



Yield and stover quality of brown mid-rib mutations in different genetic backgrounds of sorghum

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ABSTRACT

Genetic control of the lignification process through use of brown mid-rib (*bmr*) mutations has offered the most direct and productive approach to reducing lignin concentration and increasing digestibility of sorghums. Brown mid-rib genes were introgressed from IS 21888 (*bmr*-3) and IS 21891(*bmr*-8) into elite sorghum lines and the advanced progenies were evaluated in relation to normal sorghums. The *bmr* types were significantly early to flower and mature compared to normal sorghum. Normal sorghum had a significant superiority of 11 and 9% for panicle length and panicle width as compared to *bmr* sorghum. The *bmr* types recorded significantly lower grain yield than normal sorghum. However, among the *bmr* sorghums, CSV 15 × IS 21891 (*bmr*-8 mutation) derivative recorded a significant superiority over other *bmr* entries and was at par with the normal sorghum check CSV 20. For stover yields, normal sorghums were significantly superior to the *bmr* sorghums by 46%. Dry matter intake (DMI) was positively correlated with ME and IVOMD in normal sorghum. However, the correlation of DMI with IVOMD was not significant in *bmr* sorghums. In both the sorghums, IVOMD was positively correlated with TDN, relative feed value RFV, ME and negatively correlated with ADF and NDF. However, the association of IVOMD with NDF was non-significant in *bmr* sorghum. The findings of the present study indicated that *bmr* 3 and *bmr* 8 genes significantly improved the stover digestibility in specific genetic backgrounds and their interaction with recipient genetic background was evident.

Key words: Digestibility *bmr*, Sorghum stover

Todate, genetic control of the lignification process through use of *bmr* genes has offered the most direct and productive approach to reducing lignin concentration and increasing digestibility of sorghum (Gerhardt *et al.* 1994).

Lignin inhibits the fibre digestibility, which reduces milk production in dairy animals. Brown-midrib gene (*bmr*) mutants, differing in quality and quantity of lignin from normal genotypes, offer an opportunity to increase the overall digestion of plant fiber which is a major constituent of fodder, comprising 30–80% of their dry matter (Cherney *et al.* 1991b). The mutation associated with the *bmr* trait results in a change in enzyme activity associated with the process of lignin formation. The *bmr* genes are best known for their ability to reduce the amount of lignin in the stover portion of sorghum and maize (Oliver *et al.* 2005). The brown midrib mutants of sorghum have significantly lower levels of lignin content (around 51% less in their stems and 25% less in their leaves). Brown midrib sorghum silage has 17% less lignin

than regular sorghum silage making it more digestible (Grant *et al.* 1995).

Oliver *et al.* (2005) observed that the selection of compatible genetic backgrounds will be critical in determining the realized impact of *bmr* genes on its fodder value. There is also concern on possible deleterious effects of *bmr* genes on economic potential (Pedersen *et al.* 2005). Several *bmr* mutants are available, but so far the reports on forage yield and quality are confined to *bmr* 6 and *bmr* 12 genes only. To study the effect genes like *bmr* 3 and *bmr* 8 on stover yield and quality of adapted sorghum lines, brown mid-rib genes were introgressed from sources like IS 21888 (*bmr*-3) and IS 21891(*bmr*-8) into elite sorghum lines. The present study was taken up to evaluate the *bmr* (*bmr* sorghum × normal sorghum progenies selected for *bmr* trait with superior grain and stover yield traits) derivatives in relation to normal sorghums for various parameters including yields, nutritional attributes and their inter-relationships.

MATERIALS AND METHODS

The experimental material consisted of 14 dual-purpose advanced progenies (F₇ generation) out of which 7 were *bmr* types and equal number were in normal (non-*bmr*)

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background, The check variety CSV 20 sorghum was also a constituent of the normal sorghum group. The experiment was laid out in a randomized block design with three replications during rainy seasons of 2007 and 2008 at the Directorate of Sorghum Research (latitude 17°19' N, longitude 78°23' E), Rajendranagar, Hyderabad, India. Plant-to-plant and row-to-row spacing was maintained at 15 and 45 cm respectively. Recommended agronomic practices were followed throughout the crop season. Atrazine (1.0 kg/ha of active ingredient) was applied immediately after sowing. A basal fertilizer dose of 42 kg/ha N, 42 kg/ha P₂O₅ was applied just before sowing and a topdressing of 46 kg/ha N was applied 1 month after germination (floral initiation stage). In each replication, observations were recorded on 10 randomly selected competitive plants on days to 50% flowering, days to maturity, plant height, panicle length and panicle width, while grain and stover yields were recorded on whole plot basis.

