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## Research Article

### Combining ability and gene action for fodder quality improvement in brown midrib sorghum (*Sorghum Bicolor* (L.) Moench.)

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#### Abstract

Presence of brown midrib gene in sorghum is bringing an attention to utilize this crop in manipulating forage quality due to its low lignin content. Therefore efforts were taken to study the combining ability effects (general and specific) of white and brown midrib parents and  $F_1$  crosses of sorghum and gene action involved in controlling fodder quality traits. The experiment consisted of 30  $F_1$  hybrids developed by crossing six white midrib and five brown midrib genotypes. Less than unity ratio for general and specific combining ability variances for most of the traits excluding days to 50 percent flowering revealed existence of non additive gene action involved in inheritance of these traits. High mean and desired *gca* effects for grain yield, fodder yield and its contributory characters was manifested in Parbhani Moti and CSV 29R among the lines and Bmr 7-4-1 and Bmr 7-2-2 among testers. *Per se* performance and *gca* effects for *in vitro* digestibility and acid detergent lignin was significantly better in brown midrib testers; Bmr 7-4-1 and Bmr 7-2-2 than white midrib lines.  $F_1$  crosses viz., Parbhani Moti x Bmr 7-2-2, PMS 71B X Bmr 7-2-2MS 104 B x Bmr 7-4-1 and Phule Anuradha x Bmr 7-4-1 exhibited significant desirable *sca* effects and *per se* performance for grain yield, fodder yield, higher *in-vitro* digestibility and low lignin content indicating good opportunity for improving digestibility of fodder utilizing *bmr* genotypes in future breeding programme.

**Keywords:** Bmr, sorghum, combining ability, gene action, lignin, IVOMD

#### INTRODUCTION

Livestock plays an important role in livelihood security and socio-economic upliftment of poor farmers of India. The number of milch animals has increased from 62 million in 2000 to 125.34 million in 2019 with an increase of 6.0 % over the previous census (Livestock census, 2019). It is estimated that there will be a deficit of 18.4 percentage in green fodder and 13.2 percentage in dry fodder by the year 2050, even with the current rate of expansion in forage supply (Keesam Manasa *et al.*, (2024). To sustain this growth rate and to meet the demands of milk and meat production for ever growing human population livestock needs sustainable supply of quality feed material.

Sorghum crop has extra advantage of high biomass productivity potential under moisture stress, which helps to sort-out the shortage of fodder supply during dry season. In addition to quantity, quality of feed is also important to achieve better nutrition to the livestock and quality of byproducts. By providing adequate, nutritious quality feed and fodder will lead to greater milk yield than focusing on breed improvement. Breeding for sorghum is mainly focused on improving grain and fodder yield. Grain quality is another important aspect of improvement. Forage quality is determined by high protein concentration, high digestibility and low fiber concentration

which in turn confer increase in animal performance (Casler *et al.*, 2001). Therefore to increase the demand of sorghum as fodder, it is important to improve biomass quality in terms of digestibility. However, lignin is a primary indigestible plant cell walls component, which limits the digestion of cell wall carbohydrates in the rumen. Widodo *et al.* (2025) reported that BMR reveals lower fiber and lignin levels compared to conventional sorghum resulting in increase in digestibility of dry matter and fiber. The brown midrib mutants of sorghum have recorded significantly lower levels of lignin content in their stems (around 51% less) and leaves (25% less) (Umakanth *et al.*, 2014).

Line x Tester analysis is one of the most efficient procedures to study heterosis and combining ability. Performance of individual genotype across the crosses is reflected by general combining ability while performance of specific cross is estimated by specific combining ability for different polygenic traits. Padmashree *et al.* (2014) noted the importance of both GCA and SCA components in selecting superior parents for hybrid combinations. Baker (1978) and Gore *et al.* (2004) suggested GCA and SCA represents a powerful method to measure the nature of gene action involved in quantitative traits. Therefore, efforts were made to identify the potential brown and white midrib parents for improving forage yield and quality on the basis of general and specific combining ability and gene action involved in controlling the fodder quality traits.

