BIOLOGIA PLANTARUM 52 (2): 222-230, 2008

Cloning and quantification of expression levels of two MADS-box genes from *Momordica charantia*

S.M. PENG, T. LUO, J.Y. ZHOU, B. NIU, N.F. LEI, L. TANG and F. CHEN*

College of Life Science, Sichuan University, Chengdu, 610064, P.R. China

Abstract

MADS-box genes are known to be important for the development of flowers. Two MADS-box genes (*MCAG2* and *MCAG6*) were isolated from the bitter gourd (*Momordica charantia*) female bud based on the MADS-box conserved sequences. The complete cDNA sequences of *MCAG2* and *MCAG6* encode a 231 and a 247 amino acid protein, respectively. Sequence comparison and phylogenetic analysis showed that *MCAG2* and *MCAG6* had high identities of amino acid with *AG*-like and *AGL6*-like genes, respectively. The alignment of the deduced amino acid sequence of *AGL6*-like genes revealed that there were two highly conserved regions in the C-terminus, which were designated *AGL6* motif I and *AGL6* motif II. Phylogeny reconstructions suggested that *AGL6*-like genes were divided into three major clades. RT-PCR analysis of the *MCAG2* and *MCAG6* genes showed that they were both expressed in floral organs at different levels. However, *MCAG6* was also expressed highly in shoot apex. Quantitative real-time reverse transcriptase-polymerase chain reaction analysis indicated that the expression of *MCAG2* was detected at high levels in carpel, whereas *MCAG6* was detected at high levels in shoot apex. The gene expression patterns suggest that *MCAG2* and *MCAG6* have a role in regulating bitter gourd floral development.

Additional key words: *AGAMOUS*, *AGL6*, bitter gourd, expression pattern, phylogeny.

Introduction

Flower development is a very complex process which is controlled by many MADS-box genes (Theissen and Saedler 1995). The MADS-box genes compose a large gene family named after a few of its earliest members: *MCM1* (from yeast) (Passmore *et al.* 1988), *AG* (from *Arabidopsis*) (Yanofsky *et al.* 1990), *DEFICIENS* (from *Antirrhinum majus*) (Sommer *et al.* 1990, Schwarz-Sommer *et al.* 1992) and *SRF* (from *Homo sapiens*) (Norman *et al.* 1988). Genes of this large family encode transcription factors sharing a highly conserved domain of 56 amino acids, the MADS domain, involved in DNA binding and protein-protein interactions. The typical plant MADS-box gene is composed of an MADS domain, K domain, a short I region between the MADS and K domain, and the C-terminal region downstream of the K domain (Becker and Theissen 2003). During the last decades, a number of MADS-box genes were studied, and mainly derived from two dicotyledonous model species, *Arabidopsis thaliana* and *Antirrhinum majus* (Coen and Meyerowitz 1991, Schwarz-Sommer *et al.* 1990, Weigel and Meyerowitz 1994). According to the studies about MADS-box genes from *Arabidopsis* and *Antirrhinum*, floral organ identity genes have been subdivided into five different classes, termed class A, B, C, D, and E genes. Theses genes act alone or in combination to specify floral organ identity (Angenent and Colombo 1996, Theissen 2001, Theissen and Saedler 2001, Weigel and Meyerowitz 1994).

Bitter gourd is not only a vegetable crop but also an important medicinal herb (Ho *et al.* 1991). Flower development of bitter gourd is not common. Bitter gourd prosperous stage lasts about three or four months with a number of flowers and male flowers preponderate in the flower of the bitter gourd. Whereas the processes of flower development about bitter gourd also present a hermaphroditic flower stage (Wang and Zeng 2002). The

---

Received 10 April 2006, *accepted* 11 November 2006.

Abbreviations: *AG* - *AGAMOUS*; *AGL6* - *AGAMOUS LIKE6*; *MC18S rRNA* - *Momordica charantia 18S rRNA*; ORF - open reading frame; PCR - polymerase chain reaction; Mr - relative molecular mass; RACE - rapid amplification of cDNA ends; RT-PCR - reverse transcriptase polymerase chain reaction.

Acknowledgements: This work was supported by the Key Research Program from the Education Ministry of China (No. 2002061088).

* Author for correspondence; fax: (+86) 028 85417281, e-mail: cfang@263.com

flower development of the bitter gourd can also be regulated by the hormones *in vitro* (Wang *et al.* 2001). These characteristics of flower development are very prominent in the monoecious plants. In other plants, these processes are regulated by a large number of MADS-box genes. Until now, there is extremely limited information about MADS-box genes in *Cucurbitaceae* and only one MADS-box gene was isolated from bitter gourd (Yang *et al.* 2004). In order to understand and exploit the molecular mechanisms that control flower development in bitter gourd, we have begun to clone and analyze MADS-box genes from buds in bitter gourd. In this study we describe cloning and expression analyses of two MADS-box genes (*MCAG2* and *MCAG6*). Furthermore *MCAG6* is first *AGL6*-like gene in *Cucurbitaceae*.

Materials and methods

Plants of *Momordica charantia* L. grown in the field at Sichuan University (China) were used in all experiments. Tissues were separated and frozen in liquid nitrogen immediately and stored at -80 °C.

