Evaluation on the germplasm of maize (*Zea mays* L.) landraces from southwest China

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ABSTRACT. Because of their local adaptation and economic factors that limit the adoption of commercial hybrids, farmer-saved maize landraces are still grown over a considerable area concentrated in southwest China. To evaluate the potential of using maize landraces, the germplasm characteristics of 96 landraces from southwest China were evaluated at phenotypic, cellular, and molecular levels. The existence of high phenotypic variation and elite germplasm tolerant to low-N, low-P, as well as drought stress was observed. Of the total landraces, 81.25, 7.29, 5.21, and 2.08% were found with 0, 1, 2, 3, and 4 B chromosomes. Using 42 microsatellite (simple sequence repeat) loci, 246 alleles were detected among the landraces. The number of alleles per SSR locus varied from 2 to 10, averaging 5.67 allele per locus, which revealed a high level of genetic diversity of maize landraces in southwest China. Cluster analysis showed that 96 landraces could distinctly be clustered into four groups, which tended to associate with their geographic origins. We propose that the genetic diversity center of maize landraces in southwest China might be in Sichuan. A sharp genetic deviation from Hardy-Weinberg equilibrium was observed

from heterozygosity deficiency and a considerable genetic variation was revealed within, rather than among, the landraces. Based on their germplasm characteristics, the innovation and utilization of maize landraces in southwestern China for theoretical and applied research could be achieved by constructing heterosis groups, developing inbred lines with high combining ability, and maintaining the landraces with elite germplasm and B chromosomes using bulked pollen.

Key words: *Zea mays*; Germplasm characteristics; Maize landraces; Agronomic traits; B chromosome; SSR

INTRODUCTION

Maize (*Zea mays* L.) was discovered by Europeans explorers in 1492 and is grown in almost all the countries of the world today. The total production of maize grown over 135 million hectares is more than 575 million megagrams (Lana et al., 2015). The global corn production in the last century has increased many folds, with a significant reduction in hectarage. This dramatic increase has been due to the combined genetic improvements in hybrids as well as in husbandry and management practices. Under rain-fed conditions, genetic gains have accounted for 50% of the total increase in yield achieved at the farm level (Nolan and Santos, 2012). If these yield improvements are to continue, the germplasm base of corn must be maintained and expanded. However, hybrids in each era tend to concentrate on a few inbred lines and their derivatives (Nelson et al., 2016; Qin et al., 2016). Surveys conducted in the United States of the late 1970s and mid 1980s on maize inbred lines showed that pedigrees of most of the hybrids are deriver of 6-8 inbred lines (Laude and Carena, 2015). In China, the parenthood of 91.6% hybrids consists of about 20 elite inbred lines (Liu et al., 2015a). It is essential that the maize germplasm base is enhanced to prevent the loss of genetic diversity. Since its spread to the southwest regions of China, maize has widely been cultivated for almost 500 years (Zhai et al., 2016). Because of their local adaptation and economic factors that limit the adoption of commercial hybrids, farmer-saved maize landraces are still grown on a considerable area concentrated in Sichuan, Chongqing, Guizhou, and Yunnan Provinces (Yao et al., 2015; Liu et al., 2015b). According to a field survey conducted recently, these original landraces, which have long been under abiotic stress, such as nitrogen (N), phosphorus (P), and water deficiency in soil, are sources of plants with resistance to pathogens, insect pests, and other stresses, as well as of those with improved yield and quality traits (Fang et al., 2011; Yao et al., 2014). For maize breeding, systematic characterization and evaluation of this germplasm is needed to uncover its potential uses. However, as of date this remains scarcely explored. In this paper, we describe the germplasm characteristics of maize landraces from southwest China at phenotypic, cellular, and molecular levels based on field experiment, cytological observation, and microsatellite (simple sequence repeat, SSR) analysis, and thus, reveal its breeding potential.

MATERIAL AND METHODS

Plant materials

The plant materials used in this study consisted of 96 maize landraces, including 32,

33, 15, and 16 from Chongqing, Sichuan, Yunnan, and Guizhou Provinces in southwestern China, respectively (Figure 1). The origins and germplasm characteristics of these landraces are presented in Table 1.

Figure 1. Geographical distribution of the 96 landraces sampled from Southwest China.

Field trials

A field experiment was performed in the plant garden of the Life Science Department of Yangtze Normal University at Fuling, Chongqing City, China. The study was designed as a randomized complete block design with three replications and a plant density of 48,000 plants/ha. Each landrace was planted in a single row of 20 plants, and a total of 5200 plants covered an area of 0.11 ha. During the growth of plants, 16 representative individuals were sampled at corresponding growth stages to determine 15 agronomic and economic traits. These included plant height (cm), ear height (cm), days to silking (days), days to pollination (days), growth period (days), ear length (cm), sterile length (cm), ear diameter (cm), axis diameter (cm), rows per ear, kernels per row, ear weight (g), kernel weight per ear (g), fresh ear 100-kernel weight (g), and test weight (g/dm^3).

