PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2011, 38(1): 75~83
www.pibb.ac.cn

Cloning and Analysis of Two Promoters of Chalcone Synthase Gene A (*chsA*) in *Petunia hybrida**

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Abstract Chalcone synthase (CHS), coded by the *chs* gene super-family, is a key enzyme in flavonoid biosynthesis. Two independent promoters was isolated for *chs*A, named PchsA-L (550 bp) and PchsA-S (354 bp) (GenBank accession number EF199747 and EF199748 respectively), from the genomic DNA of *Petunia hybrida*. PchsA-L differs with PchsA-S mainly in that PchsA-L has a 182 bp fragment from 88～269 bp, and the sequence 103～201 bp has the characteristics of a typical intron. Both promoter sequences contain conserved sequences of TATA box, CCAAT box, cap site (CCATAA), and the flower-specific promoter sequences TACPyAT box, anther box (TAGAAGTGACAGAAAT), G-box (CACGTG), box1 element (ATGTCACGTGCCATC) and box2 element (TGTGTTGAAGGTTTGCTA). The petunia plant used for promoter cloning was a diploid with 14 chromosomes. Southern blotting showed that both promoters had multiple copies in the genome. The two promoters segregated in the offspring but the segregation did not meet the ratio of 1∶2∶1. qRT-PCR analysis showed no significant difference in *chs*A gene expression in non-UV-treated and UV-treated floral organ of plants with one or both promoters. The expression of *chs*A in the UV-treated seedling leaves was increased compared with UV-treated floral organ, and PchsA-L-driven *chs*A expression in the UV-treated seedling leaves was very significantly increased than that driven by PchsA-S while there was no *chs*A gene expression in non-UV-treated seedling leaves. The results show the presence of two independent promoters PchsA-L and PchsA-S for *chs*A in the petunia genome; the 182 bp intron-like sequence in PchsA-L promoter could significantly increased *chs*A gene expression in UV-treated seedling leaves.

Key words *Petunia hybrida*, chalcone synthase gene, promoter, cloning
DOI: 10.3724/SP.J.1206.2010.00407

Chalcone synthase (CHS) is the first key enzyme in the flavonoid biosynthetic pathway, it catalyzes the reaction of three molecules of malonyl CoA with one molecule of coumaryl CoA to generate 4, 5, 7-trihydroxy-flavanone (narigeninchalcone). Flavonoids are substances found in a wide range of secondary metabolites in higher plants. Flavonoids have a strong regulatory role in the formation of flower color and play an important role in the interactions of plant with the environment, such as prevention of UV damage, disease resistance, affecting the formation of nodules of leguminous plants[1–3]. Flavonoids are also related to the anthers of male sterile plant[4–6]. So far about 650 *chs* and related gene sequences have been cloned from the moss, ferns, gymnosperms and angiosperms, these genes often exist in multiple copies in the genome of the majority of plants. These genes, structurally similar, functionally related, but have some differences in the expression and encoding products, form a large gene family——*chs* gene superfamily[7]. Petunia (*Petunia hybrida*) *chs* gene family was among the first to be well studied, Koes *et al.*[8–9] obtained from petunia gene library eight full-length sequences of *chs* genes (*chs*A, *chs*B, *chs*C, *chs*D, *chs*E, *chs*F, *chs*G and *chs*J) and four partial sequences of the *chs* genes(*chs*H, *chs*I, *chs*K and *chs*L). In terms of expression, *chs*A and *chs*J are expressed in the floral organs, but also in UV-treated seedlings; *chs*B and *chs*G are expressed only in UV-treated seedlings but not detectable in flowers. *chs*A promoter is tissue-specific, strongly promotes petal-specific expressions[9–10]. Zhang *et al.*, Hong *et al.*, Liu *et al.* and Xia *et al.* successively cloned the promoter for *chs*A[11–14].

*This work was supported by grants from Zhejiang Natural Science Foundation (Y3080184) and Hangzhou Science and Technology Innovation Project (20091133B07).

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Received: August 7, 2010 Accepted: November 5, 2010

In this study, using one pair of specific primers designed based on the previously reported conserved sequences of petunia *chs*A promoter, we isolated two *chs*A gene promoters from the petunia genomic DNA in one PCR reaction, the functional analyses were conducted and reported.

