Genetic Variation of Storage Proteins in Sorghum Grain:
Studies by Isoelectric Focusing and High-Performance Liquid Chromatography

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ABSTRACT

The genetic variability of the storage proteins in grain of several inbreds and hybrids of sorghum (*Sorghum bicolor* (L.) Moench) and varieties representing different races was investigated by separating extracted proteins by isoelectric focusing (IEF) and reversed-phase high-performance liquid chromatography (RP-HPLC). The kafirins were extracted from the ground grain with 60% *tert*-butanol and the reduced alcohol-soluble glutelins (ASGs) were extracted with 60% *tert*-butanol containing 5% 2-mercaptoethanol. IEF was conducted with reduced-alkylated proteins in thin polyacrylamide gels containing 2% pH 6–8 ampholines and 8M urea, and the resolved proteins were visualized with silver stain. RP-HPLC was performed on a large pore column by gradient elution with increasing concentration of acetonitrile in water. Both separations indicated that kafirins and ASGs from the same genotype contain mostly the same proteins but with quantitive differences in amounts. Grains of different inbreds usually contained some different kafirin and ASG proteins; the hybrids contained proteins inherited from both parents, with those from the female predominating. Representatives of the different sorghum races varied in their component alcohol-soluble proteins, with those most widely removed from the geographical center of origin showing the greatest differences.

Sorghum (*Sorghum bicolor* (L.) Moench) is a major food grain in many arid parts of the world and is the second largest feed grain crop in the United States (Wall and Ross 1970). Although sorghum is grown mainly for the energy stored in its starch, the grain is also an important source of protein where it is a major diet or animal feed component. Varietal differences in sorghum's protein and amino acid composition have been examined (Virupaksha and Sastry 1968). The protein is generally deficient in lysine, but mutant lines having increased lysine content were discovered by Axtell et al (1974). However, very little information is available concerning the genetic regulation of variability of sorghum proteins. The poor solubility and hydrophobic nature of these storage proteins cause problems in isolation and separation of the major storage proteins, contributing to this difficulty. Two relatively new approaches to resolving sorghum proteins, isoelectric focusing (IEF) and reversed-phase high-performance liquid chromatography (RP-HPLC), were used to successfully resolve alcohol-soluble sorghum storage proteins in this study.

The proteins of sorghum endosperm are heterogeneous and vary in properties and amino acid composition (Virupaksha and Sastry 1968). The lysine-rich albumins and globulins, which are soluble in saline solutions, are minor components. The alcohol-soluble lysine-deficient kafirins are the major protein components; these are most effectively extracted from meal with 60% tertiary butanol (Jones and Beckwith 1970) and can be resolved into several components by gel electrophoresis (Sastry and Virupaksha 1967, Jones and Beckwith 1970). Most of the remaining protein, designated glutelin, is soluble in dilute alkali and was shown by Beckwith (1972) to consist mainly of polypeptide chains linked by disulfide bonds into larger molecules. Reduction of disulfides releases the polypeptide chains which, when dissolved in surfactants or urea, were shown to be heterogeneous by polyacrylamide gel electrophoresis (Beckwith 1972). Some reduced glutelin polypeptides are soluble in 60% *tert*-butanol and have amino acid compositions and electrophoretic mobilities like the kafirins (Paulis and Wall 1979).

Jones and Beckwith (1970) concluded that the electrophoretic patterns of kafirins extracted from grains of three different U.S. hybrid lines of sorghum were similar. However, Paulis and Wall (1979) found that electrophoretic patterns of kafirins from widely different genetic sources differed markedly. Taylor and Schussler (1984) demonstrated that the patterns of kafirins separated by polyacrylamide gel electrophroresis in an acidic buffer varied with the sorghum cultivar, but the resolution of component proteins was not maximized in their system. Paulis and Wall (1979) observed that the electrophoretic patterns of the alcohol-soluble fraction of reduced glutelins were similar to those of the kafirin patterns derived from the same cultivar. They postulated that the polypeptide components of the kafirins and alcohol-soluble glutelins were mostly identical.

The present study found that both IEF and RP-HPLC were effective methods of demonstrating genetic variations of kafirins and alcohol-soluble glutelins extracted from selected inbreds used for development of hybrids in the United States. The use of RP-HPLC for separation of cereal proteins was pioneered by Bietz (1983). The relationship of kafirins extracted from hybrids to those of the parental sorghum inbreds was also determined in the present study. Because the U.S. sorghums are derived from several races grown in various parts of the world (De Wet and Harlan 1971), the diversity of sorghum proteins from selected varieties representing different races was also examined. The classification of races in this study is that of Harlan and De Wet (1972).

MATERIALS AND METHODS

Sorghum Grain Extraction

Inbred TX 414, inbred CK 60 A-line, the hybrid RS 626, derived from the cross of these lines and inbred TX 415, inbred Redlan A-line, and hybrid RS 671, derived from the latter two inbreds, were used to investigate protein variation in inbred and hybrid lines. A-line refers to male-sterile plants used as the female in crosses to form hybrids. Selected varieties of different races included Standard Yellow Milo (race Caffra-Caudatum) from South Africa; Pink Kafir (race Caffra), Spur Feterita (race Caudatum), and Hegari (race Caudatum) from East Africa; Shallu (race Guinea) from West Africa; White Durra (race Durra) from India; and Manchu Brown Kaoliang (race Nervosa) from China.

The grain was ground in a Udy cyclone sample mill to pass through a 40-mesh screen. The meal was defatted upon extraction with 10 volumes/weight petroleum ether by stirring at 4°C for 1 hr. After filtering on a Buchner funnel, the meal was air-dried. To obtain kafirins, 3.5 g of defatted meal was extracted with 35 ml of 60% *tert*-butanol in water (Jones and Beckwith 1970) at room temperature for 2 hr on a reciprocating shaker. After centrifugation in an International model MC centrifuge at 2,600 rpm (1,500 $\times$ g) for 20 min and removal of the supernatant, the

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residue was resuspended in 35 ml 60% *tert*-butanol and extracted further for 1 hr. After centrifugation the resulting supernatant was combined with the previous one to yield the kafirin extract.

