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Full Length Research Paper

The effect of allelic variation on forage quality of brown midrib sorghum mutants with reduced caffeic acid *O*-methyl transferase activity

R. K. Vogler[1], T. T. Tesso[2], K. D. Johnson[1] and G. Ejeta[1]*

[1]Department of Agronomy, Purdue University, West Lafayette, IN 47907-2054, USA.
[2]Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA.

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Sorghum brown midrib (bmr) mutants have reddish-brown vascular tissues in their leaves and stems as a result of changes in lignin content and subunit composition. Past research at Purdue University has generated a set of bmr sorghum mutants via chemical mutagenesis and established some to be allelic to each other. More recently, we identified additional spontaneous mutants in true breeding lines with marked phenotype and a range of agronomic characteristics. One such mutant, bmr-26, is of particular interest because it arose in a drought-tolerant sorghum line. Analysis of testcross hybrids between this spontaneous bmr mutant and the chemically induced mutants, bmr-6 and bmr-12, showed that the bmr-26 allele was allelic to bmr-12 and not to bmr- 6. Both the bmr-12 and the bmr-26 mutations significantly reduced lignin content in leaf, blade, sheath, stem, and panicle tissue. The effect of the mutation was relatively more severe in bmr- 12 than in bmr-26. The impact of the two mutations on cell wall composition in different tissues varied. The biggest effect of the bmr-12 mutation was in reduction of lignin in the sheath, whereas lignin content in panicles was more affected by the bmr-26 mutation. This suggested an allele-specific effect in tissue lignin reduction of these mutants. Cellulose and hemicellulose concentrations were also significantly higher in certain tissue types for both the induced and spontaneous mutants. Forage quality traits including percent NDF and ADF were significantly increased by both mutations. Improvement in *in vitro* dry matter digestibility as a result of the bmr-26 mutation was relatively small and was not proportional to the reduction in the lignin content.

Key words: Acid detergent fiber (ADF), bmr, brown midrib, *in vitro* dry matter digestibility (IVDMD), lignin, neutral detergent fiber (NDF), *Sorghum bicolor*.

INTRODUCTION

Grain sorghum (*Sorghum bicolor* (L.) Moench) is a major food crop in semi-arid regions of Africa and Asia and the second most important feed grain in the United States. Although the grain is often used as a major ingredient in cattle feed, sorghum and sorghum *x* sudangrass (*Sorghum sudanense* (Piper) Stapf.) hybrids are widely used as forage, especially in the US dairy industry (Rook et al., 1977; Stalling et al., 1982; Oba and Allen, 1999; Cox and Cherney, 2001).

The value of a crop plant as forage is determined primarily by the degradability of the vegetative tissue, which in turn is affected by the property of its cell wall structure (Åman, 1993). Cellulose and hemicellulose in the cell wall provide a major energy source for ruminant animals, when they can be degraded into oligo- and mono-saccharides (Moore and Hatfield et al., 1994). The plant cell wall is a complex matrix in which cellulose microfibrils are embedded in a matrix of hemicellulose, pectin, proteins, and aromatic compounds such as lignin and hydroxycinnamic acids (Carpita and Gibeaut, 1993). The availability of cellulose and hemicellulose as a source of energy, however, depends on the overall structural properties of the cell wall, which often varies between species, genotypes, and tissue, and the interaction between these three factors. For example, the presence of the cell wall polymer lignin has been thought to impede access of hydrolytic enzymes to the cell wall polysaccharides. Cell wall digestibility of maize (*Zea mays* L.)

*Corresponding author. E-mail: gejeta@purdue.edu. Tel.: +17654944320. Fax: +1765-496-2926.

has been shown to be affected by lignin content (Wolf et al., 1993; Lundvall et al., 1994), and lignin composition (Oba and Allen, 1999; Fontaine et al., 2003), although developmental changes as a result of changes in lignin composition can account, in part, for improved digestibility (Grabber et al., 1997).

