

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

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- **Result and analysis**
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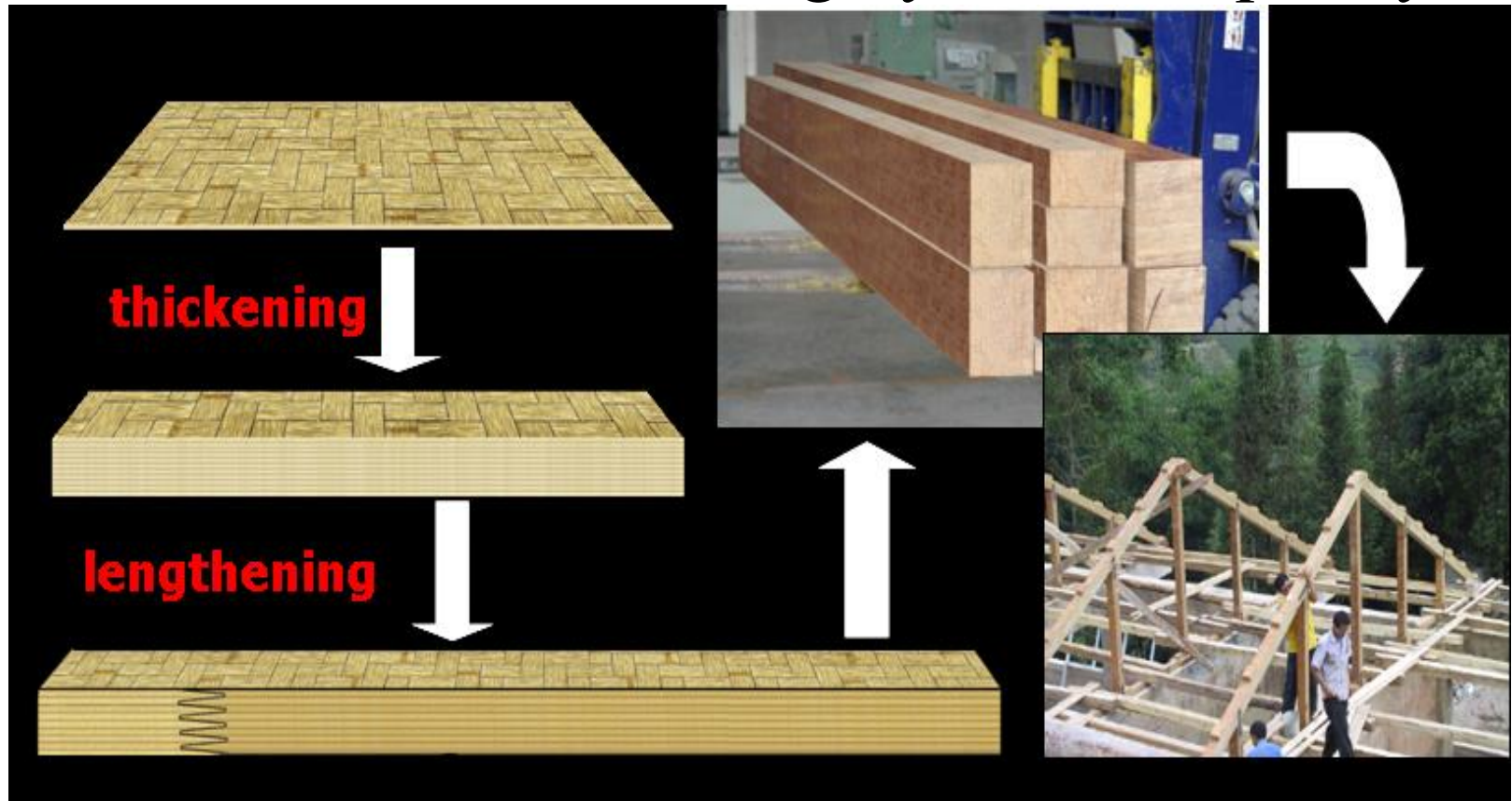


# Introduction

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

# Introduction

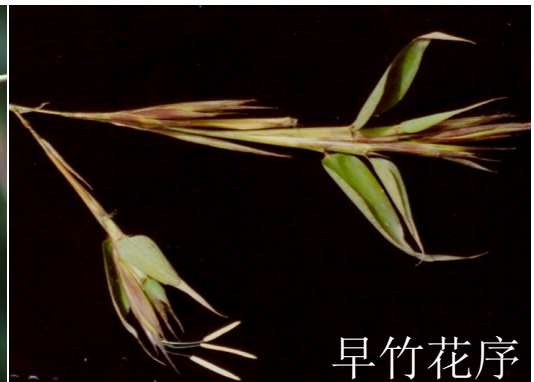
- New varieties with high yield and quality





