Indian Journal of Agricultural Sciences **91** (11): 1586–91, November 2021/Article
https://doi.org/10.56093/ijas.v91i11.118535

Unveiling combining ability and heterotic grouping of newly developed winter maize (*Zea mays*) inbreds

S B SINGH[1*], P KUMAR[2], R K KASANA[1], M CHOUDHARY[2], S KUMAR[1], R KUMAR[2],
C G KARJAGI[3], B KUMAR[3] and S RAKSHIT[2]

Regional Maize Research and Seed Production Centre, Begusarai, Bihar 851 101, India

Received: 06 March 2020; Accepted: 15 April 2021

ABSTRACT

Pooled analysis of the test-crosses evaluation of 61 newly developed maize (*Zea mays* L.) inbred lines using two inbred testers namely BML-6 and BML-7 during *rabi* 2016–17 and 2017–18 at ICAR-IIMR Regional Centre, Begusarai revealed significant variation among genotypes, general combining ability (GCA) and specific combining ability (SCA) effects for all the traits. Out of 61 inbred lines, 29 exhibited significant positive GCA effect for grain yield with maximum GCA effect value of 25.64. Inbred lines, viz. IMLSB-1299-5, IMLSB-406-2, IMLSB-334B-2, IMLSB-814-2 and IMLSB-285-1 were identified as the best general combiners on the basis of GCA effects which can be utilized to a greater extent in hybrid breeding programme. Out of 29 inbred lines with significant positive GCA effects, 12 and 17 lines also showed significant positive SCA effects with tester, BML-7 and BML-6 respectively, thus were classified into two heterotic groups 'A' and 'B', respectively. Out of 122 test-crosses, 14 exhibited significant and positive heterosis for yield over three national checks. The information generated on the heterotic grouping will help in further streamlining the available germplasm into heterotic pools and thereby augmenting the national hybrid breeding programme.

Keywords: Combining ability, Heterotic grouping, Line × tester

Maize (*Zea mays* L.) is the only billion-tonne cereal crop and third most important cereal after rice and wheat with wide adaptability under diverse ecological conditions. Diverse uses of maize as food, fodder, feed and as raw material in starch, pharmaceuticals, ethanol and various other industries is rising its demand day by day. Globally, maize is grown on 188 million ha in more than 170 countries with 1060 million MT of production. United States of America (USA) is the major producer and contributor (35.8%) of maize, whereas India stands on 4th position in terms of area with 9.2 million ha and annual production with 28 million tonnes (Anonymous 2021). The information on combining ability of the breeding material is critical for hybrid breeding programme. The estimation of GCA helps in electing the best combiner parents for the desired traits. The estimation of SCA effects helps in the heterotic grouping of inbreds lines and identification of superior single cross combinations (Revilla *et al.* 2002). Therefore, SCA and GCA are important genetic parameters which assist breeders to develop high breeding value populations.

Heterotic grouping is the commonly used method for assigning the lines into different heterotic groups as per the magnitude and direction of SCA with testers ((Melchinger and Gumber 1998, Hallauer *et al*. 1988). Heterotic group constitution is the fundamental step for exploitation of heterosis and developing superior maize hybrids (Aguiar *et al.* 2008). It helps in the streamlining of the fixed genetic material into heterotic groups for better utilization in the form of inbred derivation and generation of superior hybrids, as the selection of contrasting inbred lines for hybridization would ensure greater chances of obtaining heterotic hybrids (Singh *et al.* 2020). Legesse *et al.* (2009) carried out the heterotic grouping of highland inbreds lines derived from three different populations (Kitale Synthetic II × N3-type lines; Ecuador-573 × SC-type lines; and Pool 9A × IITA) and grouped the 23 inbreds lines into two heterotic groups. The present study was undertaken to estimate the general and specific combining ability effects of parents and hybrids, to identify the promising hybrid combinations and to classify the inbred lines into heterotic groups.

