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Full Length Article

Genetic Diversity and Genetic Structure Analysis of Maize (*Zea mays*) Landraces in Tibet

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Abstract

China is one of gigantic sources of abundant maize germplasm, however, there hasn't systematic analysis on the genetic diversity of maize landraces in Tibet so far. In this research, 40 pairs of polymorphic SSR primers were used to detect the diversity of collected maize landraces in Tibet. According to the genetic distance, 69 maize landraces were divided into four groups, this was consistent with the phenotypic identification results of cluster analysis, and there exists inherent link between phenotypic characteristics and genetic background of maize landraces. By using single DNA sampling strategy, a total of 1050 individual samples were used to analyze the genetic diversity of maize landraces. The result showed that 551 alleles were detected in 35 varieties, the average number of alleles per locus was 13.78, in which 4.64 alleles were effective, showing the abundant genetic diversity of maize landrace in Tibet of China. According to the analysis results of population genetic structure, most landraces in Tibet deviated from Hardy-Weinberg balance. The comprehensive analysis of the results showed that the genetic distance of maize landraces from Markam was closed to that of Zogang, and taking this as the center, the maize belts located in the Nujiang River Valley and the lower Lancang River valley, contained the most abundant genetic variation in China's Tibet.

Keywords: Tibet; Maize landraces; Genetic diversity; Genetic structure; SSR

Introduction

Genetic diversity analysis is the basis for maize germplasm resources utilization and protection. Genetic study on germplasm diversity and genetic structure, has important theoretical significance to broaden the genetic basis of maize and achieve efficient use of germplasm resources. In recent years, researchers focused on maize landraces, inbred lines and other germplasm resources, and adopted different means to evaluate the genetic diversity and population structure of different germplasm resources, in order to reveal the rich genetic variation and characteristics of population structures of these resources from different sides (Hagdorn *et al*., 2003; Fukunaga *et al*., 2005). In view of the population structure, the genetic basis of the hybrid maize in the United States comes from five old OVPs, including Reed, Minn13, Lancaster, Northwestern Dent, and Leaming (Triyer, 2004). Whereas, one of the main heterosis patterns used in China's maize breeding is mainly the derivatives of the superior landraces, such as "Huangzao 4" and its improved inbred lines, Lucia red cob inbred lines.

Maize landraces in China have been done numerous basic researches. The use of morphological markers, biochemical and molecular markers has revealed the abundant genetic variation of maize landraces in China in phenotype and DNA levels (Lu *et al*., 2002; Liu *et al*., 2010). By means of SSR technique, the genetic diversity of maize was investigated by previous researches (Tian *et al*., 2003; Wu *et al*., 2004; Liu *et al*., 2005; Yao *et al*., 2007). In these studies, waxy corn and popcorn of different ecological zones and the maize landraces in the whole area of Southwestern were made classification and effective reorganization. Based on molecular level evaluation, Liu *et al.* (2015) gave a clear and definite answer to the genetic variation of nine maize races in China, and revealed the genetic relationship and population differentiation characteristics between the races, which provided the basis for studying the formation and evolution of Chinese maize races (Liu *et al*., 2015).

To analyze the phenotypic traits of the core maize landraces in the flowering period, Liu *et al.* (2008) founded that the landraces from the Southwest China had high phenotypic diversity (Liu *et al.*, 2008). Based on SSR analysis, Yao *et al.* (2007) evaluated the genetic diversity and population structure of maize landraces in Southwest China, and further confirmed that maize landraces in this area had rich genetic variation in the molecular level, especially in Sichuan Province. Furthermore, the study showed that there was an inherent link between the geographical distribution and the

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genetic background of maize landraces in this area, supposing that the introduction way of maize in the area of Southwest is through "India-Tibet-Sichuan" (Yao *et al*., 2007; Yao *et al*., 2015).

Tibet is located in the southwest of China, with complex habitat. After long-term natural variation and artificial selection, it gives birth to the unique local maize germplasm. However, since the dangerous terrain, traffic inconvenience and other objective factors, the development and utilization level of the local maize germplasm are still limited. In addition, the research on genetic diversity of maize germplasm in this area has no report. To this end, based on the basis of phenotypic identification, in this paper, according to geographical origins and population heterogeneity, 69 representative maize landraces in Tibet are selected, to investigate theirs genetic structure and genetic diversity by using 40 core SSR markers covered the entire maize genome. It not only provides a reference for the improvement and utilization of maize landraces in Tibet, but also offers theoretical support for the research of the origin and the introduction way of maize.

Materials and Methods

Plant Materials

The materials in this study include 69 Tibetan maize landraces, which have a wide range of representative, and cover the traditional corn production area in Tibet. The name and origin of each maize variety are shown in Table 1.

Sampling and DNA Extraction

The seeds of tested materials were planted in the nutrition bowls, and cultured until 3-5-leaf stage. By using the bulk sampling method, 30 individual plants from each population were selected randomly, and the leaves were mixed with same quantity of each individual to compose the mixed sample, used for the SSR analysis and classification of the 69 Tibetan maize landraces. In addition, according to the geographic origin and phenotypic differences, 35 varieties were selected from 69 maize landraces in Tibet. By using individual single sampling method, 30 plants were randomly selected from each variety, that is, a total of 1050 samples were applied for the analysis of genetic diversity and genetic structure Plant genome DNA Extraction Kit (CW0531M, CoWin Biotech Co. Ltd., Beijing, China) was used for DNA extraction.

