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Assessment of the agronomic and nutritional characteristics of sorghum genotypes with and without brown midrib

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ABSTRACT. The objective was to assess the agronomic characteristics and nutritional value of silages from sorghum genotypes without *bmr* mutation and carrying the *bmr* mutation. A total of 14 genotypes were used, 7 of which were conventional (BRS 655, BRS 658, BRS 659, BRS 610, VOLUMAX, 156x947216 and 156x947030) and 7 carrying the *bmr*6 gene (2014F15641, 2014F15645, 2014F15649, 2014F15653, 2014F15661, 2014F15681 and 2014F15685). The experimental design consisted of randomized blocks – 4 blocks and 14 treatments, totaling 56 experimental plots. The means of the variables were subject to ANOVA through SISVAR; when significant was found, the treatments were compared using the Scott-Knott test at a 5% probability level. There was a difference ($p < 0.05$) for flowering days and height between genotypes. As for crude protein, mean levels ranged from 11.10 to 14.66% for 2014F15645 and BRS 610. Regardless of the mutation, there was no difference ($p > 0.05$) between the genotypes carrying the mutation and those not carrying the mutation for lignin and *in situ* dry matter digestibility. Genotypes BRS 655, BRS 658, BRS 659, BRS 610, VOLUMAX, 2014F15645, 2014F15649, 2014F15661, 2014F15681 and 2014F15685 are more suitable for silage production, as they present higher dry matter production. Regarding ISDMD, all genotypes can be used for silage production.

Keywords: composition; brown midrib; digestibility; digestible dry matter.

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Introduction

Sorghum (*Sorghum bicolor*, L. Moench) represents the 5th most produced cereal in the world, as it is largely grown in developing countries, with the exception of the United States, which is the world's largest producer of this cereal.

In recent years, efforts by researchers and companies have been directed towards improving the nutritional value of this crop, which, in addition to being tolerant to water deficit (Perazzo et al., 2014), is quite productive (Aguilar et al., 2015b; Costa et al., 2016; Lima et al., 2017). Among the factors that influence the nutritional value of forage plants, lignin content stands out, which increases along with the other components of the plant cell wall due to physiological maturity (Van Soest, 1994; Ferreira et al., 2015). In the sorghum plant, increased levels of phenolic compounds are strongly related to low ruminal degradation (Costa et al., 2016) and, consequently, can affect the productive performance of animals (Singh, Bhat, Shukla, Gaharana, & Anele, 2017). One of the advances sought by genetic engineering is the selection and exploration of sorghum genotypes, whether for grazing and/or silage, with brown pigments in the midrib, also called *bmr* mutants (Ferreira et al., 2015). The presence of these pigments is correlated with a reduction in lignin content, leading to greater digestibility of the cell wall and, consequently, greater consumption of dry matter and animal performance (Pereira et al., 2019). The *bmr* phenotype is characteristic of diploid plants and can occur spontaneously in nature or be caused by genetic engineering (Aguilar et al., 2015a).

Some studies conducted by (Ledgerwood, DePeters, Robinson, Taylor, & Heguy, 2009; Astigarraga, Bianco, Mello, & Montedónico, 2014; Ferreira et al., 2015) found that sorghum genotypes carrying this *bmr* mutation showed a reduction in lignin content, leading to greater digestibility of the cell wall (Ferreira et al., 2015).

A forage plant genetic enhancement program developed by Embrapa Maize and Sorghum has recently selected new sorghum genotypes, including normal genotypes (without mutation) and those carrying the *bmr* mutation, through seed treatment. These genotypes, especially when intended for silage production, must be nutritionally assessed for subsequent introduction into the market.

Aguilar et al. (2015b), Ferreira et al. (2015) assessed the nutritional value of plants with brown midrib used for cutting and grazing. There are few experiments that have assessed the nutritional value of silages from sorghum genotypes carrying the *bmr* mutation; therefore, it is necessary to understand whether reductions in lignin content alter the chemical composition and digestibility of silages.

In light of the foregoing, the objective was to assess the agronomic and nutritional characteristics of sorghum genotypes, with or without brown midrib, for silage production.

