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Standard heterosis and heterotic grouping of highland adapted maize (*Zea Mays* L.) inbred lines in Ethiopia

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The current study was initiated to estimate the magnitude of standard heterosis for grain yield and yield related traits in a line x tester hybrids and to classify the inbred lines into different heterotic groups. Fifty entries consisting of 48 testcrosses developed from 12 inbred lines and 4 testers using line x tester design and two commercial check hybrids used in the study. The experiment was conducted using alpha lattice design with two replications at Ambo and Holeta Agricultural Research Center in 2018 cropping season. Analysis of variance revealed highly significant mean squares due to genotypes for all traits. Site variance showed highly significant mean squares for all traits except ear height. Genotype x site interaction was significant for grain yield, days to silking, bad husk cover and ear aspect. Cross L11 x T4 exhibited maximum standard heterosis over the checks (Kolba and Jibat) for grain yield followed by L9 x T4. In addition, these hybrids showed negative standard heterosis for plant height and ear aspect. The study also proposed eight inbred lines to be assigned to one of the different heterotic groups (A and B). The current study revealed that extensive works needs to be done in broadening the genetic base for highland maize breeding program to develop higher yielding varieties for the target areas.

Key words: Heterotic grouping, Inbreed lines, standard heterosis, testers.

INTRODUCTION

Maize (*Zea mays* L.) is an important food security crop in the developing world, especially in sub-Saharan Africa (SSA) and Latin America. In Africa, maize is produced on a total area of 40.7 million ha; with the production of 81.9 million metric tons. This is about 20.64% of the total maize area of the world and 7.13% of the global production respectively (FAOSTAT, 2019). Lack of congruence between the proportion of production and the cultivated area is due to the low productivity of maize in Africa ($\leq 2t$ ha⁻¹) as compared to a global average of 5.8 tons ha⁻¹. Increasing Maize production and productivity in African countries like Ethiopia, is of significant importance due to uncertainties in future food supply because of great challenge of rapidly increasing population. Ethiopia currently leads east African countries in maize production (9.6 million metric tons) and Productivity (4.2 tons ha⁻¹)

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Leastion			Temp (°C	C)		Longitudo	Soil type	
Location	Altitude (masi)	Rainfall (mm)	Min	Max	Latitude	Longitude		
Ambo	2225	1050	10.4	26.3	8°57'N	38°7'E	Black vertisol	
Holeta	2400	1102	6	22	09°04'N	38°29'E	Nitosols and vertisols	

*Rainfall and Temp; were taken as averages of many years for each locations.

(FAOSTAT, 2019). Among the cereal crops, maize contributes the greatest share (28.5%) to the total annual crop production in Ethiopia (CSA, 2020).

Maize is cultivated in all of the major agro-ecological zones in Ethiopia. The maize production in the highland agro-ecology of Ethiopia is characterized by low productivity because of limited germplasm sources and improved varieties adapted to the agro-ecology. This calls for the development of suitable maize cultivars to increase productivity in this agro-ecology. In Ethiopia the national average grain yield increased from about 1.6 ton ha⁻¹ in 1990 (Worku et al., 2012) to 4.2 tons ha⁻¹ in 2019 (FAOSTAT, 2019). Increased yields are in part due to improved agronomic practices and increased inputs, but increased yields could not have been realized without genetic improvements (Abate et al., 2015).

This shows the presence of high potential to increase production and productivity. Information on heterosis is important in the development of maize inbred lines. Heterosis manifestation is dependent on genetic divergence of the two parental varieties (Hallauer and Miranda, 1988). Krivanek et al. (2007), declared that heterosis and combining ability are prerequisites for developing economically viable hybrid maize varieties. Assignment of maize genotypes to their respective heterotic groups has been the key to the economic success of the crop as it has allowed the exploitation of heterosis (Troyer, 2006), particularly for grain yield.

