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MULTIVARIATE ANALYSIS OF 31 PHENOTYPIC TRAITS AMONG MAJOR PARENTAL LINES OF SUGARCANE BREEDING PROGRAMS IN CHINA

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ABSTRACT

Sugarcane parental lines (*Saccharum* spp. hybrids) serve as a gene pool for sugarcane breeding and genetic improvement. Phenotypic evaluation is the most direct method to study genetic diversity among sugarcane parental lines. Morphological data of 31 phenotypic traits were collected from 130 major sugarcane parental lines for multivariate genetic analysis. The analysis revealed relatively high coefficients of variation and a wide range of frequencies. The extent of genetic diversity among the morphological traits reached a high level with the Shannon-Weiner index (*Hs*=0.858±0.026). The average genetic diversity index was greater for plant height and stem traits than all other traits. Relatively large genetic distances averaging 0.151 were observed among the 11 series of sugarcane parental lines, with the Guitang-series parental lines exhibiting the most genetic diversity (*Hs*=0.970±0.078). A dendrogram based on similarity coefficients clustered 130 major parental lines into three groups and six subgroups. The results provided key information on genetic diversity useful for the screening and selection of potential parents and the design of potential intercrosses.

Keywords: sugarcane parental line; phenotype; genetic diversity; diversity index; cluster analysis.

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INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) belongs to the *Gramineae* family, *Panicoideae* subfamily, *Andropogoneae* tribe, *Saccharinae* Benth group of *Saccharastrae* subtribe, which comprises the "*Saccharum* Complex" with *Erianthus* Michx. Sect. *Ripidium* Henrard, *Miscanthus* Anderss. Sect. *Diandra* Keng, *Narenga* Bor., and *Sclerostachya* A (Daniels and Roach 1987). The *Saccharum* genus consists of six species, *S. officinarum* L., *S. spontaneum* L., *S. robustum* Brandes and Jeswiet*ex* Grassl., *S. barberi* Jesw., *S. sinense* Roxb., and *S.edule* Hassk. These plant genetic resources have provided the foundation for sugarcane variety improvement(Liu *et al*. 2016).

Modern sugarcane varieties are primarily derived from interspecific hybridizations between the "Noble" cane *S. officinarum* and related species (Qi *et al*. 2012). Most sugarcane varieties contain genes from *S. officinarum*, *S. spontaneum*, and *S. barberi*. Some varieties also have genes from *S. sinense* or *S. robustum* (Deng *et al.* 2004). However, most sugarcane varieties grown in the world today can be traced back to only a few common progenitors from the progenies of POJ- or Co- series varieties (Deng *et al*. 2004). This narrow genetic base has hindered the progress of sugarcane improvement. Therefore, it is imperative that sugarcane breeders should investigate the genetic diversity of parental lines and broaden the genetic base by introducing superior genes from wild relatives into sugarcane varieties (Lima *et al*. 2002; Schenck *et al*. 2004; Tai and Miller 2001). Genetic diversity analysis is one of the leading methods for studying sugarcane parental lines.

With the development of biotechnology, various DNA-based molecular markers have been used in analyzing the genetic diversity of sugarcane (Qi *et al.* 2012; Cordeiro *et al*. 2000; Pan *et al*. 2004; Pan 2006; Chen *et al*. 2009; Pan 2010; Liu *et al*. 2011; You *et al*. 2016; Liu *et al*. 2018; Nayak *et al*. 2014; Wu *et al.* 2019). However, identification and description of phenotypic traits are still the most direct and basic methods to study sugarcane germplasm resources (Cai and Fan 2006). Compared to molecular markers, phenotypic traits are simple, obvious, easy to collect, economical, effective, and practical. Many researchers have carried out genetic diversity analysis of phenotypic traits in different crops. Liu *et al.* (2010) evaluated the genetic diversity of 100 sugarcane varieties with 23 phenotypic traits. Zan *et al* (2014) analyzed the genetic diversity of 104 exotic sugarcane using 29 morphological traits.

The genetic diversity of major parental lines has rarely been studied in China, which could greatly impact their effective utilization. In this study, morphological data on 31 phenotypic traits were analyzed for 130 major

sugarcane parental lines to analyze genetic diversity, genetic distance, and clustering. The objective of this study was to examine the phenotypic diversity of sugarcane parental lines of sugarcane breeding programs in China for parental selection.

MATERIALS AND METHODS

Plant materials: We chose 130 parental lines that had been used most frequently in sugarcane hybrid breeding programs in China. The parental lines were originally collected from seven provinces of China mainland, China Taiwan, and the USA. These lines were conserved in the Guangdong sugarcane germplasm resource bank at the Guangdong Provincial Bioengineering Institute (HSBS of the Guangzhou Sugarcane Industry Research Institute) and were classified into 11 series based on geographical origin (Table S1). Among these lines, 10 parental lines were bred in the 1980s, 40 were bred in the 1990s, 50 were bred in the 2000s, and 21 were introduced from China Taiwan, and USA. Twenty-one Yuetang-series parental lines (YT) were from the Guangzhou Sugarcane Industry Research Institute (Guangdong Province, China), 20 Guitang-series parental lines (GT) were from the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences (Guangxi Province, China), 15 Funong-series parental lines (FN) were from the Sugarcane Research Institute, Fujian Agriculture and Forestry University (Fujian Province, China), 15 Yacheng-series parental lines (YC) were the HSBS of Guangzhou Sugarcane Industry Research Institute (Hainan Province, China), 11 Yunzhe-series parental lines (YZ) were from the Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences (Yunnan Province, China), 8 ROC-series parental lines (ROC) were from the Taiwan Sugar Corporation (Taiwan, China), 10 Neijiang-series parental lines (NJ) were from the Neijiang Academy of Agricultural Sciences (Sichuan Province, China), eight Liucheng-series parental lines (LC) were from the Liucheng Academy of Agricultural Sciences (Guangxi Province, China), five Dezhe-series parental lines (DZ) were from the Dehong Sugarcane Research Institute (Yunnan Province, China), four Gannan-series parental lines (GN) were from the Jiangxi Sugarcane Research Institute (Jiangxi Province, China) (Figure 1). In addition, 13 CP-series parental lines (CP) were from the United States Department of Agriculture-Agricultural Research Service, Sugarcane Experiment Station, Canal Point, Florida, USA and the Sugarcane Research Unit, Houma, Louisiana, USA

Method of field test and investigation of traits: One hundred and thirty parental lines were maintained in the sugarcane parent nursery of Hainan Sugarcane Breeding Station (18°39′ north latitude and 109°15′ east longitude) during 2016-2017 (one-year Plant-cane and one-year Ratoon-cane). The plantlets had been germinated in a nursery garden in January 2016, followed by transplantation to the experimental plots in March 2016. Plant-cane was harvested in December 2016 and Ratoon-cane was planted in 2017. The experimental plots were managed using standard sugarcane parent field techniques.

