



# Dynamic expression of the nutritive values in forage sorghum populations associated with white, green and brown midrib genotypes



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## ABSTRACT

Forage sorghum (FS; *Sorghum bicolor* (L.) Moench) is growing in popularity as an important forage crop replaced corn (*Zea mays* L.) in semi-arid regions of the world. FS can further be divided into white midrib (WMR), green midrib (GMR) and brown midrib (BMR) populations based on the color of leaf midrib. Understanding expression of nutritive values in different FS populations is essential to the breeding and utilization of the new FS varieties with high quality. A field experiment was conducted in 2012 and 2013 in order to analyze the change regulation of nutritive values and compare the differences in photosynthetic indexes and anatomy parameters among WMR, GMR, BMR-6 and BMR-12 forage sorghum populations. The results showed that there were significant ( $P < 0.05$ ) differences in nutritive values and anatomy parameters between BMR and other FS populations whereas no differences ( $P > 0.05$ ) were observed in photosynthetic indexes. When compared with non-BMR populations (WMR and GMR), the mean ADL, NDF and ADF content of BMR populations (BMR-6 and BMR-12) were decreased respectively by 28.0%, 4.1% and 5.8% while the mean CP content was increased by 8.0% during the two years. Especially, BMR-12 populations had significantly ( $P < 0.05$ ) lower ADL content and higher CP content than that of BMR-6 based on their dynamic curve regression model. The nutritive values of forage sorghum populations were ranked as following order: BMR-12 > BMR-6 > GMR > WMR. In conclusion, the BMR populations, particularly BMR-12, had exhibited consistently higher nutritive values since joint stage (the average plant height was about 130 cm). This result further reinforces the advantages of BMR gene effect in populations and also will provide scientific data for rational utilizing BMR forage sorghum resources in practice.

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**Abbreviations:** WMR, white midrib; GMR, green midrib; BMR, brown midrib; PH, plant height; LN, leaf number; SD, stem diameter; DM, dry matter per plant; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; PN, photosynthetic rate; TR, transpiration rate; SC, stomatal conductance; IC, intercellular CO<sub>2</sub> concentration; TSE, thickness of stem epidermis; TS, thickness of sclerenchyma; TVB, thickness of vascular bundle sheath; TUE, thickness of upper epidermal; TUNE, thickness of under epidermal; TL, thickness of leaf.

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## 1. Introduction

In recent years, forage sorghum (FS; *Sorghum* (FS; *Sorghum bicolor* (L.) Moench) is growing in popularity as an important forage crop replaced corn (*Zea mays* L.) in semi-arid regions of the world because of declining water resources availability (Marsalis et al., 2009; Mutava et al., 2011). FS has been shown to have 27% lower evapotranspiration than corn (Howell et al., 2008) and 25% more production with the same amount of irrigation water (Bean and McCollum, 2006), however, corn requires over two times as much irrigation (550 mm vs. 250 mm) as the forage sorghum in order to produce more dry matter (Miron et al., 2007). Meanwhile, FS has stronger resistance to adjust to different climatic and edaphic conditions (Qu et al., 2014). In addition, as a C<sub>4</sub> fodder crop with good quality and high energy, FS has been used extensively to milk pro-

duction, livestock and fish farming (Bean et al., 2013; Zhan et al., 2001).

According to the color of leaf midrib, FS can further be divided into white midrib (WMR), green midrib (GMR), and brown midrib (BMR) populations (Fig. 1). WMR populations have white leaf midrib and dry stem pith, GMR populations exhibit green leaf midrib and juicy stem pith, while the BMR populations, represented by BMR-6, BMR-12 genotypes, exhibit the characteristic reddish brown coloration in leaf midrib and juicy stem pith although the intensity and persistence of the coloration varied in different BMR genotypes (Saballos et al., 2008). There are many studies on the first two populations in previous literature, which focused mainly on feeding effect, genetic breeding, cultivation and molecular biology (Oliver et al., 2004; Kenga et al., 2006; Marsalis et al., 2010; Lu et al., 2011). Nevertheless, the common problem is that they have typically higher lignin content than that of corn, and the complex linkages in lignin are detrimental to digestibility by ruminants, which ultimately limited dry matter intake and milk production (Oliver et al., 2005).

