

Fabo CHEN, Kecheng YANG, Tingzhao RONG, Guangtang PAN

## Analysis of genetic diversity of maize hybrids in the regional tests in Sichuan and Southwest China

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**Abstract** In this study, analyses of phenotypic characters, SSR molecular markers and pedigrees were done to study the genetic diversity in 186 maize hybrids that were tested in regional trials in Sichuan and Southwest China. The results showed that there were differences in the variation coefficients of different characteristics, but all of the variation coefficients changed within a narrow range. Sixty pairs of simple sequence repeat (SSR) primer distributed on the ten chromosomes of maize produced stable amplified bands and 608 alleles were detected among the hybrids. The average number of alleles per locus was 10.1 ranging from 3 to 23. The values of polymorphism information content (PIC) for each SSR locus varied from 0.5179 to 0.9256 with an average of 0.7826. The genetic similarities of SSR marker pattern among the 186 hybrids ranged from 0.6067 to 0.9162, with an average of 0.7722. There were 16499 pairs of genetic similarity, in which 96.9% were 0.70000 to 0.9256. The cluster analysis showed that the hybrids could be classified into ten clusters, with 88.2% of the hybrids included in Cluster 4, Cluster 8 and Cluster 10. The analysis of pedigree sources of 51 hybrids showed that 36 hybrids had close genetic relationships with the hybrids developed by the Pioneer Company in the late 1980s and early 1990s in the United States, such as Y78599, Y7865 and Y78698, accounting for 70.58%. Meanwhile, 13 hybrids had close genetic relationships with Y78599, accounting for 8.66%. The genetic similarities of SSR marker pattern among the 51 hybrids ranged from 0.66192 to 0.8799, with an average of 0.7686. There were 1196 pairs of genetic similarity ranged

between 0.7000 to 0.8796, accounting for 93.80% of all the genetic similarity pairs. The cluster analysis showed that 88.2% of the 51 hybrids were in Cluster 4, Cluster 8 and Cluster 10, which indicated that similarity was high and genetic diversity narrow among the 186 hybrids. This showed that it is necessary to broaden the genetic basis of breeding germplasm in maize.

**Keywords** *Zea mays* L., hybrid, genetic diversity, SSR marker, cluster analysis, pedigree

### 1 Introduction

Many maize breeders have attempted to solve the problem of narrow genetic diversity in maize. In order to broaden the genetic basis in maize breeding, it is necessary to determine the level of genetic diversity in current maize hybrids. The first study on the germplasm basis for major maize hybrids with a growing area of more than 67000 hm<sup>2</sup> during different stages shows that the major inbred lines in China can be classified into domestic inbred and exotic inland inbred lines (Wu, 1983). Maize genetic diversity has been summarized through an analysis of main maize hybrids with a growing area of more than 67000 hm<sup>2</sup>, and analysis of germplasm basis of the regional trials of maize hybrids in Henan Province from 1974 to 1985, indicating that 97.6% of the hybrids in the regional trials were concentrated on 4 inbred lines – Huobai, Mo17, Huangzaosi, and 525 – with 94.6% of main maize hybrids also with the same growing area concentrated within the four inbred lines (Wang et al., 1986). In addition, the maize germplasm base in China has also been studied through the synthetic analysis of main maize hybrids grown within a similarly sized area of more than 67000 hm<sup>2</sup> from 1978 to 1987, and through the synthetic analysis of genetic components of the regional trials of maize hybrids in China from 1984 to 1989. It is shown that utilization of the main inbred lines tends to concentrate 3 inbred lines (Zi330, Huangzaosi and Mo17) widely used as parents in hybrids for 54.33% of the total hybrids, and the

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Fabo CHEN (✉)  
Chongqing Fuling District Agricultural Science Institute, Fuling  
408000, China  
E-mail: chenfabo963@yahoo.com.cn

Kecheng YANG, Tingzhao RONG, Guangtang PAN  
Key Laboratory of Crop Genetic Resources and Improvement,  
Maize Research Institute, Sichuan Agricultural University, Ya'an  
625014, China

hybrids of the domestic inbred line  $\times$  exotic inbred line have occupied 65%–68% of the total growing area (Zeng, 1990). Simple sequence repeats (SSRs) markers are adopted in the heterotic grouping of 70 elite maize inbred lines, which can be classified by the UPGMA analysis into 6 clusters (Sipingtuo, Luda Red Cob, PA, PB, BSSS and Lancaster) (Li et al., 2003). Ninety-four elite maize inbred lines, the representative of the genetic diversity among the lines derived from the Corn Belt Dent and Southern Dent maize races, have been assayed for polymorphism at 70 SSR marker loci, with cluster analysis done on the inbred lines in nine clusters that correspond to the major heterotic groups or market classes for North American maize. The similarities may prove a valuable asset for a maize breeding program (Senior et al., 1998). There are two possible ways to study genetic diversity in maize hybrids. One is to analyze the hybrids which have been widely applied in production and those in the regional tests. Another is to analyze the parents of maize hybrids. Most of the previous studies were directed at evaluating the genetic diversity of the parents and few directed towards the hybrids. Maize (*Zea mays* L.) breeders have become aware of the necessity of maintaining both genetic diversity for crop improvement and improving the quality of genetic resource management.

## 2 Materials and methods

### 2.1 Materials

The maize materials in our study comprised 186 hybrids, including the hybrids of regional tests in Sichuan Province, the hybrids of regional tests in China (Southwest and Wuling region of China), the hybrids of introduction trials in Sichuan Province, and the CK varieties Chuandan13 and Nongda108. The materials are listed in Table 1.

