Australian Journal of Crop Science

AJCS

AJCS 6(3):455-462 (2012) ISSN:1835-2707

Heterosis for yield and agronomic attributes in diverse maize germplasm

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Abstract

Combining ability estimation are important genetic attributes for maize breeders in anticipating improvement in productivity via hybridization and selection. Maize exhibits heterosis for all traits and the extent of heterosis is significantly variable depending on the choice of parents and the trait(s) measured. We developed hundreds of S_2 lines in 2009 and out of these S_2 lines, several selected lines were crossed with three different testers in three isolations to generate testcrosses (spring 2010). The testcrosses were evaluated in a partial lattice square design with two replications to divulge combining ability and heterosis for particular traits of economic importance (summer-2010). Phenotypic data were recorded for flowering time, morphological traits and total yield to calculate total variability, combining abilities and mid parent (MPH) and better parent (BPH) heterosis. The 87 testcrosses possessed highly significant differences ($P \leq 0.01$) for the investigated traits. Proportional contribution of lines, tester and their interaction to the total variability for anthesis silking interval (ASI) was 38.33, 2.34 and 59.33%, respectively. Means yield ranged from 2547.45 to 9842.02 kg ha^{-1}. Maximum yield was observed for CMI-76, using OPV Jalal as a tester, while minimum yield was detected for CMI-190-2, using WD3x 6 as a tester. In general, tester parent OPV Jalal showed better heterotic effect with each line as compared to other testers. Based on these results we conclude that maize unveiled excessive ability for heterotic expression, even several inbred lines exhibit enough variability to find out appropriate genotypes for a successful breeding program and generating stable inbred lines for production of commercial hybrids. Genetic variability and harboring of excessive hybrid vigor makes maize as a model crop for all kind of studies. Furthermore, these results suggested that the input cost of hybrids can easily be minimized by avoiding losses of resources and time in production of desirable inbred lines, as it is obvious that the breeding community always preferred hybrids maize rather than OPV or synthetics varieties because of its high productivity.

Keywords: combining ability, heterosis, line x tester analysis, maize, yield, morphological traits.
Abbreviations: MPH (mid parent heterosis); BPH (better parent heterosis); CMI (Cereal Crop Research Institute (CCRI) maize inbred lines); OPV (open pollinated variety); ASI (anthesis silking interval); WD (white diallel), SCA (specific combining ability); GCA (general combining ability).

Introduction

Heterosis, or hybrid vigor, is the better performance of a hybrid relative to the parents, and is the outcome of the genetic and phenotypic variation. Most traits of economic importance are qualitative and controlled by several to many major genes. Generally heterosis can be divided into two broad categories, true heterosis and pseudoheterosis. In case of true heterosis, there is an increase in general vigor, yield and adaptation. In case of pseudoheterosis, the F_1 hybrid exhibits increase in vegetative growth only. It refers to the superiority of F_1 over the standard commercial check variety. So, it is also called economic heterosis or superiority over checks (Sharief et al., 2009). Recently it has been divulged that the utilization of heterosis is extremely effective for the genetic improvement of different traits and that the concepts of combining ability are the fundamental tools for enhancing productivity of different crops in the form of F_1 hybrids (Flint-Garcia et al., 2009). Heterosis occurred in the F_1 hybrids for all traits of interest, but their values varied among crosses and characters. However, Genotypes harboring desirable attributes and stable performance are vital, both as cultivars and as source of desirable germplasm for further improvement. Maize production can be boost up by providing some relevant and basic information about the pattern and genetic variability to the breeding community. Combining ability studies provide information on the genetic mechanisms controlling the inheritance of quantitative traits and enable the breeders to select suitable parents for further improvement or use in hybrid breeding for commercial purposes. In biometrical genetics two types of combining abilities are considered i.e. general combining ability (GCA) and specific combining ability (SCA). General combining ability refers to the average performance of the genotype in a series of hybrid combinations and is a measure of additive gene action whereas; specific combining ability is the performance of a parent in a specific cross in relation to general combining ability (Sharief et al., 2009). SCA is due to genes showing non-additive effects (Sprague and Tatum, 1942). Line × tester mating design was developed by Kempthorne (1957), which provides reliable information on the general and specific combining ability effects of parents and their hybrid combinations in applied breeding programs. The design has been widely used in maize breeding by several workers and continues to be applied in quantitative genetic studies in maize due to its significance (Sharma et al., 2004). Early generation testing is extremely important for efficient evaluation and production of inbred lines of maize. The use of testers in a maize recurrent selection program has been well documented (Menz et al., 1999). The use of a single-cross as a tester has been reported by Horner et al. (1976). The use of an inbred line as tester in a recurrent selection program was suggested by Russell and Eberhart, (1975) and it has been widely used by breeders. We used all these types of tester to dissect different traits and show the diversity in the germplasm. Early generation testing is a pivotal method for plant breeders to discard undesirable materials while generating inbred lines for the production of hybrids (Ali et al., 2011a). Due to early testing, the best performing inbred lines are identified, and the resulting progeny evaluated for grain yield and general performance for other parameters. The present study was aimed to evaluate and identify superior maize inbred lines (S_2) for different traits in early generation for production of stable inbred lines. We examine heterosis for multiple phenotypic traits and divulged different lines crossed with several testers of different genetic background to explore the phenomenon and provide some basic information about the diversity of maize and utilization of heterosis for further enhancement. Analysis of data for several traits in different lines provides an opportunity to make inferences about the underlying mechanisms of heterosis. Furthermore, to test the combining ability of the maize S_2 lines as potential source in the production of improved maize inbred lines and to divulge the level of heterosis at early stage.