1 Materials and methods

1.1 Experimental materials

Petunia(*Petunia hybrida*) cultivar QL01(Hangzhou Flower Nursery Company) plants were grown under natural conditions. This cultivar has flowers of different colors including purplish blue, pink and white, *etc*.

1.2 PCR amplification

The young leaves of petunia plants were used to extract genomic DNA [15]. Based on the reported sequence in the literature [10] and the sequence in the GenBank (accession number S52984), specific PCR primer pair PchsA-P1 and PchsA-P2 were designed. PchsA-P1: 5′ GGAAGCTTTTCCTGTTCAAAGCTG-ATG 3′, PchsA-P2: 5′ GCTCTAGACGATTTTTGC-TTGAAAAAAG 3′. For PCR amplification, the reaction volume was 35 μl, including 2 μl 0.1 mmol/L of dNTP mixture (Roche Co.), 2 μl of 10 pmol/L of the PCR primers, 2 U Easy-A High-fidelity PCR cloning enzyme (Stratagene Co.) and about 50 ng genomic DNA. PCR reaction parameters: 94℃ for 5 min for denaturation, PCR cycle of 94℃ 45 s, 55℃ 45 s and 72℃ 90 s for 30 cycles, extension reaction at 72℃ for 10 min, then stored at 4℃. Amplified products were subjected to 1.2% agarose gel electrophoresis, stained with ethidium bromide (EtBr), observed and photographed using Bio/Rad gel imaging system.

1.3 PCR product cloning and sequencing

The purified PCR product was linked to the sequencing vector pMD18-T (TaKaRa Co.), and was transformed into *E. coli* DH5α competent cells using freeze-thaw method. The competent cells after transformation were grown in LB plate containing 50 mg/L Amp, X-gal and IPTG to screen for positive clones. White single-colonies were grown in LB liquid medium with 50 mg/L Amp. Plasmids from positive clone were double-digested with *Hin*d Ⅲ and *Xba* Ⅰ before subjected to electrophoretic analysis. Plasmid DNA was sequenced using M13 forward and reverse universal primer sequence using ABI377 DNA sequencing instrument.

1.4 Sequencing analysis

BLAST analysis was performed on the determined sequence against international nucleic acid database; the result from BLAST analysis was then used for sequence homology similarity analysis using GenDoc software, for analysis of promoter sequence characters using DNAStar software, and for prediction of possible intron sequences using the intron prediction software Splice (http://www.fruitfly.org/seq_tools/splice.html).

1.5 Segregation ratio analysis of the two promoters in petunia progenies

The seeds from the petunia plants used in the experiment were planted in the matrix containing peat ∶ash = 2 ∶1 for germination, with daytime temperature of 25℃ and night-time temperature of 18℃. When grown to about 2 cm in height, the seedlings were transferred to pots in the artificial climate chamber in the College of Life and Environment Sciences of Hangzhou Normal University. Genomic DNA was extracted from individual petunia plant and subjected to PCR reaction using P1 and P2 primers as described in **1.2**. Amplified products were separated in 1.2% agarose gel, EtBr stained and photographed.

1.6 Analysis of parental chromosome number in petunia

Young twigs from petunia parent were cultured in MS+NAA (0.1 mg/L) liquid medium at 28℃ to induce rooting. The tips of the roots were clipped in the morning between 8:30 ～9:30, processed in 0.05% colchicines solution at 4℃ overnight. After rinsing with distilled water, the root tips were fixed in Carnoy fixative at 4℃ overnight. After rinsing with distilled water, the root tips were dissociated in 1 mol/L HCl at 60℃ for 5 min. The growth cones of the root tips were cut with a sharp blade, digested in a solution containing 1% cellulase and 0.5% pectinase at 28℃ for 4.5 h. Individual root tips were transferred from the enzyme solution with a pipette to a glass slide pre-treated with ethanol, covered with coverslip with the material placed in the center of the cover slip. The coverslip was tapped gently until the root tip was spread out. The slide was then baked for 30 s using the flame of an ethanol lamp. The coverslip was blotted to drain water around, and pressured with a glass rod rolling diagonally for 1 min. The slide was dried with an ethanol lamp and then put in a −80℃ freezer for 10 min, the coverslip was quickly removed with a blade and sample was left to dry naturally. The slide

was then stained with Giemsa dye solution for 10 min, rinsed with tap water, dried naturally and finally examined under a ZEISS microscope (Model: Axio imager).

