



Cloning and expression of an *APETALA1*-like gene from *Nelumbo nucifera*

D.Z. Kong*, X.Y. Shen*, B. Guo, J.X. Dong, Y.H. Li and Y.P. Liu

Department of Ornamental Horticulture, College of Forestry,
Henan Agricultural University, Zhengzhou, China

*These authors contributed equally to this study.
Corresponding authors: Y.H. Li / Y.P. Liu
E-mail: kzxy303@163.com / liyhany@163.com

Genet. Mol. Res. 14 (2): 6819-6829 (2015)
Received September 16, 2014
Accepted March 5, 2015
Published June 18, 2015
DOI <http://dx.doi.org/10.4238/2015.June.18.24>

ABSTRACT. The objective of this study was to clone the full-length cDNA of the *APETALA1* (*API*) gene from lotus and analyze its sequence and expression pattern. The full-length cDNA sequence of the *NnAPI* gene was amplified from the petals of *Nelumbo nucifera* 'Hongxia' using RT-PCR and rapid amplification of cDNA ends. Bioinformatic methods were used to analyze the sequence characteristics of the gene. Quantitative real-time PCR methods were used to investigate the expression pattern of *NnAPI* in various organs and during different developmental stages. The cloned full-length *NnAPI* cDNA (GenBank accession No. KF361315) was 902 bp, containing a 795-bp open reading frame encoding 264 amino acids with a relative molecular mass of 30,288.4 and an isoelectric point of 9.13. *NnAPI* had a MADS-box domain and a K-box domain, which is typical of the *SQUA/API* gene family. A protein sequence identity search showed that *NnAPI* was 75-96% similar to other plant APIs. Phylogenetic tree analysis indicated that *NnAPI* was very closely related to *API* of *Glycine max*, suggesting that they shared the same protein ancestor. Quantitative real-time PCR analysis showed that *NnAPI* was expressed in various organs during different developmental stages; it had the highest expression in blooming

flowers and had trace expression in the young vegetative and flower senescence stages. Our analysis suggests that *NnAPI* plays an important role in controlling floral meristem identity and floral organ formation.

Key words: *Nelumbo nucifera*; *NnAPI*; Gene cloning; Expression analysis

INTRODUCTION

Nelumbo nucifera Gaertn. is an aquatic perennial herb of Nymphaeaceae. It is an important aquatic ornamental plant and economic crop, and it flowers from June to September. At present, flower breeding in this ornamental plant is limited to traditional methods. With improvements in gene cloning, the study of temporal and spatial gene expression patterns provides a theoretical basis to reveal the molecular mechanisms of ornamental plant flower development, and it also enables flower engineering using many methods, such as transgenic technology. Flower development involves complex gene expression and regulation as part of a very complicated biological phenomenon. One class of genes involved in this process is the flower-timing genes, which influences flowering time and can control the morphology of flowers and inflorescence meristems (Guo et al., 1998). Research on the molecular mechanism and genetic control of flowering in *Arabidopsis* has shown that *API* is a floral meristem identity gene. This gene not only controls the transition from vegetative to inflorescence and floral meristems in plants, but it also controls the growth of the two external whorls in floral organs, sepals, and petals (Irish and Sussex, 1990).

In the classical floral organ ABC model, the *API* gene belongs to class A and can activate the expression of class B genes (Mandel and Yanofsky, 1995; Jack, 2004). Research has shown that *APETALA1* (*API*) genes are highly conserved (Peña et al., 2001). To date, researchers have identified *API*-homologous genes from lily, jujube, apple, longan and *Cymbidium ensifolium*, (Gao et al., 2006; Chen et al., 2008; Sun et al., 2009; Meng et al., 2010; Wu et al., 2013). Flowering of the inflorescence was accelerated when an *API*-homologous gene was transformed into the chrysanthemum “Yumianren” (Lü et al., 2007), and three *API*-homologous genes were shown to accelerate flowering in transgenic *Arabidopsis* (Chen et al., 2008). However, research on lotus flowering genes has been relatively limited. In a previous study, a lotus *AP2* flowering gene was cloned and its expression characteristics were explored (Liu et al., 2012). In addition, we cloned and examined the expression of *LEAFY* (*LFY*) genes from *N. nucifera* in our previous research. The *LFY* gene regulates the expression of the downstream meristem identity gene *API* and one of its effects is to activate the *API* gene. *API* has a feedback effect on *LFY* (Peña et al., 2001), thus, *API* and *LFY* have a close relationship. To date, there has been no report of research on the *API* gene in lotus.

