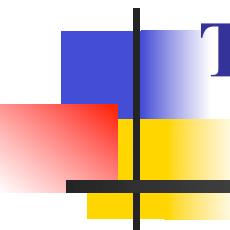


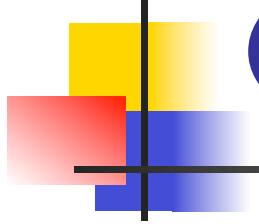


Isolation and Expression Analysis of *DlAP2* Targeted by *miR172a* in *Dendrocalamus latiflorus*



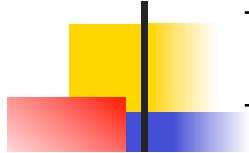
Dr. Zhimin Gao

International Center for Bamboo and Rattan
State Forestry Administration of China
19 Sep, 2015, Damyang, Korea

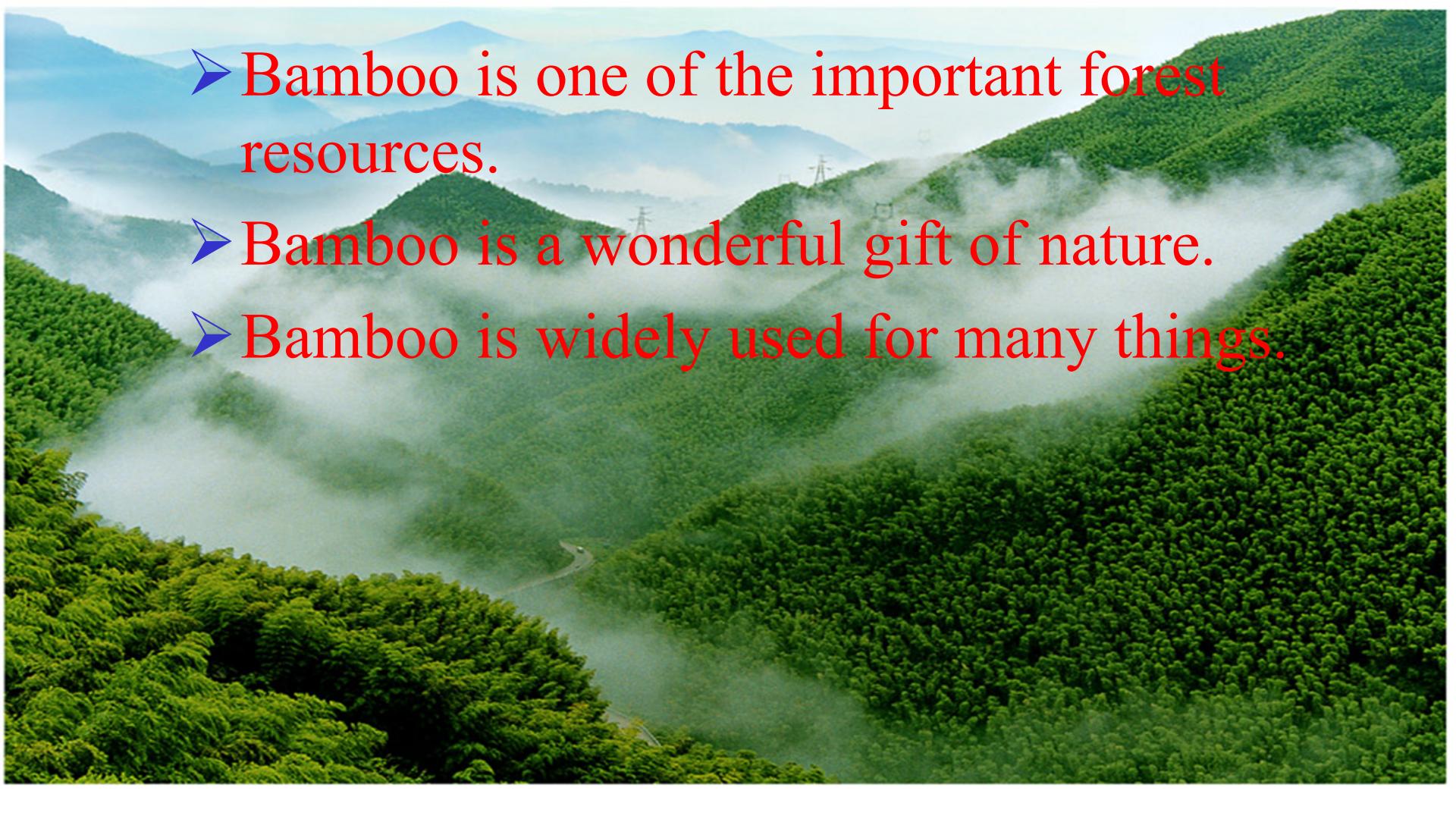


Content

- Introduction
- Materials and methods
- Result and analysis
- Conclusion



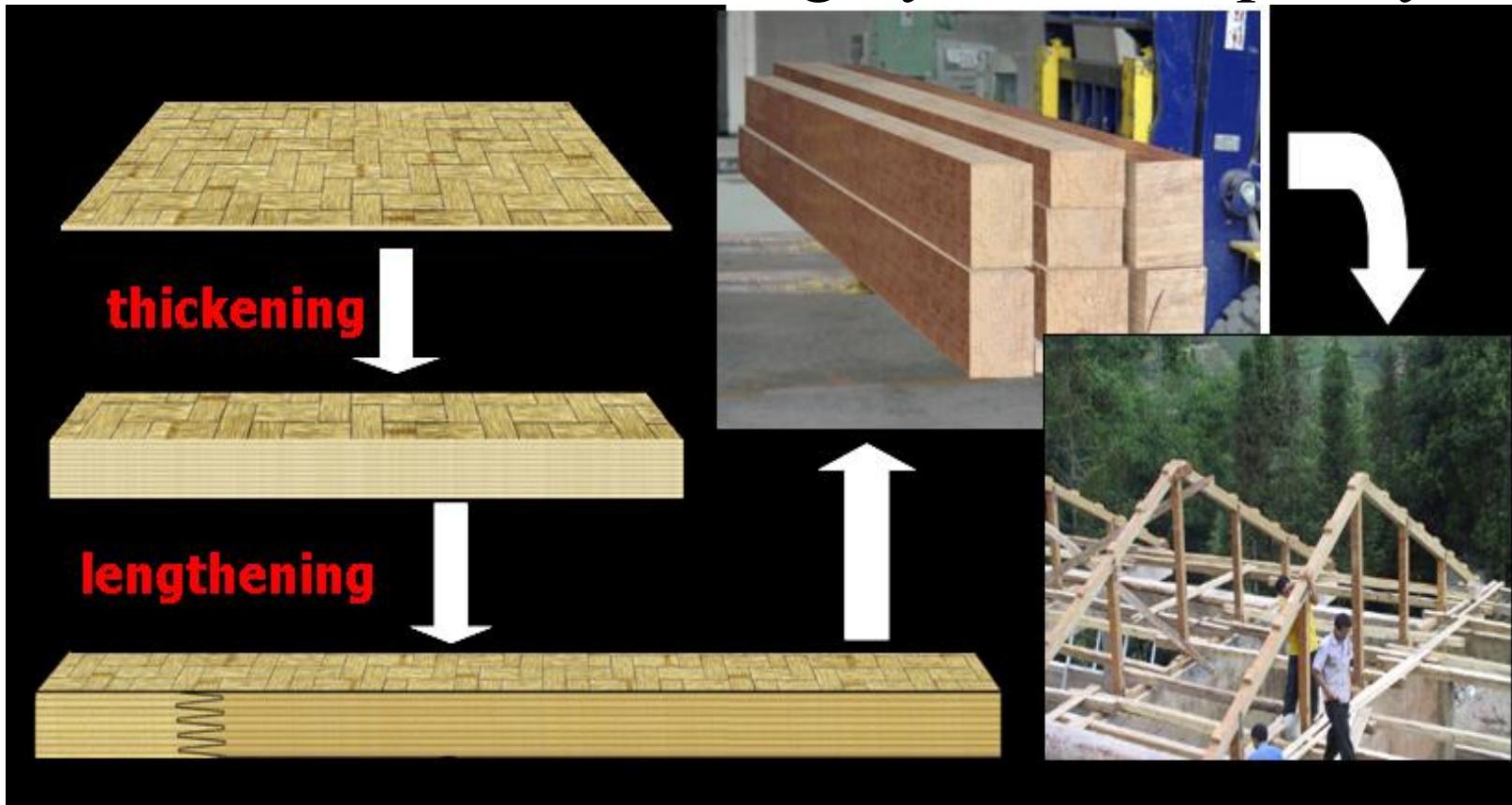
Introduction



