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RESEARCH ARTICLE

EFFECT OF GENOTYPES AND NITROGEN ON GRAIN QUALITY OF SORGHUM (*SORGHUM BICOLOR* L. MOENCH)

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ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench) is cultivated as an important food grain in the semi-arid regions of Africa. Processed grain sorghum is traditionally consumed as porridge, couscous, traditional tô or beer. The quality of such foods is highly dependent upon grain characteristics. Sorghum grain quality traits mainly include kernel hardness, kernel weight, kernel size, protein content and kernel color. Grain quality traits are often influenced by environment, genotypes, fertilizer management and their interaction. The objective of this study was to determine the impact of different levels of nitrogen application (0, 45, and 90 kg ha^{-1}) on grain quality of selected sorghum genotypes.

The field experiment was conducted at three locations in 2010 and 2011 at Manhattan. The experiment was laid in split plot randomized complete bloc design and replicated four times. The main plots were assigned to three N regimes: control (0 kg N ha^{-1}), half recommended rate (45 kg N ha^{-1}) and recommended rate (90 kg N ha^{-1}). The subplots were assigned to twelve genotypes (six hybrids and six inbred lines). Plot size was 6.1 m x 3.0 m with a row spacing of 0.75 m. After harvest, grain quality traits (hardness, weight, diameter and protein content) were evaluated using standard procedures and the data subjected to statistical design using SAS. There were significant effects of genotype for most grain quality traits across both locations in Manhattan. Inbred lines SC35 and SC599 had maximum hardness at all locations while hybrid 95207, had the lowest hardness for all locations. Also, Inbred lines SC35 and Tx340 had maximum protein content at all the locations. While hybrids 95207, 26056, 23012 had the lowest protein content.

Genotypes Tx430, SC35, had higher hardness and with higher protein content were classified as high quality. uWe conclude that application of N (45 or 90 kg ha^{-1}) significantly improved grain protein, but not other quality traits. There are opportunities to improve grain protein through fertilizer management and plant breeding.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is an important crop, usually cultivated as a feed and fodder crop by subsistence farmers in rainfed in Africa especially in Mali. In some parts of the world, it is consumed as staple food and is also used in the production of a variety of by-products like alcohol, edible oil, and sugar. In general, very little quantity of fertilizer is used for the cultivation of sorghum, probably due to high cost and poor economic condition of the farmers. Although fertilizer application increases crop production and it has universally been acknowledged that the more you pay to the crop the more you will gain", inappropriate practices that are followed during cultivation lead to low output of the applied fertilizer compared with the actual potential of fertilizer efficiency. In addition, even under the best management practices, 30%-50% of the applied N is lost through different routes (Stevenson, 1985), and hence more fertilizer has to be applied than that actually needed by the crop to compensate for the loss.

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The loss of N not only causes trouble to the farmer but also causes hazardous impact on the environment (Kessel *et al.*, 1993; Gosh and Bhat, 1998). High inputs of chemical fertilizer for sustainable crop production cause soil degradation and environmental pollution (William, 1992). Thus, it is necessary the optimum N level.

Nimje and Gandhi (1993) reported that the application of nitrogen fertilizer at the rate of 80 kg ha^{-1} significantly improves germination, seedling vigor, grain and straw yields as well as protein content. Since nitrogen is critical nutrient for growth development of crops, low nitrogen supply, besides limiting yield may also have impact on general grain quality characteristics and nutritional value of the crop. There the present study aimed at determining the impact of nitrogen fertilization on grain quality in selected sorghum genotypes.

MATERIALS AND METHODS

In summer 2010 and 2011, a two-year study was initiated to determine the effect of nitrogen levels on grain quality of selected sorghum genotypes. Test locations in 2010 and 2011were Unit 1 (Irrigated) and Unit 7 (rainfed) sites at

Ashland Bottoms Research farm near Manhattan KS. Soils at Manhattan were silt loan (Unit1) and reading silt loan (Unit7). The experiments were implemented on conventional tillage in two of the locations, Ashland bottom Unit 1 and Unit 7. The previous crop in Unit 1 was sorghum while in Unit 7 was soybean and maize, respectively for 2010. However, in 2011 the previous crop in Unit 1 was sorghum, soybean in Unit 7.

Precipitation and temperature which are the two most important climatic factors that affect crop growth during the growing season varied among the locations and years of the study.

