Food Structure

Volume 12 | Number 2 Article 3

1993

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FOOD STRUCTURE, Vol. 12 (1993), pp. 155-162 1046-705X/93$5.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

MICROSTRUCTURE OF BLACK, GREEN AND RED GRAM

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Abstract

The three most commonly consumed legumes (grams or pulses) in India, black gram (*Vigna mungo* (L.) Hepper), or urd, green gram (*Vigna radiata* (L.) Wilczek) or mung, and red gram (*Cajanus cajan* (L.) Millsp.) or tur, were examined by scanning electron microscopy. Seed coat and internal features were examined to differentiate these legumes from common beans (*Phaseolus vulgaris*), adzuki beans (*Vigna angularis*) and lentils (*Lens culinaris*). Cross-sections of the seed coats of black, green and red gram contained single layers of columnar palisade cells extending as double palisade layers at the hilum regions. The funiculi located above the hila contained storage cells; some cells were possibly collapsed during abscission from the seeds. The seed coat surface of red gram exhibited randomly distributed pits associated with surface deposits. Red gram exhibited prominent ridges above the hilum. The sub-epidermal region of red gram seed coat contained elongated pillar-shaped cells, generally called hourglass cells. Pillar cells were also present adjacent to the hilum region of both black gram and green gram seed coats, but were absent in the seed coat away from the hilum region. Starch granules embedded in a protein matrix were observed in the cross-section of cotyledons of each of the grams. Additional characterization of the microstructure of legumes will contribute to seed identification, and aid the understanding of changes that occur with development of the "hard-to-cook" phenomenon and during preparation, processing or cooking of legumes.

Key Words: Black gram (*Vigna mungo*), green gram (*Vigna radiata*), red gram (*Cajanus cajan*), legumes, scanning electron microscopy, seed microstructure, funiculus.

Initial paper received January 27, 1993
Manuscript received March 24, 1993
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Introduction

Seeds of food legumes play an important role in the diets of people around the world. India is a major developing country involved in producing and consuming legumes (Uebersax *et al.*, 1991). The protein content of the dry weight of legumes ranges from 20 to 40 percent (de Lumen, 1992). Food legumes are the main source (Toenniessen, 1985) and sometimes the only source of protein in the diets because animal foods are unavailable or expensive (Reddy *et al.*, 1982). The Hindu religion also prohibits the use of some animal foods in India. In addition to protein, legumes are good sources of carbohydrates, B complex vitamins and minerals (Hosfield, 1991). The amino acid profile of legumes complements cereal proteins (Nielson, 1991; Joseph and Swanson, 1992; Chinwe-Okeke and Obizoba, 1986). Legumes also supply a substantial amount of soluble and insoluble dietary fibers (Hughes, 1991; Schneeman, 1987), and are among desirable foods available for improving glucose tolerance (Wolever, 1990; Jenkins *et al.*, 1980, 1984 and 1986) and reducing insulin requirements for diabetics (Potter *et al.*, 1981; Tappy *et al.*, 1986). Consumption of food legumes can effectively reduce serum cholesterol concentrations (Anderson *et al.*, 1990; Shutler *et al.*, 1989; Soni *et al.*, 1979; 1982).

Black gram (*Vigna mungo* (L.) Hepper) or urd, green gram (*Vigna radiata* (L.) Wilczek) or mung, and red gram (*Cajanus cajan* (L.) Millsp.) or pigeon peas or tur, are the three most commonly consumed pulses or legumes in India. These three legume plants grow well as a main crop or an intercrop in tropical climatic conditions.

Indian legumes or pulses are smaller in size and the hulls are easily removed compared to other common dry beans. Consumption of cotyledons or dehulled legumes is a common practice in India. The Central Food Technological Research Institute at Mysore in India has developed an improved technology for dehulling on an industrial scale which reduces the time and losses required by a household process. Due to the convenient availability of dehulled pulses and milled flours, many dishes including savory and sweet desserts prepared from legumes are served at many festivals and holiday occasions in India. A typical Indian meal will contain at least two legume dishes in one form or another.

