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Heterosis and early generation testing is a pivotal method for production of hybrid

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Abstract

Flowering time is extremely crucial for reproduction and plays astonishing role in adaptation of maize to diverse environments. Several hundred S2 lines were developed in 2009 and 27 best S2 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 find out heterosis for maturity traits and develop early maturing hybrid (Summer, 2010). Significant correlation was observed among flowering time for both mid parent (MPH) and better parent heterosis (BPH). There were no significant correlation between different attributes for GCA and SCA revealing that heterosis and combining ability is trait dependent approach. The germplasm showed astonishing amount of phenotypic diversity and highly significant differences were observed except for kernel row number per ear. Negative MPH was observed among the testcrosses for flowering traits. WD 2 x 8 as a tester performed well than the other testers for ear length**,** while Jalal performed well for number of kernel row and 100-grain weight. Several lines among the total testcrosses showed negative values for GCA effect regarding flowering time and it can be concluded that these lines can be used as parental material for production of high quality inbred lines for different types of studies. The amount of variability observed for flowering traits and yield related characteristics could be used for production of high yielding early maturing hybrids. Furthermore, it can be concluded that early generation testing provides a promising tool for identification of germplasm at early stages and discards the poor combiners.

Keywords: Heterosis, early generation testing, combining ability, maize.
Abbreviations: Mid parent heterosis (MPH), better parent heterosis (BPH), specific combining ability (SCA), general combining ability (GCA), white diallel (WD), kernel row number per ear (KRN), pollen shedding (PS), CCR maize inbred lines (CMI).

Introduction

In the recent advance era of science and technology, the biggest problem in the world is food security. It has been estimated that even today almost 2 billion people suffer from hunger, malnutrition, or both (Agrios, 2005). Maize (*Zea mays* L.) is the world's most extensively grown multipurpose crop. 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). For this purpose increasing the productivity of maize is vital for the survival of human beings. Maize is an extremely diverse crop owning high potential of heterosis and combining ability. Estimation of these basic phenomena can be the best choice to increase the overall productivity of this crop. Heterosis was first applied by the purposed hybridization of complex hybrid mixtures made by farmers in the 1800s (Enfield, 1866). However, scientists East and Shull developed the concept of hybrid vigor or heterosis in maize individually in the early 1900s (Shull, 1952 and Hayes, 1963). It was understood that genetic separation or divergence of parental crosses was important for hybrid vigor expression (Collins, 1910) but the range of genetic divergence limited the expression of heterosis (Moll et al., 1965). Cultivation of hybrid can significantly increase the total production, as maize possesses extravagant hybrid vigor and can be utilized in desirable direction. Combining ability studies provide information on the genetic mechanisms controlling the inheritance of quantitative traits and enables the breeders to select suitable parents for further improvement or use in hybrid breeding for commercial purposes. It is obvious that flowering time is extremely important for different biotic and abiotic stresses (Lu et al., 2010; Ali et al., 2011). Line x tester and diallel are reliable mating designs, used for testing both general combining ability (GCA) and specific combining ability (SCA) in applied maize breeding programs (Kaushik et al., 2004). Inbred testers are the best choice for improving GCA as well as SCA in maize (Zambezi et al., 1989). While, early generation testing is a significant phenomenon in terms of time management, labors cost and other resources for the production of desirable maize inbred lines.

Early generation testing is a pivotal method for plant breeders to discard undesirable materials and generate desirable inbred lines for the production of hybrids (Ali et al., 2011). Due to early testing, the best performing inbred lines are identified at early stages, and the resulting progeny can be used for further improvement of different traits. The present study was aimed to evaluate and identify superior maize inbred lines (S2) testcrosses for different traits in early generation for production of stable inbred lines. We examined heterosis for multiple phenotypic traits, relationship between heterosis for multiple phenotypic attributes and divulged differences among lines and testers. These testers with different genetic backgrounds (WD 2 × 8, WD 3 × 6 and Jalal) were crossed to several lines for exploring the phenomenon of heterosis and provided some basic information about the diversity of maize and utilization of heterosis. Analysis of data for several traits in different lines provides an opportunity to make inferences about the underlying mechanisms of heterosis. Furthermore, we tested the combining ability of the maize S2 lines as potential source in the production of improved maize inbred lines and figured out the level of heterosis at early stage to fulfill the dire need of hybrid for global food security. The lines used in this experiment will be used as a potential source in production of improved maize germplasm for future use while those lines having negative value of heterosis for flowering time will be used for the production of hybrid with early maturity.

