Combining Ability of Highland Adapted Double Haploid Maize Inbred Lines using Line X Tester Mating Design

Diribu Tesfaye[1]*, Demissew Abakemal[2], and Ermias Habte[1]

[1]Ambo University, P. O. Box 19, Ambo, Ethiopia
[2]Ethiopian Institute of Agricultural Research, Ambo-PPRC Ethiopia, P.O. Box 37, Ambo, Ethiopia

Abstract: Development of inbred lines is a routine activity in hybrid maize breeding program. Understanding the relative importance of general (GCA) and specific (SCA) combining ability effects for different traits of newly developed maize lines is of paramount importance to design future breeding strategies for the development of hybrid. There are several types of mating designs available to study the combining abilities of maize inbred lines, of which line x tester mating scheme is one used to determine the combining abilities and also to categorize the lines into heterotic groups. The objectives of the study were, therefore, to estimate GCA and SCA of newly developed highland maize lines using line x tester mating design, to identify best promising hybrid crosses with desirable traits for further breeding and cultivar development, and to identify the heterotic groups of the lines involved in the crosses. Twelve lines and four testers were involved in the study. A total of 48 testcrosses along with two checks were made available for evaluation across locations. An alpha lattice design (10x5) with two replications at each location was used. Two lines (L_6 and L_5) showed highly significant and positive GCA effects for grain yield. Besides, L_6 also revealed positive GCA effects for the other yield related traits such as number of ears per plant (NEPP), number of rows per ear (NRPE), number of kernels per row (NKPR) and ear diameter (ED). On the other hand, crosses L_7xT_2, L_8xT_4, and L_9xT_1 had positive and significant SCA effects for grain yield. The lines and crosses with positive and significant GCA and SCA effects, respectively; could be selected for use in highland maize hybrid or/and synthetic cultivar development. The testers clearly categorized L_3 and L_6 into their respective heterotic groups in a similar pattern unlike the other lines which requires the application of molecular markers for further verification.

Keywords: General Combining ability; Specific Combining ability; line; Tester

1. Introduction

Maize (*Zea mays L*., 2n=2x=20) is among the world's most widely grown cereals indicating its adaptability to a wide range of environments. Maize is an important source of food and nutritional security for millions of people in the developing world, especially in sub-Saharan Africa (SSA) and Latin America. It is a staple food in many of the SSA countries and is commonly grown by resource poor small-scale farmers in rural areas (Shiferaw *et al.,* 2011). The need for food security and the diverse agro-climatic conditions in Ethiopia have encouraged the majority of subsistence farmers to grow various crops. One major shift is that over the past 20 years, the area dedicated to maize cultivation in Ethiopia has expanded progressively (Demissew *et al*., 2013). Increase in maize production and productivity can be achieved by identifying elite parental materials which can be used to develop high yielding hybrids by using suitable mating designs (Liaqat *et al*., 2015). There are several types of mating designs available to study the combining ability of maize lines; of which line x tester mating scheme is one. A line x tester mating design involves the crossing of a number of selections, lines, or clones to a common parent (tester) (Kempthorne, 1957). The tester is used as male parent and lines which are used as females to be tested are either male-sterile or self-incompatible, or are emasculated before pollen shed to prevent selfing. For line evaluation, a desirable tester is defined by Matzinger (1953) as one that combines the greatest simplicity in use with the maximum information on performance to be expected from tested lines with respect to general and specific combining abilities.

Apart from evaluation of cross performances at early stages of line development, several studies were conducted to study general combining ability (GCA) and specific combining ability (SCA) of lines for different traits using line x tester. The design is useful in deciding the relative ability of female and male lines to produce desirable hybrid combinations (Kempthorne, 1957). It also provides information on genetic components and enables the breeder to choose appropriate breeding methods for cultivar development. It is always important for any breeding program to generate such information for any new batch of lines generated or received outside of the program (Panhwar *et al*., 2008). Understanding the relative importance of general (GCA) and specific combing ability (SCA) effects for different traits of newly developed lines are of paramount importance to

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*Corresponding Author. E-mail: diribuz07@gmail.com

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design future breeding strategies for the development of hybrid and or synthetic varieties (Joshi *et al.,* 2002).

Heterotic classifications generated from a line x tester analysis can assist in determining the relationship existing among the different lines (Joshi *et al.,* 2002; Sharma *et al.*, 2004). A number of maize inbred lines have been developed from maize breeding programs in Ethiopia (Bayisa *et al.*, 2005; Dagne *et al.*, 2007; Worku *et al.*, 2008). However, there has been limited information on the combining ability effects and heterotic classification of the newly developed lines, which necessitated this study. Therefore, the objectives of this study were: (1) to determine general combining abilities (GCA) and Specific combining abilities (SCA) of newly developed highland maize lines using line x tester mating design and (2) to identify the heterotic groups of the newly developed lines for further use in the breeding program.

2. Materials and Methods

2.1 Description of the Study Sites

The experiments were conducted in the main cropping season of 2015 at Ambo Plant Protection and Holetta Agricultural Research Centers. Ambo is located at $8^0$57′N latitude and $38^0$7′E longitudes and an altitude of 2225 meters above sea level (masl). The long-term average annual rainfall at Ambo is 1115 mm with minimum and maximum temperature of 11.7^0c and 25.4^0c, respectively. The soil type of the experimental site of Ambo is heavy Vertisol. The location at Holetta Research Center is $9^0$00′N latitude and $38^0$30‘E longitude and an altitude of 2400 meters at sea level with soil type of Nitosol. The area receives an average annual rainfall of 1065 mm, with minimum temperature 6.4^0c and maximum 22.1^0c. Both locations represent the highland sub-humid maize growing agro-ecology of Ethiopia.

2. 2 Experimental Materials

There were twelve lines and four testers used in the present study. The testers were FS59 (tester-1), FS67 (tester-2), EC573 (tester- 3), and Kitale Synthetic II (tester- 4); the first two were lines and while the later two were populations (Table 1). Originally, tester-1 and tester-2 were derived from tester-3 and tester-4 respectively, while the twelve lines were derived from either of the population testers.

