ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 8 (3): 358-364, 2009
ISSN 1682-296X
© 2009 Asian Network for Scientific Information

Effective Improvement of Genetic Variation in Maize Lines Derived from R08×Donor Backcrosses by SSRs

Q. Shanbao, W. Yuhua, R. Tingzhao, Y. Kecheng, G. Shibin and P. Guangtang
Maize Research Institute, Key Laboratory of Crop Genetic Resources,
Sichuan Agricultural University, Ministry of Education, Ya'an 625014, Sichuan, China

Abstract: Introduction of exotic germplasm into local adapted maize (*Zea mays* L.) inbred lines might enhance genetic variability and led to greater progress from selection. Eighteen outstanding maize inbred lines with high resistance to Northern Corn Leaf Blight (NCLB) extensively utilized in Northern China were used as donor parents to improve elite line 08-641 (R08), which has being played a central role in breeding hybrids in Southwest of China. As a result, 36 backcross-derived lines (BC-derived lines) including 18 BC_1F_3 and 18 BC_2F_2, were obtained. To evaluate improvement efficiency and genetic variation for BC-derived lines, microsatellites were used to genotype these lines together with recurrent parent, R08 and 18 donor parents. Each genotype had a unique banding profile, the genetic similarity coefficient ranged from 0.398 to 0.981. Average genetic diversity (He) and Shannon's information index were 0.344 (Range: 0.036-0.774) and 0.629 (Range: 0.092-1.542), respectively. The hierarchical analysis of molecular variance (AMOVA) and the coefficient of gene differentiation (G_{ST}) values revealed that most of the variations (average: 65.8%) were presented among lines from different combinations given generation, nearly half of variations (Average: 47.6%) were found between BC_1F_3 and BC_2F_2 given combination. Four SSR markers associated with *Ht2* and *Ht3* genes were also screened in this study and the practicability of these markers assisted selection was discussed. The present study demonstrated that one generation of backcross based on the selection of right donor parents, identification of target traits and general combining ability in self-cross process would be best effective in maize backcross improvement.

Key words: Backcross-derived lines (BC-derived lines), genetic diversity, maize (*Zea mays* L.), microsatellite

INTRODUCTION

Knowledge about genetic variability and breeding stocks was expected to have a significant impact on the improvement of crop plants. In maize, a large and diverse gene pool permitted the manipulation of different genotypes that had led to improved performance of hybrids in terms of yield, resistance to diseases and other agronomic characteristics (Laborda *et al*., 2005). Exotic germplasm was the potential source of new beneficial alleles for introgression into adapted germplasm to increase the variability in qualitatively and quantitatively inherited traits (Echandi and Hallauer, 1996). Broadening and diversifying the genetic base of adapted maize germplasm through the introduction of new genetic variation could further enhance progress from selection for both grain yield and stability of performance (Menkir *et al*., 2006).

Most maize breeders in the world concerned that the genetic base of their cultivars had not become too narrow to face unexpected environmental stresses. Recently, the threat of genetic erosion led to numerous studies of maize genetic diversity had been carried out (Gauthier *et al*., 2002; Labate *et al*., 2003; Clerc *et al*., 2005). Most of these efforts had been limited in analyzing mainly populations or inbreds and it would be necessary to understand the current crop diversity situation from a multi-scale perspective (Van Etten *et al*., 2008). As highlighted by Menkir *et al*. (2006), fewer investigations had been done on Backcross Inbred Lines (BILs), maize inbred lines were regarded as the most promising sources of exotic germplasm because they had been free from deleterious recessive alleles and possessed desirable traits fixed through several generations of inbreeding and selection. Information on the genetic structure of a landrace could help limiting genetic erosion as well as conservation of

Corresponding Author: Y. Kecheng, Maize Research Institute, Sichuan Agricultural University, No. 46 Xin Kang Road, Yucheng District, Ya'an City, Sichuan Province 625014, People's Republic of China
Tel: 0086-835-2882455 Fax: 0086-835-2882154

landraces while also allowing the possible exploitation of genes for traits like resistance or tolerance to biotic and environmental stresses (Barcaccia *et al*., 2003).

