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PROTEIN CONTENT AND AMINO ACID COMPOSITION AMONG SELECTED SOUTH AFRICAN SORGHUM GENOTYPES

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ABSTRACT

The presence of genetic diversity is essential for quality improvement to achieve balanced protein and amino acid levels in sorghum. The objective of this study was to determine the genetic diversity present among selected South African sorghum genotypes for protein and amino acid content and to select candidate lines for breeding or direct production. Fifty nine selected South African sorghum genotypes grown at two localities were analysed for crude protein content using near-infrared spectroscopy (NIR). Nineteen genotypes with high crude protein content from each location were selected and analysed for amino acid profiles using protein hydrolysates. The crude protein content of the genotypes varied from 7.69 to 16.18% across the two sites with a mean of 13.07%. The genotypes that had high crude protein content at both sites were Mammopane, AS16 M1, Macia-SA, AS19, Maseka-a-swere, and AS4. The genotype AS16cyc was the best candidate for high phenylananine content at 5.99%. Overall, the studied lines had great variability in their protein and amino acid profiles. Accessions with high protein content or amino acid values can be used in sorghum breeding programmes to increase grain nutritional quality.

Keywords: amino acids, genetic diversity, near-infrared spectroscopy, protein content, sorghum.

INTRODUCTION

Food security and malnutrition are major challenges in the world today (FAO, 2010). In South Africa, there are great disparities among communities. It is estimated that 14 million people are food insecure and 1.5 million children suffer from malnutrition in South Africa (HSRC, 2004). However, in South Africa, there is a coexistence of both under- and over-nutrition across all age groups (Steyn *et al*., 2006).

Proteins are essential components of the diet needed for humans. About 63% of the world protein consumption is from grains or grain products (FAO, 2006). The protein's basic function in nutrition is to supply adequate amounts of required amino acids. These proteins are composed of numerous amino acids of which eight are essential for the human diet. In food plants, the protein quality is a measure of the amino acid levels present in a given genotype (Arun *et al*., 2009). The protein quality or its nutritive value depends on its amino acid content and on the physiological availability of specific amino acids after digestion, absorption and oxidation. Sorghum, the most important food security crop in sub-Saharan Africa, has poor protein digestibility and inadequate levels of some of the essential amino acids such as lysine compared to other cereals (FAO, 1995).

In countries where cereals are staple foods, protein malnutrition is a widespread problem. The low levels of some critical amino acids in African cereals contribute to hunger and malnutrition reported in sub-Saharan Africa (FAO, 2010). Furthermore, one of the challenges of sorghum production under a small-scale farming system in South Africa is a lack of varieties that produce stable yields which have adequate protein and amino acid contents. Hence, it is essential to characterize sorghum collections from various provinces within South Africa. Characterization and identification of suitable sorghum genotypes and development of

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improved cultivars that are more suited to the marginal areas would help in food security and alleviation of malnutrition (Slabbert *et al*., 2001). Efforts have been made to improve levels of amino acids such as lysine in sorghum via mutation breeding. Oria *et al*. (2000) reported the identification of a novel line with high protein digestibility from a cross involving the high lysine P721 opaque mutant. Sorghum lines from the African Centre for Crop Improvement and breeding lines from other sources were mutagenised with gamma irradiation and cyclotron to improve agronomic and nutritional traits (Brauteseth, 2009). Mofokeng (2015) reported sorghum genotypes with good agronomic traits as well as high protein and amino acids in sorghum. Genetic engineering has been attempted to improve sorghum protein and amino acid levels (Zhao *et al*., 2002). Information on protein content and amino acid levels among sorghum landraces are important for growers, millers, end-users and breeders. However, sorghum cultivars grown by subsistence farmers are low yielders and their protein content and amino acid levels are unknown. Hence, it is essential to assess the levels of protein and the essential amino acids present in sorghum cultivars grown by farmers. Cultivars with superior levels of protein and amino acid levels could be used in breeding programmes aimed at improving the nutritional quality of sorghum.

Various methods have been employed to assess levels of proteins and amino acids in crops (Workman and Burns, 2001; Coetzee, 2003). Near-infrared spectroscopy (NIR) is one of the methods used by researchers to assess various quality traits. NIR can be quick, affordable and accurate. It is a non-destructive method for analysing quality traits including protein and amino acids, among others (Brauteseth, 2009). NIR has been used in various studies for determination of protein and other nutritional quality traits (YoungYi *et al*., 2010; Olesen *et al*., 2011). Hence, it is an important tool for use in characterization and making selections in plant breeding programmes.

