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Heterotic grouping of maize inbred lines using line x tester analysis

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Abstract
The efficiency of the assignment of germplasm lines into heterotic groups is a prerequisite for obtaining useful heterotic patterns among germplasm lines. Therefore, this study was conducted to group lines into heterotic group besides assess the effects of both general and specific abilities. Fifteen white maize parental lines were crossed with two testers, i.e. parental line SK-12 and Single cross 120 at Agricultural Research Station in Sakha, Egypt during 2021 summer season. The resultant 30 crosses were evaluated along with four commercial hybrids in three locations during 2022. The parental inbred lines Sk5001/42, Sk5003/48, Sk5003/53, Sk5004/55 and Sk5004/56 recorded positive GCA effect on grain yield while Sk5001/41, Sk5002/46 and Sk5004/56 exhibited desirable GCA effects for earliness. Five single crosses viz. (Sk5003/53 × Sk12), (Sk5001/42 × Sk12), (Sk5004/55 × Sk12), (Sk5003/48 × Sk12) and (Sk5004/56 × Sk12) and two three way crosses (Sk5001/42 × SC.120) and (Sk5003/53 × SC.120) did not differ significantly against their respective check hybrids (SC 10 and TWC 321) though they outyield their respective checks. These hybrids could be subjected to further multi-location evaluation to assess the yield stability for commercial exploitation. Two groups of parental lines were created based on Heterotic Group Specific and General Combining Ability (HSGCA) method for grain yield trait. These groups might be employed in a breeding program to pick the best parents to make crosses in other combinations.

Keywords: Heterotic group, Zea mays, GCA, SCA, HSGCA.

INTRODUCTION

Maize (Zea mays ssp. mays) is considered the second widely cultivated crop in the world after wheat, which is cultivated on an estimated 197 million hectares (FAOSTAT, 2021). It is a versatile crop with high genetic diversity which enables its sowing in a variety of climate globally (tropical, subtropical climate worldwide and temperate) (Izhar and Ckahraborty, 2013). Maize is utilized for a variety of industrial uses, human food, and animal feed. It has the highest yield potentiality among the cereal crops. Around 1147.7 million MT of maize is being produced globally with an average productivity of 5.75 t/ha (FAOSTAT, 2020). During 2022, it was cultivated in an area of one million hectares in Egypt with production of 7.7 million metric tonnes and productivity of 7.69 ton per hectare. However, Egypt imports approximately 8 million tonnes of maize annually to tackle the production and consumption gap (Economic Sector, 2022). The expansion of corn cultivation is difficult in Egypt due to limited arable land and limited water. Therefore, increasing productivity would be solely possible by genetic improvement. Parental selection is crucial for the creation of hybrids. Breeders regularly employed the line x tester method (Kempthome, 1957) as one of the available biometrical techniques to assess combining ability (Kumara *et al.,* 2013, Suhaisini *et al.,* 2016, Patil *et al.,* 2020, Ismail *et al.,* 2022; Job and Igyuve, 2022, Subba *et al.,* 2022 and Abd El-Latif *et al.,* 2023). The potential of any inbred line in hybrid breeding to produce superior hybrids ultimately count on its ability when combined well with other lines. Hence, combining ability assessment is a crucial method for identifying potential parents which are more likely to pass on desirable traits to their offspring and to find the best specific cross(s) for yield. Breeders' selection and development of single cross hybrids are aided by knowledge of the heterotic patterns and combining ability of the parents and crosses. In order to exploit heterosis to facilitate the creation of hybrids, it is crucial to group germplasms into various heterotic groupings and patterns (Barata and Carena, 2006, Reif *et al.,* 2007, Badu-Apraku *et al.,* 2015; Meena *et al.,* 2017; Fan *et al.,* 2018). Melchinger and Gumber (1998) defined a heterotic group as a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups. Contrarily, a heterotic pattern is a unique pair of heterotic groups exhibiting desired heterosis and hybrid performance after crossing. According to Lee (1995), a heterotic group is a collection of germplasm expressing higher heterosis in combination with germplasm from an external group than when crossed with a member of its own group. Crosses derived from unrelated heterotic groups are considered to have yield potentiality than those crosses developed from lines in the same group (Melchinger 1999, Fan *et al.,* 2008; Meena *et al.,* 2017; Abd El-Latif *et al.,* 2020; Annor *et al.,* 2020; Mosa *et al*., 2021). Extensive research on assignment of lines into heterotic groups has not been conducted in the Egypt. The performance of the lines in test crosses with proven testers can be utilised as a primary criterion for grouping lines when a significant number of lines are available and there are proven testers. (Tulu *et al.,* 2021, Kumar *et al.,* 2023). The SCA effects facilitate breeders to identify heterotic patterns among populations or inbred lines to determine the desired single crosses and group them into heterotic groups (Lahane *et al.* , 2014). Heterotic patterns are crucial because they facilitate breeders to select the germplasm to be utilised in hybrid production over a long period thus simplifying management and organization of germplasm (Nepir *et al.,* 2015 and Oppong *et al.,* 2020). The current study was carried out to assess the combining abilities and gene effects of grain yield and different agronomic traits, to utilise HSGCA method for grouping the parental lines into heterotic groups and to identify promising crosses which may go into seed production.

