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Short Communication

Identification of sugarcane interspecies hybrids with RAPDs

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Identification of "*Saccharum officinarum* × *Erianthus fulvus*" F_1 hybrids was performed with random amplified polymorphic DNA (RAPD) analysis. Of 280 RAPD primers used, two primers, OPA-19 and OPN-11, were found to be the most suitable for identification of the hybrids. And the hybrids facticity-check-out rate was 70.6 and 68.3%, respectively.

Key words: Sugarcane hybrids, identification, RAPD.

INTRODUCTION

There might be existence of female parent self-pollination in the sugarcane cross breeding program. Therefore, identification of real interspecies crossbreed or elimination of self-cross offsprings is an important measure of improving efficiency of sugarcane breeding. There are a lot of studies on the facticity of sugarcane crossbreed. Random amplified polymorphic DNA (RAPD) is the simplest and fastest of DNA-based techniques in genetic similarity studies (Zhang et al., 2005). A number of scientists have used RAPD markers in identification of hybrids or cultivars in various plants (Yang et al., 2001; Ranade et al., 2002; Zhang et al., 2005).

Erianthus fulvus is one of wild sugarcane species, originated from tropic, subtropical and temperate zone, mainly distributed in south China, north India, Nepal and Pakistan. *Erianthus saccharinae* is an elite parent for sugarcane breeding, with very good agricultural character such as drought resistance, cold resistance, barren resistance and high Brix. In this paper, results of identification of sugarcane × *E. fulvus* F_1 with RAPDs were presented.

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MATERIALS AND METHODS

Plant materials included Hybrid parents Yacheng 89/9 (*Saccharum officinarum*), *E. fulvus*, and 126 hybrid F1 plants, planted in the sugarcane germplasm garden of Yunnan Agricultural University (YAU).

DNA was extracted from leaf by the CTAB method (De Riek et al., 2001). Amplification was performed in volumes of 0.02 cm^3 containing 0.002 cm^3 of the 10 x buffer, and 100 mM each of dNTPs, 0.4 mM primer, 25 ng genomic DNA, and 1 unit of polymerase (Sangon, Shanghai, China). The reaction mixture was overlaid with 0.02 cm^3 mineral oil. Amplifications were carried out using a 9600 Perkin-Elmer (Perkin Elmer, USA) thermal cycler programmed for 40 cycles as follows: 15 s at 94°C, 30 s at 36°C, 60 s at 72°C, with an initial melting of 120 s min at 94°C, and a final extension of 600 s at 72°C. Amplification products was analyzed by electrophoresis in a 1.2% agarose gel containing 0.5 µg/mL ethidium bromide, with 1× TBE buffer (90 mmol/L Tris-HBO_3, 2 mmol/L EDTA, pH 8.0), and was visualized and photographed on an Image Master VDS system (Phamacia Biotech, Sweden).

RESULTS AND DISCUSSION

Using genomic DNA of Yacheng89/9, YAU99/3 and YAU99/4 as template, 57 (20.4%) produced amplification products that were too faint to score or could not be consistently reproduced, and 132 (47.1%) produced monomorphic banding patterns. Thus 91 (32.5%) primers

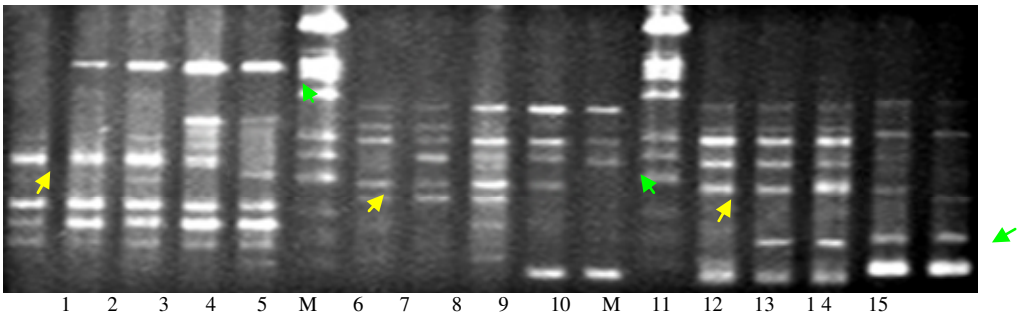

Figure 1. RAPD products obtained from sugarcane hybrids and its parents with three primers. Lane M, DNA marker□λDNA/*EcoR* □+ *Hind* □□; Line 1-5, accessions amplified by OPC-19 (Lane 1, Yacheng89/9; Lane 5, *Erianthus fulvus*; Lane 2-4, different hybrid F_1 accessions; yellow arrows in line 1 indicated maternal-specific band OPC-19_{1300}; green arrows in line 5 indicate paternal-specific band OPC-19_{3500}); Line 6-10, accessions amplified by OPE-02 (Lane 6, Yacheng89/9; Lane 10, *Erianthus fulvus*; Lane 7-9, different hybrid F_1 accessions; yellow arrows in line6 indicated maternal-specific band OPE-02_{900}; green arrows in line10 indicate paternal-specific band OPE-02_{1350}); Line 11-15, accessions amplified by OPF-04 (Lane 11, Yacheng89/9; Lane 15, *Erianthus fulvus*; Lane 12-14, different hybrid F_1 accessions; yellow arrows in line 11 indicated maternal-specific band OPF-04_{900}; green arrows in line 15 indicate paternal-specific band OPF-04_{550}).

