Genetic Resources and Crop Evolution **51:** 351–354, 2004.

Short communication

Molecular assay of novel pasturage, sugarcane-grass 94-42, using RAPD markers

Chen Liang*, Lin Nan, Huang Zhen, Weng Pin, Lu Chuanbei[1] and Shen Mingshan
*Laboratory of Cell Biology of Education Ministry in Xiamen University, Life Sciences School of Xiamen University, Xiamen 361005, China; [1]Sugarcane Institute of Fujian Province, Zhangzhou 363005, China; *Author for correspondence (e-mail: chenl304@263.net; phone: 86-592-2186050; fax: 86-592-2181015)*

Received 20 January 2003; accepted in revised form 31 October 2003

Key words: RAPD, *Saccharum robustum*, Similarity, Sugarcane-grass 94-42

Abstract

Sugarcane-grass 94-42, a novel pasturage, was derived from the cross of a sugarcane cultivar line Co419 and a wild line PT43-52 (*Saccharum robustum* Brand. et Jesw. ex Grassl). To assay the genetic diversity among Co419, PT43-52 and the F_1 hybrid sugarcane-grass 94-42, 67 RAPD markers were used to detect the genomes of three lines. Around 284 bands were produced by amplification of which 116 were shown to be polymorphic in three lines. Homology of the three lines was analyzed based on all bands. Results showed that the similarity coefficient between Co419 and PT43-52, PT43-52 and sugarcane-grass 94-42, and Co419 and sugarcane-grass 94-42 were 0.7658, 0.8009 and 0.9138, respectively. A total of 95.4% bands amplified from sugarcane-grass 94-42 were homologous with those from either Co419 or PT43-52, which proved that sugarcane-grass 94-42 was the filial generation of Co419 and PT43-52.

Introduction

In previous studies, a hybrid descendent, designated as sugarcane-grass 94-42, was obtained from a sugarcane cross between cultivar line Co419 and wild line PT43-52 (*Saccharum robustum* Brand. et Jesw. ex Grassl) (Hong et al. 1997; Lu et al. 1998). The hybrid sugarcane-grass 94-42 might be suitable to be planted as pasturage because it inherits the excellent characters of sugarcane and looks like grass.

In this paper, we reported the DNA level assay of sugarcane-grass 94-42 and its parents by using RAPD markers. The results could provide strong evidence for the genetic origin of sugarcane-grass 94-42 and be applied to its further breeding.

Material and methods

Sugarcane plants, including cultivar line Co419, wild line PT43-52 (*S. robustum* Brand. et Jesw. ex Grassl), and their F_1 hybrid sugarcane-grass 94-42 were all cultivated at the Sugarcane Institute of Academy of Agricultural Sciences of Fujian Province, China.

A total of 80 random primers (10 mer) were synthesized by Operon Tech. Inc., Shanghai Sanggong Biol. Tech. and Serv. Co. Ltd., respectively. Young leaves of 10 plants of each line were mixed to extract the total DNA by the CTAB method (Murray and Thompson 1980).

PCR amplification was carried out after (Williams et al. 1990). The amplified DNA fragments were separated by gel electrophoresis in a

Table 1. The catalogs of the amplified polymorphic bands among three lines.

	94-42	Co419	PT43-52
(1) Identical between F_1 and female parent	43	43	
Identical between F_1 and male parent	18		18
(2) Occurred in both parent but missed in F_1		3	3
(3) Occurred in F_1 but missed in both parent	11		
(4) Unique in female parent		9	
Unique in male parent			32
Total bands	72	55	53

1.4% agarose gel. The DNA fragment bands in the gel were visualized and photographed using Bio-RAD Fluor-S MultiImager.

The similarity coefficients of three lines were calculated according to the formula (Nei and Li 1979): $S = 2N_{ab}/(N_a + N_b)$, where N_a, total number of bands of a; N_b, total number of bands of b; N_{ab}, number of the identical bands of a and b.