The maize inbred line 08-641 (R08) was an elite line with good combining ability, multiple resistance to diseases, big ear and extensive adaption in Southwest of China. More than 20 varieties were released from R08 for maize production. However, the elite line would be susceptible to NCBL sometimes. Eighteen super maize inbred lines with high resistance to NCLB were thus used as donors of beneficial alleles for developing backcross inbred lines. The NCBL was reported to be controlled by *Ht2* and *Ht3* genes (Van Staden *et al*., 2001; Yin *et al*., 2003) and four microsatellites associated with the two genes were also screened in these samples. The first objective of this study was to assess the level of genetic variation retained in backcross-derived lines from diverse genetic background and different numbers of backcross using SSR markers. The second objective of this study was to explore the practicability of molecular marker assisted selection by screening the four associated microsatellites based on NCBL evaluation. Combining these estimates with the data on agronomic performance of testcrosses, we might facilitate the successful use of these backcross-derived lines to increase genetic diversity, improve performance of maize elite line R08 and determine the best effective means based on backcross numbers and right donors in maize backcross improvement.

MATERIALS AND METHODS

This study was conducted during 2006-2008 at the research station of Sichuan Agricultural University.

Genetic materials and inoculation trials: Eighteen super maize inbred lines with diverse genetic backgrounds and resistance to NCBL were chosen as donor parents in this study. The information of each pedigree was shown in Table 1. As receptor and recurrent parent, R08 was an elite inbred line but susceptible to NCBL sometimes. Crosses were made between R08 and each donor parent and each F_1 was backcrossed to R08 at the experimental farm of Sichuan Agricultural University in summer of 2006 to generate the first backcross (BC_1). Subsequently, 18 BC_1F_1 chosen by phenotypic selection based on agricultural performance were self-crossed and backcrossed to R08 to produce 18 BC_1F_2 and 18 BC_2F_1 in summer of 2007. At each generation of inbreeding from 2007 to 2008, phenotypic selection within and among lines was also made. A total of 18 uniform lines (18 BC_1F_3 and 18 BC_2F_2, three individuals were selected for each genotype), which were hereafter referred to as BC-derived lines, were selected for this study.

The single Helminthosporium-leaf spot was isolated and purified on the PDA culture medium (Yin *et al*., 2003). After propagation, the conidia were washed with sterile water containing 2% sucrose and 0.2% Tween 80. Plants at the 4-5 leaf stage were sprayed with the conidial suspension that had been adjusted to the concentration of 10-15 conidia per 10×10 microscope. Inoculated plants (BC_1F_3, BC_2F_2, donor parents and R08) were incubated for 72 h in high humidity with temperature ranged from 20 to 28°C. Inoculation trials of these samples were carried out for two times. After 14 days the plants were scored for resistance and susceptibility.

DNA isolation and microsatellite analysis: Young leaves from single plant of each genotype were collected, lyophilized and ground to powder. The DNA was extracted using the CTAB procedure (Saghai-Maroof *et al*., 1994). For the obtained DNA dilutions, DNA concentrations were determined with a spectrophotometer using as a conversion factor A 260 nm 1.0 = 50.0 ng μL^{-1}.

The SSR sequences were obtained from the Maize Genetics and Genomics Database site (http:// www. maizegdb.org). Forty SSR core primer pairs were selected on the basis of their evaluation for maize inbred lines in China and their distribution in the genome (bin location). The selected primers are shown in Table 2. The microsatellites associated with *Ht2* and *Ht3* genes used in the study were bnlg1152, bnlg1666, umc1149 and umc1202 (Van Staden *et al*., 2001; Yin *et al*., 2003).

Table 1: List of eighteen maize inbred lines used as donor parents in the study

No.	Donor	Parentage	No.	Donor	Parentage
1	Zheng58	Ye478 improved line	10	Chuan321	Xianfeng hybrid78599
2	Lu2548	Qi205×Ye478	11	Qi319	Xianfeng hybrid78599
3	Lu9801	Huang change line	12	835	8112×515 female parent:Zhaoyu2
4	Chang7-2	Huangzao4×Wei95	13	CAL73	CAL73
5	K14	K14	14	Shuang741	(Aijin525×BNP44)×(Huangzao4×A619*)
6	K12	Huang change line	15	Huangye4	(Yejihong×Huangzao4)×Dunzihuang
7	Shen137	Xianfeng6J1K111 hybrid	16	Huotanghuang	(Huobai×Tang203) Mo17 improved line
8	Wenhuang	Huangzao4×Wenqing1331	17	CA042	CA042
9	Luyuan92	Yuanqi122×1137	18	313	313

Table 2: Descriptive statistics over all SSR loci including chromosomal location (Bin), mean number of observed (no) and effective (ne) alleles per locus, Shannon's information Index (I), expected heterozygosity (He), gene differentiation (GST) and the averaged frequency of the bands that present in BC-derived lines and absent in recurrent parent, R08

