Varietal Differences in Protein, Lysine, and Leucine Content of Grain Sorghum

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Suboptimal levels of lysine limit the quality of sorghum proteins and high levels of leucine in the protein cause pellagra. 332 varieties of sorghum germ plasm from the World Genetic Stock were, therefore, screened for protein, lysine, and leucine contents. The protein per 100 g of the sample varied from 4.7 to 17.0 g; lysine per 100 g of protein, from 0.90 to 2.67 g, and leucine per 100 g protein, from 6.7 to 29.9 g. Correlation between protein and lysine in the protein was significantly negative, and that between protein and leucine content of the protein was positive. The results are discussed to identify varieties with better than average nutritional quality and less pellagragenic character using the values of leucine to lysine ratio.

The average Indian diet is predominantly cereal-based. Both quantitative and qualitative insufficiencies of protein in such diets are responsible for the widespread occurrence of protein and calorie undernutrition, especially in vulnerable segments of the population. The new strategy in agriculture, brought into focus by the high-yielding variety programs, has shown great promise in meeting the increasing food demands of the population. Phenomenal increases in the yield of food grains per unit area now achieved from this program could meet the quantitative requirements of proteins and calories. It is therefore appropriate now to make an appraisal of the possibility of improving the nutritional quality of the high-yielding cereal food grains.

The nutritive value of a protein is determined by comparing its essential amino acid pattern with that of a reference protein, such as hen's whole egg protein (Block and Mitchel, 1946; Oser, 1959). Since lysine is the most limiting amino acid in cereal proteins, their nutritive value is lower than that of proteins of animal origin (Block and Weiss, 1956). Recent discoveries of high-lysine and high-methionine mutant varieties of corn, *viz.* Opaque-2 (Mertz *et al.*, 1964) and flourey-2 (Nelson *et al.*, 1965) has stimulated genetic and biochemical research to identify and selectively propagate other such cereal varieties or mutants with genes for high lysine content.

The millet sorghum commonly known as jowar forms a major staple food in the western and southern regions of India. The proteins of this millet, like those of most other cereals, are deficient in lysine. In addition, the proteins contain high levels of leucine, as compared to other cereals. Available evidence shows that an amino acid imbalance caused by excess leucine in sorghum is responsible for the production of the disease known as pellagra, commonly seen in the jowar eaters (Gopalan, 1968). Identification and selective propagation of sorghum strains containing low levels of leucine and high levels of lysine seem to be a logical approach to the prevention of pellagra in populations subsisting largely on this millet. With this objective in view, a large collection of sorghum germ plasm was examined for its content of protein and essential amino acids, lysine and leucine.

MATERIALS AND METHODS

A world collection of the genetic stock of sorghum germ plasm, comprising mainly cultivated types from 44 countries, is maintained at the Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi. This entire collection is classified into 70 working groups on the basis of the panicle openings and on certain other qualitative and quantitative plant characters (Murty *et al.*, 1968). The material for present investigation consisted of 332 pure lines drawn from 31 of those working groups. These lines are considered as agronomically suitable for grain production. The whole material was sown in July 1965 at IARI in an augmented randomized complete block design and was harvested in January 1966. The manurial treatment per acre was 80 lb nitrogen and 40 lb each of P_2O_5 and K_2O. A composite grain sample of each variety was obtained for analysis.

All samples were finely ground and passed through 100 mesh sieve. Nitrogen was determined by conventional macroKjeldhal procedure and the protein content was calculated from percent nitrogen by multiplying with the factor 6.25. The protein values of the sample are expressed on the basis of 10% moisture, which varied between 10–12% in this material. Lysine and leucine were estimated by microbiological assays performed on the acid hydrolyzates, using *L. mesenteroides* P-60 as an assay organism (Steele *et al.*, 1949). The values of lysine and leucine are reported in terms of grams per 100 g of proteins, because the amino acid balance of the protein is important from the nutritional point of view.

RESULTS AND DISCUSSION

The frequency distributions of protein, lysine, and leucine content of the 332 varieties of sorghum analyzed are given in Table I. The sorghum varieties belonged to several groups, the average of protein, lysine, and leucine contents were calculated and the data were statistically analyzed for those groups where sufficient numbers (more than 11 varieties) were available. The data on nine such groups are given in Table II. The coefficients of correlations between protein and amino acids for different groups and also for the entire pooled data are given in Table III, along with their level of statistical significance.

Variation in Protein Content. A wide genetic variability was observed in the protein content of different sorghum varieties. The values ranged from 4.7 to 17.0 wt%. The results given in Table I indicate that in 62% of the total samples analyzed, the protein content varied within a narrow range of 11.0 to 13.0 wt%, with an average of 11.73 wt% and a standard deviation of 1.81. The number of samples containing more than 13.0% protein was 75 and these were classi-

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fied as high-protein varieties (Table IV). Variations in protein within the groups were also large (Table II) and of equal magnitude in different groups. This probably indicates that only certain genetic characters of a variety, rather than its varietal group characteristics as a whole, determine its protein content.