### 2.2 Field experiment

The field experiment was conducted in the Fenjiang Experimental Farm of Sichuan Agricultural University in Ya'an City, China, on April 4, 2005. The study was designed as a random complete-block design with three treatments, single row, 14 plants per row, one plant per nest, and a plant density of 49500 plant $\cdot$ hm $^{-2}$ . One CK was planted every ten rows, and Chuandan13 and Nongda108 as CK varieties were planted alternatively. Data were collected from the middle 12 plants in each row.

### 2.3 Investigation of traits

Silking day and pollen shedding day were investigated during plant silking and pollen shedding. Plant height

and ear height were investigated after plant silking and pollen shedding. Ear length, fertile kernel, kernel depth, cob diameter, ear diameter, row per ear, kernel per row, ear weight, cob weight, yield per plant, fresh weight per ear, water content, kernel rate, 100-kernel weight and test weight were measured after harvesting and air-drying. (Water content = (fresh weight per ear – ear weight after air-drying)/ear weight after air-drying).

### 2.4 DNA extraction

Genetic DNA of hybrids was extracted from the up-leaf tissue of one representative plant chosen from each treatment during the silking and shedding stage, according to the method 2  $\times$  CTAB used by Xia et al. (2005).

### 2.5 SSR amplification

The PCR reaction mixture consisted of 1  $\times$  buffer, 2.5  $\mu$ mol $\cdot$ L $^{-1}$  MgCl $_2$ , 150  $\mu$ mol $\cdot$ L $^{-1}$  Deoxyribonucleotide triphosphate (dNTP), 0.2  $\mu$ mol $\cdot$ L $^{-1}$  primers, 1 U Taq DNA polymerase and 100 ng DNA as a template, with a total volume of 15  $\mu$ L. The reaction mixture was overlaid with 18  $\mu$ L of mineral oil after intermixing in a pulsator. The amplification protocol included 1 cycle of denaturalization for 5 min at 95 $^{\circ}$ C used for predenaturalization, 11 cycles of predenaturalization for 40 s at 95 $^{\circ}$ C, and annealing for 30 s at 65 $^{\circ}$ C, followed by extension for 1 min at 72 $^{\circ}$ C, and 30 cycles of predenaturalization for 40 s at 95 $^{\circ}$ C, annealing for 40 s at 57 $^{\circ}$ C, and extension for 1 min at 72 $^{\circ}$ C, followed by another cycle of 1 min extension at 72 $^{\circ}$ C and keeping at 4 $^{\circ}$ C for 5 min. After amplification, the mixture was separated by electrophoresis using 6% denaturalized polyacrylamide gel under 75 W at 50 $^{\circ}$ C for 1 h (Xin, 2004).

### 2.6 Statistical analyses

#### 2.6.1 Variation coefficient analysis

Variation coefficient was analyzed, including the variation coefficients (*CV*) of plant height, ear height, days to silking, pollen shedding and ASI (silking and shedding interval), ear length, fertile kernel, kernel depth, cob diameter, ear diameter, rows per ear, kernels per row, ear weight, cob weight, yield per plant, fresh weight per ear, water content, kernel rate, 100-kernel weight and test weight among 186 hybrids, according to the mean of the three replications. It was calculated using the following equation:

$$CV(\%) = \frac{S}{\bar{x}} \times 100,$$

where  $\bar{x}$  is the mean value of a trait based on the three replications, and *S* is standard deviation (SD) of the trait.

