

# Kernel Characteristics and Protein Fraction Changes During Seed Development of High-Lysine and Normal Sorghums<sup>1</sup>

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## ABSTRACT

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Changes in seed characteristics and the proportions and electrophoretic mobilities of Landry-Moureaux (L-M) endosperm protein fractions of high-lysine opaque and normal sorghums were followed during kernel development. Seed weight and volume increased steadily through 45 days after half-bloom for normal but plateaued a week earlier for opaque. Percentage embryo of whole seed increased similarly for both lines from an average 2.1 to 8.1% during development. Normal endosperm protein declined 0.7% during seed maturation while that of opaque increased 1.2%. L-M fraction I (albumins and globulins) differed little among lines. Fraction II plus III (total kafirin) accumulation in opaque plateaued two

weeks before normal, whereas fraction IV plus V (total glutelin) increased more rapidly in opaque than normal endosperms. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of endosperm protein fractions showed few or no differences in banding patterns. It is concluded that the opaque gene (*O*) manifests itself in a pleiotropic manner with respect to endosperm proteins through quantitative rather than qualitative shifts in major fractions. The enhanced lysine content of opaque results primarily from an increase in relatively lysine-rich glutelins and decrease in lysine-deficient kafirins.

Efforts to improve protein quality of sorghum grain were aided considerably by Mohan and Axtell's (1975) discovery of a partially dominant opaque (*O*) gene conditioning a soft, floury endosperm with substantially elevated lysine content. Opaque has previously been referred to as P-721 opaque and its isogenic normal as P-721 normal. Lysine is the first limiting amino acid in sorghum grain (Pond et al 1958). The enhanced biological value of opaque compared with its normal endosperm parent was demonstrated in a 28-day rat feeding trial (Axtell et al 1974).

High-yielding opaque sorghums have been identified in selections from crosses to materials with diverse genetic background (Christensen 1978, Axtell et al 1979). However, the isogenic comparison of opaque sorghum with its 954114 normal parent, tested in three years of trials, averaged 15% less yield (VanScoyoc 1979). This yield reduction was attributed primarily to lower seed weight as a result of a premature cessation of dry matter accumulation in opaque compared with normal panicles during development. Seed number per panicle was not affected (VanScoyoc 1979).

A comparison of mature seed characteristics (Singh and Axtell 1973, Guiragossian 1977) indicated that Ethiopian high-lysine sorghum, IS-11167, conditioned by the recessive gene, *hl*, had higher whole kernel lysine than P-721 opaque (3.25 vs. 2.70 g/100 g of protein) due to a much larger embryo size (22.1 vs. 10.2% of seed weight) and lower seed weight (2.47 vs 3.09 g per 100 seeds). Endosperm percent protein and lysine as a percent of endosperm protein were virtually identical for IS-11167 and opaque. Likewise, only small differences were observed in proportions of Landry-Moureaux (L-M) endosperm fractions (Guiragossian et al 1978). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of L-M protein fractions revealed two additional fraction I bands and one more fraction IV band for opaque than for IS-11167 (Guiragossian et al 1978).

High-lysine and normal sorghums were also compared with respect to mature seed characteristics (Guiragossian 1977) and the proportions and electrophoretic mobilities of endosperm protein fractions (Guiragossian et al 1978, Paulis and Wall 1979). Although these parameters have been followed during the course

of seed development in counterpart opaque-2 and normal maize (Murphy and Dalby 1971, Moureaux and Landry 1972, Tsai and Dalby 1974, Misra et al 1975), similar data from developing seeds is not available for sorghum.

The objectives of this study were to characterize changes in kernel characteristics and the quantity and electrophoretic mobilities of L-M endosperm protein fractions of opaque and normal sorghum during seed development.

## MATERIALS AND METHODS

Selfed seed of the high-lysine mutant, P-721 opaque, and its normal endosperm parent, P-954114, were planted in a randomized complete block with six replicates at the Purdue University Agronomy Farm, West Lafayette, IN, in 1976. Plots (two per block) consisted of four rows each 45.72 m long and spaced 0.76 m apart.

Plots were overplanted and thinned (19 days after planting) to 0.61 m centers (75 plants per plot row). Main culm panicles were tagged when pollen shed had extended halfway down the head. A total of 15 panicles per genotype, all tagged on the same day, was harvested at random among the blocks at eight weekly intervals beginning at 10 days after half-bloom (DAHb).