In Manhattan, the growing season mean maximum temperatures were 28.8^{o}C and 29.3^{o}C in 2010 and 2011, respectively. While the minimum temperatures were 15.8^{o}C and 15.4^{o}C in 2010 and 2011, respectively. The rainfall was 355.4 mm, and 457.1 mm in 2010 and 2011, respectively (Figure 1)

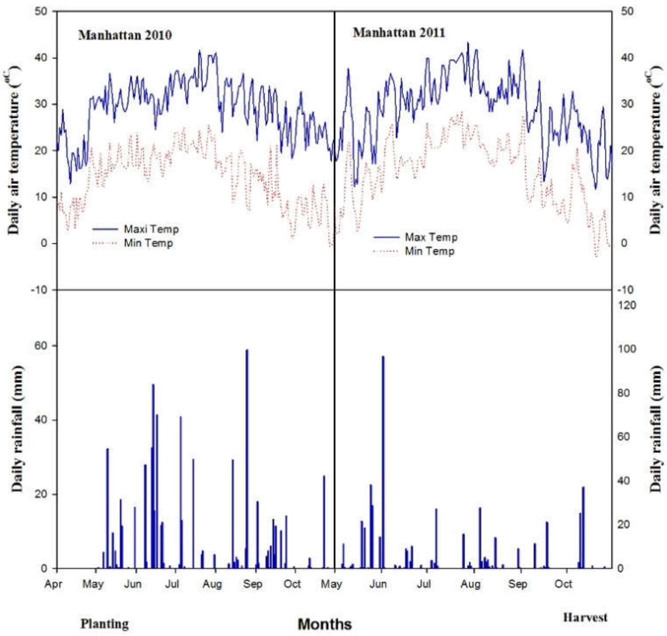

Figure 1 Daily maximum and Minimum Mean Air Temperatures and Rainfall from May to October 2010 and 2011 at Manhattan.

Experimental Details

The randomized complete block experimental design in a split plot arrangement with four replications was used. The main plots were assigned to three N levels. Control (0 kg ha^{-1}), half recommended rate (45 kg N ha^{-1}) and the recommended rate (90 kg N ha^{-1}). The sub plots were assigned to six hybrids (23012, 26056, Tx3042xTx2737, CSR1114xR45, 99480, and 95207) and six inbred lines (SC35, SC599, B35 Tx430, Tx2783, and Tx7000) of varying drought tolerance characteristics (pre-flowering and post-flowering drought tolerance. Each plots dimension was 6 m long and 4 rows unite whit row spacing of 0.75 m. The central two rows were harvested for yield estimated to eliminate any border effects, and the grain quality parameters included in this thesis were based on grain harvested from the central rows. Sorghum varieties were sown on a well-prepared seedbed. Before sowing, a composite soil sample from 0.15 and 0.60 cm soil depth was collected from the experimental plots and analyzed for physico-chemical properties.

Crop Management

The nitrogen fertilizer source was urea (46% N). The fertilizer was hand broadcast 10 d to 14 d after emergence along the rows of each plot. Planting was done in May and June across all the locations. Weeds were controlled with pre-emergence herbicides applied at labeled rates using a tractor mounted boom sprayer. At Manhattan (Unit 1), Callisto at 0.37 L ha^{-1} and Bicep at 2.75 L ha^{-1} was used. Similarly, at Manhattan (Unit 7), Lumax 2.84 at the rate of 2.9 L ha^{-1} and Bicep at 3.3 L ha^{-1} was sprayed. Hand weeding was also used when necessary to remove late emerging weeds during the growing season. Maturity the central two rows were harvested and threshed using a two row plot combine. The grain samples were collected separately for each plot. The harvested grain samples were sent to the USDA laboratory for determining grain quality parameters including protein content.

Measurements

Samples were cleaned before analysis by sieving over a screen with 2.0-mm triangular openings. Glumes, broken kernel, and foreign matter were removed by hand when necessary. Grain samples were subjected to a sequence of measurements performed by the SKCS 4100 includes weight (mg), hardness (%), and diameter (mm). Each of the measurements (weight, diameter, and hardness) were indirect and were calibrated against reference laboratory methods. Weight measurement is calibrated against mass determined using an analytical balance (AND HR-60) for single seeds with weights of 12-80 mg (U.S. method). Single characterization diameter and hardness measurement were conducted using a SKCS 4100 (Perten Instruments North America Inc., Reno, Nevada, USA). Total nitrogen in sorghum was determined by the micro-Kjeldahl method (AOAC, 1975), and the crude protein content was calculated by multiplying 6.25 with N content of grain.

