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Full Length Article

Assessment of Genetic Relationship and Diversity among Chinese Sugarcane Parental Clones using SCoT and ISSR Markers

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Abstract

Sugarcane is an allopolyploid crop with ≥120 or more chromosomes, of which~5.0–10% are *Saccharum spontaneum* and 90–95% are *S. officinarum*. The genetic characteristics of sugarcane provides a complex genetic background. The objective of this study was to use two molecular marker systems, start codon targeted (SCoT) and inter-simple sequence repeat (ISSR), to assess the genetic relationship and diversity of 75 sugarcane parental clones from Chinese sugarcane breeding program. Twenty-four SCoT primers produced 227 loci, of which 200 (88.11%) were polymorphic, whereas 15 ISSR primers resulted in a total of 146 loci, with 123 (84.25%) being polymorphic. Mean polymorphism information content values of 0.8152 and 0.8361 were detected using SCoT and ISSR primers, respectively. The genetic similarity coefficients ranged from 0.542 between ROC22 and HoCP95-988 to 0.831 between ROC25 and ROC20, with a mean value of 0.687 based on SCoT+ISSR data set. The unweighted pair group method of arithmetic averages (UPGMA) clusters and principal coordinate analysis (PCA) gave similar results. The 75 Chinese sugarcane parental clones were clustered into two main groups (A and B). Group A was primarily comprised of 42 clones from all Q-series, all CP-, HoCP-, or LCP-series, all YT-series and three *Erianthus arundinaceus* F_4 innovative parental clones, etc., while 33 parental clones of Group B contained all TT-series, all GT-series, three *Saccharum officinarum* species, etc. The genetic similarity was high among the Q-series, CP-series, HoCP-series and YT-series. The genetic relationship was close among TT-series, GT-series and three *S. officinarum* species, while the genetic similarity between YT-series and TT-series or GT-series was low. There was an abundant genetic diversity among these sugarcane parental clones however the parental clones bred by the same breeding organization have a narrow genetic basis. This information was useful for selecting crossing parents and combinations. Correlation detection between SCoT and ISSR was not significant, but highly complementary, indicating that the combination of the two marker systems could avoid biases based on a single marker. © 2017 Friends Science Publishers

Keywords: Sugarcane; Parental clone; SCoT marker; ISSR marker; Genetic diversity

Introduction

Sugarcane (*Saccharum* hybrids spp.) is the most important sugar crop in Mainland China, producing >94% of the total consumable sugar in the country and ranking third worldwide. It is also a potential renewable energy crop. Guangdong, Guangxi, and Yunnan Provinces are three major sugarcane-producing areas, with sugarcane-planting area and annual sugar production accounting for >90% in Mainland China (Chen and Zhang, 2010; Li and Yang, 2015). Sugarcane is an allopolyploid crop with ≥120 chromosomes, of which ~5.0–10% are *S. spontaneum* and 90–95% are *S. officinarum*. Sugarcane is also an aneuploid of mainly 2n + n. All of these genetic characteristics produce the complex genetic background of the species (Chen, 2003). The improvement of sugarcane varieties has contributed greatly to the sugarcane industry; therefore, research on the genetic diversity of sugarcane germplasm resources, especially parental clones, would be helpful to guide parental selection and their combinations in sugarcane cross breeding (Rachayya *et al*., 2012; Santos *et al*., 2012).

Molecular markers are widely used in plant genetic diversity as a result of not affected by environment. So far, different molecular markers, such as target region amplified polymorphism (Que *et al*., 2009), restriction fragment length polymorphism (Daugrois *et al*., 1996; Grivet *et al*., 1996), amplified fragment length polymorphism (Cai *et al*., 2005; Aitken *et al*., 2005, 2006), random amplified polymorphic DNA (RAPD) (Mudge *et al*., 1996; Tabasum *et al*., 2010), and simple sequence repeat (SSR) (Pan, 2006; Santos *et al*., 2012), have been used in studies on the genetic diversity of sugarcane. As we know, single molecular markers are more often used when developing this research; very few studies on genetic diversity of sugarcane have reported using a combination of two or more molecular markers, especially in China. The combination of two or

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more molecular marker systems can reduce the test deviation and improve the accuracy of evaluation. Therefore, it is necessary to evaluate the genetic diversity of Chinese sugarcane parents using a combination of two molecular markers.