Stover quality analysis was done at International Livestock Research Institute (ILRI) centre located in Patancheru, India, based on Near Infrared Spectroscopy reflectance (NIRS) analysis using FOSS Forage Analyzer 5000 (Blümmel *et al.* 2010, Sharma *et al.* 2010). Representative stover samples were oven-dried to estimate dry matter content. For calibration of NIRS dried samples were ground through 1mm sieve and analyzed for fibre content and dry matter digestibility. Crude protein (CP) was determined as per AOAC (2006). Cell wall fractions (NDF, ADF, etc.) were determined as per Van Soest *et al.* (1991). Digestibility of

stover (IVOMD, g/100g dry matter) was estimated by Menke's gas production technique (Menke *et al.* 1979, Menke and Steingass 1988). The following traits (Ball *et al.* 1991, Schroeder, 1994) were computed as below.

Dry matter intake (DMI) = 120 / NDF (g/100g dry matter)

Relative fodder value (RFV) = (DMI×IVOMD)/1.29

Total digestible nutrients (TDN%) = 88.9- (0.779 × ADF%)

The data pooled across two years were analysed for statistical significance using two-way ANOVA and Tukey's test for multiple comparisons.

RESULTS AND DISCUSSION

Morphological traits: The *bmr* types were significantly early to flower (75 days) and mature (113 days) in comparison to normal sorghum (80 days and 118 days) in both the years (Table 1). The entries (HC 260 × B 35)-2-1-1-1 among normal sorghums and (PC 5 × IS 21888)-8-1-1-1 in *bmr* sorghums were early to flower and mature. The latter entry was significantly early compared to all other test entries in the *bmr* group. Earlier workers reported that various brown mid-rib genes affected days to flower by up to 1 week with *bmr* 12 being the most severe. Vermerris *et al.* (2002) noted an average 7-d reduction in days to flower in two *bmr*-12 sorghum lines and essentially no change in days to flower in two *bmr*-18 sorghum lines compared with their wild-type counterparts. In general, for plant height, the normal sorghum progenies had a significant superiority of 16% compared to *bmr* sorghum. Amongst the normal sorghum, (M 11 × B 35)-1-1-2-1 was the tallest with a plant height of 373 cm while

Table 1. Mean performance of *bmr* and normal sorghum genotypes for yield and related traits during rainy seasons of 2007 and 2008

S No	Entry	Plant height (cm)	Days to flower	Days to mature	Panicle length (cm)	Panicle width (cm)	HI (%)	GY (kg/ha)	FY (kg/ha)
<i>bmr</i> sorghum									
1	(CSV 15 × IS 21891)-1-1-1-1	272	79	117	25.4	5.7	23	2227	7889
2	(CSV 15 × IS 21891)-6-1-1-1	189	76	114	28.8	6.1	24	1840	6130
3	(COS 26 × IS 21888)-1-3-1-1	242	77	115	27.6	6.4	28	1891	5831
4	(Palem 2 × IS 21891)-1-1-1-1	273	79	117	25.3	5.7	21	1981	7651
5	(PC 5 × IS 21888)-5-1-1-1	338	76	114	22.8	5.5	12	1591	11831
6	(SPV 462 × IS 21891)-3-1-1-1	247	73	111	24.2	5.4	17	1413	7013
7	(PC 5 × IS 21888)-8-1-1-1	306	68	106	21.9	5.0	18	1423	6533
	Mean	267	75	113	25.1	5.7	21	1767	7554
	CD (5%)	14	4	4	0.7	0.5	3	307	1448
Normal sorghum									
1	(SPV 462 × SPV 1474)-3-1-1-1	249	79	117	28.0	6.2	30	2313	5454
2	(SPV 1022 × CSV 17)-1-1-3-1	291	82	120	24.8	6.1	22	2492	9723
3	CSV 20	272	79	117	26.8	6.1	21	2293	9193
4	(COS 26 × I-12)-3-2-2-2	314	84	122	28.2	6.0	17	1962	10084
5	(M 11 × B 35)-1-1-2-1	373	82	120	30.7	6.4	13	2130	15117
6	(HC 260 × B 35)-2-1-1-1	330	78	116	25.6	6.0	14	1870	13039
7	(PC 5 × I-12)-2-1-1-3	349	80	118	31.4	6.5	13	1918	14501
	Mean	311	80	118	27.9	6.2	19	2140	11016
	CD (5%)	12	4	4	0.9	0.7	3	342	2352

HI-harvest index; GY- grain yield; FY-stover yield; *bmr*-brown mid-rib.

