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SCANNING ELECTRON MICROSCOPY OF THE PERICARP AND TESTA OF SEVERAL SORGHUM VARIETIES

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Abstract

Pericarp thickness (determined by Z gene) varies greatly among sorghum varieties ranging from very thin (8μm) to very thick (160μm). Pericarp thickness also varies within an individual kernel. The areas below the style and near the hilum are the thickest with the sides of the kernel being thinnest. Scanning electron microscopy was used to document differences in pericarp thickness and to explain milling differences. Varieties with a thick pericarp had starch granules in the mesocarp cell layers. Sorghums with a thin pericarp did not have starch granules in the mesocarp except near the hilum and stylar area. U.S. sorghum varieties studied had a testa thickness of 16-40μm (side of the kernel) but recently four Malian sorghums from a recent collection had very thin testae of 8-16μm. The Sudanese sorghum Shawaya had a testa ranging in thickness from 28-40μm.

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Introduction

Sorghum (Sorghum bicolor (L.) Moench) is the major cereal grain consumed by many people in Africa and parts of Asia. Many sorghum food products are made from decorticated grain which has been ground into a flour. Pericarp thickness and presence of a pigmented testa affect the processing and nutritional properties of sorghum and sorghum products.

Kernel Structure

The sorghum kernel consists of three major areas: the pericarp, the germ, and the endosperm (Figure 1). The pericarp is derived from the ovary wall (Saunders, 1955). The pericarp layers include the epicarp, mesocarp, and endocarp (Figure 2). The epicarp, usually two or three layers thick, consists of long, rectangular cells which may contain pigments. The outer surface of the epicarp is usually covered with a waxy cuticle. Thickness of the mesocarp is determined by the presence or absence of starch granules in this layer. The endocarp layer includes the cross and tube cells whose major function is transport of moisture around the kernel. The endosperm consists of the aleurone layer, the peripheral endosperm, the corneous endosperm, and the floury endosperm. The endosperm texture in sorghum can be corneous, intermediate or floury depending on the ratio of corneous to floury endosperm.

Pericarp Thickness

Pericarp thickness is controlled by the 'Z' gene. A thin "pearly" pericarp results from the dominant Z gene (Z-) and a thick "chalky" pericarp from the homozygous recessive (zz) condition (Ayyangar et al, 1934). Pericarp thickness varies within individual kernels as well as among varieties. The mesocarp varies from a thin, almost nonexistent layer (thin pericarp), to several layers of starch-filled cells (thick pericarp). Variation in pericarp thickness among varieties occurs even when the homozygous recessive condition is present. Corneous sorghums with thick pericarps usually require decreased milling time and have slightly decreased yields of milled grain compared to corneous sorghums with thin pericarps (Da et al, 1982). Native women in Africa prefer thick, chalky sorghums for traditional milling with a wooden mortar and pestle. The thick pericarps are removed in larger pieces, thus requiring less decorticating time. Since the pericarp breaks in the mesocarp layer (Freeman and Watson, 1969; Hubbard et al, 1950), the thicker pericarp (having thicker starchy mesocarps) break into flakes more easily. Using a specially modified Udy cyclone mill, G-766W, a sorghum with a thick pericarp, was shown to "peel" with large pericarp flakes produced, similar to what is seen with the traditional mortar and pestle milling system (Shepherd, 1979; Shepherd, 1981).

Pericarp thickness affects grain weathering (pre-harvest deterioration). Sorghums with thick pericarps generally appear to deteriorate more rapidly than sorghums with thin pearly pericarps. Perhaps the starch granules in the mesocarp provide a readily available substrate for the fungi (Leukel and Martin; 1943; Ellis, 1972; Glueck, 1979).

Presence of Testa

Two genes, B_1 and B_2, control the presence or absence of a pigmented testa in sorghum. If either the B_1 or B_2 gene is homozygous recessive, the pigmented testa does not form and is thought to be absorbed (Swanson, 1928; Quinby and Martin, 1954). During kernel development, the cells of the inner integument are crushed to form a single, often continuous, layer called the testa. Morrall and coworkers (1981) studied the development of the testa and reported that membrane-bound vesicles in the inner integument cells became filled with what was presumably tannin. During the milk dough stage the central vacuoles of the inner integument cells expanded to fill almost all of the cells and during the soft dough stage were filled with tannin. The testa is between the tube cell layer (innermost part of the pericarp) and the aleurone layer (outermost part of the endosperm). Remnant cells from the inner integument are sometimes observed in those kernels in which the pigmented testa is absent. Ayyangar and Krishnaswami (1941) reported the presence of a colorless layer between the pericarp and aleurone layer which they called an unpigmented testa.

Testa Thickness

Differences in thickness of the testa within individual kernels occur. The thickest area is below the style (100-140µm) and the thinnest on the sides of the kernel (means of 25 and 18µm on hilar side and opposite side) (Blakely et al, 1979). A partial testa has also been observed with the pigmented testa present only in some areas of the kernel (Blakely et al, 1979). Several varieties of local sorghums from Mali with very thin testae have been described by Dr. John Scheuring (private communication). Blakely et al (1979) also reported that testae often appeared as two overlapping layers, often of different colors. This layering of the testa can be seen in Figure 3-D.