Standard heterosis of highland maize inbred lines for grain yield and yield related traits were conducted for different sets of locally developed and introduced inbred lines (Elmyhum, 2013; Nepir et al., 2015; Shimelis et al., 2019; Abebe et al., 2020; Keimeso et al., 2020). Legesse et al. (2009), also studied the combining ability of highland inbred lines and grouped the lines to heterotic groups using specific combining effects. However, it is always important to generate such information for any new batch of inbred lines and group them to different heterotic groups for further use in developing high yielding hybrid varieties. The objective of the study was to estimate the magnitudes of standard heterosis for grain yield and yield related traits in line x tester hybrids and to classify the inbred lines into different heterotic groups.

MATERIALS AND METHODS

Description of experimental sites

The study was conducted at two locations in the highland sub-

humid agro ecology of Ethiopia, namely, Ambo and Holeta Agricultural Research Centers, in the main cropping season of 2018 (Table 1).

Experimental materials

A total of 50 entries composed of 48 test crosses, formed by crossing 12 highland maize inbred lines with four line testers (known as heterotic testers A and B), and two standard checks (Jibat and Kolba,) were investigated. Prior to this time, Ambo highland maize breeding program and CIMMYT (International Maize and Wheat Improvement Center) had developed the inbred lines from the crosses of elite by elite inbred lines. List and pedigrees of the inbred lines used in the line x tester crosses along with the testers are presented in Table 2. Two of the line testers (T3 and T4) are CIMMYT developed testers and widely used in Ethiopian maize breeding programs, while the other two are locally developed line testers (T1 and T2) commonly used by the highland maize breeding program at Ambo.

Experimental design and field managements

The experimental design was alpha lattice design (0, 1) (Patterson and Williams, 1976) with 5 plots per an incomplete block and 10 incomplete blocks with two replicates. Each entry was planted in a one row 5.25 m long plot with spacing of 0.75 m between rows and 0.25 m between plants within a row. The experimental materials were hand planted with two seeds per hill, which were later thinned to one plant to get the recommended planting density for the testing sites, 53,333 plants per hectare. Planting was conducted on the onset of the main raining season after an adequate soil moisture level was reached to ensure good germination and seedling development. Other agronomic practices were carried out as per the recommendation for the test areas.

Data collection

Data on grain yield and other important agronomic traits were collected on a plot and sampled plants bases. Data collected on a plot basis include days to 50% silking (DS), number of ears per plant (EPP), field weight (FW) (kg/plot), plant aspects (PA), ear aspects (EA) and bad husk cover (HC); while data recorded on sampled plants basis were ear height (EH) (cm) and plant height (PH) (cm). Yield (GY) in t/ha was calculated using CIMMYT fieldbook software (Banziger and Vivek, 2007).

Data analysis

All Data collected for this study were subjected to analyses of variance (ANOVA) using the PROC GLM procedure in SAS® computer program (SAS Institute, 2004). Least significant difference (LSD) was used for mean comparisons. For traits that displayed significant differences among crosses, line by tester analysis was

S/N	Lines code	Genotype name	Source (origin)
1	L1	MH1307001-4-2-1-1	EIAR- HMBP
2	L2	MH1307002-3-3-3-1	EIAR- HMBP
3	L3	MH1307002-4-1-1-2	EIAR- HMBP
4	L4	MH1307002-4-2-2-1	EIAR- HMBP
5	L5	MH1307002-9-1-1-1	EIAR- HMBP
6	L6	MH1307002-9-2-1-2	EIAR- HMBP
7	L7	MH1307002-10-1-2-1	EIAR- HMBP
8	L8	MH1307002-10-1-2-2	EIAR- HMBP
9	L9	MH1307002-10-1-2-3	EIAR- HMBP
10	L10	MH1307002-10-2-3-2	EIAR- HMBP
11	L11	MH1307002-10-2-3-3	EIAR- HMBP
12	L12	MH1307002-10-3-2-2	EIAR- HMBP
13	T1	HLM0001	EIAR- HMBP
14	T2	HLF0002	EIAR- HMBP
15	Т3	CML312	CIMMYT
16	T4	CML395	CIMMYT

Table 2. List of highland maize inbred lines and testers used for test-cross formation.

*HMBP = Highland Maize Breeding Program.

performed to further partition the variances due to crosses into lines, tester and line by tester effects using SAS program (SAS institute, 2004).