MATERIALS AND METHODS

The present study was carried out at ICAR-Regional Maize Research and Seed Production Centre, Begusarai

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Present address: [1]Regional Maize Research and Seed Production Centre (ICAR-IIMR), Vishnupur, Begusarai, Bihar; [2]ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana; [3]Unit Office, ICAR-IIMR, Pusa Campus, New Delhi. *Corresponding author email: singhsb1971@rediffmail.com.

during *rabi* 2015–16 and 2016–17. The 61 inbred lines were crossed with two testers (BML 6 and BML 7) to generate 122 test-crosses. The BML 6 and BML 7 are the parents of a commercial well-adapted maize hybrid DHM 117. Test-crosses were evaluated along with three hybrid checks (DHM 117, DKC 9081 and P 3396) and parents for yield and associated traits in Randomized Block Design (RBD) in three replications. The experimental unit consisted of a single four-meter row plot at spacing 60 cm × 25 cm. All recommended package of practices was followed to raise good plants. Observations were recorded on days to anthesis (DA), days to silking (DS), anthesis to silking interval (ASI), days to maturity (DM), grain-filling duration (GFD), plant height (PH), ear height (EH), ear length (EL), ear girth (EG), kernel rows (KR), kernels per row (K/R), shelling percentage and grain yield (GY). PH, EH, EL and EG were measured in cm while GY was recorded kg/plot and converted into q/ha. The data analysis was carried out using the INDOSTAT

Table 1 General and specific combining ability (GCA and SCA) effects, promising combiners and combinations in maize for grain yield

Parameter	Mean	GCA		Lines with high GCA	Mean	SCA		Crosses
		Maximum	Minimum			Maximum	Minimum	
Days to anthesis	109.8	7.87^{**}	-5.00^{**}	IMLSB 976-2, IMLSB 507-1, IMLSB 156-2, IMLSB 2039	104.9	3.38^{**}	-3.38^{**}	IMLSB 23-2×BML 7, IMLSB 83-1×BML 6, IMLSB 114-1×BML 7
Days to silking	113.2	7.096^{**}	-5.43^{**}	IMLSB 156-2, IMLSB 976-2, IMLSB 507-1, IMLSB 2039	108.1	3.54^{**}	-3.54^{**}	IMLSB 23-2×BML 7, IMLSB 55-2×BML 6, IMLSB 119×BML 6
Anthesis-silking interval	3.3	1.25^{**}	-0.79^{**}	IMLSB 162-1, IMLSB 231-1, IMLSB 274-1	3.2	1.12^{*}	-1.12^{*}	IMLSB 571-2×BML 6, IMLSB 166-2×BML 6, IMLSB 114-1×BML 7
Days to maturity	151.9	7.34^{**}	-5.14^{**}	IMLSB 976-2, IMLSB 93-2, IMLSB 285-1, IMLSB 55-2	148.1	4.92^{**}	-4.92^{**}	IMLSB 2028×BML 6, IMLSB 976-2×BML 6, IMLSB 119×BML 6
Grain-filling duration (GFD)	38.8	7.19^{**}	-3.60^{**}	IMLSB 156-2, IMLSB 719-1, IMLSB 2039	40.0	4.11^{**}	-4.11^{**}	IMLSB 58-1×BML 6, IMLSB 219-2×BML 6, IMLSB 976-2×BML 7
Plant height	103.1	31.13^{**}	-26.48^{**}	IMLSB 1062-2-2, IMLSB 2028, IMLSB 2039	171.0	17.36^{**}	-10.05^{**}	IMLSB 457-2×BML 7, IMLSB 457-2×BML 6, IMLSB 2039×BML 7
Ear height	40.7	22.00^{**}	-13.88^{**}	IMLSB 49-2, IMLSB 1062-2-2, IMLSB 2028	77.2	12.27^{**}	-12.27^{**}	IMLSB 507-1×BML 7, IMLSB 719-1×BML 7, IMLSB 976-2×BML 7,
Ear length (cm)	9.4	1.87^{**}	-2.56^{**}	IMLSB 164-1, IMLSB 814-2, IMLSB 1299-2	15.7	2.26^{**}	-1.63^{**}	IMLSB 49-2×BML 6, IMLSB 49-2×BML 7, IMLSB 173-2×BML 7
Ear girth (cm)	12.0	1.98^{**}	-1.31^{**}	IMLSB 83-1, IMLSB 171-2, IMLSB 1047-1-1, IMLSB 1299-2	15.3	0.97^{**}	-0.97^{**}	IMLSB 43-2×BML 6, IMLSB 171-1×BML 6, IMLSB 2166×BML 7
Kernel rows	11.8	2.37^{**}	-1.55^{**}	IMLSB 254-1, IMLSB 617-1, IMLSB 1299-2	14.3	1.30^{**}	-1.30^{**}	IMLSB 171-1×BML 6, IMLSB 507-1×BML 7, IMLSB 2083×BML 6
Kernel per row	16.5	5.42^{**}	-6.37^{**}	IMLSB 231-1, IMLSB 334B 2, IMLSB 1043-1-1, IMLSB 1299-5, IMLSB 1062-2-2	31.1	5.49^{**}	-5.49^{**}	IMLSB 457-1×BML 6, IMLSB 285-1×BML 7, IMLSB 406-2×BML 6
Shelling percentage	24.8	3.20^{**}	-4.44^{**}	IMLSB 126-2, IMLSB-301-2, IMLSB 571-2	81.4	5.26^{**}	-5.26^{**}	IMLSB 86-2×BML 6, IMLSB 83-1×BML 7, IMLSB 2083×BML 6
Grain yield (q/ha)	14.4	25.63^{**}	-15.82^{**}	IMLSB-334B-2, IMLSB 406-2, IMLSB 1299-5	93.3	23.37^{**}	-23.37^{**}	IMLSB 219-2×BML 6, IMLSB 173-2×BML 7, IMLSB 343-3×BML 7

