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Research Article

Comparison of combining ability based heterotic grouping methods and assigning a set of germplasm lines to existing testers in *rabi* sorghum[*Sorghum bicolor* (L.) Moench]

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Abstract
In the present study, 31 male lines and three females, were crossed in Line × Tester mating design and 93 hybrids were produced to assign these lines to three testers *viz.,* 104A, 401A and M31-2A. Analysis of variance exhibited significant *gca* and *sca* effects for grain yield, indicating that significant breeding progress could be achieved using both inbreeding and hybridization. Heterotic grouping based on combining ability for grain yield would be useful in planning crosses in breeding programmes. In the current study, combining ability based heterotic grouping methods such as HSGCA and SCA-PY classified sorghum lines into four groups. Further, six different potential parental combinations were determined based on the HSGCA method. These combining abilities based heterotic grouping study is an additional tool that breeders could use to identify the best parents for superior hybrids development. Among the three testers, M31-2A followed by 104A was found to be the best testers.

Keywords: Combining ability, L x T, Heterotic grouping, HSGCA, SCA-PY

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the world's fifth most significant cereal crop in terms of production and consumption, after wheat, rice, maize, and barley. Sorghum grain is a dietary staple for millions of people in Asia and Africa's semi-arid regions, where drought stress frequently causes other crops to fail. Sorghum grain is primarily consumed in Africa and Asia, although it is also fed to cattle in the United States and Australia (Reddy *et al*., 2013). Due to its versatile use as a source of food, feed, fodder and fuel, it is under cultivation in tropical, subtropical and even in temperate regions of the world as great millet. The world's 57.50 million tonnes of sorghum production in 2019–2020 comes from a 40.28 million hectare area with a productivity of 1.43 tonnes per hectare (FAOSTAT 2020). The concept of the heterotic grouping (Melchinger and Gamber, 1998) was proposed to identify genetically diverse parents for exploitation of heterosis through the development of potential hybrids. Heterotic grouping studies of germplasm lines facilitates exploitation in breeding and the choice of suitable parents for superior hybrid combinations (Akinwale *et al.,* 2014). Combining ability is the capacity of an individual to transmit a superior performance to its offspring. It provides information on gene effects in controlling the inheritance of traits of interest and helps in selecting the parents to be included in cultivar improvement or hybridization programmes. It is the best way to test the value of germplasm lines and identify the best parents to produce superior hybrids (Kanawade *et al.,*2001; Kenga *et al.,* 2004; Mindaye *et al.,* 2016). As the performance of lines crossed with testers could be used as criteria for grouping the lines (Melchinger, 1999), using combining ability information for heterotic grouping of germplasm lines may help to select superior hybrid parents to exploit

maximum heterosis. The term "heterotic grouping" refers to the finding of genetically different groups that create superior hybrids when crossed. Researchers have been using morphological *per se* performance (Barro-Kondombo *et al*., 2008; Sawadogo *et al*., 2014) and genetic relationship (Zongo *et al*., 2005; Deu *et al*., 2006; Sagnard *et al*., 2011; Billot *et al*., 2013) method to classify available germplasm lines into distinct heterotic groups and to identify suitable parents for hybridization. However, various approaches using the information of combining ability for heterotic grouping, described in maize, showed the utility of such methods in identifying suitable hybrid parents (Fan *et al*., 2009; Badu-Apraku *et al*., 2013; Akinwale *et al*., 2014; Oyetunde *et al.,* 2020; Annor *et al*.,2020). Heterotic groups specific and general combining ability (HSGCA) and Specific combining ability and pedigree yield (SCA-PY) are the quantitative methods (Fan *et al.,* 2009), uses combining ability estimates of parental lines and hybrids yield data to assign lines. An important drawback of the SCA-PY method is the significant influence of the interaction between two parents, as well as between genotype and environment, leading to assignment of the same lines into different heterotic groups in different studies (Wu *et al.,* 2007; Fan *et al.,* 2001). To address the demerits of the SCA approach, Fan *et al*. (2009) proposed the HSGCA method, which used SCA and GCA effects of grain yield; both methods utilized grain yield data to assign inbred lines into heterotic groups. The efficiencies of various methods studied in various crops. Akinwale *et al*. (2014), Badu-Apraku *et al*. (2013) and Amegbor *et al.* (2017) compared the efficiencies of different grouping methods and found the HSGCA to be the most efficient method.

