Isolation and sequence analysis of two *CYC*-like genes, *SiCYC1A* and *SiCYC1B*, from zygomorphic and actinomorphic cultivars of *Saintpaulia ionantha* (Gesneriaceae)

WANG Lei   GAO Qiu   WANG Yin-Zheng[*]   LIN Qi-Bing

(*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences*, Beijing 100093, China)

Abstract   Using the mTAIL-PCR method, we have isolated the two *CYC*-like genes, *SiCYC1A* and *SiCYC1B*, from zygomorphic and actinomorphic cultivars of *Saintpaulia ionantha* respectively in Gesneriaceae. The two genes, *SiCYC1A* and *SiCYC1B*, from the zygomorphic cultivar both contain the whole regulation domain, i.e. TCP and R domains. Therefore, they should be functional in the floral symmetry establishment, homologous with *CYC* in *Antirrhinum majus*. Unexpectedly, the two genes from the actinomorphic cultivar are identical to those from the zygomorphic in DNA sequence, respectively. Based on comparative analysis of the molecular alteration at *CYC*-like genes, which are responsible for the morphological transformation from zygomorphy to actinomorphy, we suggest that the two closely related genes *SiCYC1A* and *SiCYC1B* might be regulated by a common upstream regulator, whose change would result in silence of both *SiCYC1A* and *SiCYC1B* in controlling the development of the adaxial and lateral organs in a flower. In addition, an mTAIL-PCR method was shown to have the technological advantages of the unknown sequence for isolation.

Key words   *CYC*-like gene, whole gene, *Saintpaulia ionantha*, mTAIL-PCR, upstream regulator.

During the evolution of angiosperms, zygomorphy has very likely arisen from actinomorphy many times independently, but there also appear to have been reversals from zygomorphy to actinomorphy (Donoghue et al., 1998). In Lamiales s.l., the zygomorphic flower is believed to be ancestral while the actinomorphic one secondary (Endress, 1998). However, research in how modifications of development lead to the transformation between zygomorphy and actinomorphy during evolution is not a well unexplored field at molecular developmental level in Lamiales, except for *Antirrhinum majus* L. and a few allied species in Antirrhineae (Veronicaceae).

In the model species *Antirrhinum majus,* the asymmetrical expressions of *CYCLOIDEA/ DICHOTOMA* (*CYC/DICH*) genes control the zygomorphy of the flower (Luo et al., 1996, 1999). The *CYC* gene activity in the adaxial floral organs in a flower promotes the growth of adaxial petal in whorl two, but arrests the growth of the adaxial stamen in whorl three (Luo et al., 1996). The local expression of *DICH* accompanied with *CYC* activity determines the internal asymmetry of adaxial petals (Luo et al., 1999). Both *CYC* and *DICH* belong to the TCP gene family, including *TB1 (TEOSINTE BRANCHED1)* from maize, *PCF1* and *PCF2* from rice and *TCP1-9* isolated from *Arabidopsis* (Doebley et al., 1995, 1997; Kosugi &

Ohashi, 1997, 2002; Cubas et al., 1999a; Cubas, 2004). All members of this gene family have the TCP and R domains.

Beside the model species, a lot of *CYC*-like genes have been isolated with partial sequences of Open Reading Frame (ORF) in the clades with zygomorphic flower in Lamiales s.l., and studies have been focusing on the molecular evolution of *CYC*-like genes, such as Veronicaceae, Scrophulariaceae s.l., Gesneriaceae and Fabaceae (Möller et al., 1999; Citerne et al., 2000, 2003; Picó et al., 2002; Fukuda et al., 2003; Gubitz et al., 2003; Hileman & Baum, 2003a; Smith et al., 2004). However, the isolation of the whole ORF of these genes and studies on their expression pattern have been only limited in a few species within Antirrhineae (Veronicaceae) in Lamiales up to date (Luo et al., 1996, 1999; Cubas et al., 1999b; Hileman et al., 2003).

