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Pedigree, marker recruitment, and genetic diversity of modern sugarcane cultivars in China and the United States

Huiling Liu · Xiping Yang · Qian You · Jian Song · Liping Wang · Jisen Zhang · Zuhu Deng · Ray Ming · Jianping Wang

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Abstract Sugarcane (*Saccharum* spp) is an important crop for both sugar and biofuel production. However, the sugarcane breeding process has resulted in modern sugarcane cultivars with a narrow genetic basis. To broaden the genetic basis and promote international collaborations in sugarcane cultivar development, we documented the peidgrees of representative sugarcane culativars widely used in China and the United States of America (USA), recruited more than six thousand simple sequence repeat (SSR) markers for sugarcane, and assessed the genetic diversity and relationships beween representative sugarcane cultivars and their potential ancestry accessions. The SSR gentoyping results indicated that both the USA and Chiniese cultivars had low genetic diversity, specifically the Chinese cultivars. The USA sugarcane cultivars experienced high presure of selection for sugar content as they had the closest relationship with *S. officinarum*, followed by Chinese cultivars, *S. robustum,* and *S. spontaneum*. The sugarcane accessions assessed could be divided into five and four groups through cluster and principal component analysis, respectively. *S. spontaneum* as a potential ancestor contributing to the stress tolerance of sugarcane cultivars was grouped into distinct clusters, and *S. officinarum* was grouped with sugarcane cultivars in both countries. *S. robustum* did not seem to contribute to the sugarcane cultivar development in China, but may have contributed to the USA cultivar development. This study not only provided a

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H. Liu · J. Zhang · R. Ming · J. Wang (✉)
Center for Genomics and Biotechnology, Key Laboratory of Genetics, Breeding and Multiple Utilization of Corps, Ministry of Education, Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China
e-mail: wangjp@ufl.edu

H. Liu
e-mail: huilingliu@foxmail.com

J. Zhang
e-mail: zjisen@126.com

R. Ming
e-mail: rayming@illinois.edu

X. Yang · Q. You · J. Song · L. Wang · J. Wang
Agronomy Department, University of Forida, Gainesville, FL 32610, USA
e-mail: xipingyang@ufl.edu

Q. You
e-mail: youqian@ufl.edu

J. Song
e-mail: jsongbio@gmail.com

L. Wang
e-mail: lwang2@ufl.edu

collection of easy to use SSR markers, but also detailed genetic diversity and relationship among the cultivars in the two counties, which will be referable to promote international collaboration and broaden the genetic basis of sugarcane cultivars.

Keywords Sugarcane · *Saccharum* · SSR · Genetic diversity · Pedigree

Introduction

Sugarcane (*Saccharum* spp) belongs to the genus *Saccharum L.*, Andropogoneae tribe in the grass family (Poaceae). As a highly efficient photosynthetic C4 plant, sugarcane is not only an important sugar-bearing crop by providing 75% sugar worldwide, but also an efficient biofuel crop for bio-ethanol production, accounting for ~ 60% of global bio-ethanol production (Dahlquist 2013). It is currently grown in more than 100 countries, mainly in tropic and some sub-tropical areas.

The *Saccharum* genus is characterized as polyploid and aneuploid, thus it is one of the most complex crops for genetic studies. Classical taxonomy initially considered that the *Saccharum* genus consisted of six species, including *S. spontaneum*, *S. officinarum*, *S. robustum*, *S. edule*, *S. barberi*and *S. sinense* (D' Hont et al. 1998). A further cytogenetic investigation indicated that the *Saccharum* genus includes only two species, *S. officinarum* and *S. spontaneum* (Irvine 1999), which was further supported by the genetical characterization of the world germplasm collection (Nayak et al. 2014). These two species possess a large degree of different characteristics. *S. officinarum,* known as noble cane, can accumulate a large amount of sucrose in the stem, but has poor stress tolerance. In contrast, *S. spontaneum* has high fiber content and stress tolerance, but accumulates little sucrose (Sreenivasan and Ahloowalia 1987). Both species are remarkably different in genome composition. *S. officinarum* has a basic chromosome number of 10 and is an autopolyploid (2n = 80), while *S. spontaneum* has a basic chromosome number of 8 with a wide range of chromosome numbers from 2n = 40 to 128 (Sreenivasan and Ahloowalia 1987).

Modern sugarcane varieties were mostly derived from interspecific hybridization of *S. officinarum* and *S. spontaneum* (Bull and Glasziou 1979) followed by several rounds of backcrossing to *S. officinarum*, since the discovery of sexual fertility in 1888 in Java (Lu et al. 1994). *S. officinarum* as the recurrent female parent provides most genetic background related to sugar accumulation (Sreenivasan and Ahloowalia 1987) with *S. spontaneum* as donor parent providing the stress tolerance. Therefore, modern sugarcane cultivars as interspecific hybrids are highly polyploid and aneuploid with a chromosome number ranging from 100 to 130 (Bremer 1962), and are typically are comprised of approximately 10% chromosomes from *S. spontaneum*, 80% chromosomes from *S. officinarum,* and 10% recombinant chromosomes (D'Hont et al. 1996). The relatives of *Saccharum* genus, such as *Erianthus giganteus* (previously *S. giganteum*) and *Sorghum,* may also have contributed to some modern sugarcane cultivar development (Cordeiro et al. 2003).

Sugarcane has a long history of cultivation in China, the third largest sugarcane producer after Brazil and India. Currently, sugarcane is distributed in 12 provinces in southern China, supplying about 90% of the sugar in China (Luo et al. 2012). One argument proposed that sugarcane was introduced into China during the Zhou dynasty, approximately 3000 years ago (Yang et al. 2014). Additional pieces of evidence obtained from China archaeological finds revealed that China may be one of the centers of origin for sugarcane diversity (Daniels et al. 1975). The main sugarcane cultivars in China include ROC-serial, YT-serial, GT-serial, LC-serial, and FN-serial varieties. ROC-serial varieties have been used as the main planting varieties, occupying 85% of planting areas in China, of which 50-60% area grows only ROC22, a popular ROC-serial cultivar (You et al. 2013).

In the North America, sugarcane was introduced by the explorer Christopher Columbus in 1493 from New Guinea, where sugarcane originated (Parker 2011). The United States of America (USA) ranks 10th in sugarcane production in the world and produced ~ 28.0 million metric tons of cane with a

Q. You · Z. Deng
Institute of Sugarcane Research, Fujian Agriculture and Forest University, Fuzhou 350002, Fujian, China
e-mail: dengzuhu@163.com

R. Ming
Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-3873, USA

gross production value of $909.7 million (FAO 2014). Sugarcane production in the USA has been greatest in Florida, followed by Louisiana and Texas (Baucum et al. 2006). Within mainland USA, recurrent selection principles are mostly employed in sugarcane breeding programs. Breeders identify clones with the highest yield and acceptable stress resistance as parents to make most crosses for new cultivar selection (Todd et al. 2015). More than 90% of the USA mainland sugarcane cultivars can be traced back to only 10 origninal contributing ancestral clones (Deren 1995).

Genetic diversity in the germplasm collection or breeding lines is of incredible value to suscessful breeding programs. Vulnerability of many crops due to a narrow genetic base or similar ancestry has prompted efforts in preservation, utilization of germplasm, and evaluation of genetic diversity in breeding programs (Walsh 1981). Genetic diversity of sugarcane germplasm and cultivars has been evaluated by using different methods including morphological, cytological, biochemical and particularly molecular markers. Relationships among different accessions of the *Saccharum* complex and other related genera including Old World *Erianthus sect. Ripidium,* North American *E. giganteus, Sorghum* and *Miscanthus* were investigated using molecular makers, such as Amplified Fragment Length Polymorphism (AFLP) (Aitken et al. 2005, 2006; Besse et al. 1998), Target Region Amplification Polymorphism (TRAP) (Creste et al. 2010; Que et al. 2009), Restriction Fragment Length Polymorphism (RFLP) (Daugrois et al. 1996; D'Hont et al. 1993, 1994; Lu et al. 1994; Grivet et al. 1996), 5S rRNA ITS Marker (5sRNAITS) (Glaszmann et al. 1990; Pan et al. 2003), Random Amplified Polymorphic DNA (RAPD) (Madan et al. 2000; Mudge et al. 1996; Chen et al. 2003), Simple Sequence Repeat (SSR) (Aitken et al. 2005; Cordeiro et al. 2000, 2003; Nayak et al. 2014; Pan et al. 2003; Pinto et al. 2004) and Single Nucleotide Polymorphism (SNP) (Cordeiro et al. 2006). Of these molecular markers, SSR is relatively a simple, cheap, highly reproducible, and sensitive technique to investigate genetic diversity and has been reported as an efficient tool for screening and evaluating germplasm collections to provide information for breeding programs (Cordeiro et al. 2003).

