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Full Length Research Paper

Analysis of genetic variation in *Erianthus arundinaceum* by random amplified polymorphic DNA markers

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The polymorphism and relationships among 51 *Erianthus arundinaceum* accessions were assessed with RAPDs. One hundred and twenty-seven bands were detected, of which 89 were polymorphic (70.07%). Accession relationships were estimated through cluster analysis (UPGMA) based on RAPD data.

Key words: *Erianthus arundinaceum*, genetic diversity, RAPD, UPGMA.

INTRODUCTION

Saccharum spp. is one of the most important species for sugar production in the world. *Erianthus arundinaceum* Retz. is one of wild sugarcane species, belonging to *Erianthus.* It can be used as an elite cross parent for sugarcane breeding. It is significant to conserve the germplasm of *E. arundinaceum.* Undoubtedly, the study of the genetic diversity of *E. arundinaceum* would be important not only for germplasm conservation but also for breeding purposes. The reason is that diversity among germplasm is the key to successful breeding programs (Renganayaki et al., 2001). In plant breeding pro-grams, information on the genetic diversity within and among closely related crop species is essential for a rational use of genetic resources.

Random amplified polymorhic DNA (RAPD) is one of the simplest and fastest of DNA-based techniques in genetic similarity studies (Zhang et al., 2005). RAPDs have the advantage that the material is processed by an efficient and inexpensive technique without requiring prior knowledge of the genome. A number of scientists have used RAPD markers to study polymorphism in various plants (Ortiz et al., 1997; Gwanama et al., 2000; Ranade et al., 2002; Samal et al., 2003; Zhang et al., 2008).

In this preliminary report the RAPD procedure was used to assess the level of polymorphism, the similarities and relationships among *Erianthus arundinaceum* acces-sions from provinces of China in the sugarcane germplasm garden of Yunnan Agricultural University (YAU).

MATERIALS AND METHODS

Fifty-one *E. arundinaceum* accessions were used in the RAPD analysis. Figure 1. Plant materials planted in the sugarcane germ-plasm garden of Yunnan Agricultural University (YAU). Name and origin of the accessions are shown in Table 1.

DNA was extracted from leaves 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 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 0.04 cm^3 mineral oil. Amplifications were carried out using a 2400 Perkin-Elmer Thermal; cycles were programmed for 40 cycles as follows: 30 s at 94℃, 30 s at 36℃, 1.5 min at 72℃, with an initial melting of 6 min at 94℃, and a final extension of 6 min at 72℃. Amplification products were analyzed by electrophoresis in a 1.5% agarose gel with 1× TAE buffer (0.004 M Tris-acetate and 0.002 M EDTA).

