The Protein Content and Amino Acid Composition of Sorghum Grain

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ABSTRACT

Determinations of total protein and amino acid composition were made on a number of sorghum samples, including those from two field experiments. It was shown that the germ contained the highest proportion of protein, fqllowed in order by the whole kernel, the endosperm, and the pericarp. The amino acid composition of these parts is different, with higher proportions in the germ than the whole kernel of lysine, histidine, arginine, glycine, aspartic acid, threonine, and valine. An increase in the proportion of the germ raises the total protein and the proportion of certain essential amino acids. Earlier maturing varieties had higher protein contents. The inheritance pattern of protein content is probably not simple. Statistical analysis of the field experiments showed non-significant variations in amino acid composition between individual plants and duplicate analyses, but significant variations between two varieties and their hybrid, and between replicate plots.

In West Africa, sorghum bicolor provides a staple diet for a large part of the people. In northern Nigeria, it is the principal food crop. Agronomists have introduced higher yielding American varieties which have been hybridized with local varieties. The grain from some of these introductions contains a higher proportion of protein. Since local consumption of animal protein is low, an increase in the protein and essential amino acid contents of sorghum could lead to a major improvement in nutrition. In general, the protein content of sorghum grain lies between 8 and 16%, depending on variety and developmental conditions (1,2). Amino acid compositions have been published (3–8). It was concluded that sorghum was low in most essential amino acids, especially lysine, when compared with egg protein, and that amino acid content varied according to varieties and location. If the content and quality of sorghum protein can be improved, the nutritional level of this cereal can be raised.

In this paper, the total protein and amino acid composition of sorghum kernels have been examined statistically, and their anatomical distribution within the different parts of the kernel is established. The inheritance pattern for total protein and amino acid composition was studied in two hybridization experiments which allowed correlation with height and maturity and with effects on composition owing to variation between individual plants, varieties, and locations.

MATERIALS

Sorghum Kernels

Sorghum kernels were obtained from two field trials. In a height and maturity hybridization trial (HM trial), four American varieties–Durra, Texas Black-hull Kafir, 100-day Milo, and Combined Hegari–were hybridized with the local Shambul variety. The five parent varieties and four crosses were grown in four blocks, each containing nine plots, assigned at random to the varieties.

Samples for total protein were taken from three plants from each plot. Three varieties–Durra, Shambul, and Durra X Shambul–were used for amino acid analysis. Kernels from three plants for each variety plot in each replicate block were taken for duplicate amino acid analysis.

For studying the segregation of characteristics of an F2 population, kernels from an F2 population of Durra X Shambul were grown as an F3 population. The kernels were obtained from selfed F2 plants chosen at random. Kernels from 148 such plants were analyzed for total protein. There were 5 replicates in the F3 trial, each containing 30 rows of which either 4 or 5 were Shambul check rows.

Reagents

All the reagents used for nitrogen determination and amino acid analysis were of reagent grade. A standard amino acid mixture was obtained from Beckman's (Beckman Instruments, Inc., Palo Alto, Calif.) for calibration of the automatic amino acid analyzer.

METHODS

Analysis for Total Protein

Kernels were ground to pass through a 40-mesh screen, dried at 105°C. for 1 hr. and stored over silica gel. Kjeldahl determinations in triplicate were made on 30-mg. samples by the standard method (9).

Nitrogen-Protein Conversion Factor

Different workers have used different conversion factors. For many proteins, 6.25 is appropriate; but for most seed proteins it is too high. The FAO has recommended 5.83 for whole grain of wheat, rye, barley, and oats, and this value has been used for sorghum by Adrian and Sayerse (6), although Close and Naves (3) used 5.35. For this work, 5.83 was used, and justified from the amino acid analysis.

Direct Acid Hydrolysis

One hundred milligrams of ground sorghum kernel was hydrolyzed with constant boiling HCl, under vacuum in sealed tubes, at 100°C.±2°. Excess of HCl in the hydrolysate was removed by 3X rotary evaporation. The residue was taken up in pH 2.2 citrate buffer. Samples were stored at −32°C., and analysis was performed within a week. Sulfur-containing amino acids were determined by performic acid oxidation (10) followed by hydrolysis.

