Genetic evaluation for understanding combining ability effects and Heterotic grouping in Maize (*Zea mays* L.)

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Abstract

Combining ability of the genotypes/lines is a major factor in planning the breeding programme and for development of Heterotic hybrids. In the present study, twenty maize inbred lines were crossed to three diverse testers CM-111, GPM-549 and GPM-581 and the resultant F1 hybrids were evaluated in an alpha lattice design. General combining ability of lines which is representation of additive gene action was found to be significant for all the quantitative traits. Specific combining ability which is indication of non-additive gene action was found to be significant for the traits number of kernel rows per cob, number of kernels per row, cob girth, cob length, test weight and grain yield. Lines VL-058725, VL-1018527 and VL-108723 produced heterotic hybrids in cross combination with any of the tester due to their high GCA effects. Whereas, the lines VL-0536, SNL-1574 and VL-109086 interacted positively with their testers thus producing heterotic hybrids with high positive SCA. GGE biplot analysis was helpful in visualizing the combining ability effects and identify heterotic pattern among the inbred lines. Heterotic grouping based on SCA and mean grain yield was able to classify thirteen of the twenty inbred lines into two distinct heterotic groups i.e., Heterotic group A and B consisting of six and seven lines respectively. Heterotic group A consisted of lines with high GCA whereas, heterotic group B with low GCA lines. SCA effect showed significant positive correlation with all the quantitative traits and played a prominent role in determining the performance of hybrids, thus indicating the importance of non-additive gene action in developing heterotic hybrids.

Abbreviations

SCA_GY – Heterotic grouping based on SCA effects and grain yield	FGCA – female general combining ability effect
LGCA – mean sum of squares due to lines	SGCA – sum of parental general combining effect
TGCA – mean sum of squares due to testers	SCA – specific combining ability
L x TGCA – mean sum of squares due to lines x testers	GCA – general combining ability
MGCA – male general combining ability effect	SoCA – sum of combining ability effects

Introduction

Maize (*Zea mays* L.) is an important cereal crop after rice and wheat in India. Cultivated from the foothills of Himalayas to Kanyakumari in Tamil Nadu and from arid regions of Rajasthan to Garo hills of Assam. In India, maize is a major source of raw material to poultry and dairy industry as a feed apart from other industrial uses such as starch and glucose. In India maize is being cultivated on an area of 8.26 million-hectare area with a production of 18.73 million tons and productivity of 2965 kg/ hectare (AICRP on Maize, 2019). Among the major maize growing states Karnataka is one of the leading states in terms of area (1.3 million hectare) and

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productivity of 2900 kg/ hectare (AICRP on Maize, 2019).

In India, there is a tremendous scope for improvement of maize programme as there exists a huge gap between maize productivity (27.8 q/ha) and global maize productivity (57.50 q/ha). There are various means by which productivity can be addressed and among them exploitation of heterosis through development of high yielding heterotic hybrids, improved crop management practices, use of quality seeds and inputs and overcoming disease/pest incidence. Among these, development of high yielding heterotic hybrids is an important means by which majority of the productivity issues can be addressed. Heterosis is the increased vigour

Entry	Code	TLB Disease Score (1-9)	Pedigree			
Lines						
SNL-153296	L1	3	CML 165-B			
VL-058725	L2	4	CML 227-B*9-B-B			
VL-1012768	L3	2	(CTS013058/(AMATLC0HS167-1-1-12F/R) R-B*5/Nei402011)-B*11-B-B			
VL-1018527	L4	3	CML 317-2-B*6-B			
VL-102	L5	6	([Pop445C1F2-1-1 x Pop447c1f2] x [Pop446c1f2-358-2 x Pop445C1F2])-#-38-2-B*9-B			
VL-1033	L6	6	CA14515-B-2-B*6-B			
VL-108723	L7	4	CA00310/AMATLC0HS71-1-1-2-1-1-1-B*17-B-B			
VL-109086	L8	5	G18SeqC5F76-2-1-2-1-2-BB-B3-B-B1-BB-B			
VL-1249	L9	4	WLS-F299-2-1-2-B-2-B*6-B-B			
SNL-153280	L10	3	(DT/LN/EM-46-3-1 x CML 311-2-1-3)-B-F232-1-1-1-B*4-B			
VL-106	L11	7	(CA14502/CA14509)-F2-31-1-B*9-B			
VL-1050	L12	6	CLRCY041-B*8-B			
VL-0536	L13	6	[CML 389/CML 176]-B-29-2-2-B*6-1-B*7-B			
VL-1018798	L14	6	CLRCY018-B*5-B-B			
SNL-1563	L15	3	(DMSyn-C0)-29-1-B-1-B			
SNL-1574	L16	3	(DMSyn-C0)-40-1-B-1-B			
SNL-1559	L17	6	(DMSyn-C0)-39-1-B-1-B			
SNL-142420	L18	2	(CA14514-7-B-2-B/CA00106-9-B-2-B)-B-3-1-BB-B			
SNL-142507	L19	5	(CA14514-4-1-1-B/CA00106-9-B-2-B)-BB-1-1-BB-B			
SNL-142400	L20	5	(CA14514-4-1-1-B/CA00106-9-B-2-B)-B-16-2-BB-B			
Testers						
CM-111	-	5	Cuba-342-2-f-#-#			
GPM-549	-	6	Derived from line CML176			
GPM-581	-	8	DMR 42048-22-1			

Table 1 - Pedigree details of the inbred lines and testers used in the study.