In other studies, the protein fraction in cereal crops like sorghum was characterized by size exclusion, reverse phase HPLC and SDS–PAGE (Mokrane *et al*., 2009) and via *in vitro* protein digestibility of the extracted proteins (Mokrane *et al*., 2006). The methods used for the analysis of amino acids include ion exchange chromatography (Adeyeye, 2010), capillary electrophoresis (Waldhier *et al*., 2009), anion-exchange chromatography with integrated pulsed amperometric (IPA) detection equipped with a gold electrode (Rombouts *et al*., 2009) and high performance liquid chromatography (HLPC) (Ilisz *et al*., 2008), among others. Liquid chromatography-mass spectrometry (LC-MS) is the most widely used analytical technique for amino acids in food sources. The technique is effective and efficient for analysis of amino acids in food crops. It is fast with high throughput and provides precision and accuracy without requiring antibodies for the quantification of peptides. It also allows structurally and chemically similar peptides and proteins to be differentiated (Ewles *et al*., 2010; Ewles and Goodwin, 2011; Nowatzke *et al*., 2011). Developments in chromatographic methodology have reduced sample and reagent requirements and improved identification, resolution, and sensitivity of amino acid analyses of food samples (Peace and Gilani, 2005). The objectives of this study were to determine the genetic diversity present among selected South African sorghum genotypes, in particular, to assess their protein and amino acid composition and to select candidate lines for breeding or direct production.

MATERIALS AND METHODS

Plant materials and growing environments: Fifty nine sorghum genotypes were selected and grown at Ukulinga Research Farm (29.67'S and 30.14"E, 812 m.a.s.l) of the University of KwaZulu-Natal, and at Makhathini Research Station (27º 24' S and 32º 11' 48"E, 697 m.a.s.l) of the Agricultural Research Council. The list of sorghum genotypes used in the study is presented in Table 1. The study was conducted in the 2011/2012 growing season and March to August 2012.

Analysis of crude protein: Crude protein content was analysed using Near-Infrared Spectroscopy (NIR) (VISION, 2008) using a FOSS NIR machine, NIRSystems Composite Monochomator 6500, (FOSS NIRSystems Inc., 7703 Montpelier Rd, Laurel, MD, USA) at the Department of Plant Pathology, University of KwaZulu-Natal. About 10 g of sorghum grains of each sample from the two locations, i.e., Makhathini and Ukulinga, were placed in a sample cup that was used for scanning of the whole seeds for analysis of crude protein. The whole grains were scanned, then put into envelopes and were shaken for 5 seconds before re-scanning. The grains were scanned in triplicates. The sorghum genotypes analysed for crude protein are indicated in Table 1.

Analysis of amino acids: Nineteen sorghum genotypes

that showed high protein content were selected for the analysis of amino acids. The amino acids were analysed at the Central Analytic Facility, University of Stellenbosch, South Africa. The sorghum samples were first hydrolysed according to the AOAC (2003) method. About 0.1 g of samples were weighed using vibrator apparatus. A 6 ml of 6N HCl and 15% phenol were added into the sample inside the hydrolysis tubes.

Table 1. A list of sorghum accessions used in the study.

Serial Number	Genotype	Source/place of collection	Serial Number	Genotype	Source/place of collection
1	Mammopane	ARC-GCI	31	4891.1.1.1	Free State
2	5436.1.1.1	North West	32	5246.1.1.1	KwaZulu-Natal
3	3414.1.1.1	Eastern Cape	33	1390.1.1.1	Limpopo
4	3217.1.1.1	Eastern Cape	34	5233.1.1.1	KwaZulu-Natal
5	AS16 cyc	ACCI	35	5245.1.1.1	KwaZulu-Natal
6	05-POTCH-115	ARC-GCI	36	3416.1.1.1	Eastern Cape
7	3319.1.1.1	Eastern Cape	37	5454.1.1.1	North West
8	4442.1.1.1	Limpopo	38	05-Potch-151	ARC
9	4265.1.1.1	Mpumalanga	39	4277.1.1.1	Mpumalanga
10	3364.1.1.1	Eastern Cape	40	5393.1.1.1	North West
11	3403.1.1.1	Eastern Cape	41	1990.1.1.1	Mpumalanga
12	AS11	ACCI	42	Maseka-a-swere	ARC-GCI
13	AS21	ACCI	43	Macia-SA	ARC-GCI
14	Mamolokwane	ARC-GCI	44	4259.1.1.1	Mpumalanga
15	5287.1.1.1	KwaZulu-Natal	45	Manthate	ARC-GCI
16	M153	ARC-GCI	46	1413.1.1.1	Limpopo
17	4303.1.1.1	Limpopo	47	2985.1.1.1	Eastern Cape
18	3184.1.1.1	Eastern Cape	48	4905.1.1.1	Free State
19	4276.1.1.1	Mpumalanga	49	4154.1.1.1	Mpumalanga
20	AS16 M1	ACCI	50	1481.1.1.1	Limpopo
21	AS2	ACCI	51	05-Potch-167	ARC-GCI
22	AS16 M2	ACCI	52	2048.1.1.1	Mpumalanga
23	AS4	ACCI	53	5088.1.1.1	KwaZulu-Natal
24	5281.1.1.1	KwaZulu-Natal	54	5337.1.1.1	North West
25	MOTLERANE	ARC-GCI	55	5333.1.1.1	North West
26	1948.1.1.1	Limpopo	56	AS17	ACCI
27	AS19	ACCI	57	4909.1.1.1	Free State
28	AS1	ACCI	58	5237.1.1.1	KwaZulu-Natal
29	5258.1.1.1	KwaZulu-Natal	59	1473.1.1.1	Limpopo
30	5430.1.1.1	North West			

ACCI = African Centre for Crop Improvement, ARC-GCI = Agricultural Research Council-Grain Crops Institute.

