GENETIC DIVERSITY AND POPULATION STRUCTURE AMONG 98 MAIZE INBRED LINES INVESTIGATED WITH SSR MARKERS

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Abstract. The conversion of exotic germplasm into domestic maize breeding materials is essential to solve the narrow genetic base for maize improvement in China. The present study applied SSR markers and three complementary cluster methods (STRUCTURE, UPGMA and PCoA) to 98 foreign hybrid-selected lines for effective hybrid breeding. A total of 450 alleles, with an average of 3.98 alleles per locus, were detected**.** Among 10 chromosomes, there were 13-19 loci and 33-59 alleles with averaged alleles 2.54~3.43 per chromosome. The polymorphism information content (PIC) among 10 chromosomes ranged from 0.4504~0.5582 and 69.4% PIC variation were explained by allele number/Locus. The highest PIC was observed in chromosome 7 (0.5582) and the lowest in chromosome 8 (0.4965). The STRUCTURE clustering analysis grouped the test lines into four subpopulations (i.e. REID, Lancaster, P and Domestic) in accordance with UPGMA and PCoA clustering**.** The higher genetic diversity were detected among the inbred lines in P and Domestic subpopulations. The allele frequencies, gene diversity and population structure obtained in the present study lead us to conclude that the 98 inbred lines derived from foreign hybrid-selected lines contain extensive genetic variation and are a valuable resource for Chinese maize breeding. The result obtained in the present study will assist in effective utilization of the lines in Chinese hybrid maize breeding programs.
Keywords: *maize germplasm, hybrid-selected line, genetic variation, clustering analysis, maize breeding*

Introduction

Knowledge of genetic diversity and population structure among inbred lines and breeding materials is of great importance for maize hybrid breeding. With the popularization and application of maize hybrids over the past years, the recurrent use of a few elite germplasm lines as parental stock has led to a decrease in genetic diversity among maize breeding materials in China. The introduction of exotic germplasm, using its abundant genetic variation and good agronomic traits, is therefore essential to solve the narrow genetic base for maize improvement in China (Wen et al., 2012; Yong et al.,

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2013). However, it is necessary to make a comprehensive evaluation on the genetic diversity and population structure of exotic germplasm (Tarter et al., 2004; Šarčević et al., 2008; Živanović et al., 2012).

Molecular markers can be employed to investigate levels of genetic diversity and population structure among maize inbred lines and breeding materials. SSRs, due to its abundant, highly polymorphic, genome specific, codominant in nature, have found application in analyses of genetic diversity, population structure, gene mapping, and assisted selection for maize improvement (Phumichai et al., 2012; Wende et al., 2013; Semagn et al., 2014; Yang et al., 2013; Abakemal et al., 2015).

In the present study, 98 foreign hybrid-selected lines were analyzed using 145 SSR loci distributed over the whole maize genome. Our objectives were to estimate the levels of genetic diversity and population structure. The results will be useful to breeders in selecting the best parental combinations for maize breeding program in China.

Research Design and Methods

Plant Materials

The germplasm contained 5 tester lines (Huangzao 4, Dan 340, B73, Qi 319 and Mo17) and 98 maize inbred lines derived from foreign maize hybrid-selected lines (obtained by Liaoning Leiao seed company, China) was used in this study. The pedigree and/or origin information can be found in *Appendix 1*.

SSR Markers and Genotyping

Genomic DNA was extracted from approximately 200 mg fresh leaf tissue using the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). A total of 500 SSR primers, which were distributed evenly over the 10 maize chromosomes, were selected and synthesized according to the information available in the MaizeGDB database (http://archive.maizegdb.org/).

PCR amplifications were carried out in 10 mL reaction volumes containing 1 μL template DNA, 2 μL each of 2.5 mM primer, 5μL 2×Taq Master Mix, 0.1μL of 5 units μL^{-1} Taq DNA polymerase, 0.4μL 10 mM dNTPs and , dH2O 2μL. PCR protocols consisted of 32 cycles of 94℃ for 45s, an annealing temperature at either 45, 50, 55 or 60°C depending on the individual SSR primers for 45 s, and 72°C for 60s, and a final extension step of 72°C for 10 min. PCR products were analyzed by 8% polyacrylamide gel electrophoresis (PAGE) and visualized by silver staining.

