

Inheritance and allelism of brown midrib trait introgressed in agronomically promising backgrounds in pearl millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract: The lignin content of forage crops significantly affects the livestock's forage intake and its dry matter digestibility. The brown midrib (*bmr*) trait is known for its association with a reduced lignin content and increased digestibility. Pearl millet inbreds having the *bmr* trait were derived from two populations ICMV *bmr* and WRajPop *bmr* via continuous selfing up to 6–8 generations. The results from the crosses revealed the presence of the same single recessive gene that controlled the *bmr* in both the populations. The stable expression of the *bmr* gene in the new genetic background indicated that there was no effect of modifier genes. Four inbreds were identified with the stable expression of *bmr* (three in ICMV 155 and one in the WRajPop background) with a high biomass potential, which can serve as a *bmr* source in future pearl millet forage breeding programmes.

Keywords: brown midrib introgression; crude protein; digestibility; forage yield; forage quality

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important cereal crop grown on 30 million hectares in the marginal lands of Africa (> 18 million hectares) and Asia (> 10 million hectares) accounting for 93% of the global pearl millet production (AICPMIP 2022). This warm season C₄ crop has a short growing season and is a member of the Poaceae family. It has a high tillering ability, photosynthetic efficiency, dry matter per day productivity, and tolerance to most biotic and abiotic stresses (Singh & Nara 2023). Also, it has the ability to withstand harsh climatic conditions, such as low soil fertility and drought, where other cereal crops, like rice, wheat, maize and sorghum, are unable to grow well (Baltensperger 2002). This is an important dual-purpose crop of the semi-arid

tropics, where both grains and forage are important for human food and livestock feed, respectively. As forage, it can be grazed directly, or fed to animals as a green-chop. This can be used as single/multi-cut, and used to make stover/fodder/hay/silage (Machicek et al. 2019; Crookston et al. 2020). Many countries like the United States of America (USA) (Sheahan 2014), Australia (Hanna 1996), Canada (Brunette et al. 2014), Brazil (Dias-Martins et al. 2018) cultivate this crop as an important forage crop. Currently, pearl millet is grown on 6 hundred thousand hectares in USA (Myers 2002) and 5 million hectares in Brazil (França & Miyagi 2012) as a forage crop. Recently, it has expanded area to about 1 million hectares in India as a forage/fodder crop

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in summer as well as in the rainy season and farmers are demanding high yielding single/multi-cut cultivars with a better forage quality to meet their livestock feed demands (Amarendra Reddy et al. 2013; Bhardwaj et al. 2022).

The lignin content in plants plays important role as it enhances the cell wall rigidity, helps in the mineral transport through vascular bundles (Schuetz et al. 2014), and also protects the plants against biotic and abiotic stresses (Buxton & Casler 1993; Moura et al. 2010). Also, the lignin content is an important indicator of forage quality, where the higher content reduces its digestibility in ruminants. Therefore, it is important to identify or breed high digestibility materials with low and/or acceptable lignin content levels which could attract forage growers and milk producing farmers/dairies to grow them on a larger scale. The brown midrib trait, abbreviated as “bmr”, can offer a solution to modify the lignin quality and quantity in the plant (Cherney et al. 1991) in order to enhance the dry matter digestibility of the forage. It was first reported in maize in 1924 (Jorgensen 1931) where these mutants were reported to have brown pigmentation in the leaf midrib, stem tassel, cob and roots. Lechtenberg et al. (1972) showed that the *bmr* gene was effective in reducing lignin percentage, and enhanced *in vitro* digestibility. Later, bmr was reported in sorghum (Porter et al. 1978; Bittinger et al. 1981), and was introgressed into different genetic backgrounds. These new sorghum genotypes had less lignin and increased digestibility than their parental genotypes (Fritz et al. 1981).

Three bmr mutants have been reported in pearl millet; the first mutant, *Pbmr* derived from a diethyl sulfate mutagenised inbred line derived from the population (Tift 23D2B1/2 × PI 185642) (Cherney et al. 1988); the second mutant, SDML 89107, was a spontaneous mutant identified through selection from a germplasm (Gupta et al. 1993), and the third mutant, was spontaneous, identified from Tifton (Degenhart et al. 1991). All these mutants were found to be allelic in nature, but a stable expression was not observed in different genetic backgrounds (Gupta et al. 1993; Degenhart et al. 1995). It has been reported that the bmr mutant lines are highly digestible than normal midrib lines since it has reduced lignin content levels in pearl millet (Cherney et al. 1988, 1990; Gupta et al. 1993). On the other hand, the bmr mutant phenotype was found to be negatively associated with the forage yield in pearl millet (Hassanat et al. 2006) and sudangrass (Casler

et al. 2003). With the available bmr sources in pearl millet, bmr cultivars/hybrids have been extensively developed and utilised in countries like Canada (Hassanat et al. 2006) and the USA (Machicek et al. 2019; Ferreira et al. 2022).