**Table 1** Materials used in the study and their sources

No.	name	type of regional tests	source
1	Chundan13	CK1	Seed Management Center of Sichuan Province
2	Nongda108	CK2	Seed Management Center of Beijing City
3	2021	a	Crop Research Institute of Sichuan Agricultural Science Academy
4	2082	a	Crop Research Institute of Sichuan Agricultural Science Academy
5	2099	a	Crop Research Institute of Sichuan Agricultural Science Academy
6	CY4261	a	Sichuan Chuanfeng Seed Company
7	D0401	a	Agricultural Science Institute of Dazhou City
8	D0405	a	Agricultural Science Institute of Dazhou City
9	DH3711	a	Shandong Denghai Seed Company
10	DH3722	a	Shandong Denghai Seed Company
11	DN25301	a	Beijing Decheng Seed Company
12	DN25302	a	Beijing Decheng Seed Company
13	DT6327	a	Crop Research Institute of Sichuan Agricultural Science Academy
14	GM03-31	a	Crop Research Institute of Sichuan Agricultural Science Academy
15	GM03-34	a	Crop Research Institute of Sichuan Agricultural Science Academy
16	GM4103	a	Crop Research Institute of Sichuan Agricultural Science Academy
17	HA-20	a	Sichuan Jialing Crop Center
18	HA-30	a	Sichuan Jialing Crop Center
19	JG03-5	a	Agricultural Science Institute of Wusheng County
20	JG03-6	a	Agricultural Science Institute of Wusheng County
21	JG04-1	a	Chengdu Tianfu Crop Science Institute
22	JG04-2	a	Agricultural Science Institute of Wusheng County
23	LP301	a	Sichuan Longping high-Tec Company
24	ND128	a	Maize Improvement Center of China Agricultural University
25	R18	a	Mianyang Ruide Seed Company
26	SAU0301	a	Maize Research Institute of Sichuan Agricultural University
27	SAU0303	a	Maize Research Institute of Sichuan Agricultural University
28	SAU0304	a	Maize Research Institute of Sichuan Agricultural University
29	SAU0307	a	Maize Research Institute of Sichuan Agricultural University
30	SAU03-1	a	Maize Research Institute of Sichuan Agricultural University
31	SAU0310	a	Maize Research Institute of Sichuan Agricultural University
32	SAUQ181	a	Maize Research Institute of Sichuan Agricultural University
33	SAUQ58	a	Maize Research Institute of Sichuan Agricultural University
34	SB2405	a	Hebei Longhua Sanbei Seed Company
35	SB2420	a	Hebei Longhua Sanbei Seed Company
36	SC907	a	Chengdu Shanchuan Seed Company
37	SDZ-92	a	Sichuan Zhongzheng Seed Company
38	SL09420	a	Sichuan Shulong Seed Company
39	SL26698	a	Sichuan Shulong Seed Company
40	SL99018	a	Sichuan Shulong Seed Company
41	SZ040	a	Yaan Sanzhou Seed Company
42	TF3005	a	Sichuan Tianfeng Seed Company
43	WVE3144	a	Crop Research Institute of Sichuan Agricultural Science Academy
44	WVEH3134	a	Crop Research Institute of Sichuan Agricultural Science Academy
45	YA0412	a	Sichuan Yayu Science and Technology Company
46	YA0461	a	Sichuan Yayu Science and Technology Company
47	YAHBP15	a	Sichuan Yayu Science and Technology Company
48	ZB-128	a	Crop Research Institute of Sichuan Agricultural Science Academy
49	ZB-138	a	Crop Research Institute of Sichuan Agricultural Science Academy
50	ZB-28	a	Crop Research Institute of Sichuan Agricultural Science Academy
51	ZB-78	a	Crop Research Institute of Sichuan Agricultural Science Academy
52	ZB98	a	Crop Research Institute of Sichuan Agricultural Science Academy
53	ZH308	a	Agriculture College of Sichuan Agricultural University
54	ZH311	a	Agriculture College of Sichuan Agricultural University
55	ZH326	a	Agriculture College of Sichuan Agricultural University
56	ZK19104	a	Hunan Ketai Seed Company
57	ZL628	a	Sichuan Seed Engineering Institute
58	Ao13-8	a	Agricultural Science Institute of Wusheng County
59	Chang04-3	a	Mianyang Kuifeng Seed Company
60	Deyu1035	a	Denong Zhengcheng Seed Company
61	Deyu24-1	a	Denong Zhengcheng Seed Company
62	Deyu4-1	a	Denong Zhengcheng Seed Company
63	Deyu4-2	a	Denong Zhengcheng Seed Company

(continued)

No.	name	type of regional tests	source
64	Deyu7	a	Denong Zhengcheng Seed Company
65	Dong6007	a	Agricultural Science Institute of Wusheng County
66	Fushi1015	a	Sichuan Jialing Crop Center
67	Fushi1020	a	Sichuan Jialing Crop Center
68	Gaoyou8	a	Beijin Fenge Sangao Seed Company
69	Hai99-10	a	Liaoning Haihe Seed Company
70	Haihe2	a	Liaoning Haihe Seed Company
71	Kehua2	a	Sichuan Huafeng Seed Company
72	Huashi301	a	Sichuan Huafeng Seed Company
73	Huanshi302	a	Sichuan Huafeng Seed Company
74	Huashi304	a	Sichuan Huafeng Seed Company
75	Huanshi9421	a	Sichuan Huafeng Seed Company
76	Huaxiayu1	a	Sichuan Huaxia Seed Company
77	Huaxuan168	a	Sichuan Hualong Seed Company
78	Ji'nong3	a	Hebei Jinong Seed Company
79	Jinhai5	a	Mianyang Kexing Seed Company
80	Kaifeng65247	a	Sichuan Kaifeng Seed Company
81	Kuiyu66-7	a	Mianyang Kuifeng Seed Company
82	Liao121	a	Maize Research Institute of Liaoning Agricultural Science Academy
83	Liao123	a	Maize Research Institute of Liaoning Agricultural Science Academy
84	Luchuan9901	a	Chengdu Jinjiang Luchuan Seed Operations Department
85	Lushi1	a	Sichuan Ludan Seed Company
86	Lushi2	a	Sichuan Ludan Seed Company
87	Mian02211	a	Agricultural Science Institute of Mianyang City
88	Mian03341	a	Agricultural Science Institute of Mianyang City
89	Mian03346	a	Agricultural Science Institute of Mianyang City
90	Mian03355	a	Agricultural Science Institute of Mianyang City
91	Mian03581	a	Agricultural Science Institute of Mianyang City
92	Mian778	a	Agricultural Science Institute of Mianyang City
93	Nan005	a	Agricultural Science Institute of Nanchong City
94	Nan390	a	Agricultural Science Institute of Nanchong City
95	Nan477	a	Agricultural Science Institute of Nanchong City
96	NanC210	a	Agricultural Science Institute of Nanchong City
97	Nonghua194	a	Beijin Jinse Nonghua Seed Company
98	Shudan8	a	Sichuan Xianfeng Seed Company
99	Tunyu65	a	Sichuan Difeng Seed Company
100	Wandan14	a	Sichuan Jialing Crop Center
101	Xinshi2036	a	Agricultural Science Institute of Li County
102	Yuyu32	a	Sichuan Yufeng Seed Company
103	Yuandong18	a	Sichuan Yuandong Seed Company
104	Yuandong33	a	Sichuan Yuandong Seed Company
105	Zhengda2393	a	Sichuan Zhengda Seed Company
106	Zhengda2398	a	Sichuan Zhengda Seed Company
107	SAU0308	a	Maize Research Institute of Sichuan Agricultural University
108	Liyu10	c	Hebei Shijiazhuang Liyu Science and Technology Company
109	04-15	a	Chongqing Yimin agricultural Science Institute of Dazhu County
110	ZB-38	a	Crop Research Institute of Sichuan Agricultural Science Academy
111	ZB-38	a	Crop Research Institute of Sichuan Agricultural Science Academy
112	ZY635	a	Sichuan Seed Engineering Institute
113	JY2102	a	Sichuan Yayu Seed Company
114	T29806	a	Sichuan Meishan Yufeng Seed Company
115	WVE3151	a	Crop Research Institute of Sichuan Agricultural Science Academy
116	YA0482	a	Sichuan Yayu Science and Technology Company
117	ZY994	a	Sichuan Seed Engineering Institute
118	Danyu22	c	Sichuan Jialing Crop Center
119	Danke2151	c	Maize Research Institute of Liaoning Dandong Agricultural Science Academy
120	Dongdan11	c	Liaoning Dongya Seed Company
121	Dongdan12	c	Liaoning Dongya Seed Company
122	Dongdan225	c	Liaoning Dongya Seed Company
123	Fuyou1	c	Liaoning Dongya Seed Company
124	Huadan208	c	Liaoning Dongya Seed Company
125	Huaxuan6	c	Sichuan Hualong Seed Company
126	Huaxuan169	c	Sichuan Hualong Seed Company