Despite the existence of the World Collection of Sugarcane and Related Grasses (WCSRG), the genetic diversity of breeding materials in the sugarcane breeding programs is quite low (Tai et al. 2001; Nayak et al. 2014; Todd et al. 2014). To further promote the germplasm exchanging and utilization to thus expand the genetic basis of sugarcane breeding programs, genetic diversity evaluation of different programs across countries will provide fundmental information for genetic source selecton for long term plans of international collaboration. The objectives of this study were to 1) compare different pedigrees of sugarcane cultivars in the USA and China; 2) recruite and select a set of highly polymorohic and representative SSR markers for sugarcane breeding programs; and 3) assess the genetic diversity and similarity of sugarcane cultivars in the USA and China.

Materials and methods

Plant materials

In total, 48 sugarcane accessions (Table 1) were used in this study, including one accession of *S.officinarum* and five accessions of *S. robustum* grown in the sugarcane germplasms nursery, Yunnan, China, 12 widely grown sugarcane cultivars in China, maintained in the experiment field at the Sugarcane Research Institute, Fujian, Agriculture and Forestry University, Fuzhou, China, 10 widely used sugarcane cultivars from the United States, 10 potential ancestral germplasm accessions of *S. spontaneum* and 10 potential ancestry germplasm accessions of *S.officinarum* in WCSRG. The ancestry germplasm accessions were selected based on pedigree analysis of the common cultivars in both countries and particularly based on the previous publications on sugarcane germplasm evalutions to make sure they are prepresentitive.

Pedigree structure organization

Pedigree structure of the common cultivars in China and the USA were prepared following the principle of female parents at the left and male at the right. The relationship between cultivars and parental materials were retrieved from literature papers (Lao et al. 2008; Liu et al. 2011; Todd et al. 2015; Wen et al. 2014).

DNA extraction

Sugarcane genomic DNA was extracted from leaf tissues according to the CTAB method with minor

Table 1 Materials used in the experiment

No.	Clone name	Species name	Geographical Origin
1	Henry Creek Spont	*S.spontaneum*	Unknown
2	Coimbatore	*S.spontaneum*	Coimbatore, india
3	Tainan	*S.spontaneum*	Unknown
4	Djantoer II (2)	*S.spontaneum*	Unknown
5	PQ 84-unknown3	*S.spontaneum*	Unknown
6	S 66-unknown84	*S.spontaneum*	Unknown
7	S 66-121	*S.spontaneum*	Unknown
8	Kletak	*S.spontaneum*	Unknown
9	Moentai	*S.spontaneum*	Unknown
10	Narenga	*S.spontaneum*	Unknown
11	Yellow Caledonia	*S.officinarum*	Unknown
12	Louisiana Purple	*S.officinarum*	United States
13	Black Cheribon	*S.officinarum*	Australia
14	White Transparent	*S.officinarum*	Tamil, Nadu, India
15	Vellai	*S.officinarum*	Unknown
16	Muntok Java	*S.officinarum*	Unknown
17	Kassoer hybrid	*S.officinarum*	Unknown
18	Mialan	*S.officinarum*	Unknown
19	Falsac	*S.officinarum*	Unknown
20	Loe Thres	*S.officinarum*	Unknown
21	LCP 85-384	Cultivar_1	United States
22	HoCP 96-540	Cultivar_1	United States
23	L 99-226	Cultivar_1	United States
24	L 99-233	Cultivar_1	United States
25	CP 72-2086	Cultivar_1	United States
26	CP 80-1743	Cultivar_1	United States
27	CP 89-2143	Cultivar_1	United States
28	CP 78-1628	Cultivar_1	United States
29	L 79-1002	Cultivar_1	United States
30	CP 88-1762	Cultivar_1	United States
31	Badila	*S.officinarum*	Unknown
32	Fujian Daye	*S. robustum*	China
33	Daye1	*S. robustum*	China
34	Daye2	*S. robustum*	China
35	51 NG63	*S. robustum*	China
36	51 NG3	*S. robustum*	China
37	ROC22	Cultivar_2	China
38	ROC25	Cultivar_2	China
39	ROC16	Cultivar_2	China
40	YT83-271	Cultivar_2	China
41	YT60	Cultivar_2	China
42	YT93-159	Cultivar_2	China
43	YT94-128	Cultivar_2	China
44	GT29	Cultivar_2	China
45	GT21	Cultivar_2	China

Table 1 continued

No.	Clone name	Species name	Geographical Origin
46	LC05-136	Cultivar_2	China
47	LC03-182	Cultivar_2	China
48	FN39	Cultivar_2	China

modification (Wang et al. 2010). The integrity and quantification of the genomic DNA were checked using 1% agarose gel and Nanodrop 2000 spectrophotometer in comparison with commercial lamda DNA. The highly integrated DNA samples with more than 300 ng yield were used for SSR genotyping after diluted to 2 ng/μl working solution.

Sugarcane SSR marker recruiting and primer sequence alignment to sorghum genome

Sugarcane SSR markers were recruited by literature sesarch using the key words "sugarcane" and "SSR" in Google Scholar. The SSR markers with sucessful amplification were identified from literatures and the primer sequences of those SSR markers were retrieved. The redundant SSR primer pairs were excluded from the final SSR primer list. The unique SSR primer sequences were aligned to sorghum genome v 3.0 (Paterson et al. 2009) by using Bowtie (Langmead et al. 2009) with paired-end alignment. The allowed mismatch was set to two bases and the insert size for valid paired-end alignments ranged from 100 bp to 1000 bp.

SSR genotyping

A 12 μl PCR reaction volume contained 4 ng genomic DNA, 1 unit of GoTaq G2 Hot Start Colorless Master Mix (Promega, Madison, Wisconsin, USA) and 2 μM of each forward and reverse primers. The amplification reactions were performed following a program of 95 °C for 5 min; 10 cycles of 95 °C for 1 min, annealing for at 65 °C for 30 s, 72 °C for 30 s; and then 29 cycles of 95 °C for 1 min, annealing for 1 min at 55 °C, 72 °C for 0.5 -1 min; followed by a final elongation at 72 °C for 5 min. PCR products were separated by 9% non-denaturing polyacrylamide gel electrophoresis (PAGE) and stained with GelStain (TransGene Biotech Co., Ltd, Beijing, China) according to the modified staining protocol as described by Nayak et al. (2014) and Fountain et al. (2011). A 100 bp DNA ladder (New England Biolabs Inc.) was used to determine the size of the generated fragments. The strong bands of each DNA fragment amplified were scored as 1 for presence or 0 for absence for further data analysis.

Genotypic data analysis

After scoring in a dominant manner, each allele was transformed into a 0-1 matrix as an input file. Based on genetic similarity coefficients calculated by NTSYS-pc 2.11 W software (Numerical Taxonomy and Multivariate Analysis System) (Rohlf 2002), a dendrogram was conducted using an unweighted pair group average (UPMGA) method (Backeljau et al. 1996). Major allele frequency, number of polymorphic bands, gene diversity and polymorphism information content (PIC) were carried out by using PowerMarker V3.25 software (Liu et al. 2005). GenAlEx V6.5 software was used to calculate Nei's genetic identity and Principal Coordinates Analysis (PCoA) (Peakall and smouse 2006, 2012). All these operations used the default parameters for the particular software.