The objective of the current study was to clone the full-length cDNA sequence of the *NnAPI* gene. Using the published sequences of homologous genes, we designed degenerate primers for *N. nucifera* ‘Hongxia’ and combined this with rapid amplification of cDNA ends (RACE). Bioinformatic methods were used to analyze the sequence characteristics of the gene and the sequence information was registered in GenBank. RT-PCR and quantitative real-time PCR methods were used to analyze the expression pattern of *NnAPI* in various organs and during different developmental stages (Lenartowski et al., 2014). This research provides a basis for control of flowering time and the mechanism of floral formation using genetic engineering technology.

MATERIAL AND METHODS

Plant materials

The plant material used was *N. nucifera* Gaertn. 'Hongxiamantian', which is maintained in the landscape plant molecular biology laboratory of Henan Agriculture University. We used roots, stems, leaves, and flowers from young vegetative, early flowering, full-bloom, and flower senescence stage plants as material. All plant samples were immediately frozen in liquid nitrogen and stored at -80°C for later use.

Chemicals for DNA isolation and cloning

A RevertAid™ First Strand cDNA synthesis kit was purchased from Fermentas (Lithuania), a SMARTer™ RACE cDNA amplification kit was purchased from TaKaRa (Japan), and the pUCm-T cloning vector was purchased from Shanghai Shenggong Biological Engineering (China). A Gel DNA extraction kit was purchased from Tiangen Bio-tech (China) and Taq enzymes and DL1000 markers were purchased from TaKaRa. *Nicotiana tabacum* seeds and *Escherichia coli* strain DH5 α are maintained in our laboratory. We purchased 2X Power Taq PCR MasterMix and Power 2X SYBR Real-time PCR Premixture from BioTeke Corporation (China).

Total RNA isolation and cDNA synthesis

RNA was extracted from young petals of lotus using a modified CTAB method (Yang et al., 2009). The RNA showed typical absorption curves at A_{260}/A_{280} and A_{260}/A_{230} , demonstrating its purity, and it was then subjected to agarose gel electrophoresis. The Alpha Imager PE system was used to photograph the agarose gel and detect the quality and concentration of DNA. We used 2 μ g total RNA for reverse transcription to obtain first-strand cDNA.

Cloning of the complete *NnAPI* gene cDNA sequence

Using BLASTn, we searched for *API*-homologous nucleotide sequences in GenBank and chose sequences from species that were closely related to lotus and analyzed their homology. From this, we designed the specific primers F1 and F2 in conserved regions (Table 1). We also designed and synthesized the 3'-RACE primers F1S and F2S and the 5'-RACE primers R1A and R2A (Table 1), which were based on the amplified conserved fragments. The downstream primers were B26 (5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTTTTTT-3') and UPM (5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3'). The middle fragment and the template cDNA of 3'-RACE and 5'-RACE were obtained following protocols for the RevertAid™ First Strand cDNA synthesis and SMARTer™ RACE cDNA amplification kits (Lenartowski et al., 2014).

PCRs were performed in a 20 μ L volume including 10 μ L 2X MasterMix, 1 μ L upstream primer, 1 μ L downstream primer, 1 μ L template, and 7 μ L ddH₂O. The PCR program used was as follows: pre-denaturing at 94°C for 5 min, followed by 94°C for 50 s, annealing (temperatures and times are shown in Table 1), and 72°C for 1 min for 30 cycles, and finally 10 min of extension at 72°C. The PCR products were separated on a 1.0% agarose gel. The

fragments were ligated into the vector pUCm-T after being recovered and purified, and were then transformed into *E. coli* DH5 α . The PCR products were sequenced from both directions by the Shanghai Biotechnology Company, China.

Using the DNAMAN software (<http://www.lynnon.com/>), we combined the 5'-RACE fragment, the middle fragment, and the 3'-RACE fragment. The full coding region was analyzed on the NCBI website using Open Reading Frame Finder. We then designed the primers AP1-S and AP1-A on either side of the coding region (Table 1) to verify the result using PCR. The methods were the same as above, including the PCR program. The PCR product was recovered, purified, and sequenced to obtain the full cDNA sequence.

Table 1. Primer sequences for cloning and annealing conditions of *NnAPI* from *Nelumbo nucifera*.