The lines were introduced to Ethiopia from CIMMYT-Kenya in 2014 and test crossed with the aforementioned four testers. A total of 48 lines x tester crosses constituted from twelve lines and the four testers were made available for evaluation in 2015 cropping season. These 48 F_1 crosses and two standard checks (AMH851and AMH850) were used for this study.

2.3. Experimental Design and Field Management

An alpha lattice experimental design (10x 5) was used with two replications at each location. And also one replication consisted of five incomplete blocks within main blocks. The Spacing used was 75cm between rows and 25cm between plants. Each plot consisted of a single 5.25m long row. Each entry had an equal chance of being randomly placed to any plot per block within a replication. Planting was done on 26th of May 2015 manually using two seeds per hill after reliable moisture level of the soil had been attained to ensure good germination as well as seedling development. Later on, 35 days after planting, maize seedlings were thinned out to one plant to get 53,333 maize plant populations per hectare. The recommended rate-150 and 200 kg ha^{-1} DAP and UREA-was used, respectively. Band application of phosphorous fertilizer in the form of diammonium phosphate (DAP) was carried out at planting time. UREA was applied in two splits-at 40 days and 70 days after planting. All other agronomic management practices were done, accordingly.

2.4 Data Collection

Days to Silking (SD), Days to Tasseling (AD), and Days to Maturity (DM): The number of days from planting to when 50% of the plants in a plot produced 2-3 cm long silk, started shedding pollen and the plants in a plot reaches physiological maturity respectively. Plant height (PH), expressed in cm, measured from the base of a plant to the insertion of the first tassel branch of the same plant, ear height (EH), measured in cm from the base of a plant to the insertion of the top most ear of the same plant). Ear length (EL) was measured as the length of the ear from the base to tip, ear diameter (ED) was measured at the mid-way along ear length, and number of ears per plant (NEPP) is number of ears with at least one fully developed grain divided by the number of harvested plants). Number of rows per ear (NRPE) and number of kernels per row (NKPR) were randomly taken as averages. Thousand kernel weight (TKWT) is weight in grams of 1000 random kernels was weighed from each plot using sensitive balance and was adjusted to 12.5 % moisture level. Ear aspect (EA) was taken by judging the phenotypic appearance of the ear by giving a number scale from 1-5. Grain yield (GY) was weighed in kg/plot and adjusted to12.5% moisture content and 80% shelling percentage and then converted to tones/ha and the percentage of bare-tip ears recorded for husk cover (HC). Root lodged (RL) percentage of plants lodged per plot was recorded.

2.5 Statistical Procedures

Analyses of variance ANOVA per individual location across location ware carried out using the PROC GLM procedure of SAS 9.3(SAS, 2002). Genotypes were considered as fixed factor while replications and incomplete blocks within replications were considered as random factors. When significant difference was detected among entries at each location, a combined ANOVA was conducted. The combined analyses across locations were done for characters that showed statistically significant differences among the genotypes at two of the locations. Partitioning of the mean squares due to crosses in to lines (GCA_l), testers (GCA_t) and line x tester interactions (SCA_{lxt}) (Kempthorne, 1957) was also done using SAS (Ver. 9.3) software program. Checks were not included in the combining ability analyses. The combining ability analyses for grain yield and other agronomic traits were done using the line x tester linear model as follows: $Y_{ijk} = \mu + r(e_k) + e_k + l_i + t_j + (l \times t)_{ij} + (l \times e)_{ik} + (t \times e)_{jk} + (l \times t \times e)_{eijk} + \varepsilon_{ijk}$; where Y_{ijk} is measured trait of the genotype of i^th^ line crossed to j^th^ tester evaluated in r replications across k environments; μ is grand mean; r(ek) is the effect of replication nested within the *k* environments; l and t represent average effects of lines and of testers, respectively, which is equivalent to GCA effects of lines and testers, respectively; l x t = line x tester interaction effects that is equivalent to the SCA effects of the crosses; *e* is the environmental main effects; l x *e*, t x *e*, and l x t x *e* are the interactions of the lines, testers and the lines x testers with the environments, and ε_{ijk} is the random experimental error. The GCA of lines (GCA_l) and testers (GCA_t), and SCA_{lxt} of crosses and their standard errors were estimated (Dabholkar, 1992). Inbred line that expressed negative SCA effects when crossed to a certain tester implied that they are categorized under the same heterotic group with the tester; however, if the same line manifests positive SCA effect with the same tester, it is classified into opposite heterotic group (Vasal *et al.,* 1992). Data transformation for percentage data was done before analysis for traits such as husk cover, stalk and root lodging. Arcsine transformation method: $Y' = \text{arcsin}\ \sqrt{Y}$ for husk cover and Square root transformation methods: $Y' = \sqrt{Y} + 0.5$ for stalk and root lodging where Y’ is the transformed value, and Y is the original value.

Table 1. Descriptions of the 12 highland maize lines and 4 testers used for the research.

Serial No.	Code	Pedigree	Source	Role in the study
1	L_1	(ZEWAc1F2-134-4-1-B-1-B*4/CML540)DH-8-B-#	CIMMYT	Line
2	L_2	(G16BNSeqC4-(F22x1)-2-3-1-B*4-B/ZM523B-29-2-1-1-BBB)DH-7-B-#	CIMMYT	Line
3	L_3	ZM523B-29-2-1-1-BBB-B-B-#	CIMMYT	Line
4	L_4	(ZEWAc2F2-183-2-BBB-B/CML542) DH-3-B-#	CIMMYT	Line
5	L_5	(CML545/CML505) DH-10-B-#	CIMMYT	Line
6	L_6	(CML545/CML505) DH-29-B-#	CIMMYT	Line
7	L_7	(CML545/CML505) DH-44-B-#	CIMMYT	Line
8	L_8	(ZEWAc2F2-183-2-BBB-B/[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BBB-B) DH-11-B-#	CIMMYT	Line
9	L_9	(ZEWAc2F2-183-2-BBB-B/[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-BBB-B) DH-17-B-#	CIMMYT	Line
10	L_{10}	(ZEWAc1F2-151-6-1-B-1/ZEWAc1F2-300-2-2-B-1) DH-3012-B-B-#	CIMMYT	Line
11	L_{11}	(CML505/[INTA-2-1-3/INTA-60-1-2]-X-11-6-3-B) DH-3016-B-B-#	CIMMYT	Line
12	L_{12}	(INTA-F2-192-2-1-1-1-B*9/CML505-B) DH-3021-B-B-#	CIMMYT	Line
13	T_1	FS59	Ambo-HMBP*	Tester
14	T_2	FS67	Ambo-HMBP	Tester
15	T_3	EC573	Ambo-HMBP	Tester
16	T_4	Kitale Synthesis II	Ambo-HMBP	Tester
17	49	AMH851	Ambo-HMBP	Check 1
18	50	AMH850	Ambo-HMBP	Check 2