Results

Interaction between traits prevailing heterosis

To estimate the occurrence of heterosis for different traits in maize early generation testing and to correlate different traits of interest for heterosis, we developed S_2 lines and crossed these lines with 3 different testers of diverse genetic backgrounds. The lines crossed with each tester were referred as different population {Population-1 (Lines × WD2×8), population-2 (Lines × Jalal) and Population-3 (Line x WD3)} to calculate correlation between different traits. Correlation between the traits for mid-parent and better-parent heterosis is listed in table 2 and 3, respectively. Maximum correlation coefficient was observed among the flowering time for both mid-parent and better-parent hetorosis while there were almost no or negative correlation between the flowering time and yield related attributes. Among the yield parameters, kernel row number ear^{-1} (KRN) has a very variable correlation with other traits. A negative correlation was observed between KRN and mid-pollen shedding (PS) in population-1, followed by population-2. Regarding mid-parent heterosis highly significant correlation (0.9) was observed for days to tasseling and days to silking, followed by days to tasseling and days to pollen shedding. The correlation matrix calculated for MBH and BPH (the results of all the line × testers were taken as a whole) showed that a limited correlation exists between different traits. The figures (1 and 2) showed the extent of correlation between traits for MPH and BPH, respectively. A very similar trend was observed using correlation matrix analysis for both types of heterosis by considering each line by tester as an individual population and treating all the data as one population.

Diversity in Germplasm

Line × tester analysis was carried out to detect the combining ability effects for 87 testcrosses for various agronomic traits including morphological traits, flowering traits and yield related attributes. The germplasm showed astonishing amount of phenotypic diversity for different traits and highly significant differences among all the treatments were observed except KRN. The total variation in the germplasm was portioned into several components and their significance levels were shown in table 4. The diversity of testers is extremely important for finding out the best performing line because these lines were collected from a specific region and we observed non-significant differences among the lines for all the traits of interest except percent grain moisture at harvest (Table 4). The variability among the testcrosses was also highly significant for all traits except KRN. The total variance was further partitioned into several components, like variance due to lines, testers and their interactions (Table 5). Proportional contribution of lines, tester and line × tester to the total variance for days to mid tasseling was 18.94, 28.89 and 52.17%, respectively (Table 5). The proportional contribution of lines was 28.23; the testers showed 15.39% to the total variation and 56.38% was observed because of line × tester (Table 5). The coefficient of variation (CV) for all the traits was investigated to show the reliability of this experiment (Table 4).

Hybrid vigor

Heterosis is the superiority of an F1 hybrid produced through crossing of two genetically different individuals over the mean of its parents or the better parent. The analysis of variance for all the traits was divulged to identify variability in the germplasm for different traits (Table 4). Heterosis (%) was estimated for mid parent (MPH) and better parent (BPH) for days to mid tasseling, silking, pollen shedding, ear length, number of kernel rows ear-1 and 100 grain weight (Supplementary Table S1). Maize has great potential for heterotic expression and its utilization. This could be the reason that number of hybrid varieties is much higher than other open pollinated varieties and synthetics varieties. The heterosis and mean effect of all the parameters were presented separately to clearly identify the effect of each attribute and the genotype was divided based on the desirable values for improvement of specific traits.

Days to mid tasseling

The ANOVA results showed highly significant variability among testcrosses and variability due to tester effect as well as line × tester while variance due to line effect was non-significant for mid tasselling (Table 4). Highest mean for days to mid tasseling (62 days) was observed for testcrosses CMI-131-2 and CMI-Sl (34) using WD3×6 as a tester, and minimum value (52 days) was obtained for CMI-183, using WD2×8 as a tester. 16 S_2 lines showed a negative GCA while the maximum GCA effect was recorded for CMI-131-2, followed by CMI-Sl (34) and minimum negative GCA effect was observed for CMI-149, followed by CMI-183 (Supplementary Table S2). A positive SCA effect was observed for 42 lines and the maximum SCA effect was found for testcross CMI-82, followed by CMI-119 using WD2×8 as a tester while minimum SCA effect was observed for CMI-115, using WD3×6 as a tester (Supplementary Table S2). Heterosis was estimated for mid parent and better parent and we observed that most of the testcrosses exhibited negative mid parent heterosis for this trait (Supplementary Table S1). CMI-114 using Jalal as tester, CMI-131-2 using Jalal and WD3×6 as testers and CMI-185-2 with WD3×6 as a tester showed positive heterosis as indicated in the table

Table 1. Basic 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

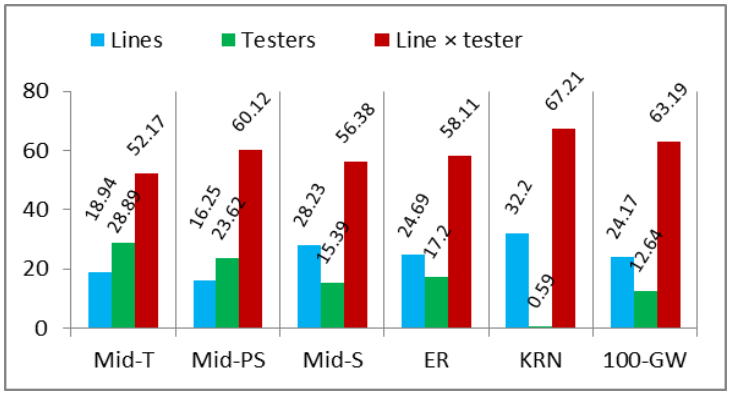

Fig 1. Contribution of lines, Tester and Line × Tester to the total variation.

while negative heterobeltiosis were observed for all testcrosses regarding this trait.