At harvest, panicles were placed in polyethylene bags and immediately transported to the laboratory where they were weighed and held at -20°C in preparation for freeze-drying. Following lyophilization, panicles were reweighed, split into equal sections with a paper cutter, and upper and lower halves were separately hand-threshed to avoid losing any seed. Threshed seed was cleaned over an air blower, reweighed, and stored in sealed plastic envelopes at room temperature.

### Seed Dry Weight, Volume, and Density

One hundred seeds per upper-panicle-half per collection date for 10 lyophilized panicles of opaque and normal parent sorghum were hand sorted to remove damaged kernels and bulked. Four 100-seed samples were withdrawn from each bulk and weighed. Percent moisture was determined on two samples gravimetrically after oven-drying for four days at 105°C. Seed volumes were determined on the remaining samples by displacement of isopropyl alcohol in a 10-ml volumetric cylinder. Seed densities (g/cm<sup>3</sup>) were calculated as the weight (g) of 100 seeds (dry weight basis) divided by their volume (cm<sup>3</sup>).

### Endosperm Preparation

Bulked kernels from the upper-panicle halves from each collection date except day 10 were hand dissected into endosperm and embryo fractions as described by Guiragossian et al (1977).

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Ten-day-old seeds were not degermed because of their small size. Approximately 10-g samples of endosperm or whole 10-day seed were ground to pass a 0.4-mm sieve on a Udy cyclone sample mill and defatted 48 hr by refluxing at 50°C with Skellysolve B in a Soxhlet apparatus. Defatted endosperm samples were air-dried and placed in open vials in a desiccator over calcium chloride. At the time of endosperm protein fractionation, vials were closed and moisture determined on subsamples.

### Seed and Endosperm Weight Determinations

Embryos and endosperms of bulked seed from upper-panicle halves (200 for day 17, 100 for other collection dates except day 10) were separated by hand dissection. Dry weights were determined gravimetrically on each seed sample after oven-drying at 105°C for four days. Dried endosperms and embryos were then defatted as described above, air-dried one day, and dried again under the above conditions. Equal numbers of whole seeds for each collection date plus 500 10-day whole seeds were treated in the same manner.

### L-M Endosperm Fractionation

Defatted endosperm and 10-day whole seed proteins were separated into five solubility classes using the fractionation sequence of Landry and Moureaux (1970). Extractions of 1-g portions were carried out entirely in 50-ml Tefzel ETFE centrifuge tubes (Nalge Co.). After agitation on a magnetic stirrer, tubes were centrifuged 10 min at 13,282 × g in a Sorvall RC2 centrifuge without removal of magnetic stir bars. Supernatants were pooled for each fraction and adjusted to 50 ml with solvent.

For total N analysis, 5-ml aliquots of each fraction were evaporated to dryness in a boiling water bath under reduced pressure in 75-ml tubes. Samples were digested with 5 ml of H<sub>2</sub>SO<sub>4</sub> and 1 g of a 15:0.7 mixture (w/w) of K<sub>2</sub>SO<sub>4</sub> and HgO in a Technicon BD-40 block digester. Temperature was programmed from ambient to 300°C, held 15 min, and raised to 405°C for 30 min. Tubes were cooled, adjusted to 75 ml with distilled water, mixed by inversion, and aliquots were taken for ammonia analysis.

Ground defatted endosperm and whole seeds (0.1 g) were digested for N analysis as above, except that the block was preheated to 300°C, then programmed to 400°C at the start of digestion, and held 1 hr.

A modification of the Berthelot indophenol blue reaction was used to quantitate ammonia content of L-M fraction digests (VanScoyoc 1979). Nitrogen content of whole seed and endosperm digests was determined with a Technicon Autoanalyzer II according to the ammonia-salicylate method of Wall and Gehrke (1975) as modified by Noel and Hambelton (1976). Percent protein was calculated from total N by multiplying by 6.25.

### SDS-PAGE

An SDS-PAGE system similar to that of Laemmli (1970) as described by Tsai (1979) was used to characterize proteins extracted by a small-scale modified L-M fractionation procedure of Tsai (1979). Pooled extracts were dialyzed against 0.05M Tris-HCl, pH 6.9, containing 0.5% SDS and 1% 2-mercaptoethanol (2-ME) overnight at room temperature. Samples containing approximately 50 µg of protein as determined by the method of Lowry et al (1951) were electrophoresed at room temperature with 25 mA through the running gel until tracking dye reached the bottom of the gel. Gels were stored overnight in Coomassie Blue (0.1% in 45% methanol and 9% acetic acid) and destained in a solution of 15% methanol and 7% acetic acid.