Primer	Primer sequence	Annealing temperatures and times
Conserved primer		
F1	5'-TGTGATGCTGAAGTCGCT-3'	50°C, 30 s
R1	5'-CCTTTGGTCCGCTGTAA-3'	
3'RACE primer		
F1S	5'-GTCGCTGTGATCGTCTTCTCCAC-3'	59°C, 30 s
F2S	5'-GAATCACAGGGAAGCTGGTCTCT-3'	55°C, 30 s
5'RACE primer		
R1A	5'-GGTCCGCTGTAATAATCTCA-3'	55°C, 30 s
R2A	5'-GCTTCCCTGGTGGAGAAGACGAT-3'	60°C, 30 s
<i>NnAPI</i> cDNA amplified primer		
AP1-S	5'-ATGGGGAGAGGTAGGGT-3'	58°C, 30 s
AP1-A	5'-TCAGATGGTGAACATGCGAGGCCG-3'	

Bioinformatic analysis of the *NnAPI* gene

Using the DNAMAN 6.0 software (<http://www.lynnon.com/>), we compared the nucleotide and amino acid sequences. A molecular phylogenetic tree was created using MEGA5.0 software (<http://www.megasoftware.net/>) with homologous amino acid sequences from GenBank obtained using the BLAST program. Protein prediction was performed on the NCBI website (<http://www.ncbi.nlm.nih.gov/>). The conserved regions were analyzed using a specialized BLAST search (CDD search). The physicochemical protein characteristics were analyzed using the ProtParam software (<http://web.expasy.org/protparam/>). Analysis of transmembrane domains and transmembrane orientation was performed by TMbase using the TMPred program (http://www.ch.embnet.org/software/TMPRED_form.html). Signal peptide prediction was performed using SignalP (<http://www.cbs.dtu.dk/services/SignalP/>). Subcellular localization prediction was done using PSORT (<http://psort.hgc.jp/form.html>) and three-dimensional structure modeling was performed using Swiss-Model Workspace (<http://swissmodel.expasy.org/>).

Analysis of *NnAPI* expression

NnAPI expression in different lotus growth stages was examined by quantitative real-time PCR. We used roots, stems, leaves, and flowers from young vegetative stage, early flowering stage, full-bloom stage, and flower senescence stage plants as material. RNA was extracted and stored as above, and reverse transcribed into cDNA.

Primers were designed to analyze gene expression using the open reading frame (ORF)

sequence of *NnAPI* (*NnAPI*-F1, 5'-GCAGGAGCAAAACAACATAC-3'; *NnAPI*-R1, 5'-TAGCAGGAAGGACGGTGA-3'). The amplified fragment length was 133 bp. The lotus *18S-rRNA* gene was used as a reference to analyze expression by RT-PCR; the primer sequences were 18S-rRNA-F 5'-CCATAAACGATGCCGAC-3' and 18S-rRNA-R 5'-CACCCATAGAAATCAAGA-3'. RT-PCR was performed following BioTeke Company protocols for the Power 2X SYBR Real-time PCR Premixture kit. PCRs were performed on a Bio-Rad iCycler iQ. The PCR program used was as follows: pre-denaturing at 95°C for 20 s, and then denaturing at 58°C for 20 s, annealing (temperatures and times are shown in Table 1), and extension at 72°C for 20 s for 40 cycles, followed by melting curves analysis. Every sample was run in triplicate and relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

RESULTS

NnAPI cloning and sequencing

From lotus petal tissue we cloned a 216 bp conserved region fragment, a 647-bp 3'-RACE fragment, and a 434-bp 5'-RACE fragment by RT-PCR and 3'/5'-RACE. The total *API* cDNA length was 902 bp and was obtained by combining the sequence fragments with DNA-MAN6.0. A 795-bp ORF fragment was obtained using PCR by designing primers from the combined total sequence (Figure 1). Sequencing of the ORF fragment confirmed the combined sequence was correct. The resulting sequence was named *NnAPI* and deposited in GenBank under accession No. KF361315 (Figure 2).

