Research Article

Genetic Diversity Analysis of Sugarcane Parents in Chinese Breeding Programmes Using gSSR Markers

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Sugarcane is the most important sugar and bioenergy crop in the world. The selection and combination of parents for crossing rely on an understanding of their genetic structures and molecular diversity. In the present study, 115 sugarcane genotypes used for parentalcrossing were genotyped based on five genomic simple sequence repeat marker (gSSR) loci and 88 polymorphic alleles of loci (100%) as detected by capillary electrophoresis. The values of genetic diversity parameters across the populations indicate that the genetic variation intrapopulation (90.5%) was much larger than that of interpopulation (9.5%). Cluster analysis revealed that there were three groups termed as groups I, II, and III within the 115 genotypes. The genotypes released by each breeding programme showed closer genetic relationships, except the YC series released by Hainan sugarcane breeding station. Using principle component analysis (PCA), the first and second principal components accounted for a cumulative 76% of the total variances, in which 43% were for common parents and 33% were for new parents, respectively. The knowledge obtained in this study should be useful to future breeding programs for increasing genetic diversity of sugarcane varieties and cultivars to meet the demand of sugarcane cultivation for sugar and bioenergy use.

1. Introduction

Sugarcane (Saccharum spp.) is the main sugar and bioenergy crop in the world. In comparison to other countries, Chinese sugar consumption is much lower and has only about 1/3 average of the world due to the different diet. However, the total sugar consumption, production, and import are in the second, third, and first positions in the world in recent years [1]. In addition, sugar from sugarcane occupies about 90%–92% of the total sugar output in China [2]. With an increasing demand for sugar, sugarcane shows more potential in China, leading to over one million sugarcane seedlings cultivated, which are produced from a total of 600–700 cross combinations every year in China [1]. The security of sugarcane cultivation is under threat from a number of diseases, especially smut disease caused by Sporisorium scitamineum and mosaic disease caused by sugarcane mosaic virus or sorghum mosaic virus. This leads to a demand for heterogeneity of cultivars. However, the heterogeneity of cultivars remains low, since the three "ROC" serial varieties account for about 85% of the total sugarcane cultivated area in China, with one (ROC22) responsible for about 50%–60% of the cultivated area in the last ten years [1]. Cross breeding is the most important way for breeding new sugarcane varieties and variety improvement, and it has played a significant role in the development of sugar industries in almost all the sugarcane-producing countries [3]. In addition, parental crosses of sugarcane always improve significantly the cane stalk yield and sugar content; thus, it is important to get the understanding of the genetic diversity of parents for crosses in breeding programs in China.

Traditional ways for sugarcane breeders to identify the relationships among varieties rely on anatomical and morphological characters [4]. In recent years, genetic diversity has been investigated for sugarcane cultivars or ancestral species by using several molecular methods, such as restriction fragment length polymorphism (RFLP) [5, 6], random amplified polymorphic DNA (RAPD) [7, 8], amplified fragment length polymorphism (AFLP) [9], intersimple sequence repeats (ISSR) [10, 11], sequence-related amplified polymorphism (SRAP) [12, 13], target region amplification polymorphism (TRAP) [14, 15], genomic in situ hybridization
2. Materials and Methods

2.1. Plant Materials. The background of the sugarcane parents used in this study was given in Table 1. Leaf samples of a total of 115 sugarcane accessions, including 64 common parents and 51 new parents, were collected. They were cultivated in Sugarcane Resources Nursery of FAFU (Fujian Agriculture and Forestry University, Fuzhou, China) and Ruili Breeding Station in Yunnan Academy of Agriculture Science (Ruili, Yunnan, China).

2.2. DNA Extraction. DNA extractions from the leaf tissues were conducted according to biospin plant genomic DNA extraction kit specification (Bioflux, Japan). Each leaf sample was collected from three independent sugarcane plants and only +1 leaf from each plant. After detection of the quality and concentration, this batch of genomic DNA was diluted to a suitable concentration and stored at −20°C.

