The effect of 5 cycles of biparental mass selection on a narrow base maize population based on phenotype, combining ability, and SSR analyses

Lujiang Li[1†], Wensheng Chen[2†], Kui Xiang[1], Lana M Reid[3], Hai Lan[1], Kecheng Yang[1*], Min Zhang[2*], Guangtang Pan[1], Tingzhao Rong[1]

[1]Maize Research Institute, Sichuan Agricultural University, Key Laboratory of Crop Genetic Resources and Improvement, Ministry of Education, Ya'an 625014, Sichuan, China
[2]College of Agronomy, Sichuan Agricultural University, Chengdu 611130, Sichuan, China
[3]Agriculture and Agri-Food Canada (AAFC), Eastern Cereal & Oilseed Research Centre, Ottawa, Ontario, Canada, K1A OC5
[†]Both authors contributed equally to this work
*Corresponding author: E-mail: lilujiang@yahoo.com.cn; yalanmin@126.com

Abstract

Five cycles of biparental mass selection (MS) were carried out to improve the narrow-base maize population P4C0. In different ecological environments, the phenotypes of the developed populations were analyzed, the combining abilities were tested according to an incomplete diallel model to study the effects of selection, and the effects of MS on genetic diversity of the populations were also analyzed by using 51 pairs of SSR markers. It was found that MS was effective in improving the main traits and general combing ability (GCA), and it was effective on maintaining the genetic diversity of the population. At the same time, the genetic structure was changed with advance of selection.

Keywords: maize, narrow base population, recurrent selection, effect of improvement, genetic diversity, SSR

Abbreviations: MS - biparental mass selection; SSR - Simple Sequence Repeat; GCA - general combing ability; PH - plant height; EH - ear height; EL - ear length; KD - kernel depth; NRE - number of rows per ear; YP - yield per plant; ANOVA - analyses of variance; LSD - least significant difference; NP - number of polymorphic loci; RP - ratio of polymorphic loci (%); I - Shannon's information index (Shannon,1949); He - Nei's (1973) expected heterozygosity; GD - genetic distance; NG - number of genotypes; SD - standard deviation

Introduction

Population improvement is an important way to broaden the genetic basis of breeding germplasm in maize. The improved populations can serve as a potential source of superior inbred lines. Recurrent selection is a key methodology used to improve maize populations (Hallauer et al, 1988; Dudley et al,1992; Lamkey,1992) and involves a process of cyclical selection in a breeding population to increase the frequency of favorable alleles, and improve mean performance (Comstock et al, 1949; Doerksen et al, 2003; Edwards et al, 2002; Holthaus et al, 1995; Hinze et al, 2005; Labate et al, 1997).

Increasing the mean performance of the populations is one of the most important goals for recurrent selection. MS have been successfully used to improve the performance of maize populations for quantitative traits. Leon et al (2002) reported that the total number of kernels per plant and dry kernel weight per plant increased significantly at a rate of 36.69 kernels cycle^{-1} and 5.60 g cycle^{-1} over 24 cycles of mass selection in the Golden Glow maize population.

Maintaining the genetic diversity of populations to facilitate long term selection is important in a recurrent selection program. Molecular genetic markers are a powerful tool for analyzing genetic diversity in maize populations. Many studies have compared the efficiency of using SSR, RFLP, AFLP, and RAPD markers (Powell et al, 1996; Pejic et al, 1998; Yuan et al, 2000). SSR markers were shown to offer advantages in reliability, reproducibility, discrimination, standardization, and cost effectiveness over other marker types and were the best one to analyze the genetic diversity of populations (Huang et al, 2004; Hinze et al, 2005).

In this study, the narrow-base population P4C0 was improved by five cycles of MS to develop five populations. In different ecological environments, the phenotypes of these populations were analyzed, the combining ability was tested according to an incomplete diallel model and the genetic diversity of the populations was also analyzed by using 51 pairs of SSR markers. The objective of the study was to determine how to increase the efficiency of improving and utilizing a narrow base synthetic population by studying the effect of MS on P4C0 as well as the effect on genetic diversity of populations as analyzed by SSR markers.

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Materials and Methods

The basic population

The basic population named P4C0 was formed by intercrossing 6 maize inbred lines such as Ye478, Shen5003, Tie7922, Cheng687, 698-3 and 32,which belonged to a same heterotic group (Zhang et al, 2006; Zou et al, 2010).