Primer	Bin	n_o	n_e	I	H_e	G_{ST}	Sequence
umc1288	4.02	2	1.504	0.492	0.316	0.178	0.380
phi402893	2.00	4	1.185	0.216	0.097	0.571	0.074
bnlg1191	9.07	4	2.078	0.625	0.362	0.513	0.259
umc1705	5.03	5	3.031	1.172	0.653	0.152	0.889
umc2163	10.04	5	1.673	0.687	0.322	0.622	0.259
bnlg439	1.03	4	1.905	0.866	0.456	0.811	0.324
phi299852	6.07	4	1.982	0.828	0.451	0.639	0.333
phi053	3.05	4	1.185	0.286	0.124	0.583	0.102
bnlg1496	3.09	4	1.690	0.522	0.275	0.556	0.222
phi116	7.06	2	1.647	0.556	0.373	0.846	0.250
bnlg2291	4.06	6	2.579	1.068	0.610	0.206	0.278
umc1741	8.03	2	1.528	0.497	0.321	0.286	0.380
bnlg125	2.02	3	1.576	0.430	0.269	0.791	0.167
phi080	8.08	4	1.860	0.764	0.420	0.451	0.435
phi072	4.01	3	1.853	0.539	0.317	0.726	0.204
bnlg161	6.00	5	3.036	1.152	0.635	0.223	0.296
bnlg2331	1.11	5	4.432	1.220	0.608	0.687	0.481
umc1225	5.08	3	1.588	0.579	0.305	0.818	0.185
bnlg1450	10.07	3	1.545	0.581	0.316	0.529	0.259
bnlg162	8.05	3	1.947	0.816	0.488	0.534	0.472
bnlg238	6.00	2	1.385	0.443	0.274	0.358	0.324
bnlg1792	7.02	2	1.432	0.372	0.222	0.483	0.185
phi308707	1.10	2	1.432	0.441	0.273	0.727	0.204
umc2246	2.00	2	1.077	0.158	0.072	0.308	0.065
phi047	3.09	3	1.651	0.679	0.372	0.767	0.222
umc1518	2.02	3	1.342	0.440	0.232	0.337	0.241
phi112	7.01	2	1.291	0.347	0.199	0.236	0.222
phi115	8.03	3	1.425	0.361	0.187	0.234	0.204
phi063	10.02	3	1.620	0.525	0.293	0.738	0.194
phi050	10.03	4	1.771	0.664	0.401	0.449	0.398
phi024	5.01	4	1.785	0.765	0.384	0.811	0.250
umc1061	10.06	2	1.224	0.308	0.169	0.385	0.157
phi085	5.06	4	1.979	0.693	0.345	0.735	0.213
phi034	7.02	4	1.439	0.487	0.248	0.428	0.204
phi448880	9.07	4	2.314	1.009	0.555	0.610	0.435
phi065	9.03	3	1.651	0.642	0.346	0.441	0.333
phi420701	8.00	4	3.834	1.341	0.736	0.431	0.481
umc2105	3.00	4	1.390	0.480	0.228	0.530	0.194
phi089	6.08	3	1.883	0.661	0.385	0.369	0.370
umc1792	5.08	3	1.382	0.454	0.227	0.518	0.222
Average	—	3.4	1.828	0.629	0.347	0.515	0.284

The PCR reaction was conducted in a volume of 15 μL in a 96 well microtiter plate using a PTC-100 thermalcycler (MJ Research, Inc.). The amplification protocol contained 100 ng of DNA, 1× reaction buffer (20 mM Tris-HCl, 50 mM KCl pH 8.4), 2.5 $\mu mol\ L^{-1}$ $MgCl_2$, 150 $\mu mol\ L^{-1}$ dNTPs, 1.0 U of Taq DNA polymerase (Invitrogen) and 0.2 uM of each primer. The PCR amplification was carried out with a first denaturing step of 5 min at 94°C , followed by 35 cycles of 1 min at 95°C, 2 min at 55°C and 2 min at 72°C and a final 10 min extension at 72°C. Amplification products were separated by electrophoresis in 1°TBE, 6% polyacrylamide gels and silver stained according to Creste *et al.* (2001).

