Indian J. Genet., **61**(3): 213-217 (2001)

Early generation identification and utilization of potential inbred lines in modified single cross hybrids of maize (*Zea mays* L.)

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(Received: June 2000; Revised: April 2001; Accepted: May 2001)

Abstract

The essential pre-requisite for commercial production of single cross maize hybrids is the development of vigorous high yielding inbred parents. A modified form of Forward and Reverse Inbreeding Process (FRIP) [1] was used to develop modified single cross hybrids. This method involves inbreeding using selfing in the early generations followed by plant-to-plant sibbing, early generation testing of lines, deriving sister lines, recombining sister lines and finally using sister line crosses to develop modified single cross hybrids. The improved performance of the inbred lines so derived and also of the experimental hybrids tested at two diverse locations in this study, substantiates the effectiveness of this methodology. Inbred testers were used in test crossing and their utility in hybrid development is discussed.

Key Words : Maize, inbred lines, modified single-cross hybrids, inbred testers.

Introduction

The discovery of the phenomenon of heterosis opened up avenues to meet the ever increasing need for enhanced maize productivity. Single cross maize hybrids have been established as the most heterotic of all the hybrid types in maize [2]. In the early stages of maize research in India, the low productivity of inbred parents was a limiting factor in the commercial seed production of single cross hybrids. Hence there was reliance on double, three way and top cross hybrids. However, of late there has been a situation of near stagnant maize production of around 10 million tonnes [3]. Hence efforts began to improve populations so as to derive potential inbred lines for commercial single cross hybrid production. The main requirement in this direction is the development of vigorous high yielding parents. The present investigation was an effort in this direction.

Materials and methods

In the present study, an improved population CMIP2-7, developed at the Maize directorate was taken up. This population had been subjected to selection for inbreeding tolerance. It also possessed relative tolerance to downy mildew, an important disease affecting maize crop in India. In this population selfing was started using around 500 plants initially. Three cycles of selfing were carried out to attain a reasonable degree of homozygosity. During the inbreeding process, selection of lines was simultaneously done based on *per se* performance for characters like standability, vigour and ear placement. In S2 generation, sister lines were developed in promising families. Continuous selfing leads to loss of vigour rapidly due to the surfacing of deleterious recessive alleles which lower the fitness value. Hence, a milder form of inbreeding namely plant- to-plant sibbing was carried out after the three initial cycles of selfing.

In order to reduce the number of lines being handled, test-crossing was done in S3 generation and each line was test-crossed to two inbred testers namely LM5 and LM6. Inbred testers were used keeping in view the ultimate aim of developing single cross hybrids, since the testers can directly serve the purpose of being one of the hybrid parents if some of their combinations proved to be highly heterotic. These two testers belong to two different heterotic pools.

In winter season (October-March) of 1995-96, the 42 test-cross hybrids were evaluated in a completely randomised block design with three replications at the Maize Research Station, Amberpet, Hyderabad. Each entry was planted in a single 5m row with a inter row space of 0.75 M and a intra row spacing of 0.25 M. The best lines were identified based on the combining ability effects for grain yield [4] and *per se* yield performance of the testcross hybrids in comparison to the check hybrid Paras. Inbred lines were raised in two seperate blocks for advancing to the next generation

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and effecting sister line crosses respectively. Sister lines were lines drawn from a common parent in an earlier generation (S2 here). Sister lines are identical for most characters and their crossing results in vigorous progeny (sisterline crosses) that can effectively serve as female parent of a potential Modified single cross hybrid.

In the rainy season (June to September) of 1996, 22 lines (11 each of inbred lines and their corresponding sister lines) were raised to be advanced further. Eleven sister line crosses of the best sister lines were also grown and used as female parents and crossed to four widely adapted good inbred testers namely CML247, CML251, CML290 and CM111 to develop 44 Modified single cross hybrids.

In the winter season of 1996-97, the 44 Modified single cross hybrids, 11 sister line crosses, 11 inbred lines and their component sister lines, 4 inbred testers and check hybrids Paras, (CM202 × CM111) and DHM103 were raised and evaluated at two diverse locations, Hyderabad and Karimnagar respectively. Hyderabad location has deep black soils while Karimnagar has red sandy loam soils. In the replicated trial, parents and hybrids were randomised separately and grown in contiguous blocks.

Data was recorded in each entry on maturity parameters like days to 50 percent tasseling, days to 50 per cent silking and on yield related characters like plant height, ear height, ear length, ear girth, number of kernel rows per ear, number of kernels per row, number of kernels per ear and grain yield per plot in Kg (5 M long with plants spaced 0.25 M apart). The final figures were computed after adjusting for plant stand (20 plants per plot) and kernel moisture (15%). For each of the characters studied the data across the two locations were pooled after carrying out the Bartlett's test and verifying the homogeneity of errors to enable combined analysis.