## MATERIALS AND METHODS

The experiment was carried out at experimental farm of Sorghum Research Station during post rainy season, 2021-22. Experimental site is located at the campus of Vasantrao Naik Marathwada Krishi Vidyapeeth, in Parbhani district of Marathwada region of East-central Maharashtra. Parbhani comes under agro-climatic zone-VII of Maharashtra and is located at 19.27°N to 76.78°E in western part of India. The site of experiment is located at 19°16' N latitude, 67° 47' E longitude and 409 meter above the sea level.

The experimental material comprised of 43 genotypes namely, 30  $F_{1s}$  hybrids produced by crossing six elite white midrib sorghum genotypes (MS 104B, PMS 71B, ICSR 196, Parbhani Moti, CSV 29R and Phule Anuradha) and five brown midrib genotypes (Bmr7-1-3, Bmr7-8-1 Bmr 7-4-1, Bmr 7-4 and Bmr 7-2-2) through hand emasculatation and pollination in line x tester mating design, the 11 parental lines, and two checks (CSH15R and CSV 22R). The experimental materials were planted in three replications in a randomized block design (RBD) with 2 rows of 4 m length and a spacing of 45 cm between the rows and 15 cm between plant. The recommended crop management practices were adopted. Observations were recorded on five randomly selected plants from each genotype in each replication for six yield and its contributing characters viz., days to 50% flowering, plant height, number of leaves per plant, L:S ratio, grain yield

and fodder yield and seven fiber quality characteristics viz., crude protein (CP), acid detergent lignin (ADL), neutral detergent fibres (NDF), acid detergent fibre (ADF), metabolizable energy (ME), *in vitro* organic matter digestibility (IVOMD) and cellulose.

For analyzing fodder quality parameters leaves including leaf sheath and stem of randomly selected five plants from each entry were chopped separately after recording the fresh forage yield at the time of physiological maturity and dried at 100°C for 48 hours in hot air oven. Then dried samples were powdered using hand mixer, sieved using 2 mm sieve and stored in a dry container for further analysis. Analysis of acid detergent lignin (ADL), neutral detergent fibre (NDF), acid detergent fibre (ADF) was done by FOSS Forage Analyzer 5000 with software package Win SI, Near Infrared Spectroscopy (NIRS), calibrated for this experiment. Crude protein (CP) concentration was estimated by auto analyzer determining total nitrogen (N) in the sample. *In vitro* organic matter digestibility (IVOMD) was estimated using *in-vitro* gas production test with manual syringes following Menke and Steingass (1988) procedure modified by Blümmel and Ørskov (1993). Cellulose and hemicelluloses were estimated as per laboratory manual of animal nutrition 1999.

The mean of data collected over the replications were subjected to analysis of variance (ANOVA). L x T analysis was carried out according to Griffing's (1956) method 1, model 2, for estimating general combining ability (GCA) and specific combining ability (SCA) effects. GCA, SCA and simple correlations were analyzed using Windostat (Indostat Services, 2004) software. Estimates of  $\sigma^2_{GCA}$  (general combining ability),  $\sigma^2_{SCA}$  (specific combining ability) were computed according to Zhang and Kang (2005) for the random-effects model to estimate  $\sigma^2_A$ ,  $\sigma^2_D$  and  $h^2$ .

