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ASSESSMENT OF GENETIC DIVERSITY OF SWEET SORGHUM COLLECTION USING PHENOTYPIC VARIATION AND SSR MARKERS

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Abstract

In this study, 32 germplasm resources of sweet sorghum were identified to evaluate the phenotypic and genetic diversity. The results showed that the diversity index (*H'*) of agronomic traits was within the range of 1.716 - 2.062, with a specially richer diversity of spike types. 899 polymorphic bands were amplified by 43 pairs of SSR primers, and the polymorphism information content (PIC) varied from 0.06 to 0.49, with the genetic diversity index (*Nei*) ranged from 0 to 0.646. Three principal components were extracted from nine agronomic traits, and the "grain yield" factors of the first principal component were negatively correlated with the "biological yield" factors of the second. 32 germplasm resources were divided into 4 groups, and group IV exhibited the wide distribution of spike types, high grain yields and brix rate. These indicated that the genetic diversity of the germplasm population was low although observing the variable phenotypes among different varieties, possibly due to the significant inbreeding within the population. The variety No.20, No.21 and No.31 exhibited a good improvement potential for breeding practice of sweet sorghum.

Key words: Sweet sorghum; Genetic diversity; Agronomic traits; SSR.

Introduction

The use of fossil fuels has increased considerably with the rapid development of the global economy, the weather is changing unpredictably, and the ecological environment has deteriorated, leading the less advantage in development of animal husbandry and fossil fuels (Melillo *et al*., 2014). Such contradiction in human being necessitates the urgent exploration of novel renewable resources to alleviate the conflict between human consumption and ecological environment imbalance and resource shortage. Sweet sorghum is a C_4 plant, it exhibits efficiency of high light energy conversion, strong abilities of photosynthetic and dry matter accumulation, rapid growth speed, and large biomass production (Zegada-Lizarazu & Monti, 2013; Yang *et al*., 2019; Rivera-Burgos *et al*., 2019). The appropriate exploitation and utilization of sweet sorghum are crucial for food security, solving energy crisis, and promoting the development of animal husbandry.

Sweet sorghum prefers warm temperature, with the greater resistance of drought, waterlogging, barren etc., showing a wide range of growth adaptation (Sasaki & Antonio, 2009; Musara *et al*., 2019). Its good flavor and high nutritional value show better quality than silage corn, and its stem have the rich sugar to extract easily, as well of seeds to brew pure-quality wine through simple process (Boboescu *et al*., 2019; Cooper *et al*., 2019; Xie and Xu, 2019). Therefore, sweet sorghum should be favored as one of the most potential resource of energy and forage crops.

Sweet sorghum varieties are rich in China, with variable phenotypes and different genetically background. In most areas, sweet sorghum cultivars are promiscuous, with weak growth vigor and low yields, to limit farmers' interest in planting sweet sorghum (Supriya *et al*., 2017). At present, resources identification of sweet sorghum mainly relies on observation of agronomic traits, which present the blindness on parents selection and group configuration in breeding process. SSR molecular markers provide an effective tool for detecting the genetic diversity of germplasm resources (Yousaf *et al*., 2015). Ali *et al*. (2008) analyzed 72 sweet sorghum materials using SSR markers, and their clustering results are consistent with the known genealogies and genetic background information. In this study, 32 germplasm resources of sweet sorghum were used to investigate the agronomic traits and morphological characteristics, to analyze their genetic diversity and correlation. SSR markers were adopted to examine the genetic variation and relationship among varieties, to provide theoretical basis for germplasm resources in breeding programme.

Materials and Methods

Materials and plant condition: 32 sweet sorghum varieties with considerably vartiable morphological traits were provided by the Crops Institute of the Chinese Academy of Agricultural Sciences (CAAS) (Supplement Table 1). In early May of both 2017 and 2018, the varieties were continuously grown in Anhui Science and Technology University (N 32°52′, E 117°33′, 43 m elevation) for two years, each variety was planted in four rows with a length of 2 m, a row spacing of 50cm, and a plant spacing of 25cm. When grown at five-leave stages(Supriya *et al*., 2017) in 2018, the leaves of six plants of each variety were randomly collected for replication (n=6), DNA was extracted by CTAB method (Sambrook *et al*., 2001).

Supplement Table 1. Name and sources of 32 sweet sorghum collection.

