

A genome-wide identification of the BLH gene family reveals BLH4 and its interacting gene KNOX6 involved the regulation of osmotic stress in *Toona sinensis*

Shuxin Chen¹, Yuhan Jia¹, Yuying Yang¹, Zhouchou Lu¹, Renying Zhuo¹, Xiaojiao Han^{1*}

(¹State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of Tree Breeding of Zhejiang Province, Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Hangzhou, Zhejiang, China, 311400)

Abstract: The BELL1-like homeobox (BLH) transcription factors are widely present in plants and belong to a subfamily of the three-amino-acid loop extension (TALE) superfamily. They play extensive roles in plant growth and development and have been reported to participate in stress responses. However, the BLH gene family in *Toona sinensis* has not yet been studied. In this study, we identified 21 BLH gene family members in the genome of *Toona sinensis*. Based on previously reported classifications in *Arabidopsis*, the BLH gene family was divided into five subfamilies, with variations in exon-intron distribution and motif composition across different subfamilies. Numerous cis-acting elements related to plant growth, development, and stress response were found in the promoters of the BLH genes. TsBLH genes exhibited tissue-specific expression, with most genes responding to drought and salt stress. Furthermore, we cloned the TsBLH4 gene and characterized its function. *TsBLH4* was highly expressed in xylem and leaves, responded to drought and salt stress in leaves. TsBLH4 is a nuclear protein by subcellular localization in tobacco leaves. Additionally, TsKNOX6 physically interacts with TsBLH4, and subcellular localization revealed that TsKNOX6 is also a nuclear protein. Transient expression of these two genes in tobacco leaves resulted in salt sensitivity and enhanced drought tolerance. This study is the first to report the function of TsBLH4 and its involvement in stress responses in *Toona sinensis*. These data provide a theoretical basis for the involvement of the BLH gene family in stress responses, particularly osmotic stress.