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蔬菜

拟南芥*At-pri-miR828* 基因的克隆及其对番茄的遗传转化

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Cloning of *Arabidopsis At-pri-miR828* Gene and Its Genetic Transformation into Tomato

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摘要 MicroRNA828（miRNA828）是一种新近发现的生物学功能还未全面研究的miRNA。为从不同角度阐明miRNA828 的生物学功能，从拟南芥中克隆到At-pri-miR828 基因并构建了该基因过量表达的植物表达载体pC2300-pOT2-At-pri-miR828，通过农杆菌介导的叶盘法将pC2300-pOT2-At-pri-miR828导入异源植物番茄品种‘Ailsa Craig’中。PCR 鉴定结果显示，外源基因At-pri-miR828 已成功整合到转基因番茄基因组中，共获得9 个转基因株系，67 株转基因植株。定量PCR 检测结果显示，与野生型番茄植株相比，转基因植株中miR828 的表达量显著增加，而生物信息学所预测的miR828 靶基因Sly-myb-like1的表达水平则相应降低。花青素含量测定结果显示，miR828 过量表达的转基因番茄植株花青素含量明显低于野生型植株，表明miR828 参与了番茄花青素的生物合成调控。

关键词： 番茄 miR828 Sly-myb-like1 花青素 实时定量PCR

Abstract： MicroRNA828（miR828）is a newly identified small RNA whose biological functions are still not very clear. To explore the biological functions of miR828，particularly in tomato，the *At-primiR828* gene was isolated from Arabidopsis thaliana，and cloned into the corresponding site of the pCAMBIA2300 to construct the plant expression vector pC2300-pOT2-At-pri-miR828. This *At-pri-miR828* overexpressing vector was then introduced into tomato（Solanum lycopersicum Mill.‘Ailsa Graig’）using Agrobacterium-mediated transformation. Total of 9 transgenic lines and 67 transgenic plants carrying the At-pri-miR828 gene under the control of the 35S promoter were generated. PCR amplification showed that At-pri-miR828 was integrated into the genome DNA of transgenic tomatoes. Real-time PCR analysis demonstrated that miR828 mRNA level in the transgenic tomatoes was increased greatly. Accordingly，expression of Sly-myb-like1，a potential target gene of miR828 predicted by informatics，was suppressed. Further anthocyanin content analysis revealed that anthocyanin content was greatly reduced in miR828 overexpressed transgenic plants，demonstrating miR828 might function in the anthocyanin biosynthesis of tomato.

Keywords： tomato, miR828, Sly-myb-like1, anthocyanin, real-time PCR

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