Quantitation of α-, β-, and γ-Kafirins in Vitreous and Opaque Endosperm of *Sorghum bicolor*[1]

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ABSTRACT

Landry-Moureaux fractionation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and enzyme-linked immunosorbent assays (ELISA) were used to investigate qualitative and quantitative differences in the protein compositions of vitreous and opaque endosperms of sorghum. Vitreous endosperm contained 1.5–2 times more protein than opaque endosperm of the same variety. Opaque endosperm contained more albumin-globulin protein and less kafirin, but the amount of glutelin protein was similar in both vitreous and opaque endosperm. The albumin-globulin fraction of opaque endosperm contained some higher molecular weight bands not seen in the albumin-globulin fraction of vitreous endosperm. The vitreous endosperm contained some lower molecular weight bands that did not appear in the opaque endosperm extracts. Both the SDS-PAGE gel of a total kafirin extract and the ELISA results indicated that the kafirin fraction of opaque endosperm has a higher percentage of β- and γ-kafirins and a lower percentage of α-kafirins compared to that of the kafirin fraction of vitreous endosperm. Because opaque endosperm had far less total kafirin than vitreous endosperm, total kafirin content may be more important than the protein-body composition in determining sorghum grain hardness.

Sorghum (*Sorghum bicolor* (L.) Moench) is a major source of nutrition in the developing countries of the semiarid tropics. Sorghum improvement programs in these areas focus on developing new varieties that are not only high-yielding and resistant to disease but also acceptable for local food preparation. Grain hardness is an important characteristic related to food quality. It affects milling and storage quality of sorghum grain (Rooney and Sullins 1977, Doggett 1981) as well as quality and texture of sorghum food products (Mukuru et al 1981, Scheuring et al 1981).

Hardness of sorghum has been attributed to its starchy endosperm structure. The endosperm of sorghum is composed of peripheral, vitreous, and opaque regions. The peripheral and vitreous endosperm have a dense and shiny appearance. The cell components are tightly packed, and a continuous protein matrix holds the starch granules and protein bodies together. Opaque endosperm, located in the center of the endosperm, has a soft and floury appearance. The cell components are loosely packed with fewer protein bodies and a discontinuous protein matrix (Hoseney et al 1974, Rooney and Miller 1981). Sorghum varieties differ in the ratio of vitreous to opaque endosperm. There is a significant correlation between percentage of vitreous endosperm and grain hardness (Kirleis and Crosby 1981).

Beyond the relationship between grain hardness and endosperm structure, little is known about the biochemical basis of grain hardness. The majority of grain hardness research has been done on wheat and comparatively little done on sorghum. Much of the current literature has focused on how protein components affect grain hardness.

Sorghum proteins, similar to other cereal proteins, are classified according to solubility (Osborne 1987, Landry and Moureaux 1970). The proteins are grouped as albumins (water-soluble proteins), globulins (salt-soluble proteins), prolamins (alcohol-soluble proteins), and glutelins (proteins soluble in dilute alkali). The prolamin fraction of sorghum, kafirin, is further divided into α-, β-, and γ-kafirins, based on differences in solubility, molecular weight, and structure (Shull et al 1991).

In sorghum, it is suggested that kafirins located in protein bodies may be responsible for grain hardness because harder, more vitreous sorghum varieties generally have a higher kafirin content than do soft, opaque varieties (Kirleis and Crosby 1981, Cagampang and Kirleis 1984). Studies by Abdelrahman and Hoseney (1984) showed that breaking strength of sorghum starch pellets could be increased by the addition of kafirin extract. These results, however, did not show a conclusive connection between kafirins and grain hardness because kafirins in situ are stored in membrane-bounded protein bodies rather than in solution.

Grain hardness is significantly correlated to the ratio of vitreous to opaque endosperm, so determining the protein compositional differences between these regions may be profitable. Previous research has suggested that prolamin composition may play a role in grain hardness. In maize, Wallace et al (1990) showed changes in the zein protein when opaque, high-lysine varieties were genetically modified to increase hardness.

In this study, vitreous and opaque endosperm were analyzed to discover the qualitative and quantitative differences in albumins-globulins, kafirins, and glutelin-type protein fractions. In addition, enzyme-linked immunosorbent assay (ELISA) was used to quantitate the relative proportions of α-, β-, and γ-kafirins in both portions of sorghum endosperm.

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MATERIALS AND METHODS

Plant Material

Three sorghum varieties, varying in hardness and percent vitreousness, were selected for this study: Mshimba (hard), P721N (intermediate), and Zanab elsha (soft). They were grown at the Purdue Agronomy Research Center at West Lafayette, IN, during the 1987 crop year. Immature sorghum kernels were collected at 30 days after half-bloom and stored at −70°C. Mature grain samples were harvested at physiological maturity and stored at room temperature.

