LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDIES OF WAXY AND NONWAXY ENDOSPERM SORGHUM VARIETIES[1]

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ABSTRACT

Microscopic studies of the endosperm of waxy and nonwaxy sorghums have indicated a change in distribution of protein and starch of the peripheral endosperm area in the waxy grain. Nonwaxy sorghums contained small starch granules embedded in a dense proteinaceous matrix in the peripheral area. In contrast, the waxy grain had a less dense peripheral endosperm with larger starch granules with considerably less protein in the peripheral endosperm area. These findings were confirmed in several waxy *vs.* nonwaxy comparisons with different genetic backgrounds. However, one waxy line had a denser peripheral endosperm area than other waxy lines. This indicates there may be differences among the waxy lines. Waxy starch granules are more susceptible to enzyme degradation than nonwaxy starch granules. The combination of increased starch susceptibility and the alteration in structure of waxy sorghum accounts for the improved feed efficiency of waxy grain over nonwaxy in feeding trials.

Digestion and feeding trials indicate waxy sorghums have improved nutritive value over nonwaxy sorghums. McCollough *et al.* (1,2) found that steers fed waxy sorghum rations had an improved feed efficiency over those fed nonwaxy rations. Sherrod *et al.* (3) reported a significant improvement in feed efficiency when steers were fed waxy sorghum. The same trend was observed by Brethour and Duitsman (4) also utilizing steers. Nishimuta *et al.* (5) and other workers (6) observed similar results with sheep. Even though the results from all the feeding trials did not indicate a statistically significant difference in feed efficiency, animals fed waxy sorghums clearly and consistently produced equal gain on less feed than those fed nonwaxy sorghum diets.

Some of the differences in feedlot performance can be explained by the type of starch in the endosperm. The nonwaxy grain contains starch that is composed of approximately 25% amylose and 75% amylopectin, whereas, waxy sorghum refers to starch that is essentially 100% amylopectin. According to Sandstedt *et al.* (7), the genes involved in producing high-amylose starch in corn produce starch that is highly resistant to enzymatic digestion, whereas, the starch properties conditioned by the waxy gene are near the maximum in susceptibility to enzymatic hydrolysis (8). Sandstedt *et al.* (7) also indicated the digestibility of the starch in high-amylose ground whole corn was only 37% that of normal corn and raw waxy sorghum starch had a greater rate of digestibility than normal sorghum starches (9).

Microscopic evaluation of selected sorghum lines indicated a modification of the peripheral endosperm area of the waxy grain (10), which may also account for part of its improved feed efficiency. The structure of the peripheral endosperm area of sorghum has been previously discussed (10,11,12,13) in detail. The interaction or arrangement of starch and protein in this area probably affects digestibility more than any other single factor. The objective of this study was to

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combine light and scanning electron microscopy techniques to gain a better understanding of differences in the structure of waxy and nonwaxy sorghum grains that might account for the improved feed efficiency of waxy sorghums.

MATERIALS AND METHODS

Grain Samples

The Kafir lines consisted of TX 3197, a nonwaxy type endosperm and TX 615, its waxy counterpart, plus two other waxy Kafir derivatives, Texioca 63 and Texioca 54. The two Redlan samples used in this study were TX 378, a nonwaxy type endosperm, and SA 413, its waxy counterpart. All six lines were grown in 1973 under comparable conditions on the Texas A&M University Plantation at College Station, Texas. The grains of the waxy and nonwaxy counterparts were essentially the same except for the waxy genes. Therefore, these comparisons provided a valid test of the effect of the waxy gene on kernel structure.

Physical and Chemical Analysis

Procedures for thousand-kernel weight, hardness, and test weights were obtained as described by Rooney and Sullins (14). Protein, fat, and ash were obtained by standard AACC methods (15).

Preparation and Examination of Endosperm Sections

Kernels prepared for thin sections were embedded in plastic as described by Sullins and Rooney (10) which included fixing in 6% acrolein, followed by dehydrating with 100% ETOH, and 100% acetone. Then, samples were embedded in Araldite/Epon plastic, polymerized, and sectioned with a glass knife. Sections were treated with hog pancreas α-amylase[3] to remove the starch as described by Wolf and Khoo (16). Sections were examined with the light and scanning electron microscope (SEM).

