

Evaluation of Potential Fodder Sorghum Genotypes for Prussic Acid, Lignin and Cellulose Content

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Abstract: Sorghum is a potential fodder crop for an alternative source of livestock feed in Kenya. A study was done to determine the levels of prussic acid, lignin and cellulose content in potential fodder sorghum varieties at Egerton University Field Station in Kenya. Twenty-five sorghum genotypes were grown in a randomized complete block design (RCBD) and replicated three times. The genotypes were sampled at 3-leaf stage and analyzed for prussic acid, lignin and cellulose. The data were subjected to statistical analysis of variance and correlation using Statistical Analysis System (SAS) program version 9.1. Prussic acid levels were significantly different even at an early stage, with local varieties producing more. Lignin and cellulose had an inverse relationship with respect to concentration. Fodder sorghum genotypes varied significantly in prussic acid, lignin and cellulose, even at an early growth stage.

Key words: Food security, prussic acid, lignin, cellulose, fodder sorghum, livestock.

1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is an important crop with a great potential to provide fodder for the growing livestock industry in the world. It is preferred over maize as it can grow in more diverse environments and is more resilient to multiple abiotic and biotic stresses [1]. Maize is the most commonly used fodder crop in sub-Saharan countries which is worrying since it is a staple food crop mainly in developing countries [2]. Therefore, maize being produced in developing countries is competed for by both human and livestock, making it difficult to bridge the food security gap created by the growing human population. In view of the growing livestock sector in Kenya, sorghum is being evaluated as an alternative fodder source with potential benefits of improving the livelihood of low-income households who are the main stakeholders in the industry [3].

In the face of climate change, fodder sorghum can be a solution to fodder shortage for the growing livestock

population. However, most sorghum varieties are not safe due to elevated prussic acid levels which are produced during the growing period. Prussic acid concentration level above a certain threshold is lethal to livestock. Sorghum contains the cyanogenic glycoside dhurrin, which, when consumed, releases HCN through mastication and enzymatic actions of grazing ruminants [4]. The prussic acid then easily enters the blood stream of livestock causing cellular asphyxiation and ultimately death. HCN is detoxified by livestock through conversion to thiocyanate and excreted as waste, but toxicity will occur if the HCN production exceeds the detoxification rate [5]. Death due to prussic acid poisoning can have great economic effect on smallholder farms. The use of sorghum as fodder has also been limited by high concentrations of lignocellulose, which is not easily digestible by livestock.

There is need to screen potential fodder sorghum for prussic acid and lignocellulose content in order to identify and recommend those with low levels, and thereby contribute to feed and nutrition security for the livestock sector in Kenya. The productivity of the livestock industry in Kenya has fluctuations due to a

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number of challenges including inadequate quality of livestock feed. Selecting sorghum genotypes with low lignin, cellulose and safe levels of prussic acid will provide year-round supply of safe fodder for the growing livestock population. This is because sorghum can thrive under different climatic conditions and also has tillering ability for biomass. Furthermore, sorghum has a wide genetic diversity with genotypes that can stay in the vegetative state throughout the year. The study therefore aimed at evaluating potential fodder sorghum genotypes for prussic acid and lignocellulose content.

2. Materials and Methods

The experiment took place at Egerton University (0°22' S; 35°55' E) which is classified as an upper-highland zone. The field was ploughed and prepared before the onset of rains to suitable tilt for the small seeded sorghum genotypes. A collection of 25 sorghum lines and varieties were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), farmers' collections and commercial varieties in the market. The experiment was laid out in a randomized complete block design (RCBD) and replicated three times. Each of the experimental plots was measured 3 m × 2.5 m and had four rows of sorghum plants. Sowing was done at a spacing of 60 cm × 10 cm with two seeds per hill. Weeds were manually controlled and all pests were taken care of by spraying using recommended pesticides. Phosphorous fertilizer was applied during planting at a recommended rate of 60 kg P₂O₅/ha after soil testing. Plants were collected at the 3rd-leaf stage for analysis of prussic acid and lignocellulose. Random sampling was done in the middle rows by cutting the plants at the base and transporting immediately to the laboratory for subsequent analysis.