Variations in Lysine and Leucine Content. Lysine is the most limiting amino acid in sorghum proteins. Hence, the nutritive value of sorghum varieties is determined by the extent of variability in the lysine content. In the present investigation, the lysine content of different varieties of sorghum ranged from 0.90 to 2.67 g. The frequency distribution of lysine in 332 varieties given in Table II showed that 68% of the samples contained 1.49 to 2.24 g of lysine. The average was 1.79 g, with standard deviation of 0.43 gram. Within each classified group of sorghum varieties, a wide variation in lysine values was observed. The differences in the mean value of each group were not statistically significant. As a result of these studies, it was possible to identify 30 varieties of sorghum having more than 2.2 g of lysine, and within the group of material analyzed, these varieties were classified as high-lysine varieties (Table V).

The leucine content in these sorghum varieties varied between 6.7 and 29.9 g. Although this range was apparently very wide, 87% of the varieties showed leucine values between 9.0 and 12.99 g with an average of 11.38 g and standard deviation of the mean 1.6 g for the entire lot. In 24 varieties only, the leucine content was less than 9.0 wt% (Table VI). Between the nine classified groups of sorghum varieties, there appeared to be no characteristic pattern in the leucine values, and the average group values were not significantly different statistically.

INTERRELATIONSHIPS BETWEEN PROTEIN, LYSINE, AND LEUCINE

Selections of varieties with high lysine and low leucine characters is important in breeding programs aimed at evolving varieties of sorghum with high nutritional quality, as well as nonpellagragenic property. However, the concentration of lysine and leucine in the proteins of sorghum is a function of protein content of the sample (Ramasastry, 1966; Virupaksha and Sastry, 1968). An increase in protein content lowers the concentration of lysine in the protein, with a simultaneous increase in its leucine content. These changes in amino acid concentrations in relation to the protein content have been attributed to changes in the alcohol-soluble proteins—the prolamins, which are the major components of the proteins in sorghum (Virupaksha and Sastry, 1968). The prolamins contain very low amounts of lysine, but high amounts of leucine, as compared to other protein fractions. Consequently, high proportion of prolamines in the grain increases the total protein, as well as its leucine content, but decreases the lysine concentration.

Table I. Frequency Distribution of Protein, Lysine, and Leucine in 332 Varieties of Sorghum

	Protein (%)			
Range	<9.99	10–11.99	12–13.99	14 and above
%	15.6	42.1	31.3	10.8
	Lysine (g per 100 g protein)			
Range	<1.49	1.5–1.99	2–2.49	2.5–2.75
%	23.5	50	25.3	1.2
	Leucine (g per 100 g protein)			
Range	<8.99	9–10.99	11–12.99	13 and above
%	7.2	42.2	41.6	9.0
	Leucine:Lysine Ratio			
Range	<4.0	4–4.5	4.5–5.0	Above 5
%	2.1	5.4	8.5	84.0

Table II. Mean Values of Protein, Lysine, and Leucine Content for Different Groups of Sorghum[a]

Group no.	No. of samples	Protein wt%	Lysine	Leucine
			g/100 g protein	
12	20	11.661 ± 1.7453	1.652 ± 0.3217	11.08 ± 1.416
22	19	12.239 ± 1.7607	1.773 ± 0.3873	11.02 ± 1.311
28	12	12.344 ± 1.5925	1.738 ± 0.3567	11.02 ± 1.056
33	41	11.464 ± 1.9344	1.545 ± 0.2654	11.28 ± 2.074
34	16	12.034 ± 2.0214	1.885 ± 0.2667	10.89 ± 1.020
38	37	12.264 ± 1.7644	1.819 ± 0.2991	11.52 ± 1.501
39	13	10.816 ± 1.2679	2.013 ± 0.2036	10.35 ± 1.119
41	92	11.415 ± 1.7978	1.946 ± 0.2863	11.44 ± 2.770
52	13	12.702 ± 1.5359	1.368 ± 0.2187	13.32 ± 4.061
All groups	332	11.734 ± 1.8147	1.792 ± 0.4328	11.35 ± 1.629

[a] Mean ± standard deviation.

Table III. Correlation Between Protein, Lysine, and Leucine in Different Groups of Sorghum Varieties

Group	Number of Samples	Protein *vs.* Lysine	Protein *vs.* Leucine	Lysine *vs.* Leucine
12	20	−0.0016	+0.1410	+0.2739
22	19	+0.0012	−0.2611	−0.0394
28	12	−0.6499[a]	−0.5769[b]	+0.4105
33	41	−0.4199[a]	−0.1085	+0.3217[b]
34	16	−0.5243[b]	+0.0442	−0.2238
38	37	−0.6102[a]	−0.2225	+0.1766
39	13	−0.2881	+0.4053	−0.2297
41	92	−0.4198[a]	−0.0488	+0.0200
52	13	−0.3813	−0.5722[b]	+0.2402
All 31 groups	332	−0.3331[a]	−0.0356	−0.0047
	326	...	+0.135[c]	...

