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MICROSTRUCTURE OF ADZUKI BEANS (*Vigna angularis* cv. Express)

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Abstract

Scanning electron microscopy (SEM) was used to study and compare the microstructure of adzuki, black and Mexican red bean seeds. The two cotyledons of the beans were separated by a deep fissure. A view of seed coat surface of adzuki beans revealed a characteristic pattern of convolutions and cracks, not observed on the seed coat surfaces of black or red beans. The cross-section of seed coat of adzuki beans exhibited a thick layer of palisade cells in the epidermal layer followed by underlaid multiple layers of tightly packed parenchyma cells. A sub-epidermal layer comprised of a single layer of hour-glass cells was observed in the black and red bean seed coats but not in adzuki beans. A cross-section of the hilum region of adzuki beans and Mexican red beans exhibited a double palisade layer, a phenomenon not observed in the black beans. The cross-section of the sub-hilar region of the adzuki and Mexican red beans revealed a sub-hilar plug, not observed in the black bean sub-hilar region. The micrographs of adzuki bean cotyledons showed spherical starch granules with an apparent pitted surface and were loosely embedded in a protein matrix. The starch granules of black and Mexican red beans were more oval in shape, with a smoother surface, more compactly packed and embedded in a protein matrix. The microstructural differences of the adzuki bean from the other common food legumes like black beans and Mexican red beans, may be useful to study the relationship between textural and functional properties of these beans.

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Introduction

Adzuki beans are among the oldest cultivated beans in the oriental countries, especially in Japan and China. Adzuki beans are dried, cooked whole or made into a paste used in soups, cakes and other confections. Adzuki beans are primarily used as a sweetened paste called "an" (Japanese for sweetened adzuki paste) which is a popular ingredient for many types of sweetened desserts (McClary et al., 1989).

Adzuki beans were second only to soybeans in total Japanese legume production in 1987. A promising Japanese market exists for Washington State produced dry edible adzuki beans (McClary et al., 1989).

The use of scanning electron microscopy (SEM) in recent studies of food microstructure has proven to be an invaluable technique, in providing excellent information about the dynamic structure of plant food products with minimal sample amount (Swanson et al., 1985; McAlear, 1972). The textural characteristics of legumes are related to the microstructure of the seeds (Sefa-Dedeh and Stanley, 1979). Sefa-Dedeh and Stanley (1979) suggested that low water-absorption rate of adzuki beans at short soaking time could be due to relative thickness and slow hydration of the seed coat. The objective of the present study was to investigate and compare the microstructures of adzuki, black and Mexican red beans.

Materials and Methods

Adzuki (*Vigna angularis* cv. express), black (*Phaseolus vulgaris* cv. black turtle soup) and Mexican red (*Phaseolus vulgaris* cv. Mexican red) beans were obtained from Washington State University Irrigated Agriculture Research and Extension Center, Prosser, WA. Adzuki and Mexican red beans have brownish-red seed coats while black beans have a black seed coat.

Surface cleaned adzuki, black and Mexican red beans were fractured with a razor blade and glued to aluminum stubs and dried for 6 hours in a desiccator. The fractured beans were then sputter-coated with 30 nm gold (in a Hummer-Technics sputter-coating unit). The gold coated specimens were examined with an Hitachi

S570 scanning electron microscope operating at 20 kV.

Results and Discussion

Leguminous seed

Typical leguminous seed consists of seed coat, two cotyledons and an embryo. The hard dry seed coat forms a protective envelope covering the cotyledons and growing embryo, while the cotyledons provide nutrient reserves for the growing embryo. The two cotyledons are generally separated by a deep fissure and attached at the site of embryo.

