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Physiochemical Properties and Identification of Elite Genotypes for Improved Sorghum Breeding in Tanzania

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Variability in physiochemical properties in sorghum is critical in cultivar development for optimum grain quality and crop resistance against fungal and insect pests. These traits are not well studied. The objective of this study was to characterize sorghum genotypes based on kernel phenotypic and biochemical traits and identify promising genotypes for better utilization of these traits in sorghum breeding. 98 sorghum genotypes comprised by the released varieties, breeding lines, hybrids and local cultivars were studied using qualitative and quantitative parameters. 75.51% of these genotypes have thick pericarp, 33.67% have testa layer, and 7.0% showed mostly-corneous endosperm texture. Results revealed a wide variability among studied genotypes in terms of phenotypic and biochemical properties (p<0.001). A cross IES11038 X A1GD 34553 recorded the highest 100 seed weight (6.2g). Pato and IESV 92174DL were the hardest genotypes with 110.33 and 108.4N respectively. Protein content ranged from 6.52 to 12.23%, of which Naco Mtama 1 and

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IESV 24030SH were the promising genotypes. Genotypes ICSA 88006 x IESV92172DL, ICSA15 x R8602 and GADAM recorded the highest starch concentration (79 g/100g). The identified elite genotypes could enable selection and hybridization of useful traits.

Keywords: Phenotypic; biochemical; genotypes; variability; sorghum; kernel.

1. INTRODUCTION

Sorghum is the main source of calories and protein to most people in Africa and Asia [1], widely grown in semi-arid areas. The crop is known to withstand harsh environmental condition including drought [2]. Sorghum have a wide genetic diversity in its physical structure and or chemical composition and therefore presenting benefits in hybridization [3]. Variation in structure, nutritional composition and phytochemical composition is critical for selection of desired traits in sorghum breeding [4]. The inheritable qualitative traits in sorghum kernel consist of pericarp color, pericarp thickness, presence of testa, testa color, and endosperm texture; while quantitative traits include grain size and weight [5,6]. Literature indicated that starch is the largest portion of sorghum grain weight made up by amylose and amylopectin molecules held by hydrogen bonds [7]. Amylopectin is made up by large branched polymer unlike the amylose structure. Sorghum starch contain 70-80% amylopectin and 20-30% of amylose; mainly for feed and industrial use [8]. Moreover, Protein concentration in sorghum grain usually varied based on the genotype, water, temperature, and soil fertility status of the soil. According to [9] drought condition is known to increase protein concentration while reducing starch content. Sorghum genotypes with higher yield is known to have smaller concentration of protein; while the application of nitrogenous fertilizer increases protein concentration particularly prolamin, kafirns and glutelins in the sorghum endosperm [10]. In addition, the germ portion comprised by albumin and globulins with highest concentration of lysine [11]. The physical appearance of sorghum kernel structure largely guided by its associated biochemical traits including the phenolic compounds. According to [12] Phenolic compounds consist of benzene ring and hydroxyl group. Plants materials contains phenolic compounds, which reflects the taste, color and appearance. Sorghums has a wide variability of phenolic acids. In addition, [12] screened a number of sorghum genotypes and found high phenolic content in high tannin sorghums. Further [13] concluded the health benefits derived from phenolic such as low digestibility,

reduction of diseases like cardiovascular, anticarcinogenic and lowering of cholesterol; This is due to antioxidant capacity of phenolic compounds as lowers amount of free radicals in the body. Some sorghum cultivars comprised by tannins or proanthocynidins which is genetically based controlled by genes B1,B2 in the testa [14]. [15] characterized sorghum as Type I (sorghums without condensed tannins), Type II sorghums are genotypes with extractable tannins using 1% acidified methanol and not the pure methanol and Type III sorghums have tannin that can be extracted using both one percent acidified methanol and the pure methanol. Sorghum tannins bind protein and makes it unavailable in the digestion through ionic, hydrogen, hydrophobic and covalent bonding [16]. These compounds were also reported to protect plants against insects [17]. For this case breeders must screen large pool of germplasm to identify genotypes with higher levels of phenolic [18].