Days to mid silking

Regarding days to mid silking highly significant results were observed with a low CV (Table 4). Maximum (67) days to mid silking was observed for CMI-187 and CMI-Sl(34), using WD3×6 as a tester, while minimum mean value of 57 days was observed for CMI-149 and CMI-152, using WD2×8 as a tester. Means and combining ability effects (GCA and SCA) of the indicated trait were also calculated and enough variation had been observed for both the traits (Supplementary Table S3). Among the total testcrosses, 15 TC showed negative values for the GCA effect and indicated that these lines could be used as parents for generation of early hybrids. Maximum positive GCA effect (1.94) was found for CMI-Sl(22) and CMI-Sl(34), while minimum negative GCA effect (-2.56) was observed for CMI-149. Forty-four testcrosses exhibited negative SCA effect and the best SCA effect was observed for CMI-82, using WD2×8 as a tester, using WD3×6 as a tester, while the least SCA effect was obtained for CMI-82, followed by CMI-115, using WD3×6 as a tester (Supplementary Table S3). The mid parent heterosis for the indicated traits ranged from -13.21 to 1.52 %. Maximum MPH was observed for CMI-187, using WD3×6 as a tester. Better parent heterosis ranged from -15.56 to 0.78 %. Maximum BPH was observed for CMI-141, using Jalal as a tester, while minimum for CMI-152, using WD 2×8 as a tester (Supplementary Table S1).

Days to mid pollen shedding

Highly significant variability was revealed for pollen shedding in the testcrosses, tester and line × tester among the breeding material (Table 4). Mostly a negative trend was observed among the testcrosses for GCA and some of the lines were observed to harbor high SCA (Supplementary Table S4). Maximum (67) days was taken by CMI-Sl (34) for pollen shedding and the earliest among the breeding material was CMI-138 while most of lines complete the mid anthesis in almost 60 days. Among the 29 testcrosses, 13 lines indicated a positive GCA and the rest showed negative effect. The lowest value regarding GCA was observed for CMI-49 and 45 testcrosses showed negative SCA. Minimum of -4.78 SCA effect was found for CMI-115, using WD3×6 as a tester. The mid parents heterosis ranged from 15.59 to 1.57% and MPH was detected for CMI-185-2, using WD3×6 as a tester, while minimum was detected for CMI-183, using WD2×8 as a tester (Supplementary Table S1). The best parent heterosis ranged from -17.29 to 12.78% and maximum BPH was observed for CMI-187, using WD2×8 as a tester while minimum for CMI-183 crossed with WD2×8. The only testcross for which positive heterobeltiosis was observed among the breeding material for the concerning trait was CMI-185-2 cross WD3×6.

Ear length (cm)

ANOVA results revealed highly significant ($P < 0.01$) differences for testcrosses, tester and line × tester, while variance due to line effect was non-significant. The Means regarding GCA and SCA effects of testcrosses ranged from 10 to 16 cm (Supplementary Table S5). The highest mean was detected for CMI-48, using WD 2 × 8 as a tester, and CMI-115 and CMI-149, using WD 3 × 6 as tester. Overall mean of the testcrosses was 12.74 cm and checks mean was 12.50 cm. The GCA effect ranged from -1.07 to 1.26 and maximum GCA effect was found for CMI-115 and CMI-190-2 while minimum for CMI-129-2 and CMI-197. In total 16 TC showed negative GCA effect while the SCA ranged from -2.26 to 3.10. The best SCA effect was detected for CMI-149, using WD 3 x 6 as a tester. The worst SCA effect was observed for CMI-115, using WD 2 x 8 as a tester. Mid parent heterosis ranged from -25.93 to 33.33% and maximum MPH was observed for CMI-48, using WD 2 x 8 as a tester, while CMI-Sl (22) exhibited the lowest MPH, using WD 3 x 6 as a tester. WD 2 x 8 as a tester performed well than the other testers for ear length. Better parent heterosis ranged from -33.33 to 27.23%. The highest BPH was observed for CMI-200, using WD 2 x 8 as a tester, while minimum was

Table 2. Correlations (r) for Mid-parent heterosis among traits for all three populations.