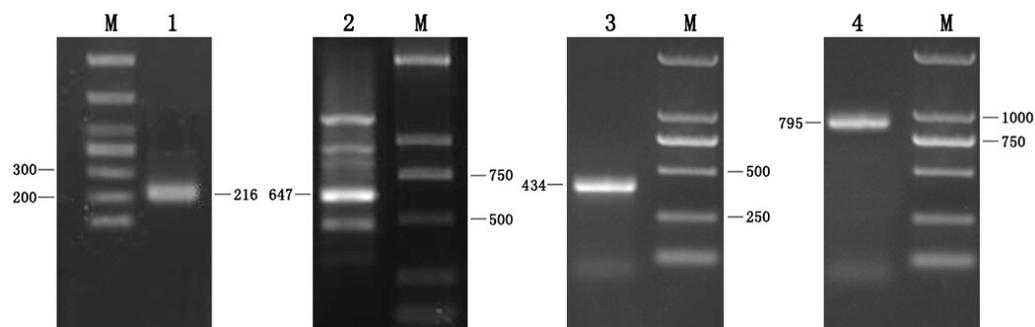


Figure 1. cDNA amplification of the *NnAPI* gene in *Nelumbo nucifera*. Lane 1 = conserved fragment; lane 2 = 3'-RACE product; lane 3 = 5'-RACE product; lane 4 = complete open reading frame; lane M = DNA size marker (units are bp).

Amino acid sequence comparison and phylogenetic analysis

Using the BLAST program, we searched for homologous amino acid sequences, which showed that *NnAPI* was 75-96% similar to APIs from other plant species sequences (Figure 3).

NnAPI was most homologous to a sequence from *Trochodendron aralioides* (approximately 96% similar). The phylogenetic tree suggested that the homologous proteins shared the same ancestor (Figure 4).

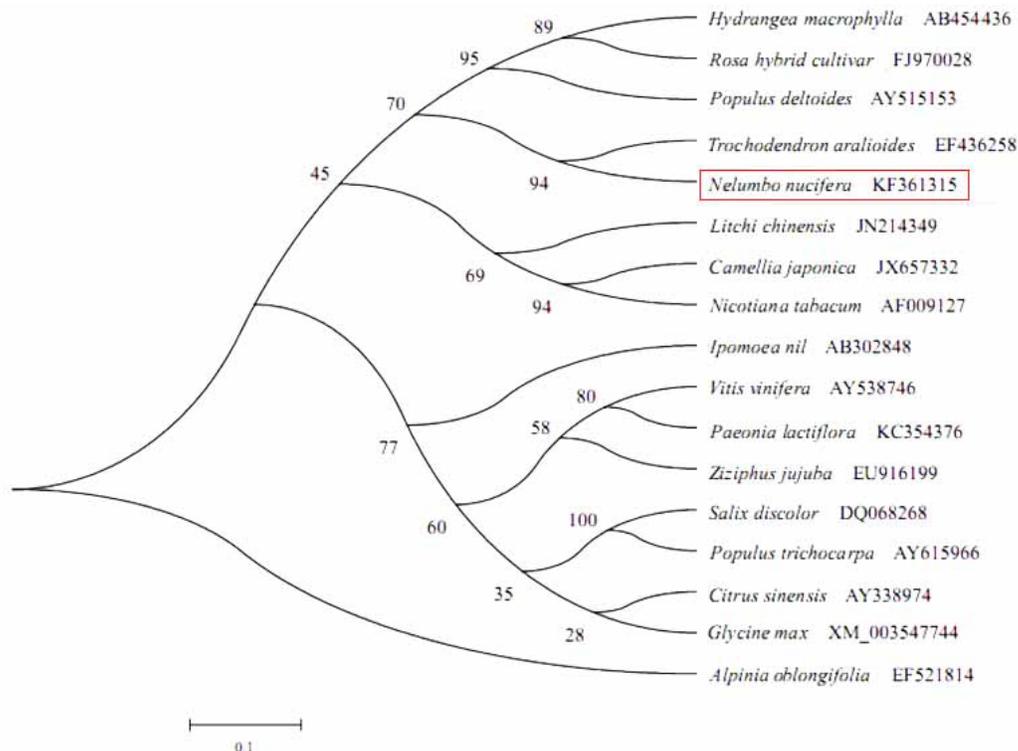


Figure 4. Phylogenetic tree of the NnAPI protein from *Nelumbo nucifera* and other APIs. NnAPI had the closest protein relationships with *Hydrangea macrophylla*, *Rosa rugosa*, *Populus deltoides*, and *Trochodendron aralioides*. It was closest to API/FUL of *T. aralioides*. Accession Nos. provided alongside species names. Figures along branches denote level of similarity (%).