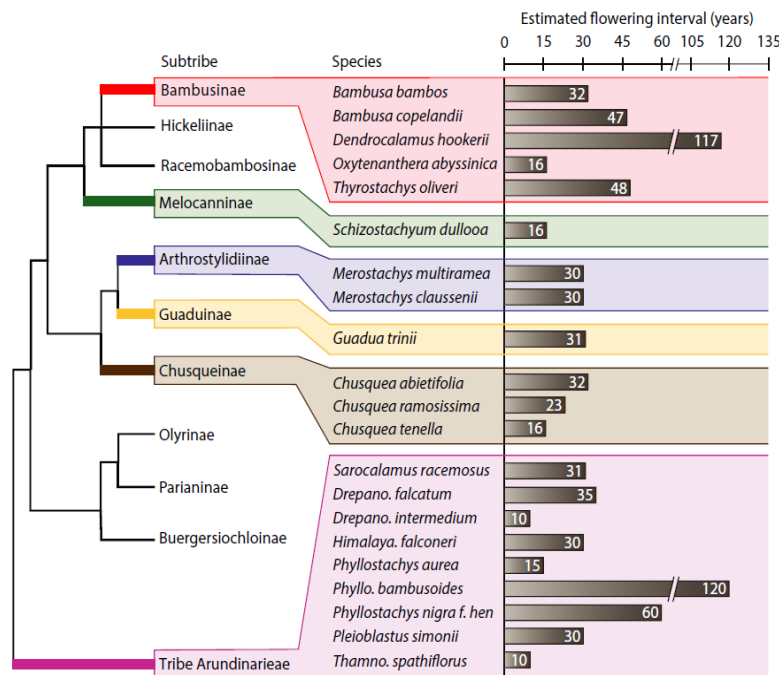
# Introduction

- Crossbreeding is an effective way to get new varieties



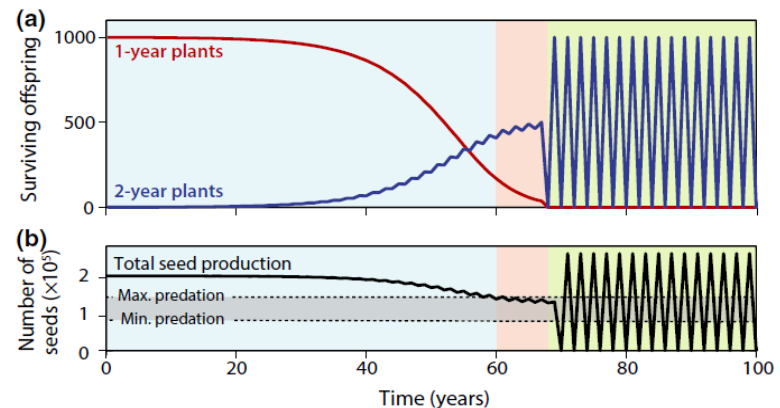
# Introduction

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



Long-intervalled flowering in bamboos

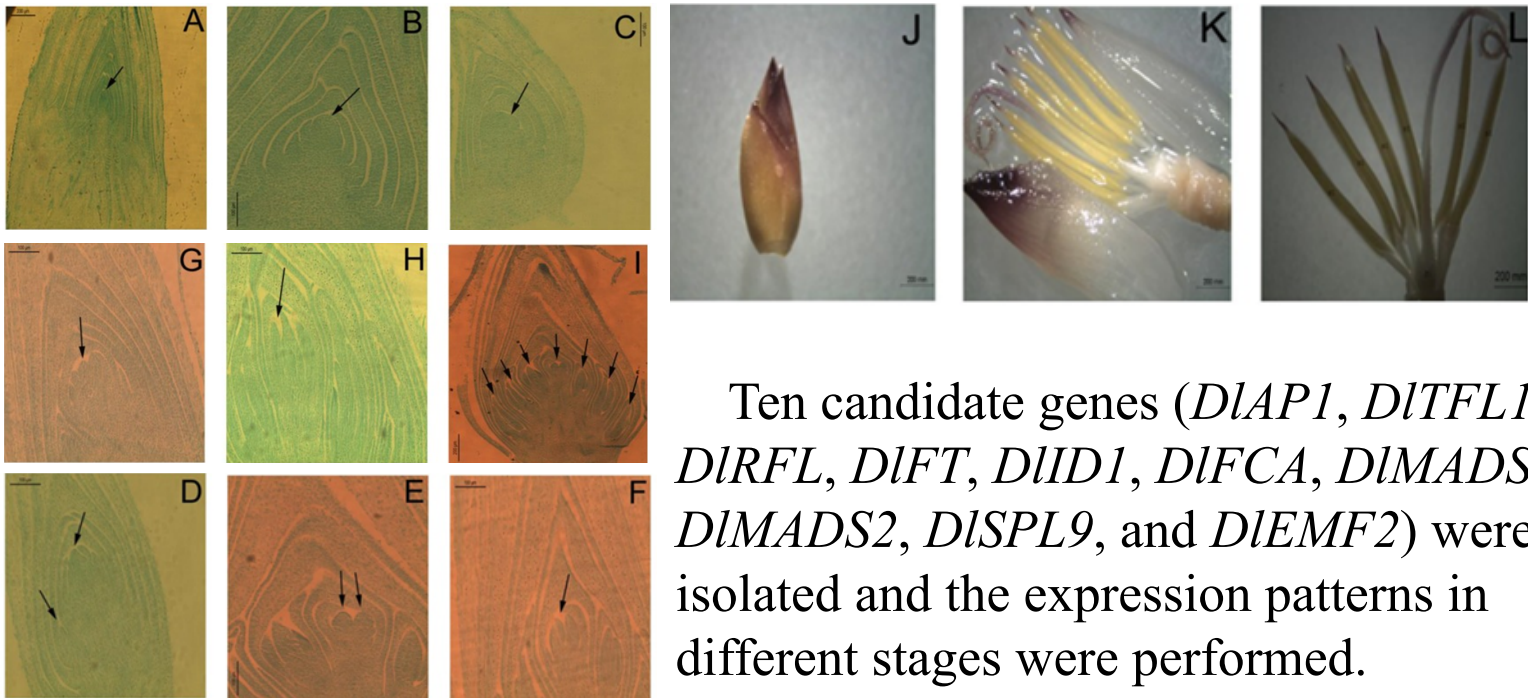
## A model of initial synchronisation in bamboos



- 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.)