Considering the high-quality forage demand, breeding efforts have been directed to breed exclusively for high nutritive forage pearl millet inbreds, hybrids and open pollinated varieties (OPVs) (Gupta et al. 2015; Ponnaiah et al. 2019; Govintharaj et al. 2018, 2021). Earlier, two popular dual-purpose varieties of pearl millet were developed by introgressing the bmr trait to improve forage quality (Yadav & Weltzein 1998; Blümmel et al. 2003). These bmr versions in two different genetic backgrounds have not been utilised properly till now. Considering the increasing demand for forage pearl millet hybrids, now there is the need to develop parental lines (inbreds) having *bmr* alleles. Hence, inbreds having the bmr trait have been developed using these two bmr populations to enable breeders to develop bmr forage hybrid cultivars in pearl millet. The present study aimed to investigate the inheritance, allelism, and stability of the expression of *bmr* alleles introgressed in new genetic backgrounds, and also assess the variability for the forage quantity and quality traits in these newly developed inbreds of pearl millet.

MATERIAL AND METHODS

Plant materials. Two pearl millet bmr source populations (ICMV 155 *bmr* and WRajPop *bmr*) were planted during the rainy season of 2015. Each population was planted in ten rows 4 m in length with 75 cm between rows with plants spaced 10–15 cm apart. The crop was fertilised with diammonium phosphate ((NH₄)₂HPO₄) at a rate of 100 kg/ha as a basal dose, and was top dressed with 100 kg per ha urea (CH₄N₂O) after 30 days of planting. The local package of practices was followed to protect the crop from diseases and pests. ICMV 155 *bmr* was developed using the ICMV 155 population. ICMV 155, a dual-purpose open-pollinated variety was developed by the random mating of 59 selfed progenies from the C4 cycle of the New Elite Composite (NELC) and released for cultivation in India in 1991 (Singh et al. 1994). Its bmr version, designated as ICMV 155 *bmr* was developed by the random mating of selected uniformly brown midrib BC₆F₄ progenies which were derived from the bmr line (*Pbmr*) developed by Cherney et al. (1988) and

introgressed into the ICMV 155 genetic background (Blümmel et al. 2003). The plants of the ICMV 155 *bmr* have brownish pigmentation of vascular bundles on both the stem and leaves. The WRajPop *bmr* was developed using the WRaj population. WRajPop was developed using 13 landrace accessions from north-western India and improved by several cycles of recurrent selection (Yadav & Weltzein 1998). Its *bmr* version, designated as WRajPop *bmr* was developed by the random mating of selected uniformly *bmr* advanced backcross progenies which were derived from the *bmr* line (*Pbmr*) developed by Cherney et al. (1988) and introgressed into the WRajPop genetic background.

Development of *bmr* inbreds. Plants with a brown midrib along with a high biomass were visually continuously selected in two populations (ICMV 155 *bmr* and WRajPop *bmr*) for several generations (from S_1 to S_{10}) during the 2015–2021 cropping seasons. The selected plants were selfed in each generation and the head to row progenies were planted in each generation following the pedigree breeding. Some of the inbreds derived from these two populations with the *bmr* trait are shown in Figure 1.

Phenotyping for brown midrib. The leaf midrib was visually observed for reddish-brown pigmentation between the 35th to 45th day after planting. The *bmr* intensity was observed in the abaxial midribs (back side of the leaves) as it was higher than the upper side of the leaves. Earlier studies also reported that the brown pigmentation is more pronounced on the abaxial surface of the leaf midrib (Porter et al. 1978; Hill-Skinner 2018). All the brown midrib plants were identified and tagged. The reddish-brown pigmentation on the leaf midribs disappeared slowly after 55 days due to the maturity of the leaves. Besides the leaf midribs, reddish brown pigmentation was also observed in the stem and stalk pith at physiological maturity (Figure 1).

Evaluation of *bmr* progenies for forage traits. A total of 36 inbreds having the *bmr* trait in the advanced generations (S_7 – S_{10}) derived from two populations (11 from ICMV 155 *bmr* and 25 from WRajPop *bmr*) were evaluated along with the base populations (ICMV 155 *bmr* and WRajPop *bmr*) including two popular pearl millet checks [Nutrifeed (PAC 981) and Nutrifast bred by Advanta Seeds Ltd.,] during the 2021 rainy season in an augmented design

