

# The Amino Acid Composition of Whole Sorghum Grain in Relation to Its Nitrogen Content

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## ABSTRACT

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Sorghum grains (12 samples from seven different lines or hybrids) with total nitrogen, *N*, content ranging from 1.5 to 3 g/100 g grain dry matter were accurately analyzed for their amino acid composition from six different hydrolysates per sample. Amino acid levels in grain increased as linear functions of *N*, with correlation coefficients close to one for most amino acids regardless of sorghum genotype or phenotype. As a result, the amino acid composition of any grain sample of normal sorghum can be predicted from its *N*. Amino acids in grain protein changed as hyperbolic functions of *N*, which increased for alanine, leucine, isoleucine,

phenylalanine, and glutamine plus glutamic acid, remained constant for serine, tyrosine, tryptophan, and asparagine plus aspartic acid, and decreased for the other amino acids. The nonprotein-to-total nitrogen ratio remained practically constant and close to 5%, and the nitrogen-to-protein conversion factor ( $k_A$ ) was close to 5.81 within the *N* range investigated. The results also showed that the composition of storage proteins accumulated in sorghum grain remained constant, with the rate of deposition of kafirins roughly 1.5 times that of glutelins.

Since 1970, sorghum has represented only about 4% of total world cereal grain production. However it ranks fifth behind wheat, corn, rice, and barley because it is widely used as food in many countries of Africa and Asia, and as major feed grain in United States, where it ranks third behind corn and wheat, reaching 8% of cereal grain production (FAO 1986). Furthermore, the discovery of the high-lysine mutant gene by Singh and Axtell (1973) has stimulated much interest in improvement of protein quality and quantity.

As in cereal grains, the total nitrogen *N* content (on a dry basis) of sorghum ranges from about 1 to at least 3% (Singh and Axtell 1973). Several studies have investigated amino acid compositions of normal sorghum genotypes (Ajakaiye 1984, Axtell et al 1975, Bressani and Rios 1962, Deosthale et al 1970, Deyoe and Shellenberger 1965, Haikerwal and Mathieson 1971, Hosney et al 1974, Jambunathan and Mertz 1973) and of high-lysine mutants (Guiragossian et al 1978, Hassen et al 1986, Paulis and Wall 1979). The influence of location or of fertilizers has been studied by Ajakaiye (1984), Deyoe and Shellenberger (1965), Eppendorfer et al (1985), and Waggle et al (1967). The amino acid composition of one or more sorghum samples has been determined for other purposes by Busson et al (1966), Chibber et al (1978), FAO (1970), Jones and Beckwith (1970), Pedersen and Eggum (1983), Pion (1971), Skoch et al (1970), Waggle et al (1966), and Wu and Wall (1980). Several of these publications have been reviewed by Chung and Pomeranz (1985), Hosney et al (1981), and Wall and Paulis (1978).

A careful analysis of this literature shows that the influence of total sorghum grain *N* on amino acid composition still calls for clarification. Many authors agree that, for a given genotype, amino acid composition can change as a function of *N*, but few (Singh and Axtell 1973, for instance) take this into account in comparing different genotypes. Relationships between amino acids and *N* were first examined by Waggle and Deyoe (1966), who showed that amino acid level in sorghum grain is linearly correlated with *N*. This means that amino acids in protein change according to quadratic relationships as a function of *N*. However, Eppendorfer et al (1985) concluded that it is not linearly correlated with *N*. The influences of genotype, culture conditions, and environment remain uncertain. However, linear relations between *N* and the levels of each amino acid in seeds, with correlation coefficients often higher than 0.99, have been shown for normal genotypes and phenotypes of wheat (Mossé et al 1985), rye, corn (Baudet et al

1987, 1986b), and legume seeds such as broad bean (Baudet and Mossé 1980), pea (Huet et al 1987), and lupin (Mossé et al 1987). These studies show that the changes of some amino acids in protein can be considerable. For example, proteins of corn grain with *N* = 1% are 50% richer in glycine and tryptophan and 60% richer in lysine than proteins of corn with *N* = 3% (Baudet et al 1986b). When *N* is doubled in pea seed from 3 to 6%, the histidine level in seed proteins remains constant, arginine increases by 53%, and tyrosine and cystine decrease by 20 and 33%, respectively (Huet et al 1987).

A similar study was thus undertaken with sorghum, using grain samples covering a wide *N* range and employing amino acid analysis conditions of highest possible accuracy.

## MATERIALS AND METHODS

### Sorghum Samples

The 12 grain samples analyzed corresponded to seven *Sorghum bicolor* (L.) Moench genotypes from field-grown sorghums. One cultivar (Monitor) was grown under different conditions in six locations. The other samples were inbred lines chosen for their wide *N* distribution. Grain sampling (from 1 kg), milling, and meal subsampling for analysis were performed as described previously (Mossé et al 1985).