Code	No. of national genebank	Origin (Province)
1	357	Hei long jiang
2	360	Hei long jiang
3	2349	Shan xi
4	2350	Shan xi
5	7322	Nei meng gu
6	7323	Nei meng gu
7	7324	Liao ning
8	7325	Liao ning
9	7329	Hei long jiang
10	7342	An hui
11	7343	An hui
12	7357	Hu bei
13	7358	Hu bei
14	10246	Ji lin
15	10264	Shaan xi
16	10265	Shaan xi
17	10288	Yun nan
18	10289	Yun nan
19	12484	Bei jing
20	12520	Bei jing
21	12630	Bei jing
22	13241	Si chuan
23	13242	Si chuan
24	13361	Gui zhou
25	13428	Hu bei
26	13443	Hu bei
27	13543	Hai nan
28	13812	Guangxi
29	13854	Gui zhou
30	14181	Han nan
31	M81	American
32	Yuexitian	An hui

Investigation of agronomic characteristics: The stigma color of sweet sorghum was investigated at the flowering stage (Supriya *et al.*, 2017), agronomic traits (plant height, main stem diameter, main stem fresh weight, photosynthetic rate, juice yield and brix) and morphological characters (main vein texture, pulse color) were determined during the grain filling stage. After harvesting, the spike length and grain weight of the main stem, and thousand kernel weight were investigated, as well as examination of spike type, awn characteristic, glume color, coating degree and grain seed color. The criteria used for morphological shape are referred to Supplement Table 2. The agronomic traits were divided into 10 levels on the basis of the average and standard deviation values, the levels ranged from the first $Xi<(x-2\sigma)$ to the tenth $Xi\geq(x+2\sigma)$ (Supplement Table 3). The relative frequency of each group was used to calculate the diversity index (Shannon & Weaver, 1949). Juice yield rate was measured by the vertical SX-300 juicer (Guangzhou, China), as brix rate measured by Brix Meter PAL-2 (ATAGO, Japan), Pn measured by a photosynthesis system (Li-6400, USA) between 9:00 and 11:00 am in sunny and windless weather. Ten plants (n=10) were used as replication for each trait in 2017 and 2018, respectively.

Diversity detection of SSR markers: SSR primers of sweet sorghum were obtained from Gramene website (http://www. gramene.org/markers/index.html.) (Kong *et al*., 2000; Schloss *et al*., 2002; Menz *et al*., 2002). A total of 276 pairs of SSR primers were evenly distributed on each chromosome of sweet sorghum, and synthesized in TaKaRa (Dalian, China). PCR reaction and procedure were according to Xavier et al. (2018). PCR products was detected via polyacrylamide gel electrophoresis (Bassam *et al*., 1991), and the results were observed under an incandescent lamp.

Data analysis: According to PCR results, the band in the same migration position was labeled as 1, and no band was recorded as 0. Double-type bands were represented by letters A, B, and C. Popgen 32 software was used to analyze polymorphic loci, the Shannon information index (*I*), the genetic diversity index (*Nei*), gene flow (*Nm*), and genetic consistency and distance among populations. SPSS 12.0 software was used to conduct statistical and cluster analysis of the agronomic traits. Broad-sense heritability (H^2 %) was calculated as follows:

$$H^2 = \frac{V_g}{V_g + \frac{V_{g\times y}}{r} + \frac{V_\varepsilon}{r \times y}}$$

V_g is the genotypic variance; $V_{g\times y}$ is the interaction variance between genotype and year; V_ε is the error variance; r and y are the number of replications and years, respectively.

Results

Variation analysis of the major agronomic traits among sweet sorghum varieties: Analysis of variance of nine agronomic traits (PH, MSD, MSFW, MSSL, MSGY, TKW, Pn, JYR and Br) showed a great and significant difference among 32 varieties , as well as five traits (PH, MSGY, TKW, JYR and Br) between the two years ($p<0.01$) (Table 1). In addition, broad-sense heritability (H^2 %) of the agronomic traits showed a relatively wide range of 74.61% to 99.86%, with MSSL and TKW displaying the highest H^2, indicating higher genetic inheritance of the two trait among the different type of germplasm. These results suggest that there is the convincing difference among sweet sorghum materials, necessitating further analysis of genetic diversity to provide a theoretical basis for useful selection of germplasm resources during breeding.

Supplement Table 2. The investigation criteria used for morphological shape.

Agronomy characteristics	Different types of distribution							
	1	2	3	4	5	6	7	8
MVT	Opaque	translucence						
MVC	White	light yellow	yellow	green				
STC	White	yellow	purple					
ST	Compact	medium compact	medium loose	side loose	loose round			
AT	Awn	awnless						
GC	White	yellow	gray	red	brownness	purple	black	
GSC	White	gray	light yellow	yellow	orange	red	brownness	black
GCD	Bare grain	1/4 coating	1/2 coating	3/4 coating	coating			

Note: MVT was main vein texture, MVC was main vein color, STC was stigma colors, ST was spike type, AT was awn type, GC was glume colors, GSC was grain seed color, and GCD was glume coating degree

Table 1. Variance analysis of main agronomy traits of sweet sorghum.