Results

Pedigree of major cultivars in the USA and China

The pedigreesof major cultivars in the the USA and China are shownrespectively in Fig. 1. ROC22 is not only the most widely grown commercial cultivar but is also frequently used as parental material for large numbers of crosses in China. For example, ROC22 was the female parent of LC 05-136 and the male parent of GT 02-761(Fig. 1a). The similar situation was noticed in the USA, CP 72-1210, which was not just a successful cultivar, but was also used as the female parent of YT 83-271and LC 03-182, and as the male parent of YT 93-159. As one of the most

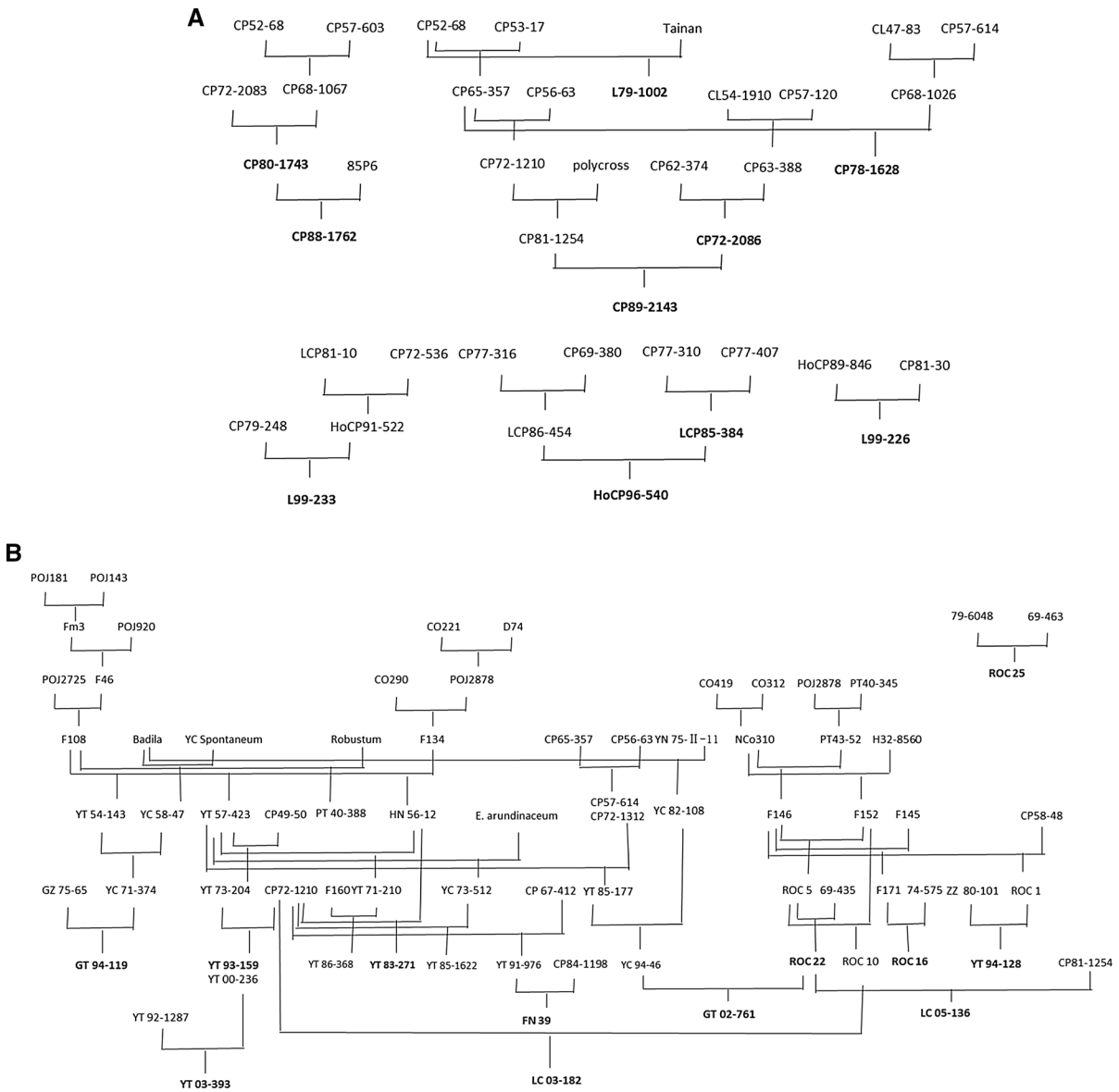

Fig. 1 The pedigree diagram of US cultivars (**a**) and Chinese cultivars (**b**). Cultivar names with bold font were included in this study. Note: YT: Yue tang; GT: Gui tang; LC; Liu cheng; YC: Ya cheng; HN: Hua nan; FN: Fu nong; GZ: Gan zhen; ZZ: Zhan

successful parents, F108 contributed genetic background to many Chinese released cultivars, including GT 94-119, YT 93-159, YT 03-393, YT 83-271 and GT02-761. F146 was the other most successful parent, contributing to ROC22, ROC16, YT94-128, LC03-182, LC05-136 and GT02-761. Several USA cultivars have been used as parental lines for developing Chinese cultivars. For example, the female and male parents of FN39 were derived from CP 72-1210, CP 67-412, and CP 84-1198.

The 10 USA cultivars in this study were mostly derived from crosses among CP- series clones, which have originated from breeding programs in either Louisiana or Florida, the two largest sugarcane producing states in the USA with distinct breeding programs in each state (Fig. 1b). Specifically, CP 80-1743 was the female parents of CP88-1762. CP89-2143 was the progeny of CP72-2086 and the grand-progeny of CP 72-1210. The male parent of HOCP96-540 was LCP85-384. CP 72-1210 and CP 78-1268 had

the same female parent, CP 65-357. The female and male parents of L99-233 and L99-226 were CP- series and HOCP- series of the USA cultivars. L79-1002 was produced by the cross of cultivar CP 52-68 × Tainan (*S. officinarum*).

Sugarcane SSR marker recruiting and their location according the sorghum genome

A total of 6,837 SSR markers were documented from 12 literature papers and the International Consortium for Sugarcane Biotechnology (ICSB) (Table 2). After removing the redundant SSR markers, 6,149 unique sugarcane SSR markers were identified (Table S1). Of the 6,149 pairs of SSR primer sequences, 1,151 (18.7%) SSR primer sequences were aligned to the sorghum reference genome with less than three base mismatches (Fig. 2). There are 229, 132, 179, 129, 33, 96, 76, 80, 117 and 80 SSR primers corresponding to sorghum chromosomes 1 to 10, respectively (Table 2). SSRs are mainly located at the two ends of each chromosome. The average SSR density is 1 SSR/500 kb (Table 3). These SSRs mapped along sorghum chromosomes can serve as a reference map given that the sugarcane genome is not available yet. Based on the reference map, 100 SSR markers (Table 4) with 10 SSR markers on each sorghum chromosome were selected for further genetic diversity analysis between ancestry species and sugarcane cultivars.

SSR genotyping and marker performance

A pilot experiment involving six genotypes with significantly different morphology was conducted to evaluate the 100 sugarcane SSR markers to select the highly polymorphic ones (Fig. S1). The six genotypes included one *S. spontaneum* accession (Tainan), one *S. officinarum* accession (Yellow Caledonia), one US cultivar (CP72-2086), two Chinese cultivars (ROC16 and ROC22), and one *S. robustum* accession (51NG3). A smaller set of 20 'core' SSR markers, two per sorghum chromosome was then selected based on two criteria: (1) More than three bands amplified; 2) Reliable amplification in repeated experiments.