The alcohol-soluble glutelin (ASG) was next extracted from the residual meal with 35 ml of 60% tertiary butanol containing 5% 2-mercaptoethanol (2-ME) by shaking for 2 hr at room temperature. After centrifugation and removal of the supernatant, the residue was shaken with 35 ml of the same solvent for 1 hr. The supernatants were combined to yield the ASG extract. Extractions were performed in duplicate.

Nitrogen contents of the extracts were determined by a semimicro-Kjeldahl procedure (AACC 1983). Protein was calculated by multiplication of the nitrogen content by 6.25.

Reduction and Alkylation of Proteins

Ten milliliters of the kafirin and ASG extracts was removed from each of the separate extracts for HPLC. To the 10 ml of kafirin extract 0.53 ml of 2-ME was added so that the solution was 5% 2-ME, and the solution was allowed to stand overnight at room temperature in order to reduce protein disulfide bonds to permit comparison with the reduced ASG. To remove any particulates, the extracts were centrifuged at 20,000 rpm with an L8-M Beckman ultracentrifuge for 20 min and the supernatants carefully decanted and used for injection on the columns.

The remaining protein extracts were placed in separate Viscose casing bags, dialyzed against 20 L of distilled water with four changes over 48 hr at 4°C, and lyophilized. To reduce disulfide bonds, 2 mg of lyophilized protein extract was added in 1.5-ml polypropylene centrifuge tubes to 100 μl of solution containing 8M urea and 1% 2-ME at pH 8.0. Reduction proceeded overnight at room temperature. Liberated sulfhydryls were alkylated by adding 20 μl of an aqueous solution containing 7.1% acrylonitrile in 8M urea. After 1 hr, alkylation was terminated by acidification by adding 10 μl of pH 3.1 aluminum lactate buffer in 8M urea (Paulis and Wall 1977). After each treatment, the solutions were agitated with a vortex mixer. After acidification the tubes were centrifuged with an Eppendorf centrifuge for 20 min. IEF was performed with the supernatant protein solutions.

Protein Separations

IEF was conducted on an LKB 2117 Multiphor unit with saturated aspartic acid and 0.1M lysine for the anodic and cathodic solutions, respectively. The IEF polyacrylamide gel slab (2 × 125 × 260 mm) was composed of 5% acrylamide and 0.183% N,N'-methylene bisacrylamide with 2% pH 6–8 LKB ampholines in 8M urea, which was polymerized by 0.02% ammonium persulfate. Paper wicks were immersed in the reduced-alkylated protein solution, excess solution was removed, and the wicks were applied to the gel surface. Focusing was carried out at 13 W constant power for 4 hr at 4°C with an EC model 500 power supply. Gels were fixed in a solution of 120 ml of methanol, 40 g of trichloroacetic acid, and 240 ml of water overnight and stained with silver using a Bio-Rad (Richmond, CA) reagent kit by following the manufacturer's directions for 2-mm gels. Densitometric analyses of the gels were conducted on a Zeineh recording scanning densitometer using a soft red laser light source (633 nm).

RP-HPLC separation of proteins was achieved on a SynChropak RP-P (C_{18}) 250 × 4.1 mm 300 Å reversed-phase column (SynChropak, Inc., Linden, IN) by a modification of the method of Bietz (1983). Gradient elution was attained by a Spectra Physics model 8700 pump and gradient system, a Spectra Physics model 8500 Dynamic mixer and a WISP 710B auto sample injector. Reduced proteins in 50 μl 60% *tert*-butanol 5% 2-ME extracts were automatically injected onto the column. Proteins were eluted at a flow rate of 1 ml/min with a continuous linear gradient formed by mixing solvent A and solvent B, in which solvent B increased from 45 to 60% of the mixture over 60 min at 70°C; solvent A was water plus 0.1% trifluoroacetic acid, and solvent B was acetonitrile and 0.1% trifluoroacetic acid. Protein peaks in the eluent were measured at 210 nm with a Beckman 165 variable dual wavelength detector. Chromatograms were recorded on a Houston Omniscribe recorder, and data were transferred to, stored in, and integrated by means of a Modcomp MAX 4 computer system. Elution of proteins was also followed by monitoring at 280 nm, but these data were not recorded on the computer.

Auxillary computer programs were used to analyze the data and plot composite elution patterns. Especially valuable was the computer program CHROCP (Butterfield 1984) for addition or subtraction of chromatographic data. In the manual mode, the operator enters any value for K in the formula $A - KB = C$, where A is data set 1, B is data set 2, and C is the resultant difference curve. When the entered value of K is negative and adjusted to relative total areas of A and B, curve C may represent the addition of A and B in any ratio, such as the 2:1 female-to-male ratio of genes contributed to cereal endosperm proteins.

RESULTS

Protein Composition

The protein contents of grains of inbred TX 414, inbred CK 60 A-line, and their hybrid RS 626, and those of inbred TX 415, inbred Redlan A-line, and their hybrid RS 671 are compared in Table I. The hybrid seeds were lower in protein content than either parental inbred. Hybrids generally exhibit lower protein contents than inbreds or open-pollinated varieties do (Miller et al 1964) because of their higher starch content. Protein contents of grains representing seven different races of sorghums varied considerably but were generally quite high (Table II).

Analyses of duplicate kafirin or ASG extracts for percent of total proteins (Tables I and II) varied slightly but the sum of the two extractions (K + ASG) was fairly consistent, suggesting that some kafirin forms disulfide cross-linked ASG. No consistent relationship was established in these studies between the percent of protein constituted by kafirins or ASGs to the total amount of protein in seeds of inbreds or hybrids (Table I) or in grains of the different races (Table II). It has been observed that some high-protein lines of grain had protein more deficient in lysine, indicating higher levels of kafirins and ASGs (Deyoe and Shellenberger 1965). Our results may reflect the difficulty of quantitatively extracting kafirin and ASG in the presence of tannins (Jambunathan and Mertz 1973). Taylor et al (1984)

TABLE I
Distribution of Protein Fractions in Grains of Hybrids and Their Parents

Genotype	Protein Content (%, N × 6.25) (as is basis)[a]	Kafirin (% of protein)[b]	ASG[c] (% of protein)[b]	Kafirin + ASG (% of protein)[b]
Hybrid RS 626	10.4 ± 0.3	19.4 ± 0.5	26.1 ± 4.0	45.5
Inbred CK 60-A-line (male-sterile)	14.6 ± 0.1	28.8 ± 8.4	25.3 ± 4.4	54.1
Inbred TX 414	13.6 ± 0.4	16.8 ± 8.4	25.3 ± 5.9	42.1
Hybrid RS 671	10.2 ± 0.2	30.2 ± 6.8	23.4 ± 2.3	53.6
Inbred Redlan A-line (male-sterile)	15.4 ± 0.2	25.0 ± 0.5	22.1 ± 4.8	47.2
Inbred TX 415	15.7 ± 0.3	19.6 ± 0.1	22.9 ± 5.0	42.5

[a] Averages of duplicate analyses.
[b] Averages of duplicate extractions. Each extract analyzed twice.
[c] ASG = alcohol-soluble glutelin.

analyzed 41 sorghum cultivars grown under different conditions and found little correlation between the total kafirin plus ASG and protein content, even though they added polyvinylpyrrolidone to their extraction solutions to minimize the effect of tannins on protein solubility.

