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Enhancement of *in Vitro* Digestibility of Sorghum (*Sorghum bicolor* (L) Moench) in Brown Midrib (*bmr*) Mutant Derivatives of *bmr*1 and *bmr*7

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ABSTRACT

Ten sorghum derivatives with brown midrib alleles (*bmr*1 and *bmr*7) were investigated for stover fodder quality traits, grain yield and the relationships between these productive traits. Potential fodder quality was assessed by laboratory analysis. Significant differences were observed among the tested derivatives of *bmr*1 allele for stover nitrogen (N) content, *in vitro* organic matter digestibility (IVOMD), and acid detergent lignin (ADL) content. In contrast, no significant genotypic differences were observed among *bmr*7 derivatives. In the *bmr*1 gene derivatives stover N content ranged from 0.63 to 0.81%, ADL content varied from 2.6 to 3.2% and IVOMD ranged from 52.9 to 55.4%. Two lines have recorded over 55% IVOMD and higher grain yield than the *bmr*1 source. In *bmr*7 derivatives IVOMD ranged between 52.9 and 54.4%. The enhanced IVOMD coupled with improved grain and biomass yield in *bmr*1 and *bmr*7 derivatives will increase overall benefits from sorghum in mixed crop livestock systems. This is the first study on utilization of *bmr*1 and *bmr*7 mutants in improving the forage quality of tropical sorghums with perceptible genetic enhancement of candidate biomass and grain yield traits.

Keywords: acid detergent lignin, correlation, grain yield, nitrogen content, stover yield

Abbreviations: ADL, acid detergent lignin; ***bmr***, brown midrib; **COMT**, caffeic acid *O*-methyltransferase; **CAD**, cinnamyl alcohol dehydrogenase; **DES**, diethyl sulphate; **IVOMD**, *in vitro* organic matter digestibility; **NIRS**, near infrared spectroscopy

INTRODUCTION

Brown midrib mutants (*bmr*) significantly reduce the level of enzyme-resistant polymer 'lignin' in plants and increase their palatability and digestibility (Rook *et al*. 1977; Cherney *et al*. 1991). Brown midrib (*bmr*) is a visible marker associated with the reduction of lignin in corn (Kuc' and Nelson 1964), sorghum (Porter *et al*. 1978), and pearl millet (Cherney *et al*. 1988). Although the intensity of the coloration cannot be taken as a measure of reduction in lignin, it is a clear indicator that the *bmr* gene(s) are present. Jung and Fahey (1983) suggested that *bmr* plants have lignin that is less polymerized and contains less phenolic monomers which can affect digestion. Brown midrib silage with and without protein supplements significantly increased milk yield of lactating cows (Frenchik *et al*. 1976; Keith *et al*. 1979; Stallings *et al*. 1982; Cherney *et al*. 1991; Oba and Allen 1999). Similarly, the rate of *in vitro* digestibility and cell wall degradation of leaf blades by rumen bacterium from *bmr*12 sorghum was shown to be significantly higher than those from their respective wildtype isolines (Akin *et al*. 1986). Allelism tests on the sorghum *bmr* mutants derived through chemical mutagenesis showed that several of the mutations are allelic, and that the total number of independent *bmr* loci was smaller than the number of mutant lines assembled (Bittinger *et al*. 1981; Saballos *et al*. 2008). The effect of the *bmr* mutations on forage quality varies and depends on the genetic background of the line in which the mutation is introduced (Cherney *et al*. 1991; Pedersen *et al*. 2006). Therefore, the effect of each mutation on forage quality and agronomic characteristics needs to be determined.

This suggests the need to either identify a suitable genetic background that allows for optimal impact of the mutation. The published literature indicates that a yield drag is associated with the *bmr* trait (Cherney *et al*. 1991; Pedersen *et al*. 2006). Most of these and other uncertainties stem from the fact that little information is in the public domain describing the characteristics and benefits of brown midrib mutant sources i.e. *bmr* 1 and *bmr*7 in forage plants. The impact of the *bmr*1 and *bmr*7 mutations, which were developed by chemically induced mutagenesis with diethyl sulphate (DES), (Porter *et al*. 1978; Vogler *et al*. 1994) on forage quality in tropical sorghums has not been studied yet. The *bmr* 7 mutant is non-allelic to *bmr* 12 and has reduced expression of caffeic acid *O*-methyltransferase (COMT). The objective of this study was to provide an assessment of the effect of the *bmr*1 and *bmr*7 mutations on fodder quality and grain yield *vis-à-vis* genetic background.