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^{o}C. After 24 hours, these were removed from the oven and allowed to cool to room temperature. The vials were transferred into two 2ml Eppendorf tubes and the remainder of each sample was discarded. One eppi was used for analysis of amino acids in the Liquid Chromatography Mass Spectroscope. The other eppi was stored at -20^{o}C. The eppi samples were subjected to the Water AccQ Tag Ultra Derivitization Kit (Waters Corporation, MA, USA). A 10 µl of 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 x 100 mm, and sample injection was of 1 µl with the ESI + source. The solvents, Eluent A2 contained 100 ml Eluent A concentrate and 900 ml water and Eluent B was supplied in the AccQ Tag Kit. The samples were run with the capillary voltage of 3.5 kilovolts (kV) and core voltage of 15 volts (V) at 120°C. The desolvation temperature, desolvation gas and core gas were 350°C, 350Lh^{-1} and 50Lh^{-1}, respectively. The list of amino acids analysed is shown in Table 2.

Table 2. Full names of the amino acids analysed and abbreviations.

Amino acid	Abbreviation
Histidine	His
Threonine	Thr
Lysine	Lys
Methionine	Met
Valine	Val
Isoleucine	Ile
Leucine	Leu
Phenylalanine	Phe

Data analysis: The spectral data of the scanned sorghum samples were entered into VISION software (VISION 2008). The data was further analysed using Unscrambler software version 3.0 (Esbesen 1994). The model used for protein predictions was adapted from Brauteseth (2009) for sorghum protein. The protein content and amino acid profiles of the accessions were compared using the analysis of variance at P≤ 0.05 and P ≤ 0.001, the means and variances were also calculated. The data were analysed in GenStat 14th edition computer package (Payne *et al*., 2011).

RESULTS

Protein content: Results of the crude protein content of the 59 sorghum genotypes across the two sites, Makhathini and Ukulinga are presented in Table 3. The protein content of sorghum lines at Makhathini ranged from 5.50 to 16.95% with a mean of 12.78% (Table 3). There was marked variation among the sorghum accessions where 4259.1.1.1 (16.18%), Manthate (16.47%), Mammopane (16.5%), Macia-SA (16.65%) and 4154.1.1.1 (16.95%). had the highest crude protein contents. Accessions 5233.1.1.1 (5.55%), 3416.1.1.1 (8.84%) and 4265.1.1.1 (8.92%) had the lowest crude protein contents.

At Ukulinga, the accessions exhibited crude protein content ranging from 8.9 to 16.8% with a mean of 13.4% (Table 3). Accessions that had high protein content were 05-POTCH-115, AS1, AS16 M1 at 16.1%, 16.2% and 16.8%, respectively. Accessions1390.1.1.1, 4259.1.1.1, and 5233.1.1.1 had the lowest crude protein contents of 8.9%, 9.8% and 9.8%, respectively.

Overall, there was a higher degree of variability among the sorghum accessions for crude protein content when tested at Makhathini than Ukulinga (Table 3). The crude protein content ranged from 7.7 to 16.2% averaged across the two sites with a grand mean of 13.1%. The accessions that showed high protein content across the two sites were AS4, followed by Maseka-a-swere, AS19, Macia-SA, AS16 M1 and Mammopane at 15.1%, 15.1%, 15.2%, 15.3%, 15.6%, and 16.2%, respectively. The lowest crude protein contents were noted in the accessions 5233.1.1.1 and 1390.1.1.1, at 7.7% and 9.7%, respectively.

Table 3. Protein content (%) of 59 sorghum accessions grown at Makhathini and Ukulinga, 2011/2012.