Result and analysis

Among 80 RAPD primers, 67 of them showed repeatable bands with lengths from 200 to 2200 bp. Band numbers amplified by each RAPD primer varied from 1 to 12. A total of 284 bands were amplified by the 67 primers, among which 116 bands showed polymorphic within the three lines. The polymorphic rate was 40.8%. According to the bands' position in the gel, the amplified polymorphic DNA fragments could be sorted into four categories (Table 1): (1) Identical in F_1 and either parent (Figure 1a, b); (2) occurring in F_1 but missing in both parents (Figure 1d); (3) occurring in both parents but missing in F_1 (Figure 1c); (4) unique band in male parent or female parent. In sugarcane-grass 94-42, 240 bands were amplified by the 67 RAPD primers, among which 229 bands (about 95.4%) were identical to those of Co419 or PT43-52 (Table 1). Similarity coefficients of the three lines were 0.7658 between PT43-52 and Co419, 0.8009 between sugarcane-grass 94-42 and PT43-52, and 0.9138 between sugarcane-grass 94-42 and Co419, respectively.

Table 2. Similarity coefficient for three lines based on RAPD analysis.

Lines	Sugarcane-grass 94-42	Co419	PT43-52
Sugarcane-grass 94-42	1	–	–
Co419	0.9138	1	–
PT43-52	0.8009	0.7658	1

Discussion

Interspecific hybrids of sugarcane cultivar line Co419 and wild line PT43-52 were hardly obtained due to difficulties in procedures such as extirpation of androecia, florescence time control, interspecific hybrid sterility and so on. In the previous study, we reported the success breeding of F_1 hybrid (sugarcane-grass 94-42) by treating the two parents with the same length of daylight, and inducing blossom by blue and far-red light (Hong et al. 1997; Lu et al. 1998).

Amplified by 67 RAPD primers, 284 repeatable DNA fragment bands detected polymorphism among the two parents and F_1 plant. These polymorphic bands have revealed much of the genomic information of the three checked lines. The similarity coefficient of the two parents, Co419 (cultivar) and PT43-52 (*S. robustum*), was 0.7658, which was consistent with the results of genetic diversity survey between 20 sugarcane cultivar lines and six wild lines using RAPD markers (Harvery and Botha 1996). Meanwhile, almost all the bands amplified from sugarcane-grass 94-42 were common with Co419 or PT43-52, which clearly proved that sugarcane grass 94-42 was a hybrid progeny of Co419 and PT43-52.

Superior to the proof of phenotype and the results of peroxidase isozyme, these DNA level polymorphic markers could directly reveal the genetic origin of sugarcane-grass 94-42. Interestingly, some amplified bands of sugarcane-grass 94-42 were unique compared with the two parents. Moreover, some bands were amplified from both parents but not from the F_1 hybrid. The reason could be due to the complex heredity background of sugarcane, which is well known for a high degree of polyploidy and frequent aneuploidy (Cordeiro et al. 2000). The genome of

