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Diversity Analysis for Grain Nutrient Content and Agronomic Traits among Newly Bred *Striga*-Resistant and *Fusarium oxysporum* f.sp. *strigae* (*FOS*)-Compatible Sorghum Genotypes

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Abstract: The parasitic weed, *Striga* species, is among the major causes of yield and quality losses of cereal and legume crops in sub-Saharan Africa. The development of nutritionally enhanced genetic resources with *Striga* resistance is an overriding consideration in sorghum breeding programs. The objective of this study was to determine the genetic variability among 12 elite and newly bred sorghum (*Sorghum bicolor* (L.) Moench) lines with variable *Striga* resistance for agronomic performance and nutritional traits. The sorghum genotypes were analyzed for protein, amino acid profiles, and selected minerals. For agronomic traits, significant variations were observed for yield per plant that varied from 51.74 to 15.12 g/plant, with a mean of 29.77 g/plant. The tested genotypes showed significant ($p < 0.05$) variation in protein, amino acid, iron, and zinc contents. The crude protein content varied from 9.59 to 13.60%, with a mean of 11.64%. The lysine values ranged from 1.13 to 3.08%, with a mean of 2.15%, while methionine content varied from 0.42 to 1.58%, with a mean of 0.87%. Iron content ranged from 35.26 to 156.32 mg/kg with a mean of 78.32 mg/kg, while zinc content varied from 14.45 to 44.46 mg/kg with a mean of 24.91 mg/kg. The following genotypes: AS1, PAN8816, 672, Macia, AS436, 3984 × 630, AS426 × 672, and 105 × 654 were identified as having superior agronomic and nutritional qualities for commercialization and sorghum breeding programs.

Keywords: amino acids; micronutrients; malnutrition; protein; sorghum; *Striga* species

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1. Introduction

Sorghum (*Sorghum bicolor* L. Moench, 2 n = 2 x = 20) is one of the most important staple crops in sub-Saharan Africa (SSA). The crop is a source of important nutrients such as vitamins, proteins, minerals, and micronutrients required for human well-being, growth, and development. The protein content of sorghum varies from 8.1 to 18.8%, which is comparable to other cereals such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.). Sufficient protein and essential amino acids are needed to build and repair body tissues [1]. In addition, sorghum provides important micronutrients such as iron and zinc, which are required for lactating women and children [2]. The parasitic weed, *Striga*, is among the major causes of yield and quality losses of cereal and legume crops in SSA. It is a major biotic constraint to sorghum productivity and food and nutrition insecurity. Higher *Striga* infestations lead to yield losses reaching up to 100% [3,4]. Most agroecosystems and climatic conditions in SSA favour the prolificacy of the parasite. Subsistence farmers who mostly depend on sorghum for food security are the most affected by the parasite as they cannot afford the recommended *Striga* control methods due to combinations of limitations. Therefore, development of *Striga*-resistant varieties is of paramount importance, especially for SSA.

Recently, the African Centre for Crop Improvement (ACCI) developed an integrated *Striga* control method based on a combination of host resistance and biological control with

the fungus *Fusarium oxysporum* f.sp. *strigae* (*FOS*) [5,6]. The method is a low-cost and readily deployable *Striga* control option. *Striga*-resistant sorghum cultivars support only a few or no *Striga* plants, ensuring higher yield production. Simultaneously, the *FOS* induces suicidal germination of *Striga* seed held in the soil seed bank and causes the premature death of *Striga* plants. *Striga*-resistant and *FOS*-compatible genotypes should have a well-balanced grain nutrient content for human well-being. Hidden hunger associated with a lack of a balanced diet affects approximately two billion people worldwide [7]. Malnutrition-related ailments are common occurrences in SAA [8]. Improving the nutritional qualities of sorghum is important to alleviate the high levels of malnutrition.

Crop biofortification using conventional and modern breeding methods can combat hidden hunger and other related nutrient deficiencies [9]. Biofortification of major cereal crops such as sorghum would deliver the necessary micronutrients to rural households. This is cost-effective and sustainable compared to other methods, such as animal-derived diets and nutrient supplementation. Public and private research institutions, including the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), have embarked on breeding sorghum to meet vulnerable groups' dietary requirements, including women and children [10]. Recently, ICRISAT released sorghum cultivars with high iron and zinc contents and low anti-nutritional properties such as phytate content [11]. Genes conditioning quality traits such as nutrient contents are highly heritable and are predominantly under additive genetic control [12]. This suggests that nutrient content can be successfully introgressed into *Striga*-resistant and elite sorghum lines. Considering the above background, this study's objective was to determine the genetic variability present among the elite and newly bred sorghum (*Sorghum bicolor* [L.] Moench) lines for agronomic traits and contents of protein, amino acids, and iron and zinc.

2. Materials and Methods

2.1. Plant Materials

The study used 12 elite sorghum genotypes acquired from ICRISAT = International Crop Research Institute for the Semi-Arid Tropics/India, African Centre for Crop Improvement (ACCI), Pannar seed company, and the Agricultural Research Council in South Africa (Table 1). The ACCI genotypes are at the F7 generation and were selected based on their outstanding resistance against the two dominant *Striga* species and for showing compatibility with *FOS*. The procedure for developing these genotypes was described in detail by [5,13], respectively.