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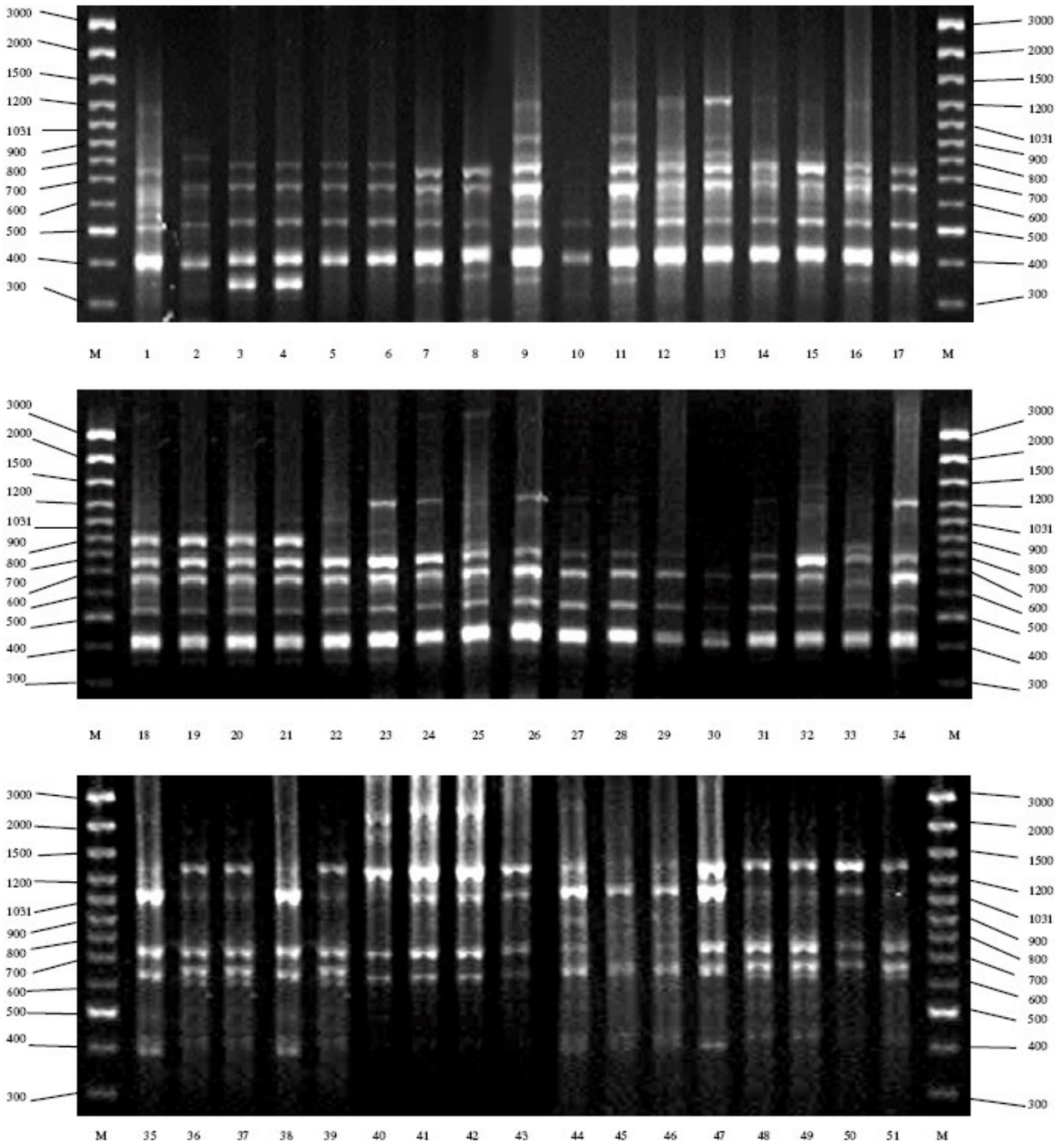

Figure 1. RAPD fingerprints of 51 *E. arundinaceum* accessions (the primer is OPA A-7). The number representing accession code was shown in the Table 1, and M is 100 bp DNA ladder.

Individual RAPD fragments for each primer-genotype combina-tion were scored as 1 (presence) or 0 (absence), and a note of their sizes was made. Only those reproducible bands were scored (repeated three times). The set of fragments co-migrating across the 51 accessions was referred to as a band. Pairwise similarity matrices were generated using Nei's coefficient of similarity (1979), and UPGMA clustering was then used to produce a dendrogram. The above procedures were performed using STATICA-pc. AMOVA analysis was performed with the software package Arlequin (Schneider et al., 2000).

RESULTS AND DISCUSSION

Of the one hundred primers were used in this study, 24 produced amplification products that were too faint to score or could not be consistently reproduced, and 62

Table 1. The 51 *E. arundinaceum* accessions used in RAPD analysis.

