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Forage-Yield and Nutrient Quality of New Brown Midrib (BMR) Mutant Lines of Sorghum

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ABSTRACT

The objective of this study was to evaluate the yield, nutrient profile and *in vitro* digestibility of new BMR mutant lines of sorghum in Indonesia. These mutant lines were GH2.1, GH2.2, GH2.3, GH4.1, GH4.2, GH4.3 and GH4.4. One sorghum mutant line (CTY) and two national sorghum varieties (Super 1 and Bioguma) were also evaluated as controls. *In vitro* digestibility and rumen fermentation were measured using Ankom Daisy Fermenter and Hohenheim gas test methods, respectively. *In vitro* measurement consisted of ten treatments with five replications following a completely randomized design. The highest stem sugar content was found in Bioguma (11.22%) and GH4.4 (9.32%) ($p<0.05$). The Bioguma variety and the GH2.3 mutant line had a higher number of stem segments and fresh forage yield than the Super 1 variety ($p<0.05$). A greater concentration of crude protein (CP) was observed for the GH.2.1, GH2.2, GH2.3 and GH4.1 lines ($p<0.05$). The GH2.3 mutant line had the lowest acid detergent lignin (ADL) content ($p<0.05$), while Bioguma had the highest level of non-fibre carbohydrate (NFC) compounds ($p<0.05$). The highest relative feed value (RFV) was observed for the GH2.3 line ($p<0.05$). Furthermore, GH4.2 and GH2.3 had greater *in vitro* true digestibility (IVTD) ($p<0.05$) but were not significantly different from Bioguma. Regarding yield characteristics, nutrient composition and *in vitro* digestibility values, the highest values were found in the Bioguma variety and the GH2.3 mutant line. Except for *n*-valerate (nC5), significant differences in all rumen fermentation parameters were observed among sorghum cultivars ($p<0.05$). Regarding the interrelationship between parameters, we found a medium correlation of DMD with the ADL and cellulose content of sorghum forage (R^2 = -0.489 and R^2 = -0.674, respectively). Based on these findings, the GH2.3 BMR mutant line should be further developed as forage sorghum.

Keywords: brown midrib; in vitro digestibility; mutant lines; sorghum

INTRODUCTION

As an archipelagic country, Indonesia has various agroecosystem characteristics, and these present challenging conditions for the livestock development, especially forage crops. Sorghum (*Sorghum bicolor* (L.) Moench) is a feed crop with potential for development in Indonesia because of its tolerance and adaptability to dry environments (Sajimin *et al.*, 2017; Wahyono *et al.*, 2019). Perazzo *et al*. (2017) and Astuti *et al.* (2019) report that because of its morpho-physiological adaptations to water stress, sorghum is an important forage source in arid and semiarid regions, as well as in environments with uneven distribution of rainfall.

As described by Li *et al*. (2015), forage sorghum can be divided into white midrib (WMR), green midrib (GMR) and brown midrib (BMR) types. The BMR type has been popularly developed commercially to support productivity (Godin *et al.*, 2016). BMR mutations are phenotypically portrayed by the presence of brown vascular tissues in the leaf edge and sheath as well as in the stem (Rao *et al.*, 2012). BMR sorghum results from a genetic mutation that contains fewer lignin compounds (Green *et al.*, 2014; Sriagtula *et al.*, 2021) and is a promising forage source because of these low-lignin characteristics (Sriagtula *et al.*, 2019). The BMR type contains less cell wall content than standard forage sorghum (Astigarraga *et al.*, 2014). The digestibility of BMR sorghum forage is higher than that of grain-class sorghum (Bean *et al.*, 2013). It has also been identified as a potential alternative to corn silage in the livestock industry (Lyons *et al.*, 2019). Sánchez-Duarte *et al*. (2019)

reviewed that cows fed BMR silage performed similarly to those fed corn silage on milk production and composition.

In the last five years, research into the forage characteristics of BMR sorghum mutant lines has been carried out in Indonesia, with mutant lines with low-lignin trait genotype and high digestibility being identified as superior both in terms of nutritional value and productivity (Puteri *et al.*, 2015). Sriagtula *et al*. (2021) reported that two BMR mutant lines (Patir 3.2 and Patir 3.7) contained less acid detergent fibre (ADF) and lignin content than non-BMR mutant lines. Furthermore, it was explained that the digestibility of the fibre fraction was noticeably higher. Wahyono *et al*. (2019) demonstrated that G5, one of the BMR lines, produced lower fibre fractions than conventional sorghum varieties. Recently, Indonesia has developed seven mutant lines belonging to the BMR type, namely GH2.1, GH2.2, GH2.3, GH4.1, GH4.2, GH4.3 and GH4.4. These mutant lines need to be compared with non-mutant sorghum and Indonesian national varieties. Super 1 and Bioguma are the two main varieties of sorghum cultivated in Indonesia. CTY is a non-BMR mutant line that is usually used as a parent. These three groups of sorghum (national varieties, non-BMR mutant line and BMR mutant lines) need further investigation. Until now, no research has provided information regarding the biomass yield, nutrient composition and digestibility value of these seven new mutant lines. The criteria indicating reliable forage are plants that have been evaluated as producing high biomass yields and nutrient values. The criteria for good BMR types can be determined by the growth and digestibility of the plants (Sriagtula *et al.*, 2017). For effective animal feed management, it is very important to provide information on the chemical content of available potential forages (Faji *et al.*, 2021). The present study was therefore designed to evaluate the yield, nutrient profile and *in vitro* digestibility of new BMR mutant lines of sorghum forage in Indonesia.

