CLASSIFYING HETEROTIC GROUPS OF 189 MAIZE INBRED LINES OF CIMMYT AND GUANGXI BASED ON SNP CHIPS

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ABSTRACT

CIMMYT maize inbred lines have good adaptability in Guangxi. At present, many new maize varieties suitable for planting in Guangxi have been successfully selected by hybridization between CIMMYT and Guangxi maize inbred lines. In order to understand the Heterosis relationship between CIMMYT and Guangxi maize inbred lines, 20 CIMMYT maize inbred lines and 169 Guangxi maize inbred lines were classified heterosis groups by 10K maize SNP chips, including 4 control inbred lines. The results showed that the heterozygosity of most of the 189 maize inbred lines were less than 10%, except A110, A134 and A186, indicating that the maize inbred lines used for analysis had high homozygosity. 189 inbred lines were divided into PB group, SPT group, Guidan 162-0810 female parent group, Guidan 162 male parent group, Guidan 0810 male parent group and other group according to evolutionary tree and principal component analysis. There were 15, 3 and 2 CIMMYT maize inbred lines belonging to SPT group, other group and Guidan 162-0810 female parent group, respectively. Guangxi maize inbred lines belong to Guidan 162-0810 female parent group, Guidan 0810 male parent group, other group, SPT group, Guidan 162 male parent group and PB group, with 54, 50, 44, 5, 10 and 6, respectively. The genetic similarity between CIMMYT and Guangxi maize inbred lines was 0.681-0.423. It could be seen that there were great genetic differences between CIMMYT and Guangxi maize inbred lines, and most of them don't belong to the same group. Therefore, the hybridization between CIMMYT and Guangxi maize inbred lines can obtain excellent maize varieties with a high probability.

Keywords: Single Nucleotide Polymorphism (SNP) chips, CIMMYT, Guangxi, Maize inbred lines, Heterotic group.

TÓM TẮT

Các dòng ngô thuần phát triển bởi CIMMYT có khả năng thích nghi tốt ở Quảng Tây. Hiện nay, nhiều giống ngô lai mới thích hợp trồng ở Quảng Tây đã được chọn tạo thành công bằng phương pháp lai giữa các dòng ngô thuần CIMMYT và dòng ngô thuần Quảng Tây. Để hiểu được mối quan hệ ưu thế lai giữa các dòng CIMMYT và Quảng Tây, 20 dòng ngô CIMMYT, 169 dòng ngô Quảng Tây và 4 dòng đối chứng đã được phân nhóm ưu thế lai bằng chip SNP 10K. Kết quả cho thấy mức độ dị hợp tử của hầu hết 189 dòng ngô dưới 10%, trừ các dòng A110, A134 và A186, cho thấy các dòng ngô dùng để phân tích có tỷ lệ đồng hợp tử cao. Dựa theo cây tiến hóa và kết quả phân tích thành phần chính cho thấy 189 dòng thuần trong nghiên cứu được chia thành sáu nhóm: nhóm PB, nhóm SPT, nhóm bố mẹ Guidan 162-0810, nhóm bố mẹ Guidan 162, nhóm bố mẹ Guidan 0810 và nhóm khác. Có 15 dòng ngô CIMMYT, 3 và 2 dòng ngô CIMMYT lần lượt thuộc nhóm SPT, nhóm bố mẹ Guidan 162-0810 và nhóm khác, tương ứng. Các dòng ngô Quảng Tây thuộc nhóm bố mẹ Guidan 162-0810, nhóm bố mẹ Guidan 0810, nhóm khác, nhóm SPT, nhóm bố mẹ Guidan 162, nhóm PB, với lần lượt là 54, 50, 44, 5, 10 và 6. Mức độ tương đồng về di truyền giữa các dòng ngô CIMMYT và ngô Quảng Tây là 0,681-0,423. Có thể thấy rằng có sự khác biệt lớn về mặt di truyền giữa các dòng ngô lai CIMMYT và ngô Quảng Tây, và hầu

hết chúng không thuộc cùng một nhóm. Do đó, việc lai giữa các dòng ngô lai CIMMYT và ngô Quảng Tây có thể thu được các giống ngô ưu tú với xác suất cao.

Từ khoá: Chip đa hình đơn nucleotide (SNP), CIMMYT, Quảng Tây, Dòng ngô thuần, Nhóm ưu thế lai.

