

Construction of a hierarchical gene regulatory network to reveal the salt tolerance mechanism and the transcription factor BpHAP51 as important regulators positively modulates salt stress in *Betula platyphylla*

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Abstract: Salinization of soil poses a significant danger to both the ecological environment and agriculture output and remains a prevalent abiotic stressor in worldwide agricultural practices. Plants have developed different adaptive strategies to regulate different physiological, biochemical and molecular responses in response to salinity. Birch (*Betula platyphylla* Suk.) is widely distributed in cold temperate regions and boreal forests of the Northern Hemisphere and is commonly used in construction and wooden furniture. Therefore, studying the salt tolerance mechanism of birch is of great significance for the selection of salt-tolerant birch varieties. Most studies in the field of salt-tolerance mechanisms of birch have only focused on regulation of salt tolerance mechanisms by a major transcription factor or functional gene. Therefore, a reliable gene regulatory network (GRN) is crucial to reveal the molecular mechanism in response to salt stress in birch. To reveal the regulatory mechanism of birch to salt stress, we constructed a GRN to infer the regulatory relationship between genes in each gene module. The GRN contains 601 potential regulatory relationships. The first and second layers include 5 and 13 transcription factors (TFs), and the bottom layer contains 103 structural genes that are involved in 10 enriched GO biological processes. Also, the authenticity of GRN was verified by chromatin immunoprecipitation (ChIP)-PCR and RT-qPCR based on transient transformation. Among the 14 regulatory relationships between the three transcription factors and the target genes in the first layer, 11 were confirmed through ChIP-PCR while 3 were not. This indicates that there were 11 direct regulatory relationships and 3 indirect regulatory relationships, resulting in a direct regulatory accuracy of 78.57% in the GRN. Three top-level transcription factors were randomly selected for functional verification through transient transformation, revealing that overexpression of these genes positively enhanced the salt tolerance of birch. The study further analyzed the salt tolerance function of the top transcription factor *BpHAP51*, with its knockout mutant exhibiting a salt-sensitive phenotype. The study also identified the cis-acting element interacting with *BpHAP51* and verified *BpHAP51* can regulate to improve the salt tolerance of birch through Luciferase complementation (LUC) experiments and combined with the downstream regulatory genes of GRN. The constructed GRN in this study enhances our understanding of potential breeding strategies for perennial woody plants like birch under salt stress and offering new insights into the regulatory mechanism of salt tolerance involving *BpHAP51*.