MATERIALS AND METHODS

Planting and Agronomy Evaluation

This research was conducted from the middle of the rainy season to the beginning of the dry season in 2021 at the Pasar Jumat Research Station, Research Centre for Isotope and Radiation Application Technology (6° 29′47″ S, 106° 77′51″ E, elevation 27 m). The climate is Schmit Ferguson Classification type D, a hot tropical area with high rainfall spread evenly throughout the year, 2000 mm mean annual precipitation, 28 °C average temperature, and 80% average air humidity. The soil type is latosol, with average pH of 5.6 and low organic-matter content. Seven BMR sorghum mutant lines (GH2.1, GH2.2, GH2.3, GH4.1, GH4.2, GH4.3 and GH4.4), one non-BMR mutant line (CTY) and two sorghum varieties (Super 1 and Bioguma) were used as treatments. Sorghum seed materials (mutant and non-mutant) were obtained from Research Centre for Isotope and Radiation Application Technology, National Research and Innovation Agency of Indonesia (BRIN). Each cultivar was sown on January 14, 2021, in five replicated plots (1.5 m x 2.5 m each unit planting area). The plots were fertilized with 100 kg N/ha, 50 kg K_2O/ha and 100 kg P_2O_5/ha. A second fertilizer dose of 100 kg N/ha (urea) was applied one month after sowing. Before being harvested at the hard dough stage (115 days after sowing), plant height was observed from the top of the soil to the highest leaf tip. All forages were manually cut at the height of 5 cm above the ground. Five samples were randomly collected from a 1 m^2 area of each replicate plot of each cultivar. The variables observed were stem diameter (mm), number of stem segments, sugar Brix (% Brix), chlorophyll content and fresh forage yield (Mg ha^{-1}).

Nutrient and Fibre Evaluation

All edible parts (stems and leaves) were separately chopped and then uniformly mixed to create a representative sample. Forage samples were placed into individual paper bags, dried at 65 °C for 72 h, milled and passed through a 1 mm sieve. The samples were then analyzed according to AOAC (2012) standards for the content of organic matter (OM), crude protein (CP) and ether extract (EE). Fibre analyzer ANKOM A200 (Ankom Technology Corp, Fairport, NY, USA) was used to analyze neutral detergent fibre (NDF) and acid detergent fibre (ADF). Acid detergent lignin (ADL) was analyzed according to Van *et al*. (1991). Hemicellulose, cellulose and non-fibre carbohydrate (NFC) content were calculated using the equations described below:

Hemicellulose (%) = NDF (%) – ADF (%)
Cellulose (%) = ADF (%) – ADL (%)
NFC (%) = OM (%) – CP (%) – NDF (%) – EE (%)

Relative Feed Value Estimation

Relative feed value (RFV) was assessed using the method described by Rohweder *et al*. (1978) and Kilic and Gulecyuz (2017). Estimation of RFV, dry matter intake (DMI) and dry matter digestibility (DMD) were calculated using the equations described below:

DMI (% LW) = 120/% NDF
DMD (%) = 88.9/(% ADF × 0.779)
RFV = (DMD × DMI)/1.29

The RFV was classified according to the Quality Grading Standard of the Hay Marketing Task Force of the American Forage and Grassland Council as follows: 1) prime (> 151); 2) premium (151–125); 3) good (124–103); 4) fair (102–87); 5) poor (86–75); and 6) reject (< 75) (Rohweder *et al.*, 1978; Wahyono *et al.*, 2019).

In Vitro Assay

In vitro digestibility was determined using *in vitro* Ankom Daisy Fermentation Equipment (Ankom Technology Corp, Fairport, New York, USA) (Ayaşan *et al.*, 2020[a]). This study was carried out following Centre for Isotope and Radiation Application Animal Ethics Committee protocols (Approved Protocol Number 001/KEPPHP-BATAN/X/2021). Rumen fluids were collected

from two fistulated Friesian Holstein cattle weighing approximately 525 kg. The animals were fed twice a day on 70% native grass and 30% concentrated diet on DM basis. Buffer solutions were prepared according to the Ankom Daisy Fermentation procedure. Approximately 500 g (DM basis) samples were inserted into F57 Ankom filter bags (Ankom Technology Corp, Fairport, New York, USA) and placed in four digestion jars. Rumen liquor (400 mL) and buffer solutions (1200 mL) were mixed into each digestion jar and incubated at 39 °C for 48 h. After incubation, bags were rinsed in tap water and dried at 105 °C for 12 h. Samples were analyzed for NDF using fibre analyzer ANKOM A200 (Ankom Technology Corp, Fairport, NY, USA). *In vitro* true digestibility (IVTD) was calculated using the equation previously reported by Wahyono *et al*. (2021), as follows:

$$IVTD\ (\%\ DM) = \{[100-(W3-(W1\times C))] / (W2\times \%DM)\} \times 100$$

where IVTD was *in vitro* true digestibility, W1 was F57 filter bag weight, W2 was sample weight, W3 was sample weight after NDF analysis, and C1 was correction factor of blank filter bag.

In vitro rumen fermentation was determined according to the *in vitro* technique described by Menke & Steingass (1988). This research consisted of ten treatments with five replications following a completely randomized design. The treatments were GH2.1, GH2.2, GH2.3, GH4.1, GH4.2, GH4.3, GH4.4, CTY, Super 1 and Bioguma. The observed parameters were pH, NH_3 concentration, single chain fatty acids (SCFAs) and methane production. Approximately 200 mg samples (DM basis) were weighed into 100 mL glass syringes (Fortuna, Labortechnik, Germany) and filled with 30 mL rumen buffer fluid. Approximately 10 mL of medium was collected after 48 h incubation to determine pH, NH_3 concentration and single chain fatty acids (SCFAs) (Sondakh *et al.*, 2017). Based on the proportions of SCFAs, methane production was estimated using the following equation (Widiawati & Thalib, 2007):

CH_4 (mM)= (0.5 × acetate concentration) + (0.5 × butyrate concentration) – (0.25 × propionate concentration)

Methane production was converted to mM/100 mg DMD.