Table 1. Seed characteristics of black, green and red gram

Seed[a]	Scientific Name	Seed coat color	Cotyledon color	Moisture Content (%, dwb[b])
Black Gram	*Vigna mungo* L.Hepper	black	white	8.57 ± 0.07
Green Gram	*Vigna radiata* L. Wilczek	green	yellow	8.35 ± 0.06
Red Gram	*Cajanus cajan* L. Millsp.	mottled, grayish red	yellow	8.45 ± 0.02

[a]Seeds were obtained from Tamil Nadu Agricultural University, India. [b]dwb = dry weight basis.

Black gram and green gram seeds are morphologically similar. The black gram seed is oval in shape, about 3 mm in length with a black seed coat, white cotyledons and a concave hilum (Kay, 1979). Seeds of green gram may have a green, brown, grey or greenish-black seed coat and yellow cotyledons. The size of the green gram seed is 2.5 to 5 mm in length and 3 to 4 mm in width with flattened ends (Kay, 1979). Red gram is the oldest cultivated crop in India and ranks fifth in importance among the edible legumes. India produces ninety percent of the world's production of red gram (Salunkhe *et al.*, 1986). Seeds of red gram may have cream-colored, various shades of brown, red, pinkish- or purplish-black or mottled seed coats with yellow cotyledons. The red gram seed is round or oval in shape, 4 to 8 mm in diameter with a raised hilum (Salunkhe *et al.*, 1986; Kay, 1979).

Black, green and red gram are consumed as whole, dehulled cotyledons, or milled flour. The pulses are commonly cooked and eaten with rice or chapatties (unleavened Indian bread). Dehulled black gram (dhal) is mainly used in fermented foods like idli, dosa (Indian pancake) and dehydrated foods like pappad which are fried and consumed as snacks. At Indian festival dinners, pappad is an important item. The flour ground from black gram cotyledons may be an alternative to eggs in cake mixtures and other baked products because of acceptable foaming functionality (Kay, 1979).

Green gram seeds are used as a weaning food for feeding children, ill and elderly persons because of easy digestibility (Adsule *et al.*, 1986). Green gram seeds are more digestible than red or black gram seeds due to the small concentration of raffinose, stachyose and verbascose, the oligosaccharides associated with flatulence production and intestinal distress (Price *et al.*, 1988).

Black gram and green gram seeds require shorter soaking times than most other food legumes. Scanning electron microscopy (SEM) of legume seed coats will help identify the seed and the role of the seed coat in water entry (Hughes and Swanson, 1986). Seed coat structure can also be used to characterize and differentiate the sub-family Papilionoideae (Lersten and Gunn, 1982).

Microstructure information for Indian legumes is limited or not available in the literature. Therefore, this study was undertaken to examine the microstructure of black, green and red gram seeds using scanning electron microscopy to compare the microstructure with other legumes such as dry beans, consumed elsewhere in the world.

Materials and Methods

Moisture content of black, green and red gram seeds was determined by drying approximately 2 grams of milled seeds in triplicate in a forced-air oven at 70 ± 3°C for 48 hours (Table 1).

Whole seeds of black, green and red gram were fractured with a razor blade, mounted on aluminum stubs, and dried for approximately 24 hours in a desiccator. The desiccated fractured seeds were sputter-coated with 30 nm gold (Hummer-Technics sputter-coating unit), and examined in an Hitachi S570 scanning electron microscope operated at 20 kV.

Results and Discussion

Seed coat surface

Randomly distributed pits with surface deposits were observed on the seed coat of red gram (Fig. 1) as observed for soybeans (Wolf *et al.*, 1981). However, the seed coats of black gram and green gram did not exhibit pits. The absence of pits is also characteristic of seed coats of mature common beans (*Phaseolus vulgaris* L.) (Hughes and Swanson, 1985) and adzuki beans (*Vigna angularis* cv. Express) (Chilukuri and Swanson, 1991). According to Hughes and Swanson (1986), an uneven surface with distinct conical papillae was observed on the seed coat surface of lentils.

