Isolation and Expression Analysis of \textit{DlAP2} Targeted by \textit{miR172a} in \textit{Dendrocalamus latiflorus}

Zhimin Gao, Lili Wang, Hansheng Zhao, Fengbo Li and Ruiguo Chen

International Center for Bamboo and Rattan, SFA Key laboratory of Bamboo and Rattan Science and Technology, Beijing 100102

Abstract

Much attention has been paid to the regulation of bamboo flowering, which has become a hot topic to reveal the regulatory mechanisms at the molecular level through methods of molecular biology. Transcription factors of APETAL2 (AP2) sub-family were involved in the growth process of plant development, such as the regulation of floral meristem, ovule and seed development etc. \textit{AP2} gene belonged to class A genes of flower development model, involving in developmental regulation of petals, while its own expression was regulated by \textit{miR172a}, which targeted \textit{AP2} gene with cutting degradation. One \textit{miR172a}-targeted gene named \textit{DlAP2} was isolated from \textit{Dendrocalamus latiflorus} with the methods of RT-PCR and RACE. Sequence analysis showed that the full length cDNA of \textit{DlAP2} was 1 729 bp including 5' UTR 81 bp, ORF 1 464 bp, 3' UTR 351 bp and 24 bp polyA. \textit{DlAP2} encoded a putative protein with 487 amino acids, which included two AP2 domains indicating that it belonged to AP2 group of AP2 subfamily in AP2/ERF family. \textit{DlAP2} had a high homology with those AP2 from other monocots, among which the highest consistency was 84.6% with that of moso bamboo (\textit{Phyllostachys edulis}) \textit{AP2} (AGH68972), and the consistency with transcription factor AP2D23-like (AAW78371) in model plant rice (\textit{Oryza sativa}) and ARGET OF EARLY ACTIVATION TAGGED1 (T NP_001189625) in \textit{Arabidopsis thaliana} were 75.1% and 49.6% respectively. There was one \textit{miR172a} complementary sites (CTGCAGCATCATCAGGATTCT) at 130 bp of the 3' end in ORF. RLM-5' RACE analysis showed that \textit{DlAP2} was regulated by \textit{miR172a} through cleavage mainly at the site between the 11\textsuperscript{th} and 12\textsuperscript{th} bases. Real-time quantitative PCR results showed that the expression of \textit{miR172a} was a declining trend from buds of 1.0 cm, 1.5 cm, 2.0 cm, top leaves of flowering branches, top leaves of branches without flowering, to that of control in leaves of normal plant, with the highest level in buds of 1.0 cm and the lowest one in leaves of normal plant,
and the similar level in leaves of flowering branches and that of not flowering branches on the flowering plant. These results demonstrated that the expression of DiAP2 was showing an opposite trend correspondingly to those of miR172, the lowest level was in buds of 1.0 cm and the highest one was in leaves of normal plant, which indicated that miR172a had regulatory effect on the expression of DiAP2.

**Key Words**

*Dendrocalamus latiflorus*, AP2 gene, miR172a, expression analysis

**Introduction**

Bamboo, being as a tribe of flowering and evergreen perennial monocots, classified in the subfamily Bambusoideae within the grass family Poaceae that includes rice, maize, wheat and other cereals (Han et al., 2009), is known to the fast-growing characteristics and one of the most important non-timber forest resources in the world (Zhou et al., 2005; Peng et al., 2010). About 2.5 billion people depend on bamboo economically, and the international trade in bamboo amounts to over 2.5 billion US dollars per year (Lobovikov et al., 2007). However, collective death in some bamboo species has occasionally occurred after flowering over a large area, which results in a large loss, even for a devastating blow to bamboo resource base. The reasons for bamboo death after flowering remain unclear. Therefore, much attention had been paid to bamboo flowering, including the study on environmental factors, nutrition factors, the specific pathways and genes involved in bamboo flowering and flower development (Tian et al., 2005; Gao et al., 2007; Lin 2009; Gao et al., 2010; Chen et al., 2013; Peng et al., 2013; Gao et al., 2014). However, few studies have focused on the characterization of microRNA (miRNA) in bamboo (Zhao et al., 2013; Zhao et al., 2014; Xu et al., 2014), little is known about how the miRNAs regulate bamboo flowering.