- Bamboo is one of the important forest resources.
- Bamboo is a wonderful gift of nature.
- Bamboo is widely used for many things.

Introduction

- New varieties with high yield and quality



Introduction

➤ Crossbreeding is an effective way to get new varieties



麻竹花序



雷竹花序



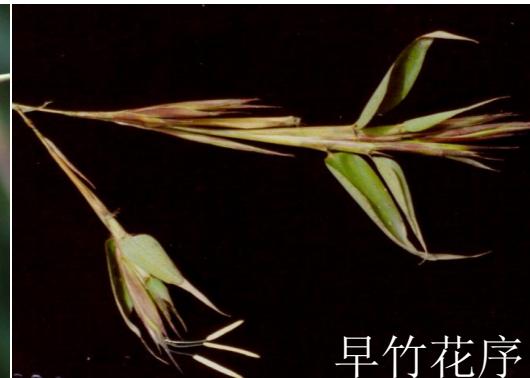
慈竹花序



毛竹花序



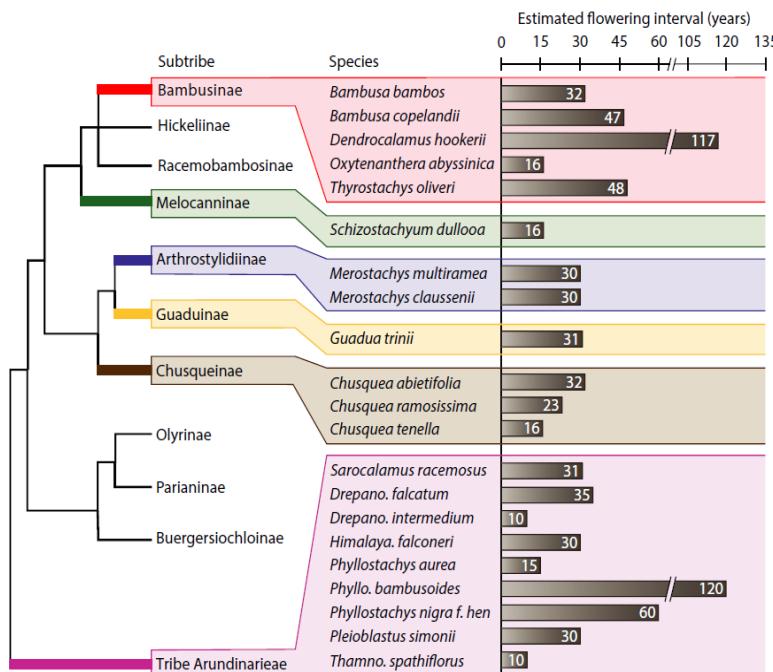
刺竹花序



早竹花序

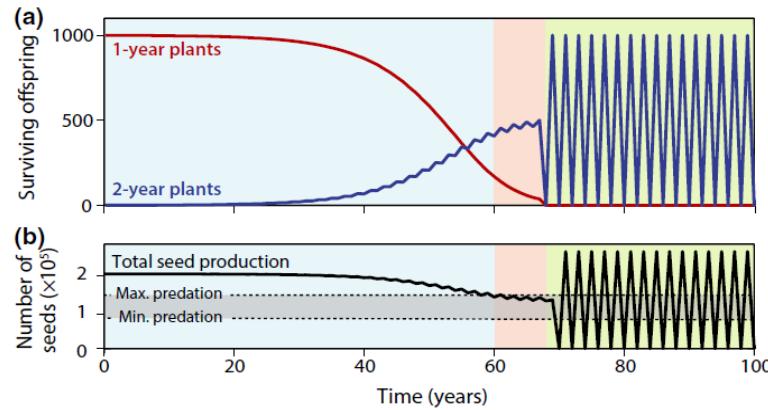
Introduction

➤ Extended flowering intervals of bamboos evolved by discrete multiplication (Veller et al., 2015. Ecol Lett.)