TLB – Turcicum leaf blight

and performance of the hybrids as compared to their parents (Shull, 1909) and exploitation of heterosis is an important objective in any crop breeding programme. When parental lines produce potent offspring, they are said to have good combining ability (Fasahat et al., 2016). This combining ability of lines can be further studied as general combining ability (GCA) and specific combining ability (SCA). Spargue and Tatum (1942) defined GCA as the average performance of a line in a series of hybrid combinations whereas, SCA as the cases in which certain combinations did relatively better or worse than can be expected on the basis of the average performance of the line involved. Further, combining ability is an important tool in classifying the lines and understanding their heterotic pattern (Melchinger and Gumber, 1998). By grouping the germplasm lines / parental lines based on their heterotic pattern exploitation of heterosis becomes more directed and efficient.

combining ability Line x Tester (Kempthorne, 1957) is the most commonly adopted method as it can be used when large number of parental lines are to be evaluated. Graphical representation of combining ability through GGE biplot technique helps to identify the best hybrid and to know the GCA status of inbred lines (Yan and Hunt, 2002).

Heterotic grouping of the germplasm is a primary step in a hybrid breeding programme. The use of elite inbred lines with known heterotic patterns as testers to classify inbred lines into heterotic groups was suggested by Melchinger and Gumber (1998). Heterotic grouping enables efficient selection of parents in hybrid breeding programme (Hallauer and Miranda, 1988). Among the various methods of heterotic grouping, grouping based on SCA effects and grain yield (SCA_GY) is widely used methodology (Hallauer and Miranda, 1988; Vasal *et al.*, 1992, Fan *et al.*, 2009).

Among the various mating designs used to estimate

Accurate prediction of hybrid performance accelerates

Source	d.f.	DTP (days)	NKRPC (number)	NKPR (number)	CG (cm)	CL (cm)	тw	
(gram)	GY (q/ha)							
Replication	2	4.93	1.48	15.75	0.07	1.1	5.37	103.32
Blocks within Replication	24	22.9*	0.68*	18.73*	0.05	2.1**	7.30**	198.26**
Genotypes	62	35.29**	2.94**	26.23**	0.19**	4.13**	17.55**	213.76**
Error	100	12.32	0.63	5.71	0.04	0.51	2.53	57.8

Table 2 - Analysis of variance of hybrids (F1 including checks) for grain yield and yield related traits.

DTP - Days to 50 per cent pollen shed (days); NKRPC - No. of kernel rows per cob; NKPR - No. of kernels per row; CG - Cob girth (cm); CL - Cob length (cm); HSW -Test weight (gram); GY - Grain yield (q/ha); *p < 0.05; **p < 0.01.

the hybrid breeding programme as field testing are expensive and time consuming. Multiple methods are used in predicting hybrid performance such as line per se performance (Smith, 1986), general combining ability (Bernardo, 1994, Charcosset, *et al.*, 1998), phenotypic BLUP (Bernardo, 1994, Panter and Allen, 1995), genetic distances estimated from markers (Bernardo, 1994) and marker effect estimate (Dudley *et al.*, 1991, Schrag, *et al.*, 2006). Among these methods, estimates of GCA effects of the parental lines provide an established and simple approach to predict hybrid performance (Melchinger *et al.*, 1987).

Hence, the present study was planned in order to understand the combining ability of the lines and heterotic grouping of the lines with tolerance to Turcicum leaf blight selected from the UAS, Dharwad (UASD) - CIM-MYT collaborative project.

Material and methods

Germplasm and evaluation of testcross hybrids

In the present investigation hundred inbred lines were procured from CIMMYT, India under UASD-CIMMYT collaborative project which had their origin from various source populations with mixed genetic composition belonging to sub-tropical and tropical germplasm. These lines were evaluated at All India Coordinated Research Project (AICRP), Maize, Dharwad (Karnataka) during kharif season of 2015 and 2016 and twenty lines were selected based on their per se performance and disease reaction against Turcicum leaf blight (TLB) of maize (Table 1). These selected lines were crossed to three genetically diverse testers CM-111, GPM-549 and GPM-581 in a line x tester method described by Kempthorne (1957) during rabi season, 2016. A total of sixty testcross hybrids were produced and were evaluated along with three hybrid checks namely, GPMH-1101 (Local check), NK-6240 (National check) and 900 MG (Private check) at AICRP Maize, University of Agricultural Sciences, Dharwad (located at 15° 26' N latitude and 75° 07' E longitude at an altitude of 678 m above mean sea level with mean annual rainfall of 740

mm) during kharif, 2017. Evaluation was carried by 9 x 7 alpha lattice design replicated thrice with nine blocks and 7 entries per block. Each entry was raised in two rows of 4.0 meter length following a spacing of 60 x 20 cm. The seeds were hand dibbled at the rate of two seeds per hill and later thinned to maintain an optimum plant population and all the recommended agronomic package of practices were followed to raise a healthy crop.

Data collection and statistical analysis

Observations were recorded on five randomly selected plants for number of kernel rows per cob (NKRPC), number of kernels per row (NKPR), cob girth (CG) and cob length (CL). Whereas, data on days to 50% pollen shedding (DTP) was collected on whole plot basis. Test weight (TW) was recorded by weighing hundred randomly selected grains and expressed in grams and grain yield (GY) was calculated by recording fresh cob weight of each entry on whole plot at the time of harvest in kilogram along with moisture percent, later converted to grain yield in quintals per hectare after accounting the shelling per cent and moisture correction.