Estimation of standard heterosis

Standard heterosis (SH) in percent was calculated for those traits that showed significant differences among genotypes as suggested by Falconer and Mackay (1996). These were computed as percentage increase or decrease of the cross performances over best standard check as follows:

$$SH(\%) = \frac{F1-SV}{SV} * 100$$

Where, F1 = mean value of a cross; SV = mean value of standard check variety.

Test of significance for heterosis was done using the t-test. The standard errors of the difference for heterosis were calculated as follows:

SE(d) for
$$SH = \pm \sqrt{2MSE/r}$$

Where, SE (d) is standard error of the difference, MSE is error mean square and r is number of replications and calculated t value was compared against the tabulated t-value at degree of freedom for error.

$$t(standard check) = F1 - \frac{SV}{SE(d)}$$

Grouping of inbred lines into different heterotic group

The inbred lines were classified into different heterotic groups based on the results from ANOVA, genotype means and SCA effect for grain yield. Heterotic grouping was determined according to the CIMMYT heterotic classification system as A, B and AB. Depending on the direction of the SCA estimate such that lines displaying positive SCA with tester A were grouped towards the opposite heterotic group, and vice versa, whereas lines exhibiting positive SCA to both testers were grouped under AB heterotic group (Vasal, 1992). Line by tester analyses was performed for traits that showed significant differences among crosses as suggested by Dabholkar (1999) and Singh and Chaudhary (1985) to partition the mean square due to crosses into lines, testers and line x tester interactions. The following mathematical model was used for the combining ability analysis of individual locations:

 $Y_{ijk} = \mu + r_k + g_i + g_j + S_{ij} + e_{ijk}$

Where, Y_{ijk} = the value of a character measured on cross of line i by tester j in kth replication; μ = population mean; r_k = effect of kth replication; g_i = general combining ability (GCA) effects of ith line; g_j = general combining ability (GCA) effect of the jth tester; S_{ij} = specific combining ability (SCA) of ith line and jth testers such that S_{ij} equals S_{ij} ; e_{ijk} = experimental error for ijkth observation.

The significance of SCA effects were tested by dividing the corresponding SCA values by their respective standard error, to obtain the calculated t values, and comparing the calculated t value with tabular t-value at the error degree of freedom.

RESULT AND DISCUSSION

The combined analysis of variance for grain yield and other related traits are shown in Table 3. The analysis showed highly significant mean squares due to genotypes for all studied traits. This indicates the presence of high genetic variation between the genotypes and the potential to develop high yielding hybrids for the targeted agro-ecology of the country. Similar results have been reported by (Tulu et al., 2018; Abebe et al., 2020). Site variance showed significant mean squares for all traits except for ear height (EH), indicating that the test environments were unique and

Source of	Dí	Trait									
variance	Di	GY	DS	PH	EH	HC	EPP	EA			
Rep(site)	3	3.42*	11.38**	81.80	121.70	53.96	0.01	0.15			
Blk(Rep*Site)	36	3.11**	4.96**	290.68*	176.78	13.59	0.04*	0.09			
Site	1	84.97**	13744.82**	1404.5**	169.28	43.92*	0.96**	15.96**			
Genotype	49	13.65**	26.22**	1072.16**	620.07**	77.61**	0.10**	0.64**			
Genotype*Site	49	2.24**	11.04**	200.81	110.68	34.78**	0.03	0.17**			
Error	62	0.86	1.80	170.27	126.16	10.93	0.02	0.06			
CV		12.07	1.27	6.29	10.49	82.71	12.43	8.30			
Grand Mean		7.68	105.95	207.57	107.04	4.02	1.21	3.04			
R ²		0.96	0.99	0.90	0.86	0.91	0.89	0.94			

Table 3. Combined analyses of variance for grain yield and yield related traits of 48 testcrosses and two hybrid checks evaluated at Ambo and Holeta.

* = Significant at 0.05 probability level; ** = significant at 0.01 probability level; GY = Grain Yield, DS = Days to Silking, PH = Plant Height, EH = Ear Height, HC = Bad Husk Cover, EPP = Ears Per-Plant, EA = Ear Aspect, CV = Coefficient of variation, R^2 = coefficient of determination.

that there is adequate variability among the inbred lines for improvements in the traits. Genotype*site interaction showed significant mean variance for grain yield (GY), days to 50% silking (DS), bad husk cover (HC) and ear aspect (EA), indicating that, the performance of these genotypes were not consistent across sites for the traits. In line with the current findings, Nepir et al. (2017) and Keimeso et al. (2020) reported similar results for GY in their study on other batches of highland adapted inbred lines.