Selection of Primers and Identification of Genotype

In this study, 40 pairs of core SSR markers evenly covered the maize chromosome complement, with good stability and high polymorphism, and had been used for the construction of maize varieties, inbred lines and resources in China. 5 'end of SSR markers were made fluorescence labeling with FAM, HEX, ROX, TAM, etc., and synthesized by Sangon Biotech (Shanghai) Co., Ltd. The name and sequence of the primers are shown in Table 2. Multiple PCR amplification was performed by using the 40 core SSR markers in the study, and the amplified products were detected by fluorescence capillary electrophoresis with DNA sequencing (3730XL). Life Science and Technology Center of China Seed Group Co., Ltd. (Wuhan) provided the relevant technical support.

Data Analysis

Using Genemapper V4.0 software to read and collect SSR data, based on which, genetic diversity analysis was conducted and the genetic distance of maize landraces in Tibet was calculated. The statistical methods and formulae used are described below:

Cluster result of the 69 maize landraces based on Rogers distances'D' between each pair of landraces.

$$D = \frac{1}{n}\sum_{i=1}^{n}\sum_{j}^{m}\frac{1}{2}(p_{ij}{}^{X} - p_{ij}{}^{Y})$$

Where, p_{ij}^{X} and q_{ij}^{Y} are the frequencies of ith allele at jth allele in landraces X and Y, respectively.

The parameters related to genetic diversity of maize landraces in Tibet:

The mean number of alleles'A': $A = \sum_{i=1}^{n} {}^{A_I}/_{n}$,

Where, A_i is the number of alleles at ith allele.

The effective allelic number 'A_e': $A_e = \sum_{i=1}^{n} A_{ei}/n = \sum_{i=1}^{n}(1/\sum_{j=1}^{m} q_j{}^2)/n$

Where, $A_{ei}$is the effective allelic number at i[th] allele, and q_i the frequency of the j[th] allele.

Ho is the observed heterozygosity: $H_O = \sum_{i=1}^{n} H_{oi}/n = \sum_{i=1}^{n}(1 - \sum_{j=1}^{m_i} q_{ij}{}^2)/n,$

Where, H_{oi} represents the observed heterozygosity of the i[th] allele, and q_{ij} is the frequency of the jth homozygous allele at i[th] allele.

H_e is the expected heterozygosity: $H_e = \sum_{i=1}^{n} H_i/n = \sum_{i=1}^{n}(1 - \sum_{j=1}^{m_i} q_{ij}{}^2)/n$

Where, H_i is the expected heterozygosity of the i[th] allele, and q_{ij} refers to the frequency of the j[th] homozygous allele at i[th] allele.

Inbreeding coefficient 'F': $F = 1 - H_o/H_e$

All the above parameters were computed using the POPGENE software version 1.2, the method refer to Yao *et al*., 2007.

Table 1: Variety name and origin of the tested maize landraces in Tibet

NO.	Variety name	Origin	NO.	Variety Name	Origin
M1	Caijia 1	Markam	M36	Raojin white maize	Zogang
M2	Shangjie red blood silk	Markam	M37	Renguo yellow maize	Zogang
M3	Zhongjin yellow blood silk	Markam	M38	Zhonglinka white maize	Zogang
M4	Zhongjin white blood silk	Markam	M39	Guba red maize	Zogang
M5	Lagen 1	Baxoi	M40	Xialinka white maize	Zogang
M6	Lagen 5	Baxoi	M41	Guba white maize	Zogang
M7	Lagen 8	Baxoi	M42	Xuri dent type 1	Zogang
M8	Zhongdu red cob	Bomi	M43	Muxu white maize 1	Markam
M9	Zhongdu white cob	Bomi	M44	Zengna 1	Zayu
M10	Shama white cob	Bomi	M45	Zengna 3	Zayu
M11	Chachang intermediate type	Bomi	M46	Zhongbai black maize	Bomi
M12	Dongba red kernel	Zogang	M47	Zhongbai yellow maize 1	Bomi
M13	Zhuka 1	Markam	M48	Suotong yellow maize	Bomi
M14	Chaba 2	Markam	M49	Suotong white maize	Bomi
M15	Riwa 1	Markam	M50	Suotong variegated	Bomi
M16	Suotong 6	Bomi	M51	Zhongbai yellow maize 2	Bomi
M17	Juelong white 88 days	Markam	M52	Zhongbai variegated	Bomi
M18	Juelonggou 2	Markam	M53	Chentangmagen	Dinggye
M19	Longgu white cob	Zayu	M54	Zhiba maize 1	Zayu
M20	Medog Baima	Medog	M55	Cona maize	Cona
M21	MedogYuanma	Medog	M56	Talin yellow maize	Zayu
M22	Luoren intermediate type	Markam	M57	Talin variegated	Zayu
M23	Quzika white maize 1	Markam	M58	Talin white maize	Zayu
M24	Luoren white maize 3	Markam	M59	Abing maize	Zayu
M25	Luoren white maize 4	Markam	M60	Dengxu flint	Zayu
M26	Jiada white blood silk	Markam	M61	Zhaen maize	Zayu
M27	Shizika 2	Zogang	M62	Zhu village chicken blood red	Medog
M28	Quzika 2	Markam	M63	Damu variegated	Medog
M29	Quzika 3	Markam	M64	Damu white flint	Medog
M30	Deqing red	Markam	M65	Deergong white 1	Medog
M31	Waxy maize	Markam	M66	Xiayadong 2	Medog
M32	Rumei white maize	Markam	M67	Wolong popcorn	Mainling
M33	Rumei yellow maize	Markam	M68	Wolong maize	Mainling
M34	Suoduoxi 2	Markam	M69	Lilong maize	Mainling
M35	Suoduoxi 3	Markam			

