Research Article

Line × Tester Analysis of Early Generation Inbred Lines for some Agronomic Traits in Maize

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Abstract | Maize (*Zea mays* L.) is the most versatile crop among cereals that grows well in a wide range of environments. Promising maize populations (and their hybrids) could be used as a source for inbred line development as well as cost-effective alternatives to single-cross hybrids. This study was aimed at determining combining ability and grain yield performance of S_1 lines derived from ten maize populations. These populations were crossed in half diallel design and the resulting 45 cross combinations were further explored for their use as source of elite inbred line development in a line × tester experiment conducted at Cereal Crops Research Institute, Pirsabak and Maize and Millet Research Institute, Yousaf wala during Kharif 2016. A total of 164 testcrosses from four testers were evaluated for yield and other traits (days to anthesis, kernels ear^{-1}, 100-grains weight) in RCB design with two replications. Analysis of variance showed significant differences among hybrids, lines and testers. Differences due to site × hybrid interaction for grain yield were also significant. Non-additive gene action was predominant for the inheritance of the studied traits. Tester, T-4 was the best general combiner with desirable GCA for kernels ear^{-1} and grain yield. The testcrosses i.e. (P8 × P10-3) × T4, (P6 × P10-1) × T4, (P8 × P10-1) × T4, (P6 × P9-2) × T4, (P2 × P3-1) × T1, (P1 × P5-2) × T1 and (P4 × P10-3) × T3 were the best performers in terms of maximum grain yield (above checks) and desirable SCA effects. Another 33 crosses exhibited positive SCA effects for 100-grains weight and grain yield and could possibly be utilized in maize breeding programs for developing high yielding maize hybrids accompanied with other desirable attributes.

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Introduction

Maize *(Zea mays* L.) is an important and versatile agricultural crop used as a staple food by several hundred million people in the developing world. It is an important crop for countries like Pakistan where the rapidly increasing population demand increased food supplies. Being a short duration crop, it gives two crops (spring and autumn maize) per year in Pakistan and can help considerably to solve the food shortage problem. In spite of potentially high yielding and easily cultivated crop than any other cereal, maize production in Pakistan is still low as compared to other important maize growing countries of the world. Maize ranks 3rd most grown crop in the world with an area of 178.69 million hectare and annual production of 1,008.99 million metric tons (USDA-FAS, 2017). In Pakistan maize is the 4th largest grown crop after wheat, cotton and rice. The area under maize in Pakistan is about 1.14 million hectares with about 4920 thousand tones production and 4301 kg ha-1 average yield (PBS, 2017). With the use of high yielding varieties/genotypes, maize production can certainly be improved. It is therefore imperative for the maize breeders to produce high yielding maize varieties that are also widely adopted, disease resistant and adjustable in the existing cropping pattern. In the past, breeders around the world used procedures for seed production including mass selection wherein seeds from good ears or plants were selected and saved each year for next year sowing and hence produced some widely used cultivars (Kutka, 2011). The deterioration in yield potential of these cultivars with the passage of time made it essential to develop and select improved modern cultivars/hybrids that are high in grain yield and accompanied with superior quality. This lead to the development of modern maize hybrids that have greater potential as compared with older cultivars/hybrids and hence corn became the highest tonnage cereal crop worldwide (Troyer and Wellin, 2009). In Pakistan, maize production has been continuously rising during last few years due the widespread use of high yielding maize hybrids. However, less attention has been paid to the development of maize inbred lines from the available diverse populations. Diverse collection of maize genotypes/populations is available with Cereal Crops Research Institute, Pirsabak, Khyber Pakhtunkhwa whose potential for inbred line development has not yet been explored. Promising populations once identified and crossed could provide unrelated and diverse elite inbreds with good GCA/SCA to be used in conventional hybrid seed industry (Carena, 2005).

Evaluation of early generation inbred progenies in testcrosses has been the primary method in maize breeding for the development of inbred lines. Information on combining ability of early generation inbred lines is vital to generate knowledge for the development of hybrid and synthetic varieties. Proper selection of parental lines is therefore very important step in hybrid development breeding program (Lippman and Zamir, 2007). Crossing promising population and testing the early selfing generations in line × tester combination provides a good source of elite inbred lines. Based on these facts, the current study was therefore conducted to estimate combining abilities of new inbred lines derived by crossing diverse populations for yield and some morphological traits in a line × tester experiment for future maize breeding programs.

Materials and Methods

Experimental materials used

A total of 166 entries including 164 testcrosses obtained from ten maize populations (Table 1) and two standard checks (Pop-8003-6 × Jalal and Pop-8003-6 × Pop-2011) were used for the study. In kharif season 2014, these ten populations were crossed in a diallel crossing block excluding reciprocals at CCRI Pirsabak and obtained 45 hybrid combinations (Table 2), following the procedure described by Russell and Hallauer, 1980. These 45 hybrids were grown and selfed in spring season of 2015. For selfing, pollen from the selected plants were bulked and pollinated these same plants to get the F_2 generation. At harvest equal amount of seeds from each selfed ear were bulked for next season sowing. The F_2 generation was then selfed to get the S_1 seeds for the development of testcrosses. Selected plants from the available S_1 lines were crossed with selected male parents used as testers to obtain testcross progenies in spring season 2016.