Seed coat cross-section

Cross-sections of black gram and green gram seed coats in the non-hilar regions were similar to the cross-section of the seed coat of adzuki beans (Chilukuri and Swanson, 1991), but distinctly different than the seed coat cross-section of black beans (*Phaseolus vulgaris* cv. Black Turtle Soup) (Hughes and Swanson, 1985). Seed coats of the black, green and red grams contained a single epidermal layer of elongated palisade cells. Palisade cell lengths for black, green and red grams were 33 to 42 μm, 47 to 49 μm and 78 to 86 μm, respectively. A sub-epidermal layer of pillar (hourglass) cells was not observed in black or green gram seed coats (Figs. 2 and 3) except adjacent to the hilum (Figs. 4 and 5). The seed coat of red gram (Fig. 1) contained an elongated layer of sub-epidermal pillar cells of 45 to 62 μm in length adjacent to the hilum. The pillar cells decreased in length in the seed coat away from the hilum (SEM data not shown). The parenchyma layer of randomly organized irregularly shaped cells was present beneath the epidermal and sub-epidermal layer of cells in the seed coats of black, green and red gram. Parenchyma thickness was 7 to 9 μm, 9 to 14 μm and 32 to 66 μm, respectively, for black, green and red grams. In black gram and green gram seeds, the palisade layer was approximately 4 to 5 times thicker than the parenchyma layer. Combined epidermal and sub-epidermal layers of red gram were greater than 2 times thicker than the parenchyma layer.

Hilar region

Cross-sections of the hilar region of black, green and red gram seeds are presented in Figs. 4, 5 and 6, respectively. In each of the grams, the single layer of epidermal palisade cells extends to the hilar region as a double palisade layer and creates a "linea lucida" or light line as observed for adzuki beans (Chilukuri and Swanson, 1991) although the "linea lucida" was previously believed to be an optical effect (Hughes and Swanson, 1986). However, in black, green and red grams, the "linea lucida" appears to be a structural feature. The shape of the hilum of black gram is concave (Fig. 4) and the shape of the hilum of green and red gram is flat (Figs. 5 and 6) as previously reported for black, green and red gram (Kay, 1979).

Contiguous tracheids composing the tracheid bar and forming a sub-hilar plug were observed in the sub-hilar region of black, green and red gram seeds, apparently to aid in the control of water entry into the seeds, as observed for adzuki beans (Chilukuri and Swanson, 1991). The black, green and red grams also contained vascular tissue in the sub-hilar region. The vascular tissue in the sub-hilar region of red gram was greater than two times thicker than the vascular tissues of black or green gram.

The hilar fissure of black and green gram was covered by a remnant of the funiculus that was not abscised from the seeds (Figs. 4 and 5). The funiculus of black and green gram is composed of storage cells, many collapsed during abscission (Figs. 7 and 8). Two prominent ridges above the hilum of red gram were observed (Fig. 6) as reported by Salunkhe *et al.* (1986) and Kay (1979). The two prominent ridges located above the hilum of red gram contain elongated cells (Figs. 6 and 9) and may also be a remnant of the funiculus.

Microstructure of Cotyledons

Observations on the microstructure of the cotyledons of legumes are available in the literature due to the interest in studying changes during maturation, storage, soaking and processing of legumes (Swanson *et al.*, 1985; Varriano-Marston and Jackson, 1981; Sefa-Dedeh and Stanley, 1979). Black, green and red gram cotyledon cells contained elliptical starch granules embedded in a protein matrix. The approximate sizes of the starch granules were 14 x 22 μm, 12 x 18 μm and 27 x 43 μm for black, green and red gram cotyledon cells, respectively (Figs. 10, 11 and 12). SEM examination of adzuki bean (Chilukuri and Swanson, 1991), African yam bean (Enwere *et al.*, 1991), and black bean (Sefa-Dedeh and Stanley, 1979; Hughes and Swanson, 1985) cotyledon cells illustrated spherical starch granules embedded in a protein matrix. The protein bodies were differentiated and more visible in the protein matrix of green gram than in black and red gram cotyledon cells.

Although the grams were not fixed with chemical fixatives, cotyledon cells of black gram were resistant to fracturing as observed in Fig. 10. Observation of fracture-resistant cells was limited to the exterior of the cytoplasm containing presumed pitfields where plasmodesmata pass through the cell wall (Hughes and Swanson, 1985). Cell walls and intercellular spaces (Figs. 10, 11 and 12) were also observed in the fractured cotyledon cells of black, green and red gram.

