Combining ability analysis and heterotic grouping for grain yield among maize inbred lines selected for the mid-altitude and highland zones of Rwanda

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Abstract

Development and identification of maize parental lines that belong to different heterotic groups is a fundamental requirement for any hybrid production programme. The objective of this study was, therefore, to determine combining ability, heterosis and heterotic patterns for grain yield among ten selected local (unknown heterotic groups) and exotic (known heterotic groups) maize inbred lines and their progenies under mid-altitude and highland conditions of Rwanda. Forty-five single crosses from a 10 x 10 half-diallel mating design plus three checks were tested in a 6 x 8 alpha-lattice design across twelve environments. General combining ability (GCA) and specific combining ability (SCA) effects were both highly significant ($P<0.001$-0.01), suggesting presence of both additive and non-additive gene effects. The percentage mid-parent heterosis (MPH) for grain yield ranged from 36.4to 267.7% with a mean of 164%, while high-parent heterosis (HPH) varied from 33.2% to 236% with a mean of 130.4%. Based on MPH, the seven local maize inbred lines were discriminated and assigned into four different heterotic groups (S4, S7, S4/S6 and S6/S7). The highest heterotic patterns were realized between tester S4 and tester S6 (hybrid S4/S6) and between group S7 and tester S4 (hybrid S2/S4). Identified patterns would be potentially useful for maize hybrid production in Rwanda. Similarly, the resulting hybrids could be recommended in sub-Saharan African regions with similar ecosystems. Significance of both additive and non-additive genetic effects in the current germplasm suggests that the Rwandan breeding programme could use both hybridization and recurrent selection methods.

Abbreviations

CIMMYT: Centro Internacional del Mejoramiento de Maiz y Trigo
FW: Field weight
GCA:General Combining Ability
m asl: metres above sea level
HPH: High-parent Heterosis
MPH: Mid-parent Heterosis
SAS: Statistical Analysis Software
SCA: Specific Combining Ability
SH: Standard Heterosis

Introduction

Maize (*Zea mays* L.) is the principal crop worldwide. It is a significant component of food security, providing food, feed and bioenergy (FAO,2012; Niyibituronsa *et al*, 2020; Oliveira *et al*, 2020).It is also the most important staple food crop on which the livelihoods of more than 1.2 billion people in sub-Saharan Africa depend on (Krivanek *et al*, 2007; FAO,2012; Niyibituronsa *et al*, 2020; Makore *et al*, 2021). Likewise, maize is important to Rwandan families who consume it in various forms which include roasted or boiled green cobs, boiled dry grain or mixed with legumes such as beans, or as ugali and uji (thick and thin porridge, respectively) prepared from dry maize flour, or brewed into local beer. As a vital component of food security across the world, maize improvement for yield potential is the focus of many genomics and breeding programmes (Hallauer and Miranda,1988; Katragadda *et al*, 2020; Makore *et al*, 2021).

In developing countries, maize yields are much lower than in developed countries. For example, average maize yields in Africa are less than 1.7 tons/ha, compa-

Table 1 - Maize germplasm selected and involved in the study

No	Name	Pedigree	Heterotic Group	Origin
S1	R10164	RM101 5-6 (64)	-	Rwanda
S2	RM8147	RMO81 9-2 (47)	-	Rwanda
S3	ACRO29	ACROSS8762 4-5 (29)II	-	Rwanda
S4	ET4	SRSYN95[KIT//N3/TUX]F1-##(GLS=2)-22-2-2-2-2-#-#-#-#-#-#	Kitale	CIMMYT
S5	ECA13	ECA16-STR 4-7 (13)	-	Rwanda
S6	ET8	[ECU/SNSYN[SC/ETO]]c1F1-##(GLS=2.5)-31-1-1-1-1-1-#-#-#-#-#	Ecuador	CIMMYT
S7	ET9	[POOL9Ac7-SR(BC2)]FS89-1-2-4-2-1-2-2-###-#-#-#	Pool 9A	CIMMYT
S8	TQX7	[TUXSEQ]C1 5-8 (7)I	-	Rwanda
S9	MZ5	ZM607-80-4-1-B*4(5)	-	Rwanda
S10	POL6	POOL32-6-1-1-B-B(6)	-	Rwanda