At the mature stage of Plant-cane and Ratoon-cane, 10 plants of each parental line were randomly selected for analysis of 31 phenotypic traits according to Cai and Fan (2006) (Table S2). Of the 31 traits, 24 were qualitative, including adventitious root (AR), internode form (IF), internode arrangement (IA), internode color unexposed (ICU), internode color exposed (ICE), growth crack (GC), bloom (Bl), growth bands form (GBF), growth bands color unexposed (GBCU), growth bands color exposed (GBCE), lodging resistance (LR), bud form (BF), bud sulcate (BSu), bud position (BP), bud size (BS), bud wing size (BWS), lateral bud (LB), leaf posture (LP), leaf color (LC), defoliation (De), leaf sheath color (LSC), No. 57 hair group (57HG), auricle (Au) and pulvinus (Pu). The qualitative trait values were recorded during careful observation and comparison. Seven were quantitative traits, including plant height (PH), stem diameter (SD), internode length (IL), Brix (Bx), leaf length (LL), leaf width (LW), and leaf aspect ratio (LAR). The quantitative traits data were presented based on the statistical analysis of two years of data.

Data analysis: Qualitative traits were assigned values according to the survey, while the membership function values were calculated based on the fuzzy membership function. The membership function values of quantitative traits were as follows:

$$\mu(\chi_i)=(\chi_i-\chi_{i\min})/(\chi_{i\max}-\chi_{i\min})\ldots\ldots\ldots..(i=1, 2, 3\ldots7)$$

where $\mu(\chi_i)$ represented the membership function value of i^{th} trait of one clone, χ_i denoted the phenotype value of i^{th} trait of one clone, $\chi_{i\max}$ or $\chi_{i\min}$ was the maximum or minimum value of i^{th} trait among all the clones, respectively. According to the formula, each quantitative trait could be defined as a closed interval [0, 1]. The membership function value of each quantitative trait was classified into 10 grades: $0\leq 1$ grade <0.1, $0.9\leq 10$ grade ≤ 1, each grade interval was 0.1 between 1 and 10 grades (Ai *et al*. 2017; Lei *et al*. 2018). The relative frequency of each grade was used for the genetic diversity indices.

Genetic diversity was analyzed from the standard quantification of all phenotypic traits of the sugarcane parental lines. The mean, minimum value, maximum value, standard deviation (SD), variation coefficient (CV), and T-test of seven quantitative traits were calculated using Microsoft Excel 2010 and SPSS 17.0 software. The phenotypic variation number and the Shannon-Weiner diversity (*Hs*) index of each morphological trait were assessed using GenAlEx 6.502 software (Peakall and Smouse 2012; 2006):

$$Hs=-1\times\sum pi\times \mathrm{Ln}\ (p_i)$$

where p_i is the frequency of the i^{th} grades of one trait. The genetic distance of each series of sugarcane parental lines was calculated by using the following formulae:

$$D=\frac{-\ln J_{xy}}{\sqrt{J_x J_y}},\ J_x=\sum_i\sum_j X_i^2,\ J_y=\sum_i\sum_j Y_i^2,\ J_x=\sum_i\sum_j X_i\ Y_i$$

Where X_{ij} is the frequency of the j^{th} grades of the i^{th} trait in the X population. Where Y_{ij} is the frequency of the j^{th} grades of the i^{th} trait in the Y population. The cluster dendrogram was constructed based on the Sequential Agglomerative Hierarchical Nested clustering (SAHN) and Unweighted Pair Group Mean Average (UPGMA) method with arithmetic averages using NTSYS-pc 2.11e (Rohlf, 2000). The cophenetic correlation analysis was assessed based on a 2-way Mantel method using NTSYS-pc 2.11e (Hegay *et al*. 2014; Mantel, 1967).

RESULTS

Variation analysis of 31 morphological traits among sugarcane parental lines: The results showed that the difference of parental lines reached an extremely significant level for the seven quantitative traits (Table S3). The CVs varied from 13.44% to 19.65%, averaging 15.97%. The internode length (IL), leaf aspect ratio (LAR) and leaf width (LW) are highly variable with CVs>19.00%. Brix (Bx) and leaf length (LL) have low and relatively stable CVs. The relatively higher CV values in phenotypic traits indicated that these sugarcane parent lines were highly variable morphologically.

The statistical analysis of the frequencies of different values of the morphological traits reflected a wide range of genetic diversity in sugarcane parents (Table 1). The valuation frequencies of quantitative traits were normally distributed and the frequencies of grades 5, 3, 4, 8, 5, 4, and 3 of plant height (PH), stem diameter (SD), internode length (IL), Brix (Bx), leaf length (LL), leaf width (LW), and leaf aspect ratio (LAR) were higher, respectively. In stem traits, the grades 1, 2, 1, 2, 1, 1, 2, 2, 1, 3, and 1 of adventitious root (AR), internode form (IF), internode arrangement (IA), internode color unexposed (ICU), internode color exposed (ICE), growth cracks (GC), bloom (Bl), growth bands form (GBF), growth bands color unexposed (GBCU), growth bands (GBCE), and lodging resistance (LR) were in the ascending order, respectively. The grades 2, 1, 2, 1, 3, and 1 of bud form (BF), bud sulcate (BSu), bud position (BP), bud size (BS), bud wing size (BWS), and lateral bud (LB) of bud traits had higher frequencies, respectively, while the grades 3, 3, 1, 3, 1, 1, and 1 of leaf posture (LP), leaf color (LC), defoliation (De) leaf sheath color (LSC), No.57 hair group (57HG), auricle (Au), and pulvinus (Pu) were higher, respectively.

Genetic diversity of morphological traits and different serials sugarcane parents: The total number of phenotypic variations of 130 sugarcane parental lines was one hundred and forty-two. Plant height (PH) had the most of phenotypic variation numbers (mean=6.0) while lodging resistance (LR) had the least (mean=1.1) (Table 1). The genetic diversity index of each morphological trait was calculated after normalization of the results of the qualitative and quantitative traits (Table 1). The genetic diversity of the morphological traits reached a high level with the Shannon-Weiner index (*Hs*=0.858±0.026). Among the 15 stem traits of sugarcane parents, the highest *Hs* value (1.591±0.105) was associated with PH. *Hs* was 0.907±0.063 for total stem traits and 0.748±0.066 for total bud traits, respectively. Among the six bud traits, the highest *Hs* value (1.052±0.062) was recorded for BF. The average *Hs* value of leaf traits was 0.851±0.079, and the highest *Hs* value (1.414±0.104) was associated with the leaf width (LW) trait.