Cellulose and lignin contents of biomass samples were analyzed using acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin

(ADL) methods [6]. During NDF analysis, approximately 1.0 g of dried biomass sample was transferred into a round-bottomed flask connected with a condensing unit, followed by 100 mL preheated neutral detergent solution (30 g sodium lauryl sulphate, 18.61 g ethylenediaminetetraacetic acid (EDTA) disodium salt, 6.81 g sodium borate decahydrate, 4.56 g disodium hydrogen phosphate and 10 mL 2-ethoxy ethanol, all dissolved in 1 L distilled water) being added into the flask. After heating the mixture for 1 h at its boiling temperature, the solution was cooled down to room temperature and filtered. The residue was washed three times with hot distilled water, followed by three times washing with acetone and vacuum dried. Further drying was conducted at 105 °C for 3 h. The percentage of weight loss was calculated from the initial and final weight difference. The ADF analysis is similar to that of NDF but using a different detergent solution, which is acid detergent solution (20 g cetyl trimethyl ammonium bromide was dissolved in 700 mL distilled water and 27.56 mL of 96.7% sulphuric acid was then added to the solution and then topped up to 1 L with distilled water). The ADL analysis utilized the residues from the ADF analysis and this was covered with 72% H₂SO₄ (at 15 °C) solution and stirred three times at hourly intervals. The mixture was then filtered, washed with hot water and then dried at 105 °C for 3 h and then cooled down to room temperature. The residue was heated in a furnace set at a temperature of 500 °C for 2 h. The hot crucibles were then transferred into a 100 °C oven for 1 h before cooling in a desiccator to room temperature and then weighed. The percentage of weight loss was calculated from the initial and final weight difference. The percentages of cellulose and lignin were calculated as follows:

$$\text{Cellulose (\%)} = \text{ADF} - \text{ADL} \text{ and } \text{Lignin (\%)} = \text{ADL} \quad (1)$$

Prussic acid content was analyzed using a digestion, distillation and titration method by Bradbury *et al.* [7]. The leaves were cut into pieces approximately one-half inch in length with a pair of grass shears. Fifty grams (50 g) of the stripped leaves will be used for a bulk

sample. The finely cut leaves were thoroughly mixed by hand before sampling for distillation. Samples were weighed out immediately and as fast as possible for moisture and for HCN determinations in order to avoid losses of moisture and HCN. The percent of moisture was determined by drying the samples in an electrically controlled oven at 105 °C under ordinary atmospheric pressure for approximately 20 h. Five grams (5 g) samples of the finely cut sorghum leaves were macerated in an iron mortar with a small quantity of pure silica sand moistened with a few cubic centimeters (cc) of distilled water. After maceration the samples were transferred to an 800 cc. Kjeldahl flask with approximately 300 cc of distilled water was used to wash out the mortar. The flask was then corked immediately, using a large rubber stopper. The macerated sorghum in the tightly stoppered Kjeldahl flask was allowed to digest overnight at room temperature. The HCN was then distilled on the ordinary Kjeldahl distilling apparatus into a receiving flask containing 10 cc of 0.05 M normal silver nitrate (AgNO₃), 1 cc of nitric acid (HNO₃) to keep the solution acid and 50 cc of distilled water was then added. The receiving flask was covered in aluminum foil to avoid reduction of silver nitrate solution by the light. The precipitate of silver cyanide was then separated from the solution in the receiving flask by filtering through a Gooch crucible. The silver nitrate remaining in solution was determined by titration with a 0.5 M standard potassium thiocyanate solution, using ferric ammonium sulphate as an indicator. The HCN percentage was calculated from the titration on a moisture free basis and converted to parts per million (p.p.m.).

The data obtained were subjected to analysis of

variance using Statistical Analysis System (SAS) program version 9.1 and means separated using least significant difference (LSD) (0.05). Correlation analysis was done between prussic acid and lignin, prussic acid and cellulose and lignin and cellulose.

3. Results and Discussion

Significant differences were observed for prussic acid content among the sorghum varieties tested (Table 1). Two local varieties B35 and GS008 X EUSS17 had the highest amount of prussic acid levels compared to the rest (Fig. 1). The difference in prussic acid level in the different varieties can be attributed to the variation in the genetic makeup [8]. It was reported that Chakwal variety produced more prussic acid compared to JS-2002, while local varieties produced higher acid levels than Chakwal and JS-2002. The low prussic acid levels in the sorghum leaves are a result of the young stage of the seedlings (3-leaf stage). The results show a large variation among the genotypes in lignin and cellulose content (Figs. 2 and 3). Correlation analysis showed no relationship between prussic acid and lignocellulose. However, an inverse relationship was observed between lignin and cellulose (Fig. 4). Since lignification is influenced by genetics, considerable variation is found among different genotypes. The low levels of lignin can be attributed to the young stage it was sampled in Carmi *et al.* [8].