1.7 Southern blot hybridization analysis

Genomic DNA was digested with *Eco*R Ⅰ at 37℃ overnight, separated in 0.8% agarose gel and transferred to a nylon membrane. The fragment PchsA-S amplified from genomic DNA using primers P1 and P2 was used to prepare the probe. Probe labeling and hybridization were performed with the DIG DNA Labeling and Detection Kit (Roche Co.) according to the manufacturer's instructions. Finally the hybridization result was scanned for record.

1.8 qRT-PCR

The flowers, as well as the leaves from plant underwent 2 h (2000 Lux) of ultraviolet irradiation were used to extract mRNA, using RNA Extraction Kit (TaKaRa Co.). RT-PCR amplification was performed using SYBR Green Realtime PCR Master Mix (TaKaRa Co.). Primers used were: for GAPDH gene (GenBank accession number GQ122207), GAPDH-F, 5′ AGCAAGGCAGTTAGTGGTGCA 3′ and GAPDH-R, 5′ TTGTGATCTCCGCTCCTAGCA 3′; for *chs*A gene(GenBank accession number X04080.1), chsA-P1, 5′ CATCGGTCAAGAGGCTCATGA 3′ and chsA-P2, 5′ TCGAGCGCCCTTGTTGTTT 3′. Quantitative PCR analysis was carried out using MX3000P fluorescent quantitative PCR instrument (Strategene). PCR conditions were 95℃ 2 min, followed by 95℃ 30 s, 59℃ 30 s and 72℃ 20 s for 40 cycles. Relative expression of *chs*A normalized to GAPDH was calculated as $2^{-(CT_{chsA}-CT_{GAPDH})}$ [16].

2 Results

2.1 Cloning and sequencing of the promoters

Petunia genomic DNA was used for PCR amplification using primers PchsA-P1 and PchsA-P2. Two very clear bands with sizes of about 550 bp and 350 bp were obtained, results were consistent after repeated for three times. Since two bands were amplified, we repeated the PCR amplification using genomic DNA from the leaves of petunia with purplish blue, pink and white flowers respectively, the PCR results remained consistent with two bands (Figure 1).

The PCR products from the purplish blue plant was purified and ligased to the sequencing vector pMD18-T, after a blue-white screening a white single colony was extracted for monoclonal plasmid DNA, digestion with *Hin*d Ⅲ and *Xba* Ⅰ followed by electrophoresis confirmed the clone as positive(Figure 2).

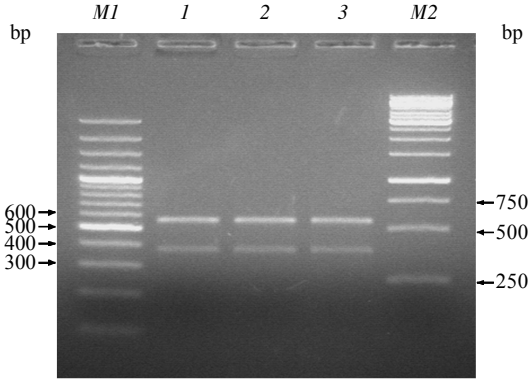

Fig. 1 PCR amplification products from genomic DNA of individual petunia plants with purplish blue, pink or white flowers respectively

M1: 100 bp ladder plus DNA molecular marker (MBI Co.); *1*: Petunia with purplish blue flower; *2*: Petunia with pink flower; *3*: Petunia with white flower; *M2*: 1 kb ladder DNA molecular marker (BBI Co.).

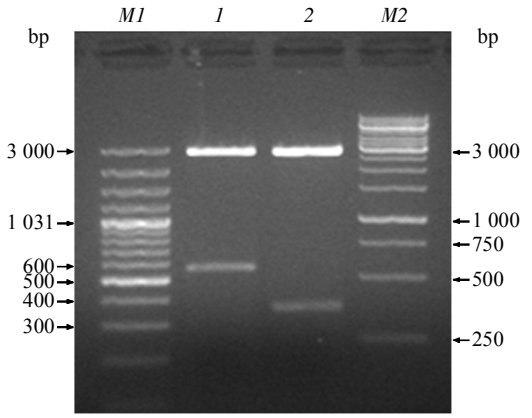

Fig. 2 Plasmid DNA clones digested by *Hin*dⅢ and *Xba* Ⅰ

M1: 100 bp ladder plus DNA molecular marker (MBI Co.); *1*: Plasmid with larger fragment; *2*: Plasmid with smaller fragment; *M2*: 1 kb ladder DNA molecular marker (BBI Co.).