The discovery of male sterility in sorghum (Stephens and Holland, 1954), the key to hybrid development, allows heterosis to be exploited to increase sorghum production. Thus, there is a need of information on combining ability based heterotic grouping, to select more potential heterotic parents to use in hybridization. The present study aimed (i) to identify the best testers for heterotic grouping, (ii) To classify the germplasm lines into the different heterotic groups and (iii) to determine the potential heterotic combinations based on the superior heterotic grouping method.

MATERIALS AND METHODS

A total 34 parents furnished in **Table 1**, were crossed in a line × tester fashion in *kharif*- 2017. The 31 germplasms and three male sterile lines were considered as lines and testers, respectively. Total of 93 hybrids along with parents were evaluated in Randomized Complete Block Design with two replications in *rabi*-2019 at Botany Garden, University of Agricultural Sciences, Dharwad. Each treatment had 2 rows of 3 m long and with the spacing of 45 × 15 cm. All the recommended packages of practices were followed to raise a good crop. The data was recorded on grain yield per plant (g) for each treatment.

Table 1. List of genotypes used for the study

S. No.	Genotypes	Origin	S. No.	Genotypes	Origin
1	IS 27912	South Africa	19	IS 8012	Japan
2	IS 30536	Korea	20	IS 29468	Lesotho
3	IS 28313	Yemen	21	IS 26617	Madagascar
4	IS 2413	Iran	22	IS 14861	Cameroon
5	IS 19389	Bangladesh	23	DSMR-8 (Restorer on *maldandi*)	India
6	IS 2933	Swaziland	24	IS 2397	South Africa
7	DSMR-4 (Restorer on *maldandi*)	India	25	IS 12302	Zimbabwe
8	IS 25249	Ethiopia	26	IS 29654	China
9	IS 12804	Turkey	27	IS 30451	China
10	IS 29392	Lesotho	28	IS 33353	Kenya
11	IS 7987	Nigeria	29	IS 26046	Mali
12	IS 31043	Uganda	30	IS 4698	India
13	IS 30466	China	31	IS 19445	Botswana
14	IS 4060	India	**Females**		
15	IS 29568	Lesotho	32	104A (*milo*)	India
16	IS 15945	Cameroon	33	401A(*milo*)	India
17	IS 15478	Cameroon	34	M31-2A(*maldandi*)	India
18	IS 5919	India			

The data were subjected to analysis of variance (ANOVA) as per the method outlined by Panse and Sukhatme (1967) to know the statistical significance of all the treatments. A line × tester analysis was used to determine the significance of GCA-line, GCA-tester, and SCA-hybrids by following Kempthorne, 1957. A tester giving a higher number of significant specific combinations in a positive direction was considered as most efficient tester. Heterotic grouping of the lines was performed by following two different methods:

1. HSGCA [HSGCA = Hybrid mean (ij) – Tester mean (j) of grain yield by following Fan *et al.*(2009) and
2. SCA effects for grain yield (SCA-PY)by following Menkir *et al*. (2004).

The resulting heterotic groups were represented by 104A (A), 401A (B), M31-2A (C)and (D) with no tester.

RESULTS AND DISCUSSION

The presence of significant variation among the parental material indicates adequate genetic differences among the lines, testers and hybrids for the effective selection of the grain yield (Saikiran *et al.*, 2021 and Patel *et al*., 2016). Furthermore, results indicate the presence of a heterotic response for the grain yield. A good outcome could be expected from these germplasm lines whether we follow hybridization or population improvement programmes for further improvement (Kenga *et al.,* 2004, Akinwale *et al.,* 2014 and Akata *et al.,* 2017). The significant values of both GCA and SCA indicate (**Table 2**) that both additive and dominance gene actions are important for the expression of the grain yield. This suggests that significant breeding progress could be achieved using both inbreeding and hybridization (Akata *et al*., 2017).

Classification of germplasm lines into an appropriate heterotic group is essential to maximize their potential usefulness for the development of productive hybrids and also to create new heterotic groups. Therefore, in the present study, HSGCA and SCA-PY methods were used for the classification of the germplasm lines.

