Isolation and Expression Analysis of *DlAP2* Targeted by *miR*172a in *Dendrocalamus latiflorus*

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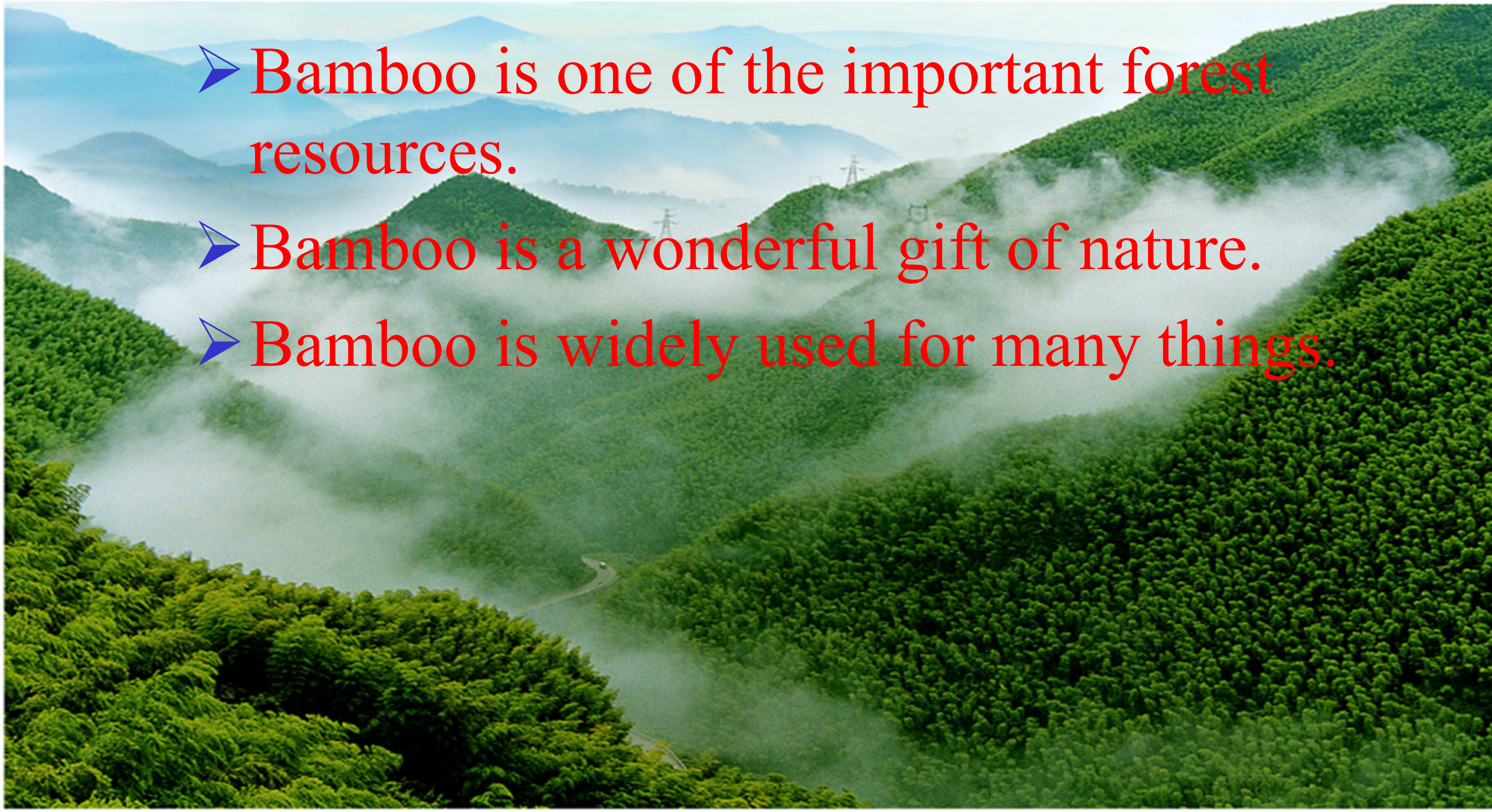
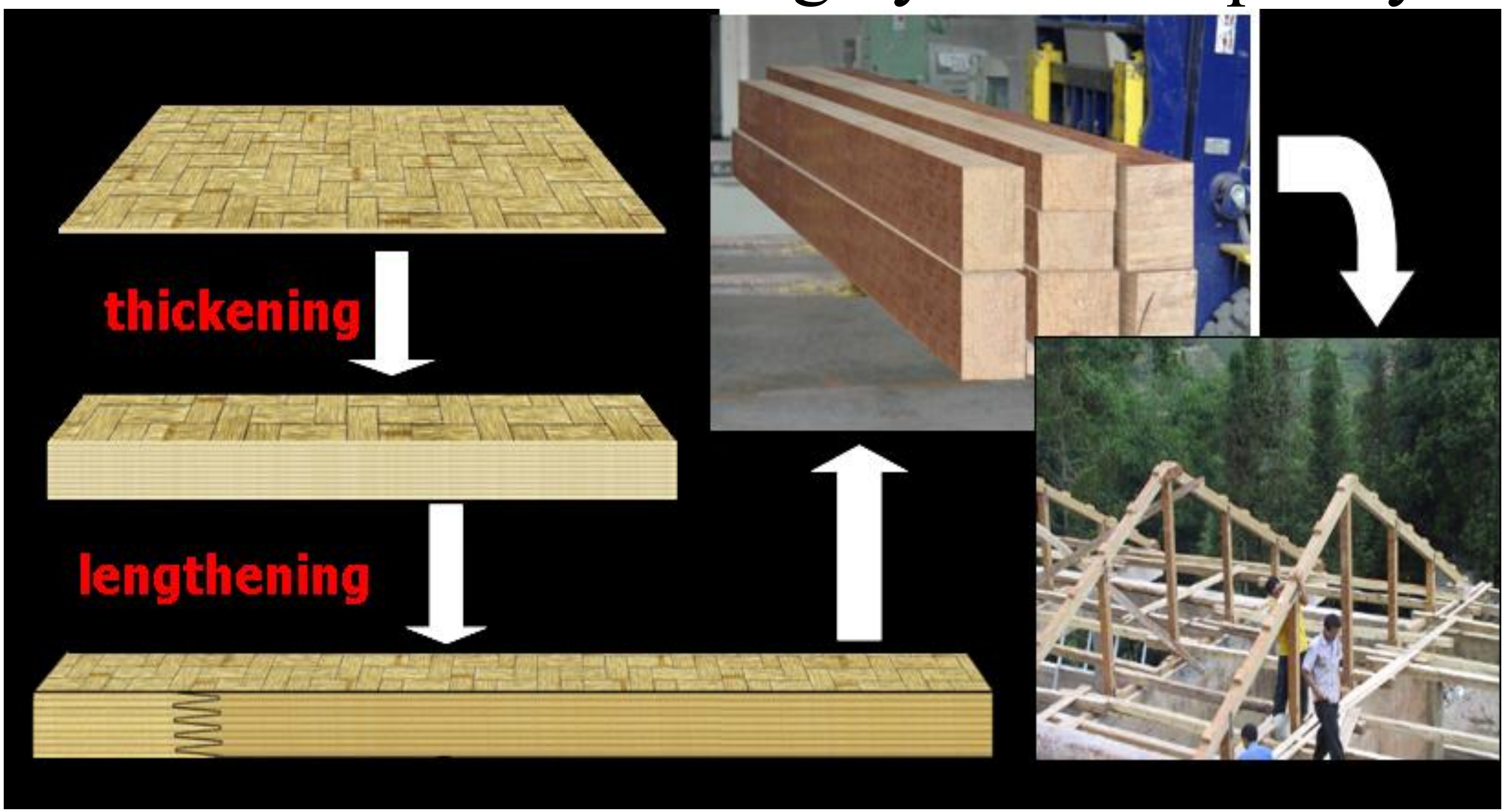
Introduction