Results

Line × Tester Analysis

Line × tester analysis was carried out to detect the combining ability effects for 87 testcrosses for various agronomic traits including morphological traits, days to anthesis silking interval (ASI) and grain yield. The significance level ($P \leq 0.01$) and mean square value of all the investigated traits are shown in Table 1. The total variance is further partitioned into several components, like variance due to lines, testers and their interactions. We observed a non-significant genetic variation among lines for all the traits except percent grain moisture at harvest. Highly significant differences were observed among testers for all traits except ASI, percent grain moisture at harvest. Line × tester interaction was highly significant for plant height, ear length, ASI and grain yield (kg ha^{-1}). The coefficient of variation (CV) for all the traits was investigated to show the reliability of this experiment (Table 1). Heterosis is the superiority of an F_1 hybrid produced through crossing of two genetically different individuals over the mean of its parents or the better parent. Mid parent (MPH) and better parent (BPH) heterosis was estimated for plant height (PH), ear height (EH), anthesis silking interval (ASI) and grain yield (kg ha^{-1}). Analysis of variance revealed highly significant differences ($P \leq 0.01$) among the genotypes for different traits (Table 1). Heterosis was obvious in different crosses for all the characters and the magnitude varied significantly for traits and crosses. The hybrid vigor can help to increase the yield by several times than OPV and will solve the problem of starvation in the developing countries.

Combining ability and performance of Germplasm

The GCA and SCA effect varies for all the characters and the mean performance of all the lines crossed with different testers are given in the concerned table for each trait. Means and combining ability effects of all the traits were calculated to show the performance of different lines regarding different traits. For plant height, testcrosses ranged from 108.7 (CMI-SL 34 with WD 2× 8 as a tester) to 173.4 cm (CMI-119 with Jalal as a tester). The overall mean for testcrosses was 147.87 cm, while mean of the check was 137.85 cm. The highest value of plant height was recorded for CMI-119 with Jalal as a tester and lowest value CMI-Sl(34) with WD3×6 as tester. In all, 82% of the testcrosses had higher plant height than the check. GCA effects can be considered as the numerical values assigned to the parents in relation to their mean performance in cross-combinations. Among the 29 testcrosses, 16 lines exhibited negative GCA effect. GCA effects ranged from -14.32 to 17.05. The SCA effects ranged from -27.34 to 32.86 while the best SCA effect was detected for CMI-82 and worst was for CMI-191, using WD 3×6 as a tester regarding plant height. Maximum ear height was detected for CMI-6, using OPV Jalal as a tester, while minimum ear height was observed for CMI-Sl (34), using WD 3 × 6 as a tester (Table 3). GCA values ranged from -8.23 to 11.90 and totally 14 lines showed negative value. The highest GCA effect was observed for CMI-187 and the lowest effect was detected for CMI-Sl(22). Out of 87 testcrosses, 44 showed positive specific combining ability effect. SCA values ranged from -16.69 to 17.97. About 9% of the testcrosses showed lower ear height when compared to S2 lines. 55.2% testcrosses possessed positive GCA effects, while 44.8% were negative while 50.6% testcrosses showed positive and 49.4% showed negative SCA effects. CIM-Sl(22) showed maximum value (5 days) for ASI, using WD2×8 as a tester, while minimum mean value (-2.28 days) was observed for CMI-149, using WD3×6 as a tester (Table 4). Grand mean for all testcrosses was -1.24 days and15 crosses out of 29 testcrosses divulged positive GCA effect for ASI (Table 4). The results revealed that 67% of the testcrosses had lower ASI than the mean of the check and overall 48.3% testcrosses showed positive GCA effects, while 51.7% were having negative GCA values. About 50.6% testcrosses showed positive SCA effects; while 49.4% testcrosses exhibited negative SCA effects. The highest GCA effect (1.26) was observed for CMI-191 and CMI-Sl(22), while SCA effect of CMI-Sl(22) was 2.28, using WD2×8 as a tester. Mean yield ranged from 2547.45 to 9842.02 kg ha^{-1} and maximum yield was observed for CMI-76, using OPV Jalal as a tester, while minimum yield was detected for CMI-190-2, using WD3x 6 as a tester (Table 5). Overall mean for testcrosses was 6204.04 kg ha^{-1}, while for check mean yield was 5296.58 kg ha^{-1}. The GCA effect was positive for 15 crosses out of 29 and the total effect ranged from -1297.31 to 1540.11. The best GCA was observed for CMI-119, while the worst effect was exhibited by CMI-82. A total of 42 testcrosses showed positive effect and the SCA effects ranged