Percent Vitreousness Determination

Weight percent. Mature sorghum was decorticated for 45 sec in a Strong-Scott barley pearler and hand-degermed. From approximately 1 g of whole endosperm, opaque endosperm was manually separated from vitreous endosperm using an adjustable-speed drill (Dremel, Racine, WI). Vitreous and opaque fractions were weighed. Percent vitreousness was calculated by dividing vitreous endosperm weight by total endosperm weight.

Area percent. Area percent was determined according to the procedure described by Kirleis et al (1984). Thirty kernels were placed in a 2.54 cm diameter plastic cup with the germ tip-to-panicle axis in a horizontal position. Spurr low-viscosity embedding medium was added to a depth of 1 in. and polymerized overnight at 65°C. Longitudinal cross-sections parallel to the germ face were prepared by sanding with 60- and 100-grit sandpaper until the largest area of the floury endosperm was brought to the surface. Areas of vitreous and opaque endosperm were determined by tracings with a Zeiss Videoplan Image Analyzer (Carl Zeiss, Thornwood, NJ).

Grain Hardness Determination

Grain hardness was determined by the Stenvert grinding resistance test (Stenvert 1974). Whole grain was conditioned in a humidity chamber to about 13% moisture (27°C, 67% rh). Samples (20 g) of each variety were prepared in triplicate and ground with a Glen-Creston 14-580 mill (Maywood, NJ). The swinging hammer mill had a grooved grinding chamber fitted with a 2.0-mm aperture screen. Milling speed was 3,600 rpm. Time required to collect 17 ml of ground meal was recorded as an index of grain hardness.

Sample Preparation

Grain was mechanically decorticated and hand-degermed. From the resultant endosperm pieces, opaque endosperm was manually separated from vitreous endosperm as described for weight percent determinations. Vitreous and whole endosperm were ground on a Tecator sample mill (Perstorp, VA) with a 1-mm screen size and then reground in a ball mill (Crescent Dental, IL) for 2 min. Protein concentrations of vitreous, opaque, and whole endosperm flour were quantified by the Kjeldahl method (method 46-11, AACC 1983) using $CuSO_4TiO_2$ as the catalyst.

Protein Fractionation

Samples of vitreous, whole, and opaque endosperm flour (200, 200, and 300 mg, respectively) were weighed into 15-ml screw-top test tubes. A Landry-Moureaux extraction scheme was used to fractionate the endosperm proteins into albumin-globulin, prolamin (kafirin), and glutelin-type protein classes. All extractions were done in triplicate. A fraction containing albumin and globulin protein, along with soluble nonprotein nitrogen, was obtained by extracting the flour with 0.5*M* NaCl. The albumin and globulin proteins were separated from the nonprotein nitrogen by precipitation with trichloroacetic acid and deoxycholate (Bensadoun and Weinstein 1976). The flour was then sequentially extracted with 60% tertiary butanol containing 2-mercaptoethanol (2-ME) (2%, v/v), yielding the kafirin fraction (Landry and Moureaux 1970). After the albumin-globulins and kafirins were extracted, the remaining protein in the flour was predominantly glutelin. This fraction was collected by washing remaining flour from extraction tubes.

For protein determination, extracts were dried in digestion tubes in a 40°C oven. Protein content was determined by Kjeldahl method.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Albumin and globulin extracts were concentrated using Centricon microconcentrators (Amicon, Danvers, MA) to a final concentration of 4 μg/μl. The concentrated albumin-globulin extracts (100 μl) were mixed with 20 μl of sample buffer (0.06*M* Tris, 2.5% [w/v] sodium dodecyl sulfate [SDS], 2.5% [v/v] 2-ME, and 0.48*M* glycine in 0.05% [w/v] bromophenol blue) and heated for 1 min in a boiling water bath. Kafirin extracts were freeze-dried and redissolved into sample buffer to a final concentration of 4 μg/μl and heated in a boiling water bath. Glutelin proteins were extracted from residual flour with sample buffer (150 μl/100 mg) on a shaker (Labindustries, Inc., Berkeley, CA) for 4 hr. Flour was pelleted by centrifugation. The supernatants were heated in a boiling water bath for 1 min.

SDS-polyacrylamide gel electrophoresis (PAGE) was conducted (Pharmacia LKB horizontal Multiphor II, Piscataway, NJ) using 125- × 260- × 2-mm gels as described by Watterson et al (1990). Gels were stained using 0.05% (w/v) Coomassie Brilliant Blue R250 stain in 25% (v/v) methanol, 10% (v/v) acetic acid. Gels were destained in 25% (v/v) methanol, 10% (v/v) acetic acid (Wilson 1986).