1. INTRODUCTION

Abundant maize germplasm resources are the basis for breeding excellent maize varieties. After decades of rapid development, China's maize germplasm resources are facing an increasingly narrow problem. The direct introduction of maize germplasm from abroad could rapidly expand maize germplasm resources, which was one of the effective means to solve the narrow of maize germplasm resources. The International Maize and Wheat Improvement Center (CIMMYT), located in Mexico, has a large number of tropical and subtropical germplasm resources. These resources contain rich genetic variation and have the advantages of high yield potential, good green retention and strong stress resistance (Fu *et al*., 2014). Guangxi Province of China and CIMMYT belong to tropical and subtropical regions. CIMMYT maize germplasm has good adaptability in Guangxi. CIMMYT maize germplasm such as tuxpeno1, tuxpeno1 p.b.c15, Amarillo dentado-2, CML161, CML285 and CML268, which were introduced and performed well in the 1970s, were improved and utilized, and a series of maize varieties suitable for planting in Guangxi were selected, such as Yumeitou 102, Yumeitou 168, Guidan series, guiding series and yellow grain population. In recent years a series of varieties approved, such as Guidan 0810, Zhaoyu 215 and Guidan 671, contain CIMMYT maize germplasm or their blood relationship. Therefore, CIMMYT maize germplasm plays an important role in maize breeding in Guangxi, and has important significance and far-reaching impact on Improving maize breeding level and maize yield in Guangxi (Chen *et al*., 2010).

It is necessary to understand the heterosis relationship between CIMMYT maize germplasm and Guangxi maize germplasm in order to utilize CIMMYT maize germplasm to breeding practice in Guangxi. Rational division of heterosis groups and construction of corresponding heterosis are conducive to expand germplasm resources, reduce the blindness of hybrid combination and improve the success rate of breeding, which has important guiding significance for maize breeding (Pan *et al*., 2020). In theory, the research methods of dividing heterosis groups include phenotypic cluster analysis, pedigree analysis, isozyme technology, molecular marker and quantitative genetic analysis. With the rapid development of research, genome sequencing and DNA chip technology, SNP (Single nucleotide polymorphism) markers have opened a new era of molecular markers. SNP markers have the advantages of good genetic stability, abundant and widely distributed loci, strong representativeness and easy to realize automatic analysis and detection. They have been applied in the construction of high-density genetic map, accurate gene location, population genetic structure analysis and phylogeny (Yan *et al*., 2010; Lu et la., 2009; Semagn *et al*., 2012).

At present, the research on heterosis between CIMMYT and Guangxi maize germplasm is mostly based on incomplete diallel hybridization of two kinds of materials, and the analysis of combining ability and heterosis through agronomic characters (Mo *et al*., 2019). This method is time-consuming and labor-consuming, and the results are greatly affected by the

environment.SNP markers can greatly shorten the breeding time and are not affected by environmental factors. Therefore, SNP markers have important application value in the analysis of genetic diversity of maize germplasm resources and the division of heterosis groups. In this study, 20 CIMMYT maize inbred lines and 169 Guangxi maize inbred lines were divided into heterosis groups by 10K maize SNP chip, so as to provide reference for the prediction of maize heterosis in Guangxi and the establishment of corresponding heterosis utilization model (Jiang *et al*., 2018).

2. MATERIALS AND METHODS

2.1. Materials

A total of 189 maize materials were tested, including 20 CIMMYT maize inbred lines numbered A1-A20, 169 Guangxi maize inbred lines numbered A21-A189, of which A73, A74, A75 and A76 were control inbred lines (CK), which were Guidan 0810 female parent, Guidan 0810 male parent, Guidan 162 female parent and Guidan 162 male parent respectively. In addition, the gene information of five Chinese backbone inbred lines (suwan1611, chang7-2, zheng58, qi319 and Mo17) was added for data analysis. The lines name and source of the tested maize materials (Table 1).

Table 1. List of 169 maize inbred lines and their source

Code	Lines name	Source
A1	CML408	CIMMYT
A2	P2-61	CIMMYT
A3	P3-97	CIMMYT
A4	DSG390	CIMMYT
A5	DSG419	CIMMYT
A6	DSG421	CIMMYT
A7	DSG426	CIMMYT
A8	DSG439	CIMMYT
A9	DSG441	CIMMYT
A10	DTMA-131	CIMMYT
A11	DTMA-191	CIMMYT
A12	DTMA-199	CIMMYT
A13	DTMA-201	CIMMYT
A14	CLYN457	CIMMYT
A15	CLYN548	CIMMYT
A16	CML228	CIMMYT
A17	CML462	CIMMYT
A18	CLYN460	CIMMYT

Code	Lines name	Source
A64	D1131-6	Guangxi
A65	D1131-5	Guangxi
A66	P9540	Guangxi
A67	D2460	Guangxi
A68	GN811	Guangxi
A69	GNV	Guangxi
A70	P1290	Guangxi
A71	NS-7	Guangxi
A72	P23	Guangxi
A73	Gui39722	Guangxi
A74	Guizhao18421	Guangxi
A75	SP221	Guangxi
A76	Xian21A	Guangxi
A77	GXT444	Guangxi
A78	GXT445	Guangxi
A79	GXT446	Guangxi
A80	GXT447	Guangxi
A81	GXT448	Guangxi