Conclusions

Indian legumes such as black, green and red gram seeds are microstructurally similar to legume seeds consumed in other parts of the world. The microstructure of red gram seeds exhibits distinct structural features such as pits with surface deposits on the seed coat, prominent ridges on either side of the hilum and pillar cells in the sub-epidermal layer of the seed coat, which are distinctly different from black and green gram seed microstructure. The microstructure of black and green gram seeds is similar to the microstructure of adzuki beans, although the starch granules of black and green gram seeds are elliptical in shape and smaller in size. The microstructure of legume seed coat surfaces, cross-sections of the seed coats, hila and cotyledons will be useful to study cultivars, storage conditions, maturity and relationships between structural and functional properties of legumes.

Acknowledgement

The authors acknowledge the use of the facilities and assistance of the technical staff of the Electron Microscopy Center, Washington State University, Pullman, WA 99164. The project was funded by Research Service Council, University of Nebraska at Kearney, Kearney, NE 68849.

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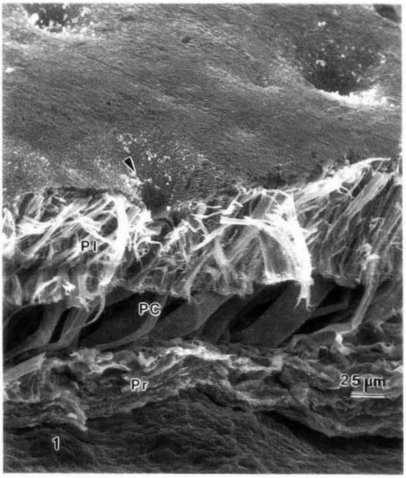
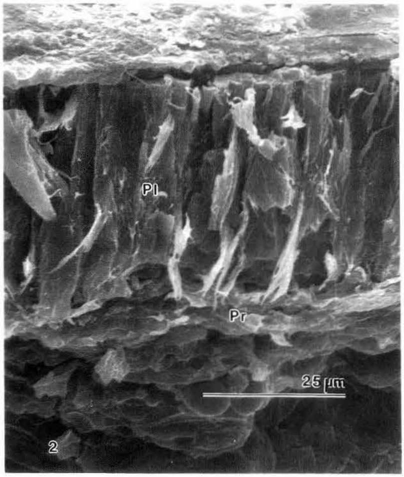
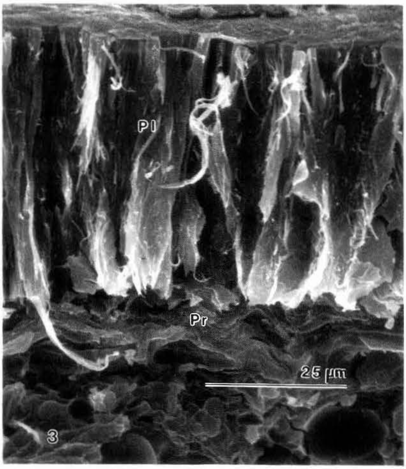