Estimation of standard heterosis

The estimates of standard heterosis over the standard checks were computed for grain yield and yield related traits that showed significant differences among genotypes in combined analysis (Table 4). None of the crosses demonstrated positive significant heterosis over the standard checks (Kolba and Jibat). Standard heterosis (SH) for GY ranged from -66.17% (L8 x T1) to 6.86% (L11 x T4) over Kolba, and -64.39 % (L8 xT1) to 12.49% (L11 x T4) over Jibat. Thirty-seven crosses showed negative and significant standard heterosis over the best hybrid check (Kolba) for grain yield, while three crosses showed positive and non-significant standard heterosis. Similarly, 31 crosses revealed negative and significant standard heterosis over Jibat, while five crosses revealed positive and non-significant standard heterosis for the same trait.

Negative and significant heterosis implies that their respective parents have resemblance and are from same heterotic group or parents are genetically less distant, whereas significantly positive heterosis in most crosses reveals parents are divergent and could be used to develop heterotically responsive hybrids. The result obtained in the study implies that inbreed lines were crossed to testers with less genetic distant. In contrast to the current findings several investigators (Zeleke, 2015; Nedi et al., 2017; Mesenbet et al., 2016; Abebe et al., 2020) have reported significant standard heterosis for GY in both directions. This may be because of the difference in materials (inbred lines) used in their study.

Estimates of standard heterosis for days to 50% silking (DS) ranged from - 4.08% (L2 x T3) to 6.95% (L2 x T4) and - 2.91% (L2 x T3) to 8.25% (L2 x T4) over standard checks Jibat and Kolba, respectively. Twenty-one testcrosses over Jibat and 27 testcrosses over Kolba showed positive and significant SH for this trait while, only five and one testcrosses revealed negative and significant SH over checks, respectively. Inbreed lines with positive and significant SH for DS can potentially be used in the breeding program to develop late maturing hybrids as compared to the standard checks and vice versa. According to Lekha et al. (2015), both positive significant SH for DS and suggested that negative SH is desirable for this trait in maize hybrid development.

The lowest SH for PH and EH were recorded on L4 x T4 (-30.35%, -28.95%) and L8 x T3 (-34.45%, -25.57%) over Jibat and Kolba respectively. Similarly, the highest SH for these traits, were recorded on L1 x T4 (4.05%, 6.14%) and L8 x T4 (11.29%, 26.35%) over standard checks Jibat and Kolba. None of the testcrosses except two over Kolba for EH showed positive and significant SH over both standard checks for both traits, whereas 28 and 16 testcrosses over Jibat, and 22 and four over kolba were revealed to have negative and significant SH for PH and EH, respectively. This implies that, most of the hybrids tested in this study were hybrids with short stature as compared to the standard checks. (Abebe et al., 2020) reported negative and positive significant SH for these traits in their study and suggested that, the negative heterosis for plant and ear height is desirable to