Whole kernels of the nonwaxy Kafir TX 3197 and waxy Kafir TX 615 were cut longitudinally with a razor blade. The sections were placed endosperm down in a spotting plate and incubated for 2 hr at 39°C in buffered hog pancreas α-amylase[3] enzyme. The half-kernels were washed, dried, and prepared for SEM examination by coating with carbon and gold. Specimens were viewed at 15 kV with a Joel JSM-U3 Scanning Electron Microscope.

Dry kernels of the samples were sectioned by the glass-knife techniques of Wolf and Khoo (16). Dry sections—4 μ thick were hydrated with distilled water, and examined with differential interference contrast (DIC) optics on a Zeiss Universal Microscope. The DIC has the ability to deform polarized light with two Wollaston prisms to give an image of an unstained specimen in color. It gives a 'relief effect' due to the difference in refractive indexes of the specimen.

The histological samples discussed in this paper represent at least a quick examination of 200 to 300 individual kernels for each sample. Individual kernels within samples of a certain sorghum line vary considerably; but the sections presented are representative of the differences inherent between the waxy and nonwaxy grains.

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RESULTS AND DISCUSSION

For all practical purposes, the six sorghums were similar in physical and chemical properties with the exception of endosperm type (Table I).

The sorghum endosperm is composed primarily of starch, protein, and cell-wall material. Protein concentration increases from the center of the endosperm toward the outside edge of the endosperm. The area around the periphery of the endosperm has the greatest concentration of protein. This peripheral endosperm area is ill defined but usually consists of the first two to six endosperm cells beneath the aleurone cell layer. The small, blocky cells contain small starch granules embedded in a dense proteinaceous matrix containing protein bodies (prolamines). With a high concentration of protein bodies in the peripheral area (Fig. 1A), the starch is relatively unavailable for enzymatic degradation. However, when the endosperm type is conditioned by the recessive waxy gene, there appears to be a modification of the protein distribution in the peripheral endosperm (Fig. 1B). There is a more even distribution of the proteinaceous matrix and protein bodies in the waxy endosperm (Fig. 1D) in contrast to the large concentration of protein bodies and matrix in the peripheral area of the nonwaxy grain (Fig. 1C).

After treating half-kernels with α-amylase for 2 hr, the concentration of protein in the peripheral area of the nonwaxy Kafir TX 3196 (Fig. 2A) can be easily contrasted to the more even distribution of the protein in the waxy Kafir TX 615 (Fig. 2B). We do not believe the waxy endosperm contains fewer protein bodies or less total protein but that the protein is more equally distributed throughout the endosperm in contrast to the greater concentration of protein in the peripheral endosperm of the nonwaxy grain. The nonwaxy Kafir (Fig. 2C) contains enough concentration of protein in the peripheral area to impede penetration of the enzymes to the starch granules. Therefore, only surface digestion is accomplished in the nonwaxy (Fig. 2C), whereas, the waxy starch granules are hydrolyzed several layers below the surface because they are more accessible to the digestive enzymes (Fig. 2D).

After establishing what was felt to be a real difference between the waxy and nonwaxy Kafir, the question arose: was this difference real for other waxy sorghums? The Texioca lines examined contained large starch granules near the aleurone cell layer and even distribution of protein in the peripheral area as did

TABLE I
Physical and Chemical Properties of Waxy and Nonwaxy Sorghums

Variety	Endosperm Type	Test Wt. lb/bu	1,000-Kernel wt (g)	Hardness Value	Protein[a] (N × 6.25) %	Ash[a] %	Fat[a] %
TX 3197	Nonwaxy Kafir	57.3	23.7	21	11.5	2.0	2.8
TX 615	Waxy Kafir	57.0	24.2	21	11.3	2.2	3.6
Texioca 54	Waxy Kafir	58.1	22.5	11	12.1	2.1	2.9
Texioca 63	Waxy Kafir	56.2	22.1	16	11.2	2.4	3.6
TX 378	Nonwaxy Redlan	58.0	26.3	11	12.7	2.0	3.0
SA 413	Waxy Redlan	55.3	23.8	13	11.6	2.1	2.3

[a]Dry weight basis.

the waxy TX 615. However, one of the Texioca lines had a thicker, denser, peripheral endosperm than that of TX 615 which indicates there is a difference among waxy lines.

