Amino Acid Composition of the Cereal Tef and Related Species of *Eragrostis* (Gramineae)

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ABSTRACT

The amino acid composition of seed proteins was determined for 11 accessions of the Ethiopian cereal *Eragrostis tef* and for 10 accessions of related wild species (*E. barrelieri, E. bicolor, E. cilianensis, E. curvula, E. diplachnoides, E. mexicana, E. minor, E. papposa,* and *E. pilosa*). The 11 cultivars of tef were fairly similar to each other but quite distinct from the various wild species. In general, the wild species had smaller seeds and a higher proportion of protein, but the cultivated tef had more lysine. The amount of lysine in the protein was positively correlated with the amounts of glycine, arginine, aspartic acid, and threonine but negatively correlated with glutamic acid, isoleucine, leucine, and proline. The seeds of the wild species possibly contain more of a protein rich in glutamic acid but of little nutritional value.

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The cereal tef (*Eragrostis tef* (Zucc.) Trotter), an ancient cultigen, is indigenous to Ethiopia and as yet is seldom grown elsewhere. The genus *Eragrostis* comprises about 300 species, the majority of which are found in East Africa, although many others occur in South Africa.

Although the seeds of tef are small (≤ 0.002 g), they constitute an important part of the staple diet of Ethiopians, particularly in urban areas. Their protein content ranges from 6 to 10%, and they are high in iron and calcium.

At least 35 cultivars of tef have been recognized (Ebba 1975), but so far little scientifically based plant breeding has been achieved, and the genetic resources of the germplasm of both the cultivars and the related wild species are largely untapped. The present work presents information on their nutritive value, which is essential to any modern breeding program, especially in a staple crop for human consumption.

Amino acid analyses were made by Jansen et al (1962), but because they used very few samples and these were unidentified varieties of tef bought from markets, their data are of little value for plant breeding and other studies. Analyses of cultivated tef have also been made recently by Tareke,[3] but wild species were not studied. The present work attempts to remedy these weaknesses of previous investigations by studying the variation in protein and amino acid content both within and between cultivars of tef and wild species of *Eragrostis*. The information produced should be valuable for planning future breeding work.

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MATERIALS AND METHODS

Seed of 11 cultivars of tef and 10 wild species of *Eragrostis* were obtained from two sources. All the tef cultivars were provided by

TABLE I
Amino Acid Composition (percent of protein recovered), Protein Composition (percent of seed flour), and Average Weight (mg) and Size (mm^2) of Seeds of Tef Cultivars and Some Wild *Eragrostis* Species