Creases	SH for GY		SH for DS SH for PH (cm)		SH for EH (cm)		SH for EPP (#)		SH for HC (%)		SH for EA (1-5)			
Crosses	Kolba	Jibat	Kolba	Jibat	Kolba	Jibat	Kolba	Jibat	Kolba	Jibat	Kolba	Jibat	Kolba	Jibat
L1xT1	-30.21**	-26.54**	1.94	0.72	-8.94	-10.73	9.09	-3.92	-18.01	-25.72**	-23.00	-25.05	3.80	-9.12
L1xT2	-20.2*	-16.00	0.00	-1.20	-16.9**	-18.53**	-9.52	-20.31*	-2.26	-11.45	-60.80	-61.87	-0.40	-12.78
L1xT3	-5.93	-0.98	-1.94	-3.12*	-16.45**	-18.1**	-9.58	-20.36*	-1.21	-10.50	57.20	53.07	-11.70	-22.72**
L1xT4	-17.7*	-13.37	7.04**	5.76**	6.14	4.05	25.18*	10.26	-29.36**	-36.01**	28.40	24.99	-10.50	-21.66**
L2xT1	-48.66**	-45.95**	3.88**	2.64*	-7.89	-9.71	5.14	-7.40	-21.43*	-28.82**	134.5**	128.31**	41.5**	23.93**
L2xT2	-35.87**	-32.49**	3.88**	2.64*	-11.01	-12.76*	0.84	-11.18	11.11	0.66	135.4**	129.12**	34.6**	17.87*
L2xT3	-11.68	-7.02	-2.91*	-4.08**	-22.35**	-23.88**	-23.41*	-32.54**	-0.55	-9.90	353.7**	341.7**	18.20	3.46
L2xT4	-22.8*	-18.73*	8.25**	6.95**	4.00	1.95	13.62	0.07	-27.04*	-33.9**	541**	524**	7.30	-6.07
L3xT1	-46.62**	-43.8**	4.13**	2.88*	-12.77*	-14.49*	-2.47	-14.10	-13.59	-21.72*	-55.40	-56.61	22.1*	6.87
L3xT2	-41.15**	-38.05**	3.4*	2.16	-9.88	-11.65*	6.15	-6.51	2.05	-7.55	-49.70	-51.02	34.2**	17.5*
L3xT3	-30.77**	-27.12**	-0.73	-1.92	-13.63*	-15.33**	-19.34	-28.95**	-11.43	-19.76*	32.60	29.04	12.10	-1.86
L3xT4	-19.37*	-15.12	5.83**	4.56**	-5.45	-7.31	6.41	-6.28	-23.95*	-31.1**	320.8**	309.62**	8.30	-5.14
L4xT1	-53.01**	-50.54**	5.58**	4.32**	-14.67*	-16.35**	0.00	-11.92	-21.37*	-28.77**	186.3**	178.68**	36.5**	19.49*
L4xT2	-53.57**	-51.12**	1.94	0.72	-28.95**	-30.35**	-24.57*	-33.56**	-8.83	-17.40	-0.30	-2.92	35.4**	18.54*
L4xT3	-32.16**	-28.59**	-2.18	-3.36*	-10.63	-12.39*	-14.50	-24.7*	-17.74	-25.47**	146.6**	140.1**	12.80	-1.26
L4xT4	-28.08**	-24.29**	6.8**	5.52**	-3.72	-5.62	1.37	-10.72	-30.66**	-37.18**	246.3**	237.11**	8.60	-4.95
L5xT1	-53.85**	-51.41**	4.37**	3.12*	-14.7*	-16.37**	-8.66	-19.55*	-14.70	-22.72*	25.30	22.01	37.6**	20.46*
L5xT2	-38.74**	-35.51**	3.4*	2.16	-10.43	-12.19*	-9.24	-20.06*	-17.33	-25.11**	177.2**	169.85**	25.1**	9.57
L5xT3	-23.26**	-19.22*	-0.73	-1.92	-7.67	-9.49	0.95	-11.09	-10.32	-18.75	-29.40	-31.29	5.60	-7.51
L5xT4	-4.36	0.68	7.77**	6.47**	0.73	-1.26	14.17	0.56	-14.08	-22.16*	419.4**	405.63**	16.10	1.65
L6xT1	-65.43**	-63.61**	3.88**	2.64*	-20.21**	-21.78**	-9.81	-20.57*	-38.7**	-44.47**	-74.70	-75.34	35.7**	18.82*
L6xT2	-44.3**	-41.37**	3.4*	2.16	-8.23	-10.04	3.00	-9.28	-17.25	-25.04**	-71.90	-72.61	33.4**	16.83*
L6xT3	-21.59*	-17.46	-0.73	-1.92	-8.95	-10.74	-21.22	-30.61**	-9.73	-18.22	-81.20	-81.66	-8.10	-19.57*
L6xT4	-38.18**	-34.93**	8.01**	6.71**	3.56	1.52	24.1*	9.30	-41.7**	-47.21**	-90.8*	-91*	-14.10	-24.83**
L7xT1	-57.74**	-55.51**	4.61**	3.36*	-20.87**	-22.42**	0.14	-11.80	-24.0*	-31.16**	-85.8*	-86.22*	37.5**	20.39*
L7xT2	-35.87**	-32.49**	2.18	0.96	-15.34**	-17.01**	-1.60	-13.33	-13.12	-21.29*	85*	80.12	25.3**	9.74
L7xT3	-25.49**	-21.56*	0.97	-0.24	-13.99*	-15.68**	-6.22	-17.40	-12.20	-20.46*	136.6**	130.34**	16.10	1.68
L7xT4	-8.80	-4.00	7.04**	5.76**	-5.15	-7.01	18.61	4.46	-2.95	-12.08	313.8**	302.84**	1.50	-11.12
L8xT1	-66.17**	-64.39**	7.04**	5.76**	-23.03**	-24.54**	-14.96	-25.1*	-26.3*	-33.21**	-100*	-100*	26**	10.30
L8xT2	-34.66**	-31.22**	1.94	0.72	-17.67**	-19.29**	-0.40	-12.27	9.26	-1.02	-76.30	-76.90	27.3**	11.43
L8xT3	-24.28**	-20.29*	0.49	-0.72	-18.98**	-20.57**	-25.6*	-34.45**	-9.63	-18.13	51.50	47.50	3.50	-9.34
L8xT4	-7.23	-2.34	7.04**	5.76**	-9.18	-10.97	26.35*	11.29	-21.3*	-28.74**	136.9**	130.66**	-12.20	-23.12**
L9xT1	-49.49**	-46.83**	2.91*	1.68	-18.95**	-20.54**	-11.78	-22.3*	-26.9*	-33.74**	-76.40	-77.07	24.8**	9.28
L9xT2	-25.86**	-21.95*	1.70	0.48	-20.55**	-22.11**	-7.42	-18.46	20.17	8.87	261.7**	252.15**	31**	14.73

