Pericarp and Endosperm Structure of Sorghum Grain Shown by Scanning Electron Microscopy[1]

R. C. HOSENEY, A. B. DAVIS, and L. H. HARBERS[2]

ABSTRACT

The structure of sorghum grain samples representing a wide genetic base was examined with a scanning electron microscope. The soft or opaque endosperm is characterized by relatively large intergranular air spaces. The starch is essentially round and covered with a thin sheet of protein. Embedded in the protein sheet are relatively large spherical protein bodies. The hard or translucent endosperm portion is characterized by a tightly packed structure with no air spaces. The starch granules are polugonal and covered with a thin protein matrix. Embedded in the protein matrix are protein bodies composed of a protein, kafrin, which is low in lysine. The hard endosperm is a result of strong adhesion between protein and starch. When the hard endosperm is fractured, many starch granules are broken rather than the starch-protein interface being broken. A dwarf variety from Sudan had relatively few protein bodies in the endosperm. Amino acid analysis confirmed that this variety contained 3.01 g. lysine per 100 g. protein, significantly more than normal in sorghum grain.

Grain sorghum ranks third among cereal crops in the U.S. exceeded only by corn and wheat. Nearly all sorghum grain grown in the U.S. is used as livestock feed; less than 1% is involved in milling operations. Worldwide, grain sorghum is the third most important food grain, exceeded only by wheat and rice (1). About half of the sorghum grain grown is used directly as human food; it forms a staple part of the diet in Africa, Asia, and Latin America. Recent reviews (2, 3, 4) have described its structure and chemistry.

We examined several varieties of sorghum grain with a scanning electron microscope to characterize the structure of the pericarp, the hard and soft portions of the endosperm, and variations in those structures of samples from wide genetic backgrounds. In addition, we hoped to confirm the relationship between the number and size of protein bodies in sorghum grain and lysine content, as recently reported by Seckinger and Wolf (5) using a transmission electron microscope. If successful, the scanning electron microscope would be a valuable tool for the selection of high-lysine sorghum grains.

MATERIALS AND METHODS

Seven grain sorghum varieties were obtained from Sudan: dwarf white milo, feterita gezera, gassabi, dabar, feterita gedarif, feterita abu diraira, and red mugud. The genetic background and conditions under which the samples were grown were not obtainable. However, they were purported to represent the types grown in Sudan. Five U.S.-grown varieties and hybrids also were used: a bulk red-seeded commercial sample, a white waxy sample (CP 622), a brown-seeded, bird-resistant sample (Acco 1023), and a yellow-seeded sample (Dekalb C-42Y).

Kernels were transversely cut with a razor blade, which produced a fracture rather than a clean cut. The cut half-kernels were mounted on aluminum stubs with Delco No. 93 colloidal silver, coated with a 150Å-thick, gold-palladium
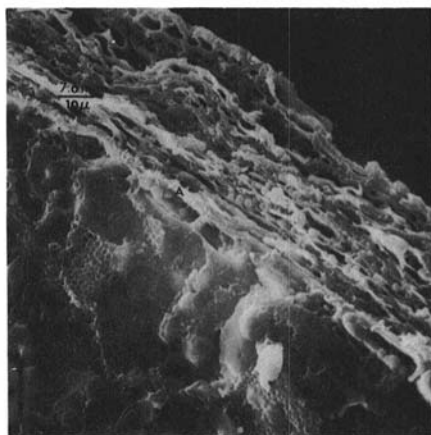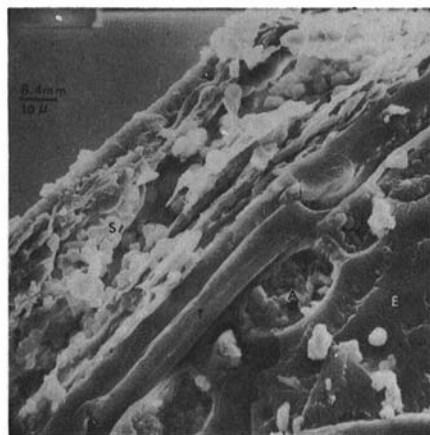

Fig. 1 (left). Scanning electron photomicrograph (SEM) of a grain sorghum pericarp from Acco 1023 containing a testa layer. S = starch, T = testa, A = aleurone, and E = endosperm.