Table 1. List and sources of sorghum genotypes used in the study.

Entry Number	Genotype/Designation	Source	Description	*Striga* Resistance	Compatibility to *FOS*
E1	105 × 654	ACCI/SA	Elite line	Resistant	Compatible
E2	672	ACCI/SA	Elite line	Resistant	Compatible
E3	105 × 672	ACCI/SA	Elite line	Resistant	Compatible
E4	675 × 630	ACCI/SA	Elite line	Resistant	Compatible
E5	AS436	ICRISAT/India	Parental line	Resistant	Compatible
E6	3484 × 424	ACCI/SA	Elite line	Resistant	Compatible
E7	3984 × 630	ACCI/SA	Elite line	Resistant	Compatible
E8	AS426 × 672	ACCI/SA	Elite line	Resistant	Compatible
E9	Macia	ICRISAT/India	Check	Susceptible	Incompatible
E10	PAN8816	PSC/South Africa	Check	Not available	Not available
E11	AS1	ACCI/SA	Check	Not available	Not available
E12	SS49	ARC/SA	Check	Not available	Not available

ACCI/SA = African Centre for Crop Improvement/South Africa, ICRISAT/India = International Crops Research Institute for the Semi-Arid Tropics/India, PSC/SA = Pannar Seed Company/South Africa, ARC/SA = Agricultural Research Council/South Africa.

2.2. Evaluation of Agronomic Performance

The study was conducted under glasshouse and field conditions at two locations: the University of KwaZulu-Natal Controlled Environment Facilities (UKZN-CEF) and the Ukulinga Research Farm of the University of KwaZulu-Natal. The glasshouse experiment was established at UKZN-CEF facilities situated at UKZN's College of Agriculture, Engineering, and Science (29.6213° S, 30.3966° E). A field study was conducted at the Ukulinga Research Farm (29.6627° S, 30.4050° E) of UKZN. For the glasshouse trial, the 12 sorghum genotypes were evaluated and laid out in a randomized complete block design with two replications. Plastic pots of 5 L capacity were used filled with pine bark. The field trial was used as the optimum environment for assessing the yield potential of the test genotypes. This experiment was conducted using a randomized complete block design (RCBD) with three replicates. The plot area consisted of 3 rows of 3 meters in length with an inter-row spacing of 0.8 m and an intra-row spacing of 0.25 m. All agronomic practices for sorghum production were strictly followed in the management of both trials.

Observations for agronomic traits were recorded on days to flowering, plant height, biomass, panicle weight, seed yield, and hundred seed weight. The days to 50% flowering were measured as the number of days from emergence until 50% of the plants in a plot showed flowering. Plant height (cm)) was measured at 50% flowering as the height from the soil surface to the panicle's tip. Total biomass (gram/plant)) was measured as the total weight of the above-ground foliage. Panicle weight was measured from the main panicle per plant and expressed in grams per the main panicle. Seed yield (gram/plant) was recorded as total grain weight in gram per plant at 12.5% moisture. The hundred seed weight was determined from the weight of one hundred randomly sampled seeds per genotype. Five plants were randomly sampled and tagged to collect data.

2.3. Nutritional Analysis

2.3.1. Determination of Protein Content

Grain protein content was analyzed using the Vario EL Cube Elemental Analyzer from Elementar (Langenselbold Germany) procedure for total nitrogen content. Sorghum grains were harvested from each genotype at 12.5% moisture content in the field trial. Each sample was dried at 110 °C for 24 h to remove moisture. Homogenous samples of the genotypes were milled to <80µm, and 0.1 g of sorghum flour was used for the analysis. Tungsten (VI) oxide was added to all the samples at a ratio of 1:1 to the sorghum sample flour. The nitrogen contents from the Vario EL Cube Elemental Analyzer were converted to crude protein content by multiplying the nitrogen content values by the Jones factor (6.25) [14].

2.3.2. Amino Acid Analysis

The profiles of the sorghum genotypes for the essential amino acids were analyzed at the Central Analytic Facility, University of Stellenbosch, South Africa. The sorghum samples were first hydrolyzed according to the method in [15]. About 0.1 g of samples were weighed. Then, 6 ml of 6 N HCl and 1.0 mL of 15% phenol were added to the sample inside the hydrolysis tubes. The hydrolysis tubes made of glass were sealed following the standard procedure for sample vacuum hydrolysis according to the manufacturer's instructions, Thermo Scientific. The hydrolysis tubes were placed inside glass beakers and put in an oven at a temperature of 110 °C. After 24 hours, these were removed from the oven and allowed to cool to room temperature. The vials were transferred into 2 mL Eppendorf tubes, and the remainder of each sample was discarded. One Eppendorf tube was used for the analysis of amino acids in the liquid chromatography–mass spectrometer. The other Eppendorf tubes were stored at −20 °C. The Eppendorf samples were subjected to the Water AccQ Tag Ultra Derivitization Kit from Waters Corporation (Massachusetts, USA). A 10 µl quantity of the undiluted sample was added to the Waters AccQ Tag Kit constituents and placed in a heating block at a temperature of 55 °C for ten minutes. The column was an AccQ Tag C18, 1.7 µm, 2.1 × 100 mm, and the sample injection was 1 µL with the ESI + source. The solvents, Eluent A2, contained 100 ml of Eluent A concentrate