Purification of Kafirin Proteins

α- and γ-Kafirin proteins were purified from whole endosperm flour according to Watterson et al (1990) and Shull et al (1992). The major β-kafirin corresponding to the M_r 20,000 polypeptide was purified. Total kafirin was extracted from whole sorghum flour (1 g of flour/1 ml of 60% 2-propanol). Extract was mixed in a 1:1 ratio with sample buffer (0.06*M* Tris, 2.5% [w/v] SDS, 2.5% [v/v] 2-ME, 0.06*M* borate in 0.05% (w/v) bromophenol blue) and loaded onto a 195- × 260- × 2-mm preparative SDS-PAGE gel (1 ml, total). The running gel consisted of 13% (w/v) total acrylamides, 0.4*M* Tris, 0.1% SDS (pH 8.8). The stacking gel consisted of 4% (w/v) total acrylamides, 0.125*M* Tris, 0.1% SDS (pH 6.8). Gels were run in an LKB horizontal Multiphor II electrophoresis system at 190-v constant voltage for 12 hr at 13°C using a tank buffer (0.025*M* Tris, 0.06*M* borate, 0.1% SDS [pH 8.8]). Immediately after electrophoresis, the kafirin bands were visualized by soaking the gel in ice-cold 0.25*M* KCl plus 1 m*M* dithiothreitol. The M_r 20,000 band was located and cut from the gel. The protein was eluted from the gel by grinding the piece in elution buffer (0.1% SDS, 0.5*M* Tris, 1 m*M* ethylenediaminetetraacetic acid, 5 m*M* dithiothreitol) (Hager and Burgess 1980). Gel pieces were removed by filtration (Whatman No. 1). The supernatant was dialyzed (Spectra Por 4 membranes, Fisher Scientific) against water and freeze-dried.

Antibody Production

Protein (300 μg) was mixed with Freund's adjuvant (Sigma Chemical Co., St. Louis, MO). Chickens were injected at several sites under both wings, and injections were repeated at two-week intervals for a total of three injections per chicken. Freund's complete adjuvant was used for the first injection; subsequent injections were made with incomplete adjuvant. Eggs were collected 6–12 weeks after the first injection (Song et al 1985). Antibodies were partially purified from the yolks according to Polson et al (1980). Antibodies were stored at −30°C until used. Preimmune eggs were collected from each hen for one week before injection. Crude antisera were used in all immunological procedures.

Immunoblotting Analysis

Total kafirin (800 ng) extracted from whole endosperm was separated by SDS-PAGE. Proteins were transferred from SDS-PAGE gels to nitrocellulose (Towbin et al 1979) using a continuous buffer system (39 m*M* glycine, 48 m*M* Tris, 0.037% (w/v) SDS, and 20% (v/v) methanol) in an LKB Multiphor II Nova Blot apparatus. Electroblotting was performed at 80 mA for 1 hr at

room temperature. After the transfer, nitrocellulose papers were agitated for 1 hr in 10 m*M* Tris (pH 7.4), 140 m*M* NaCl, and 0.15% (v/v) Tween 20 (TTBS) containing 3% (w/v) nonfat dry milk to block nonspecific binding. Membranes were washed and then incubated overnight with either anti γ-kafirin serum, anti β-kafirin serum, anti α-kafirin serum, or preimmune serum diluted 1:500 in TTBS. Membranes were then rinsed and incubated for 2 hr in rabbit-antichicken-horseradish peroxidase conjugate (Jackson Immunoresearch Laboratories, Westgrove, PA) diluted 1:3,000. After incubation, membranes were washed, and sites of antibody-protein association were visualized by reaction with 30% (v/v) H_2O_2 and 4-chloronapthanol (Lending et al 1988).