Code	Lines names	Source
A127	GXT494	Guangxi
A128	GXT495	Guangxi
A129	GXT496	Guangxi
A130	GXT497	Guangxi
A131	GXT498	Guangxi
A132	GXT499	Guangxi
A133	GXT500	Guangxi
A134	GXT501	Guangxi
A135	GXT502	Guangxi
A136	GXT503	Guangxi
A137	GXT504	Guangxi
A138	GXT505	Guangxi
A139	GXT506	Guangxi
A140	GXT507	Guangxi
A141	GXT508	Guangxi
A142	GXT509	Guangxi
A143	GXT510	Guangxi
A144	GXT511	Guangxi

Code	Lines name	Source
A19	CML284	CIMMYT
A20	DSG427	CIMMYT
A21	KJ15	Guangxi
A22	D1303-2	Guangxi
A23	Q98	Guangxi
A24	U269-7-3	Guangxi
A25	D1126-1	Guangxi
A26	D1101	Guangxi
A27	D1102	Guangxi
A28	D1110-3	Guangxi
A29	YD1	Guangxi
A30	YN3-2	Guangxi
A31	D1123-1	Guangxi
A32	D904-6	Guangxi
A33	1211	Guangxi
A34	P103	Guangxi
A35	Q933-8	Guangxi
A36	D1216-4	Guangxi
A37	LV99-5	Guangxi
A38	Q925-3	Guangxi
A39	D908-5	Guangxi
A40	P807-2	Guangxi
A41	D1113-2	Guangxi
A42	D1202-1	Guangxi
A43	D1107-2	Guangxi
A44	D1128-1	Guangxi
A45	D906-6	Guangxi
A46	D907-1	Guangxi
A47	D904-7	Guangxi
A48	D901	Guangxi
A49	U269-1-3	Guangxi
A50	D1113	Guangxi
A51	YD2	Guangxi
A52	H161	Guangxi

Code	Lines name	Source
A82	GXT449	Guangxi
A83	GXT450	Guangxi
A84	GXT451	Guangxi
A85	GXT452	Guangxi
A86	GXT453	Guangxi
A87	GXT454	Guangxi
A88	GXT455	Guangxi
A89	GXT456	Guangxi
A90	GXT457	Guangxi
A91	GXT458	Guangxi
A92	GXT459	Guangxi
A93	GXT460	Guangxi
A94	GXT461	Guangxi
A95	GXT462	Guangxi
A96	GXT463	Guangxi
A97	GXT464	Guangxi
A98	GXT465	Guangxi
A99	GXT466	Guangxi
A100	GXT467	Guangxi
A101	GXT468	Guangxi
A102	GXT469	Guangxi
A103	GXT470	Guangxi
A104	GXT471	Guangxi
A105	GXT472	Guangxi
A106	GXT473	Guangxi
A107	GXT474	Guangxi
A108	GXT475	Guangxi
A109	GXT476	Guangxi
A110	GXT477	Guangxi
A111	GXT478	Guangxi
A112	GXT479	Guangxi
A113	GXT480	Guangxi
A114	GXT481	Guangxi
A115	GXT482	Guangxi

Code	Lines names	Source
A145	GXT512	Guangxi
A146	GXT513	Guangxi
A147	GXT514	Guangxi
A148	GXT515	Guangxi
A149	GXT516	Guangxi
A150	GXT517	Guangxi
A151	GXT518	Guangxi
A152	GXT519	Guangxi
A153	GXT520	Guangxi
A154	GXT521	Guangxi
A155	GXT522	Guangxi
A156	GXT523	Guangxi
A157	GXT524	Guangxi
A158	GXT525	Guangxi
A159	GXT526	Guangxi
A160	GXT527	Guangxi
A161	GXT528	Guangxi
A162	GXT529	Guangxi
A163	GXT530	Guangxi
A164	GXT531	Guangxi
A165	GXT532	Guangxi
A166	GXT533	Guangxi
A167	GXT534	Guangxi
A168	GXT535	Guangxi
A169	GXT536	Guangxi
A170	GXT537	Guangxi
A171	GXT538	Guangxi
A172	GXT539	Guangxi
A173	GXT540	Guangxi
A174	GXT541	Guangxi
A175	GXT542	Guangxi
A176	GXT543	Guangxi
A177	GXT544	Guangxi
A178	GXT545	Guangxi

Code	Lines name	Source
A53	P808	Guangxi
A54	Z103-8	Guangxi
A55	Z103-9	Guangxi
A56	D1209-9	Guangxi
A57	D1217-2	Guangxi
A58	D1216-11	Guangxi
A59	P907	Guangxi
A60	D903	Guangxi
A61	D905-4	Guangxi
A62	Q934-5	Guangxi
A63	D1304-4	Guangxi

Code	Lines name	Source
A116	GXT483	Guangxi
A117	GXT484	Guangxi
A118	GXT485	Guangxi
A119	GXT486	Guangxi
A120	GXT487	Guangxi
A121	GXT488	Guangxi
A122	GXT489	Guangxi
A123	GXT490	Guangxi
A124	GXT491	Guangxi
A125	GXT492	Guangxi
A126	GXT493	Guangxi

Code	Lines names	Source
A179	GXT546	Guangxi
A180	GXT547	Guangxi
A181	GXT548	Guangxi
A182	GXT549	Guangxi
A183	GXT550	Guangxi
A184	GXT551	Guangxi
A185	GXT552	Guangxi
A186	GXT553	Guangxi
A187	GXT554	Guangxi
A188	GXT555	Guangxi
A189	GXT556	Guangxi

2.2. Methods

DNA of maize inbred lines was extracted by CTAB method (Rogers *et al*., 1985) and RNA was removed. The 10K SNP chips developed by ZhongYu Jin Labeling (Beijing) Biotechnology Co., Ltd. was used for genotype detection. After gene detection, the genotype was analyzed by Affymetrix (Thermo Fisher) axiom analysis suite software.