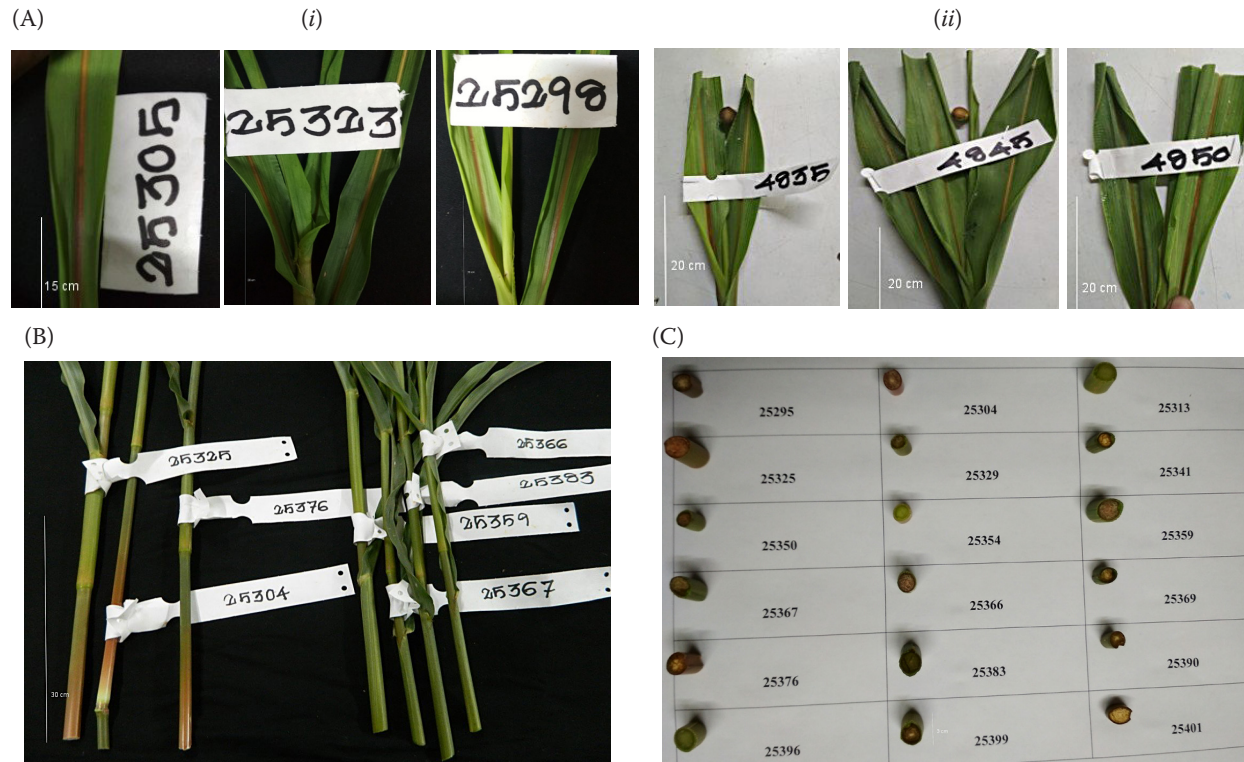


Figure 1. The brown midrib phenotype observed in the leaves in the *bmr* progenies derived from two populations: (i) ICMV 155 *bmr*; (ii) WRajPop *bmr* in pearl millet (A), *bmr* and non-*bmr* trait observed in the stem (B), *bmr* trait observed in the stem pith (C)

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in a replicated trial for the forage quantity and quality traits. The plot size was one row of 4 m in length with 75 cm between the rows and with plants spaced 10–15 cm apart. All the recommended agronomic practices were followed in the trial for good crop standing and plant growth.

The plots were harvested three times for the forage yield during the 2021 rainy season. At the time of harvest, the fresh weight of the green forage was recorded (kg) on a plot basis, oven dried for 8 h daily for three to four days at 60 °C in a Campbell dryer (Campbell Industries, Inc., Des Moines, USA), and reweighed (dry forage weight in kg). The dry matter (DM) concentration was determined by the ratio between the dry forage weight and green forage weight, and also the dry forage yield of each entry was calculated by multiplying the green forage weight and dry matter concentration. The GFY (green forage yield) and DFY (dry forage yield) were converted into t/ha. The first, second and third cut were taken at 50, 80, and 110 days after planting, respectively, from the same plot. The GFY and DFY were recorded in the second and third cuts, as described in the first cut.

The dried sub-samples of the whole plant of each entry were chopped into 10 to 15 mm pieces using a chaff cutter (Model No. 230, Jyoti Ltd., Vadodara, India) and ground in a Thomas Wiley mill (Model No. 4, Philadelphia, USA) through a 1-mm screen for chemical analysis. The ground stover samples (Approximately, 40 g of sample/entry) were analysed by near-infrared reflectance spectroscopy (NIRS) for the stover nitrogen concentration ($N \times 6.25$ equals the crude protein content), acid detergent lignin (ADL), and *in vitro* digestibility of dry matter (IVOMD) as described by Bidingier and Blümmel (2007) and Blümmel et al. (2007).

Inheritance of *bmr*. One advanced progeny (S_8) with a normal midrib (derived from the ICMV 08111 population) was crossed with two advanced *bmr* progenies (S_5 and S_8) derived from ICMV 155 *bmr* to produce two F_1 crosses. The F_1 crosses were selfed to obtain the F_2 seed. One of the F_1 was backcrossed to a non-*bmr* parent (BCP_1) to find the homozygous state of the non-*bmr* parent. These F_2 and BCP_1 populations were sown and observed for the midrib colour. Likewise, one advanced progeny (S_{12}) with a normal midrib (derived from the ICMV 08111 population) and the designated seed parent ICMB 01888 with a normal midrib were crossed with one advanced *bmr* progeny derived from the WRajPop *bmr* to produce two F_1 crosses.