2.2 Sequence analysis of the promoters

By sequencing, the two PCR amplified fragments were 566 bp and 370 bp in full-length respectively. After removing the additional restriction endonuclease sites at both ends and the protective base sequence, the lengths of the genomic DNA fragments were 550 bp and 354 bp, and were named PchsA-L and PchsA-S respectively (GenBank accession number EF199747 and EF199748 respectively). For PchsA-L, sequence structural analysis using DNAstar software revealed

one TATA box (TATAAAT) at 429～435 bp, one CCAAT box (CAAGGCCATTCA) at 53～64 bp, and one cap site (CCATAA) at 458～463 bp, these are the basic components of expression regulation in a common promoter. Also, there was one anther box (TAGAAGTGACAGAAAT) at 21～36 bp, which is the necessary regulatory element for the specific expression of target genes in young anthers. In addition, there were a box2 element (TGTGTTGAA-GGTTTGCTA) at 331～348 bp and a box1 element (ATGTCACGTGCCATC) at 363～377 bp. Box1 and box2 element are conserved sequences of gene promoters related to enzymes involved in flavonoid biosynthesis. Within the box1 element and at 390～395 bp there were two G-boxes (CACGTG), which are the binding sites of flower nuclear protein, induced and regulated by light and UV, related to flower-specific gene expressions. There were two copies of TACPyAT box (TACTAT and TACCAT) at 398～410 bp and a copy of TACPyAT box (TACTAT) at 514～519 bp, these are determining sequences of flower-specific gene expressions. All of these regulatory elements and the surrounding sequences were consistent with previous reports (Figure 3). For PchsA-S, there were a