Material and methods

The field experiment was conducted on the premises of Embrapa – National Center for Maize and Sorghum Research, located at Km 65 of the MG424 highway, in the municipality of Sete Lagoas, MG. A total of 14 sorghum genotypes were used – 7 commercial genotypes (BRS 655, BRS 658, BRS 659, BRS 610, VOLUMAX, 156x947216 and 156x947030) and 7 *bmr*6 mutants (2014F15641, 2014F15645, 2014F15649, 2014F15653, 2014F15661, 2014F15681 and 2014F15685). The 14 genotypes were planted on September 02, 2016, in 4 blocks, each consisting of 14 plots composed of 6 rows measuring 6 meters in length and spaced by 70 centimeters in between. A total of 35 seeds were sown per linear meter in each plot; each genotype was a treatment, totaling 14 treatments. Fertilization was performed in accordance with soil analysis and crop requirements, using 350 kg ha^{-1} of formula 08-28-16 (N:P:K) + 0.5% zinc at planting, and 150 kg ha^{-1} of urea in top dressing, 25 days after planting.

Harvest took place on December 13, 2020, totaling an experimental period of 102 days. The sorghum was cut 15 cm from the ground, and all materials were harvested on the same day. The cuts were made in the two central and intermediate rows of each plot (useful plot), discarding the two outer rows of each plot and 1 meter from the ends of each row (the borders).

For the agronomic assessment, the two central rows of each plot were used, and the following characteristics were assessed: plant height, which was obtained by measuring the soil level at the upper end of the plant, in 20% of the plants from each plot; flowering (days), which refers to the number of days for the sorghum plant to produce an inflorescence after planting; green matter production, which was obtained from weighing all the plants from the useful area of the plot, after cutting them 15 cm from the ground; dry matter production, which was obtained from the green matter production and the dry matter content of each genotype at the time of cutting. The two intermediates were collected and used to make the silos; subsequently, the bromatological characteristics and the quality of the silages were evaluated.

Laboratory silos made of PVC tubes measuring 100 mm in diameter and 500 mm in length were used, with the forage being chopped in a stationary chopper and pressed with a wooden socket; an average density of 600 kg m^3 was adopted. The silos were sealed at the time of ensiling with PVC lids fitted with Bunsen valves and sealed with masking tape, then opened after 56 days of ensiling. The nutritional evaluation of the silages was carried out at the Food Analysis Laboratory of the State University of Montes Claros (Unimontes) – Janaúba campus, MG.

When the silos were opened, the material was homogenized, and part of the ensiled material was placed in paper bags, weighed and then pre-dried in a forced ventilation oven at 55°C for 72 hours, or until it reached constant weight. The pre-dried samples were ground in a stationary mill with a 1 mm mesh sieve and then placed in glass jars with labeled lids for analysis of the chemical composition of the food: dry matter (DM), ash (ASH), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG). All analyses mentioned above were conducted in accordance with methodologies described by Detmann et al. (2012).

To determine the *in situ* DM digestibility (ISDMD), the silage samples were packed in TNT-type synthetic fiber bags, with a grammage of 100 microns, measuring 15 x 8 cm, following a ratio of 20 mg of DM cm^{-2} of surface area of the bag.The bags were tied and fixed to a nylon rope, then introduced into the rumen of a fistulated adult bovine. The incubation period corresponded to 144 hours, with the bags being placed in duplicate. After a total incubation period of 144 hours, all bags were removed from the rumen, washed in running water until the latter was clean, and then dried. Dry matter (DM) was determined in an oven at 55ºC for 72 hours, in accordance with methodology described by Detmann et al. (2012). *In situ* DM digestibility data were obtained by weight difference between weighings carried out before and after ruminal incubation, and expressed as a percentage.

The experimental design consisted of randomized blocks – 4 blocks and 14 treatments, totaling 56 experimental plots. The means of the variables were subjected to analysis of variance with the aid of SISVAR, as described by Ferreira (2014), and when they were significant, treatments were compared using the Scott-Knott test at a 5% probability level.

Results and discussion

Table 1 shows that there was a difference in flowering (days) and height (m), ($p < 0.05$) between the genotypes.