### Analytical Methods

Dry matter content of whole grain meal samples was determined gravimetrically (in triplicate) by heating at 105°C for 24 hr; nitrogen was determined (in triplicate) by micro-Kjeldahl analysis. Amide nitrogen of asparagine plus glutamine was determined as free NH<sub>3</sub> after a separate hydrolysis (3 hr in 2M HCl at 115°C) according to Sörensen (1973). Amino acids from five hydrolysates were analyzed by single-column ion-exchange chromatography. Four hydrolysates (15, 24, and 48 hr, plus an 18-hr hydrolysis of a sample previously oxidized by performic acid) were prepared with boiling 6M HCl to correct for partial degradation or incomplete release. Results of analyses of stable amino acids (glycine, alanine, leucine, phenylalanine, proline, lysine, arginine, aspartic acid, and glutamic acid), were used when overall variation was less than 3%. Otherwise aliquots of hydrolysates were reanalyzed or new hydrolyses were performed. Along with consistent values for stable amino acids, 15-hr results were used for serine, threonine, and tyrosine, and 48-hr results for valine and isoleucine. For sulfur amino acids, analysis of the 18-hr hydrolysate was performed in duplicate. Alkaline Ba(OH)<sub>2</sub> hydrolysis was used to determine tryptophan (in triplicate); overall variation was less than 4%. A defatted soybean preparation was used to monitor reproducibility; commercial egg-white lysozyme (Merck) and purified human serum albumin (NBC) were used to check the accuracy of amino

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acid determinations. A previous paper (Mossé et al 1985) describes all details of the methods used.

### Expression of Amino Acid Composition

In the present work,  $N$  refers to grain nitrogen content (in grams of nitrogen per 100 g of grain dry matter).

Moreover the results for the  $i$ th amino acid were expressed in five different ways as follows:  $C_i$ , grams of amino acid per 16 g of nitrogen;  $A_i$ , grams of amino acid per 100 g of grain dry matter;  $B_i$ , moles of amino acid per 100 g of grain dry matter;  $D_i$ , grams of amino acid nitrogen per 100 g of grain dry matter; and  $E_i$ , grams of amino acid residue per 100 g of grain dry matter. A residue corresponds to the anhydrous form of an amino acid, i.e., to the entity that occurs within a polypeptide chain (-HN-CHR-CO-).

The expressions  $A_i$  to  $E_i$  are related to each other as follows:

$$B_i = A_i / M_i \quad (1)$$

$$C_i = 16 \times A_i / N \quad (2)$$

$$D_i = 14 \times n_i \times A_i / M_i \quad (3)$$

$$E_i = (M_i - 18.016) \times A_i / M_i \quad (4)$$

where  $M_i$  is the molecular weight of the  $i$ th amino acid, and  $n_i$  the number of nitrogen atoms per molecule ( $M_i - 18.016$  giving the molecular weight of the  $i$ th residue).

## RESULTS

### Nitrogen Content and Amino Acid Composition of Sorghum Grain

For the six samples of Monitor,  $N$  ranged from 1.67 to 2.06; for the whole set of samples  $N$  ranged from about 1.5 to 3 (Table I). The experimental results were first obtained as grams of amino acid per 100 g of dry grain ( $A_i$ ). From these results can be deduced those including grams of amino acid per 16 g of nitrogen ( $C_i$ ) as reported in Table I.

Figure 1 shows examples of the relations of amino acid contents ( $A_i$ ) in grain to  $N$ . They can be described by linear equations such as:

$$A_i = a_i \times N + b_i \quad (5)$$

where  $a_i$  is the slope of the line for the  $i$ th amino acid, and  $b_i$  its  $A_i$  intercept. Table II indicates values of  $a_i$  and  $b_i$  obtained by linear regression analysis (as in Fig. 1), and the correlation coefficients ( $r_i$ ):  $r_i \geq 0.99$  for 11 of 19 determinations, and ranges from 0.962 to 0.983 for glycine, tryptophan, histidine, and asparagine plus aspartic acid (Asx). Only for methionine, cysteine, and lysine does  $r_i$  range from 0.876 to 0.900. It is not by chance that the analyses of these three amino acids (and tryptophan) are also always less accurate.

### Variation of Total Grain Protein Composition

The level  $C_i$  of each amino acid  $i$  in total grain protein (g/16 g of nitrogen) is obtained by combining equations (2) and (5). It follows that

$$C_i = 16 \times a_i + 16 \times b_i / N \quad (6)$$

Thus,  $C_i$  is a quadratic function of  $N$ , represented by a segment of an equilateral hyperbola of which the asymptotes are the vertical  $C_i$  axis, and the horizontal line  $C_i = 16 \times a_i$ . The extent of  $C_i$  variation depends on the magnitude of the ratio  $b_i/a_i$  (Table II), and the orientation of the concavity of hyperbola segments has the sign of this ratio (i.e., of  $b_i$ , since  $a_i$  is always positive). On this basis, amino acids may be ranged in three groups. For serine, tyrosine, tryptophan, and Asx, and even for isoleucine and proline  $b_i/a_i \sim 0$ , so  $C_i$  is practically constant ( $C_i \sim 16 \times a_i$ ). Table II shows that for glycine, valine, threonine, methionine, cysteine, lysine, arginine, and histidine,  $b_i/a_i > 0$ , so their levels in protein decrease with increasing  $N$ . An example for lysine is shown in Figure 2. In contrast, concentrations ( $C_i$ ) of alanine, leucine, phenylalanine, and glutamine plus glutamic acid (Glx) increase as a function of  $N$  ( $b_i/a_i < 0$ ) (Table II).