Table 1. Mean squares for plant height (PH), ear height (EH) anthesis silking interval (ASI) and grain yield (kg ha^{-1}) of testcrosses derived from S_2 maize inbred lines

SOV	df	PH	EH	ASI	Grain yield
Replication	1	37.24	387.22	0.10	15492716.40
Treatments	120	686.61**	243.67**	2.25**	3702690.63**
C VS (P, TC)	1	28.00 NS	23.86 ^{NS}	0.94 ^{NS}	5620355.55*
P VS TC	1	34492.19**	7488.37**	0.24 ^{NS}	75793455.92**
Checks (C)	1	364.81 ^{NS}	249.64 ^{NS}	0.25 ^{NS}	3215734.82 ^{NS}
Parents (P)	31	411.23*	158.18*	2.06 ^{NS}	2784059.63**
Testcrosses (TC)	86	404.18*	192.73**	2.38**	3178924.20**
Lines	28	414.55 ^{NS}	187.29 ^{NS}	2.80 ^{NS}	1780155.08 ^{NS}
Testers	2	3794.83**	1448.63**	2.40 ^{NS}	41253722.22**
Line x Tester	56	277.91 ^{NS}	150.60*	2.17**	2518494.54**
Error	120	259.69	92.42	1.21	1446775.80
Cv %		11.47	14.61	-------	20.59

Table 2. Means, GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from $CMIS_2$ maize inbred lines for plant height (PH).

S2 lines			Testers					
	Parent mean	GCA	WD2×8 (T_1)		Jalal (T_2)		WD3×6 (T_3)	
			PH (cm)	SCA	PH (cm)	SCA	PH (cm)	SCA
CMI-1	133.60	1.15	152.70	-0.87	153.00	-9.56	157.20	10.43
CMI-4	109.30	13.52	149.90	0.66	154.63	-0.51	151.40	-0.15
CMI-6	122.10	-4.82	147.40	-13.30	165.30	3.91	150.40	9.39
CMI-18	100.20	-6.39	132.88	-3.75	139.70	-8.72	145.50	12.47
CMI-48	115.60	9.98	130.90	-7.00	163.20	4.41	152.00	2.59
CMI-71	131.50	-2.92	143.10	-9.60	165.60	-9.39	157.00	18.99
CMI-76	129.10	0.68	140.00	5.20	145.80	15.51	138.20	-20.71
CMI-81	105.60	-10.32	152.00	7.10	163.10	0.01	122.70	-7.11
CMI-82	155.45	13.71	141.90	-7.43	151.10	-25.43	156.00	32.86
CMI-89	121.00	-1.97	144.90	-6.25	146.60	0.06	155.80	6.18
CMI-90	113.70	6.78	132.00	3.40	155.60	-9.69	149.33	6.29
CMI-115	146.45	-1.49	160.50	-4.63	162.90	11.57	155.50	-6.94
CMI-119	129.00	17.05	147.90	-3.37	173.40	5.84	144.50	-2.47
CMI-129-2	141.40	-10.55	154.00	-2.77	161.50	2.64	143.50	0.13
CMI-131-2	114.70	-9.59	125.10	-14.23	157.40	9.07	134.00	5.16
CMI-137	98.20	-0.19	123.20	-3.33	154.30	2.07	128.50	1.26
CMI-138	119.70	-2.19	134.10	-2.03	169.70	6.27	147.50	-4.24
CMI-141	122.10	-9.99	149.00	4.87	155.10	3.57	143.50	-8.44
CMI-149	118.70	-7.85	138.70	-1.77	146.60	5.34	114.50	-3.57
CMI-152	121.80	10.61	139.30	-0.63	158.40	-4.83	140.00	5.46
CMI-183	122.90	6.98	155.10	0.70	159.20	-9.79	162.50	9.09
CMI-185-2	108.70	7.75	153.10	-4.47	152.10	-6.36	160.00	10.83
CMI-187	103.20	0.61	152.40	6.67	163.80	-2.63	146.50	-4.04
CMI-190-2	116.00	-5.84	153.40	6.02	147.53	2.46	126.10	-8.48
CMI-191	122.80	-5.84	151.10	18.12	158.90	9.22	126.00	-27.34
CMI-197	123.30	1.38	156.50	5.50	157.70	-8.59	124.65	3.09
CMI -200	121.40	7.76	159.90	11.22	149.80	-0.47	128.60	-10.75
CMI-Sl(22)	128.50	-14.32	147.90	4.80	155.70	10.11	139.43	-14.91
CMI-Sl(34)	118.68	-3.72	137.40	11.20	161.60	3.91	108.70	-15.11