Long-intervalled flowering in bamboos

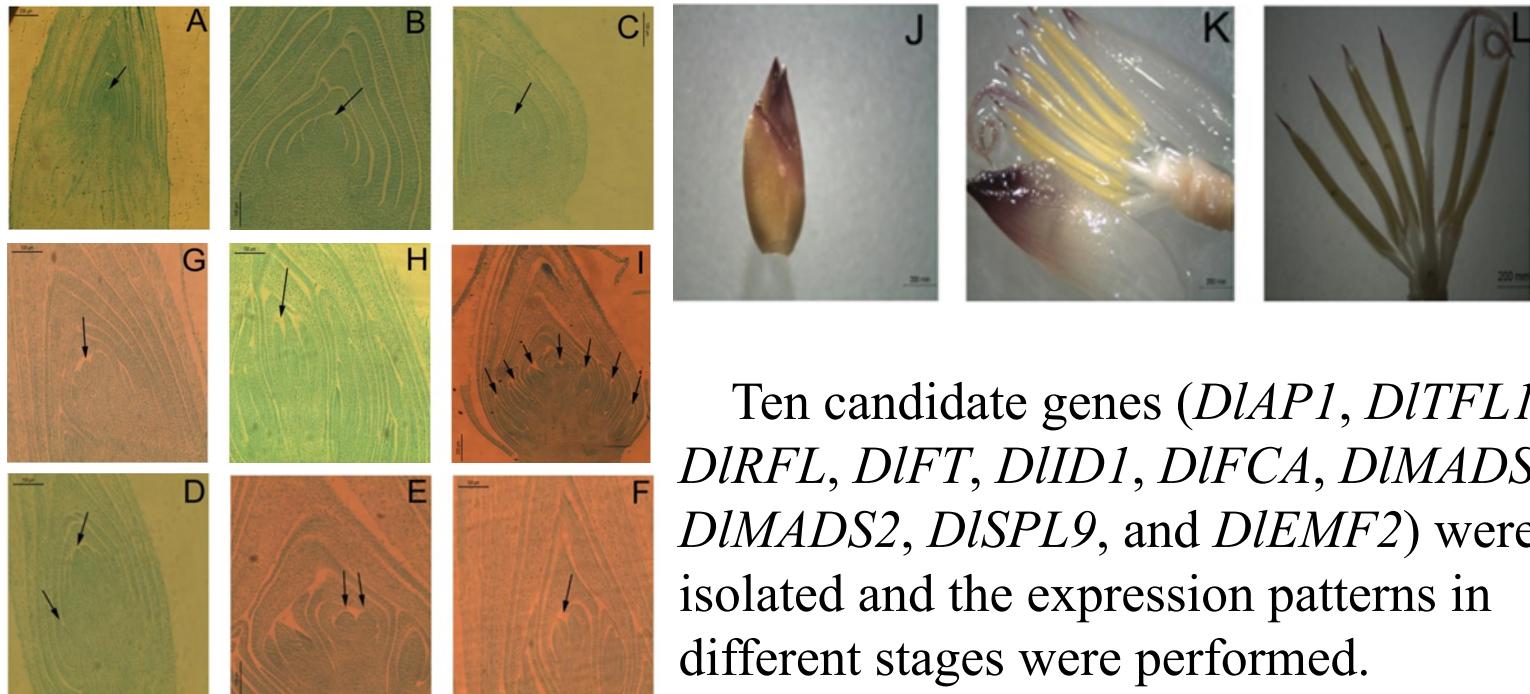
A model of initial synchronisation in bamboos



- First, an initial phase in which a mostly annually flowering population synchronises onto a small multi-year interval.
- Second, a phase of successive small multiplications of the initial synchronisation interval, resulting in the extraordinary intervals seen today.

Introduction

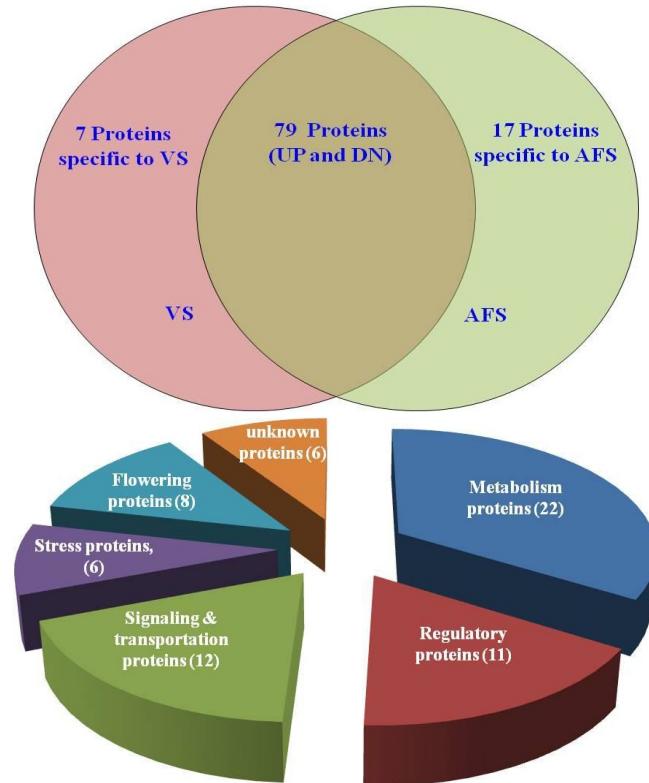
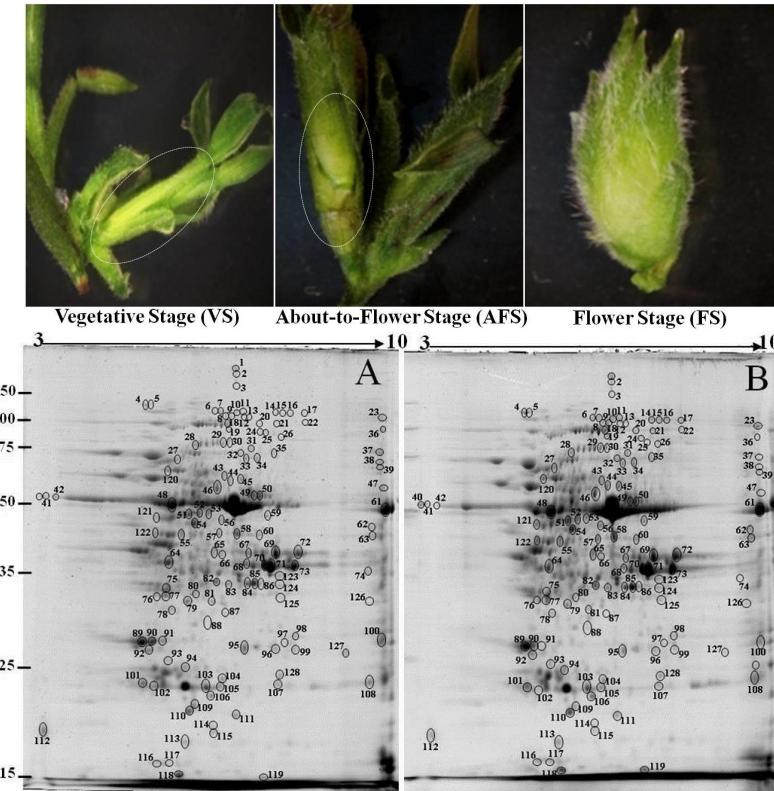
- Morphology and gene expression patterns during flowering in *Dendrocalamus latiflorus* (Wang et al., 2014. *Int J Mol Sci.*)



Ten candidate genes (*DlAP1*, *DlTFL1*, *DlRFL*, *DlFT*, *DlID1*, *DlFCA*, *DlMADS1*, *DlMADS2*, *DisPL9*, and *DlEMF2*) were isolated and the expression patterns in different stages were performed.