The presence of a pigmented testa in sorghum adversely affects the nutritional value of sorghum food and feed products. High levels of tannins are found in sorghums with a testa which depress feed intake and feed efficiency (Featherston and Rogler, 1975; Maxson et al, 1973; Cousins et al, 1981). The levels of tannins and other polyphenols in the testa and pericarp are affected by the B_1 and B_2 genes. When these two genes are dominant and a spreader gene (S) is dominant, the pericarp has high levels of polyphenols and appears brown in color. The intensity of the brown pigmentation is affected by the genetic pericarp color, whether red or white. An additional set of genes (Tp) controls the pigmentation of the testa. The

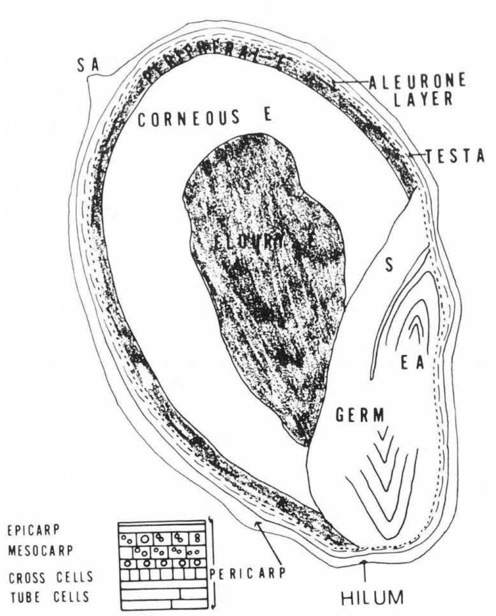

Fig. 1. Schematic longitudinal section of a sorghum kernel. SA - stylar area, E - endosperm, EA - embryonic axis, S - scutellum

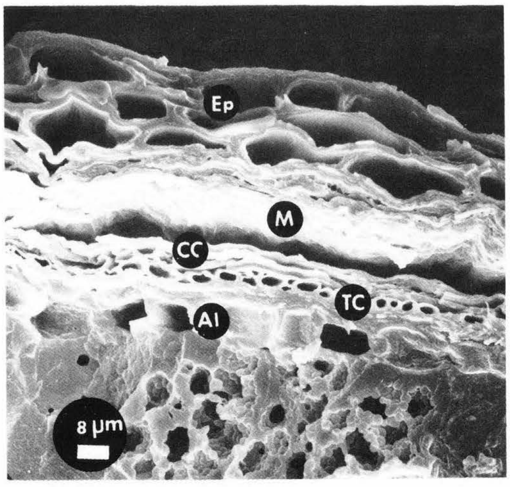

Fig. 2. Scanning electron photomicrograph of pericarp components. Ep - epicarp, M - mesocarp, CC - cross cell, TC - tube cell, Al - aleurone layer