Variation source	PH (m)	MSD (mm)	MSFW (g)	MSSL (cm)	MSGY (g)	TKW (g)	Pn ($\mu mol.m^{-2} s^{-1}$)	JYR (%)	Br (%)
Genotype (G)	1.27**	35.71**	15089.29**	257.33**	153.56**	125.26**	140.02**	0.06**	34.88**
Replication	0.05	0.82	828.86	5.97	54.25	1.05	96.09**	0.01*	3.28
Year (Y)	19.32**	0.89	192379.45	30.08	199.38**	8.84**	12.87	0.03**	23.66**
G× Y	0.03	3.96**	11606.41	3.28	140.32	0.24	2.36	0.02	0.64
Error	0.08	1.86	4676.46	8.96	32.82	0.59	10.65	0.01	2.47
H^2 %	98.20	95.63	76.45	99.00	74.61	99.86	98.20	87.80	98.24

Note: MS was mean square of statistics. PH was plant height, MSD was main stem diameter, MSFW was main stem fresh weight, MSSL was main stem spike length, MSGY was main stem grain yield, TKW was thousand kernel weight, Pn was photosynthetic rate, JYR was juice yield rate, Br was brix rate, and ** indicates the significant difference at 0.01 level ($p<0.01$)

Genetic diversity of phenotypic traits of sweet sorghum

Morphological characteristics: Eight of the major morphological characteristics of sweet sorghum were investigated to calculate the frequency distributions and obtain the diversity of morphological traits (Table 2). The results showed the extensively genetic diversity among different varieties. The diversity indexes (*H*') of glume color and spike type were higher, up to 1.668 and 1.511, respectively, and the main vein texture was the lowest, with only one feature, opaque state (1). Sweet sorghum had seven types of glume color, showing the dispersed frequency distribution of husk color, with the wider distribution of yellow types (2) and lower distribution of gray ones (3). Spike had five types, compact (1) and medium loose types (3) were dominant, while the others were less distributed. The main vein had four colors, white (1) was highly distributed, and green (4) was not observed. The stigma had three colors, white (1) and yellow (2) type exhibited high-frequency distribution, but purple (3) was rare. The frequencies of awn (1) and awnless (2) spikes were nearly half and half of each other. Grain seed color was classified into eight types, yellow (4) type had the highest frequency, while gray (2), light yellow (3), orange (5), and brownness (7) exhibited no distribution. The coating degree of glume was divided into five types, the distribution frequency of 1/2 coating (3) type was higher, while the bare grain (1) was rare.

Agronomic traits: The diversity analysis showed that (Table 3 and Supplement Table 3) the variation range of the fresh weight of main stem (MSFW) was the largest, with a maximum value of 1075g (variety No.28), a minimum value of 105g (variety No.2), and a variation coefficient of (CV) of 44.87%, which was mostly distributed within the range of 227.10 - 504.31 g, (Grades 4 - 6), with a frequency of 75% and an average MSFW of 411.9g; the variation of grain yield of main stem (MSGY) was the second largest, with a maximum value of 70g (variety No.27), a minimum value of only 9g (variety No.32), and a CV of 40.45%, which was mostly distributed within the range of 19.81 – 39.99g (grades 4 - 6), with a frequency of 63% and average MSGY of 33.5g.

These results indicate that MSFW and MSGY vary considerably among different varieties, implying a large potential to improve the fresh weight and grain yield of sweet sorghum. Simultaneously, the diversity index of the major agronomic traits was all higher, with an average of 1.841. Among which, the diversity index of thousand kernel weight (TKW) was the highest (2.062g), as well as the lowest photosynthetic rate (Pn) (1.716$\mu mol.m^{-2} s^{-1}$). Other agronomic traits, such as plant height (PH), juice yield rate (JYR), and brix rate (Br), were mostly distributed at the same 6^{th} grades, implying the close relation of the biological production in sweet sorghum with the juice yield and brix rate.

Supplement table 3. Grouping criterion of quantitative characters.