*,** Significant at P=0.05 and 0.01, respectively.

software. Line × tester analysis of variance was performed to estimate GCA and SCA effects, assuming the following statistical model (Singh and Chaudhary 1996):

Economic heterosis was calculated as per the given below formula: SH (%) = ((F_1–SH)/SH) × 100. Where, F_1=mean value of the F_1 cross; SH=mean value of the standard check. Tests for significance of heterosis were made using t-test. GY, GCA and SCA were considered for heterotic grouping. The inbred lines which possess negative SCA effect with any one of the heterotic tester was grouped with tester that was having negative SCA effect (Parentoni *et al*. 2001).

RESULTS AND DISCUSSION

Analysis of variance: The test-crosses generated by Line × Tester mating design were evaluated along with parents and checks. The mean squares due to different sources of variation, viz. treatments, lines, testers, lines effect, tester effect and their combinations were tested at significant level $P<0.001$. Mean squares due to treatments, parent × crosses, crosses and line × tester effect were significant for all the traits indicating the presence of sufficient diversity in parents involved. The mean square due to line and parents were significant for all the traits except GY indicating comparable yield performance between parents. Significant difference among lines and tester indicates that parents possess enormous diversity for yield associated traits. Mean square due to line effect was significant for all the traits except ASI and shelling percentage. The mean square due to tester effect was also significant for all the traits except EL, GFD and GY. The ANOVA revealed significant variation among the lines and crosses for GCA and SCA, respectively.

