The Relationship of Microstructure of Cowpeas to Water Absorption and Dehulling Properties[1]

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ABSTRACT

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Water absorption and dehulling characteristics are important to better utilization of legumes. Both these properties appear to be related to certain structural characteristics of legume seeds; thus, eight varieties of Ghanaian cowpeas were examined by scanning electron microscopy and the results compared to water absorption and dehulling properties. The seeds possess a highly organized cellular structure and all the varieties were characterized by similar shaped parenchyma cells (60–120 μm) containing starch granules (8–27 μm) and protein bodies (2–6 μm). Differences in the seed coats, which contain external palisade cells, accounted for the hydration and dehulling properties. Varieties with comparatively thick, smooth seed coats dehulled more satisfactorily when dry but generally had a slow initial rate of water absorption, whereas thin seed coat varieties showed a relatively high initial rate of water absorption and dehulled better after soaking. Other anatomical differences among varieties observed included micropyle size and shape and hilum size.

Reliable information on the chemical composition of specific tissues, structure of cells, and localization of chemical constituents in cells of legume seeds is a prerequisite for the explanation of physical and chemical changes taking place in the seed during mechanical, thermal, chemical, and enzymatic processes (Powrie et al 1960).

Work on the anatomy of seeds and seed coats of legumes can be placed in two main categories. First is the study of the development of seeds by embryologists. The emphasis here has been on the sequence of development of different parts of the seed (Harris and Boulter 1976, Opik 1968, Zimmerman 1936).

The second category involves studies in relation to specific functional property of the seeds (Coe and Martin 1920, Martin and Watt 1944, Rockland and Jones 1974).

Although there have been various reports on the relation between microstructure and water absorption in seeds (Coe and Martin 1920, Hyde 1951, Martin and Watt 1944, Ott and Ball 1943, Powrie et al 1960, Snyder 1936), it is not clear at this time what anatomical structures in the legume seed are responsible for water absorption. The seed coat (Coe and Martin 1920, Martin and Watt 1944, Ott and Ball 1943, Powrie et al 1960), micropyle (Snyder 1936), and hilum (Hyde 1951) all have been related to the permeability of the testa in various Papilionaceae. It is suggested that the seed coat, hilum, and micropyle together may form an integrated water absorption/removal system.

Cowpea (*Vigna unguiculata*) varieties are known to possess varied water absorption and dehulling properties. The aim of this study was to examine the structure of these different cowpea varieties in relation to their water absorption and dehulling properties.

MATERIALS AND METHODS

Cowpea Samples

The eight varieties of cowpeas used for these studies were: Adua Ayera, 1239, Ex Ada, New Era, Dark Mottled, Westbred, Kaase Market, and 3629. All samples were obtained from the Crop Science Department, University of Ghana, Legon, Ghana.

Scanning Electron Microscopy

All samples were examined in their raw, unfixed state according to the method of Sefa-Dedeh and Stanley (1979).

Water Absorption in Cowpea Seeds

The method described by Sefa-Dedeh et al (1978), based on determining the increase in weight in 10-g aliquots of cowpeas after soaking for 1, 3, 6, 12, 18, and 24 hr, was used. Determinations were done in triplicate.

Dehulling

Two traditional methods of dehulling legumes in West Africa were used. The first involved soaking the cowpeas in water and dehulling by manual rubbing. The second method of dehulling was performed on dry cowpeas. These were pounded with a mortar and pestle and the seed coat separated by winnowing.

Moisture and Protein Determinations

Moisture content of the legumes was determined by AACC method 44-15A. Total nitrogen was determined by macro-Kjeldahl method 46-13.

RESULTS AND DISCUSSION

Comparative Anatomy of Cowpea Varieties

In external topography, the important features of seeds are shape, size, seed coat surface, placement of hilum, and the presence or absence of such structures as the aril, caruncle, or elaisome. The

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[1] The data are taken from the thesis submitted by S. Sefa-Dedeh to the University of Guelph in partial fulfillment of the requirements for a PhD degree.

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eight varieties of cowpeas studied differed in color and size. A distinct elliptical hilum (2.1 to 3.0 mm) and a micropyle are visible at low magnification in all the varieties (Fig. 1). The micropyle, a characteristic of legume seeds, is situated just below the hilum (Fig. 2). Six varieties, Adua Ayera, Westbred, Ex Ada, 1239, Dark Mottled, and New Era, have a Y-shaped closed micropyle (Fig. 2A–F). The remaining two varieties, Kaase Market and 3629, however, have an open circular micropyle (Fig. 2G,H).

All the varieties showed the presence of parenchymatous cells (60 to 120 μm) (Figs. 3 and 4). These cells contain starch granules embedded in a proteinaceous matrix.

Another anatomical stucture examined was the seed coat (Fig. 5). It is apparent from these micrographs that the palisade cells of the cowpea varieties differ in thickness and shape. Six varieties, Adua Ayera, Westbred, Ex Ada, 1239, Dark Mottled, and New Era, showed the presence of a well-organized palisade layer composed of epidermal cells. Varieties 3629 and Kaase Market, however, showed an amorphous palisade layer (Fig. 5G,H) in which distinct palisade cells cannot be identified. In addition to being amorphous, the seed coats of varieties 3629 and Kaase Market were much thinner (5.84–9.92 μm) than the thick seed coat varieties (43.33–59.33 μm). Values for individual varieties have been reported earlier (Sefa-Dedeh et al 1978). Varieties classified as having relatively thick seed coats included Adua Ayera, 1239, Ex Ada, Dark Mottled, and New Era; the thin seed coat varieties included Westbred, 3629, and Kaase Market.

Examination of the surface structure of the seed coat reveals characteristic differences (Fig. 6). While the thick seed coat varieties show a relatively smooth surface, the varieties with thin seed coats show a highly rough and convoluted surface. The degree of roughness or folding seems to be related to the thinness of the seed coat. From Fig. 6, Westbred, with a seed coat thickness of 36.66 ± 1.36 shows less folding than variety Kaase Market with a seed coat thickness of 5.84 ± 0.38 (Sefa-Dedeh et al 1978).

