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RESEARCH ARTICLE

SSR-Based Molecular Identification and Population Structure Analysis for the Yunrui-Series Sugarcane (*Saccharum spp.* Hybrids) Genotypes

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Abstract The success of sugarcane breeding depends on the understanding and exploitation of new germplasm. Sugarcane breeders often choose parental clones based on phenotypes when genotypes are unknown. In this study, an SSR fingerprint profile of 104 Yunrui-series sugarcane genotypes was constructed and the genetic diversity and population structure of these genotypes were analyzed using 21 pairs of fluorescence-labeled highly polymorphic SSR markers and a capillary electrophoresis platform. One hundred and thirty-six SSR fragments were detected. The percentage of polymorphic loci averaged 98.25%, and the polymorphism information content averaged 0.88. A neighbor-joining dendrogram and population structure analysis divided the 104 genotypes into four populations with a Q value of < 0.6 for half of the genotypes. Pairwise genetic differentiation (*Fst*) between populations ranged from 0.009 to 0.023. Analysis of molecular variance (AMOVA) revealed that 98% of the variations occurred within the populations, with a significant *Fst* value of 0.018 ($p < 0.001$). The results demonstrated that Yunrui-series sugarcane genotypes shared a moderate to high level of genetic diversity, but a low level of genetic differentiation due to multiple occurrences of crossing and backcrossing involving a limited number of parents. Therefore, sugarcane breeders should consider the genetic distance and population structure information more than phenotype performance when choosing parental genotypes for crossing programs.

Keywords Breeding programs · Genetic diversity · Population structure · *Saccharum* spp. hybrids

Introduction

Sugarcane (*Saccharum spp*. hybrids) is the world's most important sugar and energy crop, accounting for 80% of the total sucrose production (Waclawovsky et al. 2010; Moore and Botha 2014). Modern sugarcane production faces a tremendous challenge due to increased human population, changing climate, increased production costs, and reduced planting area necessitating a need for greater breeding efficiency (Matsumoto 2015; Kumar 2016; Menhas et al. 2016). Diverse germplasm resources are vital for the successful breeding of any crop. In corn and rice, every major improvement in grain yield has been due to the discovery and exploitation of key germplasm resources (Wang et al. 2020). Nevertheless, sugarcane breeding is more difficult due to polyploidy and a narrow genetic base of the germplasm. For instance, more than 90% of sugarcane cultivars in the USA can be traced back to 10 ancestral genotypes (Deren 1995; Todd et al. 2015). In China, three "ROC"-series of cultivars, namely, ROC10, ROC16, and ROC22, were the major cultivars in the 1980s, 1990s, and 2000s, respectively. In China, ROC22 was planted on 50–60% of the total sugarcane fields in the last 10 years and was also the most frequently used parental genotype in cross breeding. (You et al. 2013; Liu et al. 2018). Thus, the efficiency of sugarcane breeding might be improved through the use of more diverse germplasms.

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The Ruili Breeding Station of Yunnan Sugarcane Research Institute (YSRI), Yunnan Academy of Agricultural Sciences (YAAS), Yunnan, China is a major breeding station for sugarcane crossing and hybrid seed (fuzz) production. To obtain high cane yield, high sugar content, and other favorable economic characters with improved stress tolerance, the Yunrui-series genotypes were produced by first crossing popular elite sugarcane parents with wild *S. spontaneum* germplasm collected from different regions of Yunnan province, followed by backcrossing of the hybrids with commercial cultivars (Jing et al. 2011; Zhu et al. 2014). The breeding program has released numerous new sugarcane cultivars (Jing et al. 2020). YSRI has two work sites, the Ruili Breeding Station in Ruili City, Yunnan produces and selects the Yunrui-series genotypes, and the YSRI in Kaiyuan City, Yunnan develops the Yunzhe-series genotypes. However, any Yunrui-series or Yunzhe-series genotype, once released for commercial production, receives a Yunzhe-series variety designation. For Yunrui-series genotypes, various agronomic traits, heritability, combining ability, and drought resistance of the Yunrui-series genotypes have been described (Zhu et al. 2014; Tian et al. 2017). However, molecular data is not available for the Yunrui-series genotypes to support future breeding efforts and protect intellectual property rights.

Genotype identification is one of the most important agricultural activities during breeding, seed production and trade, and inspection processes (Ali et al. 2017). Mislabeling or misidentification of sugarcane genotypes is common during the exchange or seed shipment across different test locations. The cumulative probability of this error can be higher when parental materials are vegetatively propagated across years in the form of stalk cuttings (setts) (Pan 2010). Historically, sugarcane breeders regularly use phenotypic descriptors, such as stalk shape, internode shape, bud shape, internode color unexposed and exposed to light, wax band, and growth cracks to identify varieties (Gravois 2017). However, phenotypic identification is time-consuming, less informative, and often unreliable due to genotype and environment interaction (Cordeiro et al. 2003; Dos Santos et al. 2012; Todd et al. 2018). Occasionally, isozymes and DNA in situ hybridization are used for genotype identification (D'Hont et al. 1995). Genotype pedigree information is quite reliable in assuring genotypic fidelity.