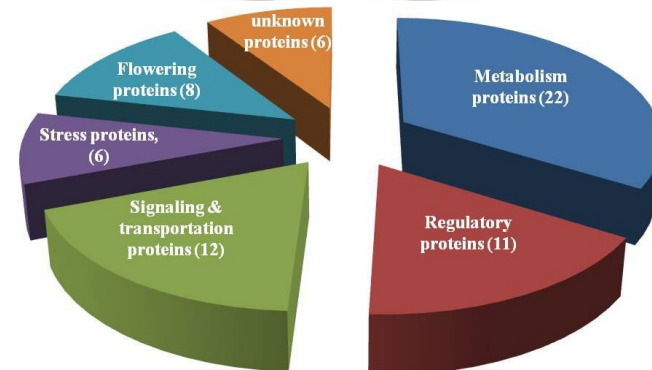
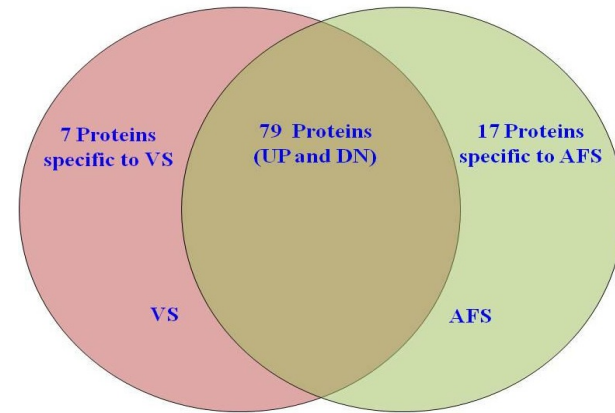
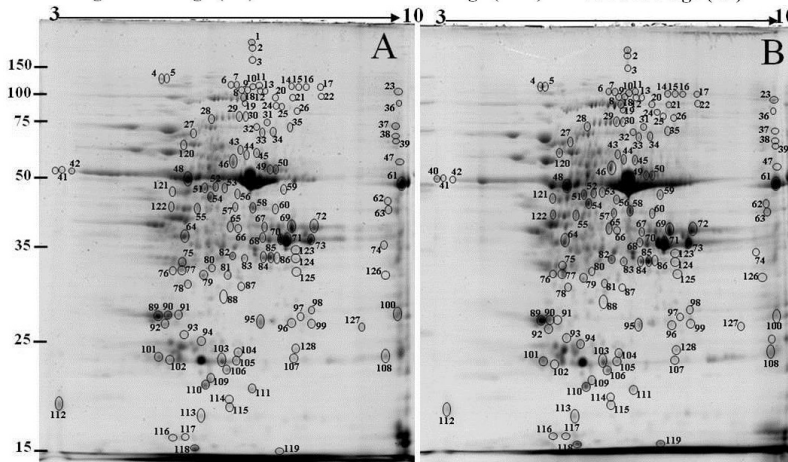


Ten candidate genes (*DlAP1*, *DlTFL1*, *DlRFL*, *DlFT*, *DlID1*, *DlFCA*, *DlMADS1*, *DlMADS2*, *DlSPL9*, and *DlEMF2*) were isolated and the expression patterns in different stages were performed.



# Introduction

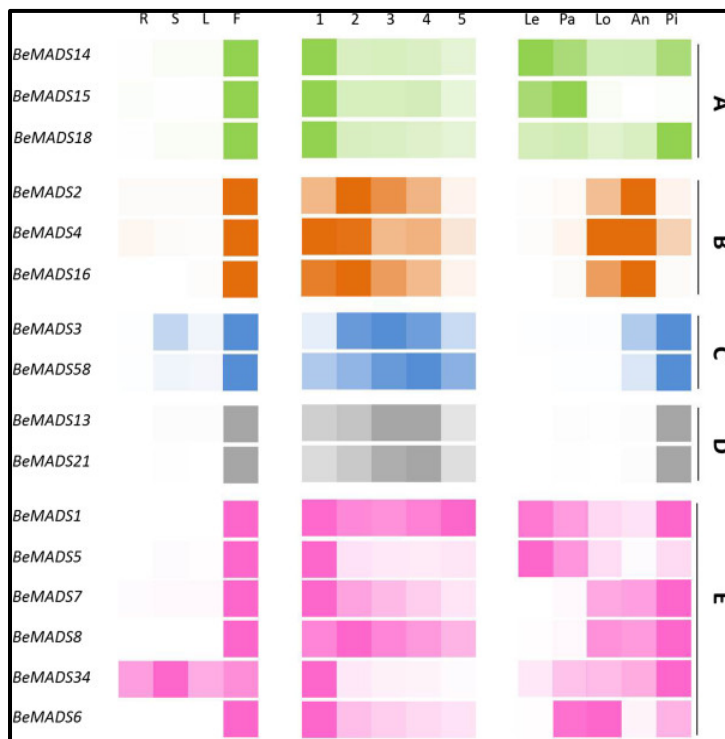
- In vitro flowering associated protein changes in *Dendrocalamus hamiltonii* (Kaur et al., 2015. Proteomics.)



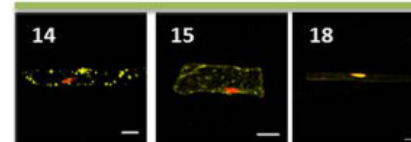


# Introduction

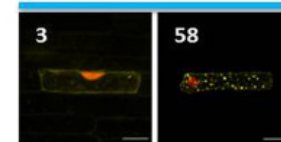
- Transcriptome and MADS genes in floral development of *Bambusa edulis* (Shih et al., 2014. BMC Plant Biol.)