Bioinformatic analysis of the NnAPI protein

The physicochemical characteristics of the NnAPI protein were analyzed using the ProtParam program. The encoded protein had 264 amino acids, a relative molecular mass of 30,288.4, a chemical formula of $C_{1318}H_{2150}N_{392}O_{409}S_8$, and an isoelectric point of 9.13. The instability coefficient of the protein was 56.39, indicating it was unstable. The fat coefficient was 82.34 and the average hydrophilic coefficient was -0.803, indicating it was hydrophilic. Its extinction coefficient was 27,180 at a depth of 280 nm in aqueous solution. The protein was composed of 20 types of amino acid, with Leu being the most abundant (12.1%). The total number of negatively charged amino acids (Asp + Glu) was 35 and the total number of positively charged amino acids (Arg + Lys) was 42.

We used TMpred to predict the transmembrane domains and transmembrane orientation. The protein had two internal to external spiral regions (located at residues 35-59 and 202-218) and one external to internal spiral region (residues 38-58). Thus, NnAPI was deduced to be a transmembrane protein. Signal peptide prediction showed that NnAPI did not have a clear signal peptide, so it was not a secretory protein (Tanaka et al., 1990). PSORT predicted that it was most likely located in the cell nucleus (76% probability), but it also predicted that it could

be located in the cytoplasmic membrane (60%), the mitochondrial plastid (36%), or the chloroplast membrane (10%). Thus, lotus *NnAPI* is likely to be mainly located within cell nuclei.

After protein analysis and prediction, the sequence was submitted to the Prosite database to analyze and predict its protein domains. The results showed that *NnAPI* has a conserved MADS region (aa 1-61) and a sub-conserved K region (aa 88-178). Its 3D structure was predicted by SWISS MODEL homology modeling. *In6jA* was identified as a template in the protein database (Liu et al., 2014) and the similarity between *NnAPI* and *In6jA* was 50.69% according to comparison with x-ray crystal diffraction (E value = $3.40e-28$; Figure 5).

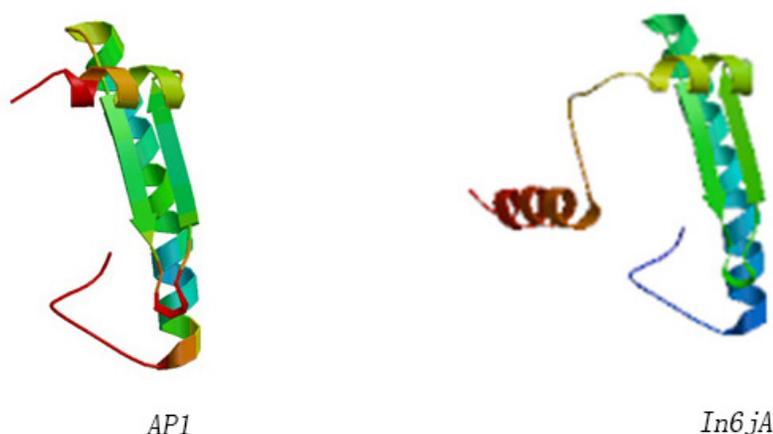


Figure 5. Three-dimensional structure of the *NnAPI* protein from *Nelumbo nucifera* and the *In6jA* template predicted by SWISS-MODEL.

Analysis of *NnAPI* expression in lotus during different developmental stages

The *18S-rRNA* gene was used as a reference gene to normalize *API* expression in lotus at different developmental stages. The results showed that *NnAPI* was expressed in the roots, stems, leaves, and flowers and had differential expression during the young vegetative, early flowering, and full-bloom stages (Figure 6). *NnAPI* had limited expression in roots, stems, and leaves during the young vegetative stage, with the highest expression observed in roots (Figure 6 I). During the young vegetative stage, the expression level of *NnAPI* in leaves and stems was 0.77 and 0.25 times that observed in roots, respectively. *NnAPI* expression level was higher during the early flowering stage (Figure 6 II) than in the young vegetative stage (Figure 6 I). During the early flowering stage, *NnAPI* expression was highest in flowers, followed by leaves, stems, and roots, with the expression level in flowers, leaves, and stems being 11, 7, and 6 times that observed in roots, respectively (Figure 6 II). During the full-bloom stage (Figure 6 III), the highest expression of *NnAPI* was observed in flowers, with lower expression recorded in the roots and stems. Thus, the gene has a close relationship with organ formation. During the flower senescence stage (Figure 6 IV), *API* expression fell to trace levels in every tissue. Comparatively, *NnAPI* expression was lower in the young vegetative and senescence stages (Figure 6 I and IV, respectively) than it was in the early flowering and full-bloom stages (Figure 6 II and III, respectively). Thus, *NnAPI* is differentially expressed during different developmental stages.