Alkaline Hydrolysis

For tryptophan analysis, direct hydrolysis with 3M barium hydroxide was carried out (11) without addition of starch.

Amino Acid Analysis of Sorghum-Grain Hydrolysates

Amino acid analysis was carried out by Moore and Stein's method (12) with a Beckman 120C automatic amino acid analyzer. Corrections were made for ammonia. Recovery of 100±2% 0.1 μM of each amino acid was obtained from calibration experiments. The error between duplicates was non-significant at the 5 and 1% levels. All determinations were made in duplicate. Values of serine, threonine, and one-half cystine decreased with time of hydrolysis; whereas values of valine, isoleucine, and ammonia increased. Corrected values for serine, threonine, and ammonia were obtained by linear extrapolation to zero time of hydrolysis. One-half cystine was determined as cysteic acid after performic acid oxidation (10). For valine and isoleucine, reciprocal extrapolation to zero was not satisfactory; the maximum values obtained after 90 hr. were taken. Tryptophan was determined after alkaline hydrolysis (11). An aqueous extract of the whole kernel was prepared using 5 g. ground kernel and 20 ml. distilled water at room temperature. The extract was diluted with pH 2.2 citrate buffer and analyzed for free amino acids without hydrolysis.

RESULTS AND DISCUSSION

Anatomical Distribution of Protein Content in Sorghum Kernels

Kernels from variety FF5683 (Guinea) were separated into three parts—pericarp, endosperm, and germ—by hand dissection under a lens after a short soaking in water. Each part was analyzed for protein content; and the results are shown in Table I.

The protein content of the germ was higher than that of the other parts. Varieties which have a greater part of the whole kernel present as germ are, other things being equal, likely to have a higher protein content in the whole grain.

Amino Acid Composition and Free Amino Acids of Different Anatomical Parts of the Kernel

Amino acid composition of the sorghum-kernel components is shown in Table II. The composition of the endosperm was similar to that of the entire kernel,

TABLE I. PROTEIN CONTENT OF THE ANATOMICAL PARTS OF SORGHUM KERNELS

Part	Percentage by Weight of Whole Kernel	Percentage of Protein in the Part
Germ	8.0	16.3
Endosperm	86.5	10.2
Pericarp	5.4	5.1
Entire kernel	100.0	10.4

TABLE II. AMINO ACID COMPOSITION OF DIFFERENT ANATOMICAL PARTS OF THE KERNEL AND THE FREE AMINO ACID POOL (mole % total)

Amino Acid	Entire Grain	Germ	Pericarp	Endosperm	Amino Acid Pool
Lysine	1.61	3.82	3.00	1.20	3.49
Histidine	1.71	2.58	1.69	1.71	0.69
Arginine	2.62	6.88	4.14	2.21	6.52
Aspartic acid	6.61	7.95	8.33	5.63	21.87
Threonine	3.69	4.51	4.86	3.40	2.32
Serine	6.48	6.95	7.16	5.52	15.91
Glutamic acid	18.58	14.03	12.43	19.48	11.72
Proline	9.71	5.19	6.44	9.94	8.02
Glycine	6.42	11.83	12.63	5.07	5.67
Alanine	14.44	10.52	11.29	12.77	10.87
½ Cystine	1.09	1.07	1.61	0.83	0.58
Valine	4.25	6.62	6.35	5.26	2.34
Methionine	1.45	1.07	1.12	1.25	0.74
Isoleucine	2.84	3.51	3.92	4.06	1.16
Leucine	12.15	7.77	8.97	14.19	3.99
Tyrosine	2.57	1.81	2.14	2.13	2.29
Phenylalanine	3.81	3.92	3.95	4.35	1.84

which was expected as it constitutes the major part of the kernel. In the germ, larger quantities of the basic amino acids lysine, histidine, and arginine were found, whereas lower quantities of glutamic acid, proline, alanine, tyrosine, and leucine were shown. The pericarp contained a high proportion of lysine, arginine, threonine, serine, valine, and glycine, with low values of glutamic acid, proline, alanine, and leucine. Amino acid composition of the pericarp more closely resembled that of the germ than of the endosperm. The free amino acid pool constituted 1.28% of the total nitrogen, and gave higher values of lysine, arginine, aspartic acid, and serine, with lower values of histidine, threonine, glutamic acid, alanine, one-half cystine, valine, methionine, isoleucine, phenylalanine, and especially of leucine, than the entire grain.