red to an average of 4.9 tons/ha globally (FARA, 2009; Shiferaw *et al*, 2011). In Rwanda, low maize yields (1.4 tons/ha) have also been reported (NIS, 2014; Rwasimitana *et al*, 2021). However, with improved inputs and agronomic practices as well as use of genetically improved varieties, it is still possible to obtain high yields in developing countries. Nonetheless, such varieties are scarcely available (Sallah *et al*, 2007; Fato *et al*, 2012; Rwasimitana *et al*, 2021) in many developing countries including Rwanda. Thus development of maize hybrids in developing countries is a valuable undertaking, but implies selection of superior parents and precise identification of heterotic patterns (Hallauer and Miranda,1988; Makore *et al*, 2021).

The phase of developing and identifying parents that form superior heterotic patterns, though fundamental to hybrid breeding, is the most costly and laborious in a maize hybrid programme. This is because *per se* performance of the parents does not predict the performance of maize hybrids for grain yield (Hallauer and Miranda, 1988; Dao *et al*, 2014; Katragadda *et al*, 2020). Heterosis will thus be an important predictor of the hybrid value in a given maize hybrid breeding programme.

Heterotic patterns are important as they guide breeders to decide on the germplasm to be used in hybrid production over a long period thus simplifying germplasm management and organization (Reif *et al*, 2005; Nepir *et al*, 2015; Oppong *et al*, 2019). Similarly, evaluation of combining ability is essential in the development of new recombinants or hybrid varieties to exploit heterosis (Fato *et al*, 2012; Nyaligwa *et al*, 2015; Oppong *et al*, 2019). Identification of inbred lines with good combining ability is a prerequisite for the success of any breeding programme aimed at hybrid development (Hallauer and Miranda,1988; Dao *et al*, 2014; Nyaligwa *et al*, 2015; Katragadda *et al*, 2020).

In Rwanda, scarcity of maize seed of improved varieties is a major challenge to small scale farmers and to the government, this has resulted in spending a lot of money in imports of hybrid seeds from outside of Rwanda.There is a need to continuously identify new sources of high performing maize hybrids using the local available breeding genetic stocks and introduced germplasm to enhance maize productivity. Both, the heterotic orientation and combining ability effects of the newly developed and introduced germplasm has not been studied as yet in Rwanda. The objectives of this study were then to determine combining ability effects and heterotic patterns for grain yield among ten maize inbred lines comprising seven locally developed and three introduced inbred lines and their progenies across the mid-altitude and highland zones of Rwanda.

Materials and methods

Germplasm

Ten inbred lines (Table 1) were crossed in a half-diallel mating design to produce 45 F1 progenies. The inbred lines comprised of seven lines developed from seven populations adapted to the mid-altitude of Rwanda as indicated in the pedigrees reported in Table 1. The other three lines (S4,S6 and S7) were highland inbred lines from CIMMYT-Ethiopia with different genetic backgrounds and these were also used as testers to guide in the discrimination of the seven maize local inbred lines into different heterotic groups.These three highland inbred lines were selected due to their adaptability to the Rwandan environmental conditions.

Experimental design and trial management

The study was carried out in four research sites representative of major Rwandan maize growing agro-eco-

Table 2 - A 10 x 10 maize diallel cross analysis for grain yield and associated traits over 12 environments in Rwanda

Source	DF	Yld‡	EPP	AD	SD	PH	EH	TLB	MSV
Environments (E)	11	443.350***	1.254***	23030.5***	24666.1***	128362.841***	41826.970***	80.959***	126.27***
E.REP	12	9.954	0.044	34.0	37.1	1054.794	462.366	4.0481	0.6509
Genotypes (G)	44	33.375***	0.104***	166.9***	180.0***	6273.414***	2328.225***	4.6875***	5.4005***
GXE	484	3.879***	0.024***	10.9***	11.2***	294.821***	132.512***	0.8768***	1.5612***
GCA	9	107.121***	0.325***	750.6***	819.1***	25142.361***	9590.9561***	16.666	18.462**
SCA	35	14.411***	0.047*	16.8ns	15.6***	1421.399ns	460.6661 ns	1.6074***	2.042***
GCA x E	99	8.196**	0.037***	17.5***	17.5***	577.400***	229.440***	1.7352*	4.4113**
SCA x E	385	2.769***	0.021***	9.2***	9.6***	222.160*	107.590 ns	0.6561***	0.8283***
Error	528	1.615	0.014	6.0	6.6	189.818	98.839	0.5481	0.4786
Mean		7.376	1.052	76.6	77.8	200.069	97.305	3.3611	1.7676
CV (%)		17.23	11.06	3.20	3.30	6.89	10.22	22.03	39.148