IEF Patterns and RP-HPLC Separations
of Inbred and Hybrid Sorghum Kafirins and ASGs

Figure 1 illustrates the IEF patterns of alcohol-soluble proteins from typical inbred and hybrid seed extracted from lines used to produce commercial grain. The kafirins of each of the inbreds CK 60 A-line, TX 414, and Redlan A-line (2K, 3K, and 5K, respectively, in Fig. 1) showed significantly different patterns which would assist identification of these inbreds. In contrast, the pattern of TX 414 kafirin is similar to kafirin from TX 415 (Fig. 1, 6K). This similarity might be anticipated, as both TX 414 and TX 415 were selected from a cross of SA 7078 and TX 09. These observations are similar to those for IEF patterns of prolamins of corn (Nucca et al 1978) and are consistent with the conclusions of Taylor and Schussler (1984) that sorghum prolamin compositions generally vary with genotype. The IEF pattern of the kafirin from hybrid RS 626 (Fig. 1, 1K) contains bands present in and unique to either of the parent strains CK 60 A-line or TX 414. For example, kafirin bands from CK 60-A-line (Fig. 1, 2K) at 4.7 and 6.2 cm are absent in TX 414 extracts (Fig. 1, 3K). Kafirins at 5.6 and 6.7 cm from TX 414 are absent in the CK 60-A-line kafirin IEF separation. However, consistent with the fact that the female strain CK 60 A-line contributes two sets of chromosomes to the endosperm, whereas the male contributes only one set, the intensities of bands resembling those in CK 60 A-line are greater than those from TX 414. A similar relationship was observed for the IEF patterns of the kafirins extracted from Redlan A-line and TX 415 and the derived hybrid from their cross, RS 671 (5K, 6K, and 4K, Fig. 1).

In an earlier study, Paulis and Wall (1979) demonstrated that electrophoretic patterns of sorghum alcohol-soluble glutelins and

TABLE II
Distribution of Protein Fractions in Grains of Genotypes from Different Sorghum Races

Genotype	Protein Content (%)[a]	Kafirin (% of protein)[b]	ASG[c] (% of protein)[b]	Kafirin + ASG (% of protein)[b]
Standard Yellow Milo	16.8 ± 0.3	20.8 ± 1.4	23.1 ± 5.6	43.9
Pink Kafir	10.1 ± 0.2	18.9 ± 0.6	23.6 ± 3.1	42.5
Spur Feterita	15.6 ± 0.4	28.8 ± 0.7	26.0 ± 2.4	54.8
Hegari	11.1 ± 0.3	24.9 ± 2.6	25.9 ± 6.1	50.8
Shallu	14.7 ± 0.1	21.4 ± 3.6	19.8 ± 1.8	41.2
White Durra	13.7 ± 0.4	22.1 ± 0.2	22.4 ± 7.1	44.5
Manchu Brown Kaoliang	16.3 ± 0.3	17.6 ± 4.1	20.3 ± 4.9	37.9
Av	14.0	22.1	23.0	45.1

[a] Average of duplicate analyses.
[b] Averages of duplicate extractions. Each extract analyzed twice.
[c] ASG = alcohol-soluble glutelin.
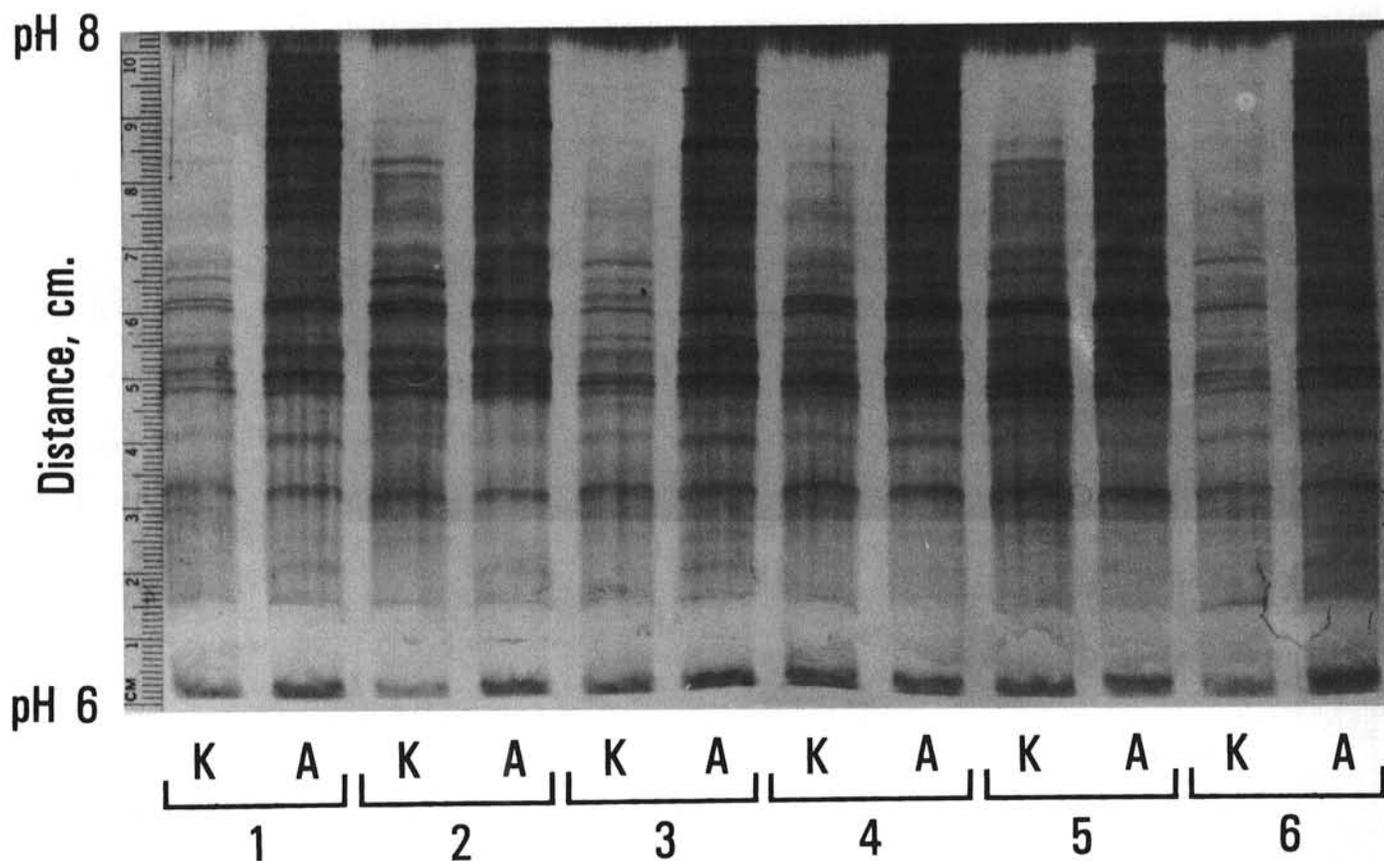
Fig. 1. Isoelectric focusing patterns of kafirins (K) and alcohol-soluble reduced glutelins (A) from hybrid RS 626 (1) and its parent inbred lines CK 60 A-line (2) and TX 414 (3) and from hybrid RS 671 (4) and its parent inbred lines Redlan A-line (5) and TX 415 (6). Conditions described under Methods.

