杨树转录因子 PdbCRF5 调控盐胁迫的分子机理研究

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摘 要:【目的】明确 *PdbCRF5* 基因在参与山新杨盐胁迫过程中发挥的功能及其调控机制，为通过林木基因工程育种改善植物的耐盐能力提供了理论依据和基因资源。【方法】前期对山新杨盐胁迫转录组分析发现，山新杨 *PdbCRF5* 基因的表达受盐胁迫诱导明显，表明其可能参与山新杨盐胁迫调控。通过培育过表达和抑制表达 *PdbCRF5* 基因的转基因山新杨植株，我们发现 *PdbCRF5* 基因负调控植物对盐胁迫的耐受性。随后我们对 *PdbCRF5* 调控的下游基因及其互作蛋白进行了进一步的分析和鉴定。【结果】1）PdbCRF5 蛋白定位在细胞核中。其表达受到高盐、干旱以及 ABA 胁迫的诱导。2）盐胁迫下过表达 *PdbCRF5* 基因负调控植物的耐盐性。进一步研究发现，抑制 *PdbCRF5* 基因的表达可以通过减少盐胁迫下植物细胞内活性氧及钠离子的积累来提高植物的耐盐性。3）通过酵母单杂交以及农杆菌瞬时转化烟草实验证明了 PdbCRF5 转录因子能结合 GCC（GCCGCC）和 DRE（ACCGAC）元件。4）对过表达及野生型山新杨进行 RNA-Seq 分析。与对照组比，过表达中有 166 个上调表达基因，41 个下调表达基因；对差异基因启动子进行分析查找，发现启动子中包含 GCC 元件的差异基因有 11 个，包含 DRE 元件的差异基因有 27 个，且这些基因大部分被报导参与了植物的非生物胁迫响应。ChIP 实验结果表明 PdbCRF5 转录因子可以与 *PdbHSP90-6* 等 13 个基因的启动子片段中含有 GCC 或 DRE 的序列结合，qRT-PCR 结果显示在盐胁迫后 *PdbHSP90-6* 的表达在转基因植株中被明显诱导，表明其在 *PdbCRF5* 调控盐胁迫的过程中可能发挥了更重要的作用。5）以前期山新杨盐胁迫转录组数据为基础，通过 GGM 算法构建了 *PdbCRF5* 基因可能参与的盐胁迫调控网络，结果表明 *PdbCRF5* 基因可能直接调控的靶基因有 18 个。进一步通过 ChIP-PCR 对 PdbCRF5 可能参与的盐胁迫调控网络进行验证，我们随机选择了 8 个可能的下游靶基因进行验证，结果表明 PdbCRF5 均能不同程度的结合这 8 个下游靶基因的启动子片段，表明该调控网络是基本准确的，可以用于后续的研究。6）转录激活试验发现 PdbCRF5 转录因子具有转录激活活性。酵母双杂交试验证明山新杨 PdbCRF5 转录因子可以与同源蛋白 PdbCRF1、2 和 6 相互作用形成同源二聚体。此外过表达 PdbCRF6 基因同样降低了转基因植株的耐盐能力。

【结论】PdbCRF5 转录因子能够参与调控植物的耐盐性，过表达 PdbCRF5 转基因植株的耐盐性降低，而抑制表达转基因植株的耐盐性增强。PdbCRF5 能够通过直接结合 *PdbHSP90-6* 等抗逆基因的启动子来参与植物的盐胁迫响应。此外，PdbCRF5 能够与 PdbCRF6 互作，且 PdbCRF6 同样负调控植物对盐胁迫的耐受性，表明 PdbCRF5 也能通过与其他转录因子互作共同调控植物耐盐性。

关键词: *PdbCRF5*；盐胁迫；酵母双杂交；酵母单杂交；BiFC；山新杨

Molecular Mechanism of Poplar Transcription Factor PdbCRF5 Regulating Salt Stress

