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SSR MARKER BASED GENETIC DIVERSITY IN SUGARCANE VARIETIES (LINES) DEVELOPED IN GUANGXI, CHINA

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ABSTRACT

It is important to identify the genetic diversity amongst sugarcane genetic resources for cultivar development and evaluation of sugarcane varieties. The genetic diversities of 125 sugarcane varieties including 117 varieties (lines) developed in Guangxi (China) and 8 others were studied using 10 SSR markers. The results showed amplification of total 110 bands ranged from 90 to 350 bp in size. The number of bands per locus ranged from 7-14, when scoring was restricted to strongly amplified fragments, and averaged 10.5 polymorphic bands per locus. The ratio of polymorphism was as high as 95.40%. The Shannon's information index (H) of amplified alleles ranged from 6.85-6.94. By UPGMA cluster analysis, all of the materials, tested using of 10 SSR markers, were distinguished completely. All the materials were clustered in one group at the genetic similarity of 0.4752, and 60.8% of sugarcane varieties (lines) were still clustered together at the genetic similarity of 0.6308, which revealed rather narrow range of variability among them. UPGMA cluster analysis was supported by PCA. Cluster analysis showed that 117 sugarcane varieties (lines) from Guangxi can be completely distinguished by 4 SSR markers. The results obtained in the present study are informative for sugarcane variety protection and parent identification.

INTRODUCATION

The assessment of varietal diversity in sugarcane is important for sustainable development of sugarcane production. The sugarcane varieties (lines) bred in the different areas are not only used for commercial cultivation, but also the important source of hybrid parent materials for many breeding programs. The accurately identification of sugarcane varieties and selection of excellent cross combinations for breeding new varieties is the main problem needs to be given attention . In this regard, it is of extremely vital significance to know the genetic diversity and genetic relationship, and to construct a scientific and reasonable fingerprint data base, which could be available to for the preservation and utilization of sugarcane germplasm resources and the protection of sugarcane varieties. At present, many kind of DNA molecular markers are being used for a assessing sugarcane genetic diversity, identification of germplasm resources, construction of genetic map and QTL localization, studying differential gene expressions and in molecular marker assisted breeding (**Lao *et al.*, 2008, 2009; Li *et al.*, 2003; Liu *et al.*, 2009, 2010; Que *et al.*, 2009**). The

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parents of sugarcane varieties, bred in Guangxi, mainly included Coseries varieties from India, CP series from United States, Yacheng series Hainan, ROC series from Taiwan and GT series from Guangxi. Until now, many sugarcane varieties with superior traits have been bred and used as new germplasm and breeding parents. However, the lack of sufficient genetic information of sugarcane varieties (lines) bred in Guangxi resulted in their inefficient utilization. In the current study, analysis of genetic diversity in sugarcane varieties (lines), bred in Guangxi, was performed by using SSR markers which will be helpful in providing the molecular basis for sugarcane hybrid parent selection and hybrid combination preparation.

MATERIALS AND METHODS

One hundred twenty five sugarcane varieties (lines),including 117 developed in Guangxi (China), two from Hainan, three from Taiwan and three from USA, were provided by Guangxi Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning (Guangxi, China) (Table 3).

Genomic DNA Extraction

Young leaves of sugarcane varieties (lines) were sampled for isolation of DNA using SDS one-step method as described by **Chen *et al.* (2010).**