2.3. SSR Analysis. A total of five highly polymorphic SSR DNA markers (SMC334BS, SMC336BS, SMC36BUQ, SMC286CS, and SMC569CS) were selected from 221 ICSB sugarcane SSR markers [24, 32]. Forward primers of all these SSR primers were labeled with FAM, the fluorescence dye. PCR amplification was performed in a 25μL reaction containing 50 ng of genomic DNA, 2.5 μL 10 × PCR buffer, 0.2μM of each primer, 200μM dNTP mixtures, and 1.0 U of rTaq polymerase. PCR comprised the following steps: the first cycle was preceded by a 3 min denaturation at 94°C, then thirty-one PCR cycles were performed in a PCR amplifier (Eppendorf 5333), with each cycle consisting of denaturation at 94°C for 30 s, annealing at either 58°C, 60°C, 62°C, or 64°C for 30 s (SMC286CS, SMC334BS, SMC569CS, and SMC36BUQ) and 62°C for 35 s (SMC336BS), and extension at 72°C for 30 or 35 s, and the last cycle was followed by a 2 min final extension at 72°C. Fragment analyses of amplified PCR products were conducted by capillary electrophoresis (CE) on ABI PRISM 377-96 DNA sequencer (Applied Biosystems) according to the manufacturer’s instructions. Each CE sample included 1.0 μL post-PCR reaction mixture, 0.5 μL of ROX-360 size standards, and 8.5 μL loading buffer of which the major ingredient contained polyacrylamide and dextran-blue. Then, PCR-amplified SSR DNA fragments were separated, and both the size standard and PCR amplified fragments were recorded automatically into individual GeneScan files.

2.4. Data Analyses. The data obtained from GeneScan files were analyzed with GeneMapper software (Applied Biosystems) to produce capillary electropherograms of amplified DNA fragments. GeneMapper parameters were set as follows: plate check module: Plate Check A; pruner module: GS PR36A-2400; run module: GS run 36A-2400; collect time: 2.5h; and lanes: 64. An SSR allele or peak was scored either as present (1) or absent (0), except for “stutters,” “pull-ups,” “dinosaur tails,” or “minus adenine” [24, 32]. The polymorphic information content (PIC) was calculated by the formula $\text{PIC} = 1 - \sum P_i^2$, where $P_i$ is the frequency of the population carrying the $i$th allele, counted for each SSR locus [21]. Then, the binary data matrices were used for genetic diversity parameter analysis. POPGENE 1.31 [33] was used to determine number of polymorphic bands (NPB); percentage of polymorphic bands (PPB); observed number of alleles (Na); and effective number of alleles (Ne). Nei’s genetic diversity ($h$), mean values of total gene diversity ($Ht$), and Shannon’s information index ($I$) were computed for each population based on allele frequencies and calculated for haploid data. In addition, gene diversity within populations ($Hs$), gene diversity between populations (Dst) by the formula $(\text{Dst} = Ht - Hs)$, gene differentiation coefficient (Gst)
| Code | Name of accession | Collection place | Code | Name of accession | Collection place |
|------|------------------|------------------|------|------------------|------------------|
| 1    | GT86-267         | FAFU             | 59   | CP65-357         | FAFU             |
| 2    | GT89-5           | FAFU             | 60   | CP67-412         | FAFU             |
| 3    | GT93-103         | FAFU             | 61   | CP72-1210        | FAFU             |
| 4    | GT94-116         | FAFU             | 62   | CP72-1312        | FAFU             |
| 5    | GT94-119         | FAFU             | 63   | CP84-1198        | FAFU             |
| 6    | GT96-44          | FAFU             | 64   | CP85-1308        | Ruili            |
| 7    | GT96-211         | FAFU             | 65   | CP88-1762        | FAFU             |
| 8    | GT73-167         | Ruili            | 66   | CP89-1509        | FAFU             |
| 9    | *GT89-7          | FAFU             | 67   | *CP92-1167       | FAFU             |