and 900 ml of water, and Eluent B was supplied in the AccQ Tag Kit. The samples were run with a capillary voltage of 3.5 ×kilovolts (kV) and a core voltage of 15 volts (V) at 120 °C. The desolvation temperature, desolvation gas, and core gas were 350 °C, 350 Lh^{-1}, and 50 Lh^{-1}, respectively.

2.3.3. Iron and Zinc Content Analyses

Iron and zinc were analyzed using the Inductively Coupled Plasma-Optical Emission Spectroscopy procedure (ICP-OES; Horiba Jobin Yvon, Longjumeau, France) [16]. Briefly, 0.5 g dried homogenous sorghum flour was weighed directly into microwave digester Teflon vessels. Then, 6 ml of nitric acid and 1 ml of hydrogen peroxide were added to the microwave digester's Teflon vessels and digested using a CEM MARS microwave digester. Digested solid samples were introduced into Thermo iCAP via the autosampler by a peristaltic pump. The samples were passed through the nebulizer, which produces a fine aerosol. The large droplets were removed by a spray chamber, and small droplets were then passed through to the plasma. The solvent was evaporated, and the residual sample was atomized and ionized. The ions excite the plasma and emit characteristic light measured by the Echelle Optical Design and Charge Injection Device (CID) solid-state detector to provide elemental analysis. The Thermo iCAP and iTEVA software, Thermo Fisher Scientific, UK were used for full control of all instrument functions and data handling.

2.4. Data Analysis

The agronomic and nutritional trait data were subjected to an analysis of variance using the general linear model (GLM) procedure of GENSTAT 18th Edition [17]. The significant differences between means were tested using Fisher's test of the least significant differences at a 5% significance level. A dendrogram was generated using the function "daisy" of the R package "vegan" and the average agglomeration method on the nutrient variables. Principal component analysis (PCA) based on the correlation matrix was performed using SPSS version 26 [18] to identify influential traits for selection. To define the trait variation among the sorghum accessions, principal components (PCs) with Eigenvalues of 1.0 were chosen. PCA biplots were plotted using the R package "FactoMineR" to determine the relationships among the studied genotypes based on the nutrient compositions. Factor analysis of mixed data (FAMD) and hierarchical cluster analysis (HCA) were conducted using the R package "FactoMineR" to group sorghum genotypes into different categories [19].

3. Results

3.1. Agronomic Performance

The analysis of variance (ANOVA) for the assessed agronomic traits is presented in Supplementary Table S1. The main and interaction effects of site and genotype were significant ($p < 0.001$) for all the studied traits. Table 2 shows the mean performance of the assessed genotypes. The days to flowering varied from 55 to 78. AS436 was the early-flowering genotype (55 days), while AS426 × 672 was the late-flowering genotype (78 days). The plant heights of the test genotypes varied from 130.40 to 202.20 cm, with a mean of 160.87 cm. Biomass ranged from 98.50 to 195.90 g/plant with a mean of 152.65 g/plant. Genotypes 3984 × 630 and AS436 produced the highest and lowest biomass, respectively. Genotypes AS1, PAN8816, and 672 were the top-yielding elite lines with 51.74, 43.31, and 41.17 g/plant, respectively. The average seed yield per plant was 29.77 g/plant. The hundred seed weight varied from 2.35 to 3.50 g, with a mean of 2.89 g (Table 2).

Table 2. Mean values for agronomic traits amongst 12 elite sorghum genotypes evaluated in this study.

Genotype	DF	PH	PW	BM	SY	HSW
105 × 654	66.00	149.50	50.28	176.80	34.40	3.10
672	74.25	202.20	58.46	191.40	41.17	2.60
105 × 672	63.25	167.60	46.16	172.00	20.47	3.24
675 × 630	70.00	175.00	34.67	110.80	24.51	2.52
AS436	55.00	175.70	26.48	98.50	17.48	2.63
3484 × 424	73.25	142.10	55.53	193.80	38.83	2.90
3984 × 630	64.25	152.60	32.59	195.90	20.25	2.58
AS426 × 672	78.00	145.00	31.50	135.30	15.12	2.35
Macia	72.75	184.10	33.22	155.00	22.19	2.64
PAN8816	62.50	160.00	67.30	139.10	51.74	3.22
AS1	65.00	130.40	83.15	124.90	43.31	3.44
SS49	71.00	146.20	56.29	138.30	27.78	3.50
Mean	67.94	160.87	47.97	152.65	29.77	2.89
CV (%)	6.25	7.27	30.9	18.69	23.43	5.83
LSD (5%)	6.22	17.14	21.73	41.84	10.23	0.25
F-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

CV = Coefficient of variation, LSD = least significant difference, DF = days to flowering, PH = plant height, BM = biomass, PW = panicle weight, SY = seed yield, and HSW = hundred seed weight.