Table 4. Estimates of standard heterosis (SH) for grain yield and other agronomic traits of 12 maize inbred lines crossed with four line testers in line x tester mating design and evaluated across sites in 2018 main cropping season.

Table 4. Contd

L9xT3	-15.11	-10.63	-0.49	-1.68	-17.71**	-19.33**	-19.46	-29.06**	-13.5	-21.62*	203.6**	195.55**	13.50	-0.63
L9xT4	4.36	9.85	5.34**	4.08**	-2.08	-4.00	11.89	-1.45	-9.91	-18.39	204.5**	196.45**	-6.60	-18.21*
L10xT1	-33.64**	-30.15**	1.94	0.72	-5.50	-7.36	3.99	-8.41	-12.8	-20.95*	1.10	-1.54	25.3**	9.69
L10xT2	-43**	-40**	2.67*	1.44	-14.84*	-16.52**	-6.98	-18.08	-0.10	-9.50	-75.00	-75.69	33.9**	17.21*
L10xT3	-16.22	-11.80	-1.46	-2.64*	-10.92	-12.67*	-10.75	-21.39*	-10.9	-19.28*	21.50	18.24	-2.30	-14.47
L10xT4	0.09	5.37	5.1**	3.84**	-6.09	-7.94	16.77	2.84	-10.4	-18.78*	161**	154.08**	-2.30	-14.46
L11xT1	-26.23**	-22.34*	1.70	0.48	0.22	-1.75	16.26	2.40	-17.1	-24.94*	-64.00	-64.92	12.60	-1.38
L11xT2	-50.14**	-47.51**	4.13**	2.88*	-10.84	-12.6*	4.77	-7.72	-11.4	-19.76*	-25.90	-27.85	35.7**	18.85*
L11xT3	-21.87*	-17.76	1.21	0.00	-20.1**	-21.67**	-6.43	-17.59	2.28	-7.34	-69.10	-69.87	5.70	-7.48
L11xT4	6.86	12.49	3.88**	2.64*	-0.62	-2.57	12.77	-0.67	-4.52	-13.50	254**	244.6**	-9.70	-20.95*
L12xT1	-39.67**	-36.49**	1.21	0.00	-6.96	-8.79	4.88	-7.62	4.37	-5.45	-85.2*	-85.58*	13.50	-0.64
L12xT2	-52.18**	-49.66**	3.16*	1.92	-17.57**	-19.19**	-6.67	-17.79	8.95	-1.30	-53.20	-54.47	30.7**	14.39
L12xT3	-20.48*	-16.29	-2.18	-3.36*	-14.43*	-16.12**	-8.10	-19.06*	7.49	-2.62	-85.2*	-85.61*	12.20	-1.75
L12xT4	-1.85	3.32	5.34**	4.08**	3.16	1.13	17.96	3.90	10.47	0.08	261.2**	251.64**	8.80	-4.74
SE(d)	0.93	0.93	1.34	1.34	13.05	13.05	11.23	11.23	0.14	0.14	3.31	3.31	0.24	0.24