(PC 5 × IS 21888)-5-1-1-1 from *bmr* progenies was the tallest (338 cm). Normal sorghums had a significant superiority of 11 and 9% for panicle length and panicle width as compared to *bmr* sorghums.

Grain and stover yield traits

With respect to grain yield, the *bmr* types recorded 17% significantly lower mean yields than normal sorghum (Table 2). Similar results for *bmr* sorghum have been reported earlier (Oliver *et al.* 2005). In the present study, highest grain yield among normal sorghums was recorded by (SPV 1022 × CSV 17)-1-1-3-1 (2492 kg/ha) and was 9% superior compared to the check CSV 20 (2293 kg/ha). However among the *bmr* sorghum, (CSV 15 × IS 21891)-1-1-1-1 recorded a significant superiority over other test *bmr* entries and yielded at par with the normal sorghum check CSV 20 and is promising.

For stover yields, normal sorghum mutants were significantly superior to the *bmr* sorghum by 46%. Among normal sorghum, (M 11 × B 35)-1-1-2-1 (151 q/ha) and among *bmr* sorghum, (PC 5 × IS 21888)-5-1-1-1 (118 q/ha) were significantly superior to the other test genotypes. *bmr* group had a significantly higher (11% higher) harvest index when compared with normal sorghum. (SPV 462 × SPV 1474)-3-1-1-1 and (COS 26 × IS 21888)-1-3-1-1 had higher harvest indices in normal and *bmr* groups respectively.

Oliver *et al.* (2005) evaluated the effect of *bmr*-6 and *bmr*-12 genes on grain yield, stover yield and stover quality in

different genetic backgrounds. They observed that *bmr* genes reduced grain yield; and residue yield in most of the genetic backgrounds; they concluded that the *bmr*-12 gene was superior to the *bmr*-6 gene in terms of potentially adding value to the stover. In sorghum, Pedersen *et al.* (2005) reviewed that the brown mid-rib mutations increased lodging, and reduced yield, re-growth, height, and tillering.

Stover quality traits: Considerable variability has been recorded for yield and stover quality traits within the material studied. However, across the introgressions made, the stover digestibility of *bmr* sorghum was comparable with normal sorghum as a group as evidenced by IVOMD values (Table 2). Nevertheless, individual introgressions with *bmr* 8 gene have recorded significantly higher IVOMD (%) values in the range of 50.4–54.0% compared to 49.5–50.8% in *bmr* 3 introgressions and 45.7–51.6% in normal sorghum. These findings are in consonance with previous studies (Fritz *et al.* 1979, Casler *et al.* 2003) on brown mid-rib sorghum digestibility.

The *bmr* types recorded higher mean values than normal sorghum for ash content, crude protein, ME and IVOMD. The NDF and ADF values were almost similar for both the types of sorghums. The entries (SPV 462 × SPV 1474)-3-1-1-1 and check CSV 20 in normal sorghum and CSV 15 × IS 21891 derivatives amongst *bmr* types were promising for CP. The variability in genotypes for crude protein and cell wall contents may be attributed to genetic, species, maturity,

Table 2. Comparative performance of normal vs. *bmr* sorghums for stover quality traits during rainy seasons of 2007 and 2008