Allelism of *bmr*. Nine (seven S_7 and two S_2) and six (four S_6 and 2 S_2) inbreds derived from WRajPop *bmr* and ICMV 155 *bmr*, respectively, were used in this study. All these parents were crossed between ICMV 155 *bmr* and WRajPop *bmr* in different combinations to generate the F_1 crosses. Three of these F_1 s were self-pollinated to produce the F_2 s. All these F_1 s and F_2 populations were sown during the rainy and summer seasons of 2019 and 2020 and observed for the midrib colour.

Data analysis. The chi-square (χ^2) test was used to test for the goodness of fit (Steel & Torrie 1980) in order to investigate the inheritance and allelism of the *bmr* trait.

RESULTS AND DISCUSSION

Variability for forage quality traits. The check hybrids Nutrifeed (PAC 981) and Nutrifast had a GFY of 14.9 and 18.6 t/ha at the first cut, 16.8 and 17.2 t per ha at the second cut, and 10.2 and 9.3 t/ha at the third cut with a DFY of 1.9 and 2.4 t/ha at the first cut, 3.3 and 3.0 t/ha at the second cut, and 1.5 t/ha each at the third cut during the 2021 rainy season. The source ICMV 155 *bmr* and WRajPop *bmr* populations had a GFY (18.0 and 20.0 t/ha at the first cut; 6.2 and 7.8 t/ha at the second cut; 2.2 and 2.4 t/ha at the third cut, respectively) and a DFY (2.5 and 3.0 t per ha at the first cut; 1.1 and 1.5 t/ha at the second cut; 0.3 and 0.6 t/ha at the third cut, respectively). Across the cuts, during the 2021 rainy season, the GFY and DFY of all 36 inbreds ranged from 0.36 to 10.3 t per ha and 0.03 to 1.7 t/ha, respectively (Table 1). The dry matter/ha values reported in this study were higher than the earlier reported values of brown midrib pearl millet (Harper et al. 2018; Machicek et al. 2019). These results indicate that significant genetic variability exists for the forage yield (GFY and DFY) in the brown midrib background in newly developed inbreds that could help derive potential cultivars. The GFY and DFY values in the inbreds were significantly lower than their parental populations and standard hybrid checks due to continuous selfing for several generations. This reduction can happen due to inbreeding depression during selection cycles for traits like plant height and the number of tillers in the *bmr* inbreds, as reported earlier in other crops (Casler et al. 2003; Hassant et al. 2006).

Previous studies have indicated that a one percent unit increase in the digestibility of sorghum and pearl millet stovers results in an increase in the livestock

Table 1. Forage traits in the bmr progenies derived from two pearl millet populations evaluated during the 2021 rainy season

S. No.	Entry	GFY (t/ha)			DFY (t/ha)			First cut (%)			Second cut (%)			Third cut (%)		
		first cut	second cut	third cut	first cut	second cut	third cut	CP	IVOMD	ADL	CP	IVOMD	ADL	CP	IVOMD	ADL
1	ICMV 155 <i>bmr</i> S1-1-1-3-2-7-1-B-B-B	6.21 ± 1.20	4.83	1.72	0.76 ± 0.19	0.78	0.30	10.58 ± 1.16	50.48 ± 1.40	3.92 ± 0.17	NA	NA	NA	13.90	54.35	3.26
2	ICMV 155 <i>bmr</i> S1-1-1-3-3-5-1-B-B-B	5.30 ± 1.20	3.40	2.27	0.71 ± 0.19	0.57	0.37	12.51 ± 1.16	49.13 ± 1.40	3.73 ± 0.18	12.21	47.07	4.09	14.34	53.68	3.15
3	ICMV 155 <i>bmr</i> S1-1-1-3-4-4-B-B	5.52 ± 1.20	2.68	1.29	0.86 ± 0.19	0.48	0.23	10.34 ± 1.16	46.78 ± 1.40	4.35 ± 0.16	12.60	51.64	3.62	14.41	51.35	3.36
4	ICMV 155 <i>bmr</i> S1-3-3-1-2-1-B-B	8.38 ± 1.20	7.12	3.45	1.46 ± 0.19	1.26	0.53	8.50 ± 1.16	47.55 ± 1.40	4.7 ± 0.16	12.13	52.38	3.67	12.65	51.95	3.55
5	ICMV 155 <i>bmr</i> S1-3-3-6-2-3-B-B	4.74 ± 1.20	2.75	NA	0.85 ± 0.19	0.55	NA	13.25 ± 1.16	46.83 ± 1.40	4.12 ± 0.17	12.02	48.95	3.84	13.10	52.51	3.49
6	ICMV 155 <i>bmr</i> S1-4-2-1-3-3-B-B	4.77 ± 1.20	1.95	1.38	0.8 ± 0.19	0.36	0.26	11.41 ± 1.16	49.32 ± 1.40	4.11 ± 0.17	12.29	48.80	4.10	13.16	52.54	3.44
7	ICMV 155 <i>bmr</i> S1-4-2-1-3-4-1-B	6.06 ± 1.20	5.15	3.06	0.83 ± 0.19	1.00	0.50	12.44 ± 1.16	46.92 ± 1.40	4.3 ± 0.16	10.90	49.42	3.94	13.15	50.95	3.58
8	ICMV 155 <i>bmr</i> S1-4-2-1-3-4-1-B-B-B	4.67 ± 1.20	2.63	0.65	0.64 ± 0.19	0.39	0.11	11.47 ± 1.16	48.39 ± 1.40	4.14 ± 0.17	13.20	49.09	3.64	14.40	50.31	3.37
9	ICMV 155 <i>bmr</i> S1-4-2-1-3-4-2-B	6.46 ± 1.20	8.17	4.62	1.18 ± 0.19	1.63	0.86	9.30 ± 1.16	47.68 ± 1.40	4.6 ± 0.16	11.41	51.93	3.90	12.65	51.24	3.69
10	ICMV 155 <i>bmr</i> S1-4-2-1-3-4-3-B	8.13 ± 1.20	4.50	3.59	1.36 ± 0.19	0.72	0.58	9.65 ± 1.16	45.43 ± 1.40	4.78 ± 0.15	12.43	49.79	3.79	12.23	51.69	3.69
11	ICMV 155 <i>bmr</i> S1-4-2-1-3-4-B-B	4.10 ± 1.20	2.55	1.44	0.80 ± 0.19	0.48	0.26	10.26 ± 1.16	48.36 ± 1.40	4.41 ± 0.16	11.57	52.38	3.73	12.37	53.05	3.57
	Minimum	4.10	1.95	0.65	0.64	0.36	0.11	8.50	45.43	3.73	10.90	47.07	3.62	12.23	50.31	3.15
	Maximum	8.38	8.17	4.62	1.46	1.63	0.86	13.25	50.48	4.78	13.20	52.38	4.10	14.41	54.35	3.69
	ICMV 155 <i>bmr</i> (original population)	18.18 ± 1.20	6.17	2.23	2.48 ± 0.19	1.13	0.31	NA	NA	NA	11.21	51.67	3.84	14.27	49.02	3.60
12	WRajPop <i>bmr</i> S1-18-4-3-2-1-2-2-B-B-B	5.47 ± 1.20	NA	NA	0.78 ± 0.19	NA	NA	11.19 ± 1.16	45.18 ± 1.40	4.54 ± 0.16	NA	NA	NA	NA	NA	NA
13	WRajPop <i>bmr</i> S1-18-4-3-2-1-4-7-B-B-B	5.99 ± 1.20	2.40	NA	0.72 ± 0.19	0.44	NA	11.87 ± 1.16	44.52 ± 1.40	4.57 ± 0.16	11.67	50.39	3.97	NA	NA	NA