Examination of cotyledon surfaces of the seven varieties shows that all have a structure similar to variety Adua Ayera, ie, wide "hills" with narrow "valleys" (Fig. 7). Complementary photomicrographs were obtained for the inner surface of the seed coat. The two varieties shown in Fig. 7 are representative of thick seed coat varieties (New Era) and thin seed coat varieties (Kaase Market). There appeared to be no anatomical difference among the varieties of cowpeas studied in their seed coat/cotyledon surface relationships.

Table I gives a summary of some of the anatomical characteristics for the eight varieties of cowpeas. Dimensions of the seeds were obtained by measuring the three major perpendicular axes as shown in Fig. 8. The varieties Kaase Market and 3629 differed from the rest in many anatomical characteristics including

TABLE I
Seed Anatomy of Eight Varieties of Cowpea

Variety	Seed Dimensions (mm)[a] Length (X)	Width (Z)	Thickness (Y)	Hilum (mm)[a]	Starch Width (μm)[b]	Starch Length (μm)[b]	Protein Bodies (μm)[b]	Cotyledon Cells (μm)[b]
Adua Ayera	6.90 ± 0.21	5.85 ± 0.24	4.25 ± 0.35	2.11 ± 0.19	8–15	10–23	3–6	60–100
Westbred	6.55 ± 0.44	5.45 ± 0.37	4.25 ± 0.35	3.00 ± 0.06	8–13	12–27	2–4	80–107
Ex Ada	8.70 ± 0.48	7.30 ± 0.59	5.10 ± 0.44	3.00 ± 0.24	8–15	10–23	2–4	67–94
1239	7.30 ± 0.67	6.40 ± 0.66	4.30 ± 0.48	2.30 ± 0.21	10–15	15–27	3–5	67–120
Dark Mottled	7.00 ± 0.30	5.20 ± 0.26	4.00 ± 0.28	2.60 + 0.20	10–15	12–23	2–3	70–94
New Era	6.05 ± 0.59	5.30 ± 0.35	3.90 ± 0.15	2.40 ± 0.21	10–15	13–27	2–3	70–107
3629	8.27 ± 0.48	6.35 ± 0.41	4.80 ± 0.71	3.00 ± 0.18	10–15	10–23	2–3	70–94
Kaase Market	8.80 ± 0.92	6.10 ± 0.32	3.90 ± 0.24	3.00 ± 0.14	10–15	15–27	3–5	85–100

[a] Mean ± standard deviation of 20 measurements.
[b] Range of 20 measurements.

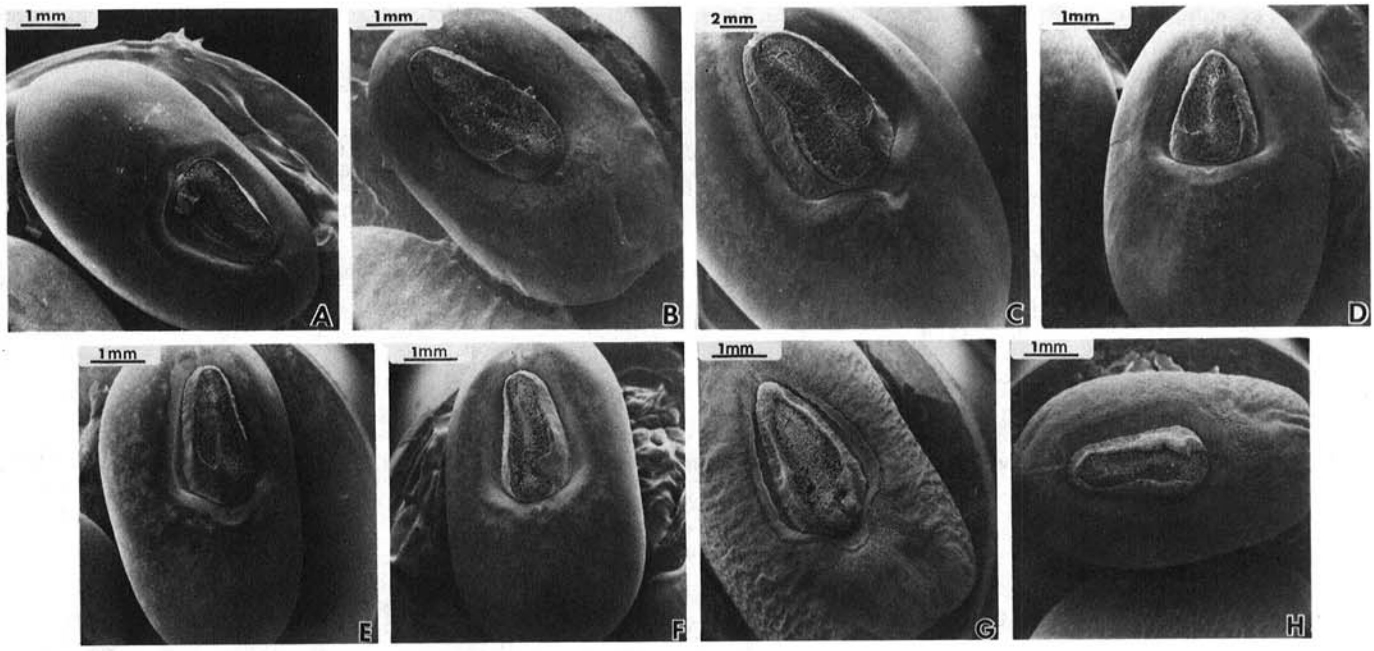

Fig. 1. Different varieties of cowpea seeds showing hilum. **A** = Adua Ayera, **B** = Westbred, **C** = Ex Ada, **D** = 1239, **E** = Dark Mottled, **F** = New Era, **G** = 3629, **H** = Kaase Market.

shape of micropyle, compactness of cotyledon, seed coat structure and thickness, and the surface structure of the seed coat. Are the observed anatomical differences responsible for water absorption properties and dehulling characteristics?