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Abstract:【Objective】Clarifying the function and regulatory mechanism of the *PdbCRF5* gene in participating in the salt stress process of *Populus davidiana*×*P. bolleana* provides theoretical basis and genetic resources for improving plant salt tolerance through tree genetic engineering breeding.【Method】Early analysis of the salt stress transcriptome of *Populus davidiana*×*P. bolleana* found that the expression of *PdbCRF5* gene was significantly induced by salt stress, indicating that it may participate in the regulation of salt stress of *Populus davidiana*×*P. bolleana*. By cultivating transgenic poplar plants that over-express and inhibit the expression of the *PdbCRF5* gene,

we found that the *PdbCRF5* gene negatively regulates plant tolerance to salt stress. Subsequently, we conducted further analysis and identification of downstream genes and their interacting proteins regulated by *PdbCRF5*. 【Result】1) The PdbCRF5 protein is located in the nucleus. Its expression is induced by high salt, drought, and ABA stress. 2) Over-expression of *PdbCRF5* gene negatively regulates salt tolerance in plants. Further research has found that inhibiting the expression of *PdbCRF5* gene can improve plant salt tolerance by reducing the accumulation of reactive oxygen species and sodium ions in plant cells under salt stress. 3) The ability of PdbCRF5 transcription factor to bind GCC (GCCGCC) and DRE (ACCGAC) elements was demonstrated through yeast one-hybrid and Agrobacterium tumefaciens transient transformation experiments in tobacco. 4) Perform RNA-Seq analysis on over-expressed and wild-type *Populus davidiana*×*P. bolleana*. Compared with the control group, there were 166 up-regulated genes and 41 down-regulated genes in the over-expression group; By analyzing and searching the differential gene promoters, 11 differential genes containing GCC elements and 27 differential genes containing DRE elements were found in the promoters, and most of these genes were reported to be involved in plant abiotic stress response. The results of the ChIP experiment showed that the PdbCRF5 transcription factor can bind to sequences containing GCC or DRE in the promoter fragments of 13 genes such as *PdbHSP90-6*. The qRT-PCR results showed that the expression of *PdbHSP90-6* was significantly induced in transgenic plants after salt stress, indicating that it might play a more important role in the regulation of salt stress by PdbCRF5. 5) Based on the previous salt stress RNA-seq data of *Populus davidiana*×*P. bolleana*, a salt stress regulatory network that *PdbCRF5* gene may participate in was constructed through GGM algorithm. The results showed that there were 18 target genes might directly regulated by *PdbCRF5*. Furthermore, ChIP-PCR was used to validate the possible involvement of *PdbCRF5* in salt stress regulation networks. We randomly selected 8 possible downstream target genes for validation, and the results showed that PdbCRF5 can bind to the promoter fragments of these 8 downstream target genes to varying degrees, indicating that the regulatory network is basically accurate and can be used for subsequent research. 6) The transcriptional activation experiment found that the PdbCRF5 transcription factor has transcriptional activation activity. The yeast two-hybrid experiment demonstrated that the transcription factor PdbCRF5 of *Populus davidiana*×*P. bolleana* can interact with homologous proteins PdbCRF1, 2, and 6 to form homologous dimers. In addition, over-expression of the *PdbCRF6* gene also reduced the salt tolerance of transgenic plants.【Conclusion】Our results demonstrated that the PdbCRF5 transcription factor can participate in regulating salt tolerance in plants. Over-expression of PdbCRF5 reduces salt tolerance in transgenic plants, while inhibition enhances salt tolerance in transgenic plants. PdbCRF5 can participate in plant salt stress response by directly binding to promoters of stress resistant genes such as *PdbHSP90-6*. In addition, PdbCRF5 can interact with PdbCRF6, and PdbCRF6 also negatively regulates plant tolerance to salt stress, indicating that *PdbCRF5* can also co regulate plant salt tolerance through interaction with other transcription factors.

Key words: PdbCRF5; Salt stress; Yeast two-hybrid; Yeast one-hybrid; BiFC; *Populus davidiana*×*P. bolleana*