* = Significant at 0.05 probability level; ** = significant at 0.01 probability level; GY = Grain Yield, DS = Days to Silking, PH = Plant Height, EH = Ear Height, EPP = Ears Per Plant, HC = Bad Husk Cover, EA = Ear Aspect. SE(d) = Standard error of difference.

enable the selection of effective shorter plant, with reduction of lodging and mechanical harvesting.

Regarding bad husk cover (HC) 19 and 20 testcross hybrids showed positive and significant SH over Jibat and Kolba standard checks. On the other hand, five and three testcross hybrids showed negative and significant SH over Jibat and Kolba, respectively. For this trait, negative SH is desirable since materials with negative SH produces tighten husk cover which is important for resisting birds and rotting of ears. This is in agreement to the Tulu et al. (2018), who reported similar result in their study over other batches of inbreed lines. None of the testcross hybrids showed positive and significant SH for EPP over both checks, while 28 and 12 testcross hybrids were showed negative and significant SH over Jibat and Kolba, respectively. Positive SH for this trait implies prolificacy of the hybrids as compared

to the standard checks. In the current study L9 x T2 which showed 20.17% and 8.87% SH over Kolba and Jibat, respectively may be used in the breeding program to develop hybrids with prolific ears. Similar findings have been reported (Abrha et al., 2013; Mesenbet et al., 2016; Abebe et al., 2020) reported similar results with the current findings.

Estimates of SH for ear aspect (EA) ranged from -24.83% (L6 x T4) to 23.93% (L2 x T1) and -14.1% (L6 x T4) to 41.5% (L2 x T1) over Jibat and Kolba, respectively. Testcrosses with negative and significant SH for this trait are in desirable direction, since lower scores are taken as good ear aspect by maize breeders. Standard heterosis is important in maize breeding and is dependent on level of dominance and differences in gene frequency. The manifestation of heterosis depends on genetic divergence of the two parental varieties (Hallauer and Miranda, 1988). In a maize breeding aimed at developing hybrids, the usage of combining ability of inbred lines and the information on heterosis is an important tool to decide whether the hybrid is selected for promotion or not.

Heterotic grouping

Grain yields averaged over two locations and estimates of SCA effects for the 48 single cross progenies are presented in Table 5. Among the testers, T1 and T2 are locally developed inbred lines by the Ethiopian highland maize breeding program having good combining ability; and have been used as testers in maize breeding for highland sub-humid agro-ecology of Ethiopia. These testers are presents of one of the

Line	T1 (HGB)		T2 (HGA)		T3 (HGA)		T4 (HGB)		
Line	GY	SCA	GY	SCA	GY	SCA	GY	SCA	нG
L1	7.53	0.65	8.61	0.88	10.15	0.37	8.88	-1.90**	В
L2	5.54	-0.13	6.92	0.41	9.53	0.96	8.33	-1.24*	В
L3	5.76	0.6	6.35	0.34	7.47	-0.58	8.70	-0.36	-
L4	5.07	0.69	5.01	-0.21	7.32	0.04	7.76	-0.52	-
L5	4.98	-0.66	6.61	0.12	8.28	-0.25	10.32	0.79	-
L6	3.73	-0.58	6.01	0.86	8.46	1.26*	6.67	-1.54*	В
L7	4.56	-0.84	6.92	0.54	8.04	-0.25	9.84	0.54	-
L8	3.65	-1.66**	7.05	0.89	8.17	-0.03	10.01	0.8	В
L9	5.45	-1.11	8.00	0.6	9.16	-0.29	11.26	0.8	AB
L10	7.16	0.78	6.15	-1.07	9.04	-0.23	10.80	0.52	A
L11	7.96	1.55*	5.38	-1.88**	8.43	-0.88	11.53	1.22	A
L12	6.51	0.71	5.16	-1.48*	8.58	-0.11	10.59	0.89	A

Table 5. Heterotic grouping of twelve inbreed lines using SCA estimates with four tester lines with defined heterotic groups.