Gesneriaceae is the most basal family of Lamiales s.l., an interesting group in the respect of evolution of floral symmetry (Endress, 1998). In this family, the corolla tends to be weakly bilateral and the adaxial stamen is less reduced than in other Lamiales (Endress, 1998, 1999). It is therefore possible that this family represents an ancient and not too elaborated form of floral bilateral symmetry within Lamiales s.l. In Gesneriaceae, several *CYC*-like genes with 60%–70% of their ORF have been isolated in up to 54 species, and analyses for the phylogeny and molecular evolution of these genes were reported (Möller et al., 1999; Citerne et al., 2000; Smith et al., 2004). These studies reveal that *GCYC* in Gesneriaceae has undergone several duplication and putative gene loss events during the evolution of Gesneriaceae. Two closely related genes, *GCYC1A* and *GCYC1B* have been isolated only in the two closely related genera, i.e. *Streptocarpus* Lindl. and *Saintpaulia* Wendl. *GCYC1A* and *GCYC1B* are probably derived from the gene *GCYC1* that is widely distributed in Gesneriaceae (Möller et al., 1999; Citerne et al., 2000). However, all these isolated genes lack the whole TCP domain and the 5′ region of ORF that are necessary for further studies in the function and expression pattern of these *CYC*-like genes in Gesneriaceae.

Saintpaulia is one of the smaller genera in the Old World Gesneriaceae (Burtt, 1958). Most of species in *Saintpaulia* have been in cultivation for many years. *Saintpaulia ionantha* Wendl. is the most popular one of species known as″ The African violet″ in the world, and its cultivated descendants are the basis of the African violet commercial sales. Its wild-type has a zygomorphic corolla with the adaxial and lateral stamens aborted. Both zygomorphic and actinomorphic cultivars are found in *S. ionantha*. To analyse if the sequence variation of *CYC*-like genes between the zygomorphic and actinomorphic cultivars is the cause for the morphological transformation, we have isolated the ORF of two *CYC*-like genes from *S. ionantha, SiCYC1A* and *SiCYC1B,* with modified TAIL-PCR. The sequences of *SiCYC1A* and *SiCYC1B,* containing complete TCP domain, R domain and 5′ region, enable us to analyse the molecular mechanism in the morphological transformation between zygomorphic and actinomorphic flowers, which would shed light on further research on their genetic control and molecular mechanism related to this transformation in floral symmetry.

1   Material and methods

1.1   DNA extraction and PCR amplification

Fresh leaves of zygomorphic cultivar (ZC) and actinomorphic cultivar (AC) of *Saintpaulia ionantha* were collected from greenhouse of IBCAS (Institute of Botany, the Chinese Academy of Sciences). Genomic DNA of ZC and AC were isolated by the CTAB method (Doyle & Doyle, 1987). The primers (forward FS 5'-ATGCTAGGTTTCGA-CAAGCC-3', reverse R 5'-ATGAATTTGTGCTGATCCAAAATG-3') were used to amplify 70% *SiCYC* sequences from zygomorphic cultivar (Möller et al., 1999; Citerne et al., 2000;

Smith et al., 2004) (Fig. 1). PCR was performed with 32–36 cycles each with denaturation at 94   for 30 s, annealing at 52   for 30 s and extension at 72   for 1 min. PCR products were run on 1% agarose gels, and bands near the expected length (ca. 620 bp for *GCYC* loci) were excised. Excised PCR products of *SiCYC* from zygomorphic cultivar were cloned and sequenced.
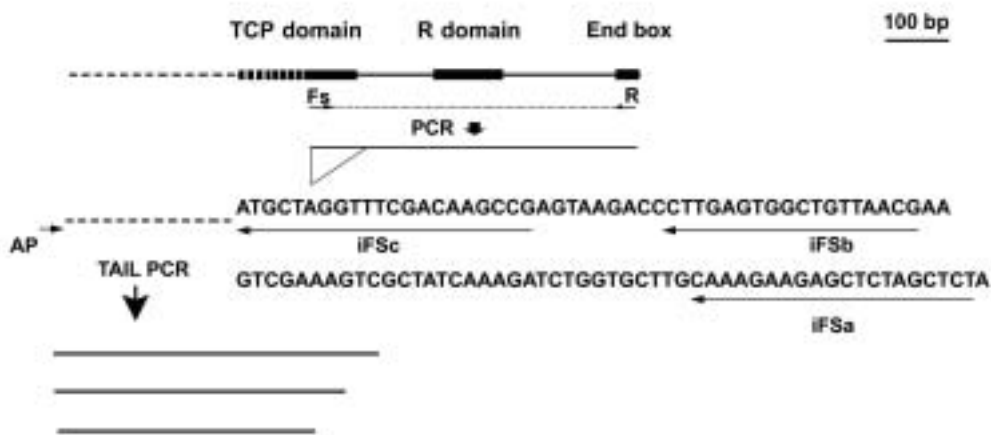
Fig. 1.   Schematic outline of the procedures used to isolate *SiCYC* 5′ flanking regions from *Saintpaulia ionantha*.   First, 70% coding region of *SiCYC* was amplified by primer pairs FS and R. On the basis of these sequence amplified, three gene-specific primers iFSa, iFSb and iFSc were designed and used in combination with a 10-mers primer (AP) for TAIL-PCR to obtain the 5′ flanking region.