Water Absorption

The amount of water absorbed per unit weight of cowpea increased with soaking time for all varieties and a concomitant swelling of the seed was observed (Fig. 9). Quast and da Silva (1977) reported a similar curve for black beans (*Phaseolus vulgaris*).

The hydration curves for cowpeas can be explained by examining the physical changes that take place during the hydration of biological samples. Imbibition is a physical process related to the properties of macromolecules. In seeds the chief component imbibing water is protein (Mayer and Poljakoff-Mayber 1975), although other components such as mucilages, cellulose, starch, and pectic substances also contribute to water absorption. Under ideal conditions, ie, constant temperature, physical and chemical homogeneity of material, and chemical stability of the absorbing substance, imbibition of water at room temperature occurs with a continually diminishing speed (Shull and Shull 1932). The initial rate of water absorption is high but falls off rapidly with each increment of moisture due to the increasing saturation of the forces that cause hydration.

This applies to imbibition by a macromolecule under ideal conditions but when studying the hydration of beans one is not dealing with ideal conditions. This is because the major water absorption component, proteins of the cotyledon, do not

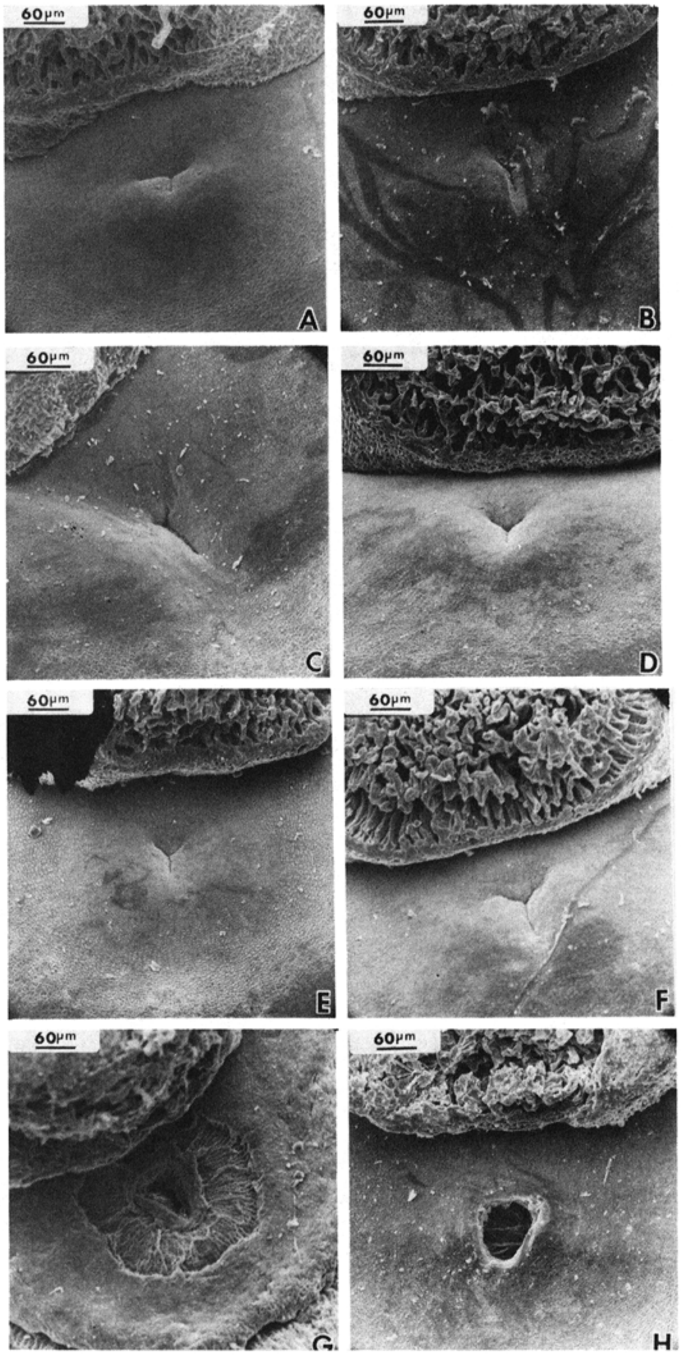

Fig. 2. The micropyle of cowpea varieties. **A** = Adua Ayera, **B** = Westbred, **C** = Ex Ada, **D** = 1239, **E** = Dark Mottled, **F** = New Era, **G** = 3629, **H** = Kaase Market.