Note: *HMBP –Highland Maize Breeding Program

3. Results and Discussions

3.1 Combining Ability Analysis

The results from combined combining ability analyses of variances for grain yield and yield related traits are presented in Table 2. The combined analyses of variance across locations revealed that mean square due to location had non-significant difference for PH, NRPE and EL while all the rest traits showed significant differences ($P < 0.01$) indicating that the two locations differed in their environmental conditions. These findings agreed with those reported by Aly and Amer (2008), Aly *et al.* (2011) and Mousa and Aly (2012). According to Gezahegn (2005), however, significant mean squares of locations for PH across four locations were reported. The mean squares due to crosses exhibited highly significant differences (P < 0.01) for most of the traits with the exception of ear length. This indicated that the crosses were sufficiently different from each other for these traits and hence, selection is possible to identify the most desirable hybrids. In line with this study several investigators reported the existence of highly significant differences among the maize crosses for almost all traits under study (Parvez *et al.*, 2007; Aly and Amer, 2008; Zoran *et al.*, 2012).

The line mean square values were highly significant for all traits indicating that the existence of variability among the set of lines used in this research (Table 2). Tester mean squares for most traits were also showed highly significant difference (P < 0.01). The significance of mean squares due to lines and testers (GCA) for most traits indicated the prevalence of additive types of gene actions in the expression of these traits. In support of the present investigation, Reza *et al.* (2013) observed that lines and testers had significant mean squares in single cross maize hybrids for all traits in the study. Mean squares from the combined analysis for Lines x tester (SCA) interaction also showed highly significant differences for most traits including GY, AD, SD, EH, HC, NKPR, ED, and TKW indicating the overwhelming contribution of dominant effect type of gene action in these traits. However, the proportion of GCA sum of squares to SCA for all the traits were greater than unity indicating that the contributions of additive gene effects were more important than non-additive gene effects in this study.

Therefore, lines used in the present study were diverse, resulting in the expression of variation among genotypes for various traits. Earlier investigators also found that line x tester mean squares were significant for grain yield and yield related traits across locations (Parveez *et al.*, 2007; Aly and Amer, 2008). Lines x location mean squares (Table 2) were significant for traits, but the magnitudes were consistently smaller than the respective line mean squares obtained from individual location analysis. This suggests the need of selecting different parental lines for hybrids in specific environment. Trait which showed non-significant variations for line x location interaction were GY, PH, PA, MD, NRPE, EL, and ED. Similar results of combined analyses were previously reported by various researchers (Bayisa *et al.*, 2008; Kanyamasoro *et al.*, 2012; Mousa and Aly, 2012) using different sets of maize inbred lines. However, this result contradicts with Ibrahim and Mousa (2011) reports of significant interaction of Line x Location mean squares for GY.

Table 2. Mean squares of combining ability from combined analysis for grain yield and other related traits of 48 Line x Tester crosses evaluated across two locations(Ambo and Holetta) in 2015.

Source of variation	Df	GY	AD	SD	PH	EH	PA	EA	RL	HC	MD	NEPP	NRPE	NKPR	EL	ED	TKW
Location	1	15.52**	20708.52**	26861.67**	405.42	2275.63**	5.03**	3**	1.22**	25986.75**	2227.68**	8.97**	1.06	781.25**	9.29	199.92**	332868.55**
REP (location)	1	4.3*	28.07**	45.38**	1371.71**	1362.52**	0.01	0.31	1.0**	33.26	75.08*	0.003	0.32	4.63	4.56	1.61	1986.9
Crosses	47	5.65**	45.00**	56.08**	1119.43**	1005.49**	0.51**	0.82**	0.22**	848.11**	51.68**	0.11**	3.80**	18.25**	10.49	24.44**	5644.88**
Line (GCA_L)	11	11.66**	131.42**	133.27**	1378.09**	2411.20**	1.53**	1.57**	0.28**	2340.95**	76.95**	0.28**	5.92**	45.01**	15.02*	40.4**	11999.32**
Tester (GCA_T)	3	22.04**	141.5**	300.01**	10010.17**	4843.47**	0.88**	5.11**	0.79**	1397.76**	98.39**	0.09	28.1**	4.76	12.43	134.7**	5174.34*
Line*Tester (SCA_{lxt})	33	2.15**	7.42**	8.18**	224.97	188.01**	0.13	0.19	0.15	300.54**	39	0.05*	1.15	10.56**	8.81	9.09**	3569.63**
Crosses*location	47	1.31	3.52**	5.71**	232.0	79.77	0.16	0.16	0.21**	333.2**	32.27	0.04**	1.19	10.56**	7.34	2.91	2988.00**
Line* location	11	1.19	6.89**	12.52**	355.11	174.7**	0.139	0.42**	0.37**	670.95**	26.39	0.01**	1.19	11.97*	9.19	1.83	4459.05**
Tester*location	3	0.19	3.45	10.31*	259.85	41.28	0.31	0.08	0.01	361.72*	54.56	0.05**	0.57	20.65**	11.90	3.91	824.49
Line*Tester*locations	33	1.46	2.4	3.02	188.43	51.63	0.15	0.08	0.17	218.03**	32.2	0.02	0.74	9.17*	6.31	3.18	2694.34*
Pooled Error	94	1.13	1.64	3.26	184.08	65.76	0.20	0.14	0.12	112.66	25.51	0.02	0.97	5.83	7.65	2.54	1623.48
R^2		0.76	0.99	0.98	0.79	0.90	0.66	0.79	0.67	0.88	0.72	0.87	0.72	0.79	0.54	0.86	0.82
GM		8.23	95	97.47	243.97	132.4	2.99	3.24	0.93	25.79	176.69	1.35	13.25	36.8	17.10	44.65	346.73
CV%		13.33	1.34	3.26	5.76	6.12	14.97	11.72	37.5	41.04	2.85	11.18	7.45	6.54	16.17	3.57	11.62