Sl = Selected line, Grand mean for testcrosses = 147.87 cm, Mean value for check = 137.85 cm

from -2963.85 to 3864.15. Maximum SCA effect was observed for CMI-119, using OPV Jalal as a tester, while minimum for CMI-119, using WD 3 × 6 as a tester. The total variation of all the testcrosses derived from S_2 lines were subdivided into several components and the percent contribution of line, tester and line × tester are presented in table 6.

Mid parent and better parent heterosis

Percent heterosis over mid parent and better parent were calculated and summarized for all the traits (Supplementary table A). MPH for plant height ranged from -17.87 to 75.09% while for ear height MPH varied from -34.54 to 86.36%. Maximum MPH was detected for OPV Jalal crossed with CMI-4 and CMI-48 for plant height and ear height, respectively. It is evident from the table that Jalal as a tester performed better than other testers for the morphological traits. The BPH varied from -23.12 to 61.71% and from -42.31 to 68.98% for plant height and ear height, respectively. Maximum BPH was observed for CMI-137, using OPV Jalal as a tester, while minimum heterosis was observed for CMI-191 regarding these two traits, using WD 3×6 as a tester. Mid parent heterosis for ASI ranged from -500 to 700 %. Maximum MPH for ASI was observed for CMI-90 and CMI-149, using Jalal as a tester. Regarding ASI, BPH ranged from -300 to 300 % and maximum value was observed for CMI-90 and CMI-149, using Jalal as a tester, while minimum for

CMI-149, using WD 3×6 as a tester. MPH ranged from -22.00 to 112.00% and 77 crosses showed positive MPH for total grain yield. The cross of CMI-6 with OPV Jalal outclassed all the testcrosses in term of grain yield, while testcross CMI-Sl(22) with WD3 × 6 was the worse among the breeding material. The BPH ranged from-34.90 to 106.76% and 65 out of 87 crosses showed BPH of positive nature. Maximum BPH was calculated for CMI-6, using OPV Jalal as a tester, while minimum was noted for CMI-82, using WD3 × 6 as a tester.

Discussion

Heterosis increases yield potential and improves adaptation to stress in maize; however, the underlying mechanisms of heterosis and combining ability remain elusive (Araus et al., 2010). Maize exhibits great potential for heterotic expression and its utilization, even less than 100 lines exhibit enough variation to figure out desirable genotypes for a successful breeding program. Genetic variation and heterosis are the basic reasons that breeding community always preferred hybrids maize rather than open pollinated varieties or synthetics varieties. The analysis of heterosis for several attributes in a small set of testcrosses revealed that maize hybrids exhibit both MPH and BPH for almost all the traits. We observed that over 67% heterosis was exhibited in a small panel while 90% heterosis was observed by Flint-Garcia et al. (2009), analyzing a large set of hybrid maize for BPH. Partitioning the total genetic variation into several components (i.e. variance due to line, tester and interaction) showed that non-significant variation was exists among all the lines for the investigated traits regarding lines. These results showed that all the lines were almost from the same genetic pool and lacking genetic diversity. The use of several broad genetic testers provides enough genetic variation to figure out a reasonable amount of heterosis even in the early generations. The overall diversity and total variation in the experimental material was obvious from the results of tester and line × tester interaction. In most cases the crosses from the same genetic background exhibited relatively low levels of heterosis, but high levels of heterosis from crosses of related lines were also observed (Flint-Garcia et al., 2009). Heterosis was observed for several combination and the lines with high values of combining ability will be further selfed to generate stable inbred lines that will be evaluated in commercial breeding programs.