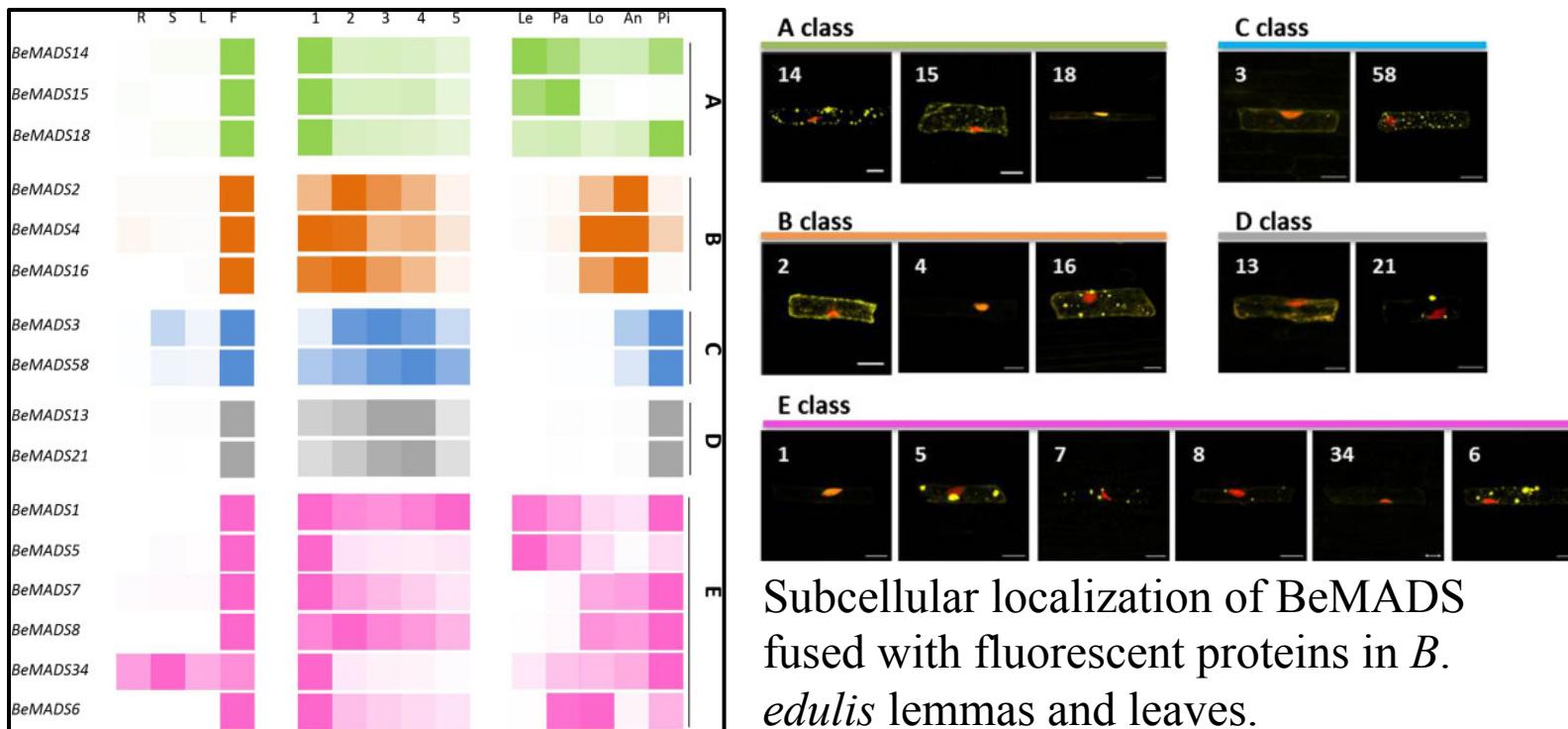
Introduction

➤ In vitro flowering associated protein changes in *Dendrocalamus hamiltonii* (Kaur et al., 2015. Proteomics.)



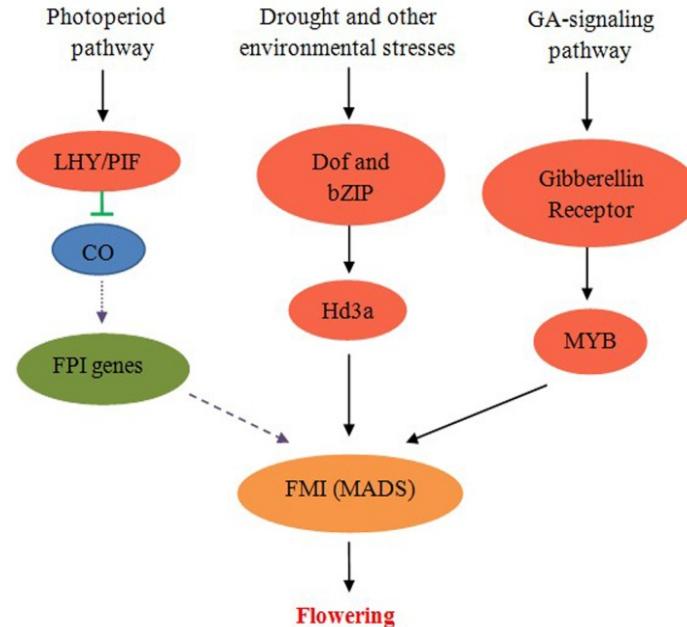
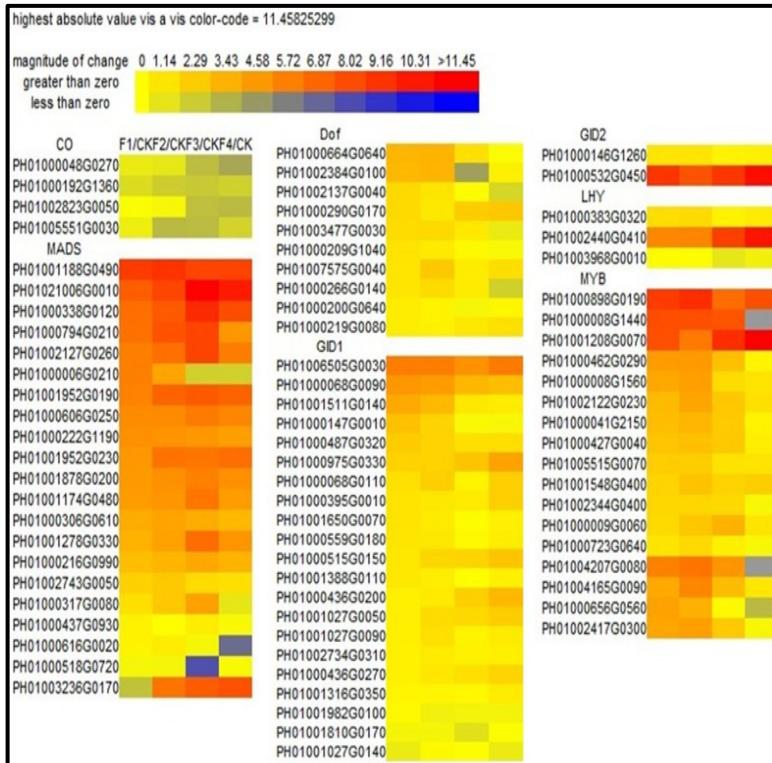
Introduction

➤ Transcriptome and MADS genes in floral development of *Bambusa edulis* (Shih et al., 2014. BMC Plant Biol.)