Enzyme-Linked Immunosorbent Assay

Total protein was extracted from vitreous and opaque endosperm flour samples with 30 mg/1 ml of 0.125*M* borate buffer, 2% SDS, and 1% 2-ME (pH 10.0) by shaking 16 hr at 37°C (Wallace et al 1990). Extracts were used within 24 hr. Total protein extracts of vitreous endosperm flour were diluted 200-fold in borate buffer; total protein extracts of opaque endosperm flour were diluted 50-fold in borate buffer. These total protein extracts were diluted again 1,250-fold in 40% (v/v) ethanol-10% (v/v) acetic acid for the γ- and β-kafirin enzyme-linked immunosorbent assay (ELISA) or 2,500-fold for the α-kafirin ELISA. Diluted total protein extract (100 μl) was added to 100 μl of 40% ethanol-10% acetic acid in the bottom well of an ELISA plate (Immulon 2, Dynatech Laboratories, Inc., Chantilly, VA). The sample was mixed immediately. Six twofold dilutions were made into adjacent wells that contained 40% ethanol-10% acetic acid. The plates were incubated for 2 hr at 30°C to allow the antigens to bind. After incubation, the solution was removed from the wells by aspiration, and the wells were rinsed three times with 25 m*M* Tris-HCl, 0.9% NaCl, and 0.05% Tween 20 (TBS-T) (pH 7.5). Then 100 μl of primary antibody solution, anti α-kafirin, anti β-kafirin, or anti γ-kafirin serum, was added to each well (1:1,000 in TBS-T). The plates were again covered and incubated for 2 hr at 30°C. After the solution was aspirated and the wells rinsed, 100 μl of rabbit-antichicken alkaline phosphatase (Jackson Immunoresearch Laboratories), diluted 1:5,000 in TBS-T, was added to each well and incubated overnight at 4°C. Aspiration and rinsing steps were repeated. Enzyme substrate (Sigma 104 phosphatase substrate tablets [para-nitrophenyl phosphate]) was dissolved in diethanolamine (9.7%, v/v, pH 9.8) as described by Clark et al (1986) and added to each well. Color was allowed to develop for 2 hr, and then absorbance was read at A_{405} on a V_{max} ELISA platereader (Molecular Devices, Menlo Park, CA).

Standard curve. α-Kafirin was extracted from mature sorghum using 90% (w/v) ethanol (3 g of flour/20 ml). β-Kafirin was obtained by extracting total kafirins from sorghum flour with 60% 2-propanol-1% 2-ME and precipitating the α- and β-kafirins by adding 1 volume of water, redissolving the pellet in 60% 2-propanol-1% 2-ME, and then precipitating β-kafirin by adding 3 volumes of 100% 2-propanol (Esen 1986). The β-kafirin pellet was redissolved in borate buffer. γ-Kafirin was directly extracted from immature sorghum with 20% 2-ME as previously mentioned. The protein content of the α-, β-, and γ-kafirin extracts were determined by the Kjeldahl method. Extracts were diluted and analyzed by ELISA, along with a total protein extract from hand-degermed P721N that was ground into a flour with the ball mill. For the ELISA standard curves, absorbance at 405 nm was graphed against protein content of the extracts. Regression analysis was performed on the linear portion of the graphs. From these lines, values (mg) of α-, β-, and γ-kafirins per 30 mg of P721N flour were determined. Analyses were done in triplicate and average kafirin values (mg) were reported.

Samples. Absorbance of vitreous and opaque endosperm protein extracts were graphed against relative antigen concentration. P721N was freshly extracted and analyzed on every ELISA plate. The antigen concentration of the samples was calculated by dividing the slope of the linear regression line by the slope of the line for P721N, and then multiplying by the amount (mg) of α-, β-, or γ-kafirins per 30 gm of P721N whole sorghum flour as determined from the standard curve. From this calculation, amounts (mg) of α-, β-, and γ-kafirins per 30 mg of vitreous and opaque endosperm flours were determined, as well as percentages of α-, β-, and γ-kafirins. Although this may be only an estimate of the true amounts (mg) of the kafirins, it gives accurate measures of the relative proportions of α-, β-, and γ-kafirins in sorghum extracts. Mean comprisons were made with Student's *t* test (Natrella 1966).

RESULTS

Grain Hardness

According to the Stenvert test, Mshimba had the longest grinding time and highest area percent vitreousness (Table I) and weight percent vitreousness (Table II). P721N and Zanab elsha had lower Stenvert grinding times and percent vitreousness.

Protein Fractionation

Vitreous endosperm contained from 1.5 to 2 times more total protein than opaque endosperm of the same variety (Table II). The highest total protein content was in the vitreous endosperm of the hard variety (Mshimba, 11.3%). The lowest protein content was in the opaque endosperm of the soft variety (Zanab elsha, 5.6%) (Table II). These differences between varieties were also reflected in total protein content of the whole endosperm. Whole endosperm flour of Zanab elsha had a lower protein content than that of P721N or Mshimba. Large differences were also noted in the prolamin or kafirin content of opaque and vitreous endosperm. Vitreous endosperm contained 5.8–8.5% kafirin, but opaque endosperm contained only 2.0–2.4% kafirin. In contrast, opaque endosperm had higher amounts of albumin and globulin proteins. The amount of glutelin protein was similar in both vitreous and opaque endosperm (Table II).

TABLE I
Stenvert Hardness and Area Percent Vitreousness for Sorghum Varieties[a]

Variety	Stenvert hardness[b] (sec)	% Vitreousness[c] (area %)	Hardness Classification
Mshimba	56.5 a	80 a	Hard
P721N	35.5 b	56 b	Intermediate
Zanab elsha	18.7 c	30 c	Soft

[a] Means in the same column not followed by the same letter are significantly different ($P < 0.01$).
[b] Average of three replicates.
[c] Average of 15 replicates.