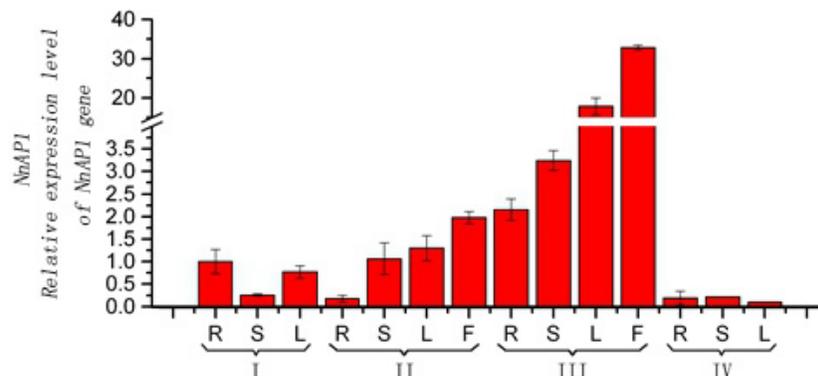


Figure 6. Relative expression of the *NnAPI* gene in different organs and during different growth stages of *Nelumbo nucifera*. I represents the young vegetative stage, II represents the early flowering stage, III represents the full-bloom stage, and IV represents the flower senescence stage. R, root; S, stem; L, leaf; F, flower.

DISCUSSION

In recent years, *API* genes have been cloned from many plants. *API* is essential for the regulation of flower development, which has been proven in the model plants snapdragon and *Arabidopsis* (Mandel and Yanofsky, 1995; Liu et al., 2009). In this study, we cloned and obtained the complete sequence of an *API*-homologous gene from lotus and analyzed its sequence characteristics and expression. The amino acid sequence of *NnAPI* included specific MADS-box and K-box domains, suggesting it belongs to the *SQUA/API* gene family, the same as was observed for Shijing mango and jujube genes (Jack, 2004). The cloned *API* amino acid sequence was queried in GenBank with the BLAST program and *NnAPI* and *API*s from other plant species showed 75-96% similarity. The MADS-box region had the highest homology and was highly conserved. *API* belongs to the MADS-box gene family, which participates in flowering transition, flower development, and organ formation (Theißen et al., 1995). *NnAPI* included a MADS-box region, which can combine with DNA in a sequence-specific way and function in transcriptional regulation (Suarez-Lopez et al., 2001; Melzer et al., 2010). MADS-box proteins can also form dimers and perform other functions (Jack et al., 1992; Shore and Sharrocks, 1995). Protein dimers can increase the variety of DNA binding proteins and the affinity between the complex and the point of DNA binding. In analysis of *Arabidopsis* MADS-box dimer proteins, *API* and *AGLI* were observed to form homogeneous protein dimers (Riechmann et al., 1996); *API* and *CAL* can also form protein dimers (Pelaz et al., 2001). Theißen and Saedler (2001) proposed the quartet molecular model: two MADS-box protein dimers can combine with the *CAR*G box to form a quartet molecule via protein interactions and ensure the development of floral organs. Thus, in *Arabidopsis*, the homogeneous dimers *API*/*AP2* and *SEP*/*API* can form a tetramer, which controls the formation of sepals, and the heterodimers *AP3*/*P1* and *SEP*/*API* can form a tetramer that controls petal development (Honma and Goto, 2001; Favaro et al., 2003). Thus, protein polymerization is important for controlling gene expression, and MADS-box genes have an essential role in flower development. Here, we found a MADS-box sequence, which is involved in developmental regulation. Sequence analysis showed that it had high homology to *API*s of other plants, thus, we can

infer similar functions from its similar structure (Xu et al., 2011).