kafirins from the same cultivar exhibited identical electrophoretic patterns in polyacrylamide gels containing aluminum lactate buffer. As seen in Figure 1, the IEF patterns of the ASGs have most bands in common with the kafirins of the same cultivar, but ASG bands with pI near 8 appear more prominent than the corresponding kafirin bands. These differences may reflect variations in extractability of the proteins as well as differences in their tendencies to form intermolecular disulfide crosslinks as well as some differences in compositions between ASG and kafirins. After reduction and alkylation of the lyophilized kafirin extracts, not all of the solids went into solution, which accounts for some of the lesser intensity of kafirin bands compared to those for ASG. In contrast, almost all of the solid ASG extract was dissolved in the
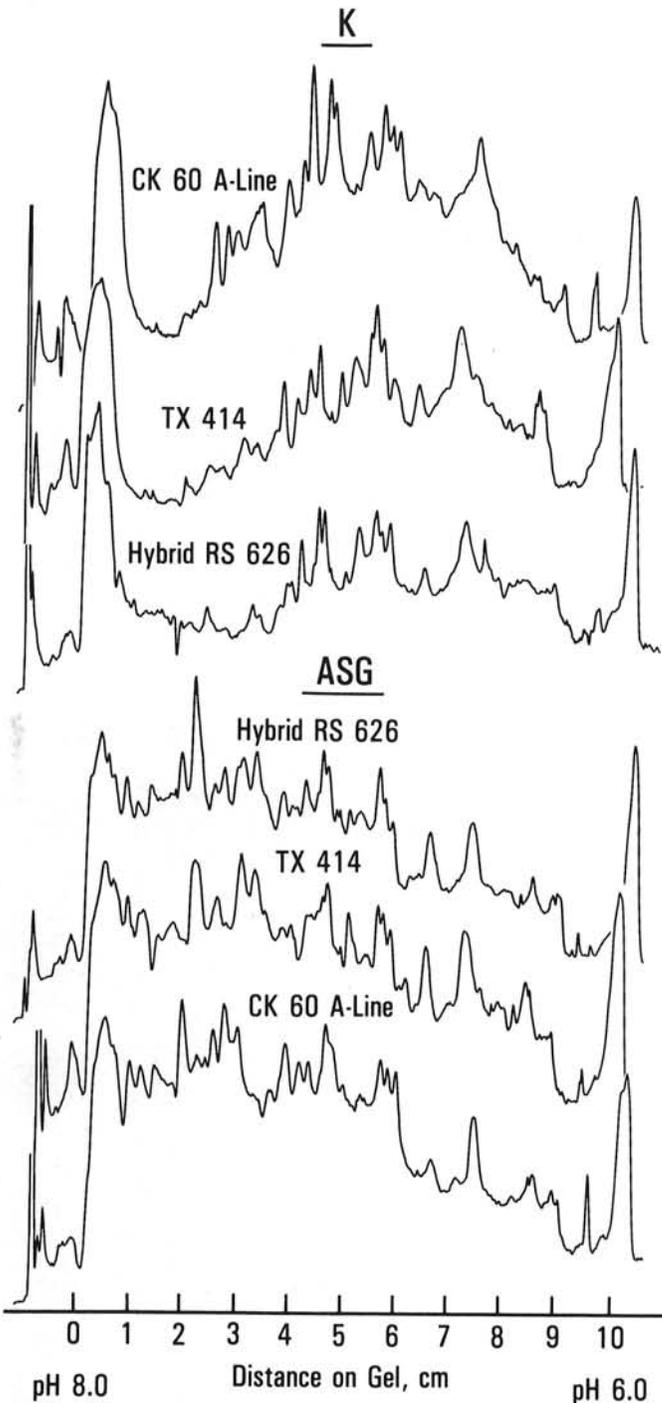
8M urea-2-ME solution. This difference may be attributable to coextraction of tannins and lipids with kafirins in the initial 60% *tert*-butanol extract and subsequent interaction of these materials upon freeze-drying of the dialyzed extracts. IEF confirms that inheritance of ASG proteins in hybrids results in proteins from both parents being expressed, with those from the female appearing more prominent than those from the male.