## RESULTS

### Kernel Characteristics

Changes in 100-seed weight, volume, and density of opaque and normal sorghum during development are presented in Figure 1. One-hundred-seed dry weights and volumes for the normal parent increased linearly through 45 DAHB, which corresponds to physiological maturity, but leveled off or decreased slightly beyond 38 DAHB for the opaque mutant. Seed densities were higher for

the normal than for the opaque endosperm line at all stages of development. Densities increased rapidly from 17 to 31 DAHB but changed little thereafter. Increases in dry weight of 100 endosperms (Fig. 2) closely followed changes in whole kernel weights for both opaque and normal lines, whereas 100-embryo dry weights did not differ between lines and increased only slightly during kernel development.

A balance sheet of changes in dry weight and percentage of whole seed, endosperm, and embryo protein is shown in Table I. A nearly identical, linear pattern of protein accumulation was observed in whole kernels of both opaque and normal through 31 DAHB. Protein accumulation in both lines reached a maximum level by 38 DAHB and showed little change thereafter. Due to larger seed weight (accumulation) of normal compared with opaque beginning at 38 DAHB, the percentage of protein in whole kernels remained essentially constant, with opaque gaining a small increase over normal by 45 DAHB. Patterns of endosperm dry weight accumulation were similar for opaque and normal lines until 45 DAHB. Percent protein in normal endosperms declined 0.7% during seed maturation whereas that for opaque increased 1.2%. Embryo protein content from both lines changed little during seed development but recoveries were erratic.

### L-M Protein Fractionation

The percentage contribution to total protein of fraction I albumins and globulins declined rapidly and steadily during development for both lines (Fig. 3). These values include both protein and nonprotein nitrogen. At each date, recoveries of albumins were very similar.

Fraction II kafirins increased at similar rates in endosperms of both lines until 24 DAHB when opaque kafirin declined, whereas normal kafirin continued to increase until 45 DAHB (Fig. 3).

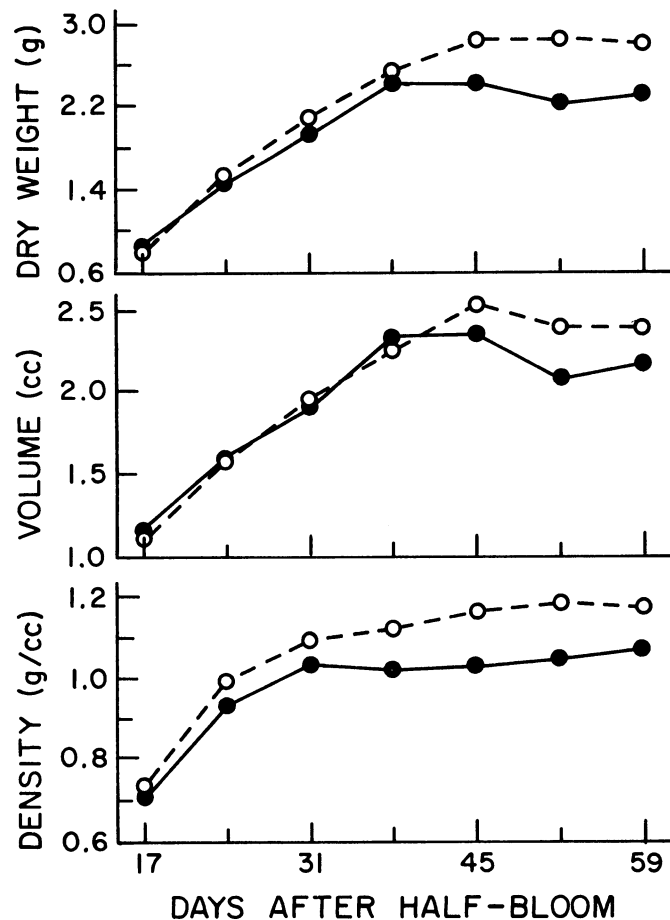


Fig. 1. Changes in 100-seed dry weight, volume, and density of opaque and normal sorghum during seed development: ● = opaque, ○ = normal.

Cross-linked kafirins (fraction III) increased continuously through endosperm development but at a faster rate for normal than for opaque (Fig. 3). The patterns of total kafirin accumulation (fraction II + III) (Fig. 3) indicated that total prolamins synthesis in normal sorghum progressed at a rapid, linear rate through 38 DAHB and then ceased, whereas production in opaque closely paralleled that of normal until 24 DAHB when further synthesis was prematurely terminated.