S No.	Entry of sorghum mutants	Ash* content	CP* content	NDF*	ADF*	Lignin*	ME (MJ/kg)	IVOMD*	DMI (%)	RFV	TDN (%)
<i>bmr</i> sorghum											
1	(CSV 15 × IS 21891)-1-1-1-1	7.93	4.18	64.45	41.16	4.61	7.65	51.4	1.86	74.29	56.88
2	(CSV 15 × IS 21891)-6-1-1-1	8.56	4.34	62.39	39.52	4.18	8.09	54.0	1.93	80.61	58.14
3	(COS 26 × IS 21888)-1-3-1-1	10.66	3.90	70.66	46.85	5.21	7.23	49.5	1.70	65.29	52.42
4	(Palem 2 × IS 21891)-1-1-1-1	7.80	3.74	66.20	42.48	5.18	7.39	50.4	1.82	71.24	55.83
5	(PC 5 × IS 21888)-5-1-1-1	7.83	3.96	65.13	41.80	4.22	7.40	50.7	1.85	72.71	56.43
6	(SPV 462 × IS 21891)-3-1-1-1	7.34	3.89	64.77	40.47	4.22	7.85	52.2	1.86	74.98	57.20
7	(PC 5 × IS 21888)-8-1-1-1	7.65	3.88	66.71	42.50	5.49	7.61	50.8	1.80	70.85	55.61
	Mean	8.25	3.98	65.76	42.11	4.73	7.60	51.3	1.83	72.85	56.07
	CD (5%)	0.80	0.83	2.95	2.42	1.16	0.26	1.2	0.08	4.58	1.87
Normal sorghum											
1	(SPV 462 × SPV 1474)-3-1-1-1	8.47	4.03	68.64	43.80	5.97	7.35	49.6	1.75	67.39	54.72
2	(SPV 1022 × CSV 17)-1-1-3-1	7.67	3.75	63.09	39.01	4.52	7.73	51.6	1.91	76.33	58.57
3	CSV 20	7.95	4.04	63.81	40.82	4.96	7.83	49.6	1.88	72.36	57.03
4	(COS 26 × I-12)-3-2-2-2	7.00	3.73	65.04	40.69	5.47	7.60	50.7	1.85	72.71	57.30
5	(M 11 × B 35)-1-1-2-1	6.82	2.54	70.35	45.31	6.18	6.77	45.7	1.71	60.69	53.65
6	(HC 260 × B 35)-2-1-1-1	7.93	3.86	63.92	41.08	4.77	7.63	51.3	1.88	74.63	56.95
7	(PC 5 × I-12)-2-1-1-3	7.44	3.89	66.33	43.40	5.27	7.33	49.4	1.82	69.56	55.02
	Mean	7.61	3.69	65.88	42.01	5.31	7.46	49.7	1.83	70.52	56.18
	CD (5%)	1.03	1.14	3.04	2.38	1.26	0.32	2.0	0.09	5.93	1.88

* Expressed as g/100g dry matter(w/w); CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ME, metabolizable energy; IVOMD, *in vitro* dry matter digestibility; DMI, dry matter intake; RFV, relative feed value; *bmr*, brown midrib; TDN, total digestible nutrients.

soil fertility and photosynthetic pathway factors, besides lignin content (Hodges and Bidwell 1993). Differences in crude protein content, NDF and ADF similar to present study were observed by Singh *et al.* (2007). For IVOMD, (SPV 1022 × CSV 17)-1-1-3-1 (51.6%) and (HC 260 × B 35)-2-1-1-1 (51.3%) in normal sorghum and (CSV 15 × IS 21891)-1-1-1-1 (51.4%), (CSV 15 × IS 21891)-6-1-1-1 (54%) and (SPV 462 × IS 21891)-3-1-1-1 (52.2%) from brown mid-rib group were significantly superior over other test entries in respective groups. The check variety CSV 20 recorded an IVOMD of 49.6 g/100g dry matter. The significant superiority (3%) of *bmr* types for IVOMD reveals the importance of brown midrib sorghum in offering opportunities to increase the digestibility of plant fibre by cattle. Nitrogen content and cell wall contents are main factors that govern the digestibility of a crop (Barriere *et al.* 2003, Seven and Cerci 2006). Increased fiber digestibility will result in greater production of rumen microbial protein, thus reducing the need for additional purchased protein. An assessment of dynamics of sorghum stover trading revealed that for 1% increase in digestibility, the price premium increased by 5% (Blümmel and Rao, 2006).

The dry matter intake and total digestible nutrients were similar in both types of sorghum owing to similar NDF and ADF values. The *bmr* types had 3% superiority for relative fodder value over the other type and the entries with high IVOMD were promising for this trait too. The entry (SPV 462 × IS 21891)-3-1-1-1 from *bmr* group was the best among the test entries for this character. These results reaffirm that genetic background effects influence the expression of *bmr* genes (Oliver *et al.* 2005).

Interrelationships: An attempt was made to unravel the relationship of yields with stover quality traits in *bmr* and normal sorghums. In general, the two sorghums did not differ much in the pattern of association of yields with stover quality traits (Table 3). Stover yield was positively and significantly correlated with digestible dry matter, crude protein and plant height. Harvest index was positively and significantly associated with ash content in *bmr* sorghum while its correlation with crude protein yield and digestible dry matter was significant and in negative direction for both types of sorghum. Dry matter intake (DMI) was positively correlated with relative fodder value, total digestible nutrients, ME and IVOMD in both sorghums. However, the correlation of DMI with IVOMD was not significant in *bmr* sorghums. Ash content was positively correlated with crude protein and NDF in normal and *bmr* sorghums respectively. In both the sorghums, IVOMD was significantly and positively correlated with TDN, RFV, ME and negatively correlated with ADF and NDF. However, the association of IVOMD with NDF was non-significant in *bmr* sorghums.