Following the discovery that the brown midrib (bm) mutants of maize had altered lignin composition (Kuc and Nelson, 1964; Gee et al., 1968) and that some of them were more digestible (Barrière and Argillier, 1993), chemical mutagenesis experiments with sorghum resulted in several brown midrib (bmr) mutants (Porter et al., 1978) that resembled those in maize. Additional spontaneous bmr mutants of sorghum were identified later in breeding populations (Vogler et al., 1994). Brown midrib mutations, both in sorghum and maize, are phenotypically characterized by the presence of brown vascular tissues in the leaf blade and sheath, as well as in the stem. The bmr phenotype becomes apparent once plants have reached the four-leaf stage and tends to begin to fade as the plants approach physiological maturity (Porter et al., 1978).

Brown midrib mutants significantly reduce the level of enzyme-resistant lignin in plants and increase their palatability and digestibility (Rook et al., 1977; Cherney et al., 1991). Brown midrib silage with and without protein supplements significantly increased milk yield of lactating cows (Frenchik et al., 1976; Keith et al., 1979; Stalling et al., 1982; Cherney et al., 1991; Oba and Allen, 1999). Similarly, the rate of *in vitro* dry matter digestibility (IVDMD) and cell wall degradation by rumen bacterium of leaf blades from bmr-12 sorghum was shown to be significantly higher than those from their respective wild-type isolines (Akin et al., 1986a, b).

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). The molecular genetic basis of many of the bmr mutations has not been clearly elucidated. The first sorghum bmr gene was recently cloned. The mutant alleles bmr-12, bmr-18 and bmr-26 were shown to contain premature stop codons in the lignin biosynthetic enzyme caffeic acid O-methyltransferase (COMT; Bout and Vermerris, 2003). These mutations ultimately result in reduced synthesis of the monolignol sinapyl alcohol. The lignin of the bmr-6 mutant was shown to contain more cinnamaldehydes (Bucholtz et al., 1980) and displayed reduced activity of the lignin biosynthetic enzyme cinnamyl alcohol dehydrogenase (Pillonel et al., 1991).

The effect of the bmr mutations on forage quality varies depending on the genetic background of the line in which the mutation is introduced (Cherney et al., 1991; Peder-sen et al., 2005). This suggests the need to either identify a suitable genetic background that allows for optimal impact of the mutation. To do this effectively, the effect of each mutation on forage quality and agronomic characteristics needs to be determined. The impact of the bmr -26 mutation on forage quality has not been studied yet. The objective of this study was to establish the allelic relationship of the spontaneous mutant bmr-26 with other known bmr mutants, and provide an assessment of the effect of the bmr-26 mutation on forage quality.

MATERIALS AND METHODS

Plant materials

The mutants, bmr -6, bmr-12 and bmr-18 were identified in a segregating population of sorghum that was obtained from seeds mutagenized with diethyl sulfate (DES) (Porter et al., 1978). A spontaneous mutant bmr-26 was later identified in a drought-tolerant stay-green sorghum inbred line, P898012 (Vogler et al., 1994). For ease of description, the parental genotype P898012 will be referred to as N-26 and similarly the wild-type versions of bmr-6, bmr-12 and bmr-18 will be referred to as N-6, N-12 and N-18, respectively. Seeds from the bmr-6, bmr-12, bmr-18 and bmr-26 lines and their respective wild-type lines were planted in the greenhouse. Allelism tests were performed by fertilizing emasculated florets of bmr-6, bmr-12 and bmr-18 with pollens collected from panicles of bmr-26 to produce F_1 hybrids. Unwanted florets on a panicle were removed using clippers leaving only emasculated florets covered with pollination bag to prevent contamination by unwanted pollen. When stigma in the emasculated florets were receptive, usually two days after emasculation, each of the emasculated panicles were dusted with pollens collected from bmr-26, and covered with a pollination bag to avoid contamination. At physiological maturity, the F_1 seeds were collected from each of the pollinated plant panicle into a labeled bag and stored separately for planting the next season. The F_1 seeds along with parental bmr lines and their wild-type counterparts were planted in separate rows at the Purdue University Agronomy Center for Research and Education near West Lafayette, IN, USA. Prior to flowering, each of the hybrids were scored for the bmr trait in the leaves (present or absent). F_2 seeds were harvested from selfed panicles of the hybrids and the F_2 populations were planted the next season and scored for segregation of the brown midrib phenotype.