Data collection and statistical analysis: The amplified bands were scored as 1 for present and 0 for absent. The raw data matrix was subjected to calculate genetic similarity coefficient (GS) between genotypes:

$$GS = \frac{2N_{ij}}{N_i + N_j}$$

where, N_{ij} was the number of common alleles in accessions i and j and N_i and N_j were the total number of alleles observed for genotypes i and j, respectively (Nei, 1978). Population genetic analysis was carried out using POPGENE version 1.31 (Yeh *et al.*, 1997).

The following parameters were estimated: the percentage of polymorphic loci (P), the observed number of alleles (no), the effective number of alleles (ne), Shannon's (Lewontin, 1972) information index (I), Nei's gene diversity (He) and the coefficient of gene differentiation (G_{ST}). Genetic Similarity (GS) values of all accessions were subjected to cluster analysis through the unweighted pair-group method (UPGMA) with NTSYS-pc v. 2.1 (Rohlf, 2000). To statistically assess genetic variation within and among lines, we performed hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) with the software GenAIEx 6 (Peakall and Smouse, 2006). Probabilities of variance components were estimated from 1000 random permutations.

RESULTS

Allelic diversity: Forty core SSRs were used to genotype 126 maize inbred lines including 108 uniform lines (54 BC_1F_3 and 54 BC_2F_2), 17 donors (The 14th donor, Shuang741, was missed) and receptor, R08. All lines could be distinguished based on the SSR loci used in the study. Results of allelic diversity at each SSR locus are shown in Table 2. A total of 136 discernible bands were obtained and all of which (100%) were polymorphic and informative. Among the polymorphic SSR fragments, 93 were present in the BC-derived lines and absent in the recurrent parent, R08. These fragments occurred at frequencies varying from 0.065 to 0.889, with 47.5% of them having low frequencies (<0.25). The remaining 43 SSR fragments were present in both the BC-derived lines and the recurrent parent at frequencies ranging from 0.111 to 0.935, with 97.5% of them having frequencies equal to or greater than 0.25. The observed number of alleles (no) per SSR marker varied from 2 to 6, with an average of 3.40. The effective number of alleles (ne) varied from 1.08 to 4.32, with an average of 1.83. The average frequency of the bands that present in BC-derived lines and absent in R08 was 0.284 (Table 2).

The number of SSR alleles scored in the BC-derived lines, which was not shared with R08 was 82 in BC_1F_3 and 84 in BC_2F_2. The bands average frequencies occurred in

BC_2F_2 (0.289) were almost the same as occurred in BC_1F_3 (0.280). The proportion of the alleles present in each BC-derived line and absent in the recurrent parent, R08, can be one measure of diversity between a BC-derived line and its recurrent parent. The number of genotypes for BC-derived lines differentiating from R08 varied from 1 to 8 per SSR and with an average 3.15 in BC_1F_3 lines (Total = 126) and 3.175 in BC_2F_2 lines (Total = 127). The expected heterozygosity (He) varied greatly among loci from 0.072 to 0.736 and was high on average (0.347). Shannon's information index (I) at each single primer pair varied from 0.158 to 1.341, with a mean of 0.629 (Table 2).

Genetic similarity and genetic distance: The genetic similarity value of each BC-derived line with its recurrent parent, R08, was also used as another measure of genetic diversity in the BC-derived lines. The GS estimates of each BC-derived line with R08 varied from 0.722 to 0.967, with a mean of 0.839, as a whole. The BC-derived lines from different source populations with the recurrent parent, R08 had the averaged GS estimate varying from 0.773 to 0.920 for BC_1F_3 and 0.738 to 0.899 for BC_2F_2 (Table 3). The averaged GS between R08 and BC_2F_2 (0.835) was less than that between R08 and BC_1F_3 (0.842), suggesting that more genetic diversity was found in BC_2F_2 lines than that in BC_1F_3. Although, the BC-derived lines derived from the same donor had a common recurrent parent, the GS estimates between BC_1F_3 and BC_2F_2 within given combination varied from 0.691 to 0.915, with an average of 0.818, suggesting that they shared less than 82% of the SSR fragments with one another.

The average GS values between pairs of lines within a given source population was higher than the average GS values between R08 and BC-derived lines from different source populations. Pairs of BC-derived lines from each source population exhibited a narrow range of GS values varied from 0.859 to 0.978 for BC_1F_3 and from 0.825 to 0.981 for BC_2F_2. Pairs of lines from different combinations of source germplasm had average GS values varying from 0.866 to 0.955 for BC_1F_3 and from 0.858 to 0.958 for BC_2F_2. The average GS for pairs of lines within each source population was 0.919 for BC_1F_3 and 0.915 for BC_2F_2. These results revealed that a larger variation was in BC_2F_2 than that in BC_1F_3 on the side.