Results and discussion

Poor *per se* vigour and consequent low grain yield of the existing inbred lines (less than 1 ton/ha) has rendered them unprofitable and ineffective for use as parents in commercial single cross hybrid production. This was the reason for the emphasis on double cross, double top-cross and three-way cross hybrids. One strategy to evolve vigorous high yielding inbred lines is to derive lines from a hybrid oriented source germplasm that has the characteristics of inbreeding tolerance, good combining ability and desirable agronomic attributes, in addition to heterotic relationship with other germplasm in producing agronomically superior high yielding F_1 crosses [8]. The present investigation was initiated from such a hybrid oriented population CMIP2-7. The objective was to develop potential vigorous inbred lines using a modified form of FRIP method and further to test these lines in hybrid combinations.

Originally the Forward and Reverse Inbreeding Process (FRIP) as given by vasal and Srinivasan [1] involved identification of superior lines based on *per se* performance in S_3 or S_4 generation, developing a large number of sister lines and selecting the better ones in each subsequent generation. Sister lines in S_6 or later generation originating from a particular S_3 or S_4 line are recombined to recover improved S_3 or S_4 lines. The lines in the advanced generation should be selected for *per se* performance, combining ability and phenotypic similarity. Crosses among lines are made to produce a new series of inbreds through renewed inbreeding.

Some modifications in the FRIP method were envisaged and carried out in the present study. In the Modified FRIP method (Fig. 1) in early generation S_3,

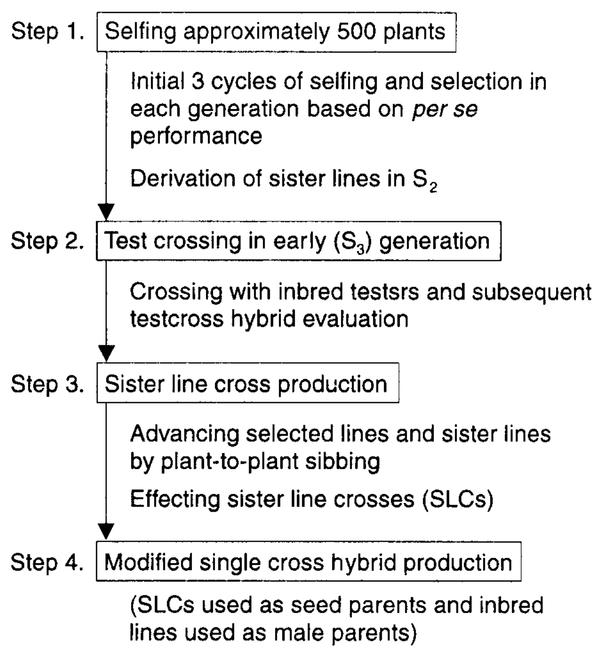

Fig. 1. Schematic representation of modified FRIP method carried out in CMIP2-7 population

the lines were tested for combining ability through development and testing of testcross hybrids. In another block, these lines were advanced further. This coupled line development with hybrid production and testing, albeit in an early generation of inbred line development.

Inbred lines were used as testers since promising hybrids if obtained could go in for Single cross hybrid development directly [5]. Inbred testers LM5 and LM6 used to develop the testcross hybrids, belong to two different heterotic pools. On crossing they give rise to a heterotic hybrid. The lines derived in this study could thus be classified into different heterotic groups [6].

The other modification carried out was in the mode of advancing the parental lines through plant-to-plant sibbing within each line subsequent to advancement by selfing for the initial three cycles since selection based on selfed progeny is expected to better mobilise additive genetic variation and can be especially used in the initial 2-4 cycles [7]. The plant-to- plant sibbing was to delay the surfacing of deleterious recessive alleles and provide for the chance of desirable gene alignments or combinations that could enhance vigour and productivity of the component lines.

The yield data recorded in the Test-cross hybrid trial showed significant differences between the lines, testers and hybrids as seen from Table 1. There were highly significant differences with regard to the lines, testers and line × Tester interaction. From Table 2 it can be seen that Specific combining ability effects of 5 of the 42 test-cross hybrids were significantly namely $P_7 \times LM_6$, $P_9 \times$ LM5, $P_{12} \times$ LM6, $P_{13} \times$ LM6 and $P_{14} \times$ LM5. The parents P_5, P_{15} and P_2 recorded positive and highly significant values for gca effects (Table 2). The top-ten test-cross hybrids varied in yield from 2.77 kg/plot ($P_{15} \times$ LM6) to 3.19 kg/plot ($P_5 \times$ LM6) (Table 3) which is 10.8 percent to 27.6 percent higher than the single cross hybrid check Paras which yielded 2.52 kg/plot. The highest yielding hybrids were having high sca effects. The parents to be advanced further were selected mainly based on their gca effects for yield and the overall performance of their hybrids. Crosses with LM6 tester were in general more heterotic than the crosses with the LM5 tester parent.

