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Full Length Research Paper

Identification of intergeneric hybrids between *Saccharum spp.* and *Erianthus fulvus* with ITSs

H. Y. Zhang[1,2], L. L. He[1], H. Q. Zhong[1], F. S. Li[1]*, S. C. He[1]and Q. H. Yang[1]

[1]College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming, Yunnan Province, 650201, People's Republic of China.
[2]Biotechnology Laboratory, Southwest Forestry College, White Dragon Temple, Kunming, Yunnan Province, 650224, People's Republic of China.

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Identification of "*Saccharum spp.* × *Erianthus fulvus*" F_1 hybrids was performed with ITS analysis. Out of 6 pairs of ITS primers, ZM-02 and ZM-04 were found to be suitable to detect the hybrids. The rate of true hybrids tested by ZM-02 and ZM-04 was 74.6 and 73.8% respectively and the amplification products of ZM-04 were similar to the ones of ZM-02.

Key words: *Saccharum spp, Erianthus fulvus*, intergeneric hybrids, identification, ITSs.

INTRODUCTION

Sugarcane (*Saccharum* spp.), a kind of tropic and semitropical plant is one of the most important sugar crops. Cultivars are the keys of development of sugarcane. So far, sexualcross and selection are the most important methods for breeding new cultivars. Before 1950, *Saccharum officinarum*, *Saccharum sinense*, *Saccharum barberi*, *Saccharum spontaneum* and *Saccharum robusrum* were used for interspecies cross and breeding of sugarcane and a lot of cultivars had been bred, such as POJ2878, Nco310 and H49/5. While after that, outstanding achievements of breeding new cultivars had not been achieved yet. And it has been attributed to consanguinity relatively close and lack of germplasm resource. Therefore, in the recent 20 years, collection, conservation and utilization of germplasm resource is the primacy of breeding work, expecting a refulgence period of sugarcane breeding, with discovering and using elite germplasm resource *Erianthus fulvus* is one of wild species, belonging to *Erianthus*, *Saccharinae*, originating from tropic, subtropical and temperate zones; mainly distributed in south China, north India, Nepal and Pakistan (Zhang et al., 2008a). It is an elite parent for sugarcane breeding, with germplasm resource is the primacy of breeding work, expecting a refulgence period of sugarcane breeding, with discovering and using elite germplasm resource. some good agricultural characters such as drought resistance, cold resistance, barren resistance and high brix (Zhang et al., 2008b). In this paper, the identification of sugarcane intergeneric hybrids (*Saccharum spp.* × *Erianthus fulvus*) were performed with ITS analysis.

MATERIALS AND METHODS

Plant materials included hybrid parents Yacheng 89/9 (*Saccharum spp.*), *E. fulvus*, and 126 hybrid F_1 plants, planted in the sugarcane germplasm garden of Yunnan Agricultural University (YAU).

Sugarcane genomic DNA was extracted from young leaves by the CTAB method as stated by Zhang et al. (2006). Samples were ground to powder in liquid nitrogen, using a mortar and pestle. The powder was transferred to a 25 ml sterile tube with 10 ml of CTAB buffer. The extraction buffer consisted of 2% (w/v) CTAB (cetyltrimethyl ammonium bromide, Sigma), 1.5 M NaCl, 20 mM EDTA, 100 mM Tris-HCl pH 9.5, and 0.2% (v/v) β-mercaptoethanol. After incubating the homogenate at 65℃ for 1 h, an equal volume of chloroform was added and centrifuged at 10,000 rpm for 20 min. DNA was precipitated with 1/10 volume (ml) of 3 M sodium acetate and an equal volume of isopropanol followed by centrifugation at 10,000 rpm for 10 min. The DNA pellet was

*Corresponding author. E-mail: lfs810@sina.com.