| 10   | *GT90-55         | FAFU             | 68   | ROC1             | FAFU             |
| 11   | *GT94-119        | FAFU             | 69   | ROC10            | Ruili            |
| 12   | *GT95-53         | FAFU             | 70   | ROC11            | Ruili            |
| 13   | *GF97-18         | FAFU             | 71   | ROC16            | FAFU             |
| 14   | YF96-835         | FAFU             | 72   | ROC20            | Ruili            |
| 15   | YF96-86          | FAFU             | 73   | ROC22            | Ruili            |
| 16   | YF00-236         | FAFU             | 74   | ROC24            | FAFU             |
| 17   | YF85-633         | Ruili            | 75   | ROC25            | Ruili            |
| 18   | YF91-967         | Ruili            | 76   | ROC26            | FAFU             |
| 19   | YF93-159         | Ruili            | 77   | *ROC2            | FAFU             |
| 20   | YF85-177         | Ruili            | 78   | *ROC7            | FAFU             |
| 21   | *YF82-882        | FAFU             | 79   | *ROC18           | FAFU             |
| 22   | *YF89-240        | Ruili            | 80   | F134             | Ruili            |
| 23   | *YF91-854        | FAFU             | 81   | *DZ93-88         | Ruili            |
| 24   | *YF91-1102       | FAFU             | 82   | *DZ93-94         | Ruili            |
| 25   | *YF96-244        | FAFU             | 83   | *DZ99-36         | Ruili            |
| 26   | *YF97-40         | FAFU             | 84   | YZ89-351         | FAFU             |
| 27   | YF71-374         | FAFU             | 85   | YZ94-375         | Ruili            |
| 28   | YF82-96          | FAFU             | 86   | *YZ92-19         | FAFU             |
| 29   | YF82-108         | FAFU             | 87   | *YZ99-91         | FAFU             |
| 30   | YF84-125         | FAFU             | 88   | *Q170            | FAFU             |
| 31   | YF89-46          | FAFU             | 89   | *Q171            | FAFU             |
| 32   | YF90-3           | FAFU             | 90   | *Q182            | FAFU             |
| 33   | YF90-33          | FAFU             | 91   | *CZ89-103        | Ruili            |
| 34   | YF92-27          | FAFU             | 92   | CZ19             | FAFU             |
| 35   | YF96-48          | FAFU             | 93   | *CN85-78         | FAFU             |
| 36   | *YF90-31         | FAFU             | 94   | ZZ74-141         | FAFU             |
| 37   | FN91-3623        | FAFU             | 95   | ZZ92-126         | FAFU             |
| 38   | FN91-4621        | FAFU             | 96   | POJ2878          | FAFU             |
| 39   | FN91-4710        | FAFU             | 97   | Co1001           | FAFU             |
| 40   | *FN81-475        | FAFU             | 98   | RB72-454         | Ruili            |
| 41   | *FN93-3608       | FAFU             | 99   | K3              | FAFU             |
| 42   | *FN94-0744       | FAFU             | 100  | GZ8              | FAFU             |
| 43   | *FN02-3924       | FAFU             | 101  | LCP85-384        | FAFU             |
| 44   | MT86-05          | FAFU             | 102  | *Nagori          | Ruili            |
| 45   | MT86-2121        | Ruili            | 103  | muck che         | Ruili            |
| 46   | MT90-55          | FAFU             | 104  | liaica82-1729    | Ruili            |
| 47   | MT92-649         | FAFU             | 105  | *YG94-39         | FAFU             |
| 48   | *MT69-421        | Ruili            | 106  | RF93-244         | FAFU             |
| 49   | *MT92-505        | FAFU             | 107  | Brazil45         | FAFU             |
| 50   | *MT93-246        | FAFU             | 108  | FR93-435         | FAFU             |
| 51   | *MT96-6016       | FAFU             | 109  | Mexi05           | FAFU             |
Table 1: Continued.

