

PdbSCL1 转录因子调控山新杨响应旱胁迫的分子机制

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摘要:【目的】山新杨是以嫩江县高峰林场的山杨为母本, 乌鲁木齐的新疆杨为父本, 于 1964 年杂交获得的品种, 具有重要的生态、观赏与经济价值, 同时由于其速生的特点, 是研究基因功能和树木生长规律的一种优良材料。然而, 当今全球气候变化下频繁发生的干旱等极端天气, 影响了山新杨的产出和发展, 因此, 挖掘具有增强山新杨抗旱能力的优质基因, 并利用其培育山新杨抗旱新品种, 是林业生产中迫切需要解决的问题。【方法】以速生良种山新杨为材料, 对其进行不同时间的干旱胁迫处理后获得转录组数据, 利用斯皮尔曼相关性算法构建多层基因调控网络, 通过 ChIP-PCR 和 qRT-qPCR 对基因调控网络的可靠性进行验证, 从中筛选出一个顶层转录因子 *PdbSCL1*, 构建其过表达和 CRISPR 载体, 将其转入山新杨并研究其耐旱性。通过 ChIP-Seq 以及 RNA-Seq 联合分析研究了其识别的顺式作用元件, 并对其可能调控的下游基因进行分析, 最后进行蛋白质翻译后修饰的检测等。【结果】构建了山新杨响应干旱胁迫的基因调控网络 (GRN), 并发现 GRN 中预测的基因之间的关系是可靠的。随机选择 GRN 中第一层转录因子基因进行瞬时转化山新杨, 生理指标结果显示, 这些基因的过表达提高了植物的耐旱能力。*PdbSCL1* 的表达可以被干旱诱导, 并具有组织表达特异性。过表达 *PdbSCL1* 的转基因山新杨通过降低活性氧、丙二醛含量、降低电解质渗透率以及提高活性氧清除酶体和超氧化物歧化酶的含量、增加维持细胞膜稳定的脯氨酸的含量来降低干旱胁迫对山新杨造成的损伤。*PdbSCL1* 结合 ABRE、PCF、NFY 等胁迫响应顺式作用元件来调控下游基因表达。*PdbSCL1* 响应干旱胁迫时会发生翻译后的乙酰化修饰, 被修饰后的蛋白可以增加对下游靶基因的调控来影响植物对于干旱胁迫的响应。进一步的研究显示, 该蛋白的第 106 和 444 位的赖氨酸位点是乙酰化位点, 该位点被突变成精氨酸后失去了被乙酰化修饰的能力, 并且发现 *PdbSCL1* 与下游元件的结合能力明显减弱, 从而降低对下游靶基因的表达调控能力。【结论】本研究通过构建山新杨响应干旱的基因调控网络找到了一个响应干旱胁迫的重要转录因子 *PdbSCL1*, 该基因可以被干旱诱导表达, 乙酰化修饰的 *PdbSCL1* 对 ABRE、PCF、NFY 等元件结合能力显著提高, 从而显著诱导了下游靶基因的表达, 进而提高山新杨抗旱能力。

关键词: 山新杨, 基因网络, *PdbSCL1* 转录因子, 乙酰化, 干旱胁迫

Molecular mechanism of transcription factor *PdbSCL1* regulating response of *Populus davidiana*×*P. bolleana* to drought stress

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Abstract: 【Objective】The *Populus davidiana*×*P. bolleana*, commonly known as Shanxin Poplar, is a hybrid species that was developed in 1964 by combining the Gaofeng Forest Farm in Nenjiang County and the Xinjiang Poplar from Urumqi County. This hybrid has significant ecological, ornamental, and economic value, making it a valuable subject for studying gene function and tree growth patterns, particularly due to its rapid growth characteristics. However, the occurrence of extreme weather conditions, such as frequent droughts resulting from global climate change, has had a detrimental impact on the productivity and development of Shanxin Poplar.

Consequently, it has become imperative to address this issue in forestry production by identifying high-quality genes capable of enhancing the drought-resistant capabilities of Shanxin Poplar. By utilizing these genes, we can cultivate new drought-resistant varieties of Shanxin Poplar, thereby mitigating the negative effects of drought and ensuring sustained production in the forestry sector. **【Method】** The transcriptome data were acquired through the application of drought stress at various time points on the fast-growing poplar. A multi-layer gene regulatory network was constructed using a Spielman correlation algorithm. The reliability of this gene regulatory network was confirmed through ChIP-PCR and RT-qPCR. The reliability of this network was confirmed through ChIP-PCR and RT-qPCR techniques, leading to the identification of a prominent transcription factor, *PdbSCL1*. Overexpression and CRISPR vectors were constructed to manipulate *PdbSCL1*, which was subsequently transferred to Shanxin Poplar for the purpose of studying its impact on drought tolerance. Furthermore, the cis-acting elements identified by ChIP-Seq and RNA-Seq were thoroughly examined, and the downstream genes potentially regulated were analyzed. Lastly, the post-translational modifications of proteins were investigated. **【Result】** The gene regulatory network (GRN) was constructed to investigate the response to drought stress, and the relationship between the predicted genes in the GRN was found to be reliable. To evaluate the impact of these genes, the first transcriptional factor genes in the GRN were randomly selected for transient transformation. The results of physiological index analysis demonstrated that overexpression of these genes significantly enhanced the plants' tolerance to drought. Specifically, the expression of *PdbSCL1*, which is induced by drought and exhibits tissue specificity, was found to be particularly beneficial. Transgenic poplar plants overexpressing *PdbSCL1* exhibited reduced damage from drought stress, as evidenced by lower levels of reactive oxygen species and malondialdehyde, decreased electrolyte permeability, increased activity of oxygen scavenging enzymes and superoxide dismutase, and higher levels of proline, which contributes to cell membrane stability. *PdbSCL1* exerts its regulatory function by binding to stress response cis-acting elements such as ABRE, PCF, and NFY, thereby controlling the expression of downstream genes. Furthermore, it was observed that *PdbSCL1* undergoes post-translational acetylation in response to drought stress. The modified protein resulting from acetylation enhances its ability to regulate downstream target genes, thereby influencing the plant's response to drought stress. Further investigation identified the specific lysine sites at positions 106 and 444 of the protein as the acetylation sites. Notably, mutation of these sites to arginine abolished their acetylation capacity. Additionally, it was observed that the binding affinity of *PdbSCL1* to downstream elements was significantly weakened upon mutation, consequently reducing its ability to regulate the expression of downstream target genes. **【Conclusion】** This study revealed the identification of a significant transcription factor, *PdbSCL1*, in response to drought stress through the construction of a gene regulatory network. It was observed that this gene is capable of being induced by drought. Moreover, the acetylation of *PdbSCL1* was found to significantly enhance its binding affinity to ABRE, PCF, NFY, and other elements, consequently leading to a substantial induction of downstream target gene expression. Consequently, the drought-resistant capability of poplar was notably enhanced.

Key words : Shanxin Poplar., gene regulatory network (GRN), *PdbSCL1* transcription factor, Acetylation, drought stress