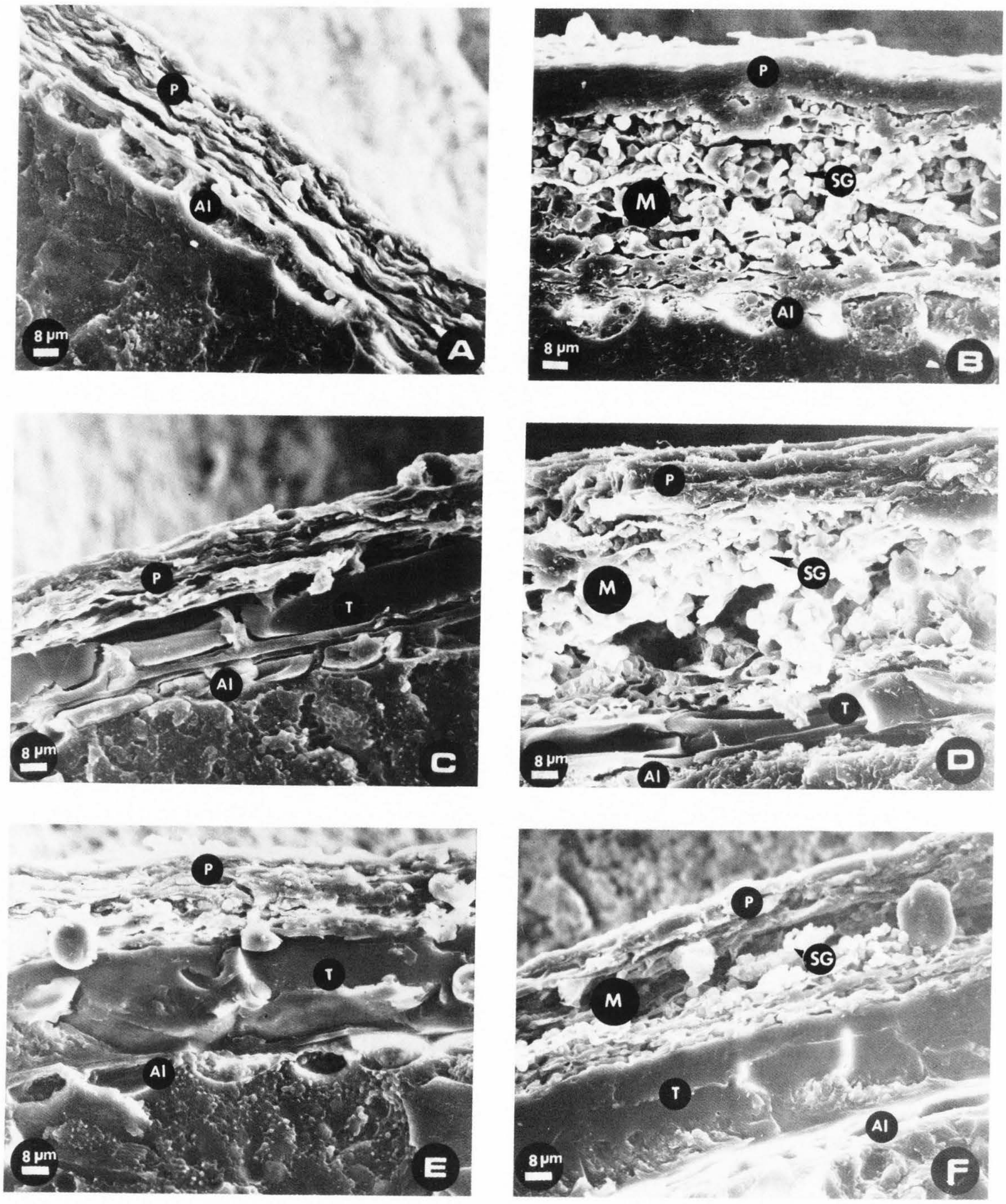

Fig. 3. Scanning photomicrographs depicting pericarp and testa thickness of several sorghum kernels.

A) Thin pericarp without testa (IS9985)
B) Thick pericarp without testa (Kende - Mali)
C) Thin pericarp with thin testa (Experimental line, College Station, Texas)
D) Thick pericarp with thin testa (Kende - Mali)
E) Thin pericarp with thick testa (GA615)
F) Thick pericarp with thick testa (ATx623 X SC0103)
P - pericarp, Al - aleurone layer, T - testa, SG - starch granule, M - mesocarp
The sorghum kernels were longitudinally cut and photomicrographs taken on the sides of the kernels in similar areas on each kernel so that differences in pericarp and testa thickness could be minimized.

testa is usually brown (Tp_) but it can be purple (tptp) (Casady, 1975). The phenolic compounds in the testa impart some resistance to field fungi thus decreasing susceptibility to pre-harvest deterioration (Glueck, 1979).

When sorghum with a testa is milled for use in food products a dark or speckled flour is produced, causing undesirable color in ogi and Tô, two African food products (Akingbala et al, 1981; Da et al, 1982). When alkali is used to process tortillas and Tô, color changes become even more pronounced (Khan et al, 1980; Johnson, 1981). These color changes are caused by the polyphenols in the pericarp and testa. Presumably, sorghums with thin testae would have lower concentrations of phenolic compounds, thus decreasing the color problem in food products, but might retain some resistance to grain deterioration. Also a thin testa may be easier to remove during milling.

Since pericarp thickness and presence of a pigmented testa affect the processing of sorghum products, the objective of this work was to document genetic related differences in sorghum pericarp thickness and how those differences affect milling characteristics.

Materials and Methods

Samples

Guineense sorghums with thick and thin pericarps, Nio-Fionto, and four thin testa varieties were grown in Mali in 1981. Kernels selected from the F_2 population of BTx623 X CS3541 were harvested in 1980 from College Station, Texas. The following six sorghums were selected to demonstrate differences in thin and thick pericarps and testae: a white sorghum with a thin pericarp without a pigmented testa (IS9985), a white Malian Kende sorghum with a thick pericarp and no pigmented testa, a red sorghum with a thin pericarp and thin testa (experimental line - College Station, Texas), a white Malian Kende sorghum with a thick pericarp and thin testa (CSM-184), a brown sorghum with a thin pericarp and thick testa (GA615), and a brown sorghum with a thick pericarp and thick testa (ATx623 X SC0103-Sel). The Shawaya sorghum with an extremely thick pericarp was received from Sudan in 1981.

Scanning Electron Microscopy

Kernels were cut in half with a razor blade and oven-dried at 45°C overnight. Samples were mounted on aluminum stubs with silver conductive paint and coated with a 200 Å layer of gold-palladium prior to viewing. Samples were examined with a JEOL JSM35 Scanning Electron Microscope with an accelerating voltage of 20kV. Care was taken to view and photograph all specimens from the same angle so as to minimize artificial distortion of pericarp thickness.

Milling

Traditional mortar and pestle milling by a Malian woman was used to mill 3 types of Malian sorghums as described in the Results and Discussion section.