Testers (male parents) used

Four testers were used for the development of testcross progenies. The testers used for the development of testcrosses were WD-2 (T1), an inbred line of full/long maturity with medium stature; WD-3 (T2), an inbred line of early maturity, short stature; WD-6 (T3), inbred line with medium maturity, medium stature and WD-3×6 (T4), an experimental hybrid with short stature and medium maturity. Each S_1 was planted in two rows with third row being the tester and a total of 164 testcross combinations were developed.

Other management

The study was conducted across two locations i.e. Cereal Crops Research Institute (CCRI) Pirsabak and Maize and Millet Research Institute (MMRI), Yousaf wala, Punjab. Testcrosses were evaluated for days to anthesis, kernels per ear, 100-grains weight and grain yield, at CCRI Pirsabak and MMRI Yousaf wala, in RCB design replicated twice during summer 2016. Two seeds per hill were sown. Fertilizers, DAP and urea were applied @ of 150 and 250 kg/ha, respectively and top dressed with additional N at 60 kg/ha at four weeks after sowing. Pre-emergence herbicide with trade name Prim extra gold @ 600 ml/acre was used to control weeds after ploughing and harrowing before sowing the maize seed. Hand weeding was also done when necessary to control weeds during the growing period and other management practices were done according to the recommendations of the

Table 1: *Characteristics of parental populations used in the study.*

No.	Name	Code	Cross/pedigree	Agronomic features			
				Type	Maturity	Grain Type	Stature
1	Pop-8003	P1	Sd(w) × babar	Breeding Population	Long	Dent	Tall
2	Pop-1325	P2	FRW4 × iqbal	Breeding population	Medium	Semi dent	Medium
3	Sarhad white	P3	Vikram(b57×b37) × akbar	Approved Variety	Long	Dent	Tall
4	Jalal	P4	Chsw×azam	Do	Long	Flint	Tall
5	Pop-2009	P5	Jalal × sd(w)	Breeding population	Medium	Flint	Medium
6	Pahari	P6	7930 × shaheen	Approved variety	Early	Flint	Short
7	Azam	P7	(7930xzia) × 7930	Do	Medium	Flint	Medium
8	Pop-2011	P8	Sd(w) × frw4	Breeding population	Early	Flint	Medium
9	SHN 107	P9	Shaheen × frw3	Do	Medium	Semi flint	Medium
10	Super-08	P10	Pahari × frw6	Do	Medium	Semi flint	Medium

Table 2: *Population hybrids after crossing the parental populations.*

S. No.	Crossing scheme			Hybrids code
1	P1	×	P2, P3…. P10	P1×P2, P1×P3, P1×P4, P1×P5, P1×P6, P1×P7, P1×P8, P1×P9, P1×P10
2	P2	×	P3, P4….P10	P2×P3, P2×P4, P2×P5, P2×P6, P2×P7, P2×P8, P2×P9, P2×P10
3	P3	×	P4, P5….P 10	P3×P4, P3×P5, P3×P6, P3×P7, P3×P8, P3×P9, P3×P10
4	P4	×	P5, P6….P10	P4×P5, P4×P6, P4×P7, P4×P8, P4×P9, P4×P10
5	P5	×	P6, P7….P10	P5×P6, P5×P7, P5×P8, P5×P9, P5×P10
6	P6	×	P7, P8, P9, P10	P6×P7, P6×P8, P6×P9, P6×P10
7	P7	×	P8, P9, P10	P7×P8, P7×P9, P7×P10
8	P8	×	P9, P10	P8×P9, P8×P10
9	P9	×	P10	P9×P10.

Crossing scheme for development of F1 population hybrids at CCRI: Male parent was planted as single row plot followed by two female rows as P1 with P2 through P10, P2 with P3 through P10, P3 with P4 through P10 and so on for feasibility in making crosses.

specific areas to raise a healthy crop. Information on general and specific combining abilities of male and female along with type of gene action involved (additive and dominance/non-additive) was obtained using North Carolina design-I as suggested by Comstock and Robinson, 1952.