3.2. Nutritional Profiles

The nutrient compositions of the 12 elite sorghum lines are presented in Table 3. Genotype effects were significant ($p \leq 0.05$) for methionine, threonine, iron, zinc, lysine, and protein contents (Supplementary Table S2). The genotype effects were not significantly different for histidine, isoleucine, leucine, phenylalanine, and valine contents. Protein content varied from 9.59 to 13.60%, with a mean of 11.64%. The genotypes that contained high crude protein contents were Macia, PAN88161, and 3484 × 424, with 13.60%, 13.37%, and 12.77%, respectively. The genotype with low protein content was AS1 at 9.59%. The lysine content of the test genotypes varied from 1.13 to 3.08%, with a mean of 2.15%. Genotype AS436 exhibited the highest lysine content (3.08%), while AS426 × 672 recorded the lowest lysine content (1.13%). Methionine content varied from 0.42 to 1.58%, with a mean of 0.87%. The genotypes AS426 × 672, SS49, and AS436 recorded the highest methionine content, with mean values of 1.58, 1.32, and 1.20%, respectively. Genotypes 675 × 630 and 672 showed low methionine content. Threonine ranged from 3.79 to 4.61%, with a mean of 4.21%. The genotypes with high threonine were Macia, 105 × 654, and SS49, with mean values of 4.61, 4.41, and 4.41%, respectively. There was a significant variation among the sorghum genotypes for iron and zinc contents. The iron levels of test genotypes ranged from 35.26 to 156.32 mg/kg, with a mean of 78.32 mg/kg. The highest iron content was 156.32 mg/kg followed by 127.41 mg/kg exhibited by Macia and AS 426 × 672, respectively. The zinc content ranged from 14.45 mg/kg to 44.46 mg/kg, with a mean of 24.91 mg/kg. Macia exhibited the highest zinc content (44.46 mg/kg).

Table 3. Mean compositions for amino acids, protein, iron, and zinc among 12 elite sorghum genotypes.

	Amino Acids								Minerals		
Genotype	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Phe (%)	Thr (%)	Val (%)	Prot (%)	Fe (mg/kg)	Zn (mg/kg)
105 × 654	2.13	3.69	11	2.07	0.65	8.63	4.41	4.91	10.81	86.51	33.66
672	2.02	3.58	10.3	2.55	0.52	8.3	3.97	4.81	11.48	55.3	19.45
105 × 672	2.48	3.52	11.28	1.92	0.65	8.48	4.25	4.90	11.39	66.95	25.44
675 × 630	1.95	3.79	11.4	2.31	0.42	8.03	4.03	4.82	12.04	57.60	23.70

Table 3. *Cont.*

	Amino Acids								Minerals		
Genotype	**His (%)**	**Ile (%)**	**Leu (%)**	**Lys (%)**	**Met (%)**	**Phe (%)**	**Thr (%)**	**Val (%)**	**Prot (%)**	**Fe (mg/kg)**	**Zn (mg/kg)**
AS 436	2.02	3.57	9.66	3.08	1.20	7.57	4.10	4.89	10.38	81.97	21.20
3484 × 424	2.09	3.68	10.10	2.11	0.91	7.77	3.79	4.67	12.77	35.26	14.45
3984 × 630	1.48	3.80	10.13	2.72	0.64	7.93	4.32	4.81	10.44	103.21	25.39
AS426 × 672	1.99	3.68	11.76	1.13	1.58	9.16	4.15	4.71	11.89	127.41	30.21
Macia	2.11	3.92	10.40	2.37	0.92	7.42	4.61	5.04	13.6	156.32	44.64
AS1	2.10	3.61	10.66	2.11	1.20	8.23	4.41	4.67	13.37	52.83	15.96
PAN 8816	2.29	3.39	11.24	1.84	1.08	8.33	4.27	4.66	9.59	40.51	16.42
SS49	2.35	3.70	10.92	1.58	1.32	8.83	4.20	4.84	11.88	75.97	28.45
Mean	2.08	3.66	10.74	2.15	0.87	8.22	4.21	4.81	11.64	78.32	24.91
CV (%)	14.17	5.07	5.30	13.13	6.55	5.27	2.60	2.08	5.91	0.90	0.99
LSD (5%)	0.47	0.29	0.89	0.44	0.09	0.67	0.17	0.16	1.07	1.1	0.38
p-value	0.330	0.015	0.077	0.002	<0.001	0.051	<0.001	0.055	<0.001	<0.001	0.009

CV = Coefficient of variation, LSD = least significant difference; His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Val = Valine; Prot = Protein; Fe = Iron; Zn = Zinc.