Results

Genetic Diversity and Cluster Analysis of 69 Maize Landraces in Tibet

Based on the SSR analysis results of 69 maize landraces in Tibet, the genetic distance of maize landraces in Tibet was calculated. Cluster analysis was carried out by using group-average method, and at the threshold of 0.35, a total of four-class groups were obtained (Fig. 1). Combined with phenotypic traits in the growth period, differentiation characteristics of various class groups were as follows: the first group included 30 varieties, of which 18 were from Markam, seven from Zogang, Zayu and Mainling each containing two, and one variety from Baxoi; on the basis of growth period, 83% identified groups belonged to the early maturing varieties. In the second group, there were 18 varieties, most of them were from Medog (seven varieties) and Zayu (five varieties), and the others were from Bomi (three varieties), Baxoi (one variety), Cona (one variety), Dinggye (one variety), respectively; and 89% groups of the identified varieties belonged to the late maturing varieties. The third group only consisted of five varieties, of which two varieties were from Zayu, and Markam, Mainling, Zogang each containing one variety. M67 from Mainling was a typical popcorn varieties showed that the group with the blood of popcorn. The last group included 16 varieties, of which nine varieties were from Bomi, the rest were from, Markam, Zayu, Baxoi, Zogang, respectively and these landraces were medium mature varieties.

Genetic Variation of SSR Locus and Genetic Structure Analysis of Maize Landraces in Tibet

1050 individuals of 35 varieties were detected 551 polymorphic loci, the average number of alleles was 13.78, and the variation amplitude of alleles ranged from 5 to 26, showing higher levels of polymorphism in the same loci compared with results of bulk DNA sampling of 69 varieties (Table 3). Ae, He and Ho ranged from 1.36 to 13.44, 0.26−0.93 and 0.09−0.79 with an average of 4.64, 0.71 and 0.51, respectively. The allele, effective allele, actual heterozygosity and expected heterozygosity detected by the SSR maker ZMGB20 showed the lowest value, while that of ZMGB05 showed the highest.

