

水曲柳响应干旱胁迫基因的多层级调控网络构建及抗旱功能分析

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摘 要:本研究通过自下而上图形高斯模型 (Bottom-up GGM algorithm) 算法构建了一个三层的水曲柳响应干旱胁迫的基因调控网络 (Gene Regulatory Network, GRN), 筛选并鉴定了 5 个干旱胁迫相关的关键基因, 分别为 *FmLOB1*、*FmLOB2*、*FmbHLH1*、*FmbHLH2*、*FmAP2/ERF-ERF2*, 均位于调控网络的顶层。对上述 5 个基因构建融合 Flag 标签的过表达载体, 瞬时转化水曲柳后进行 ChIP-PCR 和 RT-qPCR 分析。发现 67% 以上的调控关系是正确的, 验证了基因网络的可靠性。选取位于网络顶层的 *FmbHLH2* 进行抗旱功能分析。本研究通过对调控网络的构建及调控关系的验证, 为后续水曲柳基因功能的研究及分子遗传改良奠定了基础。结果表明: (1) 筛选了 265 个水曲柳干旱胁迫响应基因, 构建三层基因调控网络, 第一层为顶层, 包含 10 个转录因子, 调控 22 个下游转录因子, 共有 88 个调控关系; 第二层有 22 个转录因子, 调控 195 个下游结构基因, 共有 2273 个调控关系, 这 195 个结构基因参与了 8 个 GO 分类, 分别为响应缺水、调节气孔移动、响应渗透压胁迫、对活性氧的反应、细胞响应脱落酸刺激、响应乙烯、响应茉莉酸以及响应水杨酸。(2) 利用农杆菌介导的水曲柳高效瞬时转化技术, 得到瞬时过表达 (Over-expression, OE) 植株, 来进行 ChIP-PCR 验证顶层转录因子与下游转录因子间的调控关系, 试验结果表明第一层转录因子对第二层基因存在直接调控关系, 占调控关系的 67%。(3) 硝基蓝四唑 (NBT)、二氨基联苯胺 (DAB) 跟伊文思蓝染色三种染色结果都表明 5 个过表达瞬时表达植株中, *FmbHLH2* 的抗旱能力最强。正常生长条件下, 瞬时表达植株及对照植株的电解质渗透率、POD 酶活性表达量、叶绿素含量数据的趋势一致; 干旱胁迫的水曲柳植株, 相较于对照, 水曲柳瞬时侵染后能够有效提高 POD 酶的活性, 降低电解质渗透率, 叶绿素含量下降幅度最低, 表明 5 个基因中, *FmbHLH2* 的抗旱能力最强。(4) 对八周苗龄水曲柳敲除植株, 进行抗逆生理指标分析。结果表明: *FmbHLH2* 的敲除使植株的丙二醛、电解质渗透率显著升高, SOD、POD 酶活性显著降低。*FmbHLH2* 基因敲除后, 水曲柳植株的抗旱性降低, 说明 *FmbHLH2* 基因正调控水曲柳的抗旱性。

Construction of gene regulatory network in response to drought stress and analysis of key regulatory genes for drought resistance in *Fraxinus mandschurica*

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Abstract: In this study, a three-layer Gene Regulatory Network (GRN) was constructed based on bottom-up Ga-up GGM algorithm, and five key genes related to drought stress were screened and identified. They are *FmLOB1*,

FmLOB2, *FmbHLH1*, *FmbHLH2* and *FmAP2/ERF-ERF2*, all of which are located at the top layer of the regulatory network. Overexpression vector with fused Flag label was constructed for the above 5 genes, and then ChIP-PCR and RT-qPCR were performed after instantaneous transformation of *F. mandschurica*. More than 67% of the regulatory relationships were found to be correct, which verified the reliability of the gene network. The drought resistance function of *FmbHLH2* at the top of the network was analyzed. Through the construction of regulatory network and the verification of regulatory relationships, this study laid a foundation for the subsequent research on the gene function and molecular genetic improvement of *F. mandschurica*. The comparative experiment included:

(1) A three-layer Gene Regulatory Network (GRN) was constructed by screening 265 genes responding to drought stress in *F. mandschurica*. The first layer was the top layer, containing 10 Transcription factors (TFs) and regulating 22 downstream transcription factors. There were 88 regulatory relationships; The second layer has 22 transcription factors that regulate 195 downstream structural genes, with a total of 2273 regulatory relationships. These 195 structural genes participate in eight GO classifications, namely, responding to water scarcity, regulating stomatal movement, responding to osmotic stress, responding to reactive oxygen species, responding to abscisic acid stimulation, responding to ethylene, responding to jasmonic acid, and responding to salicylic acid. (2) Over expression (OE) plants were obtained using Agrobacterium mediated efficient instantaneous transformation technology of *F. mandschurica*, and the regulatory relationship between the top transcription factor and the downstream transcription factor was verified by ChIP-PCR. The experimental results showed that the first layer transcription factor had a direct regulatory relationship with the second layer genes. Accounting for 67% of the regulatory relationship. (3) The results of nitroblue tetrazole (NBT), diaminobenzidine (DAB) and Evans blue staining showed that *FmbHLH2* had the strongest drought resistance among the 5 transient overexpressed plants. Under normal growth conditions, the data of electrolyte permeability, POD enzyme activity expression and chlorophyll content of transient expression plants and control plants showed the same trend. Compared with the control, the instantaneous infection of *F. mandschurica* could effectively increase the POD enzyme activity, decrease the electrolyte permeability, and decrease the chlorophyll content at the lowest level, indicating that *FmbHLH2* had the strongest drought-resistant ability among the five genes. (4) The physiological indexes of stress resistance of eight-week old manchurian salix were analyzed. The results showed that the malondialdehyde and electrolyte permeability increased significantly after *FmbHLH2* elimination, and the activities of SOD and POD decreased significantly. After *FmbHLH2* gene was knocked out, the drought resistance of *F. mandschurica* plants decreased, indicating that *FmbHLH2* gene positively regulated the drought resistance of *F. mandschurica*.