首 页   本刊简介   编委会   期刊订阅	广告服务   留言板   联系我们   English	
	快速 大大	GO ag

园艺学报 » 2012, Vol. 39 » Issue (4):777-782 DOI:

新技术与新方法

最新目录 | 下期目录 | 过刊浏览 | 高级检索

<< Previous Articles | Next Articles >>

宽皮柑橘单核苷酸多态性的高分辨率熔解曲线分型

吴 波1,2,3,杨润婷1,朱世平2,钟 云3,姜 波3,曾继吾3,钟广炎3

(1 西南大学园艺园林学院,重庆 400715; 2 中国农业科学院柑桔研究所,重庆 400712; 3 广东省农业科学院果树研究所,农业部热带南亚热带果树生物学与遗传资源利用重点实验室,广州 510640)

Genotyping Single Nucleotide Polymorphisms in Mandarin Cultivars Using High Resolution Melting Analysis

WU Bol,2,3, YANG Run-ting1, ZHU Shi-ping2, ZHONG Yun3, JIANG Bo3, ZENG Ji-wu3, and ZHONG Guang-yan3,\*

(1College of Horticulture and Landscape, Southwest University, Chongqing 400715, China; 2Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing 400712, China; 3Key Laboratory of Tropical and Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, Fruit Tree Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China)

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摘要 高分辨率熔解曲线分析(High resolution melting analysis,HRM)可以检测单碱基改变引起的DNA双链熔解温度(T<sub>m</sub>)值变化,从而可以对样本在单核苷酸多态性分子标记(Single nucleotide polymorphism,SNP)上进行基因分型。通过分析NCBI数据库中宽皮柑橘的表达序列标签(Expressed sequence tag,EST)数据鉴别SNP位点,并用小片段扩增法高分辨率熔解曲线分型技术(High resolution melting analysis of small amplicons)分析11个宽皮柑橘(Citrus reticulata)品种以及柳橙(Citrus sinensis Osbeck var. 'Liucheng')的5个SNP位点的基因型。结果显示,小片段扩增法高分辨率熔解曲线分型可以快速、清楚地分辨纯合与杂合基因型,在校正温度差异后也可以很好地分辨同一个SNP位点不同的纯合型。统计分析表明样本在所有SNP位点上均存在多态性,5个SNP位点的平均多态性信息含量(PIC)为0.3190,显示样本在这组SNP位点上具有较高的杂合率。

关键词: 宽皮柑橘 高分辨率熔解曲线 单核苷酸多态性 基因分型

Abstract: High resolution melting analysis  $^{(1)}$  is capable of detecting changes in melting temperature  $^{(7)}$  of double strand DNA sequences caused by single nucleotide changes, and is suitable to genotype genetic samples using known single nucleotide polymorphic sites  $^{(5)}$ . *Citrus reticulata* ESTs from NCBI EST database were searched for SNPs, and 5 SNPs were selected to genotype 11 mandarin and 1 sweet orange cultivars using HRM genotyping of small amplicons. The results showed that, at a givenSNP site, a heterozygous genotype could be easily and clearly distinguished from a homozygous genotype by HRM, and 2 different homozygous genotypes could also be easily identified when temperature was calibrated. All 5 SNPs were found polymorphic in analyzed citrus samples, with an average polymorphic information content (PIC) value of 0.3190, indicating that these samples are highly heterozygous at these SNP sites.

Keywords: mandarin, high resolution melting, single nucleotide polymorphism, genotyping

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