Several studies attempted to characterize sorghum genotypes based on physical and biochemical composition. For instance, [19] assessed the phytochemical properties of forty five sorghum genotypes based on weight, protein and sugar content; [4] documented a wide variability in terms of nutritional and stalk sugar content in sorghum. [20] screened four improved sorghum varieties and observed considerable variability in terms of biochemical composition including mineral concentration, crude protein, starch, fat and even ash content. The current study therefore contributes to the general understanding of kernel traits related to phenotypic and biochemical properties for effective utilization of these traits. In Tanzania, many sorghum genotypes were not previously evaluated and their phenotypic and biochemical potential is not understood and or documented; therefore, it is important to characterize a broad range of sorghum genotypes. The study intended to characterize sorghum genotypes based on kernel phenotypic traits and biochemical composition to establish potential of these traits in cultivar development. The study also identified promising sorghum genotypes to be used as parental materials during hybridization.

2. MATERIALS AND METHODS

2.1 Site and Source of Materials

Ninety eight (98) sorghum genotypes collected from TARI Ilonga center, Tanzania National Plant Genetic Resource Centre (NPGRC) and International Crops Research Institute for the Semi-arid Tropics (ICRISAT); comprised by commercial varieties, hybrids, local cultivars and breeding lines (Table 1). The known agronomic properties of these materials include high yielding, midge resistance, striga resistance, anthracnose, stay green and earliness. Materials were raised at Tanzania Agricultural Research Institute Ilonga in Kilosa, Morogoro Tanzania; located at latitude 06°42'S, longitude 37°02'E and altitude of 506 meters above sea level with a bimodal type of rainfall. Materials were planted in the cropping season 2017/18. All agronomic management including supplementary irrigation, weeding, fertilizer application and insect control were applied as per recommendation. Harvested grains were cleaned and sorted for analysis of phenotypic and biochemical traits at the Nelson Mandela African Institution of science and Technology and food processing laboratory of the Sokoine University of Agriculture.

2.2 Determination of Qualitative Kernel Traits

Ten sound kernel selected randomly for each physical analysis according to procedure described by [21]. Pericarp thickness was determined by scratching sorghum kernel using scalpel and observe the pericarp thickness using a magnifying glass. The presence of testa layer and the associated color was recorded after removal of pericarp. Endosperm texture; was determined by cutting each kernel into half and observe the proportion of corneous material with the aid of magnifying glass; materials were characterized into starch, intermediate and pearly based on the score. Grain color was determined through visual examination using color chart and codes as per sorghum descriptors guide [22].

2.3 Determination of Quantitative Kernel Physical Traits

100 sound sorghum kernels were manually counted and weight measured in replicates using analytical balance TPA 500. Kernel hardness (firmness) was observed using Brookfield CT3 Texture analyzer, using probe TA41 Cylinder 6 mm D, 35 mm L; with the recommended trigger value of 50 g and Load Cell of capacity of 50 kg, test speed was set at 10 mm/s, and deformation of 0.70 mm. The average of six samples (kernels) per test was taken as hardness. Furthermore, the arithmetic mean diameters was taken as average of the major diameter, minor diameter, and intermediate diameter of sorghum kernel using automatic caliper [23].