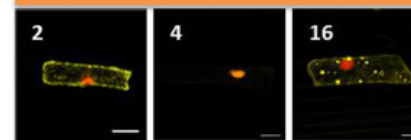
A class



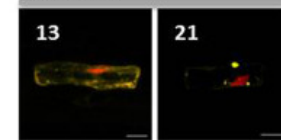
C class



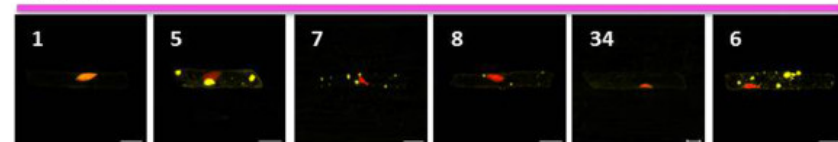
B class



D class



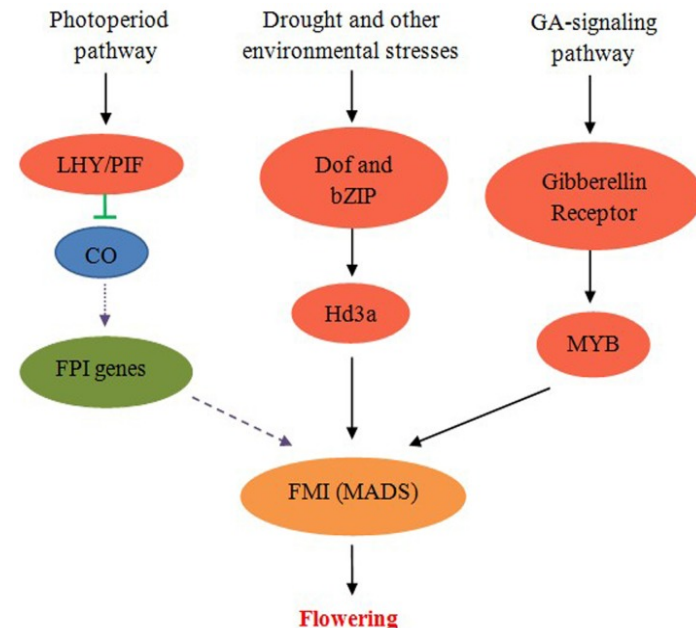
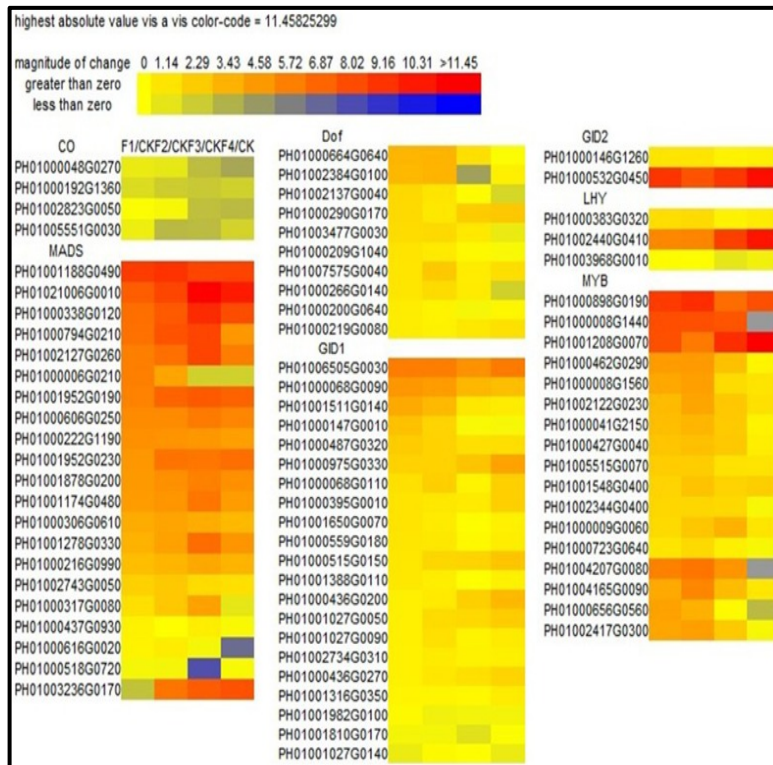
E class



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

# Introduction

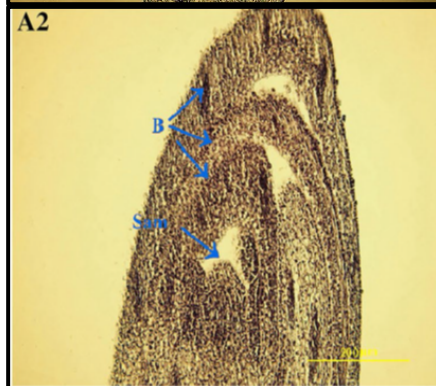
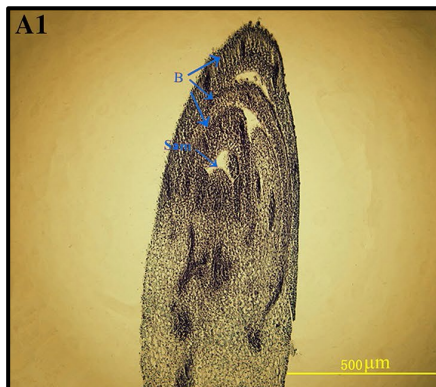
## ➤ Floral transcriptome of moso bamboo (*Phyllostachys edulis*) (Gao et al., 2014. PLoS One)



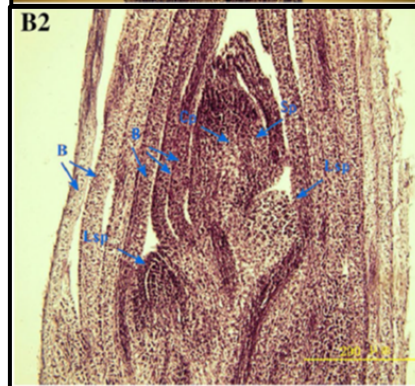
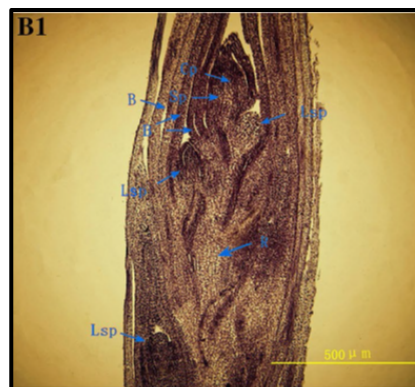
A hypothesized pathways in regulation of flowering in moso bamboo