We used quantitative real-time PCR to analyze the expression of the *NnAPI* gene during different developmental stages and in various organs, and found that the different organs had differential expression of *NnAPI* during different developmental stages (Khan et al., 2012). *NnAPI* was expressed in different organ tissues during the young vegetative stage, but the expression level was low, which may be related to flower induction (Kyojuka et al., 1997). As we expected, *NnAPI* had higher expression levels in leaves and stems, with low expression in roots. Additionally, *NnAPI* expression was clearly higher during the full-bloom stage. Wu et al. (2013) found that a homologous gene had higher expression levels in *C. ensifolium*, and the *ZjAPI* gene was found to be expressed during flowering development (Sun et al., 2009; Meng et al., 2010); thus, they share the same expression patterns. Expression analysis showed that *API* was expressed stably in stamen primordia and floral organ differentiation. Thus, *API* has an important role in controlling floral meristem identity and the formation of floral organs. In research on *Arabidopsis* flowering, *LFY* was found to be an important gene for determining floral meristem identity, like *API*. The two genes were not completely independent and expression of *LFY* preceded that of *API* (Sessions et al., 2000; Grandi et al., 2012). Thus, *API* is involved in the determination of meristem identity as a downstream gene of *LFY* (Ng and Yanofsky, 2001), and *API* affects floral meristem identity genes during its early expression, which in turn is affected by the induction of *LFY*. *API* also functions in determining floral organ characteristics in the late stage, which is affected by *LFY* indirectly (Mandel, 1992; Liljegren et al., 1999). However, there has been little research on the regulation of expression between *API* and *LFY* in lotus and its significance in the lotus bud differentiation regulation mechanism.

In this study, we identified an *API* gene that was expressed in different organs and participated in the whole process of floral bud differentiation in lotus during different periods. However, it remains unclear how *NnAPI* functions in lotus. The relationship between the expression characteristics of this *API* gene and the regulation of flower development in lotus should be further explored in depth.

ACKNOWLEDGMENT

Research funded by the Special Science and Technology Foundation of Henan Province (#072102150001).

REFERENCES

- Chen MK, Lin IC and Yang CH (2008). Functional analysis of three lily (*Lilium longiflorum*) APETALA1-like MADS box genes in regulating floral transition and formation. *Plant Cell Physiol.* 49: 704-717.
- Favaro R, Pinyopieh A, Battaglia R, Kooiker M, et al. (2003). MADS-box protein complexes control carpel and ovule development in *Arabidopsis*. *Plant Cell* 15: 2603-2611.
- Gao HY, Li Z, Jiang F, Zheng SQ, et al. (2006). Cloning and sequence analysis of *APETALA1(API)* homologous gene in Longan. *Fujian Fruits* 2:1-3.
- Grandi V, Gregis V and Kater MM (2012). Uncovering genetic and molecular interactions among floral meristem identity genes in *Arabidopsis thaliana*. *Plant J.* 69: 881-893.
- Guo H, Yang H, Mockler TC, Lin C, et al. (1998). Regulation of Flowering Time by *Arabidopsis* Photoreceptors. *Science* 279: 1360-1363.
- Honma T and Goto K (2001). Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* 409: 525-529.