2.4 Determination of Nitrogen Content

Total nitrogen and protein of sorghum genotypes was determined from grain through digestion, distillation and titration with hydrochloric acid as per Micro Kjeldahl Method [24]. Grain was grinded and sieved using 0.5mm sieve; 0.1 g was placed into a digestion tube. 1g Selenium catalyst mixture weighed and mixed with the sample; followed by addition of 5 ml of sulphuric acid (96%) into the tube. The tubes was heated slowly in the digestion apparatus until the digest is clear. The content was transferred to a 100 ml volumetric flask where distilled water was added into a 100 ml graduated flask. 5ml of boric acid indicator solution were placed into the distillation apparatus. 10ml of clear supernatant were then transferred into the apparatus where 10 ml of NaOH (46%) were added. Color change were observed when distillation drops mixed with the boric acid indicator. 150 ml of the distillate were titrated with sulphuric acids (0.0174N) where color change from green to pink was observed, the titer volume was recorded. Finally, total nitrogen was determined using the following formula:

N (percentage) = $a \times N \times Mw \times 100 \times 100\%$ b × c

Where, $a = mI$ of sulphuric acid, $N =$ Normality of sulphuric acid (0.0174), $a =$ Titer volume, Mw = Molecular weight of Nitrogen (0.014) , b = gram sample taken for analysis $(0.1 g)$ and $c = ml$ digest used for distillation (10 ml). Thus, the percentage crude protein = $6.25 \times \%$ N.

2.5 Determination of Starch Content

Starch concentration was determined using [25] official method 996.11 whereby, 100mg of finely ground sample were taken into 15ml centrifuge tubes. 0.2 ml 80% ethanol was added and vortexed. 3 ml of 10% α – amylase enzyme in mM sodium acetate buffer were added and incubated in a boiling water bath for 6 minutes with 2 minutes shaking intervals. The tubes placed in a water bath at 50°C and 0.1ml of amyloglocosidase enzyme was added; the tubes

was stirred using vortex and incubated for 30 minutes. The contents were then centrifuged for 10 minutes at 3000 rpm. A duplicate of 0.1ml aliquot was placed into 15 ml test tube. 3.0 ml of ρ-hydroxybenzoic acid and sodium azide mixture (1:1) and left to stand for 20 minutes at 20°C.

5.0 g of D-glucose powder was taken into 100 ml volumetric flask, dissolved with sodium acetate buffer to make stock solution of 50 mg/ml. Serial dilution of $0 - 40$ mg/ml prepared into 100 ml volumetric flask. 0.1 ml of diluted standard solution were taken into 15 ml test tube. 3.0 ml ρhydroxybenzoic acid and sodium azide mixture (1:1) and left to stand for 20 minutes at 20°C. Absorbencies of samples and standards was read at 510 nm using X-ma 3000 UV/Visible spectrophotometer.

2.6 Data Analysis

Qualitative data including pericarp thickness, testa presence, corneous, and endosperm color was analyzed using excel program; where frequencies and percentage presented in bar chart. Data on mean kernel diameter, 100seed weight, kernel hardness, protein and starch concentration were subjected into analysis of variance (ANOVA) using GenStat version 15 software and means were compared using Duncan new multiple range test. Pearson correlation employed to determine the association between quantitative traits. MINTAB version 14 software were used in multivariate analysis such as principal component and cluster analysis.

3. RESULTS AND DISCUSSION

3.1 Qualitative Traits

Most of sorghum genotypes studied (75.51%) had thick pericarp (Fig. 1), while the rest possessed thin pericarp. Other researchers; [26] reported a variation in pericarp thickness in sorghum using electron microscope consisting of very thin (8 to 32 μm) to very thick (40 to 160 μm).

Only 33.67% of sorghum genotypes had either purple or brown testa, while the rest of genotypes had no testa. Genotypes with testa indicates the possibility of having higher levels of tannin concentration compared to non-testa genotypes. [15] characterized sorghum into three different groups namely; Type I sorghums that lacking pigmented testa and have no tannin, Type II sorghums having pigmented testa with tannin and Type III sorghums having tannin in the testa and pericarp of the kernel. (74.49%) of the evaluated sorghum genotypes had white color endosperm, while the rest were yellowish. While, 7.14% of all genotypes had mostly corneous endosperm texture; 30.61% had intermediate corneous indicating a relative balance between floury content and corneous; while the majority of genotypes were floury or complete starch. Endosperm texture is related to kernel hardness; such that mostly corneous endosperm referring to hard kernel and floury endosperm referring to soft kernel [27]. Great variation were also observed in terms of grain color;where,45.92% of the evaluated genotypes were white in color, 24.49% were red, 23.47% of the genotypes were brown; the rest in small fraction were yellow, buff and mixed colors. However, qualitative traits in sorghum play bigger role in processing and flour quality; for instance, genotypes producing grains of uniform sizes is most preferred in milling than non-uniform because smaller kernels normally taken out with bran.