1.2   Modified TAIL-PCR procedure

In order to clone the unknown 5′ flanking sequence beyond the partial sequence of *SiCYC* from zygomorphic cultivar, a modified TAIL-PCR (mTAIL-PCR) was conducted, by using of 10 mers random primers instead of degenerate 16 mers as the short primer (Liu & Whittier, 1995; Liu et al., 1995; Terauchi & Kahl, 2000). Three gene-specific primers in nested positions close to the 5' end of the coding regions were designed and synthesized (Fig. 1). iFSA (5'-AGAGCTAGAGCTCTTCTTTG-3'), iFSB (5'-CGTTAACAGCCACTCAAG-3'), and iFSC (5'-CGGCTTGTCGAAACCTAGCAT-3'). Through the primary and secondary rounds of mTAIL-PCR, one of the arbitrary 10 mers primers (S82: 5'-GGCACTGAGG-3') for *SiCYC* was chosen from the 10 mers random primer sets (Sangon, Shanghai). mTAIL-PCR amplification was performed on Biometra TGRADIENT thermocycler. Cycling parameters for the primary, secondary, and tertiary rounds are shown in Table 1.

1.3   Isolation of the 5' region of the *SiCYC* genes in *S. ionantha*

The primer SiF (5'-TTGAGCCCTCCATCCCACA-3') was designed from 5' region sequence of *SiCYC* amplified from zygomorphic cultivar by mTAIL-PCR and was used to isolate the complete ORF of the *SiCYC* genes with reverse primer R (5'-ATGAATTTGTGGTGATCCAAAATG-3') from both zygomorphic cultivar and actinomorphic cultivar. PCR was performed with 32 cycles each with denaturation at 94 for 30 s, annealing at 52   for 30 s and extension at 72   for 1.5 min. PCR products were run on 1% agarose gels, excised bands near the expected length (900 bp). Excised PCR products were extracted and cloned into the pGEM-T Easy vector (Promega, Madison, Wis). Sixteen to 24 clones per ligation reaction were sequenced with the vector specific primers T7 and SP6. Sequencing was performed on an ABI PRISM 377 DNA Sequencer according to

manufacturer's instructions (Applied Biosystems, Foster City, Calif). Sequences alignments were performed with CLUSTAL W (Thompson et al., 1994) and adjusted manually. The secondary structure of TCP domain and R domain prediction for *SiCYC1A*/*SiCYC1B, CYC* and *LCYC* were plotted with PROTEAN 5.0.

Table 1   mTAIL–PCR cycle settings for this study

Reaction	Number of cycles	Thermal settings
	1	95 , 5 min
	5	94 , 30 s; 54 , 1 min; 72 , 2.5 min
	1	94 , 30 s; 25 , ramping to 72 in 3 min; 72 , 2.5 min
Primary	15	94 , 30 s; 54 , 3.5 min
(iFSa-S82)		94 , 30 s; 54 , 3.5 min
		94 , 30 s; 42 , 1 min; 72 , 2.5 min
	1	72 , 5 min; 4 hold
	12	94 , 30 s; 54 , 3.5 min
Secondary		94 , 30 s; 54 , 3.5 min
(iFSb-S82)		94 , 30 s; 42 , 1 min; 72 , 2.5 min
	1	72 , 5 min; 4 hold
Tertiary	30	94 , 30 s; 42 , 1 min; 72 , 2.5 min
(iFSc-S82)	1	72 , 5 min; 4 hold

Primary, secondary, and tertiary nested PCR reactions are performed sequentially. The primary PCR reaction consists of 15 TAIL cycles, while the secondary reaction contains 12 TAIL cycles. Primers iFSa, iFSb and iFSc are nested gene-specific primers while S82 is a 10-mers RAPD primer.