Start codon targeted (SCoT) polymorphism is based on the short conserved nucleotide sequence that flanks the conserved region surrounding the translation-initiation codon, ATG. Similar to RAPD, the SCoT marker involves a single oligonucleotide primer and is based on polymerase chain reaction (PCR) analysis (Collard and Mackill, 2009). As a new molecular marker method, the SCoT marker with high polymorphism and efficiency was successfully used in rice (Collard and Mackill, 2009), peanuts (Xiong *et al*., 2011), mangos (Luo *et al*., 2012), potatoes (Gorji *et al*., 2011), grapes (Guo *et al*., 2012), sugarcane (Que *et al*., 2014), and orchids (Feng *et al*., 2015).

The inter-simple sequence repeat (ISSR) genome region is a type of molecular marker that is based on inter-microsatellite DNA regions and that provides high reproducibility, polymorphism, and low cost (Zietkiewicz *et al.*, 1994). ISSR markers have been widely used for genetic diversity and cultivar identification in many species, including bananas, sorghum, *Arabidopsis* and sugarcane (Pandit *et al*., 2007; Rachayya *et al*., 2012).

In this study, SCoT and ISSR markers were applied to elucidate the genetic relationship and diversity in 75 Chinese sugarcane parental clones. Our objectives were to (1) access the genetic diversity of the parental clones to provide a scientific basis for parental selection and combination for the development of sugarcane breeding and (2) evaluate the effects of the combination of the two markers. To the best of our knowledge, this is the first report using SCoT and ISSR markers to estimate the genetic relationship and diversity of Chinese sugarcane parental clones.

Materials and Methods

Sugarcane Sample Collection

Seventy-five sugarcane parental clones, including three *S. officinarum* species and three *Erianthus arundinaceus* F_4 innovative parental clones, were collected from the sugarcane germplasm nursery of South China Agricultural University (Table 1).

DNA Extraction

Fresh and young leaves from each sugarcane parent were randomly collected and mixed for genomic DNA isolation. Genomic DNA was extracted using the cetyl trimethyl ammonium bromide method, as described by Zhang *et al*. (2006). The DNA samples were diluted to prepare a working solution of 50 ng/μL for PCR analysis and stored at -20°C for further use.

SCoT Analysis

Twenty-four SCoT primers that yielded clear, reproducible band patterns were selected to evaluate the genetic diversity of the 75 sugarcane parental clones (Table 2). The primers were commercially synthesized by the Sangon Biotech Co. Ltd (Shanghai, China) and *r*Taq DNA polymerase was purchased from the TaKaRa Bio Inc. (Dalian, China). The PCR reaction was performed in a final volume of 25 μL containing 1.0 μL genomic DNA (~50 ng), 0.2 μL *r*Taq DNA polymerase (5.0 U/μL), 2.5 μL 10 × PCR reaction buffer (with Mg^{2+}), 2.0 μL 2.5-mM dNTP mixture and 2.0 μL 5.0-μM SCoT random primer. The final volume was adjusted to 25 μL with 17.3 μL sterile distilled water. PCR amplification was performed using the MyCycler™ thermal cycler (Bio Rad, Hercules, CA, USA), with an initial denaturation at 94°C for 5.0 min, followed by 35 cycles each of denaturation at 94°C for 50 s, annealing at 52°C for 50 s and extension at 72°C for 2.0 min and a final extension phase of 72°C for 8.0 min. The amplification products were electrophoresed on 2.0% agarose gel with 0.5× TBE buffer at 100 V for 1.0 h together with the DL 2000 DNA markers. The separated DNA fragments were stained with 5.0% GoldView™ and photographed with the Gene Genius Bioimaging System. The experiment was repeated at least once.

ISSR Analysis

All ISSR primers (Table 2) were synthesized by the Sangon Biotech Co. Ltd. (Shanghai, China) and *r*Taq DNA polymerase was purchased from the TaKaRa Bio Inc. (Dalian, China). The amplification reaction system and product detection were the same as that for SCoT above. The PCR amplification procedures were as follows: an initial denaturation at 94°C for 5.0 min, followed by 35 cycles each of denaturation at 94°C for 45 s, annealing at 52°C for 45 s and extension at 72°C for 2.0 min, and a final extension phase of 72°C for 8.0 min. The experiment was repeated at least once.