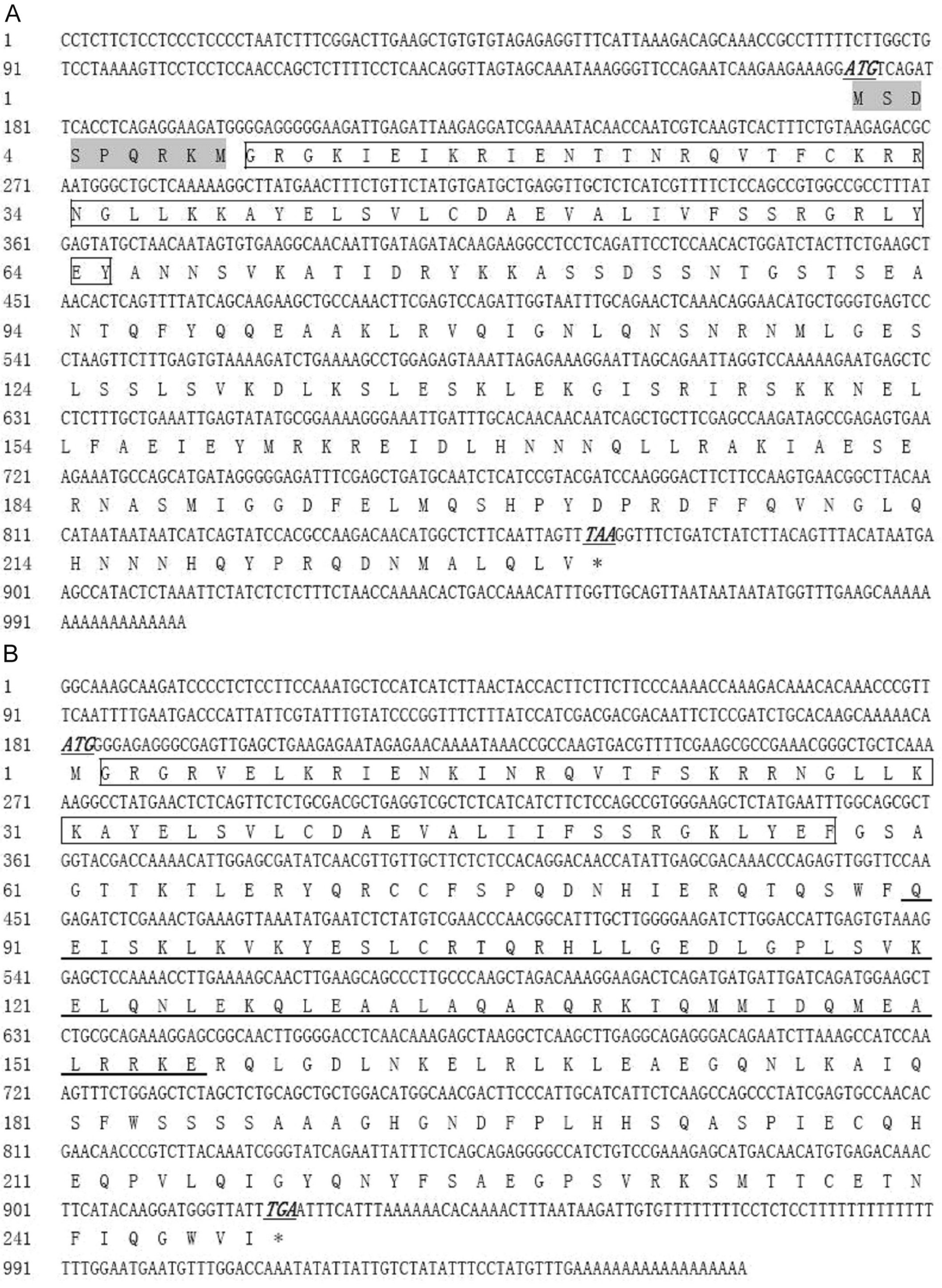
Isolation of two full-length MADS-box cDNAs: Total RNA was isolated from floral bud of bitter gourd using plant RNA extraction kit (*TaKaRa,* Dalian, China) according to the manufacture's instructions. cDNA was synthesized with the first-strand cDNA synthesis kit (*TaKaRa*) and used as a template for reverse transcriptase polymerase chain reaction (RT-PCR) amplification with two MADS-box degenerate primers: P1: 5'-CTG AAG MGR ATM GAG AAC-3'(LKRIEN) and P2: 5'-GAC MAA TMG WCA AGT YAC-3'(TNRQVT). PCR products were cloned and sequenced. Partial sequence for *MCAG2* and *MCAG6* that showed high sequence identity to *AG*-like and *AGL6*-like MADS-box genes were identified. Upstream sequences overlapping with the 3' fragments were isolated by 5' RACE method (Cold Spring Harbour Laboratory 2005). Based on the nucleotide sequence of the 3' and 5' RACE products, the gene-specific primers were designed for the amplification of full length cDNA of *MCAG2* and *MCAG6*. The gene-specific primers of *MCAG2* were P3: 5'-AGA GGG TGG AAG ATG GGG AG-3' and P4: 5'-CTC CAA AAG TAG CTG CCA ACA-3'; the gene-specific primers of *MCAG6* were P5: 5'-ACA ATG GGG AGA GGG CGA GT-3' and P6: 5'-TCA AAT AAC CCA TCC TTG TAT G-3'.

Comparison and phylogenetic analysis: Amino acid sequence analysis and multiple alignments were conducted on internet network (http://www.expasy.ch). The phylogenetic tree was constructed by software *DNAMAN 3.0* using neighbor-joining method.

RT-PCR analysis: Total RNA was extracted at different plant tissues according to the protocol described above. Single-strand cDNA was synthesized with the first-strand cDNA Synthesis kit with oligo(dT) primer. PCR was performed using undiluted and four dilutions of cDNAs as follows: the reactions were heated to 95 °C for 4 min, followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, then a single step at 72 °C for 7 min. As a marker for constitutive expression, the *MC18S rRNA* gene (GenBank accession No. AY900000) was amplified with the primers *MC18sA* (5'-CTG AGA AAC GGC TAC CAC AT-3') and *MC18sB* (5'-GAG CGT AGG CTT GCT TTG AG-3'). *MCAG2* cDNA was amplified using primers *MCAG2F* (5'-ATG CTG GGT GAG TCC CTA AGT-3') and *MCAG2R* (5'-GAT CTT GTC TTG GCG TGG ATA-3'). *MCAG6* cDNA was amplified using the primers *MCAG6F* (5'-ATT GAG CGA CAA ACC CAG-3') and *MCAG6R* (5'-GTT GTC ATG CTC TTT CGG A-3').

Quantitative real-time RT-PCR analysis: Single-strand cDNA was transcribed from 500 ng total DNA-free RNA using the ExScript^{TM} RTase (*TaKaRa*) according to protocol with a Oligo(dT) primer. The synthesized cDNA was diluted 1:2 in ddH$_2$O before proceeding to quantitative real-time RT-PCR. The fragments of *MC18S rRNA* (932 bp), *MCAG2* (324 bp) and *MCAG6* ORF (744 bp) were obtained by PCR amplified and were purified using the gel extraction kit. Copy numbers were calculated by using the fragment concentrations (quantified on the *PUXI TU-1800*, Beijing, China). Five 10-fold serial dilutions covering a range from 10^3 to 10^8 of DNA fragments were used to determine the standard curves.

