Identification of Centipedegrass Hybrids and Selection of Polymorphic Primer Combinations by Sequence-Related Amplified Polymorphism

Yiqi Zheng
Chinese Academy of Sciences, China

Jianxiu Liu
Chinese Academy of Sciences, China

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

Zheng Yiqi , Liu Jianxiu
Institute of Botany , Jiangsu Province and Chinese Academy of Sciences , Nanjing , 210014 ; E-mail : botanyzyq@ yahoo . com .cn

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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 F_1 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 (Mg^{2+} , 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 , Mg^{2+} 1 . 50mmol · L^{-1} , dNTP260 μmol · L^{-1} , primer 0 .25 μmol · L^{-1} , Taq DNA polymorphism 0 .5 U with total 20μl reaction solution . Each factors had different effect on the result of PCR . The concentration of Mg^{2+} had the greatest effect and Taq DNA polymorphism 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 F_1 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 F_1 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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