Note: *and ** refers to significant at $p \leq 0.05$ and $P \leq 0.01$ respectively, GY=grain yield (t h^{-1}), AD=days to 50% anthesis, SD=days to 50% silking, PH=plant height(cm), EH=ear height (cm), PA=plant aspect (1-5 scale), EA=ear aspect (1-5 scale), RL=root lodging (%), HC=husk cover (%), MD=days to 50% maturity, NRPE=number of row ear^{-1} (number), NEPP= number of ear $plant^{-1}$ (number), NKPR= number of kernel row^{-1} (number), EL=ear length (cm) and ED= ear diameter (cm), TKW=thousand kernel weight(g), of the genotypes used.

3.2 General Combining Ability Effects

The estimates of GCA effects of lines for grain yield and yield related traits combined across two locations are presented in Table 3. Accordingly, L_6, L_5 and L_9 showed highly significant and positive GCA effects for GY. Whereas L_2, L_7 and L_{10} had significantly negative GCA effects for GY. Consequently, the lines with positive GCA effect could be selected as good combiners for grain yield improvement. GCA of T_1 and T_4 revealed positive and highly significant effects in the combined analysis for grain yield. In contrast to this, T_2 and T_3 revealed negative and highly significant GCA effects. In line with the current study, several authors reported both positive and negative significant GCA effects of experimental maize hybrids for grain yield (Ahmad and Saleem, 2003; Pswarayi and Vivek, 2008; Legesse *et al.*, 2009; Mosa, 2010; Zeleke and Tuna, 2010). The estimates of lines' GCA effects varied from -6.28 (L_{10}) to +3.65(L_9) and -5.97(L_{10}) to +3.83(L_9) for AD and SD, respectively (Table 4). L_3, L_4, L_6, L_7 and L_9 had significant positive GCA effects for both traits while L_1, L_8 and L_{10} were highly significant and negative GCA effects for similar traits. Aminu and Izge (2013) reported highly significant negative and positive GCA effects among the tested lines for days to tasselling and silking. Lines showed varied trends of significant GCA effects for PH in the combined analysis (Table 3). L_{10} (-21.59) had negative and highly significant GCA effects for PH suggesting that this line could contribute for shorter stature trait in the improvement of maize for lodging resistance. On the other hand, L_6 (+13.46) and L_3 (+11.77) had positive and highly significant GCA effects for the same traits. Most of the lines with significant GCA effects for EH also revealed similar trend for PH with the exception of L_5 (+7.53) that showed contrasting effects (Table 3). Similar findings reported short PH and EH reduced lodging in maize (Mosa, 2010; Zeleke and Tuna, 2010; Rahman *et al.*, 2012). GCA estimates for EA from the combined analysis ranged from -0.43 to +0.34. L_3, L_9, and L_4 revealed a high and negative GCA effect which indicated a good ear character in this regard and could thus be used in breeding programs for the improvement of germplasm for the trait. Similar lines revealed significant positive and negative GCA effects for PA and EA as well. Therefore, the lines with negative GCA can be good combiners for PA, while the lines with positive GCA effects may be poor combiners for the trait. In support of this study, Girma *et al.* (2015) reported positive and negative GCA effects for ear aspect at a single location evaluation. In contrast, Wende (2013) found two lines with positive GCA effects among nine maize inbred lines evaluated across seven environments for the same trait.

3.3. Specific Combining Ability Effects

The estimates of specific combining ability effects across locations in respect to the 48 hybrids are presented in Table 4. Of all the 48 crosses evaluated, L_1xT_1, L_7xT_4 and L_4xT_4, expressed negative and significant SCA effects for grain yield, which is unwanted as these crosses showed a tendency to reduce grain yield performance. In contrast, crosses L_7xT_2, L_8xT_4 and L_9xT_1 revealed positive and significant SCA effects for GY. These crosses with high positive and significant estimates of SCA effect could be selected for their specific combining ability to use in maize improvement. In this investigation, SCA of GY across environments was negative for most crosses, which may be attributed from using lines with the same genetic background. SCA effects could be positive with superior performance for crosses involving lines from different genetic backgrounds (Betran *et al.*, 2003b). For days to 50% anthesis, nine and four crosses showed significantly negative and positive SCA effects across locations, respectively. Seven and three crosses showed significant negative and positive SCA effects across locations for days to 50% silking, respectively (Table 4). Aminu and Izge (2013) reported that negative and positive SCA estimates with respect to twenty hybrids for AD and SD in maize across environments. The SCA effects showed that crosses L_2xT_1, $L_{10}xT_4$ and $L_{11}xT_2$ revealed negative and highly significant values for PH suggesting the possibility of inheritance of shortness in maize plants to develop genotypes tolerant for lodging. These results are in contrast with those obtained by Aly (2013), who reported positive and negative significant SCA effect of top-crosses studied across two locations for the trait. Crosses L_1xT_1, L_1xT_2, L_4xT_2, L_2xT_3, L_5xT_1, L_6xT_3, L_7xT_3 and $L_{10}xT_4$ revealed negative and highly significant SCA effect for EH. In contrast to this, seven crosses revealed positive SCA effects for the trait. In line with this study, Aminu and Izge (2013) reported that negative and positive SCA estimates with respect to twenty hybrids for EH in maize across environments

Table 3: General combining ability effects (GCA) of the 12 lines and four testers for grain yield and other yield related traits combined across two locations (Ambo and Holetta) in 2015.