Data Analyses

Based on the electrophoresis results of the products amplified by SCoT PCR and ISSR PCR, the samples with DNA bands were marked as "1"; those without DNA bands were marked as "0" (only the repeatable bands in DNA electrophoresis analysis were recorded). The Jaccard similarity coefficient, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analyses, Principal Coordinate Analysis (PCA), and Mantel test were conducted using NTSYS-pc2.10 (Rohlf, 1993). Polymorphic information content (PIC) was calculated using formulas described by Botstein *et al.* (1980). Population genetic parameters, such as the observed number of alleles (N_a), effective number of alleles (N_e), Nei's gene

Table 1: Sugarcane parental clones and origins

Code	Parental clone[a]	Origin[b]	Code	Parental clone[a]	Origin[b]
1	Q190	Australia	39	YT 63-237	GSIRI/China
2	Q200	Australia	40	YT 83-271	GSIRI/China
3	Q205	Australia	41	YT 06-666	GSIRI/China
4	Q191	Australia	42	YT 99-66	GSIRI/China
5	Q189	Australia	43	YT 03-373	GSIRI/China
6	Q155	Australia	44	YT 89-113	GSIRI/China
7	Q157	Australia	45	YT 86-368	GSIRI/China
8	Q171	Australia	46	YT 91-976	GSIRI/China
9	Q179	Australia	47	YT 02-305	GSIRI/China
10	CP93-1382	USA	48	YT 08-144	GSIRI/China
11	CP72-1210	USA	49	YT 07-516	GSIRI/China
12	CP89-2143	USA	50	GT 94-119	GSRI/China
13	CP65-357	USA	51	GT 00-122	GSRI/China
14	LCP85-384	USA	52	GT 73-167	GSRI/China
15	HoCP93-746	USA	53	GT 03-48	GSRI/China
16	HoCP95-988	USA	54	GZ 95-108	JSRI/China
17	HoCP92-648	USA	55	GZ 14	JSRI/China
18	ROC1	TSRI/China	56	QZ 08-688	GSCRI /China
19	ROC25	TSRI/China	57	QZ 08-1497	GSCRI /China
20	ROC16	TSRI/China	58	QT 3	GSCRI /China
21	ROC22	TSRI/China	59	QT 5	GSCRI /China
22	ROC20	TSRI/China	60	FN 95-1702	FAFUSRI/China
23	ROC10	TSRI/China	61	YN 83-88	GAAS/China
24	ROC24	TSRI/China	62	NJ 57-614	NAAS/China
25	ROC5	TSRI/China	63	YC 71-374	HSBS/China
26	ROC23	TSRI/China	64	*E.arundinaceus* F_4-1	SCAU/China
27	LT 2	TSRI/China	65	*E.arundinaceus* F_4-2	SCAU/China
28	TY	TSRI/China	66	*E.arundinaceus* F_4-3	SCAU/China
29	ZY1	TSRI/China	67	Badila(*S. officinarum*)	SCAU/China
30	TT 89-1626	TSRI/China	68	Guangdong yellow(*S. officinarum*)	SCAU/China
31	F177	TSRI/China	69	Yunnan red (*S. officinarum*)	SCAU/China
32	F134	TSRI/China	70	N:Co310	India
33	YT 93-159	GSIRI/China	71	N:Co376	India
34	YT 03-393	GSIRI/China	72	P32-2	Unknown
35	YT 96-835	GSIRI/China	73	Brazil 618	Brazil
36	YT 91-1102	GSIRI/China	74	TH10	Thailand
37	YT 04-232	GSIRI/China	75	HoTH49	Thailand
38	YT 96-86	GSIRI/China			

Notes: [a]LT=Liangtang; TY=Taiyou; ZY=Zhanying; TT=Taitang; YT=Yuetang; GT=Guitang; GZ=Ganzhe; QZ=Qianzhe; QT=Qiantang; FN=Funong; YN=Yuenong; NJ=Neijiang; YC=Yacheng