The three testers *viz.,*104A, 401A and M31-2A were used as the representative of heterotic groups A, B and C, respectively. Based on the HSGCA heterotic grouping method, the HSGCA values were calculated for each of the lines in a combination of each tester (**Table 3**). The 31 lines were classified into four heterotic groups (**Table 4**).Ten lines were classified into heterotic group A (104A) as these lines exhibited high and positive HSGCA values with the tester 104A. Five lines were assigned to heterotic group B (401A) and 10 lines were classified into heterotic group C (M31-2A). Whereas, six lines failed to show positive HSGCA values with any of the testers and assigned to a different heterotic group of D with no tester. As these lines belong to a different heterotic group other than the testers used for the study.

In this method, the combining ability and mean grain yield of the lines in combination with the three testers *viz.,* 104A, 401A and M31-2A (**Table 3**) were used as the basis to classify the lines into heterotic groups. Eleven lines showing the highest and positive *sca* effects with 104A but having negative *sca* effects with 401A and M31-2A and with testcross mean grain yield greater than the mean yield of 104A × M31-2B, 104A × 401B and M31-2A × 401B were placed into 104A **(A)** heterotic group. In addition, four lines exhibiting the highest and positive *sca effects* with 401A and with testcross mean yield greater than the mean yield of 104A × M31-2B, 104A × 401B and M31-2A × 401B were placed into the 401A**(B)** heterotic group (**Table 5**). Similarly, eleven lines showing the highest and positive *sca* effects with M31-2A and with testcross mean yield greater than the mean yield of 104A × M31-2B, 104A × 401B and M31-2A × 401B were placed into the M31-2A**(C)** heterotic group. Whereas, five lines exhibited negative *sca* effects with all the representative testers and grain yield was also found to be low. These lines were assigned to a different heterotic group **(D)**with no tester**.**

The close correspondence in the classification of the lines into heterotic groups by the HSGCA and SCA-PY methods

Table 2. Analysis of variance for grain yield and combining ability in parents and hybrids

	Mean sum of square	
Sources of variation	**DF**	**Grain yield per plant**
Replication	1	17.71
Crosses	92	499.11**
Line effect	30	668.05**
Tester effect	2	492.64*
Line × Tester effect	60	414.86**
GCA		12.52**
SCA		174.19**
Error	92	153.86

* and ** significant @ 0.05 and 0.01 probability, respectively.

in terms of placement of the same number of lines into similar groups implied that both methods are efficient and equally effective. An important drawback of the SCA-PY method is the significant influence of the interaction between two parents, as well as between genotype and environment, leading to assignment of the same lines into different heterotic groups in different studies (Wu *et al.,* 2007; Fan *et al.,* 2001). The HSGCA method uses both *gca* and *sca* effects whereas the SCA-PY method uses only *sca* effects to classifying the lines into different

Table 3. Mean grain yield, specific combining ability effectsand HSGCA effects for different Line x tester combinations