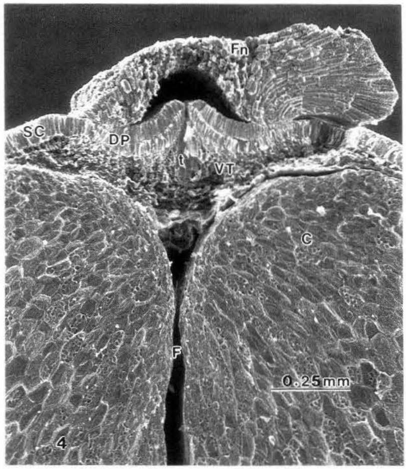
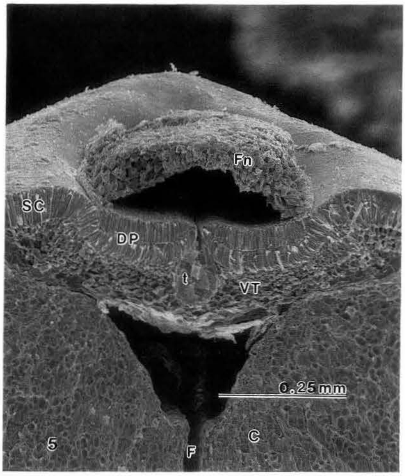
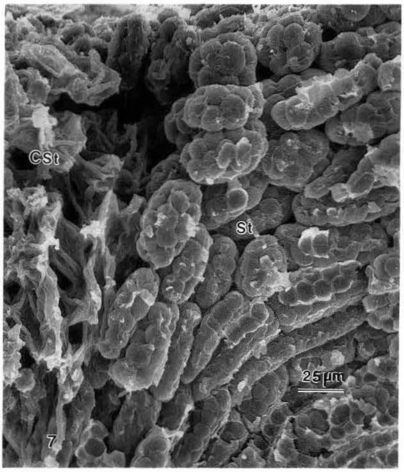


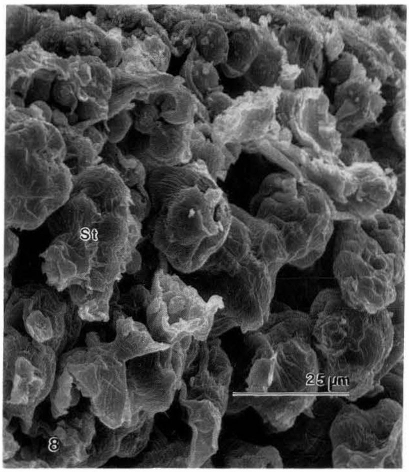
Figure 1. Cross-section of red gram seed coat. The palisade (Pl), sub-epidermal pillar cell (PC) and parenchyma (Pr) cell layers are present. The arrow (▸) points to a pit in the seed coat surface surrounded by scattered surface deposits. Note the random distribution of pits in the seed coat surface.

Figures. 2-3. Cross-section of black gram (**Figure 2**) and green gram (**Figure 3**) seed coats. The palisade (Pl) and parenchyma (Pr) cell layers are present. Sub-epidermal pillar cells are absent from the seed coats.

Figures 4-5. Cross-section of black gram (**Figure 4**) and green gram (**Figure 5**) seed coats, hila and cotyledons. The seed coats (SC) and double palisade (DP) layers of cells at the hila, the tracheid bars (t) and vascular tissue (VT) in the sub-hilar regions and







the cotyledons (C) and cotyledonary fissures (F) are visible. The structures covering the hila of black gram and green gram seeds may be remnants of the funiculi (Fn).

Figure 6. Cross-section of red gram hilum and cotyledons. The double palisade (DP) layer of cells at the hilum, the tracheid bar (t) and vascular tissue (VT) in the sub-hilar region and the cotyledon (C) and cotyledonary fissure (F) are visible. The prominent ridges (R) above the hilum may be remnants of the funiculus. The arrow (▸) points to cells of prominent ridge magnified in Fig. 9.

Figure 7-8. High magnification of funiculi covering the hila of black gram (**Figure 7**) and green gram (**Figure 8**) showing collapsed (CSt) and intact storage cells (St).