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Table 1 to be continued

S. No.	Entry	GFY (t/ha)			DFY (t/ha)			First cut (%)			Second cut (%)			Third cut (%)		
		first cut	second cut	third cut	first cut	second cut	third cut	CP	IVOMD	ADL	CP	IVOMD	ADL	CP	IVOMD	ADL
14	WRajPop <i>bmr</i> S1-18-4-3-2-1-5-B-B	7.98 ± 1.20	NA	NA	1.34 ± 0.19	NA	NA	11.21 ± 1.16	45.62 ± 1.40	4.64 ± 0.16	NA	NA	NA	NA	NA	NA
15	WRajPop <i>bmr</i> S1-18-4-3-2-2-1-22-B-B-B	8.53 ± 1.20	NA	NA	1.18 ± 0.19	NA	NA	8.96 ± 1.16	43.96 ± 1.40	5.05 ± 0.15	NA	NA	NA	NA	NA	NA
16	WRajPop <i>bmr</i> S1-18-4-3-2-2-3-1-B-B-B	6.41 ± 1.20	2.53	1.17	0.87 ± 0.19	0.47	0.22	11.48 ± 1.16	47.00 ± 1.40	4.16 ± 0.17	11.93	50.73	3.69	13.68	54.28	3.33
17	WRajPop <i>bmr</i> S1-18-4-3-2-2-5-7-B-B-B	6.94 ± 1.20	5.10	2.62	1.59 ± 0.19	0.87	0.49	10.68 ± 1.16	50.45 ± 1.40	4.18 ± 0.17	13.13	51.81	3.50	14.26	52.76	3.30
18	WRajPop <i>bmr</i> S1-18-4-3-2-3-2-1-B	7.20 ± 1.20	NA	NA	0.91 ± 0.19	NA	NA	11.86 ± 1.16	45.85 ± 1.40	4.29 ± 0.16	NA	NA	NA	NA	NA	NA
19	WRajPop <i>bmr</i> S1-18-4-3-2-3-2-2-B	6.07 ± 1.20	NA	NA	0.76 ± 0.19	NA	NA	11.91 ± 1.16	44.93 ± 1.40	4.48 ± 0.16	NA	NA	NA	NA	NA	NA
20	WRajPop <i>bmr</i> S1-18-4-3-2-3-2-3-B	1.05 ± 1.20	NA	NA	0.16 ± 0.19	NA	NA	12.44 ± 1.16	50.41 ± 1.40	3.80 ± 0.17	NA	NA	NA	NA	NA	NA
21	WRajPop <i>bmr</i> S1-18-4-3-2-3-2-4-B	2.75 ± 1.20	NA	NA	0.35 ± 0.19	NA	NA	12.51 ± 1.16	50.24 ± 1.40	4.29 ± 0.16	NA	NA	NA	NA	NA	NA
22	WRajPop <i>bmr</i> S1-18-4-3-2-3-2-5-B	6.12 ± 1.20	0.85	NA	0.75 ± 0.19	0.16	NA	11.08 ± 1.16	48.51 ± 1.40	4.17 ± 0.17	9.08	48.41	4.44	NA	NA	NA
23	WRajPop <i>bmr</i> S1-18-4-3-2-1-2-2-B-B-B	0.66 ± 1.71	NA	NA	0.09 ± 0.28	NA	NA	12.01 ± 1.65	51.09 ± 1.99	4.12 ± 0.17	NA	NA	NA	NA	NA	NA
24	WRajPop <i>bmr</i> S1-18-4-3-2-3-3-1-B-B-B	5.55 ± 1.20	NA	NA	0.79 ± 0.19	NA	NA	9.89 ± 1.16	46.05 ± 1.40	4.42 ± 0.16	NA	NA	NA	NA	NA	NA
25	WRajPop <i>bmr</i> S1-18-4-3-2-3-4-3-B-B-B	6.93 ± 1.20	1.12	0.36	0.82 ± 0.19	0.20	0.08	11.12 ± 1.16	48.97 ± 1.40	4.12 ± 0.17	10.79	45.61	4.62	13.65	52.15	3.57
26	WRajPop <i>bmr</i> S1-18-4-3-2-3-5-2-B	0.01 ± 1.71	NA	NA	0.03 ± 0.28	NA	NA	9.71 ± 1.16	48.19 ± 1.40	4.60 ± 0.16	NA	NA	NA	NA	NA	NA
27	WRajPop <i>bmr</i> S1-18-4-3-2-6-3-2-B-B-B	10.27 ± 1.20	NA	NA	1.66 ± 0.19	NA	NA	10.80 ± 1.16	45.51 ± 1.40	4.61 ± 0.16	NA	NA	NA	NA	NA	NA
28	WRajPop <i>bmr</i> S1-18-4-3-2-6-4-4-B-B-B	6.73 ± 1.20	2.78	NA	0.98 ± 0.19	0.47	NA	9.01 ± 1.16	46.68 ± 1.40	4.67 ± 0.16	12.06	50.88	3.82	NA	NA	NA