The mean number of phenotypic variations was 3.6, for YT (Table 2). Genetic diversity analysis indicated obvious differences among different series of sugarcane parental lines. Among the 11 series, the highest *Hs* value (0.970±0.078) was from the GT, followed by (0.932±0.088) of YC. The lowest *Hs* value (0.714±0.090) was for LC. The average *I* and *h* values of eight series of parental lines, namely, GT, YC, YT, NJ, FN, DZ, CP and YZ, were above the average *Hs* values of all parental lines, while the average *Hs* value of three series of parental lines, namely, ROC, GN and LC, were below the average *Hs* values of all parental lines.

Genetic distance values also varied among the 11 series of sugarcane parental lines, ranging from 0.030 to 0.329 with an average of 0.151 (Table 3). The lowest genetic distance value was 0.030 between CP and GT and the highest genetic distance value of 0.329 was between DZ and LC. The relatively low genetic distance values among different series may be the result of mutual cross-breeding among various breeding programs.

Genetic diversities of morphological traits were analyzed for 109 parental lines derived from three released eras on mainland China and 21 parental lines introduced from Taiwan, China and the USA (Figure 2). The parental lines derived from the 2000s had the most of Shannon-Weiner index value (*Hs*=1.039±0.096), followed by those released in the 1990s (*Hs*=1.009±0.091). The lowest Shannon-Weiner index value was found in parental lines released in the 1980s (*Hs*=0.878±0.078). The Shannon-Weiner index values of CP and ROC were intermediate between the 1980s and 1990s. With the released eras, the genetic diversities of parental lines were increasing. Since the 1980s, a lot of exotic species, e.g. CP and ROC, were continuously imported into China sugarcane breeding program, which partly enriched the genetic diversity level of parent resources of sugarcane of China.

Table 1. Genetic diversity indices and the frequencies of different valuations in 31 morphological traits of 130 sugarcane parental lines.

Type	Phenotypic trait	Mean number of phenotypic variation	Shannon-Weiner index (Mean±SE)	The grade with higher frequency
Stem traits	PH	6.0	1.591±0.105	5 (20.00%)
	SD	5.4	1.524±0.096	3 (26.15%)
	AR	2.7	0.841±0.077	1 (47.59%)
	IF	3.8	1.099±0.046	2 (45.28%)
	IA	2.0	0.609±0.021	1 (60.00%)
	ICU	2.6	0.635±0.068	2 (74.62%)
	ICE	3.3	1.006±0.083	1 (46.15%)
	IL	5.6	1.579±0.064	4 (26.92%)
	WC	1.5	0.207±0.075	1 (91.54%)
	IWP	3.5	1.123±0.053	2 (40.00%)
	GBF	1.9	0.555±0.061	2 (59.23%)
	GBCU	1.9	0.475±0.065	1 (74.62%)
	GBCE	2.9	0.950 ±0.045	3 (43.85%)
	LR	1.1	0.022±0.022	1 (99.23%)
	Bx	4.9	1.392±0.059	8 (36.15%)
	Mean	3.3	0.907±0.063	
Bud traits	BF	3.4	1.052±0.062	2 (40.77%)
	BD	2.1	0.444±0.061	1 (84.62%)
	BP	2.5	0.721±0.087	2 (59.23%)
	BS	2.6	0.789±0.086	1 (61.54%)
	BWS	2.9	0.988±0.035	3 (40.00%)
	LB	2.2	0.496±0.068	1(82.31%)
	Mean	2.6	0.748±0.066	
Leaf traits				
	LP	2.8	0.901±0.073	3 (43.08%)
	LC	2.5	0.667±0.063	3 (70.77%)
	LL	4.5	1.292±0.101	5 (32.31%)
	LW	4.9	1.414±0.104	4 (26.92%)
	LAR	4.5	1.303±0.098	3 (26.92%)
	De	2.6	0.746±0.093	1 (56.15%)
	LSC	3.6	1.121±0.055	3 (40.00%)
	57HG	2.7	0.735±0.070	1 (68.46%)
	Au	1.7	0.241±0.068	1 (90.77%)
	Pu	1.2	0.088±0.060	1 (96.92%)
	Mean	3.1	0.851±0.079	
Mean		4.6	0.858±0.026	

Table 2. Genetic diversity index values of different series of sugarcane parental lines.

Series of parental lines	Accession number	Mean number of phenotypic variation	Shannon-Weiner index (Mean±SE)
CP	13	3.1	0.860±0.091
DZ	5	2.8	0.878±0.074
FN	15	3.5	0.915±0.097
GN	4	2.3	0.721±0.063
GT	20	3.5	0.970±0.078
LC	8	2.6	0.714±0.090
NJ	10	3.4	0.918±0.101
ROC	8	2.7	0.750±0.084
YC	15	3.4	0.932±0.088

YT	21	3.6	0.924±0.084
YZ	11	3.2	0.859±0.096
Mean	11.8	3.1	0.858±0.026

Table 3. Genetic distance (below diagonal) of 130 sugarcane parental lines.

	CP	DZ	FN	GN	GT	LC	NJ	ROC	YC	YT	YZ
CP	0.000										
DZ	0.145	0.000									
FN	0.037	0.178	0.000								
GN	0.169	0.316	0.138	0.000							
GT	**0.030**	0.180	0.049	0.170	0.000						
LC	0.120	**0.329**	0.124	0.187	0.129	0.000					
NJ	0.117	0.267	0.101	0.252	0.108	0.234	0.000				
ROC	0.058	0.216	0.057	0.169	0.062	0.122	0.147	0.000			
YC	0.053	0.196	0.042	0.146	0.056	0.151	0.155	0.078	0.000		
YT	0.039	0.182	0.053	0.162	0.038	0.109	0.122	0.061	0.055	0.000	
YZ	0.091	0.233	0.107	0.265	0.080	0.193	0.169	0.126	0.116	0.058	0.000

Cluster analysis of sugarcane parental lines based on phenotypic traits: A phylogenetic tree was constructed, on which the 130 sugarcane parental lines were clustered into three distinct groups (I, II, and III) using the similarity coefficient of 0.407 as the threshold, and further division into six distinct subgroups at the similarity coefficient of 0.437 (Figure 3). A 2-way Mantel (Mantel 1967) test was used to test the hypothesis of a precision for phenotypic data to classify the parental lines. The result showed a highly significant correlation of the cophenetic values from the cluster analyses (r=0.472, P<0.01).