Genetic Diversity Analysis

For each SSR locus, polymorphic bands were scored as 1 or 0 for presence or absence of the bands at the same mobility, respectively. Gene diversity (PIC) was calculated for each marker according to the formula: PIC=1－∑fi2, where fi is the allele frequency for the i-th locus summed across all alleles for that locus. The program PowerMarker v3.25 and Excel was used to calculate allele number, allele frequency, and gene diversity of each locus (Liu et al., 2005).

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Population Structure Analysis

The STRUCTURE v2.3.3 were employed to assess the population structure of the 98 maize inbred lines using the Bayesian model-based approach (Pritchard et al., 2000). The number of subgroups (K), with each K repeated five times, was ranged from 1 to 12, with burn-in of 100,000 and run length of 100,000. We used the ad hoc criterion ΔK related to the second order rate of change in the log probability of data (Ln$P(D)$) to determine the most probable K value (Evanno et al., 2005).

To examine genetic relationships among the 98 maize inbred lines, the data matrices of the genetic similarity were used to create the dendrogram using UPGMA clustering with the computer software NTSYS-pc v2.2 (Rohlf 2009). Principal coordinate analysis (PCoA) was also employed to reveal relationships among the 98 inbred lines using the software JMPversion7.0 (SAS Institute Inc., Cary, NC, USA).

Data Analysis and Results

A total of 500 SSRs, randomly distributed across the maize genome, were used to evaluate the genetic diversity of the 98 maize inbred lines. Finally, 145 SSRs with clear, stable and specific bands were selected to scored on the 98 lines, with an average of 3.98 alleles per locus (range of 2-7). The PIC for all loci ranged from 0.2130 (umc1271) to 0.8316 (bnlg1666) with an average value of 0.5067 (*Appendix 2*). The higher PIC values indicated the high variability of SSRs, and also a large genetic difference among the 98 maize inbred lines.

The SSRs among 10 maize chromosomes ranged from 11 to 19 with allele number from 33-59 *(Table 1)*. The highest allele number was detected on chromosome 1 (59 alleles), followed chromosome 6 (51 alleles), and the lowest on chromosome 8 (33 alleles). For all chromosomes, there were 13-19 loci and 33-59 alleles with averaged alleles 2.54~3.43 per chromosome. The PIC among 10 chromosomes ranged from 0.4504~0.5582. The highest PIC was observed in chromosome 7 (0.5582) and the lowest in chromosome 8 (0.4965).

Table 1. *Genetic diversity at genome level of 98 maize inbred lines revealed by 145 SSR markers*

Chrom	No. of Loci	No. of Alleles	Mean allele number (range)	PIC
1	19	59	3.11	0.4897
2	13	43	3.31	0.5336
3	14	47	3.36	0.5532
4	17	51	3.00	0.5199
5	16	49	3.06	0.5036
6	16	51	3.19	0.4958
7	14	48	3.43	0.5582
8	13	33	2.54	0.4504
9	12	35	2.92	0.4507
10	11	34	3.09	0.5075

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There usually was a positive linear relationship between the polymorphism information content (PIC) and number of alleles within a given range. Simple correlation analysis indicated that the PIC was significantly and positively correlated with the number of alleles (r=0.6566, p<0.001) (*Fig. 1*). The allele number/Locus (x) could explain 69.4% PIC variation (y) estimated by a curvilinear regression equation (y =0.3286 ln (x) +0.1551 (1<x<8), R^2 =0.6937).

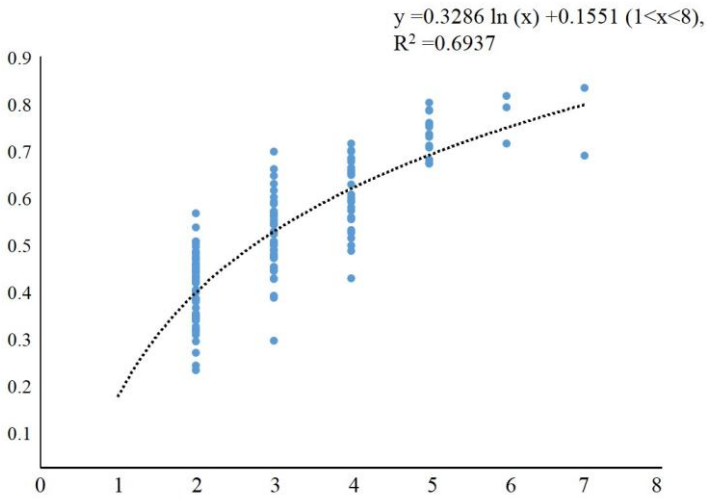

Figure 1. *Plot of PIC vs. number of alleles per SSR locus Population Structure Analysis {tc "5.2.3 Analysis of population structure of mazie core collection" \l 000003}*