Cytological identification of B chromosomes (Bs) in maize landraces

Mitotic preparations were obtained from root tips of germinating seeds. After actively growing root tips from thirty individual seedlings of each landrace were pretreated with 0.1% α-bromonaphthalene at 20°C for 3 h, they were fixed in Carnoy I (glacial acetic acid:absolute ethanol = 1:3) at 4°C for 30 min. Thereafter, they were macerated in a 1:1 mixture of 45% acetic acid and 1 M hydrochloric acid at 60°C for 2 min and squashed in 1% aceto-orcein. Thirty cells from 30 root tips were randomly observed under a microscope to test for the Bs (Kao et al., 2015). If Bs were not identified in 30 cells of a landrace, it was defined as landrace 0Bs, and landraces 1B, 2B, 3B, and 4B had 1, 2, 3, and 4 Bs in a single cell (Carlson, 2008).

Table 1. Sources and germplasm characteristics of 96 maize landraces in southwestern China.

No.	Origin (county, province)	Description of morphological characteristics	Stress tolerance characteristics
1	Wuxi, Chongqing	Intermediate maturing, yellow flint grain type with short stalk	RN, SP, RD
2	Wuxi, Chongqing	Late maturing, white flint grain type with big kernel	SN, SP, SD
3	Fengjie, Chongqing	Late maturing, yellow flint grain type with long stalk	SN, SP, SD
4	Fengjie, Chongqing	Intermediate maturing, yellow semi-dent grain type with big ear	SN, RP, SD
5	Fengjie, Chongqing	Late maturing, white semi-dent grain type with long stalk	SN, SP, SD
6	Wushan, Chongqing	Intermediate maturing, white dent grain type with big ear	SN, RP, SD
7	Wushan, Chongqing	Late maturing, white semi-dent grain type with long ear	RN, SP, RD
8	Wushan, Chongqing	Early maturing, white flint grain type with short stalk	RN, RP, SD
9	Wushan, Chongqing	Late maturing, yellow flint grain type with long stalk	SN, SP, SD
10	Yunyang, Chongqing	Intermediate maturing, white flint grain type with big kernel	SN, SP, SD
11	Yunyang, Chongqing	Late maturing, white flint grain type with big ear	SN, SP, SD
12	Yunyang, Chongqing	Intermediate maturing, yellow semi-dent grain type	SN, SP, SD
13	Yunyang, Chongqing	Late maturing, white semi-dent grain type with long stalk	RN, SP, SD
14	Yunyang, Chongqing	Intermediate maturing, white semi-dent grain type with big ear	SN, RP, SD
15	Fengdu, Chongqing	Late maturing, yellow semi-dent grain type with long ear	SN, SP, SD
16	Fengdu, Chongqing	Early maturing, white flint grain type with short stalk	RN, RP, RD
17	Fengdu, Chongqing	Intermediate maturing, yellow flint grain type with long ear	SN, RP, SD
18	Fengdu, Chongqing	Late maturing, purple flint grain type with big kernel	SN, SP, RD
19	Fengdu, Chongqing	Late maturing, white flint grain type with big ear	SN, SP, SD
20	Fengdu, Chongqing	Early maturing, variegated semi-dent grain type with short stalk	RN, RP, RD
21	Fengdu, Chongqing	Late maturing, white semi-dent grain type with big ear	SN, SP, SD
22	Fengdu, Chongqing	Intermediate maturing, white semi-dent grain type with long stalk	SN, RP, RD
23	Fuling, Chongqing	Late maturing, yellow dent grain type with long ear	SN, SP, RD
24	Fuling, Chongqing	Intermediate maturing, yellow flint grain type with short stalk	RN, SP, SD
25	Fuling, Chongqing	Late maturing, yellow flint grain type with long stalk	SN, SP, RD
26	Fuling, Chongqing	Intermediate maturing, purple flint grain type with big kernel	SN, SP, RD
27	Shizhu, Chongqing	Late maturing, blue semi-dent grain type with long ear	SN, SP, SD
28	Shizhu, Chongqing	Intermediate maturing, white semi-dent grain type	SN, SP, SD
29	Xiushan, Chongqing	Late maturing, yellow semi-dent grain type with big ear	SN, SP, SD
30	Xiushan, Chongqing	Intermediate maturing, white semi-dent grain type with long ear	SN, SP, SD
31	Pengshiu, Chongqing	Late maturing, blue dent grain type with long ear	SN, SP, SD
32	Youyang, Chongqing	Intermediate maturing, white flint grain type with short stalk	SN, SP, SD
33	Hanyuan, Sichuan	Early maturing, white dent grain type with long ear	SN, SP, SD
34	Hanyuan, Sichuan	Late maturing, yellow semi-dent grain type with long ear	RN, SP, SD
35	Hanyuan, Sichuan	Late maturing, yellow flint grain type with long stalk	SN, RP, SD
36	Hanyuan, Sichuan	Early maturing, yellow flint grain type with short stalk	SN, SP, RD
37	Hanyuan, Sichuan	Intermediate maturing, variegated flint grain type with big ear	RN, SP, SD
38	Hanyuan, Sichuan	Early maturing, yellow semi-dent grain type with short stalk	RN, SP, SD
39	Xiaojin, Sichuan	Late maturing, white dent grain type with long stalk	SN, RP, SD
40	Xiaojin, Sichuan	Intermediate maturing, white flint grain type with long ear	SN, SP, RD
41	Leimaping, Sichuan	Intermediate maturing, yellow flint grain type with long stalk	RN, SP, SD
42	Leimaping, Sichuan	Late maturing, blue flint grain type with long ear	RN, SP, SD
43	Leimaping, Sichuan	Intermediate maturing, yellow flint grain type with short stalk	SN, SP, RD
44	Leimaping, Sichuan	Intermediate maturing, white semi-dent grain type	SN, SP, SD
45	Wenchuan, Sichuan	Late maturing, yellow flint grain type with long stalk	SN, RP, SD
46	Wenchuan, Sichuan	Early maturing, white flint grain type with short stalk	RN, SP, SD
47	Danba, Sichuan	Late maturing, white flint grain type with big ear	SN, SP, SD
48	Danba, Sichuan	Early maturing, yellow semi-dent grain type with short stalk	SN, RP, SD
49	Leibo, Sichuan	Intermediate maturing, purple dent grain type with short stalk	RN, SP, SD
50	Leibo, Sichuan	Late maturing, yellow flint grain type with long stalk	SN, SP, SD
51	Linshui, Sichuan	Intermediate maturing, yellow semi-dent grain type with long ear	SN, SP, SD
52	Dazhu, Sichuan	Intermediate maturing, white flint grain type with big ear	SN, SP, RD
53	Nanchong, Sichuan	Intermediate maturing, yellow flint grain type with long stalk	SN, SP, SD
54	Yilong, Sichuan	Intermediate maturing, yellow flint grain type with long ear	SN, SP, SD
55	Gangyuan, Sichuan	Early maturing, white semi-dent grain type with big kernel	RN, SP, RD
56	Junlian, Sichuan	Intermediate maturing, white flint grain type with long stalk	SN, RP, RD
57	Junlian, Sichuan	Intermediate maturing, purple flint grain type	SN, RP, SD
58	Junlian, Sichuan	Late maturing, blue dent grain type with big kernel	RN, SP, SD
59	Junlian, Sichuan	Late maturing, white semi-dent grain type with big ear	SN, RP, SD