MATERIALS AND METHODS

Genetic materials: The genetic materials employed in this research comprised of 15 inbred lines *viz.,* Sk5001/40, Sk5001/41, Sk5001/42, Sk5001/43, Sk5001/44, Sk5002/45, Sk5002/46, Sk5003/48, Sk5003/50, Sk5003/51, Sk5003/52, Sk5003/53, Sk5003/54, Sk5004/55, Sk5004/56 used as female parents and two types of testers as male parents namely inbred line Sk-12 (T_1) and single cross SC.120 (T_2) and four checks *i.e.* two Single crosses (SC.10) and Hytech SC. 2031 and two Three way crosses TWC.321) and Pioneer TWC Fadda.

Experimental design and field managements: Fifteen female parents and two male testers were crossed at Sakha Research Station in summer season 2021 in line x tester fashion to generate 30 F_1 top crosses as out lined by Kempthorne (1957). In 2022 season, the resulting 30 F_1 topcrosses along with the four check hybrids were evaluated at three locations *viz*., Sakha, Sids and Mallawy Agricultural Research Stations, Egypt. The experiments were laid out in a randomized complete blocks design with three replications at each location. Each entry was sown in one row of 6 m long with the distance between rows being 80 cm and between plants being 25 cm. All recommended agronomic package were adopted to maintain good crop standing for all entries.Observations were recorded on days to 50% silking, plant height (cm), ear height (cm) and grain yield per plot (kg) adjusted at 15.5% grain moisture and finally converted to (ton/ha).

Statistical analysis: The combined Analysis of Variance (ANOVA) in accordance with Snedecor and Cochran (1989) was performed on the recorded data after testing the uniformity of error variance across locations for all attributes under study. Least significant difference (LSD) was performed for mean comparisons. General combining ability (GCA) and specific combining ability (SCA) effects for recorded traits were assessed based on the method described by Kempthorne (1957) using SAS software (SAS-Institute Inc. 2008). The combining ability analyses did not involve checks.

Heterotic group specific and general combining ability (HSGCA) method according to (*Fan et. al.,* 2009) was employed for grouping inbred lines into heterotic groups.

RESULTS AND DISCUSSION

Analysis of variance: The ANOVA for four attributes across three locations revealed that the locations mean squares were extremely significant, reflecting that the climate and soil conditions varied between the locations (**Table 1**). These findings were congruent with the results obtained by Ismail *et al.* (2018), Tesfaye *et al.* (2019) and Ismail *et al.* (2022). Similarly, the hybrid mean squares for all the attributes under study were identified to be highly significant, indicating that the crosses were unique from one another for these characters. Consequently, selection is feasible to pick the most desired hybrids. Similar findings were reported by Akinwale *et al.* (2014),Tulu *et al.* (2018), Abebe *et al.* (2020), Tulu *et al.*(2021). Interaction between hybrids and locations mean squares were extremely significant ($P < 0.01$) for grain yield, revealing that the hybrids performance were not stable across locations for this trait. This is similar to the findings of Nepir *et al.* (2017), Annor *et al.* (2020) and Keimeso *et al.* (2020) for grain yield.