The size of adzuki beans ranged from 0.5 to 0.8 cm in length and 0.3 to 0.5 cm in width. Black beans and Mexican red beans used were larger than adzuki beans. Black beans varied from 0.6 to 1 cm in length and 0.5 to 0.6 cm in width, while Mexican red beans measured 1 to 1.4 cm in length and 0.5 to 0.8 cm in width. The cross-sections of adzuki, black and Mexican red beans revealed typical leguminous seed structures (Figures 1 - 3) similar to those described by Corner (1951). Closer observation of the cotyledons revealed numerous spherical starch granules embedded in a protein matrix, which together provide energy and nourishment for the growing embryo.

Seed coat surface

Legume seed coats provide protection for the nutrient rich cotyledons and growing embryo from pests and microbial contaminants. The surface of the seed coat often exhibits a distinctive "fingerprint" pattern, formed during seed development due to the pressure between the seed coat and the endocarp, the innermost layer of the pod wall (Hughes and Swanson, 1985). The seed-coat surface of the adzuki beans exhibited a characteristic convoluted pattern of ridges and a mosaic pattern of cracks (Figure 4). No ridges or cracks were observed in black or Mexican red beans.

Wolf et al. (1981) characterized thirty three cultivars of soybeans on the basis of seed coat surface pits, cracks and deposits. Hughes and Swanson (1986) observed that lentil seed coat in contrast to other food legumes possess an uneven surface with distinct conical papillae. No such characteristic pits or conical papillae were observed on any of the seed coat surfaces examined in this study.

Cross-section of the seed coat

Cross-section of seed-coat of adzuki beans revealed an organized epidermal layer of palisade cells about 50 to 60 μm thick (Figure 5). The seed coat of adzuki beans, unlike that of black (Figure 6) and Mexican red beans lacked the typical sub-epidermal layer of hour-glass cells as previously observed by Sefa-Dedeh and Stanley (1979). The single layer of epidermal palisade cells in the seed coat of adzuki (Figure 7) and Mexican red (Figure 8) beans extended into the hilum region as a double palisade layer of cells. No such double palisade layer was observed in black beans (Figure 9) in this study or in the previous study of Hughes and Swanson (1985).

The appearance of a double palisade layer is generally attributed to an optical phenomenon called "linea lucida" which gives an optical impression that the palisade layer in some legumes consists of two distinct layer of cells (Corner, 1951). SEM micrographs of the seed coats of pinto and white beans (Sefa-Dedeh and Stanley, 1979), however, indicated two distinct layers of palisade cells in the seed coat. The cell layer closer to the cuticle was composed of organized cells, and a lower layer was composed of amorphous disorganized cells. Corner (1951) also observed a double palisade layer of cells in the hilum region of leguminous seeds. Thus "linea lucida" or double palisade layer may not be entirely an optical effect, but may be present in the seed coat of some legume cultivars and absent in others.

The hilum region is an important site for water imbibition in beans (Kyle and Randall, 1963; Swanson et al., 1985). Consequently the presence or absence of a double palisade layer in this region may influence the rate and quantity of water imbibed by the beans.

Sub-hilar tissue

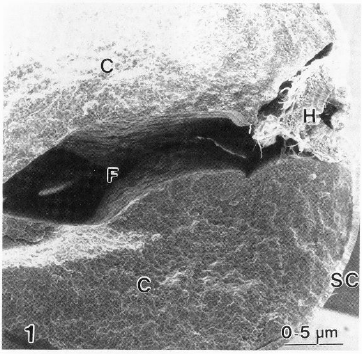
In leguminous seeds the outer integument between the endosperm and the hilum is thickened to form a sub-hilar tissue. Sub-hilar tissue is generally composed of stellate-celled aerenchyma (Corner, 1951). In some leguminous seeds there is often a hard, compact tissue of multilayered, thick-walled pitted contiguous cells immediately below the hilum which serve as a strut or sub-hilar plug against the contraction of the seed coat on to the radicle (Corner, 1951).

The cross-section of sub-hilar region in adzuki and Mexican red beans exhibited short, contiguous tracheid bars forming a sub-hilar plug (Figures 7, 8). No such sub-hilar plug was observed in black beans (Figure 9).