Grades level	PH (m)	MSD (mm)	MSFW (g)	MSSL (cm)	MSGY (g)	TKW (g)	Pn (μmol.m^{-2} s^{-1})	JYR (%)	Br (%)
1	X<2.02	X<10.27	X<42.29	X<12.42	X<6.35	X<6.20	X<15.73	X<20.69	X<9.49
2	2.02≤X<2.28	10.27≤X<11.63	42.29≤X<134.69	12.42≤X<15.98	6.35≤X<13.08	6.20≤X<8.44	15.73≤X<18.60	20.69≤X<26.25	9.49≤X<10.82
3	2.28≤X<2.54	11.63≤X<12.98	134.69≤X<227.10	15.98≤X<19.54	13.08≤X<19.81	8.44≤X<10.68	18.60≤X<21.47	26.25≤X<31.80	10.82≤X<12.15
4	2.54≤X<2.80	12.98≤X<14.33	227.10≤X<319.50	19.54≤X<23.10	19.81≤X<26.53	10.68≤X<12.92	21.47≤X<24.34	31.80≤X<37.36	12.15≤X<13.48
5	2.80≤X<3.05	14.33≤X<15.69	319.50≤X<411.91	23.10≤X<26.66	26.53≤X<33.26	12.92≤X<15.16	24.34≤X<27.21	37.36≤X<42.91	13.48≤X<14.81
6	3.05≤X<3.31	15.69≤X<17.04	411.91≤X<504.31	26.66≤X<30.22	33.26≤X<39.99	15.16≤X<17.39	27.21≤X<30.08	42.91≤X<48.46	14.81≤X<16.13
7	3.31≤X<3.57	17.04≤X<18.40	504.31≤X<596.72	30.22≤X<33.78	39.99≤X<46.71	17.39≤X<19.63	30.08≤X<32.95	48.46≤X<54.02	16.13≤X<17.46
8	3.57≤X<3.83	18.40≤X<19.75	596.72≤X<689.12	33.78≤X<37.34	46.71≤X<53.44	19.63≤X<21.87	32.95≤X<35.83	54.02≤X<59.57	17.46≤X<18.79
9	3.83≤X<4.08	19.75≤X<21.10	689.12≤X<781.53	37.34≤X<40.90	53.44≤X<60.17	21.87≤X<24.11	35.83≤X<38.69	59.57≤X<65.13	18.79≤X<20.12

Note: PH was plant height, MSD was main stem diameter; MSFW was main stem fresh weight; MSSL was main stem spike length; MSGY was main stem grain yield; TKW was thousand kernel weight; Pnwas photosynthetic rate; JYR was juice yield rate; Br was brix rate

Principal component analysis (PCA) of agronomic traits: Correlation analysis was performed on nine agronomic traits and a correlation matrix was constructed. The results showed that MSFW was positively correlated with PH and main stem diameter (MSD), with the correlation coefficients of 0.42 (P<0.01) and 0.40 (P<0.05), respectively, MSGY was positively correlated with TKW and Br (P<0.01), as well as the correlation coefficients of 0.49 and 0.41, respectively, while MSFW is negatively correlated with MSGY (P<0.01). This finding implies that variable selection indicators should be assessed according to different breeding strategies in forage breeding or new energy development of sweet sorghum.

Three principal components were extracted by analysis of the mean value of each agronomic trait in both 2017 and 2018, with comprehensive interpretation rate of 68.5% (Table 4). The first eigenvector presented bigger and positive values of traits, such as TKW, MSGY, Br etc., these traits are important factors of grain yield, and thought as "grain yield" factors. The second eigenvector showed large positive values, including MSFW, MSSL and PH, these traits are related to biological yield, and thus are called as "biological yield" factors. The "biological yield" factors are negatively correlated with "grain yield" factors. The third eigenvector related to Pn was bigger, leading to higher Br and MSFW, indicating that improving photosynthesis contributes to the formation of sugar and fresh weight of sweet sorghum.

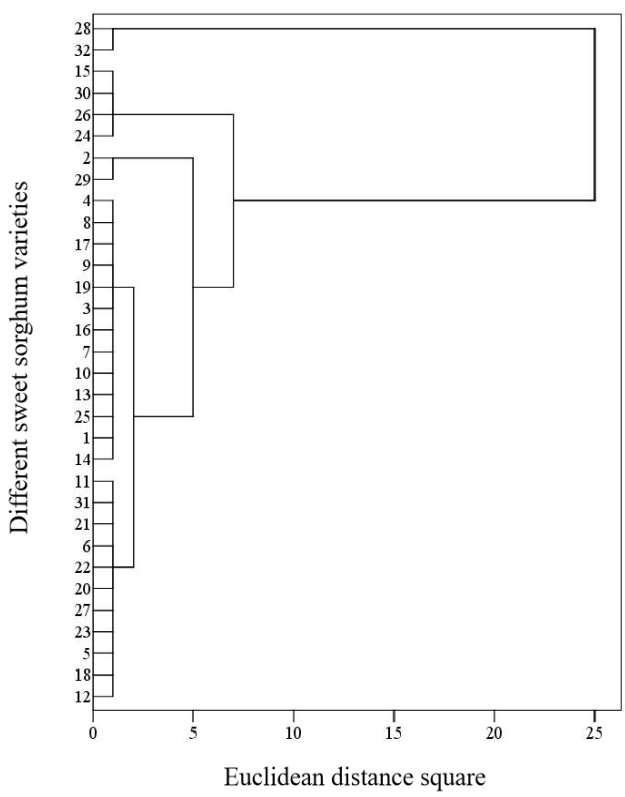

Fig. 1. Cluster analysis of 32 sweet sorghum varieties.
Note: The varieties of sweet sorghum were represented as code number, the details were as Supplement table 1.