Number	Genotype	Makhathini	Ukulinga	Overall mean
1	Mammopane	16.5	15.85	16.18
2	5436.1.1.1	11.28	15.44	13.36
3	3414.1.1.1	11.79	11.66	11.73
4	3217.1.1.1	14.73	14.12	14.43
5	AS16 cyc	15.15	13.01	14.08
6	05-POTCH-115	12.27	16.06	14.17
7	3319.1.1.1	12.29	12.83	12.56
8	4442.1.1.1	12.54	12.7	12.62
9	4265.1.1.1	8.92	11.81	10.37
10	3364.1.1.1	11.99	12.52	12.26

11	3403.1.1.1	12.65	13.13	12.89
12	AS11	12.52	14.96	13.74
13	AS21	14.47	12.31	13.39
14	Mamolokwane	12.53	14.28	13.41
15	5287.1.1.1	9.54	12.14	10.84
16	M153	12.72	15.96	14.34
17	4303.1.1.1	11.03	13.4	12.22
18	3184.1.1.1	11.46	12.44	11.95
19	4276.1.1.1	12.43	13.19	12.81
20	AS16 M1	14.32	16.81	15.57
21	AS2	12.25	15.01	13.63
22	AS16 M2	12.73	15.33	14.03
23	AS4	14.24	15.9	15.07
24	5281.1.1.1	11.56	13.49	12.53
25	MOTLERANE	12.88	15.29	14.09
26	1948.1.1.1	13.74	11.14	12.44
27	AS19	14.68	15.75	15.22
28	AS1	12.07	16.15	14.11
29	5258.1.1.1	13.53	12.79	13.16
30	5430.1.1.1	12.12	14.36	13.24
31	4891.1.1.1	12.55	12.6	12.58
32	5246.1.1.1	10.65	11.15	10.90
33	1390.1.1.1	9.38	8.92	9.15
34	5233.1.1.1	5.55	9.83	7.69
35	5245.1.1.1	12.5	13.15	12.83
36	3416.1.1.1	8.84	14.58	11.71
37	5454.1.1.1	12.34	13.03	12.69
38	05-Potch-151	11.58	14.79	13.19
39	4277.1.1.1	11.81	12.74	12.28
40	5393.1.1.1	10.88	10.8	10.84
41	1990.1.1.1	11.73	13.91	12.82
42	Maseka-a-swere	15.88	14.38	15.13
43	Macia-SA	16.65	13.97	15.31
44	4259.1.1.1	16.18	9.75	12.97
45	Manthate	16.47	13.19	14.83
46	1413.1.1.1	14.94	14.8	14.87
47	2985.1.1.1	15.56	14.42	14.99
48	4905.1.1.1	13.05	12.07	12.56
49	4154.1.1.1	16.95	11.62	14.29
50	1481.1.1.1	15.83	12.67	14.25
51	05-Potch-167	12.53	11.7	12.12
52	2048.1.1.1	15.8	13.48	14.64
53	5088.1.1.1	13.7	11.36	12.53
54	5337.1.1.1	13.73	12.42	13.08
55	5333.1.1.1	10.09	10.26	10.18
56	AS17	13.24	13.33	13.29
57	4909.1.1.1	11.82	14.96	13.39

58	5237.1.1.1	13.1	14.53	13.82
59	1473.1.1.1	9.49	14.77	12.13
	Min	5.55	8.92	7.69
	Max	16.95	16.81	16.18
	Mean	12.78	13.37	13.07
	Variance	4.89	3.14	2.51
	SD	2.21	1.77	1.58
	SE	0.31	0.27	
	F-probability	< 0.001	< 0.001	

***The amino acid composition of sorghum accessions at Makhathini*:** The selected 19 genotypes were grown, and their seed samples were profiled for 8 amino acids (Table 2). The essential amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine. The levels of amino acids were expressed as percent of the total protein (Table 4).

Percent amino acids showed significant differences among tested accessions. Levels of all amino acids in different accessions were highly significantly different at $P < 0.001$ (Table 4). Histidine content ranged between 1.81 and 2.32% with a mean of 2.10%. Accessions AS17, 2048.1.1.1 and 4276.1.1.1 had high histidine content at 2.32, 2.26 and 2.26%, respectively. Low histidine values were recorded against accessions 05-Potch-115, 05-Potch-167 and AS16cyc at 1.97, 1.91 and 1.81%, respectively. Percent lysine ranged from 1.09 to 2.17% with a mean of 1.80%.

Table 4. Amino acids composition (%) of 18 sorghum genotypes grown at Makhathini, 2011/2012.