| Code | Name of accession | Collection place | Code | Name of accession | Collection place |
|------|------------------|------------------|------|------------------|------------------|
| 52   | HoCP91-555       | FAFU             | 110  | *YZ99-601        | FAFU             |
| 53   | HoCP93-746       | Ruili            | 111  | *Ki6             | FAFU             |
| 54   | HoCP93-750       | FAFU             | 112  | *B9              | FAFU             |
| 55   | HoCP95-998       | Ruili            | 113  | *PS45            | Ruili            |
| 56   | YN73-204         | FAFU             | 114  | *LY97-151        | Ruili            |
| 57   | *YN89-525        | FAFU             | 115  | *GN95-108        | FAFU             |
| 58   | CP49-50          | FAFU             |      |                  |                  |

Sugarcane Resources Nursery of Fujian Agriculture and Forestry University (FAFU); Ruili Breeding Station in Yunnan Academy of Agriculture Science (Ruili); *represents new parents.

Table 2: The allele detection results of 5 SSR markers used for evaluation of 115 sugarcane accessions.

| Primer name | Number of alleles | Number of rare alleles | Range of allele size (bp) | Major allele Size (bp) | Frequency (%) | PIC  |
|-------------|-------------------|------------------------|--------------------------|------------------------|---------------|------|
| SMC334BS    | 19                | 6                      | 136–169                  | 147                    | 66.10         | 0.889|
| SMC336BS    | 26                | 15                     | 136–192                  | 168                    | 59.10         | 0.897|
| SMC36BUQ    | 11                | 8                      | 101–147                  | 122                    | 39.10         | 0.753|
| SMC286CS    | 15                | 6                      | 123–169                  | 146                    | 46.10         | 0.865|
| SMC569CS    | 17                | 11                     | 159–238                  | 220                    | 66.10         | 0.779|
| **Average** | **17.6**          | **9.2**                | **136–169**              | **147**                | **66.10**     | **0.837**  |

Rare allele means that the frequency of the allele is less than 5.0%; the major allele accounts for the highest proportion in all alleles.

3. Results and Analysis

3.1. SSR Markers. SSR markers were utilized to assess genetic diversity among all the 115 sugarcane parental accessions in this study, and the major values of genetic diversity parameters derived were showed in Table 2.

A total of five SSR loci were used to evaluate 115 sugarcane accessions. Distinct fragments in the size ranging from 101 bp to 238 bp were scored for analysis. The major allele of five SSR loci was observed at the sizes of 147 bp, 168 bp, 122 bp, 146 bp, and 220 bp, with the ratio of 66.1%, 59.1%, 39.1%, 46.1%, and 66.1% with the primers SMC334BS, SMC336BS, SMC36BUQ, SMC286CS, and SMC569CS, respectively. A total of 88 alleles within the data set were obtained, and alleles per locus ranged from 11 to 26, with an average of 17.6. The average number of rare alleles produced in a single individual was 9.2 (range 6–15). The highest number of alleles was scored at locus SMC336BS (26 alleles). The PIC values of five SSR loci ranged from 0.753 to 0.897 with a mean value of 0.837. The PIC value of the SMC336BS locus was the highest (0.897), while the lowest (0.753) was observed from SMC36BUQ locus.

3.2. Genetic Diversity among 64 Common Parents, 51 New Parents, and All 115 Parents. Significant genetic variation was found among all 115 parents with the genetic similarity (GS) value ranging from 0.725 to 1.000. The GS value ranged from 0.730 to 1.000 within the group of 64 common parents and from 0.722 to 0.943 within the group of 51 new parents. Of note, the GS value was 1.000 between MT90-55 and HoCP93-750, indicating that there was no genetic dissimilarity between the two parents based on the five SSR loci.

Genetic parameters for the five microsatellite loci in the two groups, common parents and new parents, were given in Table 3. A total of 88 polymorphic bands within the entire data set were scored, while taking the two groups considered separately, 82 of them were within the 64 common parents (93.18%), and 69 of them were within the 51 new parents (78.41%). Observed numbers of alleles (Na) were the same (2.000) in the two groups, and effective numbers of alleles (Ne) were higher in new parents group (1.359) than in common parents group (1.302). Nei’s gene diversity (h) was 0.178, and Shannon’s information index (I) was 0.288 in the overall sugarcane testing accessions. In contrast to the total diversity, both sugarcane parent groups of common parents and new parents had relatively high diversity, h = 0.190 and 0.223 and I = 0.308 and 0.356, respectively.

Table 4 summarized the genetic differentiation of sugarcane accessions from the two groups. The values of Ht and Dst were higher in new parents group (Ht = 0.214, Dst = 0.058) than those in common parents group (Ht = 0.190, Dst = 0.032), while the value of genetic diversity (Hs) within population was similar in two groups (0.158 for common
Table 3: The values of genetic diversity parameters for sugarcane accessions of common and new parents in different groups, estimated based on polymorphisms of 5 SSR loci.

| Group          | Clones size | NPB | PPB (%) | Na  | Ne  | hI   | I   |
|----------------|-------------|-----|---------|-----|-----|------|-----|
| Common parents | 64          | 82  | 93.18   | 2.000 | 1.302 | 0.190 | 0.308 |
| New parents    | 51          | 69  | 78.41   | 2.000 | 1.359 | 0.223 | 0.356 |
| Total          | 115         | 88  | 100.0   | 2.000 | 1.283 | 0.178 | 0.288 |

Number of polymorphic bands (NPB); percentage of polymorphic bands (PPB); observed number of alleles (Na); effective number of alleles (Ne); Nei’s genetic diversity (hI); Shannon’s information index (I).