Cluster analysis and agronomic characteristics of sweet sorghum: From the cluster analysis of agronomic traits of sweet sorghum, 32 sorghum varieties can be clearly divided into 4 groups at 2.5 of the Euclidean

distance square (Fig. 1), the fourth group, containing a large number of sweet sorghum varieties, can be further divided into two subgroups.

The total numbers and agronomic characteristics of each group are as follows (Table 5):

Group I includes two varieties: No.28 and No.32. Among 32 sweet sorghum varieties, Pn of the two varieties is high, biological yields, including PH, MSD, and MSFW, are the highest, while the MSGY and TKW are the lowest, spike type is medium loose, awnless, red and fully coating glume.

Group II comprises four varieties: PH, MSD and MSFW were higher, grain yield is medium, Br rate is higher, and morphological characteristics (e.g. grain seed and glume color) are variable, with the wide distribution frequency.

Table 2. Diversity index and frequency distribution of morphologic characters in sweet sorghum.

Characteristics	Frequency distribution								Diversity index (*H'*)
	1	2	3	4	5	6	7	8	
MVT	1.00	—	—	—	—	—	—	—	0
MVC	0.66	0.28	0.06	—	—	—	—	—	0.799
STC	0.44	0.53	0.03	—	—	—	—	—	0.803
ST	0.25	0.09	0.34	0.13	0.19	—	—	—	1.511
AT	0.59	0.41	—	—	—	—	—		0.677
GC	—	0.31	0.06	0.16	0.16	0.22	0.09	—	1.668
GSC	0.09	—	—	0.59	—	0.29	—	0.03	0.992
GCD	0.03	0.16	0.44	0.28	0.09	—	—	—	1.333

Note: Values were represented as the distributed frequency in 2017 and 2018 (n=20). MVT was main vein texture, MVC was main vein color, STC was stigma color, ST was spike type, AT was awn type, GC was glume color, GSC was grain seeds color, and GCD was glume coating degree

Table 3. Major agronomic traits and diversity indexes of 32 sweet sorghum varieties.

Traits	Average	Maximum	Minimum	Variation range	Standard deviation (SD)	Variation coefficient (%) (CV)	Diversity index (H′)
PH (m)	3.05±0.05	4.10	1.70	2.40	0.52	16.87	1.881
MSD (mm)	15.69±0.24	23.85	9.55	14.30	2.71	17.26	1.742
MSFW (g)	411.91±18.86	1075.00	105.00	970.00	184.81	44.87	1.772
MSSL (cm)	26.66±0.73	42.00	12.00	30.00	7.12	26.71	1.821
MSGY (g)	33.26±1.37	70.00	9.00	61.00	13.45	40.45	1.924
TKW (g)	15.16±0.46	22.75	5.15	17.60	4.48	29.55	2.062
Pn (μmol.m^{-2} s^{-1})	27.21±0.59	39.02	11.09	27.93	5.74	21.10	1.716
JYR (%)	42.91±1.13	70.00	3.00	67.00	11.11	25.89	1.826
Br (%)	14.81±0.27	21.50	15.80	5.70	2.66	17.95	1.826

Note: Average values were represented as means ± s.e. in 2017 and 2018 (n=20). PH was plant height, MSD was main stem diameter, MSFW was main stem fresh weight, MSSL was main stem spike length, MSGY was main stem grain yield, TKW was thousand kernel weight, Pn was photosynthetic rate, JYR was juice yield rate, and Br was brix rate

Table 4. Principal component matrix of the major agronomic traits of sweet sorgum.

Traits	Component eigenvectors		
	1	2	3
PH (m)	0.539	0.683	0.297
MSD (mm)	-0.649	0.447	0.154
MSFW (g)	-0.210	0.851	-0.220
MSSL (cm)	0.343	0.588	0.548
MSGY (g)	0.679	-0.312	0.041
TKW (g)	0.833	-0.278	0.076
Pn (μmol.m^{-2} s^{-1})	-0.083	0.339	-0.586
JYR (%)	-0.609	-0.526	0.252
Br (%)	0.587	0.087	-0.478

Note: PH was plant height, MSD was main stem diameter, MSFW was main stem fresh weight, MSSL was main stem spike length, MSGY was main stem grain yield, TKW was thousand kernel weight, Pn was photosynthetic rate, JYR was juice yield rate, and Br was brix rate

Group III has two varieties: No.2 and No.29. Biological yield, including PH, MSD, and MSFW, are the smallest, while the MSGY and TKW are higher, Br rate is the lowest, the main vein is white with an awn on the spike, and the grain seed are red.