2.3. Data Statistical Analysis

Zhongyuxin NO.1 was used to analyze the genotypes of maize inbred lines. The samples with DQC > 0.82 and CR (marker detection rate) > 97% were subjected to SNP locus quality control, and the remaining 9433 markers. The data were combined with the company's germplasm resource database data (suwan1611, Chang7-2, Zheng58, Qi319 and Mo17), filtered by miss < 0.1 and maf > 0.05, and finally 8213 available markers were obtained. The genetic similarity between inbred lines was calculated by markers, and the nj-tree model of Treebest software was used to construct the evolutionary tree (Vilella *et al*., 2009).

3. RESULTS AND ANALYSIS

3.1. Sample Heterozygosity Detection Statistics

The heterozygous rate of the sample is calculated by dividing the number of heterozygous markers of the sample by the total number of markers. The heterozygosity of samples reflects the homozygosity of materials. The higher the self intersection algebra, the lower the heterozygosity of materials. The heterozygosity rate of high generation inbred lines generally does not exceed 10% (Jiang *et al.,* 2018). Distribution map of heterozygosity of maize inbred lines (Fig.1). The map shows that except A110, A186 and A134, the variation range of heterozygosity of other maize inbred lines is less than 10%, with an average value of 2.51%, indicating that most maize inbred lines have high homozygosity.

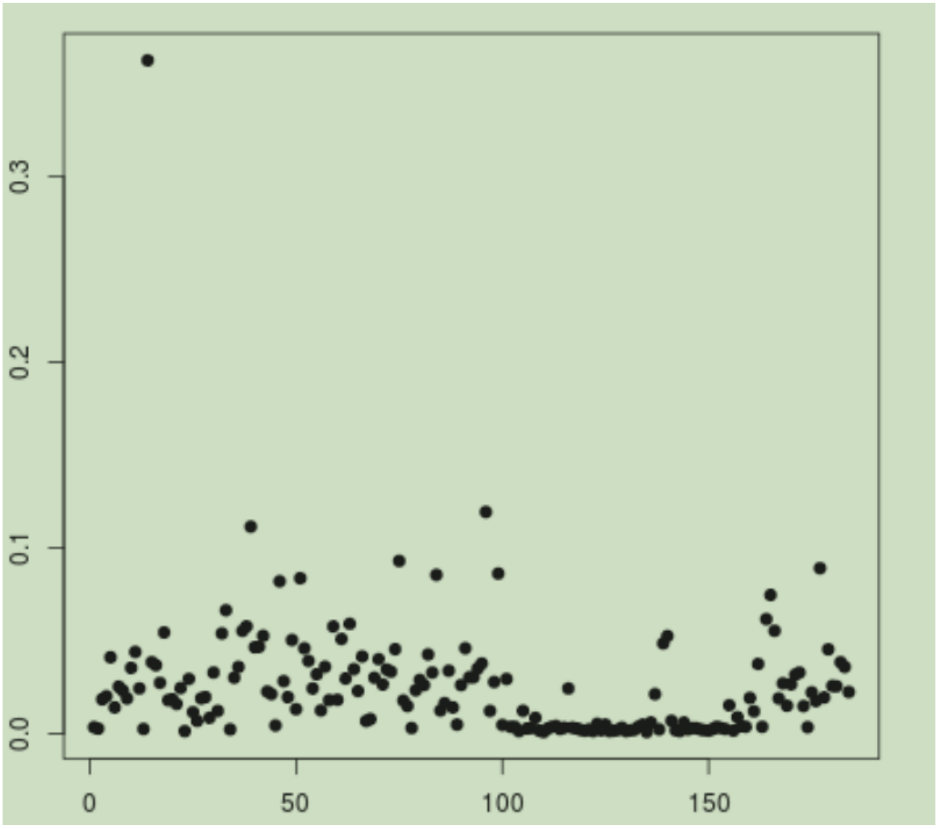

Fig.1. Distribution map of heterozygosity of maize inbred lines

Table 2. Genetic similarity between 20 CIMMYT inbred lines and the top 10 and last 10 inbred lines