## RESULTS AND DISCUSSION

**Combining ability variances and gene action:** The mean square values for replications, treatments, lines, testers, line vs testers, parents vs crosses and crosses and combining ability variances for all the traits are presented in **Table 1**. Significant differences due to treatment, parents and  $F_{1s}$  mean sum of square ( $P < 0.001$ ) showed the suitability of data for combining ability analysis. Significant and appreciable differences due to lines verses testers ( $P < 0.001$ ) were observed for all traits except number of leaves, leaf stem ratio, fodder yield and NDF % indicating significant differences between white midrib lines and brown midrib testers for these traits. Jain and Patel (2014) mentioned that presence of variability among genotypes for the trait of interest is a prerequisite for efficient selection. Mean squares of parents verses crosses were significantly high for number of leaves, plant height, grain yield and fodder yield per plant and ADL % indicating these traits have good inheritance for expression of heterosis in  $F_{1s}$ . Partitioning of genotype

Table 1. Analysis of variance for combining ability in sorghum for yield and fodder quality attributing traits

Source of variation	D.F.	Days to 50% flowering	Plant height	No. of leaves/plant	L:S Ratio	Grain yield /plant	Fodder yield/plant	Crude protein	NDF DM	ADF DM	ADL	ME	IVOMD	Cellulose
Replication	2	6.106	9.732	0.545	0.002	4.597	4.425	0.19	12.89	13.70	0.12	0.16	72.54	11.55
Treatments	40	41.82**	2229.05**	3.333**	0.009**	263.634**	681.713**	0.89**	24.2**	29.85**	0.42**	0.81**	62.65**	26.24**
Parents	10	56.933**	3106.67**	4.024**	0.005**	164.815**	531.139**	0.73**	17.08*	20.82**	0.59**	0.78**	36.08*	16.60*
Lines	5	46.99**	4015.1**	3.556**	0.003**	83.89**	688.78**	0.88**	22.3*	15.40	0.23**	0.21*	10.54	14.77
Tester	4	41.77**	2649.8**	5.567**	0.009**	63.01**	456.26**	0.53**	10.15	6.75	0.20**	0.43**	25.13	6.13
Lines vs Tester	1	167.3**	392.05**	0.198	0.198	976.7**	42.462	0.72**	18.57	104.2**	3.92**	5.04**	207.6**	67.60**
Parents vs Crosses	1	0.429	1026.1**	3.786**	3.786**	1856**	6385.9**	2.46**	6.47	3.04	0.24*	0.18	3.75	4.97
Crosses	29	38.04**	1649.5**	3.079**	0.009**	242.8**	536.94**	0.887**	27.30**	33.89**	0.36**	0.85**	73.85**	30.294**
Error	80	5.464	39.854	0.378	0.001	7.647	15.68	0.80	8.2638	7.79	0.048	0.074	24.52	7.85
$\sigma^2$ Line		1.856**	123.915	0.282	0.0005	37.454**	35.028	0.089	4.734**	6.254**	0.031	0.13*	5.16**	5.343**
$\sigma^2$ Tester		10.366**	301.615**	0.293	0.0014**	20.483	87.558**	0.065	0.506	0.184	0.019	0.043	5.23**	0.204
$\sigma^2$ GCA		6.498**	220.842**	0.288**	0.0010**	28.197**	63.681**	0.076**	2.428**	2.943**	0.025**	0.083**	5.20**	2.540**
$\sigma^2$ SCA		0.985	261.377**	0.601**	0.0015**	42.262**	103.08**	0.202**	2.678*	4.579**	0.090**	0.159**	11.1**	3.922**
$\sigma^2$ a		25.99	883.37	1.15	0.004	112.79	254.72	0.303	9.72	11.77	0.11	0.33	20.80	10.16
$\sigma^2$ D		3.93	1045.51	2.41	0.006	169.05	412.34	0.806	10.71	18.32	0.36	0.64	44.44	15.68
$\sigma^2$ GCA/ $\sigma^2$ SCA		6.597	0.845	0.479	0.667	0.667	0.618	0.376	0.907	0.643	0.278	0.522	0.468	0.648

\*\*, values significantly differ at  $P < 0.05$  and  $P < 0.01$

mean squares into GCA (lines and testers) and SCA (line x tester) variances showed highly significant variances due to lines for days to 50% flowering, grain yield, NDF, ADF, IVOMD and cellulose and due to testers for DFF, plant height, leaf stem ratio and grain yield. The variances due to testers seem to be non significant for all the fodder quality parameters except for IVOMD.