Recurrent selection methods and procedures

Biparental mass selection (MS)

Population P4C0 was improved with biparental mass selection. Six hundred individuals of population were planted in Ya'an, Sichuan Province, China in May, 2005. These plants were divided into two groups, the bulked pollen of both groups were used to pollinate the individuals of the other group. Sixty ears with superior kernel depth, row number per ear, and yield per plant were selected, and the kernels in the middle part of the ears were kept and then mixed in equal quantities to form the advanced cycle population P4MSC1. The P4MSC2, P4MSC4 were obtained in Yuanjiang, Yunnan province, in the autumn of 2005 and 2006, respectively, and the P4MSC3 and P4MSC5 were obtained in Ya'an in the spring of 2006 and 2007, respectively, following the method described above.

Field experiments

Plant materials

Two groups of materials were involved in the field experiment. The first group consisted of the six populations including the basic population P4C0 and its improved descendents, P4MSC1, P4MSC2, P4MSC3, P4MSC4 and P4MSC5. The second group consisted of 18 testcrosses developed by crossing 3 inbred lines with the 6 populations.

Experimental designs and trait evaluations

The 6 populations were evaluated in Ya'an in the spring and in Xishuangbanna in the autumn of 2008, respectively. The populations were planted in a randomized complete block design with 3 replications. Each plot contained 3 rows of 24 plants per row. Sixty plants from the centre of each plot were sampled for trait evaluations. In the spring of 2009, the 18 testcrosses were evaluated at 3 locations, Ya'an, Xishuangbanna, and Fuling City. The crosses were planted in a randomized complete block design with 3 replicates. Each plot consisted of 2 rows of 14 plants per row. Twenty plants from the centre of each plot were sampled for traits evaluations. For all experiments, the planting density was 5.8×10^4 plants per hectare and field management followed local practices for maize production. After silking, plant height and ear height were measured. After harvest, ear length, kernel depth, number of kernel rows per ear, and yield per plant were recorded after drying of samples. All measurements of phenotypic traits were recorded on an individual plant basis.

Statistical analyses for field experiments

For the populations, a combined analysis of variance (ANOVA) was carried out based on means of plots and the least significant difference (LSD) test was then carried out to compare traits tested. The linear regression of cycles means over cycles of selection (b value) were calculated based on the means of the populations. For the testcrosses, the effects of GCA of the traits for the populations were estimated according to an incomplete diallel mating design (Griffing, 1956).

SSR analyses

Thirty randomly chosen individuals for each of the 6 maize populations were used for the SSR analyses. Genomic DNA was isolated following the CTAB procedure described by Scott (1998) with minor modifications. The PCR amplification was carried out in a PTC-220 thermal cycler (Surplus Lab Inc, Michigan, USA) program at 35 cycles of 1 min at 95°C, 2 min at 55°C, and 2 min at 72°C, followed by a 10 min extension at 72°C. The PCR amplification products were separated on a 6% (w/v) denatured polyacrylamide gel and visualized using silver staining. The SSR bands were scored as present (1) or absent (0), each of which was treated as an independent character. Genetic diversity was examined based on the scores using the statistical methods and formula as follows: Genetic distance (GD) between individuals within each population was calculated according to Rogers (1972) and Nei (1978) with $GD = -\ln(2N_{ij}/N_i + N_j)$,where N_{ij} is the number of SSR alleles common to individual i and j, whereas N_i and N_j are the total number of SSR alleles observed for individual i and j, respectively.

Genetic heterosity (H) was calculated for each

Table 1 - The means of 6 major traits evaluated of 6 populations.

Populations	PH (cm) Mean ± SD	EH (cm) Mean ± SD	EL (cm) Mean ± SD	KD (cm) Mean ± SD	NER (row) Mean ± SD	YP (g) Mean ± SD
P4C0	208.33±21.44	76.55±12.97	13.84±2.16	0.978±0.135	13.59±1.95	114.93±34.98
P4MSC1	221.13±20.93	86.38±13.92	14.22±2.16	1.058±0.124	14.29±2.00	129.31±36.13
P4MSC2	229.07±21.55	90.32±13.88	14.49±2.14	1.054±0.148	14.44±2.18	131.16±37.12
P4MSC3	224.77±23.57	89.33±15.94	14.41±2.28	1.023±0.140	14.55±2.00	122.69±36.09
P4MSC4	229.84±19.84	93.55±14.15	14.49±2.19	1.052±0.131	14.70±2.09	125.55±34.72
P4MSC5	226.57±22.06	91.90±13.49	14.09±2.01	1.079±0.143	14.61±2.16	121.65±32.02
LSD0.05	6.56	4.41	0.44	0.054	0.33	6.46
LSD0.01	8.95	6.01	0.60	0.073	0.45	8.82
b	3.23	2.78	0.06	0.013	0.19	0.40

SD: standard deviation, b: the linear regression of cycles means over cycles of selection

Table 2 - The percentage of individuals with grain yield per plant ≥ 160 g, number of kernel rows per ear ≥ 16 rows, and kernel depth ≥ 1.100 cm for 6 populations.