Quantitative real-time RT-PCR was carried out using *TaKaRa SYBR* ® *ExScriptTM RT-PCR* kit and quantified the PCR amplification according to the manufacturer's protocol. Amplification was performed on an *iCycler iQTM* multicolor real time PCR detection system (*Bio-Rad*, hercules, USA). Each 0.025 cm^3 reaction mixture contains one set of primers (*MC18s Rtime1*: 5'-TGC CCG TTG CTC TGA TGA TTC-3' and *MC18s Rtime2*: 5'-CTG CTG CCT TCC TTG GAT GTG-3'; *MCAG2RTime1*: 5'-GCC GAG AGT GAA AGA AAT GCC AG-3' and *MCAG2RTime2*: 5'-ATG TTG TCT TGG CGT GGA TAC TGA-3'; *MCAG6RTime1*: 5'-CAT CAT TCT CAA GCC AGC CCT ATC-3' and *MCAG2RTime6*: 5'-ACC CAT CCT TGT ATG AAG TTT GTC TC-3') in a final concentration of 0.2 µM each primer and 0.002 cm^3 the diluted cDNA template or standard DNA fragments. No template controls were run to determine contamination and level of primer dimer formation. All standards were performed in duplicate and samples in triplicate. Standard curves and gene expression levels were analyzed using the *iCycler* system software (*Bio-Rad*). To make it possible to compare gene expression levels in the different plant tissues, they were normalized to the expression of *MC18S rRNA* in each tissue. Relative expression of *MCAG2* and *MCAG6* in different plant tissues were obtained by dividing the average number of copies by the copy number of *MC18S rRNA* for the same tissues.



```
A
1     CCTCTTCTCCTCCCTCCCCTAATCTTTCGGACTTGAAGCTGTGTGTAGAGAGGTTTCATTAAAGACAGCAAACCGCCTTTTTCTTGGCTG
91    TCCTAAAAGTTCCTCCTCCAACCAGCTCTTTTCCTCAACAGGTTAGTAGCAAATAAAGGGTTCCAGAATCAAGAAGAAAGGATGTCAGAT
1                                                                                       M  S  D
181   TCACCTCAGAGGAAGATGGGGAGGGGGAAGATTGAGATTAAGAGGATCGAAAATACAACCAATCGTCAAGTCACTTTCTGTAAGAGACGC
4      S  P  Q  R  K  M  G  R  G  K  I  E  I  K  R  I  E  N  T  T  N  R  Q  V  T  F  C  K  R  R
271   AATGGGCTGCTCAAAAAGGCTTATGAACTTTCTGTTCTATGTGATGCTGAGGTTGCTCTCATCGTTTTCTCCAGCCGTGGCCGCCTTTAT
34     N  G  L  L  K  K  A  Y  E  L  S  V  L  C  D  A  E  V  A  L  I  V  F  S  S  R  G  R  L  Y
361   GAGTATGCTAACAATAGTGTGAAGGCAACAATTGATAGATACAAGAAGGCCTCCTCAGATTCCTCCAACACTGGATCTACTTCTGAAGCT
64     E  Y  A  N  N  S  V  K  A  T  I  D  R  Y  K  K  A  S  S  D  S  S  N  T  G  S  T  S  E  A
451   AACACTCAGTTTTATCAGCAAGAAGCTGCCAAACTTCGAGTCCAGATTGGTAATTTGCAGAACTCAAACAGGAACATGCTGGGTGAGTCC
94     N  T  Q  F  Y  Q  Q  E  A  A  K  L  R  V  Q  I  G  N  L  Q  N  S  N  R  N  M  L  G  E  S
541   CTAAGTTCTTTGAGTGTAAAAGATCTGAAAAGCCTGGAGAGTAAATTAGAGAAAGGAATTAGCAGAATTAGGTCCAAAAAGAATGAGCTC
124    L  S  S  L  S  V  K  D  L  K  S  L  E  S  K  L  E  K  G  I  S  R  I  R  S  K  K  N  E  L
631   CTCTTTGCTGAAATTGAGTATATGCGGAAAAGGGAAATTGATTTGCACAACAACAATCAGCTGCTTCGAGCCAAGATAGCCGAGAGTGAA
154   L  F  A  E  I  E  Y  M  R  K  R  E  I  D  L  H  N  N  N  Q  L  L  R  A  K  I  A  E  S  E
721   AGAAATGCCAGCATGATAGGGGGAGATTTCGAGCTGATGCAATCTCATCCGTACGATCCAAGGGACTTCTTCCAAGTGAACGGCTTACAA
184    R  N  A  S  M  I  G  G  D  F  E  L  M  Q  S  H  P  Y  D  P  R  D  F  F  Q  V  N  G  L  Q
811   CATAATAATAATCATCAGTATCCACGCCAAGACAACATGGCTCTTCAATTAGTTTAAGGTTTCTGATCTATCTTACAGTTTACATAATGA
214    H  N  N  N  H  Q  Y  P  R  Q  D  N  M  A  L  Q  L  V  *
901   AGCCATACTCTAAATTCTATCTCTCTTTCTAACCAAAACACTGACCAAACATTTGGTTGCAGTTAATAATAATATGGTTTGAAGCAAAAA
991   AAAAAAAAAAAAA
B
1     GGCAAAGCAAGATCCCCTCTCCTTCCAAATGCTCCATCATCTTAACTACCACTTCTTCTTCCCAAAACCAAAGACAAACACAAACCCGTT
91    TCAATTTTGAATGACCCATTATTCGTATTTGTATCCCGGTTTCTTTATCCATCGACGACGACAATTCTCCGATCTGCACAAGCAAAAACA
181   ATGGGAGAGGGCGAGTTGAGCTGAAGAGAATAGAGAACAAAATAAACCGCCAAGTGACGTTTTCGAAGCGCCGAAACGGGCTGCTCAAA
1      M  G  R  G  R  V  E  L  K  R  I  E  N  K  I  N  R  Q  V  T  F  S  K  R  R  N  G  L  L  K
271   AAGGCCTATGAACTCTCAGTTCTCTGCGACGCTGAGGTCGCTCTCATCATCTTCTCCAGCCGTGGGAAGCTCTATGAATTTGGCAGCGCT
31     K  A  Y  E  L  S  V  L  C  D  A  E  V  A  L  I  I  F  S  S  R  G  K  L  Y  E  F  G  S  A
361   GGTACGACCAAAACATTGGAGCGATATCAACGTTGTTGCTTCTCTCCACAGGACAACCATATTGAGCGACAAACCCAGAGTTGGTTCCAA
61     G  T  T  K  T  L  E  R  Y  Q  R  C  C  F  S  P  Q  D  N  H  I  E  R  Q  T  Q  S  W  F  Q
451   GAGATCTCGAAACTGAAAGTTAAATATGAATCTCTATGTCGAACCCAACGGCATTTGCTTGGGGAAGATCTTGGACCATTGAGTGTAAAG
91     E  I  S  K  L  K  V  K  Y  E  S  L  C  R  T  Q  R  H  L  L  G  E  D  L  G  P  L  S  V  K
541   GAGCTCCAAAACCTTGAAAAGCAACTTGAAGCAGCCCTTGCCCAAGCTAGACAAAGGAAGACTCAGATGATGATTGATCAGATGGAAGCT
121    E  L  Q  N  L  E  K  Q  L  E  A  A  L  A  Q  A  R  Q  R  K  T  Q  M  M  I  D  Q  M  E  A
631   CTGCGCAGAAAGGAGCGGCAACTTGGGGACCTCAACAAAGAGCTAAGGCTCAAGCTTGAGGCAGAGGGACAGAATCTTAAAGCCATCCAA
151    L  R  R  K  E  R  Q  L  G  D  L  N  K  E  L  R  L  K  L  E  A  E  G  Q  N  L  K  A  I  Q
721   AGTTTCTGGAGCTCTAGCTCTGCAGCTGCTGGACATGGCAACGACTTCCCATTGCATCATTCTCAAGCCAGCCCTATCGAGTGCCAACAC
181    S  F  W  S  S  S  S  A  A  A  G  H  G  N  D  F  P  L  H  H  S  Q  A  S  P  I  E  C  Q  H
811   GAACAACCCGTCTTACAAATCGGGTATCAGAATTATTTCTCAGCAGAGGGGCCATCTGTCCGAAAGAGCATGACAACATGTGAGACAAAC
211    E  Q  P  V  L  Q  I  G  Y  Q  N  Y  F  S  A  E  G  P  S  V  R  K  S  M  T  T  C  E  T  N
901   TTCATACAAGGATGGGTTATTTGAATTTCATTTAAAAAACACAAAACTTTAATAAGATTGTGTTTTTTTTCCTCTCCTTTTTTTTTTTTT
241    F  I  Q  G  W  V  I  *
991   TTTGGAATGAATGTTTGGACCAAATATATTATTGTCTATATTTCCTATGTTTGAAAAAAAAAAAAAAAAAA
```