Significant ( $P < 0.001$ ) GCA and SCA variances for most of the traits showed importance of both additive and non additive type of gene action in controlling these traits. The ratio between GCA and SCA variances was ranged from 0.327 (harvest index) to 6.597 (days to 50% flowering). Less than unity ratio for the traits; plant height, number of leaves, L:S ratio, grain yield, fodder yield and all the fodder quality parameters expressed predominance of non additive gene action in governing these traits. Shiringani and Friedt, (2011) reported the importance of epistatic gene interactions in inheritance of fiber-related traits. Hence, heterosis breeding approach will be an alternate breeding strategy to exploit non additive gene effects. Tariq *et al.* (2012) also suggested the use of hybrid breeding for the characters with non additive gene effects. More than unity ratio for days to 50% flowering (6.597) pretense that the trait is under additive gene action. Cikuta *et al.* (2017) and Joshi *et al.* (2022) also reported that additive gene effects plays a major role in controlling days to 50 per cent flowering.

**Combining ability effects: General combining ability:** High mean performance coupled with good combining ability of parents provide criteria for choice of the parents for hybridization programme (Harer and Bapat, 1982). Estimates of GCA effects for yield and fodder quality parameters is presented in **Table 2**. Based on significant desirable *gca* effects ( $P < 0.001$ ) Parbhani Moti (7.254) and CSV 29R (8.495) among the lines and Bmr7-4-1 (7.815) among the tester were identified as good general combiners for grain yield. Whereas, Phule Anuradha (-2.4), Bmr 7-3-1 (-2.7) and Bmr 7-4-1(-2.64) identified as potential parents for earliness based on their significant negative *gca* effects. For fodder yield and its attributing characters like number of leaves, plant height and L:S ratio lines Parbhani Moti (11.878, 0.956 and 0.003 resp.) and CSV 29R (14.678, 0.289 and 0.002) and testers, Bmr 7-4-1 (13.31, 0.478, 0.0463) and Bmr 7-2-2(18.256, 0.7 and 0.032) showed significant *gca* effects in positive direction. Tadesse *et al.* (2008) Jain and patel (2014) identified good general combiners for grain yield, fodder yield and their contributing traits. *bmr* testers were found potential combiners than white midrib lines crude fiber, *in vitro* digestibility and acid detergent lignin.

Lines, PMS 71B (0.276 and 0.256), ICSR 196 (0.362 and 0.184) and tester Bmr 7-3-1 (0.323 & 0.297) were found good general combiner for high crude protein percentage and ash. Bmr 7-4-1 (3.056 and 53.60) and Bmr7-2-2 (2.3 and 51.16) among testers and MS 104 B (3.465 and 53.60)

among lines exhibited significant positive *gca* effects for IVOMD. Parents showing higher IVOMD also exhibited positive GCA effects for metabolisable energy (ME) and negative desirable *gca* effect for acid detergent lignin (ADL), acid detergent fibre (ADF) and neutral detergent fibre (NDF) content indicating the use of these parents as donors for breeding nutritious and more digestible fodder genotypes. Aruna *et al.* (2015) reported similar results. Lines with significant desirable *gca* effects may be used in conventional breeding programme to concentrate additive gene effect for population or inbred improvement (Khandelwal *et al.*, 2006). Gami *et al.* (2023) reported that parents with high *gca* effects are preferred for producing productive segregants in early generations.

**SCA effects for Yield and yield contributing traits:** Cross CSV 29R x Bmr 7-8-1 (-3.167) showed significantly negative *sca* effects for days to 50 % flowering. 14 other  $F_1$  crosses also exhibited negative *sca* effects indicating importance of this cross in breeding early flowering hybrids. Parents with poor *gca* effects may result in cross with significantly high *sca* effects due to cancellation of undesirable effects (Prakash *et al.*, 2010).