[b]TSRI: Taiwan Sugar Research Institute; GSIRI: Guangzhou Sugarcane Industry Research Institute; GSRI: Guangxi Sugarcane Research Institute; JSRI: Jiangxi Sugarcane Research Institute; FAFUSRI: Fujian Agriculture and Forestry University Sugarcane Research Institute; SCAU: South China Agricultural University; NAAS: Neijiang City Academy of Agricultural Sciences; GAAS: Guangdong Academy of Agricultural Sciences. GSCRI: Guizhou Subtropical Crop Research Institute; HSBS: Hainan Sugarcane Breeding Station

Table 2: Information on selected primers for the analysis of sugarcane genetic diversity

Primer	Sequence	GC content (r/%)	Primer	Sequence	GC content (r/%)
SCoT1	CAACAATGGCTACCACCA	50.0	SCoT59	ACAATGGCTACCACCATC	50.0
SCoT5	CAACAATGGCTACCACGA	50.0	SCoT68	ACCATGGCTACCAGCGTC	61.1
SCoT11	AAGCAATGGCTACCACCA	50.0	SCoT72	CCATGGCTACCACCGCCA	72.2
SCoT12	ACGACATGGCGACCAACG	61,1	SCoT76	CCATGGCTACCACTACCG	61.1
SCoT13	ACGACATGGCGACCATCG	61.1	ISSR811	GAGAGAGAGAGAGAGAC	52.9
SCoT14	ACGACATGGCGACCACGC	66.7	ISSR815	CTCTCTCTCTCTCTCTG	52.9
SCoT15	ACGACATGGCGACCGCGA	66.7	ISSR826	ACACACACACACACACC	52.9
SCoT16	ACCATGGCTACCACCGAC	61.1	ISSR827	ACACACACACACACACG	52.9
SCoT17	ACCATGGCTACCACCGAG	61.1	ISSR835	AGAGAGAGAGAGAGAGYC	52.8
SCoT22	AACCATGGCTACCACCAC	55.6	ISSR855	ACACACACACACACACYT	47.2
SCoT23	CACCATGGCTACCACCAG	61.1	ISSR857	ACACACACACACACACYG	52.8
SCoT24	CACCATGGCTACCACCAT	55.6	ISSR859	TGTGTGTGTGTGTGTGRC	52.8
SCoT28	CCATGGCTACCACCGCCA	66.7	ISSR873	GACAGACAGACAGACA	50.0
SCoT34	ACCATGGCTACCACCGCA	61.1	ISSR884	HBHAGAGAGAGAGAGAG	49.0
SCoT39	AACCATGGCTACCACCGC	66.7	ISSR890	VHVGTGTGTGTGTGTGT	49.0
SCoT40	CCATGGCTACCACCGCCG	61.1	ISSR891	HVHTGTGTGTGTGTGTG	48.0
SCoT41	ACGACATGGCGACCGCGG	72.2	ISSR104	ATGATGATGATGATGATG	33.3
SCoT48	ACAATGGCTACCACTGGC	55.6	ISSR880	GGAGAGGAGAGGAGA	60.0
SCoT49	ACAATGGCTACCACTACG	55.6	ISSR812	GAGAGAGAGAGAGAGAA	47.1
SCoT57	ACAAYGGCTACCACTACG	50.0			

diversity index (*H*), and Shannon's Information index (*I*) were used to evaluate the genetic diversity within each population using POPGENE1.31 (Yeh *et al.*, 1999).

Results

SCoT and ISSR Polymorphisms

Twenty-four SCoT and 15 ISSR primers that yielded clear, reproducible band patterns were selected to evaluate the genetic relationship and diversity of 75 sugarcane parental clones. In the SCoT analysis, the number of bands and the degree of polymorphism revealed by each primer are provided in Table 3. SCoT analysis revealed 227 distinct bands, 200 (88.11%) of which were polymorphic. The average number of amplified bands per primer was 9.5. One representative profile (SCoT28) is shown in Fig. 2. Average PIC was 0.8159, N_a was 1.8811, N_e was 1.4725, H was 0.2853 and I was 0.4342.

In ISSR analysis 146 bands were observed, of which 123 (84.25%) were polymorphic; the average number of polymorphic bands per primer was 9.7. One representative profile (ISSR 880) is shown in Fig. 3. PIC was 0.8361, N_a was 1.8425, N_e was 1.4644, H was 0.2770, and I was 0.4198. The above data indicated that both types of molecular markers were suitable for analysis of genetic diversity of sugarcane parents.