Determination of cell-wall components and *in vitro* dry matter digestibility (IVDMD)

Plant cell wall composition was analyzed by determining the neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and hemicellulose. Tissue samples from bmr-12 and bmr-26, their normal counterparts, and F_1 hybrids were collected when the plants were at the half- bloom stage. Analyses were performed on whole plants (excluding roots) and on individual tissues: stem, leaf sheath, leaf blade and panicle. For individual tissue analysis, a pool representing four plants (three for the F_1 hybrid) was analyzed, whereas for the whole-plant analysis three plants were pooled. The tissues were dried at 100°C for 48 h, and ground with a UDY cyclone mill to pass a 1- mm screen. Tissues were analyzed sequentially for NDF, ADF, and permanganate lignin concentration as described by Van Soest (1991). Hemicellulose was calculated as the loss of NDF residue upon subsequent extraction with acid detergent. Cellulose was determined as the loss of ADF residue upon extraction with sulfuric acid (72% H_2SO_4). *In vitro* dry matter disappearance (IVDMD) was determined from the ground tissue samples following the procedure described by Tilley and Terry (1963) and modified by Barnes et al. (1971). Samples were collected and analyzed in two replicates for each of the laboratory procedures.

Table 1. Midrib phenotypes of parental, F_1 and F_2 genotypes derived from crosses of bmr-6 with bmr-12 and bmr-18.

Genotype	Midrib phenotype	F_2 segregation				χ^2	
		Observed		Expected*			
		brown	white	brown	white		
bmr-26	brown						
N-26	white						
bmr-12	brown						
N-12	white						
bmr-6	brown						
N-6	white						
(bmr-6 × bmr-26)F_1	white						
(bmr-12 × bmr-26)F_1	brown						
(bmr-18 × bmr-26)F_1	brown						
(bmr-6 × bmr-26)F_2	white	72	74	64	82	1.84[a]	3.84[b]
(bmr-12 × bmr-26)F_2	brown	297	0	297	0	0.00	1.0
(bmr-18 × bmr-26)F_2	brown	165	0	165	0	0.00	1.0

[a]calculated χ^2 statistic; [b]value from χ^2 table at 1 degree of freedom.

Statistical analysis

Data were analyzed using a two-factor factorial model in a randomized complete block design. Factor I consisted of five genotypes (bmr-12, bmr-26, N-12, N-26 and bmr-12 x bmr-26) and factor II consisted of five tissues (whole plant, stem, sheath, blade and panicles). The general linear model (PROC GLM) of the statistical analysis systems (SAS, 2002) was used to analyze the data. The analysis was performed independently for different plant tissues. The main effects were tested using appropriate mean squares and a contrast statement was drawn to determine significant differences between bmr and normal genotypes.

RESULTS

The bmr-26 mutation is allelic to bmr-12 and bmr-18

The midrib phenotypes of the F_1 progenies resulting from crosses of bmr-26 with bmr-6, bmr-12 and bmr-18 along those of the parental lines are presented in Table 1. Hy-brids of bmr-12 x bmr- 26 and bmr-18 x bmr-26 expressed the mutant phenotype indicating that the bmr-26 allele is allelic to bmr-12 and bmr-18. On the other hand, crosses of bmr-6 x bmr- 26 produced hybrids with a wild-type phenotype, which indicates bmr-26 is non-allelic to bmr-6. The F_2 progeny of the cross bmr-6 x bmr-26 was there-fore expected to segregate for the bmr phenotype in a 9:7 ratio. Indeed, evaluation of 146 F_2 individuals resulted in 74 plants with a wild-type phenotype and 72 bmr mu-tants. A chi-squared goodness-of-fit test (df = 1) resulted in a value of 1.84 ($p = 0.175$), which is consistent with the 9:7 segregation ratio (Table 1).