TABLE II
Weight Percent, Percent Total Protein, and Percent Protein of Landry-Moureaux Fractions of Vitreous, Opaque, and Whole Sorghum Endosperm[a,b]

Variety	Weight Percent[c]	Total Protein[d] (%)	Albumin-Globulin[d] (%)	Kafirin[d] (%)	Glutelin[d] (%)
Mshimba					
Vitreous	90	11.3 a	0.6 c	8.5 a	2.8 ab
Opaque	10	6.7 bc	1.1 a	2.4 d	3.0 ab
Whole	100	10.9 a	0.8 bc	6.5 ab	3.1 ab
P721N					
Vitreous	80	10.4 ab	0.6 c	7.5 ab	2.4 b
Opaque	20	7.0 bc	1.1 a	2.4 d	2.9 ab
Whole	100	9.8 ab	0.7 bc	5.7 bc	2.6 ab
Zanab elsha					
Vitreous	54	10.8 ab	0.6 c	5.8 bc	3.2 a
Opaque	46	5.6 bc	0.9 ab	2.0 d	2.9 ab
Whole	100	8.0 bc	0.6 c	3.8 cd	3.1 ab

[a] Means in the same column not followed by the same letter are significantly different ($P < 0.05$).
[b] Expressed as % extracted from vitreous and whole endosperm (200 mg) and opaque endosperm (300 mg).
[c] Expressed on a percentage as is basis.
[d] Expressed on a percentage dry weight basis, average of three replicates.

SDS-PAGE

SDS-PAGE profiles of albumin and globulin fractions showed very different banding patterns for vitreous and opaque endosperm. Vitreous endosperm displayed two lower molecular weight bands (M_r 18,000 and M_r 15,000) that were less intense in albumin-globulin fractions of opaque endosperm (Fig. 1). Albumin-globulin extracts from opaque endosperm had some higher molecular weight bands (M_r 44,000 and 38,000) that did not appear in extracts from vitreous endosperm.

SDS-PAGE showed no qualitative differences between kafirin proteins extracted from vitreous and opaque endosperm flour, but visual inspection of the gel showed that, in terms of total kafirin make-up, the opaque endosperm has relatively more γ-kafirin and less α-kafirin than vitreous endosperm (Fig. 2). SDS-PAGE profiles of glutelin protein were very similar for vitreous and opaque endosperm, with the exception of a M_r 60,000 band in opaque endosperm glutelins that was either absent or present in much lower amounts in glutelin fractions of vitreous endosperm (Fig. 3).

Immunoblotting Analysis

Anti α-kafirin serum reacted specifically with M_r 25,000 and 23,000 bands. Anti β-kafirin serum reacted specifically with the M_r 20,000 band. Anti γ-kafirin serum reacted specifically with the M_r 28,000 band. No reactions were observed when preimmune serum was substituted for the primary antibody (Fig. 4).

ELISA

Standard curve. The 90% ethanol extract of sorghum was almost entirely α-kafirin (Fig. 5A). Similarly, the γ-kafirin and β-kafirin extracts were almost entirely γ-kafirin and β-kafirin, respectively (Fig. 5B and C). Using the three graphs, the α-, β-, and γ-kafirin contents of degermed P721N flour was calculated as: α-kafirin = 2.1 mg/30 mg of flour, β-kafirin = 0.18 mg/30 mg of flour, and γ-kafirin = 0.26 mg/30 mg of flour.

Samples. Opaque endosperm had a significantly lower kafirin content than vitreous endosperm in all three varieties. The vitreous endosperm of Mshimba (hard), contained more kafirin than the vitreous endosperms of either P721N (intermediate) or Zanab elsha (soft) (Table III). Vitreous endosperm contained 80–84% α-kafirin, 7–8% β-kafirin, and 9–12% γ-kafirin. Opaque endosperm contained 66–71% α-kafirin, 10–13% β-kafirin, and 19–21% γ-kafirin (Fig. 6).

DISCUSSION

Quantitative and qualitative differences in the protein content of opaque endosperm and vitreous endosperm were found. Opaque endosperm was much lower in total protein content, mainly because of the reduction in kafirin protein content. Kafirin