*, **, *** indicates significance at 0.05, 0.01, and 0.001 probability levels, respectively

‡ AD, anthesis days; AD, silking days; EPP, ears per plant; PH & EH, plant & ear height; MSV, maize streak virus; TLB, turcicum leaf blight; yld, grain yield.

logies across three seasons 2015A (Season A =from September to February), 2015B (Season B=from March to July) and 2016A(Season A =from September to February), resulting in 12 environments. Bugarama site (29°00 E, 2°28′ S, 900-1200 metres above sea level (m asl)) is located in the semi-arid mid-altitude zone with rainfall ranging from 450-900 mm during the growing seasons. Nyagatare (30°20′ E, 1°20′ S, 1450 m asl) and Rubona(29°46′ E, 2°29′S, 1650 m asl) are located in the moist mid-altitude zone with rainfall ranging from 350-900 mm), while Rwerere (29°52″ E, 1°29′ S, >1700 m asl) is located in the highlands with rainfall above 800 mm.

The 45 F1 progenies and three checks were laid out in a 6 x 8 alpha-lattice design with two replications, while the parental lines were laid out in a randomized complete block design with two replications. Plot sizes for the progenies and parental lines were one row, 4.0 - 5.0 m long, with 0.75 m inter-row spacing and 0.25 m intra-row spacing. All agronomic practices like fertilisation and weeding were followed according to recommendations for maize cropping at each site. In all the 12 environments, maize genotypes of similar vigour were used as borders.

Grain yield (t/ha), was calculated as grain mass per plot adjusted to 12.5 % moisture content. Field weight (FW) (weight of the harvested ears) per plot was multiplied by 0.80 shelling percentage to obtain grain yield (t/ha), adjusted to 12.5% grain moisture. Grain yield was computed based on the formula: Grain yield (t/ha) = field weight (kg)/[(plot size) x (100-grain moisture content) / (100-12.5) x10 x 0.8].

Statistical analysis

Data were analyzed using PROC GLM procedures in SAS statistical package version 9.3 (SAS Institute, 2002) complemented by Genstat 17th edition computer software (Payne *et al*, 2014). Bartlet homogeneity of variances were performed prior to combined analysis of variance. A mixed model was used for data analysis where genotypes were treated as fixed effects, while environments (both spatial and temporal environments) were considered as random effects.

General and specific combining ability (GCA and SCA) effects were estimated using Griffing's model 1 (fixed genotype effects), method 4 (crosses only) (Griffing, 1956). The following statistical model (Griffing, 1956; Hallauer *et al*, 2010) for the diallel analysis across environments was applied;

$$Y_{ijkl} = \mu + E_e + K(re)_K + g_i + g_j + s_{ij} + gE_{ie} + sE_{eij} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the measurement observed for the ij^{th} cross in the l^{th} environment, kth replication; μ is the grand mean; E_e is environment effect; $k(re)_k$ is the estimate of the k^{th} incomplete block within replications nested in the environment; $g_i + g_j$ are GCA effects; sij is the SCA effect; gE_{ie} is the interaction effect between GCA and the environment; sE_{eij} is the interaction effect between SCA and the environment; while ε_{ijkl} is the error term associated with the ijth cross evaluated in the k^{th} replication and E_e environment. The restrictions $\Sigma gi=0$ and $\Sigma sij=0$ were imposed on the combining ability effects. The significance of GCA and SCA effects was verified using a t-test. As the combining ability mean squares were calculated based on cross means of

Table 3 - Estimates of GCA effects and means for grain yield and other agronomic traits of ten maize parental inbred lines across 12 environments