Table 4: Genetic diversity and differentiation of sugarcane accessions between common and new parents, estimated by POPGENE (version 1.31).

| Group          | Clones size | Ht   | Hs    | Dst  | Gst  | Nm   |
|----------------|-------------|------|-------|------|------|------|
| Common parents | 64          | 0.190| 0.158 | 0.032| 0.171| 2.429|
| New parents    | 51          | 0.214| 0.156 | 0.058| 0.273| 1.335|
| Total          | 115         | 0.176| 0.159 | 0.017| 0.095| 4.762|

Mean values of total gene diversity (Ht), gene diversity within populations (Hs), gene diversity between populations (Dst), gene differentiation coefficient (Gst), and estimates of gene flow from Gst (Nm) were obtained by \((1 - \text{Gst})/2\text{Gst}\).

Table 5: Genetic diversity of sugarcane parents in 9 series released by different breeding institutions, estimated based on polymorphisms of 5 SSR loci.

| Series | Clones size | NPB | PPB (%) | Na  | Ne  | hI   | I   |
|--------|-------------|-----|---------|-----|-----|------|-----|
| GT     | 13          | 47  | 53.41   | 1.534| 1.262| 0.162| 0.250|
| YT     | 13          | 50  | 56.82   | 1.568| 1.267| 0.166| 0.258|
| YC     | 10          | 49  | 55.68   | 1.557| 1.283| 0.178| 0.275|
| FN     | 7           | 37  | 42.05   | 1.421| 1.236| 0.144| 0.219|
| MT     | 8           | 44  | 50.00   | 1.500| 1.259| 0.161| 0.247|
| HoCP   | 4           | 32  | 36.36   | 1.364| 1.268| 0.152| 0.221|
| CP     | 10          | 36  | 40.91   | 1.409| 1.222| 0.136| 0.181|
| ROC    | 13          | 46  | 52.27   | 1.523| 1.259| 0.160| 0.247|
| OTHER  | 37          | 62  | 70.45   | 1.705| 1.290| 0.177| 0.278|

parents group and 0.156 for new parents group), indicating that the genetic diversity of these two groups mainly existed within populations. The gene flow index (Nm) within groups showed that low gene flow (2.429 and 1.335, resp.) occurred in both groups, while the Gst was high in both groups—0.171 and 0.273, respectively. The gene flow between the two groups was much higher (Nm = 4.762) than those in both groups. This also indicated that the genetic variation mainly existed within populations.

3.3. Genetic Relationships of 115 Sugarcane Parents. Nine series from 115 accessions sorted by institution-based breeding programme are shown in Table 5. According to the information indicated in Table 1, we assigned them as the following nine series: GT series (13) from Guangxi Sugarcane Institute; YT series (13) from Guangzhou Institute of Sugarcane and Sugar Industry; YC series (10) from Hainan Sugarcane Breeding Station; FN series (7) from Sugarcane Research Institute of FAFU; MT series (7) from Sugarcane Research Institute, Fujian Academy of Agricultural Sciences; HoCP series (4) from Sugarcane Research Unit, Houma, Louisiana, United States Department of Agriculture, USA; CP series (10) from Sugarcane Experiment Station, Canal Point, Florida, United States Department of Agriculture, USA; and "ROC" series (13) from Taiwan Sugar Corporation. The rest of the sugarcane parents included 37 accessions from several breeding institutions different from all the above eight and were termed as OTHER.

Genetic diversity parameters for the 5 microsatellite markers in the 9 sugarcane series were presented in Table 5, indicating that except the highest NPB value (62 stands for 70.45%) observed in OTHER series, the polymorphisms among eight determinate series were as follows: YT (50, 56.82%) > YC (49, 55.68%) > GT (47, 53.41%) > "ROC" (46, 52.27%) > MT (44, 50.00%) > FN (37, 42.05%) > CP (36, 40.91%) > HoCP (32, 36.36%). Observed numbers of alleles (Na) were higher in OTHER (Na = 1.705) and YT (Na = 1.568) series compared to those of the remaining seven series. Moreover, effective numbers of alleles (Ne) were also higher in OTHER (Ne = 1.290) and YC (Ne = 1.290) series compared to those of the remaining seven determinate series. Except OTHER series (h = 0.177, I = 0.278), both the gene diversity (h) and the Shannon information index (I) were higher in YC (h = 0.178, I = 0.275) series but lower in CP series (h = 0.136, I = 0.181).