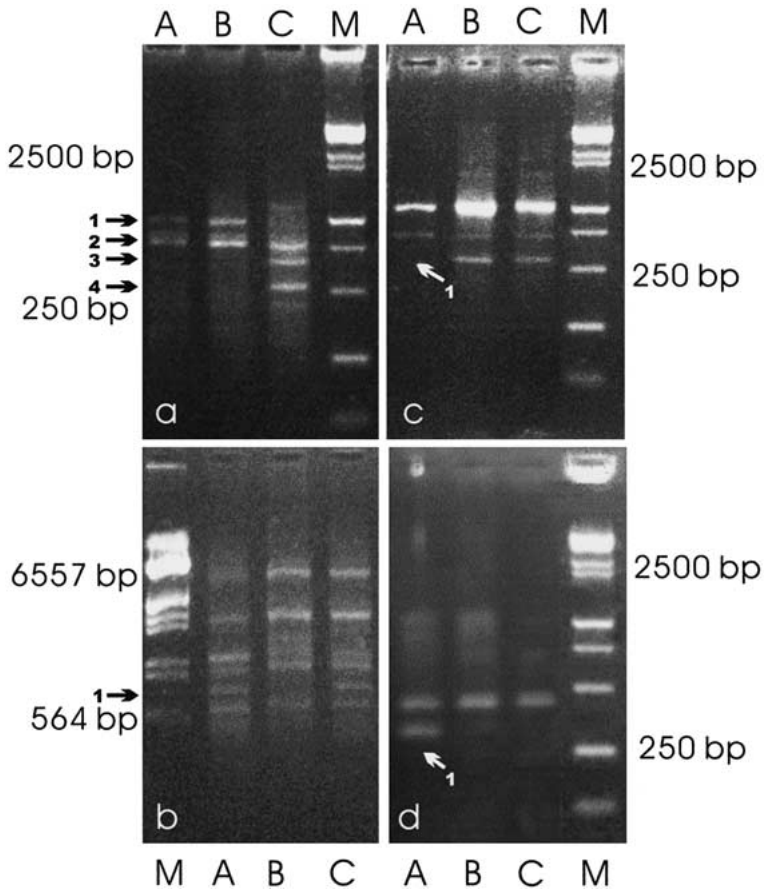

Figure 1. RAPD analysis of sugarcane-grass 94-42, Co419 and PT43-52. A, Sugarcane-grass 94-42; B, Co419; C, PT43-52; M, Ladder. (a) Primer I-11: 5′-ACATGCCGTG-3′. 1,2: Identical bands between F_1 and Co419; 3,4: Unique bands in PT43-52. (b) Primer S-24: 5′-AATCGGGGCTG-3′. 1: Identical bands between sugarcane-grass 94-42 and PT43-52. (c) Primer I-2: 5′-GGAGGAGAGG-3′. 1: Bands appeared in both two parents but absent in new species. (d) Primer I-19: 5′-AATGCGGGAG-3′. 1: Unique band in sugarcane-grass 94-42.

the sugarcane cultivar line is derived from the combination of *S. officinarum* L. ($2n = 80$) and *S. spontaneum* L. ($2n = 40$–128). The chromosome number varies from 100 to 130, of which *S. spontaneum* L. contributes 5–10% (Wen 1998). Genomic recombination easily occurred in the hybrid of these two sugarcane species, which might cause the additional or missing bands in F_1 plants.

Two years' of investigations suggested that sugarcane-grass 94-42 had higher endurance to cold and draught stress than the elite herbage *Pennisetum purpureum* Schum. (Hong et al. 1998). After treatment at −6 °C for 6 h, 88.2% of sugarcane-grass 94-42 plants survived, while all plants of *P. purpureum* Schum. died. Under the draught stress condition, the relative electric conductivity and malondialdehyde content of sugarcane-grass 94-42 increased 4.03% and 10.31%, respectively, much lower than those of *P. purpureum* Schum. Combined with the results of RAPD analysis, these characters were very likely inherited from the wild sugarcane line PT43-52, and could be kept in all plants of sugarcane-grass 94-42 due to its agamogenesis in field production. In addition, sugarcane-grass 94-42 does not flower and keeps green perennially when planted in the south of China, so it could supply feedstuff throughout the year, especially in winter and early spring.

References

Cordeiro G.M., Taylor G.O. and Henry R.J. 2000. Characterisation of microsatellite markers from sugarcane

(*Saccharum* sp.), a highly polyploid species. Plant Sci. 155: 161–168.

Harvery M. and Botha F.C. 1996. Use of PCR-based methodologies for the determination of DNA diversity between *Saccharum* varieties. Euphytica 89: 257–265.

Hong Y.Y., Lu C.B., Zheng G.D., Zeng R.Q. and Dai Y.M. 1998. Physiological appraisal of the low temperature and the drought resistance of new strain sugarcane-grass. J. Fujian Agr. Sci. 13: 1–4.

Hong Y.Y., Lu C.B., Zheng G.D., Zeng R.Q., Dai Y.M. and Huang S.Y. 1997. Preliminary studies on new sugarcane-forage varieties by breaking intergeneric barrier of hybridization. J. Agr. Sci. Fujian Acad. 12: 1–4.

Lu C.B., Hong Y.Y., Zheng G.D., Zeng R.Q., Dai Y.M. and Huang S.Y. 1998. New C_4 subtropical herbage strain 94-42 with green in the four seasons, high yield and good quality. Grassland China 5: 13–17.

Murray M.G. and Thompson W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4325.

Nei M. and Li W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273.

Wen Y. 1998. A review of cytology and genetics progress in *Saccharum* sp. Sugarcane China 5: 23–27.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531–6535.