Introduction

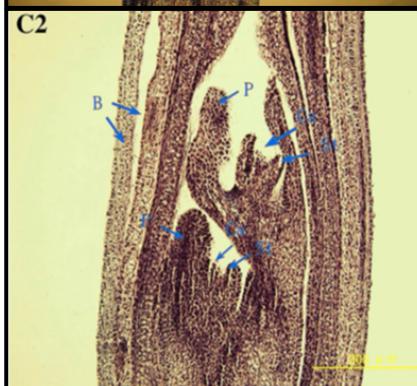
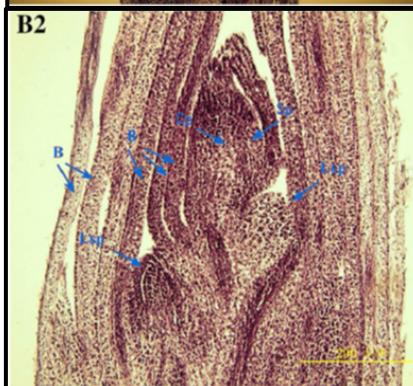
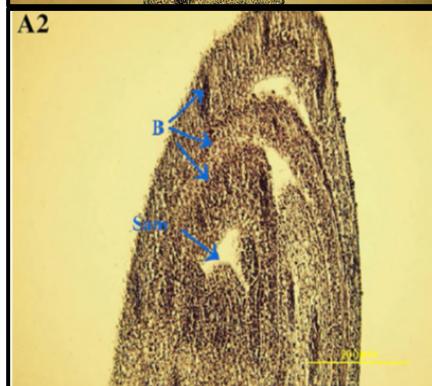
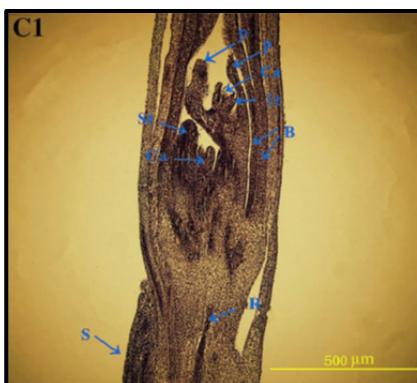
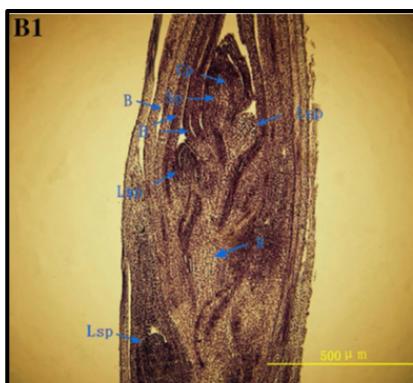
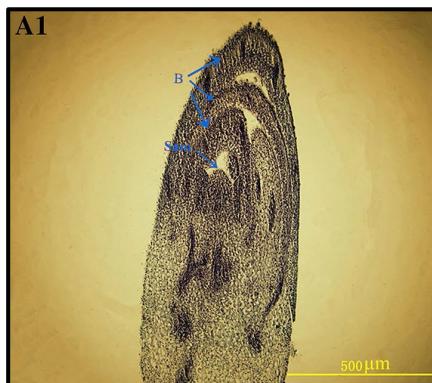
➤ Floral transcriptome of moso bamboo (*Phyllostachys edulis*) (Gao et al., 2014. PLoS One)



A hypothesized pathways in regulation of flowering in moso bamboo

Introduction

➤ miRNAs at different flowering developmental stages of moso bamboo (Gao et al., 2015. Mol Genet Genomics)

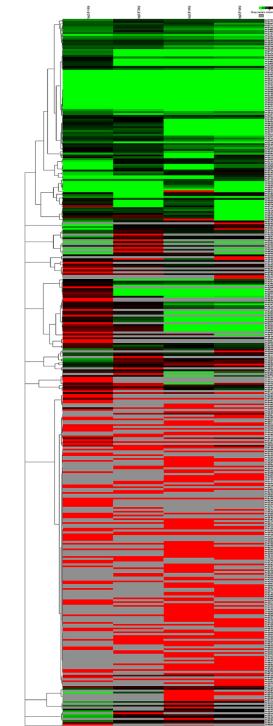


Floral bud formation

Inflorescence growing

Spikelets differentiation

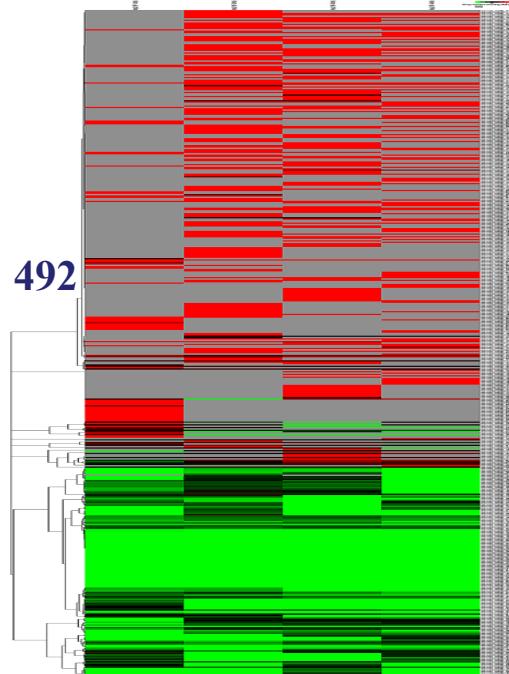
409
Known miRNAs



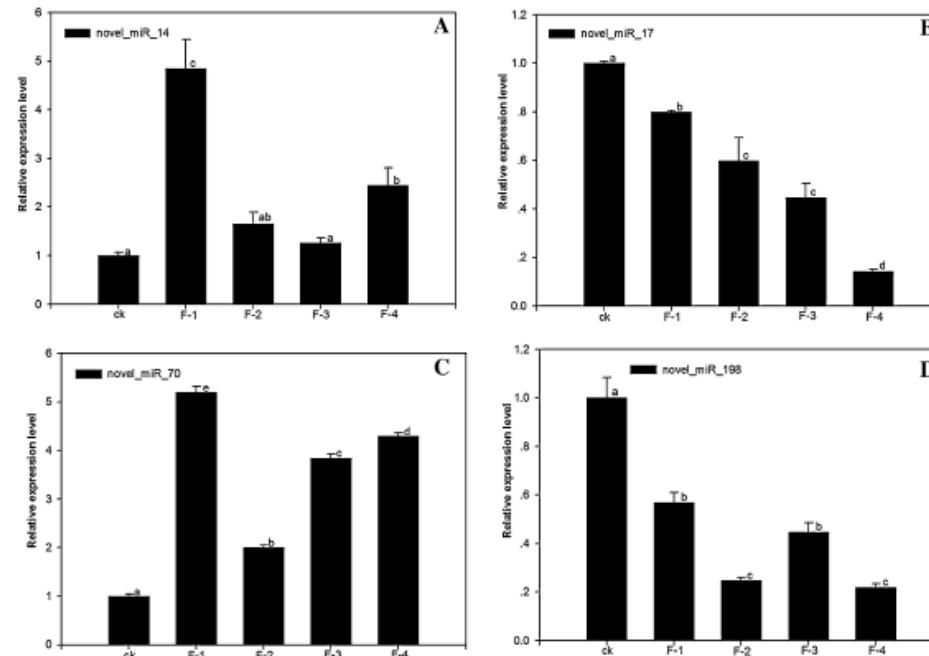
Expression Profiling

Introduction

➤ miRNAs at different flowering developmental stages in moso bamboo (Gao et al., 2015. Mol Genet Genomics)