Fig. 1. *A* - Nucleotide and deduced amino acid sequences of *MCAG2* cDNA. The MADS-box region was boxed and the N-terminal extension preceding the MADS domain was gray-shaded. The start codon (ATG) and stop codon (TAA) are in italic. *B* - Nucleotide and deduced amino acid sequences of *MCAG6* cDNA. The MADS-box region is boxed and the K domain is underlined. The start codon (ATG) and stop codon (TGA) are in italic. The positions of nucleotides and amino acids were shown on the left. (*MCAG2*: GenBank accession No. DQ299943; *MCAG6*: GenBank accession No. DQ431247).

Results

Cloning and sequencing of the full-length cDNA of *MCAG2* and *MCAG6*: A combined RT-PCR and 5'RACE strategy was used to isolate MADS-box genes from bitter gourd. Two full-length MADS-box genes designated *MCAG2* (GenBank accession No. DQ299943) and *MCAG6* (GenBank accession No. DQ431247) were isolated. *MCAG2* cDNA is 1 003 bp in length and contains an ORF of 693 bp, which encodes a 231 amino acids protein with an N-terminal extension preceding the MADS domain (Fig. 1*A*), Mr was 26.5 kDa, isoelectric point 9.59. *MCAG6* cDNA is 1 061 bp in length and contains an ORF of 741 bp, which encodes a 247 amino acids protein, Mr of 28.4 kDa, isoelectric point 9.3 (Fig. 1*B*).

Comparison analysis of *MCAG2* and *MCAG6*: The deduced amino-acid sequences of *MCAG2* and *MCAG6* were compared with other protein sequences found on the GenBank database using the *BLAST* search program. *MCAG2* had high similarities of 95, 80, 77, 68 and 64.4 % with the proteins for the published sequences of *CUM1*, *PpMADS4*, *MADS1*, *AGAMOUS* and *CsAG1* (Tsaftaris *et al.* 2005), respectively. *MCAG6* had high similarities of 80, 77, 68 and 63 % with the proteins for the published sequences of *VvMADS3*, *MdMADS11*, *GRCDS3* and *AGL6*, respectively.

Multiple sequence alignments demonstrated that the *MCAG2* and *MCAG6* proteins had the typical MIKC-type domain structure. The highly conserved MADS-domain is the major determinant of DNA-binding and performs dimerization and accessory factor binding functions (Shore and Sharrocks 1995). The less conserved K-domain is proposed to allow for the formation of an amphipathic helix involved in protein dimerization (Ma *et al.* 1991, Shore and Sharrocks 1995). The relatively weakly conserved I domain constitutes a molecular determinant for the selective

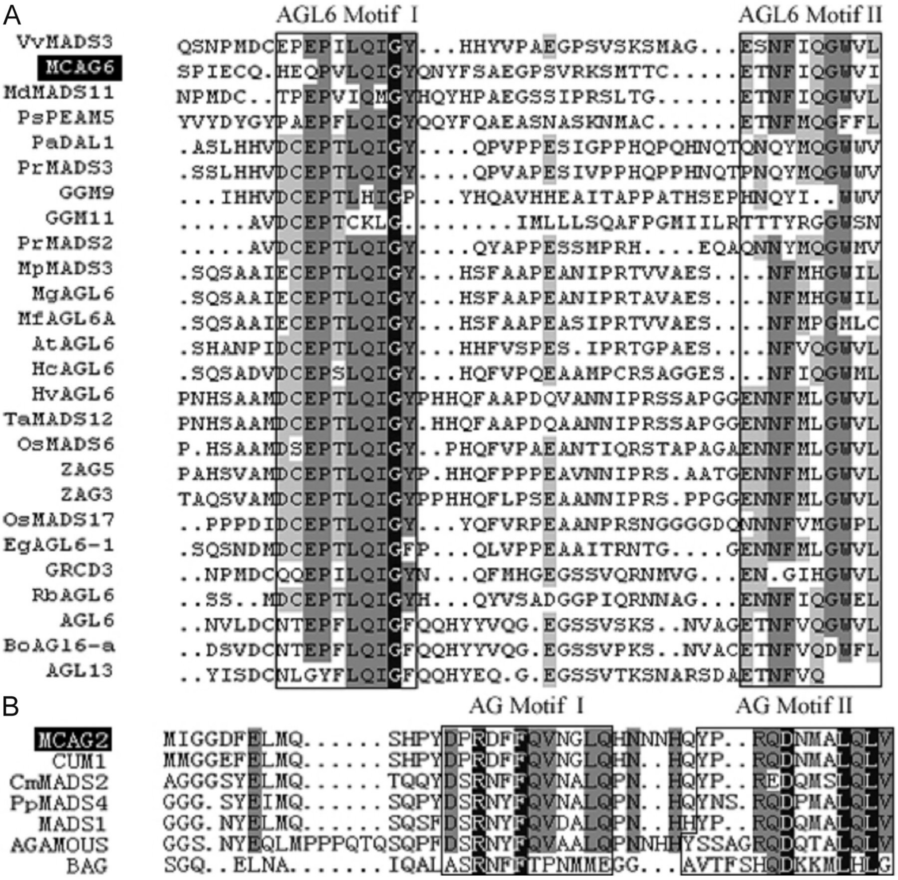


Fig. 2. *A* - Alignment of the C-terminal regions of predicted amino acid sequences for select representatives of 26 *AGL6*-like genes. Two highly conserved regions, the AGL6 motif I and AGL6 motif II, are indicated with two boxes. *B* - Alignment of the C-terminal regions of *AG*-like genes. The two highly conserved regions, the AG motif I and AG motif II, are indicated with two boxes. The alignment was generated by the DNAMAN3.0 with the multiple alignment parameters gap penalty 8, gap extension penalty 2, and PAM protein mass matrix with 100 bootstrap trails. The identical amino acid residues are indicated with black background and the different amino acid residues with white background. Gray shade indicates 70 % or more conservation among all the aligned sequences. The taxa of origin for these genes were noted in Fig. 3.