Populations	YP (≥160 g)	NRE (≥16 rows)	KD (≥1.100 cm)
P4C0	9.49	24.35	13.49
P4MSC1	20.79	35.69	15.34
P4MSC2	20.54	38.14	20.26
P4MSC3	16.10	38.97	11.97
P4MSC4	17.39	42.31	17.21
P4MSC5	12.17	35.76	27.80

population according to Nei (1978) as $H = 1-\sum F_i^2$, where F_i is the frequency of the ith allele.

The genetic diversity index (H_0) for each population and the genetic diversity index of populations (H_{sp}) were calculated according to Wachira (1995) with $H_0 = -\sum\prod_i^{\ln}\prod_i$ and $H_{sp} = -\sum\prod^{\ln}\prod$, where $\prod_i$ is the frequency of a polymorphic locus in a population and $\prod$ is the frequency of a polymorphic locus in all populations analyzed. The analyses were carried out using POPGENE32 (Yeh et al, 1997), Excel 2007, GenAL-Ex 6.2 (Peakall et al, 2006) and NTSYSpc2.1 (Rholf, 1992).

Results

The combined analyses of variance (ANOVA) showed that location effects were significant for most traits except ear length, and there were significant differences or extremely significant differences among populations for all traits (Table 1).

Direct response

The results showed that mean values for kernel depth and the number of rows per ear increased after five cycles of MS on P4C0 (Table 2). Both traits had their lowest mean values in P4C0 and highest in P4MSC5 and P4MSC4, the b value were 0.013 cm and 0.19 cm, respectively. The percentage of superior individuals with a kernel depth ≥ 1.100 cm increased with advance of selection in first two cycles, and the percentage of superior individuals with a kernel rows per ear ≥16 increased with advance of selection in the first 4 cycles of MS on P4C0 (Table 3). These results indicate that MS were effective in directly improving two traits, kernel depth and rows per ear of the original narrow base source population.

Correlated response

The direct selection for kernel depth and the number of rows per ear also resulted in significant changes in other agronomic traits. Grain yield per plant showed a trend to increase with successive MS cycles on P4C0. Maximum yield was obtained at P4MSC2 with a mean of 131.16 g and the b value was 0.40 g. The percentage of superior individuals with a grain yield per plant ≥ 160 g increased with successive selections over 5 cycles of MS on P4C0 (Table 3). Mean plant height and ear height increased significantly over the 5 cycles of MS on P4C0, the ear length of P4C0 tended to increase over 5 cycles of MS.

Indirect response to mass selection

The analyses of variance for combining ability showed that source of variation of locations and testcrosses × locations were significant for most traits and there were significant differences among testcrosses for all traits. The GCA of testers and GCA of populations were significant sources of variation for all traits except the GCA of populations for ear length (Table 4). After 5 cycles of MS on P4C0, the GCA effects of most traits increased with each cycle of selection. The results showed that the MS were effective on improving the GCA for yield and yield components (Supplementary Table 1).

Genetic diversity

Six indices of genetic diversity were calculated base on the data from 51 SSR makers (Supplementary Table 2). After 5 cycles of MS on P4C0, the number and percentage of polymorphic loci decreased with each successive cycle, the mean genetic distance (GD) also slightly declined, and the distribution of the genetic distance between individuals in a given population (Figure 1) showed that most of the genetic distance between individuals decreased with the advance of MS. The expected heterozygosity (He) and Shannon genetic diversity index (I) increased in the first 2 cycles then started to decrease from the third

Table 3 - GCA effects for 5 traits measured of 6 populations.

Population	PH	EH	KD	NRE	YP
P4C0	-6.360	-4.028	-0.064	-0.303	-9.764
P4MSC1	-2.427	-1.322	0.016	-0.190	-1.632
P4MSC2	0.019	-1.351	0.016	0.207	-0.497
P4MSC3	1.819	0.596	-0.001	0.008	4.357
P4MSC4	3.056	2.192	0.017	0.274	5.498
P4MSC5	3.893	3.913	0.016	0.004	2.038
LSD0.05	3.758	3.518	0.024	0.208	3.613
LSD0.01	5.895	5.518	0.038	0.326	5.667

Table 4 - Six indices of genetic diversity calculated from the amplification of 51 pairs of SSR markers for 6 populations.