When comparing the polymorphism parameters of the SCoT and ISSR markers, the former was higher than the latter in percentage of polymorphic bands, N_a, N_e, H and I, indicating that the ability and efficiency of SCoT markers to detect polymorphism were slightly higher than those of ISSR markers.

Genetic Similarity Coefficient and the Mantel Test

The genetic similarity coefficient detected by SCoT markers ranged from 0.524 to 0.850 (mean, 0.687), while the genetic similarity coefficient detected by ISSR markers ranged from 0.452 to 0.863 (mean, 0.658). To obtain more accurate genetic estimates, 373 bands based on the SCoT+ISSR data set were integrated into one matrix to assess the genetic relationship among the 75 sugarcane parental clones. The genetic similarity coefficients ranged from 0.542 (ROC22/HoCP95-988) to 0.831 (ROC25/ROC20) (mean, 0.687).

Correlation analysis was performed by Mantel test to compare the matrices of genetic similarity coefficients calculated by the SCoT and ISSR data sets. The low correlation coefficient (0.5807) showed that the correlation between SCoT and ISSR was not significant but highly complementary (Table 4), which provided the guidelines for the next test. The similarity coefficient matrices obtained by integrating SCoT+ISSR, SCoT and ISSR were compared using the Mantel test. A high correlation coefficient was observed between the data integrated with SCoT (r= 0.9237) and ISSR (r = 0.8480) (Table 4), showing that the combination of SCoT and ISSR produces more reliable data and avoids errors from using single SCoT or ISSR markers.

Table 3: Polymorphism analysis of SCoT and ISSR markers in sugarcane parental clones

Parameter	SCoT	ISSR	SCoT+ISSR
Number of primers	24	15	39
Total bands	227	146	373
Average bands of each primer	9.5	9.7	9.6
Polymorphic bands	200	123	323
PBB (%)	88.110	84.250	86.600
PIC	0.816	0.836	0.823
N_a	1.881	1.843	1.866
N_e	1.473	1.464	1.469
H	0.285	0.277	0.282
(*I*)	0.434	0.420	0.429

Notes: N_a: observed numbers of alleles; N_e: effective numbers of alleles; *H*: Nei's gene diversity index; *I*: Shannon's information index; *PIC*: polymorphism information index; *PPB*: percentage of polymorphic loci

Table 4: Correlation analysis between SCoT and ISSR by Mantel test

	SCoT and ISSR	ISSR and ISSR+SCoT	SCoT and ISSR+SCoT
t	27.1939	38.8044	46.0225
p	1.0000	1.0000	1.0000
r	0.5807	0.8480**	0.9237**

Note: ** Correlation analysis reached highly significant level

UPGMA Dendrogram Analysis

The genetic diversity of 75 sugarcane parental clones was assessed using SCoT+ISSR markers, and a cluster analysis was performed based on the genetic similarity coefficient (Fig. 3). From UPGMA cluster analysis, 75 sugarcane parental clones were classified into two main cluster groups with a similarity coefficient of 0.67. Group A primarily comprised 42 parental clones containing all Q-, CP- and HoCP-series; all YT-series; and three *E. arundinaceus* F_4 innovative parental clones. Group B comprised 33 parental clones containing all TT- and GT-series, three *S. officinarum* species, QZ, and GZ. The parental clones in Group A could be further divided into three subgroups (A_1, A_2, and A_3). Subgroup A_1 comprised all Q-, CP- and HoCP-series (except for HoCP92-648 alone in subgroup A_3); and YC71-374, N:Co310, N:Co376, QZ08-688, QZ08-1497, TH10 and HoTH49. Subgroup A_2 comprised the YT-series and all *E. arundinaceus* F_4 innovative parental clones. Group B could be further divided into four subgroups (B_1, B_2, B_3 and B_4). Subgroup B_1 contained ROC22 and all GT-series, YN83-88, NJ57-614, and Brazil 618. Subgroup B_2 comprised all TT-series (except for ROC22 and TT89-1626), QT5, and QT3, and subgroup B_3 comprised TT89-1626, YT08-144, YT07-516, GZ95-108, GZ14 and FN95-1702, P32-2. Subgroup B_4 comprised three *S. officinarum* species.