	Population-1 (Line x WD2×8)						Population-2 (Line x Jalal)						Population-3 (Line x WD3×6)					
	T	S	PS	ER	KRN	GW	T	S	PS	ER	KRN	GW	T	S	PS	ER	KRN	GW
T	1	0.9	0.72	-0.08	-0.28	-0.01	1	0.83	0.78	-0.01	-0.38	0.1	1	0.8	0.73	-0.3	-0.1	-0.2
S		1	0.74	0.03	-0.26	0.02		1	0.76	0.09	-0.2	0.25		1	0.78	-0.3	-0.2	0.05
PS			1	0.07	-0.41	-0.18			1	0.17	-0.38	0.23			1	-0.03	-0.08	-0.11
ER				1	0.15	0.14				1	-0.25	-0.25				1	0.16	0.23
KRN					1	0.49					1	0.14					1	0.05
GW						1						1						1

Abbreviations, T (days to mid-tasseling), S (days to mid-silking), PS (days to mid-pollen shedding), ER (ear length), KRN (kernel row number per ear) and GW (100-grain weight).

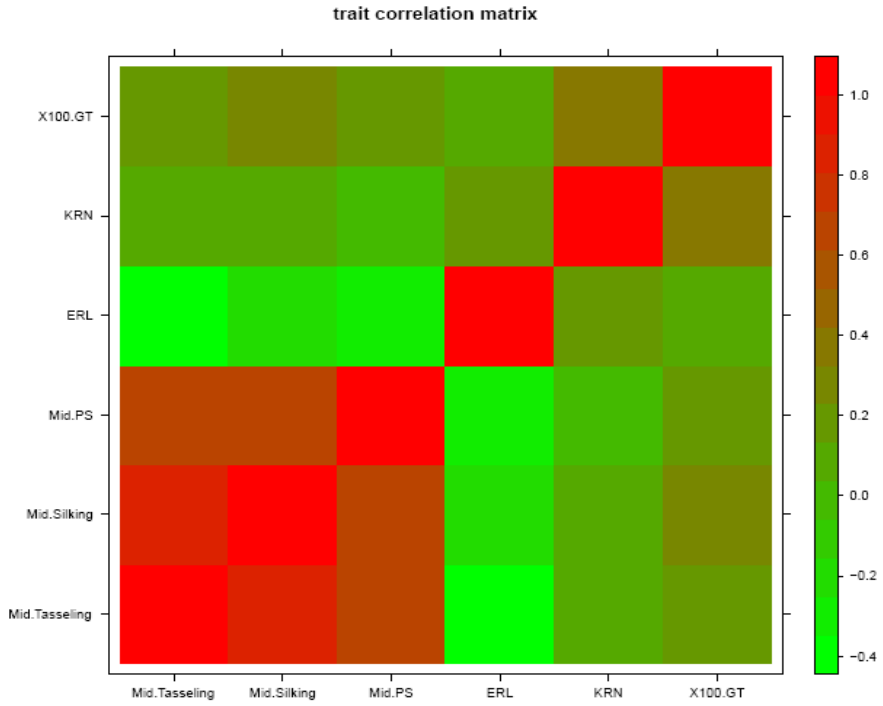

Fig 2. Better parents heterosis correlation matrix for Mid tasseling, Mid silking, Mid pollen shedding (Mid PS) , Ear row length (ERL), Kernel row number (KRN) and 100 grain weight (x100 GT).

observed for CMI-Sl (22), using WD 3 x 6 as a tester (Supplementary Table S1).

Number of kernel rows per ear

The kernel row number per ear is the most important parameter contributing towards the total yield and ANOVA results showed highly significant differences for the germplasm under investigation (Table 4). The GCA and SCA mean effects for KRN ranged from 11.50 to 18.50 and among the germplasm the best value was observed for CMI-190-2, using Jalal as a tester. CMI-Sl (22) x WD 3x6 revealed the lowest value for KRN while the grand mean for all the lines (14.64) was almost similar to the means observed for the checks used in this study (14.08). 21 testcrosses exhibited negative GCA effect and the highest value for GCA was observed for CMI-187. Of the 87 testcrosses, 46 showed positive SCA effect ranging from -15.87 to 31.63 with the highest value for CMI-6, using WD 3x6 as a tester, while minimum for CMI-6, using OPV Jalal as a tester (Supplementary Table S6). Proportional contribution for lines, testers and lines × tester interaction was 59.59, 1.35 and 39.06%, respectively (Table 5). The MPH and BPH for KRN ranged from -33.21 to 40.54% and maximum MPH values was detected for the CMI-137, using WD 3 × 6 as a tester, while minimum value was observed for CMI-149, using OPV Jalal as a tester. BPH ranged from -28.32 to 26.31%. Maximum BPH was calculated for CMI-187, using WD 3 × 6 as a tester, while minimum was noted for CMI-141, using WD 3 × 6 as tester (Supplementary Table S1).