Continued on next page

Table 1. Continued.

No.	Origin (county, province)	Description of morphological characteristics	Stress tolerance characteristics
60	Pingshan, Sichuan	Early maturing, white flint grain type with short stalk	SN, RP, SD
61	Pingshan, Sichuan	Intermediate maturing, yellow flint grain type with big ear	SN, SP, RD
62	Pingshan, Sichuan	Late maturing, yellow flint grain type with long stalk	SN, SP, RD
63	Xuyong, Sichuan	Intermediate maturing, white flint grain type with long stalk	SN, RP, SD
64	Qingchen, Sichuan	Intermediate maturing, yellow semi-dent grain type	RN, RP, SD
65	Neijiang, Sichuan	Intermediate maturing, white semi-dent grain type	SN, SP, SD
66	Jiangchuan, Yunnan	Late maturing, yellow semi-dent grain type with big ear	SN, SP, SD
67	Jiangchuan, Yunnan	Intermediate maturing, white flint grain type with long stalk	SN, SP, SD
68	Jiangchuan, Yunnan	Early maturing, white semi-dent grain type with short stalk	SN, SP, RD
69	Jiangchuan, Yunnan	Late maturing, white semi-dent grain type with big ear	SN, RP, SD
70	Chengjiang, Yunnan	Late maturing, white semi-dent grain type with long ear	SN, RP, RD
71	Chengjiang, Yunnan	Late maturing, white waxy grain type with long stalk	RN, SP, SD
72	Chengjiang, Yunnan	Intermediate maturing, yellow dent grain type with long stalk	SN, SP, RD
73	Wuding, Yunnan	Late maturing, yellow dent grain type with long ear	SN, SP, SD
74	Wuding, Yunnan	Late maturing, yellow dent grain type with big kernel	SN, SP, SD
75	Wuding, Yunnan	Late maturing, purple dent grain type with big kernel	SN, SP, SD
76	Puer, Yunnan	Late maturing, white semi-dent grain type with long ear	RN, SP, SD
77	Puer, Yunnan	Late maturing, yellow semi-dent grain type with long ear	SN, SP, RD
78	Puer, Yunnan	Late maturing, white waxy grain type with long ear	SN, SP, RD
79	Yuxi, Yunnan	Intermediate maturing, yellow flint grain type with big kernel	SN, SP, RD
80	Yuxi, Yunnan	Intermediate maturing, yellow semi-dent grain type	SN, SP, RD
81	Ziyun, Guizhou	Early maturing, white dent grain type with short stalk	SN, SP, RD
82	Wengan, Guizhou	Intermediate maturing, yellow flint grain type with long stalk	RN, SP, SD
83	Wengan, Guizhou	Late maturing, white flint grain type with big ear	SN, RP, SD
84	Bijie, Guizhou	Intermediate maturing, white flint grain type with short stalk	RN, SP, RD
85	Bijie, Guizhou	Intermediate maturing, white flint grain type with big kernel	SN, SP, RD
86	Panxian, Guizhou	Intermediate maturing, yellow semi-dent grain type with short stalk	SN, SP, RD
87	Panxian, Guizhou	Early maturing, white semi-dent grain type with short stalk	RN, SP, SD
88	Panxian, Guizhou	Late maturing, yellow semi-dent grain type with long stalk	SN, SP, RD
89	Shiqian, Guizhou	Intermediate maturing, white flint grain type with big kernel	RN, SP, SD
90	Shiqian, Guizhou	Early maturing, white flint grain type with big kernel	SN, SP, SD
91	Tongren, Guizhou	Late maturing, white dent grain type with long stalk	SN, SP, RD
92	Tongren, Guizhou	Intermediate maturing, white dent grain type with short stalk	SN, SP, SD
93	Zunyi, Guizhou	Intermediate maturing, white flint grain type with big ear	RN, SP, SD
94	Zunyi, Guizhou	Late maturing, yellow flint grain type with big kernel	SN, SP, RD
95	Xingyi, Guizhou	Early maturing, white dent grain type with short stalk	SN, SP, SD
96	Xinren, Guizhou	Intermediate maturing, yellow semi-dent grain type with big ear	RN, SP, SD