To further verify the conclusions concerning the relationships between kafirins and ASGs in these inbreds and hybrids, the IEF patterns shown in Figure 1 were examined by densitometry. Significant differences between the IEF profiles of the CK 60-A-line and the TX 414 inbred's kafirins were confirmed (Fig. 2). Hybrid RS 626 kafirin had components with isoelectric points similar to kafirins in both parental inbreds, with lesser amounts of proteins unique to the male parent TX 414 as indicated by areas under the peaks. Similarities in peak positions are evident in the kafirins and ASGs in the region extending from 3 to 8 cm along the gel separation. Differences between proteins and areas of peaks in the TX 414 and CK 60-A-line ASG profiles occur in Figure 2. The hybrid RS 626 ASG pattern is more complex than those of the parental strain and contains ASG proteins unique to either parent, but the female contribution to the hybrid pattern is greater. Accurate quantitation of the areas was not possible because of the variable background intensities of IEF patterns stained with silver.

RP-HPLC offers an alternative method of examining differences in protein components of 60% *tert*-butanol extracts of sorghum proteins because this procedure involves separation based on hydrophobic interactions. Figure 3 compares RP-HPLC elution patterns for kafirins from inbreds CK 60 A-line, TX 414,

Fig. 2. Densitometric analysis of isoelectric focusing gel in Fig. 1 showing separation and band intensities for kafirins (**K**) and reduced alcohol-soluble glutelins (**ASG**) for CK 60 A-line and TX 414 and their hybrid RS 671. Vertical axis shows relative absorbance at 633 nm.
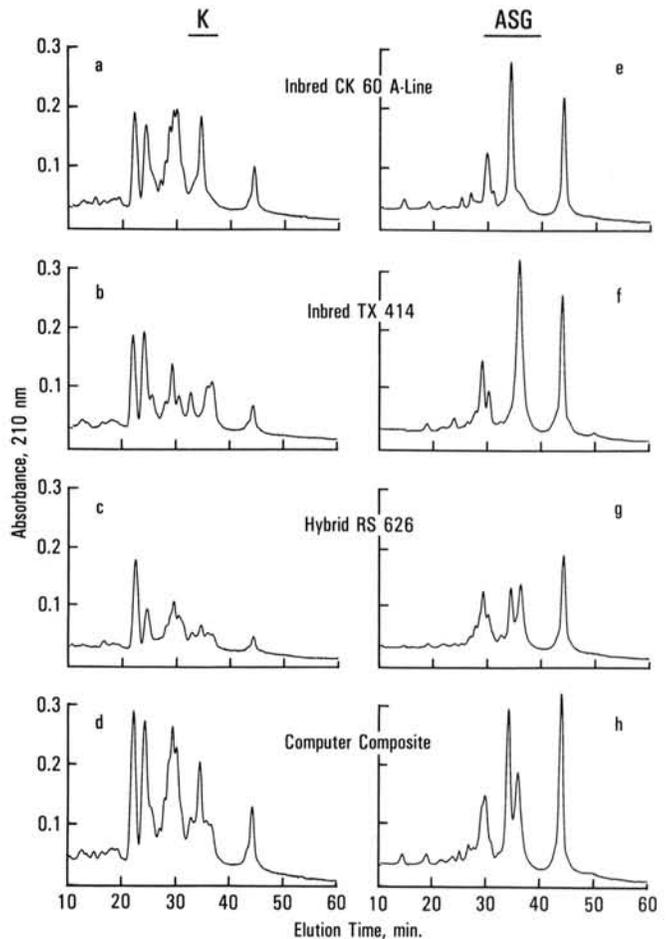
Fig. 3. Reversed-phase high-performance liquid chromatographic patterns of kafirins (**K**) and reduced alcohol-soluble glutelins (**ASG**) extracted from inbred CK 60 A-line (**a** and **e**), inbred TX 414 (**b** and **f**), and their hybrid RS 626 (**c** and **g**), and the computer-derived composites of proteins from CK 60 A-line (2×) + TX 414 (1×) (**d** and **h**). Column and elution gradient described under Methods.
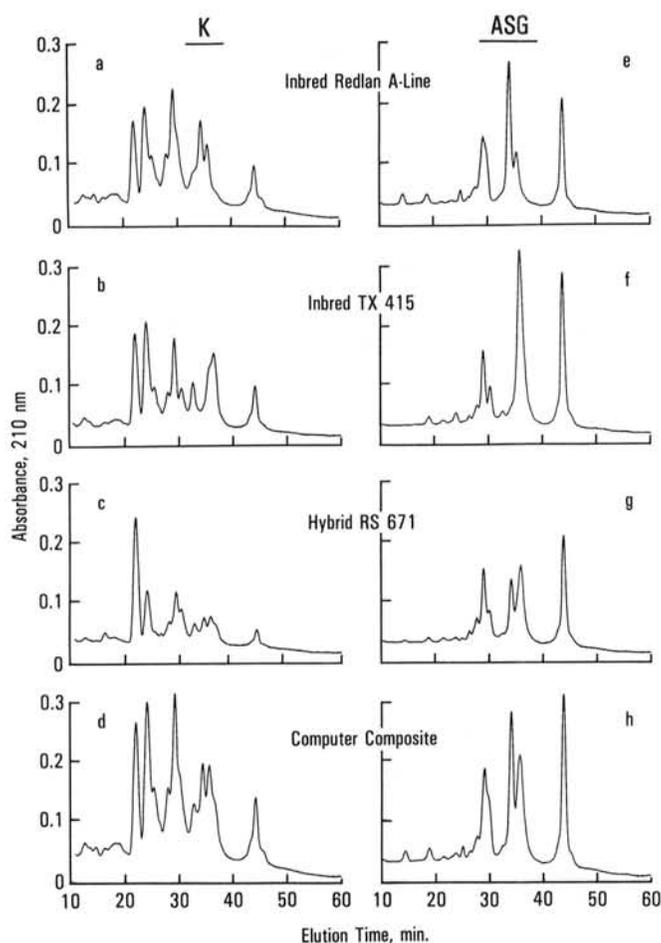
Fig. 4. Reversed-phase high-performance liquid chromatographic patterns of kafirins (**K**) and alcohol-soluble reduced glutelins (**ASG**) extracted from inbred Redlan A-line (**a** and **e**), inbred TX 415 (**b** and **f**), and their hybrid RS 671 (**c** and **g**). Patterns **d** and **h** represent computer-derived composites of proteins from Redlan A-line (2×) + TX 415 (1×).

and their hybrid RS 626 (Fig. 3a, b, and c, respectively). The patterns of kafirins from TX 414 and CK 60 A-line are significantly different. The RP-HPLC pattern of kafirins from the hybrid RS 626 (Fig. 3c) appears to consist of peaks derived from the parental lines. A composite pattern, derived by computer addition of two-thirds kafirin from the female CK 60 A-line and one third from the pollen donor TX 414 is shown in Figure 3d. This pattern indicates that both female and male parents contribute genes coding for synthesis of storage protein in the hybrid. However, the hybrid pattern is not quantitatively identical to this composite, indicating that factors other than gene numbers determine relative expression of storage protein genes. All of the kafirin chromatograms exhibit peaks at 22.0 and 24.0 min elution time, which may represent protein-pigment combinations as they do not absorb appreciably at 280 nm.