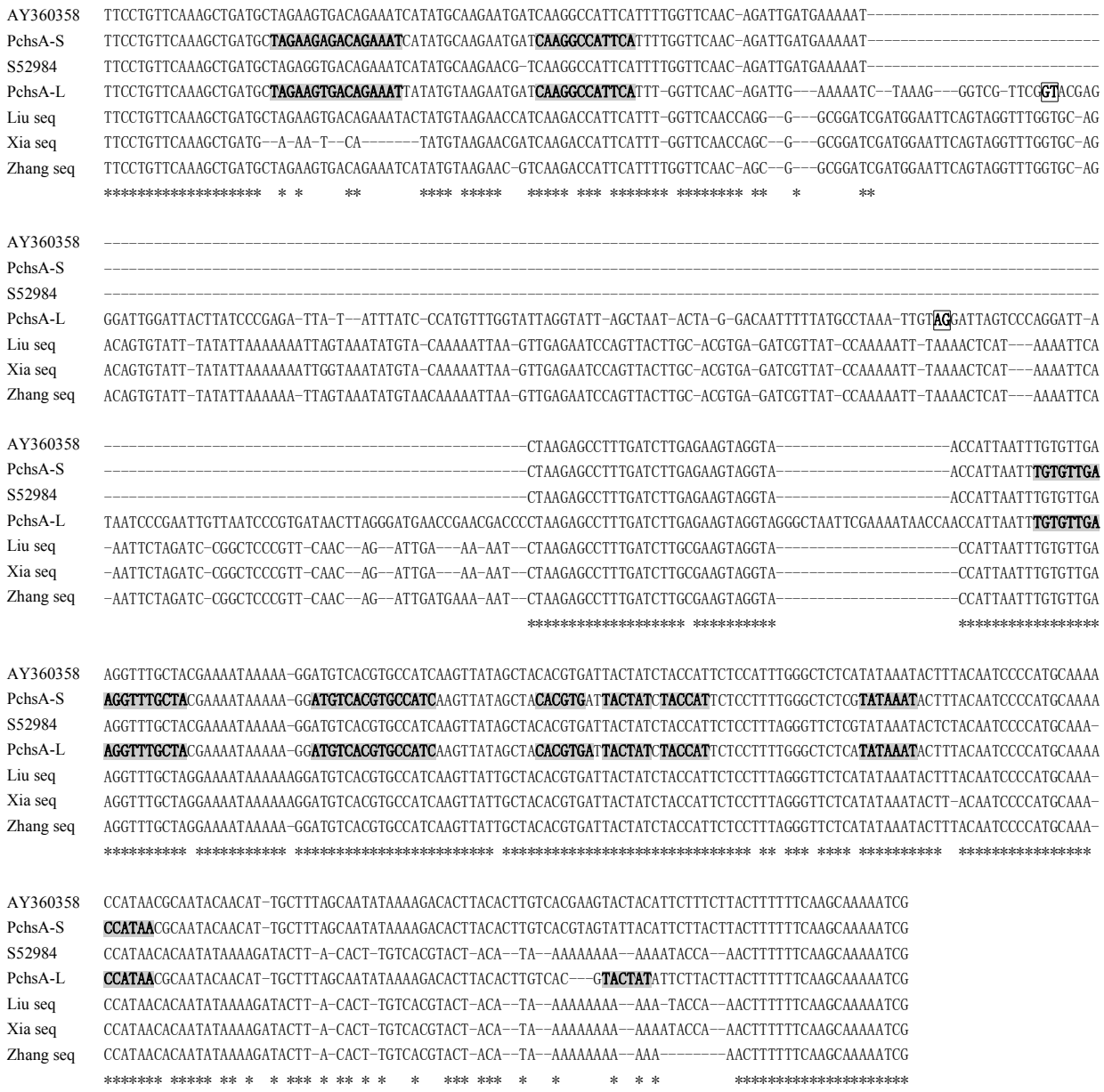

Fig. 3　Alignment of PchsA-L and PchsA-S with other *chs*A promoters from *Petunia hybrida*

Zhang seq, Xia seq and Liu seq were *chs*A promoters reported (see text). The character sequences of promoter in PchsA-L and PchsA-S were marked with shadow and bold. GT…AT, flanking at intron of PchsA-L is boxed.

TATA box (TATAAAT) at 230～236 bp, a CCAAT box (CAAGGCCATTCA) at 53～64 bp, a cap site (CCATAA) at 259～264 bp, an anther box (TAGAA-GAGACAGAAAT) at 21～36 bp, a box2 (TGTG-TTGAAGGTTTGCTA) element at 132～149 bp and a box1 (ATGTCACGTGCCATC) element at 164～178 bp. There were one G-box (CACGTG) within the box1 element, another G-box at 191～196 bp, and two copies of TACPyAT box (TACTAT and TACCAT) at 199～211 bp.

In addition, comparison between PchsA-L and PchsA-S revealed that besides the differences in individual bases, there was a 182 bp sequence only in PchsA-L (88～269 bp). The sequence has GT-AG at both ends of the 103～201 bp, typical of an intron after analysis using Splice prediction software.

Through BLAST analysis, it was found that the similarities of largest similar fragments between PchsA-L and *chs*A gene promoter sequences in the GenBank, AY360358, S52984, and X14591 were 97% (228/234), 96% (145/151) and 96% (145/151) respectively. The similarity (73/77) between the intron region 130～206 bp of PchsA-L and 2757～2833 bp of petunia gene *chs*D (GenBank accession number X14593) was 94% . In addition, the similarities between intron region 93～128 bp and flavonoid 3′, 5′-hydroxylase gene *Hf1* (GenBank accession number AB244234, AB244229, and AB244221) were all 94% (34/36); the similarity between the intron region 93～120 bp and *Hf1* (GenBank accession number AB244228) was 96% (27/28). For PchsA-S, the similarities of largest similar fragments with GenBank *chs*A gene promoter sequences AY360358, S52984, and X14591 were 98% (348/354), 96% (262/271) and 96% (262/271), respectively.

To further analyze the characteristics of the cloned promoters, the sequences of PchsA-L and PchsA-S were compared using GenDoc software with the petunia *chs*A gene promoter sequences AY360358 and S52984 in the GenBank, and the *chs*A promoter sequences reported by Zhang *et al*., Xia *et al*., and Liu *et al*. (Figure 3). Among the sequences above, AY360358 sequence was submitted by Hong *et al*.; S52984 sequence is the promoter sequence in the full *chs*A gene sequence X14591, both were submitted by the same research team; while Zhang *et al*., Xia *et al*., and Liu *et al* did not submit sequences to the GenBank.

2.3 Genetic analysis of PchsA-L and PchsA-S

Genetic analysis of the progenies of the petunia used for cloning revealed that of the 130 plants, 13 had only PchsA-S, 20 had only PchsA-L, and 97 had both promoters. In the generation, the two promoters segregated, but did not comply with the ratio of 1∶2∶1 (Figure 4).