* = Significant at 0.05 probability level; ** = significant at 0.01 probability level; HGA = Heterotic group A; HGB = heterotic group B; GY = grain yield.

commercial hybrid (Jibat) used as standard checks in this study. Whereas, testers T3 and T4 are CIMMYT inbreed lines with T3 being in heterotic group 'A' and T4 in heterotic group 'B' (Tolesa et al., 1993; Keno et al., 2017). These CIMMYT testers have been used as testers in CIMMYT and other national and international maize breeding programs in the tropics. The heterotic grouping of the locally developed inbred lines in the present study was, therefore, based on using these locally developed and CIMMYT established testers, usingan assumption that SCA and heterosis of two inbred lines from different heterotic groups is greater than those from the same group.

An inbred line expressing negative SCA effect when crossed to a certain tester implies that the line and the tester belong to the same heterotic group, while the reverse is true when the SCA effect is positive (Vasal et al., 1992). As shown in Table 5, L1 and L2 had negative and significant SCA effects when crossed to T4 and showed lowest GY when crossed to T1. This therefore, indicates that L1, L2, T1 and T4 were highly likely to be in the same heterotic group. As a result, L1 and L2 were assigned to heterotic group B. Similarly, L6 and L8 had negative and significant SCA effects when crossed to T4 and significant SCA effects when crossed to T4 and negative and significant SCA effects when crossed to T4 and t1, respectively. In addition L6 showed positive and significant SCA effect when crossed to T3 which is in heterotic group A. Consequently these inbreed lines were postulated to be in heterotic group B.

L9 had no significant SCA effects when crossed with both heterotic testers. However, all crosses of L9 with testers from both heterotic groups gave considerably high heterosis thus; L9 was postulated to be in AB heterotic group. There were no testers whose cross combination with L10 expressed significant SCA effects in both directions. But the cross combinations of this lines with T1 and T4 gave higher average grain yield. As a result this line was assigned to heterotic group A. L11 and L12 were showed negative and significant SCA effects when crossed to T2 and they had positive SCA effects and higher grain yield (L11 = 11.53 and L12 = 10.59 tons) when crossed to T4. Consequently, L11 and L12 were postulated to be in a heterotic group. In this study, some inbreed lines (L3, L4, L5 and L7) could not be assigned to any of the heterotic groups developed by CIMMYT because the testers used in our study does not gives us clear cut data to postulate these lines to one of the heterotic group.

Conclusion

The analysis of the current study, revealed the presence of high genetic variation between the genotypes and the potential to develop high yielding hybrids for the targeted agro-ecology of the country. The study also identified some testcross hybrids that have desirable standard heterosis as compared to the standard checks. L9 x T4 and L11 x T4 revealed positive standard heterosis for grain yield over both checks. In addition, these hybrids showed negative standard heterosis for PH and EA, indicating these hybrids can be used in the improvement of these traits. The two crosses identified based on their heterosis over the best standard check, (L9 x T4 and L11 x T4) had T4 as a parent in their pedigrees. This confirms that T4 is genetically distant from the rest of inbred lines and it can be used as parent in hybrid maize breeding for commercial release or for further breeding activities in highland agro-ecologies of the country. The study also proposed eight inbred lines to one of the heterotic groups. Lines L1, L2, L6 and L8 were assigned to HGB,

while lines L10, L11 and L12 were assigned to HGA and only line L9 assigned to heterotic group AB. In general, the current study revealed that extensive works should be done in broadening the genetic base for highland breeding program to develop high yielding varieties for the target areas. The information obtained from this study on the heterotic groups of the inbred lines and standard heterosis of the testcross hybrids for different traits would be useful in planning hybrid maize breeding.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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