Genotype	Amino acids							
	His	Thr	Lys	Met	Val	ILe	Leu	Phe
AS11	2.23	3.03	1.66	2.09	5.00	3.87	14.42	5.32
AS16cyc	1.81	2.26	1.09	4.28	4.28	3.26	14.14	6.86
AS17	2.32	3.23	2.17	2.03	5.09	3.76	14.18	5.39
2985.1.1.1	2.07	3.08	1.68	2.35	4.85	3.92	14.11	5.64
4905.1.1.1	2.18	3.08	1.87	2.73	4.98	3.80	13.87	5.42
5246.1.1.1	2.10	3.19	2.04	2.31	5.33	4.01	13.91	5.26
1413.1.1.1	2.14	2.93	2.02	1.70	5.06	3.84	14.14	5.15
1481.1.1.1	2.11	3.03	2.09	1.85	5.17	4.09	13.60	5.13
1948.1.1.1	2.00	2.91	1.85	1.72	5.10	4.17	14.47	5.44
4303.1.1.1	2.00	2.91	2.02	1.68	5.24	3.99	14.24	5.15
2048.1.1.1	2.26	2.93	1.89	1.81	5.09	4.11	13.95	5.34
4154.1.1.1	2.08	2.91	1.72	1.79	4.95	4.13	14.44	5.40
4259.1.1.1	2.23	3.12	1.87	2.23	4.98	3.74	13.54	5.10
4276.1.1.1	2.26	3.15	1.73	2.09	4.98	3.89	13.92	5.35
Manthate	2.23	3.00	1.98	2.42	5.19	3.70	13.40	5.26
Maseka-a-swere	2.08	2.99	1.67	1.56	5.23	3.92	14.45	5.29
05-Potch-115	1.97	3.01	1.87	1.52	4.88	3.79	14.17	5.23
05-Potch-167	1.91	3.00	1.88	1.40	4.85	3.88	14.28	5.26
Min	1.81	2.26	1.09	1.40	4.28	3.26	13.40	5.10
Max	2.32	3.23	2.17	4.28	5.33	4.17	14.47	6.86
Mean	2.10	3.00	1.80	2.10	5.00	3.90	14.10	5.40
F-probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SE	0.03	0.05	0.06	0.15	0.05	0.05	0.08	0.09
Variance	0.02	0.04	0.06	0.42	0.05	0.04	0.10	0.15

Percent amino acids showed significant differences among tested accessions. Levels of all amino acids in different accessions were highly significantly different at $P < 0.001$ (Table 4). Histidine content ranged between 1.81 and 2.32% with a mean of 2.10%. Accessions AS17, 2048.1.1.1 and 4276.1.1.1 had high histidine content at 2.32, 2.26 and 2.26%, respectively. Low histidine values were recorded against accessions 05-Potch-115, 05-Potch-167 and AS16cyc at 1.97, 1.91 and 1.81%, respectively. Percent lysine ranged from 1.09 to 2.17% with a mean of 1.80%. Accessions that had high lysine percent were Manthate, 05-Potch-115, 4905.1.1.1, 1413.1.1.1 and 2985.1.1.1 at 2.17, 2.09, 2.04, 2.02 and 2.02%, respectively. The lowest lysine percent was in genotype 4276.1.1.1, at 1.09%. Threonine ranged from 2.26 to 3.24% with a mean of 3.0%. The accessions that had a high threonine percent were 05-Potch-115, 5246.1.1.1, Maseka-a-swere and 1481.1.1.1 at 3.23, 3.19, 3.15 and 3.12%, respectively. The lowest was recorded in the genotype AS16cyc at 2.26%. Methionine levels ranged from 1.40% to 4.28% with a mean of 2.10%. The genotype that had the highest methionine percent was 2985.1.1.1 at 15.85% and the lowest was 4276.1.1.1 at 1.40%. Valine ranged from 4.28 to 5.33% with a mean of 5.0%. The genotypes that had high valine percent were 2048.1.1.1, 1948.1.1.1, AS11, 5246.1.1.1 and AS17 with 5.33%, 5.24%, 5.23%, 5.19% and 5.17%, while the lowest was noted in the genotype 4905.1.1.1 at 4.28%. Isoleucine ranged from 3.26 to 4.17% with a mean of 3.90%. The genotypes that had high isoleucine percent were 05-Potch-115, 1481.1.1.1, 1413.1.1.1 and 4259.1.1.1 with 4.17%, 4.13%, 4.11% and 4.09%, respectively. The lowest isoleucine content was recorded in the genotype 1948.1.1.1 at 3.26%. Leucine ranged from 13.40% to 14.47% with a mean of 14.10%. The genotypes that had high leucine percent were AS16cyc, Maseka-a-swere, 05-Potch-167 and 05-Potch-115 at 14.14%, 14.45%, 14.28% and 14.17%, respectively. The leucine content was the lowest in 4303.1.1.1, AS17 and AS11 at 13.6%, 13.54% and 13.4%, respectively. Phenylalanine ranged from 5.10% to 6.86% with a mean of 5.40%. The genotype Maseka-a-swere had the highest phenylalanine content at 6.86% and the lowest was noted in the genotype 4276.1.1.1 with 5.1%.

***The amino acid composition of sorghum genotypes at Ukulinga*:** Amino acid compositions of the 18 sorghum genotypes evaluated at Ukulinga Research farm are presented in Table 5. The ANOVA displayed highly significant differences ($P < 0.001$) for the eight essential amino acids.