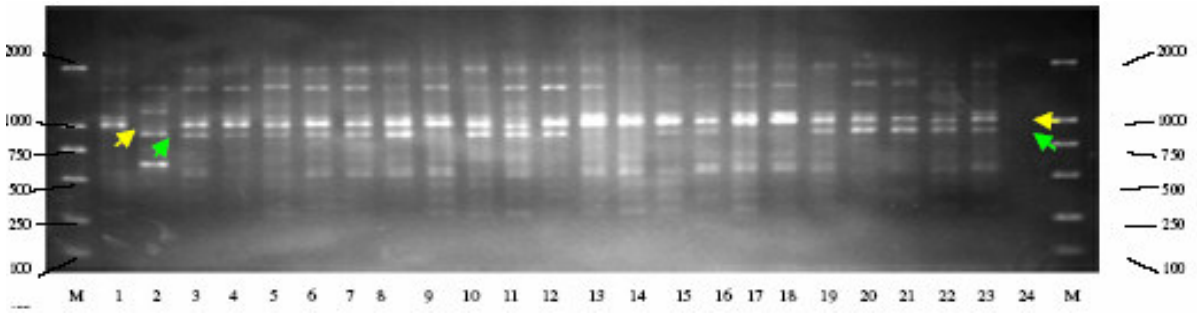

Figure 2. Results of hybrids identifications with RAPD molecular marker in "Yacheng 89/9×*Erianthus fulvus*" F_1 (Primer OPN-11). Lane M, DNA marker; Lane 1, Yacheng89/9; Lane 2, *E. fulvus*; Lane 3-23, different hybrid F_1 accessions; Lane 24, control; yellow arrows in the figure indicated paternal-specific band; green arrows in the figure indicate paternal-specific band.

could produce RAPD polymorphism bands out of 280 random primers screened. Out of 91 screened primers, five primers could produce clearly distinguishable and reproducible bands, which could be used in facticity-identification of the hybrid materials (OPA-19, OPC-19, OPE-02, OPF-04 and OPN-11). Three primers could produce parents characteristic bands respectively among hybrids, for instance, OPC-19_{3500} and OPC-19_{1300} amplified by OPC-19, OPE-2_{900} and OPE-02_{1350} amplified by OPE-02, and OPF-04_{550} and OPF-04_{900} amplified by OPF-04 showed in Figure 1). But further experiments showed that OPC-19 (5□-GTT GCC AGC C-3□), OPE-02 (5□-GGT GCG GGA A-3□), and OPF-04 (5□-GGT GAT CAG G-3□) only could be amplified in a small number of supplied hybrids. With the number of hybrids increasing, the hybrids facticity-check-out rate was decreasing greatly (32.7 - 9.8%), and without any amplification among most of other hybrids. However, the rate of OPA-19 (5'- CAA ACG TCG G -3') and OPN-11 (5'-TCG CCG CAA A-3') was 70.6 and 68.3%, respectively, and among very few hybrids without any amplification. Por-tions of gels showing typical amplification products were shown in Figures 2 and 3. It demonstrated that OPA-19 and OPN-11 were most suitable for identification of the hybrids of *S. officinarum* × *E. fulvus* among the screened primers.

At present, there are some reports about using isoenzyme to distinguish the sugarcane hybrids (Jing et al. 2001), while reports on the using of molecular marker to distinguish is not found. In this study, RAPD was employed to detect the intergeneric hybrids (*S. officenarum* × *E. fulvus*). The result showed that RAPD assay could be used to discriminate those sugar cane hybrids. It seems that RAPD is an effective tool for sugarcane hybrids identification and cultivar improvement, and sui-

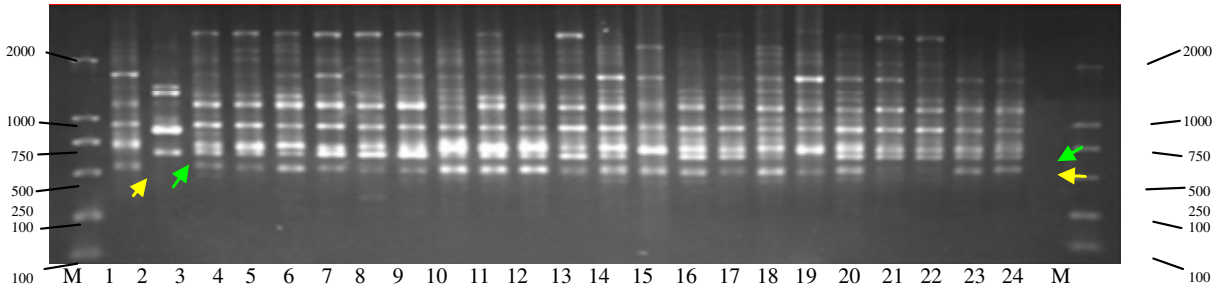

Figure 3. Results of hybrids identifications with RAPD molecular marker in "Yacheng 89/9×*Erianthus fulvus*" F_1 (Primer OPN-11). Lane M, DNA marker; Lane 1, Yacheng89/9; Lane 2, *Erianthus fulvus*; Lane 3-23, different hybrid F_1 accessions; Lane 24, control; yellow arrows in the figure indicated maternal-specific band; green arrows in the figure indicate paternal-specific band.

table to be used in the early stage of seed-seedling.

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