(continued)

No.	name	type of regional tests	source
127	Jindao7	c	Neijiang Kefeng Seed Company
128	Jinyu1	c	Heilongjiang Jincheng Agricultural Science Institute of Shuangcheng City
129	Jindan42	c	Seedling Company of Shanxi Agricultural Science Academy
130	Liaodan27	c	Maize Research Institute of Liaoning Agricultural Science Academy
131	Qianbei2	c	Guizhou Zheng'an Seed Company
132	Qiandan18	c	Land Crop Research Institute of Guizhou Agricultural Science Academy
133	Qianyu1	c	Land Crop Research Institute of Guizhou Agricultural Science Academy
134	Qianyu2	c	Land Crop Research Institute of Guizhou Agricultural Science Academy
135	Qiangsheng17	c	Seedling Company of Shanxi Agricultural Science Academy
136	Yuyu25	c	Crop Research Institute of Henan Agricultural Science Academy
137	Zhengda99	c	Hubei Xiangfan Zhengda Seed Company
138	Zhongjin368	c	Crop college of China Agricultural University
139	Zhuhuang1	c	Agricultural Test Center of Guiyang City
140	2861	b	Chongqing Agricultural Science Academy
141	961 × 168	b	Hubei Changyang Seed Company
142	SY01-3	b	Crop Research Institute of Hubei Shiyuan Agricultural Science Academy
143	TY10330	b	Shanxi Tunyu Seed Company
144	YA04273	b	Sichuan Yayu Science and Technology Company
145	Fushi1001	b	Sichuan Zhongzheng Seed Company
146	HaiS02	b	Liaoning Haihe Seed Company
147	Hang0201	b	Shijiazhuang Hangtian Agriculture Science Company
148	Xishan99	b	Hainan Sengnong Dafeng Seed Company
149	Xinghai201	b	Qianxinan Agricultural Science Institute of Guizhou Province
150	CF287	b	Beijing Jinse Nonghua Seed Company
151	CN36	b	High-Tec Agriculture Company of Sichuan Agricultural University
152	DH3633	b	Shandong Denghai Seed Company
153	DH3686	b	Shandong Denghai Seed Company
154	DH3731	b	Shandong Denghai Seed Company
155	DH3838	b	Shandong Denghai Seed Company
156	DH3861	b	Shandong Denghai Seed Company
157	HD5839	b	Shanxi Qinlong Luse Seed Company
158	ND236	b	Maize Improvement Center of China Agricultural University
159	NH1001	b	Beijing Jinse Nonghua Seed Company
160	NK6661	b	Office of Syngenta of Switzerland in Beijing
161	SB2246	b	Hebei Longhua Sanbei Seed Company
162	SB2494	b	Hebei Longhua Sanbei Seed Company
163	TF2031	b	Sichuan Tian Agriculture Science Company
164	TY30331	b	Shanxi Tunyu Seed Company
165	TY30431	b	Shanxi Tunyu Seed Company
166	X1152A	b	Liaoning Tieling Xianfeng Seed Research Company
167	YA1023	b	Sichuan Yayu Science and Technology Company
168	YF-3	b	Seed Management Center of Guizhou Province
169	Dong4243	b	Seed Test Center of Liaoning Dongya Seed Company
170	Gaoguang158	b	Henan Gaoguang Maize Research Institute
171	Heyu9566	b	Beijing Zhongnong Sanhe Agriculture Science Company
172	Liao527	b	Maize Research Institute of Liaoning Agricultural Science Academy
173	Ningyu309	b	Nanjing Chunxi Seed Research Company
174	Qiangsheng11	b	Shanxi Qiangsheng Seed Company
175	Sannong20-A	b	Henan Sannong Seed Company
176	Tiantai16	b	Shandong Pingyi Seed Company
177	Tianyuan1	b	Wuda Tianyuan Biotechnology Company
178	Yihuangan4	b	Yunnan Xuanwei Seed Company
179	Yudan11	b	Chongqing Agricultural Science Academy
180	Yuyu688	b	Hunan Longping High-Tec Longping Seed Company
181	Yunfeng103	b	Beijing Jinse Nonghua Seed Company
182	Zhongdan808	b	Crop Research Institute of China Agricultural Science Academy
183	SAU03-2	a/b	Maize Research Institute of Sichuan Agricultural University
184	YA1782	a/b	Sichuan Yayu Science and Technology Company
185	Deyu28	a/b	Denong Zhengcheng Seed Company
186	Jinyu9856	a/b	Liangyu Pang

Note: "a" and "b" denote hybrids of regional tests in Sichuan and China (Southwest and Wuling region of China), respectively; and "c" denotes hybrids of introduction trials in Sichuan Province.