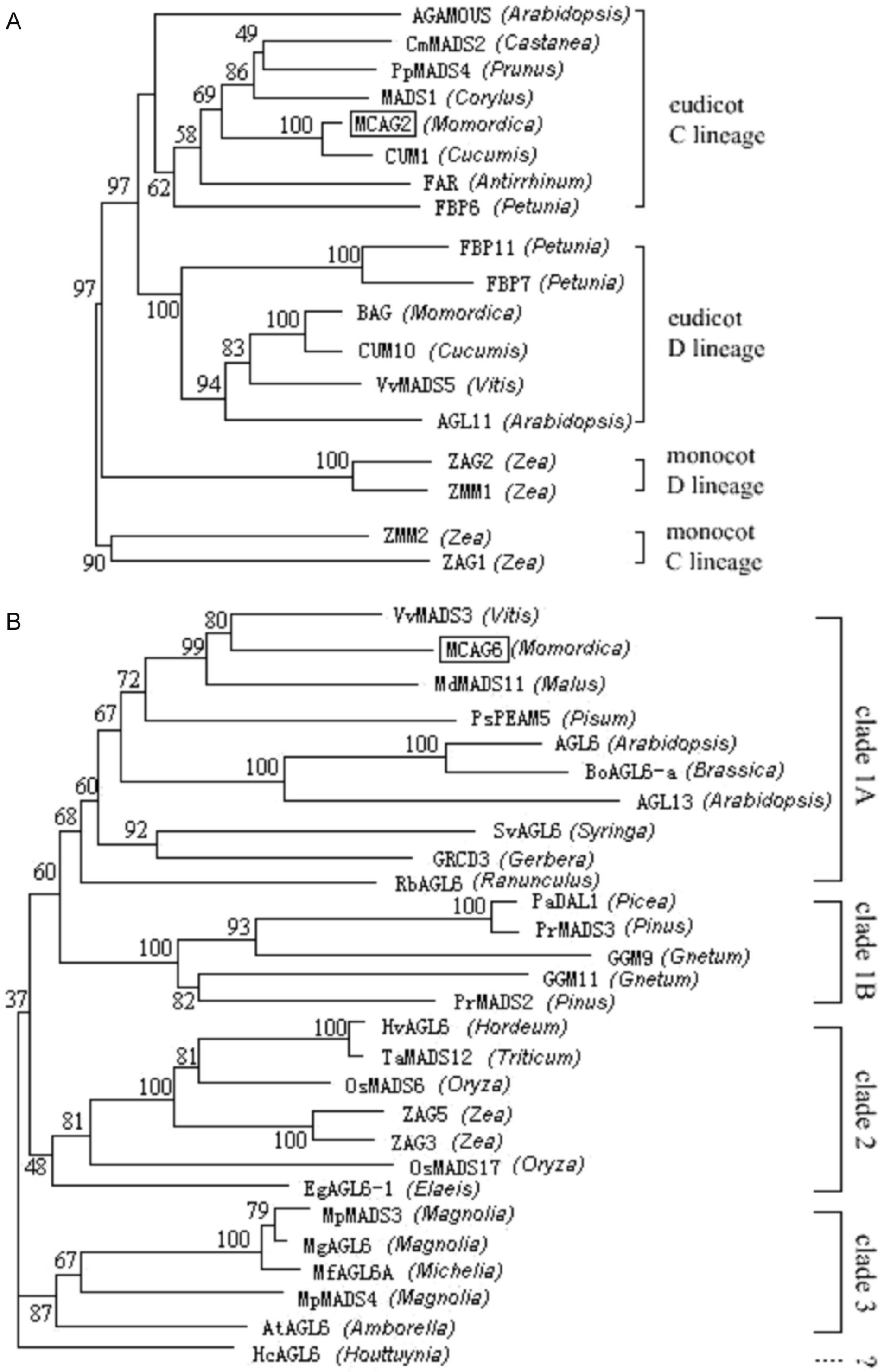


Fig. 3. *A* - Phylogenetic trees of *AG*-like genes generated by the NJ algorithm. *MCAG2* from bitter gourd is *boxed*. Four lineages are marked by the vertical line. *B* - Phylogenetic trees of *AGL6*-like genes generated by the NJ algorithm. *MCAG6* from bitter gourd is *boxed*. Three major clades are marked by the vertical line. *Clade 1A* - eudicot plants, *clade 1B* - gymnosperm plants, *clade 2* - monocot plants, *clade 3* - basal angiosperm plants. Numbers below the branches represent bootstrap values from 100 replicates. The taxon of origin are showed in *parentheses* after each gene name.

formation of DNA-binding dimmers (Riechmann and Meyerowitz 1997). The more divergent C terminal domain is involved in transcriptional activation, or the formation of multimeric transcription factor complexes (Cho *et al*. 1999, Egea-Cortines *et al*. 1999). Similarity is also found at the C-terminus of the protein. The

C-terminus of *MCAG2* contains two highly conserved regions, which are AG motif I and AG motif II (Fig. 2B). In order to investigate the similarity of *MCAG6* at the C-terminus, the C-terminal regions of predicted amino acid sequences for select representatives of 26 *AGL6*-like genes were aligned in this study. All C-terminus of *AGL6*-like proteins presented two short, highly conserved regions, which were named *AGL6* motif I and *AGL6* motif II. Both of the motifs were composed of 10 amino acid residues (Fig. 2*A*).

Phylogenetic analysis of *MCAG2* and *MCAG6*: To determine the phylogenetic position of the *MCAG2* and *MCAG6*, we conducted phylogenetic analysis of *AG*-like and *AGL6*-like genes for a data set including most of the published genes in the families. Phylogeny reconstruction indicated that the *MCAG2* fell into a group of *AG-like* genes which belong to the eudicot C lineage (Becker and Theiβen 2003; Fig. 3*A*). For *AGL6*-like genes, we used the 28 amino acid sequences of the I, K, and C domain for this analysis. The dendrogram showed that the *AGL6* homologs formed three clades. Clade 1 was distinctly divided into two groups: eudicots and gymnosperms, named clade 1A and clade 1B; the monocots formed the clade 2; and the basal angiosperms, such as *Magnolia*, formed the clade 3. In this dendrogram, *MCAG6* fell into calde 1A and was closer to *VvMADS3* than other *AGL6* homologs (Fig. 3*B*).