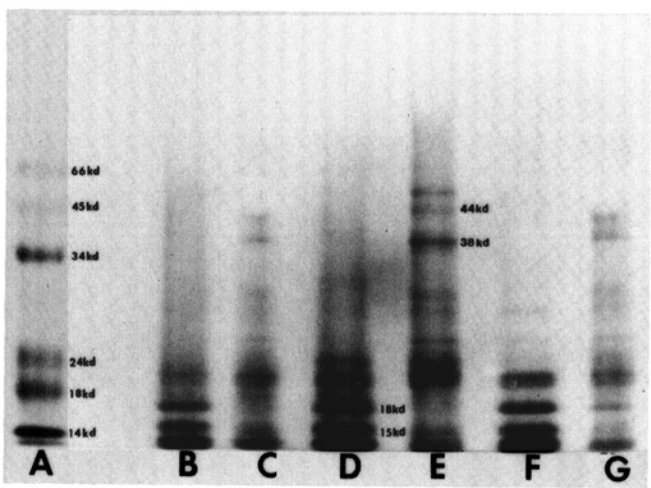

Fig. 1. Albumin and globulin protein extracts separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sorghum. **A**, molecular weight standard; **B**, vitreous endosperm of Mshimba; **C**, opaque endosperm of Mshimba; **D**, vitreous endosperm of P721N; **E**, opaque endosperm of P721N; **F**, vitreous endosperm of Zanab elsha; **G**, opaque endosperm of Zanab elsha.

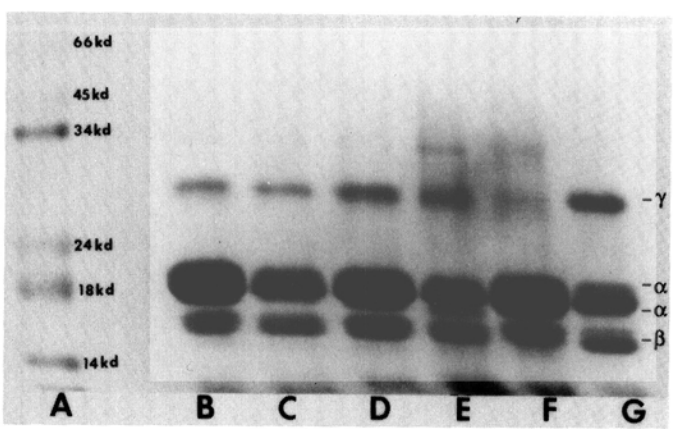

Fig. 2. α-, β-, and γ-Kafirin protein extracts separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sorghum. **A**, molecular weight standard; **B**, vitreous endosperm of Mshimba; **C**, opaque endosperm of Mshimba; **D**, vitreous endosperm of P721N; **E**, opaque endosperm of P721N; **F**, vitreous endosperm of Zanab elsha; **G**, opaque endosperm of Zanab elsha.

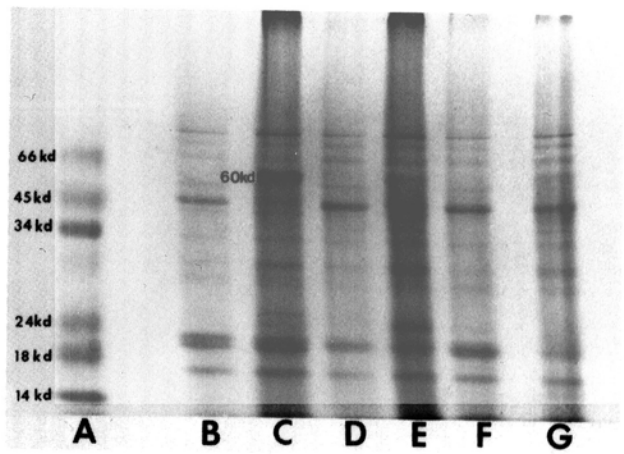

Fig. 3. Glutelin protein extracts separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sorghum. **A**, molecular weight standard; **B**, vitreous endosperm of Mshimba; **C**, opaque endosperm of Mshimba; **D**, vitreous endosperm of P721N; **E**, opaque endosperm of P721N; **F**, vitreous endosperm of Zanab elsha; **G**, opaque endosperm of Zanab elsha.

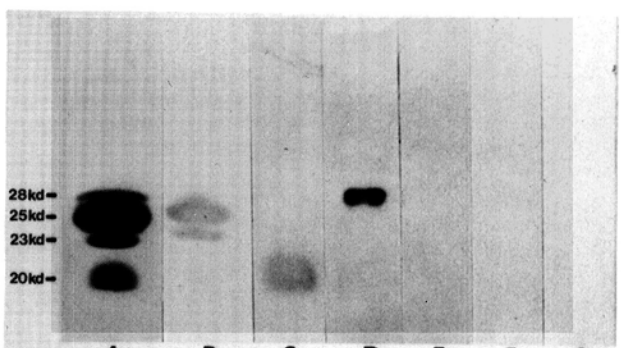

Fig. 4. Immunospecificity of α-, β-, and γ-kafirin antisera. **A**, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total kafirin. **B–G**, western blots of total kafirin reacted with: **B**, anti α-kafirin serum; **C**, anti β-kafirin serum; **D**, anti γ-kafirin serum; **E**, α-kafirin preimmune serum; **F**, β-kafirin preimmune serum; **G**, γ-kafirin preimmune serum.