Percent amino acids of the total protein showed significant differences among the tested genotypes (Table 5). All amino acids were highly significant at $P < 0.001$ (Table 6). Histidine showed variation ranging between 1.78 to 2.28% with a mean percent of 2.06. Genotypes 1413.1.1.1, 2048.1.1.1, 4259.1.1.1, 4276.1.1.1 and 2985.1.1.1 expressed high histidine levels of 2.28%, 2.25%, 2.23%, 2.23% and 2.21%, respectively. 05-Potch-167 had low histidine of 1.78%. The threonine composition showed differences ranging between 2.79% and 3.26% with an average percent of 3.05%. The genotypes 05-Potch-115 and 1948.1.1.1 had the highest threonine content of 3.26% and 3.24%, respectively. Manthate expressed the lowest threonine content of 2.79%. The lysine levels ranged from 1.71 to 2.5% with the mean of 2.05%. Accession 1481.1.1.1 had the highest lysine content of 2.5% and 4154.1.1.1 had a low content of 1.71%. The methionine values ranged from 1.7% to 2.33% with an average of 2.06%. The genotypes 05-Potch-115, 4154.1.1.1, AS16cyc, 4303.1.1.1 and 1948.1.1.1 expressed high levels of 2.33%, 2.33%, 2.32%, 2.29% and 2.27%, respectively. The lowest lysine content was observed in 2048.1.1.1 at 1.7%.

The valine content varied between 4.89% and 5.28% with an average of 5.03%. The accessions that showed high values were Macia-SA, Manthate, AS17, 1481.1.1.1 and 1413.1.1.1 of 5.27%, 5.19%, 5.19%, 5.17% and 5.15%, respectively. The lowest values were observed in 4303.1.1.1 and 2048.1.1.1 both at 4.89%. The isoleucine content among the genotypes varied from 3.63% to 4.06% with an average of 3.83%. The genotypes that had high isoleucine levels were 2985.1.1.1, 05-Potch-167 and 4276.1.1.1 at 4.06%, 4.06% and 4.01%, respectively. The lowest levels were noted in genotypes 4259.1.1.1 at 3.64% and 05-Potch-115 at 3.63%. The leucine levels ranged from 13.04% to 14.29% with the average of 13.79%. The genotypes that had high leucine were 1413.1.1.1 and 05-Potch-115 at 14.28% and 14.25%, respectively. The genotype AS17 had the lowest leucine level of 13.04%. The phenylalanine levels varied between 4.82% and 5.7% with the average of 5.17%. Manthate had the highest phenylalanine content of about 5.7% and 4259.1.1.1 had the lowest content of 4.82%. The arginine values varied between 3.11% and 4.19% with an average of 3.62%. The genotypes 05-Potch-115 and 4905.1.1.1 both expressed increased

arginine level of 4.19% each. Macia-SA had the lowest level of 3.11%. The tyrosine values ranged from 3.84% to 4.53% with an average of 4.13%. The highest tyrosine level was noted in Manthate having 4.53% and the lowest in 2985.1.1.1 having tyrosine content of 3.84%.

***Effect of environment on the amino acid composition*:** Percentage amino acids showed significant differences among the tested sorghum accessions (Table 6). The phenylalanine, lysine and leucine were significant at $P \leq 0.05$. The phenylalanine content ranged from 5.04 to 5.99 percent of the total with the mean percent of 0.23. AS16cyc had high phenylalanine of about 5.99% and 5246.1.1.1 had the lowest level of 5.04%. The lysine content ranged from 1.63 to 2.27% of the total with a mean of 1.94%. Accessions that had high lysine were 5246.1.1.1, AS17, Manthate and 1481.1.1.1 at 2.27%, 2.25%, 2.16% and 2.11% contents, respectively. The lowest lysine was recorded in AS16cyc with 1.63%. The leucine values ranged from 13.28% to 14.30% of the total with the mean of 13.94%. The genotypes that showed high leucine content were 1948.1.1.1, 4905.1.1.1, 4154.1.1.1 and 1481.1.1.1 at 14.3%, 14.3%, 14.26%, and 14.25%, respectively. Macia-SA showed the lowest level of leucine of 13.28%.

Table 5. Amino acid composition (%) of 19 sorghum types grown at Ukulinga 2011/2012.