Microscopic examination of the Kafir lines with differential interference contrast (DIC) optics again illustrated the differences in concentration of the protein bodies in the peripheral endosperm area of the nonwaxy Kafir TX 3197 (Fig. 3A) as compared to the waxy Kafir TX 615 (Fig. 3B). There is a more even distribution of protein bodies and larger starch granules near the aleurone cell layer of the waxy grain as compared to the nonwaxy.

Additional waxy *vs.* nonwaxy comparisons were made with Redlan derivatives which contained completely different genetic backgrounds from the Kafir lines. DIC microscopic examination of the Redlan lines revealed similar observations to those found in the Kafir lines. The Redlan nonwaxy (Fig. 3C) contained an abundance of protein bodies in the peripheral endosperm area with only small starch granules visible. In contrast, the waxy Redlan (Fig. 3D) had large starch granules near the aleurone cell layer and more even distribution of the protein throughout the endosperm.

We believe that the change in structure of the endosperm especially in the peripheral area of waxy sorghum and the inherent improved susceptibility of

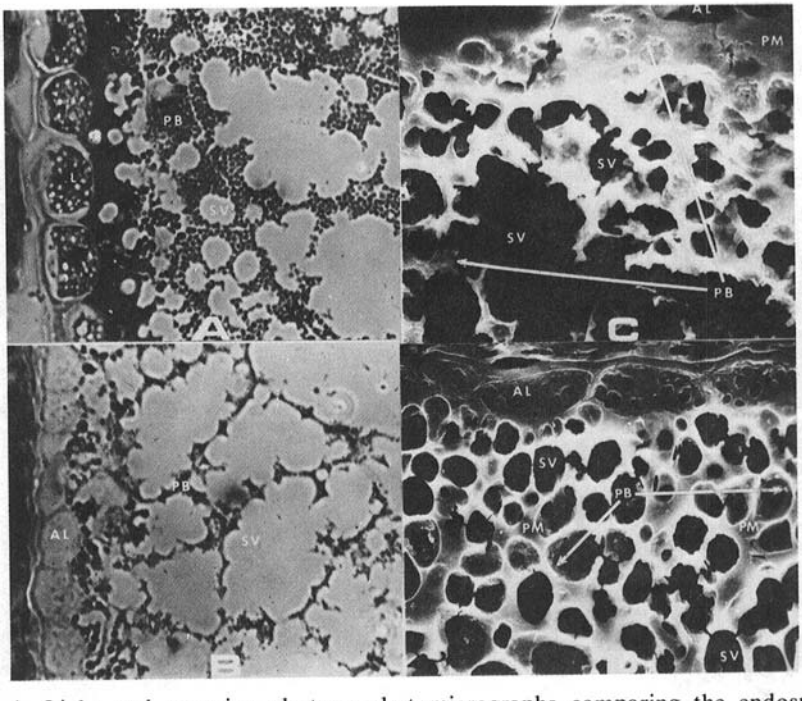

Fig. 1. Light and scanning electron photomicrographs comparing the endosperm structure of nonwaxy Kafir (A and C) and waxy Kafir (B and D) sorghum kernels. Sections were subjected to α-amylase enzyme to remove the starch. A and B = 320×; C and D = 1,000×; AL = aleurone cells; PM = protein matrix; PB = protein bodies; SV = starch voids.