## 2.6.2 SSR data analysis

The stable amplified bands produced by the 60 SSR primers based on the denaturalized polyacrylamide gel were standardized into columns of “1’s”, “0’s” and “9’s”, which represented the presence of a specific band (column) in a hybrid (row), the absence of a specific band, and one missing band in a hybrid, respectively. Polymorphism information content (*PIC*) for each SSR marker was estimated as a measure of allele diversity at a locus and its equation is described below:

$$PIC = 1 - \sum f_i^2,$$

where  $f_i$  is the frequency of the  $i$ th allele.

Genetic similarities (*GS*) were calculated based on the band data, between each pair of the hybrids, using the “Unnamed” coefficient No.1, such that  $GS = 2N_{ij}/(N_i + N_j)$ , where,  $N_{ij}$  is the number of matched bands and  $(N_i + N_j)$  is the number of all bands of hybrids  $N_i$  and  $N_j$  (Nei and Li, 1979). The 186 hybrids were clustered based on the matrix of genetic similarities using the Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) clustering algorithm.

All data manipulation and statistical analysis were performed using Excel, DPS7.55 and NTSYSpc.2.1 for Windows.

## 3 Results

### 3.1 Analysis of variation coefficient

Table 2 indicates that the variation coefficients of plant height, ear height, days to silking, pollen shedding, ASI, ear length, fertile kernel, kernel depth, cob diameter, ear diameter, rows per ear, kernels per row, ear weight, cob weight, yield per plant, fresh weight per ear, water content, kernel rate, 100-kernel weight and test weight among 186 hybrids were 5.74%, 12.06%, 2.63%, 2.36%, 8.50%, 9.68%, 5.80%, 10.57%, 9.32%, 6.02%, 7.90%, 10.54%, 15.95%, 21.51%, 16.19%, 13.85%, 10.35%, 2.46%, 10.92% and 4.93%, respectively. There were

differences in the variation coefficients of different traits, but all of the variation coefficients changed within a narrow range.

### 3.2 Analysis of SSR data

Sixty pairs of SSR primers, selected from 115 pairs of SSR primers and distributed on the ten chromosomes of maize produced stable amplified bands and 608 alleles were detected among the hybrids. As shown in Table 3, the 60 pairs of SSR primers generated a total of 608 alleles. The average number of alleles per locus was 10.1 in a range from 3 to 23. The values of polymorphism information content (*PIC*) for each SSR locus varied from 0.5179 to 0.9256 with an average of 0.7826. The genetic similarities of SSR marker patterns among the 186 hybrids ranged from 0.6067 to 0.9162, with an average of 0.7722. There were 16499 pairs of genetic similarity, of which 96.9% were 0.70000 to 0.9256.

As shown in Fig. 1, the 186 hybrids could be classified into ten clusters when the similarity was 0.7776. Cluster 1 had 15 hybrids, including the CK varieties Chuandan13 and Nongda108, which accounted for 8.1% of the total tested. Cluster 4 included 88 hybrids, accounting for 47.3% of the total hybrids tested. Cluster 8 included 47 hybrids, accounting for 25.3% of the total hybrids tested. Cluster 9 included 2 hybrids, accounting for 1.1% of the total hybrids tested. Cluster 10 included 29 hybrids, accounting for 15.6% of the total hybrids tested. There was only one hybrid tested in Cluster 2, Cluster 3 Cluster 5, Cluster 6 and Cluster 7, respectively.

The above analysis indicates that the similarities are high. The cluster analysis showed that the hybrids could be classified into ten clusters, with 88.2% of the hybrids in Cluster 4, Cluster 8 and Cluster 10. The results proved that genetic diversity was narrow among the 186 hybrids.

### 3.3 Pedigree analyses of 51 hybrids

In order to determine the genetic diversity of the hybrids more clearly, the pedigree sources of 51 hybrids were collected from companies and the internet. As shown in Table 4, 36 hybrids had close genetic relationships with

**Table 2** Variation coefficient and 20 phenotypic characters among 186 hybrids

trait	mean	SD	CV/%	trait	mean	SD	CV/%
plant height/cm	265.13	15.22	5.74	rows per ear	16.09	1.27	7.9
ear height/cm	115.78	13.97	12.06	kernels per row	34.3	3.61	10.54
days to silking/d	73.85	1.94	2.63	ear weight/g	174.61	27.9	15.95
pollen shedding/d	73.2	1.73	2.36	cob weight/g	24.96	5.37	21.51
anther silking interval/d	0.75	0.06	8.5	yield per plant/g	149.65	24.2	16.19
ear length/cm	16.81	1.63	9.68	fresh weight per ear/g	305.61	42.3	13.85
fertile kernel/cm	0.98	0.057	5.8	water content/%	0.43	0.04	10.35
kernel depth/cm	1.03	0.11	10.57	kernel rate/%	0.86	0.02	2.46
cob diameter/cm	2.82	0.26	9.32	100- kernel weight/g	28.89	3.15	10.92
ear diameter/cm	4.87	0.29	6.02	test weight/g·L <sup>-1</sup>	500.9	24.7	4.93