The number of alleles based on 5 SSR loci in different series of GT, YT, YC, FN, MT, HoCP, CP, “ROC,” and OTHER was illustrated in Figure 1. A total of 1,395 alleles...
were detected for all the 115 testing sugarcane accessions with an average of 12. The maximum number of alleles was 18 observed in YT93-159, while the minimum number was 7 in three accessions of GT90-55, YC96-48, and FN93-3608. Within the GT and FN series, the number of alleles both ranged from 7 to 15 with mean values of 11.8 and 11.1, respectively. In YT series, the number of alleles per locus ranged from 8 to 18 with an average of 12.6. Within MT series, the number of alleles ranged from 8 to 14, and the average number was 11.3. In HoCP series, the number of alleles was located between 11 and 15 with an average of 12.8. Within CP series, the number of alleles ranged from 8 to 13 with an average of 11.0. In "ROC" series, the number of alleles ranged from 10 to 16 with an average of 12.5. Within OTHER series, with an average of 12.0, the number of alleles was from 8 to 17.

### 3.4. Cluster Analysis

The measure of genetic distance (GD) can be applied to any kind of organism without regard to ploidy or mating scheme [36], with genetic distance estimates hardly affected by the sample size [37]. Therefore, in this study, a UPGMA dendrogram was constructed based on Nei's genetic distance (Figure 2), showing the genetic relationships among the various series, including single series of GT, YT, YC, FN, MT, HoCP, CP, and “ROC” and complex series of OTHER and that between two groups of common parents (64) and new parents (51). The 115 sugarcane parents were classified into three groups (Group I, Group II, and Group III) at the level of GD = 0.03. Group I consisted of 53 common parents and 38 new parents, including 10 from GT, 12 from YT, 7 from YC, 5 from FN, 6 from MT, 4 from HoCP, 9 from CP, 11 from "ROC,” and 27 from OTHER. Group II contained 3 common parents and 4 new parents, including 1 from GT, 2 from YC, 1 from CP, and 3 from OTHER. Group III contained 8 common parents and 9 new parents, including 2 from GT, 1 from YT, 1 from YC, 2 from FN, 2 from MT, 2 from "ROC,” and 7 from OTHER. At the level of GD = 0.09, Group I could be further divided into five subgroups (Subgroup Ia, Ib, Ic, Id, and le). Ia contained 15 common parents and 8 new parents, including 3 from GT, 2 from YT, 3 from YC, 2 from FN, 3 from MT, 3 from CP, and 7 from OTHER. Ib consisted of 30 common parents and 27 new parents, including 5 from GT, 10 from YT, 4 from YC, 3 from FN, 3 from MT, 2 from HoCP, 4 from CP, 10 from "ROC,” and 16 from OTHER. Ic had only two parents from YT containing 1 common parent and 1 new parent. Id contained 3 parents from each of HoCP, CP, and “ROC” and belonged to common parents. Ie contained 4 common parents and 2 new parents, including 1 from HoCP, 1 from CP and 4 from OTHER series.

It should be noted that Group I included most of the parents which came from different series. The above results demonstrate that the genotypes released by the same breeding institutions showed closer genetic relationships, except YC series released by Hainan sugarcane breeding station, which aimed at sugarcane germplasm innovation. It suggested that these parents should be useful in sugarcane cross breeding due to various genetic distances among them. Besides, a total of four testing accessions, including pairs of YT96-86 and YN73-204, plus MT90-55 and HoCP93-750, could not be distinguish based on the 5 microsatellite markers, and it may be due to their sharing of similar basis of genetic background.

### 3.5. Principal Component Analysis

PCA examined a dissimilarity matrix of pairwise differences between specimens and used eigenvalue analysis in order to take the variation between specimens and condense them into a limited number of dimensions. The maximum amount of variation was plotted as the first axis, with subsequent variation of lesser magnitude explained by each additional dimension [38]. The principal component analysis, which can be helpful for illustrating the genetic relationships of sugarcane parents as individual units, was calculated based on the SSR data matrix of the 5 loci for all 115 sugarcane accessions occupied in this study (Figure 3). The first and second principal components accounted for a cumulative 76% of the variance, including 43% for common parents and 33% for new parents, respectively. As shown in Figure 3, the 115 sugarcane parents were scattered in a limited space, covering 90% of CP series, 85% of YT and “ROC” series, 77% of GT series, 75% of MT and HoCP series, 73% of OTHER series, 71% of FN series, and 50% of YC series, respectively. We found that the distribution of sugarcane accessions in CP, YT, and “ROC” series was relatively narrow, while it was wider in YC, FN, and OTHER series. This revealed that genetic basis of the latter group was more extensive than the former group. Furthermore, the plots of two pairs of sugarcane accessions (YT96-86/YN73-204 and MT90-55/HoCP93-750) overlapped strongly (Figure 3). This analysis could not differentiate YT96-86 from YN73-204 or MT90-55 from HoCP93-750 at least at a molecular level based on the 5 SSR markers used in this study.