MiRNAs have important functions in different developmental stages and metabolic process by mediating gene silencing at transcriptional and post-transcriptional levels (Llave et al., 2002; Bari et al., 2006). In plants, miRNAs regulate leaf morphogenesis, the development of root and flowers and other key processes, such as response to biotic and abiotic stresses, via interactions with their specific target mRNAs (Palatnik et al., 2003; Chen, 2004; Wang et al.,

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MiRNAs have played key roles in the process of plant from flora induction to flora organ specification, while APETALA2 (AP2) gene is one of important factors involved in regulation of flower development. AP2 expression is regulated at the post-transcriptional level by a miRNA, mir172 (Aukerman and Sakai, 2003; Kasschau et al., 2003; Chen, 2004). The discovery of mir172 as post-transcriptional negative regulator of AP2 immediately provided a potential means to solve the apparent paradox of AP2 mRNA being ubiquitously expressed.

Ma bamboo (Dendrocalamus latiflorus) is an evergreen species locally known as ‘tropical giant bamboo’, which forms abundant forests in southern China and southeast Asia, and is a valuable natural resource used as food, building material and other human consumption (Lin et al., 2007). To reveal the expression and regulation of mir172 and AP2 gene in bamboo plants, flowering D. latiflorus was selected as material to carry out the following research. In this paper, the full-length cDNA of DLAP2 gene was isolated and characterized from D. latiflorus, the mir172a-mediated cleavage site was investigated using RLM-RACE method, the expression profiles of mir172a and DLAP2 were analyzed in different tissues of flowering D. latiflorus. This is the first report of DLAP2 targeted by mir172a in bamboo, which is a primary research of bamboo miRNA and will encourage further design strategies for the directional improvement of bamboo timber and shoots quality by molecular engineering.

**Materials and methods**

**Plant materials and strains**

The two-year seedlings of bamboo (Dendrocalamus latiflorus) from tissue culture were potted in chamber under long-day conditions (16 h light / 8 h dark) at 25°C with the light intensity of 150 µmol·m⁻²·s⁻¹. The young branches, buds leaves were collected from the normal seedlings and flowering seedlings respectively, and stored at -80 ºC for further experiment.

*Escherichia coli* strain, DH5α was used as the recipients for routine cloning experiments.

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Isolation of full length cDNA

Total RNA was isolated from different tissues of *D. latiflorus* with Trizol method according to the protocol recommended by the manufacturer (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from 500 ng of RNA using the Promega cDNA synthesis system. The 3' cDNA and 5' cDNA were synthesized using the SMART™ RACE kit (Clontech, Mountain View, CA). Primers (DIAP2-F: 5’- ATGGAGCTGGATCTGAACGTG -3’, DIAP2-R: 5’- CGGCTTCTCTACCATTGCAT -3’ were designed on the basis of the sequence of the conservative domain of AP2 gene in *Zea may* (EU974370), Gradient PCR amplification was performed with DIAP2-F and DIAP2-R to optimize the annealing temperature.

Specific primers were designed according to the obtained sequence using the procedure described above. The primers used for 5' rapid amplification of cDNA ends (RACE) were DIAP2-5-1 (5-1: 5’- GTGCAGGGTGACGCCTCTGTATTTGGA -3’) and DIAP2-5-2 (5’- GCCTGCGGGAGCGGCAAGATTCTGATGT-3’); those used for 3’ RACE were DIAP2-3-1 (5’- GATGGGAGGCTCGCATGGGCCAGTTC -3’) and DIAP2-3-2 (5’- GCGATCAATGCAATGGTAGAGAAGCCG -3’). Touch down PCR were performed with DIAP2-5-1, DIAP2-3-1 and a universal primer mix (UPM) as supplied with the SMART™ RACE cDNA amplification kit, and then the PCR amplicons were used as template for a subsequent nested PCR using primer pairs of DIAP2-5-2 or DIAP2-3-2 with the NUP primer supplied in kit. The PCR fragments were cloned into pGEM-T easy vectors (Promega, Madison, WI) using standard protocol and sequenced using an ABI 3730 sequencer (Applied Biosystems, Bedford, MA). The full length cDNA was obtained by the combination of conserved sequence with the 5' and 3' end sequences.

