COMMUNICATION TO THE EDITOR
Fractionation of Soluble Proteins of High-Lysine and Normal Sorghum Grain[1]

TO THE EDITOR:

The discovery that *opaque-2* and *floury-2* mutant strains of maize had an improved protein quality (1,2) has stimulated considerable research on the improvement of protein quality of other cereals. A mutant gene (*hl*), similar to *opaque-2*, was recently identified in sorghum by Singh and Axtell (3). After screening over 9,000 lines in the world sorghum collection, Singh and Axtell (3) were able to select two dented floury lines of Ethiopian origin, IS 11758 and IS 11167, which are high in lysine concentration at relatively high levels of protein.

When the *opaque-2* gene is incorporated into normal maize, the prolamine (zein) is reduced and the glutelins, albumins, and globulins increased, resulting in a larger amount of lysine in the whole kernel. In this communication we report the distribution of soluble protein fractions in high-lysine sorghum kernels and compare them with the fractions in the normal sorghum with similar genetic background. Phenotypically the high-lysine sorghums, IS 11167 and IS 11758, can be easily distinguished from normal sorghum by their floury and dented endosperms. By normal sorghum, we mean that which is similar to varieties and hybrids in commercial production having rounded kernels with some vitreous endosperm and low lysine content. A cross was made between high-lysine sorghum (IS 11758) and the normal variety, Redlan. Heads from five different F_1 plants of the above cross with the segregating F_2 vitreous and floury kernels were used for this study. High-lysine and normal sorghum grains from each head, along with parent Redlan and IS 11758 and another high-lysine line, IS 11167, were used in the protein fractionation procedure. Kernels were ground in a Wiley mill, defatted, and then ground to a fine powder using a small ball mill. Protein was fractionated according to the Landry-Moureaux procedure (4) as previously applied to sorghum (5). Lysine was determined on these ground samples by ion-exchange chromatography (5).

Table I indicates the differences in protein distribution and lysine levels between the high-lysine and normal sorghums. The percentage of soluble nitrogen is higher in the first fraction and lower in the second and third fractions in high-lysine sorghum when compared with normal. There is also an increase in the percentage of protein in the fifth fraction in high-lysine sorghum when compared with normal. The high-lysine kernels were 0 to 36% higher in protein and 56 to 79% higher in lysine (based on protein) than the normal kernels.

The first fraction, representing the albumins, globulins, and free amino acids soluble in sodium chloride solution has the highest amount of lysine in comparison to other protein fractions, and any increase in its percentage distribution would naturally increase the percentage of lysine in the whole kernel[2]. The classes of proteins solubilized by isopropanol (fraction II), and isopropanol containing mercaptoethanol (fraction III), are referred to as kafirins

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[2]Jambunathan, R., and Mertz, E. T. Amino acid composition of whole kernel and endosperm protein fractions of sorghum (unpublished data).

TABLE I
Nitrogen Distribution in the Whole Kernels of Normal and High-Lysine Sorghums[a]

Fraction	Redlan	Redlan × IS 11758 F_2 Kernels	IS 11758	IS 11167
I Saline	10.0	15.3[b] [22.4]	26.0	25.3
II Isopropanol	15.7	26.4 [13.7]	10.3	15.2
III Isopropanol + 2-ME	31.3	26.5 [20.2]	19.6	19.3
IV Borate Buffer + 2-ME	4.5	4.3 [4.3]	6.5	4.5
V Borate Buffer + 2-ME + SDS	29.3	22.5 [33.5]	27.2	29.5
Total Nitrogen Extracted (%)	90.8	95.0 [94.1]	89.6	93.3
% Protein	13.53	13.0 [15.6]	18.5	16.3
% Lysine[c]	1.56	1.85 [3.1]	3.27	3.10

[a]Percent of total nitrogen.
[b]Average values of kernels obtained from five different heads (55073-55077). First value is the normal sample value followed by the high-lysine sample value in brackets.
[c]Expressed as g. per 100 g. protein.

and alcohol-soluble glutelins, respectively (4), and these proteins have the lowest content of lysine. The kafirins and alcohol-soluble glutelins average 52.9% in the normal sorghum as compared with 33.9% in the high-lysine sorghum. This reduction in fractions II and III accounts for part of the increase in the lysine content in the high-lysine sorghum kernel. The fourth fraction, which contains nitrogen soluble in borate buffer and mercaptoethanol, is similar in normal and high-lysine sorghum. The fifth fraction contains nitrogen soluble in borate buffer, mercaptoethanol, and sodium lauryl sulfate. This fraction increases in high-lysine sorghum when compared with normal sorghum. Thus, the decrease in the percentage of soluble nitrogen in the second and third fractions of high-lysine sorghum is compensated by a concomitant increase in the first and fifth fractions. The distribution patterns of high-lysine Ethiopian lines IS 11758 and IS 11167 are similar to the average distribution patterns observed with the high-lysine F_2 sorghum kernels obtained by crossing Redlan and IS 11758. Studies on the ratio of normal to high lysine kernels on segregating heads (3) indicate that the high lysine character is simply inherited and can be easily transferred by standard plant breeding procedures.

Zein synthesis in *opaque-2* maize endosperms is reduced to about 50% of normal with a concomitant increase in the saline soluble and glutelin fractions. Since the protein distribution patterns obtained with high-lysine sorghum are similar to those observed in *opaque-2* maize, the gene action of these two mutants is probably similar.

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