Results and Discussion

Mean square analysis

Mean squares due to sites, lines, testers, testcrosses/genotypes and their interaction for the studied traits of both individual and combined locations were calculated to explain the observed variation. The analysis of variance, for days to anthesis, kernels per ear, 100-grains weight and grain yield are presented in Table 3. Highly significant differences ($P<0.001$) were observed among hybrids and lines for these traits indicating that the material was diverse for the traits under study. Significant differences were also observed among testers/males for all traits. This was in confirmation with the findings of Abuali et al. (2012). The site × genotype interaction was significant ($P < 0.05$) for grain yield. The existence of significant site × genotype interaction for grain yield indicated that the inbred genotypes performed differently in their respective environments and hence it makes possible to select the best specific combiners under the different environments (Khoza, 2012). Additive variances were smaller than dominance variances for all the traits implying the absence of additive gene action in the inheritance of these traits. Beyene et al. (2011) also noted the presence of non-additive gene action to condition grain yield among insect resistant maize hybrids.

Mean performance

For days to anthesis/pollen shedding, majority of the crosses showed earliness compared to the check varieties and days to anthesis ranged between 53 days to 62 days. Minimum number of days to mid pollen shed (53 days) were recorded for testcrosses; (P4 × P8-2) × T3, (P6 × P9-3) × T4, (P6 × P10-2) × T4, (P1 × P8-3) × T1, (P3 × P5-3) × T2, (P6 × P9-1) × T4, (P6 × P10-3) × T4,

Table 3: *Combined ANOVA for line × tester experiment across two sites for days to anthesis, kernels ear^{-1}, 100-grains weight and grain yield in maize.*

SV	DF	Days to anthesis	Kernels ear^{-1}	100-Grain weight	Grain yield
Site	1	2002.01**	400910.9**	3362.8**	510298034**
Rep(Site)	2	0.11	15296.9	3.7	2511832.00
Genotypes/Hybrids	163	13.01**	9876.4**	28.03**	1936022**
Male	3	16.26**	36406.8**	78.69**	15313506**
Female(Male)	160	12.95**	9378.9*	27.08**	1685194**
Site × Genotypes	163	0.13^{NS}	5680.09^{NS}	3.78^{NS}	1511003**
Residuals	326	3.44	7040.3	10.2	835800.98
Additive Variance		0.08	623.4	1.26	119920.7
Dom. Variance		8.02	1252.3	14.57	214581.5

, ** Significant at 5 and 1 % level of probability; respectively;* **NS: *non-significant;* **SV:** *source of variation.*

Table 4: *Testcrosses exhibiting desirable mean performance and their SCA effects with respect to four traits in maize.*

Trait	Mean Range	SCA range	Desirable crosses	Mean performance	SCA effects	Check 1 Mean	Check 2 Mean
Days to anthesis	53 to 62	-2.43 to 3.67	(P6 × P10-3) × T4	53 days	-2.09	57 days	60 days
			(P2 × P9-4) × T2	53 days	-1.88		
			(P6 × P9-1) × T4	53 days	-2.09		
			(P6 × P10-2) × T4	53 days	-2.24		
			(P4 × P8-2) × T3	53 days	-2.43		
Kernels ear^{-1}	317 to 597	-31.90 to 21.80	(P8 × P10-2) × T4	597 kernels ear^{-1}	19.10	528 kernels	477 kernels
			(P3 × P7-2) × T2	591 kernels ear^{-1}	19.60		
			(P2 × P9-1) × T2	588 kernels ear^{-1}	18.84		
			(P3 × P10-2) × T3	586 kernels ear^{-1}	21.80		
			(P8 × P9-5) × T4	580 kernels ear^{-1}	15.76		
100-grains weight (g)	24.4 to 37.6	-3.23 to 3.95	(P1 × P7-3) × T1	37.6 g	3.84	32.8 g	31.5 g
			(P1 × P5-4) × T1	37.5 g	3.75		
			(P2 × P8-1) × T2	36.7 g	3.95		
			(P4 × P6-4) × T3	36.0 g	3.18		
			(P4 × P9-1) × T3	35.5 g	2.87		
Grain yield (kg/ha)	5544 to 9108	-400.98 to 251.70	(P8 × P10-3) × T4	9108 kg/ha	233.52	8178 kg/ha	7439 kg/ha
			(P6 × P10-1) × T4	9093 kg/ha	230.54		
			(P8 × P10-1) × T4	9071 kg/ha	226.09		
			(P6 × P9-2) × T4	8914 kg/ha	194.95		
			(P4 × P10-3) × T3	8813 kg/ha	247.87		

T1- Tester 1 (WD-2); T2- Tester 2 (WD-3), T3- Tester 3 (WD-6) and T4- Tester 4 (WD-3×6); Check 1; Pop-8003-6 × Jalal and Check 2; Pop-8003-6 × Pop-2011.