3.3. Cluster Analysis

The tested genotypes were grouped into three clusters based on the 17 traits (Figure 1). The first cluster comprised four genotypes, while cluster II consisted of one genotype. Cluster III was the largest cluster with seven genotypes. Further, Cluster I comprised four genotypes which were separated into two sub-clusters (I-A and I-B). Macia is found in sub-cluster I-A, characterized by high contents of protein, Fe and Zn. Sub-cluster I-B comprised AS436, 675 × 630, and 3984 × 630; these were clustered based on high lysine content and low seed yield (<25 g/plant). AS426 × 672 was a singleton in the second cluster characterized by late flowering (78 days). The third cluster comprised seven genotypes, further divided into three sub-clusters based on agronomic and nutritional similarities. The sub-cluster III-A consisted of two elite genotypes (672 and 3484 × 424), which were characterized by high panicle weight, biomass, seed yield, and low Zn contents. Sub-cluster III-B consisted of three genotypes: SS49, 105 × 654, and 105 × 672. These genotypes were clustered based on high HSW and low lysine contents. Sub-cluster III-C is composed of two genotypes PAN8816, and AS1. These are commercial check varieties that exhibited high seed yield but very low Fe and Zn contents. Genotypes of different clusters denote variable heterotic groups ideal for breeding population development for sorghum biofortification programs.

3.4. Principal Component Analysis

A set of 17 traits were used to establish the principal components (PCs) and important traits for selection (Table 4). The first six PCs contributed 89.47% of the total variation among the sorghum genotypes for the assessed traits. The first principal component (PC1) accounted for 31.31% of the total variation, and the high contributing factor loading was isoleucine, iron, valine, and zinc contents; plant height, lysine, threonine panicle weight, hundred seed weight, seed yield histidine leucine, and phenylalanine. The second principal component (PC2) contributed to 20.58% of the total phenotypic variation attributed to leucine, phenylalanine, zinc, iron, methionine, threonine, histidine days to flowering, seed yield, and lysine. The third principal component (PC3) accounted for 12.34% of the total variation, mostly contributed by threonine, valine, hundred seed weight histidine contents, days to flowering, biomass, and protein. The fourth principal component (PC4) contributed to 10.87% of the total variation attributable to protein, seed yield, biomass, panicle weight, hundred seed weight, threonine, histidine, and methionine. The fifth principal component (PC5) contributed to 7.58% of the total variation due to methionine, protein contents, and a negative contribution from biomass and phenylalanine. The last principal component contributed to 6.80% of the total variation correlated with plant height and histidine content and negative contributions from biomass and threonine.