The 20 highly polymorphic SSR markers were then used to genotype 48 sugarcane accessions (Table 5) with band profiles documented for each accession (Fig. S2). In total, 310 robost bands with an average of 15.5 bands per marker were generated from 48 sugarcane accessions. Marker SCESSR0308 located on sorghum chromosome 1 produced the most number of bands (25), while SCESSR0429 located on sorghum chromosome 6 amplified the least number of bands (9) (Table 5). The major allele frequency of the 310 alleles ranged from 0.5 to 1 with an average of 0.87. The gene diversity ranged from 0.04 to 0.5 with an average of 0.2 (Table 5). The PIC values of the 20 SSR markers varied from 0.04 to 0.37, with an average of 0.17 (Table 5). Based on the source and species, the 48

Table 2 Sources of collected sugarcane SSRs

Entry	Marker Type	Number	Source
1	SSR	78	Aitken et al. (2005)
2	EST-SSR	30	Pinto et al. (2004)
3	SSR	7	Cordeiro et al. (2000)
4	EST-SSR	41	Palhares et al. (2012)
5	SSR	19	Andru (2009)
6	SSR	68	Pan et al. (2006)
7	SSR	10	Wang et al. (2010)
8	SSR	15	Chen et al. (2010)
9	SSR	78	Singh et al. (2010)
10	SSR	17	Liu et al. (2011)
11	SSR	49	Parida et al. (2010)
12	EST-SSR, SSR	6132	James et al. (2012)
13	SSR	293	International Consortium for Sugarcane Biotechnology
	Total	6837	
	Unique Markers	6149	

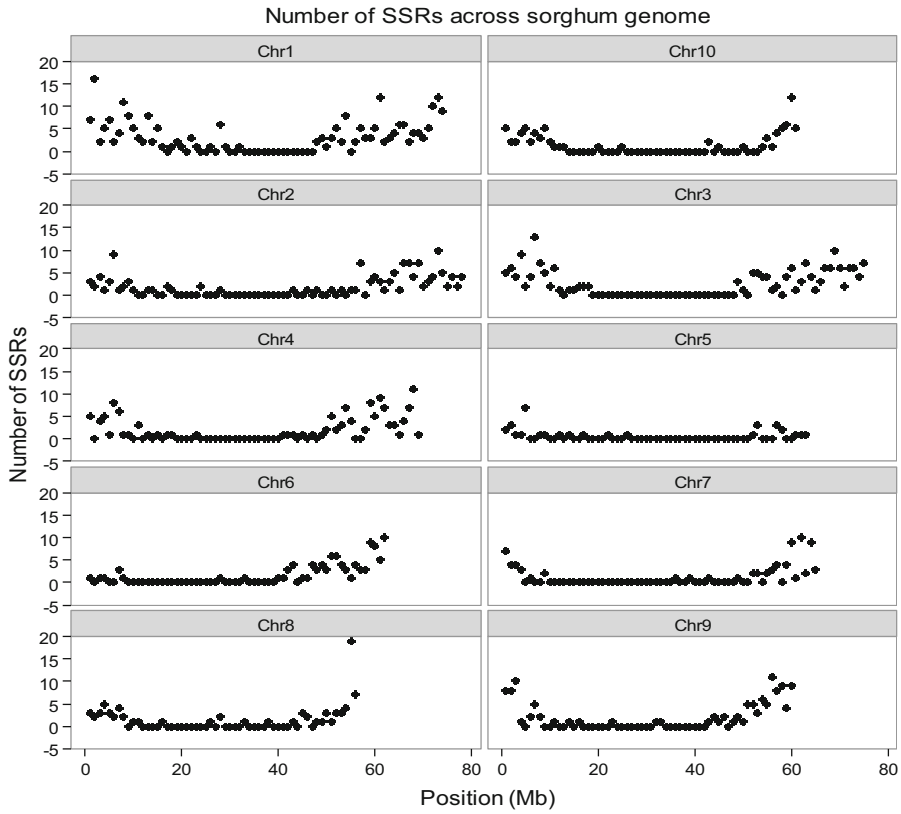

Fig. 2 The distribution of SSRs across 10 sorghum chromosomes

Table 3 Distribution of sugarcane SSRs according to sorghum genome

	Sorghum chromosome									
	1	2	3	4	5	6	7	8	9	10
Number of mapped SSRs	229	132	179	129	33	96	76	80	117	80
Chromosome size (Mb)	73.8	77.9	74.4	68	62.3	62.2	64.3	55.5	59.6	61
SSR density (SSR/Mb)	3.1	1.7	2.4	1.9	0.5	1.5	1.2	1.4	2.0	1.3

accessions were separated into five groups: 1) Chinese sugarcane cultivars including 12 accessions, (2) the USA sugarcane cultivars with 10 accessions, (3) *S. robustium* of 5 accessions, (4) *S. officinarum* of 11 accessions, and (5) *S. spontanum* of 10 accessions. The Chinese sugarcane cultivar group had the highest gene frequency (0.91), while the other groups had the gene frequency of 0.89 (Table 6). The gene diversity among the five groups ranged from 0.13 (Chinese cultivars) to 0.16 (*S. spontaneum* and *S. officinarum*) (Table 6).

Genetic identity analysis

Genetic identity between the five groups was assessed by Nei's genetic identity (Table 7). Pairwise genetic identity among the five groups ranged from 0.517 to 0.808. The highest genetic identity value of 0.808 was found between the *S. officinarum* accessions and the USA cultivars. The second highest genetic identity value of 0.762 was observed between the *S. officinarum* accessions and Chinese cultivars. The least genetic identity value of 0.517 was observed between the *S. spontaneum* accessions and the *S. robustum* accessions. The genetic identity value between the USA cultivars and Chinese cultivars was 0.760. The genetic identity between the *S. robustum* accessions

Table 4 Selected 100 sugarcane SSR primers according to the constructed reference map