Genotype	Amino acids							
	His	Thr	Lys	Met	Val	ILe	Leu	Phe
AS11	2.03	3.14	1.85	2.19	4.90	3.74	14.18	5.06
AS16cyc	1.89	3.07	2.17	2.32	4.94	3.76	14.03	5.12
AS17	1.94	3.26	2.33	2.20	4.98	3.70	13.72	4.94
2985.1.1.1	2.21	3.17	1.71	2.33	4.89	3.86	14.07	5.70
4905.1.1.1	2.05	3.06	1.95	1.88	5.00	3.94	14.28	5.35
5246.1.1.1	2.15	3.24	2.50	2.13	5.17	3.82	13.24	4.82
1413.1.1.1	2.28	2.99	1.89	1.80	5.19	4.06	14.02	5.44
1481.1.1.1	1.99	2.90	2.13	1.95	5.10	3.92	13.69	5.13
1948.1.1.1	2.04	2.96	2.34	1.72	5.19	4.01	13.49	4.97
4303.1.1.1	2.05	2.86	2.12	2.29	5.09	3.82	13.81	5.15
2048.1.1.1	2.25	2.79	1.77	2.27	5.08	3.75	13.69	5.27
4154.1.1.1	1.94	2.87	1.75	1.70	4.91	3.95	14.16	5.28
4259.1.1.1	2.23	3.17	2.04	2.02	5.15	4.06	13.89	5.27
4276.1.1.1	2.23	3.18	2.35	2.12	5.04	3.79	13.13	5.11
Manthate	2.15	3.16	2.34	2.33	5.27	3.72	13.16	5.05
Maseka-a-swere	2.04	2.95	1.77	1.96	4.95	3.64	14.06	4.98
Macia-SA	1.96	3.02	2.23	1.87	4.94	3.63	13.04	4.87
05-Potch-115	1.86	3.08	1.85	1.93	4.95	3.80	14.07	5.40
05-Potch-167	1.78	3.03	1.77	2.04	4.89	3.75	14.25	5.38
Min	1.78	2.79	1.71	1.7	4.89	3.63	13.04	4.82
Max	2.28	3.26	2.5	2.33	5.27	4.06	14.28	5.7
Mean	2.06	3.05	2.05	2.06	5.03	3.83	13.79	5.17
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SE	0.03	0.03	0.06	0.05	0.03	0.03	0.09	0.05
Variance	0.02	0.02	0.07	0.05	0.02	0.02	0.18	0.06

Among the amino acid profiles assessed, lysine was the most deficient. It was in the range of 1.63 to 2.27% with a mean of 1.94%, methionine varied between 1.67 to 3.30% with the mean of 2.07%, and histidine further varied between 1.78 and 2.26% with the mean of 2.08% (Table 6). The most abundant amino acid was leucine ranging between 13.28% and 14.30% with the mean of 13.94%.

Generally, accessions 1948.1.1.1 (14.3%), 4905.1.1.1 (14.3%), 4154.1.1.1 (14.26%) and 1481.1.1.1 (14.25%) were the best on leucine content across the two locations. 5246.1.1.1 (2.27%), AS17 (2.25%), Manthate

(2.16%) and 1481.1.1.1 (2.11%) were the best genotypes for lysine and AS16cyc (5.99%) was the best candidate genotype for phenylananine content across the two locations. Hence, these eight accessions can be used for grain quality improvement in sorghum breeding programmes.

Table 6. Mean amino acid composition (%) among 19 sorghum genotypes grown at Makhathini and Ukulinga, 2011/2012.

Genotype	Amino acids							
	Hist	Thr	Lys	Met	Val	Ile	Leu	Phe
AS11	2.13	3.09	1.76	2.14	4.95	3.81	14.30	5.19
AS16cyc	1.85	2.67	1.63	3.30	4.61	3.51	14.09	5.99
AS17	2.13	3.25	2.25	2.12	5.04	3.73	13.95	5.17
2985.1.1.1	2.14	3.13	1.70	2.34	4.87	3.89	14.09	5.67
4905.1.1.1	2.12	3.07	1.91	2.31	4.99	3.87	14.08	5.39
5246.1.1.1	2.13	3.22	2.27	2.22	5.25	3.92	13.58	5.04
1413.1.1.1	2.21	2.96	1.96	1.75	5.13	3.95	14.08	5.30
1481.1.1.1	2.05	2.97	2.11	1.90	5.14	4.01	13.65	5.13
1948.1.1.1	2.02	2.94	2.10	1.72	5.15	4.09	13.98	5.21
4303.1.1.1	2.03	2.89	2.07	1.99	5.17	3.91	14.03	5.15
2048.1.1.1	2.26	2.86	1.83	2.04	5.09	3.93	13.82	5.31
4154.1.1.1	2.01	2.89	1.74	1.75	4.93	4.04	14.30	5.34
4259.1.1.1	2.23	3.15	1.96	2.13	5.07	3.90	13.72	5.19
4276.1.1.1	2.25	3.17	2.04	2.11	5.01	3.84	13.53	5.23
Manthate	2.19	3.08	2.16	2.38	5.23	3.71	13.28	5.16
Maseka-a-swere	2.06	2.97	1.72	1.76	5.09	3.78	14.26	5.14
05-Potch-115	1.97	3.02	2.05	1.70	4.91	3.71	13.61	5.05
05-Potch-167	1.89	3.04	1.87	1.67	4.90	3.84	14.18	5.33
Macia-SA	1.78	3.03	1.77	2.04	4.89	3.75	14.25	5.38
Min	1.78	2.67	1.63	1.67	4.61	3.51	13.28	5.04
Max	2.26	3.25	2.27	3.30	5.25	4.09	14.30	5.99
Mean	2.08	3.02	1.94	2.07	5.02	3.85	13.94	5.28
Variance	0.02	0.04	0.09	0.34	0.04	0.05	0.17	0.19
STDEV	0.13	0.20	0.30	0.59	0.20	0.22	0.41	0.44
SE Mean	0.03	0.05	0.07	0.14	0.05	0.05	0.10	0.10
F-probability	0.216	0.213	0.006	0.828	0.565	0.342	0.005	0.043

Accession Maseka-a-swere, had high protein content (15.13%), and high leucine (14.45%) at Makhathini. At Ukulinga, Maseka-a-swere had high leucine (14.26%). Manthate had a high protein content of 14.83% across the two locations. At Ukulinga, Manthate showed high lysine of 2.34% and methionine of 2.33%. Across the locations, Manthate lysine of 2.16%. These two accessions have high protein content and amino acid levels useful for breeding and/or conservation purposes.