	Amino Acids																		Seed	
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Leu	Tyr	Phe	His	Lys	Arg	Protein	Weight	Size[a]
E. tef																				
T1	6.98	4.36	5.23	23.98	5.23	4.07	5.96	0.73	5.38	4.22	4.07	8.72	3.63	5.67	2.76	3.92	5.09	6.88	1.53	0.63
T23	6.56	4.25	5.22	24.79	5.47	3.77	5.71	0.85	5.47	4.13	4.01	8.51	3.89	5.71	3.16	3.52	4.98	8.23	1.88	0.56
T45	6.61	4.37	5.16	24.89	6.28	3.81	5.72	0.90	5.38	3.14	4.15	8.74	4.04	5.83	3.25	3.59	4.71	8.92	0.40	0.59
T87	6.72	4.27	5.34	23.05	5.80	3.97	5.80	1.07	5.65	4.58	3.82	8.40	3.66	5.65	3.05	3.82	5.34	6.55	0.88	0.63
T101	6.81	4.36	5.31	23.57	5.18	4.09	5.86	1.09	5.45	4.36	3.81	8.31	3.68	5.59	3.27	3.95	5.18	7.34	0.44	0.30
T105	6.80	4.33	5.56	23.80	5.41	4.02	6.03	0.93	5.10	4.48	4.17	8.66	3.86	5.87	3.55	2.94	4.48	6.47	0.88	0.38
T108	6.17	4.34	5.14	24.46	4.69	4.00	5.83	1.03	5.94	3.66	4.11	8.80	3.89	5.71	3.31	3.43	5.26	8.75	0.96	0.41
T111	6.85	4.25	5.21	23.84	5.07	3.84	5.75	0.96	5.34	4.11	3.97	8.36	3.97	5.75	3.70	3.97	4.93	7.30	0.48	0.45
T112	6.52	4.31	5.12	23.86	5.82	3.96	5.70	1.05	5.94	3.84	4.07	8.50	3.84	5.70	3.26	3.73	5.01	8.59	0.40	0.44
T119	6.89	4.31	5.06	23.36	5.92	3.98	5.49	0.97	5.17	3.88	3.88	8.40	3.98	5.38	3.23	3.77	6.24	9.29	0.48	0.56
T138	7.15	4.38	5.26	23.36	5.70	4.09	5.84	1.02	5.26	4.23	3.94	8.47	3.80	5.69	2.77	3.80	5.40	6.85	1.43	0.53
Wild *Eragrostis*																				
barrelieri	5.32	3.59	4.81	29.12	6.48	2.95	5.52	0.90	5.45	4.23	4.36	9.36	3.72	6.03	2.57	2.05	3.46	15.59	1.36	0.35
bicolor	5.30	3.91	4.61	28.96	5.83	3.22	5.30	0.96	5.48	4.17	4.09	8.26	4.70	5.57	3.13	2.43	4.09	11.50	0.72	0.33
cilianensis 2x	6.29	3.55	4.83	28.69	6.29	2.50	5.88	0.89	5.56	3.30	4.43	9.43	4.60	6.61	2.74	1.77	2.58	12.41	0.23	0.38
cilianensis 4x	5.48	3.36	4.51	30.48	6.71	2.47	5.74	0.62	5.74	3.09	4.86	9.28	4.77	6.45	2.30	1.94	2.30	11.32	0.20	0.10
curvula	5.64	3.52	4.33	30.74	6.05	2.94	5.15	1.14	5.48	3.35	4.33	9.00	4.25	5.56	2.53	2.21	3.76	12.23	0.14	0.47
diplachnoides	6.80	4.22	5.25	30.28	7.21	2.88	5.05	0.72	5.05	2.99	4.53	9.37	4.84	4.84	2.27	1.96	1.96	9.71	0.96	0.18
mexicana	5.83	3.92	4.83	26.34	6.47	2.83	5.74	1.19	5.29	3.83	4.10	8.93	6.29	5.83	2.73	2.37	3.37	10.97	1.10	0.39
minor	5.15	3.41	4.28	31.02	6.31	2.49	5.32	1.10	5.56	3.53	4.46	9.26	4.05	6.42	2.95	1.62	3.01	17.28	1.18	0.25
papposa	5.86	3.62	5.48	30.11	6.13	3.07	5.11	1.12	5.02	4.28	4.18	8.92	4.37	5.58	2.42	1.95	2.97	10.76	1.06	0.34
pilosa	6.04	4.06	4.63	27.67	5.95	3.21	5.38	1.13	5.48	3.40	4.25	8.22	4.72	5.95	2.64	2.83	4.25	10.59	1.02	0.24

[a]Area of maximum profile of seed in square millimeters.

the Debrezeit Experimental Station of the Addis Ababa University, Ethiopia, and the wild *Eragrostis* species were provided by the Department of Botany, Royal Holloway College, London Univeristy, U.K. The identification of the wild species has been confirmed on the basis of work by Clayton et al (1974).

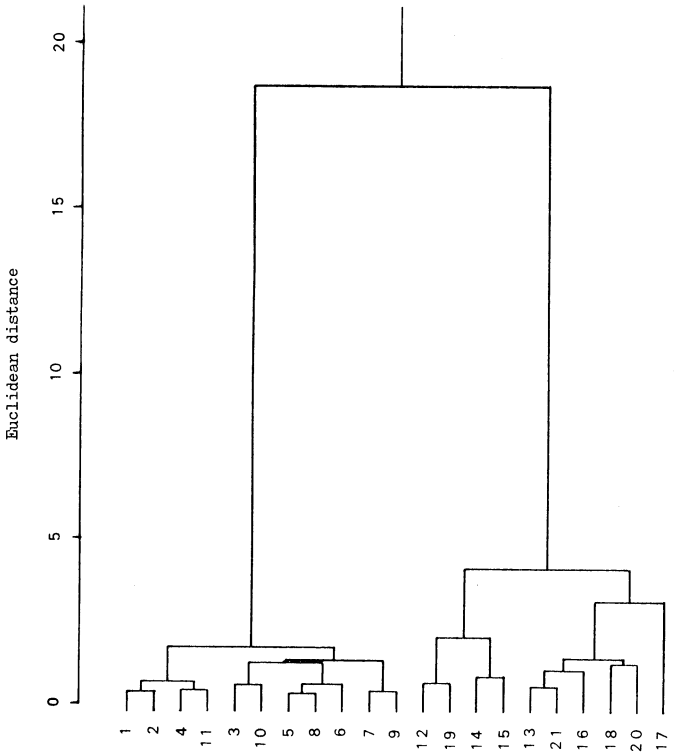

Fig. 1. Dendogram showing clustering of 21 accessions of tef and wild *Eragrostis* species based on amino acid composition, protein, and weight and size of the seed. (Euclidean distance squared and Ward's method). *E. tef:* **1,** T1; **2,** T23; **3,** T45; **4,** T87; **5,** T101; **6,** T105; **7,** T108; **8,** T111; **9,** T112; **10,** T119; and **11,** T138. *Eragrostis* species: **12,** *E. barrelieri;* **13,** *E. bicolor;* **14,** *E. cilianensis* 2x; **15,** *E. cilianensis* 4x; **16,** *E. curvula;* **17,** *E. diplachnoides;* **18,** *E. mexicana;* **19,** *E. minor;* **20,** *E. papposa;* **21,** *E. pilosa.*