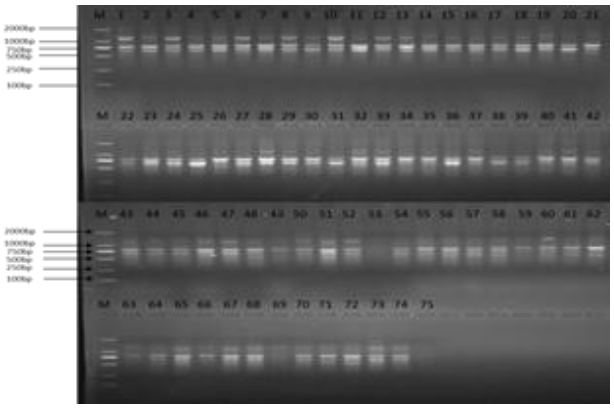

Fig. 1: SCoT amplification profile of primer SCoT28
Note: Lane M: DNA molecular standards with length (bp) on left. Lanes 1–75: 75 sugarcane samples (1–75) in Table 1

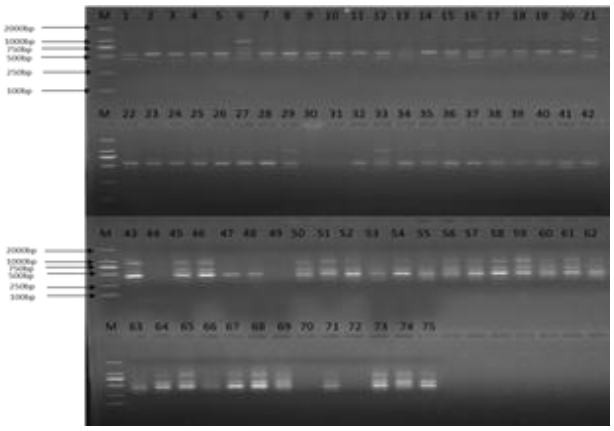

Fig. 2: ISSR amplification profile of primer ISSR880
Note: Lane M: DNA molecular standards with length (bp) on left. Lanes 1–75: 75 sugarcane samples (1–75) in Table 1

Principal Coordinate Analysis

PCA based on the genetic similarity matrix yielded by SCoT, ISSR, and SCoT+ISSR was performed to further understand the genetic relationship and diversity among the tested 75 sugarcane parental clones. The relationship among samples is represented by the positions shown in Fig. 4, which shows the distant or close genetic relationships.

Seventy-five sugarcane parental clones were divided into two groups (I and II) based on the SCoT+ISSR data set (Fig. 4). Compared with the results of the UPGMA dendrogram, group I had an increase in Guangdong yellow and Yunnan red, but a decrease in TH10 and HoTH49; the remaining parental clones was exactly the same as that of group A of the UPGMA dendrogram. Group II had a decrease in Guangdong yellow and Yunnan red but an increase in TH10 and HoTH49, with the remaining parental clones consistent with that of group B of the UPGMA dendrogram.

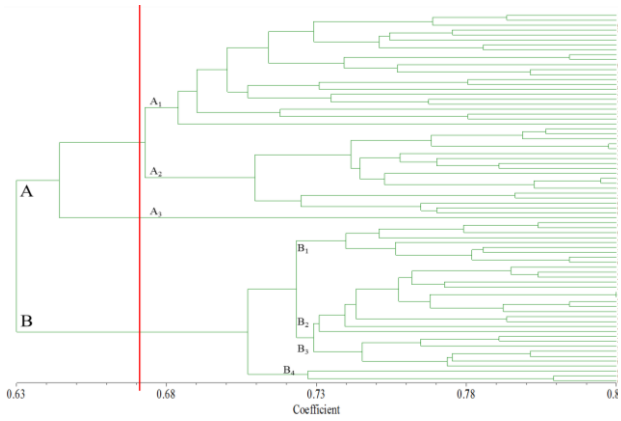

Fig. 3: Unweighted pair group method with arithmetic mean dendrogram showing relationships among sugarcane parental clones based on genetic similarities of DNA fingerprinting patterns from SCoT and ISSR markers