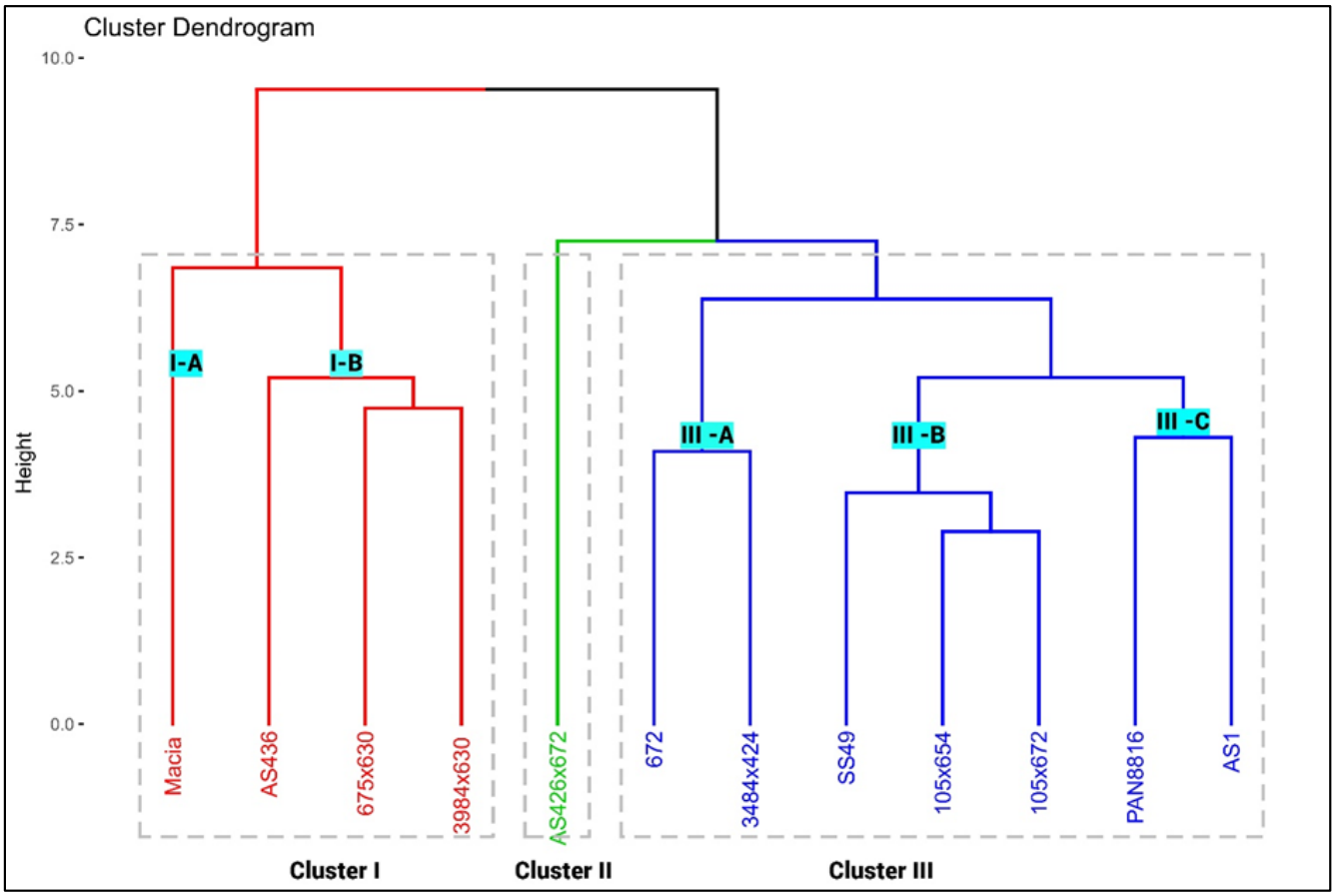

Figure 1. Dendrogram showing diversity among the sorghum genotypes generated using agronomic traits and nutritional qualities based on UPMA. (The different colors show clustering groups between the genotypes).

Table 4. Principal components (PC) for agronomic traits and nutritional compositions among 12 elite sorghum genotypes.

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Days to flowering	0.08	**0.52**	**−0.75**	0.22	−0.05	0.11
Plant height	**0.56**	−0.36	−0.04	0.26	−0.13	**0.54**
Panicle weight	**−0.86**	−0.08	0.04	**0.41**	0.04	−0.11
Biomass	0.08	−0.16	**−0.44**	**0.49**	**−0.36**	**−0.47**
Hundred seed weight	**−0.70**	0.10	**0.48**	**0.39**	0.01	−0.10
Seed yield	**−0.66**	**−0.32**	−0.13	**0.52**	0.23	−0.10
Histidine	**−0.50**	**0.33**	**0.40**	**0.35**	−0.07	**0.50**
Isoleucine	**0.80**	0.21	−0.28	0.21	0.19	−0.15
Leucine	**−0.33**	**0.75**	−0.05	−0.08	**−0.33**	0.15
Lysine	**0.47**	**−0.86**	0.20	−0.01	0.02	−0.01
Methionine	−0.25	**0.50**	0.13	**−0.37**	**0.64**	−0.02
Phenylalanine	**−0.47**	**0.67**	−0.13	−0.15	**−0.34**	−0.04
Threonine	**0.30**	**0.36**	**0.60**	**0.36**	0.13	**−0.38**
Valine	**0.76**	0.08	**0.45**	0.28	−0.26	0.17
Protein	0.25	0.23	**−0.36**	**0.54**	**0.54**	0.29
Iron	**0.78**	**0.52**	0.13	−0.04	0.12	−0.19
Zinc	**0.71**	**0.59**	0.24	0.24	−0.10	−0.07

Table 4. *Cont.*

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	5.32	3.50	2.10	1.85	1.29	1.16
Proportion of variance (%)	31.31	20.58	12.34	10.87	7.58	6.80
Cumulative variance (%)	31.31	51.89	64.23	75.10	82.67	89.47

Note: Bold font text shows significant loadings.

3.5. Principal Component Biplot

A principal component biplot was used to show the association between the genotypes and the assessed traits based on PC1 and PC2 which explained 31.30% and 20.60% of the total variation (Figure 2). Dimension vector lines with angles less than 90° between them indicated a positive correlation of the variables. Seed yield showed a high positive correlation with panicle weight and hundred seed weight; however, seed yield was negatively correlated with days to flowering and plant height. Seed yield was also correlated with some essential amino acids, namely, histidine, phenylalanine, leucine, and methionine. A high positive correlation was observed among zinc, iron, protein, threonine, isoleucine, and valine contents. The length of the vector lines shows the ability of the variable to discriminate the test genotypes. Thus, a long vector illustrates the high discriminative potential of a variable. For instance, panicle weight, and phenylalanine, iron, and lysine contents were the most discriminating traits. Desirable genotypes for a particular trait are positioned closer to the vector line and further in that specific vector's direction. For agronomic performance, PAN8816 excelled in seed yield, and 3984 × 630 had the highest plant height. AS436 was outstanding for lysine content, and Macia excelled for iron and zinc compositions. Genotypes 105 × 654, 105 × 672, and 675 × 630 were the most stable genotypes for the recorded traits.