Figures 1-3. Cross-sections of adzuki bean (**Figure 1**), black bean (**Figure 2**) and Mexican red bean (**Figure 3**) seeds showing seed coat (SC), hilum (H), fissure (F) and cotyledons (C).

Figure 4. Seed coat surface of adzuki bean showing cracks and convolutions.

Figures 5-6. Cross-sections of the seed coats of adzuki bean (**Figure 5**) and black bean (**Figure 6**) showing, palisade cells (PL), hour glass cells (HR) and parenchyma cells (PR).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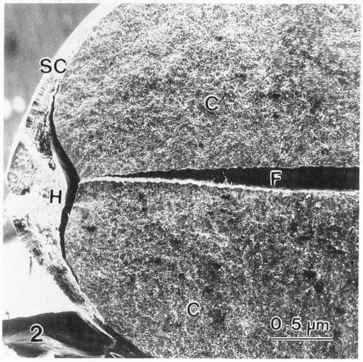
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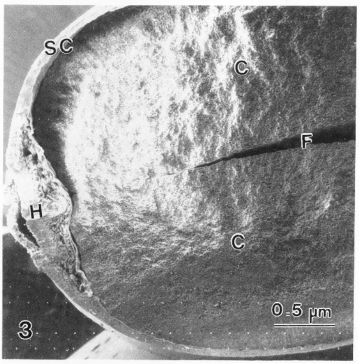
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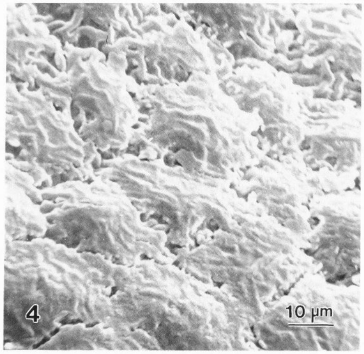
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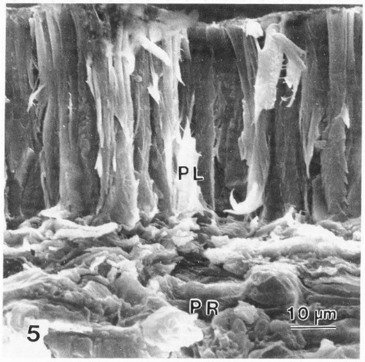
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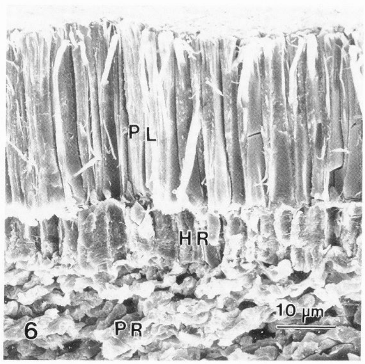
PL
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6
PR

Microstructure of cotyledons

SEM examination of black (Hughes and Swanson, 1985), adzuki cotyledon and other bean cotyledon cells (Sefa-Dedeh and Stanley, 1979), revealed spherical starch granules embedded in a protein matrix. Protein matrix is generally composed of small spherical to oval shaped individual protein bodies. The microstructure of soybean cotyledon cells observed by Wolf and Baker (1980) revealed protein bodies surrounded by a protein network in which spherosomes (lipid bodies) were embedded.

At higher magnification the microstructure of adzuki, black and Mexican red bean cotyledons revealed spherical to oval shaped starch granules embedded in protein matrix composed of small protein bodies. The starch granules of the adzuki beans were more loosely packed than the starch granules of the black beans and Mexican red beans. The surface of the starch granules of the adzuki beans exhibited characteristic pitting which was not observed in the starch granules of black beans or Mexican red beans (Figures 10 - 12). The starch granules of Mexican red beans showed slight swellings and deposits on the surface (Figure 12). Surface fissures were not observed on the starch granules of the any of the three types of beans examined in this study. These results were in contrast to the deep fissures on the surfaces of starch granules of adzuki beans (cv. Takara) reported by Tjahjadi and Breene (1984).