MATERIALS AND METHODS

Materials and experimental site

Ten improved experimental brown midrib varieties developed through pedigree method by deploying brown midrib mutants *bmr*1 (IS 21887) and *bmr*7 (IS 21890) along with one brown midrib parental control (*bmr*1- IS 21887) were grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (latitude: 17° 27' N; longitude: 78° 28' E) in the post-rainy seasons, 2007 and 2008. Both the *bmr* sources, i.e. *bmr*1 and *bmr*7 were agronomically inferior to either grain sorghum cultivars for grain yield or to forage cultivars for biomass yield (Srinivasa *et al.* 2009). The experiment was carried out in a randomized complete block design (RCBD) and each cultivar was planted in 4 rows of 4 m length in 12 m^2 plots with a spacing of 75 × 15 cm in 3 replications. Fertilizer dosage of 80 Nitrogen (N) and 40 P_2O_5 kg ha^{-1} was applied with 50% of N as basal and the bal-

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Table 1 Mean and ANOVA of improved *bmr1* (IS 21887) derivatives for stover nitrogen content (N), acid detergent lignin (ADL), *in vitro* organic matter digestibility (IVOMD) and agronomic traits.

Genotype	N (%)	IVOMD (%)	ADL (%)	Days to 50% flowering	Plant height (m)	Grain yield (t ha^{-1})
(IS 21887 x ICSB 101)-3-1-1-1-1	0.71	55.4	2.6	89	1.72	1.84
(IS 21887 x ICSB 73)-13-1-1-1-1	0.63	52.9	3.2	86.7	1.75	1.48
(IS 21887 x ICSB 93)-2-1-1-1-1	0.81	54.1	2.7	89	1.63	1.15
(IS 21887 x ICSB 93)-4-1-1-1-1	0.79	55.3	2.7	92.7	1.6	1.08
IS 21887 (*bmr* 1)	0.92	51.6	3.3	88.7	1.21	1.01
Mean	0.77	53.9	2.9	89.2	1.58	1.31
Probability	0.024	0.002	0.0004	0.0001	0.0001	0.0001
LSD ($P < 0.05$)	0.16	3.00	0.50	4.20	0.26	0.40
CV%	17.58	4.69	15.55	3.54	11.95	22.45
Genotype[a]	0.019	0.075	0.03	0.0001	0.0002	0.0004
Year[a]	0.24	0.001	0.0001	0.0001	0.0001	0.0003
Genotype x Year[a]	0.09	0.203	0.086	0.056	0.83	0.0007
h^2	0.44	0.34	0.29	0	0	0

LSD: Least significant difference at $P < 0.05$, CV%: Coefficient of variability; [a] probability values ; h^2: heritability

Table 2 Mean and ANOVA of improved *bmr7* (IS 21890) derivatives for stover nitrogen content (N), acid detergent lignin (ADL), *in vitro* organic matter digestibility (IVOMD) and agronomic traits.

Genotype	N (%)	IVOMD (%)	ADL (%)	Days to 50% flowering	Plant height (m)	Grain yield (t ha^{-1})
(IS 21890 x ICSB 88010)-5-1-1-1-1	0.7	54.3	2.8	82.3	1.82	2.17
(IS 21890 x ICSB 88010)-5-1-1-2-1	0.63	53.8	2.8	84.3	1.9	2.34
(IS 21890 x ICSB 88010)-16-1-1-2-1	0.65	53.4	2.6	86.3	1.55	2.16
(IS 21890 x ICSB 88010)-19-1-1-1-1	0.7	54.5	2.7	83	1.75	2.62
(IS 21890 x ICSB 88010)-26-1-1-1-1	0.58	53.8	2.9	85.7	1.92	1.87
(IS 21890 x ICSB 88010)-26-1-1-2-1	0.6	52.9	3.1	84.7	1.8	2.14
Mean	0.64	53.8	2.8	84.4	1.79	2.22
Probability	0.76	0.0001	0.0001	0.0001	0.018	0.0001
LSD ($P < 0.05$)	0.18	2.2	0.4	5.1	0.34	0.62
CV%	24.39	3.54	10.72	4.48	16.21	23.81
Genotype[a]	0.72	0.76	0.132	0.585	0.314	0.28
Year[a]	0.08	0.0001	0.0001	0.0001	0.0003	0.563
Genotype x Year[a]	0.95	0.033	0.023*	0.44	0.362	0.002
h^2	0	0	0	0	0.08	0

LSD: Least significant difference at $P < 0.05$, CV%: Cofficient of variability; [a] probability values ; h^2: heritability; * Significant at $P < 0.05$

ance 35 days after emergence as side-dressing. Hand weeding was done twice followed by hoeing and inter-cultivation. The crop was grown during September to January on a soil with a silt clay loam texture of about 1.0 m depth.

