Protein and Lysine Levels in Developing Kernels of Normal and High-Lysine Sorghum[1]

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ABSTRACT

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Farmers in Ethiopia consume high-lysine sorghums strictly at immature stages of grain development. The protein and lysine levels in the endosperm and germ fractions of developing kernels of eight high-lysine and normal sorghum varieties at 21, 31, and 61 days after flowering were evaluated to determine changes in protein quality and content at these stages. At 31 days after flowering (the late dough stage), high-lysine sorghums had higher endosperm protein and higher endosperm lysine contents than normal sorghums. The highest endosperm lysine content of all genotypes was also observed at the late dough stage. These findings suggest a nutritional basis for the tradition of farmers in Ethiopia to consume high-lysine varieties exclusively at the milk dough and late dough stages of grain development.

Singh and Axtell (1973) reported the discovery of two naturally occurring high-lysine sorghum mutants after screening 9,000 entries in the World Sorghum Collection. The two mutant lines, IS11758 and IS11167, are land race varieties originally collected from Wollo, Ethiopia, in the early 1960s. Average whole-kernel lysine concentrations of the mutants were 3.13 and 3.33 g/100 g of protein at 17.2 and 15.7% protein, respectively, for IS11758 and IS11167, in contrast to normal sorghums with lysine values of 2.1 g/100 g protein at about 12% protein.

The potential nutritional benefits obtained from such mutants would depend on, among other things, how well these mutants are recognized in their area of cultivation, the acreage these types occupy, the manner in which they are utilized in local food preparations, and perhaps how often they are consumed.

The authors travelled to Wollo Province, Ethiopia, in November 1973, to determine if these mutants were still in cultivation, and if they were, to assess the role that such types play in the overall nutrition of people in the area. The local farmers in Wollo, Ethiopia, recognized and identified the high-lysine mutants readily. They identified them as specialty sorghums used primarily for roasting at the late dough stage. Farmers preferred the taste of the roasted grain of the high-lysine mutants. They also recognized that total grain yield per acre of these mutants was lower than their normal sorghum varieties. As a result, everywhere the high-lysine sorghums were grown, they were planted in mixture with normal sorghum varieties, and never as pure stand. It is fascinating that such mutants with low economic yield could persist and survive the ongoing directed evolution of crop varieties towards agronomically more acceptable types. The season when these mutant types are consumed often corresponds to a critical period of food shortage. Perhaps the nutritional benefits obtained from consuming cereals at milk dough stage, a standard practice by most farmers in the region, is significant. This study is part of an attempt to learn about changes in protein quality and content as well as carbohydrate composition in the developing seed in selected normal and high-lysine sorghum varieties.

Misra and Mertz (1975) followed the levels of protein in developing endosperms of normal and high-lysine (*opaque*-2) maize. At 14, 21, 35, and 49 days post-pollination, the protein levels were 18.7, 13.7, 12.3, and 12.2%, respectively, in the normal, and 16.9, 11.8, 10.4, and 10.4%, respectively, in the *opaque*-2 maize. The sharp drop in protein between the 14th and 21st days in both genotypes is probably caused by an increased rate of starch biosynthesis during this time interval.

Reported here are the protein and lysine levels in normal and high-lysine sorghum germ and endosperm at 21, 31, and 61 days after flowering (DAF). Dry matter and carbohydrate levels are discussed in a separate paper (Ejeta and Axtell, *unpublished.*)

MATERIALS AND METHODS

Eight strains of sorghum were used for this study. These included three naturally occurring high-lysine mutants from Ethiopia, IS11758, IS11167, and YM-3, and three normal cultivars, BG-5, BG-6, and BG-10, all collected by the authors from Wollo, Ethiopia, in 1973. Also included were the chemically induced, high-lysine mutant, P-721 opaque, and its normal sib parent, P-721 normal. The eight varieties were grown at the Purdue University Agronomy Farm in the summer of 1975 in three-row plots that were 0.60-m wide and 7-m long.

Three random heads from each of the eight strains of sorghum were sampled at three different stages of maturation: 21, 31, and 61 DAF. At each stage, panicle branches were cut from central sections of the triplicate head samples, put in labeled beakers, immediately frozen in dry ice, and rushed to the laboratory to be stored at −20° C. Fifty kernels were removed from each sample, weighed, and lyophilized in a mini-lyophilizer (Virtis trap model) for 72 hr. Lyophilized samples were weighed and placed in hydrolysis vials stored in a desiccator and kept in a cold room (−20° C). These kernels were later dissected with a scalpel and separated into endosperm and germ, with the pericarp included in the endosperm fraction.

The endosperm and germ fractions of every sample in all three stages were analyzed for protein and lysine contents. The endosperm fractions were defatted before they were analyzed for total nitrogen content by the micro-Kjeldahl procedure. Percent protein was calculated by multiplying the nitrogen value by the factor of 6.25. Lysine was determined by ion-exchange column chromatography after acid hydrolysis. The method was essentially the same as that described by Spackman et al (1958). Protein is expressed as protein percent of sample and lysine is expressed as percent of protein.