Table 2: Basic information of SSR molecular markers

Locus	Primer	Chromosome location	Primer sequences	Fluorescent dye
ZMGB01	bnlg439w1	1.03	Upstream: AGTTGACATCGCCATCTTGGTGAC Downstream: GAACAAGCCCTTAGCGGGTTGTC	ROX
ZMGB02	umc1335y5	1.06	Upstream: CCTCGTTACGGTTACGCTGCTG Downstream: GATGACCCCGCTTACTTCGTTTATG	TAM
ZMGB03	umc2007y4	2.04	Upstream: TTACACAACGCAACACGAGGC Downstream: GCTATAGGCCGTAGCTTGGTAGACAC	HEX
ZMGB04	bnlg1940k7	2.08	Upstream: CGTTTAAGAACGGTTGATTGCATTCC Downstream: GCCTTTATTTCTCCCTTGCTTGCC	FAM
ZMGB05	umc2105k3	3.00	Upstream: GAAGGGCAATGAATAGAGCCATGAG Downstream: ATGGACTCTGTGCGACTTGTACCG	TAM
ZMGB06	phi053k2	3.05	Upstream: CCCTGCCTCTCAGATTCAGAGATTG Downstream: TAGGCTGGCTGGAAGTTTGTTGC	TAM
ZMGB07	phi072k4	4.01	Upstream: GCTCGTCTCCTCCAGGTCAGG Downstream: CGTTGCCCATACATCATGCCTC	TAM
ZMGB08	bnlg2291k4	4.06	Upstream: GCACACCCGTAGTAGCTGAGACTTG Downstream: CATAACCTTGCCTCCCAAACCC	HEX
ZMGB09	umc1705w1	5.03	Upstream: GGAGGTCGTCAGATGGAGTTCG Downstream: CACGTACGGCAATGCAGACAAG	ROX
ZMGB10	bnlg2305k4	5.07	Upstream: CCCCTCTTCCTCAGCACCTTG Downstream: CGTCTTGTCTCCGTCCGTGTG	FAM
ZMGB11	bnlg161k8	6.00	Upstream: TCTCAGCTCCTGCTTATTGCTTTCG Downstream: GATGGATGGAGCATGAGCTTGC	HEX
ZMGB12	bnlg1702k1	6.05	Upstream: GATCCGCATTGTCAAATGACCAC Downstream: AGGACACGCCATCGTCATCA	HEX
ZMGB13	umc1545y2	7.00	Upstream: AATGCCGTTATCATGCGATGC Downstream: GCTTGCTGCTTCTTGAATTGCGT	HEX
ZMGB14	umc1125y3	7.04	Upstream: GGATGATGGCGAGGATGATGTC Downstream: CCACCAACCCATACCCATACCAG	TAM
ZMGB15	bnlg240k1	8.06	Upstream: GCAGGTGTCGGGGATTTTCTC Downstream: GGAACTGAAGAACAGAAGGCATTGATAC	ROX
ZMGB16	phi080k15	8.08	Upstream: TGAACCACCCGATGCAACTTG Downstream: TTGATGGGCACGATCTCGTAGTC	FAM
ZMGB17	phi065k9	9.03	Upstream: CGCCTTCAAGAATATCCTTGTGCC Downstream: GGACCCAGACCAGGTTCCACC	HEX
ZMGB18	umc1492y13	9.04	Upstream: GCGGAAGAGTAGTCGTAGGGCTAGTGTAG Downstream: AACCAAGTTCTTCAGACGCTTCAGG	FAM
ZMGB19	umc1432y6	10.02	Upstream: GAGAAATCAAGAGGTGCGAGCATC Downstream: GGCCATGATACAGCAAGAAATGATAAGC	TAM
ZMGB20	umc1506k12	10.05	Upstream: GAGGAATGATGTCCGCGAAGAAG Downstream: TTCAGTCGAGCGCCCAACAC	ROX
ZMGB21	umc1147y4	1.07	Upstream: AAGAACAGGACTACATGAGGTGCGATAC Downstream: GTTTCCTATGGTACAGTTCTCCCTCGC	HEX
ZMGB22	bnlg1671y17	1.10	Upstream: CCCGACACCTGAGTTGACCTG Downstream: CTGGAGGGTGAAACAAGAGCAATG	FAM
ZMGB23	phi96100y1	2.00	Upstream: TTTTGCACGAGCCATCGTATAACG Downstream: CCATCTGCTGATCCGAATACCC	FAM
ZMGB24	umc1536k9	2.07	Upstream: TGATAGGTAGTTAGCATATCCCTGGTATCG Downstream: GAGCATAGAAAAAGTTGAGGTTAATATGGAGC	HEX
ZMGB25	bnlg1520K1	2.09	Upstream: CACTCTCCCTCTAAAATATCAGACAACACC Downstream: GCTTCTGCTGCTGTTTTGTTCTTG	ROX
ZMGB26	umc1489y3	3.07	Upstream: GCTACCCGCAACCAAGAACTCTTC Downstream: GCCTACTCTTGCCGTTTTACTCCTGT	TAM
ZMGB27	bnlg490y4	4.04	Upstream: GGTGTTGGAGTCGCTGGGAAAG Downstream: TTCTCAGCCAGTGCCAGCTCTTATTA	ROX
ZMGB28	umc1999y3	4.09	Upstream: GGCCACGTTATTGCTCATTTGC Downstream: GCAACAACAAATGGGATCTCCG	TAM
ZMGB29	umc2115k3	5.02	Upstream: GCACTGGCAACTGTACCCATCG Downstream: GGGTTTCACCAACGGGGATAGG	FAM
ZMGB30	umc1429y7	5.03	Upstream: CTTCTCCTCGGCATCATCCAAAC Downstream: GGTGGCCCTGTTAATCCTCATCTG	TAM
ZMGB31	bnlg249k2	6.01	Upstream: GGCAACGGCAATAATCCACAAG Downstream: CATCGGCGTTGATTTCGTCAG	HEX
ZMGB32	phi299852y2	6.07	Upstream: AGCAAGCAGTAGGTGGAGGAAGG Downstream: AGCTGTTGTGGCTCTTTGCCTGT	HEX
ZMGB33	umc2160k3	7.01	Upstream: TCATTCCCAGAGTGCCTTAACACTG Downstream: CTGTGCTCGTGCTTCTCTCTGAGTATT	TAM
ZMGB34	umc1936k4	7.03	Upstream: GCTTGAGGCGGTTGAGGTATGAG Downstream: TGCACAGAATAAACATAGGTAGGTCAGGTC	TAM
ZMGB35	bnlg2235y5	8.02	Upstream: CGCACGGCACGATAGAGGTG Downstream: AACTGCTTGCCACTGGTACGGTCT	HEX
ZMGB36	phi233376y1	8.09	Upstream: CCGGCAGTCGATTACTCCACG Downstream: CAGTAGCCCCTCAAGCAAAACATTC	ROX
ZMGB37	umc2084w2	9.01	Upstream: ACTGATCGCGACGAGTTAATTCAAAC Downstream: TACCGAAGAACAACGTCATTTCAGC	FAM
ZMGB38	umc1231k4	9.05	Upstream: ACAGAGGAACGACGGGACCAAT Downstream: GGCACTCAGCAAAGAGCCAAATTC	ROX
ZMGB39	phi041y6	10.00	Upstream: CAGCGCCGCAAACTTGGTT Downstream: TGGACGCGAACCAGAAACAGAC	TAM
ZMGB40	umc2163w3	10.04	Upstream: CAAGCGGGAATCTGAATCTTTGTTC Downstream: CTTCGTACCATCTTCCCTACTTCATTGC	HEX

Results of F-statistics showed that the variation amplitude of F value of population fixed index ranged from 0.71 to 0.12, with an average of 0.30, indicating that the maize landraces in Tibet were a multi-species mixture breeding system. Fis and Fit represent the amount of deviation relative to the Hardy-Weinberg equivalent of intra-population and inter-population correspondingly. In this study, the variation amplitude of Fis was -0.17−0.58, with an average of 0.03 and that of Fit was 0.12−0.71, with an average of 0.30, indicating that most Tibetan maize landraces of intra-population and inter-population were deviated from the Hardy-Weinberg equivalent, and the deviation of intra-population was greater than of inter-population. Fst is the genetic differentiation coefficient of inter-population. In this paper, variation amplitude of Fst was 0.21−0.36, with an average of 0.28, demonstrating that 28% variation of Tibetan maize landraces was from the inter-population, and 72% of the variation from the intra-population.