Variation in NDF and ADF

Significant variation in cell wall composition was observed among the genotypes in all tissue samples examined (Tables 2 and 3). Percent NDF and ADF across genotypes were significantly higher in panicle tissues followed by the leaf sheath (Table 2). This was consistent in both the bmr mutants and normal genotypes. The lowest NDF and ADF values were obtained for leaf blade tissues (Table 2). The NDF of bmr stems was signify-cantly higher than the wild-type stems, but the differences in NDF values of the other tissues were not statistically significant (Table 2). Differences between individual bmr mutants and their respective wild-type counterparts, how-ever, were in most cases significant (Table 3). The bmr-12 mutant had significantly higher NDF compared to its wild- type isoline in all tissues except the panicle. The ADF values showed a similar pattern to NDF, except that on a whole- plant basis, the difference was not significant. The impact of the bmr- 26 mutation on a whole-plant basis was considerably less than that of the bmr- 12 mutation. No significant differences were observed for NDF values, and ADF values were only different in the panicle. The F_1 bmr hybrids generally had high NDF and ADF values in all tissues except when the whole plant was evaluated.

The bmr-26 and bmr-12 mutations have different impact on permanganate lignin content

Mean permanganate lignin content was lowest in leaf blades and highest in panicle tissues across genotypes (Table 2) . Permanganate lignin content of bmr mutants averaged across tissue and genotypes was 21% lower than normal genotypes. The largest difference was observed in stem and leaf sheath tissues, where the mutants had a 30 and 27% lower lignin content, respec-

Table 2. Percent cell wall and *in vitro* dry matter digestibility (IVDMD) of normal and brown midrib sorghum genotypes.

Tissue	NDF	ADF	Lignin	Hemicellulose	Cellulose	IVDMD
Across genotypes						
Whole plant	58.1 c	31.8 c	4.8 c	26.2 c	27.4 c	59.2 b
Stem	55.0 d	31.8 c	4.9 c	23.1 d	27.6 c	59.8 ab
Sheath	64.5 b	36.3 b	5.4 b	28.2 b	31.0 b	56.2 c
Blade	53.9 e	27.6 d	3.9 d	26.3 c	23.9 d	61.3 a
Panicle	75.7 a	37.3 a	5.8 a	38.3 a	31.8 a	51.9 d
Bmr genotypes						
Whole plant	58.0 c	31.4 d	4.3 c	26.6 c	27.7 d	59.5 ab
Stem	57.5 c	32.8 c	4.2 c	24.7 d	29.1 c	56.7 bc
Sheath	65.5 b	35.8 b	4.7 b	29.7 b	31.3 b	57.0 bc
Blade	55.0 d	28.3 e	3.8 d	26.7 c	24.8 e	61.9 a
Panicle	75.9 a	36.9 a	5.4 a	39.0 a	31.8 a	54.2 c
Normal genotypes						
Whole plant	58.2 c	32.4 c	5.6 b	25.7 b	27.0 c	58.6 b
Stem	51.1 e	30.3 d	6.0 ab	20.7 c	25.4 d	64.4 a
Sheath	63.1 b	37.0 b	6.5 a	26.0 b	30.6 b	55.0 c
Blade	52.3 d	26.5 e	4.0 c	25.7 b	22.4 e	60.4 b
Panicle	75.2 a	38.0 a	6.5 a	37.2 a	31.7 a	48.5 d

Means followed by the same letter in a column within a genotype are not significantly different. NDF - neutral detergent fiber; ADF - acid detergent fiber; IVDMD *in vitro* dry matter digestibility.

pectively, than the normal genotypes. In contrast, the difference in permanganate lignin content in leaf blades was only 5%, whereas whole plant and panicle tissues showed differences of 23 and 18%, respectively.