4. Conclusions

The prussic acid, lignin and cellulose varied significantly across the genotypes. Levels of prussic acid are not lethal to livestock at this growth stage. These results demonstrate that one can select fodder sorghum genotypes with low prussic acid, lignin and cellulose.

Table 1 Means squares for prussic acid, lignin and cellulose.

Source of variation	df	Prussic acid	Lignin	Cellulose
Replicate	2	30.362	12.869	117.760
Variety	23	3.685*	247.851**	369.61**
Error	46	1.683	12.343	42.544

* and **: significant at $p < 0.05$ and $p < 0.01$.

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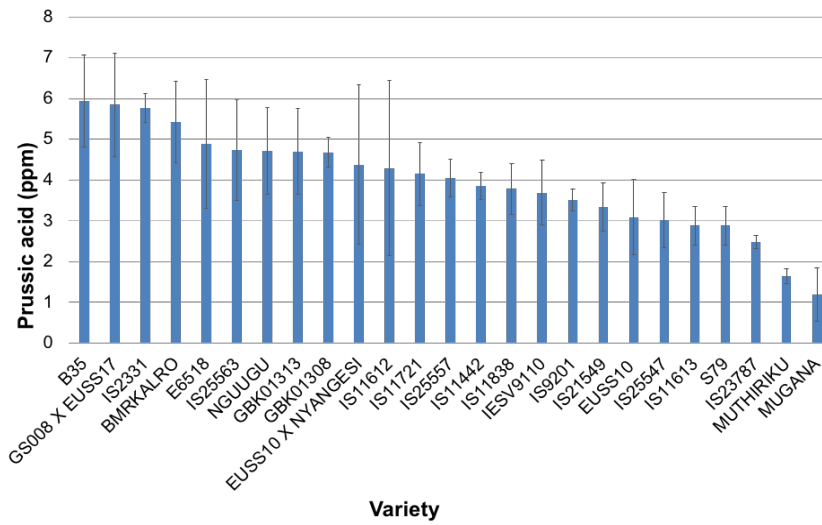


Fig. 1 Prussic acid level in selected sorghum genotypes.

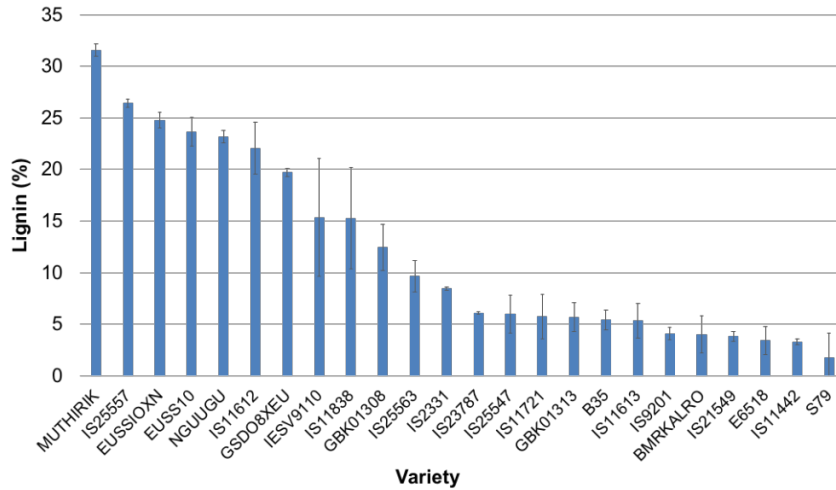


Fig. 2 Lignin level in selected sorghum genotypes.

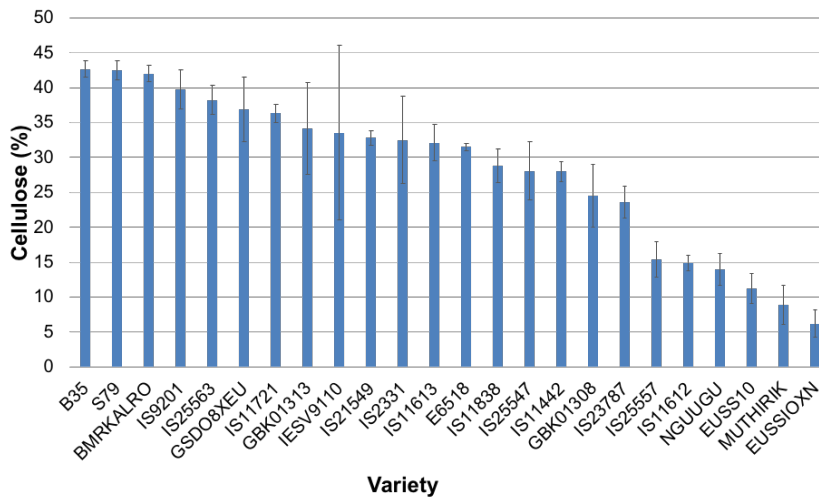


Fig. 3 Cellulose level in selected sorghum genotypes.