Genetic Relationship Analysis of Maize Landraces in Tibet

The results of genetic distance calculation for 30 individual plants from 35 varieties (Table 4) showed that the genetic distance of these maize landraces was less than 0.2: M17 and M37 (0.13), M18 and M37 (0.16), M14 and M37 (0.19), M59 and M37 (0.18), M36 and M38 (0.15), M49 and M38 (0.17), M36 and M49 (0.18), M38 and M41 (0.18), M36 and M41 (0.19), M36 and M59 (0.19), M37 and M38 (0.19), representing that in a certain extent, the genetic relationship of maize landraces from Markam and the Zogang were closed; Meanwhile, there were two varieties from Bomi and Zayualso with closed genetic distance to that of Markam and Zogang, it might be the result of farmers' mutual introduction behavior under similar climatic condition. Whereas the genetic distance was greater than 1.0 as follows: M17 and M67 (1.34), M14 and M67 (1.22), M20 and M67 (1.13), M37 and M67 (1.13), M38 and M67 (1.12), M18 and M67 (1.12), M06 and M67 (1.05), M39 and M67 (1.05) M41, and M67 (1.04) M49 and

Table 3: Genetic variation of SSR locus and genetic structure of maize landraces in Tibet

Locus	A	Ae	He	Ho	Fis	Fit	Fst	F
ZMGB01	8	2.6628	0.63	0.59	-0.0423	0.0546	0.0929	0.06
ZMGB02	20	3.2925	0.70	0.71	-0.0726	0.0069	0.0741	-0.01
ZMGB03	5	3.9009	0.75	0.88	-0.3611	-0.2063	0.1137	-0.18
ZMGB04	10	5.6012	0.83	0.93	-0.1556	-0.1028	0.0457	-0.12
ZMGB05	21	9.8776	0.91	0.84	0.0057	0.0825	0.0772	0.07
ZMGB06	3	2.0034	0.50	0.65	-0.3982	-0.3707	0.0197	-0.29
ZMGB07	7	5.6277	0.83	0.88	-0.1105	-0.0142	0.0867	-0.07
ZMGB08	18	7.1973	0.87	0.90	-0.0751	-0.0293	0.0426	-0.04
ZMGB09	11	3.367	0.71	0.90	-0.3196	-0.2369	0.0627	-0.27
ZMGB10	16	8.1176	0.88	0.90	-0.1057	-0.065	0.0368	-0.02
ZMGB11	6	3.1761	0.69	0.86	-0.2878	-0.2348	0.0411	-0.24
ZMGB12	6	2.702	0.63	0.51	0.0232	0.2385	0.2205	0.20
ZMGB13	8	4.5956	0.79	0.86	-0.1414	-0.0863	0.0483	-0.09
ZMGB14	17	7.0273	0.86	0.99	-0.1816	-0.0864	0.0806	-0.14
ZMGB15	13	7.2191	0.87	0.91	-0.1578	-0.0474	0.0954	-0.05
ZMGB16	13	2.305	0.57	0.67	-0.1989	-0.1531	0.0382	-0.17
ZMGB17	7	5.3857	0.82	0.93	-0.2376	-0.1435	0.0761	-0.13
ZMGB18	15	4.7873	0.80	0.86	-0.0874	-0.0023	0.0783	-0.07
ZMGB19	7	3.7696	0.74	0.86	-0.2588	-0.1703	0.0703	-0.16
ZMGB20	3	1.1575	0.14	0.14	-0.1585	-0.1024	0.0484	-0.06
ZMGB21	4	2.8767	0.66	0.78	-0.2895	-0.0889	0.1556	-0.19
ZMGB22	6	2.0433	0.51	0.64	-0.4459	-0.3087	0.0949	-0.24
ZMGB23	4	2.9271	0.66	0.78	-0.3287	-0.1581	0.1284	-0.18
ZMGB24	4	1.364	0.27	0.29	-0.2249	-0.072	0.1249	-0.08
ZMGB25	8	3.1384	0.69	0.80	-0.3124	-0.1686	0.1096	-0.16
ZMGB26	13	7.4044	0.87	0.87	-0.101	-0.0292	0.0652	0.00
ZMGB27	5	3.271	0.70	0.93	-0.414	-0.2913	0.0868	-0.33
ZMGB28	9	3.9642	0.75	0.67	0.0268	0.1096	0.0851	0.11
ZMGB29	7	2.426	0.59	0.61	-0.1529	-0.0822	0.0613	-0.03
ZMGB30	7	3.218	0.69	0.77	-0.2169	-0.0999	0.0961	-0.11
ZMGB31	5	2.481	0.60	0.59	-0.0404	0.018	0.0561	0.01
ZMGB32	11	5.7604	0.83	0.81	-0.1158	-0.0296	0.0773	0.02
ZMGB33	6	1.8852	0.47	0.51	-0.2208	-0.1068	0.0934	-0.07
ZMGB34	7	4.4979	0.78	0.94	-0.325	-0.2471	0.0588	-0.20
ZMGB35	7	4.6562	0.79	0.93	-0.2595	-0.1494	0.0874	-0.17
ZMGB36	11	4.1984	0.77	0.86	-0.178	-0.0838	0.08	-0.11
ZMGB37	9	3.6247	0.73	0.91	-0.2418	-0.1665	0.0606	-0.25
ZMGB38	3	1.6274	0.39	0.45	-0.4004	-0.2726	0.0912	-0.16
ZMGB39	14	5.7224	0.83	0.93	-0.1762	-0.1129	0.0539	-0.12
ZMGB40	8	4.9057	0.80	0.80	0.0325	0.1017	0.0715	0.01

M67 (1.01), M35 and M69 (1.01), where M67 was a popcorn from Mainling, showing that the genetic relationship of the maize landraces from Markam and Zogang were distant from the popcorn variety.

The genetic diversity analysis of Tibetan maize landraces showed that the average allele (A), Ae, He and Ho of the 35 species were 4.56, 2.45, 0.52, and 0.51 respectively, with a variation range of 2.83−6.48, 1.69−3.28, 0.33−0.63 and 0.34−0.64 correspondingly (Table 5). Based on the above data, it can be seen that the allele frequency and gene heterozygosity of M36 from Zogang were the highest, while that the popcorn M67 from Wolong, Mainling was the lowest. The comparison results of A, Ae, He and Ho value of the 35 varieties showed that there were 12 varieties with average allele greater than 5.0, they were from Markang (three varieties), Zogang (three varieties), Zayu (three varieties), Bomi (two varieties), Boxi (one variety). The six varieties of M06, M12, M39, M36, M44, M55 were from Nujiang River in Tibet and distributed in different river valleys. There were three varieties distributed in the lower reaches of the Lancang River valley in Tibet. It can be realized that the valley of the Nujiang River and the lower reaches of the Lancang River in Tibet contain the most abundant genetic variation.