The normality assumption of the variables (traits) was tested by Shapiro-Wilks test. Analysis of variance considering all the factors i.e., replication, blocks within replication, genotypes as fixed effects was worked using the following model.

$$Y_{ijlk} = \mu + r_l + b_{lk} + l_i + t_i + lt_{ij} + e_{ijlk}$$

where, Y_{ijk} = observed mean of the experimental unit, μ is population mean, r_l = replicate effect, b_{lk} is block within replication effect, l_i = ith line effect, t_i = jth tester effect, lt_{ij} =line*tester interaction effect and e_{ijk} = residual effect. The GCA and SCA effects were estimated as suggested by Kempthorne (1957). The line x tester analysis was carried out using 'gpbStat' package (Patil and Gangavati, 2020) of R statistical program (R core team, 2020).

The proportional contribution of line, tester and line x tester interaction towards total cross variation was cal-

Source	d.f.	DTP (days)	NKRPC (number)	NKPR (number)	CG (cm)	CL (cm)	TW (gram)	GY (q/ha)
Cross	62	35.74**	3.03**	27.04**	0.18**	4.26**	15.73**	221.14**
LGCA	19	79.54**	3.45**	39.51**	0.28**	7.81**	27.08**	285.71**
TGCA	2	28.320	11.15**	7.06*	0.26*	2.11*	7.57	563.92**
Lx T SCA	38	13.200	2.19**	21.09**	0.13**	2.26**	10.68**	166.41**

Table 3 - Line x Tester analysis of variance of F1 hybrids for grain yield and yield related traits.

DTP - Days to 50 per cent pollen shed (days); NKRPC - No. of kernel rows per cob; NKPR - No. of kernels per row; CG - Cob girth (cm); CL - Cob length (cm); HSW -Test weight (gram); GY -Grain yield (q/ha); *p < 0.05; **p < 0.01.

culated as below,

a. Contribution of lines (%) = SS (f)/ SS (c) x 100

b. Contribution of tester (%) = SS (m)/ SS (c) x 100

c. Contribution of lines x tester (%) = SS (fxm)/ SS (c) x 100

Where, SS (c) = Sum of squares due to crosses, SS (f) = Sum of squares due to lines, SS (m) = Sum of squares due to testers and SS (f x m) = Sum of squares due to lines x testers.

Classifying lines and testers based on their GCA effects

The GCA effects of lines and for the traits NKPRC, NKPR, CL, CG, TW and GY were added to obtain sum of GCA effects. Based on the sum of GCA effects the lines and testers were classified into high GCA and low GCA lines based on arbitrary value of eight and three respectivelyi.e., if the sum of GCA effects was higher than +8 or +3 it was classified as high GCA line or tester and if it was less than -8 or -3 it was classified as low GCA line or tester respectively.

Classifying inbred lines into Heterotic groups

Testers CM-111 and GPM-581 which showed diverse GCA effect and were placed in different sectors by GGE biplot were selected to classify inbred lines into distinct heterotic groups. The SCA effect and GY were used to group the lines into two heterotic group A (HG-A) and Heterotic group B (HG-B). If a cross between inbred line with tester CM-111 had a significant positive SCA effect, the line was assigned to the HG-A. Similarly, if the line recorded positive SCA effect with tester GPM-581, the line was assigned to HG-B (Vasal *et al.*, 1992).

Visualizing line x tester data through GGE biplot

GGE biplot is a multivariate statistical method to visualize two-way data. The GY of test cross hybrids was subjected to GGE biplot analysis with lines as genotypes and testers as environments (Fotokian *et al.*, 2014).



Fig. 1 - Proportional contribution of LGCA, TGCA and LTGCA towards total variation.

Parents	DTP (days)	NKRPC (number)	NKPR (number)	CG (cm)	CL (cm)	TW (gram)	GY (q/ha)	Sum of GCA effects#
			Hig	gh GCA lines				
VL-058725	0.77	-0.53*	0.3	0.06	0.23	-0.36	8.79**	8.49
VL-1018527	3.88**	0.53*	1.83*	0.29**	1.41**	2.25**	11.15**	17.46
VL-108723	0.32	0.75**	1.5	0.13*	2.19**	1.59**	10.69**	16.85
VL-1018798	1.66	0.42	3.39**	-0.06	0.21	1.95**	2.81	8.72
			Lo	w GCA lines				
VL-102	-6.01**	0.42	-0.17	-0.3	0.22	-0.16	-17.64**	-17.63
VL-109086	-2.79*	-0.91**	-3.33**	-0.5**	-1.13**	-0.54	-3.72	-10.13
VL-106	-2.34*	-0.36	-3.89**	-0.06	-0.3	0.8	-7.34**	-11.15
SNL-142420	-1.23	-0.36	-4.72**	0.08	-1.89**	-0.1	-2.5	-9.49
			Rei	maining lines				
SNL-153296	5.1**	0.31	3.17**	-0.04	1.55**	-3.04**	-7.62**	-5.67
VL-1012768	-0.46	0.42	0.78	0.15*	-0.39	1.44**	4.5	6.9
VL-1033	-3.01*	-0.97**	-1.11	0.04	0.01	-0.29	0.05	-2.27
VL-1249	4.66**	1.2**	1.77*	0.04	0.63**	0.32	1.65	5.61
SNL-153280	5.10**	0.42	-1.06	0.02	0.17	-1.02*	-3.71	-5.18
VL-1050	0.99	0.64*	0.83	0.02	0.07	-0.28	-3.27	-1.99
VL-0536	4.21**	-0.86**	1.83*	-0.02	-0.88**	-3.23**	0.72	-2.44
SNL-1563	-0.23	-0.36	1.33	-0.22**	1.48**	0.14	3.99	6.36
SNL-1574	-3.12*	-1.36**	-0.17	-0.05	-0.42	1.21**	-0.73	-1.52
SNL-1559	-2.46*	-0.25	-0.78	-0.03	-0.86**	1.18**	0.38	-0.36
SNL-142507	-0.12	1.09**	-2.17**	0.37**	-1.13**	-1.39**	1.94	-1.29
SNL-142400	-4.90**	-0.25	0.66	0.08	-1.20**	-0.46	-0.15	-1.32
C.D. (Lines) at 5%	1.17	0.26	0.79	0.06	0.23	0.53	2.53	-
No. of lines with desirable GCA effects	8	5	5	3	5	6	4	7
				Testers				
CM-111	-0.77	-0.43**	0.63*	0.06*	-0.19*	-0.29	3.62**	3.4
GPM-549	0.31	0.44**	-0.05	0.03	0.21*	-0.45*	0.32	0.5
GPM-581	0.46	-0.01	-0.58	-0.09**	-0.02	0.74**	-3.94**	-3.9
C.D. (Testers) at 5%	0.45	0.1	0.31	0.03	0.09	0.21	0.98	-