Materials code		Inbred lines with top 10 similarity									
		top1	top2	top3	top4	top5	top6	top7	top8	top9	top10
A1	Lines	A8	A70	A6	A67	A14	A3	A5	A4	A15	A116
	Similarity rate	0.733	0.681	0.662	0.632	0.627	0.627	0.627	0.625	0.624	0.621
A2	Lines	A3	A5	A67	A14	A15	A70	A4	A1	A8	A71
	Similarity rate	0.917	0.917	0.872	0.844	0.820	0.667	0.622	0.621	0.618	0.617
A3	Lines	A5	A67	A2	A14	A15	A70	A145	A4	A13	A1
	Similarity rate	0.948	0.938	0.917	0.904	0.887	0.670	0.635	0.632	0.628	0.627
A4	Lines	A13	A71	A105	A117	A116	A148	A5	A3	A101	A106
	Similarity rate	0.658	0.658	0.640	0.638	0.634	0.633	0.632	0.632	0.632	0.632
A5	Lines	A3	A67	A2	A14	A15	A70	A145	A4	A13	A1
	Similarity rate	0.948	0.937	0.917	0.904	0.887	0.670	0.634	0.632	0.627	0.627
A6	Lines	A8	A1	CK_A76	A70	A27	A26	A158	A57	A59	A13
	Similarity rate	0.672	0.662	0.634	0.625	0.623	0.622	0.621	0.619	0.619	0.619
A7	Lines	A97	A95	A103	A100	A99	A35	A20	A24	A101	A49
	Similarity rate	0.667	0.665	0.659	0.657	0.645	0.644	0.642	0.642	0.642	0.638
A8	Lines	A1	A6	A70	A13	A14	A15	A3	A5	A67	A19
	Similarity rate	0.733	0.672	0.652	0.632	0.627	0.626	0.624	0.623	0.623	0.622
A9	Lines	A10	CK_A75	A71	A163	A165	A122	A175	A164	A22	A118
	Similarity rate	0.709	0.672	0.670	0.666	0.658	0.646	0.646	0.644	0.643	0.640
A10	Lines	A9	CK_A75	A71	A163	A165	A122	A175	A164	A22	A118
	Similarity rate	0.709	0.674	0.669	0.667	0.659	0.648	0.646	0.646	0.644	0.642
A11	Lines	A12	A17	A13	A35	CK_A75	A72	A71	A4	A67	A14
	Similarity rate	0.699	0.650	0.624	0.621	0.621	0.620	0.619	0.619	0.617	0.616
A12	Lines	A11	A17	A13	A35	CK_A75	A72	A71	A4	A67	A14
	Similarity rate	0.699	0.651	0.625	0.623	0.622	0.622	0.621	0.620	0.619	0.618

Inbred lines with last 10 similarity									
last1	last2	last3	last4	last5	last6	last7	last8	last9	last10
A110	Mo17	A55	A54	Zheng58	A186	A184	A185	A134	A189
0.425	0.478	0.487	0.489	0.501	0.509	0.526	0.526	0.530	0.533
A110	Mo17	Zheng58	A55	A186	A54	A134	A184	A161	A185
0.428	0.463	0.504	0.511	0.511	0.513	0.514	0.527	0.527	0.536
A110	Mo17	Zheng58	A55	A54	A186	A134	A184	A161	A167
0.434	0.466	0.496	0.502	0.505	0.520	0.522	0.526	0.533	0.539
A110	Mo17	A55	A54	Zheng58	A186	A134	A184	A189	A185
0.450	0.479	0.488	0.490	0.511	0.512	0.534	0.536	0.547	0.550
A110	Mo17	Zheng58	A55	A54	A186	A134	A184	A161	A167
0.434	0.466	0.496	0.500	0.503	0.519	0.522	0.526	0.532	0.539
A110	Mo17	A55	A54	Zheng58	A186	A134	A161	A184	A140
0.432	0.451	0.486	0.487	0.489	0.508	0.522	0.529	0.529	0.533
A110	Mo17	A55	A54	Zheng58	A186	A140	A189	A161	A185
0.455	0.477	0.503	0.505	0.513	0.522	0.535	0.537	0.539	0.541
A110	Mo17	A55	Zheng58	A54	A186	A134	A184	A189	A167
0.438	0.467	0.495	0.497	0.497	0.512	0.530	0.533	0.535	0.536
A110	Mo17	A55	A54	Zheng58	A186	A184	A143	A134	A161
0.497	0.467	0.492	0.494	0.483	0.511	0.547	0.578	0.613	0.519
A110	Mo17	A55	A54	Zheng58	A186	A184	A143	A134	A161
0.431	0.479	0.495	0.497	0.514	0.528	0.534	0.535	0.539	0.540
A110	Mo17	A55	A54	Zheng58	A186	A134	A167	A185	A82
0.423	0.479	0.498	0.500	0.506	0.513	0.530	0.532	0.533	0.535
A110	Mo17	A55	A54	Zheng58	A186	A134	A167	A185	A82
0.423	0.481	0.498	0.500	0.508	0.513	0.531	0.532	0.534	0.535