Both bmr-12 and bmr-26 displayed significant reductions in permanganate lignin content. The impact of the bmr-26 mutation on permanganate lignin content, however, is different compared to the bmr-12 mutation (Table 3). On a whole-plant basis bmr-12 and bmr- 26 contained 22 and 16% less lignin, respectively, than their normal counterparts. Lignin reduction among stem tissues was 22 and 34%, while among leaf sheaths 29 and 23%, and among panicles 5 and 25%, respectively, in bmr-12 and bmr-26. Among leaf blades only bmr-12 results in a significant reduction in lignin content (14%). The lignin content in the bmr hybrid was, depending on the tissue, more similar to bmr-12 or bmr-26.

Variation in cellulose and hemicellulose content

Hemicellulose and cellulose contents across genotypes were significantly higher in panicle tissues both in the bmr mutants and the normal genotypes (Table 2). The lowest cellulose and hemicellulose levels were detected in stem and leaf blade tissues, respectively, in both bmr and normal genotypes, but the levels were generally higher in bmr lines than in normal genotypes. The bmr-26 mutant had higher hemicellulose content in the sheath, and slightly less in the leaf blade. Cellulose content was higher in the bmr-26 stem and blade. In contrast, the bmr-12 mutant had significantly higher hemicellulose and cellulose content than its normal counterpart in all tissues except the panicle (Table 3).

In vitro dry matter digestibility

There were significant differences in IVDMD among different tissues in both bmr and normal genotypes (Table 2). Mean IVDMD was remarkably higher in leaf blade tissues for the bmr mutants and in the stem tissues for normal genotypes, but it was lowest in the panicle tissues of both bmr and normal genotypes. Among genotypes, bmr -12 and bmr-12 x bmr-26 hybrid had the highest IVDMD for the whole plant tissue, while bmr-26 had the lowest IVDMD though not significantly different from its normal isoline (Table 3). The IVDMD of the stem their normal counterparts by 10 and 12%, respectively.

The bmr hybrid also had a low IVDMD score. In other tissues IVDMD was not significantly affected as a result of the mutations, except in the panicle tissues, where the bmr mutants had better digestibility scores than their normal counterparts.

DISCUSSION

28 bmr mutants of sorghum have been identified to date, most of which were developed via chemical mutagenesis. The number of independent loci appears to be smaller than the number of mutants, based on allelism tests per-

Table 3. Tissue cell wall and *in vitro* dry matter digestibility (IVDMD) of normal and brown midrib sorghum genotypes.

Genotype	NDF	ADF	Lignin	Hemicellulose	Cellulose	IVDMD
Whole plant						
bmr-26	60.2 a	33.1 a	4.7 b	27.1 a	29.1 a	56.1 c
N-26	60.4 a	33.7 a	5.6 a	26.6 a	28.4 a	58.3 bc
bmr-12	58.3 b	31.2 b	4.3 b	27.1 a	27.3 b	61.7 a
N-12	56.0 c	31.1 b	5.6 a	24.9 b	25.7 c	58.9 abc
F1 (bmr)	55.5 c	29.9 b	4.0 b	25.6 ab	26.7 b	60.8 ab
Stem						
bmr-26	57.4 a	33.4 ab	4.4 c	23.9 ab	29.8 a	59.5 b
N-26	56.5 a	34.3 a	6.7 a	22.1 bc	28.4 c	65.8 a
bmr-12	58.8 a	32.6 b	4.1 c	26.2 a	29.0 b	55.3 c
N-12	45.7 b	26.4 c	5.2 b	19.3 c	22.5 d	63.1 ab
F1 (bmr)	56.4 a	32.4 b	4.1 c	24.1 ab	28.5 bc	55.3 c
Sheath						
bmr-26	63.2 c	36.3 b	4.7 d	26.8 b	31.8 a	58.9 a
N-26	63.5 c	38.0 a	6.1 b	25.4 c	31.8 a	57.2 ab
bmr-12	66.1 b	34.8 c	4.8 c	31.3 a	30.3 b	56.5 ab
N-12	62.6 c	36.0 b	6.9 a	26.6 c	29.4 c	52.8 b
F1 (bmr)	67.3 a	36.3 b	4.7 d	30.9 a	31.8 a	55.7 ab
Blade						
bmr-26	53.2 c	28.5 ab	4.0a	24.6 c	24.7 ab	61.5 ab
N-26	53.0 cd	27.4 bc	3.9 a	25.5 b	23.4 c	56.9 b
bmr-12	54.9 b	27.2 c	3.5 a	27.6 a	24.1 bc	62.7 a
N-12	51.6 d	25.6 d	4.0 a	25.9 b	21.5 d	63.8 a
F1 (bmr)	57.0 a	29.1 a	3.9 a	27.9 a	25.6 a	61.4 ab
Panicle						
bmr-26	72.1 c	35.9 c	5.8 b	36.1 bc	30.5 c	54.6 b
N-26	74.2 bc	38.8 a	7.7 a	35.3 c	31.4 bc	46.7 a
bmr-12	77.7 a	37.0 bc	5.0 b	40.7 a	32.4 ab	58.1 a
N-12	76.3 ab	37.1 bc	5.3 b	39.2 ab	31.9 ab	50.3 a
F1 (bmr)	78.1 a	37.9 ab	5.3 b	40.1 a	32.6 a	49.9 a