3.2 Analysis of Variance (ANOVA)

Analysis of variance indicated a highly significant difference (p<0.001) among evaluated genotypes, showing greater genetic variability among traits under consideration (Table 1). Kernel mean diameter ranged between 2.29mm to 4.61mm. Lines ICSx152002-SB-13-2, F2Striga16 and IESH 22017 had the greater mean kernel diameter and genotypes IS 21055 had the lowest mean kernel diameter. 100 seed weight ranged between 1.81to 6.2 g. Genotypes F2Striga5, P9537A x MACIA and IES11038 x A1GD 34553 recorded the highest hundred seed weight; while genotype TZA 3983 had the least weight. Kernel hardness varied between 14.94 newton to 110.33 newton; Genotypes PATO, IESV 92174DL, and IESV 92028 DL recorded the highest kernel hardness, while genotypes IESV 92043DL, F2Striga15 and TZA3993 had the least kernel hardness. [19] reported hardness range of 3 kg to 12 kg using forty-five sorghum genotypes.

Protein concentration ranges between 6.52 to 12.23%; where genotype Naco Mtama 1 and IESV 24030SH recorded the highest concentration and genotypes F2 Striga 13 and ICS x 152 001-SB-4-2 had the lowest concentration. This finding corresponds with results from other studies. For instance, [28] reported crude protein range of 6% to 16.6%. [14] reported protein content range from 7 -15% using data from FAO and other studies. [29]

Reported protein range of 7.16- 16.18% using 59 sorghum genotypes from South Africa. However, [30] confirm the fact that protein contents varies due to environment and genotype.

The mean total starch concentration ranged between 21.88 to 79.05 g/100 g. The higher concentration observed on genotypes ICSA 88006 X IESV92172DL, ICSA15 x R8602 and GADAM; while Tegemeo and ASARECA 18-3-1 recorded the least concentration. Some genotypes recorded either lower or higher starch concentration due to high diversity of genotypes used in the present study. [14] reported starch concentration range of 60-75 g/100 g; [4] reported starch concentration range of 44.39% to 68.08% using 22 sorghum accessions mostly from Ethiopia and South Africa. However, it was suggested that starch concentration in sorghum is highly affected by genotype and environment [31]. According to [32] sorghum starch is resistant impairing digestion making it useful to people with obesity and diabetic. The higher variability among studied genotypes in terms of kernel phenotypic and biochemical traits is critical in selection of appropriate traits during cultivar development.

3.3 Correlation between Quantitative Traits

Pearson correlation analysis indicated a weak positive significant correlation between 100 seed weight and kernel hardness (r=0.250, p=0.013) (Table 2); while kernel hardness had a positive but weak significant correlation with protein concentration (r=0.225, p=0.026). Starch concentration had a weak negatively significant association with mean kernel diameter (r=-0.200, p=0.048). However, starch concentration showed a negative weak correlation with all studied parameters. This finding implies that as kernel weight increases, there is lower possibility of existence of a relationship with the increase in kernel hardness; likewise, the increase in kernel hardness has lower likelihood of existence of a relationship with the increase in protein content of the genotypes. The weak correlations observed in the present study necessitates the need for further research to confirm these findings. However, [33] found greater levels of protein content in corneous portion of the endosperm than floury endosperm in sorghum. The hard sorghum kernel is critical in resistance against fungal and insect attack such as *Sitophilus oryzae* [34]; due to presence of prolamins [17]. Hardness is also a good

determinant of grain quality relating to cooking qualities such as stiffness and the milling qualities [33].