Estimation of General Combining Ability effects: Promising lines possessing high GCA and potential crosses with high SCA have been identified (Table 1). The maximum GCA effect for GY was observed by inbred IMLSB 1299-5 (25.63 q/ha) followed by IMLSB 406-2 (25.28 q/ha) and IMLSB 334B-2 (22.46 q/ha). The promising lines for GY based on the GCA effect, viz. IMLSB 1299-5, IMLSB 406-2, IMLSB 334B-2 and IMLSB 814-2 can be used to enhance the GY in maize-breeding programme. In contrast to the current findings, Hafiz *et al*. (2015) found non-significant GCA effects for GY. Both positive and negative GCA effects for GY have been reported in maize by several investigators (Ram *et al*. 2015, Tandzi *et al*. 2015). The lines IMLSB 976-2, IMLSB 507-1, IMLSB 156-2 and IMLSB 2039 showed negative GCA effect for DA and DS. Four lines, viz. IMLSB 976-2, IMLSB 93-2, IMLSB 285-1 and IMLSB 55-2 showed negative GCA for DM which revealed the presence of genes for earliness. A total of 20 lines showed positive and significant GCA effect for PH while 18 lines had significantly negative GCA effect. Mosa (2010) and Punewar *et al.* (2017) also observed significant positive and negative GCA effects for DM and PH. The lines IMLSB 1062-2-2 and IMLSB 2028 showed significant positive GCA effects for PH and EH indicating that these lines can contribute to tallness and possess genes for greater EH. IMLSB 164-1, IMLSB 814-2 and IMLSB 1299-2 showed positive and significant GCA effects for ear length. Three lines, viz. IMLSB 254-1, IMLSB 617-1 and IMLSB 1299-2 showed positive and significant GCA effects for number of KR, which is very important yield parameter as it directly contributes to higher GY in hybrids. Five lines, viz. IMLSB 231-1, IMLSB 334B-2, IMLSB 1043-1-1, IMLSB 1299-5 and IMLSB 1062-2-2 were identified as good general combiner for K/R. Selection of parents with high GCA effect for KR and K/R would bring favourable alleles. Similarly, IMLSB 83-1, IMLSB 171-2, IMLSB 1047-1-1 and IMLSB 1299-2 for EG and IMLSB 126-2, IMLSB 301-2 and IMLSB 571-2 for shelling percentage showed positive and significant GCA effects. The GCA effects of testers revealed that BML 6 was good combiner for DA, DS, DM, PH, EG, KR, K/R and shelling percentage while BML 7 was good combiner for GY, EL and ASI.

Estimation of Specific Combining Ability Effects: A total of 26 crosses out of 122 have showed positive and significant SCA effect for GY. IMLSB 219-2×BML 6, IMLSB 173-2×BML 7 and IMLSB 343-3×BML 7 were promising for yield (Table 1). The identified good combiners for yield can be utilized for generating superior cross combinations (Shushay *et al*. 2013). The promising cross combinations with negative significant SCA effects for DA were IMLSB 23-2×BML 7, IMLSB 83-1×BML 6, IMLSB 114-1×BML 7 and for DS were IMLSB 23-2×BML 7, IMLSB 55-2×BML 6, IMLSB 119×BML 6 which indicates earliness. Three promising crosses, viz. IMLSB 2028×BML 6, IMLSB 976-2×BML 6 and IMLSB 119×BML 6 showed negative significant SCA effects for DM; suitable for earliness. The results are in conformity with earlier findings (Mosa 2010; Singh *et al*. 2010). The promising crosses for reduced plant height were IMLSB 457-2×BML 7, IMLSB 457-2×BML 6 and IMLSB 2039×BML 7. These hybrids can serve as source for deriving inbred lines that can confer lodging resistance (Asif *et al.* 2014). IMLSB 49-2×BML 6, IMLSB 49-2×BML 7 and IMLSB 173-2×BML 7 showed significant positive SCA effect for EL. Similarly IMLSB 457-1×BML 6, IMLSB 285-1×BML 7 and IMLSB 406-2×BML 6 showed significant positive SCA effects for K/R. IMLSB 43-2×BML 6, IMLSB 171-1×BML 6 and IMLSB 2166×BML 7 showed positive significant SCA effects for EG. The current findings agree with earlier findings (Ali *et al*. 2012, Singh *et al.* 2019) who reported significant positive SCA effects for ear diameter and ear girth.