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



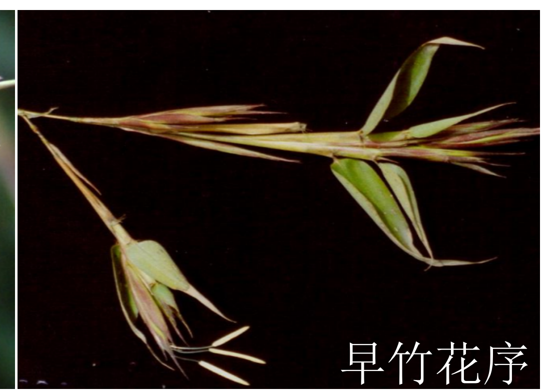
Introduction

- New varieties with high yield and quality



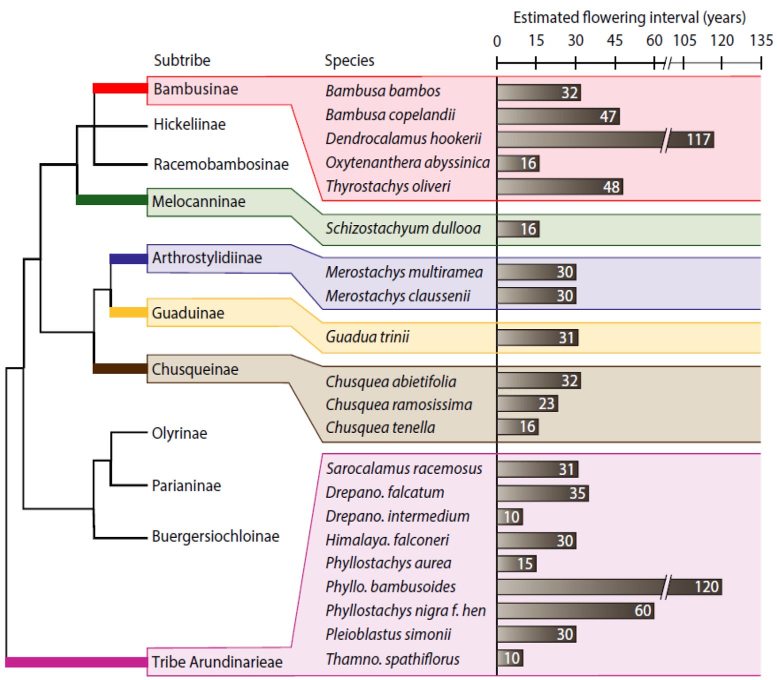
Introduction

- Crossbreeding is an effective way to get new varieties



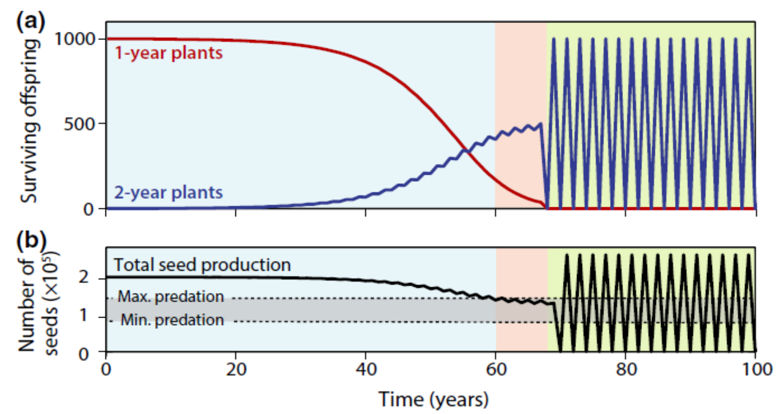
Introduction

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



Long-intervalled flowering in bamboos

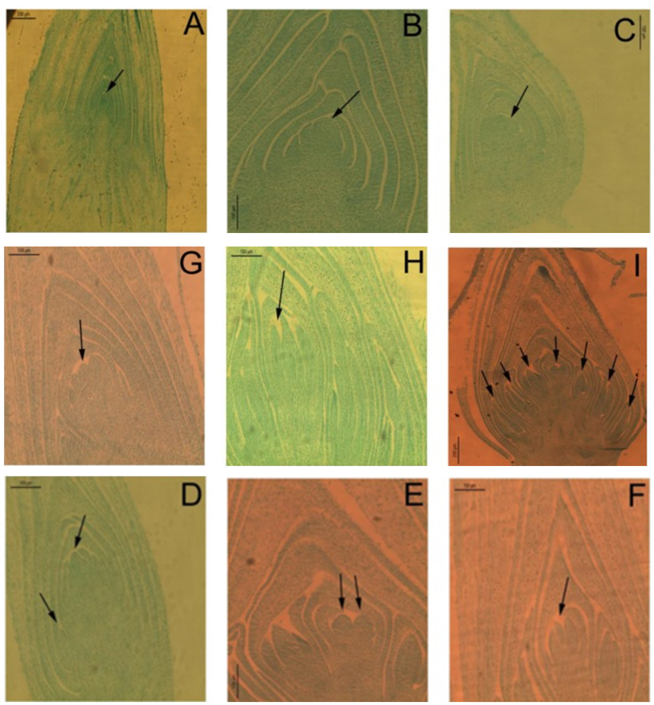
A model of initial synchronisation in bamboos

