

Tissue-specific expression analysis of PwuWRKY genes in *Populus wulianensis*

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Abstract: WRKY transcription factors are usually involved in plant defense responses. However, the role of the WRKY family in the defense of *Populus wulianensis* is limited. [Objective] To study the new important stress response genes in *Populus*, providing theoretical basis for revealing the molecular mechanism of poplar resistance to adverse conditions and breeding new resistant poplar varieties. [Methods] Based on the WRKY transcription factors in the response to drought-rehydration stress of *P. wulianensis*, five genes (including Potri.001G3524, Potri.002G193000, Potri.010G147700, Potri.013G153400 and Potri.008G103300) were screened from the *P. trichocarpa* genome using BLAST. Using these gene sequences as references and *P. wulianensis* cDNA as template, five genes (PwuWRKY28-1, PwuWRKY23-5, PwuWRKY48-9, PwuWRKY33-11, PwuWRKY48-20) were cloned. Utilize bioinformatics to analyze the structural features and phylogenetic relationships of PwuWRKY protein and its homologous proteins. Analyze the subcellular localization of PwuWRKY protein in tobacco protoplasts by transformation. Use real-time fluorescence quantitative PCR (qRT-PCR) technology to analyze the tissue-specificity and expression pattern of PwuWRKY gene under different stress conditions. [Result] In this study, evolutionary analysis indicated that PwuWRKY33-11 belongs to the I group of the WRKY family, while the other proteins belong to the IIc subfamily. All five WRKY transcription factors localize to the plant cell nucleus, and except for PwuWRKY48-20, which lacks transcriptional activation activity, the other proteins show transcriptional activation. The tissue-specific expression of five WRKY transcription factors in *P. wulianensis* was studied in response to abiotic stresses such as drought and rehydration, high salinity, high temperature and low temperature. The results showed that the expression of different members of the WRKY family had obvious tissue specificity. PwuWRKY23-5, PwuWRKY48-9 and PwuWRKY48-20 were mainly expressed in the leaves of *P. wulianensis*, and the other genes were expressed at higher levels in the roots. PwuWRKY28-1 responded to the above abiotic stresses, and the gene was rapidly up-regulated and then down-regulated. The expression of PwuWRKY48-9 and PwuWRKY48-20 was consistent with that of PwuWRKY28-1 in response to high salt treatment, and the expression trend of these two genes was almost the same in response to low temperature stress, indicating that there was functional redundancy in the response of these two genes to low temperature stress. The above five genes were all treated in response to high temperature stress. The results showed that the expression of WRKY transcription factors was affected by stress, but different WRKY family members responded differently to stress. [Conclusion] The results of this study showed that the expression of WRKY family members in *P. wulianensis* has tissue specificity, and different members have different responses to non-biotic stress. This expression pattern may be adapted to the

stress response of *P. wulianensis* in different environments. The results of this study lay a foundation for further revealing the mechanism of WRKY protein action and aim to provide a basis for genetic improvement of *P. wulianensis*.

Keywords: *Populus wulianensis*, PwuWRKY, Tissue-specific expression

五莲杨PwuWRKY基因组织特异性表达分析

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摘 要: WRKY 转录因子通常参与植物的防御反应。然而,关于 WRKY 家族在五莲杨防御中的作用的信息有限。**【目的】**研究杨树重要胁迫响应新基因,为揭示杨树抗逆分子机制、培育优良抗逆杨树新品种提供理论依据。**【方法】**基于五莲杨响应干旱-复胁迫过程的 WRKY 转录因子,通过 BLAST 在毛果杨基因组中筛选得到 5 条基因(Potri.001G352400、Potri.002G193000、Potri.010G147700、Potri.013G153400 和 Potri.008G103300),并以其序列为参考,以五莲杨 cDNA 为模板克隆得到 5 条基因 (PwuWRKY28-1、PwuWRKY23-5、PwuWRKY48-9、PwuWRKY33-11 和 PwuWRKY48-20)。利用生物信息学分析 PwuWRKY 蛋白的结构特征及与其同源蛋白的亲缘关系。通过转化烟草原生质体分析 PwuWRKY 蛋白亚细胞定位。采用实时荧光定量 (qRT-PCR) 技术,分析 PwuWRKY 基因组织特异性和不同胁迫条件下的表达模式。**【结果】**本研究中,进化分析表明,PwuWRKY33-11 属于 WRKY 家族的 I 组,其他蛋白均属于 IIc 亚家族。5 个 WRKY 转录蛋白均定位于植物细胞核,除了 PwuWRKY48-20 不具有转录自激活活性外,其他蛋白均表现出转录激活。研究五莲杨中 5 个 WRKY 转录因子表达的组织特异性表达,响应干旱与复水、高盐、高温、低温等非生物胁迫实验结果表明,WRKY 家族中不同成员的表达具有明显的组织特异性,PwuWRKY23-5、PwuWRKY48-9、PwuWRKY48-20 主要在五莲杨的叶片中表达,其余基因在根中表达水平较高。PwuWRKY28-1 均响应以上非生物胁迫,并出现基因快速上调后下调表达的趋势。PwuWRKY48-9 和 PwuWRKY48-20 在响应高盐处理与 PwuWRKY28-1 基因表达一致,且这两个基因均在响应低温胁迫过程中表达趋势几乎一致,分析这两个基因在响应低温胁迫过程中存在功能冗余。以上 5 个基因均响应高温胁迫处理。结果表明,WRKY 转录因子的表达均受胁迫的影响,但是不同 WRKY 家族成员对胁迫反应不同。**【结论】**本研究结果表明五莲杨 WRKY 家族不同成员的表达具有组织特异性并且不同成员对非生物胁迫应答反应不同。这种表达模式可能与五莲杨在不同环境下的胁迫反应相适应。本研究结果为进一步揭示 WRKY 蛋白作用机制奠定了基础,旨在为五莲杨遗传改良提供依据。

关键词: 五莲杨; PwuWRKY; 组织特异性表达

To explore the function of PdMODD in the growth and development of *Populus × euramericana*, we analyzed their sequence characteristics, subcellular localization and expression patterns in response to salt and drought stress, which will provide a theoretical basis for revealing the molecular mechanisms of stress resistance and breeding new poplar varieties. **【Method】** Based on the rice MODD amino acid sequence, Podel. 08G114100, Podel. 10G158900 and Podel. 17G098500 were selected using BLAST in the genome of *Populus deltoides* WV94 v2. 1. Using these gene sequences as references and *Populus × euramericana* 'Bofeng 3 hao' cDNA as template, three genes (PdMODD1, PdMODD2 and PdMODD3) were cloned. The structural characteristics of the PdMODD protein and its

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