### Recovery of the Amino Acid Analysis and Nonprotein Nitrogen in Sorghum Grain

The only accurate expression of analytical yield is the ratio of nitrogen recovered from amino acids to total nitrogen of the sample analyzed. Thus, both amino acids and amide nitrogen must be determined. Nitrogen recoveries (R) of analyses are indicated in

TABLE I  
Amino Acid Composition of Sorghum Samples (g/16 g nitrogen)

Component	Variety <sup>a</sup>											
	1	2	3	4	5	6	7	8	9	10	11	12
$N^b$	1.51	1.67	1.74	1.86	1.86	1.88	2.06	2.25	2.34	2.66	2.86	2.97
Glycine	3.4	3.15	3.05	3.05	3.1	2.9	2.85	2.8	3.0	2.75	2.55	2.5
Alanine	9.1	9.2	9.2	9.4	9.5	9.1	9.6	9.5	9.3	9.8	9.7	10.0
Valine	5.2	5.5	5.3	5.6	5.6	5.2	5.6	5.3	5.3	5.3	5.2	5.1
Leucine	12.7	13.9	13.6	13.8	14.2	13.6	14.4	13.8	13.3	14.2	14.1	14.7
Isoleucine	4.1	4.3	4.2	4.3	4.4	4.1	4.3	4.2	4.2	4.4	4.3	4.4
Serine	4.7	4.6	4.5	4.6	4.7	4.5	4.5	4.7	4.6	4.7	4.5	4.5
Threonine	3.35	3.25	3.15	3.3	3.3	3.2	3.15	3.2	3.2	3.2	3.1	3.0
Tyrosine	3.9	4.2	4.1	4.2	4.3	3.9	4.3	3.9	4.0	4.0	4.0	4.1
Phenylalanine	5.2	5.5	5.4	5.5	5.5	5.2	5.4	5.5	5.3	5.5	5.6	5.7
Tryptophan	1.08	1.03	1.00	1.01	1.11	0.84	1.00	1.05	1.05	1.02	1.00	1.00
Proline	8.1	8.8	8.5	8.6	8.9	8.2	8.9	8.3	8.1	8.4	8.3	8.4
Methionine	2.00	1.65	1.55	1.60	1.70	1.35	1.55	1.35	1.65	1.60	1.40	1.40
Half cystine	2.15	1.90	1.70	1.75	1.90	1.75	1.65	1.85	1.90	1.60	1.45	1.50
Lysine	2.5	2.3	2.3	2.3	2.3	2.1	2.15	2.05	2.3	1.95	1.95	1.65
Histidine	2.3	2.2	2.15	2.25	2.3	2.2	2.15	2.3	2.1	2.05	2.0	2.0
Arginine	4.1	4.0	4.0	4.0	4.1	3.8	3.8	3.7	4.2	3.7	3.7	3.1
Asx <sup>c</sup>	6.7	7.0	7.0	7.0	6.9	6.7	7.0	7.7	7.3	7.3	7.1	6.7
Glx <sup>c</sup>	20.3	21.0	20.8	21.4	21.7	20.8	22.0	22.4	21.2	22.6	22.3	22.9
Amide NH <sub>3</sub>	2.45	2.7	2.7	2.75	2.7	2.5	2.85	2.85	2.65	2.95	2.85	3.05
R <sup>d</sup>	92.9	95.3	94.0	95.7	97.3	91.1	96.1	95.6	94.1	96.0	94.2	94.6

<sup>a</sup> 1, BIL 1; 2 to 7, Monitor; 8, SA 10015; 9, F3OR; 10, IS 2926; 11, Iowar Man Majra; 12, B 4359.

<sup>b</sup>  $N$  = Grain nitrogen content (g/100 g dry matter).

<sup>c</sup> Asx and Glx were evaluated in grams of aspartic acid and glutamic acid.

<sup>d</sup> Percentage of nitrogen recovered by amino acid analysis.

Table I.  $R$  depends on  $N$  (Mossé et al 1985), and can be calculated from the amino acid level  $D_i$  (expressed in grams of amino acid nitrogen per 100 g of grain dry matter) provided by equation (3).