Parent	†Yld		EPP		AD		SD		PH		EH		TLB		MSV	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
S1	3.42	0.270	1.06	0.038*	80	-1.446*	81	-1.674	126.11	-9.061**	56.88	-3.405	3.2	0.073	1.5	0.053
S2	1.67	-0.936***	0.86	-0.066+	75	-3.066**	76	-3.346**	89.87	-20.066***	44.20	-9.338***	4.1	0.656***	1.3	0.001
S3	3.10	0.026	1.02	0.029*	84	0.752	86	0.883	131.55	0.317	58.79	-1.917	3.3	-0.104	1.9	0.053
S4	2.68	1.358***	1.03	0.068+	87	1.700	86	1.524	149.03	14.958***	82.84	15.992***	3.6	0.021	1.8	0.261*
S5	3.93	1.184***	1.02	0.011*	85	2.075+	87	1.982+	167.46	18.222***	70.84	4.762**	3.5	-0.234**	3.0	0.480***
S6	2.23	-0.356	1.02	-0.041*	86	1.169	88	1.347	132.60	-2.037	63.17	1.233	3.3	-0.260**	1.1	-0.494
S7	2.34	-0.504**	1.03	-0.037*	87	2.075+	89	2.451*	137.10	-0.785	66.68	0.723	3.3	-0.385***	1.1	-0.551***
S8	1.78	-0.564**	0.94	0.017*	86	0.799*	86	0.680	143.26	2.950	55.25	-4.612**	4.9	0.125	1.5	0.053***
S9	3.48	-0.161	0.96	0.002**	81	-1.196**	83	-1.007	163.53	5.311+	77.71	2.724+	3.6	-0.089	1.5	0.058
S10	3.94	-0.318	1.03	-0.023*	77	-2.863**	78	-2.841*	133.05	-9.809***	65.32	-6.161***	3.7	0.198*	2.0	0.084

+,*, **, and ***, indicate significance of GCA effects at 0.1, 0.05, 0.01 and 0.001 probability, respectively

† AD, anthesis days; SD, silking days; EPP, ears per plant; Yld, grain yield (t/ha); PH, plant height; EH, ear height; TLB, Turcicum leaf blight; MSV, maize streak virus

each genotype from each location, error mean square was used for *GCA* and *SCA* significance. The standard errors of the *GCA* and *SCA* effects were estimated as the square root of the *GCA* and *SCA* variances (Griffing, 1956). To determine gene action model, the relative importance of additive and non-additive effects (*GCA* and *SCA*, respectively) were estimated according to *GCA* and *SCA* mean squares ratios (Baker, 1978). Ratio close to the theoretical maximum of one (unity) indicated the importance of additive genetic effects while ratio much lower than unity implied the importance of dominance genetic effects for a given trait. The formula used was as follows:

$$\frac{2MS_{GCA}}{(2MS_{GCA}+MS_{SCA})}$$

where MS_{GCA} and MS_{SCA} were the mean squares for *GCA* and *SCA* effects, respectively.

Mid-parent heterosis (*MPH*) as the performance of the hybrid compared to the average parental performance was calculated as follows (Hallauer *et al*. 2010):

$$MPH = \frac{(F_1 - MP)}{MP} \times 100$$

Further, high-parent heterosis (HPH) as the performance of the hybrid compared to its best parent performance was calculated as follows:

$$HPH = \frac{(F_1 - HP)}{HP} \times 100$$

where F_1 is the mean performance of the cross and *MP* is mean of the two inbred parents and *HP* is the mean value of the highest performing parent. Standard heterosis *(SH)* in addition to mid-parent heterosis *(MPH)* was computed as:

$$SH = ((F1 - MT)/ MT) *100$$

where *MT* = Mean of the testers, best hybrid or the trial mean, *F1* = *F1* hybrid mean performance. Heterotic groups were defined using *MPH*.