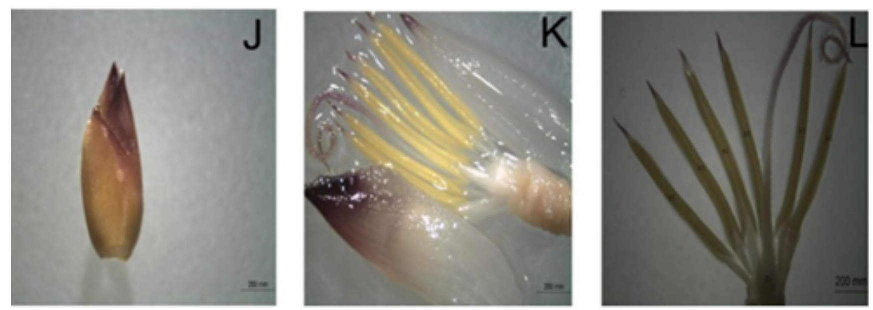


- First, an initial phase in which a mostly annually flowering population synchronises onto a small multi-year interval.
- Second, a phase of successive small multiplications of the initial synchronisation interval, resulting in the extraordinary intervals seen today.

Introduction

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

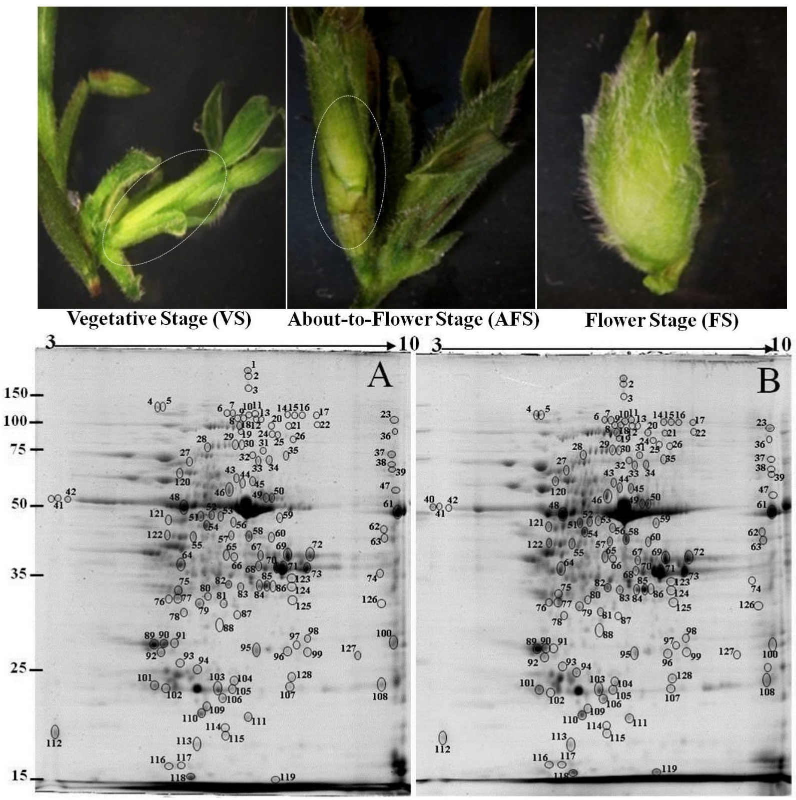


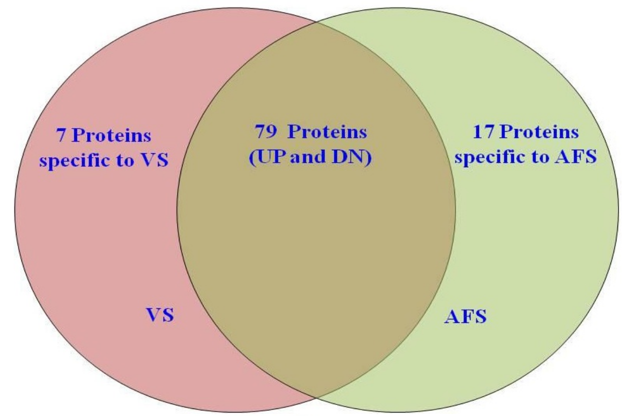


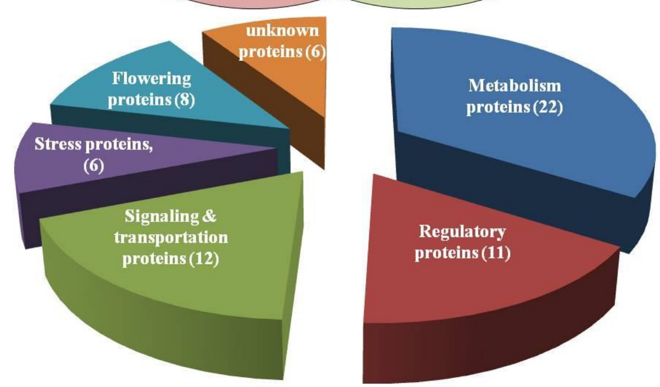
Ten candidate genes (*DlAP1*, *DlTFL1*, *DlRFL*, *DlFT*, *DlID1*, *DlFCA*, *DlMADS1*, *DlMADS2*, *DlSPL9*, and *DlEMF2*) were isolated and the expression patterns in different stages were performed.

