

林学—研究报告

XL-90等美洲黑杨杂交子代ISSR分子鉴别

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摘要:

为了探讨ISSR分子标记在美洲黑杨杂交子代分子鉴别和分子标记辅助育种中的应用, 笔者以15个黑杨无性系为研究对象, 进行ISSR分子标记研究。①经单因素对比实验, 建立适合黑杨无性系的ISSR-PCR分子反应体系, 即在25 μL反应体系中加入引物1.0 μmol/L, 模板30 ng, Taq酶1.5 U, dNTP 0.25 mmol/L, Mg<sup>2+</sup>2.0 mmol/L。反应程序为94℃加热3 min, 使模板DNA变性, 然后进入下列温度循环: 94℃变性45 s、56℃退火30 s、72℃延伸1 min, 共计35个循环。循环结束后在72℃延伸5 min, 以保证DNA延伸彻底。②筛选出10个ISSR引物对15个黑杨无性系进行ISSR分析。共检测到63个位点, 各无性系的多态位点百分率在20.63%~30.16%之间。多态位点百分率最高的为XL-77、XL-83、XL-101、2KEN8和I-69; 多态位点百分率最低的为XL-92、XL-90。③通过各无性系的Nei遗传距离与UPGMA聚类分析, 除欧美杨A65/31外, 其他所有美洲黑杨无性系聚为1个类群3个亚群, 美洲黑杨杂交子代间遗传分化及亲缘关系通过ISSR-PCR特异性标记谱带可以得到准确鉴别。

关键词: 美洲黑杨; ISSR-PCR; 多态位点; 遗传距离

Identification of Hybrid Progenies as Populus deltoides cl. 'Xianglin 90' etc. Based on ISSR Molecular Markers

Abstract:

In order to explore the application of ISSR markers for molecular identification and marker-assisted breeding of Populus deltoides, 15 populus deltoides clones were analyzed by ISSR. ①The reaction volume of ISSR-PCR which was fit for populus deltoids was set up by single analysis of variance test. The reaction volume was 25 μL and adds with primer for 1.0 μmol/L, template for 30 ng, Taq enzyme for 1.5 U, dNTP for 0.25 mmol/L, Mg<sup>2+</sup> for 2.0 mmol/L. ISSR program was: 3 min at 94℃ for calefaction to DNA denaturalized, then 35 cycles of 45 s at 94℃ for denaturalization, 30 s at 56℃ for annealing, 1 min at 72℃ for extension and extension at 72℃ for 5 min finally. ②15 populus deltoides clones were analyzed by 10 primers of ISSR. The number of the fragments examined were 63, the percentage of polymorphism fragments of each clone was between 20.63% and 30.16%, the highest of which were XL-77, XL-83, XL-101, 2KEN8 and I-69, the lowest of which were XL-92, XL-90. ③The clones except A65/31 belonged to the same group which included 3 subgroups, and the genetic differentiation and genetic relationship among the hybrid progenies of populus deltoides can be identified accurately by the specific makers bands of ISSR-PCR.

Keywords: Populus deltoides ISSR-PCR polymorphism fragments genetic distance

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