Fig. 2 (right). SEM of a grain sorghum pericarp from CP 622 without a testa layer. A = aleurone.
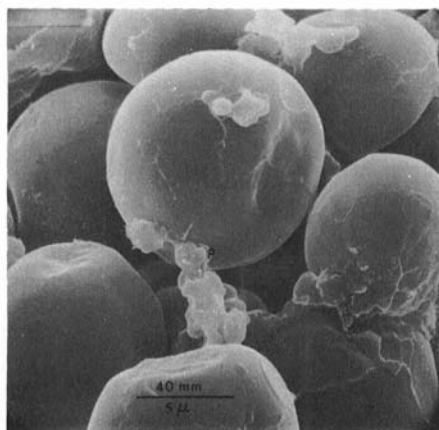
Fig. 3. (left) SEM of the soft endosperm of grain sorghum from bulk hybrid. P = protein bodies.
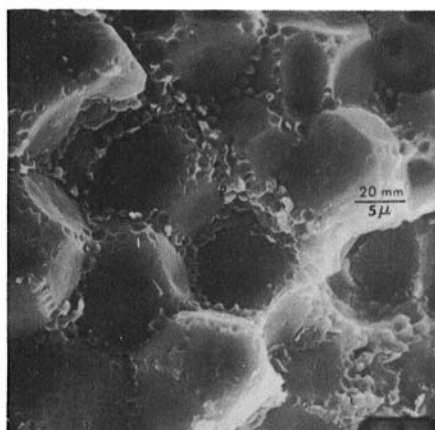
Fig. 4. (right) SEM of the hard endosperm of grain sorghum from bulk hybrid. P = protein bodies, I = indentions.
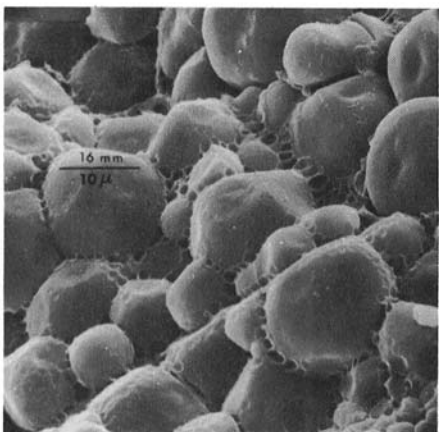

Fig. 5. (left) SEM of grain sorghum endosperm from bulk hybrid treated for 3 hr. with 70% ethyl alcohol at 60°C.
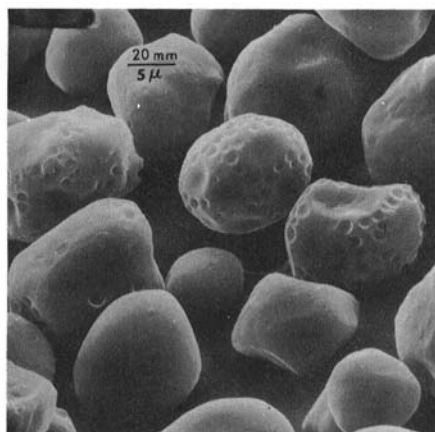
Fig. 6. (right) SEM of isolated grain sorghum starch from bulk hybrid. I= indentions.

layer, and viewed and photographed in an ETEC Autoscan scanning electron microscope at an accelerating voltage of 20 kv.

Amino acid composition was determined using a 120B Beckman amino acid AutoAnalyzer. Samples were hydrolyzed for 22 hr. with 6N HCl at 110°C. in sealed tubes.

RESULTS AND DISCUSSION

Sorghum grain pericarp (Fig. 1) is composed of an outer layer or epicarp, a

Fig. 7 (left). SEM of grain sorghum from dabar showing fractured starch granules and exposed hila. 1 and 2 = hila.
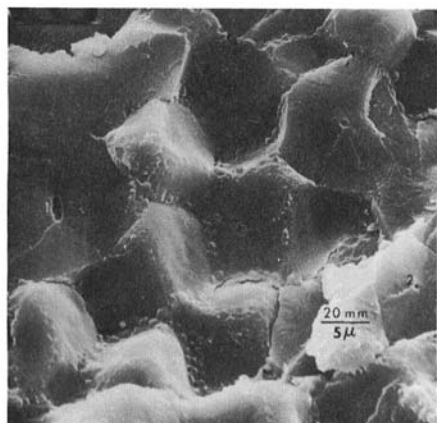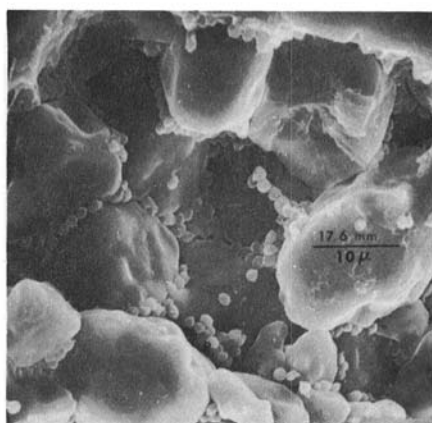
Fig. 8. (right) SEM of a soft or opaque endosperm variety (mugud) of grain sorghum. P = protein bodies.