Table 4 - Estimates of general combing ability effects of lines and testers for yield and yield related traits.

DTP - Days to 50 per cent pollen shed (days); NKRPC - No. of kernel rows per cob; NKPR - No. of kernels per row; CG - Cob girth (cm); CL - Cob length (cm); TS -Test weight (gram); GY -Grain yield; (q/ha)'*p< 0.05; **p < 0.01.# -sum of GCA effects for traits NKRPC, NKPR, CG, CL, TS and GY

Principle components PC1 and PC2 were derived from environmental (column) centered data. The GGE biplot is based on genotype focused scaling where singular values are partitioned entirely into the genotype eigenvectors. GGE biplot was generated using 'GGEBiplots' package (Dumble, 2017) of R programme. The statistical model used is,

$$X_{ij} - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where, Y_{ij} is the genotypic value of the cross (F1 hybrid) between line i and tester j of the trait, β_j is the mean

of all combinations involving Tester j; λ_1 and λ_2 are singular values of PC1 and PC2, respectively; ξ_{i1} and ξ_{i2} are the PC1 and PC2 eigenvectors, for line i; η_{j1} and η_{j2} are PC1 and PC2 eigenvectors, respectively, for tester j; and ϵ_{ij} is the residual of the model of the cross line i and tester j.

Results and Discussion

Shapiro-Wilks test suggested all the traits followed normal distribution. Analysis of variance (ANOVA) for grain yield and its component traits among the hybrids

Linos	GCA	c	CM-111	G	PM-549	GPM-581	
LITES	SCA Grain yield (q/ha) SCA Grain y		Grain yield (q/ha)	SCA	Grain yield (q/ha)		
			Heterotic G	iroup A			
VL-102	-17.64**	10.35*	48.63	-9.77*	25.21	-0.58	30.14
VL-1033	0.05	8.9*	64.87	-3.21	49.46	-5.69	42.72
VL-109086	-3.72	10.08*	62.29	1.73	50.63	-11.82**	32.83
VL-1249	1.65	11.01*	68.58	-5.57	48.7	-5.43	44.59
SNL-153280	-3.71	0.25	52.46	10.28*	59.19	-10.54*	34.12
SNL-142420	-2.5	8.86*	62.28	-4.94	45.18	-3.92	41.94
			Heterotic G	iroup B			
VL-058725	8.79**	-8.72*	56	3.53	64.94	5.19	62.35
VL-1018527	11.15**	-5.21	61.87	4.63	68.4	0.57	60.09
VL-1018798	2.81	-4.62	54.11	-0.89	54.54	5.51	56.69
SNL-1563	3.99	-4.76	55.15	-4.99	51.62	9.76*	62.12
SNL-1574	-0.73	-6.32	48.87	-12.53**	39.36	18.85**	66.49
SNL-1559	0.38	-12.68**	43.62	3.6	56.6	9.08*	57.82
SNL-142507	1.94	-7.34	50.53	5.18	59.75	2.15	52.46
			Heterotic Gr	roup AB			
SNL-153296	-7.62**	3.28	51.58	-4.44	40.55	1.15	41.9
VL-108723	10.69**	8.47	75.08	-9.42*	53.88	0.95	60.01
VL-106	-7.34**	0.35	48.93	-0.79	44.49	0.45	41.47
			Rejected	lines			
VL-1012768	4.5	-0.11	60.3	1.63	58.75	-1.52	51.35
VL-1050	-3.27	-3.8	48.85	6.46	55.8	-2.65	42.44
VL-0536	0.72	-0.34	56.31	10.65*	63.99	-10.31*	38.78
SNL-142400	-0.15	-7.65	48.12	8.85*	61.31	-1.2	47.01
Mean	0	0	55.92	0	52.62	0	48.37
	C.D. for	GY at 5%	10.64	1	C.D. for SC	A at 5%	8.72

Table 5 - Specific combining ability and grain yield (GY) of line x tester hybrids.