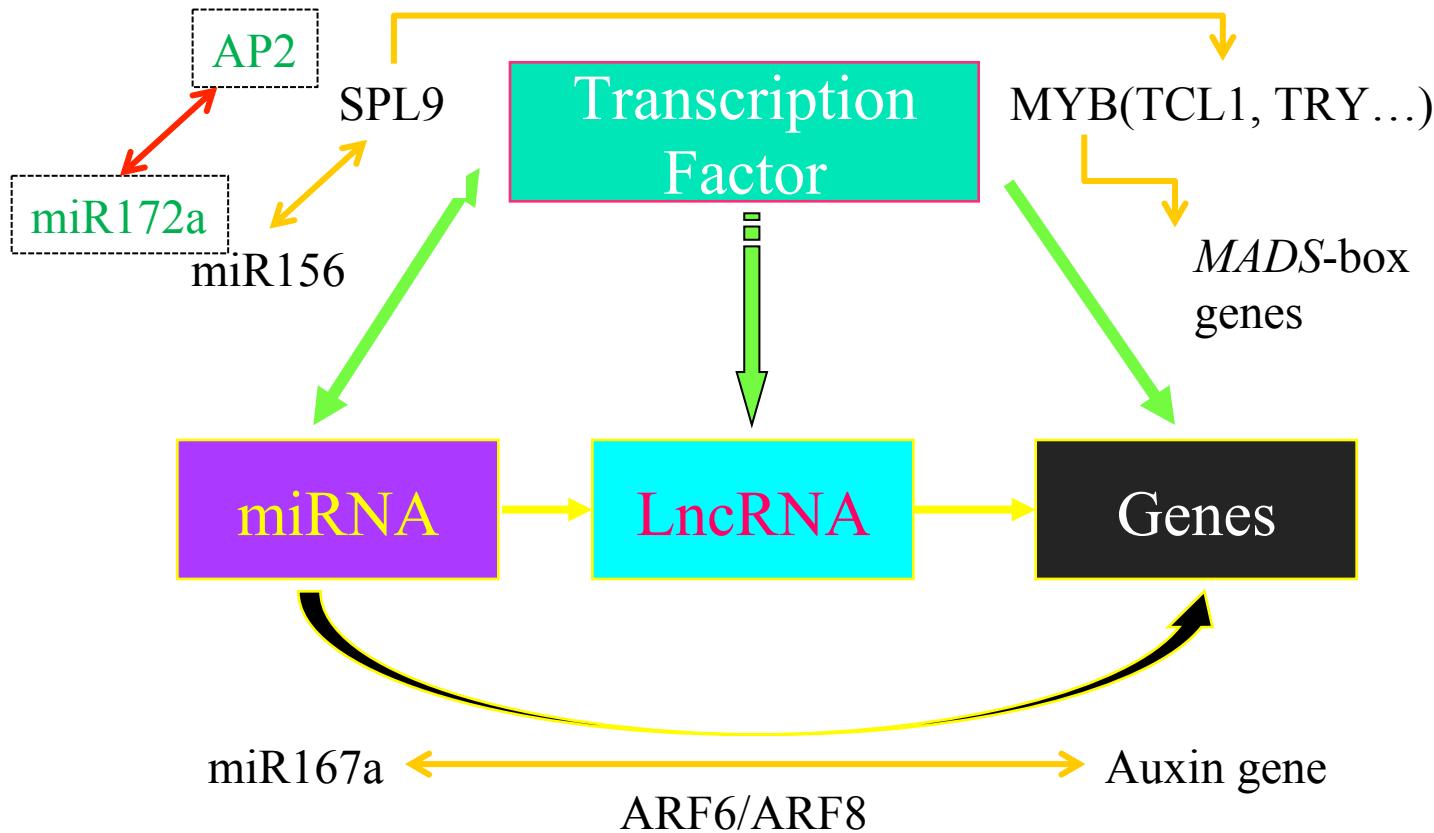
Expression profiles of novel miRNAs



Expression validation of four novel miRNAs in flower at different stages

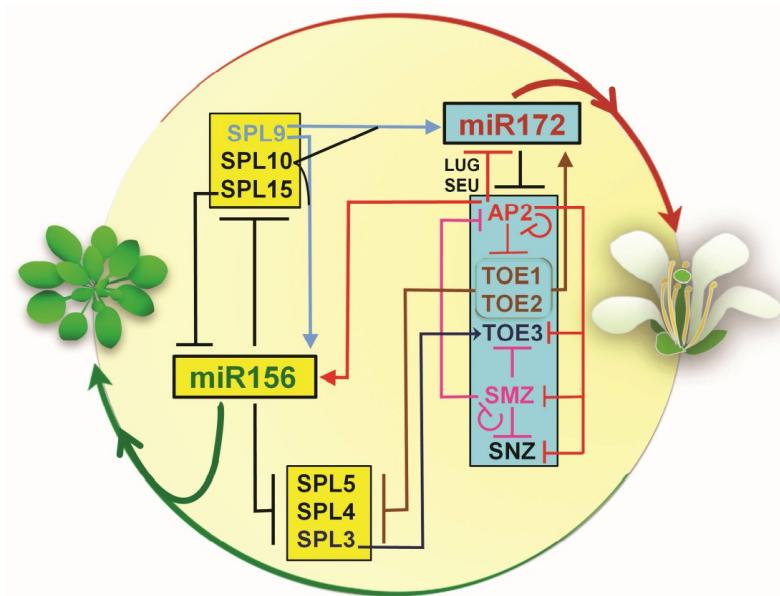
Introduction

➤ Regulation model of miRNA-TF-Gene



Introduction

- Transcription factors of APETALA2 (**AP2**) is a super family, involved in many growth processes of plant development, such as the regulation of floral meristem, ovule and seed development etc.
- *AP2* belonged to class A genes of flower development model, involving in developmental regulation of petals, while its own expression was regulated by miR172a.