The experimental design was a split plot in time with four fixed factors: kinds (normal and high lysine), varieties (8) nested within kinds, three stages of maturation, and three plants sampled at each stage. The analysis of variance and the separation of significant differences between means for the variables evaluated in this experiment were performed according to procedures outlined in Steel and Torrie (1960).

RESULTS AND DISCUSSION

Protein Levels in Germ

In Table I one can see that the protein level in the embryo varied from 15.7 to 18.9% in the high-lysine mutants and from 16.2 to 19.0% in the normal genotypes at 21 DAF. At 31 days, the protein in mutants dropped to a range of 14.5–16.6% but then rose to 20–22.8% at 61 days. The normal genotypes stayed in the range of

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16.6–18.9% protein at 31 days and then increased at 61 days to 20.4–23.5%, which was similar to that of the mutants.

Lysine Levels in Germ

The lysine levels in the germ of the mutants and the normal genotypes were in the 3–4% range at 21 days, increased moderately at 31 days, and at 61 DAF were in the 4.5–5.3% range (Table II). Mature normal germ had the same amount of lysine as mature mutant germ, so the increased lysine in the mutants must have come from the endosperm.

Protein Levels in Endosperm

Protein levels in the endosperm are outlined on Table III. At 21 days, levels ranged from 12.8 to 13.9% in the Ethiopian mutants and from 12.0 to 12.4% in the Ethiopian normals. P-721 opaque was significantly lower in protein (9.7%) than its normal counterpart, P-721 (11.2%). At 31 days, in all but one case, the protein level increased in the mutants and in all cases fell in the normal genotypes. At 61 days, all mutants had increased protein levels again. The Ethiopian mutants had a high-protein content (14.6–15.7%) probably because of disruption in starch synthesis, and the Ethiopian normal genotypes were lower at 11.3–12.2%. P-721 opaque was 2% lower than its normal counterpart (10.6% compared with 12.6%).

Mean values of percent protein across maturity dates showed differential response for P-721 opaque and the high-lysine (*hl*) varieties from Ethiopia, when contrasted with their respective normal controls. The Ethiopian *hl* varieties showed significantly higher levels of endosperm protein at all stages than the normal varieties; the maximum endosperm protein value of 15.33% (average of three *hl* lines) was obtained at 61 DAF. Unlike the *hl* lines, P-721 opaque had lower than normal protein content but increased protein quality. The high-lysine varieties from Ethiopia showed a consistent increase in protein content in the endosperm, the maximum amount of protein being accumulated in the final stages of grain development. The normal varieties, however, showed a decrease in protein synthesis between 21 and 31 DAF and then increased in the final period. When considered with the dry matter accumulation differences (not reported here), in the period between 21 and 31 DAF the normal varieties accumulated more carbohydrates and less protein. At 61 DAF, the average total protein content of the *hl* varieties (15.3%) remained significantly higher than the normal varieties (11.8%).

Lysine Levels in Endosperm

In contrast to *opaque*-2 maize (Tossello 1974, Misra 1975) the high-lysine sorghum mutants increased in protein content between 21 and 61 DAF. Normal sorghum in most cases showed a drop similar to that observed for normal maize between the 21st day and day 61 in the case of sorghum and day 49 for maize. At 21 days, the Ethiopian endosperm mutants were no higher in lysine than the Ethiopian normals (Table IV). By the 31st day the lysine in mutants either stayed the same or was increasing, whereas that in normal varieties, with the exception of BG-10, was dropping. However, at the 61st day, all normals had dropped, and the mutants had substantially higher levels of lysine (2–2.4 g in mutants versus 1.4–1.5 g in normals). P-721 opaque showed an increase over P-721 normal for lysine levels in all three stages. These data indicate that, similar to corn (Barbosa 1971, Ruschel 1972), the high-lysine genes in both the Ethiopian and Purdue high-lysine sorghums exert their effect on protein synthesis in the endosperm and not in the germ. These data also suggest that the protein quality of 21-day-old Ethiopian high-lysine sorghum was essentially the same as 21-day-old Ethiopian normal sorghum. Therefore, at the late dough stage when the farmers in Wollo, Ethiopia, normally consume the mutants (31 DAF), the genotypes are most likely exhibiting their maximum protein quality. These data indicate that there may be a nutritional basis for the tradition of consuming the high-lysine varieties exclusively at the milk dough and late dough stages.