With respect to the final evaluation trial of experimental hybrids, inbred lines, sister lines (SL), sister line crosses (SLCs), testers and checks at Hyderabad (HYD) and Karimnagar (KRM), the ANOVA exhibited significant differences for all the characters studied.

Seperate randomisation of parents and hybrids enabled the orthogonality for hybrids alone to be

Table 1. ANOVA for line x tester analysis for yield (kg/plot) of Testcross hybrids

Source	DF	Mean sum of squares
Replications	2	0.67
Lines (L)	20	1.12**
Testers (T)	1	2.71**
L × T	20	0.30**
Error	82	0.10

**Significant at 1 per cent level

Table 2. Combining ability effects of test cross hybrids for yield

Parent	SCA effects of crosses with		GCA effects
	LM5	LM6	
P_1	0.23	–0.24	0.59**
P_2	–0.06	0.06	0.48**
P_3	0.05	–0.05	–0.36**
P_4	–0.17	0.17	–0.03
P_5	0.06	–0.07	0.76**
P_6	–0.20	0.20	0.36**
P_7	–0.38*	0.38*	0.24*
P_8	0.04	–0.05	0.02
P_9	0.37*	–0.37*	–0.53**
P_{10}	0.20	–0.20	–0.63**
P_{11}	–0.12	0.12	–0.58**
P_{12}	–0.31*	0.31*	–0.27*
P_{13}	–0.30	0.30*	–0.23*
P_{14}	0.44**	–0.45**	–0.53**
P_{15}	0.25	–0.25	0.53**
P_{16}	0.21	–0.21	0.33**
P_{17}	–0.04	0.04	–0.32**
P_{18}	0.07	–0.07	–0.35**
P_{19}	–0.18	0.18	0.14
P_{20}	–0.04	0.04	0.37**
P_{21}	–0.09	0.09	–0.05
LM5			–0.15**
LM6			+0.15**

*Significant at P = 0.05; **Significant at P = 0.01

Table 3. Mean values of test cross hybrids for yield (kg/plot)

Parent (@)	Test cross hybrid yield Cross with		Over all mean
	LM5	LM6	
P_1 (A1)	3.03	2.85	2.94
P_2 (A2)	2.62	3.04	2.83
P_3	1.89	2.08	1.98
P_4 (A3)	2.03	2.67	2.35
P_5 (A4)	3.03	3.19	3.11
P_6 (A5)	2.35	3.06	2.71
P_7 (A6)	2.06	3.12	2.59
P_8 (A7)	2.22	2.50	2.36
P_9	2.03	1.59	1.81
P_{10}	1.77	1.65	1.71
P_{11}	1.48	2.03	1.75
P_{12}	1.61	2.52	2.06
P_{13}	1.66	2.56	2.11
P_{14}	2.11	1.51	1.81
P_{15} (A8)	2.98	2.77	2.88
P_{16} (A9)	2.74	2.61	2.68
P_{17}	1.84	2.21	2.02
P_{18}	1.92	2.06	1.99
P_{19} (A10)	2.15	2.82	2.49
P_{20} (A11)	2.53	2.91	2.72
P_{21}	2.06	2.53	2.30
Check (Paras)			2.52

Overall hybrid mean = 2.24; Standard error of hybrids = 0.22; CV = 12.67%; @- (The parentheses indicate those parents selected for further use.)

evaluated separately. The top seven Sister line crosses (SLCs) varied in grain yield from 1.97 to 2.85 kg/plot (Table 4). The testers varied in yield from 2.0 kg/plot (CM111) to 2.31 kg/plot (CML290). Among the modified single cross hybrids the top ten crosses varied in grain yield from 3.5 kg/plot to 4.45 kg/plot outperforming the best check DHM103 by 17.4 to 49.15 percent. (Table 4).

observed that the lines and sister lines do not differ much and the SLCs resulting from them are invariably better yielding than their corresponding parents. Moreover, the SLCs $(A_1 \times A'_1)$, $(A_8 \times A'_8)$, $(A_9 \times A'_9)$ and $(A_{10} \times A'_{10})$ out yielded elite inbred testers used as male parents of the EHs and hence these SLCs are recommended as female parents of the Modified single cross hybrids. A purview of the cross combinations

Table 4. Mean performance of high yielding experimental hybrids vis-a-vis their component parents across the locations