Data Analysis

All data were analyzed using a one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate means when significant differences were observed at $p<0.05$ (Steel & Torrie, 1960). An orthogonal set of contrasts was used to identify the differences between national varieties x mutant lines (NV*M), non-mutant line x mutant lines (NM*M), and mutant lines (M). A simple linear correlation analysis was performed to determine the relationship between parameters. Data were analyzed using SPSS 23.0 software (IBM, Armonk, New York, USA).

RESULTS

Agronomy Characteristics

Agronomy characteristics and total fresh forage yield (kg ha^{-1}) are presented in Table 1. GH2.2 and GH2.3 mutant lines were taller than Super 1 and CTY ($p<0.05$). Both GH2.3 and Bioguma had more internodes and greater fresh forage yield than Super 1 as a control variety ($p<0.05$). The average stem diameter of GH2.1 and GH2.2 was higher than that of CTY as a non-BMR mutant control ($p<0.05$). Except for GH4.4, there were no significant differences in chlorophyll contents between cultivars. Stem sugar content was greater in GH4.4 and Bioguma than in the other cultivars, at 9.32 % Brix and 11.22 % Brix, respectively ($p<0.05$).

Nutrient and Fibre Characteristics

Nutrient composition and fibre fractions from the ten cultivars of forage sorghum are shown in Table 2. Among cultivars, Ash, OM, CP, NDF, ADF, hemicellulose and NFC contents were significantly different ($p<0.001$). Cellulose was significantly influenced by cultivar ($p<0.01$). Ether extract (EE) and ADL were also significantly different among cultivars ($p<0.05$). The GH2.3 mutant line had higher OM content ($p<0.05$) than the other BMR mutant lines, while GH4.2 had higher EE than Super 1, Bioguma, and CTY ($p<0.05$), and GH2.1 had the highest CP percentage ($p<0.05$). However, there were no differences in GH2.2. Concerning fibre composition, Bioguma and GH2.3 had the lowest NDF percentage ($p<0.05$), while GH2.3 had the lowest ADF and ADL content of 38.99 and 3.56% DM, respectively ($p<0.05$). The ADL content of BMR mutant lines in this experiment varied between 3.56% and 7.80% DM. Bioguma had the highest NFC content (15.05% DM), followed by GH2.3 (13.11% DM).

Relative Feed Value and Digestibility

Given the differences in nutrient and fibre composition in sorghum cultivars, IVTD of forage sorghum was determined to investigate nutrient value. Results for RFV prediction and IVTD are presented in Table 3. The highest values of DMI and DMD ($p<0.05$) were obtained for GH2.3, with 1.81% and 58.53%, respectively. Except for Bioguma and GH2.3, all cultivars were included in the reject class due to their RFV score being less than 75. The average RFV obtained from BMR mutant lines was estimated at 70.34. The highest and lowest values of IVTD ($p<0.05$) were found in GH4.2 (62.27%) and GH2.1 (53.10%).

In Vitro Fermentation and the Estimated Methane Production

In vitro rumen fermentation products of BMR mutant lines and control varieties are shown in Table 4. Total SCFAs, pH, N-NH_3, acetate, propionate, iso-butyrate and isovalerate contents were significantly different among cultivars ($p<0.01$). The ratio of acetate propionate

Table 1. Yield characteristics of new brown midrib mutant lines and national varieties sorghum in Indonesia

Cultivar	Characteristics					
	Height (cm)	Internodes number	Stem diameter (mm)	Chlorophyll content	Sugar stem content (% Brix)	Fresh forage yield (Mg ha^{-1})
Variety						
Super 1	279.40^{b}	11.00^{a}	17.00^{ab}	28.90^{ab}	5.38^{ab}	49.58^{abc}
Bioguma	296.80^{bc}	14.80^{c}	18.63^{abc}	24.58^{a}	11.22^{c}	78.44^{e}
Non-BMR mutant line						
CTY	244.00^{a}	11.20^{ab}	16.85^{a}	30.08^{ab}	5.48^{ab}	32.98^{a}
BMR mutant line						
GH2.1	288.20^{bc}	11.80^{ab}	21.08^{c}	28.78^{ab}	5.40^{ab}	65.84^{cde}
GH2.2	305.00^{c}	13.00^{abc}	20.89^{bc}	25.50^{ab}	5.58^{ab}	67.26^{cde}
GH2.3	305.00^{c}	13.20^{bc}	20.50^{abc}	32.70^{ab}	5.22^{ab}	74.99^{de}
GH4.1	296.00^{bc}	12.00^{ab}	18.24^{abc}	33.40^{ab}	3.40^{a}	41.93^{abc}
GH4.2	290.60^{bc}	12.00^{ab}	18.76^{abc}	31.90^{ab}	5.76^{ab}	68.81^{cde}
GH4.3	288.40^{bc}	12.80^{abc}	17.27^{abc}	34.90^{ab}	6.62^{b}	66.53^{cde}
GH4.4	252.00^{a}	12.00^{ab}	17.10^{ab}	41.64^{b}	9.32^{c}	56.45^{bcd}
SEM	3.233	0.239	0.407	1.553	0.402	2.655
p-value	0.001	0.01	0.07	0.45	0.001	0.001
Contrast						
V*M	0.001	0.43	0.15	0.13	0.15	0.001
NV*M	0.67	0.29	0.09	0.10	0.001	0.84
NM*M	0.001	0.07	0.04	0.45	0.70	0.001
M	0.001	0.41	0.01	0.14	0.25	0.05

Note: BMR=brown midrib; V*M=varieties x mutant lines; NV*M=national varieties x mutant lines; NM*M=non-BMR mutant line x mutant lines; M=mutant lines (GH2.1 vs GH2.2 vs GH2.3 vs GH4.1 vs GH4.2 vs GH4.3 vs GH4.4). Means in the same column with different superscripts differ significantly ($p<0.05$).