The nature of proteins in the different anatomical parts is different, and factors which alter their relative proportions would alter the amino acid composition of the whole kernel. An increase in the proportion of the germ would give a higher content of lysine, histidine, arginine, threonine, and valine, but would lower the content of leucine. As their would still be plenty of leucine combined with the

TABLE III. ANALYSIS OF VARIANCE OF PROTEIN CONTENT FROM THE HM TRIAL

Source of Variation	Degrees of Freedom	Mean Squares
Between varieties	8	16.5**
Between blocks	3	1.63
Between plants	2	2.50
Varieties X blocks	24	2.74*
Varieties X plants	16	1.12
Blocks X plants	6	1.08
Varieties X blocks X plants	48	1.32
Total	107	...

high protein content of the germ, a considerable gain in nutritional value would occur.

Protein Content of Kernels from the HM Trial

Protein content ranged from 6.5 to 14.2%. The variance between varieties was highly significant at both 5 and 1% levels (Table III), whereas the variance between blocks and between plants was not significant. A real variation in protein content for different varieties was clearly established when they were grown under the same conditions. The variety X block interaction was significant at the 2.5% level; and it may be that different blocks, although close together, were somewhat more suitable for some varieties than others.

A comparison between mean protein content for the different varieties with mean days to anthesis, number of leaves, and height, is shown in Table IV. Good inverse correlations exist between protein content and days to anthesis or number of leaves. The earlier maturing types, mainly from the U.S., outyielded the local sorghum (13,14). Early flowering plants had a smaller number of leaves and a higher protein content in the kernels. The protein content of hybrids was intermediate between those of the parents, except for Milo X Shambul for which the protein content was lower than both the parents. In all cases the values were closer to that of the Shambul variety. The hybrid Durra X Shambul showed the greatest increase over the value of Shambul.

TABLE IV. PROTEIN CONTENT (%), CORRELATION WITH GROWTH MEASUREMENTS

Variety	Days to Anthesis	Number of Leaves	Height (in.)	Protein %
Durra	64	16	145	11.76
Shambul	86	22	264	8.93
Durra X Shambul	74	21	236	10.19
Hegari	66	17	36	11.27
Hegari X Shambul	81	22	226	8.98
Kafir	64	16	74	11.34
Kafir X Shambul	79	21	237	9.72
Milo	77	21	44	11.10
Milo X Shambul	81	22	216	8.78

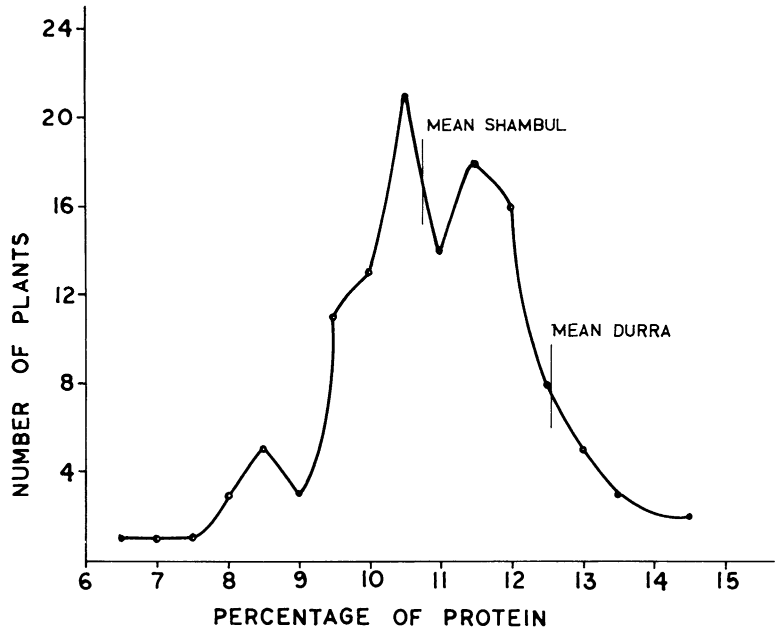

Fig. 1. Frequency distribution of the protein level in an F_3 population of Durra X Shambul.