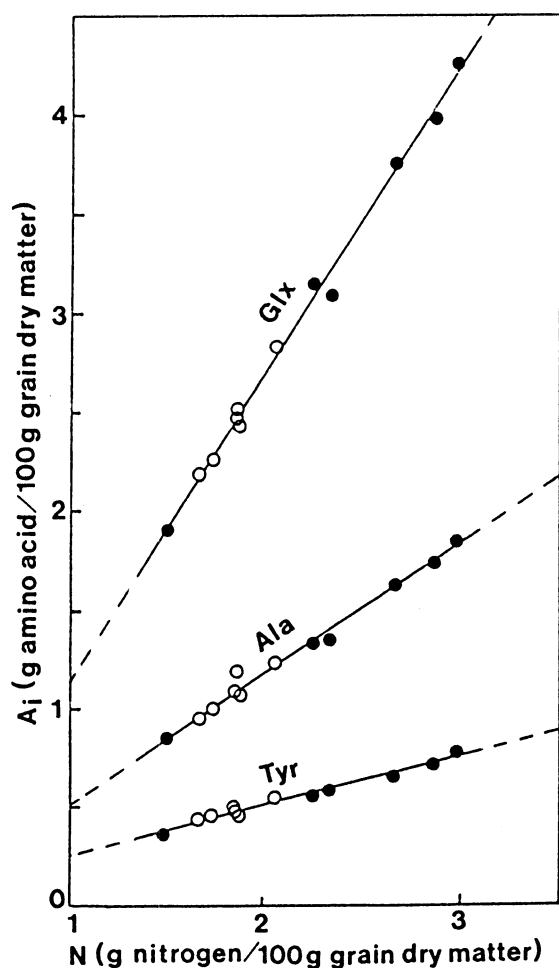


Fig. 1. Relationships between glutamine plus glutamic acid (Glx) alanine (Ala) and tyrosine (Tyr) levels in sorghum grain ( $A_i$ ) and total nitrogen content ( $N$ ).  $\circ$  = Cultivar Monitor;  $\bullet$  = all other cultivars.

Like  $A_i$ ,  $D_i$  is a linear function of  $N$ . The same is true for the sum:

$$\Sigma D_i = p \times N + q \quad (7)$$

for all amino acids and amide nitrogen. The ratio of this sum (giving nitrogen recovered from analyses in grams per 100 g of grain dry matter) to  $N$  determined by the Kjeldahl procedure provides the value of the analytical recovery (Mossé et al 1985):

$$R = p + q/N \quad (8)$$

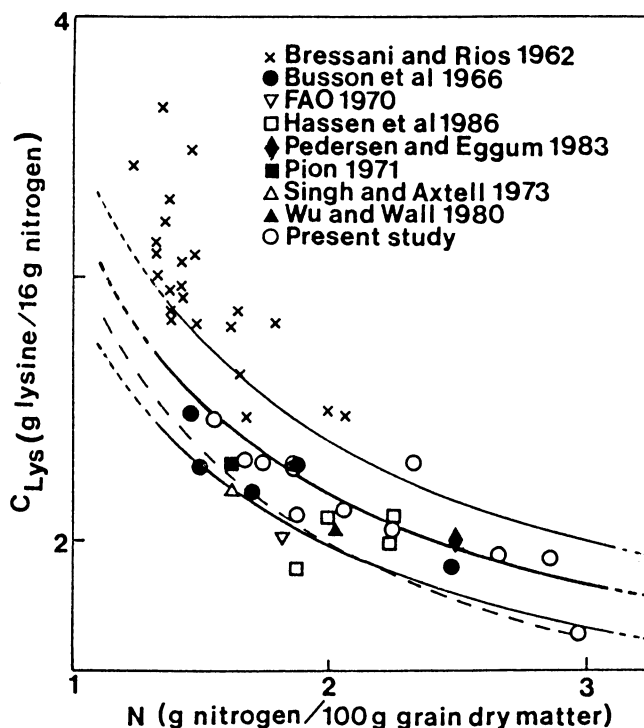


Fig. 2. Variation of lysine in sorghum protein ( $C_{Lys}$ ) as a function of total nitrogen content  $N$  of grain. Solid line = Predicted values  $\pm$  half standard deviation; dotted line = predicted values from Waggle and Deyoe (1966). Our experimental values are indicated by open circles; all other results are from the literature.

TABLE II  
Slope  $a_i$ , intercept  $b_i (\pm SD)$ , Correlation Coefficient  $r_i$  Between  $A_i$  and  $N$ , and Correlation Coefficient  $r'_i$  Between  $C_i$  and  $N$

Component	$a_i \pm SD$	$b_i \pm SD$	$r_i^a$	$r'_i^b$	$r'_i^c$	$b_i/a_i$
Glycine	0.110 $\pm$ 0.010	0.148 $\pm$ 0.022	0.962	-0.90	-0.87	1.34
Alanine	0.666 $\pm$ 0.015	-0.156 $\pm$ 0.032	0.998	0.85	0.72	-0.23
Valine	0.306 $\pm$ 0.012	0.056 $\pm$ 0.026	0.993	-0.47	-0.07	0.18
Leucine	0.960 $\pm$ 0.035	-0.192 $\pm$ 0.076	0.994	0.60	0.45	-0.20
Isoleucine	0.283 $\pm$ 0.007	-0.034 $\pm$ 0.016	0.997	0.52	0.82	-0.12
Serine	0.280 $\pm$ 0.007	0.014 $\pm$ 0.015	0.997	-0.28	-0.01	0.05
Threonine	0.176 $\pm$ 0.006	0.049 $\pm$ 0.013	0.995	-0.79	-0.78	0.28
Tyrosine	0.250 $\pm$ 0.011	0.010 $\pm$ 0.025	0.990	-0.09	0.44	0.04
Phenylalanine	0.368 $\pm$ 0.011	-0.059 $\pm$ 0.024	0.995	0.57	0.67	-0.16
Tryptophan	0.062 $\pm$ 0.005	0.004 $\pm$ 0.011	0.969	-0.11	-0.39	0.06
Proline	0.501 $\pm$ 0.021	0.054 $\pm$ 0.046	0.991	-0.29	0.20	0.11
Methionine	0.071 $\pm$ 0.011	0.054 $\pm$ 0.024	0.900	-0.55	-0.23	0.76
Half cystine	0.063 $\pm$ 0.011	0.095 $\pm$ 0.024	0.876	-0.76	-0.48	1.51
Lysine	0.071 $\pm$ 0.012	0.131 $\pm$ 0.026	0.883	-0.88	-0.79	1.85
Histidine	0.105 $\pm$ 0.007	0.061 $\pm$ 0.015	0.980	-0.80	-0.55	0.58
Arginine	0.171 $\pm$ 0.022	0.141 $\pm$ 0.048	0.927	-0.73	-0.67	0.82
Asx <sup>d</sup>	0.455 $\pm$ 0.027	-0.033 $\pm$ 0.058	0.983	0.29	-0.06	-0.07
Glx <sup>d</sup>	1.564 $\pm$ 0.038	-0.436 $\pm$ 0.083	0.997	0.85	0.88	-0.28
NH <sub>3</sub>	0.212 $\pm$ 0.009	-0.084 $\pm$ 0.020	0.991	0.79	nd <sup>e</sup>	-0.40