**Table 3** The number, the ratio of polymorphic loci and the PIC for each SSR primer detected in 186 hybrids

primer	No. of alleles amplified	chrom.	PIC	primer	No. of alleles amplified	chrom.	PIC	primer	No. of alleles amplified	chrom.	PIC
bnlg439	8	1.03	0.8077	phi096	5	4.04	0.5786	phi069	9	7.05	0.842
phi109275	13	1.03	0.8194	phi079	11	4.05	0.8721	bnlg1200	18	7.1	0.7839
phi339017	3	1.03	0.5972	phi093	5	4.08	0.5308	phi076	11	7.1	0.7564
phi227562	8	1.1	0.8498	bnlg589	17	4.11	0.9115	phi1304	14	8.02	0.8426
phi308707	8	1.1	0.8532	phi006	10	4.11	0.8357	phi115	9	8.03	0.8213
phi109642	7	2	0.8984	nc130	12	5	0.8646	bnlg162	11	8.05	0.8531
phi1555	8	2.04	0.8033	phi024	20	5.01	0.9256	bnlg240	9	8.05	0.8862
bnlg2039	15	2.05	0.8957	phi331888	10	5.04	0.7892	phi100175	4	8.06	0.8456
nc133	16	2.05	0.8754	bnlg238	14	5.06	0.8976	phi23376	7	8.06	0.7825
umc211	18	2.05	0.9142	phi087	7	5.06	0.7369	phi1279	5	9	0.5721
phi101049	4	2.1	0.7916	phi085	12	5.07	0.8304	phi022	10	9.03	0.8934
phi104124	14	3.01	0.7343	phi126	7	6	0.7238	phio65	5	9.03	0.564
phi0293	5	3.04	0.7422	umc1143	10	6.01	0.7936	phi032	7	9.04	0.7628
phi083	11	3.04	0.8071	phi423796	8	6.02	0.7524	phi448880	7	9.05	0.6953
umc0071	6	3.05	0.5846	phi078	20	6.05	0.8792	phi063	11	10.02	0.7542
phi046	6	3.08	0.6675	phi070	18	6.07	0.8637	phi059	9	10.02	0.7642
phi053	4	3.08	0.5179	phi299523	7	6.08	0.6483	p-phi052	14	10.02	0.7294
umc2081	9	3.08	0.7018	phi299852	4	6.08	0.9009	phi050	19	10.03	0.8699
phi213984	14	4.01	0.8635	umc2160	11	7.01	0.7732	phi062	23	10.04	0.9143
umc1228	8	4.01	0.6359	phi114	4	7.02	0.8441	phi084	9	10.04	0.7105

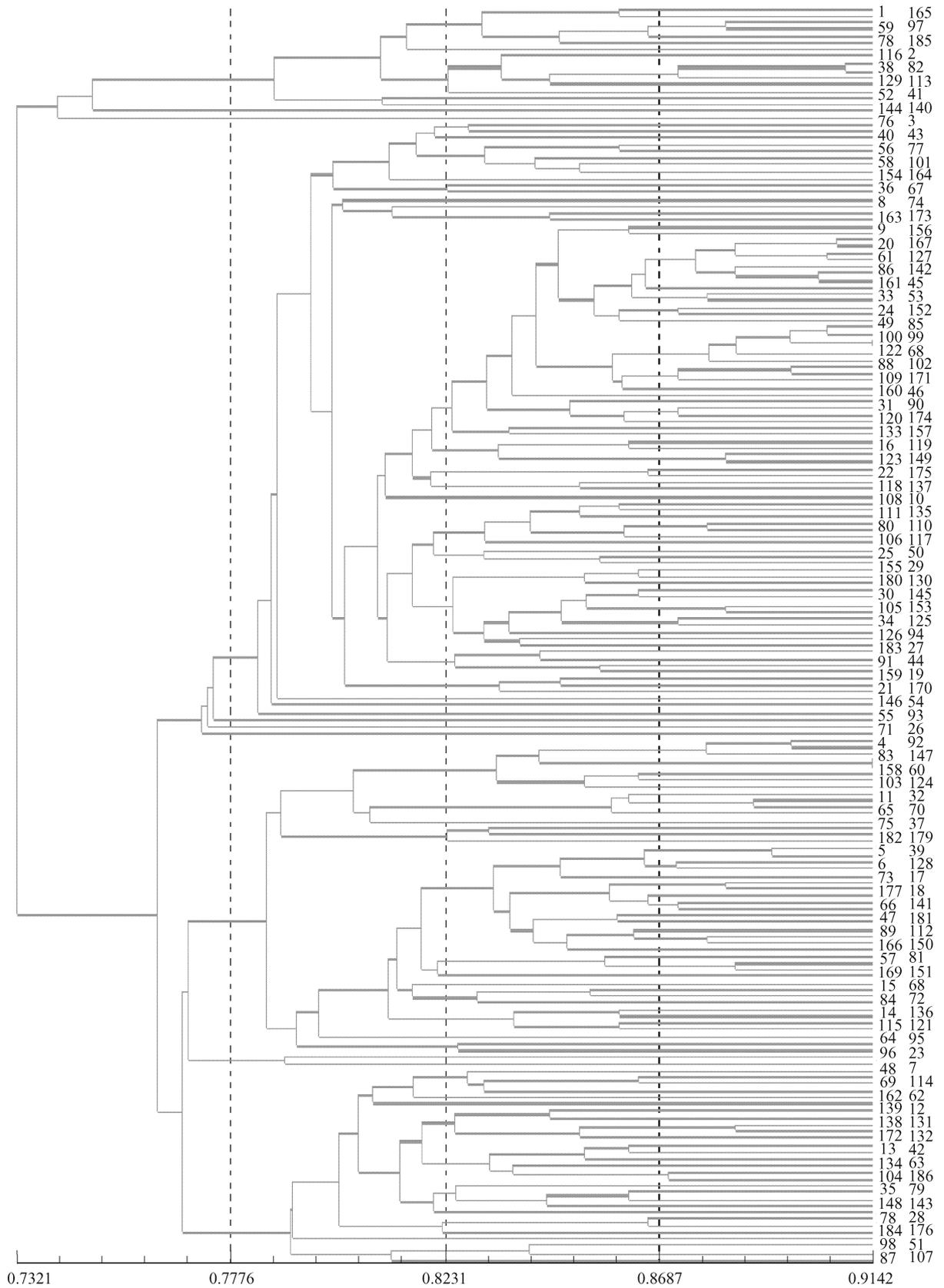
the hybrids developed by the Pioneer Company (USA) in the late 1980s and early 1990s in the US, such as Y78599, Y7865, Y78698, accounting for 70.58%. Meanwhile, 13 hybrids showed close genetic relationships with Y78599, accounting for 8.66%. From Table 3, we can see that the genetic similarities of SSR marker pattern among the 51 hybrids ranged from 0.66192 to 0.8799, with an average of 0.7686. There were 1196 pairs of genetic similarities ranged between 0.7000 to 0.8796, accounting for 93.80% of all the genetic similarity pairs. The cluster analysis showed the hybrids No.1, No.2, No.59, No.82 and No.129 were included in Cluster 1, with the hybrid No.140 forming a cluster, and the hybrids Nos. 16, 19, 21, 22, 30, 33, 36, 40, 50, 54, 100, 108, 118, 119, 120, 123, 125, 135, 155, 175 and 183 were included in Cluster 4, the hybrids Nos.16, 65, 70, 92, 95, 95, 112, 121, 24, 128, 136, 141, 158, 166, 169, 179 and 182 were included in Cluster 8, the hybrids Nos.13, 51, 131, 138, 139 and 186 were included in Cluster 8, with 88.25% of the 51 hybrids in Cluster 4, Cluster 8 and Cluster 10. It also indicated that genetic diversity was narrow among the hybrids.