Protein Content of an F3 Population of Durra X Shambul

Protein content was measured on the kernels of 148 plants from the trial. The values ranged from 6.5 to 14.5%. A plot of frequency distribution of the total protein is shown in Fig. 1. There is some evidence of segregation of plants into two major groups. The inheritance pattern of protein content in sorghum may not be simple, in contrast to that for the days of anthesis and the length of the internode (13,14).

Amino Acid Composition of Kernels from HM Trial

Kernels from three plants within each of the four field replicates for the three varieties, Shambul, Durra, and Durra X Shambul, were subjected to duplicate amino acid analysis. They were subjected to analysis of variance using a split/split block design (Table V). Varieties X Replicates X Plots were divided into plants and then split further into duplicates. Four variance ratios (VRA, VRB, VRC, VRD) were calculated, each being the ratio of a particular mean square for a particular amino acid to the mean square for errors A, B, C, or D, where error A is Varieties X Replicates, error B is Varieties X Replicates X Plants, error C is Varieties X Replicates X Plants X Duplicates, and error D is the sum of errors A, B, and C (Table V).

The standard errors (SE), least significant differences (LSD), and coefficients of variation (CV) were calculated on the error D values. Values for tryptophan were not included. Replicate and Variety treatment means for each amino acid, with their LSD values at the 5 and the 1% levels were also calculated (Table VI).

TABLE V. ANALYSIS OF VARIANCE (AS MEAN SQUARES) OF AMINO ACID CONTENT OF SORGHUM KERNELS FROM THE HM TRIAL

Amino Acids	Replicates R (3)	Variety V (2)	Error A R×V (6)	Plants P (2)	Plants × Varieties (4)	Plants × Replicates (6)	Error B R×V×P (12)	Duplicate D (1)	Error C R×V×P×D (35)
Lysine	0.922*	0.0034	0.163*	0.049	0.0639	0.0734	0.0427**	0.0808*	0.0139
Histidine	0.269*	0.015	0.0666*	0.0027	0.0302	0.0233	0.0202**	0.0814**	0.0052
Arginine	0.926*	0.0356	0.147	0.0479	0.142	0.129	0.0922**	0.0171	0.0171
Aspartic acid	1.37*	2.08*	0.325*	0.0428	0.174	0.0555	0.088	0.149	0.0638
Threonine	0.053	0.0103	0.0163	0.0151	0.0200	0.0169	0.0147	0.0003	0.0084
Serine	0.779*	0.150	0.125	0.024	0.0129	0.0245	0.0915**	0.0153	0.0178
Glutamic acid	4.03*	0.249	0.559	0.211	0.369	0.420	0.272	0.107	0.164
Proline	1.37	0.953	0.704	0.779	0.575	0.555	0.535	0.119	0.294
Glycine	15.56	6.46	9.28*	0.461	0.126	0.699	0.839	0.384	0.479
Alanine	2.00	2.23	0.844*	0.0938	0.065	0.170	0.234**	0.0350	0.0439
½ Cystine	0.070**	0.115*	0.017	0.0016	0.0453	0.0112	0.0472	0.0226	0.0289
Valine	2.34*	1.35*	0.388*	0.131	0.0428	0.143	0.120	0.0017	0.0581
Methionine	0.482	0.126	0.255*	0.0902	0.170	0.150	0.0758	0.257	0.0780
Isoleucine	1.57*	1.90*	0.194	0.119	0.0597	0.0549	0.114**	0.0002	0.0364
Leucine	1.59	0.401	0.535	0.0071	0.582	0.491	0.226	0.0115	0.111
Tyrosine	0.0063	0.0005	0.0828*	0.0157	0.0549	0.0182	0.0200*	0.00003	0.0081
Phenylalanine	0.0972**	0.218**	0.0080	0.0345	0.0160	0.0196	0.0299	0.0111	0.0160