Since 1990, DNA-based markers have been developed and broadly used for gene mapping, genetic diversity, and population structure studies, marker-assisted selection, and genotype identification (Aitken et al. 2005; Chang et al. 2012; Wang et al. 2020). Among PCR-based markers, SSR is considered the most valuable and robust marker due to high polymorphism, good repeatability, and co-dominant inheritance (Cordeiro et al. 2001; Ul Haq et al. 2016). Several sugarcane research groups in China have reported studies on fingerprint construction, genetic diversity assessment, and population structure analysis (Liu et al. 2010, 2016; Qi et al. 2012). These studies mainly focused on released cultivars and wild *Saccharum* species using polyacrylamide gel electrophoresis and silver staining. Pan (2010) developed an SSR-based fluorescence-capillary electrophoresis detection (SSR-CE/FD) protocol for sugarcane genotypes identification. Chandra et al. (2014) and Fu et al. (2016) demonstrated an SSR-CE/FD protocol which was effective for identifying Indian and Chinese sugarcane genotypes. Ali et al. (2017, 2019) also applied this protocol to establish the molecular identities of 91 nationally or provincially released Chinese sugarcane cultivars, 79 accessions of *Saccharum spp*., six *Erianthus arundinaceus* accessions, and 30 *Saccharum spp*. hybrids. The researchers used the molecular identity information to assess the genotypic diversity and population structure of the *Saccharum* and *Erianthus* germplasm.

The objectives of this study were to: (1) construct an SSR fingerprint profile; (2) assess genetic diversity and genetic distance; and (3) analyze the population structure by implementing genetic differentiation and analysis of molecular variance (AMOVA). The results should enable the molecular identification of the Yunrui-series genotypes and provide guidance in the optimal selection of parental genotypes and the design of cross combinations to enhance future sugarcane breeding programs.

Materials and Methods

Materials

A total of 104 Yunrui-series sugarcane genotypes, commonly used as crossing parents, were selected for this study (Table 1).

Genomic DNA Extraction and Quality Assessment

Healthy apical leaves were sampled in the field during the seedling stage. Leaves were cut and ground to powder in liquid nitrogen. Genomic DNA was extracted using Tiangen's DNAsecure Plant Kit (Product No. DP320-02, Beijing, China) following the manufacturer's instructions. A NanoDrop1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure DNA concentration and quality, and DNA quality was rechecked by 1% agarose gel electrophoresis. Working DNA samples (20 ng/μL) were prepared by dilution with sterile deionized water. All DNA samples were stored at − 20 °C prior to PCR.

Table 1 The name, parents and utilization in sugarcane breeding programs of 104 Yunrui-series genotypes

Genotype name	Parents (female × male)[a]	Utilization in breeding	Genotype name	Parents (female × male)[a]	Utilization in breeding
YR95-128	F172 × YR88-1081	Parent	YR05-690	ROC10 × YR03-315	Parent
YR99-113	YR95-113 × ROC10	Clone/parent	YR05-704	ROC10 × YR03-315	Variety/parent
YR15-1112	YT93-124 × YZ89-7	Clone	YR05-724	ROC10 × YR03-315	Parent
YR15-1035	ROC25 × LC03-1137	Clone	YR11-256	YR08-99 × YR08-472	Innovation germplasm
YR15-1095	ROC22 × ROC25	Clone	YR05-733	DZ93-88 × YR99-490	Parent
YR12-399	YT93-159 × YR05-171	Clone/parent	YR05-744	DZ93-88 × YR99-490	Parent
YR99-48	YR91-2008 × YR93-3148	Innovation germplasm	YR05-747	DZ93-88 × YR99-490	Parent
YR99-601	ROC10 × YR95-113	Clone	YR05-762	DZ93-88 × *E.arund* 84–250	Innovation germplasm
YR99-67	BMZ 7 × *S.spon* 82–114	Innovation germplasm	YR05-767	DZ93-88 × YR03-405	Parent
YR09-895	YT96-86 × ROC22	Clone	YR05-768	CP65-357 × YR03-405	Parent
YR03-11	YR99-23 × YR99-248	Parent	YR05-770	DZ93-88 × YR03-409	Parent
YR03-315	ROC10 × YR95-1458	Parent	YR15-103	FN03-35 × YR10-688	Parent
YR03-392	DZ93-88 × YR99-634	Parent	YR16-26	YR14-165 × YR10-914	Innovation germplasm
YR03-393	DZ93-88 × YR99-634	Parent	YR05-781	YR03-417 × ROC10	Parent
YR03-394	DZ93-88 × YR99-634	Parent	YR05-782	YR03-417 × ROC10	Parent
YR03-409	YR99-546 × ROC10	Parent	YR05-784	ROC10 × YR03-403	Parent
YR03-417	YR99-546 × ROC10	Parent	YR05-785	CP65-357 × YR03-405	Parent
YR03-425	ROC10 × YR99-546	Parent	YR05-790	DZ93-88 × YR99-490	Parent
YR03-78	YR99-248 × YR97-105	Parent	YR06-189	ROC22 × YR99-113	Variety
YR03-80	YR99-248 × YR97-105	Parent	YR16-42	YR10-902 × YR14-189	Innovation germplasm
YR03-809	YR99-248 × YR97-105	Parent	YR06-2416	DZ93-94 × YR99-917	Parent
YR12-248	CP94-1100 × YR95-128	Clone	YR16-81	YR13-33 × YR14-170	Innovation germplasm
YR12-263	Hocp95-988 × YR05-770	Clone	YR06-3226	YR03-13 × ROC22	Parent
YR04-1051	ROC10 × YR03-80	Parent	YR06-3227	YR03-13 × ROC22	Parent
YR11-450	YR05-283 × RB85-5156	Clone	YR06-3603	YR91-3696 × YC58-47	Parent
YR04-52	YR99-248 × YR03-117	Clone	YR16-82	YR13-33 × YR14-170	Innovation germplasm
YR12-450	YR05-785 × YT93-159	Clone	YR06-4674	DZ93-88 × YR99-546	Parent
YR05-189	YR99-601 × DZ93-88	Parent	YR06-6003	YR04-1051 × DZ93-88	Parent
YR15-1086	GT94-119 × ROC22	Parent	YR06-8270	YR03-393 × MT90-1022	Parent
YR15-1117	FN02-6427 × YT99-66	Parent	YR06-8362	CP65-357 × YR03-917	Parent
YR10-330	FR96-405 × YR05-189	Parent	YR08-1276	YR03-11 × RB72-454	Parent
YR05-282	DZ93-88 × YR03-373	Parent	YR09-311	CL69-52 × YR05-285	Parent
YR05-283	DZ93-88 × YR03-373	Parent	YR09-315	CL69-52 × YR05-285	Clone
YR05-285	DZ93-88 × YR03-373	Parent	YR09-751	CP86-1664 × YR05-628	Parent
YR05-292	DZ93-88 × YR03-373	Clone/parent	YR09-753	CP86-1664 × YR05-628	Parent
YR05-346	CP65-357 × YR99-113	Clone	YR10-1055	CP72-1210 × YR03-7	Parent
YR05-407	YR04-1001 × YR99-634	Parent	YR10-172	FR96-405 × YR05-189	Parent
YR05-440	CP65-357 × *E. arund* 250	Innovation germplasm	YR10-248	DZ93-88 × YR05-458	Parent
YR05-457	ROC20 × *E. arund* 250	Innovation germplasm	YR10-291	CP72-1210 × YR03-7	Parent
YR05-458	YR04-1051 × YR03-315	Parent	YR10-299	CP72-1210 × YR03-7	Parent
YR10-336	FN71-745 × YR05-776	Parent	YR10-495	YR05-768 × FN94-0403	Parent
YR05-566	CP65-357 × YR03-917	Parent	YR10-509	YR06-2412 × DZ93-88	Parent
YR09-155	Barwilspt × HNXCZ	Parent	YR10-550	YR05-782 × YR03-80	Parent
YR09-176	Pansahi × Zopitala	Parent	YR10-648	YR05-606 × GT94-119	Parent
YR05-596	ROC10 × YR03-394	Variety	YR10-725	Q121 × YR05-576	Parent
YR05-606	YR03-315 × DZ93-88	Parent	YR10-736	Q151 × YR05-770	Parent
YR05-628	DZ93-88 × YR03-417	Parent	YR13-33	YR08-100 × YR10-927	Innovation germplasm