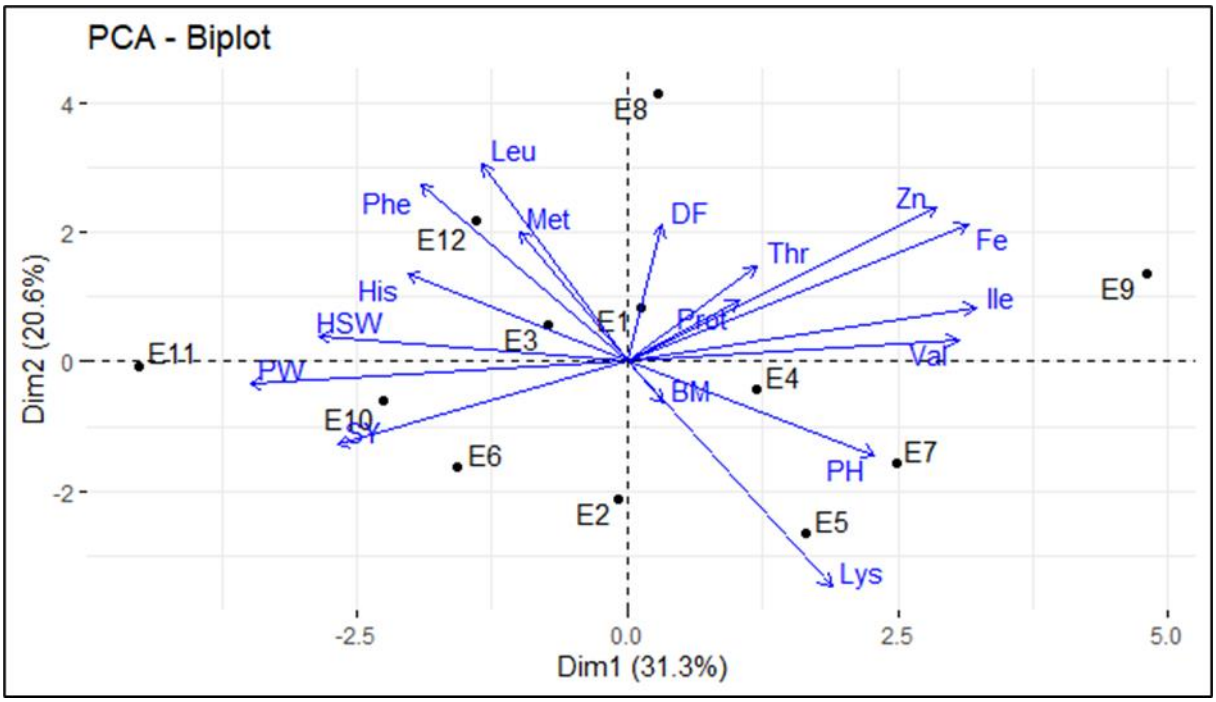

Figure 2. Principal component biplot showing the distribution and overall variation among 12 sorghum genotypes (denoted E1 to E12) based on combined agronomic and grain quality parameters. Note: see Table 1 for entry codes. His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Val = Valine; Fe = Iron; Zn = Zinc; Prot = Protein, DF = days to flowering, PH = plant height, BM = biomass, PW = panicle weight, SY = seed yield and HSW = hundred seed weight.

4. Discussion

The analysis of variance revealed higher genetic diversity of the assessed sorghum lines for the assessed traits except for biomass production. The newly developed *Striga*-resistant and *FOS*-compatible genotypes produced promising seed yields comparable with the check varieties (Table 2). Elite lines designated as 105 × 654, 672, and 3484 × 630 were among the high yielders (>30 g/plant). Other elite genotypes (105 × 672 and 3984 × 630) had average yields (>20 g/plant) with desirable traits such as high biomass. The elite lines were selected for genetic advancement and release.

Significant variations were recorded among the assessed genotypes for protein content (Table 3). The crude protein content varied from 9 to 13%. The current results agree with those reported by Ng'uni et al. [20] when assessing some South African sorghum accessions and Abdelhalim et al. [21] in wild grain sorghum from Sudan. Conversely, the protein contents of the presently assessed elite lines were lower than those reported by Mofokeng et al. [22] and Ng'uni et al. [23] among southern African sorghum genotypes. This variation can be attributed to variable genetic compositions of test genotypes and environmental effects [24,25].

Amino acids are vital for protein synthesis, the maintenance of metabolic activities, and normal growth and development [26]. Sorghum is usually deficient in two important amino acids: lysine and methionine. Although these two amino acids are limiting, the currently tested elite lines exhibited wide variation for the two amino acids. The lysine content was higher than that reported by other studies [22,24]. The genotype AS436 displayed a relatively higher lysine content (3.08%) than other genotypes evaluated in the present study. Interestingly, AS436 was highly preferred by birds, presumably due to the high lysine content. This genotype's panicles had to be bagged using three layers of mesh bags to protect them from bird damage in the field. In the present study, methionine content was lower than that reported by Mokrane et al. [27] and Mofokeng et al. [22] but higher than that reported by Ebadi et al. [24]. The wide variations in this amino acid content could be attributable to the genetic diversity present in the assessed sorghum genetic pool. The isoleucine and valine contents found in the current study were comparable to those reported by Afify et al. [28], varying from 3.49 to 3.58 g/100 g of protein.