(P2 × P9-4) × T2 and (P6 × P7-1) × T4. Of these, crosses (P6 × P10-3) × T4, (P2 × P9-4) × T2 and (P6 × P9-1) × T4 produced more yield than the high yielding check which gave 8178 kg/ha grain yield (Table 4) and are good from the maturity and yield point of view. Mean number of kernels ear^{-1} for the 164 testcrosses ranged from 317 to 597 kernels. The highest kernels per ear were observed in testcrosses (P8 × P10-2) × T4, (P8 × P9-5) × T4 and (P8 × P9-3) × T4 which also produced good yield (8363, 8782 and 8391 kg/ha, respectively). Compared to the check varieties, 43 crosses were superior in per se performance than check-1 (528 kernels) and 95 crosses were better performer than check-2 (477 kernels) for total number of kernels ear^{-1}. The range observed for grain yield among 164 testcrosses was 5544 to 9108 kg/ha and 30 crosses produced more grain yield than the high yielding check (8178 kg/ha). The most outstanding

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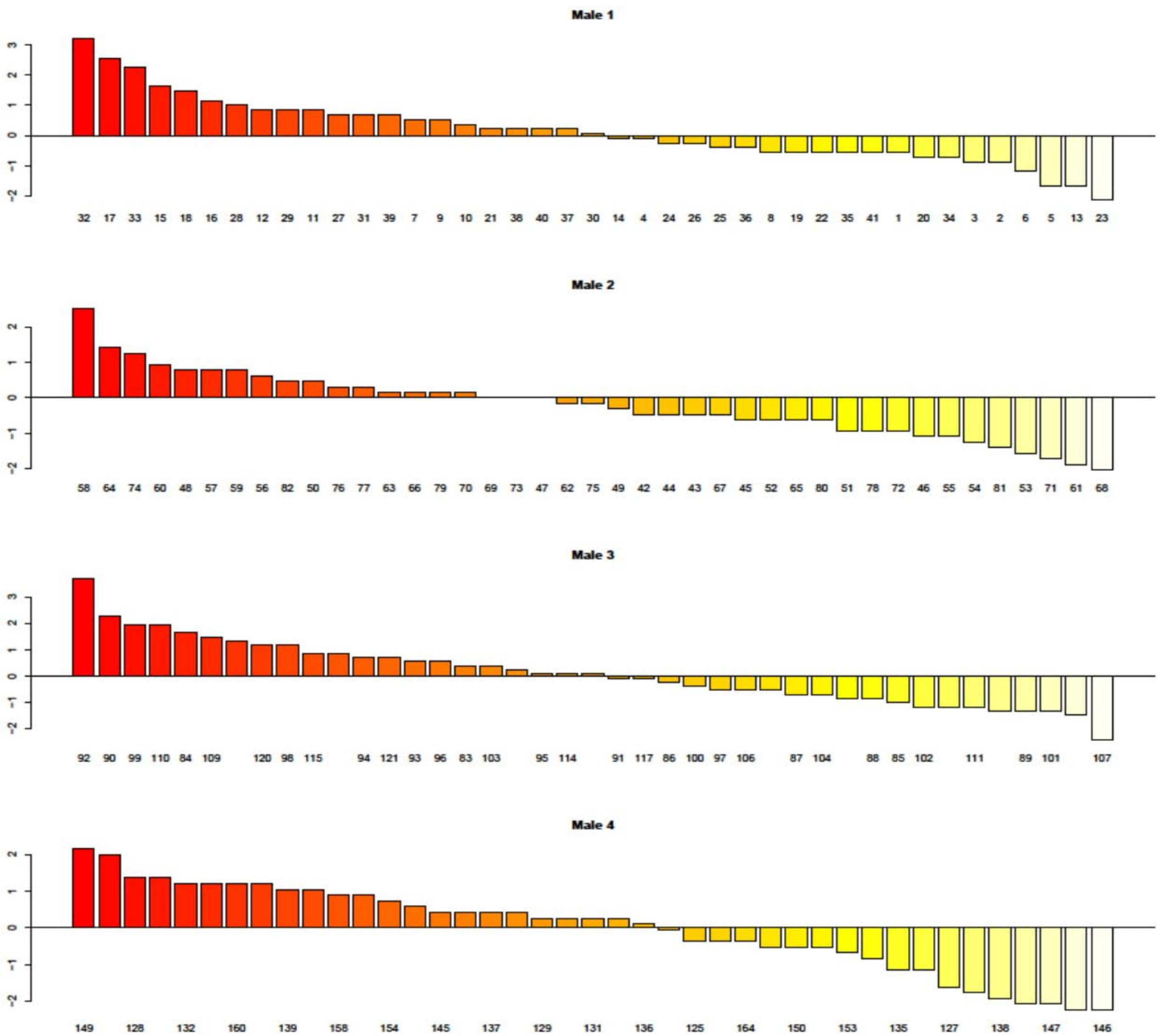
crosses for grain yield included (P8 × P10-3) × T4, (P6 × P10-1) × T4 and (P8 × P10-1) × T4 (9108, 9093 and 9070.6 kg/ha, respectively). The 100-grain weights of these crosses were also comparatively high. Another four crosses i.e. (P1 × P10-1) × T1, (P4 × P5-5) × T3, (P4 × P7-5) × T3 and (P5 × P10-1) × T4 were superior from grain yield and 100-grains weight point of view and could be considered for their use in hybrid development. The number of crosses that performed better for grain yield and other traits considered in this study could be used for inbred line development in future maize hybrid breeding program (Menkir et al., 2015).