Morphological traits

Plant height and ear height are important morphological traits affecting the final yield of maize crop. Extremely dwarf varieties have the problem of crowded canopy, aeration and transmission of sun light to the lower parts resulting in drastic reduction in yield while the high stature plants are highly susceptible to lodging (Ali et al., 2011b). Greater ear height is undesirable because the ear placement at a greater height from the ground level exerts pressure on plant during grain filling and physiological maturity and causes lodging, which could ultimately affect the final yield. Keeping in view the importance of morphological traits, we have observed varying degree of combining ability and the contribution of line × tester interaction was relatively higher for plant height compared to the other traits. Evaluating different germplasm in different studies, high levels of combining ability were observed (Carena, 2005) and it has been mentioned that GCA effects always dependent on the breeding material or germplasm involved in the evaluation (Konak et al., 2001). The magnitude of heterotic effect appeared to vary for the morphological traits and 9% of the testcrosses showed lower ear height when compared to S_2 lines. The negative/least estimates of heterosis for ear height are preferred because low cob bearing is desired in maize breeding. Yao et al. (2011) also concluded that selection for plant height and its components would be effective in early generation and improvement in these traits will be promising to develop new varieties with desirable traits, most importantly lodging resistance.

Flowering and Yield

In multiple cropping systems and for regions with short growing seasons, early maturing varieties are needed. Days required to tasseling along with other maturity traits are commonly used by plant breeders as basis of determining maturity of maize. Anthesis silking interval revealed the time span or heat units required between anthesis to pollination. It is a trait used mostly in screening genotypes for tolerance to stresses especially for drought resistance (Lu et al., 2010). It is directly correlated with seed setting percentage in a reciprocal way. Lesser the gap between tasseling to silking in a cross, greater will be the probability of grain setting hence, more will be grain yield while maximum grain yield is the prime objective in most breeding programs In order to develop high yielding early maturing hybrids, information regarding GCA and SCA inheritance pattern can facilitate breeders in improving genetic architecture of the maize in particular direction in the long run. Many researchers reported that genetic variance among testcross progenies using inbred testers was about twice, when broad base testers were used (Sharief et al., 2009). Rahman et al., (2009) suggested that selection in early generations would be fruitful in improving several traits while analyzing the genetic behaviour of yield related traits in mungbean genotypes. Semel et al. (2006) report that better-parent heterosis was observed primarily for reproductive traits related to yield. In this study we have divulged significant level of heterosis for the reproductive traits. About 73% of the testcrosses showed higher grain yield and 52% testcrosses showed positive GCA effects; while 48% were having negative GCA effects. In all, 48.3% testcrosses showed positive SCA effects, while 51.7% were having negative SCA effects. Paul and Debenth (1999) reported that GCA effects were significant for anthesis silking interval and that additive gene action played a major role in the inheritance of this trait. Perez-Velasquez et al. (1996) reported that maize grain yield and flowering traits were mostly under the control of non-additive (SCA effect) type of gene action. In general, tester parent OPV Jalal showed better heterotic effect with each line as compared to other testers. Over all maize possess extravagant variation for all traits and this variability can be easily used by the plant breeders to develop high yielding and early maturing genotypes for global food security. In the near future the increasing world's population will require more food and maximum part of this food will come from maize crop (Ali and Yan, 2012). It has been estimated that more than half of the increased demand in the world food in term of cereals as a whole will be produced from maize farmers and consumers (Yan et al., 2011).

Material and methods

The experiment was conducted at Cereal Crop Research Insttute (CCRI) and Agricultural Research Farm, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan,

Table 3. Means, GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from $CMIS_2$ maize inbred lines for ear height (EH).