Means followed by the same letter in a column within tissue type are not significantly different. NDF - neutral detergent fiber; ADF - acid detergent fiber; IVDMD *in vitro* dry matter digestibility.

formed by Bittinger et al. (1981) . The results from the tissue in bmr-26 and bmr-12 was significantly lower than allelism tests reported here are consistent with this finding where bmr- 12 and bmr- 18 are reported to be allelic and different from bmr-6, as well as with recent sequence and expression data reported by Bout and Vermerris (2003).

The most dramatic effect of the bmr -12 and bmr-26 mutations appears to be on the lignin content. Lignin concentration measured as permanganate lignin was consistently reduced in bmr mutations compared to the wild-type iso- lines. However, the degree to which the lignin content was reduced varied among tissues. The effect of the bmr-26 mutation was most severe in the panicle tissue and the least severe in the leaf blade, whereas the impact of the bmr-12 mutation was most severe in the sheath and least severe in the panicle. On whole plant basis, lignin content appeared to have been severely reduced in bmr-12 than bmr- 26. These results are in agreement with previous reports from studies conducted on same mutations (Oliver et al., 2004, 2005). Such differences may indicate the possibility of multiple mutant alleles occurring at the same locus. In this respect, it is of interest to note that the bmr-12 and bmr-26 mutations are in the first and second exon of the COMT gene, respectively (Bout and Vermerris, 2003), perhaps resulting in differential expression of the mutations. Further analyses of the effect of these two mutations in the same genetic background may produce additional information on background effect on the degree of expression of mutations. Such information may be used to selectively alter lignin content and subunit composition in specific parts of the plant and also provide means for addressing the potential negative agronomic attributes of

the mutations such as lodging and susceptibility to pests and diseases supposedly associated with modified lignin content or composition. It is also interesting to note that there is a differential effect on cellulose and hemicellulose contents of the mutants. In the bmr-12 mutant, the degree of reduction in lignin content is paralleled with an increase in cellulose and hemicellulose contents. The reduction in lignin content is likely to have an effect on the overall structural integrity of the cell wall. The in-crease in cellulose and hemicellulose contents may re-flect the existence of a mechanism that compensates for the reduction in lignin content. This has been shown to occur in aspen (Hu et al., 1999) and rice (Li et al., 2003). Alternatively, the reduction in lignin content may make the polysaccharide fractions more susceptible to che-mical or enzymatic degradation, which could account for an increased release of sugars.