STRUCTURE V2.3.3 software was employed to assess the population structure of 98 inbred lines based on 145 whole-genome unlinked SSR markers. Ln$P(D)$ progressively increased as K (2-10) increased and no obvious inflexion point was observed, which Ln$P(D)$, in this case, may not be suitable to estimate the true K value (*Fig. 2*). The peak of ΔK was observed at K=4, suggested that the 98 inbred lines were fell into four sub-populations (*Fig. 2*).

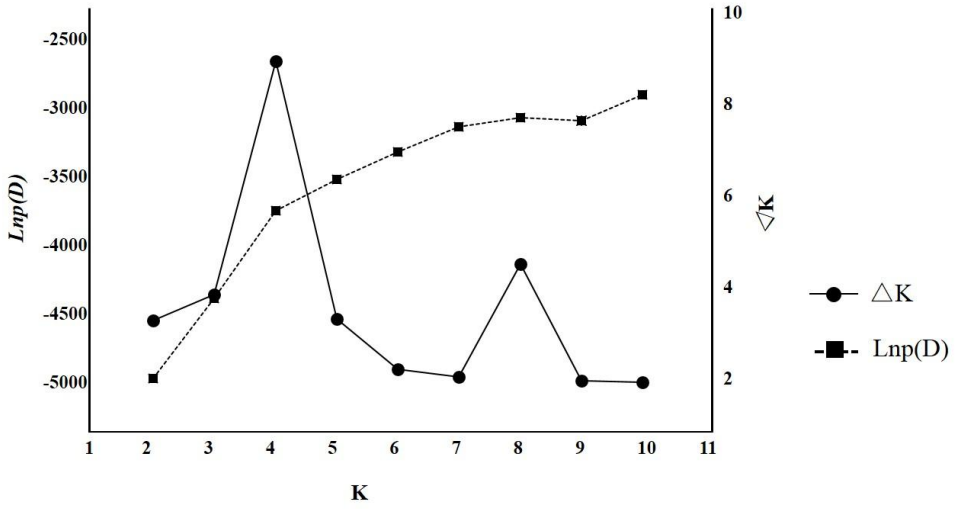

Figure 2. *Plot of LnP (D) and △K calculated for K ranging from 1 to 10*

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Based on the maximum membership probability, 98 inbred lines were assigned into 4 subpopulations (Reid, Lancaster, P, Domestic) (*Fig.3*). Reid included 35 inbred lines closed to B73 (Reid) genetic background. Lancaster had 26 inbred lines related to Mo17 (Lancaster) genetic background. P comprised 22 inbred lines, most derived from Pioneer hybrids (P). The other 15 inbred lines were closed to Dan 340 or Huangzao 4 background, which named as Domestic subgroup described by Xie et al. (2008).

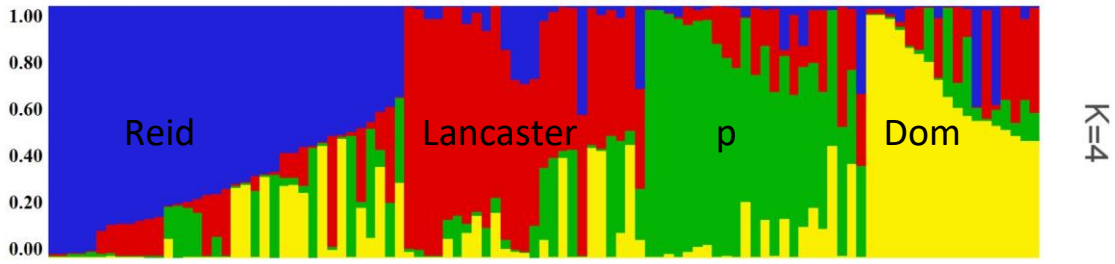

Figure 3. *Population structure of the 98 inbred lines (K=4). Note: The horizontal coordinate represents the 98 inbred lines, and the vertical coordinate of each subgroup indicates the membership coefficients for each individual. Blue zone: Reid; Red zone: Lancaster; Green zone: P; Yellow zone: Domestic*