[a] Significant at 1% level. [b] Significant at 5% level. [c] Significant at 2% level.

In the present investigation, a significant ($P < 0.01$) inverse correlation was found between the protein and lysine content. Such a correlation was also observed consistently in varietal groups of sorghum. On the other hand, no significant correlation could be seen between the protein and leucine or leucine and lysine content in any of the groups of sorghum varieties or in the entire pooled sample. When the extreme values of leucine in six varieties were excluded, a significant correlation between protein and leucine ($r = 0.135, P < 0.02$) was observed for the entire data, which fits with the observations of earlier workers regarding the direct relationship between protein and leucine.

High levels of leucine in sorghum proteins may interfere with conversion of tryptophan to niacin (Belavady *et al.*, 1963) and also inhibit the synthesis of nicotinamide nucleotides (Reghuramulu *et al.*, 1965). These metabolic disturbances lead to niacin deficiency in jowar eaters. Such a situation can be corrected by supplementation with niacin (Belavady *et al.*, 1967) or tryptophan (Krehl *et al.*, 1946). These observations, therefore, lend strong support to the view that proper balance between leucine and tryptophan in sorghum proteins would be a more suitable index than the leucine content alone for selection of nonpellagragenic sorghum strains. Since there is a positive correlation between lysine and tryptophan content of sorghum protein (Deosthale, 1969), the ratio of leucine to lysine would give equally meaningful information, not only regarding the pellagragenic character of a sorghum strain, but also about its nutritional quality in terms of lysine content. Hence, in the present investigation the leucine to lysine ratio was used as a criterion for assessment of the nutritional as well as pellagragenic character of the sorghum varieties.

The entire collection of sorghum analyzed here was, therefore, first classified on the basis of their protein, lysine, and leucine content. The high-protein (>13.0 g %), high-lysine (>2.2 g), and low-leucine (<9.0 g) groups of varieties were then further classified on the basis of their leucine to lysine ratio. As a result of this method, it was possible to identify sorghum varieties having some useful combination of high nutritional quality and nonpellagragenic character.

Cereals such as rice, wheat, bajra, etc., are known to be nonpellagragenic. In these cereals the ratio of leucine to lysine varies from 2.0 to 3.8. A safe level of leucine to lysine ratio could therefore be fixed by comparison of the values of this ratio in pellagragenic and other food grains. The amino acid composition of nine such cereals and millets, *viz.*, rice, wheat, bajra, varagu, samai, ragi, Italian millet, maize, and sorghum, was available (Ramachandran and Phansalkar, 1956). From these results, the values of leucine to lysine ratios were calculated. A direct correlation was found between the values of the ratio and leucine content, and an inverse correlation with lysine content. Regression equations for these relationships, fitted by the method of least squares, are presented graphically in Figure 1. At the point of intersection, the value of the leucine to lysine ratio was 3.6. This would suggest that any food grain variety with a leucine to lysine ratio more than 3.6 would contain relatively too much leucine and would be pellagragenic. On the other hand, at lower values of this ratio, the proportion of lysine and of tryptophan would be more, resulting in better protein quality. Making some allowance for the variation in the value of the leucine-to-lysine ratio, and in the light of the above observations, the ratio below 4.6 could perhaps be considered a safe level.

In the varieties of sorghum reported here, the ratio of leucine to lysine varied from 3.57 to 16.5, with an average value of 6.97. The results presented in Table I indicate that 84% of the total samples showed leucine to lysine ratios of more than 5.0, and only in 22 sorghum lines (Table VII) was it within the safe limits (below 4.6). In three of these varieties, *viz.*, IS 4234, 516, and 4642, the combination of leucine and lysine appeared to be the best, as the ratio of these amino acids was very near 3.6. IS 4234 appeared to be the superior line, as it contained more protein than other two varieties.

The low value of the ratio in 22 lines of sorghum listed in Table VII was due to both high lysine and low leucine contents; at least seven of those varieties had average protein, content. Such varieties could form a suitable parental material in breeding programs aimed at combining high yielding character with desirable nutritional attributes. Among the high-protein lines (Table IV), four varieties, *viz.*, IS 5262, 8298, 5478, and 3950, were identified as containing high lysine values also. The leucine content of these was lower than the average value, and the leucine to lysine ratio was just outside the safe limits. In view of their high-protein, high-lysine characters and probably less pellagragenic potential, these four varieties could be recommended to improve dietary protein quantity as well as quality in population groups subsisting on this millet.

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