3.4 Principal Component Analysis

Principle component analysis (PCA) grouped five traits into five components. Retention of PCs were based on proportion of variance criterion described by [35]. Four components can be retained based on adequate cumulative amount of variance explained (>80%). About 85.9% of the variances contained in the dataset were retained by the first four principal components. The first component explained 32.7% of the total variation. The high contributing factor loading are 100 seed weight, kernel hardness, mean kernel diameter (MKD), and protein content (Table 3). The second principle component (PC2) accounted 20.1% of the total variation; mainly a function of starch concentration and kernel hardness with negative loadings. With similar logic, in the third component (PC3) protein content have higher positive loading and 100Swt with the largest negative loading. PC4 accounted 15.8% of the total variation with high negative loadings from starch concentration and the mean kernel diameter. According to [35] loading greater than ±0.40 were considered to best represent the corresponding PC axis. The first and second components accounted over fifty percent of the variation demonstrating existence of relationship among traits. [36] reported large contribution of the first two components using forty sorghum accessions. Similar findings has been reported by [4] using 22 sorghum accessions.

Further, the score plot for the first two components (Fig. 2) indicate existence of genetic variation among sorghum genotypes in terms of studied physiochemical traits. The scattered genotypes across all quadrants indicate a high genetic variability among them. Genotypes from different origin and or type were scattered. The closer genotypes in the PC axes indicate the close genetic relationship, which can be explained by the shared traits. Genotypes ICSx152002-SB-4-1, IESH 22023, and ICSA75 x ICSR38 were the extremely genotypes; therefore some of these lines can be selected for hybridization of traits of interest to improve sorghum cultivars.

3.5 Cluster Analysis

Cluster analysis for the phenotypic kernel traits and biochemical parameters indicated a clear

separation of the evaluated sorghum genotypes (Fig. 3). Four main clusters was observed namely; cluster I, II, III and IV formed at 59.68% similarity level. (Table 4) indicates cluster means, explaining the differences among groups of the evaluated genotypes. Cluster I grouped twenty (20) sorghum genotypes formed based on the lowest concentration of starch and small mean kernel diameter, and highest hundred seed weight, kernel hardness and protein content.

Cluster II grouped seven (7) genotypes consisting of hybrids, breeding lines and a local cultivar (Mbangala white) with the average protein content, highest mean kernel diameter, and starch concentration. Cluster III grouped sixty seven (67) sorghum genotypes based on average mean kernel diameter, 100 seed weight, kernel hardness, protein content and starch concentration. Cluster IV grouped four (4) sorghum genotypes originated from ICRISAT and Tanzania namely IESV92043DL, IS 21881, ICSx152001-SB-2-2 and TZA3993 these genotypes had the lowest mean kernel diameter, 100 seed weight, kernel hardness and protein content. Dendrogram shows that genotypes from the same origin and or the same type; were not necessarily assembled within similar clusters.

Fig. 1. Frequencies and percentages among qualitative kernel traits

Table 1. Simple statistics

MKD = mean kernel diameter, 100Swt =100 seed weight;

SE= Standard error of mean, CV= coefficient of variation,

Table 2. Pearson correlation among the studied traits in terms of phenotypic and biochemical properties

**significant at p<0.05*

Fig. 2. Score plot of first and second principle components explaining kernel phenotypic and biochemical variation among the evaluated sorghum genotypes

Fig. 3. Dendrogram showing various clusters among 98 sorghum genotypes evaluated in terms of physiochemical properties

Variable	PC ₁	PC ₂	PC ₃	PC4	PC ₅
MKD	0.452	0.386	0.256	-0.761	0.042
100Swt	0.492	-0.051	-0.657	0.076	0.563
Hardness	0.484	-0.485	-0.205	-0.066	-0.697
Protein	0.425	-0.394	0.677	0.300	0.342
Starch	-0.373	-0.677	-0.051	-0.567	0.281
Eigenvalue	1.6346	1.0060	0.8639	0.7907	0.7048
% variance	32.7	20.1	17.3	15.8	14.1
Cumulative % variance	32.7	52.8	70.1	85.9	100