middle layer or mesocarp containing small starch granules, and an inner layer or endocarp composed of cross and tube cells. The testa or subcoat layer also is clearly visible beneath the pericarp and outside of the aleurone cells. In certain varieties the testa layer is missing (Fig. 2). Of the samples examined, only the bird-resistant Acco 1023 and the three feterita samples contained a prominent testa layer. Immediately below the aleurone cells is the area generally referred to as the peripheral endosperm.

Sorghum grain has both hard and soft endosperm, similar in that respect to the structure of corn. Recent reports, for both corn (6) and sorghum grain (7), have shown protein contents of hand-dissected soft and hard endosperm portions of the same sample to be essentially equal, which seems to rebut reports (5, 8, 9) that protein content varies from the exterior to the interior of the kernel. Those reports were on milled samples, so the fate of the protein-rich aleurone layer was not clear. It is also possible that protein does vary from the outer part to inner part of the hard endosperm and that the average protein value is about equal to the protein of the soft endosperm.

The soft or opaque endosperm (Fig. 3) is characterized by relatively large intergranular air spaces. Starch in that portion of the endosperm is essentially round and covered with a thin layer of protein. Occasionally embedded in the protein layer are small spherical protein bodies (prolamines). By contrast, in the soft endosperm of corn, either no or extremely small protein bodies have been noted (6, 10).

The hard or translucent endosperm portion of sorghum grain (Fig. 4) is characterized by a tightly packed structure with no air spaces. The starch granules are polygonal and appear to be covered with a thin protein matrix. Embedded in the protein are numerous small spherical protein bodies. The protein bodies are clearly visible. The small protein bodies are prolamines (Fig. 5) because they are soluble in 70% ethyl alcohol at 60°C. Indentations in the
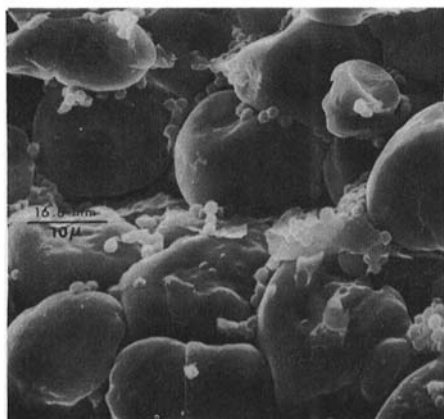
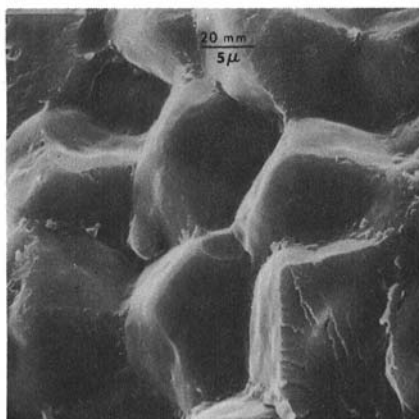Fig. 9. (left) SEM of a soft endosperm variety (gassabi) of grain sorghum.

Fig. 10. (right) SEM of a Sudanese dwarf grain sorghum containing few protein bodies.

starch granules are also observed (Fig. 4); these seem to occur when the protein bodies are removed as the kernel is fractured. The indentations also are clearly visible in isolated grain sorghum starch (Fig. 6). They likely are responsible for the "fuzzy edge" appearance of some grain sorghum starch granules under the light microscope.

From the foregoing, several conclusions concerning the structure of sorghum grain endosperm appear warranted. In the hard or translucent endosperm portion, shrinkage of the matrix protein resulting from water lost during maturation forces the round, relatively soft starch granules together and into their polygonal shape. The relatively small protein bodies are thus forced to the interfacial edges of the starch granules where they are concentrated and become indented at the edges of the polygonal-shaped starch granules. Indentations in the starch indicate that the starch is relatively soft at this stage.