TABLE I
Protein Levels in the Germ During Development in Selected High-Lysine and Normal Sorghum Strains[a]

Genotype	Days After Flowering[b] 21	31	61
IS-11758	15.7 b	14.5 c	20.0 b
IS-11167	18.2 a	15.9 b	20.0 b
YM-3	18.9 a	16.6 b	22.8 a
P-721 opaque	16.5 b	15.9 b	21.0 b
P-721 normal	16.2 b	17.5 ab	23.5 a
BG-5	19.0 a	18.9 a	22.0 a
BG-6	18.5 a	18.1 a	21.0 b
BG-10	17.7 ab	16.6 b	20.4 b

[a] Percent protein in germ (dry basis).
[b] Means within a column followed by a common letter do not differ significantly at the 0.01 level as determined by Duncan's multiple range test.

TABLE II
Lysine Levels in the Germ During Development in Selected High-Lysine and Normal Sorghum Strains[a]

Genotype	Days After Flowering[b] 21		31		61	
IS-11758	3.4 bc	(0.53) b	3.9 ab	(0.56) c	5.1 a	(1.01) ab
IS-11167	3.2 c	(0.57) b	3.6 b	(0.57) c	4.8 b	(0.94) b
YM-3	3.1 c	(0.59) b	3.2 b	(0.53) c	5.1 a	(1.20) a
P-721 opaque	3.6 b	(0.59) b	4.2 a	(0.66) bc	4.6 b	(0.98) b
P-721 normal	3.5 b	(0.57) b	4.6 a	(0.70) b	4.5 b	(1.07) ab
BG-5	3.8 ab	(0.73) a	4.3 a	(0.80) a	5.0 a	(1.09) a
BG-6	4.0 a	(0.74) a	4.1 a	(0.74) ab	5.2 a	(1.10) a
BG-10	4.1 a	(0.73) a	4.2 a	(0.69) b	5.3 a	(1.10) a

[a] Grams of lysine per 100 g of germ protein. Lysine as percent of germ (dry basis) is shown in parentheses.
[b] Means within a column followed by a common letter do not differ significantly at the 0.01 level of significance as determined by Duncan's multiple range test.

TABLE III
Protein Levels in the Endosperm During Development in Selected High-Lysine and Normal Sorghum Strains[a]

Genotype	Days After Flowering[b] 21	31	61
IS-11758	13.9 a	13.1 a	14.6 a
IS-11167	13.8 a	13.9 a	15.7 a
YM-3	12.8 ab	14.3 a	15.7 a
P-721 opaque	9.7 d	10.2 c	10.6 d
P-721 normal	11.2 c	11.0 bc	12.6 b
BG-5	12.4 b	11.6 b	12.2 bc
BG-6	12.4 b	11.3 bc	11.8 bc
BG-10	12.0 bc	10.7 bc	11.3 cd

[a] Percent protein in endosperm (dry basis).
[b] Means within a column followed by a common letter do not differ significantly at the 0.01 level of significance as determined by Duncan's multiple range test.

TABLE IV
Lysine Levels in the Endosperm During Development in Selected High-Lysine and Normal Sorghum Strains[a]

Genotype	Days After Flowering[b] 21		31		61	
IS-11758	2.8 ab	(0.39) a	3.1 a	(0.40) b	2.0 a	(0.30) b
IS-11167	2.6 bc	(0.36) ab	3.4 a	(0.47) a	2.2 a	(0.34) a
YM-3	3.1 a	(0.40) a	3.0 a	(0.43) ab	2.4 a	(0.37) a
P-721 opaque	2.7 ab	(0.26) c	2.6 bc	(0.27) de	2.2 a	(0.24) c
P-721 normal	2.1 c	(0.23) c	1.4 e	(0.15) f	1.5 b	(0.19) d
BG-5	2.9 ab	(0.36) ab	2.6 c	(0.30) cd	1.4 b	(0.17) d
BG-6	2.9 ab	(0.35) ab	2.1 d	(0.23) e	1.4 b	(0.17) d
BG-10	2.9 ab	(0.34) b	3.1 a	(0.33) c	1.5 b	(0.17) d

[a] Grams of lysine per 100 g of endosperm protein. Lysine as percent of endosperm (dry basis) is shown in parentheses.
[b] Means within a column followed by a common letter do not differ significantly at 0.01 level of significance as determined by Duncan's multiple range test.

In general, the trends associated with the mean value for lysine as percent of protein and lysine as percent of sample were similar for P-721 and the Ethiopian mutants. Nevertheless, as indicated earlier, P-721 opaque had a lower protein content than its normal parent in all stages of grain development. This low protein content in P-721 opaque could be a result of some kind of feedback control on protein synthesis by the increasing concentration of free amino acids. The Ethiopian high-lysine varieties, on the other hand, have an additional advantage in that percent protein in the endosperm increased even as the amino acid concentration increased. Therefore, the excellent performance in feeding trials on the biological value of the high-lysine mutants (Singh and Axtell 1973, Mohan and Axtell 1975) may be attributed to their significantly higher percent of germ, higher absolute protein content, and production of lysine-rich proteins. This combination of desirable components makes the Ethiopian high-lysine mutants not only superior to the normal varieties, but also unique relative to any of the endosperm mutants in corn and sorghum.

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