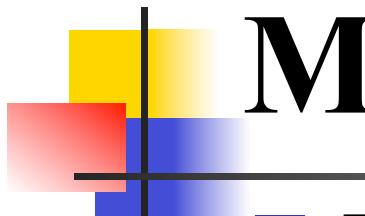


Materials and methods

➤ Plant materials



Ma bamboo seedlings (*Dendrocalamus latiflorus*)



Materials and methods

■ Methods

- Bioinformatics
- Molecular biology



Result and analysis

➤ *DIAP2* cloning

- ✓ FL cDNA: 1729 bp
- ✓ ORF: 1464 bp
- ✓ 5' UTR: 81 bp
- ✓ 3' UTR: 184 bp

Two conserved domains of AP2/ERF

miR172a target site in the coding frame near the 3' end of the 130 bp

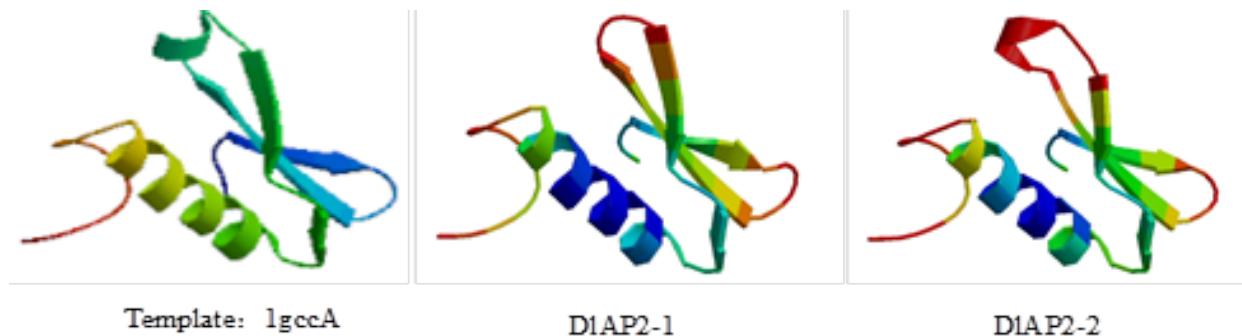
GenBank No: KM267641

1 ACGCGGGGGCTGGTTTGGGTTGAGGGTGTGATTGGAGTTGGATTGACTTGGTTGAGGAGGTGGAGGAGATGGAGCTGGATCTG
M E L D L
97 AACGTGGGGACGGGGCGCCGGAGAACCGGAACGGATGGCGCGAGCAGTCCGGCACGTCGACTGCCGGTCTGAACCGGAGGGCTCCGGC
N V A D G A P E K P E A M A R S D S T S D S P V L N A E A S G
193 GCGGGAGGCCGGCGGGCTGGCGCCGGAGGGGGCTCCAGCTCGACGCCCGCCGCTGGCGGTCTGAGTCAGCATGAGGAAGTCGGCG
G G G A A G A P A E E G S S S T P P P L A V L E F S I M R S S A
289 TCGCCGAGGGCGAGAAAGACGTGGGGTTCGGCATGACGAGGAGGGCACCCGTCGCCCTCTCGCCGGCAGCAGCTGTACCCAGCAGTC
S A E G E K D V G V A D D E E A T P S P L R R Q L V T Q Q L
385 TTCCGGTCAAGCGGCCGGCGCCGCGCCGGCGCCGCGCCGCGCCGCGCCGAGCTGGTTCTTACGCCCGAGCCGCCGGCGCAGCGGACATC
F P V D A G P P R P V P Q P G A E L G F L R P E P P G P Q P D I
481 AGAACCTTGGCGCTCCCGCAGGCGCACGCGCCGCCGCGCAGCCGAGGTACCAAGAACAGGCCCGCCGCCGCGCTCCCGCAGCTCGCAGTC
R I L P L P Q A H A P P A Q P Q A T K K S R R G P R S R S S Q Y
577 CGCGGCGTCACCTTCTACCGCCGACCGCCGCTGGAAATCCATTGGATTGGCGCAAGCAAGTGTACTTAGGTGGAATTGACACTGCTCAT
R G V T F Y R R T G R W E S H I W D C G K Q V Y L G G F D T A H
673 GCTGCTGAAGGGCGTATGATCGAGCGGCGATCAAGTTCCGGCATCGACACGGACATAAACTCGATCTTAGTGAACAGGAGCAGATGAAG
A A A R A Y D R A A I K F R G I D T D I N F D L S D Y E D D M K
769 CAGGTGAAGAGCCTATCCAAGGGAGGTTCTGTCAGCTCGCGACAGCTCTCGCGAGGAGCTCCAAATACAGAGGGCTAAC
Q V K S L S K E E F V H V L R R Q S T T G F S R G S S K Y R G V T
865 CTGCAAAAGTGGCCGATGGGGAGCTCGCATGGCCAGTCTCTCGGAAGAAGTACATATCTGGGATTTCGACAGCGAAGTAGAGGCTGCA
L H K C G R W E A R M G Q F L G K Y I Y L G L F D S E V E A A
961 AGGGCTTATGATAAGGTGCGATCAAATGCAATTGGTAGAGAACGGCTGACGAACCTCGAGCTAGCACATATGATGGGGAGATGCTTAAGGTT
R A Y D K A A I K C N G R E A V T N F E P S T Y D G E M L T E V
1057 GGTGCTGAAGGTGAGATGTCGATCTGAACCTGGCATATCTCAACAGCTTGCAGAGGCCAAAGGGATAAGACTCCCTGGCTCGAGCTG
G A E G A D V D L N L S I S Q P A L Q S P Q R D K N S L G L Q L
1153 CACCATGGATTATGATGGCTCTGAAGTGAAGAACAGCTAAAGTGTGCTCCCTGAACTGGCTGGCCGCCATCGTTCCCTCTTCGACCC
H H G L F D G S E V K R A K I D A P S E L A G R P H R F P L L T
1249 AAGCATCCACAGTCTGGCTGCCAATCTCACCCCATATTCAAATAATGAGGTGATCTAGAGATCATAAACAGGAGGCCAGGGGGACCC
K H P P V W P A Q S H P I F S N N E D A S R D H N R R P E G S T
1345 GGGGGTGTCCAGCTGGCATGGAAAGTGAAGGCCACCTCCACCAACTACCATTCGCGCTGTTCTCGCTGTTGCACTCGTCATCGCAGCA
G G V P S W A W K V S H P P P T L P L P L F S S L S S S S A A A
1441 TCATCAGGATTCTCCAGAACCGTCAAGATAGCTATCTCCACCACTACCATTCGCGCTGTTCTCGCTGTTGCACTCGTCATCGCAGCA
S S G F S R T V K I A I S T T P S T S L Q F D P M A P S S N H
1537 CACCGCTGAATAGAACCCACACTGTAATTGGCCGGAAAGCCGGCATCTTTCCTCCGACCTTCAACGCTCTCGTTGGCCGGGTGG
H R
1633 TTTCTGTAGTGGATTGGATTGACTGACTGATTTGATGCTGCCAATTGAAATGTTCTATTGACCGCACAAAAA