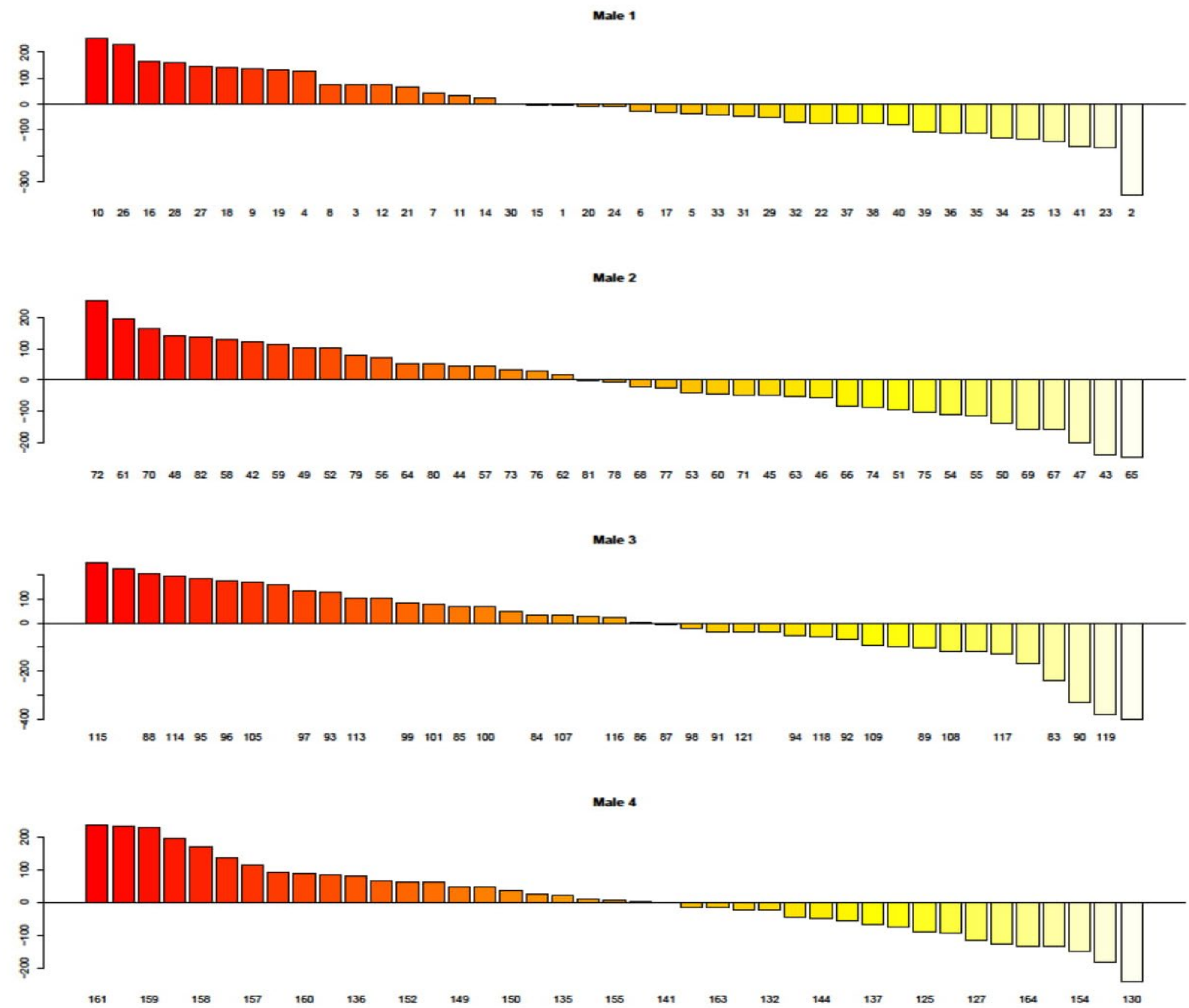
Combining ability effects

Combining ability analysis of the current study showed the importance of non-additive variances involved in the exploitation of breeding behavior of the genetic potential of these inbred lines for the important agronomic traits considered in this study. In this study, SCA effects for some traits may not be high at this stage as the parental populations used to produce the S_1 lines were diverse populations. Several studies indicated that high SCA is exhibited by narrow based inbred lines upon crossing as it is easy to estimate gene frequency in such parents. (Assefa et al., 2017). For days to anthesis, higher values of negative SCA and



Male 1- T1 (WD-2); Male 2- T2 (WD-3); Male 3- T3 (WD-6); Male 4- T4 (WD-3×6).