Results

The study was effective in discriminating maize hybrids for grain yield. The analysis of variance for individual locations displayed significant differences (P<0.001-0.01) among crosses for grain yield (data not displayed) in all the twelve testing environments except for Rwerere 15B site. Within sites across the three seasons, highly significant differences were observed among crosses in all the sites (data not displayed) and effects of season, season x crosses were also significant. Similarly, when all environments were combined (Table 2),a highly significant difference was observed among the genotypes for. The trend was similar for GCA and SCA effects. In addition, the environment variance, GCA x E, SCA x E, genotype x environment interaction were significant. However, the magnitude of these interactions were lower compared to the main effects. The proportions of GCA effects for were larger than SCA effects for the combined environments.

Table 4 - Means, mid-parent and high parent heterosis for grain yield over 12 environments in Rwanda

		†YLD					YLD		
		Heterosis					Heterosis		
No	Cross	Mean	MPH(%)	HPH(%)	No	Cross	Mean	MPH(%)	HPH(%)
1	S1/S2	5.90	131.5	72.4	24	S3/S10	7.65	117.4	123.7
2	S1/S3	7.81	139.6	128.4	25	S4/S5	9.70	193.5	147.0
3	S1/S4	9.46	209.7	176.5	26	S4/S6	9.03	267.7	236.8
4	S1/S5	8.71	137.0	121.8	27	S4/S7	8.26	229.0	208.0
5	S1/S6	7.57	167.8	121.4	28	S4/S8	7.41	231.9	176.3
6	S1/S7	7.68	166.4	124.5	29	S4/S9	8.59	178.7	146.6
7	S1/S8	7.76	198.1	127.0	30	S4/S10	8.40	153.6	145.6
8	S1/S9	7.29	110.9	109.1	31	S5/S6	8.38	172.0	113.3
9	S1/S10	6.37	73.1	86.4	32	S5/S7	8.68	177.0	121.0
10	S2/S3	6.25	161.9	101.6	33	S5/S8	7.93	177.9	102.0
11	S2/S4	7.72	254.8	188.0	34	S5/S9	8.33	124.7	112.1
12	S2/S5	8.45	201.7	115.1	35	S5/S10	7.86	99.6	129.7
13	S2/S6	6.37	226.5	185.5	36	S6/S7	3.12	36.4	33.2
14	S2/S7	6.16	207.0	163.2	37	S6/S8	6.76	236.7	202.9
15	S2/S8	5.53	220.3	210.3	38	S6/S9	7.61	166.2	118.3
16	S2/S9	6.59	155.5	89.0	39	S6/S10	7.48	142.4	118.8
17	S2/S10	5.94	111.6	73.7	40	S7/S8	7.19	249.0	207.5
18	S3/S4	8.67	200.2	180.0	41	S7/S9	6.94	138.4	99.2
19	S3/S5	7.83	123.0	99.4	42	S7/S10	7.09	125.7	107.2
20	S3/S6	7.22	170.8	133.0	43	S8/S9	6.03	129.1	73.2
21	S3/S7	7.24	166.5	133.9	44	S8/S10	6.59	130.3	92.8
22	S3/S8	6.66	173.0	115.1	45	S9/S10	6.46	73.9	88.8
23	S3/S9	7.26	120.5	108.2					

Combining ability effects

Significant differences among the inbred lines for GCA effects for grain yield were observed. The GCA effects for the combined environments ranged from -0.936 t/ha to 1.184 t/ha (Table 3). Sixty percent of the inbred lines displayed negative GCA effects, with inbred line S5 showing the highest positive GCA effects (1.184),while inbred line S2 had the lowest GCA effects (-0.936). Similarly, 50% of the inbred lines exhibited significant variations ($P < 0.01$-0.0001) for GCA effects with inbred lines S4, S5, S2 displaying the highest or lowest GCA effects. Furthermore, in relation to other traits studied, GCA effects among the inbred lines showed different trends depending on the inbred line and considered trait.