For grain yield per plant, eight crosses among all, registered significant *sca* effects in positive direction along with higher mean performance in g/plant (**Fig. 1a**). Cross CSV 29R x Bmr 7-4-1 (5.287 & 58.33) expressed significantly highest positive *sca* effects and high mean for grain yield followed by MS 104B x Bmr 7-8-1 (10.178 and 38.25), Parbhani Moti x Bmr7-4 (7.53 and 49.92) and Phule Anuradha x Bmr7-4-1 (5.004 and 46.24). One of the parent in these crosses exhibited high *gca* effect. Involvement of one parent with high GCA in these crosses showed significant *sca* effects in desirable positive direction. This indicates the prevalence of dominant genes in inheritance of the grain yield. Hence, this combinations with high x high *gca* effect might be used for bi parental mating or inter-mating in later generations to produce desirable segregants. This conclusion is in conformity with reports of Prabhakar (2002) and Hariprasanna *et al.* (2012).

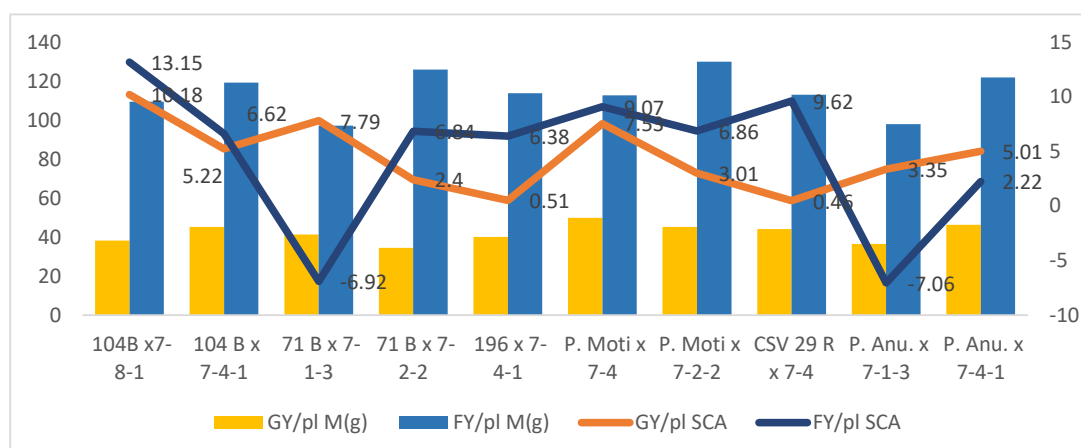
Among all, 10 crosses exhibited significantly positive *sca* effects and higher mean for fodder yield per plant (**Fig. 1a**) and ICSR 196 x Bmr 7-1-3 (18.9 & 103.36 g), MS104B x Bmr 7-8-1 (13.15 & 112.69 g), Phule Anuradha x Bmr 7-8-1(11.77 & 114.95 g), CSV 29 R X 7-4 (9.62 & 112.96 g) and Parbhani Moti x 7-4 (9.07 & 112.69 g) were top performing. These crosses accommodated at least one of the parents with high *gca* effects. This indicated additive genetic component in good combiner parent and complementary epistatic effect in other parent are responsible for enhancing the fodder yield in all the above crosses. Among other crosses with significantly positive *sca* effects Parbhani Moti x Bmr 7-4-1, Parbhani Moti x Bmr 7-2-2, CSV29R x Bmr 7-2-2 and CSV 29R x Bmr 7-2-2 were resulted from the involvement of high x high GCA parents. Prakash *et al.* (2010) and

Table 2. Estimates of general combining ability effects (GCA) of parents for yield and fodder quality parameters