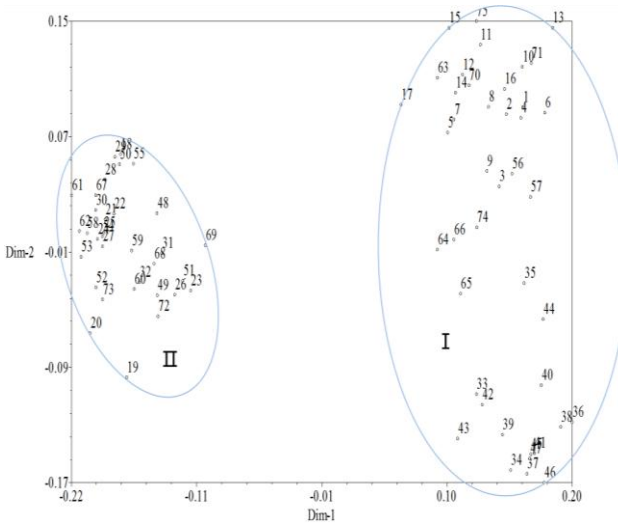

Fig. 4: Principal coordinate analysis of 75 sugarcane parental clones based on SCoT and ISSR markers

PCA and UPGMA, used in the analysis of the genetic relationship and diversity among the tested sugarcane parental clones, produced generally similar results. The principal coordinate analysis showed that the classification position of different sugarcane parents can directly reflect the genetic relationship among different sugarcane parental clones, and could be regarded as an intuitive interpretation of clustering results.

Discussion

Each molecular marker has its own advantages and disadvantages, and the combination of two markers can focus on the advantages of them to extensively cover different regions of the entire genome, thus achieving a

more reasonable and effective result (Rachayya *et al*., 2012; Feng *et al*., 2015). In the breeding research on many crops, both single markers or a combination of different markers were used (Kim *et al*., 2002; Cai *et al*., 2014), but in the research on the genetic diversity of sugarcane, the SCoT or ISSR marker was usually used alone (Rachayya *et al*., 2012; Que *et al*., 2012; Hsie *et al*., 2015), and the use of SCoT+ISSR has not yet been reported. In this study, using the correlation between two simple matching coefficient values resulted in a low correlation between SCoT and ISSR data (r = 0.5807), indicating that the two markers could reveal the genetic diversity of sugarcane from different aspects of the genome, and that the correlation between them, although not significant, was highly complementary. Comparing the similarity coefficient matrices obtained by the integration of SCoT+ISSR, SCoT and ISSR using the Mantel test, a high correlation coefficient was observed between the integrated data with SCoT (r = 0.9237) and ISSR (r = 0.8480), demonstrating that the information revealed by the SCoT+ISSR markers was more accurate; therefore, the integration of the SCoT and ISSR markers, in theory, was expected to decrease the effect of their independent inaccuracies and be more effective in detecting genomic variation and in realizing a better or more complete analysis of genetic diversity by covering different regions of the entire genome. This conclusion was similar to that of previous reports (Li *et al*., 2011; Luo *et al*., 2013; Rachayya *et al*., 2012). This is the first report to integrate SCoT and ISSR data to elaborate on the genetic relationships and diversity among sugarcane parental clones, which can help reveal the genetic diversity of sugarcane parental clones from different aspects, and provide a more scientific basis for the establishment of an effective system for the evaluation of genetic diversity in sugarcane.

The genetic similarity coefficient detected by SCoT markers ranged from 0.524 to 0.850 (mean, 0.687), while the genetic similarity coefficient detected by ISSR markers ranged from 0.452 to 0.863 (mean, 0.658). Similarly, the genetic similarity coefficients ranged from 0.542 to 0.831 (mean, 0.687) based on the SCoT+ISSR data set. There was a wide range of genetic similarity in this study, which showed that genetic diversity of the tested parental sugarcane clones was abundant; these results concur with those of Que *et al*. (2014). However, the results of cluster analysis based on this study showed that genetic similarity was higher among sugarcane parental clones bred by the same breeding organization owing to their close ancestry relationship and narrow genetic basis. The genetic basis of sugarcane varieties bred by the same breeding organization was similar and may be related to less parental regeneration (Rachayya *et al*., 2012; Que *et al*., 2014); therefore, it is necessary to accelerate parental regeneration and expand the genetic differences of parental combinations for breeding breakthrough varieties in the future.

