2004; Kim et al., 2006). As the transposon insertion affects a *cis*-acting region relating to *CYC* expression, the change in *cis*-elements may also result in the cell cycle-related genes uncoupling from the *TsCYC* regulatory pathway in *Tengia*. It merits further studies in function and regulatory network to determine how developmental and evolutionary modifications in the regulatory pathway have led to

the uncoupling between the *TsCYC* gene expression and the androecial development, especially the regulatory interaction between *TsCYC* and *cyclinD3* genes, in *Tengia*.

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