The similarity coefficient among 98 maize inbred lines ranged from 0.2692 to 0.9825 and the average was 0.6564. When the similarity coefficient was 0.57, UPGMA cluster analysis also clearly grouped 98 inbred lines into four subpopulations (*Fig.4*). Reid, Lancaster P and Domestic subpopulation comprised 36, 32, 13 and 17 inbred lines, respectively. The assignments of 77 inbred lines (78.6% of the total) by UPGMA clustering were consistent with their assignments using STRUCTURE.

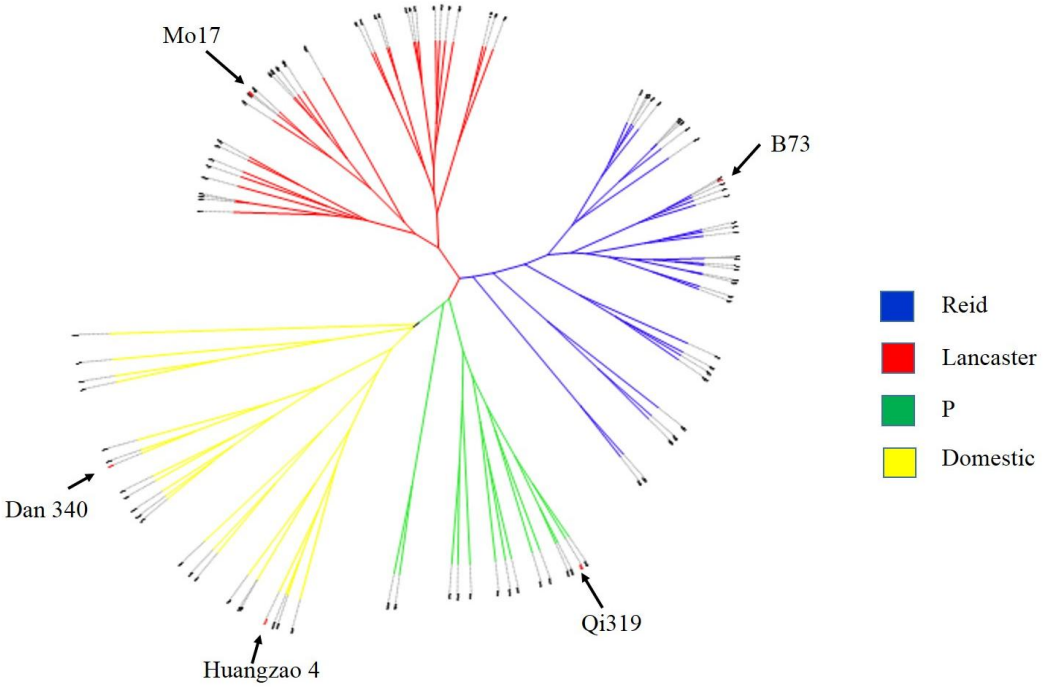

Figure 4. *Dendrogram of 98 maize inbred lines based on UPGMA cluster analysis*

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The principal coordinate analysis (PCoA) based on 145 SSR markers also separated the 98 maize inbred lines into four major groups (*Fig.5*). As inferred by STRUCTURE analysis, the inbred lines in Reid were mainly distributed in the lower left of the plot resulting, Lancaster distributed in the upper right, P in the upper left, and Domestic in the upper right. Most individuals within Reid and Lancaster subpopulations were grouped more closely. The inbred lines in P and Domestic were widely scattered, indicating that higher genetic diversity resided in P and Domestic subpopulations.