Table 3. Principle component analysis of quantitative physiochemical traits in 98 sorghum genotypes

PC= principal component, MKD = mean kernel diameter, 100Swt =100 seed weight;

Table 4. Cluster means of the phenotypic and biochemical traits in the evaluated sorghum genotypes

MKD = mean kernel diameter, 100Swt =100 seed weight;

3.6 Identification of Elite Genotypes for Breeding

Few sorghum genotypes performed better In terms of 100 seed weight; these include genotype IES11038 X A1GD 34553 (6.20 g), P9537A X MACIA (5.49 g), F2Striga5 (5.30 g), ICSA44 X IESV 91104 DL (5.30 g) , ATX623 X AIGD34533 (5.12 g), P9507A X IESV 91131 DL (5.12 g) and F2 Striga 14 (5.03 g). Lines F2Striga5 and F2Striga14 can be recommended for crop improvement in terms of yield. However, genotypes with highest average mean kernel diameter were ICS x 152 002-SB-13-2, F2 Striga 16, IESH 22017, IS 15443, F2 Striga 18, N13 and WAHI recorded 4.61, 4.54, 4.07, 3.82, 3.65, 3.62, and 3.60 mm respectively.

Lines with the highest Kernel hardness include PATO, IESV 74 DL; IESV 92028 DL, Mbangala white and F2 Striga 11 which recorded 110.33, 108.43, 103.90, 101.11 and 100.72 N. The highest protein content were recorded in genotype NACO Mtama 1 (12.23), IESV 92174 DL (12.18), IESH 22023 (11.58), IESV 92028 DL (11.21) and ASARECA 15-3-1 (11.04). These genotypes can be potential source of hardness and protein content in breeding programs. Hence, hardness and protein correlated with corneous portion in the endosperm; the later play significant role in resistance against pests including storage weevils. Improvement of these traits in commercial released varieties could be

necessary for sustainable management of storage insects. Nevertheless, more research is needed; a multi-location study is recommended to confirm potentiality of these genotypes.

4. CONCLUSION

The present study revealed a wide variability for the qualitative and quantitative parameters studied. Analysis of variance for the mean diameter, 100 seed weight, kernel hardness, protein and starch concentration showed a high significance difference (p<0.001). Crosses performed better in terms of yield possibly due to heterosis. The best genotypes in terms of 100 seed weight were IES11038 x A1GD 34553 and P9537A x MACIA. However, lines F2Striga5 and F2Striga14 can be recommended to improve yield component. Promising genotypes in terms of mean kernel diameter were ICS x 152 002-SB-13-2, F2Striga16 and IESH 22017. Lines with the upper most kernel hardness include PATO, IESV 74 DL; IESV 92028 DL, and Mbangala white; representing potential sources of kernel hardness. Genotype NACO Mtama 1, IESV 92174 DL, IESH 22023 and IESV 92028 DL could be potential parental materials to improve protein content in sorghum cultivars. However, weak correlation among these traits indicate the need for multi-location or multi-season study to confirm potentiality of these genotypes while accounting the effect of genetic environmental interaction. The studied materials were clustered

into four main clusters at 59.68% similarity level; genotypes clustered together indicates the possibility of easy selection during hybridization. Physiochemical traits are useful in determination of food quality, processing and kernel protection against pests in sorghum. Variability identified in the present study could aid selection of useful traits for breeding precision.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Table 5. Analysis of variance for the quantitative traits

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MKD = mean kernel diameter, 100Swt =100 seed weight;

SE= Standard error of mean, SED = Standard of error of differences of means, LSD = Least significance difference of means (5% level), CV= coefficient of variation $_$, and the set of th

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