The largest genetic distance (GD = 0.602) was recorded in BC_1F_3 lines derived from 4th combination with the 4th donor, while the smallest one (0.033) was recorded in BC_1F_3 lines derived from 5th combination and the recurrent parent, R08. The genetic distance of BC_1F_3 and BC_2F_2 from their recurrent parent was recorded to be largest in 14th (0.235) combination and 13th (0.278) combination, respectively, suggesting that the BC-derived lines from the two combinations had the most genetic diversity among the eighteen combinations in the study. Genetic structure: To quantify the significance of the genetic separation of the 18 combinations and two generations, AMOVA was carried out. The results indicated that the molecular diversity were significantly different ($p<0.001$) among all combinations within given generation and also between BC_1F_3 and BC_2F_2 within given combination. As shown in Fig. 1, the most parts of the genetic diversity (67.17% in BC_1F_3 and 64.33% in BC_2F_2), could be attributed to differences among combinations. The genetic differentiation varied greatly among combinations and less than 40% variation was within the BC-derived lines from each source population. The highest and the lowest values were obtained for the two generations in the 12th (70.50%) and 14th (19.90%)

Table 3: Dice's (1945) Genetic Similarity (GS) estimates among BC-derived lines within and between populations and generations calculated from SSR markers

		Population																		
Generations	GS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Average
BC_1F_3	Avr	0.919	0.911	0.923	0.916	0.908	0.953	0.942	0.866	0.955	0.896	0.931	0.913	0.932	0.910	0.889	0.898	0.952	0.930	0.919
	Max	0.934	0.929	0.933	0.963	0.929	0.963	0.945	0.881	0.978	0.903	0.941	0.922	0.947	0.918	0.912	0.929	0.958	0.951	0.935
	Min	0.911	0.885	0.911	0.885	0.888	0.941	0.940	0.859	0.937	0.885	0.915	0.896	0.918	0.906	0.873	0.872	0.944	0.911	0.904
BC_2F_2	Avr	0.924	0.933	0.893	0.866	0.955	0.907	0.925	0.928	0.953	0.879	0.953	0.958	0.907	0.858	0.867	0.882	0.935	0.942	0.915
	Max	0.959	0.944	0.918	0.878	0.981	0.926	0.941	0.948	0.963	0.911	0.967	0.963	0.918	0.895	0.921	0.899	0.947	0.954	0.935
	Min	0.903	0.922	0.859	0.844	0.941	0.892	0.904	0.911	0.937	0.829	0.937	0.955	0.898	0.836	0.825	0.863	0.918	0.918	0.894
Between	Avr	0.815	0.791	0.787	0.794	0.850	0.863	0.762	0.774	0.873	0.815	0.878	0.796	0.824	0.812	0.826	0.775	0.860	0.829	0.818
BC_1F_3 and	Max	0.860	0.822	0.863	0.851	0.915	0.885	0.796	0.825	0.892	0.866	0.903	0.829	0.842	0.866	0.862	0.808	0.890	0.851	0.857
BC_2F_2	Min	0.749	0.758	0.691	0.767	0.791	0.843	0.732	0.736	0.836	0.766	0.844	0.747	0.811	0.776	0.786	0.737	0.829	0.806	0.778
Between	Avr	0.542	0.674	0.742	0.408	0.673	0.789	0.708	0.706	0.767	0.683	0.766	0.741	0.595	—	0.517	0.636	0.725	0.942	0.683
BC_1F_3 and	Max	0.560	0.680	0.755	0.426	0.709	0.791	0.720	0.729	0.777	0.695	0.777	0.755	0.603		0.526	0.677	0.725	0.772	0.687
donors	Min	0.533	0.669	0.732	0.398	0.627	0.784	0.695	0.695	0.755	0.658	0.747	0.729	0.588		0.505	0.608	0.721	0.723	0.657
Between	Avr	0.581	0.714	0.744	0.536	0.705	0.738	0.772	0.720	0.773	0.703	0.794	0.762	0.612	—	0.523	0.647	0.715	0.925	0.704
BC_2F_2 and	Max	0.631	0.725	0.781	0.582	0.713	0.743	0.781	0.729	0.781	0.729	0.803	0.770	0.621		0.535	0.673	0.721	0.747	0.710
donors	Min	0.548	0.703	0.721	0.476	0.694	0.728	0.755	0.710	0.770	0.680	0.781	0.755	0.601		0.513	0.633	0.706	0.695	0.675
Between	Avr	0.844	0.819	0.817	0.896	0.920	0.835	0.860	0.773	0.861	0.871	0.889	0.797	0.832	0.784	0.833	0.843	0.882	0.792	0.842
BC_1F_3 and	Max	0.848	0.829	0.836	0.934	0.967	0.840	0.865	0.796	0.866	0.900	0.911	0.807	0.840	0.787	0.851	0.870	0.894	0.811	0.858
R08	Min	0.838	0.799	0.784	0.837	0.884	0.833	0.849	0.751	0.855	0.829	0.855	0.781	0.925	0.765	0.800	0.800	0.874	0.778	0.824
Between	Avr	0.812	0.833	0.845	0.792	0.867	0.887	0.787	0.839	0.861	0.843	0.897	0.848	0.738	0.799	0.846	0.790	0.899	0.846	0.835
BC_2F_2 and	Max	0.832	0.836	0.877	0.818	0.881	0.896	0.802	0.866	0.896	0.877	0.911	0.863	0.749	0.818	0.853	0.823	0.921	0.870	0.855
R08	Min	0.772	0.825	0.810	0.768	0.859	0.877	0.771	0.803	0.885	0.803	0.889	0.840	0.722	0.749	0.842	0.773	0.877	0.826	0.816