Table 1

YR11-101	POJ213 × YR09-F19	Parent	YR14-161	YC58-47 × YR09-167	Parent
YR11-103	POJ213 × YR09-F19	Parent	YR14-190	YR09-163 × YR08-474	Innovation germplasm
YR05-662	ROC10 × YR03-117	Parent	YR14-195	YR09-167 × YR08-474	Innovation germplasm
YR05-668	ROC20 × *E. arund* 5	Parent	YR14-211	YR99-67 × YC97-47	Parent
YR05-679	ROC20 × YR03-394	Parent	YR14-221	50uahipele × YR08-92	Parent

[a]YR, Yunrui; ROC, Taiwan, China; DZ, Dezhe; YT, Yuetang; FN, Funong; GT, Guitang; YC, Yacheng; MT, Mintang; *S. spon*, *Saccharum spontaneum*; *E. arund*, *Erianthus arundinaceus*; BMZ and HNXCZ, landraces; Barwilspt, 50uahipele, Zopitala, and Pansahi, *S. officinarum* clones

PCR Reaction and Genotyping

Twenty-one pairs of highly polymorphic SSR primers developed by the International Consortium of Sugarcane Biotechnologists were adopted for this study (Table 2) (Pan 2010). PCR was performed in a 20 μL volume consisting of 2 μL DNA, 2 μL of 10X reaction buffer, 1.6 μL of 10 mM dNTPs, 1 μL each of 2 μM forward and reverse primers, 0.3 μL of TransGen Phi29 DNA Polymerase, and 12.1 μL of sterile deionized water. PCR was programmed using a Bio-Rad thermal cycler (Hercules, CA, USA) at 94 °C for 4 min, then 35 cycles of 30 s at 94 °C, 90 s at appropriate

Table 2 Primer sequence and targeted fingerprints of the 21 SSR markers used in this study