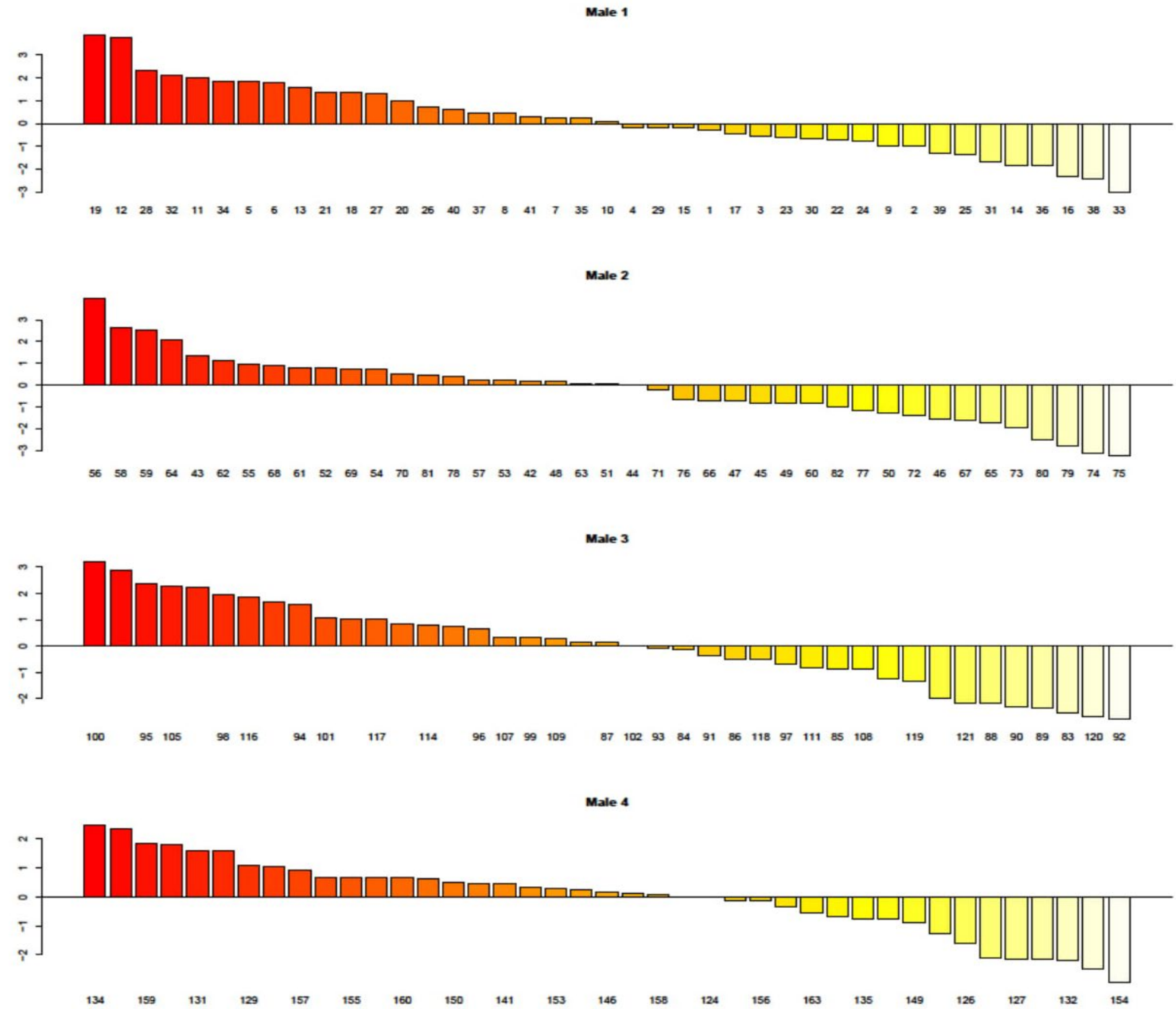
Figure 1: *Specific combining ability (SCA) effects of each set of lines with specific male parents/ testers for days to anthesis.*



Male 1- T1 (WD-2); Male 2- T2 (WD-3); Male 3- T3 (WD-6); Male 4- T4 (WD-3×6).