SSR Amplification

Eighty seven SSR markers developed by **Pinto *et al.* (2004)** and **Pan *et al.* (2006)** were used in the present study. Each 20 µL reaction mixture contained 2µL 10 × PCR buffer, 2 µLdNTPs (2.0 M each),0.4 µL of each SSR forward and reverse primer (7.5 µM), 20~30 ng genomic DNA, and 0.75 U*Taq* DNA polymerase (TaKaRa Biotechnology (Dalian) Co. Ltd., Dalian, China). DNA amplification was performed in a Biometra T1 96 ThermoBlock Cycler programmed at 94°C for 5 min for initial denaturation, followed by 32 cycles at 94°C for 40 s, 52-60°C for 1 min, and 72°C for 50 s, and 72°C for 5 min for a final extension. The PCR product was separated on 7% polyacrylamide gel and visualized by silver staining. The reproducibility of the DNA profile was tested by repeating PCR amplification. For each marker, presence/absence of each fragment (product length variant) and banding pattern (fragment combination) in each sugarcane variety was recorded. Products of the same size in different sugarcane varieties (lines) were considered as homologous and recorded as "1", otherwise "0". Polymorphism showed as a percentage of polymorphic fragments, which was calculated as N/K, where N is the polymorphic loci and K is the total fragments amplified by one marker. Shannon (Shannon-wiener) index was analyzed by DPSv8.01 software. Genetic similarities and genetic distances between varieties (lines) were estimated, and the distance matrices were subjected to complete linkage cluster analysis, to assess and group the tested varieties. The principal component analysis (PCA) was performed by NTSYSpc2.10 software.

RESULTS

Polymorphism Analysis

Among the 87 SSR markers tested on the 10 different sugarcane germplasm accessions from different regions (two from Guangxi, two from Hainan, three from Taiwan and three from America), 10 produced clearly polymorphic bands (Table 1). And these were used to assess genetic diversity among 125 sugarcane varieties (lines). Ten SSR markers produced a total of 110 bands, of which 95.4% were polymorphic. The number of alleles amplified by per marker ranged from 7 to 14 with an average of 10.5 polymorphic bands per locus. The Shannon's information index (H) of the amplified alleles ranged from 6.85 to 6.94 with an average of 6.91 (Table 2). The fragment sizes ranged from 90 to 350 bp. The data indicated that 125 sugarcane varieties (lines) possessed rich diversity at 10 SSR loci.

Cluster analysis

The genetic similarity coefficients of 125 sugarcane genotypes were ranged from 0.4752 to 0.9756. With these polymorphic SSR markers, the corresponding clustering dendrogram was constructed (Fig.1). Based on SSR alleles, the 125 sugarcane genotypes can be classified into eight categories on the genetic similarity coefficients of 0.6308, which contained two broad groups (A and F) and six small groups (B, C, D, E, G and H). 60.8% of all the materials were included in A group, which implicated narrow genetic basis of sugarcane germplasm bred in Guangxi. Cluster analysis also showed that the 117 sugarcane varieties (lines) developed in Guangxi were completely distinguished by the 4 SSR markers SMC278CS, SMC1493CL, SMC16SA and SMC2017FL.

Table 1. Sequences of 10 SSR markers

Primer code	Forward primer sequence	Reversed primer sequence	Annealing temperature(°C)	Bands (bp)
SMC1047HA	TGAGCCTAAGCCAGA AAGAAG	GGAACTAATTTCCTACGAGAACAC	60	110-150
SMC2017FL	CACAAGTGAAGATAATAGTGTCCCT	GATCCCAAATCCCTTGATCTC	60	205-310
SMC24DUQ	CGCAACGACATATACACTTCGG	CGACATCACGGAGCAATCAGT	60	120-170
SMC278CS	TTCTAGTGCCAATCCATCTCAGA	CATGCCAACTTCCAA ACAGACT	58	140~200

Table 2.The amplification results using 10 SSR primers

Primer code	No. of alleles	No. of polymorphic alleles	Ratio of polymorphic alleles (%)	Shannon's information index (H)	Bands (bp)
SMC1047HA	10	10	100	6.93	110-155
SMC2017FL	14	14	100	6.94	204-310
SMC24DUQ	11	11	100	6.93	120-173
SMC278CS	10	10	100	6.87	125-193
mSSCIR68	7	7	100	6.85	190-225
SMC119CG	7	6	85.7	6.92	96-160
SMC1237FL	12	12	100	6.93	105-160
SMC1493CL	12	10	83.3	6.93	100-160
SMC16SA	14	14	100	6.93	90-150
SMC2055FL	13	11	84.6	6.89	240-350
Total	110	105	-	-	90-350
Average	11	10.5	95.4	6.91	-