<sup>a</sup> Significance level of  $r_i$ : 0.823 ( $P=0.001$ );  $r'_i$ : 0.708 ( $P=0.01$ ).

<sup>b</sup>  $r'_i$  from the present study.

<sup>c</sup>  $r'_i$  from Eppendorfer et al (1985).

<sup>d</sup> Asx and Glx were evaluated in grams of aspartic acid and glutamic acid.

<sup>e</sup> nd = Not determined.

A regression calculation gives  $p = 0.95 \pm 0.02$ , and  $q = -0.014 \pm 0.045$  ( $r = 0.998$ ). Thus,  $q$  does not differ significantly from zero, and  $R \sim p \sim 0.95$  is practically constant in sorghum grain.  $R$  is always less than one because a small fraction of total grain nitrogen is not aminated and thus cannot be detected by amino acid analysis. Knowledge of  $R$  therefore enables calculation of the level of nonprotein nitrogen (NPN) in grain:

$$D_{\text{NPN}} = N - \sum D_i = N \times (1 - R) \quad (9)$$

In sorghum,  $D_{\text{NPN}} = 0.05N$ . Thus, grain NPN level is proportional to  $N$ ; but as a percentage of total grain nitrogen, NPN concentration remains nearly constant (about 5%).

#### Degree of Amidation and Nitrogen-to-Protein Conversion Factors

The molar ratio (asparagine + glutamine)/(Asx + Glx), as calculated (Mossé et al 1985) from data in Table II, indicates degree of amidation of protein. The amount of  $\text{NH}_3$  released by amide groups of asparagine and glutamine and the amount of Asx and Glx expressed in number of moles per 100 g of grain dry matter according to equation (1) are:

$$B_{\text{NH}_3} = 10^{-5} \times (1,245 N - 493),$$

$$\text{while } B_{\text{Asx}} + B_{\text{Glx}} = 10^{-5} \times (1,405 N - 321).$$

The ratio of these two equations equals the degree of protein amidation, which increases significantly from 69% for  $N = 1\%$  to 83% for  $N = 3\%$ .

Nitrogen-to-protein conversion factors and their variations can also be determined from the data in Table II. As explained elsewhere (Mossé et al 1985), three conversion factors can be distinguished:  $k_p = \sum E_i / N$ , not including  $\text{NH}_3$  of amide groups, gives the amount of polypeptide chains;  $k_A = \sum E_i / \sum D_i$  gives the ratio of true protein to grain amino nitrogen  $\sum D_i$ , obtained from amino acid analyses; and  $k_N = \sum E_i + 6.25 \times D_{\text{NPN}} / N$  provides an assessment of crude proteins (true proteins plus NPN compounds). The calculation shows that  $k_p = 5.55 - 0.09 / N$  slightly increases from 5.46 to 5.52 when  $N$  increases from 1 to 3%. It is almost constant, and the true proteins of sorghum grain have a nitrogen

content ( $100/k_p$  equal to  $18.2 \pm 0.1\%$ ). The other two coefficients are practically independent of  $N$  and are similar to each other:  $k_A = 5.81$ , and  $k_N = 5.83$ .

## DISCUSSION

### Comparison of Predicted Values with Literature Data

It is interesting to compare experimental results (Table I) and predicted values (Table II) with data from the literature. In Figure 2 are shown 51 lysine determinations of sorghum proteins including 12 from the present work and 39 from other publications; 25 of these determinations were microbiological (Bressani and Rios 1962). Although a little high in lysine concentration or in  $N$ , these 25 data give an idea of lysine variation at low  $N$  values. The plot of the 51 points (Fig. 2) suggests a curvilinear relationship of  $C_{\text{Lys}}$  to  $N$ . This can also be observed in plots of Singh and Axtell (1973) and Eppendorfer et al (1985). Similar curvilinear relationships have also been found in barley (Chung and Pomeranz 1985, Mossé and Baudet 1969), wheat (Mossé et al 1985), rye (Baudet et al 1987), and corn (Baudet et al 1986b, Eppendorfer et al 1985). If the microbiological determinations are excepted, 22 of the 26 remaining points are within an area (Fig. 2) corresponding to half the standard deviation interval calculated in Table II. The variation of lysine with  $N$  of Waggle and Deyoe (1966) (Fig. 2, dotted line) is also inside or close to this area. As an example, Table III compares the amino acid composition of Pedersen and Eggum (1983) for  $N = 2.5\%$  and the average for 522 lines of Axtell et al (1975) for  $N = 2.02\%$  with the predicted compositions calculated from Table II. The compositions are nearly identical, having relative differences not exceeding 5% for most of the amino acids and exceeding 10% for sulfur amino acids only. Thus, many types of comparisons reveal good agreement between our results and others from the literature.