### 4. Discussion and Conclusions

Improvement of sugarcane by genetic manipulation has been ongoing since 1888, following the observation in 1858 that sugarcane produced viable seed [1, 39]. According to the studies of Chen et al. (2011) and of Baver (1963), the
Figure 2: The UPGMA dendrogram of 115 Sugarcane parents based on 5 pairs of SSR primers.

Figure 3: Principal coordinates analysis (PCA) of 115 sugarcane parents using 5 pairs of SSR markers based on genetic similarity.
contribution based on genetic improvement to increase the yield of cane sugar was estimated to be 75% of the yield increase attained by the Hawaiian sugar industry in the 1950s and more than 60% in the Chinese sugar industry in the last three decades [1, 40]. In Hawaii, the yield has improved every decade except in the 1970s, when disease problems plagued the sugar industry [40]. Although the degree to which varietal improvement has contributed to increase yield potential has varied widely from nation to nation, undoubtedly all nations have benefited to some degree by converting to newer, improved varieties from cross breeding. In addition, sugarcane is a potential bioenergy crop due to its high yield and high biomass. The world record and average in Hawaii (1978–1982) are 24.2 and 11.9 metric tons/ha/year, respectively. The 11.9 metric tons/ha/year represents a sugarcane dry matter yield of only 0.07 mt/ha/day, which is much lower than the theoretical maximum of 0.7 mt/ha/day estimated by Loomis and Williams [41].

In China, approximately 400 sugarcane varieties have been released in the last 50 years by cross breeding [42]. However, most of the sugarcane cultivars in the world can be dated back to only a few common ancestors [1, 19]. This may be due to the problem that the genetic basis of the sugarcane is limited; thus, new cultivars with interesting traits are difficult to be developed [43]. A similar situation has occurred in China, where the major cultivars in the 1980s, 1990s, and 2000s were ROC10, ROC16, and ROC22, respectively. Thus, till now, the heterogeneity of cultivars has been very low since the variety ROC22 takes about 50%–60% of the total sugarcane planting area. This limits any further increase of sugar yield per unit and has many potential risks of suffering from common diseases [1]. Sugarcane cross breeding largely depends on broadening the genetic basis and the selection of parents for crossing. The Hainan Sugarcane breeding station is responsible for sugarcane hybridization in China, innovation targets of parents, and introduction of new parents into sugarcane hybridization programs. An increase in the genetic diversity of parental accessions should be helpful to broaden the genetic basis of the sugarcane [26, 44].

In the present study, the genetic diversity of 115 sugarcane parents was evaluated based on 5 microsatellite loci. These SSR markers were highly robust and codominant as characterized by high PIC value (0.84 on average), but exhibited the lower level of polymorphism described by Liu (2011) who reported average PIC value = 0.70 [24]. However, the level of polymorphism obtained in our and Pan’s studies was much higher than other SSR markers reported by Filho et al. (2010), who reported mean PIC value = 0.57 [45]. Genetic diversity of different series including eight determinate and one complex (OTHER) series showed that YC series had higher genetic diversity ($h = 0.188$ and $I = 0.275$) except OTHER ($h = 0.177$ and $I = 0.278$) and that CP and FN series had lower ones ($h = 0.136$ and 0.144, $I = 0.181$ and 0.219, resp.). This is consistent with the results reported by Li et al. (2005) and Lao et al. (2008) [46, 47].

In the present study, all 64 accessions in common parents group showed relatively lower diversity, compared with the higher diversity exhibited by 51 accessions of new parents group. The result was based on the value of Nei’s genetic diversity ($h = 0.190 < 0.223$) and Shannon’s information index ($I = 0.308 < 0.356$), indicating that the innovation of parents has showed a positive role in sugarcane breeding programs in China, since the group of new parents has higher genetic diversity, and thus, it will to some degree benefit the broadening of the genetic basis in sugarcane hybridization.