Fig. 4. RT-PCR analysis of the expression pattern of *MCAG2* and *MCAG6*. Specific primers were used to amplify a 324 bp (*MCAG2*) and 467 bp (*MCAG6*) fragment with cDNA from root, stem, leaf, shoot apex, male bud, female bud and young fruit. 1~5 denote the five 5-fold cDNA serial dilutions from high to low separately. *MC18S rRNA* (392 bp) gene was used as an RT-PCR positive control.

RT-PCR analysis of *MCAG2* and *MCAG6*: Transcripts of *MCAG2* were present in flower buds and young fruits but none in other tissues by using the RT-PCR analysis (Fig. 4). The level of *MCAG2* transcripts, judged by the amount of RT-PCR product obtained and normalized by *18S rRNA*, was obviously higher in female buds compared with male buds and young fruits. The transcripts of *MCAG6* were detected in male buds, female buds and shoot apexes (Fig. 4). And the level of *MCAG6* was obviously higher in shoot apexes compared with male buds and female buds.

Quantitative real-time RT-PCR analysis of *MCAG2* and *MCAG6*: To determine the detail expression patterns of the *MCAG2* and *MCAG6* in different tissues, we examined the mRNA levels by quantitative real-time RT-PCR (Fig. 5). The PCR special normalization of gene expression showed that the expression of *MCAG2* was very low or none in petal, calyx, and shoot apex, high in carpel and moderate in stamen and young fruit (Fig. 5). The transcripts of the *MCAG6* were found very low or none in young fruit, carpel, and stamen, high in shoot apex, and moderate in calyx and petal (Fig. 5).

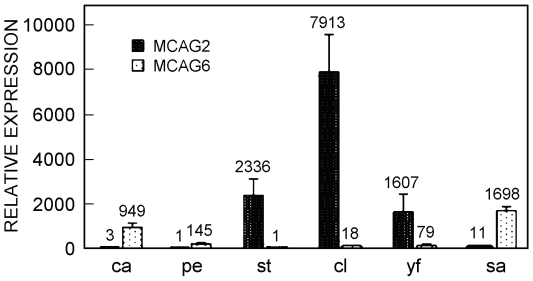


Fig. 5. Quantification of expression levels of the *MCAG2* and *MCAG6* genes in different tissues of bitter gourd as determined by quantitative real-time RT-PCR analysis. The housekeeping gene *MC18S rRNA* was used to normalize the amount of sample cDNA added to the reaction. Relative quantification was performed using the comparative C_T method, in which simplified arithmetic formulas ($2^{-\Delta\Delta C_t}$) are used to obtain the same result as the one yielded by the relative standard curve method when the target gene and the reference control gene have approximately equal amplification efficiency (≥ 95 %). The PCR efficiency for *MCAG2*, *MCAG6*, and *MC18S rRNA* were above 95 % (data not show). The lowest number in this case (*MCAG2* in petal; *MCAG6* in stamen) was set to a value of 1 and subsequently expression levels are relative to this number. The relative quantification values of different tissues are showed above each vertical bar. *Bars* indicated standard deviation from triplicate amplification of the *MCAG2* and *MCAG6* genes samples respectively. ca - calyx, pe - petal, st - stamen, cl - carpel, yf - young fruit, sa - shoot apex.

Discussion

Bitter gourd is an important economic crop. To study the floral-organ identity genes of bitter gourd, two cDNA clone of *MCAG2* and *MCAG6* were isolated from the female bud based on the MADS box conserved sequences. *BLAST* search and phylogeny reconstruction revealed that the deduced protein sequence of *MCAG2* shared high sequence identity with the *AG-like* genes, such as *CUM1*, *PpMADS4*, *MADS1* and *AGAMOUS*. Phylogenetic analysis revealed that *MCAG2* fell into the eudicot C lineage. Zahn *et al*. (2006) and Kramer *et al*. (2004) discussed the evolutionary relationship and the conserved structures among the *AG-like* genes. Almost all the function C genes have two unique features in their sequences. First, an N-terminal extension preceding the MADS domain is present. Second, the genomic sequence of a function C gene contains 8 introns. Sequence analysis of *MCAG2* showed that two conserved *AG* motif I and *AG* motif II were present at the C-terminus and N-terminal extension was present before the MADS domain. These data suggest that *MCAG2* is an *AG-like* gene and might belong to class C gene.

Previously, *BAG*, another bitter gourd *AG*-like gene, was isolated and belonged to the class C gene (Yang *et al.* 2004). Among the model plants, only one class C gene (*AGAMOUS*) was isolated from *Arabidopsis*, while two class C genes, *PLENA* (*PLE*) and *FARINELLI* (*FAR*), were cloned from *Antirrhinum* (Davies *et al.* 1999). The duplication of the class C gene goes not singly but in pairs. Cucumber (Kater *et al*. 1998, Treves *et al.* 1998), gerbera (Yu *et al.* 1999), maize (Theissen *et al.* 1995), petunia (Kater *et al.* 1998, Tsuchimoto *et al.* 1993), poplar (Brunner *et al.* 2000), tobacco (Kempin *et al.* 1993, Zhang 1998), and tomato (Pnueli *et al.* 1991, 1994) have two *AG* homologues. Comparison and phylogenetic analysis suggested that *MCAG2* had high similarities (95 %) with the proteins of *CUM1* and same N-terminal extension region, and *BAG* had 95 % similarities with the proteins of *CUM10* while both had no N-terminal extension region. Therefore, *MCAG2* is closer to *CUM 1*, while *BAG* is closer to *CUM10*. The expressions of *CUM1* and *CUM10* were restricted to whorl 3 in male and whorl 4 in female cucumber flowers. Ectopic expression of the cucumber *AG* homologue, *CUM1*, caused a complete homeotic conversion of sepals into carpels and petals into stamens, while the other *AG* homologue, *CUM10* resulted in plants with partial transformations of the petals into antheroid structures, indicating that *CUM10* is also able to promote floral organ identity (Kater *et al.* 1998). The two bitter gourd *AG* homologues, *BAG* and *MCAG2*, were both expressed in male (stamen) and female reproductive organs (carpel), especially expressed highly in carpel (Yang *et al.* 2004). However, *BAG* was restricted to be expressed in carpel and stamen (Yang *et al.* 2004), while *MCAG2* was expressed not only in carpel and stamen but also in young fruit. Thus, *MCAG2*, the second class C gene, is redundant to identify the carpel while different effective to identify the stamen, and young fruit in bitter gourd.