### Linearity Between Amino Acid Level in Grain and Nitrogen Content

Waggle and Deyoe (1966) suggested a similar conclusion but had some problems. Their data resulted from analyses of 77 samples, of which only the mean and range are given; it is thus impossible to check reliability of their analyses. They did not determine several amino acids (tyrosine, tryptophan, cysteine) and amide nitrogen.

TABLE III  
Amino Acid Composition of Sorghum Grain (g/16 g nitrogen): Comparison of Predicted Values with Data from Other Studies

Amino Acid	N 2.2%		N 2.5%	
	Axtell et al 1975	Predicted Values	Pedersen and Eggum 1983	Predicted Values
Glycine	2.98	2.93	2.99	2.71
Alanine	9.61	9.42	9.55	9.66
Valine	5.20	5.34	5.07	5.25
Leucine	13.86	13.84	14.48	14.13
Isoleucine	3.96	4.26	4.30	4.31
Serine	4.39	4.59	4.79	4.57
Threonine	3.17	3.20	3.02	3.13
Tyrosine	4.37	4.08	4.23	4.06
Phenylalanine	5.04	5.42	5.35	5.51
Tryptophan	(1.27) <sup>a</sup>	1.02	nd <sup>b</sup>	1.02
Proline	7.93	8.44	8.03	8.36
Methionine	1.75	1.56	1.83	1.48
Half cystine	(1.46) <sup>a</sup>	1.76	1.75	1.62
Lysine	2.07	2.17	2.11	1.97
Histidine	1.95	2.16	2.06	2.07
Arginine	3.49	3.85	3.98	3.64
Asx <sup>c</sup>	7.61	7.02	7.09	7.07
Glx <sup>c</sup>	22.55	21.57	22.17	22.23
Total <sup>d</sup>	102.7	102.7	102.8	102.8

<sup>a</sup> Trp and Cys data from Axtell et al (1975), between brackets, correspond to average for 9 and 3 lines, respectively, instead of 522 lines for the other amino acids.

<sup>b</sup> nd = Not determined.

<sup>c</sup> Asx and Glx were evaluated in grams of aspartic acid and glutamic acid.

<sup>d</sup> Data from Pedersen and Eggum (1983) and Axtell et al (1975) were adjusted to 102.8 and 102.7 recovery basis (as total grams of amino acids per 16 g of nitrogen), respectively.

The parameters  $a_i$ ,  $b_i$ , and  $r_i$  are given for only five amino acids, including lysine, with which our results agree (Fig. 2). It is striking that none of all the publications in this field (except Hoseney et al 1981) compared results with the predicted compositions of Waggle and Deyoe (1966), or echoed their conclusions on linear relationships between  $A_i$  and  $N$ . Even their own group did not consider these original results in further publications (Waggle et al 1966, 1967).

The basic conclusions of Waggle and Deyoe (1966) have not been well understood: e.g., Eppendorfer et al (1985) drew different conclusions from a similar investigation. From analysis of 44 samples of one sorghum cultivar grown with varied rates of different applications, Eppendorfer et al (1985) concluded that  $C_i$  varies linearly with  $N$ . By assuming such a linear relationship, the corresponding correlation coefficients  $r_i$  were calculated from our work and compared with those of Eppendorfer et al (1985) (Table II). It can be seen that in both studies several amino acids (valine, serine, tryptophan, proline, methionine, aspartate plus asparagine) are characterized by  $r_i$  values that are devoid of significance. As all protein amino acids are deposited in grain (through storage protein accumulation) at rates depending on one another because they are bound in polypeptide chains, it is impossible that all these amino acids are not highly correlated with  $N$  in their accumulation. Hence the weakness of  $r_i$  values for some of them exclude the linearity of the relationships between  $C_i$  and  $N$ . The interpretation of Eppendorfer et al (1985) is as much strange as in previous studies on phenotypic variation of amino acid and  $N$ ; Eppendorfer and Bille (1974) concluded that, for amino acid concentration expressed as grams per 16 g of nitrogen (i.e.,  $C_i$  in this paper), correlations were generally not as close as for concentration expressed as percent of dry matter (i.e.,  $A_i$ ). In the understanding of this question, the significant progress to which Eppendorfer et al (1985) contributed is the confirmation that in different species, for a given genotype, any kind of application changed amino acid only indirectly through its effects on  $N$ , as previously suggested by Mossé and Baudet (1969).

#### Range Over Which the Linear Relationships Are Valid

The number of varieties used in the present study may seem small. Because six determinations were performed for each sample, however, and because results are available for other species, this number of genotypes seemed adequate. In recent studies on wheat (Mossé et al 1985), corn and rye (Baudet et al 1986b, 1987), barley, triticale, pearl millet, oats (*unpublished results*), broad bean (Baudet and Mossé 1980), pea (Huet et al 1987), and lupin (Mossé et al 1987), many more genotypes were examined. However, no significant deviation could be found between values of  $a_i$ ,  $b_i$ , and  $r_i$  determined from different or from the same genotype(s).