GCA- General combining ability; SCA – Specific combining ability; *p < 0.05; **p <0.01.

revealed the existence of significant difference among the genotypes for the quantitative traits indicating that the experimental material had sufficient variation (Table 2). Variance of the blocks within replication was significant for DTP, NKRPC, NKPR, CL, TW and GY suggesting blocking (local control) was effective to explain variation among the hybrids for these traits. The overall variation among the test hybrids was further divided into LGCA, TGCA and LxT SCA. LGCA was found to be significant for all the traits (Table 3) and TGCA was significant for NKRPC, NKPR, CG, CLand GY indicating importance of additive gene action. LxT SCA which is an indication of interaction between alleles of lines and tester was found significant for traits NKRPC, NKPR, CG, CL, TW and GY indicating the importance of nonadditive gene action.

Contribution of lines towards total cross variation was more than that of testers for traits DTP, CG, CL, NKPR and TW (Figure 1) indicating lines were majorly responsible for variation among hybrids for these traits. Whereas, variation due to tester was higher than lines for only NKRPC and GY indicating that either testers had favorable alleles for these two traits only.

General combining ability of inbred lines

Among the twenty inbred lines GCA for GY ranged from -17.64 to 11.15 (Table 4) with VL-1018527 and VL-102 recording highest and lowest value respectively for grain yield. Though inbred line SNL-142507 recorded highest GCA effect for NKRPC and CG, it had low GCA effect for GY due to negative GCA effect for NKPR, CL and TW. The line VL-1018527 showed significant positi-

Sl. No.	Genotypes	GY (q/ha)	DTP (days)	NKPRC (number)	NKPR (number)	CG (cm)	CL (cm)	TW (gram)		
	Test cross hybrids									
1.	VL-108723 x CM-111	75.08	60.00	15.67	39.17	4.55	18.85	29.81		
2.	VL-1249 x CM-111	68.58	66.00	15.33	38.0	4.73	17.75	29.96		
3.	VL-1018527 x GPM-549	68.40	68.00	14.00	34.67	4.75	18.74	26.75		
4.	SNL-1574 x GPM-581	66.49	56.67	13.67	37.0	4.47	18.06	27.84		
5.	VL-058725 x GPM-549	64.94	66.00	14.67	31.33	4.78	16.93	25.49		
6.	VL-1033 x CM-111	64.87	61.33	12.83	34.83	4.70	16.92	29.03		
7.	VL-0536 x GPM-549	63.99	70.33	13.67	36.5	4.37	15.67	25.07		
8.	VL-058725 x GPM-581	62.35	61.33	14.33	39.67	4.37	16.78	23.76		
9.	VL-109086 x CM-111	62.29	59.00	14.33	31.00	4.38	16.11	24.86		
10.	SNL-142420 x CM-111	62.28	59.00	14.33	29.33	4.70	14.43	28.36		
				Checks						
1.	GPMH-1101 (L.C)	46.09	60.33	13.33	35.67	4.70	17.59	26.32		
2.	NK-6240 (N.C)	54.76	63.33	14.33	36.33	5.15	18.46	32.83		
3.	900 MG (P.C)	63.08	65.33	15.00	37.50	4.67	17.27	22.34		
Mean	54.05	62.79	14.67	34.54	4.50	16.84	25.43			
Max.	75.08	70.33	17.67	40.17	5.15	19.69	32.83			
Min.	25.21	55.00	12.00	27.67	3.45	14.05	18.53			
CV %	14.06	6.04	5 41	7.66	1 98	4 69	6.48			

Table 6 - The top ten hybrids along with mean, range, of test cross hybrids including checks for grain yield and yield related traits.

NKRPC - No. of kernel rows per cob; NKPR - No. of kernels per row; CG - Cob girth (cm); CL - Cob length (cm); TS -Test weight (gram); GY -Grain yield; CV - Coefficient of variation; CD- Critical difference; LC - Local check; NC-National check; PC- Private check.

7.66

1.98

ve GCA effects for all the traits indicating the accumulated additive genes for yield and yield related traits.

4.38

5.41

10.64

The GCA effects for all the traits except DTP were added to get sum of GCA effects and based on sum of GCA effects lines and testers were grouped into high and low GCA group. Lines VL-058725, VL-1018527, VL-108723, VL-1018798 and CM-111 fell in high GCA group whereas, lines VL-102, VL-109086, VL-106, SNL-

Table 7 - Correlation between hybrid performance and combining ability.

Trait	FGCA	MGCA	SGCA	SCA
NKRPC	0.659**	0.297**	0.727**	0.655**
NKPR	0.669**	0.152	0.686**	0.727**
CG	0.710**	0.255**	0.754**	0.360**
CL	0.788**	0.125	0.798**	0.603**
TW	-0.278*	0.079	-0.251	0.644 **
GY	0.644**	0.308*	0.714**	0.70**

NKRPC - No. of kernel rows per cob; NKPR - No. of kernels per row; CG - Cob girth (cm); CL - Cob length (cm); TS -Test weight (gram); GY -Grain yield; FGCA, Female parent GCA effects; MGCA, Male parent GCA effects; SGCA, Sum parental GCA effects; SCA, Specific combining ability effects; *p < 0.05; **p < 0.01.