2   Results and discussion

2.1   Floral morphology

Flowers in the zygomorphic cultivar are identical to those of the wild species of *Saintpaulia ionantha*. The bilabiate corolla has a lower (abaxial) trilobed lip that is longer than the upper (adaxial) bilobed lip (Fig. 2: A). The androecium consists of two abaxial stamens and three staminodes in lateral and adaxial positions (Fig. 2: A, C). The actinomorphic cultivar has five almost equal petals with a short corolla tube, and five fertile stamens, i.e. one adaxial, two lateral and two abaxial stamens. Among the five stamens, the two abaxial stamens are larger than the adaxial and lateral ones (Fig. 2: B, C).

2.2   Analysis of DNA and deduced amino acid sequences

We conducted mTAIL-PCR to amplify the 5' region sequence of *CYC*-like genes from zygomorphic cultivar in *S. ionantha* base on their partial sequences in the coding region (Citerne et al., 2000). The sequence of a
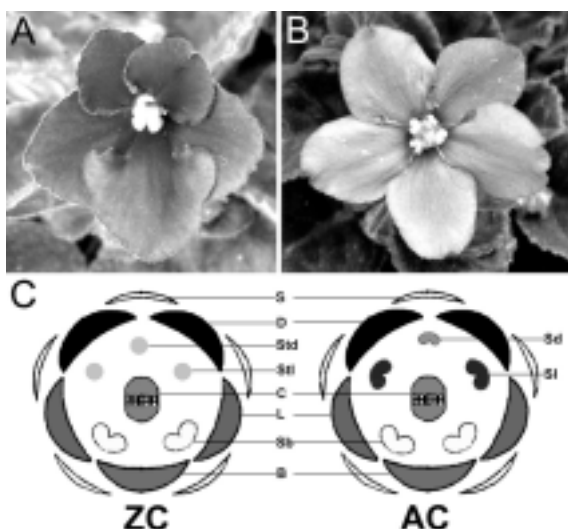
Fig. 2.   Two types of floral morphology (A, B) and diagram (C) in zygomorphic cultivar (ZC) and actinomorphic cultivar (AC) of *Saintpaulia ionantha*. In a ZC flower, the upper lip with two adaxial petals is shorter than the lower lip with one abaxial and two lateral petals, with two abaxial stamens plus three staminodes (A, C, ZC). In an AC flower, there are five almost equal petals with five stamens (B, C, AC). C, carpel; B, abaxial petal; D, adaxial petal; S, sepal; L, lateral petal; Sb, abaxial stamen; Sd, adaxial stamen; Sl, lateral stamen; Std, adaxial staminode; Stl, lateral staminodes.

420 bp fragment obtained from 5' upstream by mTAIL-PCR was 20 bp overlapping with the 5'-end of the partial sequence of *SiCYC* from zygomorphic cultivar of *S. ionantha*. The longer *SiCYC* sequences with a complete 5' ends were further amplified and cloned, using the primers which were designed based on the two partial sequences. Sequencing results show that there are two copies of *CYC*-like genes in the zygomorphic cultivar of *S. ionantha,* i.e. *SiCYC1A* and *SiCYC1B.* The two genes *SiCYC1A* and *SiCYC1B* are further cloned from the actinomorphic cultivar. It is unexpected that the DNA and deduced protein sequences of *SiCYC1A* and *SiCYC1B* from the actinomorphic cultivar are identical to those of zygomorphic cultivar, respectively (Fig. 3). The total length of sequence is 951 bp in *SiCYC1A* and 963 bp in *SiCYC1b*. The protein sequences of *SiCYC1A* and *SiCYC1B* are 316 aa and 320 aa, respectively. Like other *CYC* homologues, *SiCYC* sequences lack intron in the coding regions (Luo et al., 1996, 1999; Cubas et al., 1999b).