Statistical Analyses

Analyses of variance were performed for the dependent variables, (kernel hardness, weight, diameter, and protein content) content using the SAS version 9.1 with GLM at an alpha level of 0.05. Data for the two years 2010 and 2011 experiments were analyzed separately due to contrasting climate conditions between the years during the growing season. For significant variables, means separation was accomplished using LSD test procedure. Whenever interactions were significant, main effects were ignored and interactions effects were discussed.

RESULTS

Nitrogen Effects on Grain Quality Traits in 2010

Manhattan Unit 1

Grain physical property and protein content as affected by applied nitrogen rate

There were no significant effects of N levels for all traits except grain diameter which was significant different ($P<0.05$). At 45 kg N ha^{-1} (56.76%) kernel hardness was lower compared to 90 kg N ha^{-1} (57.18%) and 0 kg ha^{-1} (58.89%). At 45 kg N ha^{-1} similar response was obtained for crude protein content. But crude protein values increased slightly with increasing N fertilizer levels from 45 to 90 kg N ha^{-1}. On average, the highest protein content 10.62 % was produced at 90 kg ha^{-1} of N. For grain kernel weight, weight values were similar at 45 kg N ha^{-1} (25.80 mg) and 90 kg N ha^{-1} (25.95mg) when compared to 0 kg N ha^{-1} (26.26mg) mm). Similar response was obtained for grain diameter (Table 1).

Table 1 Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 1 in 2010.

N levels (N)	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
0	58.89	26.26	2.24a	10.57
45	56.78	25.80	2.19b	10.45
90	57.18	25.95	2.20b	10.62
LSD (0.05)	NS	NS	0.03	NS

Manhattan Unit 7

Grain physical property and protein content as affected by applied nitrogen rate

There were significant (P<0.05) effects of N regimes on all the variables except gain weight. Sorghum grain hardness ranged from 74.69 to 78.78. Grain hardness values increased slightly with increasing N fertilizer levels from 0 to 90 kg ha^{-1}. Crude protein also increases significantly from 0 to 90 kg ha^{-1}. (Table 2).

Table 2 Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 7 in 2010.

N levels (N)	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
0	74.69b	26.62	2.17b	9.35c
45	77.60a	27.18	2.22a	9.80b
90	78.78a	27.24	2.19ba	10.44a
LSD (0.05)	1.89	NS	0.038	0.37

Genotype by N interaction effect on grain quality

Genotype by N interaction was significant (P<0.05) for crude protein content. Genotypes CSR1114R45, and Tx2783 had similar response for protein but was significantly higher at 45 kg ha^{-1}. Whereas at 45 kg N or 90 kg N ha^{-1}, genotypes 23012, SC599, and Tx430 had similar response. While genotypes SC35, SC599, and Tx340 had higher crude protein content at 45 kg N ha^{-1} or 90 kg N ha^{-1}. Genotypes 95207and 99480 has similar response for crude protein content at 45 kg N ha^{-1}. Overall, when averaged across the genotypes, the lowest crude protein content was produced at 0 kg N ha^{-1} for genotype 23012 and Tx3042Tx2737 (Figure 2).

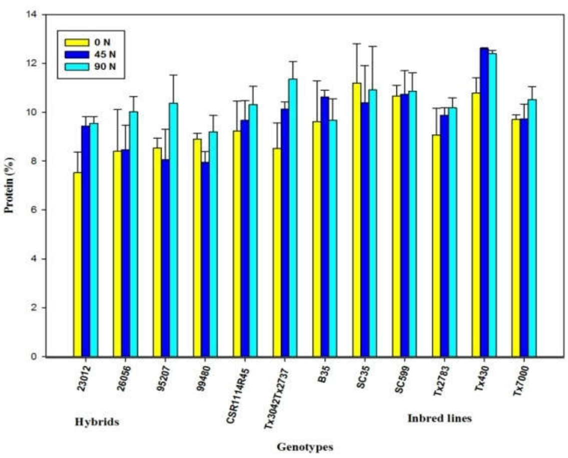

Figure 2 Interaction of genotypes and N rates on protein (%) at Manhattan (Unit 7) in 2010

For grain hardness, genotype by N interaction was significant (P<0.05). Grain hardness was apparent at all the N regimes among genotypes 23012, and Tx3042Tx2737 but was significantly higher at 90 kg N ha^{-1}. The highest grain hardness value was obtained at 90 kg N ha^{-1} for genotypes SC599 and Tx3042Tx2737. While averaged across the genotypes, the lowest hardness value was produced at 0 kg N ha^{-1} for genotypes Tx7000 (Figure 3).