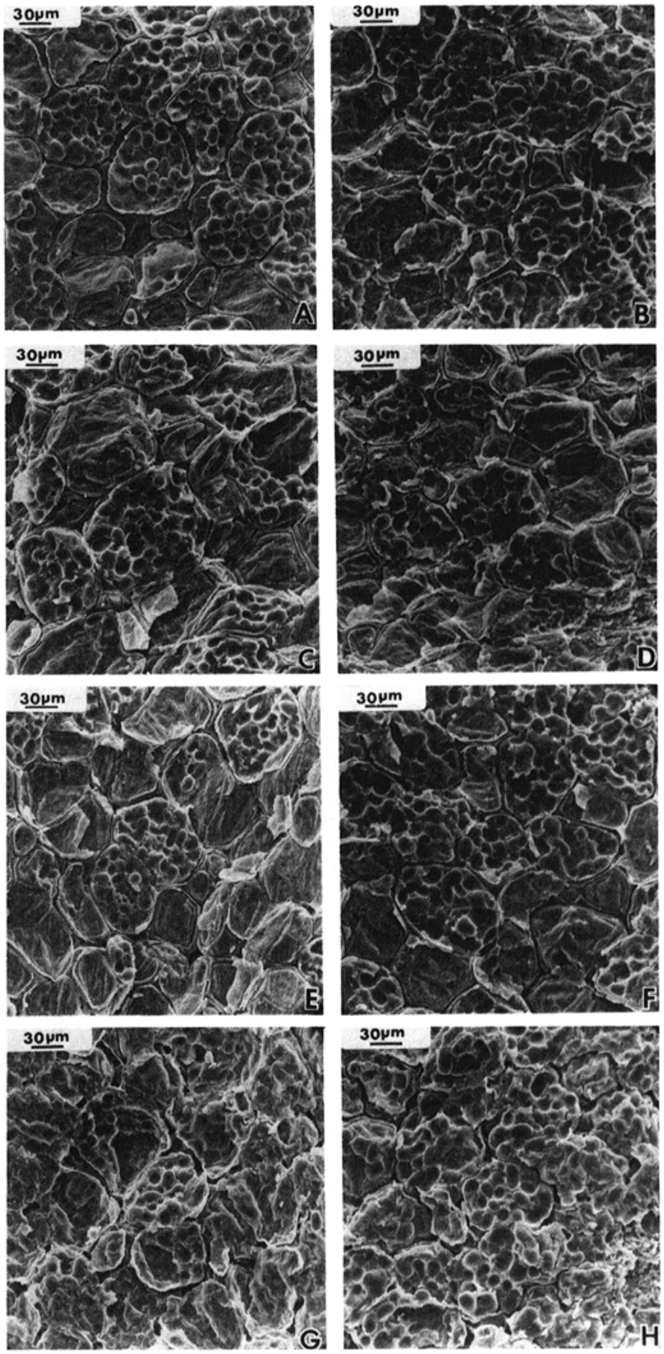

Fig. 3. Cross-sections of raw, unfixed cowpea cotyledons of different varieties. **A** = Adua Ayera, **B** = Westbred, **C** = Ex Ada, **D** = 1239, **E** = Dark Mottled, **F** = New Era, **G** = 3629, **H** = Kaase Market.

immediately come in contact with water due to the barrier presented by the seed coat. Shull and Shull (1932) indicated that, for biological materials such as seeds, irregularities in absorption occur. The causes of these irregularities were given as: (a) formation of internal cavities during imbibition, (b) osmotic activity of the seed coat, and (c) lack of physical homogeneity in the absorbing material.

The hydration curves for the cowpea varieties Adua Ayera and New Era show this irregular pattern (Fig. 9) in the form of a slow initial rate followed by a rapid increase in water absorption and, lastly, a reduction in the rate of water absorption. The slow initial rate of water absorption may be related to the thick seed coat, small hilum, and micropyle of these varieties. Kyle and Randall (1964) reported that the micropyle is the most important area of water entry in Great Northern beans (*P. vulgaris*). The size of the micropylar opening was important in determining the amount of water absorbed. Thus it is not surprising that the cowpea varieties Kaase Market and 3629, with a relatively thin amorphous seed coat (5 to 10 μm) and an opened micropyle (Fig. 2), tended to show a relatively rapid water uptake within the first hour of soaking (Fig. 9).

In these two varieties, the proteins and other compounds in the cotyledons responsible for absorbing water could achieve a high initial rate of water absorption because there seems to be little resistance to entry of water through the amorphous and thin seed coats. It was observed that the seed coats of these two varieties

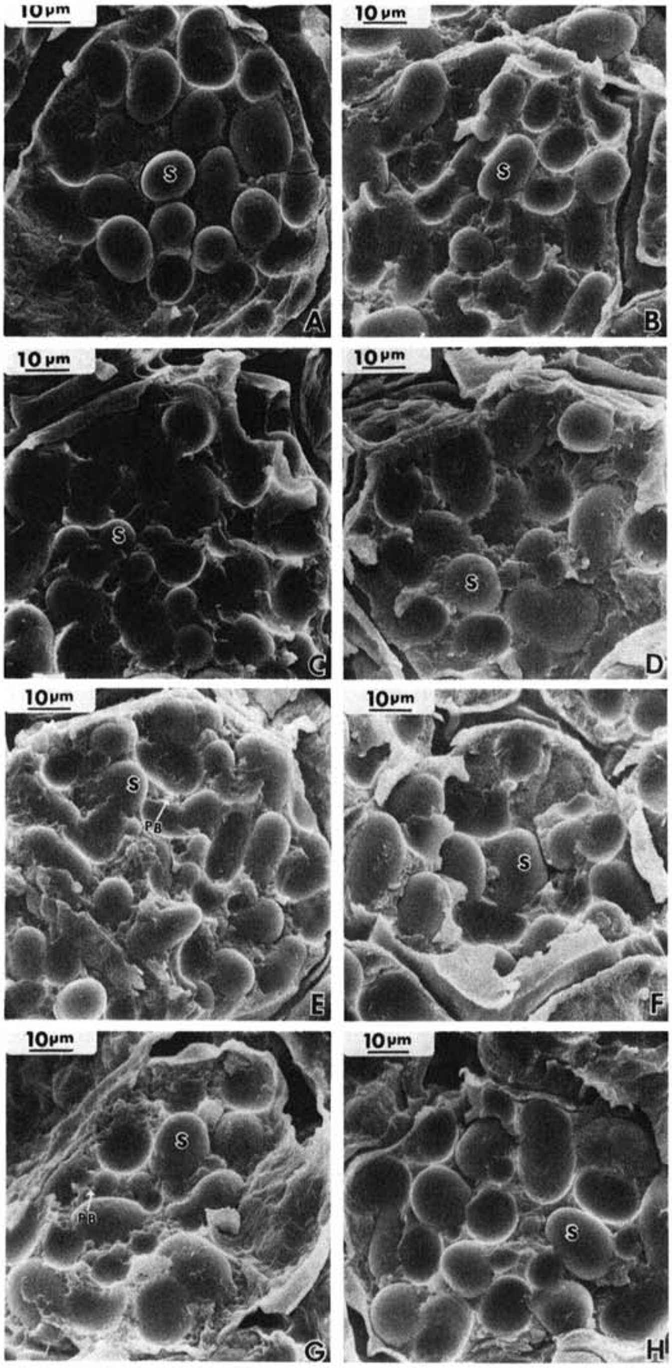

Fig. 4. Cross-sections of raw, unfixed cowpea cotyledons showing single cells. **A** = Adua Ayera, **B** = Westbred, **C** = Ex Ada, **D** = 1239, **S** = Starch granule, **PB** = Protein body, **E** = Dark Mottled, **F** = New Era, **G** = 3629, **H** = Kaase Market.