S_2 line	Parent mean	GCA	WD2×8 (T_1) EH (cm)	WD2×8 (T_1) SCA	Jalal (T_2) EH (cm)	Jalal (T_2) SCA	WD3×6 (T_3) EH (cm)	WD3×6 (T_3) SCA
CMI-1	63.90	-3.30	77.00	4.34	76.00	-5.91	61.10	1.57
CMI-4	46.50	9.06	69.70	0.98	62.28	-4.60	71.00	3.61
CMI-6	57.30	3.30	73.20	-9.86	87.70	5.29	74.40	4.57
CMI-18	43.20	-6.23	67.80	1.87	64.60	-8.98	68.50	7.10
CMI-48	52.10	3.10	61.70	-2.26	80.90	7.49	63.50	-5.23
CMI-71	55.30	0.30	65.70	-9.56	82.60	-1.01	73.50	10.57
CMI-76	55.90	-3.65	65.10	4.59	67.30	3.49	58.70	-8.08
CMI-81	47.20	-7.06	72.20	3.91	69.45	-0.85	53.20	-3.06
CMI-82	72.38	2.80	64.60	-1.36	67.10	-9.71	66.00	11.07
CMI-89	70.00	0.58	70.00	-4.84	71.10	0.51	69.88	4.33
CMI-90	53.83	5.57	64.00	0.67	75.60	-3.98	67.17	3.30
CMI-115	64.40	4.14	81.10	3.31	80.90	7.95	72.50	-11.26
CMI-119	62.10	8.74	72.90	-2.69	86.30	3.55	64.50	-0.86
CMI-129-2	74.00	-5.60	71.10	-6.66	78.80	9.19	64.00	-2.53
CMI-131-2	51.40	-7.29	54.30	-7.07	72.43	-0.09	60.00	7.16
CMI-137	46.10	-3.17	59.20	4.02	71.70	2.56	52.00	-6.58
CMI-138	49.00	-3.90	68.60	7.34	79.30	7.89	56.88	-15.23
CMI-141	64.70	-5.93	73.50	4.17	75.30	4.52	49.50	-8.70
CMI-149	50.70	3.40	71.40	-6.46	66.80	-11.51	65.25	17.97
CMI-152	53.70	3.84	66.10	0.71	72.80	-5.25	75.25	4.54
CMI-183	57.20	7.17	74.70	-0.83	72.10	-7.88	80.00	8.70
CMI-185-2	47.67	3.47	73.90	-3.93	67.80	-8.48	77.50	12.40
CMI-187	51.90	11.90	78.60	0.34	78.40	-7.71	84.20	7.37
CMI-190-2	54.30	-3.91	71.70	-2.54	71.70	1.60	65.60	0.94
CMI-191	57.50	-3.94	66.05	4.83	79.43	11.86	61.75	-16.69
CMI-197	60.30	-2.50	71.20	4.54	71.50	-2.81	50.40	-1.73
CMI -200	60.50	3.21	75.90	2.33	74.40	-0.62	54.80	-1.71
CMI-Sl(22)	56.10	-8.23	71.50	-2.13	70.30	13.82	61.43	-11.70
CMI-Sl(34)	54.08	-5.86	58.90	12.21	84.30	-0.35	40.00	-11.86

Sl = Selected line, Grand mean for testcrosses = 63.35 cm, Mean value for check = 63.40 cm

during 2009-10. Standard cultural practices including irrigation, fertilizer application, hoeing and thinning were carried out throughout the growing seasons.

Genetic material

Preparation of Genetic Material

Improvement in maize germplasm has been initiated in Plant Breeding and Genetics Department of Agriculture University, Peshawar with the collaboration of Cereal Crop Research Institute (CCRI) Pakistan, using different breeding procedure for increasing the overall average production of maize and release of hybrids for commercial use. Inbred lines were generated from different varieties (Azam, Sarhad white (SW), Baber and land races collected from different parts of the country. Thousands of inbred lines were generated using manual self-pollination procedure during 2008-09 (Russell and Hallauer, 1980). Out of these lines, several lines were selected on the basis of their performance for disease resistance and seed setting to generate advance lines for hybrids and varieties production. During this experiment the CMIS2 (CCRI maize inbreds S2) lines were evaluated to find out the combining ability of different lines for yield and related attributes. In the spring season (February – June) S_2 lines was out crossed with three testers viz; WD 2 × 8, WD 3 × 6 and Jalal variety at three isolations. The first two testers were high yielding single cross hybrid derived from white diallel and Jalal is the high yielding well known open pollinated white maize variety. The pedigree of the germplasm along with their relative information is provided in table 7. In the second season (July – October), performance of the testcrosses were evaluated along with their S_2 parents, testers and check varieties. The experiment was laid out in lattice square design with two replications. The genotypes were grown in single row plots, with row length of 3m, having row to row and plant to plant distance of 0.75 and 0.25m, respectively. Two seeds per hill were planted and were thinned to one plant per hill at 4-5 leaf stage. Data were recorded for maturity, morphological and other agronomic traits as and when appropriate.

Plant height (cm)

Plant height in each plot was recorded after completion of male flowering, as the distance between the ground surface and node bearing the flag leaf, using five randomly selected plants in each row. Average data in cm were used for further analysis (Ali et al., 2011b).

Ear height (cm)

Ear height was measured in cm from the ground level to the base of apical ear as an average of five randomly selected

Table 4. Means, GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from CMIS$_2$ maize inbred lines for ASI (Anthesis silking interval).