Introduction

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

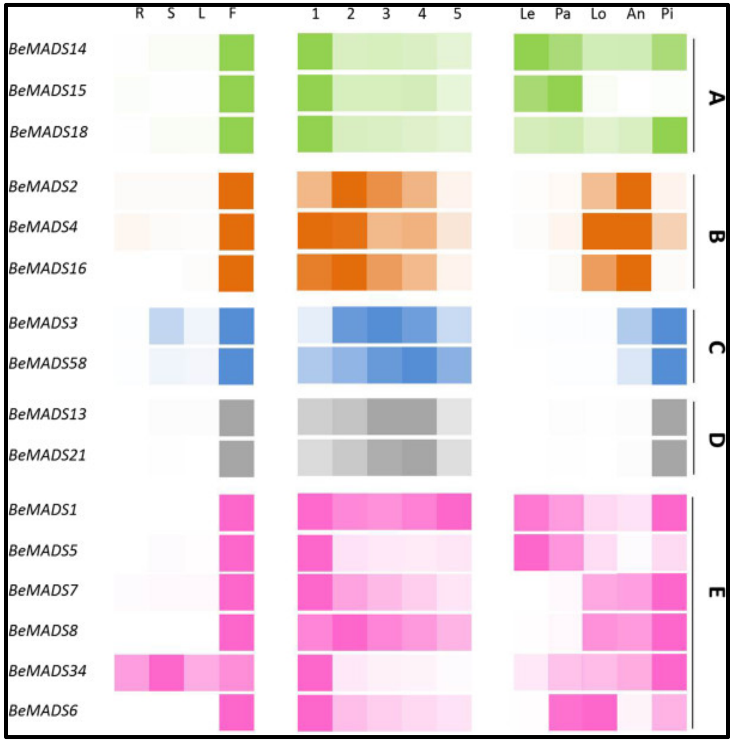


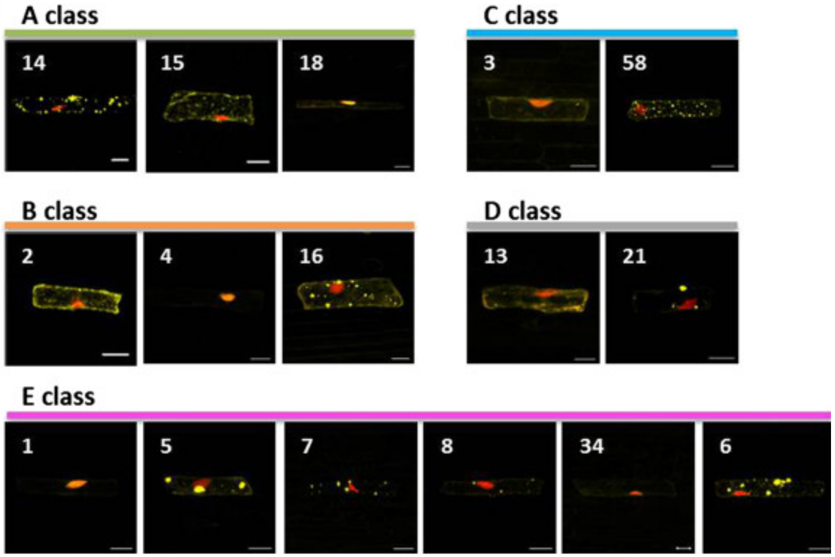




Introduction

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

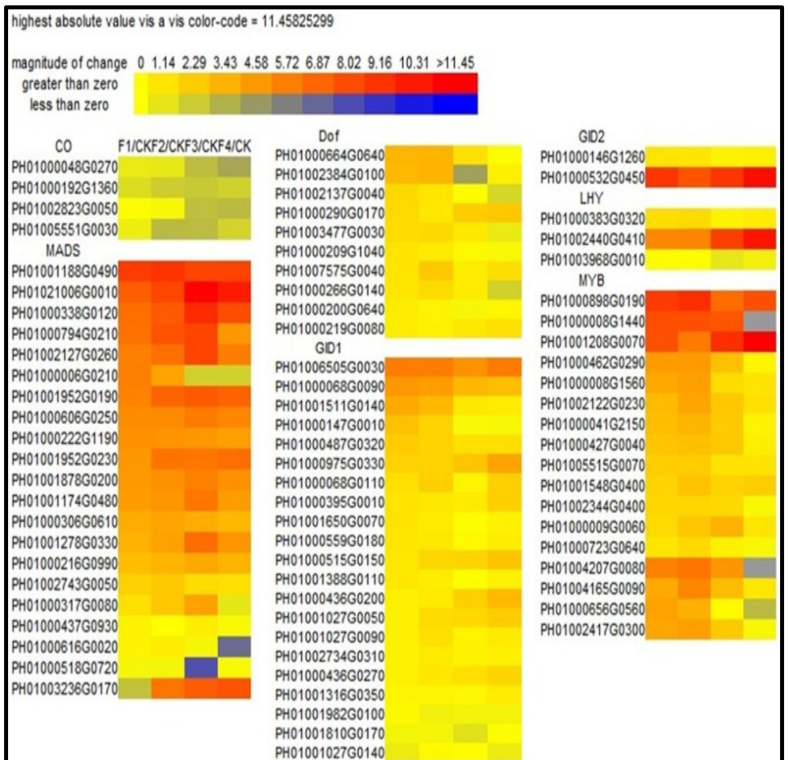


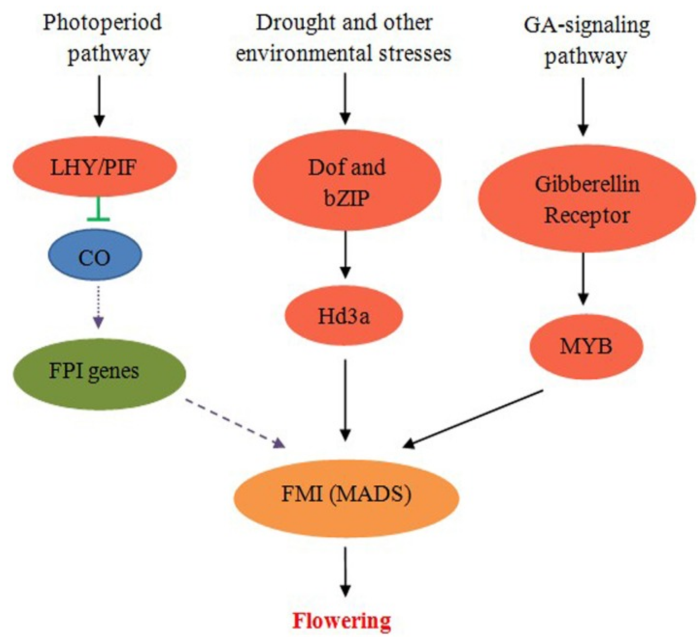


Subcellular localization of BeMADS fused with fluorescent proteins in *B. edulis* lemmas and leaves.