Code	SS marker[a]	Forward primer (5′–3′)	Reverse primer (5′–3′)	Targeted SSR fingerprints[a] (bp)
1	SMC119CG	TTCATCTCTAGCCTACCCCAA	AGCAGCCATTTACCCAGGA	106, 112, 118, 128, 131
2	SMC1604SA	AGGGAAAAGGTAGCCTTGG	TTCCAACAGACTTGGGTGG	109, 112, 115, 118, 121, 124
3	SMC18SA	ATTCGGCTCGACCTCGGGAT	AGTCGAAAGGTAGCGTGGTGTTAC	137, 140, 144, 147, 150
4	SMC24DUQ	CGCAACGACATATACACTTCGG	CGACATCACGGAGCAATCAGT	126, 128, 131, 135, 137, 142
5	SMC278CS	TTCTAGTGCCAATCCATCTCAGA	CATGCCAACTTCCAAACAGACT	140, 153, 166, 168, 170, 174, 176, 178, 182
6	SMC31CUQ	CATGCCAACTTCCAATACAGACT	AGTGCCAATCCATCTCAGAGA	138, 150, 160, 162, 163, 165, 167, 171, 173, 177, 179
7	SMC334BS	CAATTCTGACCGTGCAAAGAT	CGATGAGCTTGATTGCGAATG	146, 149, 151, 161, 163, 164
8	SMC336BS	ATTCTAGTGCCAATCCATCTCA	CATGCCAACTTCCAAACAGAC	141, 154, 164, 166, 167, 169, 171, 173, 175, 177, 183
9	SMC36BUQ	GGGTTTCATCTCTAGCCTACC	TCAGTAGCAGAGTCAGACGCTT	112, 118, 121
10	SMC486CG	GAAATTGCCTCCCAGGATTA	CCAACTTGAGAATTGAGATTCG	224,227, 237, 239, 241
11	SMC569CS	GCGATGGTTCCTATGCAACTT	TTCGTGGCTGAGATTCACACTA	167, 170, 210, 219, 222
12	SMC7CUQ	GCCAAAGCAAGGGTCACTAGA	AGCTCTATCAGTTGAAACCGA	158, 162, 164, 166, 168, 170
13	SMC597CS	GCACACCACTCGAATAACGGAT	AGTATATCGTCCCTGGCATTCA	144, 148, 154, 157, 159, 161, 163, 164, 165, 168, 174
14	SMC703BS	GCCTTTCTCCAAACCAATTAGT	GTTGTTTATGGAATGGTGAGGA	201, 206, 208, 210, 212, 214, 216, 220, 222
15	SMC851MS	ACTAAAATGGCAAGGGTGGT	CGTGAGCCCACATATCATGC	128, 130, 132, 134, 136, 141
16	mSSCIR66	AGGTGATTTAGCAGCATA	CACAAATAAACCCAATGA	127, 130, 132, 134
17	mSSCIR3	ATAGCTCCCACACCAAATGC	GGACTACTCCACAATGATGC	141, 145, 171, 173, 175, 177, 178, 180, 182, 187
18	SMC1751CL	GCCATGCCCATGCTAAAGAT	ACGTTGGTCCCGGAACCG	140, 144, 147, 151, 154
19	SMC22DUQ	CCATTCGACGAAAGCGTCCT	CAAGCGTTGTGCTGCCGAGT	125, 148, 151, 154, 157, 160, 163
20	mSSCIR43	ATTCAACGATTTTCACGAG	AACCTAGCAATTTACAAGAG	206, 209, 233, 235, 237, 239, 248, 250, 252
21	mSSCIR74	GCGCAAGCCACACTGAGA	ACGCAACGCAAAACAACG	217, 220, 223, 226, 229
			Total	144

[a]Pan (2010)

Table 3 Polymorphism index of the 21 SSR primer pairs

Name of SSR primer pair	Number of expected fragments[a]	Number of detected fragments[b]	NPB[c]	PPB[d] (%)	PIC[e]
SMC119CG	5	5	5	100	0.90
SMC1604SA	6	6	6	100	0.93
SMC18SA	5	5	4	80	0.87
SMC24DUQ	6	6	6	100	0.93
SMC278CS	9	8	8	100	0.98
SMC31CUQ	11	11	11	100	0.98
SMC334BS	6	6	6	100	0.95
SMC336BS	11	10	10	100	0.98
SMC36BUQ	3	3	3	100	0.81
SMC486CG	5	5	5	100	0.78
SMC569CS	5	4	4	100	0.83
SMC7CUQ	6	6	5	83	0.63
SMC597CS	11	10	10	100	0.94
SMC703BS	9	8	8	100	0.83
SMC851MS	6	6	6	100	0.93
mSSCIR66	4	4	4	100	0.90
mSSCIR3	10	8	8	100	0.94
SMC1751CL	5	5	4	100	0.57
SMC22DUQ	7	7	6	100	0.91
mSSCIR43	9	8	8	100	0.96
mSSCIR74	5	5	5	100	0.86
Total/Mean	144	136	6.29	98.25	0.88

[a]Number of SSR fragments expected (Table 2)

[b]Number of SSR fragments detected among 104 Yunrui-series genotypes

[c]Number of polymorphic bands

[d]Percentage of polymorphic bands

[e]Polymorphism information content

annealing temperature (range from 48 to 62 °C depending on the primer) and 1 min at 72 °C, with a final extension step at 72 °C for 5 min. After PCR, amplified SSR fragments along with the size standards ROX500 were subject to capillary electrophoresis in an ABI 3730XL Sequencer to produce the GeneScan files following the manufacturer's protocol (Shanghai Biowing Applied Biotechnology Co. Ltd, Wuxi City, China).

Allele Scoring and Fingerprint Profile Construction

The GeneScan files were processed with GeneMarker™ version 2.7.0 (Soft Genetics LLC, State College, Pennsylvania, USA). A panel for each SSR marker was created using the distinctive sizes of targeted SSR fragments shown in Table 2. The sizes of detected SSR fragments were calibrated against the ROX500 size standards before the SSR fragments were displayed in the sample plot window for manual scoring and interpretation. Only true SSR fragments were scored while non-specific fragments such as stutters and zig-zag dinosaur tails were ignored (Pan et al. 2003, 2007; Pan 2010). Presence of a true SSR fragment was given a score of "1" and its absence a score of "0". The resulting "1" or "0" binary data sheet was used for subsequent fingerprint construction and population structure assessment.

Data Analysis

The PIC of each SSR primer pair was calculated by the method of Milbourne et al.(1997). Genetic distance was computed based on Nei's standard distance (Nei 1972) by NTSYSpc version 2.10 (Rohlf 2000), and the dendrogram was created by MEGA X software (Kumar et al. 2018) using the neighbor-joining method based on Nei's genetic distance matrix.