Table 2. Nutrient profiles and fibre contents (%DM) of new brown midrib mutant lines and national varieties sorghum in Indonesia

Cultivar	Nutrient profile and fibre content									
	Ash	OM	EE	CP	NDF	ADF	Hemicellulose	Cellulose	ADL	NFC
Variety										
Super 1	8.19^{bc}	91.81^{cd}	3.93^{a}	7.30^{de}	71.35^{bcd}	43.99^{c}	27.36^{c}	36.57^{abc}	7.42^{b}	9.23^{bcd}
Bioguma	7.77^{abc}	92.23^{cde}	3.80^{a}	6.77^{bc}	66.61^{a}	41.80^{b}	24.81^{a}	34.30^{a}	7.51^{b}	15.05^{f}
Non-BMR mutant line										
CTY	9.15^{de}	90.85^{ab}	3.42^{a}	6.53^{a}	70.31^{b}	43.47^{c}	26.84^{bc}	37.48^{bc}	5.99^{b}	10.60^{d}
BMR mutant line										
GH2.1	7.98^{bc}	92.02^{cd}	3.54^{a}	7.36^{f}	72.14^{bcde}	46.59^{d}	25.56^{ab}	38.89^{c}	7.70^{b}	8.98^{bcd}
GH2.2	6.95^{a}	93.05^{e}	4.90^{ab}	7.21^{def}	71.01^{bc}	45.25^{cd}	25.76^{ab}	38.76^{c}	6.49^{b}	9.94^{cd}
GH2.3	9.90^{e}	90.10^{a}	3.65^{a}	7.17^{de}	66.18^{a}	38.99^{a}	27.18^{c}	35.43^{ab}	3.56^{a}	13.11^{e}
GH4.1	8.52^{bc}	91.48^{bc}	4.06^{a}	7.07^{d}	72.87^{cde}	45.19^{cd}	27.68^{c}	37.89^{bc}	7.30^{b}	7.48^{ab}
GH4.2	7.96^{bc}	92.04^{cd}	6.01^{b}	6.83^{c}	73.23^{de}	45.87^{d}	27.36^{c}	39.31^{c}	6.56^{b}	5.98^{a}
GH4.3	7.34^{ab}	92.66^{de}	4.35^{ab}	6.83^{c}	73.74^{e}	46.35^{d}	27.39^{c}	38.55^{c}	7.80^{b}	7.75^{ab}
GH4.4	7.42^{ab}	92.58^{de}	5.02^{ab}	6.65^{ab}	72.55^{cde}	45.00^{cd}	27.55^{c}	37.51^{bc}	7.49^{b}	8.36^{bc}
SEM	0.147	0.147	0.191	0.042	0.406	0.358	0.185	0.329	0.278	0.406
p-value	0.001	0.001	0.04	0.001	0.001	0.001	0.001	0.001	0.01	0.001
Contrast										
V*M	0.62	0.62	0.03	0.001	0.001	0.01	0.10	0.02	0.96	0.001
NV*M	0.91	0.91	0.17	0.69	0.001	0.001	0.001	0.01	0.27	0.001
NM*M	0.05	0.05	0.07	0.001	0.23	0.15	0.84	0.55	0.19	0.10
M	0.02	0.02	0.08	0.001	0.001	0.001	0.001	0.29	0.03	0.001

Note: Dry matter (DM); organic matter (OM); ether extract (EE); crude protein (CP); neutral detergent fibre (NDF); acid detergent fibre (ADF); acid detergent lignin (ADL); non fibre carbohydrate (NFC); BMR=brown midrib; V*M=varieties x mutant lines; NV*M=national varieties x mutant lines; NM*M=non-BMR mutant line x mutant lines; M=mutant lines (GH2.1 vs GH2.2 vs GH2.3 vs GH4.1 vs GH4.2 vs GH4.3 vs GH4.4). Means in the same column with different superscripts differ significantly ($p<0.05$).

Table 3. The estimated relative feed value and in vitro true digestibility of new brown midrib mutant lines and national varieties sorghum in Indonesia

Cultivar	Indicators				
	Dry matter intake (% Live weight)	Dry matter digestibility (%)	Relative feed value	*In vitro* true digestibility (%)	Quality grading standard
Variety					
Super 1	1.68^{bcd}	54.63^{b}	71.28^{bc}	55.81^{ab}	Reject
Bioguma	1.80^{e}	56.34^{c}	78.70^{d}	58.81^{bc}	Poor
Non-BMR mutant line					
CTY	1.71^{d}	55.04^{b}	72.91^{c}	58.98^{bc}	Reject
BMR mutant line					
GH2.1	1.66^{abcd}	52.61^{a}	67.89^{ab}	53.10^{a}	Reject
GH2.2	1.69^{cd}	53.65^{ab}	70.36^{abc}	55.77^{ab}	Reject
GH2.3	1.81^{e}	58.53^{d}	82.29^{e}	59.55^{bc}	Poor
GH4.1	1.65^{abc}	53.70^{ab}	68.61^{ab}	54.85^{ab}	Reject
GH4.2	1.64^{ab}	53.17^{a}	67.55^{ab}	62.27^{c}	Reject
GH4.3	1.63^{a}	52.79^{a}	66.60^{a}	58.86^{bc}	Reject
GH4.4	1.65^{abc}	53.84^{ab}	69.70^{ab}	58.10^{bc}	Reject
SEM	0.01	0.279	0.775	0.558	
p-value	0.001	0.001	0.001	0.01	
Contrast					
V*M	0.01	0.01	0.01	0.59	
NV*M	0.001	0.001	0.001	0.81	
NM*M	0.25	0.15	0.21	0.52	
M	0.001	0.001	0.001	0.07	

Note: BMR= brown midrib; V*M= varieties x mutant lines; NV*M= national varieties x mutant lines; NM*M= non-BMR mutant line x mutant lines; M= mutant lines (GH2.1 vs GH2.2 vs GH2.3 vs GH4.1 vs GH4.2 vs GH4.3 vs GH4.4). Means in the same column with different superscripts differ significantly ($p<0.05$).

is also significantly different ($p<0.05$) among cultivars. The pH value of sorghum cultivars varied between 6.77 and 6.89. The highest N-NH_3 concentration was obtained from the Super 1 variety (56.44 mg 100 mL^{-1}) followed by CTY (51.09 mg 100 mL^{-1}). The greatest total SCFA concentrations were produced by GH4.3 (115.21 mM) and GH4.4 (110.13 mM). Except for valerate, the SCFA profiles were detected as different ($p<0.05$), with GH4.3 and GH4.4 producing the highest acetate concentration. The highest methane production was obtained from GH4.4, GH4.3 and Super 1 (Figure 1) ($p<0.05$), while the lowest was obtained from CTY, Bioguma and GH2.2.