							Traits									
Line	GY	AD	SD	PH	EH	PA	EA	RL	HC	MD	NEPP	NRPE	NKPR	EL	ED	TKW
L_1	-0.18	-2.28**	-2.53**	-0.16	-11.58**	0.16*	-0.15	0.01	-16.7**	1.36	0.05	-0.52*	-0.86	-1.39	-1.64**	14.31
L_2	-1.07**	-0.78	-0.53	-7.66	0.72	0.06	0.25	0.21	14.95*	0.17	-0.06	0.13	0.43	-0.4	-2.05**	-48.98**
L_3	0.44	3.28**	1.77*	11.77**	11.91**	-0.27**	-0.43**	0.2	-16.8**	3.8**	0.04	-0.94**	0.17	0.9	-0.29	49.92**
L_4	-0.51	1.65**	2.58**	3.58	5.91	-0.18*	-0.3*	-0.03	-14.62*	-2.07	-0.02	0.05	4.35**	0.03	0.01	-11.15
L_5	0.9**	0.65	-0.41	-2.22	7.53*	-0.18*	0.19	0.02	9.76	1.55	0.11*	-0.23	-2.17**	0.16	-0.15	43.3**
L_6	1.88**	2.4**	2.58**	13.46**	18.84**	-0.08	-0.15	-0.09	3.24	2.61*	0.28**	0.71**	1.65*	-0.56	2.19**	-18.16
L_7	-0.98**	1.53*	2.77**	-1.41	5.03	-0.08	0.25	0.16	-1.8	0.61	-0.11*	-0.46	-0.65	2.5**	-0.03	5.07
L_8	-0.08	-3.03**	-2.97**	-2.84	-6.96*	0.34**	0.34*	-0.07	10.12	-2.51*	-0.04	0.01	-1.14	-0.3	0.71*	10.314
L_9	0.55*	3.65**	3.83**	3.65	-0.52	-0.27**	-0.43**	-0.16	-6.42	-1.38	-0.01	0.51	-0.19	-0.01	2.04**	-7.95
L_{10}	-0.81**	-6.28**	-5.97**	-21.59**	-28.71**	0.78**	0.34*	-0.11	10.23	-2.88*	0.08	0.09	-1.26	-1.07	-2.54**	-20.93
L_{11}	-0.18	-0.15	-1.09	-3.53	-5.15	-0.24**	-0.24	-0.15	-6.34	0.98	-0.17**	1.22**	0.1	0.06	2.13**	-13.56
L_{12}	0.06	-0.65	-0.03	6.96	2.97	-0.02	0.31*	0.01	14.48*	-2.26	-0.13	-0.57*	-0.41	0.08	-0.36	-2.17
$SE_{(gi)}$	0.26	0.62	0.84	4.51	3.16	0.08	0.15	0.14	6.2	1.22	0.05	0.26	0.82	0.72	0.32	15.98
Tester																
T_1	0.83**	-0.94**	-0.76	3.75	1.6*	-5.98*	-0.34**	-0.06	-0.01	-1.23	0.02	0.05	0.02	0.12	-0.07	5.06
T_2	-0.46**	-0.86**	-2.16**	-18.82**	-12.83**	1.06	0.01	-0.08	-0.01	1.19	0.03	-1.08**	-0.36	-0.12	-1.85**	9.05*
T_3	-0.62**	-0.76**	-0.68	-0.93	-0.42	6.83**	0.42**	0.2**	0.03**	-1.23	-0.01	0.36**	-0.06	-0.58	-0.28	-14.67**
T_4	0.25**	2.57**	3.6**	16**	11.61**	-1.91	-0.06*	-0.04	-0.02*	1.28	-0.05	0.66**	0.4	0.58	2.21**	0.55
$SE_{(gi)}$	0.05	0.23	0.4	2.01	0.8	2.37	0.03	0.07	0.01	0.92	0.04	0.09	0.56	0.43	0.24	3.58

Note: *and ** refers to significant at $P \leq 0.05$and at $P \leq 0.01$ respectively, L_{1-12} = Line, T_{1-4}= Tester, GY=grain yield (t h^{-1}), AD=days to 50% anthesis, SD=days to 50% silking, PH=plant height (cm),EH=,ear height (cm), PA=plant aspect (1-5 scale), EA=ear aspect (1-5 scale), RL=root lodging (%), HC=husk cover (%), MD=days to maturity, NEPP= number of ear plant^{-1} (number), NRPE=number of row ear^{-1} (number), NKPR=number kernel row^{-1} (number), EL=ear length (cm) and ED= ear diameter (cm), TKW=thousand kernel weight (g), of the genotypes used; See Table 1 for descriptions of lines and testers codes

SCA effects for PA varied from -0.38 to +0.55. Cross L_3xT_2 manifested negative and significant SCA effect for PA indicating that it was good specific combiner. On the other hand, the cross combinations of L_1xT_2 and L_3xT_1 expressed positive and significant SCA effects. Hybrids such as L_3xT_4, L_8xT_1, L_9xT_2 and $L_{11}xT_3$ had significantly negative SCA effects for ear aspect (EA) while L_2xT_1, L_3xT_3, L_7xT_1, and $L_{11}xT_4$ had significantly positive SCA effects for the same trait (Table 4). Girma *et al.* (2015) obtained negative and positive SCA effects for ear aspect, while Wende (2013) reported similar results for plant aspect. Crosses of L_1xT_3 and L_2xT_3 showed positive and highly significant SCA effects for root lodging (RL) suggesting that they were undesirable crosses. Similarly, Bhatnagar *et al.* (2004) reported significant SCA effect for RL. Cross L_9xT_2 had significant and negative SCA effect for HC. Other hybrids with significant and positive SCA effects for HC included L_7xT_3, L_5xT_4 and $L_{12}xT_1$. These results were at par with the findings of Girma *et al.*, (2015) who reported positive and negative significant SCA effects of maize crosses for HC.