Results and Discussion

Screening of sorghum samples indicated a large variation in pericarp thickness (Table 1). York (1977) proposed that the genes denoting pericarp thickness be reclassified to reflect his belief that genes other than the Z locus influence the thickness of the starchy mesocarp. The thin pericarps contained few, if any, starch granules (Figures 3-A,C,E), whereas thick pericarps contained abundant starch granules in the mesocarp area (Figures 3-B,D,F). When present in the thin pericarp, starch granules were in the mesocarp below the style and around the hilum area which are the two thickest parts of the pericarp. The ranges in pericarp thickness observed in these and other samples are reported in Table 1. The upper value of the thin and intermediate pericarps was that measured below the style and next to the hilum.

Table 1. Pericarp thickness of sorghum varieties

Thin	8-32μm
Intermediate	28-48μm
Thick	40-160μm

The testa in Figure 3-C was thinner (16μm) than in the varieties in Figures 3-E and F (32μm), but it still was not as thin as the Malian sorghum in Figure 3-D (8μm). The Malian sorghums had much thinner testae than those of the U.S. sorghums studied.

Pericarp Thickness Among Varieties

Photomicrographs were taken of sorghum kernels from the F2 population of the cross BTx623 X CS3541 which were segregating for pericarp thickness. All the F2's had white pericarps without pigmented testae. Some of the progeny had a thin pericarp without starch granules in the mesocarp resembling the parent CS3541 (Figure 4-A); another had a thick pericarp with considerable starchy mesocarp resembling the parent BTx623 (Figure 4-C); the last F_2 had an intermediate pericarp thickness (Figure 4-B) with relatively few starch granules in the mesocarp. All photomicrographs were taken at the sides of the kernel in the thinnest areas of the pericarp in approximately the same place on each kernel.

Pericarp Thickness Within Kernel

Photographs of Nio-Fionto grain were taken at different points around the periphery of the kernel to observe variations in pericarp thickness within individual kernels. The pericarp next to the hilum (80 μm) (Figures 5 (point D) and 6-D) was the thickest, with that near the top of the kernel (64 μm) (Figures 5 (point B) and 6-B) and near the style (56 μm) (Figures 5 (point A) and 6-A) intermediate, and that at the side of the kernel the thinnest (48 μm) (Figures 5 (point C) and 6-C). The waxy cutin layer on the kernel next to the style is much thicker than in other areas of the pericarp. Pericarp thickness measurements did not include the cuticle. These data suggest that it is possible to compare pericarp thickness among sorghum varieties only if the comparisons are based on the examination of similar locations on the kernel periphery.

Pericarp Thickness and Milling

When sorghum is milled, the endosperm type

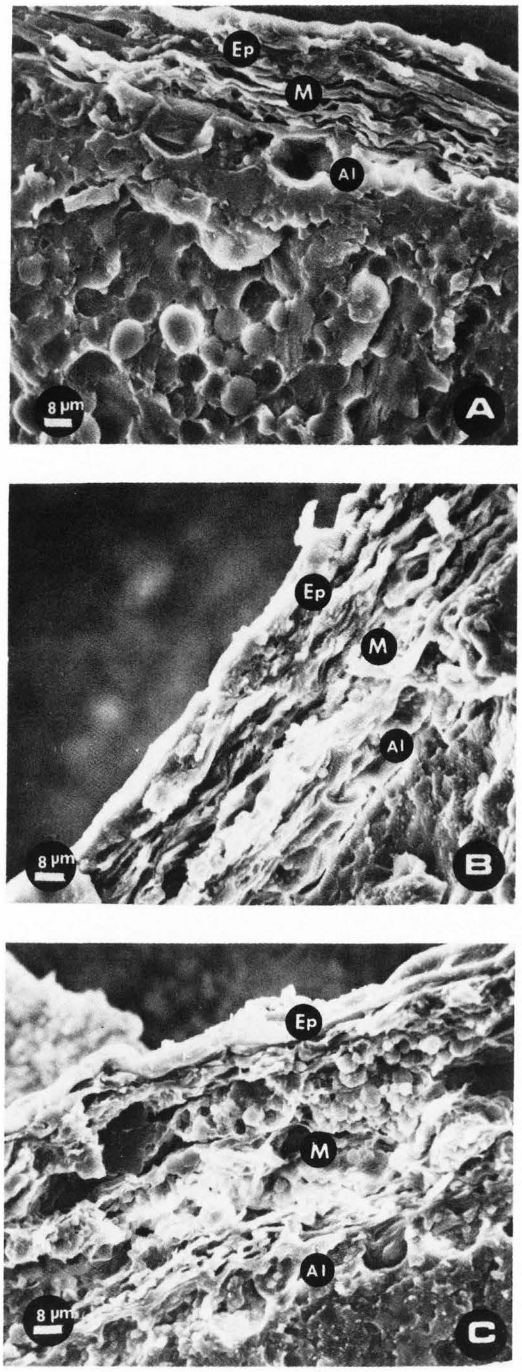

Fig. 4. Scanning electron photomicrographs of sorghums with varying pericarp thickness. Samples were the F_2 population from the cross BTx623 (thick pericarp) X CS3541 (thin pericarp).
A) Thin pericarp
B) Intermediate pericarp
C) Thick pericarp
Pictures indicate thicknesses along side of kernel and not near the style or hilum where pericarp thickness varies. Ep - epicarp, Al - aleurone layer, M - mesocarp