Interrelationship Between Nutrient Composition, Ruminal Fermentation and Digestibility

Figure 2 shows the interrelationship between nutrient composition, IVTD and ruminal fermentation in the new BMR sorghum mutant lines. We found a strong correlation ($p<0.01$) of RFV with the NDF, ADF and cellulose contents (R^2 = -0.973, R^2 = -0.970 and R^2 = -0.687, respectively). Although not significant ($p>0.05$), we found weak correlations of IVTD with the NDF and ADF content (R^2 = -0.201 and R^2 = -0.202, respectively). Furthermore, we found medium correlations of DMD with the ADL and cellulose content of sorghum forage (R^2 = -0.489 and R^2 = -0.674, respectively). In contrary, there was a strong positive correlation between DMD and NFC ($p<0.01$; R^2 = 0.723). There was a positive correlation between methane and NDF ($p<0.05$; R^2 = 0.293), as well as between methane and hemicellulose ($p<0.05$; R^2 = 0.387). On the contrary, there was a weak positive correlation between methane and NFC ($p<0.01$; R^2 = 0.372).

DISCUSSION

Plant height, number of stem segments and stem diameter are the parameters used to represent forage biomass. In our findings, plant heights of BMR mutant lines are between 252 cm and 305 cm. Except for GH4.4, there are no significant differences in plant height between BMR mutant lines and the Bioguma control variety. Previous studies have reported no difference in plant height between BMR and conventional types (Miron *et al.*, 2005). The absence of plant height difference between genotypes was consistent at flowering and dough physiological age (Li *et al.*, 2015). Plant height positively correlated with sorghum biomass production (Silungwe, 2011). As well as high stem sugar value, the Bioguma variety exhibited a greater plant size. High stem sugar content places a sorghum variety in the bioethanol category. According to genetic traits, bioethanol sorghum varieties are larger than BMR cultivars. Bioethanol types also produce greater average height than BMR sorghum (Puteri *et al.*, 2015). Stem height, stem diameter, variety and harvesting time affect the production of stem juice. We assume that the high juice production of sorghum also has a positive effect when applied as a forage. The number of internodes and stem diameter represent the amount of sugar in the form of energy in sweet sorghum.

Table 4. *In vitro* rumen fermentation product of new brown midrib mutant lines and national varieties sorghum in Indonesia

Cultivar	*In vitro* rumen fermentation products									
	pH	N-NH_3 (mg 100 mL^{-1})	Total SCFAs (mM)	C_2	C_3	iC_4	nC_4	iC_5	nC_5	C_2:C_3
				(mol% SCFAs)						
Variety										
Super 1	6.82^{ab}	56.44^{c}	94.16^{abc}	48.40^{abc}	17.04^{ab}	1.70^{ab}	20.00^{b}	4.88^{abcd}	2.14^{ns}	2.85^{c}
Bioguma	6.77^{a}	41.99^{b}	74.80^{a}	40.61^{a}	14.79^{a}	1.61^{a}	11.54^{a}	4.19^{a}	2.05^{ns}	2.74^{bc}
Non-BMR mutant line										
CTY	6.77^{a}	51.09^{c}	81.67^{ab}	44.37^{ab}	16.30^{ab}	1.89^{ab}	12.65^{a}	4.41^{ab}	2.06^{ns}	2.73^{abc}
BMR mutant line										
GH2.1	6.86^{bc}	36.24^{b}	85.64^{ab}	44.61^{ab}	16.70^{ab}	2.75^{abc}	14.04^{ab}	5.27^{abcde}	2.27^{ns}	2.67^{abc}
GH2.2	6.86^{bc}	41.72^{b}	82.15^{ab}	42.69^{ab}	16.56^{ab}	3.20^{abcd}	12.78^{a}	4.69^{abc}	2.23^{ns}	2.58^{ab}
GH2.3	6.86^{bc}	39.72^{b}	99.04^{bcd}	51.70^{bcd}	20.16^{bc}	4.15^{bcd}	14.87^{ab}	5.39^{bcde}	2.76^{ns}	2.56^{ab}
GH4.1	6.82^{ab}	28.35^{a}	86.59^{ab}	43.53^{ab}	17.13^{ab}	4.10^{bcd}	13.71^{ab}	5.68^{cde}	2.44^{ns}	2.54^{a}
GH4.2	6.85^{bc}	40.65^{b}	101.01^{bcd}	51.26^{abcd}	19.96^{bc}	4.67^{cd}	16.43^{ab}	6.14^{e}	2.55^{ns}	2.57^{ab}
GH4.3	6.89^{c}	43.13^{b}	115.21^{d}	59.43^{d}	22.37^{c}	8.04^{e}	16.31^{ab}	6.30^{e}	2.76^{ns}	2.66^{abc}
GH4.4	6.85^{bc}	42.39^{b}	110.13^{cd}	58.56^{cd}	21.95^{c}	5.58^{d}	15.95^{ab}	5.87^{de}	2.22^{ns}	2.67^{abc}
SEM	0.009	1.475	2.852	1.447	0.563	0.405	0.684	0.156	0.074	0.023
p-value	0.001	0.001	0.001	0.01	0.001	0.001	0.22	0.001	0.22	0.03
Contrast										
V*M	0.001	0.001	0.02	0.04	0.001	0.001	0.68	0.001	0.06	0.01
NV*M	0.001	0.001	0.06	0.09	0.02	0.001	0.79	0.01	0.06	0.02
NM*M	0.04	0.001	0.13	0.21	0.18	0.001	0.18	0.05	0.22	0.11
M	0.68	0.79	0.02	0.03	0.03	0.01	0.03	0.01	0.70	0.96