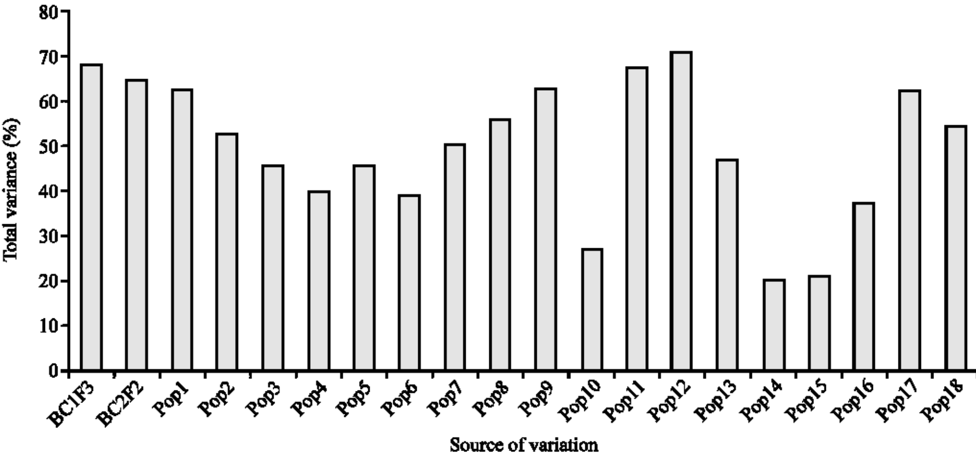

Fig. 1: AMOVA analysis for the partitioning of SSR variation of BC-derived lines

combinations, with an average of 47.6% within given combination. Similarly, the coefficient of gene differentiation (G_{ST}) varied from 0.152 to 0.846, with an average of 0.515 (Table 2). The results also indicated that most of the genetic variation (51.5%) resided among the combinations given generation or between two generations given combination.

NCBL analysis: Four SSR primer pairs linked with *Ht2* and *Ht3* genes controlling NCBL were assayed in the samples of BC_1F_3, BC_2F_2, donor parents and R08. A total of 14 discernible alleles were obtained and all of which were polymorphic (100%) and informative. However, no specific amplifications were found in all of the primers. The linked markers could not distinguish all the donors from the recurrent parent, R08 and there were 1, 3, 6 and 6 donors that were not polymorphic at such loci bnlg1152, bnlg1666, umc1149 and umc1202 compared with R08, respectively. On the other hand, a low level of correlation was found between SSR assays on BC-derived lines and evaluation of NCBL phenotype. The consistency was relatively higher in BC_1F_3 (43.85%) than that in BC_2F_2 (32.35%) and diverse genetic background BC-derived lines with diverse consistency given generation. The best performance was presented in the 8th combination, which BC_1F_3 and BC_2F_2 assays showed high prediction of 100% accuracy with phenotype resistance to NCBL.