For amino acid analysis, seeds were pulverized, and samples were hydrolyzed under vacuum in 6*M* HCl at 110° C for 24 hr. The hydrolysates were then neutralized with 2*M* NaOH and analyzed with a Locarte amino acid analyzer, using a single column for acidic, neutral, and basic amino acids. Tryptophan is entirely destroyed by acid hydrolysis and was not measured. Up to 50% of the cystine may be lost on hydrolysis, but this effect should be similar for all samples. For most amino acids, the assay error is about ±2% of the values given. The amount of each amino acid was initially expressed as a percentage of the fresh weight of the seed flour; because the amino acid recovery was over 95% (except for cystine), the sum of these values represented the protein content with little error. Subsequently the content of each amino acid was expressed as a percentage of the protein (Table I). Unfortunately only one sample of each accession was analyzed: replication was financially impossible.

Direct measurement of tef seeds is difficult because they are very small. Measurements were therefore taken from scanning electron micrographs at comparable magnifications, and the total area in maximum profile was called the seed size. Seeds of different species vary considerably in size and shape. Single-seed weights were derived from 100-seed weights.

The results were analyzed in two ways, using the CLUSTAN 1C numerical taxonomy computer package (Wishart 1978). Similarities of accessions according to their amino acid composition and other characters were calculated using Euclidean distance squared (d^2), and clustering (Fig. 1) was achieved by Ward's method (Clifford and Stephenson 1975). Correlation coefficients between all the amino acids and other characteristics were computed (Table II).

RESULTS AND DISCUSSION

Considerable variation in amino acid composition and other qualities was found between the seeds of *E. tef* and those of the related species (Table I).

Cluster analysis (Fig. 1) showed a clear distinction between the cultivars of tef and the wild species; the former were more tightly clustered than the latter, indicating less variation between the cultivars of tef than between the various wild species of *Eragrostis*. The clustering pattern is closely related to the content of protein and some of the amino acids (Table I). Differences between the cultivars of tef and the wild species were obvious, yet *t*-tests showed

TABLE II
Correlation Coefficients of Amino Acid and Protein Composition and Weight and Size of Seeds of *Eragrostis*

	Amino Acids																	Seed	
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ileu	Leu	Tyr	Phe	His	Lys	Arg	Protein	Weight	Size
Asp	1.00																			
Thr	0.84	1.00																		
Ser	0.78	0.72	1.00																	
Glu	−0.79	−0.87	−0.67	1.00																
Pro	−0.40	−0.56	−0.40	0.69	1.00															
Gly	0.76	0.90	0.70	−0.92	−0.76	1.00														
Ala	0.49	0.45	0.40	−0.72	−0.54	−0.52	1.00													
Cys	−0.17	−0.01	−0.10	−0.09	−0.23	0.08	−0.18	1.00												
Val	−0.27	−0.11	−0.37	−0.06	−0.24	−0.00	0.28	0.04	1.00											
Met	0.27	0.36	0.54	−0.54	−0.57	0.58	0.35	0.27	−0.17	1.00										
Ileu	−0.58	−0.73	−0.56	0.81	0.67	−0.82	−0.34	−0.46	0.10	−0.71	1.00									
Leu	−0.44	−0.67	−0.34	0.69	0.65	−0.75	−0.23	−0.35	−0.02	−0.52	0.80	1.00								
Tyr	−0.39	−0.37	−0.37	0.45	0.55	−0.63	−0.28	0.11	−0.18	−0.43	0.38	0.28	1.00							
Phe	−0.45	−0.57	−0.47	0.24	0.08	−0.45	0.36	−0.04	0.42	−0.18	0.44	0.35	0.07	1.00						
His	0.40	0.60	0.37	−0.72	−0.72	0.69	0.51	0.20	0.15	0.43	−0.64	−0.61	−0.43	−0.04	1.00					
Lys	0.76	0.87	0.58	−0.94	−0.71	0.94	0.56	0.06	0.11	0.46	−0.81	−0.77	−0.54	−0.35	0.66	1.00				
Arg	0.59	0.76	0.42	−0.88	−0.76	0.91	0.47	0.25	0.13	0.54	−0.86	−0.80	−0.58	−0.29	0.70	0.92	1.00			
Protein	−0.86	−0.85	−0.78	0.82	0.57	−0.83	−0.54	0.11	0.12	−0.41	0.63	0.64	0.31	0.47	−0.46	−0.81	−0.64	1.00		
Weight	0.07	0.20	0.25	−0.12	−0.14	0.16	0.04	−0.03	−0.23	0.42	−0.20	−0.05	−0.11	−0.13	−0.11	0.08	0.12	−0.04	1.00	
Size	0.54	0.54	0.41	−0.68	−0.46	0.67	0.39	0.13	−0.02	0.43	−0.70	−0.42	−0.45	−0.23	0.44	0.69	0.73	−0.50	0.21	1.00