Group IV contains 24 varieties. For these varieties, Pn is low, biological yields, including PH, MSD, and MSFW, are medium. MSGY and TKW are the largest, Br is high, JYR is acceptable, and the distribution of spike types is wide range. The group is divided into two subgroups: Subgroup IV-1 has 13 varieties, the main vein and stigma are mostly white, with the most awn spikes and 1/2 coating glume types; Subgroup IV-2 comprises 11 varieties, the main vein is light yellow, the stigma is mainly yellow, half of the spike has awns, and grain seed color varies considerably.

Genetic diversity of sweet sorghum by SSR Markers: 276 pairs of SSR primers were selected to test the polymorphism of sweet sorghum, and identify 43 pairs of primers with abundant polymorphism in the 32 varieties. The results

showed that 40 pairs of primers exhibited effective polymorphism, detecting a total of 1430 PCR bands, and 899 PCR bands exhibited polymorphic, accounting for 62.9%. The polymorphic bands between 100 bp and 200 bp were counted to analyze allele loci. Due to the small recognition interval for PCR band, 1-3 alleles were detected for each pair of primers, and 72 effective alleles were found, with an average of 1.7 per pair of primers. The polymorphism information content (PIC) ranged from 0.06 to 0.49, with an average of 0.27. The Shannon's information index (*I*) was between 0 and 1.069, with a CV of 7.9%. The genetic diversity index (*Nei*) averaged 0.347, with a range of 0 to 0.646 (Table 6). These results indicate that these sweet sorghum varieties exhibit the relatively rich molecular genetic diversity among the SSR marker loci.

The inbreeding coefficient (*Fis*) in the population ranged from -0.867 to 1.000, with an average of 0.633, the total inbreeding coefficient (*Fit*) was between -0.778 and 1.000, with an average of 0.762. The positive average values of *Fis* and *Fit* indicate that the inbreeding frequency of the sweet sorghum population is seriously high at 43 SSR loci, and these varieties are basically homozygous, only a few loci (primers S34, S142, and S218) are hybridized (Nagylaki, 1998). The genetic differentiation coefficient (*Fst*) ranged from 0.000 to 0.861, with an average of 0.352, suggesting that 35.2% of the genetic variation occurred among populations, and 64.8% of the genetic variation within the sweet sorghum populations. Gene flow (*Nm*), an important factor affecting population differentiation, ranged from 0.040 to 5.583, with an average of 0.461, implicating the extremely low gene flow of these SSR loci, and the entire population was possibly differentiated via genetic drift. The *Nm* levels of 17 loci in the population were greater than 1, accounting for 50%, the *Nm* levels of primers S182 and S120 loci were greater than 5, and sufficient to resist genetic differentiation caused by genetic drift in the population.

In accordance with the genetic distance of *Nei*, the cluster analysis via UPGMA showed that the 32 sweet sorghum varieties were also divided into 4 groups. The genetic distance is distributed between 0.092 and 0.340, as well as the genetic consistencies between 0.712 and 0.912 (Table 7). Groups I and III had the larger genetic distances, whereas the small genetic consistencies, suggesting that the two groups have more distant genetic relation. This finding is basically consistent with the clustering results of the agronomic traits.

The ratio of polymorphic loci in the four groups was high, with an average of 59.53%. The two subgroups of Group IV had the most abundant polymorphic loci, whereas the Group I had the fewest loci (i.e., only 11 loci). The Shannon's information index (*I*) of different groups ranged from 0.181 to 0.561, and the genetic diversity indexes (*Nei*) were between 0.128 and 0.364, suggesting that the genetic information among different groups varied considerably, whereas the difference of genetic diversity is small (Table 8). The *Nei* of the four groups were ranked as follows: IV>II>III>I, implying that Group IV has the rich genetic resources, and could be adopted to primarily use for sweet sorghum breeding.

Discussion

The genetic structure of plant population is affected by a large of factors, such as the bottleneck effect of parents, genetic drift, and human interference (Wang *et al*., 2020). Therefore, the agronomic traits and morphological characteristics of different varieties were analyzed to explore the genetic difference and genetic structure of the sweet sorghum, to accurately reflect the genetic diversity of the population for high efficient breeding. In this study, the differences among 32 sweet sorghum germplasm resources were highly significant, the CVs of various indicators were large, and the diversity was rich, especially with the higher diversity index of the spike morphological traits. The evaluation of SSR molecular markers showed that the population exhibited the seriously inbreeding, as well of a moderate diversity among different varieties at the molecular level, only a few varieties with a considerable genetic differences. That is speculated that 32 sweet sorghum varieties may have a relatively closer source and the artificial-selfing during production, leading to a simple genetic basis, only a part of sweet sorghum (No.20、No.21 and No.31) exhibit a good improvement potential.