DISCUSSION

The development of maize varieties and hybrids with high yield potential and improved adaptation to the major stresses was important to increasing productivity in the diverse production environments (Menkir *et al*., 2006). During the past 50 years, intensive plant breeding has led to undeniable genetic advances based on using the genetic diversity available (Clerc *et al*., 2006). In this study, we first explored the way in which genetic diversity had been affected during maize backcross improvement. The BC_1F_3 and BC_2F_2 lines derived from R08 and 18 predominant varieties were fingerprinted using 40 core maize SSRs. The set of donor parents used in the study were believed to be resistance to the maize Northern corn leaf blight in China. The ability to detect multiple alleles at a single locus makes microsatellites more appropriate for many diversity studies (Barbosa *et al*., 2003; Menkir *et al*., 2005; Van Etten *et al*., 2008).

Despite the importance of the marker type in analyzing variation, the choice of statistical coefficients was a rather fundamental step in studying genetic diversity (Laborda *et al*., 2005). The GS estimates could be useful in breeding programs to maximize the level of variation in segregating populations by crossing lines with diverse genetic backgrounds (Menkir *et al*., 2005). In our study, the GS estimates showed that the BC-derived lines shared a large fraction (= 80%) of the SSR fragments with the recurrent parent and no significant difference was found in BC_1F_3 and BC_2F_2 lines. These results suggested that only a small fraction of the SSR genetic diversity captured in the BC-derived lines was not derived from the recurrent parent, R08.

The BC-derived lines evaluated in this study were at the BC_1F_3 and BC_2F_2 stage and would require additional generations by selfing to develop highly homozygous lines. However, Tarter *et al*. (2004) argued that further inbreeding and selection could result in the loss of exotic alleles in highly homozygous lines due to selection against alleles contributing to or linked to poor adaptation

to a target environment. Allelic richness, in terms of number of alleles and genotypes occurred in BC_2F_2 were almost as same as that occurred in BC_1F_3, which consistent with the findings of GS analysis in the study. All these results showed that the genetic diversity in BC_1F_3 stage was almost as much as that in BC_2F_2 stage. This might be the result of the strict selection according to the resistance to NCBL phenotype after genetic recombination. Thus the effective selection could speed up the progress of genetic improvement in backcross breeding.

The observed substantial amount of genetic diversity among the BC-derived lines in our study derived mainly from combinations. The AMOVA showed that average 65.75% of the total molecular variation was explained by the variation among combinations. Some differentiations were perceptible between BC_1F_3 and BC_2F_2 generations in combination 12 compared to those in combination14, whereas very low differentiation was found between the two generations. These results suggested that the importance of using diverse maize lines as donor parents in backcross populations to enhance the probability of extracting productive inbred lines with good combining ability and other agronomic performance.

However, the SSR markers linked with *Ht2* and *Ht3* genes controlling NCBL were not practicability in present study. The donor parents might be multi-resistance to NCBL and it was not suitable to assay some character controlled by multiple genes using single-gene markers. The capacity of these assays can not work on a broad range of any one maize sample with prediction of high accuracy. So, it is necessary to identify specific molecular markers for special population or novel maize varieties.

In conclusion, the present study demonstrated that backcross once based on the selection of right donor parents, identification of target traits and general combining ability in self-cross process would be best effective in maize backcross improvement. These results could be used as guidelines in maize breeding by backcross improvement. In combination with phenotypic and molecular data, it might be possible to find putative linkages without mapping by directly selecting lines carrying pertinent alleles. This was especially useful in identifying favorable alleles of genes whose phenotypes were difficult to screen or whose effects were masked by an agronomically poor background (George *et al*., 2004). The high level of resistance fixed in these BC-derived lines could be exploited readily in breeding programs as parents of hybrids and synthetics and also as sources of favorable alleles to improve resistance to NCBL in locally adapted germplasm.

ACKNOWLEDGMENTS

This study was supported by the grants of National High Technology Research and Development Program of China (No. 2006AA100103), the International Agricultural Science and Technology Introduction Program of Ministry of Agriculture, People's Republic of China (No. 2003-Q03) and program for Changjiang Scholars and Innovative Research Team in University (No. IRT0453).

REFERENCES

Barbosa, A.M.M., I.O. Geraldi, L.L. Benchimol, A.A.F. Garcia, C.L.J. Souza and A.P. Souza, 2003. Relationship of intra-and interpopulation tropical maize single cross hybrid performance and genetic distances computer from AFLP and SSR markers. Euphytica, 130: 97-99.

Barcaccia, G., M. Lucchin and P. Parrini, 2003. Characterization of a flint maize (*Zea mays* var. indurata) Italian landrace, II. Genetic diversity and relatedness assessed by SSR and Inter-SSR molecular markers. Genet. Res. Crop Evol., 50: 253-271.