Primer	Chr.	Location (kb)	Forward primer	Reverse primer
SCESSR0177	1	279	TCCCCAAAACAAAACCCTAGC	AGCGGAGGAACCGAGGAG
UGSuM281	1	4556	TTTACTGGAGAACCACCTGA	GGGAAGACCATCACATCC
SCESSR2333	1	6789	GTACGGCTGGGGCACGTACT	GTGGTGCAGGTCCCTTACCC
UGSuM56	1	9950	TAATACTTTCACCAGCCAA	GGAGCAGCAACGCACAGG
SCESSR0308	1	15,945	AGTACTACCAGGCGGGGCAC	GATCCCCAATCCAGAGGGTC
LAPSSR0147	1	52,654	TAGCTGTATCTGGAACTTGTAG	AATGTGTTACTATGGAGGATGTC
SCESSR0425	1	62,941	AGGGAGAGAGGAAAGGACCG	TTCATGACTGGTGCGCTCAT
SMC1120HA	1	65,199	TTCGTAGCATCCCTGTTCG	CATGGGACAGAGATTACAAGGC
SCESSR2566	1	68,327	TGGTAAATTCGACGTGTCTTCTGA	TGCAAAATTCATCTGCATCCC
UGSM629	1	70,240	CAAGAACCGCCTCCTCTC	TTCCAACCAACAGACACAG
SMC662CS	2	1321	GACTGCATGGCTTGCTGATCG	GGACCTTGGCGGTGATGGG
LAPSSR1206	2	27,088	GGAAACAAGTGGTGGTGGTG	TCTGTCAGCACAGGTTCATC
SCESSR1334	2	52,004	ACCAACCCAGCCTCGTCAT	TCGTAGAAGCGGTAGGCGG
SCESSR0865	2	55,214	TGGAGGAAGTACGGCCAGAA	TTCGATTCCACTTGGGAGGA
mSSCIR37	2	66,456	ATTCTGTCTGTCGTTCTCC	ACTTTCTTGGTTCTTCATA
SCESSR2288	2	68,224	GGGCAAAGTGTATCGGCATC	AGCAGCTAGAGAGGCCCCTAA
SCESSR0983	2	68,677	CGGTCGGTGCACATACAGAG	GATGCACCTAGCATCACCGA
mSSCIR31	2	70,227	ATTTGGGTAAGGATGGAT	CCTAATGATACGCTTTGA
UGSM60	2	73,798	CGACTCCACACTCCACTC	CCGAACACCACCTTCTTG
UGSM690	2	76,024	ATCTATCGGTCTTCTGGAGATT	CACTTCCTCCTTATTATACCACTT
SMC519MS	3	697	CGATGGACGCCAATGCAA	GTGCCGCCGCACCTCATA
SCESSR1017	3	1399	AGCGGTTACAGCCAAGCTCA	CAGAAACCTGGCCAAGCAGT
UGSM399	3	6855	TACTATAATGATAGATCTCCTCCG	GTAATAGGACTGGATTGGAATG
mSSCIR11	3	53,200	CCACCATCTTTTCGCACCAG	GCAGCACCAACCATAATCAT
SCESSR0583	3	63,685	CTCGATGATGCATCCGCTC	TACTCGTAGTCCCCCACCCC
SCESSR0573	3	65,296	GAGGAGGAGGCGGAGGACT	GGGTTGAAGGACCCGAACTT
SCESSR1133	3	69,954	CAGGGAGCAGCAGCAGAAAC	GCCATGTAGCCCCGGAAC
SCESSR2281	3	70,834	GGCTTTGAGACTGAGGTCAAGTG	GAATCTTTGCGCCTGCAGAT
SCESSR1743	3	72,765	CCCTCTTCCTCCGCTCTTCT	GGTTGACATCGAACGGCCTA
UGSuM150	3	74,338	ACACTGACCGATGGATCCTCTT	ATCAACGTGGACCAGATCTTCTT
SCESSR1978	4	3897	CTTCCTCGCCTCCCCCTC	GCAAGCAGGATCCGGTAGGA
SCESSR1360	4	7159	ACCTTCAGGAGGTCGGGCT	CAGCACCAGCAGCTTCCTCT
LAPSSR0648	4	22,731	GGAGAGGACGGAAGGCAATG	CCGTAGAGGAGGTCGTCAATGTT
SCESSR2456	4	42,960	TCCTCGCATCTCGATCCATT	GGACCACCTCTGTTGCGTTC
SCESSR1845	4	49,252	CGGCGGATCGAGATCTACAC	CCTCCACCTCCACCTTTCCT
SCESSR0607	4	53,666	TAGCCAGGCAGGAGATGGAG	GTGATGAGCTCCTCGTCGGT
MCSA116D08	4	57,529	CAGTCGCCCCACACGCCGAT	CCATGCTGTCGCCGACCACG
mSSCIR65	4	58,450	ACGGGCTGGAGGAAGGA	AAATCAGGGTCACGAGTTCA
SCESSR2177	4	63,107	GCGGGCAGAACACTAACCAC	CTGCGCTCCATTTCCATTTC
MOLSSR1941	4	67,555	CTACAAAATGGAAGCAGGGAAGTG	GAGGTCAGAATGGGATGATGAGAC
SCESSR2185	5	1480	GCTAATTGCAGAGATGTCGCC	TTCCCTGAAAGTTAGGGATCACA
SCESSR0069	5	2717	GAGCAAGGCAAGGCTAAGCA	GAGTTTGGTGGCTGCTGTCC
SMC1572CL	5	4335	GAGGATATGGTTTTCATTGCC	ACACCTTCTCACCACTTAGGTTC
ESTB100	5	4828	CCACGGGCGAGGACGAGTA	GGGTCCTTCTTCGCCTCGTG
LAPSSR1144	5	8492	TGCAAAAACACTCCAATGACTTGT	GTTCATGACACTTTGACTCGATGG

Table 4 continued

Primer	Chr.	Location (kb)	Forward primer	Reverse primer
LAPSSR1205	5	16,845	CAGCAGCTTCCATATTGCCTACTC	ATCAATGGACTCAAACCAGAAACC
SMC528MS	5	25,823	CCCTGCACCTCCTTGAGACTA	CCGAAGTGCTTGTAGTAGGGGT
LAPSSR0021	5	56,586	AACACCGGTGCTCTGCTTC	GTGCCGTGCCTTGACATCGA
SCESSR2313	5	57,866	GGTGAACCCTACCGCCTACC	GTTCGCCACGGCTAGTTGAC
LAPSSR0640	5	62,278	CAGGTACTACTACTGCCGGCTCAG	AGAGCTTGTTCTTGCTCTCCTTGA
SCESSR0612	6	2645	CTCCTCGCTCTTCTCCCACC	CCCTCCGTCACCTTGTTCAG
LAPSSR0173	6	7156	ATTGAACCGAAGAAGGAGAAG	ATCAGGCTCTGTAGCACATAG
SCESSR0429	6	42,781	GTCCCAAGTGAGTGGCAAGG	GAGCAGCACCACCAGCAGTA
UGSuM197	6	48,130	GAAGGAGCAGCAGCGCCAGT	GATTTGCCGTCCTAGGGTTT
SCESSR2573	6	50,514	TGTCGTCATCGTCTGCACAA	AGCGACTCCTCCAGCTCCTC
mSSCIR46	6	53,173	ATGCTCCGCTTCTCACTC	AAGGGGAAAATGAAAACC
SCESSR0345	6	55,270	CGACCTGCTGGATCTCGG	GAGGACCTCCTCGATGACCA
SCESSR0914	6	58,420	GCCGAAGAAGCATCACCATC	GCTTTCCTATCCGGCGAACT
SCESSR0209	6	58,888	CTCCCTCCCATTCCGATCAT	TGTGCACCTCGTTCCAGAGA
SCESSR1251	6	60,755	AAATTGCAATGGCTGCTGCT	TGCTTCTCCTCCAGCTCCAC
SMC483BS	7	724	GACTGCACACAACCATAGAACAT	CATGTCAATACTTATCCGAGGAA
SCESSR1415	7	1809	AGATGCGGGATCTGGAGGAC	GGCCGGGTAGAAGCCGTAG
SCESSR1665	7	3594	CCAACCCTAGCCAATCCTCC	GTTGTGTCCGTGGTGCCC
SCESSR1364	7	3938	ACGACCTCGTCGAACCCTTT	AGATCGAACCGCTCATCCAG
SCESSR1027	7	5851	GGAGCGGAGGAAGATGATGA	GAGCTCCTCGAAGGAGAGGC
SCESSR0890	7	8872	GCGCCACCACCAACTACAAC	GACCGCTACCGTCACTGCC
MOLSSR2288	7	42,750	TTACTATGGAGGATGTCAACACGG	TGGTATTAGAGGTGTTCCTGGGAT
MOLSSR2576	7	56,580	ATGCGATTGCCATTAGTTGCTAGT	TTGGGAGAATCATTTTTGCATTTC
SC118I15-12a	7	59,234	GTCCCTCCGTCCTGCACATA	TCCAAGAAAGCCAGTCGAGC
LAPSSR0062	7	62,807	GGAGGTTGAGGTCCTTGGA	GTATGCTCATGCCGTCTC
SCESSR0092	8	2983	AAGGACAAGCAGCCCAAGG	GCAGCTTCATGCCCTTCATC
LAPSSR1032	8	3562	AGCAGGCAGTTAGCCAACAGTG	GTTGTTGTCGACGAGGACGAG
SCESSR0484	8	6820	CCTGGTAGTTTGGGCAACCA	TGCTGCTGAGTTTGTGGCAT
SCM15	8	7821	GGAGATGTTTGAGAGGGAA	AGAGTAGCATAAAGGAGGCAG
mSSCIR58	8	9737	CTCACTCAGGCACAAGAAT	TGGGGTCTAACAATCAACT
SCESSR0908	8	32,308	GATCGAGAAGCAGCTCGCC	ACCGCACCTTCACATCCACT
MOLSSR1752	8	37,421	CAACAAGAAGAGCCTCAACCAAAG	AATAAGAGTTGCATGCCTTGCTCT
SCESSR2223	8	50,082	GGAACCCTAGTCGAGGTCGG	AGCTCCGGAAAGAGCAAACC
mSSCIR72	8	54,425	ACATTTCCCCTTCAAGTGG	GCCACCTCCAAGTTCTTT
SCESSR0128	8	55,174	GAGCTCGTGCACCTCACATTC	GGATCTCCCCGGAGAAGAAA
SCESSR1551	9	842	CAACCAACCAACCAACCACC	GTATACCTTGCCGCCGATGA
mSSCIR49	9	1531	CAAGAGAAAACACAAAAATA	CAGCAGCGTTATGAGGTC
SCESSR2390	9	2960	GTTGAAGACGTCGTCGGGAT	AGACACACTTGGGGCAGCAT
SCESSR2120	9	5068	GCAGGAGGCGGACAAGGTA	TGCTCTTGGTCCTCCTGACC
SMC01BUQ	9	5586	AAGGTTCTTGGATTTGGCATCT	GGCAATTAGGGTGGCTTCC
SCESSR1597	9	6411	CACGCTCCTCATCTGGAAGG	AGCCGAGGTGCGTGAAGAG
UGSuM15	9	6775	GTTTAAGACAAGATGGTGTAGATG	TACATATTTACATTGTTACTCCGC
SCESSR1518	9	31,747	ATGAGAAGCTACGCCCCTCC	GGTAAGCACTGCCCTTGTGC
SMC720BS	9	51,265	CGCACCGACGCACGTCT	GCCAATGGAACGGGTCTA
SCESSR1631	9	56,125	CAGGTCGCTCGGCCTCTAC	CGGTCGTCTCTTCCTCCTCC