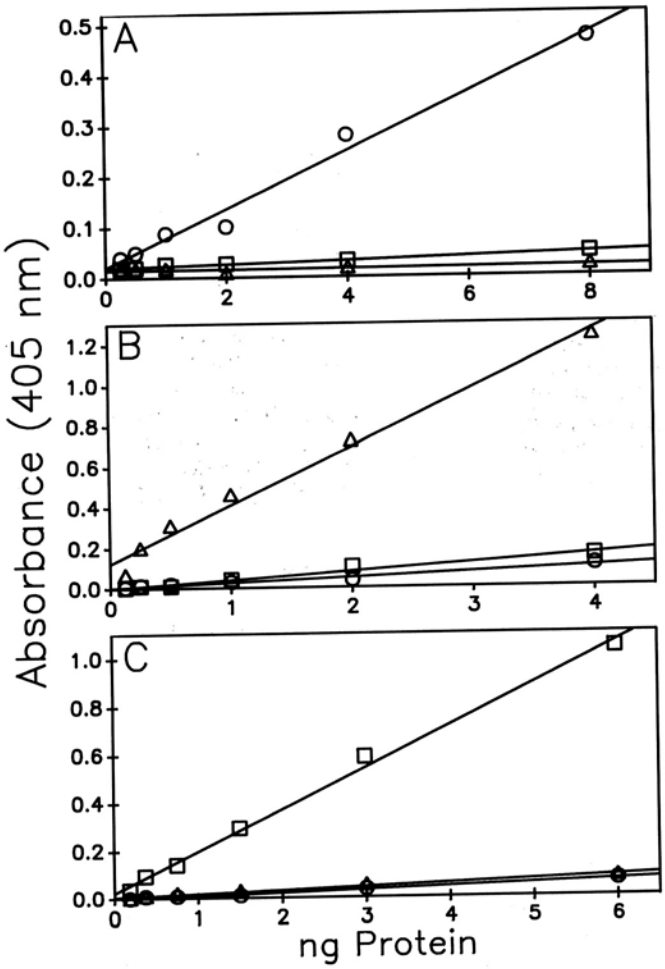

Fig. 5. Enzyme-linked immunosorbent assays of α-, β-, γ-kafirin. **A**, 90% ethanol extract of sorghum flour; **B**, partially purified β-kafirin from sorghum flour; **C**, 20% 2-mercaptoethanol of immature sorghum. ○ = α-kafirin. △ = β-kafirin. □ = γ-kafirin.

TABLE III
Amount of α-, β-, and γ-Kafirins in Vitreous Endosperm and Opaque Endosperm of Mshimba, P721N, and Zanab elsha Sorghum[a-c]

Variety	α-Kafirin	β-Kafirin	γ-Kafirin	Total Kafirin (α + β + γ)
Mshimba				
Vitreous	2.25 a	0.22 a	0.35 a	2.82 a
Opaque	0.44 c	0.09 b	0.14 c	0.67 c
P721N				
Vitreous	2.22 a	0.20 a	0.28 ab	2.70 a
Opaque	0.60 c	0.09 b	0.16 c	0.85 c
Zanab elsha				
Vitreous	1.70 b	0.14 ab	0.18 bc	2.02 b
Opaque	0.55 c	0.08 b	0.17 c	0.80 c

[a] Means in the same column not followed by the same letter are significantly different ($P < 0.01$).
[b] Expressed as mg per 30 mg of flour.
[c] Average of two replicates.

content of opaque endosperm was low in all three varieties. It was independent of varietal differences in grain hardness. In contrast, prolamin content of vitreous endosperm varied with hardness of the grain. Differences in kafirin content between hard and soft sorghum varieties was previously reported (Cagampang and Kirleis 1984). Our study indicates that hard sorghum varieties are higher in kafirin proteins because they contain lower amounts of opaque endosperm, which is low in kafirin, and higher percentages of vitreous endosperm, which contains relatively higher amounts of kafirins, compared to vitreous portions of softer varieties.