For flowering days, values ranged from 66 to 87 days for BRS 655 and 2014F15685, respectively. The normal genotypes BRS 655, BRS 658, BRS 659, BRS 610, VOLUMAX, 156X947216 and the *bmr* mutants 2014F15641, 2014F15645, 2014F15649, 2014F15653 obtained lower values (66 to 76 days), 2014F15661 obtained intermediate values (79 days), and the *bmr* genotypes 2014F15681 and 2014F15685 were the latest (85 and 87 days). This fact may explain the superiority of later materials for DMP, since, according to Almeida Filho et al. (2014), genotypes that have a later cycle tend to be more productive due to their longer vegetative phase.

When it comes to height, the mean values ranged from 1.98 to 2.56 m for the normal genotype BRS 655 and the *bmr* mutant 2014F15645. The normal genotypes BRS 655, BRS659, BRS610, VOLUMAX, 156X947216, 156X947030 and the mutant 2014F15641 obtained lower means of 1.98; 2.16; 2.02; 2.09; 2.04; 1.67 and 2.12 m compared to genotype BRS 658 and the *bmr*6 mutants 2014F15645, 2014F15649, 2014F15649, 2014F15653, 2014F15661, 2014F15681 and 2014F15685 (2.32; 2.56; 2.55; 2.31; 2.50; 2.30 and 2.40 m). Plant height is a characteristic that generally determines green matter and dry matter production. The results show that most of the mutant genotypes were superior in height and had a positive correlation with dry matter production (BRS 658, 2014F15645, 2014F15649, 2014F15661, 2014F15681 and 2014F15685). Aguilar et al. (2015b) assessed the agronomic characteristics of twenty hybrid sorghum genotypes, nine of which were normal, and eleven, *bmr* mutants, and found mean heights of 1.28 to 1.72 m in the first cut, made 55 days after sowing, and another cut 42 days after sowing. Plant height is correlated with cutting time, and in the present study the plants in the second cut were still developing, which may have directly influenced their growth.

Regarding green matter production (GMP), the mean values ranged from 31.09 to 56.77 t ha^{-1} for genotypes 2014F15641 and 2014F15685, respectively (Table 1). Genotype 2014F15685 was the most productive, followed by 2014F15645, 2014F15649 and 2014F15661, with means of 56.77; 45.91; 45.87 and 45.27 t ha^{-1}. Almeida and Sabundjian (2020) analyzed agronomic characteristics of sorghum hybrids with different doses of urea and found mean green matter production (GMP) values of 312 to 314 kg ha^{-1} for doses ranging from 0 to 200 kg of N.ha^{1}, which are lower than those in the present study.

Table 1. Mean values for height in meters and days of flowering (inflorescence) of normal sorghum genotypes and those with *bmr* mutation.

Genotypes	Inflorescence (Days)	GMP (t ha^{-1})	DM (%)	DMP (t ha^{-1})
BRS 655	66 D	37.49 C	21.01 B	7.91 C
BRS 658	70 D	34.64 C	23.95 A	8.27 C
BRS 659	69 D	34.92 C	26.30 A	9.20 B
BRS 610	76 C	37.69 C	24.12 A	9.09 B
VOLUMAX	76 C	36.31 C	21.86 B	7.91 C
156x947216	70 D	26.35 D	23.99 A	6.33 D
156x947030	70 D	21.09 D	21.23 B	4.47 D
2014F15641	72 D	31.09 D	23.46 A	7.35 C
2014F15645	76 C	45.91 B	23.06 A	10.58 B
2014F15649	75 C	45.87 B	20.18 B	9.28 B
2014F15653	72 D	29.69 D	21.29 B	6.35 D
2014F15661	79 B	45.27 B	20.06 B	9.12 B
2014F15681	85 A	39.55 C	21.24 B	8.41 C
2014F15685	87 A	56.77 A	22.72 A	13.00 A
MEAN	75	37.32	9.58	8.37
CV (%)	3.70	14.95	18.94	18.77

Means followed by different letters in the column differ from each other by the Scott-Knott test at a 5% probability level. CV = Coefficient of variation.