Measurements and biochemical analysis

For each plot days to flowering (DF) was recorded at 50% anthesis and plant height (PHT) was measured to the top of the mature panicle before harvest. Ten mature plants were randomly selected from the centre four rows of each plot, and the panicles were cut for estimations of grain yield. The data were adjusted for each cultivar for a moisture content of 14.5%. Stover quality analyses were conducted on samples harvested from each plot. All samples were analyzed by near infrared spectroscopy (NIRS) calibrated for this experiment against conventional chemical and *in vitro* laboratory analyses. The NIRS instrument used was a FOSS Forage Analyzer 5000 with software package Win SI. Stover nitrogen (N) was determined by an Auto Analyzer (VAP 4500, Gerhardt) and acid detergent lignin (ADL) content were analyzed according to Goering and Soest (1970). *In vitro* organic matter digestibility (IVOMD) contents were determined and calculated according to Menke and Steingass (1988) as modified by Blümmel and Ørskov (1993).

Statistical analysis

General linear model (GLM) procedure was used for analysis of variance and to calculate significant differences among improved varieties with the SAS (1988) computer program (version 6.03). GraphPad Prism (1994) was used for simple linear regression analysis between traits.

RESULTS

ANOVA for agronomic and biochemical traits

The results for stover N, ME, IVOMD, DF, PHT and grain yield of *bmr*1 derivatives along with the parent, 'IS 21887' are presented in **Table 1**. All the nutritional and agronomic measurements differed consistently and significantly among cultivars. The year-wise differences were also significant for most of the traits studied except for stover N. The genotype × year interaction was non-significant ($P \leq 0.01$) for all the traits except for grain yield. Similarly, the data on above parameters for *bmr*7 derivatives are shown in **Table 2**. No significant differences were observed among the cultivars for agronomic and forage quality parameters. However, year and genotype × year interactions were significant for majority of the traits revealing the high influence of the environment.

Correlation among characters studied

The correlation coefficients of agronomic characters with candidate forage digestibility traits in *bmr*1 derivatives is shown in **Table 3**. Plant height was significantly negative correlated with stover N (-0.96). The data indicated that better results can be achieved by selecting late maturing lines in the breeding program to improve IVOMD as the late flowering entries recorded higher IVOMD. In case of *bmr*7 derivatives (**Table 3**) no significant association among the traits was observed. Relationships between the agronomic parameters i.e. grain yield, DF and PHT with forage quality traits *viz.*, IVOMD and N are shown in **Fig. 1A-F**. Significant relationships could not be established between the above agronomic traits with quality traits as the sample size is too small except for the negative relationship of plant