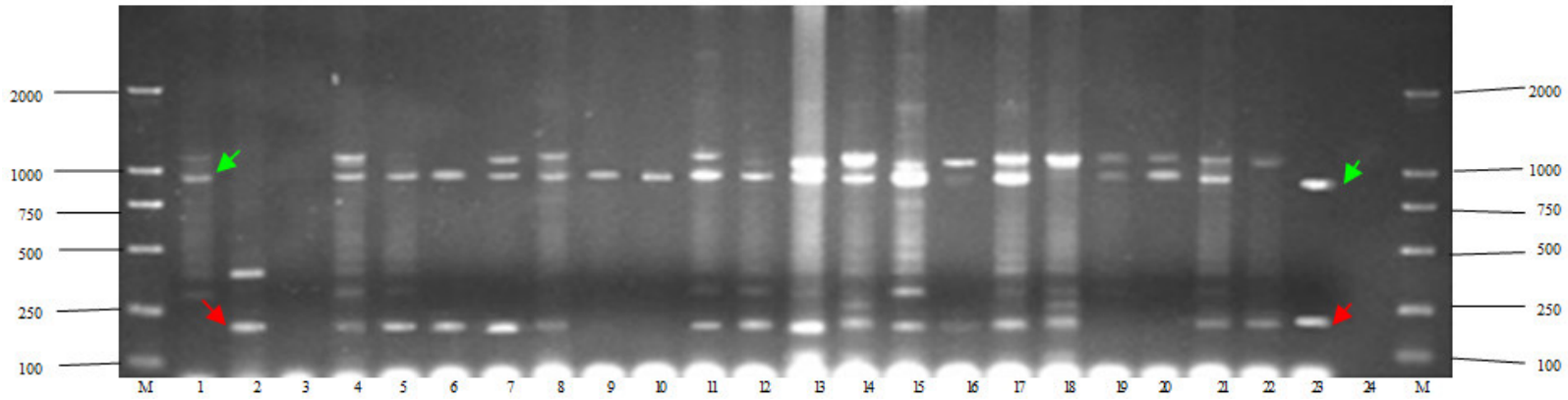

Figure 1. Results of hybrids identifications with ITS molecular markers in "Yacheng89/9 × *E. fulvus*" F_1 (Specific primer ZM-02). Note: Lane M, DNA marker; Lane 1, Yacheng89/9; Lane 2, *E. fulvus*; Lane 3-23, different hybrid F_1 accessions; Lane 24, control; green arrows in the figure indicated maternal-specific band; red arrows in the figure indicate paternal-specific band.

washed with 70% ethanol, air-dried, and resuspended in TE-buffers (10 mM Tris pH 8.0 and 0.1 mM EDTA). DNA quantity was estimated spectrophotometrically by measuring absorbance at 260 nm. DNA samples were diluted in sterile deionized water andmaintained at -20℃.Amplification was performed in volumes of 0.02 cm^3 containing 0.002 cm^3 of the 10x buffer, and 100 mM each of dNTPs, 0.4 mM primer, 25 ng genomic DNA and 1 unit of polymerase (Sangon, 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. Amplifications were programmed for 40 cycles as follows: 50 s at 93℃, 20 s at 52℃, 40 s at 72℃, with an initial melting of 300 s/min at 95℃, and a final extension of 600 s at 7. Amplification products were 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, pH8.0), and were visualized, photographed and analyzed on an Image Master VDS system (Phamacia Biotech, Sweden).

RESULTS AND DISCUSSION

Based on the ITS sequence of *E. fulvus* (Chen et al., 2003), 6 pairs of primers were synthesized. All 6 pairs of primers used in this study, ZM-05 and ZM-06 produced amplification products that were too faint to score and ZM-01, ZM-02, ZM-03 and ZM-04 could produce paternal-specific band about 200 bp and maternal-specific band about 950 bp. Among the other supplied materials such as *S. sinensis*, *S. officinarum*, *S. spontaneum*, *Saccharum narenga*,*Erianthus arundinaceus* and *Miscanthus floridulus*, only ZM-02 and ZM-04 could not produce the specific bands. Hence, only ZM-02 and ZM-04 were used to verify true hybrids. The amplification products of ZM-02 showed that there were 7 hybrid progenies without any amplification, 15 hybrid progenies just with paternal-specific band $ZM\text{-}02_{200}$, 19 hybrid progenies just with maternal-specific band $ZM\text{-}02_{950}$, and the other 79 hybrid progenies with both paternal-specific and maternal-specific bands. Portion of gels showing typical amplification products are shown in Figure 1. The true hybrids rate verified by ZM-02 was 74.6% (Table 1). The amplification products of ZM-04 were similar to the ones of ZM-02, and the one checked out by ZM-04 was 73.8% (Table 1). Portion of gels showing typical amplification products are shown in Figure 2. Results showed that some hybrids were just with paternal-specific band (without maternal-specific band). However, these accessions were still inferred to be the true hybrids. The reason was that there might be a certain maternal-specific marker just on one homologous chromosome, while the other one without this marker, among the highly heterozygous genome of *Saccharum* spp., and that the recombination of genes and chromosome happened in the period of meiosis might cause the marker missing in some of the female gametophytes. Identification of hybrids was very important for breeding programes. Up to date, there were some reports about using isoenzyme (Jin et al., 2001), or RAPD (random amplified polymorphic DNA) markers

Table 1. ITSs among the "Yacheng89/9 × *E. fulvus*" F_1 progenies.