Fig. 1 An SSR fingerprint profile of 104 Yunrui-series sugarcane genotypes. The names of 21 SSR primer pairs and the sizes of 144 SSR fragments were listed on the right side. The names of genotypes (Table 1) were shown on the bottom. Black and white boxes indicate the presence and absence of SSR fragments, respectively

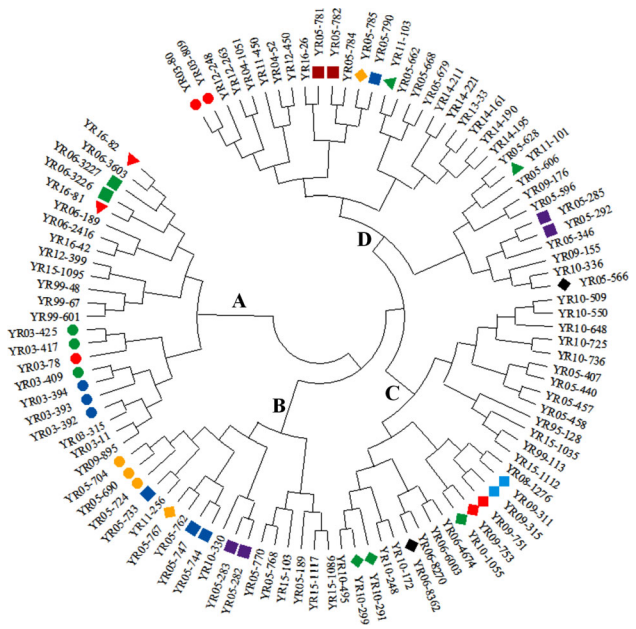

Fig. 2 A dendrogram of 104 Yunrui-series sugarcane genotypes based on the genetic distance matrix of SSR data set using the Neighbor-joining method. Genotypes marked with the same symbol by both shape and color were selected from the same cross according to their pedigrees shown in Table 1

To further elucidate the genetic composition and genetic structure among the Yunrui-series genotypes, a population structure analysis was performed using STRUCTURE version 2.3.3 (Pritchard et al. 2000) with the number of clusters (K value) from 1 to 10, 10 independent runs, and a burn-in period of 10,000 followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations. An optimum K value was identified by the log probability of data LnP (D) for each K value using a web-based software STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/structureHarvester/). A Q-Plot was drawn with CLUMPP version 1.1.2 (Kopelman et al. 2015) and DISTRUCT version 1.1 software (Rosenberg 2016). The frequency data of *Q* values were input into an Excel spreadsheet, which was converted to a figure depicting the distribution of *Q* values in each population. Finally, pairwise genetic differentiation *(Fst)* and AMOVA was conducted using the Excel-based GenAlEx 6.5 (Peakall and Smouse 2012).

Results

Polymorphism of SSR Primer Pairs

A total of 136 SSR fragments were detected out of the 144 expected (Table 3). The number of polymorphic bands (NPB) ranged from 3 (SMC36BUQ) to 11 (SMC31CUQ) with a mean of 6.29 per primer pair. Percentage of polymorphic bands (PPB) averaged 98.25%, with a range of 80%-100%. The PIC averaged 0.88, ranging from 0.57 (SMC1751CL) to 0.98 (SMC278CS, SMC31CUQ and SMC336BS). The genetic similarity coefficients among the 104 Yunrui-series genotypes averaged 0.69, with a range of 0.49–0.96, indicating a medium to a high level of genetic similarity among these genotypes.

SSR-Based Fingerprint Profile and Pedigree Validation of Two Genotypes

An SSR-based molecular fingerprint profile was constructed for the 104 Yunrui-series genotypes (Fig. 1). The profile showed the presence (black box) or absence (white box) of the expected 144 distinctive fragments. The profile showed that each Yunrui-series genotype had a unique SSR fingerprint.

For this study, only two genotypes (YR05-458 and YR10-550) had their parental genotypes (YR04-1051, YR03-315, YR05-782, and YR03-80) included in the profile. When the SSR fingerprints of these six genotypes were compared, 54 SSR fragments from YR05-458 and 60 SSR fragments from YR10-550 could be found in their parents, either maternal or paternal or both (Fig. 1). However, YR05-458 had five additional SSR fragments: SMC119CG_118bp, SMC24DUQ_131bp, SMC278CS_166bp, SMC31CUQ_162bp, and SMC336BS_167bp, respectively, which were absent in either YR04-1051 or YR03-315. On the other hand, YR10-550 had nine additional SSR fragments: SMC18SA_147bp, SMC24DUQ_135bp, SMC24DUQ_137bp, SMC278CS_140bp, SMC31CUQ_138bp, SMC336BS_141bp, SMC597CS_154bp, SMC851MS_130bp, and mSSCIR66_134bp, that were absent in either YR05-782 or YR03-80. Therefore, YR05-458 and YR10-550 were probably not the true progenies of the designated crosses.