Sequence analysis

Sequence analysis was carried out with the DNASTAR software package, and the cDNA sequences were subjected to a similarity search against the NCBI database (http://www.ncbi.nlm.nih.gov) using the Blastx algorithm with default parameters. The target sites of miRNA were analyzed using the online software

Theme: Biology, Morphology and Taxonomy
(http://plantgrn.noble.org/psRNATarget/). On the basis of this assembled sequences, the putative amino acid were analyzed using the software of Motif Scan (Sigrist et al., 2010), ProtScale (Gasteiger et al., 2005) and SOMPA (Geourjon and Deléage, 1995) to predict the physicochemical properties, structure and functional domains. Three-dimensional model analysis was carried out by using AP2 template of Arabidopsis. A neighbor joining (NJ) tree was constructed using the MEGA4.0 software package and the CLUSTAL algorithm in conjunction with the homologue sequences of AP2.

**MiR172a-mediated cleavage site analysis of the target gene**

Primers for 5′RACE were designed on the basis of prediction for target miRNA sites, outer primer 172F1 (5′-CCAATCCACTACAAGAAACCACCCCGG -3′) and Inner primer 172F2 (5′-CTTCCCGGAAAT TTACAGTGTCGC -3′) were synthesized respectively. The cDNA for RLM-RACE was synthesized using Full RACE Kit with TAP (Code No.6107) followed the instruction manual of the kit. Outer PCR was carried out using outer primer and 172F1 with final volume of 20 µL containing 10×GC Buffer || 2 µL, 1×cDNA Dilution Buffer || 8 µL, cDNA template 2 µL, 172F1 (10 µM) 1 µL, 5′RACE Outer Primer(10 µM) 1 µL, LA Taq (5 U·µL⁻¹) 0.25 µL and ddH₂O 5.75 µL. The PCR procedure involved in an initial denaturation period at 94°C for 3 min, followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72°C for 30 s, then with a final extension period at 72°C for 10 min.

After that, inner PCR was performed inner primer and 172F2 with final volume of 20 µL containing 5×PrimerSTAR Buffer (Mg²⁺ plus) 4 µL, dNTP mix (2.5 mM each) 1.6 µL, outer PCR reaction product 1 µL, 172F2 (10 µM) 1 µL, 5′RACE Inter Primer (10 µM) 1 µL, PrimerSTAR HS DNA Polymerase (2.5 U·µL⁻¹) 0.2 µL and ddH₂O 11.2 µL. The inner PCR procedure involved in an initial denaturation period at 98°C for 3 min, followed by 30 cycles at 98 °C for 30 s, 55 °C for 30 s, and 72°C for 30 s, then with a final extension period at 72°C for 10 min. The PCR fragments were cloned into pGEM-T easy vectors and sequenced.

**Real time-PCR analysis**

The stem-loop primer (5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTGCAGCA-3′) for cDNA synthesis from miRNA was designed using stem-loop primer method, and the quantitative analysis of expression miR172 was carried out with specific

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primer (miR172a- F: 5’- AGCAGCATATATAGAATCCTGATG -3’) and universal primer (miR172a- R: 5’- CTCAACTGGTGCTGGAGGTA GC -3’). Primers were designed from non-conserved region of the isolated DlAP2 using ABI Primer express 3.0 (F1: 5’- TTACTGAAGTGGTGCAGGT -3’; R1: 5’- TTACTGAAGTGGTGCAGGT -3’).

The cDNA templates were synthesized using the total RNA isolated from buds of 1.0 cm, 1.5 cm, 2.0 cm, top leaves of flowering branches, top leaves of branches without flowering and the leaves of normal plant, respectively. Real time fluorescent quantitative PCR amplification and analysis were carried out using Light Cycler® 480 Real-Time PCR System with LightCycler® 480 SYBR Green I Master kit (Roche Applied Science). The final volume was 10 μl containing 2×SYBR Premix Ex Taq 5 μl, 0.4 μl of each primer (5 μM), 1 μl of cDNA and 3.2 μl of nuclease-free water. Amplification was performed according to the procedure of an initial denaturation period at 95°C for 2 min, followed by 45 cycles at 95 °C for 30 s and 58 °C for 60 s. Bamboo NTB gene (Fan et al., 2013) was selected as a reference gene for DlAP2, as well as U6 snRNA for miRNA (Zhao et al., 2013).