RN = tolerant to low-N; SN = sensitive to low-N; RP = tolerant to low-P; SP = sensitive to low-P; RD = tolerant to drought; SD = sensitive to drought.

DNA isolation and microsatellite analysis

Genomic DNA was isolated from a bulk sample of 30 individual plants to analyze the genetic diversity of the 96 landraces. To decipher the genetic structure within landraces, five individual plants for each landrace were separately chosen to isolate genomic DNA of an individual sample. DNA samples from a total of 480 individuals were used.

PCR amplification of DNA was performed in a PTC-220 thermal cycler (Surplus Lab Inc., USA). The PCR mixture consisted of 1X buffer, 2.5 μM $MgCl_2$, 150 μM dNTPs, 0.2 μM each primer, 1 U Taq DNA polymerase, and 100 ng template DNA in a total volume of 15 μL. The reaction mixture was overlaid with 18 μL mineral oil after vortexing. The amplification protocol was as follows: 35 cycles of 1 min at 95°C, 2 min at 55°C, and 2 min at 72°C, followed by a 10-min extension at 72°C. After amplification, the mixture of DNA products was separated by electrophoresis on 6% denatured polyacrylamide gel at 75 watts and 50°C for 1 h and visualized by silver staining.

Data scoring and analysis of SSRs

After amplification using the primer sets, the presence or absence of SSR bands was scored as 1 or 0, respectively. The statistical methods and formulae used are described below (Yao et al., 2012; Fang et al., 2013).

1) The index of genetic similarity (GS): $GS = 2N_{ij}/(N_i + N_j)$, where N_{ij} is the number of SSR bands common to landraces i and j, whereas N_i and N_j are the total number of SSR bands for landraces i and j, respectively. A dendrogram was constructed by the unweighted pair-group method with arithmetic mean (UPGMA) clustering and principal coordinates analysis (PCA) was also used to reveal the genetic relationship among the landraces using software NTSYS-pc version 2.10 (Exeter Software, USA).

2) The observed heterozygosity (H_O): $H_O = \sum_{i=1}^{n} H_{oi}/n = \sum_{i=1}^{n}(1-\sum_{j=1}^{m} q_{ij}^2)/n$, where H_{oi} represents the observed heterozygosity of the *i*th allele, and q_{ij} is the frequency of the *j*th homozygous allele at the *i*th allele.

3) The index of gene diversity, also known as the expected heterozygosity (H_E) (Nei, 1973): $H_E = \sum_{i=1}^{n} H_i/n = \sum_{i=1}^{n}(1-\sum_{j=1}^{m} q_{ij}^2)/n$, where H_i refers to the expected heterozygosity of the *i*th allele, and q_{ij} is the frequency of the *j*th homozygous allele at the *i*th allele.

4) Polymorphic information content (PIC): $PIC = 1 - \sum_i P_i^2 - \sum_{i=1}\sum_{j=i+1} 2P_i^2 P_j^2$, where p_i and p_j are the frequencies of the *i*th and *j*th alleles.

5) Inbreeding coefficient (F): $F = 1 - H_o/H_E$, where F is 1 only if landraces are genetically heterozygous. F_{IT}, F_{IS}, and F_{ST} are Wright F-statistics parameters (Wright, 1978; Tao and Ren, 2004). F_{IT} and F_{IS} were defined as genetic deviation from Hardy-Weinberg equilibrium within and among the landraces, respectively. When F_{IT} and F_{IS} are 0, the landraces arrive at Hardy-Weinberg equilibrium. F_{ST} is an estimate of gene differentiation between the landraces, representing genetic variation among them.

H_O, H_E, PIC, F, F_{IT}, F_{IS}, and F_{ST} were computed by the POPGENE software (Yeh et al., 1997) to analyze the genetic structure of maize landraces using co-dominant markers.

RESULTS

Phenotypic characteristics of maize landraces

The maize landraces from the southwest of China exhibited a large phenotypic variation (Table 1). Their growth period varied from the early to late maturing and the grain types were composed of flint, semi-dent, and dent. The grain color trait was quite polymorphic, with the white and yellow type being predominant, accounting for 49.0 and 40.0%, respectively. The rarest grain colors were variegated (2.1%), followed by blue (4.2%) and purple (5.2%). Nineteen landraces with long stalks had higher plant heights and were sensitive to lodging. Based on a previous stress trial, 25, 21, and 31 landraces tolerant to low-N, low-P, and drought stress, respectively, were also identified.