Note: N-ammonia (N-NH_3); single chain fatty acids (SCFAs); acetate (C2); propionate (C3); iso butyrate (iC4); n butyrate (nC4); iso valerate (iC5); n valerate (nC5): acetate propionate ratio (C2:C3); BMR=brown midrib; V*M=varieties x mutant lines; NV*M=national varieties x mutant lines; NM*M=non-BMR mutant line x mutant lines; M=mutant lines (GH2.1 vs GH2.2 vs GH2.3 vs GH4.1 vs GH4.2 vs GH4.3 vs GH4.4). Means in the same column with different superscripts differ significantly ($p<0.05$); no significant (ns).

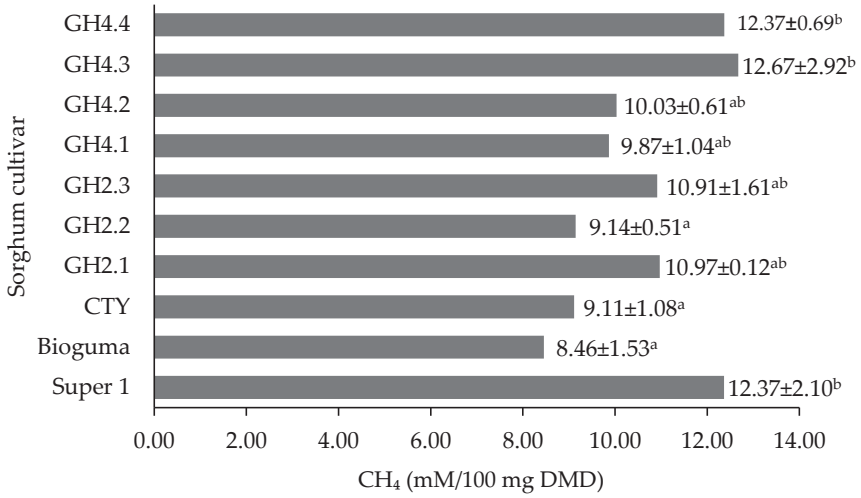

Figure 1. The predicted methane production of new brown midrib mutant lines and national varieties sorghum in Indonesia. Note: Different superscripts mean significantly differences ($p<0.05$).

All BMR genotypes produced yields that tended to be the same or higher than the Super 1 and CTY controls. However, Bioguma was still superior to the BMR type (except GH2.3). Varying results were also reported by previous researchers. Bean *et al*. (2013) reported that the reduced lignin was associated with the reduced yield; however, genetic background as well as the environment, may have had a substantial impact. On the other hand, GH 2.3 still produced high forage yields even though the lignin content was also low. This may be due to the low grain production of GH2.3, which is associated with high forage yield production. Grain yields of BMR lines were relatively low compared to the other sorghum breeding lines (Vietor *et al.*, 2010). BMR-12 and BMR-6 genotypes have better agronomic superiority (relative fresh weight and relative dry weight) than wild parent sorghum forage under salinity stress in both glass house and field studies (Vinutha *et al.*, 2018). BMR lines and sweet sorghum have many equal yield and agronomy characteristics. Therefore, BMR sorghum