S. No.	Days to 50% flowering	Plant height	No. of leaves/plant	L:S Ratio	Grain yield/plant	Fodder yield/plant	CP	NDF	ADF	ADL	ME	IVOMD	Cellulose
<b>Lines</b>													
MS 104 B	0.267	1.744	-0.444**	0.012	-4.59**	-4.566	0.110	-2.976**	-2.03**	-0.109	0.547**	3.465*	-1.921*
PMS 71B	0.467	-8.256 **	-0.378*	0.035**	-2.87**	1.379	0.256**	-1.967*	-3.19**	-0.209 **	0.168*	1.009	-2.984**
ICSR 196	-1.067	-11.456 **	0.022	-0.006	-4.97**	-9.79**	0.184*	0.161	-0.356	-0.045	-0.070	-0.873	-0.310
Parbhani Moti	1.067	11.878 **	0.956**	0.003	7.254 **	5.418**	0.046	0.193	-0.487	-0.059	0.106	1.760	-0.429
CSV 29 R	1.667**	14.678 **	0.289	0.002	8.495 **	5.166**	-0.597**	3.540**	3.655**	0.319**	-0.532**	-3.852 **	3.335**
Phule Anuradha	-2.400**	-8.589 **	-0.444**	-0.04**	-3.31**	2.394*	0.000	1.049	2.412**	0.102	-0.219**	-1.508	2.309**
SE (m)	0.604	1.621	0.159	0.008	0.714	1.023	0.073	0.742	0.720	0.057	0.070	1.278	0.723
CD @ 1%	1.6074	4.3177	0.4228	0.0213	1.9017	2.7235	0.194	1.977	1.919	0.151	0.187	3.405	1.927
<b>Testers</b>													
Bmr 7-1-3	-2.700 **	-25.967**	-0.189	-0.05**	-0.319	-4.655	0.29**	0.002	-0.128	-0.077	-0.063	-2.152	-0.050
Bmr. 7-8-1	-0.367	-5.522**	-0.578**	-0.013	-4.07**	-6.526**	0.029	0.421	-0.926	0.199**	-0.077	0.607	-1.125
Bmr 7-4-1	-2.644**	13.311**	0.478**	0.042**	7.815**	9.894**	0.077	-1.092	-0.289	-0.160**	0.263**	3.056*	-0.129
Bmr 7-4	0.411	-0.078	-0.411 **	-0.010	-1.599*	-9.123**	-0.426 **	1.399*	1.223	0.109*	-0.289 **	-2.596 *	1.115
Bmr 7-2-2	5.3**	18.256**	0.7**	0.032**	-1.83**	10.409**	0.023	-0.731	0.120	-0.070	0.166*	2.30*	0.189
SE (m)	0.551	1.480	0.145	0.007	0.652	0.934	0.067	0.677	0.658	0.052	0.064	1.167	0.660
CD @ 1%	1.4674	3.9415	0.3860	0.0194	1.7360	2.4862	0.177	1.805	1.752	0.138	0.171	2.38	1.759

\* \*\*, values significantly differ at  $P < 0.05$  and  $P < 0.01$





**Fig.1a. Specific combining ability (SCA) effects of high performing  $F_1$ s for grain and fodder yield**

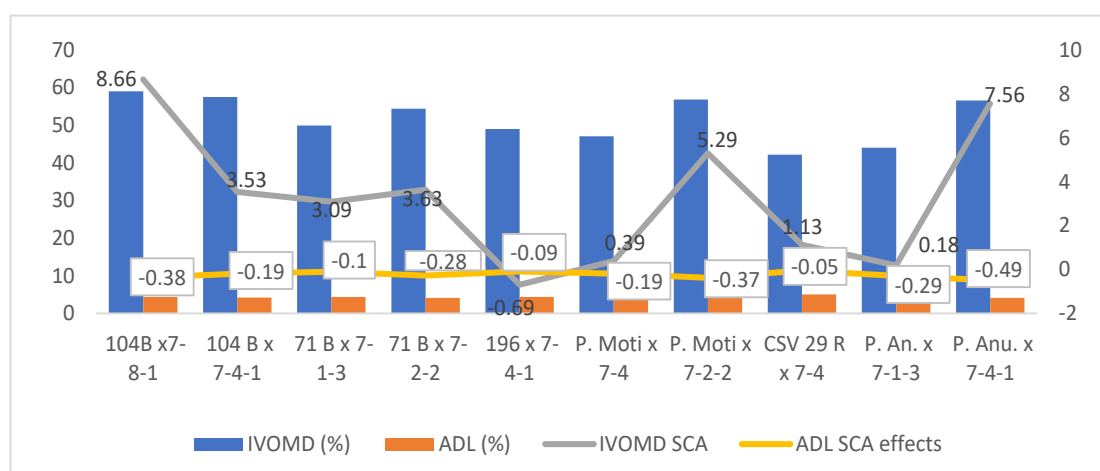
Cikuta *et al.* (2017) summarized the similar results in their studies.