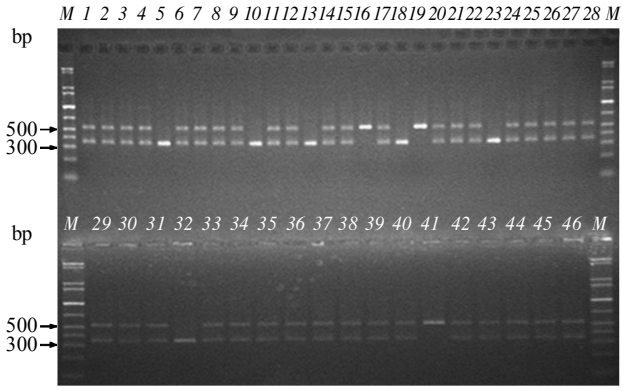

Fig. 4 PCR analysis of genomic DNA from individual offsping of *Petunia hybrida*

M: 1 kb plus DNA ladder molecular marker (Tiangen Co.); *1～46*: Individuals of *Petunia hybrida*.

In addition, chromosome analysis showed that the petunia plant used for promoter cloning was a diploid with 14 chromosomes (Figure 5). Southern blotting showed that the two promoters all had multiple copies in the genome (Figure 6).

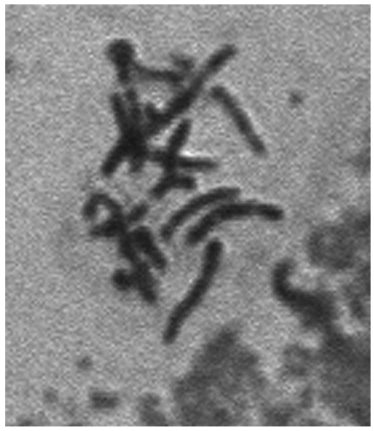

Fig. 5 Karyotic analysis of the parent *Petunia hybrida* individual

Fig. 6 Result of Southern blot
1: PchsA-S; *2*: PchsA-L; *3*: Both PchsA-S and PchsA-L.

2.4 qRT-PCR analysis

qRT-PCR analysis showed that there was no significant difference in *chs*A gene expression in non-UV-treated and UV-treated floral organ of plants with only PchsA-S, or PchsA-L promoter or with both PchsA-S and PchsA-L. The expression of *chs*A in the UV-treated seedling leaves was increased compared with UV-treated floral organ, and PchsA-L-driven *chs*A expression was very significantly increased than that driven by PchsA-S. Besides, there was no *chs*A gene expression in non-UV-treated seedling leaves (Figure 7). The results showed that the 182 bp intron-like sequence in PchsA-L could significantly increase *chs*A gene expression in UV-treated leaves.

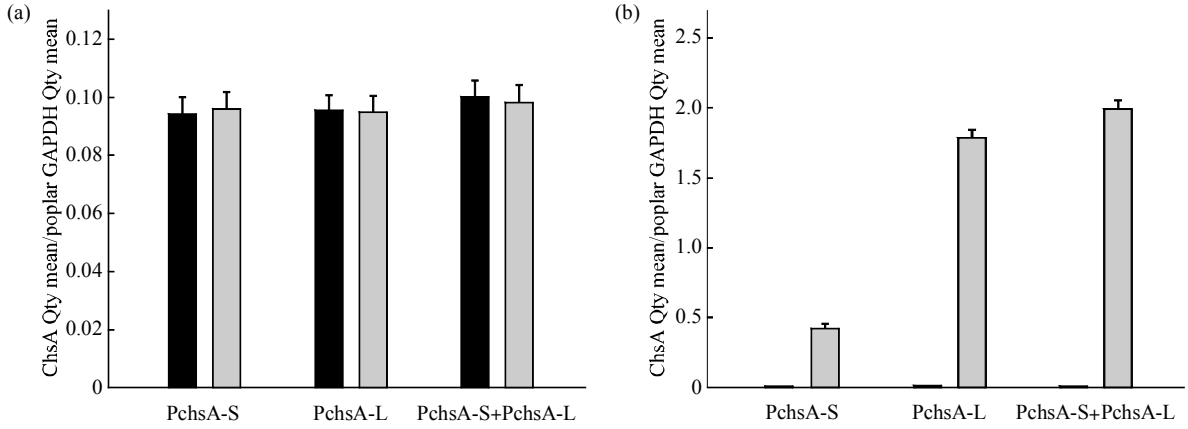

Fig. 7 Gene expression levels of *chs*A under only PchsA-S, or PchsA-L or both PchsA-S and PchsA-L
(a) Expression in floral organ. (b) Expression in seedling leaves. ■: Non-UV-treated; □: UV-treated.