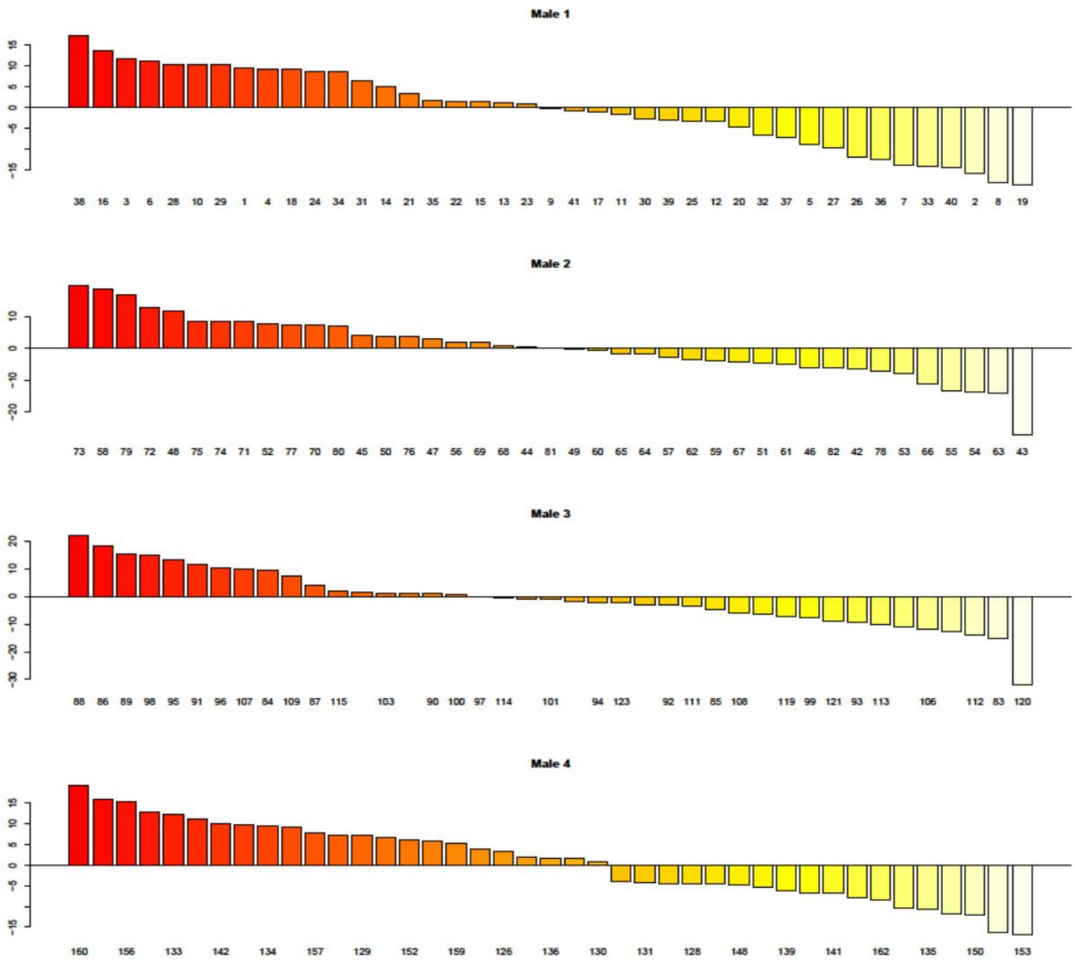
Figure 2: *Specific combining ability (SCA) effects of each set of lines with specific male parents/ testers for grain yield.*

GCA are desirable in parents to ensure early maturity. A total of 80 testcrosses exhibited SCA in desirable direction for mid pollen shedding and ranged from -0.05 to -2.43. Highest SCA in negative direction was estimated for the testcrosses (P4 × P8-2) × T3 (-2.43), (P6 × P9-3) × T4 (-2.24) and (P6 × P10-2) × T4 (-2.24). SCA effects in negative direction were manifested in 20 lines in cross combination with tester T-1, 22 lines with tester T-2, 20 lines with tester T-3 and 18 lines with tester T-4 for days to anthesis (Figure 1). SCA in the positive direction ranged from 0.1 to 21.8 and 81 testcrosses exhibited positive SCA for kernels ear^{-1}. Highest positive SCA was recorded in testcross (P3 × P10-2) × T3 followed by (P3 × P7-2) × T2 (19.6) and (P8 × P10-2) × T4 (19.1) and could be selected in maize breeding for the improvement of this trait. Crosses (P1 × P7-3) × T1, (P1 × P10-1) × T1, (P2 × P9-1) × T2, (P2 × P9-2) × T2, (P4 × P5-5) × T3, (P4 × P7-5) × T3, (P4 × P7-3) × T3, (P5 × P9-1) × T4 and (P8 × P10-3) × T4 exhibited SCA effects in positive direction for 100-grains weight and grain yield suggesting that these are good specific combiners for these traits. On the other hand, (P3 × P4-2) × T2, (P3 × P5-2) × T2, (P3 × P9-2) × T3, (P3 × P9-4) × T3, (P5 × P6-2) × T3, (P5 × P8-3) × T4 and (P8 × P9-1) × T4 exhibited significant SCA effects in negative direction (i.e. poor combiners) for the above-mentioned traits. Out of the 164 crosses tested here, positive SCA effects for grain yield were displayed by 16 crosses in cross combination with tester T-1, 19 with tester T-2 and 21 crosses with tester T-3 and T-4 (Figure 2) whereas, for 100-grains weight, 20 crosses showed positive SCA effects with tester T-1, 19 with tester T-2, 21 with tester T-3 and 23 crosses with tester T-4 (Figure 3). Similarly, positive SCA was observed in 20, 18, 16 and 22 crosses using testers T-1, T-2, T-3 and T-4, respectively (Figure 4) for kernels ear^{-1}. Among the testers, T1 and T2 were good general combiners for 100-grains weight and days to anthesis, respectively, whereas T4 was good general combiner for both kernels ear^{-1} and grain yield (Figure 5). The results obtained in the current study are in confirmatory with the earlier findings of Barh et al., 2015, Abrha et al., 2013, Assefa et al., 2017 and Shams et al., 2010, who worked with testcrosses of varying maturity and heterotic group in maize.