In the present study, the cluster analysis and PCA showed that the Q-series accessions, CP-series, HoCP-series, YT-series, and N: Co-series were grouped into the same cluster A, indicating that they had a close genetic relationship. The CP- and HoCP-series were bred by sugarcane-breeding institutes in America with the advantages of maturing early, having high sugar content, having strong ratooning properties, and being fast growing (Deng and Li, 2007; Shen *et al*., 2008). Since the early 1980s, dozens of CP or HoCP varieties have been introduced and studied by sugarcane breeding organizations in China; however, they are usually directly used as the cross parents rather than as production varieties because of their thin stems. Among all the institutions, the Guangzhou Sugarcane Industry Research Institute used the introduced CP or HoCP varieties as parents and successfully bred sugarcane varieties of the YT-series (Deng and Li, 2007; Wen *et al*., 2014); therefore, the YT-series and the CP- or HoCP-series have a closer genetic relationship or ancestry basis, which is consistent with the results of our study. Co-series varieties bred by Coimbatore Sugarcane Breeding Institute of India are early stage sugarcane varieties that have been widely used as cross parents by many sugarcane breeding institutes throughout the world, while the N:Co-series varieties were bred by Sugarcane Breeding Institute of South Africa with hybrid seeds from India (parents from the Co-series). From the previous studies, we find that the Q-, CP-, and HoCP-series are nearly all offspring of the Co-series (Zhou and Li, 2012; Liu *et al*., 2013). This analysis supports our conclusion that the genetic similarity among series of N:Co, Q, CP and HoCP is high and the ancestry foundation is close.

E. arundinaceus is an important closely related genus of *S. officinarum* (Deng *et al.*, 2010). Interestingly, in the present study, three *E. arundinaceus* F_4 innovation parental clones derived from YC73-226 (female) and YC06-111 (male) clustered into the same subgroup of YT-series rather than into their own single groups. In theory, *E. arundinaceus* F_4 progeny contains only 1/16 of the ancestry of *E. arundinaceus* (Chen, 2003), which means that the content of the ancestry of *E. arundinaceus* in three *E. arundinaceus* F_4 innovation parental clones is rather low. In addition, their parents, YC73-226 and YC06-111, are rich in the Co- and CP-series ancestry (Zhang, 1996; Liu *et al*., 2011) and the YT-series is also rich in the Co- or CP-series ancestry (Deng and Li, 2007; Wen *et al.*, 2014); therefore, it is understandable that the genetic relationship between the three *E. arundinaceus* F_4 innovation parental clones and the YT-series is relatively close.

The parents of sugarcane hybrids have strong differences or heterogeneity, which can be conducive to the breeding of excellent varieties (Rachayya *et al*., 2012; Que *et al*., 2014). The findings in the present study show a close genetic relationship among varieties bred in the same breeding institutions, while there is some degree of genetic difference among varieties bred by different breeding

institutions. Meanwhile, there was a large degree of genetic difference between the YT-series and TT-series or GT-series; therefore, we suggest that it is important to cross breed using parents from different breeding institutions to improve the genetic diversity of the species in the sugarcane breeding programs of China.

Sugarcane cultivar ROC22 was bred by Chinese Taiwan Sugar Industry Research Institute and introduced to sugarcane areas in Southern China in the 1990s. Over the past 10 years, ROC22 has been developed into a main cultivar with the largest annual planting area, accounting for >60% of the total sugarcane planting area in Mainland China (Zhou *et al*., 2012; Li and Yang, 2015). In this study, we found that cultivar ROC22 is genetically distant from any other ROC-series and constitutes a unique genetic basis. ROC22 (ROC5 × 69-435) and ROC10 (ROC5 × F152) are the offspring of cultivar ROC5, but ROC22 clusters in subgroup B_2, which is different from its female parent ROC5 and half-sisters ROC10 (subgroup B_1), indicating that the genetic differences between ROC22 and ROC5 or ROC10 were relatively large. This indicates that the choice of male plant was equally important as the choice of female plant in selecting and combining breeding parents, and that we should select parents that have a large heterogeneity and complementary traits for hybridization in sugarcane breeding in the future (Santos *et al*., 2012; Que *et al*., 2014).

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