In Figure 4, RP-HPLC patterns of kafirins from inbreds Redlan-A-line, TX 415, and their hybrid RS 671 are shown (Fig. 4a, b, and c, respectively). The protein elution patterns are different for these two inbreds; also, these patterns differ from that of kafirin of inbred CK 60 A-line (Fig. 3a), but TX 414 (Fig. 3b) kafirin has an elution pattern similar to that of TX 415, consistent with their selection from the F2 generation of the same cross. The HPLC pattern of the hybrid RS 671 kafirin is qualitatively similar but not quantitatively identical to the computer composite pattern of the female and male parents' kafirins added in a 2:1 ratio (Fig. 4d).

Figures 3 and 4 also illustrate the RP-HPLC patterns of the ASG extracts obtained from the inbreds CK 60 A-line (Fig. 3e), TX 414 (Fig. 3f) and their hybrid RS 626 (Fig. 3g) and from inbreds Redlan A-line (Fig. 4e), TX 415 (Fig. 4f), and their hybrid RS 671 (Fig. 4g). Comparison of adjacent kafirin and ASG patterns of the same inbred or hybrid indicates that the kafirin and ASG patterns may contain some of the same polypeptide units, as evidenced by similar peak elution positions, but there are considerable quantitative differences in peak heights that differentiate the two patterns. For example the CK60 A-line kafirin and ASG chromatograms have peaks at 27.0, 29.0, 31.0, 34.5, and 45.0 min, whereas TX 414 kafirin and ASGs (Fig. 3b,f) have peaks in common at 26.5, 27.5, 29.0, and 30.5 min. In addition, the kafirin patterns have more prominent peaks that elute earlier, whereas the ASG has larger peaks that elute in the later, more hydrophobic
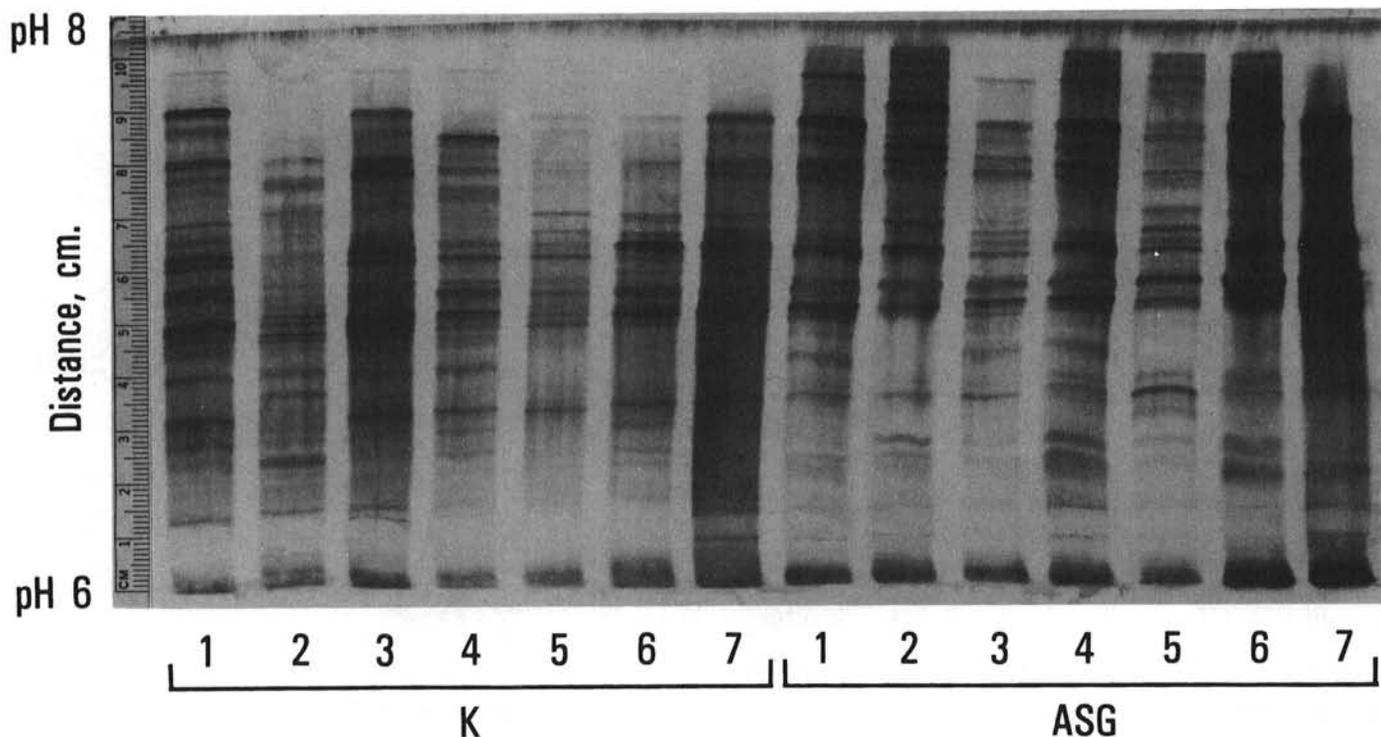
Fig. 5. Isoelectric focusing patterns of kafirins (**K**) and alcohol-soluble reduced glutelins (**ASG**) from varieties representing different races of sorghum. Standard Yellow Milo (**1**), Pink Kafir (**2**), Spur Feterita (**3**), Hegari (**4**), Shallu (**5**), White Durra (**6**), and Manchu Brown Kaoliang (**7**).

eluent region (34–50 min). Computer-derived composites from inbred ASG data (Figs. 3h and 4h) added in a 2:1 female-to-male ratio qualitatively resemble hybrid patterns (Figs. 3g and 4g) and indicate that ASG polypeptides inherited from both parents are expressed in hybrids.