Hybrids displayed significant differences for SCA effects and estimates of SCA of the 45 hybrids averaged across the 12 testing environments (data not displayed). The SCA effects for grain yield ranged from -3.399 (hybrid S6/S7) to 0.883 (hybrid S7/S8). Hybrids S7/S8 (0.883), S2/S5 (0.821), S6/S10 (0.781) and S6/S9 (0.749) displayed the highest positive SCA effects. Conversely, hybrids S6/S7 (-3.399), S1/S10 (-0.954), S1/S2 (-0.813) and S4/S8 (-0.761) displayed the lowest SCA effects for grain yield. Overall, around 56% of the hybrids had positive SCA effects for grain yield, but only a few of them showed significant SCA effects. When averaged across seasons within sites (data not shown), SCA effects for grain yield did not exhibit any clear consistent pattern. However, most of the SCA effects were not significant in all sites. Hybrid S2/S5 displayed the highest positive (1.933) but not significant SCA effects at Rubona station, while hybrid S7/S8 displayed the consistent highest but not significant SCA effects at three sites (Nyagatare, Rubona and Rwerere stations).

Heterosis and Heterotic alignment

Significant variation for levels of heterosis between the lines was observed in the study. Mean performance of

Table 5 - Top 15 maize hybrid yield across 12 environments with standard heterosis higher than 6% of trial mean

		Relative yield to				
No	**Cross**	**Trial mean (%)**	**Best check(%)†**	**Tester 1(S4) mean (%)**	**Tester 2(S6) mean (%)**	**Tester 3(S7) mean (%)**
1	S4/S5	31.4	-39.2	119.9	164.3	152.1
2	S1/S4	28.1	-19.5	191.3	250.1	234.0
3	S4/S6	22.4	-2.6	252.6	323.7	304.2
4	S1/S5	18.0	-10.2	224.8	290.4	272.4
5	S5/S7	17.6	-22.0	182.4	239.3	223.7
6	S3/S4	17.5	-20.9	186.2	244.0	228.2
7	S4/S9	16.5	-20.0	189.4	247.8	231.8
8	S2/S5	14.4	-24.9	171.7	226.5	211.5
9	S4/S10	13.8	-34.3	137.7	185.6	172.5
10	S5/S6	13.5	-35.6	132.9	179.9	167.0
11	S5/S9	12.9	-20.4	188.0	246.1	230.2
12	S4/S7	11.9	-13.0	214.9	278.5	261.1
13	S5/S8	7.5	-34.3	137.6	185.5	172.4
14	S5/S10	6.4	-36.6	129.5	175.9	163.2
15	S3/S5	6.1	-43.0	106.3	147.9	136.5

† The highest performing check.

the hybrids and heterosis for grain yield across the four testing locations in all the three seasons is summarized in Table 4. The percentage mid-parent heterosis (MPH) for grain yield ranged from 36.4% (S6/S7) to 267.7% (S4/S6) with a mean of 164%, while high-parent heterosis (HPH) varied from33.2% (S6/S8) to 236% (S4/S8) with a mean of 130.4%.In general, 91% of the crosses exhibited MPH≥100%, whereas 78% of the crosses displayed HPH≥100%. In the top 10 crosses showing high MPH, around 50% of them comprised parent 4 (S4) and 5 (S5), the same parents were involved in the highest yielding cross S4/S5 (9.70 t/ha).Differences for standard heterosis (SH) were also observed, SH was calculated based on relative trial mean (%), relative best check hybrid mean (%), and relative mean of the testers (%) (Table 5). Most of the crosses displayed positive SH except for heterosis relative to the highest performing check (best check) where all the crosses exhibited negative SH.

New heterotic patterns among inbred lines and their alignment with testers were observed. Heterotic patterns are shown in Table 5. Among the top 10 hybrids, 60% had S4 as progenitor, 30% were between lines from S4 with the others from the 2 groups (S6 and S7). In addition, the cross S4/S6 was ranked third among the top 10 hybrids, while the cross S4/S7 appeared among the top 12. Since most of the SCA effects were not significant for grain yield, heterotic alignment was performed based on mid-parent heterosis (Table 6). Three varieties (Table 1) with known heterotic groups were considered as testers (S4, S6 and S7) and were included in the diallel study to determine heterotic divergence and guide in the discrimination of the seven maize local inbred lines into different heterotic groups.All the lines displayed positive heterosis with all the three testers; however, most of the inbreds were inclined towards tester S7 or displayed similar levels of heterosis with both S6 and S7 testers (Table 6), while the remainder aligned with either S4 or S4/S6. The highest (267.69%) mid-parent heterosis was realized in the cross: S5/S6. On the contrary, the lowest (125.67%) mid-parent heterosis was observed in the cross: S7/S10.