Genetic Relationship and Cluster Analysis of Yunrui-Series Genotypes

To examine the genetic relationships among the 104 Yunrui-series sugarcane genotypes, we constructed a dendrogram using neighbor-joining method based on Nei's genetic distance matrix. As a result, 104 Yunrui-series genotypes were placed into four major clusters: A, B, C and D (Fig. 2). Cluster A consisted of 23 genotypes, of which, YR16-82 and YR16-81 (labeled with red inverted triangle) were selected from the cross YR13-33 × YR14-170. YR06-3226 and YR06-3226 (green square box) were selected from the cross YR03-13 × ROC22. YR03-425, YR03-417, and YR03-409 (green dot) were selected from the cross YR99-546 × ROC10, and YR03-392, YR03-393,

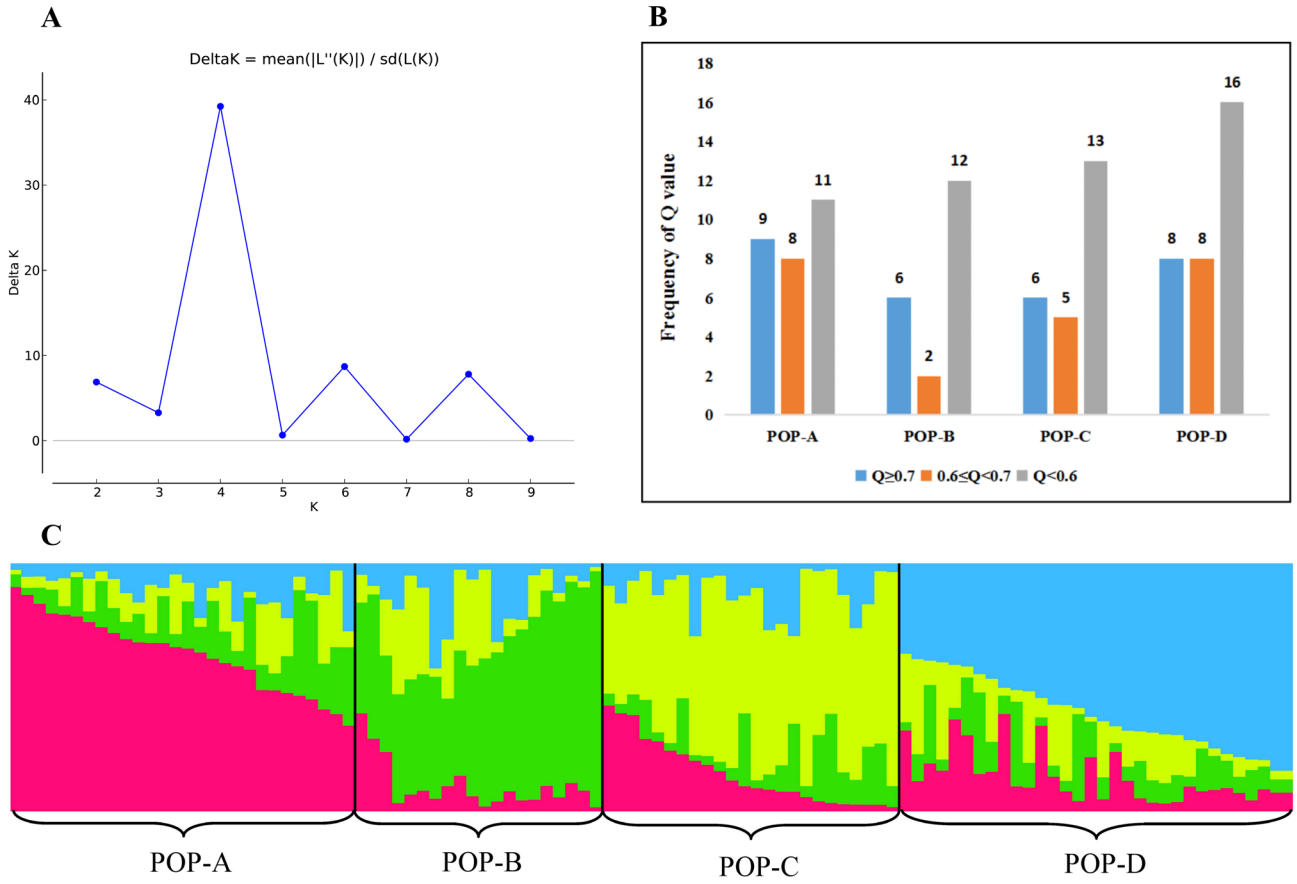

Fig. 3 Genetic composition and population structure of the 104 Yunrui-series sugarcane genotypes. **A** assessment of the best *K* value; **B** Frequency distribution of *Q* value in each population; **C** population structure of the 104 Yunrui-series sugarcane genotypes on $K = 4$

and YR03-394 (blue dot) were selected from the cross DZ93-88 × YR 99–634. Cluster B contained 18 genotypes, of which nine were progenies of genotype DZ93-88. In addition, YR05-704, YR05-690, and YR05-724 were selected from the cross ROC10 × YR03-315; YR05-733, YR05-744 and YR05-747 were selected from the cross DZ93-88 × YR99-490; and YR05-282 and YR05-283 were selected from the cross DZ93-88 × YR03-373. Twenty-eight genotypes were assigned to Cluster C, of which YR10-291, YR10-299, and YR10-1055 were selected from the cross CP72-1210 × YR03-7. YR09-751 and YR09-753 were selected from the cross CP86-1664 × YR05-628. YR09-311 and YR09-315 were selected from the cross CL69-52 × YR05-285. Cluster D contained 35 genotypes, among which YR05-285 and YR05-292, YR11-101 and YR11-103, YR05-781 and YR05-782, and YR03-809 and YR03-80 had the same pedigrees (Table 1), respectively. Most of these Cluster D genotypes were placed close to each other on the same branch, except for YR11-101 and YR11-103. However, six genotypes were not assigned into the same cluster even though these genotypes were derived from the same cross. For example, YR05-566 and YR06-8362 were selected from the cross CP65-357 × YR03-917, but they were assigned into cluster D and C, respectively. YR05-785 and YR05-768 were selected from the cross CP65-357 × YR03-405, but they were assigned into cluster D and B, respectively. Similar results also occurred for YR03-78 and YR05-790 as these two genotypes were scattered in different clusters than their respective sister lines.