Table 4 continued

Primer	Chr.	Location (kb)	Forward primer	Reverse primer
SMC280CS	10	6904	TGATCGCACGTTGTATCCAACA	TTTGACCACGCCACGGTAGAT
SCESSR1561	10	8373	CAAGCCCAACTACGGGTTCC	ACCCAGAGCCGTAGCTCTCC
SCESSR2412	10	9878	TAGGCGTTGTTCCTGCCATC	TAGCAGTGATTGGGGATCGG
SCESSR0218	10	12,590	TGCTGTTTTGGGAGATTGACC	CGACGATGGTGGTGGAGAG
SCESSR1306	10	49,385	ACAAACCAACCGGAAGGACC	GCTGTCGCAGAAGAGCGAGT
UGSM333	10	56,966	CTGAGGTGAAATTATCGTGTGT	GCAACGTCTAAATATAATTGCTAA
SCESSR1149	10	57,488	GCAATCTCGTCACGCCCTAC	GGGAAGCCAAGCTGTCAGAA
SCESSR0582	10	59,377	ACGCCATGGAGAAGTTCCAG	GGACGAGCAGGACGCATTTA
mSSCIR55	10	59,811	ATATGTAGGAGTAGGACCAA	CAACAGGTTTCAGTATATTT
mSSCIR15	10	60,417	CTTGGACCCGTTCTTGGATG	AGCACTGAGGCGACTTACCC

and US cultivars, and between the *S. robustum* accessions and Chinese cultivars was 0.631 and 0.633, respectively.

Cluster analysis

A dendrogram of the 48 accessions (Fig. 3) revealed five major groups at the cutoff of L1 (genetic similarity coefficient = 0.784 at L1), named G1, G2, G3, G4, and G5. The 10 *S. spontaneum* clones were separated into the G1and G2 groups. The G4 and G5 groups consisted of four *S. robustum*a accessions. All the *S. officinarum* accessions and cultivars were clustered as the largest group, G3, which was further classified into three distinct subgroups at L2 (genetic similarity coefficient = 0.821), named G3-1, G3-2 and G3-3. Subgroup G3-1 contained six *S. officinarum* accessions and three US cultivars. Subgroup G3-2 contained two *S. officinarum* accessions, Fujian Daye, seven US cultivars and 12 Chinese cultivars. The remaining three *S. officinarum* accessions were placed in subgroup G3-3. Fujian Daye showed a closer relationship with *S. officinarum* accessions and the commercial cultivars than with the other *S. robustum* accessions. Chinese cultivars were grouped in a separate branch, separated from ROC25, which was clusterd together with CP-series of the USA cultivars.

Clusters analysis created by DARwin (Fig. 4) also revealed five major groups, I, II, III, IV and V. Similarly, the 10 *S. spontaneum* accessions were placed inI and II groups. All *S. officinarum* accessions and modern cultivars were divided into the III group, which was further classified into three distinct subgroups, III-1, III-2 and III-3. In III-1 group, all *S. officinarum* accessions and four modern cultivars from the USA were clustered closely, whereas all of the cultivars clustered in the III-3 group were Chinese cultivars. The majority of the USA cultivars were grouped in III-2. In addition, the *S. robustum* accessions were clearly classified into two small clusters (IV and V), except Fujian Daye, which was placed in the III-1 group.

Principal coordinates analysis (PCoA) however illustrated four distinct groups, A, B, C and D (Fig. 5). One remarkably dense cluster was observed in the C group including all *S. officinarum* clones, all cultivars from the two countries and two *S. robustum* accessions (Fujian Daye and 51NG3), accounting for 72.9% of the total accessions. Ten *S. spontaneum* accessions were clearly classified into two groups (A and B). The D group comprised of three *S. robustum* accessions.

Discussion

Since 1888, with the improvement of understanding of sugarcane genetic structure (Stevenson 1965), interspecific and intergeneric hybridizations have been used to develop new cultivars with high sugar yield and stress tolerance. It takes 12–15 years and many generations of selection to produce a new cultivar through hybridization. Selection of breeding materials or germplasm accessions for crossing plays an important role in cultivar development. However, it has been widely acknowledged that the genetic basis of sugarcane breeding programs all over the world is very narrow (Todd et al. 2015). In the USA, only 10 origninal ancestors contributed germplasm to more

Table 5 Parameter list of 20 core SSR primers used for assessment genetic diversity of 48 sugarcane clones