Primer	Sequence (5'→ 3')	Without any amplification	Only with paternal-specific band	Only with maternal-specific band	With both paternal-specific and maternal-specific bands	True hybrids rate
ZM-02	TCGTAACAAGGTTTCCGTAG CTTAGGCGTCAAGGAACACT	7	15	25	79	74.6%
ZM-04	GCAAAATGCGATACCTGGTG CTTAGGCGTCAAGGAACACT	7	15	26	78	73.8%

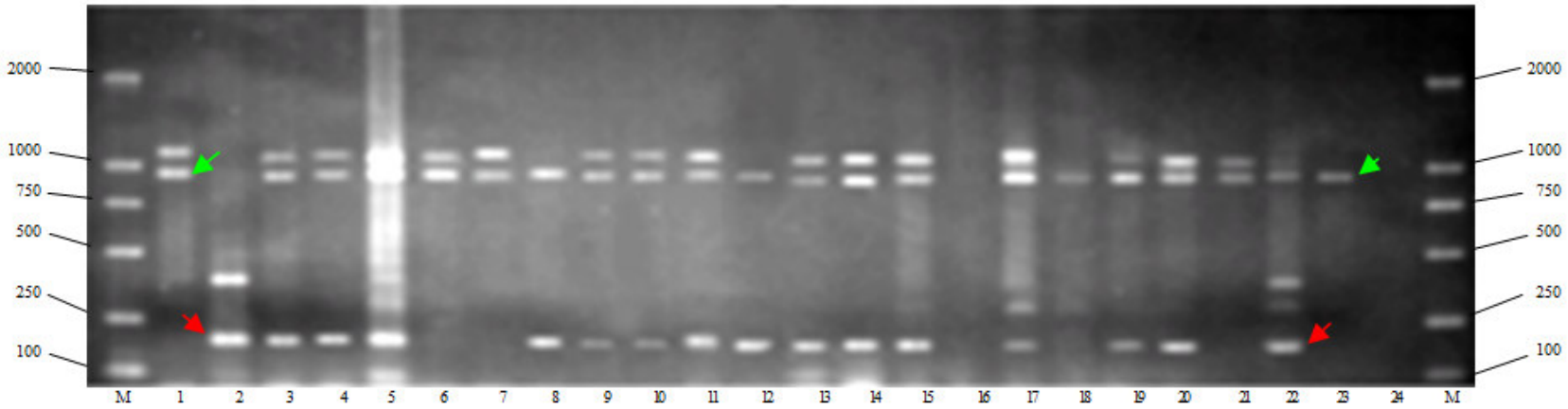

Figure 2. Results of hybrids identifications with ITS molecular markers in "Yacheng89/9 × *E. fulvus*" F_1 (Specific primer ZM-04). Note: Lane M, DNA marker; Lane 1, Yacheng89/9; Lane 2, *E. fulvus*; Lane 3-23, different hybrid F_1 accessions; Lane 24, control; green arrows in the figure indicated maternal-specific band; red arrows in the figure indicate paternal-specific band.

to distinguish the sugarcane hybrids (Zhang et al., 2008b). The RAPD technology is one of the simplest and fastest of DNA-based techniques in genetic similarity studies (Zhang et al., 2005), and is suitable for the identification of hybrids (Zhang et al., 2008b). A number of scientists have used RAPD markers in identification of hybrids in various plants (Sheng et al., 2000; Yang et al., 2001; Rajora and Rahman, 2003; Bang, et al., 2007; Saito et al., 2007). However, there are also some problems in using RAPD markers, such as poor reproducibility, faint or fuzzy products and difficulty in scoring bands (Liu and Zhang 2008). ITS (internal transcribed spacer) analysis requires small amounts of DNA and it is easy to generate, simple, fast, does not require the use of isotopes, and without any problem as RAPDs mentioned above, its genetic techniques is suitable for the identification of species (Kollipara et al., 1997, Powers et al., 1997, Parani et al., 2000). In this study, ITSs were employed to detect intergeneric hybrids (*Saccharum spp.* × *E. fulvus*).

The manipulations were proved to be easy and shorter and the results were reliable and reproducable. The methods also proved to be suitable to detect the facticity of hybrids in the early stage of seed-seedling. It is worth noting that the ITSs

were first employed to identify intergeneric hybrids.

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