The means, ranges, variances, and coefficients of variation of 15 agronomic and economic traits are listed in Table 2. The variances in these 15 traits in the maize landraces were significant at 0.01 level. The ranges in the agronomic traits, including plant height, ear height, total leaves, days to silking, days to flowering, and growth period were 119.60 cm, 77.23 cm, 7.57, 22.00 days, 23.00 days, and 30.00 days, respectively. For economic traits, namely ear length, sterile length, ear diameter, axis diameter, rows per ear, kernels per row, ear

weight, 100-kernel weight, and test weight, the ranges were 7.51 cm, 0.87 cm, 2.15 cm, 1.20 cm, 7.54, 12.55, 62.40 g, 12.07 g, and 305.56 g/dm^3, respectively. The rank of the traits based on coefficients of variation (CV) was sterile length, ear weight, 100-kernel weight, ear height, kernels per row, ear length, rows per ear, ear diameter, total leaves, days to silking, days to flowering, plant height, axis diameter, test weight, and growth period. The CVs of economic traits were higher than those of the agronomic traits.

Table 2. Variance analysis of traits in the 96 maize landraces.

Traits	Mean	Range	CV (%)	DF			MS			F-value	
				Block	Treatment	Error	Block	Treatment	Error	Block	Treatment
Plant height(cm)	273.64	119.60	6.35	2	95	95	4218.24	1526.31	136.85	315.47**	14.05**
Ear height(cm)	141.86	77.23	20.03	2	95	95	3153.87	1294.17	51.45	112.68**	4.58**
Total leaves	20.33	7.57	11.72	2	95	95	415.27	147.62	13.65	3.58	2.50**
Days to silking (days)	81.92	22.00	7.45	2	95	95	1578.42	546.21	46.35	117.31**	2.19**
Days to flowering (days)	81.38	23.00	6.84	2	95	95	1432.18	528.56	57.38	134.16**	3.86**
Growth period (days)	132.42	30.00	1.12	2	95	95	2965.30	988.91	92.21	326.35**	8.71**
Ear length (cm)	14.25	7.51	14.95	2	95	95	287.73	97.30	85.69	217.31**	3.36**
Sterile length (cm)	1.31	0.87	43.51	2	95	95	26.45	8.62	0.92	3.85	2.51**
Ear diameter (cm)	3.96	2.15	12.34	2	95	95	76.52	26.57	2.55	8.54**	2.82**
Axis diameter (cm)	2.17	1.20	4.91	2	95	95	53.75	19.14	1.97	4.16**	2.67**
Rows per ear	12.61	7.54	14.31	2	95	95	264.95	53.96	6.41	13.13	3.43**
Kermes per row	268.34	12.55	16.07	2	95	95	423.48	120.53	11.97	31.16**	4.27**
Ear weight (g)	87.39	62.40	32.36	2	95	95	1678.35	534.61	49.73	115.26**	8.20**
100-kernel weight (g)	23.98	12.07	21.43	2	95	95	523.18	185.29	17.28	39.27**	3.15**
Test weight (g/dm^3)	627.48	305.56	4.32	2	95	95	8478.51	2749.17	917.59	420.13**	14.93**

*,**Significant levels at $P < 0.05$ and 0.01, respectively.

Bs of maize landraces in Southwest China

Bs are found in more than 1800 species of plants and animals (Huang et al., 2016); variation in these chromosomes is a part of characterization of germplasm and biological diversity. Based on cytological observations, Bs were identified in maize landraces from Southwest China. The number of Bs in a cell ranged from 0 to 4 (Figure 2). Of the 96 landraces, 81.25, 7.29, 5.21, and 2.08% were found to have 0, 1, 2, 3, and 4 Bs. With regard to the geographic origin of the B chromosomes, the landraces collected from Sichuan had 1, 2, 3, and 4 B chromosomes, respectively. One and 2 Bs existed in the landraces from Yunnan and Guizhou, respectively. We found that Bs were distributed in the landraces from three regions, the exception being the landraces from Chongqing.

Genetic diversity among maize landraces

Using 42 SSR loci across 10 chromosomes of maize, a total of 246 alleles were detected in the bulked DNA samples from ninety-six landraces. The number of alleles per SSR locus varied from 2 to 10 with an average of 5.67 per locus, which, on the whole, revealed a high level of genetic diversity of the maize landraces in southwestern China. A total of 75 unique alleles were detected, of which 26, 23, 15, and 11 alleles were detected in the maize landraces from Sichuan, Chongqing, Yunnan, and Guizhou, respectively. Cluster analysis based on the matrix of genetic similarities with the UPGMA clustering algorithm showed that the 96 landraces could distinctly be clustered into four clusters when the genetic similarity was 0.60 (Figure 3). The biggest group (Cluster III) consisted of 18, 7, 13, and 5 landraces from Sichuan, Chongqing, Guizhou, and Yunnan, respectively.