Clerc, V.L., F. Bazante, C. Baril, J. Guiard and D. Zhang, 2005. Assessing temporal changes in genetic diversity of maize varieties using microsatellite markers. Theor. Appl. Genet., 110: 294-302.

Clerc, V.L., V. Cadot, M. Canadas, J. Lallemand, D. Guerin and F. Boulineau, 2006. Indicators to assess temporal genetic diversity in the French Catalogue: No losses for maize and peas. Theor. Appl. Genet., 113: 1197-1209.

Creste, S., A. Tulmann Neto and A. Figueira, 2001. Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. Plant Mol. Biol. Reporter, 19: 299-306.

Dice, L.R., 1945. Measures of the amount of ecological association between species. Ecology, 26: 297-302.

Echandi, C.R. and A.R. Hallauer, 1996. Evaluation of US Corn Belt and adapted tropical maize cultivars and their diallel crosses. Maydica, 41: 317-324.

Excoffier, L., P.E. Smouse and J.M. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics, 131: 479-491.

Gauthier, P., B. Gouesnard, J. Dallard, R. Redaelli, C. Rebourg, A. Charcosset and A. Boyat, 2002. RFLP diversity and relationships among traditional European maize populations. Theor. Appl. Genet., 105: 91-99.

George, M.L., E. Regalado, M. Warburton, S. Vasal and D. Hoisington, 2004. Genetic diversity of maize inbred lines in relation to downy mildew. Euphytica, 135: 145-155.

Labate, J.A., K.R. Lamkey, S.E. Mitchell, S. Kresovich, H. Sullivan and J.S.C. Smith, 2003. Molecular and historical aspects of corn belt dent diversity. Crop Sci., 43: 80-91.

Laborda, P.R., K.M. Oliveira, A.A.F. Garcia, M.E.A.G.Z. Paterniani and A.P. Souza, 2005. Tropical maize germplasm: what can we say about its genetic diversity in the light of molecular markers?. Theor. Appl. Genet., 111: 1288-1299.

Lewontin, R.C., 1972. The Genetic Basis of Evolutionary Change. Columbia University Press, New York and London.

Menkir, A., J.G. Kling, B. Badu-Apraku and I. Ingelbrecht, 2005. Molecular marker-based genetic diversity assessment of Striga-resistant maize inbred lines. Theor. Appl. Genet., 110: 1145-1153.

Menkir, A., M.O. Olowolafe, I. Ingelbrecht, I. Fawole, B. Badu-Apraku and B.I. Vroh, 2006. Assessment of testcross performance and genetic diversity of yellow endosperm maize lines derived from adapted ×exotic backcrosses. Theor. Appl. Genet., 113: 90-99.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

Peakall, R. and P.E. Smouse, 2006. GENALEX 6: Genetic analysis in excel: Population genetic software for teaching and research. Molecular Ecol. Notes, 6: 288-295.

Rohlf, F.J., 2000. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 2.1, Exeter Publications, New Yark.

Saghai-Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang and R.W. Allard, 1994. Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations and population dynamics. Proc. Natl. Acad. Sci. USA., 91: 546-547.

Tarter, J.A., M.M. Goodman and J.B. Holland, 2004. Recovery of exotic alleles in semiexotic maize inbreds derived from crosses between Latin American accessions and a temperature line. Theor. Applied Genet., 109: 609-617.

Van Etten, J., M.R.F. Lopez, L.G.M. Monterroso and K.M.P. Samayoa, 2008. Genetic diversity of maize (*Zea mays* L. ssp. mays) in communities of the western highlands of Guatemala: geographical patterns and processes. Genet. Resour. Crop Evol., 55: 303-317.

Van Staden, D., C.A. Lambert and A. Lehmensiek, 2001. SCAR markers for the *Ht1*, *Ht2*, *Ht3* and *HtN1* resistance genes in maize. Maize Genet. Conf. Abst., 43: 134-134.

Yeh, F.C., R.C. Yang, T. Boiley, Z.H. Ye and J.X. Mao, 1997. POPGENE, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada.

Yin, X.Y., Q.H. Wang, J.L. Yang, D.M. Jin, F. Wang, B. Wang and J.R. Zhang, 2003. Fine mapping of the *Ht2* (Helminthosporium turcicum resistance 2) gene in maize. Chinese Sci. Bull., 48: 165-169.