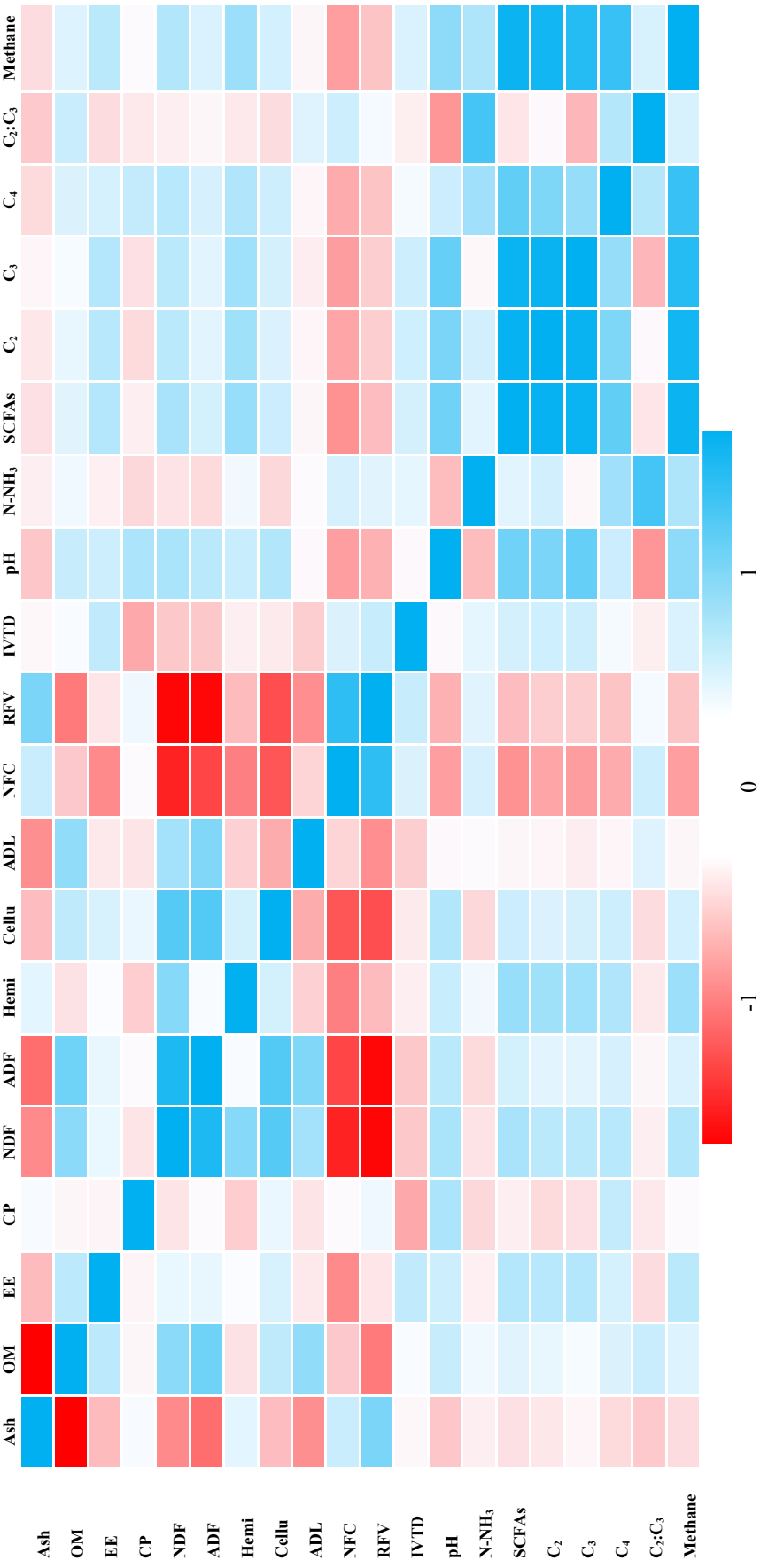

Figure 2. Interrelationship between nutrient composition, ruminal fermentation and *in vitro* true digestibility. Note: Organic matter (OM); ether extract (EE); crude protein (CP); neutral detergent fibre (NDF); acid detergent fibre (ADF); acid detergent lignin (ADL); non fibre carbohydrate (NFC); relative feed value (RFV); *in vitro* true digestibility (IVTD); N-ammonia ($N\text{-}NH_3$); single chain fatty acids (SCFAs); acetate (C_2); propionate (C_3); butyrate (C_4); acetate propionate ratio (C_2:C_3).

has the potential to produce high-energy forage for livestock. From the point of view of energy conversion, high yield combined with high levels of chemical characteristics will aid in decision-making as to which materials have the greatest potential for the future development of bioenergy (Almeida *et al*., 2019). Therefore, it is necessary to observe the composition of nutrients and fibre in this study.

Forage nutritive value is determined by nutrient composition, particularly NFC, NDF, ADF and CP, as reported by Lyons *et al*. (2019) and Wahyono *et al*. (2019). Non-fibre carbohydrate includes rapidly fermentable carbohydrates (starch and soluble sugars) (Kondo *et al*., 2015) and is calculated through a formula that uses ash, EE, CP and NDF (Table 2) (Ayaşan *et al*., 2020b). In the present findings, the GH2.3 mutant line was the only genotype with higher NFC than the Super 1 control variety (the control variable is being classed as sweet sorghum). For example, high levels of NFC in Bioguma varieties are also accompanied by high stem sugar content (Table 1). Furthermore, harvesting sorghum in the dough phase will increase sugar accumulation in the stems (Qu *et al*., 2014; Sriagtula *et al*., 2017; Wahyono *et al*., 2019). High levels of NFC may be associated with low lignin content in plants (Wahyono *et al*., 2019).

Neutral detergent fibre (NDF) is the primary chemical component in forages and measures cell-wall composition. This fraction is composed of hemicellulose, cellulose and lignin components. Lower NDF content is associated with lower lignin content (Wahyono *et al*., 2019). Low NDF is correlated with high partitioning factor and microbial biomass production in the rumen (Raju *et al*., 2021). In the present study, the NDF content of BMR sorghum ranged from 66.18% to 73.74%. This range is higher than the NDF value in several previous studies, which reported the NDF range of BMR sorghum as being around 55.88%–59.85% (de Aguilar *et al*., 2014), 48.70%–54.1% (Godin *et al*., 2016), 45.11%–63.31% (Wahyono *et al*., 2019) and 48.01%–69.69% (Sriagtula *et al*., 2021). Variation in mean NDF among experiments may be due to differences in characteristics of genotypes, rainfall, growing season and/or planting treatment. However, in our findings, the GH2.3 mutant line contained lower NDF content than the control variety. BMR types had consistently higher nutritive values than green midrib (GMR) and white midrib (WMR) sorghum types (Li *et al*., 2015).

Most BMR mutant genotypes showed the lowest cellulose and lignin amounts (de Aguilar *et al*., 2014; Sattler *et al*., 2015), representing ADF content. However, only GH2.3 produced lower ADF and ADL values than the control varieties. These results allow us to initiate investigations into genetically similar BMR lines to sweet sorghum in further studies. In the present study, the variation pattern in ADF and ADL contents among mutant lines was similar to the variation of NDF (Wahyono *et al*., 2019). ADF and ADL ranges for BMR mutant lines were from 38.99% to 46.59% and 3.56% to 7.80%, respectively. These values follow Vinutha et al. (2018), who reported a range of ADF (%) for BMR mutants of 42.67% to 49.53% under normal environmental conditions. Conversely, this value is lower when compared to the findings of Godin *et al*. (2016) and Lyons *et al*. (2019), i.e. 26.40%–29.90% and 26.00%–30.70%, respectively. However, the value is lower than the ADF range in non-BMR sorghum, 51.94%–57.72% (Raju *et al*., 2021). The low levels of ADL in GH2.3 prove that the BMR type tends to produce low amounts of lignin. All BMR mutants had significantly lower ADL values than wild-type ones (Scully *et al*., 2016). Compared with non-BMR types, the mean ADL content was lower by 28.0% (Li *et al*., 2015). The genotypes of BMR sorghum types show significantly lower lignin content than conventional types (Almeida *et al*., 2019), making these genotypes more promising for the enzymatic conversion process. Interestingly, the range of ADL among BMR mutants is high, i.e. 3.02%–6.09% (Rao *et al*., 2012). Variations in ADL content may be due to cultivation management. For example, additional water could increase lignin content (Qu *et al*., 2014). There are negative relationships between NDF, ADF and ADL content and nutrient digestibility (Faji *et al*., 2021; Kondo *et al*., 2015; Puteri *et al*., 2015; Wahyono *et al*., 2019).