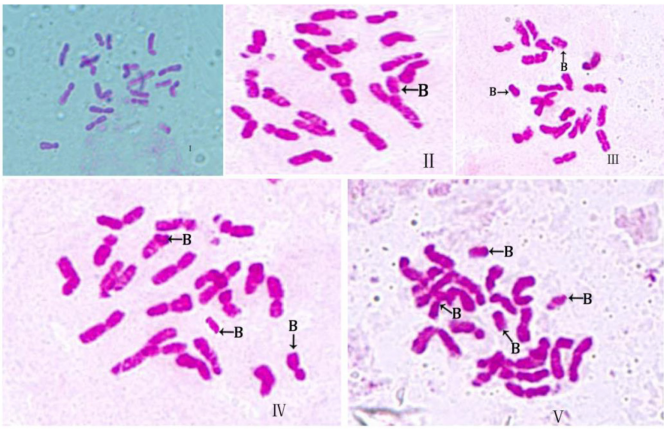

Figure 2. Chromosomes in four maize landraces from Southwest China. I: maize landrace from Youyang, Chongqing has no B chromosome (2n = 20), II: maize landrace from Puer, Yunnan has one B chromosome (2n = 21), III: maize landrace from Yuxi, Yunnan has two B chromosomes (2n = 22), IV: maize landrace from Dazhu, Sichuan has three B chromosomes (2n = 23), V: maize landrace from Gangyuan, Sichuan has four B chromosomes (2n = 24).

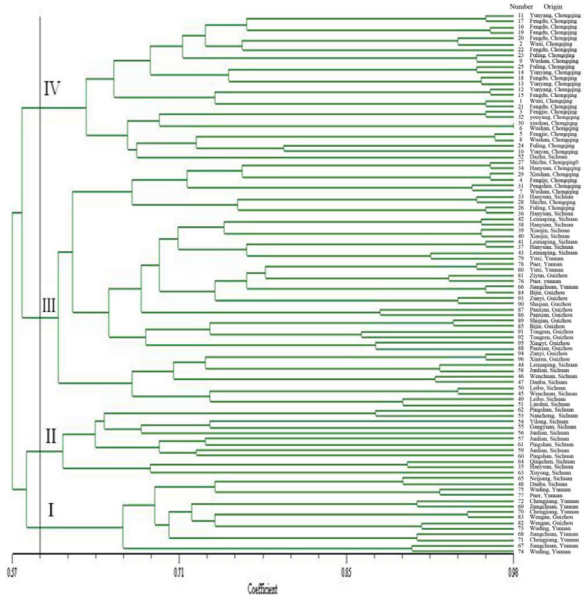

Figure 3. Dendrogram of 96 landraces constructed from SSR marker-based genetic similarity.

Cluster IV included 25 landraces from Chongqing and one from Sichuan. Twelve landraces from Sichuan were clustered in Cluster II. Cluster I encompassed 14 landraces, of which, 2, 10, and 2 were from Sichuan, Yunnan, and Guizhou, respectively. A comparison of the landraces with the same geographic origin showed that most landraces were clustered together and had similarity coefficients of over 0.65. It was obvious that the maize landraces from Southwest China tended to associate with their geographic origins, and each of the clusters included maize landrace from Sichuan.

To establish the genetic relationships among the 96 maize landraces, principal component analysis was performed based on the SSR data. The first three principal components explained 87.65, 4.35, and 0.76% of the genetic variation, respectively. The PCA plot (Figure 4) analysis revealed that all the landraces could be separated into four clusters according to the nearest genetic relationship. Cluster I, II, and IV included 12, 9, and 23 landraces. The biggest cluster (Cluster III) contained 52 landraces from the four regions in southwestern China. The results of PCA were consistent with those of the cluster analysis. Further analysis of PCA showed that most of the maize landraces from Sichuan were in the central region of the plot.

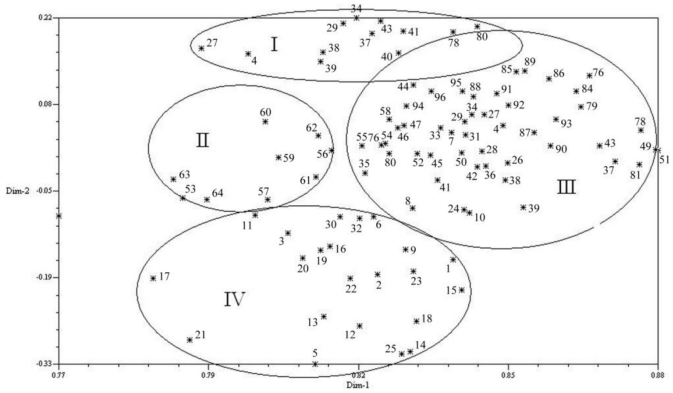

Figure 4. PCA plot of the first two principal components from the principal component analysis (PCA) based on SSR data. Numbers at each dot corresponding to those displayed in Table 1.

Genetic variation of SSR loci within the maize landraces

The information on the genetic variability at SSR loci is summarized in Table 3. A total of 256 alleles were detected at the 42 SSR loci. A large variation was observed at the SSR loci. The number of alleles 'A' ranged from 3 to 8 with an average of 6.10. Compared to the bulked DNA samples, the alleles detected in individual DNA samples were higher at the same 42 loci. The average 'H_E' was 0.69, ranging from 0.57 to 0.81. 'H_O' exhibited a small range in variation from 0.25 to 0.65 with a mean of 0.38. The highest and lowest PIC values were 0.71 (for nc130) and 0.41 (for phio46), respectively, with an average value of 0.52.