few of these differences to be significant, partly because of the small sample size and the large variance within the groups. The tef cultivars have less protein (6.47–9.29%, $P < 0.2$) but higher proportions of lysine ($P < 0.05$), arginine ($P < 0.2$), glycine ($P < 0.02$) aspartic acid ($P < 0.3$), threonine ($P < 0.1$), serine ($P < 0.4$), and histidine ($P < 0.3$). The wild species have more protein (9.71–17.28%, $P < 0.2$) and higher proportions of glutamic acid ($P < 0.02$), isoleucine ($P < 0.4$), leucine ($P < 0.5$), tyrosine ($P < 0.4$) and proline ($P < 0.4$). The highest protein contents were found in *E. minor* (17.28%) and *E. barrelieri* (15.59%). Highest proportions of lysine (about 3.8% of the protein) were found in the T1, T87, T101, T111, T138 cultivars of *E. tef*. The differences between the wild species and the cultivars are probably meaningful but may have been influenced by different cultural conditions at the two sources of the seed.

Protein content was negatively correlated with seed size (Table II, r = −0.50), suggesting that selection for larger seeds in the cultivated varieties of tef has increased the proportion of starch and thereby reduced the proportion of protein. This is of considerable significance to breeding programs aimed at improving the protein content of tef (Bekele 1978). If the endosperm is mostly starch and most of the protein is in the embryo, then breeding programs might use selection for larger embryos in relation to endosperm in order to obtain higher protein content.

The correlation coefficients between the proportions of each amino acid, the percentage of protein, and the size of the seed showed some remarkably high values (Table II), but seed weight showed relatively low correlation with any other characteristic. Very high correlations were shown between lysine and glycine (r = 0.94), arginine (r = 0.92), threonine (r = 0.87), aspartic acid (r = 0.76), histidine (r = 0.66), serine (r = 0.58), alanine (r = 0.56) and methionine (r = 0.46). However, lysine showed much lower correlation values with valine (r = 0.11) and cystine (r = 0.06) and very high negative values with glutamic acid (r = −0.94) and some other amino acids.

Glutamic acid showed high correlations with isoleucine (r = 0.81), leucine (r = 0.69), and proline (r = 0.69) and lower correlations with tyrosine (r = 0.45) and phenylalanine (r = 0.24). Cystine, valine, and weight showed little correlation with either lysine or glutamic acid or with each other.

The high positive correlation of protein with glutamic acid and their high negative correlation with lysine is very interesting. Glutamic acid is one of the main components of *Eragrostis* seed proteins, constituting approximately 23–25% of the protein in tef and 26–31% in the wild species. Corresponding values for isoleucine, leucine, and proline are 3.8–4.1 and 4.1–4.8%, 8.3–8.8 and 8.3–9.4%, and 4.7–6.3 and 5.8–7.2% of the protein, respectively. The protein was 6.5–9.3 and 9.7–17.3% of the seed fresh weight. The wild species, which have a higher protein content, also appear to have higher proportions of these four amino acids. During domestication of tef selection has somehow been made against certain amino acids, including glutamic acid, resulting in an increase in the proportion of other amino acids, including lysine (from about 1.6–2.4 to 3.0–4.0% of the protein). This is of great nutritional significance. The average amount of glutamic acid in the seed flours of the 10 wild species was calculated as 3.59%; in the 11 cultivars it was 1.85%. The corresponding values for lysine were 0.26 and 0.28%.

Further work is needed to determine whether the correlations of amino acids are due to the presence of a few major proteins that should be selected appropriately in plant breeding projects. Prolamins and glutelins are the main protein constituents of most cereals (Rhodes and Jenkins 1978). Because prolamins contain only small amounts of lysine, changes in the relative amounts of this protein fraction, and specifically the partial suppression of prolamin synthesis, may result in higher lysine, as in *Hordeum* (Miflin and Shewry 1979) and in *Sorghum* (Paulis and Wall 1979).

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