For each condition, the qRT-PCR experiments were performed as biological triplicates and expression levels were normalized according to that of the internal control. The relative value of the gene expression was calculated using the 2−ΔΔCt method (Livak and Schmittgen 2001). Statistical tests were performed on the qRT-PCR data using SPSS (Statistical Product and Service Solutions) 18.0 software. Error bars representing the standard deviation were derived from the three experiments in triplicate.

**Results**

**Gene isolation and sequence analysis**

AP2 homologue genes have high similarities among monocotyledon plants which facilitate the design of primers for RT-PCR to clone DlAP2. As a result of the RT-PCR with DIAP2-F and DIAP2-R, a 990 bp nucleotide fragment was obtained, which had high identities with those in Oryza sativa, Zea mays and Triticum aestivum. Subsequently, a 387 bp fragment from the 5'-end region and a 774 bp fragment from 3'-end region were obtained by 5' and 3'-RACE, respectively. After analysis of the obtained sequences, a 1729 bp full length cDNA

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was identified containing a 1464 bp ORF, a 81 bp 5’ untranslated region (UTR) and a 184 bp 3’ UTR. There was a high degree of matching target site for miR172a in the coding frame near the 3’end of the 130 bp. The ORF encoded a putative peptide of 487 amino acids including two conserved domains of AP2/ERF which indicated it belonged to the AP2 subfamily of AP2/EREBP family. The theoretic isoelectric point (pl) and calculated molecular mass of DIAP2 were 6.859 and 52.75 kDa, respectively. The GenBank accession numbers of DIAP2 is KM267641 (Figure 1).

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1  ACGCGGGGGTCTGGTTGTTTGGGTTTGAGGGGTGTTGATTGGGAGTTGGGATTGATCTTGGTTGAG
67  GAGGTGAGAGGAGAGATGGAGCTGGATCTGAAGCTGGACGGACGGGCAGACGACGAGCCAGCGAGCAG
M E L D L N V A D G A P E K P E A
133  ATGGCGGAGGCTTTGCTCCGCGTGGCTCCGCGTGGCTCCGCGTGGCTCCGCGTGGCTCCGCGTGGCT
M A R S D S G T S D S P V L N A E A S G G G
199  GGCAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGG
265  TTTACAGACCCAAGGCTGCCGCTCAACCGAACTGGGTCGGCTCCGCGTGGCTCCGCGTGGCTCCGCG
F S I M R S S A S A E G K D V G V A D E
331  GAGAGGACCCAGCGCTTCGCTTCGCGCGAGCACGCTGGTCAACCCGAGCTCGTCTCCGTTCCGCGTG
E E A T P S P L R R Q Q L V T Q Q L F P V D
397  GCCGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGG
A G P P R P V P Q P G A E L G F L R P E P P
463  GGCAGGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACGACG
G P P Q P D I R I L P L P Q A H A P A Q P Q
529  GCTACCAAGAAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
A T K K S R R G P R S R S Q Y R G V T F Y
595  GCCCGGCAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
C G P P Q P D I R I L P L P Q A H A P A Q P Q
661  GACAGTGCCTGCTGCTGTCAGGCGGATGAGGCAGGCGGATGAGGCAGGCGGATGAGGCAGGCGGATGAG
D T A H A A A R A Y D R A A I K F R G I D T
727  GACATAAATGCTACATGTTATGACTACAGAAGGAGCATGAAAGACTGCTGTAATACGCAAGAGAG
D I N F D L S D Y E D M K Q V K S L S K E
793  GAGTTCGCTGACGCTGCTGACGCTGCTGACGCTGCTGACGCTGCTGACGCTGCTGACGCTGCTGAC
E F V H V L R R Q S T G F S R G S S K Y R G
859  GTCACCGCTCAAGGACGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
V T L H K C G R W E A R M Q G F L G K K Y I
925  TATCTTGGCTATTTGACTAGCTAGCAGAGGCGTGGCTAGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
Y L G L F D S E V E A R A Y D K A A I K C
991  AATGGTAAAGGCGGCGGCGGAGCATGCTGCAAGGAGCATGCTGCAAGGAGCATGCTGCAAGGAGCATG
N G R E A V T N F E P S T Y D G E M L T E V
1057  GGCTGCAAGGCTGCAAGGCTGCAAGGCTGCAAGGCTGCAAGGCTGCAAGGCTGCAAGGCTGCAAGG
G A E G A D V D L N L S I S Q P A L Q S P Q
1123  AGGATCAAGAGACTCCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
R D K N S L G L Q L H H G L F D G S E V K R
1189  GCTAGATTGTAATGATCTCCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
A K I D A P S E L A G R P H F P L L T K H
1255  CCACACGTGTGGCCTGCCCAATACTCCACCCCATATTTCAAAATAATGAGGATGACATCTAGATCAT
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Protein properties analysis and structure prediction