Many authors still interpret their results as suggesting an influence of genotype on amino acid composition (Ajakaiye 1984, Bressani and Rios 1962, Deosthale et al 1970, Deyoe and Shellenberger 1965, Haikerwal and Mathieson 1971). There are two reasons for this interpretation, with which our results disagree. Comparisons of different genotypes generally do not consider  $N$ , except in detection of high-lysine mutant sorghums by Singh and Axtell (1973). Also, amino acid determinations may not be sufficiently reliable to prevent analytical deviations from creating most of the observed differences.

Because the protein amino acids are highly correlated with  $N$  in their accumulation (Huet et al 1987; Mossé et al 1986, 1987), they should be characterized by equal correlation coefficients,  $r_i$ . However, Table II shows that  $r_i$  values are not all equal. Only two kinds of events can explain the variation of  $r_i$  values: either some of these amino acids occur in a free state so that a small amount escapes the regulation of storage protein accumulation, or there are deviations in their analytical determination. The first kind of event might explain the value  $r_{\text{Arg}} = 0.927$  of arginine, as already shown in pea (Huet et al 1987) where it is predominant among free amino acids, but the total level of free amino acids is so minute that their concentration in a free state can be neglected. In contrast, methionine, cysteine, and lysine show the lowest  $r_i$  values (0.876–0.900) and are among those that are often affected by

analytical errors in their determination. This does not exclude small genetic variations of composition; some of the polypeptide chains of storage proteins differ by their primary structure and by their amino acid composition from one genotype to another. But it is easy to calculate that such a cause of variation results in modifications of whole grain protein composition that are negligible compared with analytical deviations.

These considerations suggest that for all sorghum samples, any genotypic or phenotypic modification of  $N$  results from the same variation in amino acid composition. Similar results have also been observed over wider  $N$  ranges for other species, for instance for  $N$  values as low as 1.1% for pearl millet (*unpublished results*) or 1% for corn (Baudet et al 1986b), and for  $N$  values as high as 4% for barley (*unpublished results*) or 4.4% for rye (Baudet et al 1987). Therefore, this may be a general phenomenon, shared by seeds of all species, and valid for  $N$  values ranging from around 1 to 4% for cereal grains.

Only biochemical mutants, such as those found by Singh and Axtell (1973), introduce significant genotypic variation leading to modifications of the coefficients  $a_i$  and  $b_i$ , just as in another species. With regard to phenotypic variations, only severe sulfur starvation could originate a break in the correlations between  $A_i$  and  $N$ , as was shown for corn (Baudet et al 1986a).

#### Practical and Nutritional Consequences

It is obvious that the linear relationships between  $A_i$  and  $N$  permit prediction of amino acid composition of any grain sample from only its moisture and nitrogen content. This is also possible with mixtures of samples with different  $N$  values, or with mixtures of cultivars. The mean value of  $A_i$  will correspond to the mean  $N$  value. As elementary as this statement may seem, it must be emphasized that it no longer applies to the amino acid levels in protein  $C_i$  because relationships between  $C_i$  and  $N$  are often not linear. It is still common in nutritional calculation of grain protein to average  $C_i$  values, but this results in inaccurate data; only  $A_i$  values can be averaged. As long as cultivated sorghum genotypes are not altered significantly, the results in Table II may be used instead of amino acid analyses. It might therefore be more practical to market sorghum according to protein quality, as determined from  $N$ , which can change to a great extent according to year, location, climate, and fertilization. Moreover, it must be emphasized that any experiments designed to show that different genotypes have different amino acid compositions ought to take into account the relationships demonstrated here.

From a nutritional viewpoint, knowledge of  $C_i$  variation in sorghum also enables prediction of essential amino acid scores as a function of  $N$  and comparison with those of other cereals. For example, variations of these scores for sorghum and corn are presented in Figure 3. This confirms that lysine is the most limiting essential amino acid in both cereals, sorghum having the lowest score. However, sorghum protein of  $N = 1.5\%$  is higher in lysine concentration than is corn protein of  $N = 3\%$ . Tryptophan is seen not to be limiting in sorghum, although it is in corn. The second limiting amino acid in sorghum is threonine (more limiting than in corn), as shown by Eppendorfer et al (1985). Only sulfur amino acids are also limiting in sorghum grains of  $N > 1.75\%$ , in contrast to corn. All other essential amino acids are not limiting in sorghum whatever  $N$  may be.

Sorghum nitrogen-to-protein conversion factor variation as a function of  $N$  was studied here for the first time. Sorghum is unique among cereals:  $k_N$  and  $k_A$  are higher than in wheat (Mossé et al 1985), corn (Baudet et al 1986b), and other cereals (*unpublished results*). Thus, at equal  $N$ , sorghum grain is a little richer in protein than any other cereal.  $K_P$  of sorghum is also higher than in most cereals, except foxtail millet and high-protein corn. These coefficients change significantly with  $N$  in most cereals, while remaining nearly constant in sorghum.