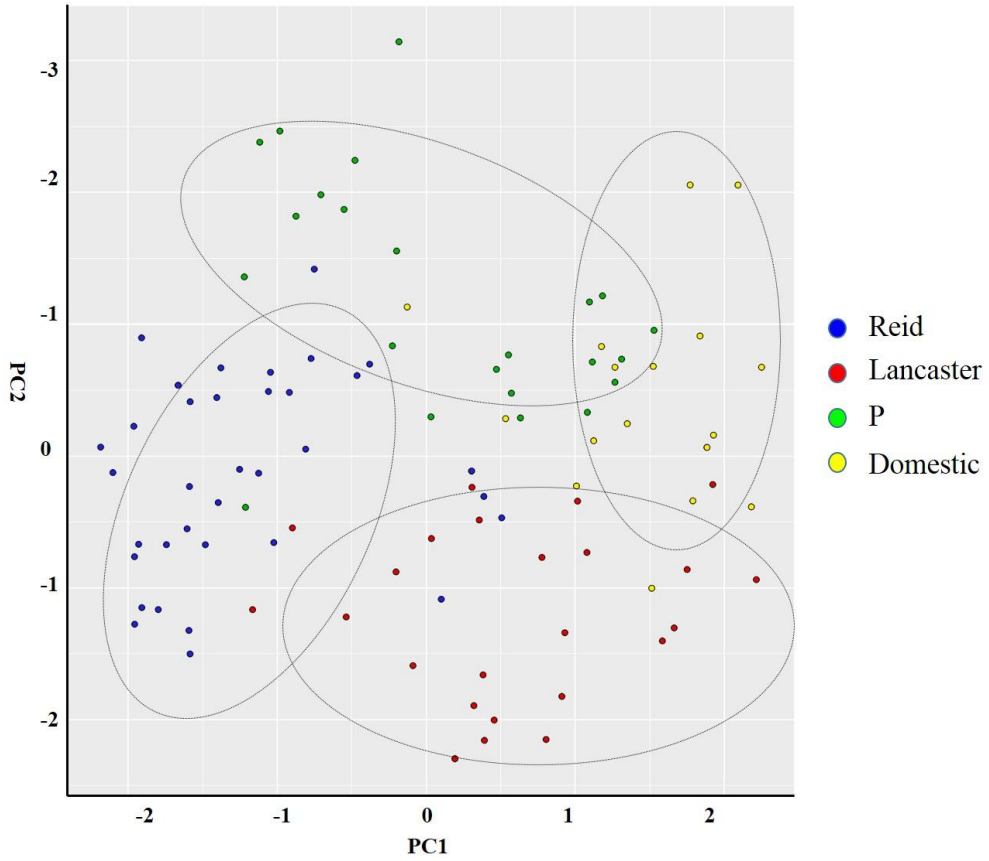

Figure 5. *Principal coordinate analysis of 98 maize inbred lines based on 145 SSR markers. Reid (blue), Lancaster (red), P (green) and Domestic (yellow) are the four subgroups identified by STRUCTURE assigned with the maximum membership probability*

Discussion and Conclusion

SSR markers, due to their abundance, co-dominance, and locus specificity, have been extensively used to assess genetic diversity in maize genotypes (Šarčević et al., 2008; Inghelandt et al., 2010).

In the present study, all the 98 tested maize inbred lines were derived from foreign hybrid-selected lines. We used 145 SSR markers to screen the population and a total of 450 alleles, with an average of 3.103, were detected. The average polymorphic information content (PIC) value was 0.5067, which was lower than that in Chinese important inbred lines with PIC over 0.6 (Wang et al., 2008; Xie et al., 2008). The number of alleles found in this study is also in agreement with other studies (Wang et al., 2008; Park et al., 2015). Wang et al. (2008) reported a total of 1,365 alleles with an

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average of 9.4 alleles per locus by screening 95 inbred lines using SSR markers. Park et al. (2015) genotyped 174 maize inbred lines by 150 SSR markers and detected a total of 1082 alleles with an average of 7.21 alleles per locus. In our study, alleles were obtained at the whole genome level (*Table 1*). Chromosome 1 showed the highest allele number (59 alleles) and chromosome 8 the lowest (33 alleles). Therefore, we have thus determined that there is a higher level genetic diversity in the 98 foreign hybrid-selected lines, which has the potential to enhance the genetic diversity of Chinese maize breeding materials.

Population structure in the present study was also investigated using three complementary analysis methods STRUCTURE, UPGMA and PCoA based on SSR data. We selected maximum membership probability as the subgroup subdivision criterion, 98 maize inbred lines were assigned into four subpopulations which was in agreement with the assignments obtained by UPGMA and PCoA clustering. Nevertheless, for the 98 tested foreign hybrid-selected lines, the pedigree information was not in accordance with their clustering. In our data, the Domestic subgroup closed to Dan 340 or Huangzao 4 background. This finding can be partially explained by complex genetic background in foreign hybrids. Therefore, it is of significant importance to understand population structure and relationships among inbred lines is for maize improvement.

