

Uncovering miRNA-mediated in Wood Formation of *Pinus massoniana* Under Brassinosteroid Treatment

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Abstract: 【Objective】 This research aims to provide a theoretical foundation for comprehending the miRNA regulatory mechanisms involved in the BR response in *P. massoniana*. **【Method】** The experimental material comprised one-year-old seedlings of *P. massoniana* from a single family line, grown in a substrate mixture of natural soil and perlite. Based on previous experiments conducted by the research team, treatments of 1 mg/L BR, 1 mg/L PCZ, and deionized water (CK) were selected to investigate the effects of BR on the growth and development of *P. massoniana* seedlings. The treatments were administered by foliar spraying once every 7 days. The treatments were carried out for a total of 120 days, with at least three biological replicates for each treatment. **【Result】** In this study, *P. massoniana* seedlings were subjected to BR treatment along with the Brassinolide inhibitor PCZ. The results showed the net height growth in the BR-treated group was higher than in the control group, while the PCZ-treated group had lower height growth compared to the control. Additionally, the net growth of stem diameter in the BR-treated group increased by 143% compared to the control group, which was statistically significant; BR application significantly promotes the thickness of the xylem and phloem, as well as the number of xylem cell layers in *P. massoniana* stems. The BR treatment increased lignin content by 13% compared to the control group, which was significantly higher. In contrast, PCZ treatment did not significantly reduce lignin content compared to the control. High-throughput sequencing was employed for analyzing differential miRNA expression under different treatments and predicting the target gene functions. This study aims to identify miRNAs responsive to BR in *P. massoniana*. A total of 18 miRNA families were identified, with 49 miRNA members exhibiting significant differential expression in response to BR and 30 miRNAs showing significant differential expression in response to PCZ. Among them, 15 miRNAs were found to be coordinately expressed under both BR and PCZ treatments, with their target genes, such as *HD-ZIP*, *HSP90A*, and *LAT52*, being closely related to plant cell growth and development. Two novel miRNAs were specifically upregulated under BR treatment but downregulated under PCZ treatment; their target genes are involved in lignin synthesis and phosphate transport. It is noteworthy that miR159 was significantly upregulated under PCZ treatment, suggesting a potential crosstalk between the BR and GA signaling pathways mediated by this specific *miR159-GmMYB33* regulatory network associated with GA signaling and metabolism. miR396 has been demonstrated to play a crucial role in plant growth, development, and abiotic stress responses in other plants. Our study reveals that overexpression of miR396 leads to stunted growth and reduced leaf size in tobacco plants, which is consistent with previous research findings. In addition, the *miR396-GRF* regulatory module is also involved in GA signaling, suggesting that the coordination between BR and GA relies on the regulation of various miRNAs. Previous research at the mRNA level has shown involvement of the BR response module in BR signaling and GA synthesis. The responsive miRNAs identified in this study can shed light on potential molecular mechanisms of *P. massoniana*'s response to BR stimulation at the post-transcriptional level.

【Conclusion】 This study provides a comprehensive compilation of tissue-specific BR-responsive miRNAs in *P. massoniana* seedlings. The differential expression of miR396 was functionally validated. This study provides a valuable reference for further analysis of differential gene expression and the molecular mechanisms in *P. massoniana* in response to BR treatment.

Keywords: *Pinus massoniana*, Brassinolide, MicroRNAs, Wood formation