RAPD 技术在药用植物绞股蓝鉴别中的初步研究

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摘 要: 运用 RAPD 技术对绞股蓝(*Gynostemma pentaphyllum*)及其伪品进行 DNA 指纹图谱的鉴别研究。采用改进的 CTAB 法提取七叶绞股蓝、五叶绞股蓝、乌蔹莓(*Cayratia japonica*) 3 种植物的总 DNA, 主要以七叶绞股蓝 DNA 为模板, 采用随机引物 WGS001 进行 PCR 扩增, 对反应体系包括模板、Mg^{2+}、*Taq* 酶、牛血清白蛋白(BSA)、退火温度进行优化。优化的反应体系总体积 25 μL, 含 $MgCl_2$ 2 mmol/L、dNTP 0.2 mmol/L、引物 0.4 μmol/L、模板 60 ng、*Taq* 酶 1 U、BSA 2 μg/μL, 退火温度 58℃。用 10 条含 20 个碱基的随机引物对以上 3 种植物总 DNA 作 PCR 扩增, 进行引物筛选。筛选得到的两条随机引物(WGS001、WGS004)可扩增得到识别这些物种基因组 DNA 的多态性片段。这些片段可以有效地鉴别绞股蓝和乌蔹莓。

关键词: 绞股蓝; 基因组 DNA; RAPD; 鉴别

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Initial Research on Authentication of Medicinal Plant *Gynostemma pentaphyllum* by RAPD Method

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Abstract: The random amplified polymorphic DNA (RAPD) analysis was applied to identify the resources of medicinal plant *Gynostemma pentaphyllum* and its spurious breed with DNA fingerprints. The modified CTAB method was used to extract the genomic DNA of several plants, including seven leaves Jiaogulan, five leaves Jiaogulan, *Cayratia japonica*. With the DNA extracted from seven leaves Jiaogulan as template, the random primer WGS001 for PCR has been done meanwhile RAPD reaction system has been optimized so that it could be determined for the suitable annealing temperature and concentrations of template, Mg^{2+}, primer, *Taq* DNA polymerase, BSA. In the optimized system of 25 μL, $MgCl_2$ 2 mmol/L, primer 0.4 μmol/L, template 60 ng, *Taq* DNA polymerase 1 U, BSA 2 μg/μL and annealing temperature 58℃ were used. Based on the optimized system, ten primers of 20 bp were applied to do random amplification with total DNAs of the above mentioned three plants, respectively. The polymorphic fragments of DNA fingerprints of the above mentioned three plants were obtained with two screened random primers (WGS001, WGS004). These polymorphic fragments can be used to distinguish *G. pentaphyllum* with *C. japonica* obviously.

Key words: *Gynostemma pentaphyllum*; Genomic DNA; RAPD; Authentication

绞股蓝[*Gynostemma pentaphyllum* (Thunb.) Makino]为葫芦科绞股蓝属植物。自 20 世纪 70 年代以来, 从该种植物中分离出了 84 种绞股蓝皂苷, 其中 6 种与人参皂苷的结构完全相同, 具有降血脂、抗衰老、抗肿瘤等多种生理功效, 由此引发了对绞股蓝植物的科研和开发热潮[1]。药材市场上常见的伪品有乌蔹莓[*Cayratia japonica*

(Thunb.) Gagnep]。由于绞股蓝为草类药材, 其特点是质地松脆, 在采收加工、干燥、运输及储藏的过程中易干瘪皱缩、破裂, 给鉴别带来困难[2]。本实验应用 RAPD(随机扩增多态性 DNA, random amplified polymorphic DNA)技术对绞股蓝及其伪品乌蔹莓进行了分子水平的鉴定, 为绞股蓝的准确鉴定提供了理论依据。