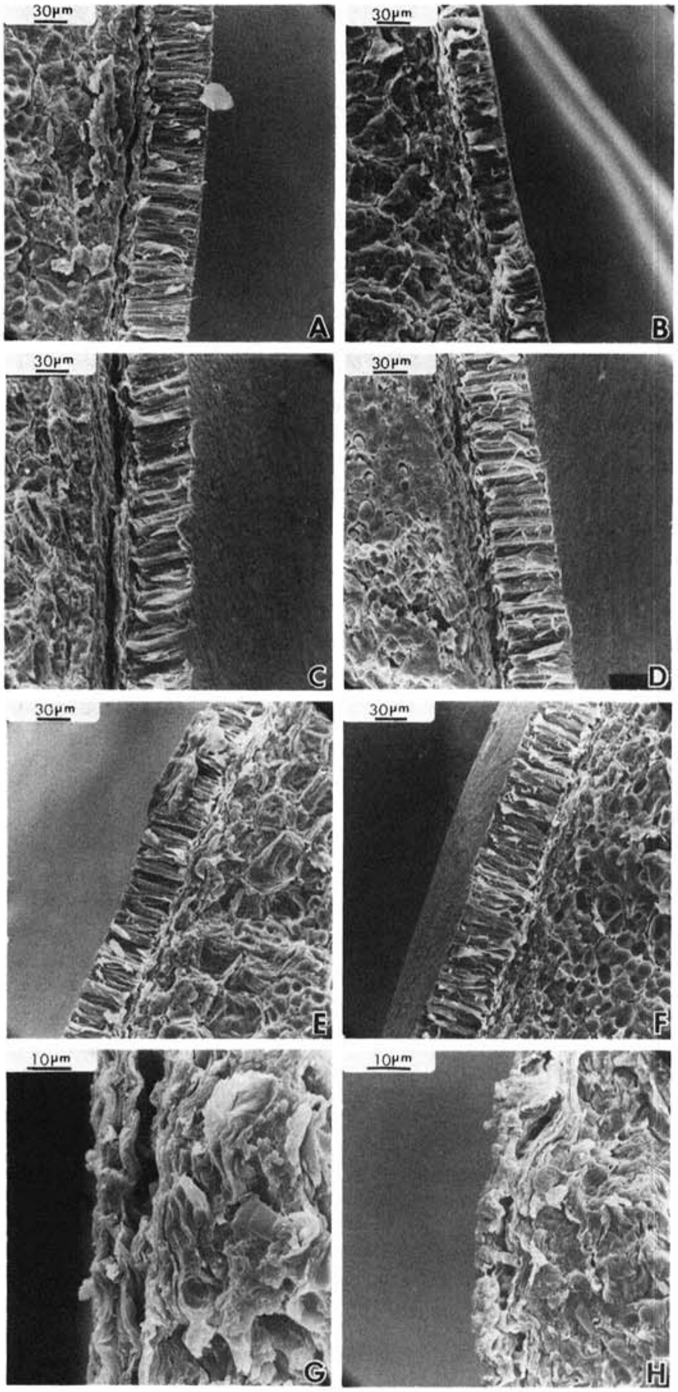

Fig. 5. Cowpea seed coats of different varieties. **A** = Adua Ayera, **B** = Westbred, **C** = Ex Ada, **D** = 1239, **E** = Dark Mottled, **F** = New Era, **G** = 3629, **H** = Kaase Market.

absorb water easily and swell rapidly (within 10 min of soaking) and thus are able to separate from the cotyledon leaving a space that is filled with water. By this process, the cotyledons with their proteins and other water-absorbing components are directly in contact with the water allowing rapid imbibition. Another anatomical characteristic that may explain the rapid absorption of water by the varieties Kaase Market and 3629 is their porous cotyledon structure (Fig. 3G,H). This would allow for the rapid channeling of water in the cotyledon that later becomes physically entrapped.

Other cowpea varieties that showed hydration curves similar to variety 3629 were Dark Mottled, 1239, and Ex Ada (Fig. 9). Interestingly, varieties Ex Ada and 1239, although possessing relatively thick seed coats, tended to show a rapid initial rate of water absorption. It is possible that the seed coats of these varieties possess a high hydration rate and, once hydrated, the water is able to pass quickly to the water-absorbing components in the cotyledons. The seed coats of varieties Ex Ada and 1239, however, did not exhibit the swelling characteristic observed in Kaase Market and 3629.

