

两种SNP分型方法的比较及其在柚品种鉴定中的应用

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Comparison of Allele-specific PCR and High Resolution Melting Analysis in SNP Genotyping and Their Application in Pummelo Cultivar Identification

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摘要 用两种常用的SNP分型方法, 等位基因特异性PCR法 (Allele-specific PCR, AS-PCR) 和高分辨率熔解曲线分析 (High resolution melting analysis, HRMA), 对16个柚栽培品种和8个柚杂种后代材料进行了7个SNP位点的分型研究。结果表明这两种方法所得的分型结果相同, 都将24个样本分成了22种基因型。值得一提的是, 样本‘八朔’与‘红八朔’, ‘红甘夏’与‘川野夏橙’之间在所测位点无差别, 表明其应当是同一来源的无性系变异。‘早熟真龙柚’和‘中熟真龙柚’的分型结果表明它们的基因型不同, 看来并非是起源同一基因型的不同芽变品种, 也不存在亲子关系。可见, AS-PCR和HRMA均适用于柚类品种的区分和鉴定。AS-PCR法是一种准确、低成本的SNP分型方法, 适合普通实验室使用, 惟对PCR反应体系要求严格。HRMA分型方法具有准确、快速、简便、分析量大的特点, 但需要专门的设备, 试剂成本也高。

关键词: 柚 单核苷酸多态性 等位基因特异性PCR 高分辨率熔解曲线分析 基因分型

Abstract: Allele-specific PCR (AS-PCR) and high resolution melting analysis (HRMA) are two widely used SNP genotyping methods but no research has been done to compare them in terms of genotyping efficiency. In this study, 16 pummelo cultivars and 8 pummelo hybrids were genotyped using AS-PCR and HRMA respectively on two different sets of 7 SNP loci. It was shown that both methods generated the same genotyping results in which 24 accessions were assigned into 22 genotypes. It was noteworthy that Hassaku and Red Hassaku were identical at all SNP loci, indicating both accessions should have originated asexually from the same mother cultivar, as was the same case for the two Japanese summer orange cultivars, Beni Amanatsu and Kawano Natsudaikai. Interestingly, the early- and middle-season ‘Zhenlong’ pummelo cultivars possessed different genotypes, indicating clearly that they unlikely had the same origin as bud mutations as was thought before, and further analysis of HRMA results showed there is no direct hereditary relationship between them. Our results showed that both AS-PCR and HRMA were suitable methods for the identification of pummelo-related accessions. AS-PCR is a reliable and low-cost SNP genotyping method and easily accessible to ordinary laboratories though the method was found to be sensitive to changes in PCR conditions. HRMA is proven to be a reliable, quick, simple and high throughput SNP genotyping method; However, it uses special equipment and expensive reagents.

Keywords: pummelo, single nucleotide polymorphism, allele specific-PCR, high resolution melting analysis, genotyping

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