Extraction of fraction IV glutelin-like proteins gave low recoveries (8%) of equal size for both opaque and normal (Fig. 3). However, true glutelin (fraction V) constituted a greater percentage of total protein for opaque than normal beyond 24 DAHB (Fig. 3). This was due to a continual moderate increase in true glutelin in opaque endosperms throughout seed development and a slight decline in normal endosperms beyond 17 DAHB. Total glutelin (fractions IV + V) accumulation showed a pattern for opaque and normal similar to that for fraction V alone (Fig. 3).

The amounts of N per endosperm contributed by the various L-M fractions of opaque and normal endosperms during development are shown in Figure 4. Patterns of N per endosperm for the fraction I albumins and globulins were very different from those expressed as a percentage of total protein (Fig. 3). Opaque and normal endosperm N increased through 31 DAHB, then decreased through 52 DAHB. The increase at 59 DAHB has no real significance because physiological maturity occurred at 45 DAHB. Trends were parallel for both lines, with opaque having more N per endosperm at all dates beyond 17 DAHB. Although otherwise similar, trends of L-M fraction II + III total kafirin accumulation (Fig. 4) expressed as N per endosperm indicated a later cessation of net increase for opaque (38 vs. 24 DAHB) as well as for normal (52 vs. 38 DAHB) compared with total kafirin accumulation expressed as a fraction of total protein (Fig. 3). Glutelin-like L-M fraction IV protein N per endosperm (Fig. 4) accumulated at a gradual, linear, almost identical rate for both lines through 45 DAHB, whereas there was essentially no accumulation when expressed as a fraction of total protein (Fig. 3). True glutelin (fraction V) expressed as N per endosperm increased steadily through 38 DAHB in normal (Fig. 4), in contrast to accumulation patterns expressed as a fraction of total protein, where the normal endosperm protein content declined slightly

throughout development. However, opaque endosperms still accumulated much more true glutelin N per endosperm between 24 and 45 DAHB than normal when expressed as a percent of total protein.

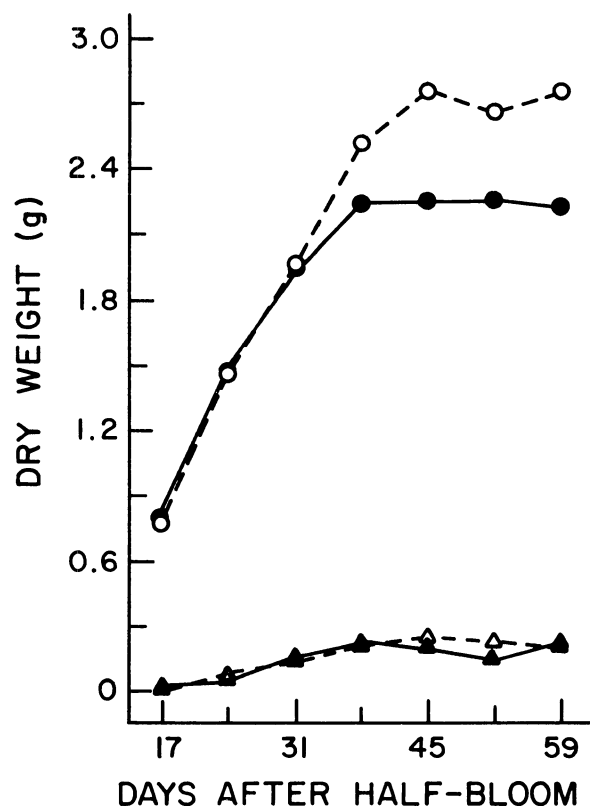


Fig. 2. Dry weight increases in opaque and normal endosperms and embryos during development: ● = opaque endosperm, ○ = normal endosperm, ▲ = opaque embryo, △ = normal embryo.

TABLE I  
Comparison of Opaque and Normal Seed Characteristics During Seed Development