Conclusions

Adzuki bean microstructure, while generally similar to the common black beans and Mexican red beans, shows some interesting differences. The apparent differences in seed coat structure, cotyledon microstructure, and the hilum region may provide useful information for seed identification purposes. Microstructural differences could also be used to study relations between structural, textural and functional properties of the beans. Further research is in progress to characterize different cultivars of adzuki beans based on seed microstructural differences.

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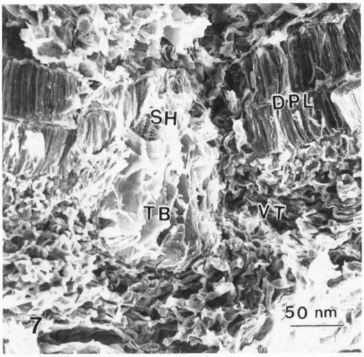
Figures 7-9. Cross-sections of the hilar and sub-hilar (SH) regions of adzuki bean (**Figure 7**), Mexican red bean (**Figure 8**), and black bean (**Figure 9**) showing, double palisade layer (DPL). tracheid bars (TB) and vascular tissue (VT).

Figure 10. Adzuki bean starch granules at higher magnification showing pitted surface. Starch granules (S), protein matrix (PM).

Figure 11. Black bean starch granules at higher magnification showing smooth surface. Starch granules (S), Protein matrix (PM).

Figure 12. Mexican red bean starch granules showing slight swellings on the surface. Starch granules (S), Protein matrix (PM).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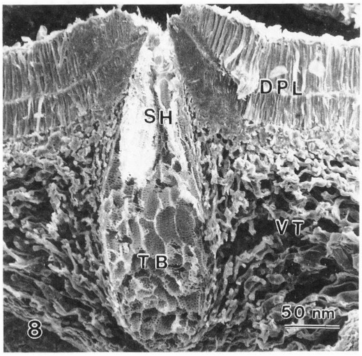
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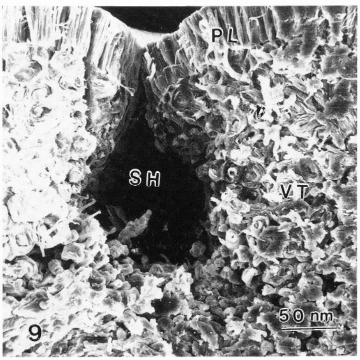
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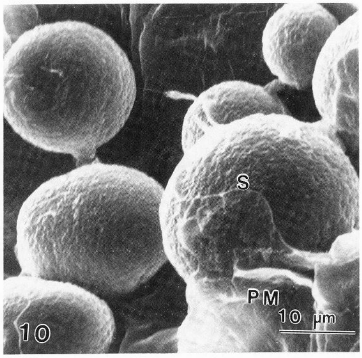
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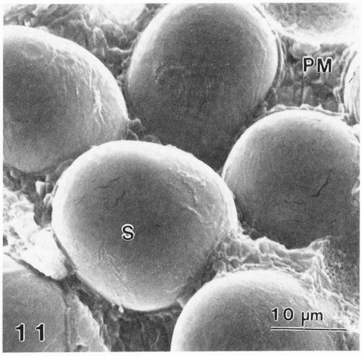
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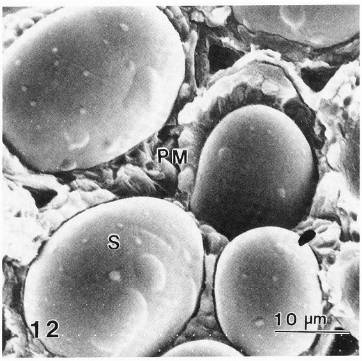
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