Discussion

In this study, 551 alleles were detected in 1050 DNA samples of 35 landraces, the average number of alleles per locus was 13.78, which was higher than the overall level of genetic diversity of nine races of maize landraces in China (11.5/site) (Lu *et al*., 2002; Liu *et al*., 2015), showing higher genetic diversity of maize landraces in Tibet. It is inferred that this result is related to the special geographical climate of Tibet on the one hand. The distribution area of Tibetan maize is with large span altitude and complex habitat environment which breeds a variety of maize germplasm resources. On the other hand, it may be related to the experimental method. Multiplex real-time PCR and capillary electrophoresis techniques were combined in this experiment, and compared with the conventional polyacrylamide gel electrophoresis, the detection efficiency of the amplified products in this method was increased by 11 times, which can accurately analyze and quantify the fragment length and the abundance of amplified product, had the advantages of high accuracy, efficiency and resolution, to play an important role in improving the efficiency of genetic diversity analysis (Ying-hui *et al.*, 2010; Aci *et al.*, 2013; Xia *et al.*, 2015).

In order to fully reveal the genetic information of the maize landraces in Tibet, the experimental cost and efficiency are also considered, in this study, we made full use of the advantages of bulk DNA sampling and single DNA sampling strategy to reflect the genetic basis of maize landraces in Tibet to an extreme. The strategy of bulk DNA sampling from 30 individuals had the advantages of saving manpower, rapid and efficient, and which clearly revealed the genetic differences between populations based on the heterotic grouping of 69 maize landraces in Tibet. According to geographic origin and phenotypic differences, 35 varieties were selected, and 30 single DNA sampling strategy were used to analyze the genetic diversity and genetic structure. The results showed that the number of alleles detected in the single DNA samples was higher than in the mixed DNA samples. This is consistent with the research results of Yao *et al.* (2007), suggesting that the mixed DNA samples of 30 individuals representing a maize population were feasible in the use of SSR technique to evaluate genetic diversity of maize landraces, while in the analysis of the number of alleles, genotype heterozygosity and other genetic structure information, single DNA sampling strategy was appropriate.

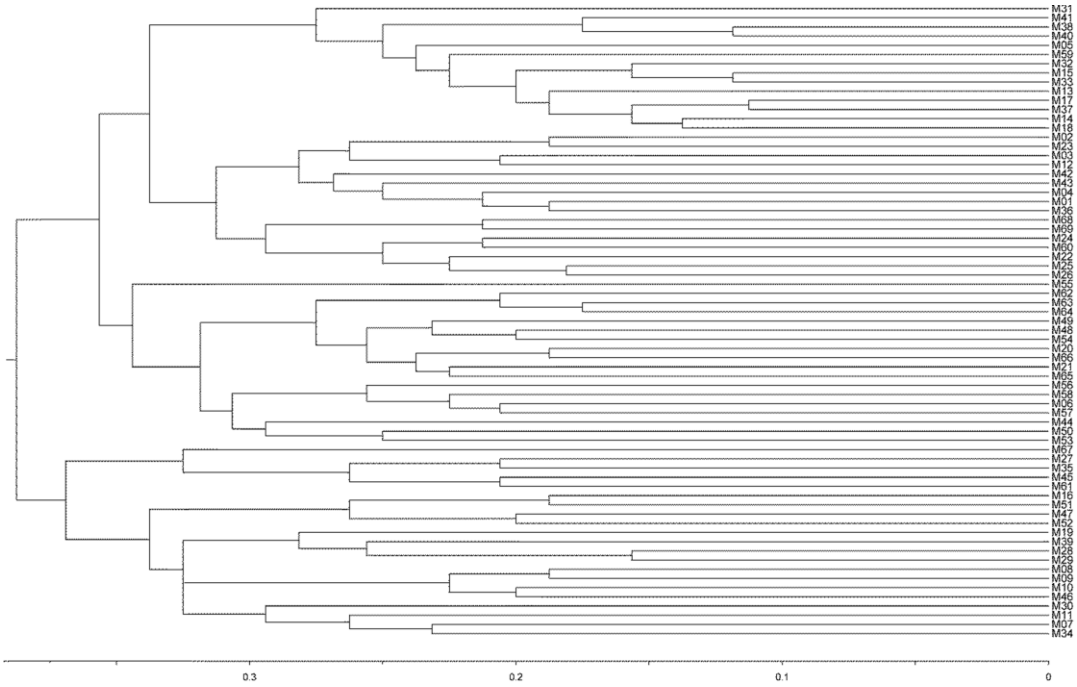

Fig. 1: Cluster result of the 69 maize landraces in Tibet

Through the analysis of genetic distance and genetic variation within the variety, it was found that the genetic distance from Markam and Zogang was close and this as the center, the maize belt which was located in the Nujiang River Valley and Lancang River valley, had better polymorphism and contained the more abundant hereditary basis. This may be related to the introduction and genetic evolution of the maize landraces in Tibet. The temperature of the maize belt in the valley is more abundant, which can better meet the needs of maize growth. Moreover, in this area, it has long introduction and cultivated history, with abundant genetic variation, to form the enrichment center of various types of germplasms, providing the good potentiality in genetic improvement and utility value.