Introduction

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

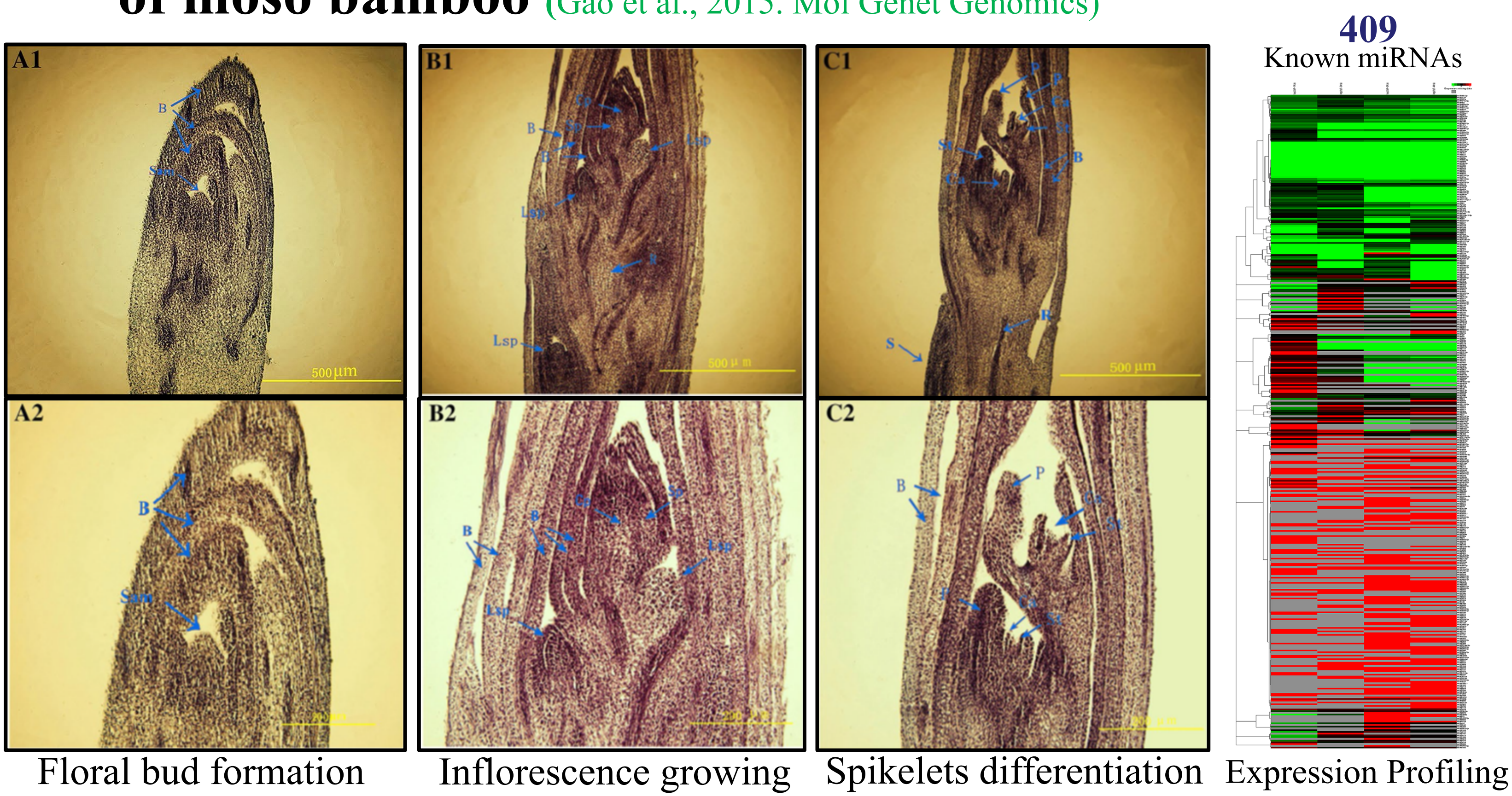




A hypothesized pathways in regulation of flowering in moso bamboo

Introduction

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



Introduction

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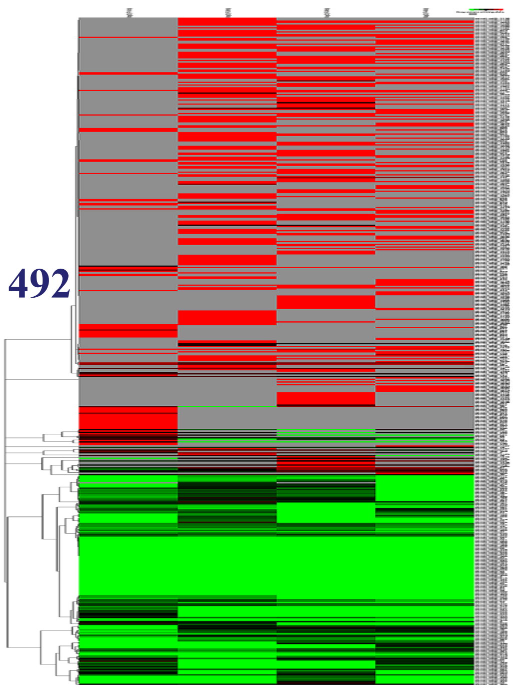
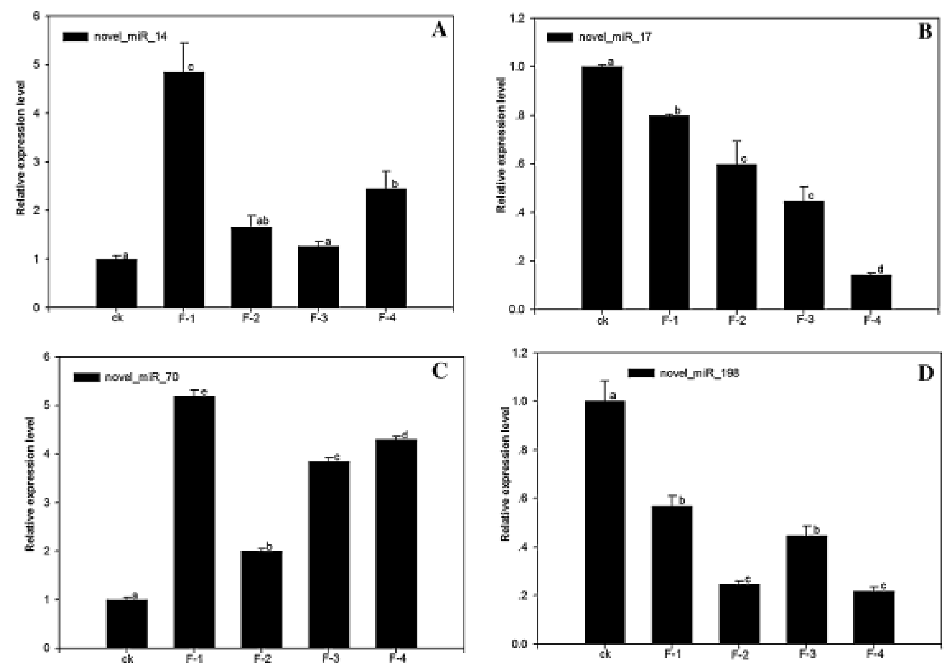