# Introduction

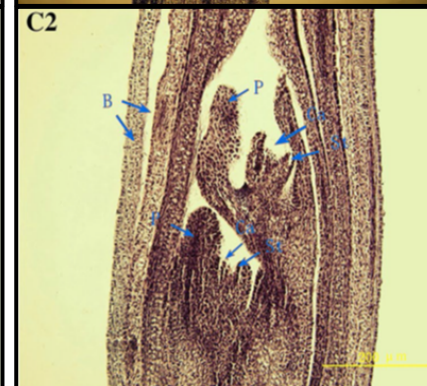
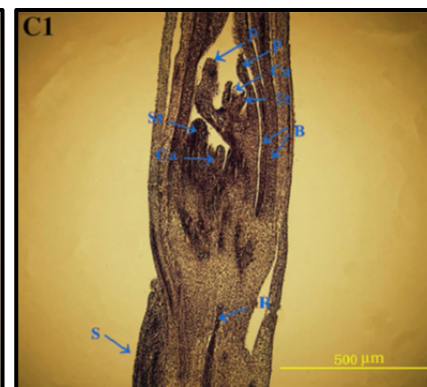
- miRNAs at different flowering developmental stages of moso bamboo (Gao et al., 2015. Mol Genet Genomics)



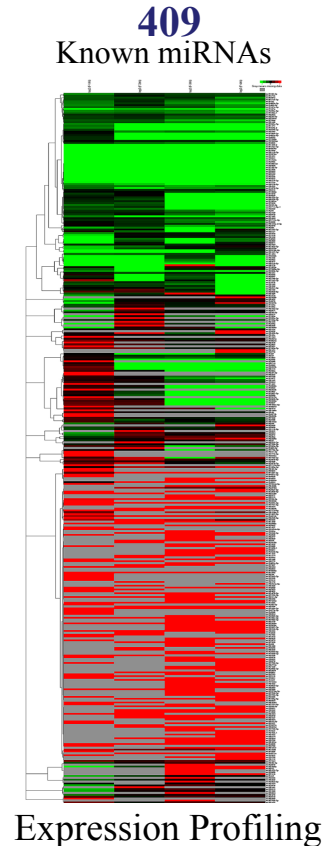
Floral bud formation



Inflorescence growing

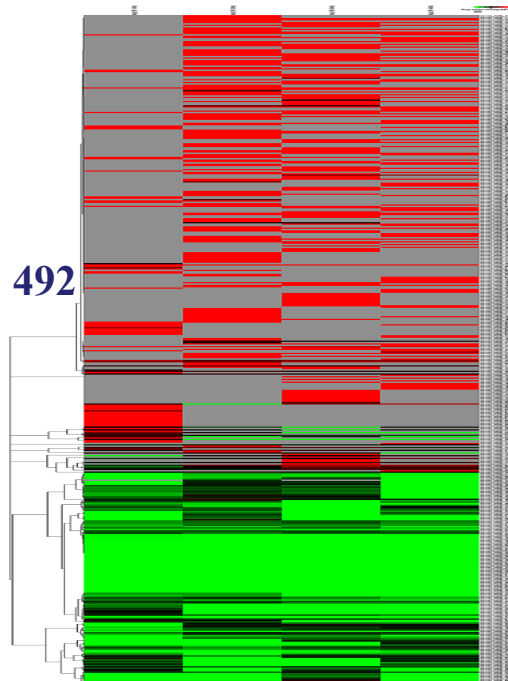


Spikelets differentiation

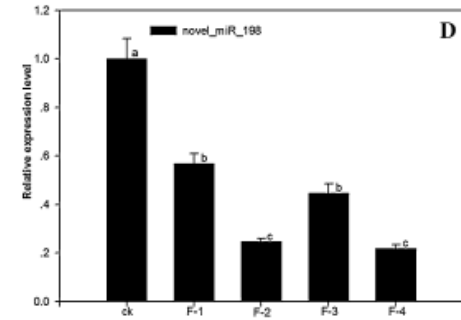
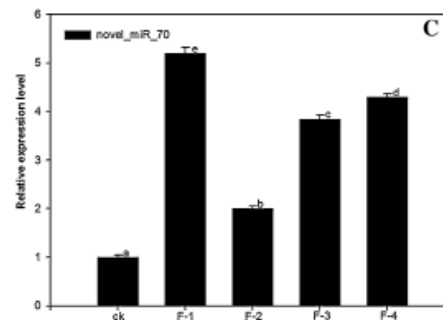
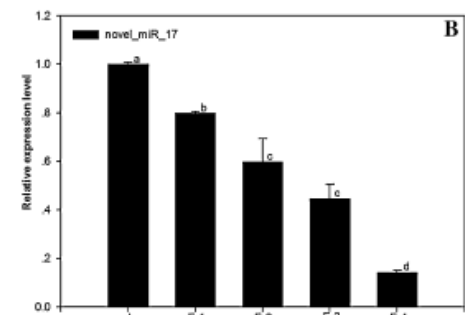
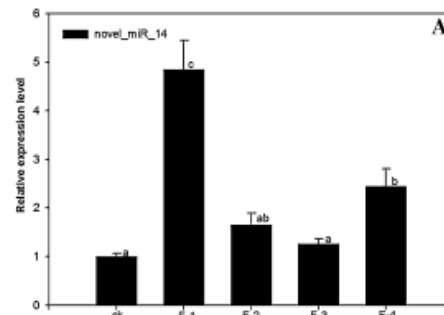