The assessed genotypes presented desirable agronomic characteristics for silage production, and the genotype that presented the highest green matter production was among those with the greatest height.

There was no difference between the genotypes in terms of dry matter content at the time of cutting, with the mean found being 21.16% (Table 1). The values obtained in this experiment are close to the results described by Ribeiro et al. (2017), with dry matter values between 28 and 29%. According to McDonald, Henderson, and Heron (1991), dry matter content is considered good when it is above 25%. And according to this same author, with dry matter above 20%, it is possible to produce silage. In the present study, the genotypes presented low values, but above the 20% recommended by the author mentioned above. The low dry matter content of the plant can be explained by the high proportion of sorghum stems and/or the moment of cutting.

With respect to dry matter production, difference was found, as the normal genotypes BRS 655, BRS 658, BRS 659, BRS 610, VOLUMAX and the *bmr* mutants 2014F15645, 2014F15649, 2014F15661, 2014F15681 and 2014F15685 were superior and similar to each other, with means of 791; 7.83; 9.20; 7.75; 7.58; 8.79; 9.28, 8.89, 8.41 and 11.97 t ha^{-1} (Table 2).

Table 2. Mean levels for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG) and *in situ* dry matter digestibility (ISDMD) of silages from normal sorghum genotypes and from those with *bmr* mutation.

Genotypes	CP (%)	NDF (%)	ADF (%)	LIG (%)	ISDMD
BRS 655	12.36 B	70.14 A	42.12 A	4.17 A	53.78 A
BRS 658	12.68 B	6661 A	36.00 A	4.57 A	60.72 A
BRS 659	12.59 B	66.19 A	34.48 B	4.61 A	63.74 A
BRS 610	14.66 A	66.03 A	37.37 A	4.73 A	63.97 A
VOLUMAX	13.49 A	63.87 A	32.87 B	4.85 A	58.69 A
156x947216	13.92 A	63.63 A	33.44 B	4.22 A	56.42 A
156x947030	13.30 A	62.87 A	31.70 B	4.88 A	57.64 A
2014F15641	12.99 B	65.42 A	37.47 A	4.70 A	60.48 A
2014F15645	1110 C	69.03 A	3666 A	5.01 A	63.96 A
2014F15649	12.46 B	61.95 A	29.13 B	5.20 A	60.51 A
2014F15653	14.02 A	63.44 A	33.85 B	4.64 A	65.06 A
2014F15661	11.07 C	68.98 A	38.20 A	5.03 A	58.38 A
2014F15681	12.30 B	68.77 A	38.28 A	5.20 A	58.96 A
2014F15685	12.78 B	68.76 A	38.56 A	5.00 A	59.92 A
MEAN	-	66.12	-	4.77	59.23
CV (%)	7.94	6.28	9.33	18.16	14.93

Means followed by different letters in the column differ from each other by the Scott-Knott test at a 5% probability level. CV = Coefficient of variation.

Among the parameters that evaluate the productive potential of a forage, such as green matter production (GMP), dry matter production (DMP) and digestible dry matter production (DDMP), the most important are DMP and DDMP, as they concern the fraction of the food that can be utilized by animals, disregarding its water content. The dry matter production potential increases with height, and the percentage of panicles decreases with increasing plant height. This decrease occurs at a lower rate in small- and medium-sized hybrids, and at a higher rate when the plant height exceeds three meters. Among the materials assessed, genotypes 2014F15685, 2014F15661, 2014F15649 and 2014F15645 presented green matter production greater than 40 t ha^{-1}, and all with a height of less than 3 meters, suggesting a good proportion between stem, leaf, and panicle, which improves the digestibility of these genotypes.