Harvest Date	Whole Seed <sup>a</sup>			Endosperm				Embryo			
	Dry Wt (μg)	Protein		Dry Wt (μg)	% of Whole Seed	Protein		Dry Wt (μg)	% of Whole Seed	Protein	
		Dry Wt (μg)	%			Dry Wt (μg)	% of Endosperm			Dry Wt (μg)	% of Embryo
Day 10											
Opaque	1,602	222	13.9								
Normal	1,570	226	14.4								
Day 17											
Opaque	8,355	1,063	12.7	8,170	97.8	1,038	12.7	185	2.2	25	13.5
Normal	7,910	1,049	13.3	7,745	97.9	982	12.7	160	2.1	67	41.9
Day 24											
Opaque	15,750	2,068	13.1	14,900	94.6	1,891	12.7	850	5.4	178	20.9
Normal	15,660	2,155	13.8	14,800	94.5	1,833	12.4	860	5.5	322	37.4
Day 31											
Opaque	21,390	2,982	13.9	19,700	92.1	2,356	12.0	1,690	7.9	626	37.0
Normal	21,400	2,809	13.1	19,910	93.0	2,317	11.6	1,490	7.0	492	33.0
Day 38											
Opaque	24,740	3,573	14.4	22,510	91.0	2,963	13.2	2,230	9.0	610	27.4
Normal	27,340	3,914	14.3	25,170	92.1	3,047	12.1	2,170	7.9	867	40.0
Day 45											
Opaque	24,660	3,617	14.7	22,590	91.6	3,060	13.6	2,070	8.4	557	26.9
Normal	30,050	4,175	13.9	27,700	92.2	3,358	12.1	2,350	7.8	817	34.8
Day 52											
Opaque	24,460	3,491	14.3	22,790	93.2	3,149	13.8	1,670	6.8	664	39.8
Normal	28,990	3,961	13.7	26,700	92.1	3,267	12.2	2,270	7.9	694	30.3
Day 59											
Opaque	24,680	3,500	14.2	22,390	90.7	3,113	13.9	2,290	9.3	387	16.9
Normal	29,650	4,008	13.5	27,590	93.1	3,301	12.0	2,060	7.0	707	34.3

<sup>a</sup> Average replicate analyses for seeds at day 10; 200 seeds, endosperms, and embryos at day 17, and 100 seeds, endosperms, and embryos for the other dates.

## SDS-PAGE

SDS-PAGE patterns of albumin, globulin, kafirin, and glutelin proteins from 10-day seeds and 24-, 31-, 38-, and 45-day endosperms of opaque and normal are depicted in Figure 5. Opaque and normal endosperms have two or three major albumin bands as well as a number of minor bands which migrate more slowly (Fig. 5A). The major band nearest the origin stained more intensely for opaque than normal after 31 DAHB. A third possible major band was present at the extreme cathode edge of the gels for 38 and 45 DAHB, but this may be an artifact. All three major bands were absent from both lines at 10 DAHB.

Minor differences between banding patterns of globulins were seen at any developmental stage for the two lines (Fig. 5B). However, nearly all globulin bands were absent at 10 DAHB and only a few were discernible at 24 DAHB. Evidently, new globulin proteins are synthesized in the endosperm during seed development.

One, or possibly two partly overlapping major kafirin bands in addition to three minor bands were present in equal intensity at all developmental stages except 10 DAHB for both lines (Fig. 5C). None of the 5-6 bands were visible in 10-day whole seeds.

Except for the increased intensity of one band for normal endosperm at 38 and 45 DAHB, no discernible differences were seen between opaque and normal glutelin banding patterns (Fig. 5D). Glutelin gels streaked at 10 DAHB and no discrete bands were visible, but otherwise intensities were about the same for most bands at all developmental stages.

## DISCUSSION

Changes in 100-seed weight, volume, and density (Fig. 1) as well as endosperm and embryo dry weight (Fig. 2) of lyophilized opaque and normal materials were, as expected, very similar to patterns observed for air-dried seed collected from space planted

main culms the same year (VanScoyoc 1979). Patterns of 100 seed dry weight increases for opaque and normal (Fig. 1) closely resembled those for panicle dry matter accumulation (VanScoyoc 1979) and support the conclusion that the difference in yield between opaque and normal sorghum is primarily due to reduced seed weight of opaque.

Opaque and normal mature seed characteristics determined by Guiragossian (1977) agree fairly well with present values averaged over the last three collection dates (45, 52, and 59 DAHB) (Table I).

The constant percent protein values for opaque and normal whole seeds during development (Table I) indicated that protein content increased proportionally with seed weight. A lack of change in percent protein values with seed development was also reported for the cultivar Combine Kafir-60 by Kersting et al (1961) who found no change in percent N in seeds beyond 17 days.

The decline in true kafirin content as a percent of total protein for opaque after 24 DAHB (Fig. 3) and after 31 DAHB when expressed as N per endosperm (Fig. 4) may be due to its conversion to disulfide bridge cross-linked kafirin (Figs. 3, 4) which continued to increase beyond these harvest dates. The net effect of this hypothesized conversion was the plateauing of total sorghum kafirin accumulation at 24 DAHB when expressed as percent of total protein (Fig. 3) and 38 DAHB when expressed as N per endosperm (Fig. 4).