Among all ICSR 196 x 7-8-1 (25.344) showed highest significant and positive *sca* effects for plant height. While crosses CSV 29R x Bmr 7-1-3 (1.044 and 0.063) and ICSR 196 x 7-1-3 (1.589 and 0.062) recorded significant *sca* effects in positive direction along with the higher means for plant height, number of leaves and leaf: stem ratio.

**SCA effects for fodder quality parameters:** Significantly positive *sca* effects for *in-vitro* digestibility and negative *sca* effects for acid detergent lignin with desirable high mean performance was observed in crosses viz., MS 104B x Bmr 7-8-1 (8.66 & -0.38), Phule Anuradha x Bmr 7-4-1 (7.57 & 0.49), PMS 71B x Bmr 7-2-2 (3.63 & -0.28) and Parbhani Moti x 7-2-2 (5.29 & 0.37) (Fig. 1b) indicating good opportunity for improving digestibility of fodder utilizing these crosses in future breeding programme. These crosses showed low x low parental combination

signifying the role of non allelic interactions and former two crosses showing high x low and low x high cross combinations in respect to *gca* effects indicating role of dominant and non allelic gene effects in expression of the ADL and IVOMD percentage. Results are in conformity with the findings of Aruna *et al.* (2015). Mean performance in these crosses for acid detergent lignin was ranged from 4.10 to 4.37 % and *in vitro* organic matter digestibility (IVOMD) ranged from 54.42 to 59.01 %. In addition these crosses also showed negative *sca* effects, lower mean values for NDF, ADF and cellulose % and significant positive *sca* effects for metabolizable energy indicating good opportunity for improving the fodder digestibility in these crosses in future breeding programme.

In addition, MS 104 B x Bmr 7-8-1 (0.615), Phule Anuradha x Bmr 7-4-1 (0.643), Parbhani Moti x Bmr 7-2-2 (0.564), ICSR 196 x Bmr 7-4-1 (0.799), ICSR 196 x Bmr 7-8-1 (0.401), 71 B x 7-1-3 (0.347) and CSV 29R x Bmr 7-2-2 (0.344) showed highly significant and positive *sca* effects for protein percentage.



**Fig.1b. Specific combining ability (SCA) effects of high performing  $F_1$ s for IVOMD and ADL**

Brown midrib (BMR) lines Bmr 7-4-1 and Bmr 7-2-2 can serve as potential donors of low lignin content and improved digestibility in future sorghum breeding programmes aimed at developing high-yielding genotypes with enhanced fodder quality. The crosses such as MS 104B x Bmr 7-8-1 PMS 71 B x Bmr 7-2-2 which exhibit good *per se* performance and significant positive *sca* effects, hold potential for heterosis breeding, might be exploited directly as both of the lines are male sterile. Biparental mating in early segregating generation of crosses can be performed in potential  $F_1$  crosses derived from either high x low, low x high or low x low parental combination. The results of the present study indicated that both additive and non-additive gene effects play significant roles in controlling grain and fodder yield, as well as in determining fodder quality parameters. Hence, recurrent selection can be practised in these crosses for simultaneous exploitation of both additive and non additive gene action.

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