- Irish VF and Sussex IM (1990). Function of the *apetala-1* gene during *Arabidopsis* floral development. *Plant Cell* 2: 741-753.
- Jack T (2004). Molecular and genetic mechanisms of floral control. *Plant Cell* 16: S1-S17.
- Jack T, Brockman LL and Meyerowitz EM (1992). The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS-box and is expressed in petals and stamens. *Cell* 68: 683-697.
- Khan S, Al-Qurainy F, Nadeem M and Tarrum M (2012). Development of genetic markers for *Ochradenus arabicus* (Resedaceae), an endemic medicinal plant of Saudi Arabia. *Genet. Mol. Res.* 11: 1300-1308.
- Kyozuka J, Harcourt R, Peacock WJ and Dennis ES (1997). Eucalyptus has functional equivalents of the *Arabidopsis API* gene. *Plant Mol. Boil.* 35: 573-584.
- Lenartowski R, Suwińska A, Prusińska J, Gumowski K, et al. (2014). Molecular cloning and transcriptional activity of a new *Petunia calreticulin* gene involved in pistil transmitting tract maturation, progamic phase, and double fertilization. *Planta* 239: 437-454.
- Liljegrén SJ, Gustafson-Brown C, Pinyopich A, Ditta GS, et al. (1999). Interactions among *APETALA1*, *LEAFY*, and *TERMINAL FLOWER1* specify meristem fate. *Plant Cell* 11: 1007-1018.
- Liu C, Xi W, Shen L, Tan C, et al. (2009). Regulation of floral patterning by flowering time genes. *Dev. Cell* 16: 711-722.
- Liu S, Lv Y, Wan XR, Li LM, et al. (2014). Cloning and expression analysis of cDNAs encoding ABA 8'-hydroxylase in peanut plants in response to osmotic stress. *PLoS One* 9: e97025.
- Liu Z, Gu C, Chen F, Jiang J, et al. (2012). Identification and expression of an *APETALA2*-like gene from *Nelumbo nucifera*. *Appl. Biochem. Biotechnol.* 168: 383-391.
- Lü JH, Wu YL, Sun L and Zhang Q (2007). Genetic transformation of *Chrysanthemum morifolium* cv. 'Yu Ren Mian' with *API* gene mediated by *Agrobacterium tumefaciens*. *Sci. Sil. Sin.* 43: 128-132.
- Mandel MA and Yanofsky MF (1995). A gene triggering flower development in *Arabidopsis*. *Nature* 377: 522-524.
- Mandel MA, Gustafson-Brown C, Savidge B and Yanofsky MF (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. *Nature* 360: 273-277.
- Melzer R, Wang YQ and Theißen G (2010). The naked and the dead: the ABCs of gymnosperm reproduction and the origin of the angiosperm flower. *Semin. Cell Dev. Biol.* 21: 118-128.
- Meng YP, Cao QF and Sun HF (2010). Cloning and expression analysis of floral meristem identity gene *ZjAPI* from Chinese jujube. *J. Shanxi Agric. Sci.* 38: 6-11.
- Ng M and Yanofsky MF (2001). Activation of the *Arabidopsis* B class homeotic genes by *APETALA1*. *Plant Cell* 13: 739-753.
- Pelaz S, Gustafson-Brown C, Kohalmi SE, Crosby WL, et al. (2001). *APETALA1* and *SEPALLATA3* interact to promote flower development. *Plant J.* 26: 385-394.
- Peña L, Martín-Trillo M, Juárez J, Pina JA, et al. (2001). Constitutive expression of *Arabidopsis LEAFY* or *APETALA1* genes in citrus reduces their generation time. *Nat. Biotechnol.* 19: 263-267.
- Riechmann JL, Krizek BA and Meyerowitz EM (1996). Dimerization MADS domain homeotic *APETALA1*, *APETAL3*, *PISTILLATA* and *AGAMOUS*. *Proc. Natl. Acad. Sci. U. S. A.* 93: 4793-4798.
- Sessions A, Yanofsky MF and Weigel D (2000). Cell signaling and movement by the floral transcription factors *LEAFY* and *APETALA1*. *Science* 289: 779-782.
- Shore P and Sharrocks AD (1995). The MADS-box family of transcription factors. *Eur. J. Biochem.* 229: 1-13.
- Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, et al. (2001). *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410: 1116-1120.
- Sun HF, Meng YP, Cao QF and Liang AH (2009). Cloning and expression analysis of *APETALA1 (API)* homologue in Chinese Jujube. *J. Shanxi Univ. (Nat. Sci.)* 32: 266-272.
- Tanaka A, Mita S, Ohta S, Kyozuka J, et al. (1990). Enhancement of foreign gene expression by a dicot intron in rice but not in tobacco is correlated with an increased level of mRNA and an efficient splicing of the intron. *Nucleic Acids Res.* 18: 6767-6770.
- Theißen G and Saedler H (2001). Plant biology: Floral quartets. *Nature* 409: 469-471.
- Theißen G, Strater T, Fischer A and Saedler H (1995). Structural characterization, chromosomal localization and phylogenetic evaluation of two pairs of *AGAMOUS*-like MADS-box genes from maize. *Gene* 156: 155-166.
- Wu JH, Wu SH, Yang C and Zhang ZZ (2013). Cloning and expression analysis on *API* homologous gene from *Cymbidium ensifolium* and interaction analysis between *API* and MADS-box transcription factors. *Acta Hort. Sin.* 40: 1935-1942
- Xu ZL, Ali Z, Yi JX, He XL, et al. (2011). Expressed sequence tag-simple sequence repeat-based molecular variance in two *Salicornia* (Amaranthaceae) populations. *Genet. Mol. Res.* 10: 1262-1276.
- Yang F, Li C, Liu YP, He MZ, et al. (2009). Extraction methods of the total RNA in the petals of lotus. *Henan Sci.* 27: 1226-1228.