lines	Mean grain yield with 104A	Mean grain yield with 401A	Mean grain yield with M31-2A	*sca* effects with 104A	*sca* effects with 401A	*sca* effects with M31-2A	Yield based heterotic group	HSGCA effct with 104A	HSGCA effct with 401A	HSGCA effct with M31-2A	HSGCA based heterotic group
IS 27912	69.80	77.34	78.75	-8.60	4.32	4.28	No tester	-16.63	-4.20	-4.49	No tester
IS 30536	69.59	66.43	102.73	-14.77	-12.55	27.32**	M31-2A	-16.84	-15.11	19.49	M31-2A
IS 28313	92.06	90.82	98.70	-4.91	-0.76	5.68	M31-2A	5.63	9.28	15.46	M31-2A
IS 2413	61.86	75.28	77.23	-12.71	6.10	6.61	No tester	-24.57	-6.26	-6.01	No tester
IS 19389	99.59	80.70	76.40	10.92	-2.59	-8.33	104A	13.16	-0.84	-6.84	104A
IS 2933	110.68	75.88	93.73	14.14	-15.28	1.13	104A	24.25	-5.66	10.49	104A
DSMR-4	80.02	69.32	75.69	1.90	-3.42	1.52	No tester	-6.41	-12.22	-7.55	No tester
IS 25249	109.90	95.44	74.18	16.94*	2.88	-19.82*	104A	23.47	13.90	-9.06	104A
IS 12804	103.84	93.50	85.68	8.79	3.84	-12.62	104A	17.41	11.96	2.44	104A
IS 29392	96.60	84.95	108.41	-4.50	-10.76	15.26	M31-2A	10.17	3.41	25.17	M31-2A
IS 7987	79.94	92.90	106.82	-18.06*	0.29	17.77*	M31-2A	-6.49	11.36	23.58	M31-2A
IS 31043	97.50	102.62	64.93	6.04	16.54*	-22.58**	401A	11.07	21.08	-18.31	401A
IS 30466	97.91	93.84	79.38	3.76	7.07	-10.83	401A	11.48	12.30	-3.86	401A
IS 4060	95.03	88.92	95.76	-1.18	-1.91	3.09	M31-2A	8.60	7.38	12.52	M31-2A
IS 29568	96.40	82.46	99.30	0.57	-7.98	7.42	104A	9.97	0.92	16.06	M31-2A
IS 15945	91.11	58.24	61.38	17.76*	-9.73	-8.03	104A	4.68	-23.30	-21.86	104A
IS 15478	98.01	70.96	90.68	11.68	-14.98	3.30	104A	11.58	-10.58	7.44	104A
IS 5919	91.87	86.78	89.64	-0.67	-0.37	1.05	M31-2A	5.44	5.24	6.40	M31-2A
IS 8012	71.34	80.79	93.16	-10.20	4.64	5.56	M31-2A	-15.09	-0.75	9.92	M31-2A
IS 29468	120.43	83.86	91.80	18.62*	-12.56	-6.06	104A	34.00	2.32	8.56	104A
IS 26617	49.42	101.82	77.22	-29.84**	27.94**	1.90	401A	-37.01	20.28	-6.02	401A
IS 14861	30.20	63.29	75.30	-29.17**	9.30	19.87*	No tester	-56.23	-18.25	-7.94	No tester
DSMR-8	83.04	91.28	113.07	-15.04	-1.42	16.46*	M31-2A	-3.39	9.74	29.83	M31-2A
IS 2397	91.95	77.70	87.08	-0.50	-3.72	4.22	M31-2A	5.52	-3.84	3.84	104A
IS 12308	74.58	52.24	79.20	2.80	-14.16	11.36	No tester	-11.85	-29.30	-4.04	No tester
IS 29654	86.06	80.24	51.50	10.35	9.91	-20.26*	104A	-0.37	-1.30	-31.74	No tester
IS 30451	86.74	66.74	69.32	9.37	-5.26	-4.11	104A	0.31	-14.80	-13.92	104A
IS 33353	84.30	89.59	61.66	2.67	13.35	-16.02	401A	-2.13	8.05	-21.58	401A
IS 26046	93.37	85.64	87.61	1.39	-0.96	-0.43	104A	6.94	4.10	4.37	104A
IS 4698	72.86	71.29	92.77	-9.23	-5.41	14.63	M31-2A	-13.57	-10.25	9.53	M31-2A
IS 19445	93.41	97.00	41.50	21.67*	17.64*	-39.30**	104A	6.98	15.46	-41.74	401A
104B		75.43									
401B			80.17								
M31-2B	81.23										

-104B, 401B and M31-2B lineswere not included in line × tester analysis.
* and ** significant @ 0.05 and 0.01 probability, respectively.

Table 4. Heterotic grouping of lines based on HSGCA method in *rabi* sorghum

S. No.	Lines	Groups
1	IS 19389, IS 29335, IS 25249, IS 12804, IS 15945, IS 15478, IS 29468, IS 2397, IS 30451and IS 26046	104A (A)
2	IS 31043, IS 30466, IS 26617, IS 33353 and IS 19445	401A (B)
3	IS 30536, IS 28313, IS 29392, IS 7987, IS 4060, IS 29568, IS 5919, IS 8012, DSMR-8 and IS 4698	M31-2A (C)
4	IS 27912, IS 2413, DSMR-4, IS 14861, IS 12308 and IS 29654	No tester (D)

Table 5. Heterotic grouping of lines based *scaeffects*and test cross grain yield method in *rabi* sorghum

S. No.	Lines	Groups
1	IS 19389, IS 29335, IS 25249, IS 12804, IS 15945, IS 15478, IS 29468, IS 29654, IS 30451, IS 26046 and IS 19445	104A (A)
2	IS 31043, IS 30466,IS 26617 and IS 33353	401A (B)
3	IS 30536, IS 28313, IS 92392, IS 7987, IS 4060, IS 29568, IS 5919, IS 8012, DSMR-8, IS 2397 and IS 4698	M31-2A (C)
4	IS 27912, IS 2413, DSMR-4, IS 14861 and IS 12308	No tester (D)