Materials code		Inbred lines with top 10 similarity									
		top1	top2	top3	top4	top5	top6	top7	top8	top9	top10
A13	Lines	A4	A101	A8	A35	A103	A15	A63	A100	A64	A3
	Similarity rate	0.658	0.636	0.632	0.631	0.631	0.629	0.629	0.629	0.629	0.628
A14	Lines	A67	A5	A3	A15	A2	A70	A4	A16	A1	A8
	Similarity rate	0.910	0.904	0.904	0.849	0.844	0.662	0.629	0.627	0.627	0.627
A15	Lines	A3	A5	A67	A14	A2	A70	A13	A4	A8	A1
	Similarity rate	0.887	0.887	0.880	0.849	0.820	0.653	0.629	0.628	0.626	0.624
A16	Lines	A42	A56	A68	A25	A28	A47	A65	CK_A75	A61	A27
	Similarity rate	0.668	0.666	0.655	0.653	0.653	0.651	0.650	0.649	0.648	0.647
A17	Lines	A12	A11	A35	A67	A5	A4	A103	A3	A70	CK_A75
	Similarity rate	0.651	0.650	0.628	0.624	0.623	0.622	0.621	0.621	0.620	0.620
A18	Lines	A19	CK_A76	A8	A72	A35	A4	A1	A13	A14	A59
	Similarity rate	0.899	0.626	0.621	0.617	0.616	0.615	0.615	0.614	0.613	0.613
A19	Lines	A18	CK_A76	A8	A72	A35	A4	A1	A13	A27	A87
	Similarity rate	0.899	0.626	0.622	0.618	0.616	0.616	0.615	0.614	0.613	0.613
A20	Lines	A7	A95	A16	A33	A97	A34	A103	CK_A74	A42	A99
	Similarity rate	0.604	0.588	0.604	0.601	0.592	0.581	0.591	0.592	0.599	0.594

Inbred lines with last 10 similarity									
last1	last2	last3	last4	last5	last6	last7	last8	last9	last10
A110	Mo17	A55	A54	Zheng58	A186	A161	A134	A185	A140
0.445	0.479	0.502	0.505	0.507	0.514	0.534	0.537	0.538	0.538
A110	Mo17	Zheng58	A55	A54	A186	A134	A184	A161	A167
0.432	0.471	0.488	0.494	0.495	0.509	0.518	0.524	0.531	0.531
A110	Mo17	A55	A54	Zheng58	A186	A184	A134	A183	A167
0.432	0.471	0.494	0.495	0.488	0.509	0.524	0.518	0.540	0.531
A185	A161	A162	A187	A188	A186	A58	A61	A51	A78
0.593	0.564	0.579	0.587	0.568	0.594	0.546	0.647	0.648	0.632
A110	Mo17	A55	Zheng58	A54	A186	A134	A161	Qi319	A167
0.432	0.460	0.491	0.493	0.495	0.507	0.523	0.537	0.538	0.539
A110	A55	A54	Mo17	Zheng58	A186	A161	A134	A184	A185
0.437	0.481	0.483	0.487	0.494	0.500	0.512	0.513	0.519	0.521
A110	A55	A54	Mo17	Zheng58	A186	A134	A161	A184	A185
0.437	0.481	0.483	0.486	0.495	0.501	0.512	0.512	0.518	0.521
A110	A55	A54	Mo17	Zheng58	A186	A134	A161	A184	A185
0.437	0.481	0.483	0.486	0.495	0.501	0.512	0.512	0.518	0.521

3.2. Genetic Similarity Analysis

The higher the genetic similarity between materials, the closer the blood relationship. Table 2 shows that there were 7, 7, 7, 3, 7, 3, 1, 4, 1, 1, 4, 5, 4, 8, 8, 0, 5, 6, 5 and 2 CIMMYT inbred lines in the top 10 of A1-A20 similarity respectively. A3 and A5 had the highest similarity, reaching 0.948. The highest similarity between A1-A20 and Guangxi inbred lines was A3 and A67, up to 0.937. According to the pedigree, the inbred line comes from the improved inbred line of CML161. Therefore, after excluding A67, the highest similarity between A1-A20 and Guangxi inbred lines was A1 and A70, only 0.681. In addition, there were relatively high similarities between Guangxi control inbred lines A74, A75 and A76 and some CIMMYT inbred lines. The last 10 in the similarity ranking of A1-A20 belong to Guangxi inbred lines and Chinese backbone inbred lines Mo17, Zheng58 and Qi319. The lowest similarity between A1-A20 and Guangxi inbred lines were A11 and A110, A12 and A110, both of which were 0.423. It could be seen that CIMMYT inbred lines had high genetic similarity with each other, and CIMMYT inbred lines have low genetic similarity with most maize inbred lines in Guangxi and Chinese backbone inbred lines, especially with three Chinese backbone inbred lines Mo17, zheng58 and Qi319.