Proteins in Races of Sorghums

Inbred lines of grain sorghums used to produce commercial hybrids are derived from combinations of varieties selected from the sorghum races originating in Africa and Asia. As sorghum spread from its proposed center of origin in Ethiopia, different races became dominant in separate regions, and thus races distant from the origin exhibit greater differences in morphology and possible biochemical characteristics (De Wet and Harlan 1971).

Figure 5 illustrates IEF patterns of kafirins and ASGs from seven varieties representing different races of sorghum. All of the kafirin patterns differ, but some cultivars show some bands in common, possibly indicating closer relationship. For example Standard Yellow Milo, Spur Feterita, and Hegari (1, 3, and 4, respectively, Fig. 5) have a number of bands with similar isoelectric points at 3.2, 4.0, 5.0, 5.5, 5.7, 6.1, 6.2, 6.5, 7.7, 8.0, and 8.7 cm. Although Yellow Milo (1, Fig. 5) is derived from a cross of Kafir and Feterita types, its protein most closely resembles the Feterita. The Pink Kafir (2, Fig. 5) and Shallu (5, Fig. 5) kafirin patterns are quite different from the Milo, Feterita, and Hegari. Durra (6, Fig. 5) and Manchu Brown Kaoliang (7, Fig. 5) kafirin IEF patterns have many bands in common, i.e., 6.3 and 8.0 cm, but differ significantly from the aforementioned sorghum's kafirin IEF patterns by absence of several bands such as 3.2 and 4.0 cm.

The IEF patterns of the ASGs of Standard Yellow Milo, Pink Kafir, Spur Feterita, and Hegari (1–4, Fig. 5) resemble their respective kafirin IEF patterns except that the ASGs have additional bands or more intense bands at the pH 8.0 region.

The RP-HPLC separation patterns of the kafirins from representatives of the different races are shown in Figs. 5 and 6 and demonstrate considerable variation. The chromatographs of the kafirins from all these varieties exhibit the peaks at 19- and 21-min elution times. Kafirins from Yellow Milo (Fig. 6a) also exhibit peaks at elution times of 25.0, 26.5, 27.5, 29.0, 31.0, 34.5, and 42.0 min. Pink Kafir kafirin (Fig. 6b) differs, with peaks at 27.0, 28.5, 29.5, and 32.5 min. Spur Feterita kafirin (Fig. 6c) has peaks at the same elution position as the Yellow Milo but these differ in area. Hegari kafirin (Fig. 6d) resemble the elution pattern of Yellow Milo but lacks a peak at 29.0 and 31.0 min and has a peak at 32.0 min. The pattern for Shallu kafirins (Fig. 7a) differs with peaks at 25.5, 28.0, 31.5, and 34.0 min elution time. The kafirin elution patterns of White Durra and Manchu Brown Kaoliang (Fig. 7b and c) have peaks at 27.0, 33.0, 34.0, and 35.0 min, but the Durra protein has a peak at 29.5 min, whereas that of Kaoliang has a 28.5-min peak. These observations indicate that the protein compositional differences are consistent with relationships between races evident through morphological similarities.

The major differences between the kafirins and the ASG from a single variety are the lack of peaks eluting at 19.0 and 21.0 min and more intense peaks among the more hydrophobic components in the ASGs. However the ASGs of each variety had components with elution times similar to most kafirins from that variety. For example Yellow Milo kafirin and ASG (Fig. 6a and e) had peaks at 25.0, 26.5, 27.0, 29.0, 34.5, and 42.0 min elution time. Again Yellow Milo ASG (Fig. 6e) had components present in both Spur Feterita and Hegari ASGs (Fig. 6f and h). Pink Kafir ASG (Fig. 6f) had a different pattern than these others. Shallu ASG (Fig. 7d) was distinguished from the others by the peaks at 28.0 min. ASGs from White Durra and Manchu Brown Kaoliang (Figs. 7e and f) exhibit similarities except for peaks in the former at 29.5 and the latter at 28.5 min.
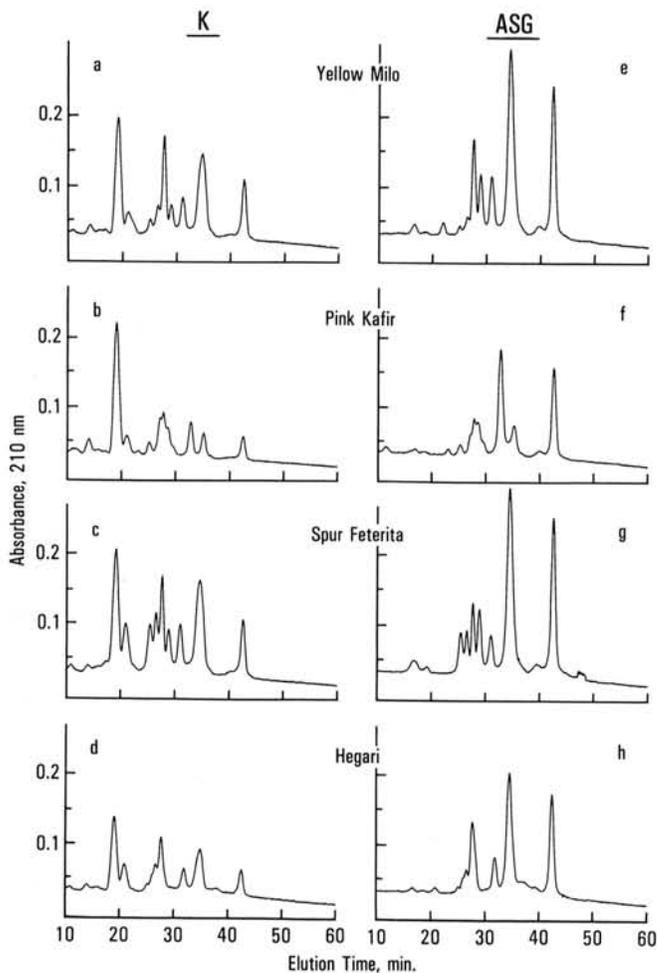
Fig. 6. Reversed-phase high-performance liquid chromatography elution patterns of kafirins (**K**) and alcohol-soluble-reduced glutelins (**ASG**) extracted from Standard Yellow Milo (**a** and **e**), Pink Kafir (**b** and **f**), Spur Feterita (**c** and **g**), and Hegari (**d** and **h**) sorghum grains. Column and elution gradient described under Methods.
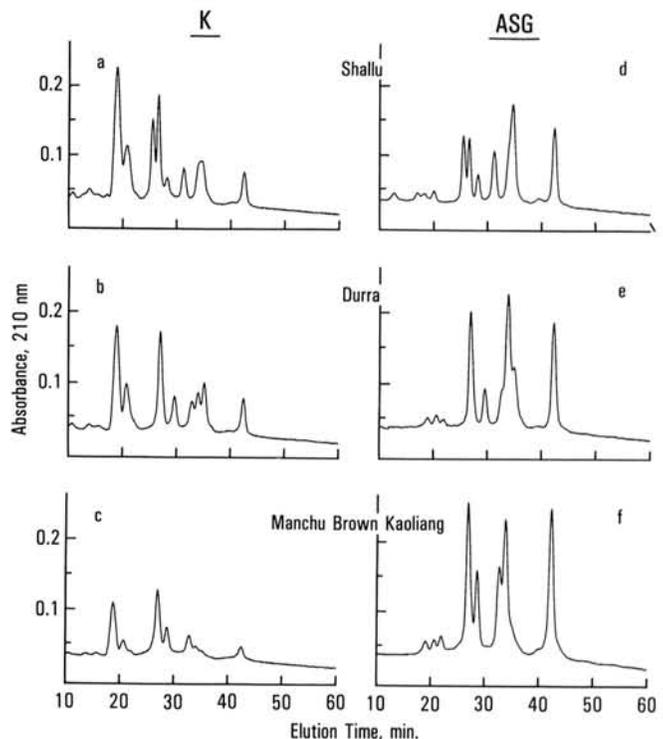
Fig. 7. Reversed-phase high-performance liquid chromatography elution patterns of kafirins (**K**) and alcohol-soluble-reduced glutelins (**ASG**) extracted from Shallu (**a** and **d**), White Durra (**b** and **e**), and Manchu Brown Kaoliang (**c** and **f**).