Heterotic grouping: Grain yield, GCA and SCA effects were considered to group the lines into different heterotic groups. Twenty nine of the 61 lines showed positive GCA and SCA effects and higher GY over grand mean. Based on the positive SCA effects against the testers, 17 and 12 inbred lines were assigned to HG 'B' and 'A' respectively (Table 2). Thus, heterosis can be exploited by crossing between inbred lines belonging to opposite heterotic groups. The inbreds IMLSB 219-2, IMLSB 406-2 and IMLSB 814-2 belonging to HG 'B' and IMLSB 114-1, IMLSB 334B-2

Table 2 Heterotic grouping of inbred lines based on the positive GCA and SCA effects

Inbred	Yield (q/ha)		GCA	SCA		Heterotic Groups
	BML 6	BML 7		BML 6 (B group)	BML 7 (A group)	
IMLSB 23-2	74.12	81.20	-15.622**	-3.298	3.298	
IMLSB 43-2	81.16	77.94	-13.729**	1.848	-1.848	
IMLSB 49-2	77.29	101.60	-3.837	-11.920**	11.920**	
IMLSB 54-2	80.51	78.02	-14.021**	1.483	-1.483	
IMLSB 55-2	100.14	105.57	9.571**	-2.478	2.478	
IMLSB 58-1	82.55	81.53	-11.239**	0.748	-0.748	
IMLSB 81-1	82.54	83.56	-10.232**	-0.272	0.272	
IMLSB 83-1	82.49	96.51	-3.779	-6.772*	6.772*	
IMLSB 86-2	79.63	80.30	-13.321**	-0.097	0.097	
IMLSB 91-2	80.87	86.40	-9.647**	-2.527	2.527	
IMLSB 93-2	83.91	71.00	-15.827**	6.697*	-6.697*	
IMLSB 100-1	106.72	86.55	3.354	10.322**	-10.322**	B
IMLSB 107-2	89.56	86.11	-5.447*	1.963	-1.963	
IMLSB 114-1	85.48	115.41	7.161**	-14.728**	14.728**	A
IMLSB 119-1	92.02	92.60	-0.972	-0.048	0.048	
IMLSB 126-2	104.53	89.15	3.558	7.928*	-7.928*	B
IMLSB 128-1	78.83	93.91	-6.911**	-7.300*	7.300*	
IMLSB 156-2	86.41	103.14	1.491	-8.128*	8.128*	A
IMLSB 162-1	80.03	91.72	-7.406**	-5.608	5.608	
IMLSB 164-1	86.75	95.56	-2.124	-4.167	4.167	
IMLSB 166-2	101.30	96.47	5.601*	2.655	-2.655	B
IMLSB 171-2	99.00	92.79	2.616	3.343	-3.343	B
IMLSB 173-2	63.71	101.98	-10.437**	-18.897**	18.897**	
IMLSB 181-2	87.81	106.60	3.924	-9.155 **	9.155**	A
IMLSB 190-1	84.85	79.83	-10.946**	2.748	-2.748	
IMLSB 219-2	123.95	77.68	7.529**	23.373**	-23.373**	B
IMLSB 231-1	114.54	98.49	13.236**	8.263*	-8.263*	B
IMLSB 254-1	95.15	106.11	7.348**	-5.242	5.242	A
IMLSB 274-1	92.07	98.66	2.086	-3.057	3.057	A
IMLSB 285-1	96.99	118.44	14.434**	-10.488**	10.488**	A
IMLSB 301-2	111.72	91.03	8.093**	10.580**	-10.580**	B
IMLSB 306-1	103.60	77.36	-2.801	13.357**	-13.357**	
IMLSB 334B-2	115.27	116.24	22.469**	-0.247	0.247	A
IMLSB 342-1	108.18	95.91	8.761**	6.372	-6.372	B
IMLSB 343-3	79.00	112.13	2.284	-16.328**	16.328**	A
IMLSB 406-2	127.20	109.93	25.283**	8.873**	-8.873**	B
IMLSB 428-2	100.22	76.38	-4.982*	12.158**	-12.158**	
IMLSB 457-2	88.96	87.74	-4.934*	0.850	-0.850	
IMLSB 507-1	69.37	92.34	-12.429**	-11.248**	11.248**	
IMLSB 508-1	80.56	100.41	-2.797	-9.690**	9.690**	
IMLSB 561-1	70.68	87.45	-14.221**	-8.147*	8.147*	
IMLSB 571-2	92.55	90.02	-1.996	1.502	-1.502	
IMLSB 592-2	98.79	91.13	1.678	4.072	-4.072	B
IMLSB 617-1	95.71	80.82	-5.014*	7.683*	-7.683*	

Contd.