The nonsignificant negative associations of grain and stover yields with most important traits of digestibility demonstrate the possibility of improving the nutritional

Table 3. Phenotypic correlation for yield and stover quality traits in *bmr* and normal sorghums

	GY		FY		HI		DMI		RFV		TDN		Ash		CP		NDF		ADF		ME		IVOMD	
	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal	<i>bmr</i>	Normal
PH	-0.01	0.02	0.63*	0.81**	-0.62*	-0.84**	-0.08	-0.15	-0.25	-0.33	-0.22	-0.39	-0.41	-0.49	-0.22	-0.40	0.08	0.17	0.22	0.40	-0.38	-0.51	-0.35	-0.47
GY			0.39	0.29	0.20	0.19	0.05	0.12	-0.17	-0.03	-0.26	-0.19	-0.02	-0.03	-0.14	-0.02	-0.04	-0.11	0.26	0.19	-0.31	-0.19	-0.38	-0.19
FY					-0.75**	-0.83**	0.22	0.11	-0.07	-0.12	-0.14	-0.12	-0.38	-0.50	-0.07	-0.24	-0.22	-0.10	0.14	0.34	-0.33	-0.32	-0.38	-0.33
HI							-0.25	-0.03	-0.07	0.14	-0.12	0.07	0.58*	0.48	-0.08	0.20	0.27	0.02	0.12	-0.28	0.10	0.30	0.15	0.28
DMI									0.88**	0.92**	0.75**	0.77**	-0.52	-0.01	0.27	0.35	-1.00**	-1.00**	-0.75**	-0.74**	0.69**	0.80**	0.48	0.72**
RFV											0.83**	0.89**	-0.36	0.09	0.36	0.43	-0.88**	-0.93**	-0.86**	-0.86**	0.91**	0.90**	0.83**	0.93**
TDN													-0.41	0.10	0.42	0.45	-0.76**	-0.78**	-0.98**	-0.99**	0.85**	0.89**	0.70**	0.90**
Ash															0.21	0.70**	0.54*	0.00	0.41	-0.16	-0.21	0.12	-0.08	0.17
CP																	-0.30	-0.35	-0.42	-0.32	0.39	0.33	0.36	0.46
NDF																			0.76**	0.75**	-0.69**	-0.81**	-0.47	-0.73**
ADF																					-0.85**	-0.85**	-0.73**	-0.85**
ME																							0.89**	0.88**

GY, grain yield; FY, stover yield; HI, harvest index; DMI, dry matter intake; RFV, relative feed value; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ME, metabolizable energy; IVOMD, *in vitro* dry matter digestibility; *bmr*, brown midrib.

quality of stover. We could succeed in breeding a genotype (SPV 1022 × CSV 17)-1-1-3-1 which among normal sorghums had all the desirable traits like high grain yield, acceptable stover yields coupled with high digestibility, lower NDF and ADF values. In the *bmr* group, (CSV 15 × IS 21891)-1-1-1-1 had all the desirable traits mentioned above apart from higher CP and offers promise. Another *bmr* derivative is (PC 5 × IS 21888)-5-1-1-1 with high stover and digestible dry matter yields, that can be assessed for its suitability as single-cut forage. (CSV 15 × IS 21891)-6-1-1-1 recorded the highest IVOMD and crude protein values amongst all the test entries across different sorghums and offers promise as a source of high digestibility and protein content. It is worthwhile assessing the improvement in milk yield and body weight gain of cattle by including the *bmr* sorghum in their diets. These lines might pave way to add value to crop and animal systems with enhanced stover digestibility by the use of brown midrib sorghum, without significantly compromising on grain and stover yield.

The findings of the present study indicated that *bmr* 3 and *bmr* 8 genes significantly improved the stover digestibility in specific genetic backgrounds and their interaction with recipient genetic background was evident. It would be appropriate to introgress these genes in more diverse genetic backgrounds to identify beneficial combinations. Reduced height and reduction in grain and stover yields in many genetic backgrounds were noticed in the present study. Therefore, while dealing with *bmr* sorghum with premium on stover quality, it is imperative to determine the acceptable levels of grain and stover yields as compared to normal sorghum.

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