Table 1 to be continued

S. No.	Entry	GFY (t/ha)			DFY (t/ha)			First cut (%)			Second cut (%)			Third cut (%)		
		first cut	second cut	third cut	first cut	second cut	third cut	CP	IVOMD	ADL	CP	IVOMD	ADL	CP	IVOMD	ADL
29	WRajPop <i>bmr</i> S1-18-4-3-2-6-5-B-B-B	7.89 ± 1.20	NA	NA	1.14 ± 0.19	NA	NA	10.37 ± 1.16	45.06 ± 1.40	4.66 ± 0.16	NA	NA	NA	NA	NA	NA
30	WRajPop <i>bmr</i> S1-19-7-3-3-2-2-1-B-B-B	8.35 ± 1.20	5.02	1.70	1.10 ± 0.19	0.87	0.28	11.01 ± 1.16	48.62 ± 1.40	4.09 ± 0.17	11.81	50.74	3.69	13.53	50.39	3.65
31	WRajPop <i>bmr</i> S1-19-7-3-3-2-3-B-B-B	5.47 ± 1.20	NA	NA	0.83 ± 0.19	NA	NA	11.39 ± 1.16	43.44 ± 1.40	4.74 ± 0.16	NA	NA	NA	NA	NA	NA
32	WRajPop <i>bmr</i> S1-19-7-3-3-2-5-B-B-B	2.37 ± 1.71	NA	NA	0.37 ± 0.28	NA	NA	8.99 ± 1.65	45.12 ± 1.99	4.97 ± 0.15	NA	NA	NA	NA	NA	NA
33	WRajPop <i>bmr</i> S1-19-7-3-3-5-B-B-B	6.77 ± 1.20	NA	NA	0.90 ± 0.19	NA	NA	9.57 ± 1.16	44.74 ± 1.40	4.81 ± 0.15	NA	NA	NA	NA	NA	NA
34	WRajPop <i>bmr</i> S1-21-4-1-2-2-4-B-B	9.14 ± 1.20	2.12	2.11	1.28 ± 0.19	0.33	0.32	9.74 ± 1.16	45.07 ± 1.40	4.56 ± 0.16	12.60	50.50	3.64	12.19	47.30	3.97
35	WRajPop <i>bmr</i> S1-9-7-1-2-1-1-B	5.82 ± 1.20	2.45	1.25	0.78 ± 0.19	0.41	0.12	10.95 ± 1.65	47.21 ± 1.99	3.95 ± 0.17	13.00	50.55	3.55	15.21	51.19	3.37
36	WRajPop <i>bmr</i> S1-9-7-1-2-1-2-B	5.09 ± 1.20	2.55	1.25	0.62 ± 0.19	0.45	0.23	11.92 ± 1.16	49.64 ± 1.40	3.73 ± 0.18	11.29	46.11	4.44	14.75	53.51	3.23
	Minimum	0.66	0.85	0.36	0.03	0.16	0.08	8.96	43.44	3.73	9.08	45.61	3.50	12.19	47.30	3.23
	Maximum	10.27	5.10	2.62	1.66	0.87	0.49	12.51	51.09	5.05	13.13	51.81	4.62	15.21	54.28	3.97
	WRajPop <i>bmr</i> (original population)	20.35 ± 1.20	7.8	2.36	2.99 ± 0.19	1.53	0.55	NA	NA	NA	12.67	52.68	3.69	14.50	53.69	3.22
Checks																
	Nutrifed (PAC 981)	14.87 ± 1.20	16.77	10.21	1.91 ± 0.19	3.35	1.54	10.26 ± 1.16	45.57 ± 1.40	4.73 ± 0.16	12.43	51.26	3.77	11.90	51.77	3.84
	Nutrifast	18.61 ± 1.20	17.20	9.27	2.38 ± 0.19	2.99	1.50	9.16 ± 1.16	45.96 ± 1.40	4.69 ± 0.16	11.48	51.19	4.12	12.93	51.84	3.76