100-grain weight (g)

The highest value of 100-grain weight along with some other important yield related components can dramatically increase the total production of maize. Variability among the breeder material and picking the desirable germplasm to improve this trait is the objective of maximum breeding programs. We observed a high variability for this important trait and ANOVA results revealed highly significant mean squares differences for grain weight among the testcrosses, tester and line × tester, while non-significant for line effect (Table 4). The mean values for GCA and SCA ranged from 27.30 to 48.53 g and among the material maximum value was observed for CMI-129-2, using OPV Jalal as a tester, while minimum mean was detected for CMI-131-2, using WD 3 × 6 as a tester. Overall mean for the testcrosses was 36.17 and check mean was 36.09 g. Out of 29 testcrosses, 15 showed positive GCA effect and the best GCA effect was observed for CMI-191, whereas minimum effect was observed for CMI-149. Forty-six out of 87 testcrosses exhibited positive

Table 3. Correlations (r) for Better-parent heterosis among traits for all three populations.

	Population-1 (Line x WD2×8)						Population-2 (Line x Jalal)						Population-3 (Line x WD3×6)					
	T	S	PS	ER	KRN	GW	T	S	PS	ER	KRN	GW	T	S	PS	ER	KRN	GW
T	1	0.64	0.52	0.16	-0.26	0	1	0.75	0.8	0.03	-0.24	0.25	1	0.73	0.73	-0.39	0.01	-0.38
S		1	0.31	0.32	-0.06	0.01		1	0.7	0.14	-0.18	0.14		1	0.83	-0.29	-0.18	-0.37
PS			1	-0.02	-0.18	0.04			1	0.02	-0.30	0.21			1	-0.32	-0	-0.41
ER				1	0.34	0.17				1	-0.29	-0.26				1	0.2	0.30
KRN					1	0.43					1	0.2					1	0.14
GW						1						1						1

Abbreviation, T (Days to mid-tasseling), S (Days to mid-silking), PS (Days to mid-pollen shedding), ER (Ear length), KRN (kernel row number per ear) and GW (100-grain weight).

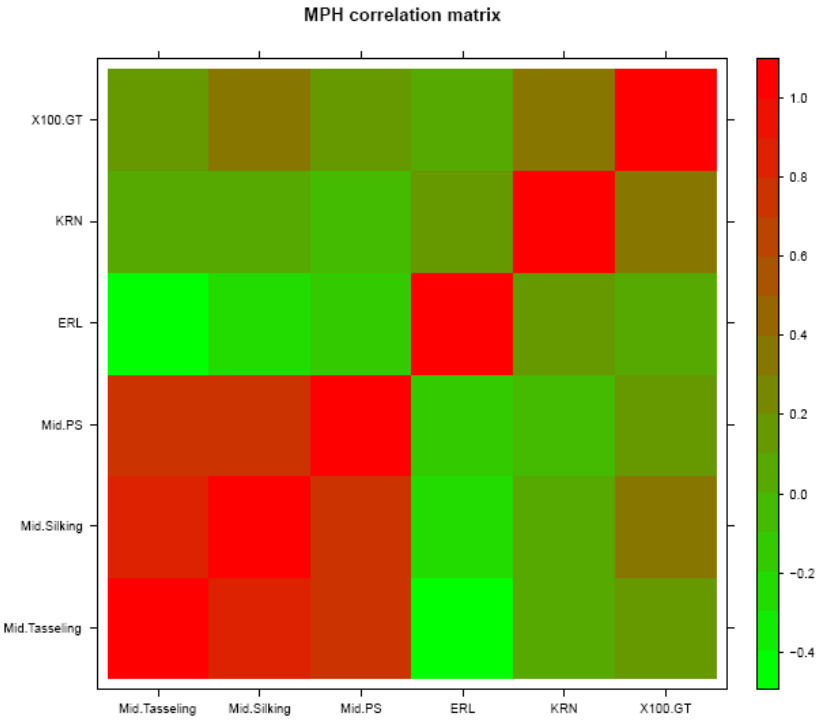

Fig 3. Mid parents heterosis correlation matrix for Mid tasseling, Mid silking, Mid pollen shedding (Mid PS) , Ear row length (ERL), Kernel row number (KRN) and 100 grain weight (x100 GT).

SCA effect. The SCA effect ranged from -7.45 to 7.18. CMI-129-2 showed the highest SCA effect, using OPV Jalal as a tester, while lowest was found for CMI-200, using OPV Jalal as a tester (Supplementary Table S7). The MPH and BPH for 100-kernel weight are presented in table 6. Mid parent heterosis ranged from -27.76 to 72.09%. Maximum MPH was observed for CMI-129-2, using OPV Jalal as a tester, while minimum was detected for CMI-131-2, using WD 3 × 6 as a tester. Better parent heterosis ranged from -28.78 to 69.11%. Maximum BPH was detected for CMI-129-2, using OPV Jalal as a tester, while minimum was found for CMI-131-2, using WD 3 × 6 as a tester.