Genetic structure of maize landraces

To analyze the genetic structure of maize landraces, a summary of Wright's F-statistics (Wright, 1978) including estimates of heterozygosity deficiency within population (F_{IS}), between populations (F_{IT}), and fixation index (F_{ST}) is presented in Table 3. *F* varied from 0.08 (phi299852) to 0.61 (umc1447), which indicated that the landraces had a typical mixed-mating system and were deficient in heterozygotes. F_{IS} averaged 0.47 ranging from 0.18 (phi051) to 0.75 (umc1545) whereas the average F_{IT} was 0.50, varying from 0.23 (bnlg1023) to 0.78 (umc1545). This suggested that a sharp genetic deviation from Hardy-Weinberg equilibrium occurred both among and within the landraces. The overall F_{ST} range was from 0.03 for phi034 to 0.11 for phi069 with a mean of 0.07, implying that the among-landrace and within-landrace genetic variation was 7 and 93%, respectively.

Table 3. Genetic variation of SSR loci and landrace genetic structure parameters.

Locus	Chromosome	N_A	H_E	H_O	PIC	F_{IS}	F_{IT}	F_{ST}	F
bnlg1429	1.02	4	0.62	0.33	0.59	0.51	0.48	0.04	0.47
bnlg1023	1.06	8	0.81	0.45	0.63	0.21	0.23	0.09	0.44
bnlg1617	6.05	4	0.68	0.56	0.57	0.26	0.31	0.08	0.18
nc130	5.00	6	0.80	0.65	0.71	0.39	0.43	0.07	0.19
nc133	2.05	8	0.71	0.36	0.58	0.65	0.68	0.09	0.49
phi008	5.03	6	0.64	0.25	0.49	0.55	0.57	0.07	0.61
phi014	8.04	5	0.76	0.47	0.62	0.56	0.59	0.05	0.38
phi029	3.04	7	0.65	0.36	0.51	0.42	0.46	0.07	0.45
phi031	6.04	6	0.70	0.42	0.54	0.51	0.53	0.07	0.40
phi034	7.02	5	0.76	0.38	0.60	0.50	0.52	0.03	0.50
Phi041	10.00	5	0.67	0.32	0.55	0.57	0.63	0.09	0.52
phi046	3.08	3	0.64	0.27	0.41	0.60	0.64	0.08	0.58
phi051	7.05	6	0.73	0.35	0.45	0.18	0.25	0.09	0.52
phi057	7.01	7	0.62	0.34	0.42	0.37	0.43	0.07	0.45
phi062	10.04	6	0.67	0.45	0.60	0.47	0.47	0.06	0.33
phi065	9.03	5	0.72	0.30	0.55	0.60	0.61	0.06	0.58
phi069	7.05	7	0.70	0.44	0.61	0.53	0.60	0.11	0.37
phi072	4.00	7	0.67	0.49	0.52	0.46	0.49	0.06	0.27
phi075	6.00	5	0.73	0.35	0.48	0.44	0.45	0.07	0.52
phi076	4.11	7	0.68	0.45	0.50	0.59	0.62	0.07	0.34
phi083	2.04	6	0.67	0.46	0.52	0.52	0.54	0.06	0.31
phi090	2.08	6	0.68	0.40	0.47	0.37	0.39	0.05	0.41
phi092	4.08	7	0.71	0.35	0.46	0.36	0.41	0.07	0.51
phi096	4.04	8	0.66	0.50	0.59	0.49	0.54	0.09	0.24
phi115	8.03	6	0.70	0.42	0.57	0.57	0.61	0.09	0.40
phi127	2.08	7	0.69	0.37	0.53	0.44	0.49	0.09	0.46
phi102228	3.06	6	0.67	0.42	0.54	0.53	0.57	0.08	0.37
phi108411	9.05	6	0.78	0.40	0.56	0.61	0.63	0.07	0.49
phi109188	5.03	4	0.57	0.25	0.39	0.27	0.33	0.08	0.56
phi299852	6.07	7	0.63	0.58	0.60	0.39	0.41	0.07	0.08
phi308707	1.10	6	0.69	0.30	0.47	0.21	0.26	0.06	0.57
umc1161	8.06	6	0.65	0.29	0.45	0.50	0.53	0.08	0.55
umc1297	9.00	7	0.75	0.34	0.50	0.61	0.62	0.07	0.55
umc1304	8.02	5	0.67	0.34	0.48	0.51	0.52	0.07	0.49
umc1332	5.04	7	0.59	0.29	0.42	0.37	0.40	0.06	0.51
umc1432	10.02	6	0.71	0.32	0.51	0.48	0.52	0.07	0.55
umc1447	5.03	7	0.72	0.28	0.46	0.36	0.42	0.09	0.61
umc1501	3.05	7	0.71	0.46	0.57	0.52	0.54	0.06	0.35
umc1545	7.00	6	0.69	0.29	0.47	0.75	0.78	0.07	0.58
umc1719	1.04	7	0.73	0.39	0.52	0.43	0.45	0.03	0.47
umc1877	10.07	7	0.72	0.38	0.50	0.58	0.63	0.08	0.47
umc2359	9.07	5	0.67	0.28	0.43	0.39	0.42	0.07	0.58
Mean		6.10	0.69	0.38	0.52	0.47	0.50	0.07	0.45

N_A = mean number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; PIC = polymorphic information content; F_{IT} = genetic deviation from Hardy-Weinberg equilibrium within landraces; F_{IS} = genetic deviation from Hardy-Weinberg equilibrium among landraces; F_{ST} = estimate of gene differentiation between landraces, representing genetic variation among the landraces; F = inbreeding coefficient.