CONCLUSIONS

Both IEF in polyacrylamide gels (pH 6–8) and RP-HPLC with a linear gradient of increasing acetonitrile are effective methods for separation of sorghum alcohol-soluble proteins. Data presented establish that RP-HPLC is useful for separating sorghum proteins as was previously shown for wheat, corn, and other cereals (Bietz 1983). In addition to providing an independent method of resolution based on hydrophobic bonding, RP-HPLC provides a quantitative measure of the separated proteins. This information is useful not only in comparing compositions of extracts from different varieties, but in demonstrating differences and similarities between the kafirins and ASGs. Recourse to densitometry is necessary for estimation of amounts of individual proteins in the IEF patterns; densitometry of IEF patterns was conducted in these studies, and the data confirmed the conclusions reached by direct examination of the IEF separation.

Previously it was demonstrated by N-terminal amino acid analysis of kafirins isolated from 60% *tert*-butanol extracts of sorghum that different kafirins have a high degree of homology and thus must constitute a family of closely related proteins coded by a multigene system (Bietz 1982). Allelic genes coding for synthesis of kafirins show some significant differences among most of the genotypes investigated in this study.

The crossing of inbreds to produce hybrids results in combining the parental genes in the hybrid endosperm in the combination of two from the female parent and one from the male. The codominance of these allelic genes is evidenced by the expression of the genes so that in endosperm tissue the proteins inherited from the female A-line are present in a larger amount than those from the pollen donor. The computer program permitting addition of RP-HPLC chromatograms in the desired 2:1 ratio did not indicate that the observed ratio of female and male storage proteins contribution in hybrids was exactly 2:1. This variation from expectation may be the result of differences in expression of the various genes or variations in the extractability of proteins.

The variations between protein patterns in the inbred lines investigated here indicate that alcohol-soluble protein patterns may be helpful as a means of identifying sorghum inbreds. Because of the rapidity of analysis, RP-HPLC may be preferred for sorghum analyses as for wheat (Bietz et al 1984). It must be emphasized that the chromosomes on which the genes coding for alcohol-soluble sorghum storage proteins are located have not yet been identified. They may be concentrated on only a couple of chromosomes, as in corn (*Zea mays* L.) (Soave et al 1980). If that is the case, some inbreds having different phenotypic characteristics coded on other chromosomes may not differ significantly in protein pattern, as evidenced by inbreds TX 414 and TX 415.

The diversity of protein IEF and RP-HPLC patterns was greatest among the varieties representing the different sorghum races, consistent with the evolutionary divergence of sorghum races as they spread west and south in Africa and into Asia from East Africa. The fact that both kafirins and ASGs from Standard Yellow Milo had components similar to those of Spur Feterita and Hegari is consistent with the conclusion that the milos are not a distinct race but are derived from a cross of varieties from the races Caffra and Caudatum (Harlan and DeWet 1972).

The IEF and RP-HPLC data indicate that the kafirins and ASG fractions from the same genotype differ in amounts of constituent polypeptides and possibly in the nature of some of the component proteins. However, most of the ASGs have counterparts to the kafirins in isoelectric point and hydrophobicity as determined by IEF and HPLC respectively. These latter observations are generally consistent with the earlier findings of similarity in these fractions by Paulis and Wall (1979), who used polyacrylamide gel electrophoresis in aluminum lactate buffer, and with observations of Taylor and Schussler (1984) who used an acetate buffer for polyacrylamide gel electrophoresis. It is possible that the greater resolving power of IEF and the improved quantitative analysis of HPLC patterns better characterized differences in kafirins and ASGs. Alternatively, the extraction scheme employed here may have resulted in the observed variation in composition of the two protein fractions.

In another paper we discuss how IEF and RP-HPLC can be used to explore the inheritance of genes coding for the high-lysine character and for sorghum storage proteins in progeny of crosses of high-lysine sorghums with inbred lines used to produce high-yielding hybrid sorghums (Sastry et al 1986).

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