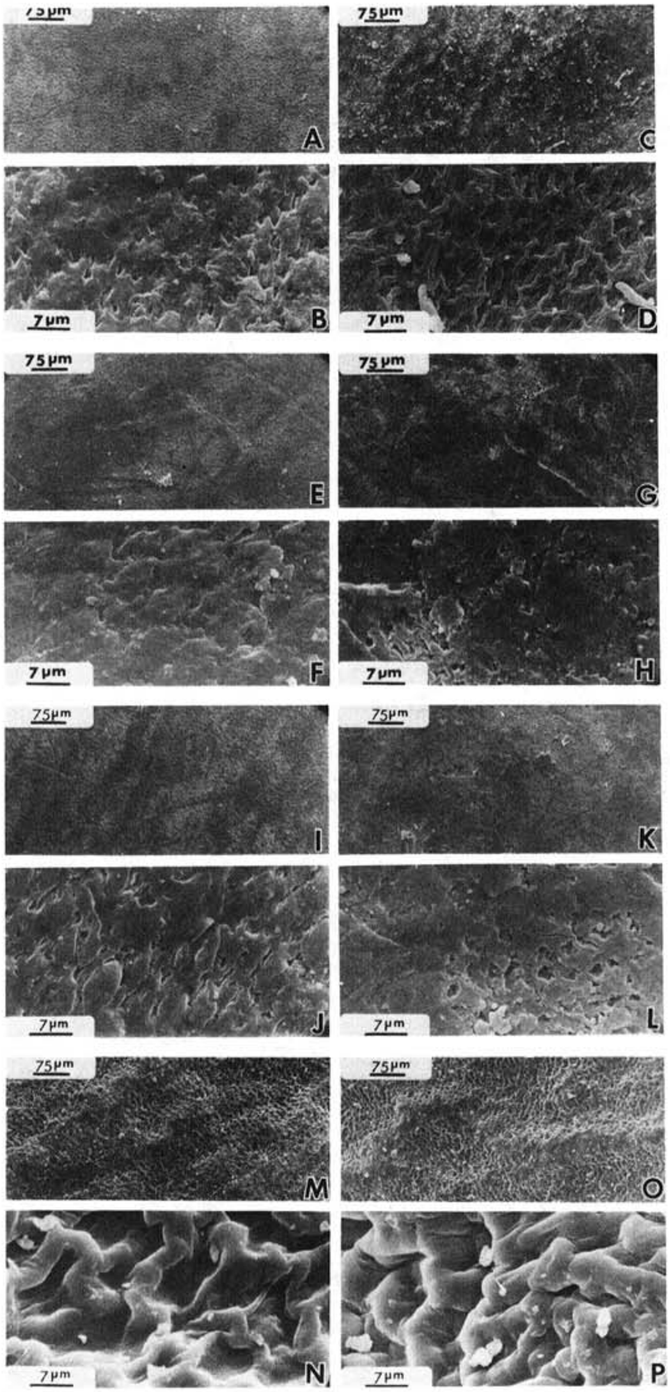

Fig. 6. Seed coat surfaces of eight cowpea varieties. **A** and **B** = Adua Ayera, **C** and **D** = Westbred, **E** and **F** = Ex Ada, **G** and **H** = 1239, **I** and **J** = Dark Mottled, **K** and **L** = New Era, **M** and **N** = 3629, **O** and **P** = Kaase Market.

The variety Westbred, though possessing a relatively thin seed coat (36.66 ± 1.36 μm), showed a reduction of the initial rate of water absorption (Fig. 9) as compared with Kaase Market and 3629 varieties. This may be due to the fact that the seed coat of variety Westbred is about five times thicker than that of Kaase Market and this, coupled with their nonamorphous structure and closed micropyle, may reduce initial water absorption. The seed coat of Westbred did not show the rapid initial swelling observed for Kaase Market and 3629.

Table II shows the moisture and protein contents of the cowpea varieties. Although the chief component that imbibes water in seeds is protein (Mayer and Poljakoff-Mayber 1975), it is difficult to make generalizations from Table II. Of the eight varieties, New Era had the highest protein content. In contrast, this variety absorbed

TABLE II
Moisture and Protein Contents of Cowpea Varieties

Variety	Moisture (%)	Protein[a] (%)
Adua Ayera	10.16 ± 0.48	23.54 ± 0.22
Westbred	10.16 ± 0.19	23.63 ± 0.18
Ex Ada	9.29 ± 0.19	25.13 ± 0.31
1239	10.97 ± 0.11	24.24 ± 0.08
Dark Mottled	9.37 ± 0.01	23.51 ± 0.38
New Era	9.06 ± 0.32	25.59 ± 0.19
3629	9.65 ± 0.08	23.96 ± 0.14
Kaase Market	9.02 ± 0.03	22.24 ± 0.14

[a] Dry basis (N × 5.7).

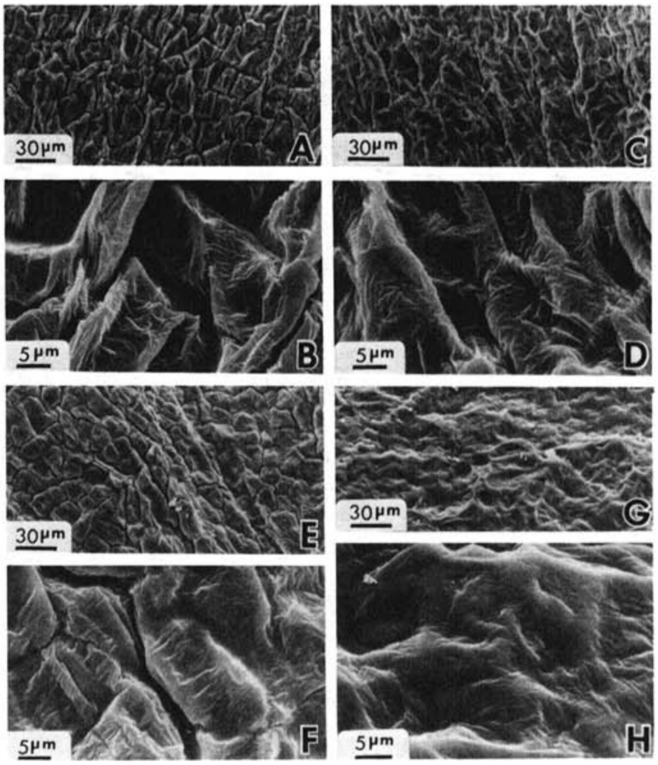

Fig. 7. Cotyledon surface (A, B, E, F) and inner surface of seed coat (C, D, G, H) of two cowpea varieties. **A, B, C,** and **D** = New Era; **E, F, G,** and **H** = Kaase Market.

