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植物生产层

红豆草ISSR体系优化及其在航天诱变种质鉴定中的应用 沈紫微, 陈本建, 康俊梅, 魏小兰, 张蕴薇

摘要:

采用正交设计法优化适合于红豆草(Onobrychis viciaefolia)的ISSR体系,对影响ISSR PCR的 多个因素,包括dNTP、引物浓度、Taq酶浓度、Mg2+浓度进行了4因素3水平的比较、优化,建立 了红豆草的ISSR最佳反应体系。运用该体系进行引物及最佳退火温度的筛选,并应用ISSR分子标记 技术初步分析红豆草航天搭载诱变效应。结果表明,红豆草ISSR PCR最佳反应体系为: 2.5 μL 10 ▶加入我的书架 ×PCR buffer、50 ng模板DNA、dNTP 0.2 mmol/L、Taq DNA 聚合酶1.5 U、引物0.4 μmol/L、Mg2+2.0 mmol/L,总体积为25 μL。试验从53个ISSR引物中筛选出扩增条带清晰、多 态性丰富的12个引物,并依次筛选出各引物的退火温度。12个引物标记结果显示共获得多态性位点 142个,多态性比率为73.6%。地面对照的多态位点比率(P)、Nei基因多样度(h)和Shannon多样 性指数(1)均低于航天诱变种,太空环境能够诱导红豆草发生基因变异。航天诱变可作为红豆草种质资 源创新和品种选育的方法之一。

关键词: 红豆草; ISSR标记; 体系优化; 引物筛选; 航天诱变

PCR system and its application in the identification Optimization of ISSR of sainfoin germplasm of space irradiation

SHEN Zi wei, CHEN Ben jian, KANG Jun mei, WEI Xiao lan, ZHANG Yun wei

Abstract:

Based on the orthogonal design, the optimized ISSR PCR system of sainfoin (Onobrychis viciaefolia) germplasm was established by comparing the 4 factors (dNTP, Taq polymerase, primer and Mg2+) and the 3 levels. This established system was used to select the primer combination and the optimum Tm and the ISSR Marker System was applied to identify the variation of hereditary substance of sainfoin offspring from space irradiation seeds. The results of this study showed that the optimized ISSR PCR system for sainfoin was 2.5 _µL 10×PCR buffer, 50 ng template DNA, dNTP 0.2 mmol/L, Taq DNA polymerase 1.5 U, primer 0.4 umol/L, Mg2+ 2.0 mmol/L in a total of 25 _UL reaction solution. 12 primer combinations were selected with abundant polymorphism from 53 primers. 201 DNA bands were amplified by 12 ISSR primers, in which 142 bands were polymorphic, and polymorphic ratio (PPB) was 73.6%. This study also indicated that the percentage of polymorphic loci(P), Nei gene diversity(h) and Shannon index(I) in Sainfoin seeds of the control was lower than that of the seeds carried by spaceflight, implying that space environment caused the variation of hereditary substance DNA of Sainfoin. This study suggested that the space radiation would be an effective approach to developing new sainfoin germplasm.

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红豆草; ISSR标记; 体系 优化; 引物筛选; 航天诱变

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Keywords: Onobrychis viciaefolia; ISSR markers; optimization of system; selection of primers; space radiation

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