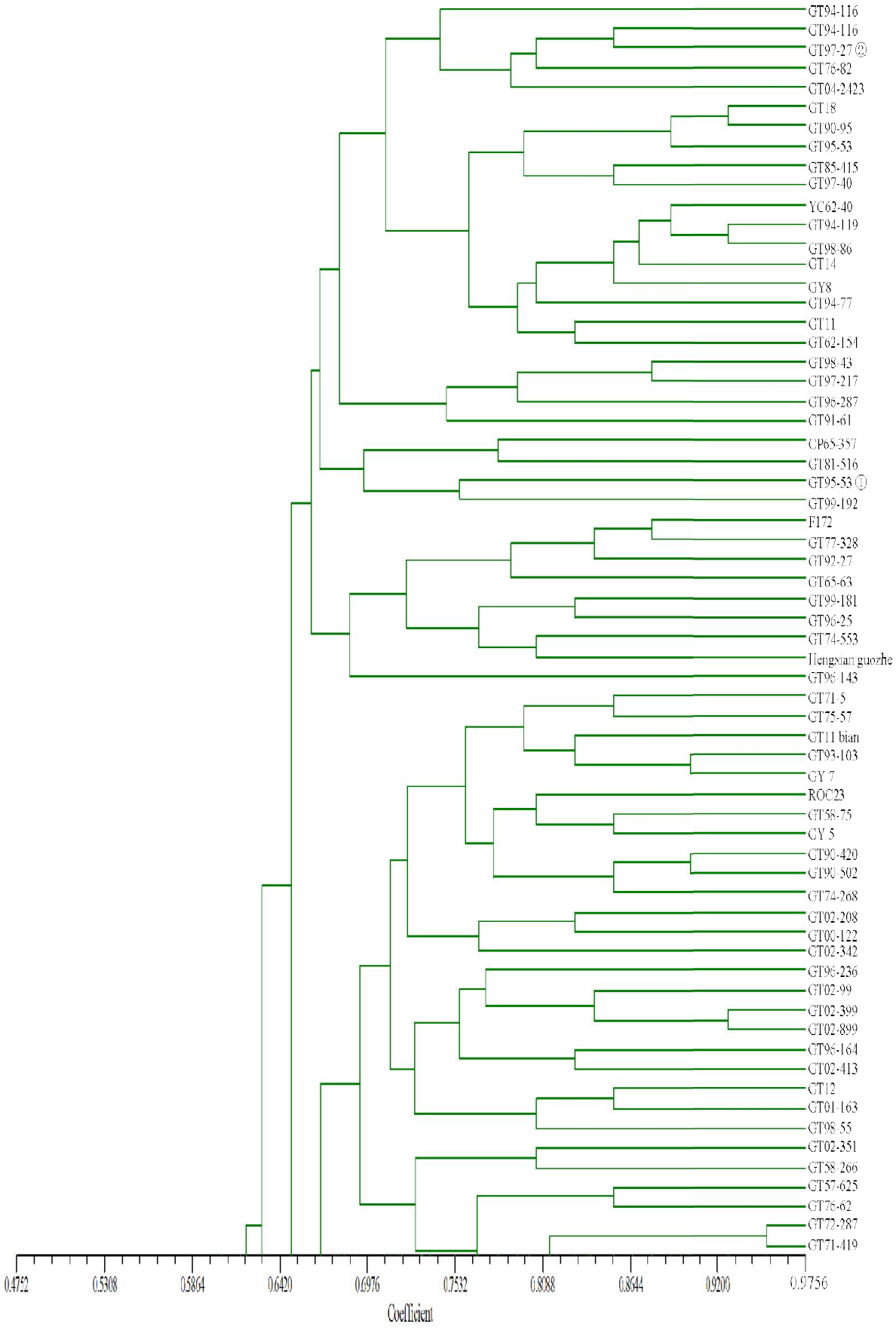

Fig. 1.Dendrogram of 125cultivars based on SSR analysis

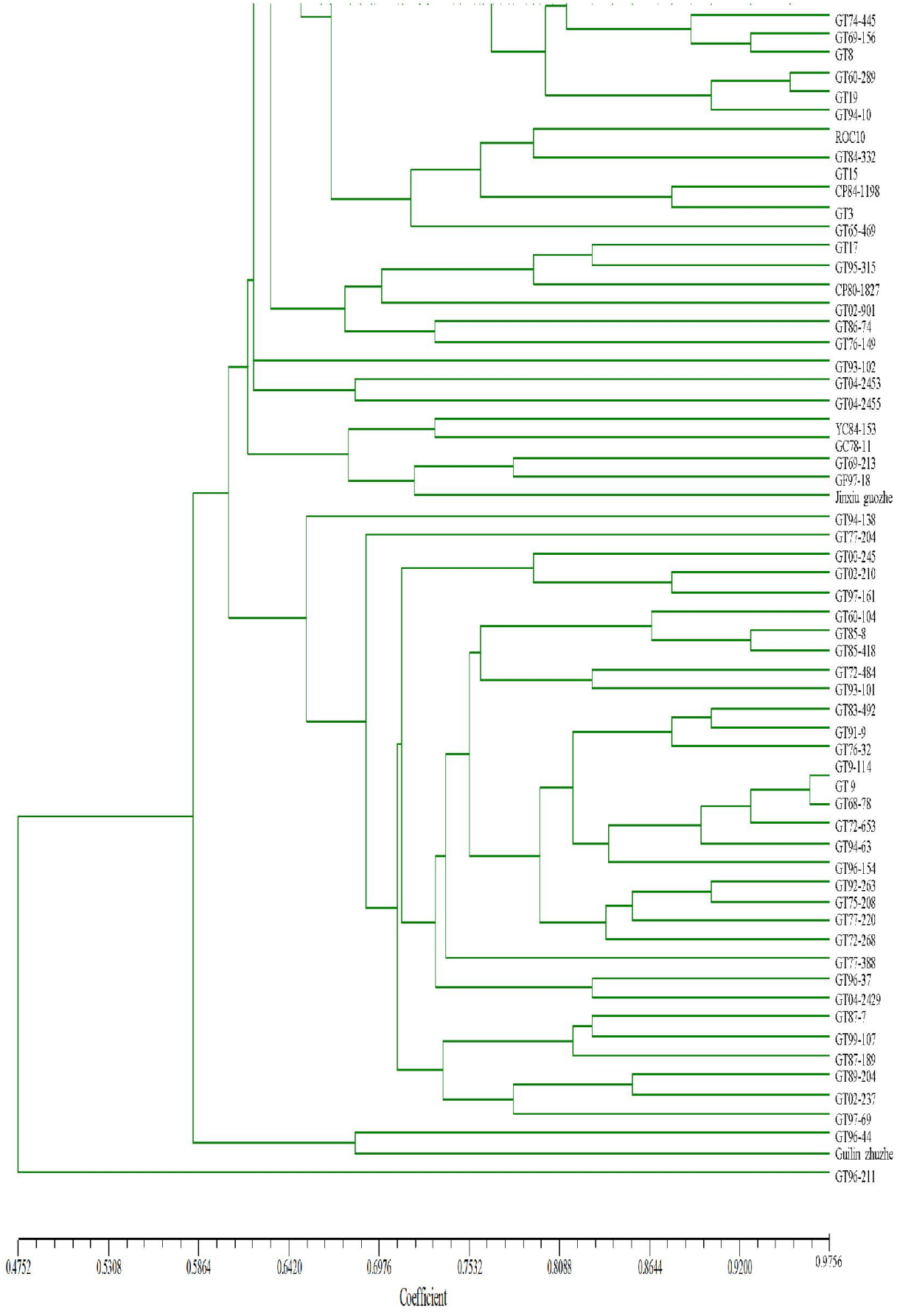

Fig. 1. (Contd.) Dendrogram of 125cultivars based on SSR analysis

Table 3. Sugarcane varieties used in the experiment to assess the genetic diversity