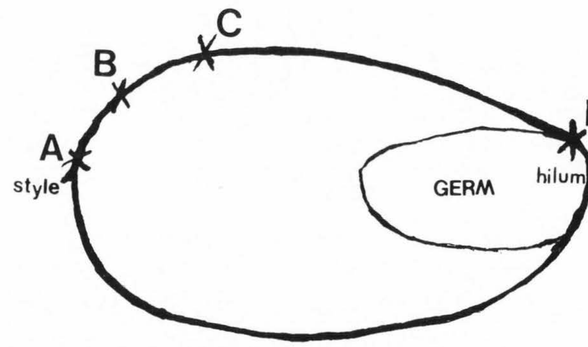

Fig. 5. Approximate location on kernel where Figure 6 photomicrographs were taken.

(percentage of corneous or floury endosperm) influences how well the kernel can be decorticated. More kernel breakage and incomplete milling occur in varieties with more floury endosperm (Maxson et al, 1971). The following milling trials were made with corneous to intermediate texture sorghums to permit comparisons based on pericarp thickness.

Milling trials performed on several sorghum samples from Mali and Upper Volta showed large differences in milling times between the thin and thick Guineense and the very thick Nio-Fionto grains (Table 2). Using a wooden mortar and pestle, average milling times for 2 kg lots of grain were 27.7, 19.8, and 11.0 minutes for the thin Guineense, the thick Guineense, and the Nio-Fionto, respectively. The milling was performed by native Malian women with three replications for each of four Guineense varieties and eight replications for the very thick Nio-Fionto sorghum. In Figure 7, differences in mesocarp thickness of these varieties is evident. Photographs B, D, and F were taken at approximately the same place on the kernels as indicated by the arrows in A, C, and E. The thin Guineense has virtually no mesocarp. The thick Guineense has a starchy mesocarp. The Nio-Fionto grain also has a thick, starchy mesocarp; the mesocarp is a layered structure with abundant starch granules. The photographs illustrate that the very thick, layered pericarp of the Nio-Fionto sorghum would easily be torn or peeled from the endosperm, thus decreasing milling time with the traditional pounding method of milling.

Testa Thickness Among Varieties

Variations in testa thickness were studied using four Malian sorghums and one (Shawaya) from Sudan. There was considerable variation in testa thickness among the Malian varieties (8-16µm) (Figures 3-D, 8-A,B,C). However, the testae of these varieties were thinner than those of the other varieties studied (eg. Figures 3-E and 3-F). The opposite extreme was seen with the Sudanese Shawaya (testa thickness 24-40µm) (Figure 9). This grain has a thick testa, the thickest pericarp encountered in our studies (160µm). It was approximately 3x the thickness

Table 2. Average milling time and endosperm recovery of sorghums with different pericarp thickness

Grain Type	Pericarp Thickness	Average Dehulling Time (minutes)[1]	Endosperm Recovery (%)[1]
Nio-Fionto	Very thick	11.0[a]	66.3[a]
Malian Guineense (thin pericarp)	Thin	26.4[c]	68.6[a]
Malian Guineense (thick pericarp)	Thick	19.4[b]	71.7[a]
Voltaic Guineense (thin pericarp)	Thin	29.0[d]	-*
Voltaic Guineense (thick pericarp)	Thick	20.0[b]	-*

*NA - data not available.

[1] numbers in same column with different superscripts are significantly different (P>.05)