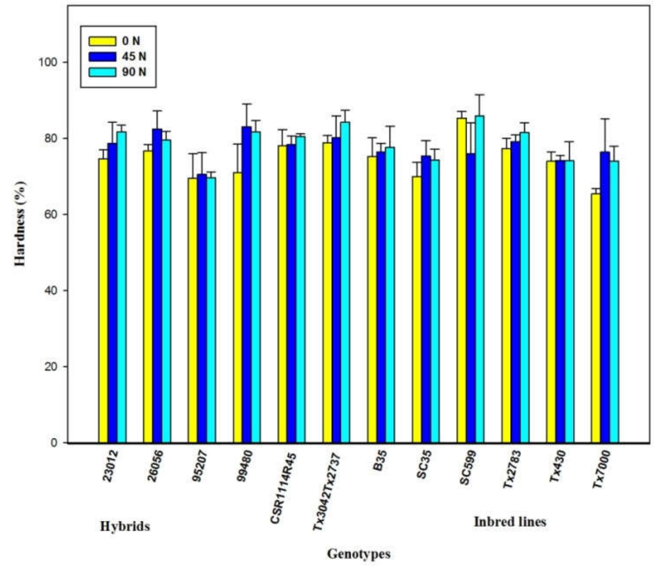

Figure 3 Interaction of Genotypes and N Rates on Hardness (%) at Manhattan (Unit 7) in 2010.

Nitrogen Effects on Grain Quality Traits in 2011

Manhattan Unit 1

Grain physical property and protein content as affected by applied nitrogen rate

There were no significant (P>0.05) effects of N regimes on all the variables except grain protein which was highly significant (P<0.0001). Sorghum grain hardness ranged from 77.06 at 0 kg N ha^{-1} to 78.64% at 90 kg N ha^{-1}. Grain hardness values increased slightly with increasing N fertilizer levels from 0 to 90 kg N ha^{-1}. Maximum grain hardness was obtained at 90 kg n ha^{-1} when compared to 0 kg N ha-1 or 45 kg N ha^{-1}. Similar responses were observed for grain protein content (Table 4).

Table 4 Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 1 in 2011.

N levels (N)	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
0	77.06	28.39	2.32	10.01c
45	78.39	28.31	2.30	10.52b
90	78.64	28.57	2.32	11.04a
LSD (0.05)	NS	NS	NS	1.98

Manhattan Unit 7

Grain physical property and protein content as affected by applied nitrogen rate

Effect of N regimes was significant (P<0.05) on all the variables except kernel weight. Grain hardness ranged from 80.08 at 0 kg N ha^{-1} to 82.87% at 45 kg N ha^{-1}. Kernel weight values decreased slightly with increasing N fertilizer levels from 0 to 90 kg N ha^{-1}. Similar response was also obtained for grain diameter. Kernel weight increases from 0 kg N ha^{-1} to 90 kg N ha^{-1}. Crude protein content was ranged from 11.52% at 0 kg N ha^{-1} to 12.29% at 90 kg N ha^{-1}. Maximum protein content was obtained at 90 kg N ha^{-1} (12.29%) (Table 5).

Table 5 Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 7 in 2011.

N levels (N)	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
0	80.08b	27.90	2.29a	11.52c
45	82.87a	27.47	2.26ba	11.96b
90	81.51ba	27.35	2.25b	12.29a
LSD (0.05)	1.60	NS	NS	0.31

Principal Component Analysis (PCA)