S_2 lines	Parent mean		Testers					
			WD2×8 (T_1)		Jalal (T_2)		WD3×6 (T_3)	
		GCA	ASI	SCA	ASI	SCA	ASI	SCA
CMI-1	2.00	-0.07	1.50	0.11	0.50	-0.61	1.50	0.51
CMI-4	3.50	-0.07	0.50	-0.89	1.00	-0.11	2.00	1.01
CMI-6	1.00	-1.24	2.00	1.78	-1.00	-0.95	-1.00	-0.83
CMI-18	3.00	-0.57	1.00	0.11	0.00	-0.61	1.00	0.51
CMI-48	3.00	0.09	1.00	-0.56	2.00	0.72	1.00	-0.16
CMI-71	1.50	0.26	2.50	0.78	1.00	-0.45	1.00	-0.33
CMI-76	0.00	0.43	1.00	-0.89	2.00	0.39	2.00	0.51
CMI-81	1.50	-0.91	1.00	0.44	-1.00	-1.28	1.00	0.84
CMI-82	0.50	-0.91	1.00	0.44	1.00	0.72	-1.00	-1.16
CMI-89	1.00	-1.07	0.50	0.11	-1.00	-1.11	1.00	1.01
CMI-90	0.50	-0.07	0.00	-1.39	2.00	0.89	1.50	0.51
CMI-115	1.50	-0.24	0.00	-1.22	1.00	0.05	2.00	1.17
CMI-119	2.00	-0.74	0.50	-0.22	1.00	0.55	0.00	-0.33
CMI-129-2	-0.50	-0.57	0.00	-0.89	1.00	0.39	1.00	0.51
CMI-131-2	1.00	0.26	1.50	-0.22	1.00	-0.45	2.00	0.67
CMI-137	1.50	-0.07	1.00	-0.39	2.00	0.89	0.50	-0.49
CMI-138	2.50	1.09	2.50	-0.06	3.50	1.22	1.00	-1.16
CMI-141	0.50	0.59	3.00	0.94	1.50	-0.28	1.00	-0.66
CMI-149	0.50	-0.74	1.50	0.78	2.00	1.55	-2.00	-2.33
CMI-152	1.50	0.26	1.00	-0.72	3.00	1.55	0.50	-0.83
CMI-183	-1.00	-0.07	2.00	0.61	0.00	-1.11	1.50	0.51
CMI-185-2	2.00	0.09	2.00	0.44	1.50	0.22	0.50	-0.66
CMI-187	2.50	0.93	2.00	-0.39	1.50	-0.61	3.00	1.01
CMI-190-2	2.50	0.09	1.50	-0.06	1.00	-0.28	1.50	0.34
CMI-191	1.50	1.26	3.00	0.28	2.50	0.05	2.00	-0.33
CMI-197	1.00	0.93	2.00	-0.39	1.50	-0.61	3.00	1.01
CMI -200	1.00	0.09	2.00	0.44	0.50	-0.78	1.50	0.34
CMI-Sl(22)	1.50	1.26	5.00	2.28	1.50	-0.95	1.00	-1.33
CMI-Sl(34)	1.00	-0.24	0.00	-1.22	2.00	1.05	1.00	0.17

Sl = Selected line, Grand mean for testcrosses = 1.24 days, Mean value for check = 1.75 days

plants per plot and then means were calculated.

Anthesis silking interval (ASI)

ASI was calculated by the following formula:
ASI = Days to 50% silking – Days to 50% pollen shedding

Grain yield (kg ha^{-1})

Field weight of each entry taken at the time of the harvest was converted to grain yield ha^{-1} with the help of the following formula:

Grain yield (kg ha^{-1}) =

$$\frac{Field\,weight\,(kg) \times (100 - MC) \times 10000\,(m^2) \times 0.8}{85 \times plot\,area\,(m^2) \times ha}$$

Where:

Field weight = Fresh weight of the ears row^{-1}
0.8 = Shelling coefficient
85 = Grain moisture standard value at 15% MC = Moisture content (%) in grains at harvest

Statistical analysis

The data were subjected to ANOVA, appropriate for line × tester design using computer programme SAS by an interactive macro program for line × tester analysis (Bartolome). Analysis for GCA and SCA was carried out following Singh and Chaudhary (1979). General combing ability effects were calculated using the expression:

$$gi = \frac{Xi\,..}{tr} - \frac{X\,...}{ltr}$$

l = number of lines
t = number of testers
r = number of replications

Specific combining ability effects were calculated using the expression:

$$si = \frac{Xij}{r} - \frac{Xi...}{tr} - \frac{X.j.}{lr} + \frac{X...}{ltr}$$

Mid-parent heterosis was calculated as percent increase (+) or decrease (-) exhibited by the F_1 hybrids over mid parents:

$$Mid\ parent\ heterosis = \frac{F_1 - mid\ parent}{Mid\ parent} \times 100$$

Table 5. Means, GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from CMIS$_2$ maize inbred lines for grain yield (kg ha^{-1}).