The BMR traits have aroused extensive interest by many breeders all over the world. These mutants, originally induced and described in mutagenized chemically sorghum (Porter et al., 1978), have been characterized as having lower lignin content and higher digestibility than non-BMR counterparts, even as to a level close to that of corn (Cherney et al., 1986; Gerhardt et al., 1994; Casler et al., 2003; Oliver et al., 2004). More studies on nutritive quality for BMR genotypes were primarily focused on comparing individual BMR genes and their isogenic FS, or several cultivars of forage sorghum. Oliver et al. (2005) determined the impact of BMR-6 and BMR-12 genes on forage quality in near-isogenic versions of four silage sorghum lines. Vogler et al. (2009) reported the effect of allelic variation on forage quality of BMR sorghum mutants. Aguilar et al. (2014) evaluated nutritional traits of BMR mutant and normal genotypes in silage sorghum. Ledgerwood et al. (2009) appraised on digestibility of a BMR mutant of sudangrass (*S. sudanense* (Piper) Stapf.). Beck et al. (2013) compared the nutritive quality of BMR and non-BMR sudangrass. Astigarraga et al. (2014) quantified nutritive quality in the BMR and conventional variety of sorghum-sudan hybrid [*S. bicolor* (L.) Moench × *S. sudanense* (Piper) Stapf.] for grazing dairy cows. Bean et al. (2013) compared nutrient composition and fiber digestibility of different FS classes as BMR, non-BMR, photoperiod sensitive (PS) and non-PS. These studies provide valuable information regarding BMR genes with the less lignin content and higher forage quality at harvest stage. However, to date, there has been very limited data on comparing nutritive values among FS populations associated with different genotypes of leaf midrib, such as WMR, GMR and BMR. Also there has been little research on the expression of nutritive value in BMR populations under the different growing stages, such as joint, head, flower, milk and dough stage.

This study based on the level of populations. All cultivars of FS populations associated with WMR, GMR and BMR genotypes were selected separately as the experimental materials. Within this context, specifically, we mainly focused on the change regulation of the lignin content and other nutritive values under different phenological stages during the two consecutive growing seasons, in 2012 and 2013. Meanwhile, we also compared the photosynthetic indexes and anatomy parameters among the WMR, GMR and BMR forage sorghum populations. The objective of this research was to answer the following questions.

a Whether is there any significant differences in expression of nutritive values, photosynthetic indexes and anatomy parameters among the WMR, GMR and WMR forage sorghum based on the populations?

b What is the change regulation of lignin content between BMR-6 and BMR-12 genotypes in BMR forage sorghum populations? Whether can this difference have any influences to the protein-synthesis and photosynthesis?

c Which stage is different in expression of nutritive values among the WMR, GMR and BMR forage sorghum populations? whether the BMR forage sorghum populations always have lower lignin content in all critical stages?

## 2. Materials and methods

### 2.1. Site location and climate data

Field trials were conducted in 2012 and 2013 at the Dryland Farming and Water-saving Station of Hebei Academy of Agricultural and Forestry Sciences in Hengshui, Hebei province, China (37°44'N and 115°42'E, elevation 20 m). This site were located at the Haihe plain of China, where is not only the principal agricultural cultivation areas in the North China Plain, but also is the production regions of forage sorghum. This region is characterized as semi-arid with mean annual precipitation of 484 mm (65% in July and August), evaporation of 1670 mm, and average temperature of 13.2 °C, sunshine duration of 2546 h, relative humidity of 63%, frost-free period of 206 days in long-term (1981–2013). The meteorological parameters (2012–2013) of the research plot were similar to the long-term except for total seasonal precipitation (average 525 mm) exceeded that of average for the 33 years (Fig. 2). Soil type in this area is typically silty loam, with PH of 7.88, salt of 0.053%, organic matter of 16.5 g kg<sup>-1</sup>, alkali-hydrolysable N of 60.4 mg kg<sup>-1</sup>, available P of 12.5 mg kg<sup>-1</sup>, available K of 201.7 mg kg<sup>-1</sup> within the top 20 cm soil. The previous crop was corn.

### 2.2. Experimental design and cultivation

The objective of this trial was mainly to compare nutritive values of the forage sorghum populations associated with different genotypes of leaf midrib. However, in the world, the different types of sorghum which used for cutting or silage, such as silage sorghum, sudangrass and the sorghum-sudan hybrid, were all called forage sorghum. Therefore, the selection of experimental materials should meet the following demands: Firstly, cultivars should be selected in every forage sorghum population with WMR, GMR, BMR-6 and BMR-12 genotypes. Secondly, the cultivars of each population should be composed of silage sorghum, sudangrass and the sorghum-sudan hybrid. Thirdly, in order to compare scientifically the differences in nutritive values of FS populations, the cultivars of each type of FS should have similar agronomic traits (i.e., growing days, plant height, stem diameter, leaf number, leaf length, leaf width, panicle length) except for the color of leaf midrib. Before conducting experiment, these cultivars were picked out at milk stage in breeding nursery except for SS2 and F8423 introduced from Pacific seed company in Australia, XS2 registered by the national forages variety appraisal committee in China (Table 1). The information and data for agronomic characteristics of tested cultivars at milk stage are shown in Table 1.

All cultivars were sown on May 28, 2012 and Jun 1, 2013, respectively, and planted at a depth of 3–5 cm. Complete randomized block design was arranged with three replications. Plot area was 18 m<sup>2</sup> (6 m × 3 m), row and plant spacing were 50 and 20 cm, respectively. Before sowing, all plots were irrigated by supplemental water (600 m<sup>3</sup> ha<sup>-1</sup>) and tilled to depth of 20 cm soil. A pre-plant NPK fertilizer (15-15-15) was applied at a rate of 750 kg ha<sup>-1</sup> with an additional 300 kg N ha<sup>-1</sup> urea applied 43 days after planting (joint stage). In order to control weeds, 38% atrazine (2-chloro-4-(ethylamine)-6-(isopropylamine)-s-triazine)

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