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## Research paper

# Poplar CBF1 functions specifically in an integrated cold regulatory network

Ying Li<sup>1,2</sup>, Yuepeng Song<sup>1,2</sup>, Baohua Xu<sup>1,2</sup>, Jianbo Xie<sup>1,2</sup> and Deqiang Zhang<sup>1,2,3</sup>

<sup>1</sup>National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Technology, Beijing Forestry University, No. 35, Qinghua East Road, Beijing 100083, PR China; <sup>2</sup>Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants, College of Biological Sciences and Technology, Beijing Forestry University, No. 35, Qinghua East Road, Beijing 100083, PR China; <sup>3</sup>Corresponding author (DeqiangZhang@bjfu.edu.cn)

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The C-repeat binding factors (CBFs), also termed dehydration-responsive element-binding protein 1 (DREB1) family members, play crucial roles in the acquisition of stress tolerance, but in trees, the underlying mechanisms of stress tolerance remain elusive. To gain insight into these mechanisms, we isolated five *CBF1* orthologs from four poplar sections (*Populus* spp.) and assessed their expression under drought, cold, heat and salt stress conditions. Globally induced expression in response to cold suggested a correlation between poplar *CBF1* expression and the acquisition of cold tolerance. Responses that varied between sections may reflect section-specific stress tolerance mechanisms, suggesting an effect of ecological context on the development of *CBF1*-mediated stress tolerance in poplar. We then used a genome-wide search strategy in *Populus trichocarpa* to predict 2263 putative CBF target genes; the identified genes participate in multiple biological processes and pathways. Almost all of the putative target genes contained multiple *cis*-acting elements that mediate responses to various environmental and endogenous signals, consistent with an important role of CBF1s in an integrated cold regulatory network. Finally, analysis of an association population of 528 individuals of *Populus simonii* identified six single-nucleotide polymorphisms (false discovery rate  $Q < 0.10$ ) significantly ( $P < 0.005$ ) associated with malondialdehyde production and electrolyte leakage, suggesting the potential importance of *PsCBF1* in the regulation of some membrane-related functions. Our findings provide new insights into the function of *PsCBF1* and shed light on the CBF-mediated regulatory network in poplar.

**Keywords:** abiotic stress, cold, DREB1/CBF, target gene.

## Introduction

Transcription factors (TFs) have important functions in stress responses and tolerance. They specifically bind to *cis*-elements in the promoter regions of target genes and regulate gene expression (Nakashima et al. 2009). In *Arabidopsis thaliana*, the C-repeat (CRT) binding factors (CBFs), also known as dehydration responsive element (DRE) binding protein 1 (DREB1) family members (Nakano et al. 2006), contain a conserved AP2 domain and specifically bind to the DRE/CRT promoter element of target genes, regulating their expression and thus enhancing plant tolerance to low temperature, drought and salt stresses (Liu et al. 1998, Sakuma et al. 2002, Shinozaki et al. 2003).

Three CBF members, namely *CBF1*, *CBF2* and *CBF3*, were first isolated from *A. thaliana* (Stockinger et al. 1997). Their expression increases under cold conditions and induces the expression of cold-responsive genes (Gilmour et al. 1998, Jaglo-Ottosen et al. 1998).

Previous reports indicated that cold-responsive expression of *CBF1* was conserved between herbaceous *Arabidopsis* and perennial woody trees, such as poplar and birch (Benedict et al. 2006, Welling and Palva 2008). However, perennial woody plants, such as poplar, encounter various kinds of abiotic stresses throughout their lifespan and most of these stresses occur in different combinations. Moreover, such stresses likely vary with the ecological