Discussion

Heterosis in maize

Breeders are always interested in hybrids having the ability to perform extravagantly in yield and other traits of economic importance. Heterosis describes the superior phenotypes detected in F1 hybrids relative to their parental inbred lines. Maize possesses a high level of heterosis for a wide range of attributes, however the magnitude of heterosis is highly dependent on the parental lines and the trait(s) measured. Melchinger (1999) concluded that evaluating the performance of crosses among groups based upon genetically diverse parents is essential to identify promising heterotic patterns. Due to the complexity encountered in multi-trait and multi-stage selection for economically important traits, the inconsistency between genotypic and phenotypic (e.g., testcross, diallel) data shows that extensive testing of phenotypic data should be priority of maize breeding programs (Barata and Carena, 2006). For this purpose we selected several inbred lines and testers of diverse natures to collect maximum phenotypic data and explore the mechanism of heterosis and combining ability with different approaches. The analysis of heterosis for different traits in these three diverse types of testcrosses revealed that maize hybrids exhibit better-parent and mid-parent heterosis for nearly any trait in nearly every hybrid. For the entire flowering trait a negative value was observed of almost all the hybrid showing the margin of decreasing the total days to flowering and early maturity is extremely high in maize. Furthermore, in multiple cropping systems and for regions with short growing seasons, early maturing varieties are needed and diverse germplasm can be used to solve this problem for food security. The reason to use maize crop for food security is its high yield potential and enough variability for all the traits of interest. Furthermore, it has been revealed that in future maize crop will be the main source of staple food and no staple crop exhibits enough hybrid vigor like maize (Ali et al., 2012). The correlation between different

Table 4. Mean squares for days to mid tasseling, pollen shedding, silking and anthesis silking interval of testcrosses derived from S_2 lines of maize.

SOV	Tasseling	PS	Silking	ER	KRN	100-GW
Rep	16.40	25.79	29.16	0.81	41.41	29.46
Treat	9.19**	11.45**	11.93**	3.30**	45.80 NS	36.93**
C VS (P, TC)	0.78 NS	1.82 NS	5.39 NS	0.14NS	0.03 NS	2.12 NS
P VS TC	152.62**	290.38**	307.20**	1.40 NS	124.6 NS	433.68**
Checks (C)	1.00 NS	1.00 NS	2.25 NS	1.00 NS	40.11 NS	0.96 NS
Parents (P)	7.16 NS	9.49 NS	8.18**	2.70**	8.83 NS	43.97**
Testcrosses	8.44**	9.14**	10.04**	3.60**	58.81 NS	30.60**
Lines	4.91 NS	4.56 NS	8.71 NS	2.73 NS	58.16 NS	22.71 NS
Testers	104.90**	92.85**	66.42**	26.64**	14.86 NS	166.35**
Line x Tester	6.77 NS	8.44**	8.69**	3.22**	60.70 NS	29.69 **
Error	4.93	4.81	5.01	1.24	44.84	17.20
Cv %	3.82	3.60	3.60		-----	11.73

** = Highly significant at 1%of probability, *= Significant at 5% of probability, NS = Non significant, CV = Coefficient of variation.

Table 5. Proportional contribution of lines, testers and line × tester interaction to total variance of testcrosses derived from S2 lines in maize.

Parameters	Contribution (%)		
	Lines	Testers	Line × tester
Tasseling	18.94	28.89	52.17
PS	16.25	23.62	60.12
Silking	28.23	15.39	56.38
Ear length	24.69	17.20	58.11
KNR	32.20	0.59	67.21
Grain weight	24.17	12.64	63.19

trait prevailing heterosis was mostly non-significant and all the traits exhibited different values for different traits in different population. This idea may be interpreted that heterosis is trait dependent phenomenon and the performance of different traits is controlled by different genes.If there is certain genes specifically controlling heterosis then we must be able to observe high hybrid vigor for specific lines for almost all the traits. Stupar et al. (2008) had investigated the heterosis and gene expression profiles for a set of maize hybrids with varying levels of parental genetic diversity and they had observed that the distributions of better parent heterosis among a series of 25 maize hybrids generally didnot exhibit significant correlations between different traits.

Flowering Traits

Understanding the heterotic responses among different germplasm source is useful to determine the best method for finding germplasm with desirable attributes in populations that are agronomical and fit in the existing cropping pattern of any specific region in the world. Days required to silking and tasseling along with other maturity traits are commonly used by plant breeders as basis of determining maturity of maize. The earliest among the testcrosses was CMI-183, using WD 2 × 8 as a tester regarding mid tasseling. About 56.3% testcrosses took minimum days to 50% tasseling when compared to checks, while 10.3% TSs were similar to the check. Testcrosses that tasseled in fewer days than their respective S2 parents are a good source of generating early maturing hybrid for different zones and this phenomenon can be used in a more diverse population to produce hybrids for different environments in the world. About 47% testcrosses showed positive GCA effects, while 53% testcrosses were having negative GCA effects. About 48% testcrosses showed positive SCA effects, while 52% testcrosses exhibited negative SCA effects. Torrecilla et al. (2000) concluded that GCA effects could be used to measure the divergence among the maize population. We have concluded that lines and tester variability was significant for the flowering traits in this germplasm. The amount of variability observed for flowering traits can be used for production of high yielding early maturing hybrids. Contribution of line × tester interaction was relatively higher for days to mid tasseling (52.17%) and it is obvious in the literature that the interaction for this trait is always high (Mendoza et al., 2000 and Konak et al., 2001). The negative estimates of heterosis for flowering time are preferred in maize hybrid production, because it leads to earliness and help the spring crop from pollen desiccation from high temperatures during the month of May in the sub-tropic and tropic regions of the world. The heterosis over better parent for flowering time was estimated by taking the performance of the parents with earlier flowering parent. Heterotic values indicated that lines involving OPV Jalal as a tester were early silked than other testers used as parents. Among all the testcrosses, 91% took fewer days to 50% pollen shedding than the checks. Similar results of negative heterosis regarding flowering traits were observed by many scientists using different kind of germplasm in different kind of environments (Mendoza et al., 2000; Dickert and Tracy, 2002). Evaluating the heterosis for flowering time and agronomic traits among early open-pollinated sweet corn cultivar, Dickert and Tracy (2002) observed that hybrids differed for flowering data and yield related traits except row number.