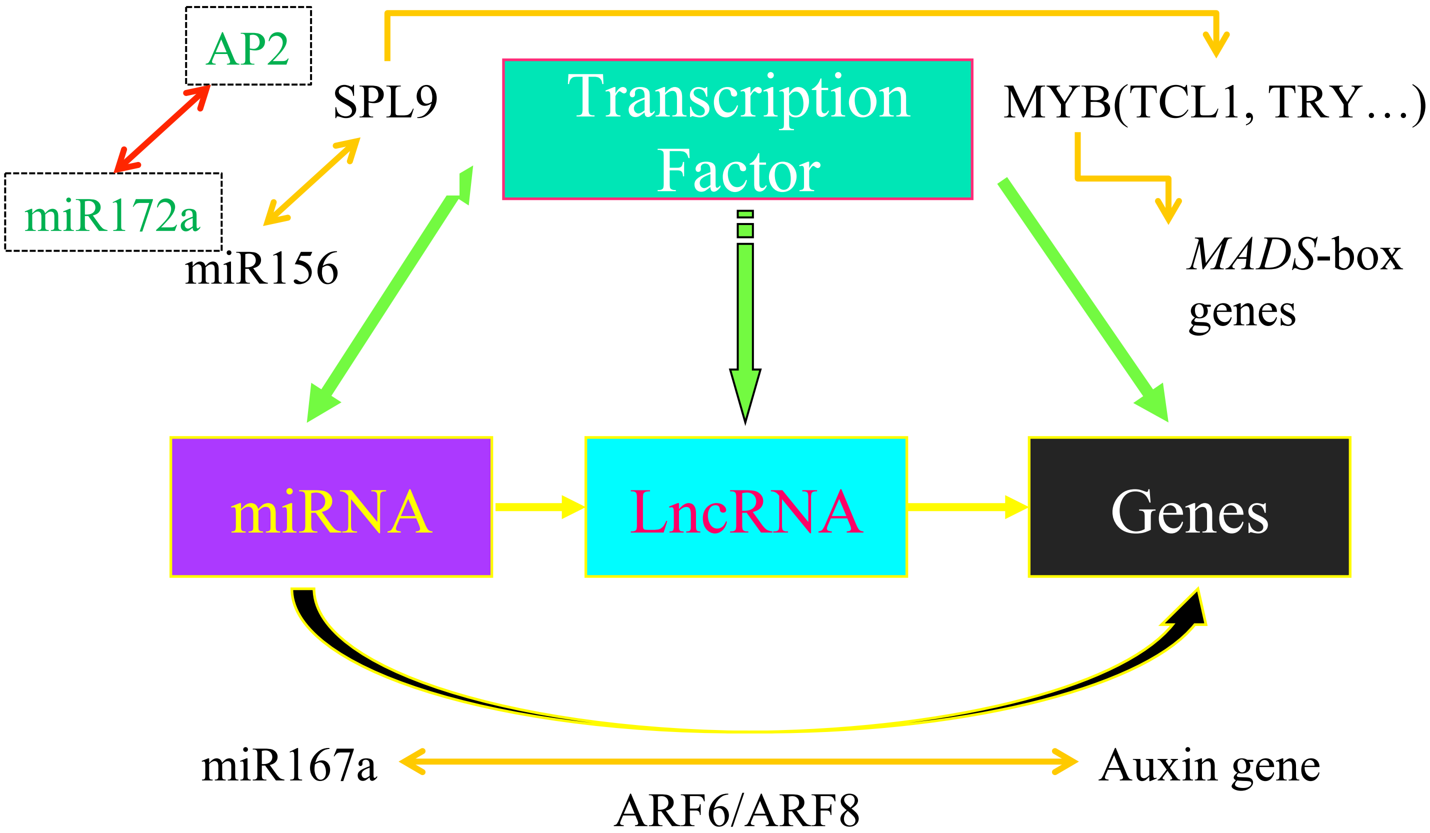
Expression profiles of novel miRNAs



Expression validation of four novel miRNAs in flower at different stages

Introduction

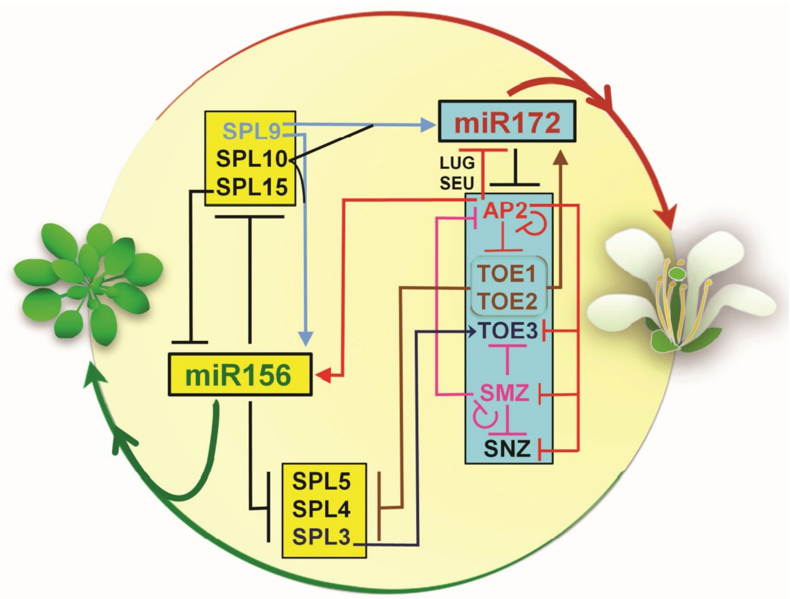
- **Regulation model of miRNA-TF-Gene**



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

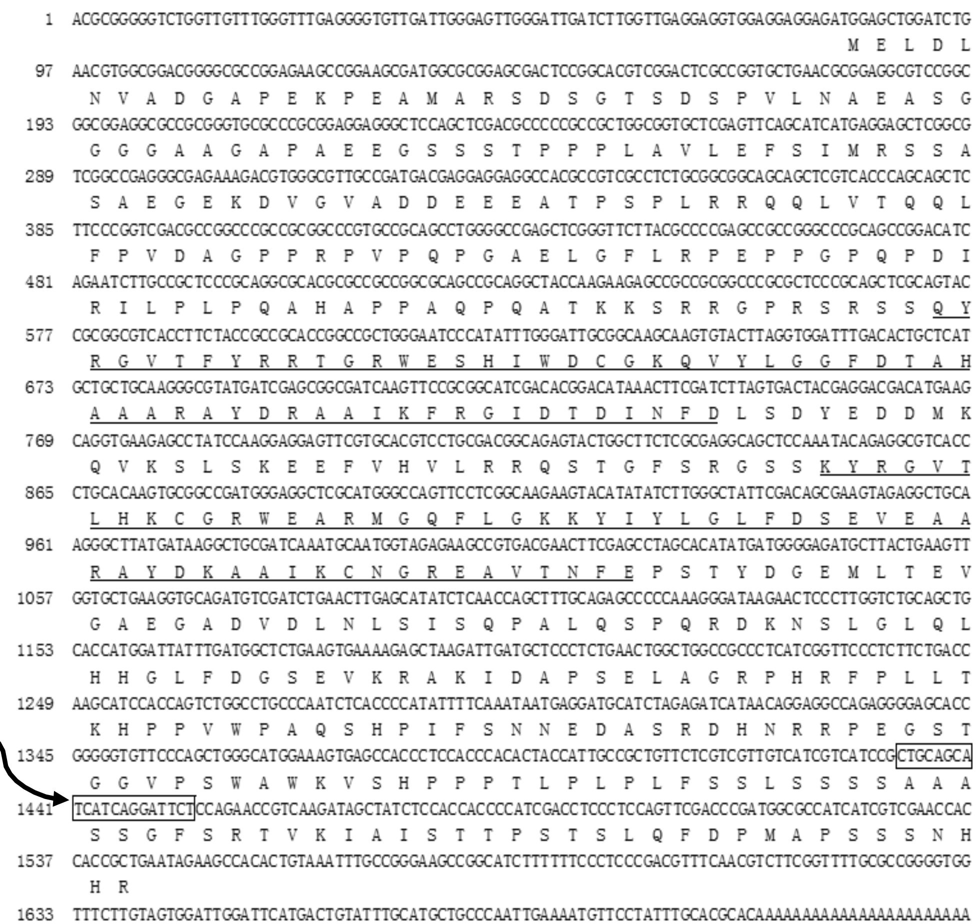
Result and analysis

- ***DlAP2* cloning**
  - ✓ FL cDNA: 1729 bp
  - ✓ ORF: 1464 bp
  - ✓ 5′ UTR: 81 bp
  - ✓ 3′ UTR: 184 bp

Two conserved domains of AP2/ERF