On the basis of genetic distance calculation, 69 representative maize landraces in Tibet were divided into four groups. Combined with the phenotype of each group, the characteristics of growth period was identified: the first group was mainly from Markam and Zogang, belonging to the early-mid maturing group; the second group was mainly from Zayu and Metok, it was the mid-late maturing group; in third group, the geographical distributions of varieties were relatively decentralized, in which included popcorn variety, belonging to popcorn group; the fourth group was from Bomi, belonging to mid-maturing group. Combined with the results of phenotypic identification, the results of this study verified the accuracy of material selection and the results analysis from the DNA level and phenotypic level respectively. In addition, it showed that there was an inner link between the phenotypic characteristics and genetic background of maize landraces in Tibet, and the differentiation characteristics of the growth period were more obvious.

Genetic diversity and genetic structure of crop population are influenced by many factors during the process of evolution. The main factors influencing the genetic structure include gene mutation, natural and artificial selection, individual migration and genetic drift (Dreisigacker *et al.*, 2005). The genetic structure analysis of this study showed the lack of heterozygote of maize landraces in Tibet, intra-population and inter-population were all deviated from the Hardy-Weinberg equivalent, and the deviation of intra-population was larger than that of inter-population, indicating that is not a completely random mating population. This is because two main aspects: the first one is that the farmers only select a small number of excellent maize as the breeding objects in the process of reserving seed for planting, causing highly homozygous within the varieties group, and the heterozygous ratio is decreased (Pinedahidalgo *et al.*, 2013). The second one is that the selected maize landraces in this study were collected from the corn ear in the original habitat, not taken artificial propagation, leading to relatively small degree of inter-population deviated from Hardy-Weinberg equivalent, but larger deviation degree of intra-population (Chen *et al.*, 2016). This study also found that the source of variation of maize landraces in Tibet was mainly derived from intra-population. This is mainly because the maize itself is a cross pollinated crop, the outcrossing rate of inter-individual within a population is larger, and the variation of inter-population is lower, which enriches the genetic variation of intra-population. In addition, in various climate conditions,

Table 4: The genetic distance between 35 maize landraces in Tibet

	M04	M06	M09	M12	M14	M15	M17	M18	M19	M20	M21	M25	M35	M36	M37	M38	M39
M06	0.46																
M09	0.60	0.70															
M12	0.57	0.48	0.77														
M14	0.51	0.38	0.68	0.70													
M15	0.43	0.30	0.73	0.62	0.21												
M17	0.52	0.37	0.63	0.66	0.17	0.21											
M18	0.43	0.43	0.60	0.69	0.18	0.26	0.17										
M19	0.50	0.59	0.61	0.59	0.66	0.56	0.60	0.55									
M20	0.67	0.51	0.65	0.86	0.51	0.48	0.53	0.59	0.75								
M21	0.42	0.42	0.63	0.45	0.40	0.39	0.52	0.46	0.58	0.51							
M25	0.37	0.48	0.69	0.63	0.50	0.40	0.47	0.47	0.59	0.63	0.50						
M35	0.62	0.64	0.78	0.55	0.84	0.79	0.91	0.93	0.65	0.78	0.59	0.73					
M36	0.28	0.28	0.54	0.51	0.23	0.18	0.23	0.23	0.42	0.44	0.34	0.31	0.64				
M37	0.44	0.30	0.57	0.61	0.19	0.20	0.13	0.16	0.51	0.47	0.41	0.42	0.90	0.20			
M38	0.43	0.27	0.58	0.61	0.21	0.22	0.20	0.26	0.55	0.43	0.42	0.44	0.77	0.15	0.19		
M39	0.55	0.46	0.85	0.64	0.64	0.55	0.72	0.73	0.55	0.60	0.51	0.62	0.58	0.43	0.59	0.47	
M41	0.36	0.35	0.64	0.62	0.23	0.24	0.28	0.28	0.58	0.47	0.45	0.48	0.80	0.19	0.25	0.18	0.52
M43	0.30	0.57	0.55	0.67	0.66	0.58	0.71	0.64	0.61	0.76	0.45	0.54	0.67	0.37	0.57	0.58	0.62
M44	0.33	0.54	0.75	0.57	0.53	0.44	0.53	0.51	0.67	0.62	0.42	0.50	0.60	0.35	0.43	0.46	0.48
M46	0.53	0.59	0.34	0.60	0.59	0.64	0.65	0.62	0.58	0.62	0.49	0.64	0.67	0.46	0.58	0.55	0.63
M48	0.41	0.43	0.60	0.59	0.42	0.35	0.37	0.45	0.54	0.36	0.37	0.40	0.63	0.30	0.33	0.35	0.56
M49	0.39	0.34	0.64	0.61	0.25	0.26	0.30	0.36	0.54	0.43	0.42	0.42	0.69	0.18	0.28	0.17	0.51
M50	0.44	0.71	0.74	0.84	0.66	0.52	0.70	0.71	0.73	0.52	0.57	0.65	0.69	0.40	0.60	0.56	0.65
M51	0.50	0.76	0.81	0.67	0.84	0.79	0.91	0.86	0.60	0.83	0.66	0.74	0.67	0.52	0.84	0.83	0.56
M52	0.40	0.66	0.52	0.52	0.69	0.67	0.72	0.68	0.60	0.83	0.50	0.69	0.75	0.43	0.67	0.59	0.56
M53	0.48	0.51	0.48	0.68	0.46	0.43	0.46	0.45	0.57	0.52	0.47	0.50	0.84	0.31	0.38	0.38	0.60
M54	0.38	0.33	0.60	0.56	0.32	0.28	0.30	0.36	0.45	0.48	0.37	0.43	0.67	0.22	0.28	0.24	0.47
M55	0.53	0.77	0.68	0.93	0.92	0.88	0.85	0.87	0.65	0.81	0.80	0.72	0.94	0.58	0.78	0.71	0.74
M57	0.34	0.43	0.55	0.64	0.41	0.35	0.41	0.42	0.53	0.50	0.33	0.48	0.66	0.26	0.35	0.33	0.60
M59	0.41	0.30	0.60	0.60	0.28	0.25	0.22	0.25	0.42	0.53	0.42	0.45	0.74	0.19	0.18	0.21	0.50
M62	0.46	0.44	0.70	0.74	0.41	0.39	0.44	0.45	0.76	0.30	0.40	0.44	0.78	0.35	0.40	0.35	0.56
M66	0.45	0.35	0.54	0.65	0.36	0.38	0.42	0.41	0.59	0.27	0.40	0.52	0.69	0.35	0.34	0.31	0.50
M67	0.63	1.05	1.02	0.83	1.22	1.09	1.34	1.12	0.84	1.13	0.67	0.89	0.76	0.86	1.13	1.12	1.05
M69	0.72	0.80	0.91	0.82	0.62	0.67	0.68	0.66	0.77	1.05	0.81	0.77	1.01	0.52	0.62	0.67	0.91
	M41	M43	M44	M46	M48	M49	M50	M51	M52	M53	M54	M55	M57	M59	M62	M66	M67
M43	0.51																
M44	0.42	0.54															
M46	0.56	0.56	0.62														
M48	0.42	0.52	0.49	0.43													
M49	0.23	0.50	0.46	0.55	0.33												
M50	0.62	0.52	0.48	0.60	0.50	0.49											
M51	0.74	0.59	0.58	0.44	0.53	0.66	0.55										
M52	0.57	0.56	0.54	0.34	0.49	0.58	0.60	0.22									
M53	0.42	0.56	0.47	0.56	0.54	0.40	0.53	0.79	0.61								
M54	0.26	0.49	0.52	0.49	0.30	0.27	0.60	0.64	0.49	0.47							
M55	0.73	0.66	0.77	0.77	0.69	0.75	0.81	0.82	0.70	0.78	0.59						
M57	0.41	0.47	0.47	0.53	0.38	0.38	0.52	0.72	0.53	0.48	0.33	0.71					
M59	0.32	0.56	0.48	0.54	0.42	0.27	0.53	0.71	0.54	0.41	0.30	0.77	0.31				
M62	0.47	0.66	0.54	0.63	0.33	0.34	0.47	0.80	0.68	0.52	0.38	0.70	0.49	0.45			
M66	0.32	0.56	0.48	0.44	0.39	0.34	0.48	0.65	0.52	0.40	0.40	0.68	0.43	0.41	0.34		
M67	1.04	0.78	0.77	0.81	0.81	1.01	0.82	0.79	0.76	0.93	0.91	0.87	0.70	1.15	0.88	0.86	
M69	0.66	0.67	0.87	0.74	0.76	0.72	0.86	0.85	0.70	0.76	0.55	0.93	0.78	0.74	0.95	0.75	1.04

