

杨树花发育基因 *PagAG2* 基因编辑突变体创制及其基因组变异分析

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摘要: 【目的】林木在提高城市绿化水平中发挥重要功能,但雄株散落的花粉在引起了一定的植源性污染问题,是人体重要的致敏源之一,迫切需要培育少花粉甚至无花粉的环保型优良杨树品种改善北方城市花粉环境问题。【方法】利用 CRISPR/Cas9 基因编辑技术对雄花发育过程关键基因 *PagAG2* 进行精准编辑,采用实时荧光定量 PCR 方法分析靶标基因及其上下游相关基因表达量;采用 sanger 测序及重测序技术手段以评估基因编辑的脱靶情况及此次基因编辑事件对全基因组变异的影响;转录组和蛋白组高通量组学测序技术评估编辑植株 *PagAG2* 的突变对营养生长关键过程调控作用。【结果】本研究采用 CRISPR/Cas9 基因编辑技术获得了杨树 *PagAG2* 基因编辑植株,在靶位点附近造成了 6bp 碱基的缺失和 2bp 碱基的替换。编辑植株 *PagAG2* 基因的表达量在根、茎、叶中的低于野生型,生长速率高于野生型。同时, *PagAG2* 突变可以调控其下游基因 (*STK1*、*STK2*、*SPL16*、*SPL25*) 的表达,但对 *AG* 基因可能产生拮抗作用的上游基因 *AP2* 影响较小。Sanger 测序及重测序对基因编辑脱靶情况的分析结果显示本研究设计的 sgRNA 具有高特异性,未发现脱靶事件。野生型植株与基因编辑植株的基因组变异相似,大多数突变为野生型植株和基因编辑植株所共有 (SNPs 为 55.9%, indels 为 36.1%),这主要是由于组织培养中的体细胞无性系变异,Cas9 诱导的突变在‘84K’杨中表现出高度特异性,而野生型植株与基因编辑植株在转录和蛋白质水平的差异主要是由于自发突变和 Cas9 核酸内切酶诱导的突变。多组学分析结果显示位于基因组外显子上的变异所对应的基因、转录组的差异基因及蛋白组的差异蛋白主要在类黄酮生物合成、昼夜节律-植物、半乳糖代谢等生物途径被富集,这可能也是造成野生型植株与编辑植株表型差异的原因。【结论】本研究采用 CRISPR/Cas9 基因编辑技术创制了雄花发育关键基因银腺杨的纯合突变体,且在营养生长阶段未表现出不良影响,基因编辑事件并未对编辑植株基因组变异产生显著影响,为利用基因编辑技术培育无花粉或少花粉的杨环保型优良品种奠定重要基础。

关键词: CRISPR; 花发育基因; *PagAG2*; 全基因组测序; poplar; 转录组分析; 蛋白质组分析

Production and Analysis of Flower development gene *PagAG2* Editing Mutant in *Populus alba* × *P. glandulosa* ‘84K’

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Abstract: **【Objective】** Trees play an important role in improving the level of urban greening, but pollen scattered by male plants causes certain plant-derived pollution, which is one of the important allergens for human body. It is urgent to cultivate environmentally friendly poplar varieties with little or no pollen to improve the pollen environment in northern cities. **【Method】** The key gene *PagAG2* in male flower development was accurately edited using CRISPR/Cas9 gene editing technology, and the expression levels of the target gene and its upstream and downstream related genes were analyzed by real-time quantitative PCR. Sanger sequencing and WGS techniques were used to evaluate the off-target situation of gene editing and the effect of the gene editing event on the whole genome variation. Transcriptome and proteome high-throughput sequencing techniques to evaluate the role of *PagAG2* mutation in edited plants in regulating key processes of vegetative growth. **【Result】** In this study, CRISPR/Cas9 gene editing technology was used to obtain poplar *PagAG2* gene editing plants, which caused 6 bp base deletion and 2 bp base substitution near the target site. The expression of *PagAG2* gene in the root, stem and leaf of the edited plants was lower than that of the wild-type, and the growth rate was higher than that of the wild-type. Meanwhile, *PagAG2* mutation can regulate the expression of its downstream genes (*STK1*, *STK2*, *SP L16*, *SPL25*), but has little effect on the upstream gene *AP2*, which may have an antagonistic effect on the *AG* gene. The analysis results of Sanger sequencing and WGS on gene editing off-target showed that the sgRNA designed in this study had high specificity and no off-target event was found. The genome variation of wild-type plants was similar to that of gene-edited plants, and most of the mutations were shared by the wild-type plant and the gene-edited plant (SNPs was 55.9%, indels was 36.1%), which was mainly due to somaclonal variation in tissue culture. Cas9-induced mutations showed high specificity in *Populus alba* × *P. glandulosa* ‘84K’. The differences in transcription and protein levels between wild-type plants and gene-edited plants were mainly due to spontaneous mutations and the mutations induced by Cas9 endonuclease. The results of the multi-omics analysis showed that the genes corresponding to the variation on the genome exon, the differential genes of the transcriptome and the differential proteins of the proteome were mainly enriched in biological pathways such as flavonoid biosynthesis, circadian rhythm-plant, and galactose metabolism, which may also be the reason for the phenotypic differences between wild-type plants and edited plants. **【Conclusion】** In this study, CRISPR/Cas9 gene editing technology was used to create a homozygous mutant of the key gene of male flower development, and it showed no adverse effects in the vegetative growth stage, and the gene editing event did not have a significant effect on the genome variation of the edited plants, which laid an important foundation for the use of gene editing technology to develop environmentally friendly poplar varieties with no pollen or little pollen.

Keywords: CRISPR; flower development gene; *PagAG2*; whole-genome sequencing; poplar; transcriptome; proteome