The values of Nei’s genetic diversity and Shannon’s information index were much lower in other series than those in two groups. However, the level of diversity obtained in our research (two groups) was similar to previous research, which reported Nei’s genetic diversity $h = 0.222$ and Shannon’s information index $I = 0.328$ [13]. Since gene flow can resist the effect of genetic drift within populations and prevent the differentiation of populations with $N_m > I$, the genetic drift would lead to genetic differentiation among populations as the value of $N_m < I$ [48]. The $N_m$ value in this study was 4.762, indicating that there was no significant genetic differentiation between the two groups or nine series. The low genetic differentiation ($G_{st}$) among populations was primarily caused by the high level of gene flow. However, compared to wild sugarcane ($G_{st} = 0.209$) [13] and weedy rice ($G_{st} = 0.387$) [38], the $G_{st}$ (0.095) of 115 sugarcane parents was still at a low level.

It is interesting that, in this study, both cluster and PCA analyses of individuals (including all the nine series) exhibited similar results: OTHER, YC, and GT series fell into three different groups and HoCP only belonged to Group I. Furthermore, a limited space covered 90% CP series, 85% YT and “ROC” series, and only 50% YC series, respectively. It was obvious that the distribution of accessions in CP, YT, and “ROC” series was relatively narrow while it was broader in YC, FN, and OTHER series. The results revealed that the genetic basis of YC, FN, and OTHER was more extensive than CP, YT, and “ROC” series, which also suggested that more attention should be made on the application of new parents in sugarcane hybrid breeding in the future. It was not difficult to find in the dendrogram (Figure 2) and PCA (Figure 3) that the clusters or components were closely related to their breeding institutions.

It was also apparent that there were two pairs of four accessions (YT96–86 and YN73–204 at the level of $G_{st}$ = 0.50 and MT90–55 and HoCP93–750 at the level of 0.59) which the analysis failed to differentiate. Furthermore, the PCA analysis indicated that the plots of YT96–86 and YN73–204 or MT90–55 and HoCP93–750 overlapped entirely. This shows that the analysis could not differentiate between these accessions at the molecular level based on the five testing SSR loci and indicated that more SSR loci would be necessary for differentiation from MT90–55 to HoCP93–750 and from YT96–86 to YN73–204. For example, based on the pedigree, HoCP93–750 evolved from CP84–0272 and LCP81–030, while MT90–55 derived from CP57–614 and YC84–153 (Figures 4 and 5). From the pedigree of HoCP93–750 and MT90–55, it is obvious that we could not find the same parents between the two sugarcane clones within five generations. Therefore, it is inaccurate to analyze the genetic structures, genetic diversity, or genetic relationships only by pedigree records. If we want
to further identify the four sugarcane clones, more SSR loci should be applied.

According to previous reports, gSSR markers produce polymorphisms based on the difference in the number of DNA repeat units in regions of the genome and derive from genomic DNA libraries at a high price, while EST-SSR detect variations in the expressed portion of the genome and can be mined from the EST databases at low price [20, 49, 50]. EST-SSR technology has been widely used in many plants, such as rice [51], sorghum [52], wheat [53], and several other plant species. However, the usefulness of EST-SSRs varies in different varieties of sugarcane, as the level of polymorphism (PIC = 0.23) was lower than that of anonymous SSR markers (PIC = 0.72) in sugarcane cultivars. It was also reported that
EST-SSRs had higher level of polymorphism across ancestral species (PIC = 0.66 > 0.62) [20]. In other research, the number of alleles of gSSRs loci (7–9) was more than EST-SSRs loci (4–6), and about 35% of the gSSRs had PIC values around 0.90 in contrast to 15% of the EST-SSRs (50). What should also be stressed is that the two types of SSR, gSSR and EST-SSR, made no significant difference at the average genetic similarity (GS) based on Dice coefficient and were in good agreement with pedigree information for genetic relationships analysis [50]. These results demonstrated that, in the future, EST-SSRs should be used together with gSSRs for genetic relationship analysis in sugarcane.

From the above discussion, identifying useful gSSRs is significant, but in sugarcane, this can be a lengthy and difficult process due to their complexity and their abundance within the sugarcane genome [20, 50, 54]. Therefore, there is further work required to promote this technique. This paper used only 5 pairs of gSSR primers in the genetic diversity analysis of 115 sugarcane parents in spite of the testing SSR loci being selected from a batch of gSSR loci (221 ICSB sugarcane SSR markers) and having shown to be robust and polymorphic. This suggests that more basic Saccharum species, more gSSR markers, and more molecular methods like EST-SSRs can be utilized in further study.

**Conflict of Interests**

The authors declare no conflict of interests.

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