Male 1- T1 (WD-2); Male 2- T2 (WD-3); Male 3- T3 (WD-6); Male 4- T4 (WD-3×6).

Figure 3: *Specific combining ability (SCA) effects of each set of lines with specific male parents/ testers for hundred grain weight.*



Male 1- T1 (WD-2); Male 2- T2 (WD-3); Male 3- T3 (WD-6); Male 4- T4 (WD-3×6).

Figure 4: *Specific combining ability (SCA) effects of each set of lines with specific male parents/ testers for kernels ear^{-1}.*

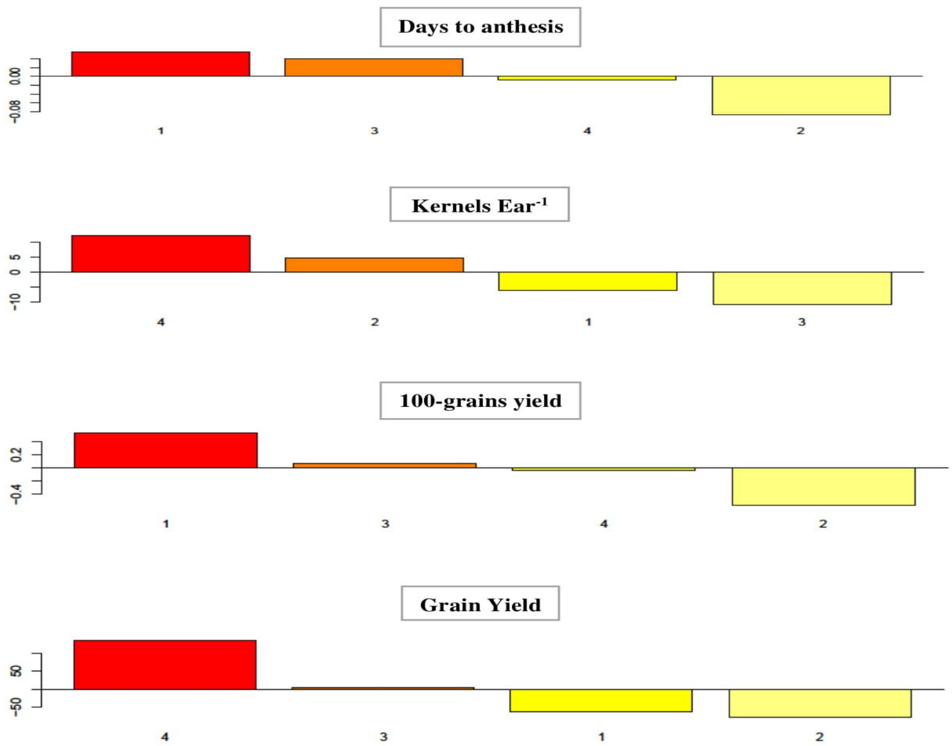


Figure 5: *Estimates of general combining ability effects of testers for different characters across two environments (CCRI Pirsabak and MMRI yousaf wala) in maize.*

Conclusions and Recommendations

The analysis of variance for Line × Tester with two locations exposed significant differences among lines, testers and their crosses for the traits considered in this study suggesting there were differences among the S_1 lines. Five best crosses with significant SCA effects and good per se performance for the studied traits are listed in Table 4. Crosses with good SCA were also superior in their mean performance in majority of the cases. Many of the crosses expressed significant SCA effects for different characters while some crosses were found desirable simultaneously for all the studied characters. Therefore, crosses (P4 × P5-5) × T3, (P1 × P10-1) × T1 and (P4 × P7-5) × T3 showed high SCA values for 100-grains weight and grain yield. Generally, results of the current study showed that 9, 18, 17 and 19% of the lines were comparatively superior in performance for days to anthesis, kernels ear^{-1}, 100-grains weight and grain yield, respectively and selected for further evaluation across locations to identify the desirable lines for inbred line development.

Author's Contributions

MUR and HUR designed the experiment and wrote first draft of the manuscript; MI helped to conduct the field experiment; IHK and ZS helped in data analysis and review the manuscript. The final draft of this manuscript was read and approved by all the authors.

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