DISCUSSION

Genetic diversity and structure of maize landraces

Abundant germplasm plays a crucial role for future breeding progress (Disasa et al., 2016). The evaluation on the basis of the germplasm characteristics is a key step in maize breeding (Wu et al., 2016). In this study, we estimated the genetic diversity in maize landraces from Southwest China and looked for innovative ways of their utilization. Among the 96 maize landraces, 5.67 alleles per locus were detected using primers for 42 SSR loci. The total

number of alleles was higher than the numbers previously reported by Sharma et al. (2010) and Ignjatovic-Micic et al. (2015). This difference could probably be attributed to the number of landraces and diversity of germplasm used in the present study. The estimates of H_O, H_E, PIC, F, F_{IT}, F_{IS}, and F_{ST} were in agreement with the results obtained by Yao et al. (2007), who used 54 landraces from Southwest China and the same SSR primers. In general, landraces showed a relatively high level of genetic diversity (N_A = 6.10, H_O = 0.69, H_E = 0.38, and PIC = 0.52). The F_{IS} is a measure of the extent of inbreeding within subpopulations and the mean reduction in heterozygosity of an individual due to non-random mating within a subpopulation (Wright, 1978). A positive inbreeding coefficient was observed across loci (F_{IS} = 0.47), indicating the shortage of heterozygotes. The reason for the shortage of heterozygotes could be non-random mating within a landrace. The fixation index F_{ST} is the mean reduction in heterozygosity of a subpopulation due to genetic drift among subpopulations and a measure of the extent of genetic differentiation among subpopulations (Wright, 1978). As expected for an out-crossing species, a low overall differentiation among the maize landraces was observed (F_{ST} = 0.07) and a considerable genetic diversity was revealed within, rather than among the landraces. A low differentiation among landraces means that most of the genetic variation is maintained within a landrace rather than among the landraces, probably due to more admixtures between or among the landraces. The fixation index F_{IT} is the mean reduction in heterozygosity of an individual relative to the total population based on contributions from non-random mating within demes F_{IS} and effects of random drift among demes F_{ST} (Wright, 1978; Arteaga et al., 2016). A positive fixation index was estimated across all the loci (F_{IT} = 0.50), implying heterozygosity deficiency.

The cluster results showed that all the maize landraces could be classified into four groups. It was obvious that maize landraces in Southwest China tended to associate with their geographic origins, and each of the clusters included maize landrace from Sichuan. The PCA showed that most of the maize landraces from Sichuan were in the central region of the plot. The results supported our previous study that the genetic diversity center of maize landrace in Southwest China may be in Sichuan and the maize landrace in Southwest China was first introduced to Sichuan from India via Tibet (Yao et al., 2015).

Breeding potential and utilization of maize landraces

Knowledge of the amount and the distribution of genetic variation within and among the maize landraces will provide a guide for predicting the degree of inheritance, variation, and level of heterosis, which are essential for maize breeding (Laude and Carena, 2015; Nankar et al., 2016). Maize landraces in southwestern China exhibited a large variation at phenotypic, cellular, and molecular levels. High variation in the phenotypes was reflected by wide ranges for most of the morphological traits, such as grain color, plant height, and ear weight. Higher number of alleles per locus and PIC suggested a broad genetic base of the maize landraces at molecular level. Elite germplasm was also identified. Among them, 21, 19, and 20 landraces tolerant to low-N, low-P, and drought stress, respectively, were chosen from the materials studied. Additionally, 81.25, 7.29, 5.21, and 2.08% of the total landraces were found with 0, 1, 2, 3, and 4 Bs, respectively. However, most of the landraces exhibited short plant height and low resistance to lodging. In this study, the observed and expected heterozygosities showed obvious deviations from Hardy-Weinberg expectation that resulted from heterozygote deficiency. The within-landrace genetic variation (93%) was higher than

the among-landrace variation (7%). These results were expected because farmers tend to plant maize landraces with seeds from a small number of ears, which could have led to the deficit of heterozygous individuals. As the ear size is noticeably affected by human selection, it is possible that farmers could unintentionally select ears from the most heterozygous plants. This means that a high level of genetic diversity within landraces is maintained and genetic drift among the landraces is prevented. Overall, the landrace germplasm has a huge potential for use in genetic improvement programs for development of novel maize hybrids. However, for maize breeding, it is difficult in direct utilization of the landrace germplasm (Meseka et al., 2015; Yao et al., 2015). Based on their germplasm characteristics, the innovation and utilization of maize landraces in southwestern China would be to 1) construct the heterosis group using the landraces with high genetic variations; 2) develop inbred lines with high combining ability by recurrent selection between the landrace and tropic maize germplasm; and 3) maintain the landraces with elite germplasm and Bs, using bulked pollen both for theoretical and applied research.

Conflicts of interest

The authors declare no conflict of interest.

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