3 Discussion

In this study, a pair of specific primers was designed based on the conserved sequences in the previously reported petunia *chs*A promoters, and two different promoters PchsA-L and PchsA-S were amplified from the genomic DNA in petunia. Besides differences in a few individual bases, PchsA-L differs with PchsA-S mainly in that PchsA-L has a 182 bp fragment starting from the 88 bp, which has the characteristics of a typical intron. With regard to *chs*A gene, van der Meer *et al*.[10] by way of library screening first discovered *chs*A gene promoter, and later *chs*A gene promoter sequences were also cloned through PCR amplification by Zhang *et al*., Hong *et al*., Liu *et al*., Xia *et al*.[11–14]. Significantly, by comparison, the PCR primers used by us were the same in sequence with the ones used by Zhang *et al*., Hong *et al*., Liu *et al*., and Xia *et al*. and differed only in the 5′ end where the additional restriction endonuclease sites were added, however, two promoters were amplified only by us. Of the two promoters we obtained, PchsA-S was consistent with the promoter reported by van der Meer *et al*. and Hong *et al*.; PchsA-L was consistent with the promoter reported by Zhang *et al*., Liu *et al*. and Xia *et al*.. It was thought that the longer promoter (consistent with PchsA-L in this study) with more than 100 bp than the sequence reported by van der Meer *et al*. was due to the difference in the template materials used for PCR amplification[11, 13–14]. However,

the two promoters PchsA-L and PchsA-S cloned in the present study both have the characteristics and sequences of anther-specific gene expression promoter. Progeny analysis revealed that the two promoters segregated in the offspring. Of the 130 plants analyzed, 13 had only PchsA-S, 20 had only PchsA-L, and 97 had both; the segregation ratio did not meet the ratio of 1∶2∶1. Karyotyping showed that the petunia plant used for promoter cloning was a diploid with 14 chromosomes. Southern blotting showed that both promoters had multiple copies in the genome, this explains why the segregation ratio was not 1∶2∶1.

In petunia, van Tunen *et al.* [17-19] reported that chalcone flavanone isomerase (CHI, another enzyme in the biosynthetic pathway of flavonoids) gene *chi*A has two promoters P_{A1} and P_{A2} in tandem, in which P_{A1} controls *chi*A gene expression in the receptacle and P_{A2} controls *chi*A gene expression in mature anther. The result in our study showed that there are also two promoters PchsA-L and PchsA-S for *chs*A gene in petunia, as for *chi*A genes. However, the two promoters PchsA-L and PchsA-S are independent in genome since they segregate in the offspring.

chs gene is a super-gene family, so far full sequences of 8 *chs* genes (*chs*A, *chs*B, *chs*C, *chs*D, *chs*E, *chs*F, *chs*G and *chs*J) and partial sequences of 4 *chs* genes (*chs*H , *chs*I, *chs*K and *chs*L) have been obtained from the petunia, among which, *chs*A and *chs*J genes are specifically expressed in flowers [8-9]. Koes *et al.* [9] compared the promoter sequences of *chs*A and *chs*J, while the two are highly homologous, the *chs*J promoter is not the same as PchsA-L or PchsA-S in this study. Moreover, in the report by van der Meer *et al.*[10], they determined the sequence of the 2 400 bp upstream of the 5′ end of *chs*A gene transcripts, but we did not find PchsA-L sequence in that. In addition, through BLAST analysis, we fail to find any sequences in the GenBank similar to PchsA-L and are promoter for the other members of the *chs* gene family. It is worth mentioning that the study by van der Meer *et al.* [10] showed the existence of a silencer between 2 400 bp to 800 bp upstream of the 5′ end of the *chs*A gene transcript.