PCA is a multivariate technique for examining relationships among several quantitative variables and is especially a valuable analytical technique in exploratory data analysis. The PCA was carried out to identify the principal components of grain quality (hardness, weight, diameter, and protein content) that best described the genotypes with high and poor grain quality. Similarly, the response of genotypes for nitrogen level was done. The PCA identified the grain qualities that best separated the genotypes for their grain quality traits. However, the response of nitrogen on grain quality was not separated and all the levels of N are in on principal component vector. The first four principal component vectors (PC1, PC2, PC3 and PC4) accounted for 98.6 % of the total variability. The PC1 eigenvector contrasted genotypes with high positive loadings for variables hardness, and protein. The PC2 eigenvector contrasted genotypes with high positive loadings for all the variables. The PC3 eigenvector contrasted genotypes with high positive loadings for variables diameter, and protein. The PC4 eigenvector contrasted genotypes with high positive loadings for variables hardness, and diameter (Table 24). On PC1, hardness had a loading of 0.99 and protein had 0.03. However, in PC2, it had 0.09 and 0.11, respectively. The seed weight had the highest loading of 0.98 in PC2. Highest loading of protein content was observed in PC3 (0.993). Similarly, diameter loading was highest in PC4 (0.0998).

The biplot is a simply and specially scaled combination of PC scores and loadings (eigenvectors) that allow the approximate similarities and differences of the genotypes (the scores) to be displayed simultaneously and allow the different response variables (eigenvectors) to be associated with genotypes. A biplot of PC1 against PC2 revealed that there is considerable variation among genotypes in their response to nitrogen, with genotype score ranging from −9.5 to about 5.73. The PCA separated the genotypes based on grain quality and the genotype. The genotypes with higher grain quality were placed on the right of the biplot while genotypes with low values were placed on the left of the biplot (Figure 4). The genotypes were divided into four groups based on the scores of the first two principal components (Figure 8): group 1 genotypes as high grain quality with positive scores for PC1 and PC2, group 2 as moderately high grain quality with positive PC1 and negative PC2 scores, group 3 as moderately low grain quality with negative PC1 and positive PC2 and finally group 4 as low grain quality with negative PC1 and PC2 scores.

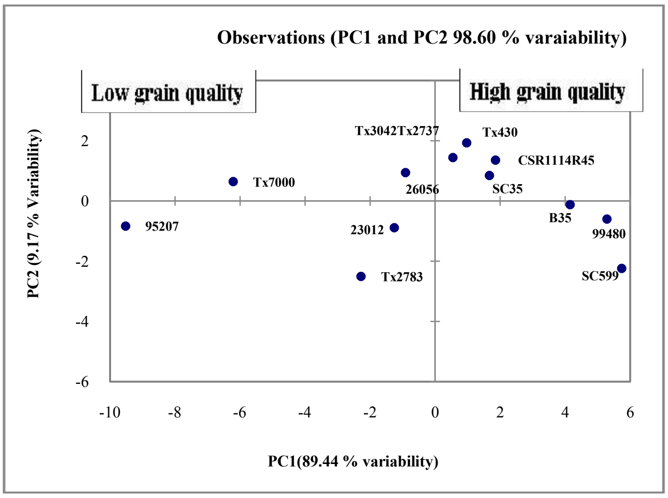

Figure 4 First and Second Principal Component Scores (PC1 and PC2) for the Identification of Sorghum Genotypes for Grain Quality Traits.

DISCUSSION

Effect of Nitrogen Levels on Grain Quality Traits

Most of the studies have long recognized the close association between nitrogen fertilizer and sorghum grain yield, and protein content but not often with hardness, diameter, and weight. The study shows variability among genotypes (hybrid and inbred lines) and the types in all the traits that were measured. Sorghum genotypes responded positively to N fertilizer application. This study did not show a significant effect of nitrogen on grain hardness, kernel weight, and kernel diameter. Many study reported increasing of nitrogen in cultivars of remarkably caused to increase in kernels weight. They found that increasing nitrogen fertilization rates led to a significant increase in grain weight and grain fertilizer as compared with control treatment. Similarly, conclusion was reached by Said *et al*. (1996).

In our study the effect of nitrogen on kernel weight was generally not significant in 2010 and 2011 averaged across all environments. The variability of the seed weight observed in this current study might be due to decrease seed filling duration as result of the high temperatures (> 32°C) during the growing season especially in 2011. Despite the fact that environmental conditions were favorable at the time of flowering, but stress occurring 10 - 15 d before flowering, has the tendency to reduce seed weight. This condition prevailed in 2011. Thus, seed weight may be reduced if drought stress occurs immediately after seed set because of reduction of seed filling. As indicated in the results, 2011 was a dry year and this resulted in a reduction of seed weight for most of the genotypes.