S_2 line	Parent mean	GCA	WD2×8 (T_1) Grain yield	WD2×8 (T_1) SCA	Jalal (T_2) Grain yield	Jalal (T_2) SCA	WD3×6 (T_3) Grain yield	WD3×6 (T_3) SCA
CMI-1	4423.98	615.66	5906.41	-963.45	6763.92	-1823.87	7644.44	2787.32
CMI-4	4655.69	68.26	5595.90	253.57	6386.20	-669.55	6386.20	415.97
CMI-6	3344.61	-423.04	6004.71	-657.00	6917.44	699.36	5234.73	-42.36
CMI-18	3697.30	726.33	9741.18	1393.95	6438.90	-1098.29	5783.96	-295.66
CMI-48	5290.76	480.00	6607.06	74.47	7414.38	-77.56	6107.19	3.09
CMI-71	6810.14	-504.31	5906.41	-381.52	7078.79	782.88	5159.04	-401.36
CMI-76	5071.69	787.18	6437.65	-2278.65	9842.02	66.72	8594.82	2211.93
CMI-81	5569.68	-108.94	6990.70	602.69	7197.76	-487.91	5294.12	-114.78
CMI-82	6866.82	-1297.31	5582.52	781.08	5510.16	-953.27	4705.88	172.19
CMI-89	5673.20	192.15	5552.94	-1328.90	9148.24	81.46	5537.25	1247.44
CMI-90	7025.88	1054.37	5806.01	696.97	5897.14	-2368.46	8847.06	1671.49
CMI-115	5217.25	-228.49	5513.73	-912.26	9411.76	1699.09	4580.39	-786.83
CMI-119	5992.16	1540.11	6908.24	-900.31	9827.45	3864.15	4278.07	-2963.85
CMI-129-2	6298.75	-140.71	5284.28	526.78	6955.29	470.47	4335.12	-997.25
CMI-131-2	7570.59	571.71	6040.64	-513.78	8114.51	908.64	5772.55	-394.87
CMI-137	3441.05	786.26	5068.41	-980.57	5552.24	-2527.66	8847.06	3508.23
CMI-138	5362.82	-960.29	5613.59	193.98	6461.96	154.49	4238.78	-348.47
CMI-141	4031.82	-761.52	4871.92	45.65	7105.88	82.66	5228.76	-128.30
CMI-149	3487.22	113.67	5457.03	-1555.83	6002.02	-902.11	8522.88	2457.95
CMI-152	4680.78	-286.92	6370.51	1131.63	6824.58	6.58	4517.65	-1138.20
CMI-183	5741.96	-880.75	4312.30	653.21	6090.46	87.89	3973.86	-741.10
CMI-185-2	6094.64	-1082.36	5732.05	152.01	7914.25	-9.90	4768.63	-142.11
CMI-187	3609.41	-684.91	5861.53	133.07	7609.41	476.55	4304.31	-609.62
CMI-190-2	3781.85	834.17	5601.88	-1253.32	7408.94	2884.77	2547.45	-1631.45
CMI-191	5189.02	26.98	6447.06	249.60	7750.27	398.19	5513.73	-647.79
CMI-197	3887.45	-181.81	5172.71	-515.79	6794.40	1449.69	4505.10	-933.90
CMI-200	4149.96	298.79	6255.20	-45.74	6617.52	-355.60	3079.53	401.34
CMI-Sl(22)	4567.84	-772.19	8121.31	1972.41	4947.90	-434.04	3285.15	-1538.37
CMI-Sl(34)	4439.50	217.92	9210.98	3426.05	5249.52	-2405.36	3750.59	-1020.69

Sl = Selected line, Grand mean for testcrosses = 6204.04 kg ha$^{-1,}$ Mean value for check = 5296.58 kg ha^{-1}

Table 6. Proportional contribution of lines, testers and line × tester interaction to total variance of testcrosses derived from S$_2$ maize inbred lines.

Parameters	Contribution (%) Lines	Testers	Line × tester
Plant height	33.39	21.83	44.77
Ear height	31.64	17.48	50.88
ASI	38.33	2.34	59.33
Grain yield	18.23	30.18	51.59

Table 7. Relative description of the genetic material used in the experiment.

Genotypes	Type	Origin	Kernel type	Stature	Maturity	Pedigree
Azam	OPV	Pakistan	Flint	Medium	Medium	Pirsabak 7930 x Zia x pirsabak- 7930
SW	OPV	Pakistan	Semi-Dent	Tall	Medium	[Vikram (B11 x B37)] x Akbar
Jalal	OPV	Pakistan	Flint	Tall	Long	Azam x CHSW
WD 2 x8	Hybrid	Pakistan	Semi-Flint	Medium	Medium	FRH -22 (F$_2$)-5 x FRHW-20-4
WD 3 x 6	Hybrid	Pakistan	Semi-Dent	Tall	Long	FRHW-22 x FRHW-20

Better-parent heterosis was calculated as percent increase (+) or decrease (-) exhibited by the F_1 hybrids over better parents:

$$Better\ parent\ heterosis = \frac{F_1 - better\ parent}{Better\ parent} \times 100$$

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