GFY – green forage yield; DFY – dry forage yield; CP – crude protein; IVOMD – *in vitro* organic matter digestibility; ADL – acid detergent lignin; NA – not available

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productivity (milk, meat and draught powers) in the range of 6–8% (Kristjansson & Zerbini 1999). In the materials under investigations in this study, the *bmr* linked important positive (IVOMD) and negative (ADL) forage quality traits ranged from 43.5 to 54.35%, and 3.15 to 5.05%, respectively, across the three cuts (Table 1). The mean values for ADL were lower and the mean values for IVOMD were higher in the *bmr* inbreds under investigation than those earlier reported that had normal midrib forage inbreds in pearl millet (Cherney et al. 1990; Mustafa et al. 2004; Hassanat et al. 2006). Additionally, the forage quality trait crude protein (CP) varied 8.50 to 15.21% across the three cuts in the inbreds. The results suggested that observed feed protein in these newly developed inbreds is more than the minimum (7%) requirement of rumen microbes (Blümmel et al. 2007). It was already reported in earlier studies that the CP was higher in *bmr* lines than the normal midrib of pearl millet inbreds (Cherney et al. 1998; Ferreira et al. 2020, 2022) as well as in sorghum (Pupo et al. 2022). This study helped to identify inbreds with a stable *bmr* coupled with improved forage yield and quality traits. Three and two ICMV 155 *bmr* (S. No. 4, 9 and 10) and WRajPop *bmr* (S. No. 17 and 30) samples were identified as promising sources of *bmr* with superior forage quantity and quality traits, respectively. Of these, three ICMV 155 *bmr* progenies and one WRajPop *bmr* progeny have now been used in a breeding programme as the donor parent for the introgression of *bmr* alleles into promising genetic backgrounds.

Inheritance of *bmr*. All the F₁ plants derived from four crosses involving normal midrib parents and brown midrib parental lines had normal midribs (Table 2) indicating that the *bmr* in pearl millet is controlled by recessive allele(s). The F₂ segregation data for the midrib colour in the ICMV 155 *bmr* crosses are presented in Table 2. In both the F₂s, the *bmr* colour segregation fitted well to a 3:1 (normal: brown) ratio indicating the single recessive gene control of *bmr*. All the normal plants observed in BCP₁ (backcross between F₁ and non-*bmr* parent) indicated the non-*bmr* parent used in this study to be in a homozygous state for the non-*bmr* trait. Earlier studies involving a *Pbmr* source (Cherney et al. 1988) also indicated the *bmr* in pearl millet to be controlled by a single recessive gene (Gupta 1995). The same study and another in sorghum (Vangala et al. 2020) also reported that some *bmr* × non-*bmr* crosses could not confirm the monogenic recessive

Table 2. Segregation for the midrib colour in F₁ and segregating generations derived from the crosses between the brown midrib and normal midrib lines in pearl millet

Crosses	Genera- tions	Observed (O)		Expected ratio		Expected (E)		O – E		(O – E) ²		(O – E) ² /E		χ ²	P value
		normal	brown	normal	brown	normal	brown	normal	brown	normal	brown	normal	brown		
ICMV 08111 S1-1-1-4-1-8-1-3-5 × ICMV 155 <i>bmr</i> S1-4-2-1-3-4-1-B-B	F ₁	30	0	–	–	–	–	–	–	–	–	–	–	–	–
	F ₂	90	32	3	1	91.5	30.5	–1.5	1.5	2.25	2.25	0.025	0.074	0.098	0.75
	BCP ₁	74	0	–	–	–	–	–	–	–	–	–	–	–	–
ICMV 08111 S1-1-1-4-1-8-1-3-5 × ICMV 155 <i>bmr</i> S1-4-2-1-3-5	F ₁	10	0	–	–	–	–	–	–	–	–	–	–	–	–
	F ₂	137	34	3	1	128.5	42.5	8.75	–8.75	76.56	76.56	0.597	1.791	2.388	0.12
	BCP ₁	83	0	–	–	–	–	–	–	–	–	–	–	–	–
ICMV 08111 S1-1-1-4-1-7-2-6-2-B-B-B × WRajPop <i>bmr</i> S1-19-7-3-2-2-1-B-B-B-B	F ₁	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	F ₂	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	BCP ₁	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ICMB 01888 × WRajPop <i>bmr</i> S1-19-7-3-2-2-1-B-B-B	F ₁	120	0	–	–	–	–	–	–	–	–	–	–	–	–
	F ₂	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	BCP ₁	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Significance limit of χ^2 ($P = 0.05$, $df = 1$) = 3.84