No.	Accession	Origin	No.	Accession	Origin
1	82—8	Yunnan	27	91—43	Jiangxi
2	82—28	Yunnan	28	91—51	Fujian
3	82—30	Yunnan	29	91—58	Fujian
4	89—80	Yunnan	30	91—67	Fujian
5	82—139	Yunnan	31	91—68	Fujian
6	82—135	Yunnan	32	91—83	Guangdong
7	82—138	Yunnan	33	91—84	Guangdong
8	82—143	Yunnan	34	91—85	Guangdong
9	83—162	Yunnan	35	91—87	Guangdong
10	83—168	Yunnan	36	91—94	Guangdong
11	83—180	Yunnan	37	91—96	Guangdong
12	92—52	Yunnan	38	91—101	Guangxi
13	92—54	Yunnan	39	91—109	Guangxi
14	91—2	Shichuan	40	91—119	Guangxi
15	91—18	Shichuan	41	91—125	Guangxi
16	91—39	Shichuan	42	93—34	Hainan
17	91—43	Shichuan	43	93—36	Hainan
18	91—77	Shichuan	44	93—38	Hainan
19	91—79	Shichuan	45	93—59	Hainan
20	91—82	Shichuan	46	93—66	Hainan
21	91—83	Shichuan	47	93—77	Hainan
22	91—84	Shichuan	48	93—78	Hainan
23	91—85	Jiangxi	49	92—13	Hainan
24	91—89	Jiangxi	50	92—37	Hainan
25	91—33	Jiangxi	51	92—39	Hainan
26	91—40	Jiangxi			

Table 2. Sequence of RAPD primers used for genetic variation analysis of *E. arundinaceum*, and results obtained.

Primer	Sequence (5'-3')	Total bands	Polymorphic bands	Polymorphism (%)
OPAA-02	GAGACCAGAC	12	8	66.67
OPAA-07	CTACGCTCAC	10	10	100
OPAA-08	TCCGCAGTAG	5	3	60
OPAK-10	CAGTGTGCTC	11	7	63.64
OPAB-02	GGAAACCCCT	7	5	71.43
OPAB-06	GTGGCTTGGA	9	7	77.78
OPAE-10	CTCAAGCGCA	15	11	73.33
OPAG-20	TGCGCTCCTC	4	2	50
OPAK-19	TCGCAGCGAG	6	5	83.33
OPAN-07	TCGCTGCGGA	10	8	80
OPAN-08	AAGGCTGCTG	13	5	38.46
OPAN-15	TGATGCCGCT	9	7	77.78
OPAP-17	ACGGCACTCC	13	9	69.23
OPAQ-05	ACGGAGCTGA	3	2	66.67
Total		**127**	**89**	**70.07**