3.3. Phylogenetic Tree Analysis

Phylogenetic tree (also known as evolutionary tree) is a branch graph or tree that describes the evolutionary order between populations, which is used to represent the evolutionary relationship between populations. According to the similarities or differences in physical or genetic characteristics of populations, their genetic relationship can be inferred.Figure 2 shows that 189 inbred lines could be divided into PB group represented by Qi319, SPT group represented by sw1611 and Chang7-2, Guidan162-0810 female parent group represented by sp221 and Gui39722, Guidan162 male parent group represented by Xian 21A, Guidan0810 male parent group represented by Guizhao 18421 and other groups that do not belong to the above groups.It could be seen from Figure 2 that there were 6 inbred lines in PB group, including A55, A54, A184, A183, A182 and A69, all of which were Guangxi maize inbred lines. There were 20 inbred lines in SPT group, including A5, A3, A67, A14, A2, A15, A145, A8, A1, A70, A6, A19, A18, A4, A13, A12, A11, A17, A34 and A146, of which 15 were CIMMYT maize inbred lines and 5 were Guangxi maize inbred lines. There were 56 inbred lines in Guidan162-0810 female parent group, including A21、A49、A150、A71、A72、A75、A10、A9、A163、A175、A98、A140、A142、A139、A144、A143、A141、A73、A115、A117、A120、A126、A116、A114、A119、A111、A112、A118、A52、A29、A66、A164、A165、A135、A136、A138、A110、A107、A108、A105、A106、A109、A92、A53、A30、A178、A180、A179、A181、A104、A60、A25、A33、A42、A28 and A56, of which 2 were CIMMYT maize inbred lines and 54 were Guangxi maize inbred lines. There were 10 inbred lines in Guidan 162 male parent group, including A76、A159、A158、A82、A169、A168、A43、A81、A23 and A189, all of which were Guangxi maize inbred lines. There were 50 inbred lines in Guidan 0810 male parent group, including A48、A64、A65、A22、A44、A37、A68、A74、A121、A122、A46、A38、A62、A155、A152、A156、A151、A153、A154、A157

、A167、A134、A131、A128、A127、A132、A125、A123、A124、A129、A130、A170、A171、A174、A176、A177、A172、A173、A35、A24、A49、A91、A83、A89、A90、A85、A86、A88、A84 and A87, all of which were Guangxi maize inbred lines. There were 46 inbred lines in other group, including A166、A20、A7、A59、A133、A137、A147、A148、A93、A94、A97、A99、A96、A100、A101、A95、A102、A103、A16、A45、A36、A57、A26、A27、A41、A50、A77、A79、A78、A80、A63、A51、A58、A31、A32、A39、A47、A40、A61、A162、A188、A187、A186、A161、A160 and A185, of which 3 were CIMMYT maize inbred lines and 43 were Guangxi maize inbred lines. It could be seen that the population categories of CIMMYT maize inbred lines and Guangxi maize inbred lines were very different.

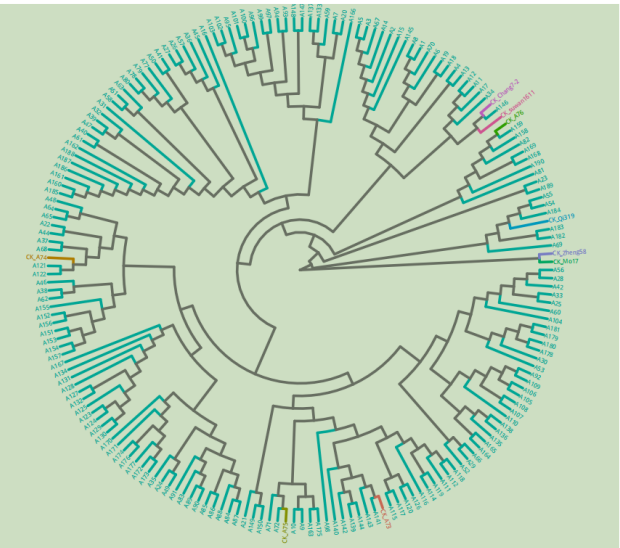

Fig. 2. Phylogenetic tree of 189 maize inbred lines based on SNP markers

4. DISCUSSION

Maize heterosis group and its model are important basis for guiding maize breeding practice. SNP markers can analyze the genetic distance and genetic similarity between maize inbred lines, which is the basis of cluster analysis and heterosis group division. In this study, 5 maize backbone inbred lines (Qi319, sw1611, Chang7-2, Zheng58 and Mo17) commonly used in China were selected as the reference inbred lines (CK) for the division of heterosis groups. These reference inbred lines are the backbone parent resources used in maize production in China at present or in history, covering three main heterosis groups (PB group represented by Qi319, SPT group represented by sw1611 and Chang7-2, LAN group represented by zheng58 and Mo17). And 4 Guangxi backbone inbred lines (Gui 39722, Guizhao 18421, SP221 and Xian 21A) were selected as reference inbred lines, which were the female and male parents of Guidan 0810 and Guidan 162, the main maize varieties in Guangxi. Guangxi maize inbred lines have more tropical blood, while Chinese Backbone inbred lines have more temperate blood. The Backbone Inbred Lines of China and Guangxi are selected as the reference inbred lines, which can ensure the breadth and accuracy of grouping.