In the soft endosperm the matrix protein probably differs, as reported for corn (11). During drying the sorghum protein ruptures, leaving relatively loosely packed, round starch granules and intergranular air spaces. The opaque appearance of the soft endosperm is caused by air spaces diffracting light, as Duvick reported for corn (12). The hard endosperm is translucent because it has no air spaces.

Air spaces in the soft endosperm result in a less dense material; thus, kernels with predominantly soft endosperm are less dense than hard kernels. The round, loosely packed starch granules and weakly adhering protein matrix, pliant under external pressure, are responsible for the kernel's soft character. The tightly packed, hard endosperm, on the other hand, has strong protein-starch adhesion (evidenced by frequent fracturing through starch granules rather than at the starch-protein interface) that results in a hard endosperm. The broken starch granules and the starch hilum, in two dimensions, are clearly shown in Fig. 7. The hilum appears to be about 1 μ in diameter and 2.5 μ long.

Genetic Variation

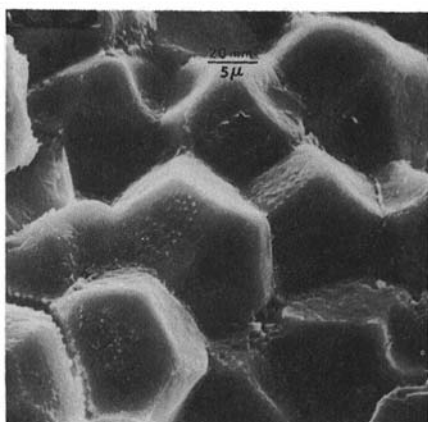The structure of sorghum grain endosperms varies widely. Mugud, a red,

Fig. 11. SEM of C-42Y (a Dekalb hybrid) grain sorghum containing numerous but extremely small protein bodies.

TABLE I. AMINO ACID COMPOSITION (g./100 g. PROTEIN)
OF INDICATED GRAIN SORGHUM VARIETIES AND HYBRIDS

Amino Acid	Bulk C-42Y	Hybrid	Gezera	Dabar	Gassabi	Mugud	Gedarif	Diraira	Dwarf	CP 622	Acco 1023
Lysine	2.54	2.24	1.67	2.09	2.10	2.35	1.71	1.86	3.01	2.54	1.77
Histidine	2.32	1.71	2.10	2.02	2.16	2.35	2.18	2.04	2.32	2.64	2.10
Ammonia	2.62	2.95	2.13	2.09	2.07	2.12	2.17	2.05	2.61	2.58	2.40
Arginine	4.03	3.18	3.40	3.46	3.86	4.42	3.38	3.31	4.79	4.12	3.63
Aspartic acid	6.55	6.94	6.26	6.65	6.31	6.67	6.40	6.27	7.12	6.65	6.70
Threonine	3.04	3.64	2.89	3.06	3.10	3.14	2.83	3.01	3.34	3.40	3.21
Serine	4.31	4.73	4.30	4.35	4.37	4.37	4.36	4.30	4.49	4.51	4.36
Glutamic acid	20.80	22.27	23.00	21.86	22.00	21.38	23.14	22.75	19.64	21.41	22.91
Proline	8.78	7.19	8.00	7.89	7.93	7.91	8.00	7.88	7.56	7.49	7.39
Glycine	3.02	3.40	2.73	2.97	3.13	3.35	2.68	2.88	3.83	3.13	2.70
Alanine	8.58	9.11	9.11	8.73	8.87	8.40	9.11	8.77	8.46	8.54	9.51
Half-cystine	2.39	1.73	2.01	2.51	2.23	2.24	1.87	2.10	3.06	2.07	1.42
Valine	4.51	4.51	4.47	4.77	4.59	5.09	4.07	5.39	4.90	5.02	4.69
Methionine	1.39	1.23	1.46	1.65	1.71	1.44	1.33	1.53	1.56	1.05	1.39
Isoleucine	3.69	3.77	3.59	3.64	3.62	3.67	3.67	3.53	3.55	3.67	3.67
Leucine	13.11	13.11	13.97	13.51	13.09	12.73	14.18	13.84	11.43	12.32	13.52
Tyrosine	3.58	3.41	4.03	3.88	3.90	3.67	3.95	3.80	3.68	3.90	3.86
Phenylalanine	4.75	4.89	4.89	4.87	4.97	4.77	4.98	4.71	4.66	4.95	4.78
Protein[1]	9.2	10.6	13.1	9.9	8.9	8.6	12.2	10.6	7.4	8.8	13.3

