白桦 *BpPIN3* 参与调控叶片近轴面卷曲的分子机制

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摘 要:【目的】叶片是植物的营养器官之一，对植物的生长发育至关重要。PIN-FORMED（PINs）基因作为 IAA 运输载体，在叶片发育过程中发挥着关键作用，为了明确 *BpPIN3* 基因的功能，揭示白桦 *BpPIN3* 在叶片极性建成中的功能。【方法】本实验采用农杆菌介导法开展该基因的遗传转化研究，以 *BpPIN3* 过表达、抑制表达株系为材料，进行叶片解剖观察、叶片栅栏组织细胞密度测定、叶片内源 IAA 的含量分析以及组织定位，并基于 RNA-seq 分析进行差异基因挖掘。【结果】研究发现，RE 株系表现出叶缘近轴向上卷曲的特征，*BpPIN3* 表达量较低，卷曲程度较大。IAA 在生长素 GUS 报告系统中的组织定位证明，RE 中的生长素主要分布在叶缘区域的次生叶脉、栅栏组织和表皮细胞中。叶缘区域的生长素含量显著大于主脉组织。发现 RE 株系卷曲叶缘的栅栏组织细胞密度和栅栏组织与海绵组织的比值显著降低。RNA - seq 分析发现，与 OE 和 WT 株系相比，RE 激素信号通路基因显著富集；尤其是生长素响应相关基因 SAURs (即 *SAUR23*、*SAUR24*、*SAUR28* 和 *SAUR50*)和 *GH3.10* 被发现显著上调。qRT - PCR 分析表明，在 RE 株系中，*BpPIN3* 在叶缘的表达量显著低于主脉附近。相比之下，SAURs 和 *GH3.10* 的表达量显著高于中脉附近。【结论】*BpPIN3* 参与白桦叶片极性的形成。*BpPIN3* 表达量的降低影响生长素的极性运输，从而导致叶片中生长素浓度的差异,生长素含量的增加促进了白桦叶片中生长素响应相关基因的表达。叶缘过量的生长素通过影响生长素响应相关基因，改变了栅栏组织细胞的结构，导致叶缘向上卷曲。

关键词: 白桦，*BpPIN3*，IAA 极性运输,，叶片，近-远轴面

Molecular mechanism of *BpPIN3* involved in regulating leaf adaxial surface curling in Betula pendula

Abstract:【Objective】Leaves are one of the vegetative organs of plants that are essential for plant growth and development. PIN-FORMED (PINs) gene is an indoleacetic acid (IAA) transporter that plays a critical role in leaf development. In order to clarify the function of *BpPIN3* gene and reveal the function of BpPIN3 in leaf polarity formation of Betula pendula. 【Method】In this study, the genetic transformation of *BpPIN3* was carried out by Agrobacterium-mediated method. The *BpPIN3* overexpression and *BpPIN3*-reduced expression lines were used as materials to observe the leaf anatomy, measure the cell density of leaf palisade tissue, analyze the content of endogenous IAA in leaves and tissue localization, and perform differential gene mining based on RNA-seq analysis. 【Result】Research findings：The RE lines displayed the characteristics of leaf margin adaxial upward curling, with lower expression of *BpPIN3* resulting in greater rolling. Tissue localization of IAA in the auxin GUS reporter system proved that auxin in the RE was mainly distributed in the secondary veins, palisade tissues, and epidermal cells in the leaf margin area. The auxin content in the leaf margin area was significantly greater than that in the main vein tissue. The cell density of the palisade tissue and the ratio of palisade tissue to spongy tissue in the curled leaf

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margin of the RE lines were found to be significantly decreased. RNA-seq analysis revealed that the RE hormone-signaling pathway genes were significantly enriched compared with those of the OE and WT lines; in particular, the auxin response-related genes SAURs (i.e., *SAUR23*, *SAUR24*, *SAUR28*, and *SAUR50*) and *GH3.10* were found to be significantly upregulated. qRT-PCR analysis indicated that *BpPIN3* expression at the leaf margin was significantly lower than that near the main vein in the RE lines. In contrast, the expression levels of SAURs and *GH3.10* were significantly higher than those near the midrib. 【Conclusion】*BpPIN3* is involved in leaf polarity formation in birch. The decrease in *BpPIN3* expression led to affected the polar transport of auxin, resulting in a difference in auxin concentration in the leaves. The increase of auxin content promoted the expression of auxin response related genes in birch leaves. The excessive auxin at the leaf margin changed the structure of PT cells by affecting auxin response-related genes, leading to upward curling at the leaf margin.

Key words: Betula pendula, *BpPIN3*, IAA transport, leaf, Adaxial-Abaxial.