Discussion

Combining ability effects

Significant combining ability effects and their interaction with environments have implications for the breeding strategy. Analyzed across seasons in four environments, GCA effects for grain yield were significant and their mean squares were higher than SCA mean squares, suggesting that additive gene action was more important than non-additive in controlling grain yield. These findings are consistent with previous studies (Musila *et al*, 2010; Sibiya *et al*, 2011, 2012, 2013; Rovaris *et al*, 2014; Nepir *et al*, 2015; Oppong *et al*, 2019). This implies that selection processes such as recurrent selection utilizing GCA could be applied in the base populations from which the inbred lines were derived to obtain lines with traits in consideration. Ho-

Table 6 - Heterotic grouping of the inbred lines using mid-parent heterosis (%)

		Heterosis with Testers (%)			
Line	**Pedigree**	**S4**	**S6**	**S7**	**Alignment with testers†**
S1	R10164	209.70	167.78	166.38	S6/ S7
S2	RM8147	254.84	226.48	206.97	S7
S3	ACRO29	200.17	170.83	166.53	S6/ S7
S5	ECA13	193.53	267.69	229.03	S4
S8	TQX7	231.90	236.71	248.99	S4/S6
S9	MZ5	178.74	166.17	138.41	S7
S10	POL6	153.58	142.44	125.67	S7

† S4, S6, and S7 heterotic grouping.

wever, SCA effects were also significant, implying that non-additive effects also played a role in controlling this trait and suggests the breeding programme in Rwanda could also benefit from hybridization. The significance of GCA x E and SCA x E indicated that effects associated with for genotypes varied with the environment in the current study. Additionally, the highly significant differences observed among genotypes implies the presence of large differences among the performance of the genotypes under this study, while the higher magnitude of mean squares for G and GCA than G x E and GCA x E justifies that environment effects had less influence on the genotypes and additive gene action. A similar trend was reported by other researchers for various crops (Musila *et al*, 2010; Sibiya *et al*, 2011; Rovaris *et al*, 2014; Wegary *et al*, 2014; Nepir *et al*,2015; Mafouasson *et al*, 2017). In the current study, lines S4 and S5 displayed significant, consistent positive GCA effects for yield which are desirable, implying the existence of positive attribute as good combiner parents in contributing to increased grain yield in their crosses. Hallauer and Miranda (1988), indicated that maize inbred lines which have superior GCA effects should be retained for further use in a breeding programme. This, therefore confirms, suitability of S4 and S5 inbred lines for inclusion in the Rwanda maize breeding programme and can be used directly for hybrid production. This is in agreement with other studies where positive and significant GCA effects were also reported for lines useful for use in hybrid production (Sibiya *et al*, 2011, 2012; Fato *et al*, 2012; Rovaris *et al*, 2014; Nepir *et al*, 2015; Annor *et al*, 2019; Elmyhun *et al*; 2020).

The SCA effects across environments for grain yield were positive and significant for crosses S7/S8 and S2/S5. However, lines S2, S7 and S8 had negative GCA effects for the same trait. This indicated that high yielding hybrids could be gained not only by relying on crossing good x good GCA lines but also by crossing bad x good GCA lines. It was earlier stated (Nepir *et al*, 2015) that high SCA values indicate the significance of non-additive gene action and thus it is manifested between crosses of two genetically divergent parental lines, mainly due to the preponderance of dominance gene effects. Significantly variable SCA effects observed under the current study among the crosses implied that a breeding strategy based on SCA effects like hybridization could be used to select good hybrids.