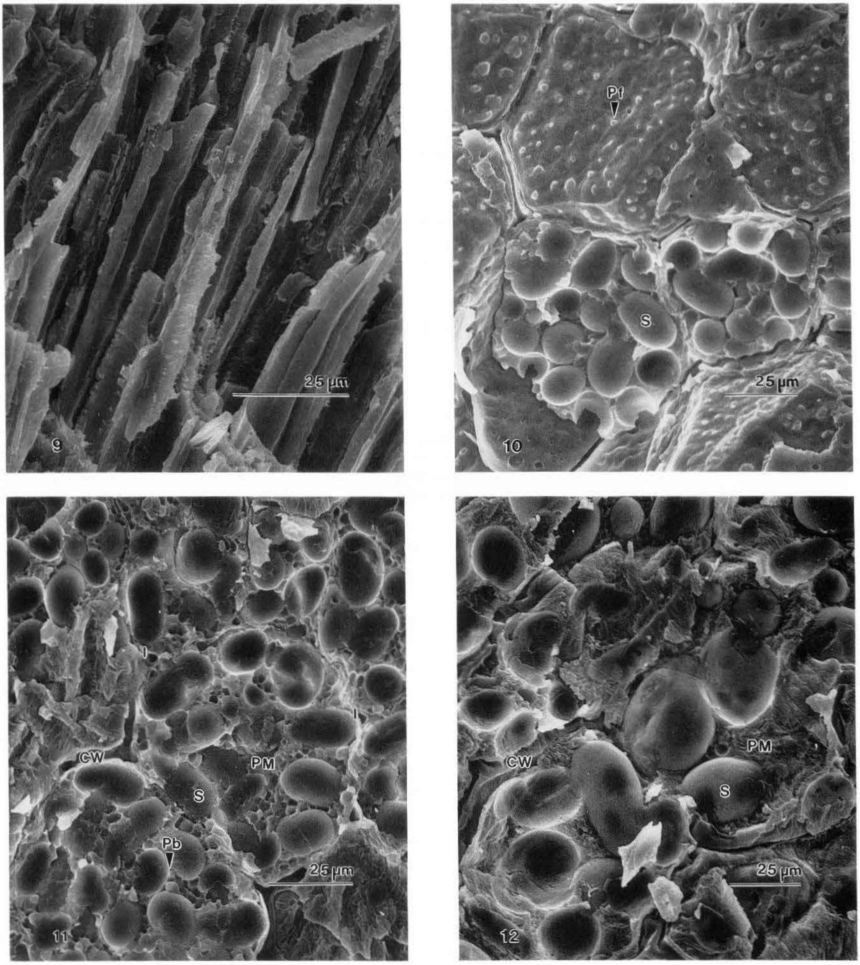


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Figure 9. High magnification of prominent ridge located to the right and above the hilum of red gram (see arrow in Fig. 6). The elongated cells may be storage or transport cells of the funiculus.

Figure 10. Cross-section of black gram cotyledon cells. Fracture-resistant cells exhibit the exterior of the cytoplasm containing presumed pitfields (Pf) where groups of plasmodesmata pass through the cell wall. The fractured cells contain elliptical starch granules (S) in a protein matrix.

Figure 11. Cross-section of green gram cotyledon cells. Elliptical starch granules (S), protein bodies (Pb), cell walls (CW) and intercellular spaces (I) are visible.

Figure 12. Cross-section of red gram cotyledon cells. Elliptical starch granules (S), cell walls (CW) and protein matrix (PM) are visible.

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Discussion with Reviewers

J.S. Hughes: Is there any information to indicate that "hardness" or the "hard-to-cook" phenomenon is a problem among the Indian legumes (grams) investigated in this research?
Authors: The "hard-to-cook" phenomenon is apparently not a serious problem among the Indian legumes. Because the legumes are consumed daily and used quickly, long term storage of legumes is not typical in India.

J.S. Hughes: I understand that in India legumes are commonly consumed without their seed coat. Is it possible that this practice developed in order to reduce the problems caused by "hardness" in Indian legumes?
Authors: In India, legumes are consumed with seed coat as well as without the seed coat. In the northern part of India, more legumes are consumed with seed coat; and in the southern part of India, legumes are consumed mostly without seed coats. The practice of using whole or dehulled legumes is based upon the type of dishes common to each region. The seed coats of Indian legumes are easily removed in the home.

J.S. Hughes: Are any of the legumes examined in this study consumed in significant quantities in other countries besides India?
Authors: Yes. These Indian legumes are consumed in significant quantities in Pakistan, Bangladesh, Thailand, Malaysia, Hong Kong, Japan, China, and Africa.

R.D. Phillips: India is a large country with a population approaching $1x10^9$ and many ethnic groups. Is the statement "A typical Indian meal will contain at least two legume dishes in one form or other" true for the entire nation or one or more sub-groups?
Authors: The statement is true for the entire nation. As animal foods are very expensive and not acceptable to many Indians based on religion, legumes play an important role in Indian meals. Dehulled legumes or legume flours are used for preparing savory items, as well as desserts.