# Introduction

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



Expression profiles of novel miRNAs

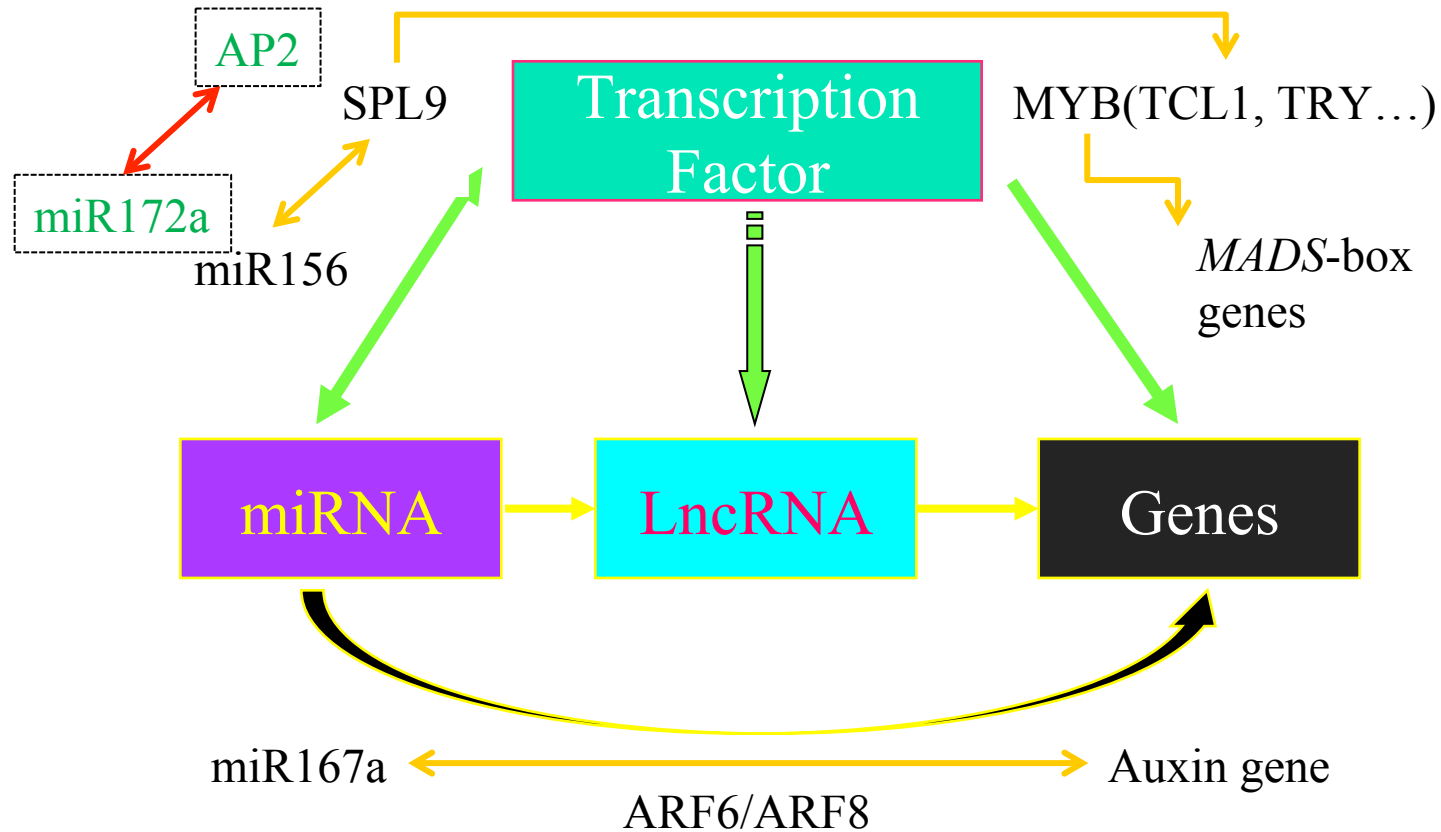


Expression validation of four novel miRNAs in flower at different stages



# Introduction

## Regulation model of miRNA-TF-Gene



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# Materials and methods

## ➤ Plant materials



Ma bamboo seedlings (*Dendrocalamus latiflorus*)

# Materials and methods

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## ■ 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