#### Composition of Storage Proteins of Sorghum Grain

As shown previously (Huet et al 1987; Mossé et al 1986, 1987), the linear relationships between  $A_i$  and  $N$  permit assessment of storage protein composition in mature sorghum grains, as  $N$  (and

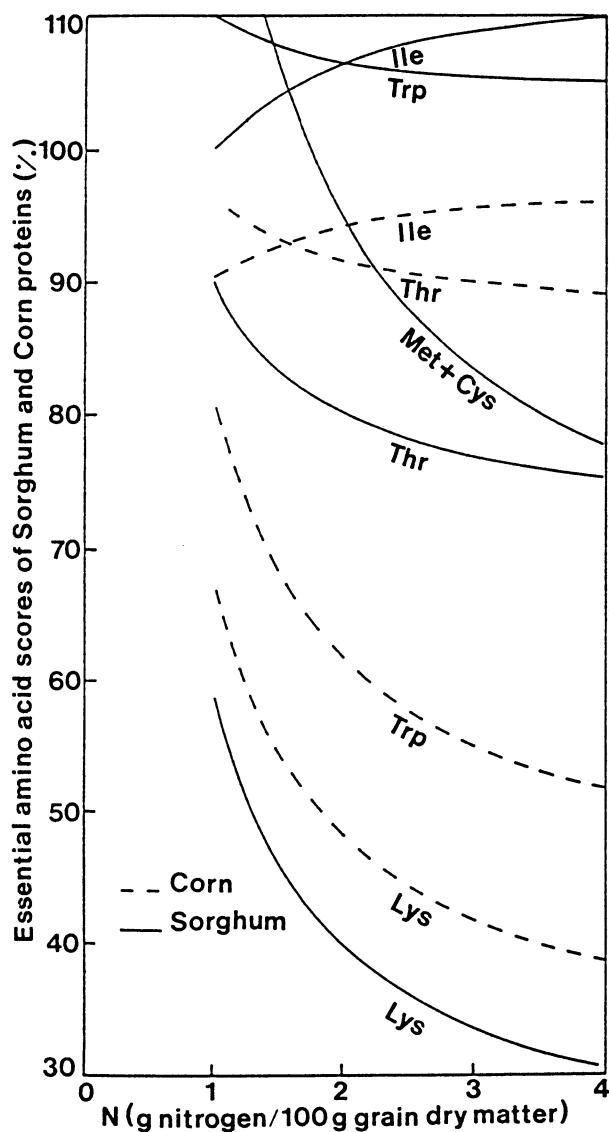


Fig. 3. Variation of limiting essential amino acid score (%) in sorghum and corn (Baudet et al 1986b) as a function of grain nitrogen content  $N$ : isoleucine (Ile), tryptophan (Trp), threonine (Thr), methionine plus cysteine (Met + Cys) and lysine (Lys). Essential amino acid scores are from FAO (1973).

protein content) becomes higher. The results from the present study make it clear that two sorghum samples differing by their nitrogen content,  $N_1$  and  $N_2 > N_1$ , contain amino acid levels equal to  $A_i(N_1)$  and  $A_i(N_2)$ , respectively. The additional proteins characterizing the second sample in comparison with the first have a composition equal to:

$$16 \times [A_i(N_2) - A_i(N_1)] / (N_2 - N_1) = 16 \times a_i$$

in grams of amino acid per 16 g of nitrogen. Because  $a_i$  is independent of  $N$ , these extra proteins have a constant amino acid composition, whatever the protein content of grain be. The data presented in Table IV suggest that the additional proteins synthesized as  $N$  increases consist of approximately 0.6 part prolamin plus 0.4 part glutelin, at least under the conditions used by Guiragossian et al (1978) for extracting these fractions. Therefore, the ratio of prolamin to glutelin might be a little lower in storage proteins of sorghum grain than in those of corn grain (Mossé et al 1986).

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TABLE IV  
Amino Acid Composition of Kafirins (K), Glutelins (G),  
a Calculated Mixture (CM = 0.6K + 0.4G),  
and Extra Proteins Accumulated in Sorghum Seeds (EP = 16 a)  
(g of amino acid per 16 g nitrogen)

Amino Acid	K <sup>a</sup>	G <sup>a</sup>	CM	EP
Glycine	1.45	3.7	2.4	1.8
Alanine	10.6	9.2	10.0	10.7
Valine	4.3	6.0	5.0	4.9
Leucine	17.8	12.0	15.5	15.4
Isoleucine	4.2	4.4	4.3	4.5
Serine	4.0	3.9	4.0	4.5
Threonine	2.3	3.3	2.7	2.8
Tyrosine	4.8	4.4	4.6	4.0
Phenylalanine	6.2	4.7	5.6	5.9
Proline	10.0	10.3	10.1	8.0
Methionine	1.05	1.45	1.2	1.1
Half cystine	0.9	0.55	0.75	1.0
Lysine	0.15	2.0	0.90	1.1
Histidine	1.05	3.0	1.8	1.7
Arginine	1.50	3.6	2.3	2.7
Asx <sup>b</sup>	6.2	5.9	6.1	7.3
Glx <sup>b</sup>	26.0	24.0	25.2	25.0

<sup>a</sup> Recalculated from Guiragossian et al (1978) and adjusted to the same total of amino acid recovered (g/16 g nitrogen), Trp being excluded.

<sup>b</sup> Asx and Glx were calculated as grams of aspartic acid and glutamic acid.

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