Using 10K maize SNP chips developed by ZhongYu Jin Labeling (Beijing) Biotechnology Co., Ltd., 20 CIMMYT and 169 Guangxi inbred lines were labeled, and the heterosis groups were divided according to genetic distance. All materials were divided into 6 groups, including PB group, SPT group, Guidan 162-0810 female parent group, Guidan 162 male parent group, Guidan 0810 male parent group and other groups. Among them, 75% (15) CIMMYT inbred lines were divided into SPT group, and only 3% (5) Guangxi maize inbred lines belonged to this group. Most maize inbred lines in Guangxi were divided into Guidan 162-0810 female parent group, Guidan 0810 male parent group and other groups, with 54, 50 and 44 respectively, accounting for 32%, 30% and 26%, while CIMMYT inbred lines were divided into 2, 0 and 3 respectively, accounting for 10%, 0 and 15%. It could be seen that most of CIMMYT and Guangxi maize inbred lines do not belong to the same group, and the probability of obtaining excellent maize hybrids is high。

Zhao *et al*. believe that foreign high-quality germplasm resources can form complementary advantages with regional variety resources after improvement (Zhao *et al*., 2018). On the basis of maintaining the original advantages, the introduced materials also have strong heterosis and can play an important role in variety breeding. The breeding practice in Guangxi had confirmed that CIMMYT maize material played a very important role in maize breeding in Guangxi (Chen *et al*., 2011). The results of this study obtained the grouping results between CIMMYT and Guangxi maize inbred lines, which provided a theoretical basis for the scientific and rational use of CIMMYT and Guangxi inbred lines for hybrid matching, avoiding a lot of blind matching work, and improving the utilization efficiency of CIMMYT inbred lines.

In addition, SNP chips can also judge the purity and genetic similarity of maize inbred lines. The results showed that the heterozygosity of A110, A134 and A186 inbred lines were higher than 10%, indicating that the purity of these three inbred lines were poor and not stable enough, so they need to continue inbreeding. Genetic similarity analysis showed that A67 had high similarity with many CIMMYT inbred lines. Through pedigree investigation, it was found that A67 was a backcross improved type from the early introduced CIMMYT inbred line CML161. Therefore, genetic similarity analysis can identify the authenticity of the sources of different materials.

REFERENCES

Chen, T.Y., Lu, S.Q., Li, Y.Q., *et al*. (2010). Application of CIMMYT germplasm in maize breeding in Guangxi. Guangxi Agricultural Sciences. 41(8): 745-748.

Chen, T.Y., Wu, Y.S., Ma, L., *et al*. (2011). Heterosis among CIMMYT lines and Guangxi key inbred lines in white maize. Southwest China Journal of Agricultural Sciences. 24(6), 2038-2043.

Fu, Z.J., Yang, H., Qi, Z.Y., *et al*. (2014). Combining ability of ear traits of 6 maize inbred lines from CIMMYT. Hubei Agricultural Sciences. 53(14): 3241-3243.

Jiang, S.Q., Guo, R., Zhao, A., *et al*. (2018). Heterotic grouping by core SNP markers for maize inbred lines widely used in Liaoning China. Journal of Maize Sciences. 26(4): 17-23.

Lu, Y., Yan, J., Guimaraes, C., *et al*. (2009). Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. Theoretical and Applied Genetics. 120: 93-115.

Mo, R.X., Huang, K.J., Huang, A.H., *et al*. (2019). Analysis on combining ability of main agronomic traits of 20 low nitrogen tolerance maize inbred lines from CIMMYT. Southwest China Journal of Agricultural Sciences. 32(12): 2732-2739.

Pan, G.T., Yang, K.C., Li, W.Z., *et al*. (2020). A review of the research and application of heterotic groups and patterns of maize breeding in southwest China. Journal of Maize Sciences. 28(1), 1-8.

Rogers, S.O., Bendich, A.J., (1985). Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol. 5: 69-76.

Semagn, K., Magorokosho, C., Bindiganavile, S.V., *et al*. (2012). Warburton ML: Molecular characterization of diverse CIMMYT maize inbred lines from eastern and southern Africa using single nucleotide polymorphic markers. BMC Genomics. 13(1): 113.

Vilella, A.J., Severin, J., Ureta-vidal, A., *et al*. (2009). Compara GeneTrees: Complete, duplication-aware phylogenetictrees in vertebrates. Genome Research. 19(2): 327-335.

Yan, J., Yang, X., Sha, H.T., *et al*. (2010). High-throughput SNP genotyping with the Golden-Gate assay in maize. Mol. Breed. 25: 441-451.

Zhao, J.R., Li, C.H., Song, W., *et al*. (2018). Genetic diversity and population structure of important Chinese maize breeding germplasm revealed by SNP-Chips. Scientia Agricultura Sinica. 51(4): 626-644.