Table 3. Phenotypes of the F₁ hybrids for the allelism study

Brown midrib mutants	ICMV 155 <i>bmr</i> S1-4-2-1-3-4-B	ICMV 155 <i>bmr</i> S1-1-1-3-4-4-B	ICMV 155 <i>bmr</i> S1-3-3-1-2-2-B	ICMV 155 <i>bmr</i> S1-1-1-3-2-2-B	ICMV 155 <i>bmr</i> -4-2	ICMV 155 <i>bmr</i> -3-3
WRajPop <i>bmr</i> S1-9-7-7-1-2-1-B	<i>bmr</i>	–	–	–	–	–
WRajPop <i>bmr</i> S1-18-4-3-2-2-B	–	<i>bmr</i>	–	–	–	–
WRajPop <i>bmr</i> S1-18-4-3-2-6-1-B	<i>bmr</i>	<i>bmr</i>	<i>bmr</i>	–	–	–
WRajPop <i>bmr</i> S1-19-7-3-2-2-B	<i>bmr</i>	–	–	–	–	–
WRajPop <i>bmr</i> S1-18-4-3-2-3-B	<i>bmr</i>	–	–	–	–	–
WRajPop <i>bmr</i> S1-18-4-3-2-2-5-B	–	<i>bmr</i>	–	–	–	–
WRajPop <i>bmr</i> S1-18-4-3-2-3-4-B	–	–	–	<i>bmr</i>	–	–
WRajPop <i>bmr</i> -9-2	–	–	–	–	<i>bmr</i>	<i>bmr</i>
WRajPop <i>bmr</i> -7-4	–	–	–	–	–	<i>bmr</i>

– indicates that the cross was not developed

inheritance of *bmr* when this allele was introgressed in different genetic backgrounds. This was indicative that there can be the presence of some modifier genes which can change the expression of the *bmr* trait in different genetic backgrounds. In few crosses, the *bmr* was also found to be controlled by two genes (Gupta 1995). The finding of the recessive inheritance of the *bmr* in this study was, as expected, as the same *bmr* source (*Pbmr*) used in the introgression into two populations, was reported to be under recessive inheritance in previous studies (Cherney et al. 1988; Gupta 1995). The present study also found that there was no effect of modifier genes on the inheritance of *bmr* in the inbreds derived from the ICMV 155 *bmr* genetic background, hence leading to the stable expression of *bmr*.

Allelism of *bmr*. All the 12 F₁ plants derived from the ICMV 155 *bmr* × WRajPop *bmr* crosses had a brown midrib (Table 3), and all the F₂ plants derived from three of these crosses had the *bmr* trait (Table 4). The results suggested that the gene(s) controlling the trait in these *bmr* inbreds that were derived from two different genetic backgrounds are allelic as both the *bmr* parental populations were derived using the same source of *Pbmr* as reported by Cherney et al. (1988). Earlier studies also concluded that crosses between *Pbmr* with other reported *bmr* sources SDML 89107 and a spontaneous mutant from Tifton in pearl millet are allelic (Gupta et al. 1993; Degenhart et al. 1991). Likewise, in sorghum, two *bmr* genes *bmr30-1* and *bmr30-2* (isolated from an ethyl methane sulfonate (EMS)-mutagenised TILLING population BT×623) were cross-pollinated, and the F₁ progeny (*bmr30-2* × *bmr30-1*) plants have the *bmr* phenotype (allelic nature) (Tetreault et al. 2021).

CONCLUSION

Newly developed *bmr* inbreds from two genetic backgrounds (ICMV 155 *bmr* and WRajPop *bmr*)

Table 4. Segregation for the midrib colour in the F₂ cross combinations derived from the crosses between the *bmr* lines in pearl millet

Crosses	No. of plants in F ₂	
	non <i>bmr</i>	<i>bmr</i>
WRajPop <i>bmr</i> -9-2 × ICMV 155 <i>bmr</i> -4-2	0	138
WRajPop <i>bmr</i> -9-2 × ICMV 155 <i>bmr</i> -3-3	0	152
WRajPop <i>bmr</i> -7-4 × ICMV 155 <i>bmr</i> -3-3	0	67

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showed the stable expression of the *bmr* trait in the advanced generation progenies along with better forage quality traits. These identified promising sources can be used to develop better forage quality cultivars in pearl millet. The monogenic recessive inheritance revealed for *bmr* suggests that breeders need to develop both the hybrid parental lines (seed and pollinator parent) with *bmr* so as to develop a new series of quality forage *bmr* hybrids in pearl millet. These identified sources with *bmr* alleles are being maintained and seeds can be supplied on request for research purposes.

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