Yield related traits

Yield is a quantitative trait controlled by many genes and is hard to accumulate all the desirable genes in one germplasm, however through conventional breeding and crossing several genes can be combined in one germplasm to observe super high productive maize. Furthermore, it has been recommended that yield is a multiplicative trait that integrates quantitative variation for other traits (Williams,

1959; Lippman and Zamir, 2007). According to this hypothesis, the lower levels of heterosis observed for other traits may interact in a non-linear fashion to produce higher heterosis levels for yield (Flint-Garcia et al., 2009). The best mid parent heterosis was observed for CMI-48, using WD 2 x 8 as a tester whereas the highest better parent heterosis was observed for CMI-200, using WD 2 x 8 as tester. The range of magnitude of heterotic effect among various crosses ranged from -25.93 to 33.33% and -33.33 to 27.23% against their mid and better parental values, respectively. Regarding kernel row numbers, the best mean value was observed for CMI-6, using WD 3 × 6 as a tester whereas the lowest was recorded for CMI-191, using WD 3 × 6 as a tester. Our results showed that several lines exhibit a high level of heterosis for most of the yield related traits. About 44.8% testcrosses showed positive GCA effects, while 55.2% testcrosses were having negative GCA effects and 43.5% testcrosses showed positive SCA effects for kernel row number. Regarding 100-grain weight 52% testcrosses showed positive GCA effects and 53% testcrosses exhibited positive SCA effects revealing the potential of maize inbred lines for production of high yielding hybrids. Moreover, this early generation testing provides basic information to select desirable inbred lines for further selfing and generate several lines of different genetic behavior to produce different kinds of hybrids for wide range of environments. Different inbred lines of maize have been divided into several "heterotic groups" based upon the level of grain yield heterosis and generally, crosses within heterotic groups have lower grain yield heterosis than crosses between groups. Moreover, lines of the testcrosses CMI-6, CMI-82, CMI-115, CMI-119, CMI-187, CMI-190-2 and CMI-191 are good general combiners for important traits viz; plant height, ear height, kernel rows ear-1, grain weight and grain yield. Lines of the testcrosses CMI-6, using WD 3x6 as a tester (kernel rows ear-1), CMI-129-2 and CMI-119, using OPV Jalal as a tester (grain weight) are good specific combiners. Regarding heterosis several lines have been observed to have better performance and these S2 lines can be used in future breeding programs for production of high yielding early hybrids and varieties. The heterotic groups are used as a common approach and not as an exact predictor of heterotic response (Tracy and Chandler, 2006). There is a correlation between different trait(s) heterosis and genetic diversity such that increasing genetic diversity produces increasing level of grain yield heterosis (Melchinger and Gumber, 1998). However, when the parents become highly diverse this relationship is no longer observed (Melchinger, 1999; Moll et al., 1965).

Material and methods

The experiment was conducted at Cereal Crop Research Institute (CCRI) and Agricultural Research Farm, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan, during 2008-2010. All the standard cultural practices including irrigation, fertilizer application, hoeing and thinning were carried out throughout the growing seasons to provide favorable condition for growth.