the least amount of water (73 g/100 g dry beans) after 24 hr of soaking. This shows that an explanation for the water absorption characteristics of cowpeas is not simple. The cowpea seed is a nonhomogeneous material and the conditions during soaking were not as ideal as described by Shull and Shull (1932). Irregularities can be expected and factors such as protein content, hilum size, initial water content, seed coat thickness, and seed volume can show their influence on water absorption. Thus, a stepwise multiple regression analysis was performed on the data for the eight cowpea varieties to relate these factors to the amount of water absorbed at each soaking time. Table III shows the regression equations obtained at the soaking times used. The contribution of each independent variable to the multiple R^2 was plotted (Fig. 10). Regression analysis indicated that for the initial soaking periods (1 to 3 hr), the equations obtained could explain 85 and 82% of the total variation in water absorption (Table III). For both periods, the seed coat thickness contributed over 40% to multiple R^2. The contribution of the seed coat decreased sharply after 3 hr of soaking (Fig. 10); other important factors during the early soaking period were seed volume and initial moisture content of the seed, accounting for 25 and 19% of the variation, respectively. Regression analysis of the 6 and 12 hr data indicated that a total of 85 and 92% of the variation could be explained with the hilum size being the most important variable (Fig. 10). It is at these soaking periods that protein content begins to show a major influence on water absorption (Fig. 10). The protein content of the seed accounted for most of the variations in cowpea seed water absorption after 12 hr of soaking. These data indicate that the

TABLE III
Regression Equations for Predicting Water Absorption in Cowpeas

Soaking time (hr)	Equation[a]	R^2
1	$Y = -141.44 + 22.09X_1 + 0.55X_2 - 1.36X_3 - 17.73X_4 + 0.30X_5$	85.3
3	$Y = -187.21 + 28.16X_1 - 2.70X_2 - 1.08X_3 + 15.47X_4 + 0.22X_5$	82.0
6	$Y = -52.32 + 28.07X_1 - 15.76X_2 + 0.34X_3 + 75.74X_4 + 0.14X_5$	84.6
12	$Y = 55.17 + 21.65X_1 - 13.03X_2 + 0.41X_3 + 48.24X_4 + 0.03X_5$	92.3
18	$Y = 97.57 + 13.98X_1 - 9.26X_2 + 0.17X_3 + 33.45X_4 + 0.01X_5$	97.6
24	$Y = 201.05 + 11.68X_1 - 12.18X_2 + 0.28X_3 + 27.52X_4 - 0.03X_5$	95.23

[a] Y = Water absorbed (g H_2O/100 g dry beans), X_1 = initial water content, X_2 = protein content (%), X_3 = seed coat thickness (μm), X_4 = hilum size (mm), X_5 = seed volume (mm^3).

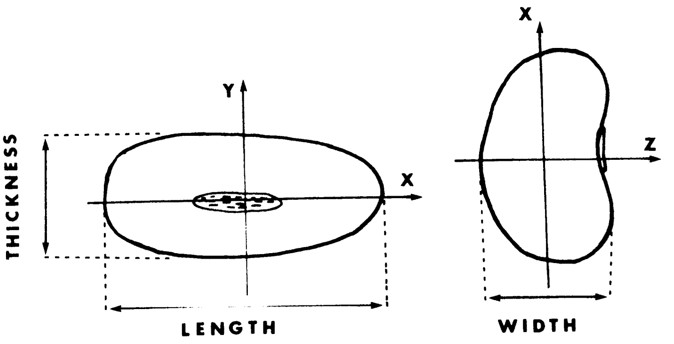

Fig. 8 Principal dimensions of cowpea seeds.

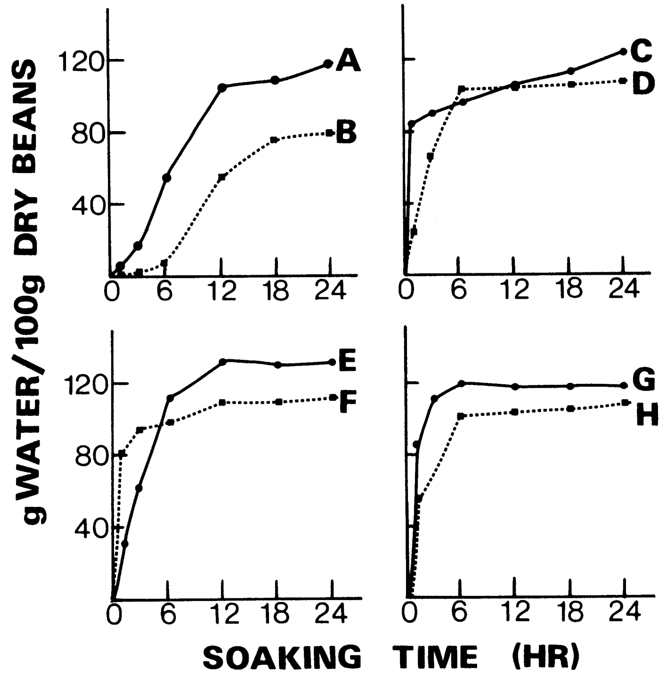

Fig. 9. Water absorption during hydration of cowpea varieties. **A** = Adua Ayera, **B** = New Era, **C** = Kaase Market, **D** = Dark Mottled, **E** = Westbred, **F** = 1239, **G** = 3629, **H** = Ex Ada.

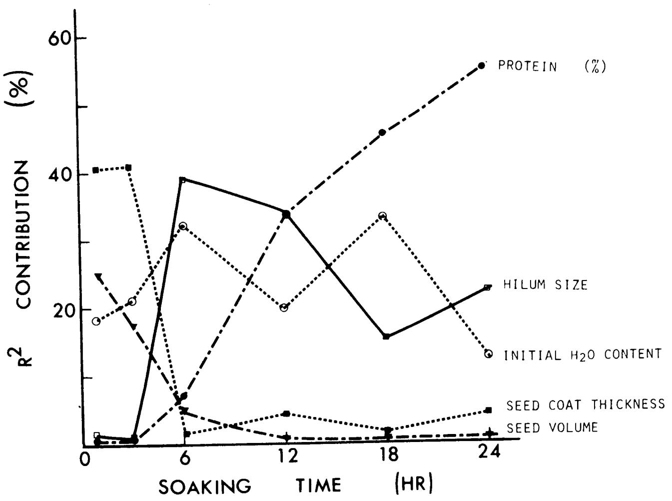

Fig. 10. Changes in R^2 contribution with soaking time for five independent variables.