Population Structure of Yunrui-Series Sugarcane Genotypes

In the STRUCTURE analysis, a sharp peak of ΔK was present at $K = 4$ (Fig. 3A), indicating that the 104 genotypes were segregated into four populations. If we considered genotypes with a *Q* value score higher than 0.6 as pure and those with a *Q* value score lower than 0.6 as admixture, 52 genotypes could be classified as admixtures. Based on the *Q* value scores, 28 genotypes were assigned to POP-A, of which 17 were pure and 11 were admixtures ($Q < 0.6$). Twenty genotypes were assigned to POP-B with 8 pure and 12 admixtures. Twenty-four genotypes were assigned to POP-C with 11 pure and 13 admixtures. Thirty-

Table 4 Consistency analysis between cluster results and population structure analysis results

Populations	Cluster[a] A	Cluster B	Cluster C	Cluster D
POP-A[b]	YR06-189	YR11-256, YR15-1117, YR15-1086	YR10-172, YR06-4674, YR09-753, YR09-751, YR15-1112, YR99-113, YR15-1035, YR95-128, YR05-407, YR10-725, YR10-509, YR10-550	YR12-248, YR16-26, YR05-784, YR05-785, YR11-103, YR05-668, YR13-33, YR14-161, YR14-190, YR11-101, YR09-155,YR10-336
POP-B	YR03-393	YR05-690, YR05-733, YR05-762, YR05-747, YR05-744, YR10-330	YR10-495, YR10-1055, YR05-458, YR05-440, YR10-648	YR04-1051, YR11-450, YR05-782, YR05-662, YR05-606, YR05-596, YR05-346, YR05-566
POP-C	YR06-2416, YR12-399, YR03-78, YR03-394, YR03-392	YR05-704, YR05-724, YR05-767, YR05-283, YR05-282, YR05-770, YR05-189	YR10-248, YR06-8362, YR06-8270, YR06-6003, YR09-311, YR09-315	YR12-263, YR12-450, YR05-790, YR14-195, YR05-285, YR05-292
POP-D	YR16-82, YR06-3603, YR06-3226, YR06-3227, YR16-81, YR16-42, YR15-1095, YR99-48, YR99-67, YR99-601, YR03-425, YR03-417, YR03-409, YR03-315, YR03-11, YR09-895	YR05-768, YR15-103	YR10-299, YR10-291, YR08-1276, YR05-457, YR10-736	YR03-80, YR03-809, YR04-52, YR05-781, YR05-679, YR14-211, YR14-221, YR05-628, YR09-176

[a]Cluster *A*, *B*, *C* and *D* were from the dendrogram;

[b]POP-A, POP-B, POP-C and POP-D were from the population structure analysis

Table 5 Pairwise *Fst* values among the four populations

POP	*A*	*B*	*C*	*D*
A	0.000			
B	0.014	0.000		
C	0.019	0.009	0.000	
D	0.022	0.017	0.023	0.000

two genotypes were assigned to POP-D with 16 pure and 16 admixtures (Fig. 3B, C).

There was no consistency between genetic relationships and structural analyses (Table 4). For instance, 28 genotypes from POP-A were assigned into four clusters in the dendrogram, of which 12 were assigned to C and D, respectively, three genotypes were assigned to B, and one was assigned to A (Table 4). Twenty genotypes from POP-

Table 6 The AMOVA analysis of the 104 Yunrui-series sugarcane genotypes

Source of variance	*df* [a]	SSD [b]	MSD [c]	Variance component	Percentage of total variance	*Fst* value	Probability (*p*)
Among populations	3	131.040	43.680	0.552	2	0.018	< 0.001
Within populations	100	2947.681	29.477	29.477	98		
Total	103	3078.721		30.029	100		

[a]Degrees of freedom

[b]Sum of squared deviation

[c]Mean squared deviation

B were scattered into cluster A, B, C, and D, respectively. The genotypes from POP-C were almost equally divided among clusters. Most genotypes from POP-D were assigned to cluster A, then D, while cluster B only contained two genotypes. It was worth noting that 52 genotypes (50%) were admixtures, suggesting that these genotypes could be assigned to any population. The results of genetic relationship analysis and structure analysis indicated that 104 genotypes were genetically complex and high similarity, and shared various degrees of introgression among them.

Genetic Differentiation and AMOVA

Pairwise *Fst* values varied from 0.009 to 0.023 (Table 5), suggesting a low level of genetic differentiation among populations. The highest level of genetic differentiation (*Fst* = 0.023) was observed between POP-C and POP-D. The lowest level of genetic differentiation was detected between POP-B and POP-C with a *Fst* value of 0.009. A similar result was obtained from AMOVA analysis with a highly significant *Fst* value of 0.018 at $p < 0.001$ (Table 6), suggesting an overall extremely low level of genetic differentiation among and within the four populations. Genetic variation among the four populations was only 2%, while variation within populations was 98%, possibly due to the fact that only a few genotypes had been repeatedly used as male or female parents in the early breeding stages at Ruili Breeding Station.