Plant material

Improvement in maize germplasm has been initiated in Plant Breeding and Genetics Department (PBG) Agricultural University, Peshawar with the collaboration of Cereal Crop Research Institute (CCRI) Pakistan, using different breeding procedure for increasing the average production of maize and releasing hybrids for commercial use. This program is based to provide some basic information about the most important phenomenon of hybridization and the use of early generation testing. Inbred lines were generated from different varieties (Azam, Sarhad White (SW), Baber and land races collected from different parts of the country. Hundreds of S_1 inbred lines were generated using manual self-pollination procedure (February–June, 2008). Among these S_1 lines, several lines were selected on the basis of their performance for disease resistance, flowering time and seed setting to generate advance S_2 lines (July–October, 2008) for hybrids and varieties production. The production of inbred lines procedure is defined by Ali et al. (2011). During this experiment the $CMIS_2$ (CCRI maize inbred S_2) lines were evaluated to find out the combining ability of different lines for flowering time and several agronomic traits of interest. 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 experimental materials were planted in three different places far apart from all maize fields to allow an ideal spatial isolation. The inbred lines were sown in 2:1 ratio (2 inbred lines with one tester). The inbred lines were manually detesseld well before the appearance of mail inflorescence to avoid any kind of contamination. All the standard cultural practices were carried out to produce good and pure (uncontaminated) inbred lines. Manual pollination was carried out based on the requirement of the lines and to generate proper amount of seed. As these lines were generated from different sources therefore, the flowering time were adjusted by planting the late material early than the early genotypes to achieve a synchronize flowering in all the isolations. The first two testers are high yielding single cross hybrid derived from white diallel cross and being released as commercial hybrids in Pakistan. Jalal is the high yielding well known open pollinated white maize variety with the most promising attributes among all the known OPVs. The pedigree of the germplasm along with their relative information is provided in table 1. In the second season (July – October), performance of the testcrosses were evaluated along with their S_2 parents, testers and check varieties. A partial lattice square design was used 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 75 cm and 25 cm, respectively. Two seeds per hill were planted and were thinned to one plant per hill at 4-5 leaf stage. Phenotypic data were recorded for five plants per plot. Plant data collected in the field included: flowering time as days to tasseling, days to siliking, days to pollen shedding and yield related traits as Ear length (cm), Kernel row number per ear and 100- grain weight (g). Ear and kernel (kernels are referred to as ‘‘fruit’’ in the plant ontology database; http://www.plantontology.org) data were recorded from open-pollinated ears (July – October, 2009).

Days to mid tasseling

Through daily visual observation during the flowering period, days from planting to 50% tasseling were counted when 50% of the plant had extruded tassels in each plot.

Days to mid silking

Days were counted from sowing date to the day when 50 % of each lines showed silks based on daily visits to field.

Days to mid pollen shedding

Data on days to 50% pollen shedding was worked out by visual observation when 50% of plants in a plot shed pollen. The days were counted from date of sowing.

Ear length

Five ears were randomly selected from each row and ear length was taken with the help of meter rod. The means data of all the five cobs in each replication was used for further analysis.

Kernel rows per ear

Number of kernel rows per cob (KRN) was counted for 5 randomly selected cobs after harvesting and the average data was used for the analysis of variance.

100-Grain weight

Grains were collected randomly from each harvested plot and 100 grains were counted from the lot of each plot. With the help of electrical balance, weight was measured and subjected to analysis.

Statistical analysis

The phenotypic data collected were used for different kind of analysis. The data were subjected to ANOVA; appropriate for line × tester design using computer package SAS by an interactive macro program for line ×tester analysis (Bartolome). Analysis for GCA and SCA was carried out following Singh and Chaudhary (1979). Correlation coefficients (r) for all the traits were calculated separately for Mid-parent and better-parent heterosis using SAS. The relationships between different lines using the three testers were investigated by constructing a Hap-map by R software. The expression used for GCA and SCA is given below

(GCA) $$gi = \frac{Xi..}{tr} - \frac{X...}{ltr}$$

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

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

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

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

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

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

Conclusion

The lines possess high specificity and general combining ability can be used for future breeding and also as a source of germplasm for different studies. Furthermore, we concluded that heterosis and combining ability could be trait dependent approaches and if the genetic material harbors genes for specific trait can be combined in the progeny by crossing, so for that specific trait, heterosis can be observed and those lines will be good combiner. On the other hand if certain lines have no gene for a specific trait then their hybrids will not be best performers. Depending upon the correlation of different traits for MPH and BPH, the phenomenon of heterosis can be explained to find out that either one/several gene(s) in a specific line control heterosis or these are the actual genes for each trait which yields into hybrid vigor or combining ability. If a specific gene control heterosis then there must be a network of those genes in crop plants to control all the genes for different traits and yield in hybrid vigor. To confirm this idea a lot of molecular work will be required to answer the exact question but here we just give an idea based on the phenotypic data for several traits and their heterosis.

Supplementary Material (Online Only)

Table S1. Heterosis (%) values over mid-parent (MPH) and better parent (BPH) of flowering traits and yield related attributes for all testcrosses with three testers derived from S2 lines of maize.
Table S2. GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from S_2 lines of maize for days to mid tasseling.
Table S3. GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from S_2 lines of maize variety Azam for days to mid silking.
Table S4. GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from S_2 lines days to mid pollen shedding (PS).
Table S5. GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from S_2 lines of maize for ear length.
Table S6. GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from S_2 lines of maize for kernel rows number ear^{-1} (KRN).
Table S7. GCA effects of parents and SCA effects of 87 testcrosses with three testers derived from S_2 lines of maize for 100-grain weight (100-GW).

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