Alignment of DNA and amino acid sequences shows that the similarity is 88% between *SiCYC1A* and *SiCYC1B* at DNA level, while 84% at protein level. The substitutions between the two genes usually occur outside the conserved regions, such as TCP domain, R domain and End box. The length of TCP domain is 59 aa and R domain is 18 aa both in *SiCYC1A* and *SiCYC1B* respectively, which have the same length as the ones from *CYC* and *LCYC* in Antirrhineae (Luo et al., 1996; Cubas et al., 1999a, b). Alignment of the amino acid sequence from *Antirrhinum majus CYC*, *Linaria vulgaris LCYC* and *SiCYC1A*/*SiCYC1B* shows that there are five amino acid substitutions in TCP domain of *SiCYC* (Fig. 4)*.* The secondary structure prediction for *SiCYC1A*/*SiCYC1B*, *CYC* and *LCYC* plotted with PROTEAN 5.0 shows that the amino acid substitutions in TCP domain *SiCYC* do not change the secondary structure (data not shown).

Homology assessment from phylogenetic analysis suggests that *CYC*-like genes in Gesneriaceae have undergone several duplication and putative gene loss events during the evolution of Gesneriaceae (Citerne et al., 2000). According to the phylogenetic analysis, the *SiCYC* homologues *GCYC1A* and *GCYC1B* limited within the two closely related clades of the African *Streptocarpus* and *Saintpaulia* have been suggested as a secondary and more recent duplication, which might have evolved from the gene *GCYC1*, a gene widely distributed in Gesneriaceae (Möller et al., 1999; Citerne et al., 2000; Smith et al., 2004). Therefore, we consider that *SiCYC1A*/*SiCYC1B* might have functionally differentiated from *GCYC1* as two redundant genes in controlling the floral symmetry during evolution.

2.3   The morphological transformation from zygomorphy to actinomorphy in *Saintpaulia ionantha*

In the zygomorphic cultivar, flowers are identical to those of the wild plants of *S. ionantha* in floral symmetry. However, there are five almost equal petals and five fertile stamens in the actinomorphic cultivar of *S. ionantha*. This morphological change from zygomorphic to actinomorphic flower should be related to partial or complete loss of function of *SiCYC1A*/*SiCYC1B* during the floral development. Nevertheless, the identical sequence of both *SiCYC1A* and *SiCYC1B* between zygomorphic and actinomorphic cultivars raises an interesting question about what lead to this morphological transformation without sequence alteration at the two *CYC*-like genes. In the model species *Antirrhinum majus*, the transposon insertion at cis or trans-regulation region makes *CYC* complete loss of function, disrupting the normal expression of *CYC* transcription in the adaxial and lateral regions, which leads to the peloric mutants (Luo et al., 1996, 1999). However, the loss of function of *LCYC*, the only homologue of *Antirrhinum CYC* in *Linaria* Miller, in actinomorphic variation is not caused by a cis-regulator disrupting or DNA change but is correlated with extensive methylation (Cubas et al., 1999b). In another case, the single *GCYC1* gene from the actinomorphic cultivar of *Sinningia speciosa* Benth. & Hook. (Gesneriaceae) carries a frame-shift mutation that gives
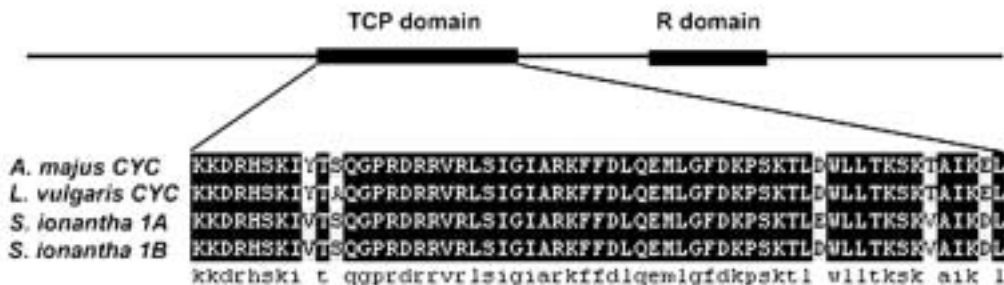
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ZC S. ionantha 1A   MFGKNSYLHPPQVSQPLQTQGPTSSVDLSDGAEILLHHHQDD-VLSGHYLAENTPFLGV
AC S. ionantha 1A   MFGKNSYLHPPQVSQPLQTQGPTSSVDLSDGAEILLHHHQDD-VLSGHYLAENTPFLGV
ZC S. ionantha 1B   MFGKNSYLHPPQVSQPLQTRGPTSSIDLIDEAEILLHHHQDGVLASHYLATNTPFLGV
AC S. ionantha 1B   MFGKNSYLHPPQVSQPLQTRGPTSSIDLIDEAEILLHHHQDGVLASHYLATNTPFLGV
                    *******************:*****:**.*.**********::*.**:.****.*******:


ZC S. ionantha 1A   TLYSQDLGVGSEDPSSLSNTLSRRQVVKKDRHSKIVTSGPRDRRVRLSIGIARKFFDL
AC S. ionantha 1A   TLYSQDLGVGSEDPSSLSNTLSRRQVVKKDRHSKIVTSGPRDRRVRLSIGIARKFFDL
ZC S. ionantha 1B   TLYSQDAGEGTEDPSALANALSRRQTVKKDRHSKIVTSGPRDRRVRLSIGIARKFFDL
AC S. ionantha 1B   TLYSQDAGEGTEDPSALANALSRRQTVKKDRHSKIVTSGPRDRRVRLSIGIARKFFDL
                    ****** *.*:****:*:*:*****.**********************************
                                                          TCP domain

ZC S. ionantha 1A   EMLGFDKPSKTLEWLLTKSKVAIKDLVLAKKSSSSRSTSSPSECEVVLNGETFEDGSCL
AC S. ionantha 1A   EMLGFDKPSKTLEWLLTKSKVAIKDLVLAKKSSSSRSTSSPSECEVVLNGETFEDGSCL
ZC S. ionantha 1B   EMLGFDKPSKTLDWLLTKSKVAIKDLVLTKKSSSSKSTSSPSECEVVLNGEAFQDGNCL
AC S. ionantha 1B   EMLGFDKPSKTLDWLLTKSKVAIKDLVLTKKSSSSKSTSSPSECEVVLNGEAFQDGNCL
                    ************:***************:******:*******************:*:**.**


ZC S. ionantha 1A   PPDFKRKS---ASTARDPAQSASTLAKESRAKARARARERTKEKLCIKKLNESRNMNNN
AC S. ionantha 1A   PPDFKRKS---ASTARDPAQSASTLAKESRAKARARARERTKEKLCIKKLNESRNMNNN
ZC S. ionantha 1B   HPDSRKSSSTNANKCKDPAQSASNLAKESRAKARARARERTKEKMCIKKLNESRNMDNN
AC S. ionantha 1B   HPDSRKSSSTNANKCKDPAQSASNLAKESRAKARARARERTKEKMCIKKLNESRNMDNN
                    **.::.*..*...:*******.*****************:************:**
                                           R domain

ZC S. ionantha 1A   FASNSQPVLHCPITNEATATQQDLIQESSVIKRMLRHHQS-FFGFHCAALPSPDVDENW
AC S. ionantha 1A   FASNSQPVLHCPITNEATATQQDLIQESSVIKRMLRHHQS-FFGFHCAALPSPDVDENW
ZC S. ionantha 1B   FASNSRPVLQCPITNEATATHEDLIQESSVIKRMLRHHQSSFFGYHCG-LPSPNVNENW
AC S. ionantha 1B   FASNSRPVLQCPITNEATATHEDLIQESSVIKRMLRHHQSSFFGYHCG-LPSPNVNENW
                    *****:***:*********::************** ***:**.****:*:***


ZC S. ionantha 1A   AGSLTSQSNLCDILDQHKFNH
AC S. ionantha 1A   AGSLTSQSNLCDILDQHKFNH
ZC S. ionantha 1B   VGSLTSQSNLCDILDQHKFNH
AC S. ionantha 1B   VGSLTSQSNLCDILDQHKFNH
                    .*******************.
                            End box
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Fig. 3. Alignment of the protein sequence of *SiCYC1A, SiCYC1B* in zygomorphic cultivar (ZC) and actinomorphic cultivar (AC) of *Saintpaulia ionantha*. The length of TCP domain is 59 aa and R domain is 18 aa both in *SiCYC1A* and *SiCYC1B* (underlined).

rise to a truncated protein, which might be responsible for the loss of function of *GCYC1* in this actinomorphic cultivar (Citerne & Cronk, 1999; Möller et al., 1999; Citerne et al., 2000; Cubas, 2004).

