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植物生产层

垂穗披碱草ISSR反应体系的正交优化

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摘要：

以垂穗披碱草（Elymus nutans）基因组DNA为模板，对ISSR PCR反应体系中5个因素（TaqDNA聚合酶、Mg2+、模板DNA、dNTPs、引物）进行优化试验，建立垂穗披碱草稳定的ISSR PCR反应体系及最佳扩增程序。在25 μL反应体系中，最佳反应体系为2.5 μL 10×buffer （不含Mg2+）、1.0 U Taq酶、2.0 mmol/L Mg2+、30～120 ng模板DNA、0.25 mmol/L dNTPs、0.25 μmol/L 引物。在优化体系的基础上筛选出11条多态性丰富，扩增稳定的引物，并通过设置退火温度梯度、循环次数梯度、延伸时间梯度，得到垂穗披碱草ISSR PCR最佳扩增程序。反应程序：94℃ 预变性2 min，94℃变性1 min，51℃退火1 min（视不同引物而定），72℃延伸1.5 min，共41个循环，72℃延伸10 min。通过对ISSR PCR最佳反应体系和最佳扩增程序的验证，发现该反应体系和扩增程序具有较高的稳定性和较好的重复性。

关键词： 垂穗披碱草；ISSR PCR；正交优化

Optimization of the ISSR reaction system of Elymus nutans by orthogonal design

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Abstract:

Five factors（DNA polymerase, Mg2+, template DNA, dNTPs, primer) in ISSR PCR reaction system were carried on the optimized experiment by taking the genomic DNA of Elymus nutans as template. The optimum reaction system and reaction process of E. nutans ISSR PCR analysis were established. The results of this study showed that the volume of reaction system was 25 μL. It included 2.5 μL 10×buffer (except Mg2+), 1.0 U TapDNA polymerase, 2.0 mmol/L Mg2+, 30—120 ng template DNA, 0.25 mmol/L dNTP and 0.25 μmol/L primer. Eleven primers with stable amplification and rich polymorphism were obatined based on the optimal PCR reaction system.The reaction program was devised for 2 min at 94℃ in the first circle, 1 min at 94℃, 51℃ for 1 min (depended on different primer), 72℃ for 1.5 min for 41 cycles, and 72℃ for 10 min for extending, which was obatined across the gradient PCR experiments, including temperature gradient,cycle gradient and extending time gradient experiments. Validation of the optimistic ISSR PCR reaction system and amplification program showed that the reaction system and amplification program had high stability and good reproducibility.

Keywords: Elymus nutans ISSR PCR orthogonal design

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