As for crude protein (CP), there was a difference ($p < 0.05$) between the genotypes. Mean levels ranged from 11.10 to 14.66% for 2014F15645 and BRS 610, respectively. The normal genotypes BRS 610, VOLUMAX, 156x947216, 156x947030 and the mutant 2014F15653 showed higher values (14.02 to 14.66%). Followed by BRS 655, BRS 658, BRS 659, 2014F15641, 2014F15649, 2014F15681 and 2014F15685 (12.36 to 12.78%), with intermediate values. The mutants 2014F15645 (11.10%) and 2014F15661 (11.07) obtained lower values than the others. The evaluated genotypes presented ideal CP indices to meet the nitrogen requirements of the ruminal flora and for the good functioning of the rumen, which is at least 7%. However, a decrease in protein content may occur with the physiological maturity of the plant. The increase in CP levels is related to the dry matter accumulation of the genotypes. Aguilar et al. (2015b), while working with mutant sorghum genotypes and their isogenic pairs, found a mean of 13.78% for crude protein.

The values observed may be related to the cutting age, and the low dry matter content of the plants at the time of cutting indicates a less advanced physiological maturity stage, which explains the higher protein content.

For neutral detergent fiber (NDF), there was no difference ($p > 0.05$) between the genotypes, with means at 66.12%, which allows for stating that the *bmr* mutation did not interfere with NDF proportions. NDF content is related to the consumption, digestibility, passage rate and chewing activity of ruminant animals, while ADF is more related to the digestibility potential of a given roughage. Diets for ruminants with NDF levels above 55% of DM tend to have lower energy density, limiting dry matter consumption through rumen filling, and animal performance may be compromised. However, more important than NDF content is the quality of the fiber or the proportion between hemicellulose, cellulose and lignin. High hemicellulose content indicates good digestibility of the fiber, even if the NDF content is high. Now, values above 40% ADF can compromise digestibility, but this will depend on the levels of lignin and its ester-type bonds with hemicellulose. There was a significant difference ($p < 0.05$) in ADF levels. Genotypes BRS 655, BRS 658, BRS 610, and the mutants 2014F15641, 2014F15645, 2014F15661, 2014F15681 and 2014F15685 were superior, ranging from 42.12 to 36.00%. The others (BRS 659, VOLUMAX, 156x947216, 156x947030, 2014F15649 and 2014F15653) were inferior, with means ranging from 29.13 to 34.48%. Since the assessed materials were harvested with a low dry matter content (21.16), the physiological maturation stage of the plants was possibly less advanced, which suggests lower lignin levels, making the fiber more digestible. Experiments assessing the chemical composition of mutant sorghum hybrids are not very frequent in the literature and present conflicting results.

Regardless of the mutation, there was no difference ($p > 0.05$) between the mutant and normal genotypes, which may suggest that the *bmr* mutation did not interfere with lignin proportions, with a mean of 4.77%. The lignification process is recognized as the main factor that leads to a reduction in the digestibility of the plant cell wall (Van Soest, 1994); therefore, a change in lignin content could directly interfere with DM digestibility. Research carried out by Tolentino et al. (2016), on the evaluation of the quality of silages from different sorghum genotypes grown in Sete Lagoas, reported lignin content values of 5.74 and 6.78% in the BRS 655 and Volumax hybrids, respectively.

Considering the *in situ* digestibility of the 14 sorghum silages, there was no statistical difference between the genotypes ($p > 0.05$). The data are available in Table 2, where the overall mean of the genotypes stood at 59.23. Silages with ISDMD between 40 and 55% are classified as being of satisfactory quality, and silages with 55 to 65% are classified as being of good quality. When assessing the digestibility data, we found a mean value of 59.23%, which suggests the good quality of the silage produced. The mutation and fiber quality of the genotypes used in terms of their type and composition may influence the digestibility coefficient of the plant as a whole, since the mutation caused by the *bmr* gene, which alters the cellular composition and causes an OMT enzyme (O-methyltransferase) deficiency, decreases lignin content. The present study suggests that the use of different genotypes belonging to the same genus subjected to a conservation process presented a similar *in situ* DM digestibility; thus, producers will be able to opt for the forage plant with the highest production and that best adapts to the climatic conditions of the region.

Conclusion

Regarding agronomic characteristics, genotypes BRS 655, BRS 658, BRS 659, BRS 610, VOLUMAX, 2014F15645, 2014F15649, 2014F15661, 2014F15681 and 2014F15685 are the most suitable for silage production, as they were the ones with the highest dry matter production. Regarding ISDMD, all genotypes can be used for silage production.

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