Experimental hybrids Pedigree	Yield (kg/plot)	Superiority over Check(%)	Sisterline crosses Days to 50%			Testers Days to 50%		Inbred lines Days to 50%		Sister lines Days to 50%	
			Tassel	Silk	Yield (kg/plot)	Tassel	Yield (kg/plot)	Silk	Yield (kg/plot)	Tassel	Yield (kg/plot)
$(A_1 \times A'_1) \times$ CM 111	4.45	49.15	73	75	2.49	75	2.08	77	2.21	72	2.24
$(A_{10} \times A'_{10}) \times$ CML290	4.29	44.00	71	73	2.77	74	2.31	74	2.63	75	2.50
$(A_9 \times A'_9) \times$ CML251	3.99	33.75	68	69	2.20	72	2.25	75	1.98	72	1.97
$(A_1 \times A'_1) \times$ CML290	3.96	32.80	72	73	2.49	75	2.31	74	2.21	72	2.24
$(A_{11} \times A'_{11}) \times$CML 290	3.85	29.08	72	73	2.26	75	2.31	74	1.96	77	2.07
$(A_3 \times A'_3) \times$ CML 290	3.80	27.55	68	69	1.97	69	2.31	74	1.66	73	1.77
$(A_7 \times A'_7) \times$ CM111	3.78	26.93	73	74	2.31	73	2.08	77	1.81	73	1.90
$(A_7 \times A'_7)$ times CML290	3.74	25.54	72	73	2.31	73	2.31	74	1.81	73	1.90
$(A_7 \times A'_7) \times$ CML 251	3.57	19.76	70	72	2.31	73	2.25	75	1.81	72	1.90
$(A_8 \times A'_8) \times$ CML 290	3.50	17.40	69	70	2.85	71	2.31	74	2.61	72	2.54
$(A_1 \times A'_1) \times$ CML247	3.46	15.97	73	74	2.49	75	2.19	74	2.21	72	2.24
DHM 103 (Check)	2.98	-	72	74	-	-	-	-	-	-	-

Overall mean of Modified single cross hybrids = 3.13 kg/plot; LSD hybrids (0.05 level) 0.58; (0.01 level) 0.77; cv = 13%

Vasal *et al.* [8] reported that tropical and subtropical maize populations show a high level of inbreeding depression, a trait which needs to be improved to make them suitable as hybrid oriented source germplasm. In this study, the modification carried out was that no inbreeding was done subsequent to recombining sister lines and sister line crosses were themselves used as female parent of the Modified single cross hybrids developed using established inbred lines as male parents. Sister lines are more or less similar to the corresponding inbred lines with minor differences in respect of gene fixation. These minor genetic differences would lead to residual heterozygosity in the sister line crosses (SLCs), that enhances their performance, thus endowing the SLCs with suitability as likely female parents of potential single cross hybrids.

The enhanced yield levels of inbred lines, sister lines and sister line crosses derived using modified version of FRIP reveals the efficacy of the methodology used to enhance the parental performance. The synchrony of male and female parents was also appropriate (Table 4) signaling the feasibility of hybrid seed production.

From Table 4 depicting the yield performance of elite experimental hybrids and their parents it can be with high heterosis shows that the testers drawn from diverse sources have given heterotic crosses in combination with the SLCs developed. Thus the importance of genetic divergence to increase heterotic response is reiterated [9].

When the yield levels of the top ten testcross hybrids (ranging from 2.7 kg/plot to 3.1 kg/plot) developed initially are compared to modified single cross hybrid yields (ranging from 3.5 kg/plot to 4.45 kg/plot) developed subsequently, the latter significantly out yielded the testcross hybrids. Hence Modified Single Cross Hybrids developed here can be tried as effective means to get higher maize productivity.

The results on the *per se* performance of line crosses vis- a-vis experimental hybrids revealed that a majority of the high yielding SLCs recorded high values for various yield related traits. The lines developed showed improved *per se* performance. Most of the lines and sister lines developed, out yielded the inbred testers. The sister line crosses were superior to their component lines and sister lines. The grain yield levels of some Modified Single Cross Hybrids were significantly superior than the best check hybrid Deccan 103. The high heterosis of the experimental hybrids and good *per se* performance of the parents derived namely

SLCs, lines and sister lines indicate the effectiveness of utilising the Modified Forward and Reverse Inbreeding Process method in deriving superior parents that can be used for enhancing line development and ultimately hybrid production. Further, the lines and sister lines derived can themselves be used as female parents with various other testers and subsequently their hybrid performance can be assessed. The best heterotic hybrids developed from this study have been entered into the National Maize Co-ordinated Trials for further testing on a large scale.

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