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

GenBank No: KM267641

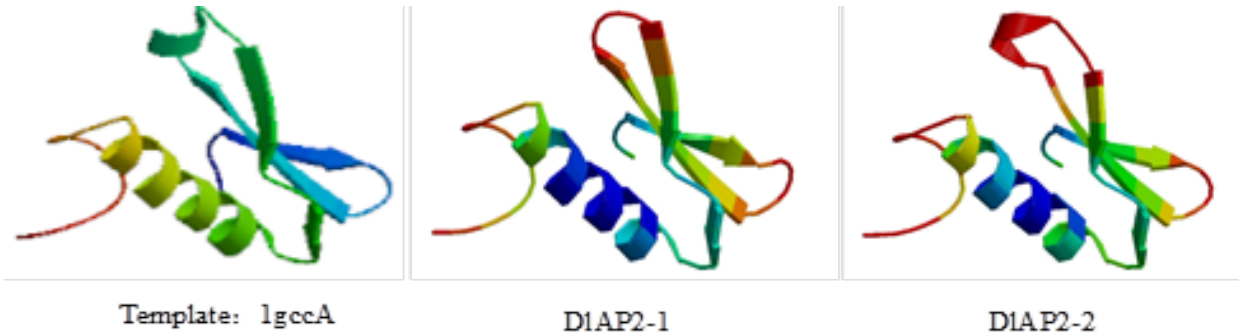
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1  ACGCGGGGCTCGTTGTTGGGTTTGAGGGTGTGATTGGGAGTTGGGATTGATCTTGGTTGAGGAGTGGAGGAGATGGAGCTGGATCTG
M E L D L
97  AACGTGGCGGACGGGGCGCGGAGAAAGCGGAAGCGATGGCGCGGAGCGACTCCGGCACTCGGAGTCCGCGGTGCTGAACGGGAGGCGTCCGGC
N V A D G A P E K P E A M A R S D S G T S D S P V L N A E A S G
193  GCGGAGGCGCGCGGGTGCCTCGCGGAGGAGGCTTCAGCTCGACGCCCGCGCGTGGCGGTCTGAGTTGAGTATCAGGAGCTCGGCG
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  TCGGCGGAGGCGGAGAAAGACGTGGGCGTTGCCGATGACGAGGAGGAGGCCACGCCGTGCGCTCTCGGCGGCGACGAGCTGTCACCCAGCAGCTC
S A E G E K D V G V A D D E E E A T P S P L R R Q Q L V T Q Q L
385  TTCGGGTGACGCGCGCGCGCGCGCGCGTGGCGCACTGGGCGCGAGCTGGGTTCTTACGCCCGAGCGCGCGCGCGCGCGAGCGCGACATC
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  AGAATCTTGGCGTCCCGCAGGCGCACGCCCGCGCGCGAGCGCTACCAAGAAGAGCGCGCGCGCGCGCGCGTCCCGCAGCTCGCAGTAC
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  CGCGGCGTCACTCTTACCGCGCACCGGCGCGTGGGAATCCCATATTGGGATTGCGGCAAGCAAGTGTACTTAGTGGATTGACACTGCTCAT
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  GCTGCTGAAGGGCGTATGATCAGCGGCGGATCAAGTTCCGCGCGCATGACACGGACATAAACTTCGATCTTAGTGACTACGAGGACGACATGAAG
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  CAGGTGAAGAGCCTATCCAAGGAGGAGTTCGTGCACGTCCTGCGACGCGAGAGTACTGGCTTCTCGGAGGCGAGCTCCAAATACAGAGGCGTCAAC
Q V K S L S K E E F V H V L R R Q S T G F S R G S S K Y R G V T
865  CTGCACAAGTGGGCGGATGGGAGGCTGCATGGGCCAGTTCTCAGCAAGAAGTACATATATCTTGGGCTATTGACAGCGAAGTAGAGGCTGCA
L H K C G R W E A R M G Q F L G K K Y I Y L G L F D S E V E A A
961  AGGGCTTATGATAAGGCTCGCATCAAAATGCAATGGTAGAGAAGCCGTGACGAACTTCGAGCTAGCACATATGA TGGGAGATGCTTACTGAAGTT
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  GGTGCTGAAGGTGCAGATGTCGATCTGAACCTTGAGCATATCTCAACCAAGCTTTCGAGAGCCGCCAAAGGGA TAAAGACTCCCTTGGTTCGACGCTG
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  CACCATGGATTATTGATGGCTCTGAAGTGAAAAGAGCTAAGATTGATGCTCCCTCTGAAGTGGCTGGCGCGCCCTCATCGGTTCCCTCTTCGACC