Code	Variety/Clone	Code	Variety/Clone	Code	Variety/Clone
1	GT94-116	43	GT72-287	85	GT93-101
2	GT71-5	44	GT71-419	86	GT85-418
3	Yacheng84-153	45	GT04-2455	87	GT89-204
4	GT18	46	GT85-415	88	GT77-388
5	CP65-357	47	GT58-266	89	GT76-62
6	F172	48	GT74-445	90	GT99-107
7	GT17	49	GT76-149	91	GT97-69
8	Yacheng 62-40	50	GT04-2423	92	GT02-237
9	GT11	51	GT95-315	93	GT96-211
10	GT02-351	52	GT90-502	94	GT65-63
11	CP80-1827	53	GT91-61	95	GT60-289
12	ROC10	54	GT74-553	96	GT96-287
13	GT02-901	55	GT92-27	97	GT75-57
14	ROC23	56	GT74-268	98	GT98-55
15	CP84-1198	57	GT58-75	99	GT99-192
16	GT3	58	GT14	100	GT97-27 ②
17	GT77-328	59	GT96-44	101	GT69-213
18	GT96-236	60	GT96-143	102	GT90-95
19	GT95-53	61	GT93-102	103	GT93-103
20	GT02-99	62	GT60-104	104	GT94-10
21	GT02-399	63	GT83-492	105	GT69-156
22	GT97-27	64	GT77-204	106	GT96-164
23	GT98-43	65	GT9-114	107	GT76-82
24	GT02-208	66	GT72-653	108	GT02-413
25	GT97-217	67	GT02-210	109	GT01-163
26	GT02-899	68	GT92-263	110	GT97-40
27	GT94-138	69	GT85-8	111	GT00-122
28	GT90-420	70	GT91-9	112	GT19
29	GT62-154	71	GT75-208	113	GT65-469
30	GT04-2453	72	GT9	114	GT8
31	GT02-342	73	GT68-78	115	GT94-119
32	GT11 changed	74	GT76-32	116	GT98-86
33	GT12	75	GT96-154	117	Guiyin 5
34	GT99-181	76	GT87-7	118	Guiyin 7
35	GT86-74	77	GT87-189	119	Guiyin 8
36	GT84-332	78	GT72-484	120	Guilin cane
37	GT95-53 ①	79	GT77-220	121	Gongcheng78-11
38	GT57-625	80	GT96-37	122	Jinxiu cane
39	GT94-77	81	GT97-161	123	Hengxian cane
40	GT15	82	GT04-2429	124	Guifu97-18
41	GT81-516	83	GT72-268	125	Guifu96-25
42	GT00-245	84	GT94-63		