DISCUSSION

Assessing local sorghum genotypes for protein and amino acids is essential for exploiting the existing potential residing in the local landraces for improved human nutrition. There was variation present among the sorghum accessions studied based on the crude protein and amino acid profiles. Shegro *et al*. (2012) also found genetic variation among the sorghum landraces when analysing protein and other mineral elements. In their report, the protein content varied between 8.08 and 15.26%. Nguni *et al*. (2012) further reported grain protein content ranging between 9.7 and 16.3% in Southern African sorghum genotypes. In the present study, the crude protein content varied between 7.69% and 16.18%, which is similar to the crude protein reported by Shegro *et al*. (2012) and Nguni *et al*. (2012). Pepo *et al*. (2011) reported protein levels ranging

between 9.43 and 17.7% among sorghum cultivars and single hybrids. Perdesen and Kofoid (2003) reported crude protein ranging from 106 to 128 g/kg with a mean of 117 g/kg for sorghum lines without testa and 107 to 124 g/kg protein with testa containing sorghum lines when assessing the sorghum conversion lines for protein content. Mokrane *et al*. (2010) reported protein content of about 16% in various Algerian sorghum cultivars. Douglas *et al*. (1990) found crude protein levels in sorghum lines to be higher than maize, ranging from 8.8 to 15.0%. Crude protein in the range of 6% to 16% has been reported by other researchers (Youssef, 1998; Afripro, 2003).

Protein content and amino acid compositions are highly variable due to differences in genotypes, environments and genotype x environment interaction. The protein content of a crop is influenced by the production environment. In this study, the protein content varied across locations and genotypes. The genotypes that exhibited high protein content in this study have potential to be selected for breeding, conservation or direct production at the target agro-ecology. More studies are needed in different agro-ecologies to select genotypes with stable protein expression.

There were significant differences among the sorghum accessions based on the amino acid composition. The amino acid levels were different for lysine, isoleucine and phenylalanine across the two locations. Lysine and methionine were in low levels than other amino acids whereas leucine content was found in higher levels. These results concur with the reports of other researchers who found low levels of lysine and methionine in sorghum (Azevedo *et al*., 1997; Amjad *et al*., 2003). Ebadi *et al*. (2005) also found low levels of lysine and methionine in high tannin sorghums. Hicks *et al*. (2002) reported genetic variation among the sorghum inbred lines and hybrids for crude protein and other quality traits. Mokrane *et al*. (2010) found different levels of amino acids analysed in Algerian sorghum cultivars. The amino acid profiles had an amino acid score of 1.0-2.6 of the human protein requirement. Moreover, the amino acids contents ranged from 0.9 to 2.6 g/100g except for lysine, methionine, and cysteine. Genetic variation was also observed among high lysine sorghum genotypes from India and MASSA 03 based on protein and amino acids. There were high lysine and threonine soluble concentrations observed among the sorghum accessions which could serve as potential food sources due to a better balanced amino acid profile.

A large part of the differences observed in amino acid profiles was due to genetic effects. Hence, breeding for enhanced amino acid profiles is feasible. The best sorghum accessions can be used as parents to develop superior cultivars and/or hybrids with improved protein and amino acids.

Generally, the sorghum accessions studied showed a wide variation in crude protein content and amino acid profiles. High crude protein content recorded at Makhathini and Ukulinga were for accessions Mammopane (16.18%), AS16 M1 (15.57%), Macia-SA (15.31%), AS19 (15.22%), Maseka-a-swere (15.13%) and AS4 (15.07%). Hence, these lines can be recommended for further grain quality improvement in sorghum breeding or direct production. The candidate genotypes with superior levels of leucine were 1948.1.1.1 (14.3%), 4905.1.1.1 (14.3%), 4154.1.1.1 (14.26%) and 1481.1.1.1 (14.25%). Accessions 5246.1.1.1 (2.27%), AS17 (2.25%), Manthate (2.16%) and 1481.1.1.1 (2.11%) were the best for high lysine, whereas AS16cyc (5.99%) was the best candidate for phenylalanine. Manthate and Maseka-a-swere were best candidates for high protein and good amino acid composition. The presence of genetic diversity among the sorghum accessions studied is imperative to meet the current and future needs of sorghum improvement programmes as well as for improved human nutritional value.

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