Kernel hardness (endosperm texture) affects the processing properties of the grain and the resulting products. An increased N supply has been associated with increased kernel hardness (Kaye *et al*., 2007). Irrigation has been shown to result in softer kernels (Taylor *et al*., 1997). In general, dry milling and alkaline cooking for human food products is better with hard kernels (Johnson, 2005; Shandera *et al*., 1997), while wet millers and brewers prefer softer kernels with lower protein concentrations (Fox *et al*., 1992). The determination of grain yield and hardness of food-grade sorghum hybrids grown in different production environments would assist grain merchandisers, farmers, and food processors in targeting environments and hybrids for value-added end-use markets. In our study, in 2011 drought and high temperature stress was severe during the growing period had the highest hardness compared to 2010. Research with sorghum and maize has shown that kernel density is greater under dryland conditions than irrigated conditions Kaye *et al*., 2007. Kniep and Mason, 1989; Bauer and Carter, 1986; Duarte *et al*., 2005). Johnson (2005) found harder sorghum kernels produced under drier Texas growing conditions than in Kansas and Nebraska.

Protein content is one of the major components determining the quality of fodder crops and was influenced significantly by application of nitrogen. The result indicated that all the levels of nitrogen significantly affected the grain protein contents during 2010 and 2011. Crude protein contents showed linear increase with an increase in nitrogen level because a large proportion of the N in grain is remobilized from leaves and stems after anthesis rather than being taken up from the soil. Ercoli *et al*. (2008) found that dry matter and nitrogen increased up to maturity when fertilizer was not applied. They concluded that nitrogen in the grain was derived primarily by translocation from leaves and stems rather than by uptake from the soil during the period of grain formation. Knowles and Watkins (1993) found that most of the N that was taken up by wheat plants was translocated to the grain either directly or by mobilization from other plant parts. Other studies have shown that the relationship between grain protein concentration and N translocation or N-translocation efficiency is not consistent (Dordas, 2009; Asseng and Milroy, 2006). Conversely, Gooding *et al*. (2005) and Robert *et al*. (2001) have reported that protein concentration in grain might be improved by selecting genotypes that translocate a higher percentage of N from the vegetative organs to the grain. Positive correlations have been observed in wheat between grain protein concentration and nitrogen harvest index (Saint Pierre *et al*., 2008). A progressive increase of grain protein content with increase of N level may be also due to the reason that fertilizer enhanced the amino acid formation. Nimji and Gandhi (1993) reported that the application of nitrogen fertilizer at the rate of 80 kg ha^{-1} significantly improves germination, seedling vigor, grain and straw yields as well as grain protein

Principal Component Analysis

The PCA is perhaps the most useful statistical tool for screening multivariate data with significantly high correlations (Johnson, 1998). The cluster analysis applied to the principal components divided the genotypes into four distinct groups. The PC1 eigenvectors for variables hardness and protein content have high positive loadings, while variables weight and diameter have high negative loadings. The PC1 vectors indicated that genotypes with high weight and optimum diameter do not necessarily have high grain hardness or high protein content. But, good grain quality will result only from high hardness and high protein content. Based on the biplot PC1 vs PC2 genotypes Tx430, SC35, Tx3042xTx2737 and CSR1114/445 had higher hardness and with higher protein content were classified as high quality, and genotypes 95207, 23012, and Tx2783 were classified as low grain quality.

CONCLUSION

In summary, grain sorghum genotypes vary in their response to nitrogen fertilizer. Sorghum genotypes responded positively to N fertilizer application. There was a significant effect of genotypes on grain quality traits. Increasing nitrogen fertilization rates led to a significant increase in grain protein content as compared with control. Grain quality traits of inbred lines were comparable with hybrids. Besides application of N significantly improved grain protein, but not other quality traits. There was a significant difference between sorghum hybrid and inbred lines in term of grain protein content. The study showed that mostly inbred lines performed better than hybrid in terms of grain crude protein content. The maximum grain protein content was obtained at the optimum N regime, followed by the half recommended rate and the least was the control.

Based on the result of this study there were no significant different for the entire trait except crude protein content which is easily comprehensive because of the richness of the soil in high residual N. In contrast the same study will be very useful for farmers in Africa especially in Mali where most of the soil has been used for long time without a substantial contribution of nitrogen and other nutrient such as phosphorus, potassium. There are opportunities to improve grain protein through fertilizer management and plant breeding. For grain hardness and crude protein content genotypes Tx430, SC35, SC599, and B35 were superior. These genotypes can be used in breeding program.

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