BLAST searches and phylogeny reconstruction revealed that the deduced protein sequence of *MCAG6* shared high sequence identity with the *AGL6*-like genes. Only few *AGL6*-like genes have been isolated from diverse angiosperms and gymnosperms (Favaro *et al.* 2002, Boss *et al*. 2002, Hecht *et al*. 2005) (Fig. 2*A*). All *AGL6*-like genes seem to have a common feature: expression in reproductive organs. By the RT-PCR analysis, the transcripts of *MCAG6* were detected in male flower bud, female bud, and shoot apex (Fig. 4). The feature of expression in reproductive organs coincides with those of other *AGL6*-like genes.

By the quantitative real-time RT-PCR, the transcripts of the *MCAG6* were found very high in shoot apex. High expression of *MCAG6* in shoot apex was very interesting because expression in vegetative organs was not common in other organisms *AGL6*-like genes. Expression in vegetative shoots was also detected in *DAL1* which might have a regulatory role in the juvenile-to-adult transition in Norway spruce (Carlsbecker *et al*. 2004). In model species, *Arabidopsis AGL6* was preferentially expressed in flowers and faintly expressed in stems (Ma *et al*. 1991) and *AGL13* was expressed in the inflorescence and siliques and at low levels in leaves and seedlings (Rounsley *et al.* 1995). This expression pattern strongly supported that *MCAG6*, *DAL1*, *AGL6*, and *AGL13* fell into the same clade (Fig. 3).

References

Angenent, G.C., Colombo, L.: Molecular control of ovule development. - Trends Plant Sci. **1**: 228-232, 1996.

Becker, A., Theissen, G.: The major clades of MADS-box genes and their role in the development and evolution of flowering plants. - Mol. Phylogenet. Evol. **l29**: 464-489, 2003.

Boss, P.K., Sensi, E., Hua, C., Davies, C., Thomas. M.R.: Cloning and characterisation of grapevine (*Vitis vinifera* L.) MADS box genes expressed during inflorescence and berry development. - Plant Sci. **162**: 887-895, 2002.

Bowman, J.L., Smyth, D.R., Meyerowitz, E.M.: Genetic interactions among floral homeotic genes of *Arabidopsis*. - Development **112**: 1-20, 1991.

Brunner, A.M., Rottmann, W.H., Sheppard, L.A., Krutovskii, K., DiFazio, S.P., Leonardi, S., Strauss, S.H.: Structure and expression of duplicate *AGAMOUS* orthologues in poplar. - Plant mol. Biol. **44**: 619-634, 2000.

Carlsbecker, A., Tandre, K., Johanson, U., Englund, M., Engstrom, P.: The MADS box gene *DAL1* is a potential mediator of the juvenile-to-adult transition in Norway spruce (*Picea abies*). - Plant J. **40**: 546-557, 2004.

Cho, S., Jang, S., Chae, S., Chung, K.M., Moon, Y.H., An, G., Jang, S.K.: Analysis of the C-terminal region of *Arabidopsis thaliana APETALA1* as a transcription activation domain. - Plant mol. Biol. **40**: 419-429, 1999.

Coen, E.S., Meyerowitz, E.M.: The war of the whorls, genetic interaction controlling flower development. - Nature **353**: 31-37, 1991.

Davies, B., Motte, P., Keck, E., Saedler, H., Sommer, H., Schwarz-Sommer, Z.: *PLENA* and *FARINELLI*, redundancy and regulatory interactions between two *Antirrhinum* MADS-box factors controlling flower development. - EMBO J. **18**: 4023-4034, 1999.

Egea-Cortines, M., Saedler, H., Sommer, H.: Ternary complex formation between the MADS-box proteins *SQUAMOSA*, *DEFICIENS* and *GLOBOSA* is involved in the control of floral architecture in *Antirrhinum majus*. - EMBO J. **18**: 5370-5379, 1999.

Favaro, R., Immink, R.G., Ferioli, V., Bernasconi, B., Byzova, M., Angenent, G.C., Kater, M., Colombo, L.: Ovule-specific MADS-box proteins have conserved protein-protein interactions in monocot and dicot plants. - Mol. Genet. Genomics **268**: 152-159, 2002.

Hecht, V., Foucher, F., Ferrandiz, C., Macknight, R., Navarro, C., Morin, J., Vardy, M.E., Ellis, N., Beltran, J.P., Rameau, C., Weller, J.L.: Conservation of *Arabidopsis* flowering genes in model Legumes. - Plant Physiol. **137**: 1420-1434, 2005.

Ho, W.K.K., Liu, S.C., Shaw, P.C., Yeung, H.W., Ng, T.B., Chan, W.Y.: Cloning of cDNA of α-momorcharin, a new ribosome-inactivating protein. - J. mol. Biol. **214**: 625-626, 1991.

Kater, M.M., Colombo, L., Franken, J., Busscher, M., Masiero, S., Van Lookeren Campagne, M.M., Angenent G.C.: Multiple *AGAMOUS* homologs from cucumber and petunia differ in their ability to induce reproductive organ fate. - Plant Cell. **10**: 171-182, 1998.

Kempin, S.A., Mandel, M.A., Yanofsky, M.F.: Conversion of perianth into reproductive organs by ectopic expression of the tobacco floral homeotic gene *NAG1*. - Plant Physiol. **103**: 1041-1046, 1993.

Kramer, E.M., Jaramillo, M.A., Di-Stilio, V.S.: Patterns of gene duplication and functional evolution during the diversification of the *AGAMOUS* subfamily of MADS box genes in angiosperms. - Genetics **166**: 1011-1023, 2004.

Ma, H., Yanofsky, M.F., Meyerowitz, E.M.: *AGL1-AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. - Genes Dev. **5**: 484-495, 1991.

Norman, C., Runswick, M., Pollock, R., Treisman, R.: Isolation and properties of cDNA clones encoding *SRF*, a transcription factor that binds to the *c-Fos* serum response element. - Cell **55**: 989-1003, 1988.

Passmore, S., Maine, G.T., Elble, R., Christ, C., Tye, B.K.: *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating of MAT-alpha cells. - J. mol. Biol. **204**: 593-606, 1988.

Pnueli, L., Abeid, M.A., Zamir, D., Nacken, W., Sommer, Z.S., Lifschitz, E.: The MADS box gene family in tomato, temporal expression during floral development. - Plant J. **1**: 255-266, 1991.

Pnueli, L., Hareven, D., Rounsley, S.D., Yanofsky, M.F., Lifschitz, E.: Isolation of the tomato *AGAMOUS* gene *TAG1* and analysis of its homeotic role in transgenic plants. - Plant Cell **6**: 163-173, 1994.