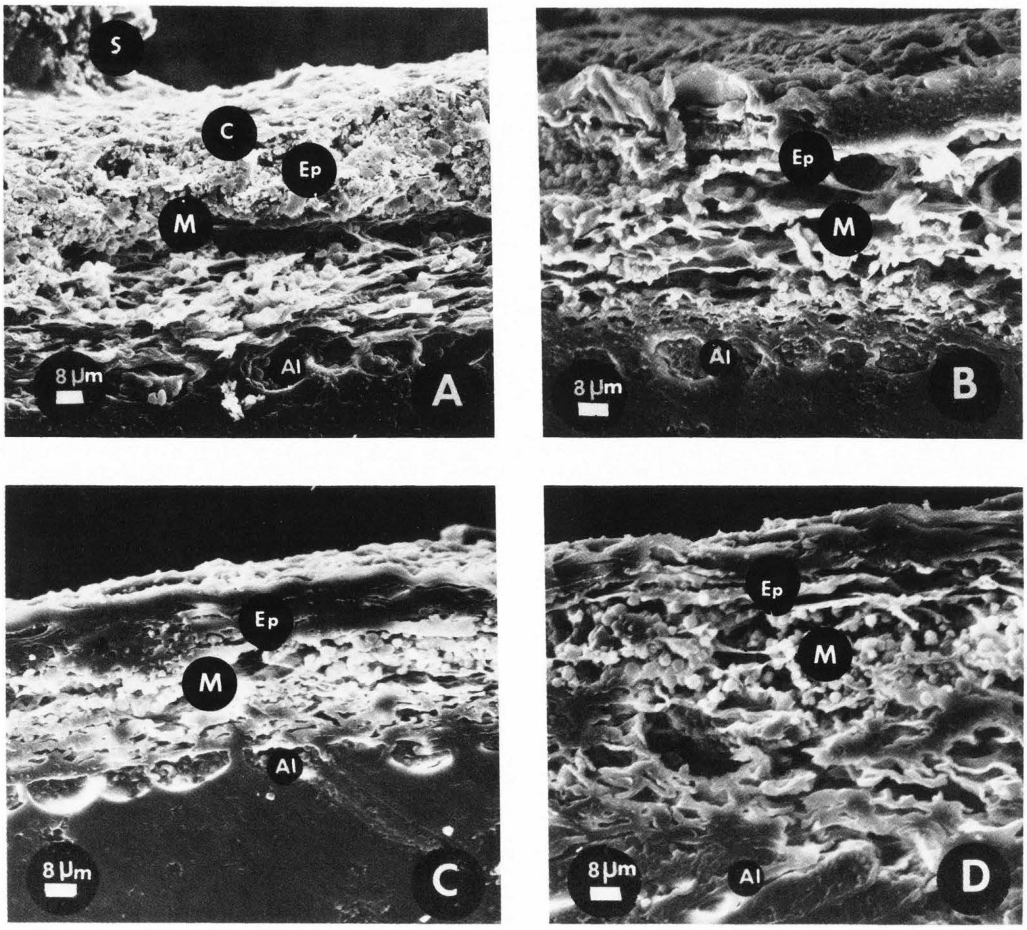

Fig. 6. Scanning electron photomicrographs of Nio-Fionto sorghum at several locations around kernel periphery depicting variation in pericarp thickness. Thickness of the pericarp at the following locations was:
A) Below style - 56µm
B) Near top of kernel - 64µm
C) Side of kernel - 48µm
D) Near hilum - 80µm
Al - aleurone layer, S - style, C - cuticle, Ep - epicarp, M - mesocarp