#### 4 Discussion

Maize breeders have paid great attention to the genetic diversity of hybrids. It has helped them make decisions on breeding objectives and strategy, improve the quality and maximize use of genetic resources through studies of changes in genetic diversity of current hybrids. Most of the previous studies indicated that the narrow genetic diversity in maize is a ubiquitous problem (Wu, 1983; Wang et al., 1986; Zeng, 1990; Yang and Ma, 1994;

Huang and Li, 2001; Li et al., 2000; Yuan et al., 2000; Fan et al., 2003; Fan et al., 2004). Our research showed that the variation coefficients of all traits changed in a narrow range, the genetic similarities of SSR marker pattern among the 186 hybrids showed an average of 0.7722 and there were 96.9% of genetic similarities from 0.70000 to 0.9256. Meanwhile, the analysis of pedigree sources of 51 hybrids showed that 70.58% hybrids had close genetic relationships with hybrids developed by the Pioneer Company in the late 1980s and early 1990s in the US. The genetic similarities became higher and maize breeding depended on few inbred lines and genetic germplasm, in contrast to what was discovered in previous studies (Jammes et al., 2002; Liu et al., 2003; Forrest, 2004; Xia et al., 2004; Nei et al., 2005; Liu et al., 2005). Although breeders have made great progress in the improvement of maize genetic germplasm, the problem of narrow genetic diversity in maize breeding still exists in China.

The result of cluster analyses based on SSR data had no relationship to the geographical origin of hybrids. The hybrids from different breeding units in different areas were also similar to each other. Meanwhile, the hybrids from the same breeding units were more easily contained in a cluster, which may have relationships to the hybrids depending on the similarity of the material and the standard of breeding process. The analyses of pedigree indicated that the hybrids from the same background were more easily contained in one cluster. For instance, the parents of Chuandan 13 and Nongda108 both come from America hybrid 78599, and the parents of Liaodan27 and Fuyou1 were both from Dan598. The patterns of cluster analyses based on SSR data were



**Fig. 1** Dendrogram of cluster analysis of 186 hybrids based on SSR data

Note: The order of the number on the right of the figure is from left to right, then from up to down, and each branch notes a hybrid.