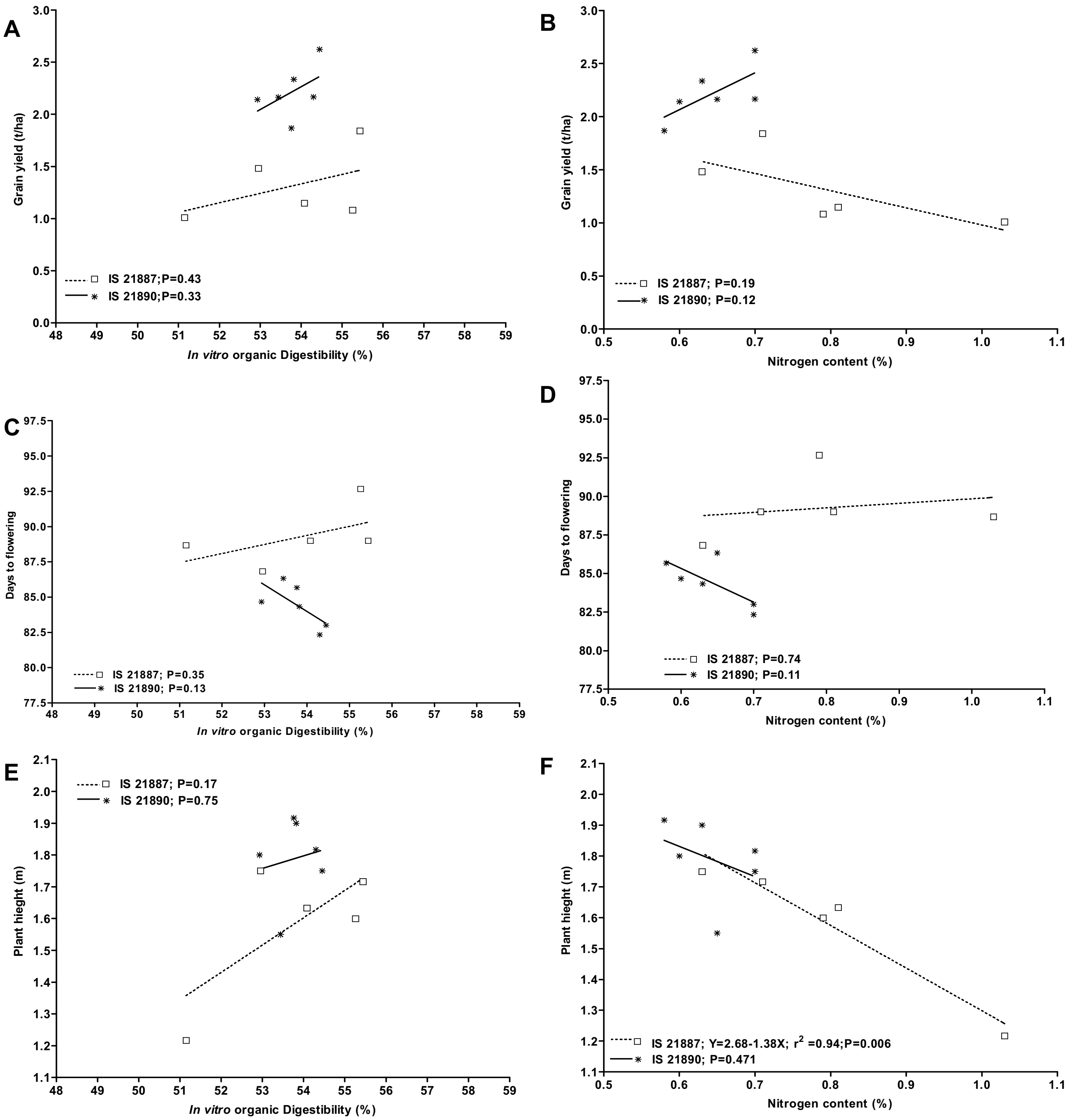

Fig. 1 Relationship between agronomic and fodder quality traits in *bmr*1 and 7 derivatives. Relationships between: (**A**) grain yield and IVOMD, (**B**) grain yield and nitrogen content, (**C**) days to 50% flowering and IVOMD, (**D**) days to 50% flowering and nitrogen content, (**E**) plant height and IVOMD, (**F**) plant height and nitrogen content.

height with stover N in *bmr*1 derivatives. There was a negative association of grain yield with N (**Fig. 1B**) albeit non-significant. Similarly, the association of days to 50% flowering with N seems to be positive (**Fig. 1D**). More studies with larger sample size would certainly help to establish any relationships between these traits.

DISCUSSION

The data (**Table 1**) revealed that *bmr*1 has background effects on ADL, IVOMD and ME, i.e. here three lines 'ICSB 73', 'ICSB 101' and 'ICSB 93' into which *bmr* gene was transferred, similar to the observations of Oliver *et al.* (2005) in *bmr*6 derived lines.

The importance and high monetary value of (non-sweet) sorghum stover as livestock fodder was highlighted by Blümmel and Rao (2006) based on their survey from 2004 to 2005 on sorghum stover trading in Hyderabad in India. Higher quality stover fetched premium prices and it was

Table 3 Correlation of agronomic traits with candidate forage digestibility traits in *bmr*1 and *bmr*7 derivatives.

Trait	Nitrogen content (N %)	*In vitro* organic dry matter digestibility (IVOMD %)	Acid detergent lignin (ADL %)
Trait association in *bmr* 1 derivatives			
Days to 50% flowering	0.21	0.54	-0.09
Plant height (m)	-0.96**	0.71	-0.53
Grain yield (t ha^{-1})	-0.69	0.46	-0.34
Trait association in *bmr* 7 derivatives			
Days to 50% flowering	-0.69	-0.68	-0.44
Plant height (m)	-0.4	0.16	0.5
Grain yield (t ha^{-1})	0.69	0.48	-0.44