Riechmann, J.L., Meyerowitz, E.M.: MADS domain proteins in plant development. - Biol. Chem. **378**: 1079-1101, 1997.

Rounsley, S.D., Ditta, G.S., Yanofsky, M.F.: Diverse roles for MADS box genes in *Arabidopsis* development. - Plant Cell **7**: 1259-1269, 1995.

Schwarz-Sommer, Z., Hue, I., Huijser, P., Flor, P.J., Hansen, R., Tetens, F., Lonnig, W.E., Saedler, H., Sommer, H.: Characterization of the *Antirrhinum* floral homeotic MADS-box gene *DEFICIENS*, evidence for DNA binding and autoregulation of its persistent expression throughout flower development. - EMBO J. **11**: 251-263, 1992.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H., Sommer, H.: Genetic control of flower development by homeotic genes in *Antirrhinum majus*. - Science **250**: 931-936, 1990.

Shore, P., Sharrocks, A.D.: The MADS-box family of transcription factors. - Eur. J. Biochem. **229**: 1-13, 1995.

Sommer, H., Beltran, J.P., Huijser, P., Pape, H., Lonnig, W.E., Saedler, H., Schwarz-Sommer, Z.: *DEFICIENS*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*, the protein shows homology to transcription factors. - EMBO J. **9**: 605-613, 1990.

Theissen, G.: Development of floral organ identity: stories from the MADS house. - Curr. Opin. Plant Biol. **4**: 75-85, 2001.

Theissen, G., Saedler, H.: MADS-box genes in plant ontogeny and phylogeny, Haeckel's 'biogenetic low' revisited. - Curr. Opin. Genet. Dev. **5**: 628-639, 1995.

Theissen, G., Saedler, H.: Floral quartets. - Nature **409**: 469-471, 2001.

Theissen, G., Strater, T., Fischer, A., Saedler, H.: Structural characterization, chromosomal localization and phylogenetic evaluation of two pairs of *AGAMOUS*-like MADS-box genes from maize. - Gene **156**: 155-166, 1995.

Treves, R.P., Kahana, A., Rosenman, N., Xiang, Y., Silberstein, L.: Expression of multiple *AGAMOUS*-like genes in male and female flowers of cucumber (*Cucumis sativus* L.). - Plant Cell Physiol. **39**: 701-710, 1998.

Tsaftaris, A.S., Pasentsis, K., Polidoros, A.N.: Isolation of a differentially spliced C-type flower specific *AG*-like MADS-box gene from *Crocus sativus* and characterization of its expression. - Biol. Plant. **49**: 499-504, 2005.

Tsuchimoto, S., Van der Krol, A.R., Chua, N.H.: Ectopic expression of *pMADS3* in transgenic petunia phenocopies the petunia blind mutant. - Plant Cell **5**: 843-853, 1993.

Wang, Q.M., Zeng, G.W.: Studies on expression of sex differentiation program in *Momordica charantia*. - J. Zhejiang Univ. (Agr. Life Sci.). **28**: 243-248, 2002.

Wang, S., Tang, L., Chen, F.: *In vitro* flowering of bitter melon. - Plant Cell Rep. **20**: 393-397, 2001.

Weigel, D., Meyerowitz, E.M.: The ABCs of floral homeotic genes. - Cell **78**: 203-209, 1994.

Yang, M.Y., Zhao, M.J., Xu, Y., Tang, L., Bai, J., Chen, F.: [Cloning and expression of a MADS box gene from bitter melon (*Momordica charantia* L.).] - J. Beijing Forest Univ. **26**: 30-34, 2004. [In Chin.]

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., Meyerowitz, E.M.: The protein encoded by the Arabidopsis homeotic gene agamous resembles transcription factors. - Nature **346**: 35-39, 1990.

Yu, D., Kotilainen, M., Pollanen, E., Mehto, M., Elomaa, P., Helariutta, Y., Altert, V.A., Teeri, T.H.: Organ identity genes and modified patterns of flower development in *Gerbera hybrida* (*Asteraceae*). - Plant J. **17**: 51-62, 1999.

Zahn, L.M., Leebens-Mack, J.H., Arrington, J.M., Hu, Y., Landherr, L.L., dePamphilis, C.W., Becker, A., Theissen, G., Ma, H.: Conservation and divergence in the *AGAMOUS* subfamily of MADS-box genes, evidence of independent sub- and neofunctionalization events. - Evol. Dev. **8**: 30-45, 2006.

Zhang, J.S., Wu, Y.H., Li, Q., Li, Y.: Molecular cloning and characterization of *NFBP6*, a putative floral homeotic gene, from tobacco. - Sex Plant Reprod. **11**: 113-116, 1998.

Campbell, A., Anderson, W.W, Jones, E.W. (ed.): **Annual Review of Genetics. Vol. 410**. - Annual Reviews, Palo Alto 2007. 564 pp. ISBN 978-0-8243-1241-1

The Introductory chapter deals with the work and life of Allan Campbell who proposed in 1962 a model for integration of λ prophage into the bacterial chromosome. The readers of Biologia Plantarum may find interesting the reviews: “Epigenetic control of centromere behavior” (K. Ekwall), “The origin and establishment of the plastid in algae and plants” (A. Reyes-Prieto, A. P.M. Weber, D. Bhattacharya), “Biotin sensing: universal influence of biotin status on transcription” (D. Beckett), “Mendelian genetics of apomixes in plants” (P. Ozias-Akins, P.J. Van Dijk), and “Orthology and functional conservation in eukaryotes” (K. Dolonski, D. Botstein). Other reviews covered in this volume: “Bacterial gene regulatory networks”, “SNA12 function in humans and mice”, “Cell turnover and adult homeostasis”, “Immunoglobulin somatic hypermutation”, “Specificity in two-component signaling” “Chromosome fragile sites”, “Genetics of *Candida albicans*”, “Mating-type switching and silencing in fission yeasts”, “MHC, TSP, and the origin of species”, “Intercellular transport of RNAi”, “Eukaryotic DNA transposons”, “Mammalian meiotic recombination hot spots”, “Regulation of sterol synthesis in eukaryotes”, “Systems biology of Caulobacter”, and “Use of Y chromosome and mitochondrial population structure in tracing human migrations”.

Annual Reviews is a nonprofit scientific publisher, so that the Annual Reviews are reasonably priced. Current individual subscriptions include online access to full-text articles, PDFs, reviews in advance (as much as 6 months ahead of print publication), bibliographies, and other supplementary material in the current volume and the prior 4 year´s volumes. All articles are written by the foremost experts in the field. The Annual Review of Genetics is online at http://genet.annualreviews.org

T. GICHNER (*Prague*)