Changes in percent contribution to total N and amounts of N per endosperm in fractions of opaque-2 and normal maize during development (Murphy and Dalby 1971) are not directly comparable to values for opaque and normal sorghum (Figs. 3 and 4) due to differences in fractionation procedures.

A direct comparison of protein fraction changes in opaque and normal sorghum developing endosperms with those in maize is possible using data of Misra et al (1975). These workers

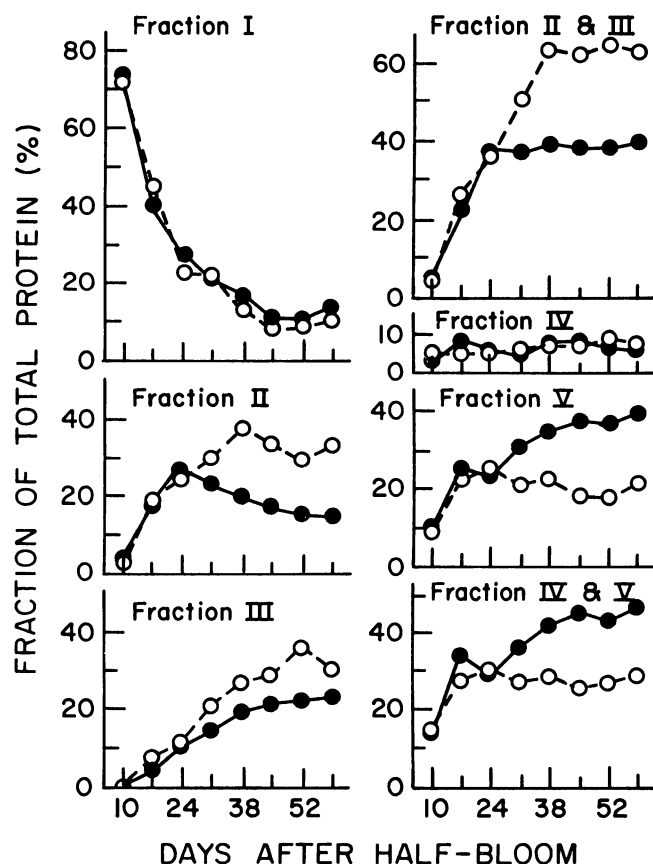


Fig. 3. Percentage contribution to total protein of various Landry-Moreaux endosperm fractions of opaque (●) and normal (○) sorghum during development. Ten-day values refer to fractions derived from whole seeds.

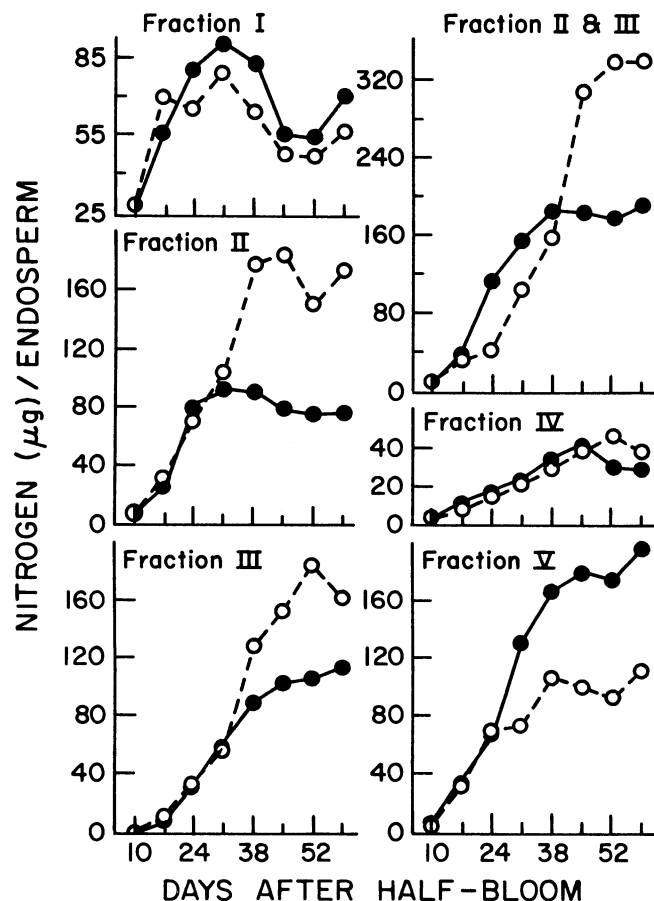


Fig. 4. Amount of nitrogen per endosperm contributed by various Landry-Moreaux endosperm fractions of opaque (●) and normal (○) sorghum during development. Ten-day values refer to fractions derived from whole seeds.