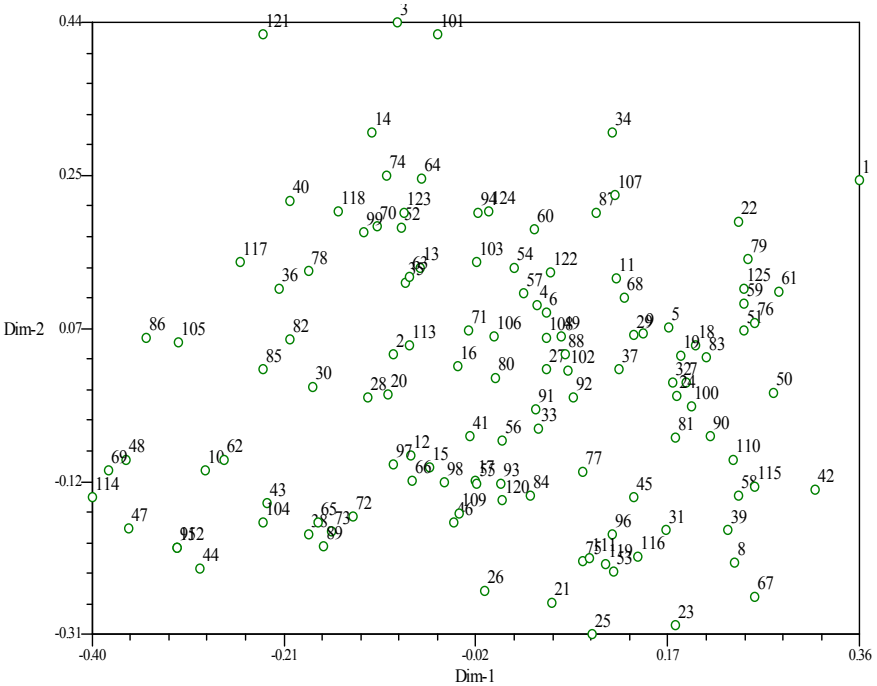

Fig. 2. Principal coordinate analysis with 125 sugarcane varieties (lines). The codes of accessions corresponding with those are mentioned in Table 1

The principal Component Analysis (PCA)

PCA analysis was performed by NTSYSpc2.10 software for all the resources and the 2D cluster figure was obtained (Fig.2). The results showed the first three contribution rates were 9.55, 8.16 and 7.00%, respectively (total only 24.72%), and experimental Guangxi sugarcane varieties (lines) in space were mainly distributed in the middle, only a few parts in both ends up and down, which indicated the inconsistent and narrow genetic basis of 125 sugarcane varieties (lines). The PCA was basically consistent with the results of cluster analysis.

DISCUSSION

The genetic similarity coefficient (or genetic distance) reflected the genetic differences between varieties, which is one of the key components in the process of breeding. It showed superior advantages to analyze genetic similarity coefficient of sugarcane by DNA molecular markers with regard to hybrid, environmental impact, time, information and the accurate result, which will vastly promote sugarcane genetic breeding. In the present study, the sugarcane varieties (lines), bred in Guangxi, were analyzed by using SSR markers which provided the molecular basis for breeding of sugarcane, selecting parent combination, efficiently dividing heterosis, improving varietal quality and breeding efficiency. The genetic diversities of 125 sugarcane varieties were studied using 10 SSR markers with high polymorphism. The ratio of polymorphism was as high as 95.40% and the variation was from 7 to 14, which inferred the higher genetic diversities of sugarcane varieties (lines) bred in Guangxi. The results of the study were similar to **Pan (2006)**, except a slightly high variation. The materials used in the study were bred in Guangxi many years ago, which caused the relative larger year span among them. Because of gene loss or gene introduction in the long time, the degree of genetic difference became larger.

Most of the research results indicated that the genetic difference, genetic distance and genetic diversity of sugarcane can be more truly understood by use of molecular marker technology, sequentially it should help to select the matched parents and to improve the breeding efficiency (**Cordeiro *et al.*, 2003; Alwala *et al.*, 2006**). The parent matching is the key step in sugarcane breeding. The phenotypic and agronomic traits and the geographic differences have always been considered in the selection of parents for a long time. The repeated utilization of few core germplasm materials and artificial selection resulted in the loss of diversified genes and the accordant traits of different parents. So it is very important to match and utilize sugarcane parent for improving the breeding level. In recent years, with the application of molecular markers, the methods of selecting the pairing sugarcane parents have also been developed. Studies conducted by **Lao (2008, 2009)** indicated the genetic similarity coefficient between two parents for making a cross should be in the scope of 0.6723±0.0600 and it results in easy development of good varieties when making a cross with parents from different groups. Genetic diversity and genetic relationship among Yacheng (Hainan), Guangdong, Fujian sugarcane series, wild sugarcane species and sugarcane proto-species were analyzed with AFLP, ISSR and SSR markers (**Lao *et al.*, 2008, 2009; Liu *et al.*, 2008; Wang *et al.*, 2007; Zhuang *et al.*, 2005; You *et al.*, 2008**) which provided important references for the use of sugarcane varieties. A lot of sugarcane germplasm accessions used in the present study were preserved in gene bank, and applied as important parents in making a cross. On the basis of the results, it may be concluded that germplasm with narrow genetic relationship, similar genetic composition or similar clustering should be avoided to use as parents in breeding programs. Contrastingly, the germplasm with wider genetic relationships should be given more emphasis.

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