Sorghum genotypes with high iron and zinc contents can significantly reduce 'hidden hunger', especially in regions where sorghum is the main staple. In the current study, iron content varied from 127.41 mg/kg to 156.32 mg/kg, with a mean of 78.32 mg/kg. Comparably similar results were reported by Shegro et al. [29] among Ethiopian sorghum genotypes. The iron content recorded in the present study was higher than that reported in other studies [20,30]. There were significant variations among the genotypes for zinc concentrations. Phuke et al. [31] reported variations in iron and zinc contents among recombinant sorghum inbred lines across environments. The zinc concentrations of test lines ranged from 14.45 to 44.46 mg/kg, with a mean of 24.91 mg/kg. These results were comparable to those reported by Kumar et al. [32] but were greater than those reported by Ng'uni et al. [20].

The cluster analysis grouped the genotypes into three main clusters (Figure 1). Genotypes with similar agronomic and nutritional traits were found in the same cluster. Genotypes in cluster I displayed low seed yield, panicle weight, and high levels of amino acids, especially lysine. Two genotypes in this cluster share a common parentage (105 × 654, and 105 × 672), and the other two (Macia and AS436) share a geographic origin. Genotype AS426 × 672 was a singleton in cluster II and unique from the other genotypes. This genotype displayed high Fe and Zn contents but low grain yield. Genotypes in cluster III exhibited desirable agronomic traits such as high seed yield and panicle weight. Genotype clustering showed significant variation, which is useful for selection programs in sorghum biofortification in *Striga*-infested environments. Selection and hybridisation of genotypes from different clusters can combine desirable genes for grain yield and nutritional qualities [33].

A principal component analysis was performed to determine the magnitude of the total variation involving several traits. An eigenvalue greater than 1 is considered significant, and component loadings greater than ±0.3 were deemed meaningful. The current study found that isoleucine, iron, valine, and zinc contents contributed highly to PC1, showing its significance in explaining genetic variation among the sorghum genotypes (Table 4). These traits should be targeted for diversity analysis and selection programs in sorghum breeding. Gerrano et al. [34] reported the importance of iron and zinc contents correlated in PC1 explaining genetic diversity for nutritional content. Grain yield had a low contribution in the first two PCs, contrary to the reports of Hamidou et al. [35] and Abraha et al. [36], who pinpointed that grain yield significantly contributed to the variation in the first two PCs. The test elite lines showed adequate genetic diversity for nutritional traits valuable in sorghum biofortification.

A principal component analysis biplot (Figure 2), a multivariate technique, was used to understand the correlation between the agronomic traits in the test genotypes. A significant correlation was found between panicle weight, seed yield, and hundred seed weight, and this association is necessary for indirect selection [37]. The PCA biplots demarcated the genotypes across the four quadrants. Small angles between vector lines indicate a high association among traits, and the winning genotypes are positioned at the vector lines' vertices. The PCA-biplot for nutritional traits shows a positive association between protein, zinc, and iron contents. These results agreed with those reported by Shego et al. [29] and Abdelhalim et al. [21]. Furthermore, Kumar [38] reported a highly significant correlation between iron and zinc contents. This indicates that these vital nutrients show genetic associations. Increasing the concentrations of nutrients such as iron and zinc may increase protein content simultaneously. Two genotypes were allocated in quadrant I and were associated with higher threonine, valine, iron, zinc, isoleucine, and protein contents. SS49 and 103 × 672 genotypes were grouped in quadrant II and were associated with essential amino acids, namely, histidine, methionine, and leucine. Genotypes in quadrant III were characterized by high seed yield and panicle weight. Genotypes located in quadrant IV were associated with high lysine content, a rare amino acid in sorghum. Nutritional traits with vector lines situated closer to the origin had little impact on explaining the genetic variation among the test genotypes.

5. Conclusions

The tested sorghum genotypes showed marked genetic variation in agronomic traits and nutrient compositions. Genotypes with high crude protein content were Macia, AS1, and 3484 × 424, with 13.60%, 13.37%, and 12.77%, respectively. Hence, these genotypes could be considered for sorghum nutritional improvement. The genotype AS436 had the highest lysine content (3.08%). Genotype AS426 × 672 was the highest in methionine content at 1.58%. Macia exhibited high iron (156.32 mg/kg) and zinc (44.64 mg/kg) concentrations. Overall, the selected *Striga*-resistant and *FOS*-compatible elite genotypes displayed substantial variation in nutritional qualities comparable to the check entries. The following genotypes were selected for displaying high nutritional contents: Macia, AS436; 3984 × 630; AS426 × 672; 105 × 654; and AS1. These are useful genetic resources for quality breeding programs in sorghum.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/xxx/s1; Table S1: Partial analysis of variance and significant tests for agronomic traits of the 12 sorghum genotypes assessed in two sites; Table S2: Analysis of variance and significant tests for the contents of major amino acids and mineral content measured from 12 sorghum genotypes.

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