Identification of bHLH gene family of *Liriodendron chinense* and its expression analysis in response to cold stress

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Background: bHLH is one of the largest transcription factor families in plants, which plays an important role in plant growth and development, especially in plants responding to abiotic stresses such as low temperature[1]. *Liriodendron chinense* is an important landscape, timber and medicinal tree species, mainly distributed in southern China[2]. Hence, it is important to characterize bHLH family of *Liriodendron chinense*, and further elaborate the functions of bHLH genes in responding to cold stress.

Methods: This study combines the methods of bioinformatics prediction and experiment to prove our point of view. The methods involved are as follows: Identification of bHLH Family Genes in *L.Chinese*; Bioinformation Analysis;Gene Cloning; Gene Expression Analysis.

Results: In this research, we performed the genome-level identification of the bHLH gene family of *Liriodendron chinense*, and phenotypic analysis of two members under low temperature stress. The results showed that there were 91 bHLH members in *Liriodendron chinense*, and these members were divided into 33 subgroups according to 50% of the bootstrap supported by guidance[3], which was more than 22 subgroups in rice and 21 subgroups in *Arabidopsis*[4]. By comparing the gene structure, conserved motifs, phylogenetic relationships and collinearity of bHLH gene families from different species, it is indicated that the functional differences of bHLH gene families in the evolutionary process may be caused by the structural domain changes caused by the sequence differences of some members. In addition, the genome distribution of bHLH gene in *Liriodendron chinense* strongly supports the hypothesis that whole genome and tandem replication promote the expansion of bHLH gene family, which accords with the evolutionary theory of gene family. RNA-seq and qRT-PCR analysis showed that the expressions of some members including LcbHLH54, 39, 24 and 69,were differentially expressed in response to cold stress in seedling leaves of *Liriodendron chinense*. Two members, LcbHLH24 and LcbHLH69, which have high homology with ICE in *Arabidopsis thaliana*, were transferred into *Arabidopsis thaliana* respectively, and it was found that they partly enhanced the tolerance to low temperature stress.

Conclusion: This research will lay a foundation for further analyzing the functions of bHLH gene family members, and also provide genetic resources for mining low temperature response genes and cultivating cold-tolerant germplasm.

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