Group I comprised of 4 lines, which were further divided into two subgroups (A and B). Subgroup A contained four NJ (NJ03-218, NJ04-70, NJ07-13 and NJ08-5) sharing medium plant height, large stalk, medium internode length, low Brix, short and wide leaf, and low aspect ratio. All lines had more aerial root, drum internode form, no internode wax powder, expansion growth bands form, kelly growth bands, hang down loosely and green leaf (Table S4). Subgroup B consisted of one DZ (DZ03-83) with the features of tall plant height, long internode length, large stalk, low Brix, medium-long and wide leaf, and moderate aspect ratio (Table S4).

Group II comprised of four parental lines that were further divided into only one subgroup C at the similarity coefficient of 0.437. Subgroup C contained one CP (CP80-1827), one GT (GT96-154), one YC (YC06-61), and one YT (YT89-240), with traits like short plant height, large stalk, long internode length, high Brix, long and narrow leaf, and high aspect ratio (Table S4). All parental lines of subgroup C had more aerial root, yellow internode color before exposed, no water crack, no bud ditch, low bud position, and no lateral bud (Table S4).

Group III consisted of 121 sugarcane parental lines, which were further clustered into three subgroups (D, E and F). Subgroup D included ten parental lines with traits such as medium plant height, medium-large stalk, short internode length, medium Brix, short leaf. Most of the parental lines of subgroup D had green internode color before exposed, red internode color after exposed, no expansion growth bands form, grey orange growth bands color after exposed, no bud ditch, reach bud position, middle bud size, no lateral bud, green leaf, degeneration auricle. Subgroup D had one GN, two GT, one NJ, three YC, two YT, and one YZ. Subgroup E contained forty-one accessions, including three CP, three DZ, five FN, one GN, eight GT, four LC, one NJ, four ROC, six YC, four YT, and two YZ, with traits like medium plant height, medium-large stalk, medium internode length, medium Brix, long leaf (Table S4). Most of the parental lines of subgroup E had a zigzag pattern of internode arrangement, thin internode wax powder, grey orange growth bands after exposed, no bud ditch, reach bud position, small bud size, no lateral bud, and green leaf. Subgroup F contained the largest number of 70 parental lines, including nine CP, one DZ, ten FN, two GN, ten GT, three LC, four NJ, four ROC, five YC, 14 YT, and eight YZ, with the characteristics of medium plant height, medium-large stalk, medium internode length, medium Brix, and long and wide leaf (Table S4). Most parental lines of subgroup F had yellow growth bands after exposed, kelly growth bands, no bud ditch, no lateral bud, green leaf, and degeneration auricle.

Thus, there were substantial differences among different groups of parental lines. Only four NJ (NJ03-218, NJ04-70, NJ07-13 and NJ08-5) were clustered into one subgroup. Most of the parental lines that belonged to the same series usually clustered into different groups, indicating that there was no correlation between genetic divergence and geographical origin. These results will facilitate the selection of parents for crossing.

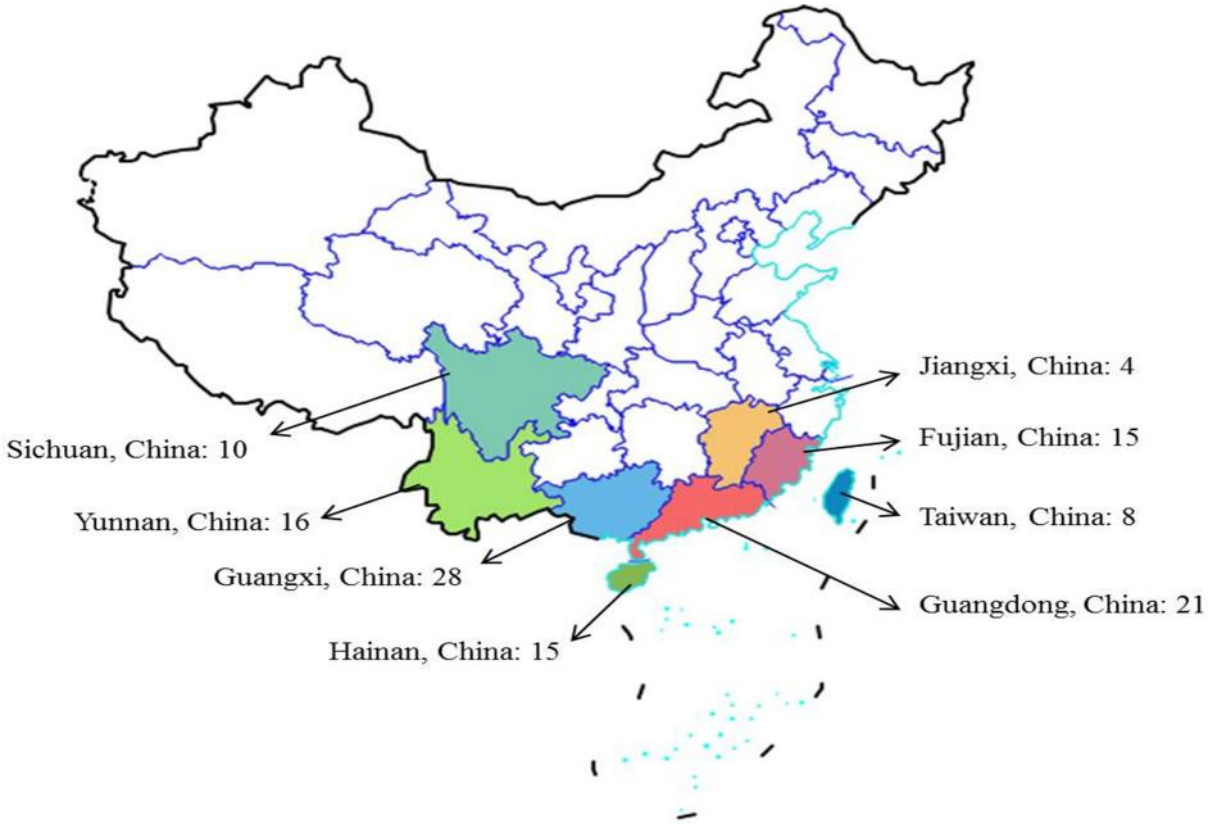

Figure 1: Geographic origin of 117 sugarcane parental lines from eight provinces in China: Guangdong (21 lines), Guangxi (28), Fujian (15), Hainan (15), Yunnan (16), Taiwan (8), Sichuan (10), Jiangxi (4)