The EE content of BMR mutant lines in the present study ranged from 3.54% to 6.01%. Other studies have examined the EE content of BMR types in Indonesia, including Puteri *et al*. (2015), Sriagtula *et al*. (2017) and Wahyono *et al*. (2019) and reported values of BMR sorghum of 0.34%–1.84%, 1.16%–2.27% and 2.03%–2.38%, respectively. These differences might be due to differences in the lipid content of sorghum grain or differences in the wax content of leaves (Wahyono *et al*., 2019). Among all BMR mutant lines in this study, only GH2.1 produced greater CP content than control varieties. In several previous studies, there were variations in the content of CP between BMR and non-BMR lines. Bean *et al*. (2013) stated that the BMR trait did not affect CP content (average 7%). In contrast, Godin *et al*. (2016), Wahyono *et al*. (2019) and Sriagtula *et al*. (2021) reported that BMR mutant lines had higher CP than non-BMR lines. Apparently, there is still speculation regarding the effect of the BMR trait on CP content in sorghum. Different CP content could be attributed to different soil types, cultivation methods, and plant genotypes (Raju *et al*., 2021). Differences in the interaction of ecological conditions, total rainfall and temperature during the planting period may also cause differences in nutrient content (Ayaşan *et al*., 2020b). CP ranged from 6.65% to 7.36% for all BMR mutant lines. This range is similar to CP in BMR sorghum found in other studies, ranging from 5.80% to 9.28% (Bean *et al*., 2013; Lyons *et al*., 2019; Wahyono *et al*., 2019; Sriagtula *et al*., 2021).

RFV is used to compare similar forage types for two important qualities: how well they will be consumed and how well they will be digested (Jahansouz *et al*., 2014). BMR sorghum line GH2.3 and the sweet sorghum Bioguma produced the highest RFVs, related to the low NDF and ADF contents in both genotypes. Figure 2 shows that there is a strong negative correlation ($p<0.01$) between RFV and NDF, as well as with ADF content ($R^2 = -0.973$ and $R^2 = -0.970$, respectively). Basaran *et al*. (2017) reported that RFV is important for estimating the value of forage instead of measuring nutrient utilization in feed. Therefore, nutrient digestibility

needs to be directly measured. We suggest that the RFV pattern in these ten genotypes will be the same as the IVTD value. However, some BMR mutant lines, i.e., GH4.2, GH4.3 and GH4.4, also produced a fairly high IVTD. Apparently, this phenomenon may be related to the high sugar content of the stems in these three mutant lines (Table 1). However, this speculation needs to be further investigated. Many previous studies reported that BMR types in forage had consistently higher digestibility than conventional varieties (Sriagtula *et al.,* 2021; Wahyono *et al.,* 2019). A high IVTD value in the GH2.3 mutant line relates to low lignin levels (Table 2). De Aguilar *et al*. (2014) reported that most BMR mutant genotypes show low lignin (3.08%–5.14%) and cellulose (22.88%–24.86%) contents.

Rumen fermentation and methane characteristics mainly indicate the degradation patterns of substrates by ruminal microbes (Wahyono *et al.,* 2019). In the present study, the average pH was in the normal range (5.5–6.5) (McDonald *et al.,* 2010). However, the value is similar to research by Wahyono *et al*. (2019), which reported that the rumen pH value with BMR sorghum substrate was around 6.75–6.77. It is interesting to note that GH4.2, GH4.3 and GH4.4 produced high total SCFAs (> 100 mM). This pattern is related to the high IVTD value of these three mutant lines (Table 3). This can be explained by the high NDF content as the source of carbohydrates to be digested. Total SCFA production represents the degradation pattern of carbohydrates (Sugoro *et al.,* 2015; Zhong *et al.,* 2016). Nevertheless, forage quality must also be observed from an environmental point of view, namely the emission of enteric methane. Enteric methane emission could be explained quite accurately by short chain fatty acids composition (Jayanegara *et al.,* 2013). Methane production represents the efficiency of rumen fermentation and is associated with high NDF content (Jayanegara *et al.,* 2009; Su-jiang *et al.,* 2016). There was a positive correlation between methane and NDF ($p<0.05$; $R^2 = 0.293$; Figure 2). Despite producing high IVTD and SCFA, it appears that GH4.3 and GH4.4 produce high enteric methane emissions, and this needs to be considered in their uses as environmentally friendly forage.

CONCLUSION

The yield and nutrient quality of the seven new BMR mutant lines appeared to be highly variable. GH2.3 had the highest yield and *in vitro* digestibility. GH2.3 also tended to represent a low lignin content, which is a characteristic of BMR types. Other findings were that the Bioguma variety, non-BMR sorghum, produced greater yield and nutritive value. Regarding fresh yield, nutrient profile and *in vitro* digestibility, the highest values were found in Bioguma and the GH2.3 mutant line. Based on these findings, GH2.3 needs to be further developed as forage sorghum. Based on the interrelationship analysis, the main parameters contributing to the quality of sorghum mutant lines are NDF, ADL and cellulose. Further research is also needed to investigate the GH2.3 BMR mutant forage in terms of palatability, intake, *in vivo* digestibility and growth performance in ruminants.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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