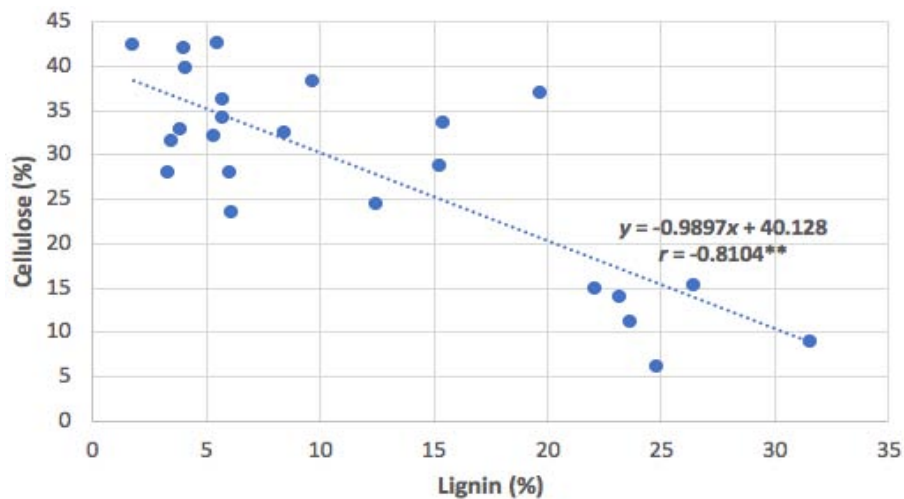


Fig. 4 Inverse relationship between lignin and cellulose levels in sorghum genotypes.

5. Recommendations

Progressive monitoring of prussic acid, lignin and cellulose throughout different growth stages is required for the selection of the suitable genotype(s). The selected genotype(s) can then be evaluated for biomass and regeneration for the best fodder material.

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References

[1] Reddy, B. V. S., Ramesh, S., and Reddy, P. S. 2004. "Sorghum Breeding Research at ICRISAT—Goals, Strategies, Methods and Accomplishments." *International Sorghum and Millets Newsletter* 45: 5-12.

[2] Suttie, J. M. 2000. *Hay and Straw Conservation—For*

Small-Scale Farming and Pastoral Conditions. Roma: FAO Plant Production and Protection Series, 29.

[3] Mitaru, B. N., Mgonja, M. A., Rwomushana, I., and Opio, F. 2012. "Integrated Sorghum and Millet Sector for Increased Economic Growth and Improved Livelihoods in Eastern and Central Africa." In *Proceedings of the ECARSAM Stakeholders Conference*, November 20-22, 2006, Dar es Salaam, Tanzania.

[4] Patel, H. B., Singh, R. D., Mody, S. K., Modi, C. M., Kamani, D. R., Parekar, S., and Chukeware, A. 2014. "Cyanide Poisoning in Animals." *AGRES-An International e-Journal* 3: 202-16.

[5] Knight, A. P., and Walter, R. G. 2001. "Plants Causing Sudden Death." In *A Guide to Plant Poisoning of Animals in North America*. Ithaca, NY: International Veterinary Information Service.

[6] Omar, R., Idris, A., Yunus, R., Khalid, K., and AdiaIsma, M. I. 2011. "Characterization of Empty Fruit Bunch for Microwave-Assisted Pyrolysis." *Fuel* 90: 1536-44.

[7] Bradbury, J. H., Egan, S. V., and Lynch, M. J. 1991. "Analysis of Cyanide in Cassava Using Acid Hydrolysis of Cyanogenicglucosides." *Journal of Food and Agriculture* 55: 277-90.

[8] Carmi, A., Aharoni, Y., Edelstein, M., Umiel, N., Hagilali, A., Yosef, E., Nikbachat, M., Zanou, A., and Miron, J. 2006. "Effects of Irrigation and Plant Density on Yield Composition and *in Vitro* Digestibility of a New Forage Sorghum Variety, Tal, at Two Maturity Stages." *Journal of Animal Feed Science and Technology* 131: 120-33.