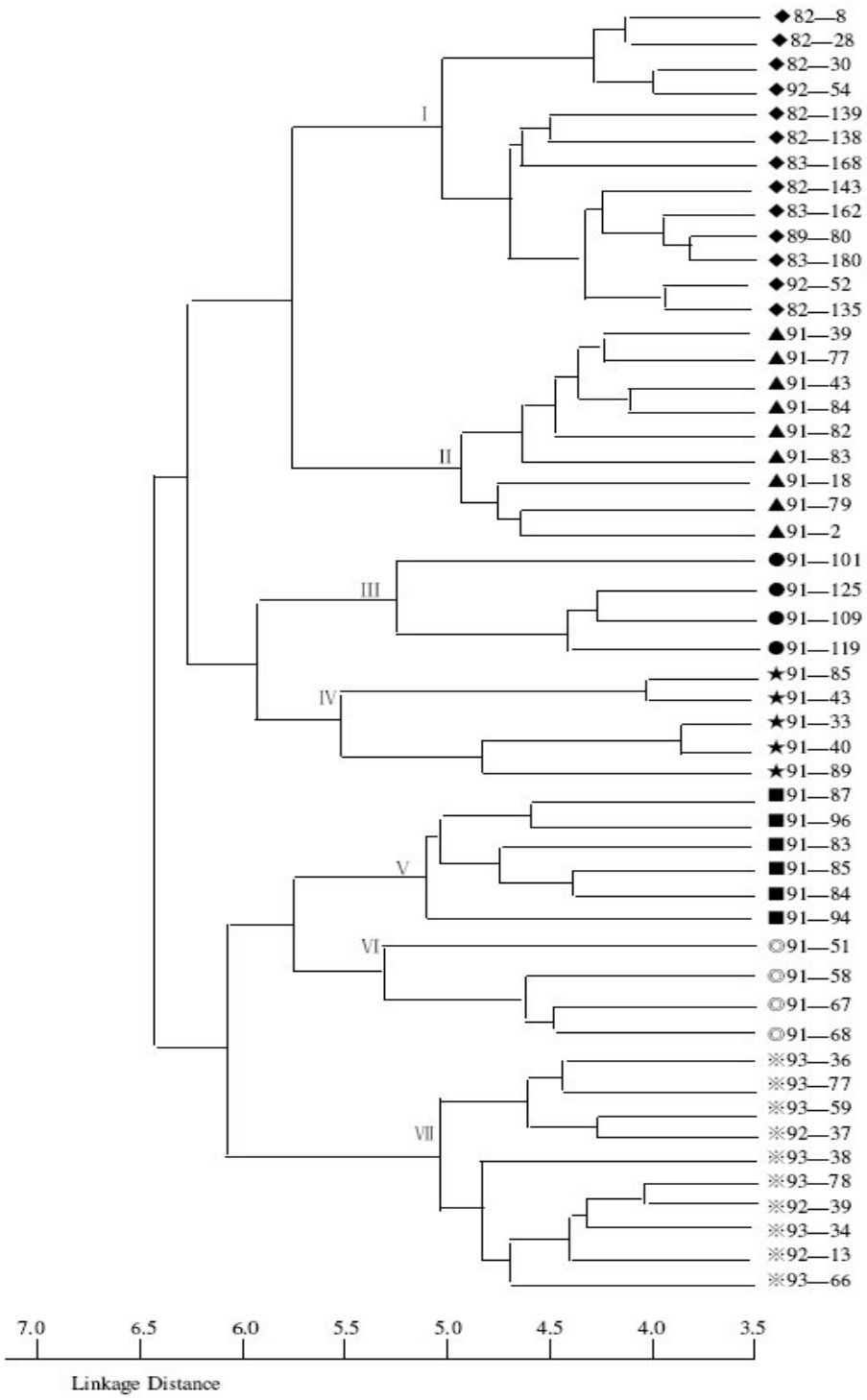

Figure 2. Dendrogram of the 51 *E. arundinaceum* accessions based on cluster analysis (UPGMA) of the genetic distances calculated from 94 polymorphic RAPD fragments. Symbols indicate province of origin: ◆ = Yunnan. ▲= Shichuan. ●= Guangxi. ★ = Guangxi. ■= Guangdong. ◎= Fujian. ※= Hainan

produced monomorphic banding patterns. Thus only 14 out of 100 primers were scored. A total of 127 bands were scored from the comparison of amplifications with 14 primers of DNAs from 51 *E. arundinaceum* accessions (Table 2), with an average of 9.07 bands scored per primer. Three to fifteen bands generated by a single pri-

mer of variable lengths were detected. The polymorphic bands were 89 (70.07%), and in average the band polymorphism per genotype was 17.3%.

A pairwise matrix of percent agreement between all the analyzed plants was obtained (data not shown), and between 69.31 and 95.98% of all fragments were shared between plants of the same group. It suggested the existence of a great number of genetic variations among *E. arundinaceum* accessions.

The relationships among the plants are shown by the dendrogram (Figure 2), which revealed seven clusters. The dendrogram indicated a clear pattern of division among the *E. arundinaceum* accessions based on geographic origin, as seen in some other crops (Paul et al., 1997; Zhang et al., 2006).

When the genetic variation of the cultivars was partitioned by AMOVA, 67.54% of the variation was found among the accessions that had different origins while 32.46% was found among the ones that had the same origins. Both of the variation figures, within and among the origins, were highly significant ($P < 0.001$) (data not shown). Characterization and quantification of genetic diversity has long been a major goal in germplasm conservation. The results showed that RAPD is an effective tool for *E. arundinaceum* germplasm management.

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