TABLE VI. VARIETY AND REPLICATE MEANS AND THEIR LSD VALUES FOR THE AMINO ACID COMPOSITION (MOLE %) OF SORGHUM KERNELS FROM THE HM TRIAL

	Variety Means					Replicate Means					
Amino Acid	Durra X Shambul	Durra	Shambul	LSD 5%	LSD 1%	Rep. I	Rep. II	Rep. III	Rep. IV	LSD 5%	LSD 1%
Lysine	1.80	1.78	1.80	0.121	0.159	1.61	1.80	1.66	2.11	0.139	0.184
Histidine	1.59	1.64	1.63	0.077	0.102	1.65	1.52	1.53	1.78	0.089	0.118
Arginine	2.74	2.67	2.71	0.143	0.189	2.73	2.63	2.46	3.00	0.165	0.218
Aspartic acid	7.15	7.49	6.91	0.181	0.240	6.87	7.54	7.13	7.19	0.209	0.277
Threonine	3.41	3.45	3.41	0.063	0.083	3.37	3.46	3.38	3.48	0.072	0.096
Serine	5.66	5.76	5.61	0.117	0.156	5.38	5.81	5.82	5.70	0.180	
Glutamic acid	19.38	19.46	19.26	0.292	0.387	19.39	19.37	19.93	18.77	0.337	0.447
Proline	9.02	9.01	9.36	0.378	0.500	8.73	9.36	9.27	9.15	0.436	0.578
Glycine	4.84	5.80	5.66	0.669	0.886	4.09	6.08	5.51	6.50	0.772	1.023
Alanine	13.89	14.25	13.65	0.235	0.311	13.91	14.03	14.29	13.49	0.271	0.359
½ Cystine	0.77	0.86	0.73	0.099	0.132	0.70	0.79	0.83	0.84	0.115	0.152
Valine	4.77	4.49	4.96	0.189	0.251	5.24	4.52	4.43	4.78	0.219	0.290
Methionine	0.95	1.09	1.06	0.190	0.252	1.10	0.79	1.14	1.11	0.220	0.291
Isoleucine	3.33	2.95	3.51	0.153	0.202	3.68	3.08	3.04	3.24	0.176	0.233
Leucine	12.96	12.82	13.07	0.276	0.366	13.33	12.66	13.04	12.78	0.319	0.423
Tyrosine	2.81	2.82	2.82	0.083	0.111	2.84	2.81	2.80	2.82	0.096	0.128
Phenylalanine	3.79	3.68	3.87	0.079	0.104	3.89	3.74	3.77	3.73	0.091	0.120

In broad summary: There are no significant differences in amino acid composition found between duplicate hydrolyses, or between plants; almost all amino acids show significant differences between replicates; and a number (especially aspartic acid, serine, glycine, alanine, one-half cystine, valine, isoleucine, and phenylalanine) between varieties.

Consideration of the replicate means confirms the significance of the replicate differences. For all amino acids except tyrosine and one-half cystine, at least three out of the six possible differences (four replicates) are significant; and except for methionine and phenylalanine, four, five, or even six are significant. The variety means confirm the variation between varieties. Out of the three possible differences (three varieties), all three were significant for lysine, histidine, arginine, threonine, glutamic acid, proline, methionine, leucine, and tyrosine; and two out of three for phenylalanine, aspartic acid, alanine, valine, and isoleucine. For all amino acids, replicate I showed the lowest figure; and in 15 out of 17 cases, replicate III showed the highest, and replicate II the 2nd position, while replicate IV occupied third position in 16 cases. Thus, the order III > II > IV, I was clearly established.

These experiments have established statistically that there is a significant variation in the amino acid composition of different varieties of sorghum. In addition, the significant variation between replicates indicates that growing conditions even between experimental plots within one location are capable of inducing a variation. Since this experiment was designed to eliminate differences of growing conditions as far as possible, the outcome needs further consideration. In the field, there was no evidence of variation in the availability of water, nutrient, or fertilizer which might be responsible; but clearly, some such factor must have been at work.

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