fractionated proteins of inbred Oh43 normal and opaque-2 maize by the L-M procedure at weekly intervals during seed development. They expressed their protein recovery data as a percentage of total endosperm protein. The general shape and parallel relationship of fraction I recoveries of opaque-2 and normal maize are quite similar to that observed for sorghum (Fig. 3). However, the difference in amount of recovered fraction I protein throughout development was consistently larger between opaque-2 and normal maize than between opaque and normal sorghum. Opaque-2 maize, at maturity, had about 7% more fraction I protein than normal maize. This is roughly twice the difference between opaque and normal sorghum.

Total prolamin recoveries (fraction II + III) of Oh43 opaque-2 and normal maize developing endosperms showed generally upward and parallel trends from day 14 onward (Misra et al 1975), with normal maize having larger recoveries than opaque-2 at all developmental stages. This sharply contrasts with total prolamin recoveries for opaque and normal sorghum developing endosperms (Fig. 3). Here, the progress curves overlapped until 24 DAHB, at which time net kafirin synthesis plateaued for opaque but continued to increase at a rapid rate through 38 DAHB for normal. Assuming a close similarity in maize between prolamin and total dry matter accumulation (DMA) progress curves, as found for opaque and normal sorghum (VanScoyoc 1979), the parallel slope of the zein accumulation curves for Oh43 maize may be cultivar specific. Makonnen and Bauman (1976) observed some opaque and normal counterpart maize inbreds with kernel DMA that closely resembled the zein progress curves for Oh43 (Misra et al 1975). Other inbreds, however, had DMA curves similar to those of opaque and normal sorghum presented in Figure 3.

The general trends of total glutelin (fraction IV + V) accumulation for opaque and normal developing endosperms of maize

(Misra et al 1975) and sorghum (Fig. 3) are similar, with recoveries for opaque mature endosperms 16–20% higher than for normal counterparts. However, total glutelin increased steadily in opaque sorghum endosperms throughout development, while plateauing after 17 DAHB in normal counterpart endosperms. On the other hand, total glutelin did not increase after 28 DAHB in opaque-2 maize (Misra et al 1975) and declined somewhat in normal counterpart endosperms after this developmental stage.

SDS-PAGE showed few or no differences in banding patterns for any protein fraction and only occasional differences in staining intensities between opaque and normal for all developmental stages except 10-day whole seeds where nearly all bands were absent (Fig. 5). Ten-day seeds were not degermed due to their tiny size, and because gels were loaded on an equal protein basis, embryo proteins would have diluted the endosperm proteins. However, the absence of any appreciable DMA in 10-day sorghum seeds (Fisher and Wilson 1975) and their hollow appearance after lyophilization support the electrophoretic data of little or no endosperm proteins at all at this developmental stage.

The similarities in protein patterns for all fractions are to be expected because opaque and normal differ in only a single gene. The absence of differences in number or in intensity of total kafirin bands for normal and opaque gels indicates that the opaque gene conditions quantitative rather than qualitative differences in kafirin levels. Of course, since the gels were loaded on an equal protein basis, these quantitative differences are not seen but they are apparent from the fractionation recoveries (Figs. 3 and 4). Qualitative and quantitative similarities in kafirin and cross-linked kafirin banding patterns of mature normal and opaque endosperms were also observed by Paulis and Wall (1979). The lack of any differences in patterns between the kafirin and cross-linked kafirin (alcohol-soluble reduced glutelin) fractions led these