The lack of significant improvement in IVDMD as a result of the bmr mutations are somewhat unexpected. The maize bm3 mutant, which also has a mutation in the COMT gene (Vignols et al., 1995), was shown to result in significantly better intake and digestibility in animal feeding studies (Oba and Allen, 1999), and similar results were expected for bmr -12 and bmr -26 mutants. One explanation for improved intake would be increased palatability, which may primarily reflect taste and physical attributes such as breaking strength of the tissue. Digestibility, however, has been shown to depend on chemical composition of the cell wall (Jung and Buxton, 1994; Lundvall et al., 1994; Fontaine et al., 2003), although the exact relationship is quite complex. The IVDMD analysis is performed *in vitro*, and may therefore not be an accurate reflection of *in-vivo* digestibility in this particular case. It may be worth performing animal feeding studies with these two specific mutants to develop a better understanding on their effect on dry matter digestibility. However, feeding studies conducted on dairy cows have shown that bmr sorghum silage has significantly improved dry matter digestibility and resulted in higher milk yield compared to conventional sorghum silage (Oliver et al., 2005; Dann et al., 2008) . The bmr mutants had 4% higher NDF than the normal lines, though this varied according to tissue type and the genetic background in which the mutations are constituted. The mutant bmr-12 had consistently higher NDF in all tissue types than N-12, while bmr-26 was exceeded by its normal counterpart in the whole plant and leaf sheath tissues. There was no noticeable difference for mean ADF between bmr mutants and the normal lines. Both N-12 and N-26 had slightly higher ADF than their mutant counterparts in all tissues except the stem and leaf blade for bmr- 12 and the leaf blade tissue for bmr-26. This result is in agreement with previous work by Cox and Cherney (2001) who reported significantly higher NDF in maize brown midrib hybrids than normal hybrids of different backgrounds. Porter et al. (1978) also reported significantly higher percent ADF in normal genotypes than their mutant counterparts, but the difference in the current study was comparatively small. Variation in cellulose and hemicellulose contents in both backgrounds followed a similar pattern. The bmr mutants generally tended to have higher cellulose and hemicellulose contents though there were few exceptions for certain tissue types in the bmr-26 background. This again agrees with the report by Porter et al. (1978) who observed consistently higher cellulose and hemicellulose concentrations in bmr -12 than its normal counterpart. But this is not always the case for all genotypes. Similar to the bmr -26 in this study, many other mutants have been shown to have lower concentrations of cellulose, hemicellulose or both than their normal counterparts. However, the result for lignin content was very different. Almost all previous reports revealed significantly lower concentration of lignin in mutants compared to their normal sister lines in both sorghum (Porter et al., 1978) and corn (Cardinal et al., 2003). The results of our current study also corroborates these findings as we found the average lignin content combined across tissue types of bmr mutants to be 21% lower than the normal lines. This varied for different tissue types with the range spanning from only 6% difference in the leaf blade tissue to 30% in the stem. This variation, however, was not reflected in the difference in IVDMD between the mutants and the normal lines in this study. The mutation that reduced the lignin content of the genotypes may have altered the composition of lignin that renders them undigestable in certain tissues. Previous studies have shown that in the cell walls of bmr-6, the concentration of certain phenolic compounds, such as aldehydes and ferulates, were increased while the content of p-coumarate decreased (Akin et al., 1986b; Pillonel et al., 1991). In bmr-12 and bmr-18, the concentration of syringyl moieties and p-coumarate were reduced (Akin et al., 1986) and in a related cereal, pearl millet (*Pennisetum glaucum* (L.) R. Br.), p-coumarate was again reduced but the concentration of the guaiacyl units were increased (Cherney et al., 1991). This decrease in one and increase in another component of cell wall, though ultimately resulting in lower lignin content, might have altered its composition such that in certain tissues and at a certain growth stage, it becomes more resistant to degradation. However, previous feeding studies have shown that brown midrib mutants in both corn and sorghum have resulted in improved milk yields in dairy cows and growth in sheep (Frenchik et al., 1976; Keith et al., 1979; Stalling et al., 1982; Aydin et al., 1999; Grant et al., 1995; Oba et al., 1999) . This could be due to the combined effects of improved digestibility of the cell wall associated with reduced lignin content and increased intake as a result of improved palatability of the brown midrib genotype.

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