Table 1. Analysis of variance for DS, PH, EH and GY traits across three locations

Source	d.f	DS	PH	EH	GY
Location (Loc)	2	1307.3**	180229.0**	88068.5**	719.98**
Rep/Loc	6	51.9	2197.1	1146.0	1.60
Hybrids (H)	33	5.49**	1650.5**	780.6**	8.06**
H × Loc	66	2.97	216.8	142.3	3.38**
Error	198	2.45	174.8	106.0	0.77

** indicated significant at 1% level of probability.
DS= Days to 50% silking (day), PH= Plant height (cm), EH= Ear height (cm), GY= Grain yield (ton/ha).

Table 2. Mean performance of DS, PH, EH and GY characters of 30 hybrids of maize across three locations

Hybrid	DS (day)	PH (cm)	EH (cm)	GY (ton/ha)
Sk5001/40 × Sk12	65.7	236.7	126.8	9.27
Sk5001/41 × Sk12	65.1	213.8	110.3	10.01
Sk5001/42 × Sk12	65.7	233.6	116.9	10.95
Sk5001/43 × Sk12	66.3	224.1	114.2	10.11
Sk5001/44 × Sk12	65.7	233.2	119.1	9.42
Sk5002/45 × Sk12	66.3	229.6	118.8	9.08
Sk5002/46 × Sk12	64.9	233.8	117.7	9.72
Sk5003/48 × Sk12	65.8	250.4	124.8	10.57
Sk5003/50 × Sk12	66.0	232.1	118.0	9.05
Sk5003/51 × Sk12	65.6	222.6	118.7	9.04
Sk5003/52 × Sk12	66.6	210.0	106.6	8.66
Sk5003/53 × Sk12	65.6	249.2	129.1	11.05
Sk5003/54 × Sk12	66.6	236.2	124.1	10.15
Sk5004/55 × Sk12	65.8	235.6	115.4	10.79
Sk5004/56 × Sk12	64.2	240.6	126.0	10.55
Sk5001/40 × SC120	65.1	252.2	137.0	7.68
Sk5001/41 × SC120	64.0	230.9	120.2	8.07
Sk5001/42 × SC120	64.9	246.3	124.1	10.65
Sk5001/43 × SC120	66.4	245.1	131.4	9.32
Sk5001/44 × SC120	65.7	234.8	123.3	8.26
Sk5002/45 × SC120	66.4	238.9	125.6	9.05
Sk5002/46 × SC120	64.2	247.1	133.3	8.60
Sk5003/48 × SC120	65.9	256.6	136.2	9.69
Sk5003/50 × SC120	65.1	232.9	118.6	8.47
Sk5003/51 × SC120	64.8	251.1	132.6	9.51
Sk5003/52 × SC120	64.1	226.3	116.1	8.31
Sk5003/53 × SC120	65.3	262.2	143.6	10.87
Sk5003/54 × SC120	65.9	248.9	133.1	7.90
Sk5004/55 × SC120	64.0	243.7	126.0	9.64
Sk5004/56 × SC120	64.4	256.3	138.4	9.62
Check SC.10	66.0	265.2	142.1	10.48
Check SC. 2031	66.4	254.9	132.6	9.98
Check TWC.321	65.9	254.3	136.2	10.62
Check TWC. Fadda	65.8	261.7	137.7	9.85
LSD 0.05	1.45	12.21	9.51	0.81
LSD 0.01	1.90	16.01	12.47	1.07

DS= Days to 50% silking (day), PH= Plant height (cm), EH= Ear height (cm), GY= Grain yield (ton/ha).