Heterosis and heterotic groups

Mid-parent heterosis analysis of grain yield in the present study revealed that all hybrids were superior to their parents, suggesting the potential of these inbred lines in hybrid development to exploit hybrid vigor and suggests the positive role of non-additive gene effects. A similar trend was also realized for HPH, highlighting that the newly bred hybrids can perform better than their high parent in grain yield which could be recommended for hybrid production. Consequently, hybrids selected based on both MPH and HPH can be selected for release and/or for further breeding in the maize programme in Rwanda. The level of mean based on mid parent (164%) and high parent (130.4%) heterosis shown for grain yield in the current study was however lower than that previously reported by Nepir *et al.* (2015). This difference in levels of heterosis might have resulted in dissimilarities of germplasm involved in the two studies. Furthermore, standard heterosis revealed that most of the crosses displayed positive SH except heterosis relative to the best check where all the crosses exhibited negative SH. This implies that selection should be done based on other advantages when comparing the hybrids of the current study and the checks. In addition to this, not only is grain yield a polygenic trait, it depends also on a large number of other related traits and environments. Therefore, selection along with its component characters and specific environments could be more effective and reliable (Fasahat *et al*, 2016).

Heterotic groups A and B at CIMMYT have been aligned similar to some of the well-known heterotic patterns across the globe. It was cited by previous researchers (CIMMYT, 2000; Pswarayi and Vivek, 2008) that group A is expected to exhibit heterosis similar to Kitale, Tuxpeño, N3, and Reid, while group B would exhibit heterosis similar to Ecuador, ETO, SC, Blanco, and Lancaster. Similarly, for the seven local lines and three basic testers of different background, it was possible to demonstrate some heterotic patterns. The seven lines were assigned to four major heterotic groups based on mid-parent heterosis magnitude when crossed to the testers. Hence, a cross between a line and a tester revealing low mid-parent heterosis level had the line assigned to the same heterotic group as the tester. Although, theoretically no heterotic patterns are expected from crosses of inbred lines from the same group some heterotic patterns have been realized within groups (Fato *et al*, 2012; Nepir *et al*, 2015; Richard *et al*,2016). It was earlier reported that sufficient MPH could exist between parents of high GCA within the same heterotic groups. This is because in general, tropical maize germplasm is known to have an intragroup diversity that is sufficient to exploit heterosis contributed by additive genetic effects (Pswarayi and Vivek, 2008). On the other hand, lines exhibiting high magnitude of mid-parent heterosis were aligned to different heterotic groups, implying that good heterotic patterns are expected from crosses of lines identified in different groups (Pswarayi and Vivek, 2008;Oppong *et al*,2019; Elmyhun *et al*, 2020) as realized in cross S5/S6. As maize programme in Rwanda is geared towards development of three-way hybrids, this could be a better opportunity where hybrids could be developed using the two heterotic groups (e.g. A x A' crossed to a line from the group B). Single crosses with higher yield can be developed from higher-yielding as well as good combining inbred lines that belongs to the same heterotic group by largely exploiting additive variance, while retaining the dominance effects to be fully exploited in the final cross of a three-way cross hybrids (Fato *et al*, 2012; Nepir *et al*, 2015;Elmyhun *et al*, 2020).

Nevertheless, as heterotic patterns are specific to the group of parents being tested, changes might be expected in the heterotic behavior observed in the current study. It was earlier stated (Rawlings and Thompson, 1962) that lines belonging to the same heterotic group may not have absolutely identical heterotic patterns because of small differences in the alleles they may be carrying. Similarly, in this study, lines that were derived from the same genetic background were not necessarily assigned to the same heterotic group. On the other hand, lines derived from different genetic background may have absolutely identical heterotic patterns (Dao *et al*, 2014). This indicates that genetic diversity of constituent parents of a hybrid is not necessarily correlated with hybrid performance.

Conclusions

The results of this study revealed the presence of high variability among hybrids for grain yield. Therefore, it would be possible to select maize hybrids that are suitable for the mid and high altitudes of Rwanda. Maize inbred lines S4 and S5 displayed consistently positive GCA effects in all environments with line S4 qualifying as the best combiner. Among the top10 crosses showing high heterosis, 50% of them comprised parent 4 (S4), the same parent was also involved in the highest yielding cross S4/S5 (9.70 t/ha). This hybrid and others would be used directly as single cross hybrids or as potential single-cross testers for development of three-way hybrids in the maize programme for the mid and highland ecologies of Rwanda. Three maize inbred lines (S4, S6 and S7) that were considered as testers discriminated the seven local lines into three heterotic groups that could form the basis of the maize hybrid programme in Rwanda

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