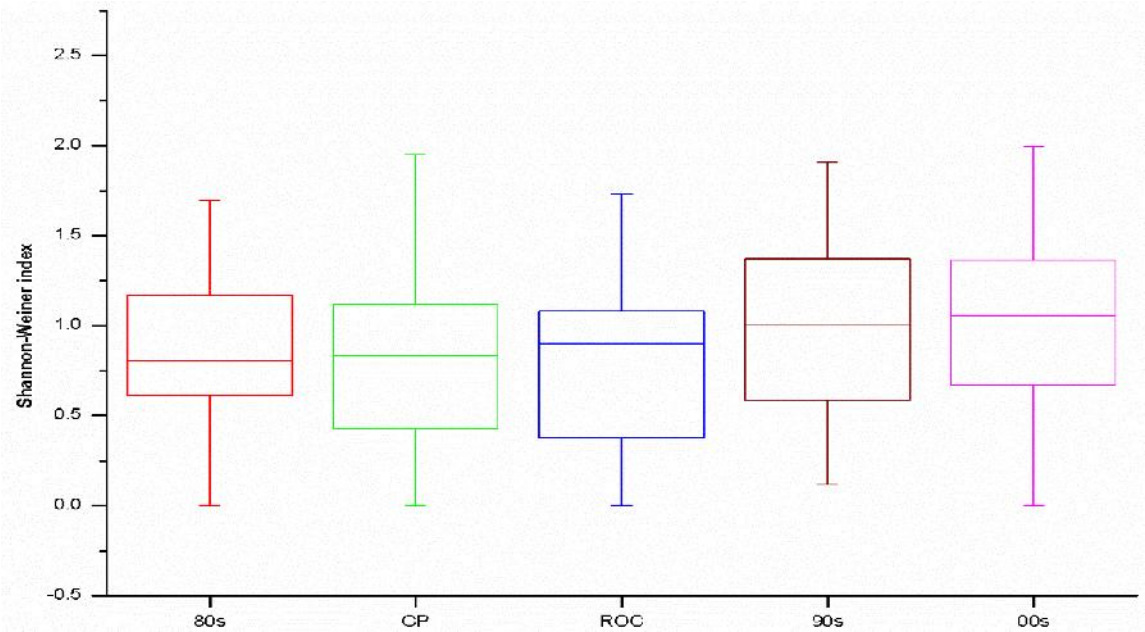

Figure 2: Genetic diversity of morphological traits for parental lines derived from different eras of breeding and introduced location

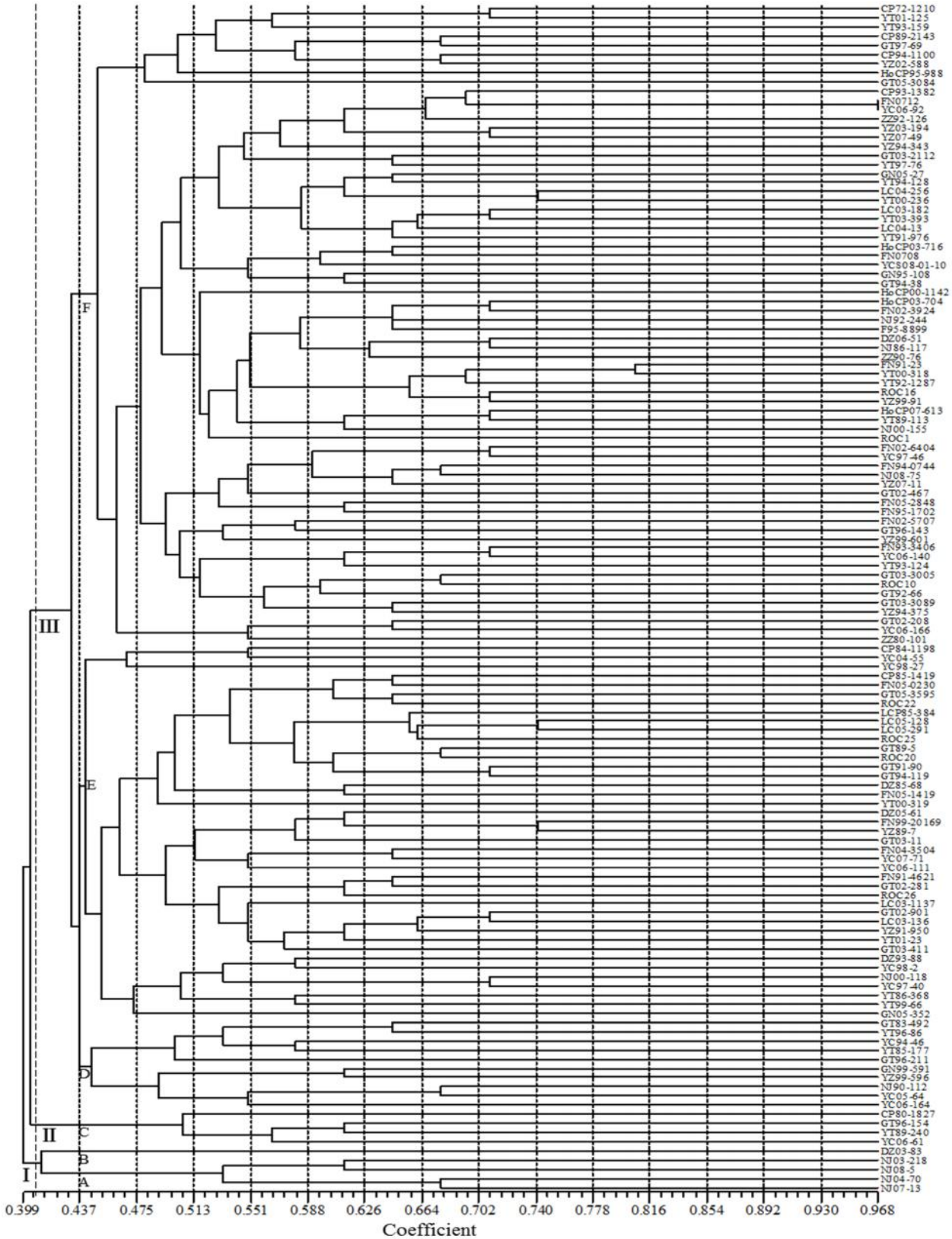

Figure 3: The cluster dendrogram of 130 parental lines was constructed based on the Sequential Agglomerative Hierarchical Nested clustering (SAHN) and unweighted pair group method with arithmetic averages (UPGMA). Three major groups (I, II, and III) and six subgroups (A to F) are shown