Mean Performance: Mean performance of the 30 crosses along with four checks (two single crosses and two three way cross) for all the measured traits are presented in **Table 2**. For days to 50 % silking, the hybrid Sk5004/56 × Sk12 (64.2 day) was significantly earlier than the earliest check SC.10 (66 day). Similarly, the three way crosses namely Sk5001/41 × SC120, Sk5002/46 × SC120, Sk5003/52 × SC120 and Sk5004/55 × SC120 were significantly earlier than the earliest check TWC. Fadda. A total of 13and 10 single crosses recorded significantly low values than the best check SC. 2031 for plant and ear heights, respectively. Meanwhile, five and seven three way crosses exhibited significant lowest values for plant height and ear height when compared to the top check TWC 321, respectively. The lowest value amongst the single crosses was recorded in the cross Sk5003/52 × Sk12 for plant and ear heights. Among the three way crosses, the lowest value for plant height was recorded by Sk5001/41 × SC120. While the cross Sk5003/50 × SC120 recorded the lowest value for ear height. With respect to grain yield , five single crosses and two three way crosses exceeded insignificantly the best check hybrids SC.10 (10.48 ton/ha) and TWC.321, (10.62 ton/ha) respectively. The best yield recorded by the cross Sk5003/53 × Sk12 (11.05 ton/ha) followed by Sk5001/42 × Sk12 (10.95 ton/ha) for single crosses while the three way cross Sk5003/53 × SC120 (10.87 ton/ha) followed by Sk5001/42 × SC120 (10.65 ton/ha) obtained the best yield amongst the three way crosses. These findings confirmed that the inbred lines Sk5001/42 and Sk5003/53 gave the best single and three way crosses. Therefore, these hybrids could be used for commercial cultivation after being subjected to additional multi-location evaluation to determine the yield stability across various environments.

Line x Tester analysis of variance: ANOVA for lines (L) and Testers (T) revealed significance of mean squares for all the traits suggesting that prenatal lines and testers had greater diversity among them. These findings are congruent with the earlier study of Girma *et al.* (2015), Chandel *et al.* (2019), Diviya *et al.* (2022), Lal *et al.* (2022), Ismail *et al.* (2022) and Tabu *et al.* (2023). The L × T interactions mean squares was highly significant for grain yield. The significant differences observed among the lines, testers and line-by-tester suggested both additive and non-additive gene effects exist in controlling these traits (Kumar *et al.,* 2015, Ismail *et al.,* 2022 and Tabu *et al.,* 2023). Significant L × Loc mean squares was observed for days to 50% silking. Meanwhile, L × Loc, T × Loc and L ×T × Loc mean squares for grain yield were highly significant, indicating that L and T and L ×T behaved somewhat differently from one location to another for this trait. The presented results showed that the variance due to lines and testers (additive gene effects) was greater than that due to L ×T interaction variance (non-additive gene effects) for all the measured traits, indicating that the predominance of additive effects in the inheritance of these traits (Dhasarathan *et al.,* 2015 and Tabu *et al*., 2023).

General combining ability effects: The general combining ability effects (ĝi) of the 15 parental lines and two testers for the measured traits are furnished in **Table 4**. From the breeder's perspective, high negative values for silking emergence, plant height, and ear height together with high positive values for yield,, would be beneficial for maize breeding programs.

The tester Sk-12 expressed the desirable (ĝi) effects for plant height, ear height and grain yield. While the parental tester SC.120 possessed the negative desirable values solely for earliness. For days to 50% silking, the parental lines Sk5001/41, Sk5002/46 and Sk5004/56 expressed negatively significant (ĝi) effects. These lines could play vital role for breeding to earliness. For plant height, the desirable (ĝi) effects were recorded by the lines Sk5001/42 and Sk5003/48. Hence, these lines could be considered as good combiner for short stature hybrids breeding. The inbred lines Sk5001/41and Sk5003/52 could play a vital role due to their significant negative GCA effects for ear hight, reflecting their potential to decrease lodging and subsequently increasing grain yield indirectly. Five inbred lines *viz*. Sk5001/42, Sk5003/48, Sk5003/53, Sk5004/55 and Sk5004/56 showed significant desirable GCA effects for grain yield. Hence these lines could be exploited in breeding program for high grain yield trait.