The SCA effects for MD ranged from -5.82 to +6.4. Crosses L_6xT_1, $L_{10}xT_2$ and $L_{11}xT_4$ manifested negative and significant SCA effects indicating that these crosses were good specific combinations for early maturing. Hence, such cross combinations could effectively be exploited in hybrid breeding program in maize research for reduced days to maturity. On the other hand, cross combinations L_6xT_2 and L_9xT_4 expressed positive and significant SCA effects for days to 50% maturity, which were undesirable as these crosses showed a tendency to mature late (Table 4). This result disagrees with the findings of Melkamu *et al.* (2013) who reported non-significant QPM crosses for the same trait at a single location evaluation. Twelve crosses showed significant estimates of SCA effects for NEPP (Table 5). Cross L_1xT_3, L_2xT_2, L_5xT_4, L_6xT_1, L_7xT_1, L_8xT_4, and $L_{11}xT_4$ were good positive specific combiners while L_1xT_2, L_5xT_1, L_6xT_4, L_7xT_4 and L_8xT_3 were found poor. The highest SCA effect for GY in the combined analysis was obtained from L_8xT_4 cross. It was, therefore, demonstrated that high yielding crosses showed high SCA values, indicating the importance of SCA effects in predicting hybrid performance for each trait. This observation is similar with the study of Betran *et al.* (2003b) and Gezahegn (2005) who suggested that SCA predicts hybrid performance for NEPP. SCA estimates of combined data of the crosses ranged from -0.9 to +0.84 for NRPE. Crosses L_1xT_3, L_4xT_2, $L_{11}xT_3$ and $L_{12}xT_4$ showed positive and significant SCA effects which was attractive for this trait because genotypes with good SCA can contribute to increased grain yield in hybrid combinations. On the other hand, L_2xT_2, L_3xT_3 and $L_{11}xT_4$ revealed negative and significant SCA effects. However, Aly (2013) reported non-significant SCA among twenty-two top-crosses studied across two locations for NRPE. Only cross L_7xT_4 revealed positive and significant effects for number of kernels per row (NKPR). In contrast to this investigation, Aly (2013) reported positive and negative significant SCA effects among twenty-two top-crosses studied across two locations for number of kernels per row. Eight crosses showed positive and significant estimates of SCA effects for ED (Table 4). Cross L_1xT_1, L_2xT_4, L_4xT_2, L_7xT_3, L_9xT_2, $L_{10}xT_2$, L_{11} xT_1 and $L_{12}xT_4$ were found with good positive SCA while nine crosses were the poorest of all for the same trait. These results contradict with the results of Aly (2013) who reported non-significant ED. The highest positive SCA effect for the GY was contributed by L_3xT_3 from the across locations analysis. In general, it was demonstrated that high yielding crosses showed high SCA values in this study indicating the importance of SCA effects in predicting the performance hybrids. Ear diameter and number of kernel rows per ear could be given attention during selection for grain yield improvement in maize (Manivannan, 1998). SCA effects of EL and TKW ranged from -2.39 to 6.04 and -75.81 to -45.62, respectively (Table 4).

Cross L_2xT_2 and L_7xT_4 expressed significant negative SCA effects and were found to be poor combiners for TKW while cross L_7xT_4 showed positive and highly significant effect for EL. These results were in agreement with the investigation of Pal *et al.* (1986) who reported positive and negative significant SCA effects for TKW, and Aly (2013) found similar results for ear length.

Table 5 shows putative heterotic grouping of the lines used. In general, from the SCA values in Table 5, one can depict that the four testers: tester-1(FS59), tester-2(FS67), tester-3(ECU573) and tester-4(KITSYN II) of the two major heterotic groups (Ecuador and Kitale) used in the highland maize breeding program were able to categorized seven of them (L_2, L_3, L_4, L_6, L_8, L_9, and L_{12}) among the 12 lines into the already known heterotic groups (Ecuador and Kitale), whereas there was no clear discrimination in to heterotic groups for the remaining five lines (L_1, L_5, L_7, L_{10} and L_{11}) by the testers.

Particularly, both groups of testers clearly categorized L_3 and L_6 into their respective heterotic groups in a similar pattern unlike for the other lines where the testers groups had not shown similar patterns of classifications, though the SCA values were not statistically significant in some cases.

Table 4: Estimation of Specific Combining Ability effects of the 48 Line x Tester crosses for grain yield and other related traits evaluated across locations in 2015.