142420 and GPM-581 fell in low GCA group. The lines under high GCA group also recorded positive GCA effect for DTP indicating late flowering genotypes are relatively high yielding and also these lines possess novel alleles for increased grain yield. The high negative GCA of VL-102 for grain yield maybe due to its early flowering character as evident from its high negative GCA for DTP. Crosses between and among the high and low GCA groups lines have high probability of producing heterotic hybrids.

4.69

6.48

The GCA effect mainly relates to additive genetic effects (Kang, 1994) and genes with such additive effects accumu-late as cyclic selection progresses (Hallauer and Miranda, 1988). The differences in GCA effects are attributable to differences in frequencies of genes with the additive effects (Falconer and Mackay, 1996). Grain yield is the result of interaction between various yield related traits, thus negative GCA for yield related traits resulted in negative GCA for GY as evident from lines VL-106 and SNL-142420. Similarly, positive GCA for yield related traits resulted in high GCA effect for GY.

Specific combining ability of test cross hybrids

The SCA effect of test cross hybrids for GY ranged from

1. 2. 3. 4. 5. 6. 7. 8. 9.

C.D. at 5%



Figure 2 - Classifying inbred lines into two distinct heterotic groups.

Fig. 2 - Classifying inbred lines into two distinct heterotic groups Classifying inbred lines into two distinct heterotic groups

-11.82 to 18.85 (Table 5) with cross SNL-1574 x GPM-581 (18.85) recording the highest SCA effect followed by VL-1249 x CM-111 (11.01) and VL-0536 x GPM-549 (10.65). The SCA effect of a hybrid is attributed to interaction of alleles coming from male and female parents and their complementation and it is an indication of non-additive gene action (Kang, 1994). High SCA effects indicate the parents possess complementary alleles, which when brought together show inter and intra gene action, thus improving the trait. Inbred lines VL-1012768 and VL-106 expressed low non-significant SCA effects with all the three testers indicating lack of interaction between the alleles of parents. Similarly, none of the test hybrids expressed significant SCA effects for DTP.

Grain Yield

Grain yield among the test cross hybrids ranged from 25.21 q/ha to 75.08 q/ha with mean of 52.30 q/ha (Table 5). Seven test cross hybrids recorded higher grain yield than the best check 900 MG (63.08 q/ha). The cross combination of VL-108723 x CM-111 recorded highest grain yield (75.08 q/ha) with 15.98 % increased yield over the best check hybrid 900 MG (63.08 q/ha) this was followed by cross combinations of VL-1249 x CM-111(68.58 q/ha) and VL-1018527 x GPM-549 (68.40 q/ha) with 8.71 and 8.43 % respectively increased yield over best check hybrid (Table 6). Yield related traits such as NKRPC, CL and CG showed relatively low

variation among the hybrids. The best test hybrid (VL-108723 x CM-111) was early by five days for DTP as compared to the best yielding check hybrid 900 MG and the higher yield was manifested through the yield contributing characters as evident with higher NKPRC, NKPR, CL and TW. For earliness the cross combination of SNL-1574 x GPM-581 was the best hybrid with 56.0 days for DTP and it also recorded higher GY of 66.49 q/ ha as compared to best yielding check hybrid.

Heterotic grouping

The mean GY of lines with tester CM-111 and GPM-581 was 55.92 q/ha and 48.37 q/ha respectively (Table 5). The SCA_GY method classified 13 out of 20 inbred lines into two distinct heterotic group, HG-A with six lines and HG-B with seven lines. Inbred lines SNL-153296, VL-108723 and VL-106 showed positive SCA effects with both the tester thus were grouped separately in heterotic group-AB. Whereas, the lines VL-1012768, VL-1050, VL-0536 and SNL-142400 recorded negative SCA effects with both the testers were not assigned to any group.

Similarity can be seen between grouping of lines based on sum of GCA effects and SCA_GY method as three high GCA a lines VL-058725, VL-1018527 and VL-1018798 were grouped in HG-B, whereas, three low GCA lines VL-102, VL-109086, SNL-142420 were placed in HG-A (Table 5). Thus, in general GPM-581 produced better hybrids when paired with lines having



Fig. 3 - The black dot indicated average tester cordinate (ATC).

high GCA. Whereas, CM-111 could produce better hybrids even with lines having low GCA. The results indicate that tester CM-111 has complementary alleles and ability to uncover desirable allele from the lines for grain yield and is a potential tester for maize hybrid breeding in the tropical environments. This is also evident from the fact that CM-111 was extensively used as tester in Indian maize breeding programme in the 1990's. Three lines SNL-1563, SNL-1574 and SNL-1559 derived from synthetic population DMSyn-C0 were together grouped in HG-B. Whereas, three white seeded inbred lines VL-058725, VL-1018527 and VL-0536 of African origin were not placed into single heterotic group, indicating that grouping of lines was not based on origin of the lines but on the heterotic reaction between lines and testers.

Lines from each group could be recombined separately followed by selection and further recombination to develop two new heterotic populations A (HP-A) and heterotic population B (HP-B) (Figure 2). These two new populations can exhibit a high level of heterosis between them and also serve as superior hybrid-oriented germplasm for further hybrid work (Vasal *et al.*,1992). These two heterotic populations could be improved using reciprocal recurrent selection to synthesize more genetically diverse superior inbred lines. Further, lines VL-1018527, SNL-1563, SNL-1574, SNL-142420, SNL-153280 possess alleles for resistance against Turcicum



Fig. 4 - Polygon (Which Won Where) view of biplot based on line x tester data for grain

leaf blight which can be recombined to form a heterotic populations for Turcicum leaf blight tolerance and further to derive the inbred lines.