*miR*172a target site in the coding frame near the 3 'end of the 130 bp

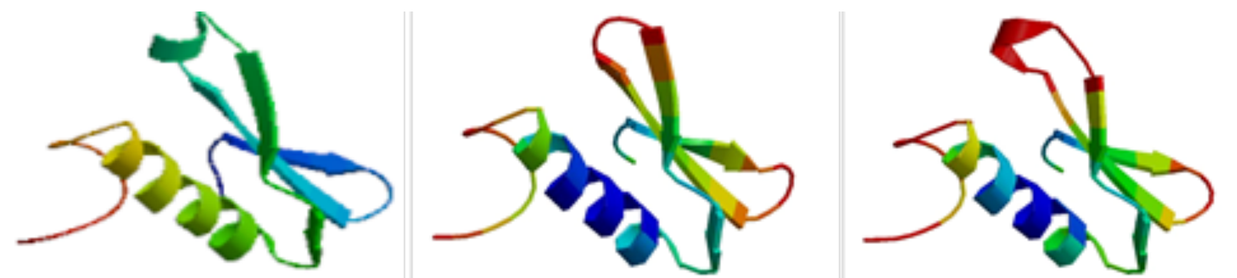
GenBank No: KM267641



Result and analysis

➢ Protein properties analysis and structure prediction

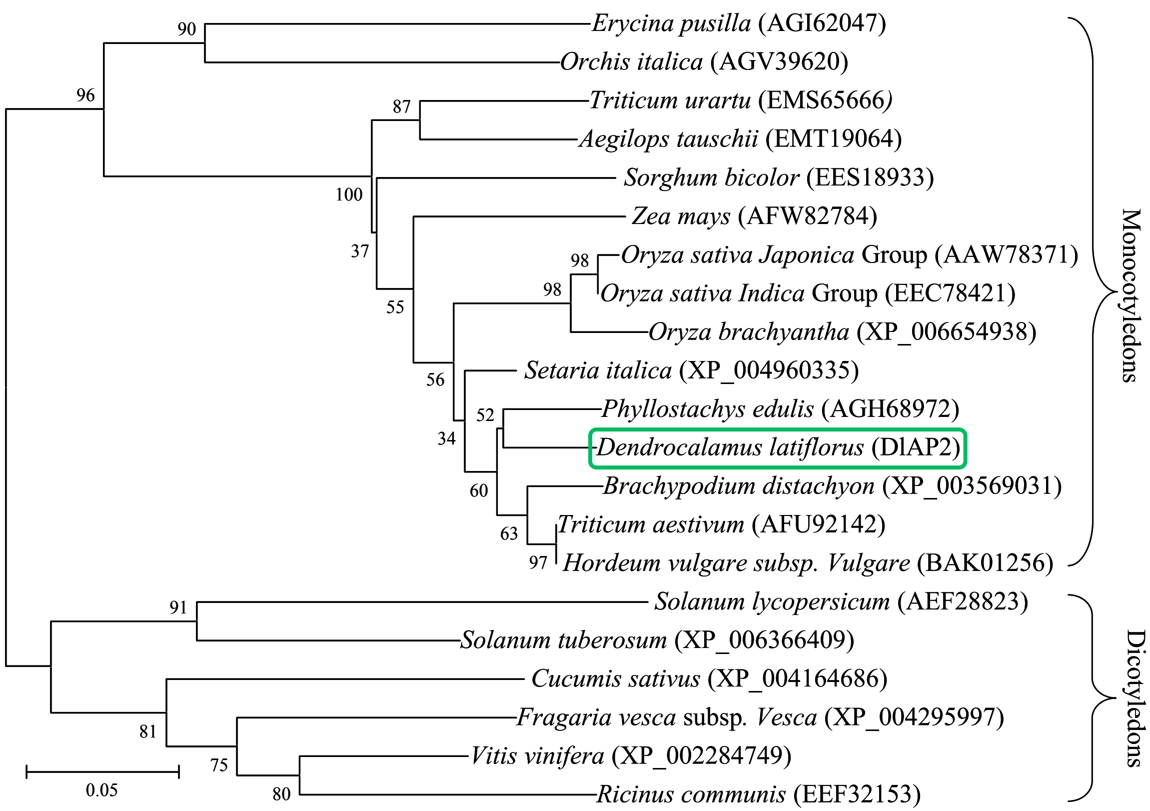
- ✓ *DlAP2* encoded a putative peptide of 487 amino acids
- ✓ Theoretic isoelectric point: 6.859
- ✓ Molecular weight: 52.75 kDa
- ✓ Hydrophilicity / hydrophobicity: strong hydrophilicity
- ✓ Typical structure of AP2: with 3 α helix and 3 β folds