The allele frequencies, gene diversity and population structure obtained in the present study lead us to conclude that the 98 inbred lines derived from foreign hybrid-selected lines contain extensive genetic variation and are a valuable resource for Chinese maize breeding.

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APPENDIX

Appendix 1. *Germplasm sources and pedigrees of 103 maize acessions*

ID	Inbred line	Pedigree/source
1	Huangzao4	Landrace Tangsipingtou
2	Mo17	C103×187-2
3	Dan340	Baigulu9×Z. mays-tunicata
4	Qi319	selected from Pioneer hybrid ‘‘78599’
5	B73	Iowa Stiff Stalk Synthetic C5 (BSSS)
6	Y005	selected from American hybrid “DK516”
7	Y025	selected from American hybrid “DK516”
8	Y052	selected from American hybrid “DK516”
9	Y057	selected from American hybrid “DK516”
10	Y058	selected from American hybrid “DK516”
11	Y087	selected from American hybrid “DK516”
12	Y088	selected from American hybrid “DK516”
13	Y097	unknown
14	Y099	unknown
15	Y101	unknown
16	Y102	unknown
17	Y113	selected from American hybrid “DK007”
18	Y136	selected from American hybrid “DK007”
19	Y187	selected from American hybrid “DK007”
2	Y195	selected from American hybrid “DK007”
21	Y199	selected from American hybrid “DK007”
22	Y210	selected from American hybrid “DK007”
23	Y219	selected from American hybrid “DK008”
24	Y220	selected from American hybrid “DK008”
25	Y255	selected from American hybrid “DK008”
26	Y263	selected from American hybrid “DK008”
27	Y275	selected from American hybrid “DK008”
28	Y282	selected from American hybrid “DK008”
29	Y295	selected from American hybrid “DK008”
30	Y302	selected from an American hybrid
31	Y316	selected from an American hybrid
32	Y323	selected from an American hybrid
33	Y354	selected from American hybrid “3425”
34	Y361	selected from American hybrid “3425”
35	Y362	selected from American hybrid “3425”
36	Y386	selected from American hybrid “3425”
37	Y393	selected from Pioneer hybrid ‘‘XY335”
38	Y403	selected from Pioneer hybrid ‘‘XY335”
39	Y415	selected from Pioneer hybrid ‘‘XY335”
40	Y416	selected from Pioneer hybrid ‘‘XY335”
41	Y420	selected from Pioneer hybrid ‘‘XY335”
42	Y421	selected from Pioneer hybrid ‘‘XY335”
43	Y426	selected from Pioneer hybrid ‘‘XY335”
44	Y431	selected from Pioneer hybrid ‘‘XY335”
45	Y437	selected from Pioneer hybrid ‘‘XY335”
46	Y443	selected from Pioneer hybrid ‘‘XY335”
47	Y464	selected from Pioneer hybrid ‘‘XY335”