During the genetic diversity analysis of sweet sorghum, the morphological characteristics of hull color and spike types showed the higher genetic diversity index (*H′*), as well as the higher *H′* of main stem grain yield (MSGY) and thousand kernel weight (TKW). These suggest that the traits related to spike morphology and grain yield are used as the key selection indicators in breeding programme. According to the principal component analysis (PCA), the first principal component of the grain yields related to MSGY, TKW, brix etc. had a increased trend, while the second of biological yields related to main stem fresh weight (MSFW), main stem spike length (MSSL), plant height (PH) etc. showed a reduced appearance, indicating that it is hard to obtain an excellent variety by simultaneously improving the two component indicators, and necessary to select a optimal breeding strategy in the production practice.

Table 5. Cluster analysis and agronomic characteristics of 32 sweet sorghum varieties.

Group	Sweet sorghum varieties	Total No.	Agronomic characteristics
I	No.28 and No.32	2	High Pn, the highest biological yields and lowest grain yields
II	No.15, 24, 26 and 30	4	higher biological yields, medium grain yields and higher Br
III	No.2 and No.29	2	The smallest biological yields, higher grain yields and the lowest Br
IV	(1) No.1, 3, 4, 7, 8, 9, 10, 13, 14, 16, 17, 19 and 25	13	Low Pn, medium biological yields, the largest grain yields, higher Br, acceptable JYR and wide range of spike type distribution
	(2) No.5, 6, 11, 12, 18, 20, 21, 22, 23, 27 and 31	11	

Note: PH was plant height, MSD was main stem diameter, MSFW was main stem fresh weight, MSSL was main stem spike length, MSGY was main stem grain yield, TKW was thousand kernel weight, Pn was photosynthetic rate, JYR was juice yield rate, and Br was brix rate

Table 6. Molecular diversity and genetic differentiation at 43 SSR loci in 32 sweet sorghum varieties.

No.	SSR primers	PIC values	*I*	*Nei*	*Fis*	*Fit*	*Fst*	*Nm*	No.	SSR primers	PIC values	*I*	*Nei*	*Fis*	*Fit*	*Fst*	*Nm*
1.	S1	0.28	0.612	0.343	0.887	0.912	0.222	0.878	23.	S146	0.36	0.568	0.353	0.688	0.887	0.638	0.142
2.	S8	0.34	0.436	0.238	1.000	1.000	0.731	0.092	24.	S154	0.42	0.580	0.440	0.760	0.807	0.194	1.039
3.	S15	0.40	0.850	0.530	0.636	0.800	0.449	0.306	25.	S162	0.46	0.628	0.411	0.228	0.372	0.186	1.096
4.	S20	0.41	1.018	0.626	0.876	0.910	0.272	0.669	26.	S179	0.19	0.688	0.498	1.000	1.000	0.221	0.882
5.	S23	0.20	0.423	0.180	1.000	1.000	0.800	0.063	27.	S181	0.12	0.856	0.522	1.000	1.000	0.200	1.000
6.	S24	0.37	0.464	0.366	0.837	0.880	0.261	0.707	28.	S182	0.19	0.673	0.464	1.000	1.000	0.044	5.426
7.	S25	0.06	0.683	0.469	1.000	1.000	0.164	1.274	29.	S184	0.32	0.662	0.496	0.775	0.900	0.556	0.200
8.	S27	0.38	--	--	--	--	--	--	30.	S191	0.29	0.215	0.219	1.000	1.000	0.215	0.914
9.	S29	0.22	0.199	0.225	1.000	1.000	0.176	1.173	31.	S202	0.21	1.070	0.651	0.660	0.763	0.302	0.577
10.	S34	0.22	0.688	0.492	-0.831	-0.760	0.039	6.204	32.	S207	0.17	--	--	--	--	--	--
11.	S36	0.30	0.464	0.293	-0.294	-0.218	0.059	4.020	33.	S210	0.22	0.658	0.486	0.747	0.758	0.042	5.703
12.	S37	0.34	0.436	0.320	1.000	1.000	0.150	1.417	34.	S211	0.48	0.666	0.465	1.000	1.000	0.677	0.120
13.	S39	0.16	0.224	0.140	1.000	1.000	0.368	0.429	35.	S214	0.12	0.602	0.433	0.942	0.960	0.308	0.561
14.	S40	0.17	0.206	0.064	1.000	1.000	0.211	0.938	36.	S216	0.22	0.778	0.476	1.000	1.000	0.214	0.920
15.	S43	0.38	0.000	0.105	1.000	1.000	0.286	0.625	37.	S218	0.30	0.955	0.554	-0.671	-0.482	0.113	1.962
16.	S45	0.22	0.827	0.510	0.766	0.803	0.155	1.360	38.	S224	0.38	0.621	0.405	1.000	1.000	0.451	0.305
17.	S49	0.17	0.778	0.426	0.682	0.885	0.638	0.142	39.	S228	0.30	0.898	0.537	0.808	0.957	0.775	0.073
18.	S75	0.16	0.613	0.347	0.845	0.876	0.201	0.996	40.	S246	0.17	0.547	0.375	0.932	0.947	0.220	0.887
19.	S76	0.38	0.199	0.135	1.000	1.000	0.358	0.449	41.	S249	0.40	0.224	0.137	1.000	1.000	0.060	3.933
20.	S78	0.49	0.956	0.595	0.555	0.784	0.515	0.235	42.	S250	0.21	0.692	0.498	0.875	0.915	0.322	0.527
21.	S139	0.14	0.778	0.509	0.837	0.874	0.224	0.866	43.	S329	0.12	0.266	0.098	0.817	0.848	0.172	1.204
22.	S142	0.30	0.122	0.035	-0.333	-0.053	0.211	0.938									