Table 3. Line x tester analysis for DS, PH, EH and GY over three locations

Source	d.f	DS	PH	EH	GY
Line	14	7.5**	1860.0**	841.4**	11.52**
Tester	1	26.1**	11059.2**	7032.9**	49.21**
Line ×Tester	14	2.5	229.2	86.4	2.39**
Line × Loc	28	4.0*	227.1	144.9	4.04**
Tester × Loc	2	1.2	35.5	246.4	3.08**
Line ×Tester × Loc	28	2.6	149.1	108.2	2.28**
Error	174	2.5	170.2	103.8	0.70

*, ** indicated significant at 5% and 1% levels of probability, respectively.
DS= Days to 50% silking (day), PH= Plant height (cm), EH= Ear height (cm), GY= Grain yield (ton/ha).

Table 4. General combining ability effects ($\hat{g}_i$) of 15 inbred lines and two testers for the measured traits across three locations

Lines	DS	PH	EH	GY
Sk5001/40	-0.01	5.96	7.69**	-0.99**
Sk5001/41	-0.84*	-1.66	-8.92**	-0.42*
Sk5001/42	-0.12	-20.32**	-3.70	1.33**
Sk5001/43	0.99**	17.23**	-1.37	0.25
Sk5001/44	0.27	4.07	-2.98	-0.62**
Sk5002/45	0.99**	1.12	-2.03	-0.40*
Sk5002/46	-0.84*	9.96**	1.30	-0.31
Sk5003/48	0.43	-16.16**	6.30**	0.66**
Sk5003/50	0.16	1.46	-5.92*	-0.70**
Sk5003/51	-0.23	-3.88	1.41	-0.19
Sk5003/52	-0.07	-4.49	-12.87**	-0.98**
Sk5003/53	0.04	-4.27	12.13**	1.49**
Sk5003/54	0.82*	1.96	4.41	-0.44*
Sk5004/55	-0.51	15.01**	-3.48	0.74**
Sk5004/56	-1.07**	-5.99	8.02**	0.61**
LSD gi 0.05	0.73	6.03	4.71	0.39
LSD gi 0.01	0.96	7.93	6.20	0.51
Tester Sk-12	0.31*	-6.40**	-5.10**	0.42**
Tester SC120	-0.31*	6.40**	5.10**	-0.42**
LSD gi 0.05	0.27	2.20	1.72	0.14
LSD gi 0.01	0.35	2.90	2.26	0.19

*, ** indicated at 5% and 1% levels of significance.

DS= Days to 50% silking (day), PH= Plant height (cm), EH= Ear height (cm), GY= Grain yield (ton/ha).

Specific combining ability effects: Specific combining ability effects of the 30 crosses for all measured traits is furnished in **Table 5**.

The single crosse (Sk5003/54 x SK-12) and the three way crosse and (Sk5003/51 x SC.120) had significant and positive SCA effects for grain yield trait. These crosses could be exploited for heterosis breeding.

Heterotic group: The inbred lines were grouped in to heterotic groups based on specific and general combining ability (HSGCA) effects for grain yield according to Fan *et al.* (2009). The lines Sk5001/42, Sk5003/51, Sk5003/52 and Sk5003/53 were grouped with the tester Sk-12 and the inbred lines Sk5001/40, Sk5001/41, Sk5001/44, Sk5003/48, Sk5003/54 and Sk5004/56 were grouped with the tester SC.120. Nevertheless, this method was unable to classify the inbred lines Sk5001/43, Sk5002/45, Sk5002/46, Sk5003/50 and Sk5004/55. In the context, Lee (1995), Mosa *et al.,* 2017 and Ismail *et al.,* 2022 stated that, the heterotic group is a collection of closely related inbred lines which tend to result in vigorous hybrids when crossed with lines from a different heterotic group, but not when crossed to other lines of the same heterotic group. Hence, Heterotic group method could be recommended in breeding programs for selecting the diverse parents to make crossing between them. These findings could be exploited in making crosses between the two group and to utilize the heterosis.