Result and analysis

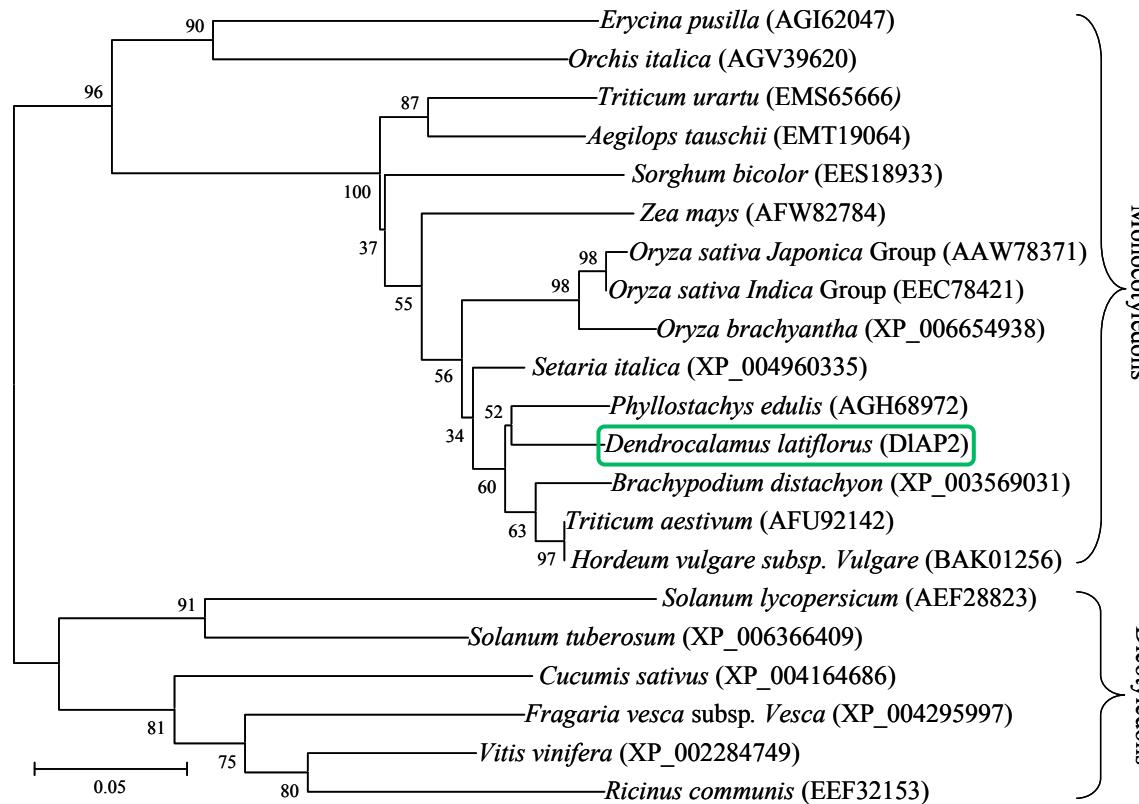
➤ Protein properties analysis and structure prediction

- ✓ *DlAP2* encoded a putative peptide of 487 amino acids
- ✓ Theoretic isoelectric point: 6.859
- ✓ Molecular weight: 52.75 kDa
- ✓ Hydrophilicity / hydrophobicity: strong hydrophilicity
- ✓ Typical structure of AP2: with 3 α helix and 3 β folds



Result and analysis

➤ Phylogenetic analysis of DIAP2



Result and analysis

➤ ***MiR172a*-mediated cleavage site identification**

RNA ligase-mediated 5' RACE

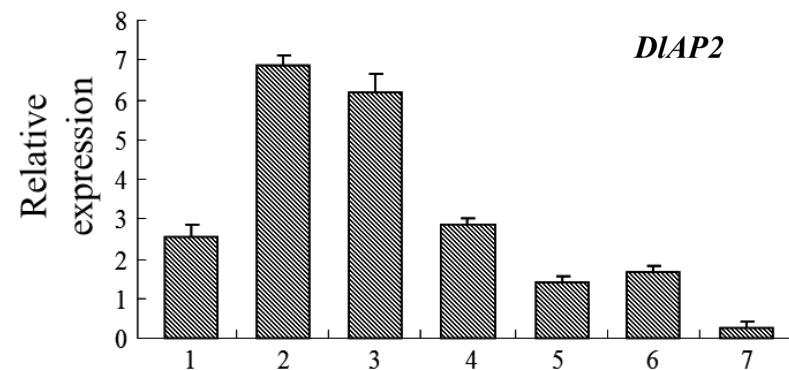
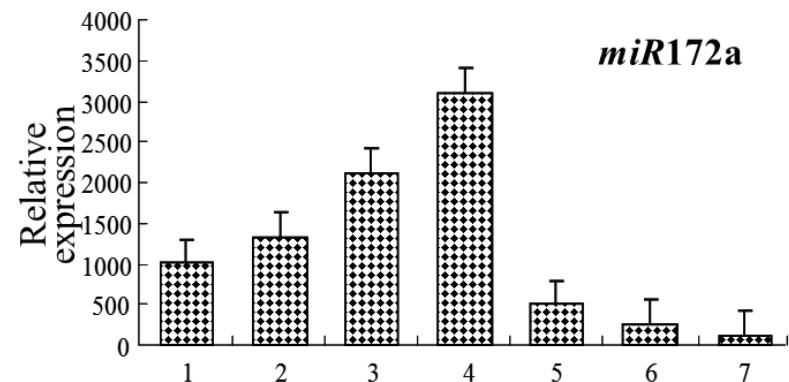
DIAP2 sequence: C T G C A G C A T C A T C A G G A T T C T

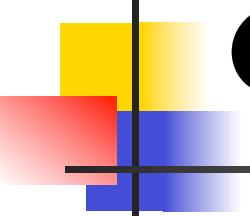
miR172a: G A C G U C G U A G U A G U C C U A A G A

----The result of sequencing showed that there were nine samples with cleavage sites between the 11th-12th bases, and only one sample with a cleavage site between the 12th -13th bases in the ten sequenced samples.

Result and analysis

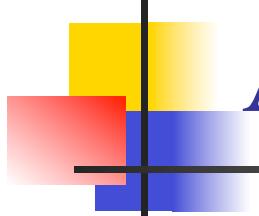
➤ Expression of *miR172a* and *DLAP2* in different tissues





Conclusion

- Full length cDNA of *DlAP2* was cloned from *D. latiflorus* by RT-PCR and RACE.
- RLM-5' RACE analysis showed that *DlAP2* was regulated by *miR172a* through cleavage mainly at the site between the 11th and 12th bases.
- qRT-PCR results showed that the expression pattern of *DlAP2* was opposite to that of *miR172a* in flower buds, which indicated that *miR172a* played a regulatory role in regulating the expression of *DlAP2*.



Acknowledgements

- The work was supported by the Sub-Project of National Science and Technology Support Plan of the Twelfth Five-Year in China [No. 2015BAD04B01 and No. 2015BAD04B03].
- The introduction project of “948” by the State Forestry Administration of China [No. 2011-4-55].



Thank you for your
attention!