Template: 1gccA D1AP2-1 D1AP2-2

Result and analysis

- **Phylogenetic analysis of DlAP2**



Result and analysis

➢ ***MiR*172a-mediated cleavage site identification**

RNA ligase-mediated 5 ′RACE

9/10 ↓ ↓ 1/10

DIAP2 squence：**C T G C A G C A T C A T C A G G A T T C T**

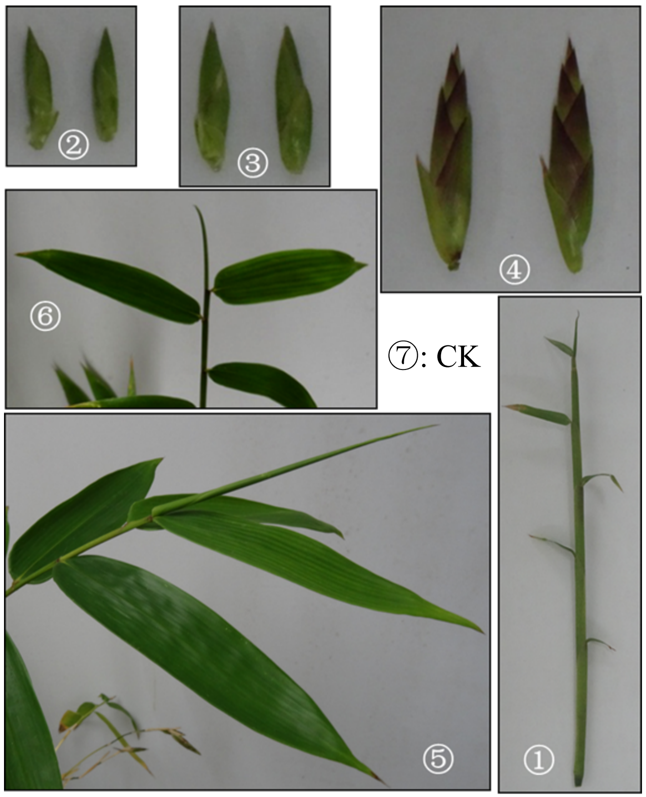
| | | | | | | | | | | | | | | | | | | | |

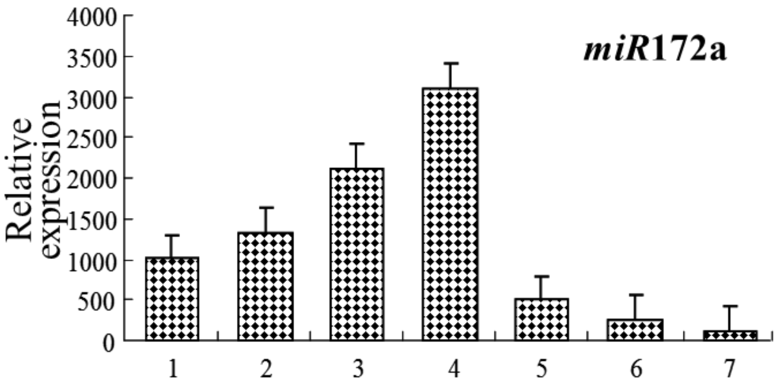
*miR*172a：**G A C G U C G U A G U A G U C C U A A G A**

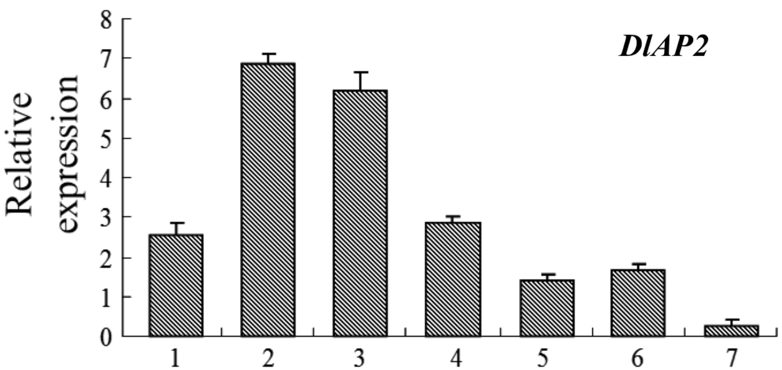
----The result of sequencing showed that there were nine samples with cleavage sites between the 11^{th}-12^{th} bases, and only one sample with a cleavage site between the 12^{th} -13^{th} bases in the ten sequenced samples.

Result and analysis

- **Expression of *miR*172a 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 *miR*172a through cleavage mainly at the site between the 11^{th} and 12^{th} bases.
- qRT-PCR results showed that the expression pattern of *DlAP2* was opposite to that of *miR*172a in flower buds, which indicated that *miR*172a played a regulatory role in regulating the expression of *DlAP2*.

Acknowledgements

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Thank you for your attention!