No.	Primer name	Sorghum gene ID	Chr.	Forward primer	Reverse primer	NPB.	Major Allele Frquency		Gene diversity		PIC	
							Range	Mean	Range	Mean	Range	Mean
pri 4	UGSuM56	Sb01g011060	1	TAATACTTTCACCAGCCAA	GGAGCAGCAACGCACAGG	13	0.65–0.98	0.91	0.04–0.46	0.14	0.04–0.35	0.12
pri 5	SCESSR0308	Sb01g016170	1	AGTACTACCAGGCGGGGCAC	GATCCCCAATCCAGAGGGTC	25	0.54–0.98	0.86	0.04–0.50	0.21	0.04–0.37	0.18
pri 14	SCESSR0865	Sb02g022290	2	TGGAGGAAGTACGGCCAGAA	TTCGATTCCACTTGGGAGGA	22	0.67–0.98	0.89	0.04–0.44	0.17	0.04–0.35	0.15
pri 16	SCESSR2288	NA	2	GGGCAAAGTGTATCGGCATC	AGCAGCTAGAGAGGCCCCTAA	21	0.52–0.98	0.89	0.04–0.50	0.17	0.04–0.37	0.15
pri 25	SCESSR0583	Sb03g035680	3	CTCGATGATGCATCCGCTC	TACTCGTAGTCCCCCACCCC	17	0.54–0.98	0.86	0.04–0.50	0.22	0.04–0.37	0.19
pri 26	SCESSR0573	NA	3	GAGGAGGAGGCGGAGGACT	GGGTTGAAGGACCCGAACTT	17	0.52–0.98	0.89	0.04–0.48	0.16	0.04–0.36	0.14
pri 31	SCESSR1978	Sb04g027100	4	CTTCCTCGCCTCCCCCTC	GCAAGCAGGATCCGGTAGGA	17	0.52–0.98	0.84	0.04–0.50	0.22	0.04–0.37	0.18
pri 37	MCSA116D08	NA	4	CAGTCGCCCCACACGCCGAT	CCATGCTGTCGCCGACCACG	17	0.50–1.00	0.85	0.00–0.50	0.19	0.00–0.37	0.15
pri 42	SCESSR0069	Sb05g027470	5	GAGCAAGGCAAGGCTAAGCA	GAGTTTGGTGGCTGCTGTCC	22	0.52–0.98	0.84	0.04–0.50	0.23	0.04–0.37	0.19
pri 43	SMC1572CL	NA	5	GAGGATATGGTTTTCATTGCC	ACACCTTCTCACCACTTAGGTTC	23	0.73–0.98	0.90	0.04–0.39	0.18	0.04–0.32	0.15
pri 53	SCESSR0429	NA	6	GTCCCAAGTGAGTGGCAAGG	GAGCAGCACCACCAGCAGTA	9	0.58–0.96	0.77	0.08–0.49	0.31	0.08–0.37	0.25
pri 55	SCESSR2573	Sb06g023320	6	TGTCGTCATCGTCTGCACAA	AGCGACTCCTCCAGCTCCTC	15	0.54–1.00	0.89	0.00–0.38	0.16	0.00–0.37	0.14
pri 64	SCESSR1364	NA	7	ACGACCTCGTCGAACCCTTT	AGATCGAACCGCTCATCCAG	13	0.60–1.00	0.88	0.00–0.48	0.18	0.00–0.36	0.16
pri 65	SCESSR1027	Sb07g004560	7	GGAGCGGAGGAAGATGATGA	GAGCTCCTCGAAGGAGAGGC	10	0.60–0.98	0.90	0.04–0.48	0.15	0.04–0.36	0.13
pri 71	SCESSR0092	NA	8	AAGGACAAGCAGCCCAAGG	GCAGCTTCATGCCCTTCATC	15	0.56–0.98	0.87	0.04–0.49	0.19	0.04–0.37	0.16
pri 73	SCESSR0484	Sb08g005250	8	CCTGGTAGTTTGGGCAACCA	TGCTGCTGAGTTTGTGGCAT	19	0.54–0.98	0.94	0.04–0.44	0.20	0.04–0.37	0.17
pri 86	SCESSR1597	NA	9	CACGCTCCTCATCTGGAAGG	AGCCGAGGTGCGTGAAGAG	12	0.60–0.98	0.86	0.04–0.46	0.20	0.04–0.36	0.17
pri 88	SCESSR1518	NA	9	ATGAGAAGCTACGCCCCTCC	GGTAAGCACTGCCCTTGTGC	13	0.67–0.98	0.81	0.04–0.44	0.28	0.04–0.35	0.23
pri 93	SCESSR2412	NA	10	TAGGCGTTGTTCCTGCCATC	TAGCAGTGATTGGGGATCGG	14	0.58–0.98	0.82	0.04–0.46	0.26	0.04–0.35	0.21
pri 98	SCESSR0582	Sb10g029610	10	ACGCCATGGAGAAGTTCCAG	GGACGAGCAGGACGCATTTA	16	0.60–0.98	0.91	0.04–0.48	0.15	0.04–0.36	0.14
Mean								0.87		0.20		0.17

PIC polymorphism information content, *NPB* number of polymorphic band

Table 6 The genetic diversity of five populations based on 20 SSRs marker

Population	Sample size	Major allele frquency	Genotype number	Allele number	Gene diversity	PIC
Cul_1	10	0.89	1.47	1.47	0.15	0.12
Cul_2	12	0.91	1.42	1.42	0.13	0.10
So	11	0.89	1.50	1.50	0.16	0.13
Sr	5	0.89	1.37	1.37	0.15	0.12
Ss	10	0.89	1.57	1.57	0.16	0.14

Ss, *S.spontaneum*; So, *S. officinarum*; Sr, *S. robustum*; Cul_1, The USA cultivars; Cul_2, Chinese cultivars

PIC polymorphism information content

Table 7 Pairwise population matrix of nei genetic identity

	Ss (10[a])	So (11)	Cul_1 (10)	Sr (5)	Cul_2 (12)
Ss (10)	1.000				
So (11)	0.552	1.000			
Cul_1 (10)	0.550	0.808	1.000		
Sr (5)	0.517	0.615	0.631	1.000	
Cul_2 (12)	0.545	0.762	0.760	0.633	1.000

Ss, *S.spontaneum*; So, *S. officinarum*; Sr, *S. robustum*; Cul_1, The USA cultivars; Cul_2, Chinese cultivars

[a]Number indicated number of accessions for each group

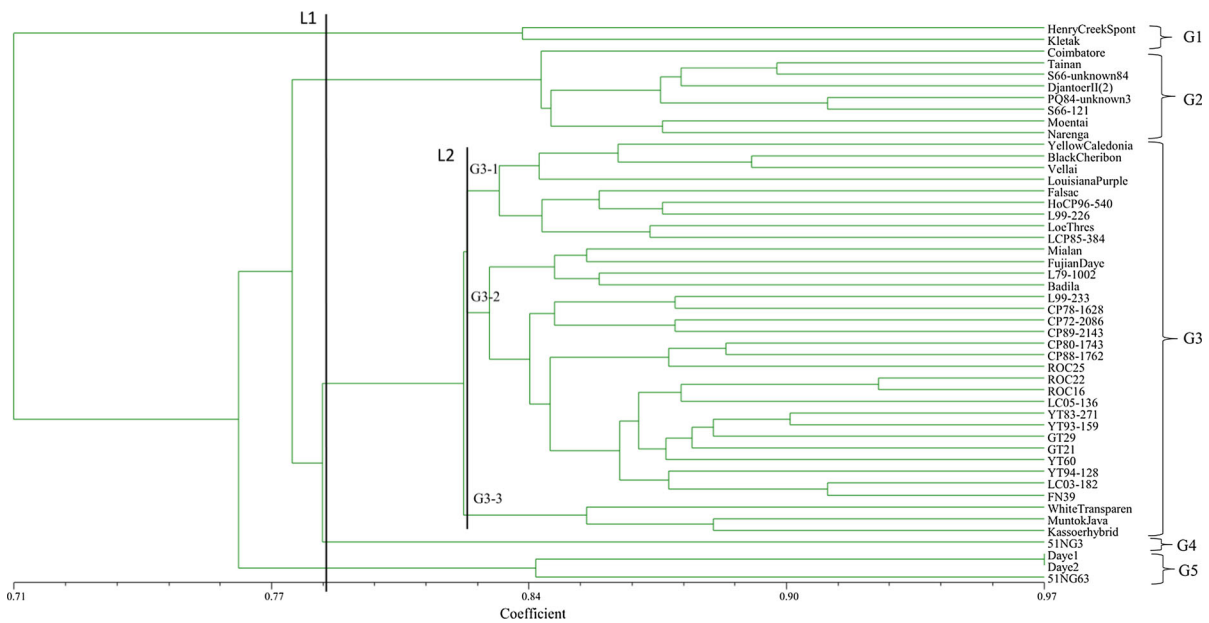

Fig. 3 The phylogenetic tree based on SSR amplification patterns obtained by using the UPGMA method showing the genetic relationships beween the *Saccharum* genus and cultivars

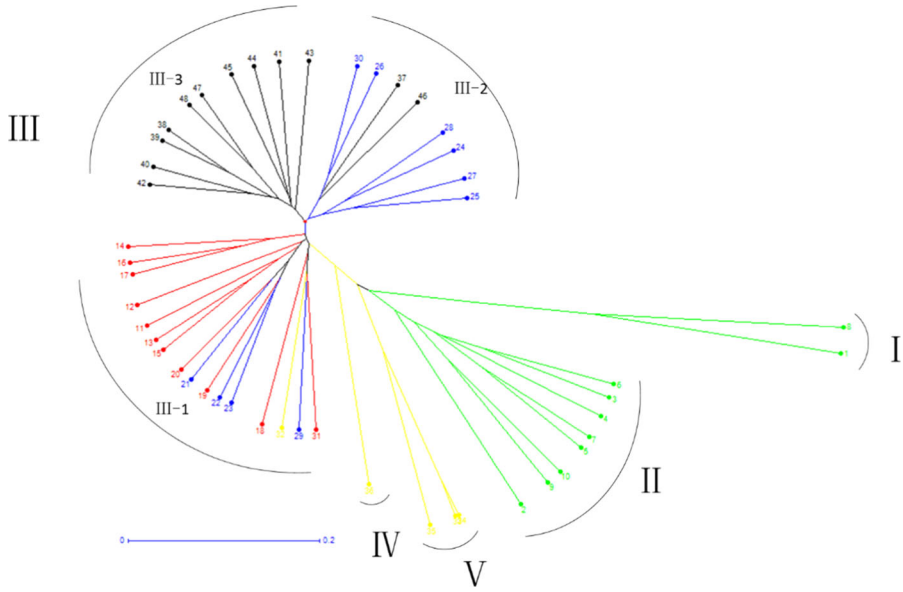

Fig. 4 Clusters analysis of 48 sugarcane accessions based SSRs data. Note: Green: *S. spontaneum*; Red: *S. officinarum*; Yellow: *S. robustum*; Blue: US cultivars; Black: Chinese cultivars. Numbers of the accession in the figure were corresponding to the numbers in Table 1. (Color figure online)