The analysis result showed that there contained 56 strongly basic amino acids, 59 strongly acidic amino acids, 146 hydrophobic amino acids, 119 polar amino acids in the protein encoded by \( DlAP2 \). Protein hydrophilicity / hydrophobicity analysis indicated \( DlAP2 \) had strong hydrophilicity.

The prediction of protein structure demonstrated that there also contained a proline-rich region (103-148) and a serine-rich region (444-483) besides the two AP2/ERF domains. There had one amidation site, one N-terminal glycosylation site, one cAMP- and cGMP-dependent on protein kinase phosphorylation site, six casein kinase phosphorylation sites, 8 N- myristoylation sites and 5 protein kinase C phosphorylation sites. The secondary structure analysis indicated \( DlAP2 \) protein contained four kinds of structure such as no rules of curling, \( \beta \) fold, extension chain and \( \alpha \) helix, in which the no rules of curling was the most one with 315 aa covering 64.68\% of \( DlAP2 \), followed by \( \alpha \) helix with 97 aa (19.92\%), next extension chain with 53 aa (10.88\%) and \( \beta \) fold with 22 aa (4.52\%). Homology modeling was used for the AP2 domains in \( DlAP2 \). Using the Arabidopsis AtERF1 the GBD (GCC-box binding domain) protein three-dimensional configuration as a template (1gccA), the three-dimensional configuration of AP2 domains in \( DlAP2 \) was predicted. They all had typical structure of AP2 with 3 \( \alpha \) helix and 3 \( \beta \) folds, which had similarity to the template with 45.16\% and 32.79\%, respectively (Figure 2).
Fig. 2 Three-dimensional configuration prediction of AP2 in DIAP2

Phylogenetic Analysis of DIAP2

The deduced amino acid sequence of DIAP2 was analyzed with AP2 from other plant species using Blastp in NCBI database. The results showed that there were 91 protein sequences with more than 50% homologous to DIAP2, among which AP2 (AGH68972) of Phyllostachys edulis had the highest identities (84.6%), and those of transcription factor AP2D23-like (AAW78371) in Oryza sativa and ARGET OF EARLY ACTIVATION TAGGED1 (NP_001189625) in Arabidopsis thaliana were 75.1% and 49.6% respectively.

Construction of phylogenetic tree showed that AP2 homologous proteins from monocots and dicots were clustered into two large branches, DIAP2 and AP2 of P. edulis clustered together nearby the homologous proteins from monocots such as Hordeum vulgare, Triticum aestivum, Brachypodium distachyon, Setaria italic, Zea mays and Sorghum bicolor, while farther away from those of dicots (Figure 3).

Fig. 3 Phylogenetic tree analysis based on AP2 homologue proteins. Numbers on major nodes indicate bootstrap values.

Theme: Biology, Morphology and Taxonomy
branches indicated bootstrap estimates for 1000 replicate analyses.

**MiR172a-mediated cleavage site identification in DlAP2**

RLM-5’RACE and sequencing methods were used to confirm the miR172a-mediated cleavage site in DlAP2. The result of sequencing showed that there were nine samples with cleavage sites between the 11\textsuperscript{th}-12\textsuperscript{th} bases, and only one sample with a cleavage site between the 12\textsuperscript{th}-13\textsuperscript{th} bases in the ten sequenced samples (Figure 4). This result was consistent with that of AP2 mediated by miR172a in Arabidopsis, which cleavage sites were mainly between the 11\textsuperscript{th}-12\textsuperscript{th} bases. We conclude that miR172a regulates its target gene DlAP2 in *D. latiflorus* by a translational mechanism.

**miR172 target sequence in DlAP2:**  
\[\text{CTGCAAGCATACTCAAGGATTC}\]

**miR172a:**  
\[\text{GACGUAGUAGUCUAAAGA}\]

Fig. 4 Target sites for miR172 in three AP2-like transcripts. Cleavage sites are indicated by arrows. The numbers of 5’ RACE clones sequenced that correspond to each cleavage product are indicated.