In all three cases mentioned above, alteration of *CYC*-like gene expression or function is responsible for the morphological transformation from zygomorphy to actinomorphy. The first is the disruption or change in their cis or trans-regulation elements, as observed in peloric mutants in the model species snapdragon. Another is the modified coding region of *CYC*-like genes without DNA change, such as the methylation of the *LCYC* gene sequence in the actinomorphic variation of *Linaria*. And the third is due to the substitution, insertion or

Fig. 4. Alignment of the amino acid sequence of TCP domain of *Antirrhinum majus CYC*, *Linaria vulgaris LCYC* and *Saintpaulia ionantha SiCYC1A*/*SiCYC1B*.

deletion taking place in *CYC*-like genes at DNA level, such as the deletion resulting in the frame-shift in *GCYC1* sequence from the actinomorphic cultivar of *Sinningia speciosa*.

Because the DNA and protein sequences of both *SiCYC1A* and *SiCYC1B* are identical between zygomorphic and actinomorphic cultivars of *S. ionantha*, it is possible that the methylation of the *SiCYC* gene sequence leads to the morphological transformation from zygomorphic to actinomorphic flowers. However, since the two redundant genes *SiCYC1A* and *SiCYC1B* are recently duplicated from *GCYC1* (Citerne et al., 2000), they should have a very similar function in controlling zygomorphy like *CYC/DICH* in snapdragon. Therefore, the morphological transformation from zygomorphy to actinomorphy might be resulted from double silence of the two genes, *SiCYC1A* and *SiCYC1B*. If this is true, the above explanation would require an additional hypothesis that the methylation of *SiCYC* genes as two separate events would co-occur at the same time. Alternatively and most likely, these would be a single factor which is responsible for this morphological transformation. Therefore, we consider that the two closely related genes *SiCYC1A* and *SiCYC1B* might be regulated by a common upstream regulator, whose change would result in silence of both *SiCYC1A* and *SiCYC1B* in controlling the adaxial and lateral organs in a flower. It is necessary to carry out an intensive study on expression pattern and upstream regulator of the two genes *SiCYC1A* and *SiCYC1B,* which would be helpful to understand the developmental pathways and molecular mechanism for the morphological transformation from zygomorphy to actinomorphy in *S. ionantha*.

2.4   Technological advantages of the modified TAIL-PCR

Modified PCR procedures to isolate the DNA fragments adjacent to known sequences are powerful strategies for chromosome walking. Several modified PCR methods are available for this purpose, for example: inverse PCR, ligation-mediated PCR (LM-PCR) and randomly primed PCR (RP-PCR) (Frohman et al., 1988; Ohara et al., 1989; Huang, 1994; Sterky et al., 1998; Akiyama et al., 2000; Dai et al., 2000). The TAIL-PCR (thermal asymmetric interlaced PCR) method developed by Liu and Whittier (1995) is a simple, but sometimes not efficient, technique for genomic walking which does not require any restriction or ligation steps. In this study, using the random 10 mers originally developed for RAPD analysis (Williams et al., 1990) as the short arbitrary primers instead of three degenerate 16-mer primers as described in the original TAIL procedure, we have successfully isolated the 5′-flanking regions of *SiCYC* genes. The mTAIL-PCR is more efficient and controllable. Using the random 10 mers primers, we are able to obtain the right size of the prospective PCR products from 200 to 1500 bp. Therefore, the random 10 mers primers may be much suitable to isolate adjacent fragments from some new genes, where the sequence information around

the conserved region is often limited.

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CYC

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GCYC   ,   mTAIL-PCR   *Saintpaulia ionantha*   *CYC*   5′   ,

Gesneriaceae   : *SiCYC1A*   *SiCYC1B*   ,
SiCYC1A   *SiCYC1B*   ,   (TCP domain   R domain)
          Antirrhinum majus   *CYC*   ,   ,

                                                        CYC   ,
, *SiCYC1A*   *SiCYC1B*   ,
                              ,   TAIL-PCR(mTAIL-PCR)

     ,
     CYC   ;   ;   ; mTAIL-PCR;