

利用 CRISPR/Cas9 系统定点敲除元宝枫 *AtruPDS* 基因的研究

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摘 要: 元宝枫 (*Acer truncatum*) 是中国特有树种, 兼具观赏、药用及食用价值, 其基因编辑研究尚未见报道。为建立元宝枫基因编辑体系, 以元宝枫胚芽为外植体建立再生体系, 以元宝枫八氢番茄红素脱氢酶(PDS)的编码基因 *AtruPDS* 为靶基因, 在其第一外显子和 3' 端分别设计 sgRNA, 构建 pCambia1300-psgR-Cas9-sgRNA (target 1)-pAtU6-sgRNA (target 2) 转入根癌农杆菌。以根癌农杆菌介导法转化元宝枫胚芽进行基因编辑。结果表明, 元宝枫胚芽诱导不定芽最佳配方为: DKW+ZT 0.05 mg/L+6-BA 0.25 mg/L+NAA 0.1 mg/L +蔗糖 30 g/L+琼脂粉 8 g/L, PH 5.8; PCR 和表型检测初步筛选获得基因编辑阳性苗。综上, 该研究建立了以元宝枫胚芽为外植体的再生体系, 并利用 CRISPR/Cas9 技术实现了对 *AtruPDS* 基因的靶向敲除, 为元宝枫功能基因组研究和种质创新奠定了基础。

关键词: 元宝枫; *AtruPDS*; CRISPR/Cas9; 基因敲除

The research of CRISPR/Cas9-mediated targeted mutagenesis of *AtruPDS* gene in *Acer truncatum*

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Abstract: *Acer truncatum* is a unique tree species in China, with ornamental, medicinal, and edible values. Its gene editing research has not meant reported yet. To establish a gene editing system for *Acer truncatum*, a regeneration system was established using embryos as explants. The phytoene dehydrogenase gene (*AtruPDS*) of *Acer truncatum* was used as the target gene. Designed sgRNAs at the first exon and 3' end, and pCambia1300-psgR-Cas9-sgRNA (target 1) - pAtU6-sgRNA (target 2) was constructed. Using *Agrobacterium*-mediated transformation of *Acer truncatum* embryos for gene editing. The results showed that the optimal formula for inducing adventitious buds from *Acer truncatum* embryos was DKW+ZT 0.05 mg/L+6-BA 0.25 mg/L+NAA 0.1 mg/L+sucrose 30 g/L+agar powder 8 g/L, pH 5.8; preliminary screening by PCR and phenotype detection resulted in gene edited positive seedlings. In summary, this study established a regeneration system using *Acer truncatum* embryos as explants and achieved targeted knockout of the *AtruPDS* gene using CRISPR/Cas9 technology, laying the foundation for functional genomics research and germplasm innovation of *Acer truncatum*.

Keywords: *Acer truncatum*; CRISPR/Cas9; *AtruPDS1*; gene editing

基金项目: 中央财政林业改革发展资金(林业科技推广示范补助)项目“元宝枫良种繁育及丰产栽培技术示范(鲁[2020]TG01号)”和“槭属主要树种繁育及栽培技术推广示范项目(鲁[2023]TG 018-02号)”, 山东省农业良种工程项目课题“元宝枫抗逆高产无性系早期鉴定及神经酸分离纯化技术(2020LZGC0090301)”, 科技创新合作项目“元宝枫种质资源收集评价及种质挖掘(2020KJCX01)”和山东省自然资源厅“珍贵树种保育利用创新团队项目”(LZYZZ202398)。