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Identification of centipedegrass hybrids and selection of polymorphic primer combinations by Sequence-related amplified polymorphism

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Key words: centipedegrass, SRAP, optimized SRAP-PCR system, polymorphic primer selection, hybrid identification

Introduction Centipedegrass is a warm-season perennial turfgrass which is native to China and South-east Asia. It is popular as a turfgrass due to its adaptation to low fertility conditions and its low maintenance requirements. Centipedegrass is used primarily for lawns, parks, golf course roughs, roadside and soil conservation. With the development of molecular biology, molecular markers have been used for genetic diversity analysis, genetic linkage map construction, QTL mapping, cultivar identification and so on. However, few molecular markers had been used in centipedegrass compared to other turfgrass. Sequence-related amplified polymorphism (SRAP) is a novel molecular marker technique designed to amplify open reading frames (ORFs). The SRAP-PCR system was set up and applied to the polymorphic primer combinations selection and centipedegrass hybrids identification.

Materials and methods The F₁ hybrids of centipedegrass used in this investigation were derived from two crosses between four heterozygous highly polymorphic genotypes, ♀ E102 (brown stolon) and ♂ E092(1) (green stolon), ♀ E142 (few seed head) and ♂ E022 (dense seed head). The orthogonal design was used to optimize SRAP-PCR system with four factors (Mg²⁺, dNTP, primer and Taq polymerase) at three levels respectively. In addition, the concentration of template DNA was also selected. Four parents, i.e. E102, E092(1), E142 and E022, were screened by SRAP technique with 400 primer combinations. 183 hybrids were identified in this study using SRAP primer pair which had paternal characteristic bands, 96 from E102×E092(1) and 87 from E142×E022.

Results and discussion The optimized SRAP-PCR system for centipedegrass was: 2 μl 10× PCR buffer, 60 ng template DNA, Mg²⁺ 1.50 mmol·L⁻¹, dNTP 260 μmol·L⁻¹, primer 0.25 μmol·L⁻¹, Taq DNA polymerase 0.5 U with total 20 μl reaction solution. Each factor had different effect on the result of PCR. The concentration of Mg²⁺ had the greatest effect and Taq DNA polymerase had the least effect on the result.

A total of 236 polymorphism primer combinations were identified between parents E102 and E092(1) and 269 between parents E142 and E022. A total of 443 polymorphic fragments were detected from E102 and E092(1) and 592 from E142 and E022. The average polymorphism detected per primer combination were 1.88 for E102 and E092(1), and 2.20 for E142 and E022. Some primer combinations which had paternal characteristic bands were selected to test the hybrid status of F₁ populations.

In E102×E092(1), there were 89 real hybrids tested by 6 SRAP primer pairs, i.e. Me3+Em16, Me17+Em15, Me3+Em15, Me20+Em9, Me12+Em17, Me18+Em18. In E142×E022, there were 83 real hybrids tested by 5 SRAP primer pairs, i.e. Me1+Em12, Me8+Em15, Me19+Me10, Me+Em2, Me18+Em1.

Conclusions The optimized SRAP-PCR system of centipedegrass was established by orthogonal design. Many polymorphic primer combinations of SRAP were also screened. These polymorphic primer combinations and real F₁ hybrids tested in this study could be applied to construct the molecular genetic linkage map and identify QTL which could be used in marker-assisted selection to improve economically important traits of centipedegrass.

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