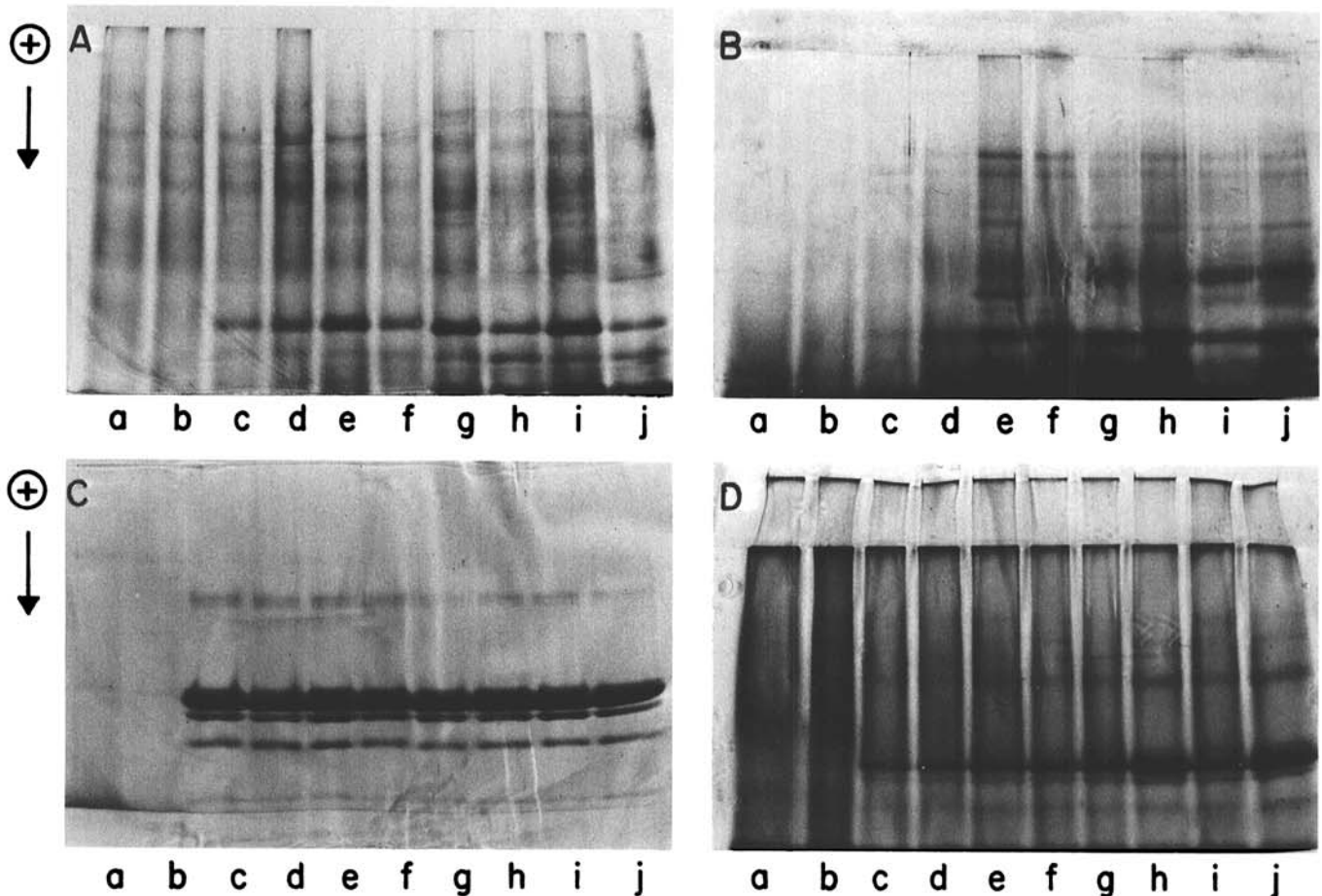


Fig. 5. A–D, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of Landry-Moreaux fraction proteins from 10-day whole seeds and 24-, 31-, 38-, and 45-day endosperms of opaque and normal sorghum: A = albumins; B = globulins; C = kafirins; D = glutelins; a = opaque (O), 10-day; b = normal (N), 10-day; c = O, 24-day; d = N, 24-day; e = O, 31-day; f = N, 31-day; g = O, 38-day; h = N, 38-day; i = O, 45-day; j = N, 45-day.

workers to conclude that there was no reason that further screening for new mutants by electrophoresis could not be done on a combined kafirin extract (alcohol plus reducing agent).

## CONCLUSIONS

The protein fractionation, amino acid, and electrophoretic data suggest that the opaque gene manifests primarily in a pleiotropic manner with respect to endosperm proteins through quantitative rather than qualitative shifts in the major fractions. Thus, improved lysine content is really an indirect effect of protein class changes that favor the relatively lysine-rich albumins, globulins, and glutelins over the lysine-deficient kafirins.

The absence of large differences between the amino acid (Van Scoyoc 1979) and electrophoretic patterns of opaque and normal endosperm fractions also supports the conclusion that the observed reductions in kafirin, total protein, and dry matter content in the opaque developing endosperm are similar to trends previously observed in opaque-2 maize. They are consistent with the hypothesis advanced by Tsai et al (1980) that prolamins storage protein plays an important role in DMA in the developing seed as a nitrogen sink.

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