From this study, Sk5001/43, Sk5002/45, Sk5002/46, Sk5003/50 and Sk5004/55 had desirable GCA effects for grain yield across research environments. While, the inbred lines Sk5001/41, Sk5002/46 and Sk5004/56 possessed desirable GCA effects for earliness. Favourable alleles from these inbreds might be introgressed to develop maize hybrids with high yielding and early maturity. Three single hybrids Sk5001/42 × Sk12, Sk5003/53 × Sk12 and Sk5004/55 × Sk12 and two three way crosses Sk5001/42 × SC120 and Sk5003/53 × SC120 had registered high grain yield. As a result, these hybrids could be used for commercial cultivation after being put through additional multi-location evaluation to determine their yield stability in a variety of environments. The HSGCA heterotic grouping method was able to classify 10 inbred lines out of 15 lines. These two groups could be used in the breeding program for picking the wanted parents for making crosses.

Table 5. Estimates of specific combining ability (*sca*) effects of 30 cross combination for four studied traits

Lines	DS		PH		EH		GY	
	Testers		Testers		Testers		Testers	
	SK.12	SC.120	SK.12	SC.120	SK.12	SC.120	SK.12	SC.120
Sk5001/40	-0.03	0.03	-1.38	1.38	-0.01	0.01	0.37	-0.37
Sk5001/41	0.24	-0.24	-2.16	2.16	0.16	-0.16	0.54	-0.54
Sk5001/42	0.08	-0.08	0.01	-0.01	1.49	-1.49	-0.27	0.27
Sk5001/43	-0.37	0.37	-4.10	4.10	-3.51	3.51	-0.03	0.03
Sk5001/44	-0.31	0.31	5.62	-5.62	2.99	-2.99	0.16	-0.16
Sk5002/45	-0.37	0.37	1.73	-1.73	1.71	-1.71	-0.41	0.41
Sk5002/46	0.02	-0.02	-0.27	0.27	-2.73	2.73	0.13	-0.13
Sk5003/48	-0.37	0.37	3.34	-3.34	-0.62	0.62	0.01	-0.01
Sk5003/50	0.13	-0.13	6.01	-6.01	4.83	-4.83	-0.13	0.13
Sk5003/51	0.08	-0.08	-7.88	7.88	-1.84	1.84	-0.66*	0.66*
Sk5003/52	0.91	-0.91	-1.77	1.77	0.33	-0.33	-0.25	0.25
Sk5003/53	-0.20	0.20	-0.10	0.10	-2.12	2.12	-0.33	0.33
Sk5003/54	0.02	-0.02	0.07	-0.07	0.60	-0.60	0.70*	-0.70*
Sk5004/55	0.58	-0.58	2.34	-2.34	-0.17	0.17	0.15	-0.15
Sk5004/56	-0.42	0.42	-1.49	1.49	-1.12	1.12	0.04	-0.04
LSD Sij 0.05	1.03		8.52		6.66		0.55	
LSD Sij 0.01s	1.36		11.22		8.76		0.72	

* indicated significant at 5% level of significance.

Table 6. Estimates of heterotic groups based on specific and general combining ability method (HSGCA) for grain yield across the three locations

Lines	Mean Performance (ton/ha)		HSGCA	
	SK.12	SC.120	SK.12	SC.120
Sk5001/40	9.27	7.68	-0.63	-1.36#
Sk5001/41	10.01	8.07	0.35	-0.74#
Sk5001/42	10.95	10.65	-1.26#	-0.71
Sk5001/43	10.11	9.32	1.46	1.52
Sk5001/44	9.42	8.26	-0.29	-0.60#
Sk5002/45	9.08	9.05	0.34	1.16
Sk5002/46	9.72	8.60	0.75	0.48
Sk5003/48	10.57	9.69	-0.42	-0.44#
Sk5003/50	9.05	8.47	1.20	1.46
Sk5003/51	9.04	9.51	-0.41#	0.91
Sk5003/52	8.66	8.31	-0.88#	-0.37
Sk5003/53	11.05	10.87	-0.74#	-0.07
Sk5003/54	10.15	7.90	0.39	-1.01#
Sk5004/55	10.79	9.64	0.81	0.51
Sk5004/56	10.55	9.62	-0.67	-0.75#

means that this inbred line belongs to tester group.

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