

BpbZIP1 调控白桦耐盐机制研究

迟瑶, 王超*

(林木遗传育种全国重点实验室 东北林业大学 黑龙江 哈尔滨 150040)

摘要:解析 *BpbZIP1* 转录因子调控白桦响应盐胁迫的功能及其调控途径, 为研究 bZIP 转录因子家族基因的耐盐调控机制及白桦耐盐新品种选育奠定基础以及提供理论依据。利用实时荧光定量 PCR (RT-qPCR) 分析 *BpbZIP1* 在盐以及 ABA 处理下的表达模式, 进一步通过 pBI121-*BpbZIP1*-GUS 载体稳定转化白桦, 进行 *GUS* 基因表达及组织化学分析, 验证其盐胁迫响应; 利用叶盘转化法获得 *BpbZIP1* 过表达及抑制表达白桦植株, 在 200mM NaCl 胁迫处理下进行生长表型观察、组织化学染色以及生理指标测定进行基因功能鉴定; 通过 RNA-Seq 分析, 结合 RT-qPCR 以及顺式作用元件分析筛选 *BpbZIP1* 下游靶基因, 利用酵母单杂交 (YIH)、染色质免疫沉淀 (ChIP) 及双荧光素酶 (dual-LUC) 试验进行验证; 利用酵母双杂交 (Y2H) 及双分子荧光互补 (BiFC) 试验鉴定 BpbZIP1 互作蛋白。*BpbZIP1* 基因响应盐胁迫以及 ABA 处理, pBI121-*BpbZIP1*-GUS 转基因白桦在盐胁迫处理下染色较深, *GUS* 基因显著诱导; *BpbZIP1* 过表达转基因植株在盐胁迫处理后, 与野生型相比长势较好并表现出较高的超氧化物歧化酶 (SOD), 过氧化物歧化酶 (POD) 活性、叶绿素含量及脯氨酸 (Pro) 含量, 较低的过氧化氢 (H_2O_2) 以及丙二醛 (MDA) 含量, 而 *BpbZIP1* 抑制表达植株与之相反; 在 *BpbZIP1* 过表达株系及野生型转录组中, 鉴定出差异表达基因 440 个, 其中有 153 基因上调表达, 287 个基因下调表达, GO 注释表明这些差异表达基因与代谢过程、生长发育、非生物胁迫应答等相关, 其中 *BpFLZ* 基因表达显著上调。*BpbZIP1* 分别结合 ABRE 元件、含 A BRE 元件的 *BpFLZ* 启动子片段激活其表达; BpbZIP60、BpZFP 分别与 BpbZIP1 互作且激活 *BpFLZ* 基因的表达。*BpbZIP1* 基因显著受盐胁迫诱导, 可与 BpbZIP60, BpZFP 相互作用, 结合 *BpFLZ* 启动子调控其表达, 通过清除 ROS、减少膜质过氧化程度以及细胞死亡来提高 *BpbZIP1* 转基因植株的耐盐能力。

Regulation of salt tolerance by *BpbZIP1* in *Betula platyphylla*

Chi Yao Wang Chao*

(State Key Laboratory of Tree Genetics and Breeding Northeast Forestry University Harbin 150040, China)

Abstract: To analyze the function and regulatory pathway of BpbZIP1 transcription factor in regulating the salt stress response of *Betula platyphylla* and provide a theoretical basis for studying the salt tolerance regulation mechanism of bZIP transcription factor family and the salt-tolerant breeding of birch. RT-qPCR was used to analyze the expression pattern of *BpbZIP1* under salt and ABA treatment, and then the *GUS* gene expression and histochemical analysis were performed to verify the response of *BpbZIP1* to salt stress by stable transformation of birch with pBI121-*BpbZIP1*-GUS vector. Using the leaf disc transformation method, *BpbZIP1* was overexpressed and suppressed in birch plants, and the growth phenotypes were observed under 200mM NaCl stress treatment, and the tissues were stained with histochemical methods and physiological indicators were measured to identify the gene function. The downstream target genes of BpbZIP1 were screened by RNA-Seq analysis, combined with RT-

qPCR and *cis*-element analysis, and validated by yeast one-hybrid (YIH), chromatin immunoprecipitation (ChIP) and dual-LUC assays. Yeast two-hybrid (Y2H) and bimolecular fluorescence complementation (BiFC) assays were used to identify BpbZIP1 interacting proteins. *BpbZIP1* responds to salt stress and ABA treatment. pBI121-*BpbZIP1*-GUS transgenic birch was deeply stained and *GUS* gene was significantly induced under salt stress treatment. Transgenic plants with overexpression of BpbZIP1 showed better growth and higher superoxide dismutase (SOD), peroxide dismutase (POD) activity, chlorophyll content and proline (Pro) content, and lower hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) content compared with wild type (WT) after salt stress treatment. The reverse phenotype was observed in *BpbZIP1*-RNAi plants. In the transcriptome of *BpbZIP1* overexpression lines and WT lines, 440 differentially expressed genes were identified, among which 153 genes were up-regulated and 287 genes were down-regulated. GO annotation showed that these differentially expressed genes were related to metabolic processes, growth and development, and abiotic stress responses, among which *BpFLZ* gene expression was significantly up-regulated. *BpbZIP1* binds ABRE element and *BpFLZ* promoter fragment containing ABRE element to activate its expression. BpbZIP60 and BpZFP interact with BpbZIP1 and activate the expression of *BpFLZ* gene, respectively. BpbZIP1 is significantly induced by salt stress, which can interact with BpbZIP60 and BpZFP, regulate its expression by binding the *BpFLZ* promoter, and improve the salt tolerance of *BpbZIP1* transgenic plants by scavenging ROS, reducing the degree of membrane peroxidation and cell death.