**Table 4** Pedigrees of 51 hybrids

No.	name	pedigree	remark
1	Chuandan13	478 × 18 – 599	478 from 8112 × 5003; 18-599 from America hybrid 78599
2	Nongda108	HuanghC × 178	HuangC from the synthetic cultivar of Yugoslavia germplasm, Huang162,Zi330 and tropic Population Mobei-1; 178 from America hybrid 78599
6	CY4261	C42 × C16	C42 from (18-599 × America lang Ear maize)/18-599; C16 from Germen hybrid
13	DT6327	Shen141 × Cheng613	Cheng613 from Germen hybrid
16	GM4103	Y731 × 095	Y731 from America hybrid; 095 from Landrace Beibei Huangmaya
19	JG03-5	18-599 × JG02-15	JG02-15 from inbred 1572
21	JG04-1	R08 × JG02-18	R08 from America hybrid 78641
22	JG04-2	JG02-1 × JG02-2	JG02-1 from Germen hybrid KX5461; JG02-2 from Tie7922/(Shen5003/Liaolun753) × Dan9195
30	SAU03-1	R08 × SCML202	SCML202 from tropic germplasm
33	SAUQ58	ES40 × 698-3	ES40 from Landrace of Sichuan Province; 698-3 from America hybrid 78698
36	SC907	LSC107 × LSC99-1	LSC107 from 87-1 × 86-1, 87-1 from America hybrid 78599; LSC99-1 from 7916 × Mobei961
40	SL99018	65232 × L902	
50	ZB-28	chengzi6982 × chengzi108	Chengzi6982 from America hybrid 78698 Chengzi108 from America maize 6JK108
51	ZB-78	cheng698-3 × Jinhuang96C-2	Cheng698-3 from America hybrid 78696; Jinhuang96C-2 from “Zhongzong” population
54	ZH311	K236 × 21-ES	K236 from America maize 3163; 21-Es from Landrace of Sichuan Province
59	Chang04-3	Chang04 × Y3	Chang04 from 9313
65	Dong6007	H811 × Dan340	Dan340 from radialization treatment of Baiguli9 × wild pod-corn
70	Haihe2	LS02 × LS06	LS02 from Landrace of Sichuan Province 78599; LS06 from wild pod-corn
82	Liaodan121	Liao2361 × Dan598	Liao2361 from Liao5114 × Dan9046, Dan9046 from Shen5003 × Tie7922
92	Mian778	P953 × 18-599	P953 from Pin95-3
95	Nan477	273 × Nan381	273 from overseas; Nan381 from Landrace d351
100	Wandan14	286-5 × 75-1	286-5 from 21-3 × 81565, 21-3 from Yugoslavia hybrid BC8241Ht; 75-1 from 77 × Wu151
108	Liyu10	618 × HuangC	618 from America hybrid 78575 × Landrace Qiongyahuang
112	ZY635	Z0682-6 × 18-599	Z0682-6 from 65232 × (3382 × Y7865)
118	Danyu22	Dan1324 × Dan337	Dan1324 from NN14BHt2 × Mo17
119	Danke2151	Dan717 × Dan598	Dan717 from Danhuang19 × Danhuang17; Dan598 from the synthetic cultivar of Ho43, Dan340, Danhuang02, Danhuang11 and 78599
120	Dongdan11	LD143 × LD61	LD143 from 5003 × 2-83; LD61 from Dan340
121	Dongdan12	LD808*LD81 × LD61	LD61 from Dan340
123	Fuyou1	C8065-2 × Dan598	C8065-2 from 7922 × 5003
124	Huadan208	Hai98-18 × 96Hong	Hai 98-18 from variation of Dan598
125	Huaxuan6	G931-122 × 78599-141	G931-122 from K12 × 138e, K12 from Huangzaosi × Weichun
128	Jinyu1	96008 × 96002	96008 from Huangzaosi × Dan340, 96002 from “Pinzong-1” population
129	Jindan42	Zhongmiao928 × Zhongmiao929	Zhongmiao928 from America hybrid 78599 × 78638; Zhongmiao929 from K12 × 196. K12 from Huangzaosi
130	Liaodan27	2309 × Dan340	Dan340 from Baiguli9 × wild pod-corn
131	Qianbei2	X986 × X858	X986 from synthetic cultivar of 78607,78599 and 78601; X858 from the variation of Huang-C
135	Qiangsheng17	928 × 929	
136	Yuyu25	Zhen653 × BT1	Zhen653 from 5003 × Zhong31, 5003 from America hybrid 3147; BT1 from 8058(S3) × Thailand maize
138	Zhongjin368	112 × 036	
139	Zhuhuang1	S37 × SL97-6	SL97-6 from tropic germplasm TI5604
140	2861	Wan286-4 × Yu561	Wan286-4 from Nan21-3 × 81565; Yu561 from Nongda202 × 095
141	961 × 168	961 × 168	961 from America hybrid 78575; 168 from (Chang72 × 332) × Huang-C
153	DH3686	DH41 × Shen137	Shen137 from America hybrid 6JK111
155	DH3838	DH08 × line-8	DH08 from 8112 × 65232.
158	ND236	W499 × W89	W499 from America hybrid; W89 from 31 × Zhong31
166	X1152A	PH6WC × PH5AD	Both PH6WC and PH5AD from Pioneer Seed Company of America
169	Dong4243	A801 × LD61	A801 from Dan9042 × (Dan9046 × Mohuang-9)
175	Sannong20-A	143-2 × B815	143-2 from 1141 × 78599; B815 from variation of Nongxi531
179	Yudan11	549 × 51	549 from 78698 × 5005, 5005 America hybrid 8147; 51 from Jiaomaerhuangzao Landrace of Guizhou Province
182	Zhongdan808	CL11 × NG5	CL11 from 78599; NG5 from America hybrid 95236 × 95167
183	SAU03-2	SCML202 × Huangjin96B	Jinhuang96B from “Zhongzhong” population
186	Jinyu9856	PS098 × PS056	PS098 from K22 × 78698, K22 from K11 × 478; PS056 from 78599 × Dan341

essentially consistent with the known pedigrees. Therefore, some hybrids from the same background cannot be contained in a cluster, which may result in the obvious dissimilarity to another parent of the hybrid.

The American Association of Seed Trade has been investigating the genetic diversity of maize nine times every five years since 1956 to supervise maize breeding (James et al., 2002). Although there are more than 1000 hybrids tested in regional trials in China every year, only 186 hybrids in the regional trials in Sichuan and Southwest China were tested in the present study. Further research is needed to study the genetic diversity of maize hybrids tested in other regional trials. At the same time, the DNA fingerprint of maize hybrids should be constructed.

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