Table 6. Expected superior parents of different heterotic groups based on HSGCA method

+ve A (104A group) x –ve B (401A group)		
(Higher positive HSGCA)	**(Lower negative HSGCA)**	**Single cross hybrids**
IS 29468	IS 12308	5 x 5 diallel=25
IS 2933	IS 15945	
IS 25249	IS 14861	
IS 12804	IS 30536	
IS 19389	IS 30451	
+ve A (104A group) x –ve C (M31-2A group)		
IS 29468	IS 19445	5 x 5 diallel=25
IS 2933	IS 29654	
IS 25249	IS 15945	
IS 12804	IS 33353	
IS 19389	IS 31043	
+ve A (401A group) x –ve C (M31-2A group)		
IS 31043	IS 19445	5 x 5 diallel=25
IS 26617	IS 29654	
IS 19445	IS 15945	
IS 25249	IS 33353	
IS 12804	IS 31043	
-ve A (104A group) x +ve B (401A group)		
IS 14861	IS 31043	5 x 5 diallel=25
IS 26617	IS 26617	
IS 2413	IS 19445	
IS 30536	IS 25249	
IS 27912	IS 12804	
-ve A (104A group) x +ve C (M31-2A group)		
IS 14861	IS 19445	5 x 5 diallel=25
IS 26617	IS 29654	
IS 2413	IS 15945	
IS 30536	IS 33353	
IS 27912	IS 31043	
-ve A (401A group) x +ve C (M31-2A group)		
IS 12308	IS 29468	5 x 5 diallel=25
IS 15945	IS 2933	
IS 14861	IS 25249	
IS 30536	IS 12804	
IS 30451	IS 19389	

heterotic groups. Based on the present results and previous reports of Fan *et al.*, 2009; Akinwale *et al.*, 2014; Badu-Apkaru*et al.*, 2013; Amegbor *et al.*, 2017 and Annor *et al.*, 2020) the HSGCA method was found superior. The failure of the HSGCA and SCA-PY grouping methods to classify some lines into three heterotic groups represented by three testers suggested that those lines belong to other heterotic groups. In this study, all the lines were assigned to same heterotic groups by both methods whereas, the lines IS 29568 and IS 2397 were assigned to high yielding heterotic groups of M31-2A **(C)** and 104A**(A)**, respectively based on HSGCA (**Table 3**) but failed to assign to these groups by SCA-PY method. Hence, further potential heterotic combinations were identified based on the HSGCA method **(Table 6)**. According to Fan *et al.* (2009), the lines with higher HSGCA (where the maximum frequency of positive alleles for yield and yield traits prevailed in the genotypes) and lower HSGCA (these parents harbored diverse alleles for yield and yield traits) on crossing would accommodate highest possible heterozygosity in terms of yield contributing traits (Fan *et al.,* 2009). These combinations can be hybridized in the diallel fashion to assess their grain yield performance and further can be exploited by converting the desired parents into male sterile versions and other counter parents into restorers. These lines also can be subjected to recurrent selection to increase the positive allelic frequency for grain yield.

In the present study, the efficiency of the testers was determined based on their positive and significant *sca* effects with lines. In both the methods the testers *viz.,* 104A and M31-2A represented the same number of lines and gave high HSGCA and *sca* effects along with high yield. These two could be considered as good testers for discrimination of the lines. On the other hand, M31-2A exhibited positive specific combinations with the highest number of germplasm lines and could be considering as the best tester. Hence, the ranking based on the discriminating ability of the testers was as follows: M31-2A>104A>401A. These testers could be utilized for cost effective classification of other germplasm lines into heterotic groups, assess the combining ability and identify superior hybrid combinations for grain yield.

Both methods effectively classified the germplasm lines into four different heterotic groups. The identified potential heterotic combinations can be exploited further by transferring the male sterile background and the other groups with restoring ability to take the advantage of male sterility system for heterosis breeding. Testers M31-2A and 104A were identified as the most efficient for classifying other lines into heterotic groups, assessing the combining ability and developing superior hybrids.

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