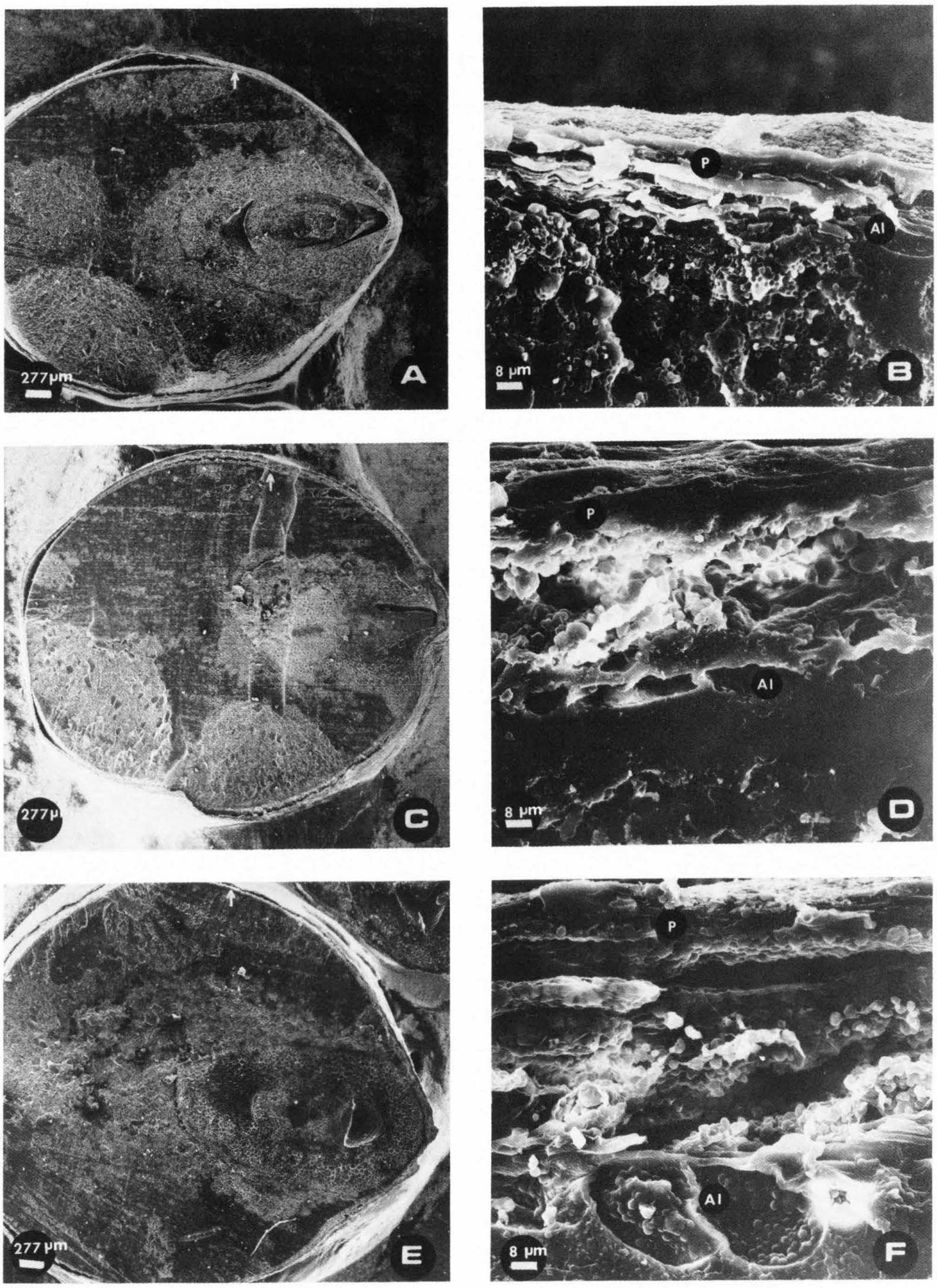

Fig. 7. Scanning photomicrographs of three sorghums from Mali.
A&B) Thin pericarp (Guineense)
C&D) Thick pericarp (Guineense)
E&F) Nio-Fionto grain with a much thicker pericarp and considerable starch in the mesocarp
P - pericarp, Al - aleurone layer. The arrows on A, C, E indicate where B, D, F sections were photographed.

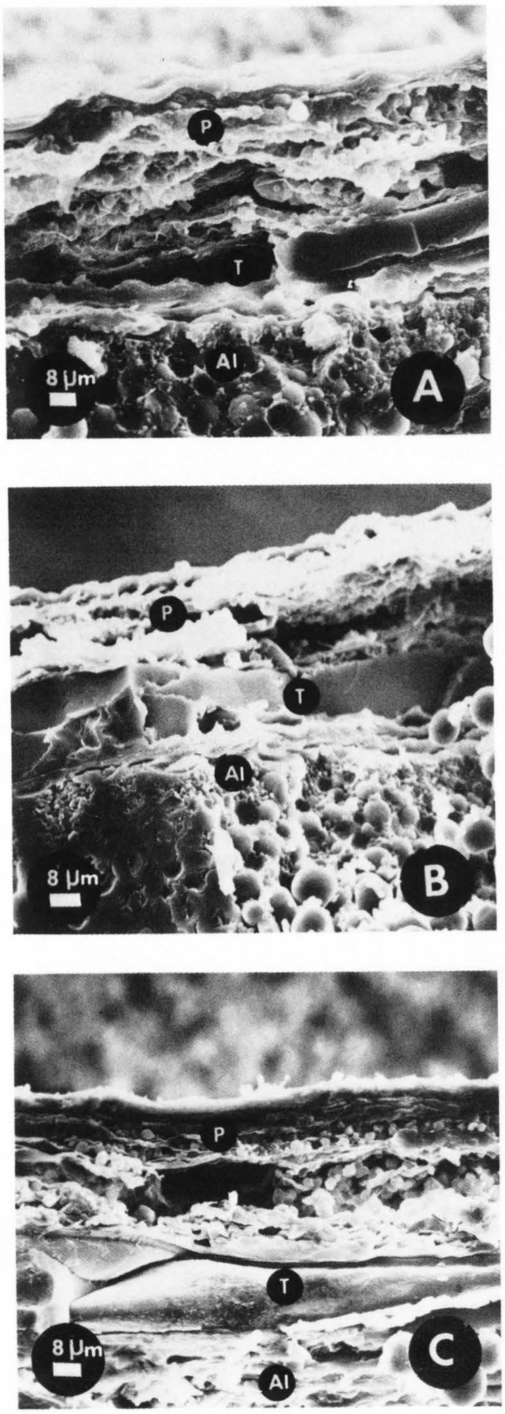

Fig. 8. Three Malian sorghums with thin testae. P - pericarp, T - testa, Al - aleurone layer