GGE biplot analysis of Line x Tester data

The mean vs stability view of biplot corresponds to combining ability effects of genotypes. The GCA and SCA effects of the genotypes are determined by Average Tester Coordinate (ATC). The ATC is established with its abscissa passing through the origin and average tester (the black dot). The ordinate is drawn perpendicular to the ATC abscissa and passing through origin (Yan and Hunt, 2002).

The two principal components of the GGE biplot (Fig.

3) together explained 79.02% of the total variation for grain yield. The ATC ordinate divides the inbred lines with positive and negative GCA effects. The inbred lines lying on the left side of ATC ordinate show negative GCA effects whereas, inbred lines on the right-hand side of ATC ordinate show positive GCA effects. The GCA effects of the lines is approximated by their projections on to the ATC abscissa. Among the inbred lines, VL-1018527 showed highest GCA effect for grain yield followed by VL-058725, VL-108723 and VL-1012768, on the contrary lines VL-102 recorded lowest GCA effect for GY which is in agreement with calculated GCA effects. The SCA effects of lines onto ATC ordinate



Fig. 5 - Partitioning combining ability effects into sum of parental GCA and SCA of top ten crosses based for grain yield (q/ha)

and observed lines have tendency to produce superior hybrids with specific testers (Yan and Hunt, 2002). For GY, inbred line SNL-1574 manifested highest positive and negative SCA effect followed by lines VL-109086, SNL-153280 and VL-0536.

The present biplot was divided into six sectors and all the three testers fell in different sectors. Inbred lines located at the vertex of polygon such as VL-058725, VL-1018527, VL-102, VL-109086, VL-0536 and SNL-1574 (Figure 4) are highly responsive to change in their mating partners. The three testers fell in different sectors indicating they are genetically divergent from each other and showed unique interaction with lines. The polygon view of the biplot successfully identified the best mating partner for testers GPM-549 and GPM-581 as VL-1018527 and SNL-1574 respectively. Though biplot suggested VL-0536 to be best mating partner for CM-111 it differed from that of conventional tester data analysis which showed VL-108723 to be the best mating partner. The three white seeded inbred lines VL-058725, VL-1018527 and VL-0536 were present on the right-hand side of ATC ordinate and formed the vertex of polygon indicating these lines can be highly competitive for grain yield in tropical region.

Partitioning combining ability for grain yield of top ten test cross hybrids

Graphical representation of partitioned combining ability for grain yield of top ten hybrids is presented in Figure 5. Total combining ability effect is divided into FGCA, MGCA, SoCA (FGCA+MGCA) and SCA effects. The best performing cross VL-108723 x CM-111 (first) with grain yield of 75.08 q/ha was a high x high GCA type of cross were both the parents showed high significant GCA effects but non-significant positive SCA effect. Whereas, crosses VL-1018527 x GPM-549 (third), VL-058725 x GPM-549 (fifth) and VL-058725 x GPM-581 (eighth) were of high x low GCA type in which one of the parents showed high significant GCA effect and other had negative or near zero GCA effect. This is confirmation with the studies of Arunachalam and Reddy (1981) wherein they observed from their study that the GCA status of a heterotic hybrid was usually high x high or high x low type. The high grain yield of VL-1249 x CM-111 (second), SNL-1574 x GPM-581 (fourth), VL-1033 x CM-111 (sixth), VL-0536 x GPM-549 (seventh), VL-109086 x CM-111 (ninth) and SNL-142420 x CM-111 (tenth) cross can be attributed to high SCA effect rather than GCA effects. The cross combination of SNL-1574 x GPM-581 was unique in the sense that parental GCA effects were in negative direction for GY however, the SCA effect was high which may be due to complementation of male and female alleles leading to non-additive gene action in the desired direction. In general, it can be observed that all the top performing crosses showed positive SCA effect indicating positive SCA irrespective of GCA effects indicating importance of non-additive gene action in determining the performance of hybrids.

Correlation between hybrid performance and combining ability

Pearson's correlation coefficient between mean performance of crosses with their respective FGCA, MGCA, SGCA and SCA was estimated for traits NKRPC, NKPR, CL, TW and GY (Table 7). Results revealed that the hybrid mean performance significantly correlated with FGCA and SCA. FGCA showed positive significant correlation with all the traits except HSDW whereas SCA showed significant positive correlation with all the observed traits. Previously, hybrid performance was predicted by GCA of the parental lines by Cockerham, (1967) and Melchinger et al., (1987). But as evident the SCA constitute an important role in hybrid performance and should be included in prediction of hybrid performance. Bernardo (1994) observed that correlations between predicted and observed single cross GY were consistently greater when both GCA and SCA effects were included in the model rather than only when GCA effect was included. But negative significant correlation was observed between FGCA and HSW. Whereas, MGCA was significantly associated only with NKRPC, CG and GY.