							Traits								
Crosse	GY	AD	SD	PH	EH	PA	EA	RL	HC	MD	NEPP	NRPE	NKPR	EL	ED	TKW
L_1XT_1	-0.01	1.32*	1.32	5.43	-6.2*	-0.08	-0.22	-0.22	2.7	-0.32	-0.01	-0.17	0.29	0.41	2.53**	41.17
L_1XT_2	-1.08*	-2.51**	-2.02**	-3.48	-6.72*	0.55**	0.01	-0.07	-0.29	2.48	-0.19**	-0.2	-0.97	-0.56	-0.44	9.99
L_1XT_3	0.9	0.13	0.01	-1.13	13.6**	-0.23	-0.01	0.45**	-0.53	-2.57	0.15*	0.84*	0.35	0.58	-0.31	-9.89
L_1XT_4	0.19	1.05	0.7	-0.81	-0.68	-0.23	0.22	-0.16	-1.88	0.4	0.05	-0.45	0.33	-0.43	-1.76*	-41.27
L_2XT_1	0.25	0.32	-0.17	-17.06**	3.73	0.01	0.36**	-0.13	2.6	3.364	-0.09	0.32	2.15	1.16	-0.92	12.03
L_2XT_2	0.01	-0.01	0.72	8.51	-0.78	0.02	-0.14	-0.15	5.17	-3.07	0.14*	-0.7*	1.19	0.33	-1.79*	-75.81**
L_2XT_3	0.19	-0.36	-0.75	-2.13	-6.95*	-0.01	-0.04	0.34**	0.32	0.114	-0.001	0.17	-1.6	-0.35	0.43	29.34
L_2XT_4	-0.45	0.05	0.2	10.68	4.00	-0.01	-0.18	-0.05	-8.19	-0.4	-0.05	0.21	-1.74	-1.14	2.28**	34.42
L_3XT_1	-0.71	-0.48	1.26	-0.01	1.29	0.34*	-0.06	0.33	2.73	-0.01	-0.03	-0.09	-0.25	-0.41	0.45	-20.98
$L3XT_2$	0.71	0.92	0.66	5.82	4.02	-0.38*	-0.07	-0.27	-2.6	1.05	0.1	0.54	-0.79	-0.99	0.19	-8.46
L_3XT_3	-0.72	-1.67*	-1.56*	-8.31	-4.89	0.07	0.39**	0.12	-0.4	-3.01	-0.04	-0.9*	-0.16	0.35	-1.16	2.55
L_3XT4	0.72	1.23	-0.35	2.49	-0.43	-0.04	-0.24*	-0.19	0.27	1.96	-0.02	0.46	1.21	1.04	0.52	26.89
L_4XT_1	0.24	-0.11	-0.29	6.68	5.79	-0.12	0.057	0.04	1.05	-0.88	-0.05	-0.42	0.72	0.97	-0.77	5.47
L_4XT_2	0.45	-0.44	-1.15	-7.23	-7.97**	-0.09	0.17	-0.03	-3.39	-0.32	0.05	0.71*	2.02	0.99	1.63*	31.03
L_4XT_3	0.56	0.19	0.11	1.36	6.35*	0.11	-0.23	0.01	-5.86	3.36	0.08	0.09	-2.44	-0.71	-0.36	-22.76
L_4XT_4	-1.26*	0.36	1.32	-0.81	-4.12	0.1	0.01	-0.02	8.21	-2.15	-0.08	-0.37	-0.3	-1.25	-0.49	-13.74
L_5XT_1	-0.89	-0.36	-0.04	-10.5	-7.57*	0.01	-0.19	0.23	-9.55	-0.51	-0.18**	0.19	0.6	-0.78	0.31	-5.59
L_5XT_2	-0.14	1.05	-0.15	6.32	4.40	0.15	0.04	-0.08	4.35	-3.44	0.01	0.16	-1.08	0.24	-1.62*	11.46
L_5XT_3	0.79	0.69	1.11	5.68	6.98*	-0.13	0.14	-0.13	-2.31	2.23	0.01	0.21	0.76	-0.15	0.53	-26.25
L_5XT_4	0.24	-1.38*	-0.92	-1.5	-3.80	-0.01	0.01	-0.01	7.52	1.71	0.16**	-0.58	-0.27	0.68	0.77	20.37
L_6XT_1	-0.03	-1.36*	-0.29	-1.94	2.35	-0.08	-0.09	0.01	-5.05	-5.82*	0.13*	0.06	1.17	0.32	0.71	4.96
L_6XT_2	0.12	0.55	0.34	6.89	2.58	0.05	0.39**	0.02	11.24	5.48*	0.02	-0.62	-0.69	0.43	-0.03	-13.24
L_6XT_3	-0.69	0.69	0.86	-4.5	-6.07*	-0.23	-0.14	-0.14	-1.05	-2.07	0.02	0.25	0.63	-0.34	-0.9	-10.02
L_6XT_4	0.6	0.11	-0.92	-0.44	1.13	0.26	-0.15	0.1	-5.12	2.4	-0.17**	0.29	-1.1	-0.42	0.3	18.3
L_7XT_1	-0.01	1.76**	0.76	0.18	-0.32	-0.08	0.24*	-0.24	1.03	1.92	0.15*	0.09	-2.32	-2.39*	-1.98*	-23.5
L_7XT_2	1.09*	0.67	0.66	6.76	8.1**	0.05	-0.14	0.06	-10.23	1.48	0.09	-0.26	-1.61	-2.06*	0.65	38.36
L_7XT_3	0.25	-3.17**	-2.81**	-5.63	-16.01**	0.01	-0.04	0.04	13.06*	-0.57	-0.08	0.53	0.99	-1.58	1.6*	30.77
L_7XT_4	-1.34**	0.73	1.39	-1.31	8.19**	0.01	-0.05	0.12	-3.85	-2.84	-0.15*	-0.35	2.94*	6.04**	-0.27	-45.62*

Table 4. continued

L_8XT_1	-0.48	0.82	1.26	-1.38	-3.57	-0.02	-0.34**	-0.13	-11.11	0.8	-0.01	-0.05	1.84	1.01	-0.99	20.3
L_8XT_2	-0.15	-0.76	-0.83	-0.29	-0.34	-0.13	0.14	0.14	3.46	1.61	-0.05	0.41	-1.29	0.12	1.33	-12.03
L_8XT_3	-0.41	-1.61*	-2.06**	-4.69	-5.26	0.07	0.1	-0.16	-10.3	-0.69	-0.12*	-0.69	-0.84	0.67	1.22	-6.31
L_8XT_4	1.05*	1.55*	1.64*	6.36	9.19**	0.07	0.09	0.15	17.95**	-1.71	0.19**	0.33	0.29	-1.81	-1.57*	-1.95
L_9XT_1	1.54**	-0.11	-2.04**	10.36	3.73	-0.02	0.05	-0.04	0.34	-1.32	0.02	0.28	-1.62	0.94	0.34	4.3
L_9XT_2	-0.67	-0.44	0.09	-10.79	-3.28	-0.01	-0.32**	0.1	-12.76*	-4.51	-0.03	0.41	0.67	-0.34	1.54*	30.65
L_9XT_3	-0.78	1.44*	1.61*	3.30	-2.95	-0.04	0.26*	-0.07	8.34	-0.57	-0.08	-0.53	1.38	0.98	-1.35	-25.85
L_9XT_4	-0.08	-0.88	0.32	-2.88	2.5	0.07	0.01	0.01	4.06	6.4**	0.09	-0.16	-0.43	-1.58	-0.54	-9.1
$L_{10}XT_1$	-0.35	0.07	-0.23	2.11	-1.07	0.04	0.02	0.12	-5.83	1.17	0.08	-0.13	-2.31	-0.88	0.01	-13.73
$L_{10}XT_2$	0.34	-0.26	-0.33	2.95	3.15	-0.06	-0.1	0.04	6.45	-5.01*	-0.1	0.01	1.81	0.97	1.82*	21.11
$L_{10}XT_3$	0.03	1.63*	1.68*	7.05	8.73**	0.24	-0.01	-0.13	3.7	2.17	0.09	-0.45	-0.47	0.54	-0.09	24.12
$L_{10}XT_4$	-0.02	-1.44*	-1.1	-12.13*	-10.8**	-0.22	0.09	-0.03	-4.31	1.65	-0.07	0.58	0.97	-0.62	-1.73*	-31.49
$L_{11}XT_1$	0.29	0.44	0.64	6.05	0.8	-0.05	-0.01	0.24	6.5	1.8	-0.06	-0.26	-1.77	-0.58	1.93**	12.05
$L_{11}XT_2$	-0.22	0.11	0.78	-13.35*	0.58	-0.03	-0.01	0.09	9.8	1.61	-0.07	0.21	-0.54	0.18	-1.59*	-21.44
$L_{11}XT_3$	-0.16	1.01	1.3	5.74	2.67	0.04	-0.29*	-0.21	-8.78	1.3	-0.04	0.75*	1.73	0.63	0.01	-0.72
$L_{11}XT_4$	0.1	-1.57*	-2.73**	1.55	-4.11	0.04	0.31*	-0.11	-7.52	-4.71*	0.17**	-0.7*	0.59	-0.23	-0.35	10.11
$L_{12}XT_1$	0.16	-2.3**	-2.17**	0.05	0.98	0.09	0.18	-0.21	14.49*	-0.19	0.06	0.19	1.5	0.21	-1.63*	-36.49
$L_{12}XT_2$	-0.45	1.11	1.22	-2.1	-3.78	-0.13	0.04	0.14	-11.19	2.61	0.03	-0.66	1.29	0.66	-1.69*	-11.63
$L_{12}XT_3$	0.03	1.01	0.49	3.24	3.78	0.07	-0.1	-0.12	3.83	0.3	0.01	-0.28	-0.32	-0.61	0.47	15.04
$L_{12}XT_4$	0.25	0.17	0.45	-1.19	-0.99	-0.04	-0.11	0.19	-7.13	-2.71	-0.11	0.75*	-2.47	-0.26	2.84**	33.08
$SE_{(sij)}$	0.5	0.64	0.72	5.69	2.97	0.16	0.12	0.17	6.12	2.35	0.06	0.35	1.25	1.02	0.73	21.51