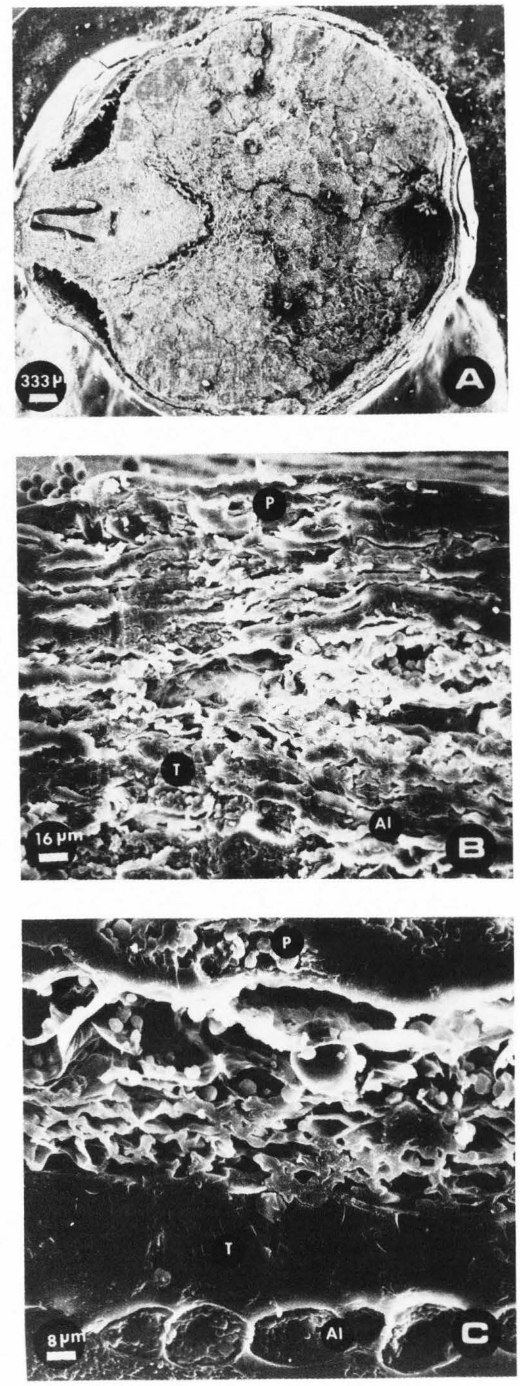

Fig. 9. Shawaya sorghum from Sudan with an extremely thick pericarp and a thick testa. P - pericarp, T - testa, Al - aleurone layer

of other thick pericarp sorghums studied.

The thin testa Malian varieties would be desirable in food products utilizing the entire ground kernel. Milling studies need to be performed before any conclusions can be drawn concerning the ease of removal of the testa from these varieties.

Based on the thickness of its pericarp, Shawaya would appear to be ideally suited to traditional milling methods. However, the grain is black in color. Shawaya may be a genetically red sorghum with a photosensitive pigment in the epicarp. Unless the pericarp and thick testa were removed prior to processing, the benefits of ease in milling would be outweighed by the development of undesirable colors. The very thick pericarp would also result in lower yield of milled grain.

Conclusions

Pericarp and testa thickness varies among sorghum varieties ranging from very thin to very thick. These differences affect the milling properties of the grain. Pericarp thickness affects mechanical and traditional hand milling systems differently. Our microscopic techniques can be used by plant breeders to select for pericarp and testa thickness.

Acknowledgements

The authors wish to thank Dr. John F. Scheuring, with ICRISAT in Mali, for use of the milling data and for the sorghum varieties used in the study of pericarp thickness and thin testa varieties. This research was supported in part by Grant AID/DSAN/XII/G-0149 from the Agency for International Development, Washington, DC 20523.

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Discussion with Reviewers

C.W. Glennie: Did you observe any difference in the size of the starch granules in the pericarp (mesocarp) as compared to those in the endosperm?
Authors: There appears to be a large difference in the sizes of the starch granules in the two areas. The starch granules in the pericarp are approximately 2-8μm while the starch granules in the endosperm vary depending on whether in the peripheral endosperm (6-10μm), the corneous endosperm (7-13μm), or the floury endosperm (13-30μm). There is a large variation in size of sorghum starch granules. These are some general values I have selected and by no means are they absolute values.
C.W. Glennie: Would the authors care to comment on their suggested photo-sensitive pigment mentioned in the last paragraph of Results and Discussion?
Authors: At this point we believe that the pigment is an anthocyanin. The young seed is red and gradually becomes darker, in some cases black. The same purplish-black color has been attributed to anthocyanins in the leaf-sheath and glume of some sorghum varieties. We presently have some crosses in the green house with these black seeded varieties and hope to get enough seed to do some extraction and identification of the flavonoid compounds soon.
C.W. Glennie: Were all testae observed as two cells thick?
Authors: No, they were not. The sorghums with testae seem to fall into two categories: 1) those sorghums with a red, white, or yellow pericarp with pigmented testae and 2) those sorghums with pigmented testae and a dominant spreader gene causing the phenotypic pericarp color to be brown, regardless of the genetic color of the pericarp (white, yellow, or red). The sorghums in the first group appear to have the two layered testae usually two different colors. The sorghums with the pigmented testae and spreader appear to have a more continuous testa layer. We are presently doing some additional microscopy work looking at sorghums with testae to see if the two-layer testa phenomenon holds true only for the one type of sorghum.
A. Shepherd: Is testa thickness chosen because of its ease of measurement? One might think that tannin content or some other measure of pigmentation would be more relevant. Please explain the choice. Do you have any hope of finding a single gene responsible for testa thickness?
Authors: It is quite possible that there is a single gene responsible for testa thickness. Until we received the thin testa Malian sorghum varieties and the thick testa Sudanese Shawaya we had seen very little difference in testa thickness among varieties so no work has been done to identify a gene for pericarp thickness. We undertook this work to verify that the Malian sorghums did indeed have thinner testae than the U.S. sorghums we have studied. The initial quantity of samples available was very small, thus we have not yet been able to do any tannin analyses.
E. Varriano Marston: Why did you oven-dry the kernels before viewing in the SEM? The samples should be sufficiently dry without drying any further. At what temperature were they dried?
Authors: The humidity here is so high that we cannot coat the kernels until the samples are dried to reduce the excess moisture. They were dried at 45°C.
Editor: What do a, b, c, d in Table 2 signify?
Authors: These are referenced by the superscript 1. This is the usual way of referencing means that are significantly different as determined by Duncan's Multiple Range Test.