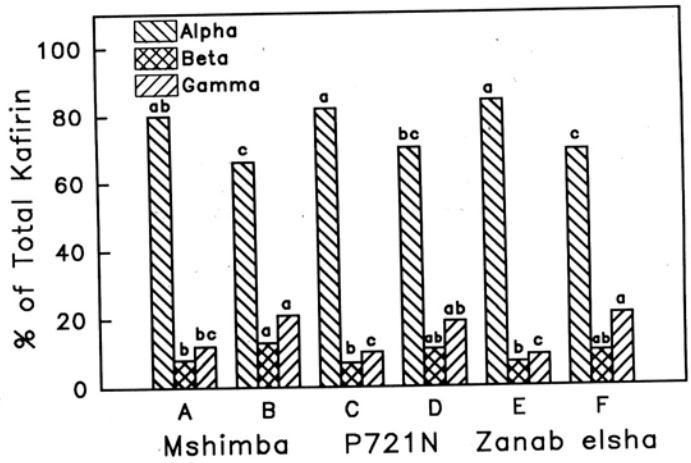

Fig. 6. Distribution of α-, β-, and γ-kafirin in vitreous and opaque endosperms (calculated by dividing mg of α-, β-, and γ-kafirin by mg of total kafirin as determined by enzyme-linked immunosorbent assays). **A**, vitreous endosperm of Mshimba; **B**, opaque endosperm of Mshimba; **C**, vitreous endosperm of P721N; **D**, opaque endosperm of P721N; **E**, vitreous endosperm of Zanab elsha; **F**, opaque endosperm of Zanab elsha. Mean percentages within the same kafirin class not followed by the same letter are significantly different ($P < 0.01$).

More differences were noted in the kafirin fraction when protein extracts of vitreous and opaque endosperm were analyzed by ELISA developed for the quantitation of α-, β-, and γ-kafirins. The kafirin fraction of opaque endosperm contained less α-kafirin and almost twice as much γ-kafirin than that of vitreous endosperm of the same variety (Fig. 6). Similar results were shown by the SDS-PAGE gel of the total kafirin extracts. These results suggest that the protein bodies of the vitreous endosperm differ in composition from those of the opaque endosperm. Similar results were reported by Shull et al (1992). In that study, we observed, with transmission electron microscopy, that the protein bodies of the central endosperm of sorghum contained more dark-staining inclusion than those located more peripherally in the endosperm. Immunocytochemistry showed that these dark-staining regions were composed of γ- and β-kafirins. α-Kafirin existed predominantly in the light-staining regions of the protein bodies.

Qualitative and quantitative differences between albumin-globulin fractions of the opaque and vitreous endosperms were found. Opaque endosperm had a higher albumin-globulin content. The albumin-globulin extract from opaque endosperm contained several higher molecular weight proteins either not present or present in reduced amounts in vitreous endosperm extracts. Vitreous endosperm extracts contained two low molecular weight bands present in reduced amounts in extracts from opaque endosperm. Glutelin protein contents of opaque and vitreous endosperm were very similar both qualitatively and quantitatively.

In sorghum, it has been suggested that differences in hardness between vitreous and opaque endosperms are due to the formation of a continuous protein matrix in vitreous endosperm that entraps protein bodies and starch granules (Shull et al 1990). This protein matrix is composed of albumin-globulin and glutelin-type proteins (Seckinger and Wolf 1973). Therefore, it may be expected that the total amount of albumin-globulin and glutelin-type proteins would be higher in vitreous endosperm than in opaque endosperm. However, as reported, the amount of glutelin protein per gram of endosperm was the same in both vitreous and opaque endosperm, but the amount of albumin and globulin was actually higher in opaque endosperm. These findings may not be contradictory to the role previously attributed to the continuous matrix. Wall et al (1988) suggested that the glutelin proteins that make up the protein matrix are derived from cytoplasmic albumin and globulin proteins that form disulfide linkages during the latter stages of development. In addition, Shull et al (1990) observed, with transmission electron microscopy, that the protein matrix appeared to be composed of cytoplasmic material that had deteri-

orated, condensed, and been compressed by the enlarging starch granules and protein bodies. Our study shows that opaque endosperm had much less kafirin protein than vitreous endosperm, indicating a reduction in the amount of protein bodies in this area. It is possible that, in the opaque endosperm, the cytoplasmic proteins deteriorate and form disulfide bonds similar to those of the vitreous endosperm. However, opaque endosperm lacks enough protein bodies and tightly packed starch granules to compress the cytoplasmic protein and form it into a matrix that entraps or adheres to starch granules and protein bodies.

How the noted qualitative differences in albumin and globulin content or protein body composition may affect adhesion between endosperm components is unclear. If the extracted albumins and globulins are from the protein matrix, then it is possible that the presence or absence of a particular protein from the matrix may affect its adhesion capabilities. Increased amounts of γ-zein in maize are thought to increase grain hardness. The high cysteine content of γ-zein would, theoretically, enable γ-zein to form disulfide bonds with other endosperm proteins (Wallace et al 1990). We found, however, that the percentage of γ-kafirin was actually higher in opaque endosperm. Opaque endosperm has far less total kafirin than vitreous endosperm, suggesting that, in sorghum, the total kafirin content or protein body amount may be more important than the protein body composition in determining grain hardness.

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