Note: *and ** refers to significant at $p \leq 0.05$ and highly significant at $p \leq 0.01$respectively, L_{1-12} = Line, T_{1-4} = Tester, GY=grain yield(t h^{-1}), AD=days to 50% anthesis, SD=days to 50% silking, PH=plant height (cm),EH=,ear height (cm), PA=plant aspect (1-5 scale), EA=ear aspect (1-5 scale), RL=root lodging (%), SL= stem lodging (%), HC=husk cover (%), MD=days to maturity, NRPE=number of row ear^{-1} (number), NEPP= number of ear plant^{-1} (number), NKPR= number of kernel row^{-1}(number), EL=ear length (cm) and ED= ear diameter (cm), TKW=thousand kernel weight (g), of the genotypes used.

Table 5. Grain yield mean and SCA effects of highland maize lines evaluated across locations using different tester groups.

	Tester-1 (FS59)		Tester-2 (FS67)		Tester-3 (EC573)		Tester-4 (KITSYN II)	
Line	GY	SCA	GY	SCA	GY	SCA	GY	SCA
L_1	8.87	-0.01	6.5	-1.08*	8.33	0.9	8.5	0.19
L_2	8.25	0.25	6.7	0.01	6.72	0.19	6.95	-0.45
L_3	8.8	-0.71	8.94	0.71	7.34	-0.72	9.65	0.72
L_4	8.8	0.24	7.7	0.45	7.65	0.56	6.7	-1.26*
L_5	9.07	-0.89	8.53	-0.14	9.3	0.79	9.63	0.24
L_6	10.94	-0.03	9.77	0.12	8.8	-0.69	10.97	0.6
L_7	8.07	-0.01	7.87	1.09*	6.87	0.25	6.15	-1.34**
L_8	8.5	-0.48	7.53	-0.15	7.1	-0.41	9.45	1.05*
L_9	11.17	1.54**	7.65	-0.67	7.37	-0.78	8.95	-0.08
L_{10}	7.9	-0.35	7.3	0.34	6.83	0.03	7.65	-0.02
L_{11}	9.17	0.29	7.35	-0.22	7.25	-0.16	8.4	0.1
L_{12}	9.3	0.16	7.37	-0.45	7.7	0.03	8.8	0.25

Based on these classifications, the two tester groups could not be suggested as most contrasting candidate testers for assigning the lines into heterotic groups. On the other hand, absence of distinct categories or classifications for the remaining five lines could be attributed, in part, to the limited discriminating power of the testers which restricts the development of good testers for the highland breeding program. Therefore, it is imperative to further confirm these results through applications of molecular techniques in addition to multi-environment combining ability tests. Because no one method can absolutely be used to estimate heterotic grouping, so that, the integration of different methods can be the best solution since each method has its own limitations as suggested by Duvick *et al.* (2004).

4. Conclusions

Maize breeding relies on the extent of genetic variability available among genotypes. The lines and testers used in the present study were diverse, resulting in the expression of variation among genotypes for various traits. The genetic variability observed among the lines and testers used in this investigation for various traits implied great potential for the development of improved hybrids for the highland agro-ecologies.

From the combined analysis, GCA accounted for 73.20% while for SCA was 26.80% of the total sum of squares for grain yield showing the importance of additive genetic effect over the non-additive. Line like L_5 and L_6 showed highly significant and positive GCA effects for GY across locations. In addition, L_6 had revealed positive GCA effects for other yield related traits such as NEPP, NRPE, NKPR and ED. Consequently, these lines can selectively be used to make progress in yield improvement in the highland maize breeding program.

Crosses such as L_7xT_2, L_8xT_4 and L_9xT_1 manifested significant and positive SCA effects for grain yield. Besides, the significant and desirable SCA effects by some of the crosses for increasing grain yield, number of ear per plant, number of rows per ear, number of kernels per row, ear diameter, ear length and thousand kernel weight, reduced days to maturity and plant height indicated the prevalence of dominance gene effects that could be exploited through hybrid breeding method.

The two major heterotic groups (Ecuador and Kitale) used in the highland maize breeding program were able to categorized seven lines (L_2, L_3, L_4, L_6, L_8, L_9, and L_{12}) into the already known heterotic groups. Particularly, L_3 and L_6 were classified into their respective heterotic groups in a similar pattern showing that the restricted power of the testers to discriminate. Consequently, the applications of molecular techniques in addition to multi-environment tests are important to confirm further highland maize breeding program.

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