qRT-PCR analysis showed no significant difference in *chs*A gene expression in non-UV-treated and UV-treated floral organ of plants with one or both promoters. The expression of *chs*A in the UV-treated seedling leaves was increased compared with UV-treated floral organ, and PchsA-L-driven *chs*A expression in the UV-treated seedling was very significantly increased than that driven by PchsA-S while there was no *chs*A gene expression in non-UV-treated seedling leaves. Recently intron structures were found in the 5′-UTR of a number of eukaryotic genes, and they can regulate gene expressions. For example, Garbarino *et al.* reported the finding of an intron structure in the polyubiquitin gene promoter in *Arabidopsis* that can enhance polyubiquitin gene expression [20]. Polyubiquitin gene promoter sequence with or without the intron sequence isolated from potato tubers were combined with GUS gene to construct a series of fusion genes into potatoes, and the expression of the fusion gene containing the intron was more than 10 times higher than that without the intron in transient expression studies [21]. In *Chlamydomonas reinhardtii*, the intron structure contained in the IC70 gene promoter is essential for IC70 gene expression[22]. Patel *et al.* [23] reported that the 5′-UTR of *AhRbcS1* gene had a strong enhancing effect on *gusA* expression in transgenic *Flaveria bidentis*. In this study, the 182 bp sequence with intron characteristics in PchsA-L promoter has a similar function, significantly increased *chs*A gene expression in UV-treated seedling leaves. Further studies are needed to examine whether the 182 bp intron sequence in PchsA-L functions to offset the gene silencer 2 400 bp to 800 bp upstream of the 5′ end of the *chs*A gene transcript.

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矮牵牛中查尔酮合成酶基因 A (*chs*A) 2 个启动子的克隆和分析 *

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摘要　查尔酮合成酶(chalcone synthase, CHS)是类黄酮类物质生物合成途径中的第一个关键酶，其控制基因 *chs* 为超家族基因. 根据前人报道的矮牵牛查尔酮合成酶基因 A (*chs*A)启动子的保守序列设计 1 对特异性引物，从矮牵牛基因组 DNA 中通过 1 次 PCR 同时扩增出长为 550 bp 和 354 bp 的启动子(分别命名为 PchsA-L 和 PchsA-S，GenBank 登录号：EF199747 和 EF199748), 其中 PchsA-L 与 PchsA-S 相比，除个别碱基有差异外，在 88～269 bp 多出一段 182 bp 的序列，其中 103～201 bp 含有典型的内含子特征. 应用 DNAStar 软件分析表明 2 条序列均含有普通启动子的保守序列 TATA box、CCAAT box、cap site (CCATAA)，并含有花中特异表达启动子的特征序列 TACPyAT box、anther box (TAGAAGTGACAGAAAT)、G-box (CACGTG)、box1 元件(ATGTCACGTGCCATC)和 box2 元件(TGTGTTGAAGGTTTGCTA). 对克隆启动子所用的矮牵牛后代群体进行分析，130 个单株中只含有 PchsA-S 的有 13 株，只含有 PchsA-L 的有 20 株，同时含有 2 个启动子的有 97 株. 2 个启动子在后代中发生了分离，但其分离比并不符合 1∶2∶1. 克隆启动子所用的矮牵牛有 14 条染色体，为二倍体. DNA 印迹表明 2 个启动子在基因组中均是多拷贝. qRT-PCR 分析显示：未经过紫外光处理的花中以及经过紫外光处理的花中，PchsA-L 启动子驱动的 *chs*A 基因与 PchsA-S 启动子驱动的 *chs*A 基因表达都未见明显差异；在紫外光处理的幼苗叶片中表达量相应地比紫外光处理的花中的表达量增高；在紫外光处理的幼苗叶片中，PchsA-L 启动子驱动的 *chs*A 基因比 PchsA-S 启动子驱动的 *chs*A 基因表达量显著增高；而未经过紫外光处理的幼苗叶片中，PchsA-L 启动子驱动的 *chs*A 基因、PchsA-S 启动子驱动的 *chs*A 基因都没有检测到明显的表达信号. 结果表明：在矮牵牛中 *chs*A 基因存在 2 个独立的启动子 PchsA-L 和 PchsA-S；启动子 PchsA-L 中 182 bp 类内含子特征的序列具有显著提高 *chs*A 基因在紫外光处理的幼苗叶片中表达量的功能.

关键词　矮牵牛，查尔酮合成酶基因，启动子，克隆

学科分类号　Q943，Q75　　**DOI**: 10.3724/SP.J.1206.2010.00407

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* 浙江省自然科学基金资助项目(Y3080184)和杭州市科技创新项目(20091133B07).

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收稿日期：2010-08-07，接受日期：2010-11-05