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  AAGCATCCACGAGTCTGGCGTGGCCATCTCAACCCCATATTTCAAATAATGAGGATGCATCTAGAGATCA TAAACAGGAGGCCAGGCGGAGCACC
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  GGGGGTGTCCAGCTGGGATGGAAGTGAGCCACCTCCACCCACACTACCATGCGCGTGTCTGCTGTTGTATCGTATCCGCTGACGCA
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  TCATCAGGATCTCCAGAACCGTCAAGATAGCTATCTCCACCAACCCATCGACCTCCCTCCAGTTCGACCGATGGCGGCATCATGTCGAACCA
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 S N H
1537  CACCGCTGAATAGAAGCCACACTGTAAATTTGCGGGAAGCGCGCATCTTTTTCCTCCGACGTTTCAACGTTCTCGGTTTTCGCGCGGGTGG
H R
1633  TTTCTGTAGTGATTGGAATCATGCTGATTTCGATGCTGCCAATTGAAAAATGTTCTATTTCGACGCAAAAAA
  
```

# Result and analysis

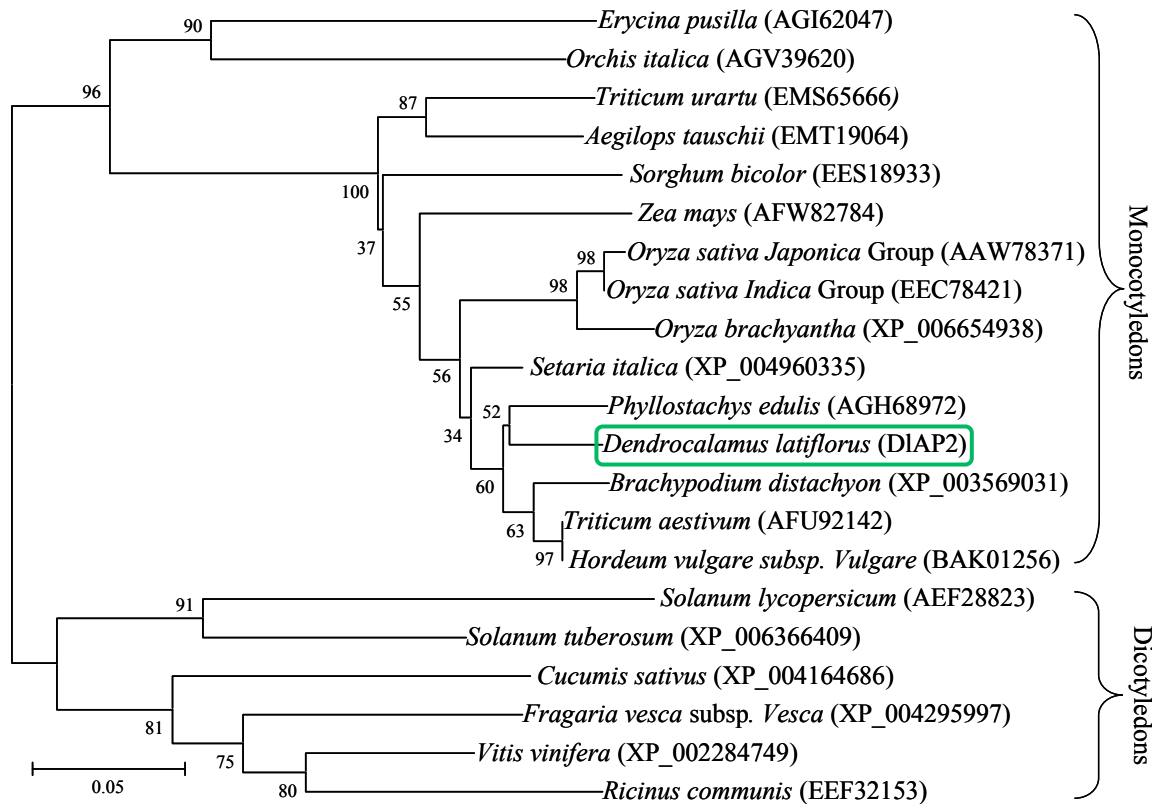
## ➤ Protein properties analysis and structure prediction

- ✓ *DIAP2* 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  $\alpha$  helix and 3  $\beta$  folds



# Result and analysis

## Phylogenetic analysis of DIAP2





# Result and analysis

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

RNA ligase-mediated 5' RACE

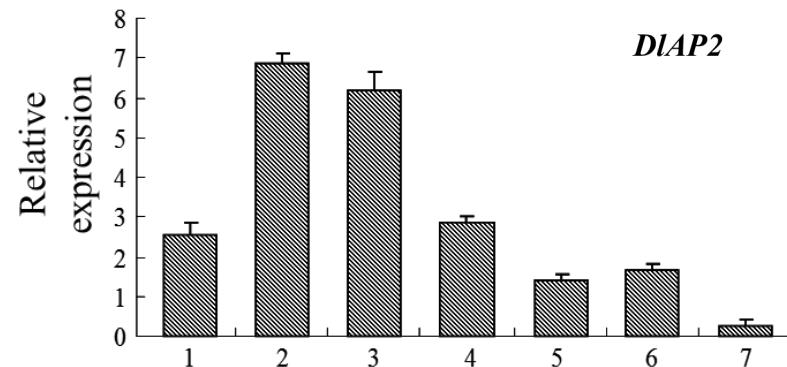
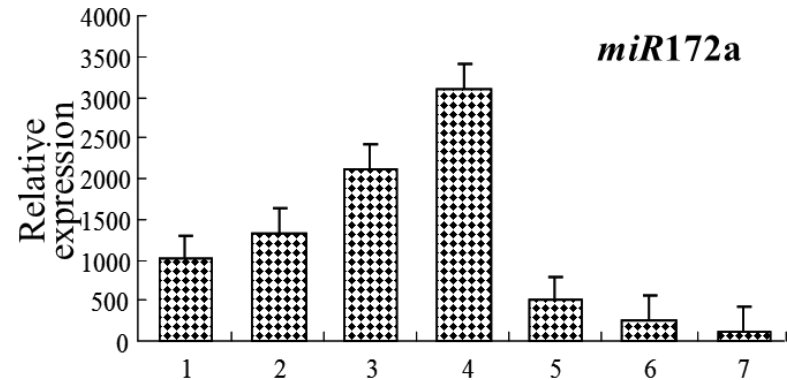
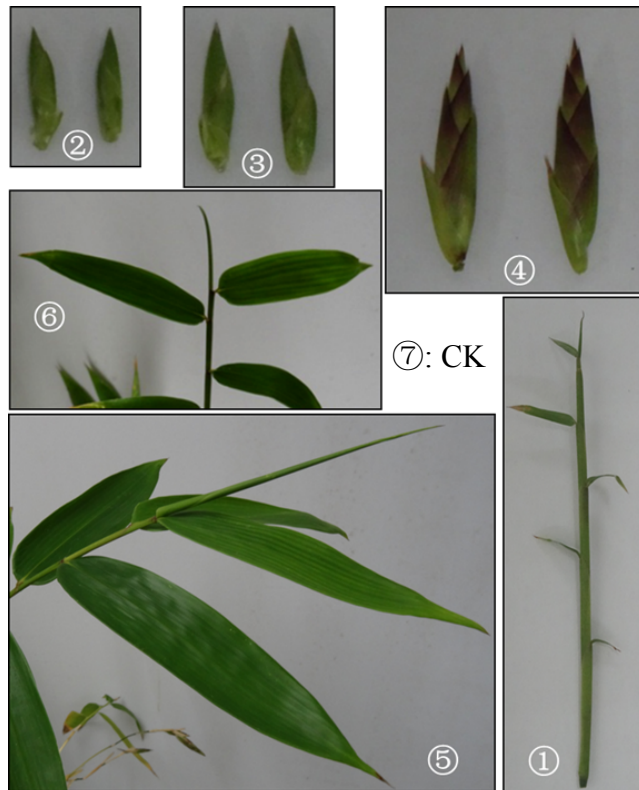


----The result of sequencing showed that there were nine samples with cleavage sites between the 11<sup>th</sup>-12<sup>th</sup> bases, and only one sample with a cleavage site between the 12<sup>th</sup> -13<sup>th</sup> bases in the ten sequenced samples.



# Result and analysis

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





# Conclusion

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- 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 11<sup>th</sup> and 12<sup>th</sup> 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

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- 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].
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**Thank you for your  
attention!**