Populations	NP	RP (%)	I ± SD	He ± SD	GD ± SD	NG
P4C0	206	90.75	0.995±0.311	0.5706±0.1366	0.5916±0.0380	270
P4MSC1	199	87.67	1.016±0.326	0.5832±0.1406	0.5670±0.0363	257
P4MSC2	197	86.78	0.998±0.316	0.5717±0.1385	0.5601±0.0380	261
P4MSC3	198	87.22	0.976±0.343	0.5575±0.1498	0.5687±0.0351	251
P4MSC4	193	85.02	0.972±0.338	0.5586±0.1504	0.5600±0.0361	255
P4MSC5	192	84.58	0.965±0.308	0.5553±0.1325	0.5526±0.0315	240

NP - number of polymorphic loci, RP - ratio of polymorphic loci (%), I - Shannon's information index (Shannon, 1949), He - Nei's (1973) expected heterozygosity, GD - genetic distance, NG - number of genotypes, SD - standard deviation.

cycle of selection, but there were no significance difference among different cycles. The number of genotypes selected decreases with selection presented the trend of declining with the advance of selection and related to the location where the populations were developed.

Analysis of molecular variance

The results of a principle coordinates analysis (Figure 2) showed that the individuals were distributed over a larger range in P4C0 and the early cycles of MS than in the more advanced MS cycles. The results showed that directional changes occurred in the distribution of the individual with the advance of selection.

Discussion

The effect of different recurrent selection methods for improving maize populations have been compared by many researchers; different recurrent selection methods could improve the populations effectively, but the effect varies from method to method (Holthaus et al, 1995; Li et al, 2008; Weyhrich et al,1998; Wardyn et al,2009). When the effect of each cycle of selection is compared, mass selection was considered the most effective method (Hallauer 1981). The response to selection for seven different methods, including mass, modified ear-to-row, half-sib with inbred tester, full-sib, S_1-progeny, S_2-progeny, and reciprocal full-sib selection, were compared in the BS11 maize population (Weyhrich et al, 1998). The results showed that all selection methods were successful in significantly improving the population per se performance for grain yield. S2-progeny selection had the greatest response for grain yield of 4.5% per cycle and mass selection had the lowest response of 0.6% per cycle. In the current study, the effect of MS on a narrow base maize population, P4C0 was evaluated, Mass selection was found to be effective at improving the yield and the yield components and their GCAs.

The effects of recurrent selection on genetic diversity were analyzed. Mass selection was showed to be the most effective method for maintaining the genetic diversity of populations. The genetic variance could last for 30 cycles of mass selection, but could be exhausted by 14 cycles and 11 cycles in S_1-progeny selection and S_2-progeny selection, respectively. Tabanao (2005) reported that using multiple parents may help sustain genetic variability in advanced cycle breeding. In our study, the genetic diversity of the population displayed no significant change after 5 cycles of MS, this could be because it is easy to evaluate and select more progeny to recombine the advanced cycle populations in a mass selection program and there was more opportunity for exchange and recombination of alleles. The genetic structure was changed with advance of recurrent because specific types of individuals were selected to form the advanced cycle population.

In conclusion, MS was effective in improving the main traits and general combing ability (GCA), and MS was effective on maintaining the genetic diversity of the population. The genetic structure was also changed with advance of selection.

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Figure 1 - The distribution of the genetic distance between individuals in each of 6 populations.

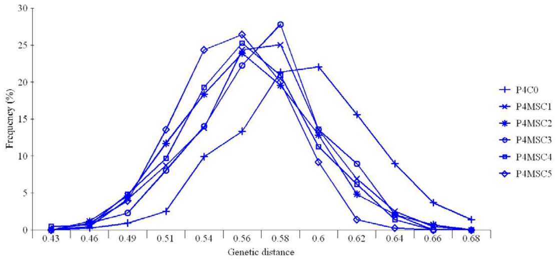
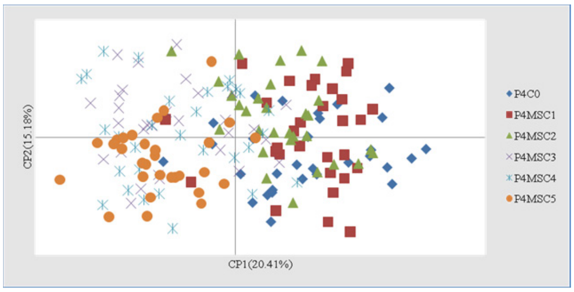


Figure 2 - Principle coordinates analysis for the populations based on the SSR marker analysis.



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