Overexpression of an *AP2/ERF* family gene, *BpERF13*, in birch enhances cold tolerance through upregulating *CBF* genes and mitigating reactive oxygen species

Kaiwen Lv1 Jiang Li2 Kai Zhao1 Su Chen1 Jeffff Nie3 Wenli Zhang4 Guifeng Liu1 * Hairong Wei5*

(1 State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Harbin 150040, PR China; 2 Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, Beijing 100083, PR China; 3 Center of Medical Physics and Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui, 230031, PR China; 4 State Key Laboratory for Crop Genetics and Germplasm Enhancement, JiangSu Collaborative Innovation Center for Modern Crop Production, Nanjing Agricultural University, No.1 Weigang, Nanjing, Jiangsu, 210095, PR China; 5 College of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI, 49931, United States)

Abstract: The AP2/ERF (APETALA2/ethylene-responsive factor) family of transcription factors (TF) is involved in regulating biotic and abiotic stress responses in plants. To explore the role of AP2/ERFs in cold tolerance in woody plants, *BpERF13* was cloned and characterized in *Betula platyphylla* (white birch), a species primarily found in Asia in temperate and boreal climates. Based on phylogenetic analysis, BpERF13 is a member of the IXb subfamily of ERFs. Using qRT-PCR, we found that *BpERF13* was diffffferentially expressed in diffffferent tissues, and its expression could be induced by cold treatment (4 °C). BpERF13 protein, fused with GFP, was exclusively localized to nuclei. To further assess the role of *BpERF13* in cold tolerance, *BpERF13* overexpression (OE) transgenic lines were generated in *B. platyphylla* and used for cold stress treatment and biochemical/physiological studies. *BpERF13* overexpression lines had signifificantly increased tolerance to subfreezing treatment and reduced reactive oxygen species. Using a TF-centered yeast one-hybrid (Y1H) experimental system, we showed that BpERF13 could bind to LTRECOREATCOR15 and MYBCORE cis-elements to activate a reporter gene. ChIP-seq and ChIP-PCR experiments further demonstrated that BpERF13 bound to these cis-elements when present in the 5′ proximal regions of superoxide dismutase (*SOD*), peroxidase (*POD*), and C-repeat-binding factor (*CBF*) genes. qRT-PCR was employed to examine the expression levels of these genes in response to cold stress; SOD, POD, and CBF genes were signifificantly upregulated in BpERF13 transgenic lines compared to wild-type plants in response to cold stress. These results indicate that the transcription factor BpERF13 regulates physiological processes underlying cold tolerance in woody plants.