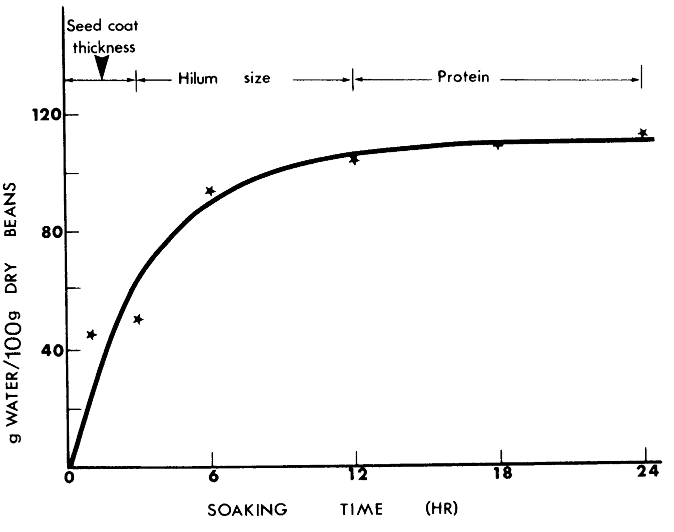

Fig. 11. Generalized water absorption curve of cowpea varieties showing components accounting for most of the variation in water absorption.

factors affecting water absorption of cowpeas contribute unequally during the soaking process.

Figure 11 shows a generalized water absorption curve for the eight cowpea varieties plotted from their mean values. The components accounting for most of the variation in water absorption for the specified time period are indicated. Seed coat thickness is an important factor during the first 3 hr of soaking since it is the first barrier the soaking water encounters. The contribution of seed coat thickness to the variation in water absorption drops sharply after 3 hr of soaking. After this period, the hilum size accounts for most of the variation in water absorption. Both seed coat and hilum are external structures and it is expected that they are important during the initial stages of soaking.

In the later stages of soaking, protein content becomes increasingly important and the changes in curve shape at this point are due to this component. The absolute value for water uptake increases only slightly during the final period of soaking but what variations occur are mainly due to the amount of protein present and likely to the propensity of these molecules to bind water.

In summary, our observations indicate that water uptake by cowpeas is a complex process in which several anatomical and compositional factors become sequentially important—seed coat structure and thickness, seed size, hilum size, and protein content.

Dehulling Characteristics

The dehulling characteristics of legume seeds play an important role in their selection for use in foods. It was observed that the cowpea variety Adua Ayera dehulled better in the dry state using a mortar and pestle. The same dehulling characteristics were observed for the varieties 1239, New Era, Ex Ada, and Dark Mottled. The varieties 3629, Kasse Market, and Westbred, however, were difficult to dehull dry. These varieties dehulled easily by hand rubbing after a period of soaking (10 min to 2 hr).

The microstructure of legume seeds (Figs. 5 and 7) and that of the seed coat/cotyledon relationship reported by Sefa-Dedeh and Stanley (1979) can be used to explain some of the dehulling properties observed. For the varieties Adua Ayera, 1239, New Era, Ex Ada, and Dark Mottled, their relatively thick seed coats did not swell rapidly, but remained attached to the cotyledon throughout the soaking period. Because of the interlocking structure of their cotyledon surface and inner surface of the seed coat, the swelling associated with soaking might have produced a tighter cotyledon surface/seed coat structure, thus making dehulling after soaking by rubbing difficult. However, in the dry state, because their seed coats are thick, they can withstand shearing when pounded with a mortar and pestle resulting in easy dehulling.

The thin seed coated varieties Kaase Market, 3629, and Westbred, however, cannot withstand shearing; when pounded the cotyledons break with the seed coats tightly attached to the fragments. In water, their amorphous structure and relative thinness allow the seed coats to hydrate rapidly resulting in expansion. This expansion of the seed coat relative to the cotyledon may cause separation and make dehulling by hand rubbing possible.

CONCLUSIONS

The eight varieties of cowpeas studied showed similar cotyledon structure but differences in seed coat structure and thickness, micropyle, and hilum size were observed. These different anatomical structures could be used to explain functional properties such as water absorption and dehulling characteristics. From these studies, it is suggested that the structure and thickness of seed coat may have a significant effect on water absorption and dehulling characteristics. Varieties with relatively thick seed coats showed a slow initial rate of water absorption as compared with the thin seed coat varieties. The water absorption of cowpea varieties are influenced by external structures such as seed coat thickness and hilum size at early stages of soaking. The protein concentration becomes more important at later stages of soaking. The varieties with thin seed coats dehulled easily after a short soaking period. This may be due to the easy penetration of water that results in an expansion of the seed coat and to their easy separation from the cotyledon surface.

In the processing of cowpeas in West Africa, a high rate of water absorption and easy dehulling are desired quality attributes. Thus, the thin-coated varieties will be more desirable and it might be appropriate to suggest the breeding and cultivation of such varieties. It should be noted, however, that their thin and amorphous seed coats that promote these properties may make them more susceptible to insect attack.

In most West African areas, insect infestation of the cowpea seed is a major problem. It is known that the varieties Kaase Market and 3629 are easily infested with cowpea weevils. Insect infestation of the seeds leads to reduced food value and an economic loss to the farmer. From this work, it can be suggested that the weevils are able to attack these varieties partly because of their thin and amorphous seed coats, which provide minimal protection from insect attack. The variety Adua Ayera and others with relatively thick seed coats, however, have a reduced degree of insect infestation partly because of the thick, highly organized palisade layer of their seed coats.

Since good water absorption properties are associated with thin seed coats, it might be appropriate to suggest the breeding of such varieties. Because of their susceptibility to insect attack, however, it is proposed that a combination of an organized palisade structure as a component of the thin-coated variety may provide cowpea strains that can absorb water readily yet be resistant to insect attack.

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