**Expression analysis of miR172a and DlAP2 in different tissues**

The expression patterns of miRNAs could provide clues to their functions (Yao et al., 2007). Real time PCR was employed to detect the expression level of both miR172a and DlAP2 in different tissues of flowering bamboo (*D. latiflorus*).
Fig. 5 miR172a (A) and DIAP2 (B) expression in different tissues. 1: Young branch of flowering plant; 2: 1.0 cm bud; 3: 1.5 cm bud; 4: 2.0 cm bud; 5: Top leaf on the flowering branch; 6: Top leaf on the unflowering branch of flowering plant; 7: leaf of unflowering plant.

The result demonstrated that the expression of miR172 was a declining trend from buds of 1.0 cm, 1.5 cm, 2.0 cm, top leaves of flowering branches, top leaves of branches without flowering, to that of control in leaves of normal plant, with the highest level in buds of 1.0 cm and the lowest one in leaves of normal plant, and the similar level in leaves of flowering branches and that of not flowering branches on the flowering plant (Figure 5A). Meanwhile the expression of DIAP2 was showing an opposite trend correspondingly to those of miR172, the lowest level was in buds of 1.0 cm and the highest one was in leaves of normal plant (Figure 5B), which indicated that miR172 had regulatory effect on the expression of DIAP2.

Discussion

Unlike other MADs-box gene family, AP2 had unique AP2 domains and were important factors in the regulation of floral development in plants, which were divided into three subfamilies including AP2, EREBP and RAV1/RAV2 (Lin et al., 2007). There is a miR172 binding site in AP2 gene of Arabidopsis using large-scale sequencing, and it was confirmed that the expression of AP2 was regulated by miR172 using the transgenics of miR172 and the apetala2 mutant (Jones-Rhoades et al., 2006; Zhao et al., 2007). There was one miR172a complementary sites (CTGCAGCATCATCAGGATTCT) at 130 bp of the 3′ end in ORF of DIAP2. RLM-5′RACE analysis showed that DIAP2 was regulated by miR172a through cutting mainly at the site between the 11th and 12th bases.

The expression of miR172a in flowering tissues of D. latiflorus was significantly higher than that of unflowering leaves, which is similar to miR172 over-expression can lead to early flowering tomato (Martin et al., 2009). With the elongation of the flower bud, the expression of miR172a was up-regulated, while that of DIAP2 gene was down-regulated, which may be explained by the finish tend of the development of flower buds reached 2.0 cm (the differentiation is complete in each part of its floral organ). Little expression changes of miR172a and DIAP2 were found in young branches, top leaves of flowering branches and top leaves of branches without flowering, this may be that they are in a state of equilibrium in vegetative organs. This was

Theme: Biology, Morphology and Taxonomy
confirmed in Arabidopsis, in which any member of the AP2 family, including TOE2, SNZ and SMZ mediated by *miR172*, expressed constitutively would result in delayed flowering during the transition from vegetative growth to reproductive growth (Jung et al., 2007).

It was reported that *miR172* exercised regulatory functions together with other miRNAs besides regulating target gene only by itself. For example, *miR172* was not only involved in developmental regulation infancy, but also in the regulation of maize inflorescence meristem under an indirect effect *miR156* (Chuck et al., 2007; Chuck et al., 2008). Transcript cleavage and translational inhibition both play a role in AP2 regulation by *miR172*, although assessing the relative importance of the two processes is confounded by a negative feedback loop in which AP2 represses its own transcription (Schwab et al., 2005; Mlotshwa et al., 2006). Recent report indicated that 84 conserved miRNAs belonging to 17 families, and 81 novel miRNAs were identified in *D. latiflorus*, and one hundred and sixty-two potential targets were predicted for the 81 novel bamboo miRNAs. Several targets for the novel miRNAs are transcription factors that play important roles in plant development (Zhao et al., 2013). Therefore, *miR172a* regulation of growth and development for *D. latiflorus* will be a complex regulatory network, further study on the mutual adjustment mechanism of other miRNAs and their target genes in *D. latiflorus* should be carried out, in order to make a comprehensive analysis of its growth and development.

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