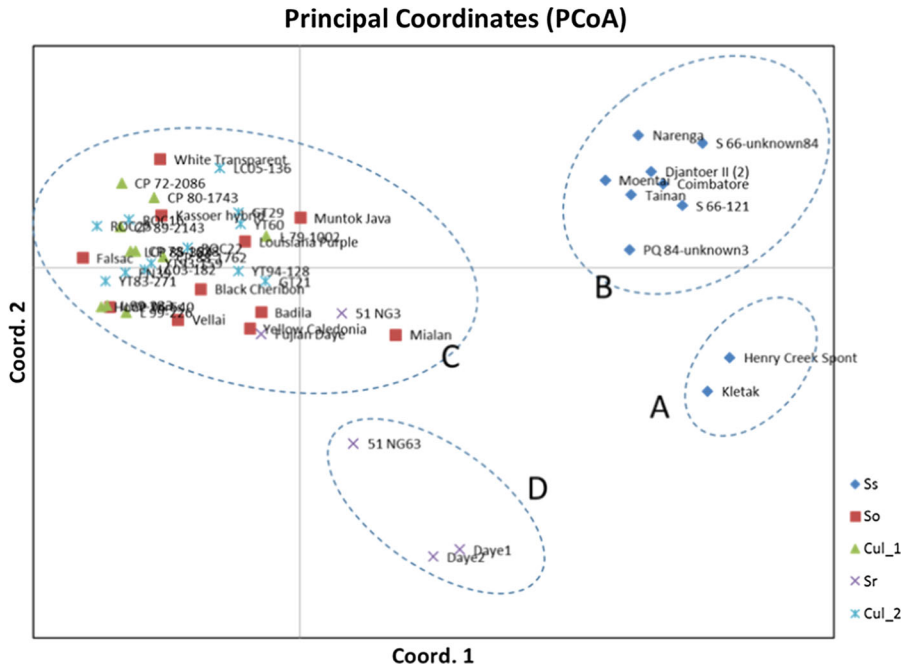

Fig. 5 Principal Coordinates Analysis (PCoA) of 48 sugarcnae accessions collected from US and China based on codominant genotypic distance. Note: Ss, *S. spontaneum*; So, *S. officinarum*; Sr, *S. robustum*; Cul_1, US cultivars; Cul_2, Chinese cultivars

than 90% of the USA mainland sugarcane cultivars (Deren 1995). In China, sugarcane cultivars mainly included ROC- series, YT- series, GT- series, LC-series and FN- series with 85% of sugarcane planting area being the ROC-series, especially ROC22, which occupies 50–60%. ROC22 is not only the most widely grown cultivar but also the most frequently used parental material in sugarcane breeding programs in China (You et al. 2013). To broaden the genetic basis and promote the international collaboartion on sugarcane cultivar development, it is essential to evaluate the widely used cultivars and their ancestries from different countries.

Despite the advances of next generation sequencing technology, SSRs are still the marker of choice in many laboritories with limited bioinformatic capacity

to quickly evaluate the genetic background of breeding materials. In this study, a total of 6,149 unique sugarcane SSR primers were identified from published sugarcane literatures. A set of 20 highly polymorohic SSR markers randomly distributed in the genome were selected to analyze the genetic diversity and the population structure of 48 sugarcane cultivars and progenitor accessions. The primer sequences and band pattern images of each marker were documented, which will be a useful marker resource for the sugarcane community. The average of PIC of these SSR markers was 0.17 and an average of gene diversity of the 48 accessions was 0.2, which were lower than the PIC value of 0.245 and gene diversity of 0.304 in the WCSRG (Nayak et al. 2014). The main reason was that the plant accessions in the WCSRG are highly diverse with over 1,000 accessions collected from more than 45 countries, whereas only 48 accessions with a focus on the cultivars from two countries were included in this study.

Genetic diversity of 48 accessions based on 20 core SSR markers divided the 48 accessions into five groups according to cluster and PCoA anslyses. The strong distinction between the two major species in *Sacharrum*, *S. spontaneum* and *S. officinarum* indicated the effectiveness of the selected 20 markers, though the number seems small. In practice, the smaller the number of markers to distingshish difference germplasm accessions, the more efficient and powerful the selected markers are. Compared with *S. spontaneum, S. robustum* had a closer relationship with *S. officinarum,* which is consistent with the morphology, physiology, and cytology of these two species (Alwala et al. 2006; Irvine 1999), and also was demostrated in other studies (Alwala et al. 2006; Nair et al. 1999; Nayaka et al. 2014).

There were no distincted subgroups found within G3 or Group III, which was not a suprise as sugarcane cultivars had a closer relationship with *S. officinarum* than other *Saccharum* species due to the backcrossing to *S. officinarum* and selection of high sugar content contributed by *S. officinarum* during the cultivar development. The closest relationship between progeny and parent pairs were found in the dendrogram, which was in agreement with their pedigree information, such as CP88-1762 and CP80-1743, CP89-2143 and CP72-2086, LC05-136 and ROC22, and LC03-182 and FN39, except for HoCP96-540 (progeny), which was more closely related to L99-226 than to LCP85-384 (one of the parents for HoCP96-540 according to the pedigree). However, LCP85-384 was still the next closest in relation to its progeny HoCP96-540, followed by L99-226 and Falsac (*S. officinarum*). In this study, all the parents of the 12 China major cultivars showed a very close relationship with ROC25 indicating the significant germplasm contribution of ROC25 to the Chinese sugarcane cultivar development. These results provided additional support that these 20 SSR markers had ability to distinguish various sugarcane accessions, even closely related ones. *S. robustum* seemed not contibute to the modern sugarcane cultivars in China since four of the five *S. robustum* accessions were clustered as an outgroup of all the accessions assessed. FujianDaye, which was derived from Fujian sugarcane breeding program, though named as *S. robustum*, might be a hybrid from *S. robustum*. FujianDaye even cluster closer with the USA cultivars than with Chinese cultivars indicating that the FujianDaye as a hybrid may have parental relationship with the USA cultivars.

The genetic similarity coefficient is an important index to measure the degree of genetic differentiation among individuals. The highest Nei's genetic identity among five groups was obtained between *S. officinarum* and the USA cultivars (0.808) followed by *S. officinarum* and Chinese cultivars (0.762), US cultivars and Chinses cultivars (0.760). The results demonstated that the USA breeding program implemented a much higher selection pressure than Chinese breeding programs to improve sucrose content in the process of "nobilization" by selecting high genetic background of *S. officinarum*. The gene diversity of Chinese cultivars was much smaller than that of the USA ones indicating a much narrower gene pool in Chinese cultivars even though the American cultivars were included in the pedigree of Chinese cultivar development. It is urgent to increase the genetic diversity and broaden the gene pool by introducing related species in *Saccachrum* genera into the breeding programs and engage in international germplasm exchanging and utilization.

In summary, we have documented the pedigree information of popular sugarcane cultivars in China and the USA, recruited more than six thousand SSR markers, and provided the sorghum genome locations of one thousand of them. The selected 20 core SSR markers randomly distributed in the sugarcane genomes were applied to assess the genetic diversity of the

sugarcane cultivars in China and USA along with their potential ancestors. The results of the diversity analysis provided a reference for the breeding programs in broaden the genetic basis and collaborations in developing cultivars with sustainablity and high productivity.

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