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$

also shown that stover *in vitro* digestibility accounted for 75% of the variation in stover price. Yearly mean stover IVOMD of 46.9 to 51.7% were associated with prices of 3.1 to 3.9 Indian Rupees (US$ = 0.066-0.083) per kg dry stover (Blümmel and Rao 2006). In the present study the improved varieties (IS 21887 × ICSB 101)-3-1-1-1-1 and (IS 21887 × ICSB 93)-4-1-1-1-1 had exhibited the highest IVOMD of 55.4 and 55.2%, respectively compared with parental control IS 21887 (51.55%). Among the *bmr7* derivatives (IS 21890 × ICSB 88010)-5-1-1-1-1 recorded the highest IVOMD of 54.3% and 8.10 MJ kg^{-1} ME, while lowest values for these traits was recorded by the line (IS 21890 × ICSB 88010)-26-1-1-2-1 with 52.9% IVOMD and 7.8 MJ kg^{-1} ME (**Table 2**). The white midribbed control, RS 29 (data not shown), recorded the lowest means for N (0.55), ME (7.0) and IVOMD (58.5%). However, it recorded the highest grain yield (3.3 t ha^{-1}) and ADL (3.3%). Fritz *et al.* (1981) found that the NDF digestibility of *bmr* sorghum-sudangrass hybrids was 5% greater than non *bmr* hybrids when fed to non-lactating dairy cows. Comparison of *bmr*6 and *bmr*12 in multiple genetic backgrounds indicated that the effects of individual brown midrib genes within specific sorghum lines – and extrapolating within hybrids – are not uniform. The detailed study on *bmr*6 and *bmr*12 mutants in the backgrounds of 'RTx 430' and 'Wheatland' sorghum varieties showed significant differences in soluble and cell wall-bound aromatics, like *p*-coumaric acid, ferulic acid and caffeic acids as well as histochemical staining with diazotized nitroaniline (Palmer *et al*. 2008). Conforming to the above, in this study *bmr*1 and *bmr*7 showed varying levels of lignin content in different backgrounds. The range was 2.58 to 3.22 in *bmr*1 derivatives while it was 2.63 to 3.10 in *bmr*7 incorporated lines. These results points to significant impact of *bmr*1 alleles in reducing the lignin content and improving the IVOMD. The grain yield is also high in these lines, i.e. 1.0- 1.8 t ha^{-1} in *bmr*1 derivatives (*bmr* 1: 1.0 t ha^{-1}) while 1.8-2.6 t ha^{-1} in the lines with *bmr*7 alleles. However, the grain yield is much lower compared with white midrib grain sorghum control, RS 29 (3.3 t ha^{-1}) but much higher than that of the *bmr* sources. Considering the vast differences of background effects, end users of brown midrib sorghum, i.e. farmers, should always evaluate the performance of specific hybrid/gene combinations (Bout and Vermerris 2003; Sattler *et al*. 2010). In such scenario the improved lines, IS 21890 × ICSB 88010)-19-1-1-1-1 (IVOMD 54.4% and grain yield 2.6 t ha^{-1}) and (IS 21890 × ICSB 88010)-5-1-1-2-1 with IVOMD 53.8% and grain yield of 2.3 t ha^{-1} can be recommended for cultivation owing to their superiority for IVOMD though the grain yields are marginally lower.

In the present work, it has been shown that the IVOMD was much higher in *bmr*1 derivatives to an extent of 4% as in (IS 21887 × ICSB 101)-3-1-1-1-1 with 55.4% and (IS 21887 × ICSB 73)-13-1-1-1-1 with 55.2% compared with parental line IS 21887, which will likely to fetch more price in the fodder market (Blümmel and Rao 2006). Similarly, among the six *bmr*7 derivatives studied, (IS 21890 × ICSB 88010)-19-1-1-1-1 and (IS 21890 × ICSB 88010)-5-1-1-1-1 with 54.4% and 54.3% IVOMD, respectively, could fetch and high price due to better quality.

CONCLUSION

The results of this study indicated that there is considerable background noise on the effect of *bmr* 1 mutant alleles. There appears to be consistent pattern emerging that showed that brown midrib in sorghum has a major impact upon stover quality. This came at no penalty in total yield of plant material but with much greater digestibility, higher protein level, and significantly increased palatability. All of these components contribute significantly to weight gains in animals and thereby increased milk yields. The brown midrib trait in tropical sorghum too appears to have a very bright future not only as better forage but also as a feedstock for ligno-cellulosic ethanol production, considering the tedious methods of pre-treatment and associated costs. This is the first study on utilization of *bmr*1 and *bmr*7 mutants in improving the forage quality of tropical sorghums with perceptible genetic enhancement of candidate biomass and grain yield traits.

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