DISCUSSION

The significance of genetic diversity of sugarcane parental lines: Genetic diversity is a critical part of biodiversity, and the genetic information of the organism is of great theoretical and practical significance. Genetic diversity is a necessary condition for improving yield or resistance to biotic and abiotic stresses (Kefyalew *et al*. 2000). It is very important to morphologically characterize and classify plant germplasm for genetic improvement, and gene resource preservation and exploitation (Harrison *et al*. 2000). Nowadays, the data to evaluate the genetic diversity of germplasm resources primarily originate from molecular markers (Bassil *et al*. 2018; Gitau *et al*. 2017; Xiong *et al*. 2016) and phenotypic traits (Lei *et al*. 2018; Hegay *et al*. 2014; Kefyalew *et al*. 2000). Molecular markers are widely used in genetic diversity studies because of their large information content, co-dominance, and environmental stability; however, molecular marker studies are rather difficult to conduct, and expensive. Phenotypic traits are the direct manifestations of biodiversity resulting from long-term natural and artificial selection (Dong *et al*. 2016), and an intuitive and direct means for studying the genetic diversity of plant germplasm resources. In order to promote the utilization and innovation of sugarcane parent resources, morphology genetic diversity of major parental lines of sugarcane breeding programs in China was conducted using the Shannon-Weiner diversity (*Hs*) index and Nei' genetic distance.

Genetic diversity of major sugarcane parental lines: Sugarcane plants are allopolyploids with highly complex genomes. After long-term hybridization, and artificial and natural selection, a wealth of genetic diversity has accumulated (Li *et al*. 2003). The genetic relatedness/distance among sugarcane parental lines is the foundation for the richness of breeding resources and plays a significant role in parental selection (Hu *et al*. 2012). In the present study, genetic diversity of 130 sugarcane parental lines was evaluated based on 31 highly variable phenotypic traits. These traits showed relatively high CV values and a wide range of frequency distribution, demonstrating that the 130 sugarcane parental lines can provide excellent genetic resources for breeding of sugarcane varieties. In the previous studies, You *et al.* (2016) reported that the Shannon's information index (*Hs*) of 14 series ranged from 0.23 to 0.32 while Que *et al.*(2014) reported the *Hs* of 12 series varied from 0.2411 to 0.5298 which the results were much lower than our present result (*Hs*=0.858±0.026). Higher genetic diversity values than our results were reported by Liu *et al.* (2010) on traits such as bud shape (*Hs*=1.1826), internode color unexposed (*Hs*=1.6865), internode color exposed (*Hs*=1.3642), bud furrow (*Hs*=1.0660), and hair group 57 (*Hs*=1.0299). The genetic distance values among the 11 series of sugarcane parental lines ranged from 0.030 to 0.329 with an average of 0.151, which indicated a high level of genetic diversity. Different from our results, Liu *et al.* (2018) reported that pairwise genetic identity values among the five groups of sugarcane parental populations ranged from 0.517 to 0.808. Pairwise genetic similarity coefficients of 0.375 to 0.881 among 107 sugarcane accessions were reported by Que *et al.* (2014) and of 0.56 to 0.916 among 40 sugarcane accessions by Chen *et al.* (2009). The difference among the results may be due to the difference in the materials and methodology involved in the studies.

Breeding application of cluster analysis: Geographical factors play an important role in the evolution of plant species (Jaradat and Shahid 2006). Phenotyping-based cluster analysis can roughly reflect the genetic relationship among accessions. However, in our study, the relationship between morphology and geographical origins did not show consistent trends for the130 sugarcane parental lines. Similar results have been reported in the literature. Based on neighbor-joining cluster analysis, 181 parental lines involved in China's sugarcane breeding programs could be clustered into seven groups (You *et al*. 2016). Similarly, three groups were reported for 115 sugarcane parental lines (You *et al*. 2013). Another study showed there were five groups within 96 parental sugarcane cultivars (Qi *et al*. 2012). In a report by Chen *et al.* (2009), 35 sugarcane cultivars and five clones of related wild species clustered into five groups. In this study, cluster analysis of 130 sugarcane parental lines based on genetic similarity coefficient values resulted in three groups and six subgroups that comprised of mixed series of parental lines. Among the six subgroups, the F subgroup contained the largest number of parental lines with the YT being the primary materials. Six subgroups had obvious morphological characters, particularly quantitative ones (Table S4). Based on cluster analysis, parental lines with different morphological features can be chosen for intercrossing. For example, most of the parental lines in subgroup C showed high Brix but short plant height, while almost all the parental lines in subgroup B had low Brix but taller plant height. Therefore, parental lines of subgroup C and B may be intercrossed to develop a segregating population from which new varieties with high sugar and tall plant height may be selected. This strategy may help improve sugar and cane yield through pyramid breeding. The selection of distant relative parents is an effective method to construct generation groups with abundant genetic variation. In this study, the highest genetic distance was between DZ and LC. So in the sugarcane cross-breeding, we should give priority to select parents in DZ and LC for broadening the genetic base. We should configure hybrid combination using the parents with far genetic distance to increase our options. Comprehensive

cluster analysis to reveal the genetic diversity of sugarcane parental lines is valuable in broadening the genetic base of Chinese sugarcane breeding programs.

Innovation of sugarcane parents in China: In China, more than 200 sugarcane varieties have been released from cross-breeding programs during the last 30 years. However, several issues such as a limited genetic base due to low heterogeneity had impeded the improvement of sugarcane varieties (Chen 2011). Further improvement of sugarcane varieties depends largely on genetic base broadening, parental line selection, and cross combinations. To date, more than 2,000 accessions, including *S. officinarum*, *S. barberi*, *S. sinense*, *S. spontaneum*, *S. robustum*, landrace, chewing cane, advanced hybrids, *E. arundinaceus*, *Miscanthus*, *Narenga*, *P. schumach*, and *P. typhoideum*, have been collected in two Sugarcane Germplasm Resource Bank at the HSBS of Guangzhou Sugarcane Industry Research Institute (Hainan Province, China) and the Sugarcane Research Institute of Yunnan Academy of Agricultural Sciences (Yunnan Province, China). The HSBS is the primary sugarcane crossing facility in Mainland China, which undertakes sugarcane crossing, germplasm collection and conservation, and parental line innovation and introduction (Li *et al*. 2003). Good results have been achieved for the exploitation of *S. officinarum, S. robustum,* and *S. spontaneum*, as well as, *E. arundinaceus* (Qi *et al*. 2012). Some new parents have succeeded in breeding and utilization of such as YC71-374, YC05-164, YC07-71, YCS08-01-10, and YC97-46 via introgression breeding with *S. spontaneum* and *E. arundinaceus*, resulting in the release of more than 30 commercial varieties from the YC (Deng *et al*. 2004). More than 100 YC have been used in sugarcane cross breeding programs in China every year, accounting for more than 30% of all the crossing parental lines (Qi *et al*. 2012). The YC have the highest level of genetic diversity based on several molecular marker studies (You *et al*. 2016; 2013). Due to the germplasm introduction, the genetic diversity of parental lines of sugarcane breeding programs in China was constantly increasing. Some exotic species, e.g. CP72-1210 and ROC22, greatly promoted the development of sugarcane breeding in China. The evaluation of genetic diversity of YC, along with other parental lines such as CP, YT, GT, FN, YZ, ROC, NJ, LC, DZ, and GN from the sugarcane breeding programs in China will be beneficial to the effective utilization of these sugarcane parental lines by increased efficiency of cross hybridizations.