Note: *PIC* was polymorphism information content; *I* was Shannon's information index; *Nei* was genetic diversity index; *Fis* was inbreeding coefficient; *Fit* was total inbreeding coefficient; *Fst* was genetic differentiation coefficient; *Nm* was gene flow

Table 7. Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Groups	I	II	III	IV
I	****	0.748	0.712	0.738
II	0.290	****	0.901	0.912
III	0.340	0.104	****	0.905
IV	0.304	0.092	0.100	****

Table 8. Genetic diversity of 4 sweet sorghum groups.

Group	Samples	Polymorphic loci numbers	Ratio of polymorphic loci (%)	*I*	*Nei*
I	4	11	25.58%	0.181	0.128
II	7	29	67.44%	0.463	0.312
III	4	19	44.19%	0.312	0.223
IV	22	32	74.42%	0.469	0.297
	18	37	86.05%	0.561	0.364
Average	11	25.6	59.53%	0.397	0.265

Note*: I* was Shannon's information index, *Nei* was genetic diversity index

The genetic diversity index (*Nei*) of the population was low, primarily due to the large inbreeding coefficient (*Fis*) of the sweet sorghum population and the low gene flow (*Nm*) level in more than 50% of the genetic loci. The inbreeding trend within the varieties was evident, it is presumed that the 32 sweet sorghum varieties had close sources, apparent directional selection, to produce a narrow genetic range. The 32 germplasm materials were divided into 4 categories, Groups II and IV had a bigger genetic consistency (0.912), Group IV exhibited high genetic diversity index, wide spike type distribution, good grain yields, and high brix and juice yields rate. The germplasm materials in this group can be hybridized with other sweet sorghum varieties of distant genetic relationship to achieve satisfied characteristics.

It was generally inconsistent with the relationship between individual genetic variation detected by SSR data and phenotypic variation reflected by agronomic traits (Carputo *et al*., 2013; Zheng *et al.*, 2020). In this study, the phenotypic traits diversity of the 32 varieties was rich, while SSR molecular identification showed the moderate diversity and simple genetic basis. However, the classification based on agronomic traits was similar to that of SSR molecular marker, and both could be divided into four groups. This is possibly due to the susceptibility of agronomic traits to environmental conditions and dominant (recessive) genes, with the instability of genetic expression. The detected target of SSR molecular marker is the non-functional area in the genome, the detection results are stable in various tissues and different developmental stages of plants (Li *et al*., 2020). This study only investigated several major agronomic traits of sweet sorghum, and the sequence of functional genes corresponding to relative molecular marker was unclear, this scenario may lead to the differences between phenotypic characteristics and molecular identification.

Conclusion

The agronomic traits among 32 sweet sorghum varieties were significantly different, with a richer diversity of spike type and glume color, which could be used as the rapid selection criterions during breeding programmes. SSR markers analysis showed that these sweet sorghum varieties exhibited the relatively rich genetic diversity at the molecular level, yet a high inbreeding coefficient within the population, which was speculated that the varieties were planted with severely directional selection during breeding practice, resulting in the relatively narrow genetics range within the population. 32 sweet sorghum varieties were divided into 4 groups, group IV contained a large number of varieties, performed a wide distribution of spike type, the high grain yields and brix rate, a good juice yield and rich genetic diversity, and the variety No.20, No.21 and No.31 may be used as the key materials for breeding.

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