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48	Y467	selected from Pioneer hybrid ‘‘XY335”
49	Y505	selected from Pioneer hybrid ‘‘PR3394”
50	Y509	selected from Pioneer hybrid ‘‘PR3394”
51	Y525	selected from Pioneer hybrid ‘‘PR3394”
52	Y531	selected from Pioneer hybrid ‘‘PR3394”
53	Y532	selected from Pioneer hybrid ‘‘PR3394”
54	Y569	unknown
55	Y570	unknown
56	Y594	selected from American hybrid‘‘3382”
57	Y625	selected from American hybrid ‘‘3382”
58	Y645	selected from American hybrid ‘‘3382”
59	Y647	selected from American hybrid ‘‘3382”
60	Y648	selected from American hybrid ‘‘3382”
61	Y649	selected from American hybrid ‘‘3382”
62	Y683	selected from Pioneer hybrid ‘‘XY222”
63	Y700	selected from Pioneer hybrid ‘‘XY222”
64	Y721	selected from Pioneer hybrid ‘‘XY222”
65	Y728	selected from Pioneer hybrid ‘‘XY222”
66	Y733	unknown
67	Y751	unknown
68	Y753	unknown
69	Y760	selected from Pioneer hybrid ‘‘78599’
70	Y764	selected from Pioneer hybrid ‘‘78599’
71	Y769	selected from Pioneer hybrid ‘‘78599’
72	Y777	selected from Pioneer hybrid ‘‘78599’
73	Y793	selected from Pioneer hybrid ‘‘78599’
74	Y815	selected from Pioneer hybrid ‘‘78599’
75	Y829	selected from Pioneer hybrid ‘‘XY508”
76	Y837	selected from Pioneer hybrid ‘‘XY508”
77	Y839	selected from Pioneer hybrid ‘‘XY508”
78	Y851	selected from Pioneer hybrid ‘‘XY508”
79	Y882	selected from Pioneer hybrid ‘‘XY508”
80	Y886	selected from Pioneer hybrid ‘‘XY508”
81	Y889	selected from Pioneer hybrid ‘‘XY508”
82	Y895	selected from Pioneer hybrid ‘‘XY508”
83	Y897	selected from Pioneer hybrid ‘‘XY508”
84	Y918	selected from Pioneer hybrid ‘‘33G35”
85	Y919	selected from Pioneer hybrid ‘‘33G35”
86	Y920	selected from Pioneer hybrid ‘‘33G35”
87	Y963	selected from Pioneer hybrid ‘‘33G35”
88	Y966	selected from Pioneer hybrid ‘‘33G35”
89	Y1026	selected from Pioneer hybrid ‘‘33G35”
90	Y1043	selected from Pioneer hybrid ‘‘32T24”
91	Y1045	selected from Pioneer hybrid ‘‘32T24”
92	Y1052	unknown
93	Y1069	unknown
94	Y1071	unknown
95	Y1081	unknown
96	Y1084	selected from Pioneer hybrid ‘‘33F20”
97	Y1092	selected from Pioneer hybrid ‘‘33F20”
98	Y1094	selected from Pioneer hybrid ‘‘32D22”
99	Y1099	selected from Pioneer hybrid ‘‘32D22”
100	Y1115	selected from Pioneer hybrid ‘‘32D22”

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101	Y1124	selected from Pioneer hybrid ‘‘32D22”
102	Y1161	selected from Pioneer hybrid ‘‘32D22”
103	Y1183	selected from Pioneer hybrid ‘‘32D22”

Appendix 2. *Chromosome locations for 145 SSRs, and their allele numbers and PIC value detected in 98 inbred lines of maize*

Primer Name	**Bin**	**No. of alleles**	**PIC**	**Primer Name**	**Bin**	**No. of alleles**	**PIC**
umc985	1.06	6	0.7085	umc2400	5.04	3	0.4399
umc1166	1.02	2	0.4301	bnlg389	5.09	4	0.5027
umc1243	1.04	2	0.3888	bnlg1046	5.03	3	0.4637
umc1292	1.01	3	0.4136	bnlg1118	5.07	3	0.4752
umc1358	1.07	2	0.3291	bnlg1237	5.05	5	0.6998
umc1383	1.08	2	0.3082	bnlg2323	5.04	2	0.3004
umc1568	1.02	3	0.5166	umc1250	6.05	4	0.4149
umc1590	1.06	4	0.5438	umc1296	6.06	2	0.3681
umc1706	1.07	3	0.5156	umc1413	6.05	4	0.6408
umc1744	1.11	2	0.3860	umc1474	6.05	2	0.2240
umc1972	1.06	2	0.3636	umc1490	6.07	2	0.3697
umc2012	1.01	4	0.6776	umc1653	6.07	5	0.7820
umc2025	1.05	3	0.4921	umc1656	6.02	3	0.4869
umc2240	1.08	2	0.3318	umc1887	6.03	2	0.4445
bnlg439	1.03	4	0.6564	umc1918	6.04	3	0.5316
bnlg1014	1.01	3	0.4587	umc2006	6.04	3	0.4398
bnlg1025	1.07	4	0.5626	umc2059	6.08	2	0.4585
bnlg1866	1.03	6	0.8137	umc2165	6.07	4	0.5976
bnlg2238	1.04	2	0.4073	umc2312	6.01	3	0.3758
umc1026	2.04	2	0.4426	bnlg107	6.01	4	0.5818
umc1265	2.02	2	0.3364	bnlg161	6.00	5	0.7235
umc1419	2.00	3	0.4313	bnlg1538	6.01	3	0.4943
umc1755	2.06	3	0.5509	umc1066	7.01	2	0.4273
umc1845	2.03	3	0.5324	umc1159	7.01	4	0.6501
umc1875	2.06	4	0.6769	umc1213	7.02	2	0.4040
umc2094	2.01	5	0.7983	umc1407	7.05	3	0.6391
umc2129	2.07	4	0.6692	umc1593	7.03	7	0.6825
umc2214	2.10	2	0.3260	umc1642	7.00	3	0.6911
umc2372	2.06	4	0.5199	umc1718	7.03	2	0.2511
bnlg1017	2.02	3	0.4619	umc1782	7.04	5	0.7452
bnlg1258	2.08	5	0.7293	umc1799	7.06	4	0.6917
bnlg1520	2.09	3	0.4612	umc1944	7.04	2	0.4672
umc1501	3.05	2	0.3027	umc1983	7.02	2	0.4719
umc1539	3.05	4	0.6191	umc2057	7.07	3	0.4930
umc1886	3.02	3	0.5347	bnlg1666	7.04	7	0.8316