In this study, the phenotypic diversity of sugarcane parental lines based on morphological data was conducted. To know better of genetic relationship of parental lines, the genetic diversity of sugarcane parental lines based on molecular markers should be carried out.

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TableS1: The amount, series, and origin of sugarcane parents

NO.	Clone name	Bred era	Series	Origin
1	CP72-1210	Introduced	CP	USA
2	CP80-1827	Introduced	CP	USA
3	CP84-1198	Introduced	CP	USA
4	CP85-1419	Introduced	CP	USA
5	CP89-2143	Introduced	CP	USA
6	CP93-1382	Introduced	CP	USA
7	CP94-1100	Introduced	CP	USA
8	HoCP00-1142	Introduced	CP	USA
9	HoCP03-704	Introduced	CP	USA
10	HoCP03-716	Introduced	CP	USA
11	HoCP07-613	Introduced	CP	USA
12	HoCP95-988	Introduced	CP	USA
13	LCP85-384	Introduced	CP	USA
14	DZ03-83	00s	DZ	Yunnan, China
15	DZ05-61	00s	DZ	Yunnan, China
16	DZ06-51	00s	DZ	Yunnan, China
17	DZ85-68	80s	DZ	Yunnan, China
18	DZ93-88	90s	DZ	Yunnan, China
19	FN02-3924	00s	FN	Fujian, China
20	FN02-5707	00s	FN	Fujian, China
21	FN02-6404	00s	FN	Fujian, China
22	FN04-3504	00s	FN	Fujian, China
23	FN05-0230	00s	FN	Fujian, China
24	FN05-1419	00s	FN	Fujian, China
25	FN05-2848	00s	FN	Fujian, China
26	FN0708	00s	FN	Fujian, China
27	FN0712	00s	FN	Fujian, China
28	FN91-23	90s	FN	Fujian, China
29	FN91-4621	90s	FN	Fujian, China
30	FN93-3406	90s	FN	Fujian, China
31	FN94-0744	90s	FN	Fujian, China
32	FN95-1702	90s	FN	Fujian, China
33	FN99-20169	90s	FN	Fujian, China
34	GN05-27	00s	GN	Jiangxi, China
35	GN05-352	00s	GN	Jiangxi, China
36	GN95-108	90s	GN	Jiangxi, China
37	GN99-591	90s	GN	Jiangxi, China
38	GT02-208	00s	GT	Guangxi, China
39	GT02-281	00s	GT	Guangxi, China
40	GT02-467	00s	GT	Guangxi, China
41	GT02-901	00s	GT	Guangxi, China

42	GT03-11	00s	GT	Guangxi, China
43	GT03-2112	00s	GT	Guangxi, China
44	GT03-3005	00s	GT	Guangxi, China
45	GT03-3089	00s	GT	Guangxi, China
46	GT03-411	00s	GT	Guangxi, China
47	GT05-3084	00s	GT	Guangxi, China
48	GT05-3595	00s	GT	Guangxi, China
49	GT83-492	80s	GT	Guangxi, China
50	GT89-5	80s	GT	Guangxi, China
51	GT91-90	90s	GT	Guangxi, China
52	GT92-66	90s	GT	Guangxi, China
53	GT94-119	90s	GT	Guangxi, China
54	GT94-38	90s	GT	Guangxi, China
55	GT96-143	90s	GT	Guangxi, China
56	GT96-154	90s	GT	Guangxi, China
57	GT96-211	90s	GT	Guangxi, China
58	GT97-69	90s	GT	Guangxi, China
59	LC03-1137	00s	LC	Guangxi, China
60	LC03-136	00s	LC	Guangxi, China
61	LC03-182	00s	LC	Guangxi, China
62	LC04-13	00s	LC	Guangxi, China
63	LC04-256	00s	LC	Guangxi, China
64	LC05-128	00s	LC	Guangxi, China
65	LC05-291	00s	LC	Guangxi, China
66	NJ00-118	00s	NJ	Sichuan, China
67	NJ00-155	00s	NJ	Sichuan, China
68	NJ03-218	00s	NJ	Sichuan, China
69	NJ04-70	00s	NJ	Sichuan, China
70	NJ07-13	00s	NJ	Sichuan, China
71	NJ08-5	00s	NJ	Sichuan, China
72	NJ08-75	00s	NJ	Sichuan, China
73	NJ86-117	80s	NJ	Sichuan, China
74	NJ90-112	90s	NJ	Sichuan, China
75	NJ92-244	90s	NJ	Sichuan, China
76	F95-8899	Introduced	ROC	Taiwan, China
77	ROC1	Introduced	ROC	Taiwan, China
78	ROC10	Introduced	ROC	Taiwan, China
79	ROC16	Introduced	ROC	Taiwan, China
80	ROC20	Introduced	ROC	Taiwan, China
81	ROC22	Introduced	ROC	Taiwan, China
82	ROC25	Introduced	ROC	Taiwan, China
83	ROC26	Introduced	ROC	Taiwan, China
84	YC04-55	00s	YC	Hainan, China
85	YC05-64	00s	YC	Hainan, China
86	YC06-111	00s	YC	Hainan, China
87	YC06-140	00s	YC	Hainan, China
88	YC06-164	00s	YC	Hainan, China
89	YC06-166	00s	YC	Hainan, China
90	YC06-61	00s	YC	Hainan, China
91	YC06-92	00s	YC	Hainan, China
92	YC07-71	00s	YC	Hainan, China
93	YC94-46	90s	YC	Hainan, China
94	YC97-40	90s	YC	Hainan, China
95	YC97-46	90s	YC	Hainan, China
96	YC98-2	90s	YC	Hainan, China
97	YC98-27	90s	YC	Hainan, China
98	YCS08-01-10	00s	YC	Hainan, China
99	YT00-236	00s	YT	Guangdong, China
100	YT00-318	00s	YT	Guangdong, China
101	YT00-319	00s	YT	Guangdong, China
102	YT01-125	00s	YT	Guangdong, China
103	YT01-23	00s	YT	Guangdong, China