Conclusions

Careful analysis of combining ability effects helps in revealing the gene action of inbred lines and hybrids for the given trait. The LGCA and TGCA were higher than LxT SCA indicating the prominent role of additive gene action for all the traits. From the present study, we have observed that both GCA and SCA play a prominent role in production of heterotic hybrids. The hybrids derived from lines VL-058725, VL-1018527 and VL-108723 had higher GCA effect indicating the contribution of additive gene action towards grain yield. These inbred lines could be further used to develop promising hybrids or used in developing synthetic population as these lines are source of additive genes. It can be noted that female lines VL-0536, SNL-1574 and VL-109086 could produce superior hybrid for grain yield due to non-additive gene action when crossed with a suitable complementary tester. CM-111 was relatively a better tester as evident it was able to throw out high yielding and heterotic hybrids in cross combination with majority of the female lines. Further, lines VL-058725 and VL-0536 when used as female parent produced resistant hybrids irrespective of disease the reaction of male parent (data not shown). The white seeded inbred lines from African origin were better performing and also showed resistance to Turcicum leaf blight of maize which can be a source for breeding for TLB. Heterotic grouping helped in identifying the heterotic pattern among the parental lines and provided framework for future breeding programme. Biplot presents a worthwhile view of combining ability effects but should be verified through conventional line x tester analysis. From association and partitioning studies of combining ability effects we have observed that SCA plays a prominent role in determining the performance of a hybrid, indicating the importance of non-additive gene action. Thus, the breeders focus in exploiting nonadditive gene action for developing superior hybrids through planned crossing programme.

References

- All India Coordinated Research Project on Maize. (2019). Annual Progress Report Kharif Maize 2016.Retrieved from https://iimr.icar.gov.in/ attachments/article/29/Maize%20AICRP%20 REPORT%20KHARIF%202016.pdf
- Arunachalam V, Reddy BB, 1981. Evaluation of heterosis through combining ability in Pearl Millet II. Multiple crosses. Indian Journal of Genetics and Plant Breeding, 41: 66-74
- Bernardo R, 1994. Prediction of Maize Single-Cross Performance Using RFLPs and Information from Related Hybrids. Crop Science, 34: 20-25
- Charcosset A, Bonnisseau B, Touchebeuf O, Burstin J, Barrière Y, Gallais A, Denis J, 1998. Prediction of Maize Hybrid Silage Performance Using Marker Data: Comparison of Several Models for Specific Combining Ability. Crop Science, 38: 38-44
- Cockerham CC, 1967. Prediction of double crosses from single crosses. Der Züchter, 37: 160-169
- Dudley JW, Maroof MA, Rufener GK, 1991. Molecular Markers and Grouping of Parents in Maize Breeding Programs. Crop Science, 31: 718-723
- Dumble S, 2017. GGEBiplots: GGE Biplots with 'ggplot2'. R package version 0.1.1. https:// CRAN.R-project.org/package=GGEBiplots
- Falconer DS, Mackay TF, 1996. Introduction to Quantitative Genetics. Harlow Pearson, Prentice Hall.
- Fan XM, Zhang YM, Yao WH, Chen HM, Tan J, Xu CX, 2009. Classifying maize inbred lines into heterotic groups using a factorial mating design. Agronomy Journal, 101:106–112.
- Fasahat P, Rajabi A, Rad JM, Derera J, 2016. Principles and utilization of combining ability in plant breeding. Biom. Biostat. Int. J. 4: 85.
- Fotokian MH, Agahi K, 2014. Biplot Analysis of Genotype by Environment for Cooking Quality in Hybrid Rice: A Tool for Line × Tester Data. Rice Science, 21(5): 282-287.
- Hallauer AR, Miranda FB, 1988. Quantitative

Genetics in Maize Breeding, Second edition, Iowa State University Press, Ames, Iowa.

- Kang MS, 1994. Applied Quantitative Genetics. Baton Rouge, M.S. Kang Publisher, Louisiana
- Kempthorne O, 1957.An Introduction to Genetic Statistics. John Wiley and Sons, New York
- Melchinger AE, Geiger HH, Seitz G, Schmidt GA, 1987. Optimum prediction of three-way crosses from single crosses in forage maize (*Zea mays* L.). Theoretical and Applied Genetics, 74: 339-345.
- Melchinger AE Gumber RK,1998. Overview of heterosis and heterotic groups in agronomic crops. In: Lamkey, K. R., Staub J. E., (eds) Concept and breeding of heterosis in crop plants. CSSASP, publication no. 25, Madison, Wis. pp 29–44.
- Panter DM, Allen FL, 1995. Using Best Linear Unbiased Predictions to Enhance Breeding for Yield in Soybean: II. Selection of Superior Crosses from a Limited Number of Yield Trials. Crop Science, 35: 405-410
- Patil, NL, Gangavati LR, 2020. Statistical analysis of Plant Breeding data. R package version 0.3.1. https://cran.r-project.org/package=gpbStat

- R Core Team 2013. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Schrag TA, Melchinger AE, Sorensen AP, Frisch M, 2006. Prediction of single-cross hybrid performance for grain yield and grain dry matter content in maize using AFLP markers associated with QTL. Theoretical and Applied Genetics, 113: 1037-1047
- Shull GH, 1909. A pure line method of corn breeding. American Breeders Assoc. Rep., 5: 51-59
- Smith OS, 1986. Covariance between line per se and test cross performance. Crop Sci. 26: 540-543.
- Sprague GF, Tatum LA, 1942. General vs specific combining ability in single crosses of corn. Agron. J., 34: 923-932
- Vasal SK, Srinivasan G, Han GC, Gonzalez F, 1992. Heterotic patterns of eighty-eight white subtropical CIMMYT maize lines. Maydica, 37: 319–327.
- Yan W, Hunt LA, 2002. Biplot analysis of diallel data. Crop Science, 42: 21-30.