[1]Estimated from amino acid recovery.

large-seeded variety from Sudan, has essentially an all-soft endosperm. In contrast to the soft endosperm of opaque or floury corn, where protein bodies are absent or very small, protein bodies are prominent in the outer portion, near the aleurone cells, of mugud sorghum (Fig. 8). Because of its soft character, the starch is not polygonal and the protein bodies make practically no indentation in the starch granules. Another all-soft-endosperm variety from Sudan (gassabi) also had numerous protein bodies (Fig. 9).

Number and relative size of protein bodies varied widely in varieties with a hard endosperm portion. In contrast to the numerous large bodies in the bulk

sample (Fig. 4), few protein bodies were found in a "dwarf" type from Sudan (Fig. 10) and numerous but very small protein bodies (Fig. 11), in C-42Y (a Dekalb hybrid).

Because the protein bodies are composed of kafrin, a protein reported to be essentially free of lysine (13), the percentage of lysine per gram of protein should vary inversely with the number and size of the protein bodies. Such a relationship was recently reported by Seckinger and Wolf (5). Amino acid analyses of the samples (Table I), generally confirm the SEM observations. The Sudanese "dwarf" contained 3.01 g. lysine/100 g. protein and significantly less glutamic acid and leucine than the other samples. Waggle and Deyoe (14) have given a statistical relationship between lysine and the protein content of sorghum grain. Only data from the Sudanese "dwarf" of the samples tested were outside 95% confidence level then calculated. The amino acid values for the other samples in the study are considered to be within the normal variation encountered.

Further research on the Sudanese "dwarf" is necessary to ascertain if its higher lysine content is genetic. This report shows that the scanning electron microscope is useful in quickly surveying sorghum endosperm for low kafrin samples and thus those potentially higher in lysine.

Literature Cited

1. MATZ, S. A. Cereal science. Avi Pub. Co.: Westport, Conn. (1969).
2. ROONEY, L. W., and CLARK, L. E. The chemistry and processing of sorghum grain. Cereal Sci. Today 13: 259 (1968).
3. ROONEY, L. W., JOHNSON, J. W., and ROSENOW, D. T. Sorghum quality improvement: Types for food. Cereal Sci. Today 15: 240 (1970).
4. WALL, J. S., and ROSS, W. M. Sorghum production and utilization. Avi Pub. Co.: Westport, Conn. (1970).
5. SECKINGER, H. L., and WOLF, M. J. Sorghum protein ultrastructure as it relates to composition. Cereal Chem. 50: 455 (1973).
6. ROBUTTI, J. L., HOSENEY, R. C., and WASSOM, C. E. Modified *opaque-2* corn endosperms. II. Structure viewed with a scanning electron microscope. Cereal Chem. 51: 173 (1974).
7. PALMER, G. H. Morphology of starch granules in cereal grains and malts. J. Inst. Brew. 78: 326 (1972).
8. ROONEY, L. W., FRYAR, W. B., and CATER, C. M. Protein and amino acid contents of successive layers removed by abrasive milling of sorghum grain. Cereal Chem. 49: 399 (1972).
9. NORMAND, F. L., HOGAN, J. T., and DEOBALD, H. J. Protein content of successive peripheral layers milled from wheat, barley, grain sorghum, and glutinous rice by tangential abrasion. Cereal Chem. 42: 359 (1965).
10. INGLETT, G. E. Corn proteins related to grain processing and nutritional value of products. In: Symposium: Seed proteins. Avi Pub. Co.: Westport, Conn. (1972).
11. ROBUTTI, J. L., HOSENEY, R. C., and DEYOE, C. W. Modified *opaque-2* corn endosperms. I. Protein distribution and amino acid composition. Cereal Chem. 51: 163 (1974).
12. DUVICK, D. N. Protein granules of maize endosperm cells. Cereal Chem. 38: 374(1961).
13. VIRUPAKSHA, T. K., and SASTRY, L. V. S. Studies on the protein content and amino acid composition of some varieties of sorghum grain. J. Agr. Food Chem. 16: 199 (1968).
14. WAGGLE, D. H., and DEYOE, C. W. Relationship between protein level and amino acid composition of sorghum grain. Feedstuffs 38(51): 18 (1966).