104	YT03-393	00s	YT	Guangdong, China
105	YT85-177	80s	YT	Guangdong, China
106	YT86-368	80s	YT	Guangdong, China
107	YT89-113	80s	YT	Guangdong, China
108	YT89-240	80s	YT	Guangdong, China
109	YT91-976	90s	YT	Guangdong, China
110	YT92-1287	90s	YT	Guangdong, China
111	YT93-124	90s	YT	Guangdong, China
112	YT93-159	90s	YT	Guangdong, China
113	YT94-128	90s	YT	Guangdong, China
114	YT96-86	90s	YT	Guangdong, China
115	YT97-76	90s	YT	Guangdong, China
116	YT99-66	90s	YT	Guangdong, China
117	ZZ80-101	80s	YT	Guangdong, China
118	ZZ90-76	90s	YT	Guangdong, China
119	ZZ92-126	90s	YT	Guangdong, China
120	YZ02-588	00s	YZ	Yunnan, China
121	YZ03-194	00s	YZ	Yunnan, China
122	YZ07-11	00s	YZ	Yunnan, China
123	YZ07-49	00s	YZ	Yunnan, China
124	YZ89-7	80s	YZ	Yunnan, China
125	YZ91-950	90s	YZ	Yunnan, China
126	YZ94-343	90s	YZ	Yunnan, China
127	YZ94-375	90s	YZ	Yunnan, China
128	YZ99-596	90s	YZ	Yunnan, China
129	YZ99-601	90s	YZ	Yunnan, China
130	YZ99-91	90s	YZ	Yunnan, China

Table S2: Descriptor and valuation of morphological traits evaluated in 130 sugarcane parental lines.

Type	Trait	Trait code	Descriptor	Valuation
Stem traits	Plant height	PH	The height of stem in mature stage	Measured by using ruler
	Stem diameter	SD	The diameter of stem in mature stage	Measured by using caliper
	Adventitious root	AR	The roots on aerial stems	None=1, less=2, more=3
	Internode form	IF	The form of stem between leaf scar and growth bands	Cone=1, cylinder=2, drum=3, slender waist=4, curved=5, inverted taper=6
	Internode arrangement	IA	The overall shape of stem in mature stage	Zigzag pattern=1, upright pattern=2
	Internode color unexposed	ICU	The color of stem between leaf scar and growth bands unexposed	Yellow=1, green=2, red=3, purple=4
	Internode color exposed	ICE	The color of stem between leaf scar and growth bands exposed	Yellow=1, green=2, red=3, purple=4
	Internode length	IL	The length of stem between leaf scar and growth bands	Measured by using ruler
	Growth cracks	GC	The crack in stem between leaf scar and growth bands	None=1, have=2
	Bloom:	Bl	A white, powder like coating sometimes found on stem surface	None=1, thin=2, middle=3, thick=4
	Growth bands form	GBF	The form of growth bands which above root zone	Non-expansion=1, expansion=2
	Growth bands color unexposed	GBCU	The color of growth bands unexposed	Yellow green=1, green=2
	Growth bands color exposed	GBCE	The color of growth bands exposed	Yellow green=1, green=2, grey orange=3
	Lodging resistance	LR	Whether to fall when the wind blows	Low resistance=1, highly resistance=2
	Brix	Bx	The average of the sugarcane-juice brix in mature stage	Measured by using Hand held refractometer
Bud traits	Bud form	BF	The form of lateral bud	Roundness=1, oval=2, triangle=3, obovate=4, rhombus=5, elliptic=6, rectangle=7
	Bud sulcate:	BSu	The groove of lateral bud above bud	None=1, shallow=2, deep=3

	Bud position	BP	The position of lateral buds on stem	Low=1, reach=2, high=3
	Bud size	BS	Bud size	Small=1, middle=2, big=3
	Bud wing size	BWS	Bud wing size	None=1, narrow=2, wide=3
	Adventitious bud	AB	Growth situation of lateral bud in mature stage	None=1, less=2, more=3
Leaf traits	Leaf posture	LP	The posture of leaf in mature stage	Erect=1, reptent=2, spreading=3
	Leaf color	LC	The color of leaf in mature stage	Yellow green=1, light green=2, green=3
	Leaf length	LL	The length of +3 leaf in mature stage	Measured by using ruler
	Leaf width	LW	The width of +3 leaf in mature stage	Measured by using ruler
	Leaf aspect ratio	LAR	The aspect ratio of length and width of leaf	LL/LW
	Defoliation	De	The degree of leaf sheath enclosing the stem in mature stage	Hard=1, medium=2, hard=3
	Leaf sheath color	LSC	The color of leaf sheath in mature stage	Yellow green=1, light green=2, green=3, green with purple spots =4, purple=5, pink=6
	No.57 hairgroup	57HG	The hair group in reverse side of leaf sheath	None=1, sparse=2, thick=3
	Auricle	Au	A small earlike lobe or appendage	Degeneration=1, lanceolate=2, triangle=3
	Pulvinus	Pu	A light color band structure on the outside of the junction of leaf blade and sheath	None=1, have=2

Table S3: Variations among 130 sugarcane parental lines for seven quantitative traits.

Trait	Average	Min	Max	SD	CV (%)	*T*-test for parental lines[a]
PH	301.55	200	385	40.52	13.44	84.85**
SD	3.04	1.8	4.8	0.54	17.76	64.18**
IL	12.11	6.5	20.0	2.42	19.97	57.09**
Bx	23.03	14.8	26.7	2.31	10.02	113.79**
LL	134.52	90	170	16.02	11.91	95.75**
LW	5.02	2.4	8.5	0.95	19.01	59.98**
LAR	27.63	15.3	44.4	5.43	19.65	58.02**

a "**" means significant at 0.01 levels.

Table S4: Statistics of seven quantitative traits in different subgroups.

Subgroup	PH (cm)	SD (cm)	IL (cm)	Bx (%)	LL (cm)	LW (cm)	LAR (%)
A	291.3	4.0	10.3	20.4	120.0	6.0	20.4
B	340.0	3.7	15.0	20.9	130.0	5.3	24.5
C	263.3	2.8	8.6	24.4	137.5	4.2	33.9
D	290.0	3.2	11.5	22.8	126.0	5.1	25.0
E	299.1	3.0	12.2	23.4	135.3	4.9	27.9
F	306.8	3.0	12.4	23.0	136.0	5.0	27.9
Mean	301.5	3.0	12.1	23.0	134.5	5.0	27.6