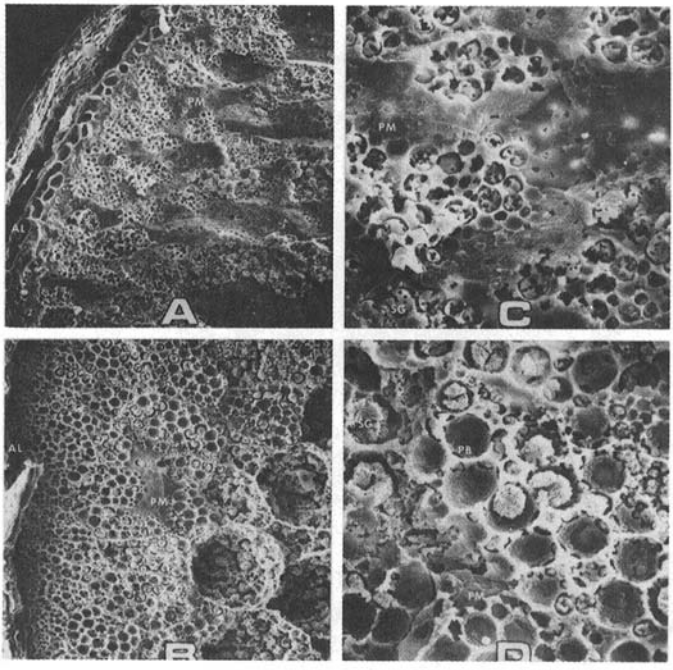

Fig. 2. Scanning electron photomicrographs of nonwaxy Kafir (A and C) and waxy Kafir (B and D) sorghum kernels. Half-kernels were subjected to α-amylase enzyme for 2 hr, washed, air dried, and examined. A and B = 250×; C and D = 1,000×; AL = aleurone cells; PB = protein bodies; PM = protein matrix; SG = starch granule.

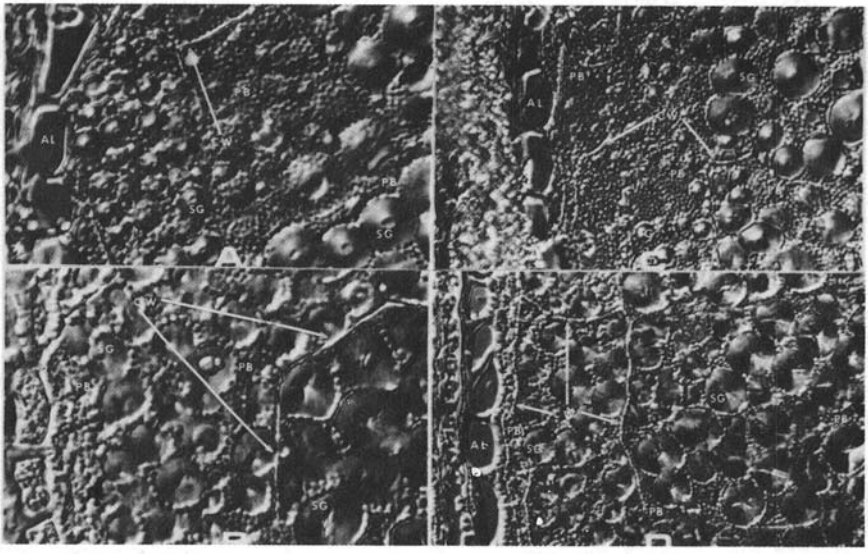

Fig. 3. Sections of untreated nonwaxy and waxy sorghum endosperm viewed with the light microscope by differential interference contrast optics. A, B, C, and D = 320×; AL = aleurone cells; CW = cell wall; PB = protein bodies; SG = starch granules. Photographs: A = nonwaxy Kafir; B = waxy Kafir; C = nonwaxy Redlan; D = waxy Redlan.

waxy starch granules to amylose attack account for the increased digestibility and better feed efficiency of waxy sorghum.

Additional experiments are needed to attempt to determine which factor is most important. The sorghum kernel contains a high proportion of peripheral area which affects availability. For instance, to achieve maximum feed efficiency of steam-flaked sorghum the flakes must be much thinner compared to other grains. The peripheral endosperm area is more completely disrupted in a thin flake, which permits digestion of starch and protein in the peripheral area. Thus, waxy grain with the less dense peripheral endosperm would probably require less energy for processing. In addition, we may be able to select nonwaxy sorghum that have improved digestibility by looking for varieties with similar alterations.

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