Table 2 (*Concluded*)

Inbred	Yield (q/ha)		GCA	SCA		Heterotic Groups
	BML 6	BML 7		BML 6 (B group)	BML 7 (A group)	
IMLSB 719-1	79.82	90.18	-8.284**	-4.943	4.943	
IMLSB 758-1	83.54	90.58	-6.226**	-3.282	3.282	
IMLSB 763-1	97.17	77.20	-6.096**	10.222**	-10.222**	
IMLSB 800-1	101.03	100.11	7.291**	0.698	-0.698	B
IMLSB 814-2	115.45	111.55	20.218**	2.192	-2.192	B
IMLSB 883-1	91.64	91.13	-1.896	0.495	-0.495	
IMLSB 975-2	110.52	97.92	10.934**	6.538*	-6.538*	B
IMLSB 976-2	84.53	99.49	-1.272	-7.238*	7.238*	
IMLSB 1043-1-1	92.04	101.15	3.313	-4.320	4.320	A
IMLSB 1047-1-1	99.02	90.98	1.721	4.258	-4.258	B
IMLSB 1062-2-2	97.77	108.40	9.803**	-5.077	5.077	A
IMLSB 1299-2	99.22	83.29	-2.026	8.202*	-8.202*	
IMLSB 1299-5	117.54	120.30	25.638**	-1.138	1.138	A
IMLSB 2028	105.30	96.09	7.411**	4.842	-4.842	B
IMLSB 2039	101.83	90.07	2.668	6.118	-6.118	B
IMLSB 2083	105.84	85.53	2.399	10.393 **	-10.393**	B
IMLSB 2166	76.70	87.06	-11.406**	-4.942	4.942	
Average	93.04	93.52				

*, ** Significant at P=0.05 and 0.01, respectively.

and IMLSB 1299-5 belonging to HG ‘A’ resulted >10% yield superiority over best check when crossed with BML 6 and BML 7 respectively. Hundera (2017) and Singode *et al.* (2017) have reported heterotic grouping studies based on SCA effects for grain yield.

Economic heterosis: The economic heterosis was estimated over national checks, viz. DHM 117, DKC 9081 and P-3396 for grain yield. Out of 122 crosses eight, one and five crosses were significantly superior over DHM 117, DKC 9081 and P-3396 respectively. Similar findings were observed for economic heterosis on grain yield over the checks (Rushwandi *et al*. 2015, Karim *et al*. 2018). Hence the identified superior hybrids need to be further exploited for multilocation trials.

Heterotic grouping of inbred lines enhances the efficiency of hybrid-breeding programme by increasing the frequency of heterotic hybrids. The study indicated the presence of sufficient genetic variability to initiate future breeding programme. The lines namely, IMLSB 334B 2, IMLSB 406-2 and IMLSB 1299-5 were identified as good general combiners for grain yield can be exploited for the development of heterotic single cross hybrids. The study has identified several superior crosses, viz. IMLSB 219-2×BML 6, IMLSB 173-2×BML 7 and IMLSB 343-3×BML 7 for grain yield. However, multi-location testing would help to identify widely adaptable maize hybrids. Based on the information generated for heterotic grouping, the inbred lines of these two groups can also be used for the development of heterotic pools. The information on heterotic pattern among different lines will aid in making pedigree crosses between selected inbred lines within heterotic groups to develop new and improved inbred lines which will save time, increase the efficiency and also accelerate the pace of the breeding programme.

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