the target characters of seeds selected by farmers are different, which preserves the characteristics of the rich genetic diversity of landraces. Furthermore, since the own reserving maize varieties are excellent individual with high heterozygosity mostly, the degree of variation of intra-population is increased correspondingly (Yao *et al.*, 2007).

Conclusion

According to the analysis of genetic diversity, Tibet maize landraces had higher level of diversity than from other areas of China. It can be concluded that maize landraces in southwest China were initially introduced to Tibet than Sichuan and from there to adjacent areas. The comprehensive analysis of the results showed that the genetic distance of maize landraces from Markam was closed to that of Zogang, and taking this as the center, the maize belts located in the Nujiang River Valley and the lower Lancang River valley, contained the most abundant genetic variation in China's Tibet.

Table 5: Genetic diversity within 35 maize landraces in Tibet

Landrace	A	Ae	He	Ho
M04	6.23	3.10	0.62	0.61
M06	5.03	2.33	0.51	0.48
M09	3.60	1.92	0.40	0.43
M12	5.30	2.50	0.57	0.55
M14	3.90	2.07	0.43	0.41
M15	4.35	2.37	0.51	0.49
M17	3.85	2.10	0.44	0.44
M18	4.33	2.12	0.43	0.40
M19	4.10	2.47	0.54	0.58
M20	2.95	1.92	0.39	0.41
M21	4.15	2.48	0.55	0.58
M25	5.70	2.69	0.59	0.57
M35	3.75	2.65	0.58	0.44
M36	6.48	3.28	0.62	0.52
M37	3.95	2.25	0.46	0.40
M38	4.35	2.14	0.45	0.42
M39	5.75	2.53	0.56	0.57
M41	4.95	2.40	0.50	0.55
M43	5.65	2.93	0.62	0.64
M44	6.00	3.03	0.63	0.57
M46	4.25	2.60	0.56	0.59
M48	5.10	2.85	0.60	0.58
M49	5.55	2.45	0.52	0.53
M50	3.20	2.19	0.50	0.48
M51	4.88	2.50	0.56	0.55
M52	4.68	2.63	0.58	0.62
M53	4.20	2.74	0.58	0.56
M54	4.93	2.63	0.57	0.55
M55	3.00	2.05	0.45	0.35
M57	5.00	2.58	0.55	0.56
M59	5.08	2.61	0.57	0.48
M62	4.68	2.40	0.51	0.55
M66	4.63	2.44	0.55	0.58
M67	2.83	1.69	0.33	0.34
M69	3.20	2.06	0.46	0.43

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