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umc2049	3.01	2	0.3834	bnlg2132	7.00	2	0.3683
umc2118	3.00	2	0.4951	umc1130	8.05	2	0.4149
umc2263	3.04	3	0.4672	umc1139	8.01	2	0.3220
umc2266	3.06	2	0.4086	umc1161	8.06	2	0.4546
umc2273	3.07	3	0.5603	umc1384	8.07	3	0.5105
umc2369	3.03	2	0.4614	umc1530	8.03	3	0.4855
bnlg197	3.06	5	0.6653	umc1638	8.09	2	0.5568
bnlg1350	3.08	4	0.6432	umc1663	8.09	3	0.4956
bnlg1496	3.09	5	0.6669	umc1728	8.06	2	0.2907
bnlg1754	3.09	5	0.7816	umc1846	8.05	4	0.4745
bnlg2241	3.06	5	0.7553	umc1984	8.03	2	0.4445
umc1017	4.01	3	0.5118	bnlg666	8.05	4	0.5909
umc1132	4.08	3	0.6059	bnlg1863	8.03	2	0.4831
umc1294	4.02	2	0.3234	bnlg2046	8.04	2	0.3315
umc1716	4.11	3	0.5778	umc1107	9.04	3	0.5137
umc1738	4.10	2	0.4949	umc1271	9.03	2	0.2130
umc1821	4.04	2	0.4281	umc1277	9.07	2	0.2976
umc1847	4.07	2	0.2759	umc1366	9.06	3	0.3711
umc1943	4.02	3	0.4133	umc1492	9.04	2	0.4905
umc2027	4.06	3	0.5921	umc1505	9.08	2	0.3351
umc2039	4.03	2	0.4409	umc1570	9.04	2	0.3240
umc2046	4.09	3	0.5153	umc2128	9.03	3	0.5502
umc2188	4.08	2	0.5254	umc2337	9.03	3	0.4308
bnlg1265	4.05	3	0.4761	umc2343	9.05	5	0.7501
bnlg1444	4.08	5	0.7055	bnlg1191	9.07	4	0.4862
bnlg1621	4.06	4	0.5840	bnlg1401	9.02	4	0.6455
bnlg1890	4.11	4	0.6944	umc1196	10.07	3	0.5811
bnlg2162	4.08	5	0.6742	umc1319	10.01	3	0.5145
umc1019	5.06	6	0.7887	umc1380	10.00	2	0.4580
umc1072	5.07	3	0.6536	umc1477	10.05	4	0.5459
umc1389	5.03	2	0.3367	umc1506	10.05	3	0.5425
umc1478	5.01	2	0.4445	umc1873	10.04	2	0.4724
umc1624	5.04	2	0.3501	umc1877	10.07	3	0.2772
umc1692	5.03	2	0.4182	umc2069	10.02	4	0.5141
umc2036	5.01	3	0.5573	umc2350	10.04	2	0.4190
umc2136	5.08	3	0.6215	bnlg987	10.03	4	0.5482
umc2216	5.06	4	0.5698	bnlg2190	10.06	4	0.7093
umc2386	5.05	2	0.4352				

Note: Bin numbers were determined from maizeGDB

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