Discussion

Sugarcane genotyping is a powerful tool to guide breeding programs. Yunrui-series sugarcane genotypes have been increasingly used for sugarcane breeding in China, especially in Yunnan province. Previous studies of the Yunrui-series genotypes mainly focused on phenotypic traits and stress tolerance. This study provided a systematic study of the genetic diversity for a large collection of Yunrui-series genotypes that are commonly used as parents for crossing. An SSR fingerprint profile of 104 Yunrui-series genotypes was constructed that confirmed the value and effectiveness of the 21 SSR primer pairs (Pan 2010). These primer pairs were highly polymorphic and helped identify genotypes from the same cross. However, six genotypes from the same cross were not assigned to the same cluster in the dendrogram. For example, YR06-8362 and YR05-566 shared the same parents, but YR06-8362 was assigned to cluster C while YR05-566 was assigned to cluster D. A similar situation was reported by Fu et al. (2016). We speculated that this may be caused by various degrees of introgression among Yunrui-series genotypes which resulted in a high similarity. Besides, genotype misidentification or mislabeling is possible in a breeding program because the early-stage hybrid selection is mainly based on plant phenotype and field performance. The clones with good field performance were likely to be selected. Furthermore, the SSR fingerprints of sugarcane genotypes were reproduceable across locations and years (Pan 2010; Pan et al. 2007), and in very rare cases, a non-parental SSR fingerprint was observed (Pan et al. 2015; Lu et al. 2015). Since 2003, SSR genotyping became an effective tool for sugarcane breeders to identify mislabeled sugarcane genotypes in field trials and crossing carts (Pan et al. 2003; Todd et al. 2020; Pan, unpublished). We also found the pedigrees of two Yunrui-series genotypes (YR05-458 and YR10-550) were probably incorrect based on their parental SSR fingerprint and suggested that these two genotypes were probably not the progenies of designated crosses (YR04-1051 × YR03-315) and (YR05-782 × YR03-80). Even though genotypes YR04-1051 and YR05-782 were most likely their respective maternal parents, the paternal or pollen parents were unknown. These findings point to a common problem of misidentification, mislabeling, and pollen control in sugarcane breeding programs (Pan 2010; Todd et al. 2020). Therefore, it is a requisite to integrate molecular marker and phenotypic information into the breeding program even if the genetic distance and pedigree records are agreed in most cases (Wang et al. 2020). However, considering that sugarcane has a highly complex genome and that not all markers show Mendelian inheritance, additional research is needed to identify the source of new, yet rarely occurring, marker fragments and trace the specific bands of males in progeny.

Genetic diversity, either phenotype-based or molecular marker-based, is a crucial parameter for breeding and has long been investigated in sugarcane (Chang et al. 2012; Govindaraj et al. 2014; Zan et al. 2020). Previous studies mainly targeted the genetic diversity among sugarcane varieties and related *Saccharum* species (You et al. 2013; Liu et al. 2016; Ali et al. 2017, 2019). This study assessed the genetic diversity of the Yunrui-series genotypes that have played a key role in the Chinese sugarcane breeding programs, most recently the breeding programs in Yunnan province. Although moderate to high values of PPB and PIC were found among the Yunrui-series genotypes, the two values were lower than the reported earlier for Chinese sugarcane varieties (Ali et al. 2017). This is because PIC values can vary among different germplasm sources (Arkova et al. 2015). In addition, breeders' preference has a huge effect on the genetic diversity of the germplasm. For instance, ROC-series sugarcane varieties and DZ 93-88 have been used repeatedly as parents at Ruili Breeding Station for many years, causing a relatively high level of genetic similarity among the germplasm resources.

Besides genetic diversity, genetic distance and genetic structure also are important reference parameters for the choice of parent clones. In this study, the 104 Yunrui-series genotypes were divided into four populations by both cluster and population structure analyses. For STRUCTURE analysis, the *Q* values of these genotypes exhibited a similar trend in that 52 (half of the genotypes) had *Q* values less than 0.6 (Fig. 3), suggesting a complex genetic composition of the Yunrui-series genotypes. From a genome perspective, sugarcane is a highly complex polyploid and aneuploid hybrid ($2n = 8 \times$ or $10 \times = 100$–130 chromosomes) derived from inter-specific hybridization between *S. officinarum* and *S. spontaneum* (D'Hont et al. 1996; Grivet and Arruda 2002). The complex genetic composition of the Yunrui-series genotypes can also be explained from the perspective of the breeding process or pedigree. Most Yunrui-series genotypes used in this study are BC_3 to BC_6 progenies developed by the breeders at Ruili Breeding Station, who first crossed superior sugarcane cultivars with indigenous *S. spontaneum* genotypes, then selected the excellent F_1 hybrids for backcrossing with cultivars.*Fst* is an important parameter to assess genetic differentiation among populations, and an *Fst* value ranged from "0" to "1". A value of "0" suggests no differentiation, while a value of "1" suggests a complete differentiation between the populations (Aesomnuk et al. 2021). In this study, a low level of *Fst* was found among the four populations (Table 5) that also coincided with the AMOVA results (Table 6), where 98% of the total variations were within-population, while only 2% of the total variation was accounted for among population. This high level of genetic homogeneity was reasonable given the limited number of superior sugarcane cultivars available as shown by the pedigree information in Table 1. In comparison to the results from 91 nationally or provincially released Chinese sugarcane varieties (Ali et al. 2017), the 104 Yunrui-series genotypes shared a relatively higher level of homogeneity. Therefore, the breeders should explore and introduce more diverse sugarcane germplasm resources. Furthermore, the results also offer a powerful support for the utilization of Yunrui-series sugarcane resources in future breeding programs. When designing sugarcane crosses, genetic divergence and genetic structure of the parental genotypes should be considered to maximize genetic variation among the hybrids. The fingerprint profile, dendrogram, and genetic structure plots from this study would offer useful molecular information for exploration and utilization of the key Yunrui-series genotypes in the future breeding programs.

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