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MICROSTRUCTURAL CHANGES IN MATURING SEEDS OF THE COMMON BEAN (Phaseolus vulgaris L.)

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Abstract

Seeds of Phaseolus vulgaris L. beans were collected at weekly intervals throughout maturation and examined by scanning electron microscopy (SEM). No major structural changes were observed on the surface of the seed coat during the seven week study period. A cross-sectional examination of the seed coat revealed a substantial increase in thickness of the parenchyma cell layer in young seeds followed by a dramatic decrease in thickness as the seed approached maturity. In the cotyledons, the diameter of the storage cells and starch granules increased over time, with distinct protein bodies becoming visible only in the later stages of maturity. An extensive vascular system responsible for rapid delivery of water and nutrients to the cotyledons was observed in both immature and mature beans.

Introduction

In recent years, both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have been used to study the microstructure of legume seeds. The susceptibility of legume seeds to hardening is one reason for research interest in legume microstructure. Two different types of hardness have been observed in legumes—hardseed or hardshell and hard-to-cook. Hardseed or hardshell legumes are seeds that do not imbibe water in a reasonable length of time (18-24 h). Hard-to-cook legumes, on the other hand, imbibe water but do not soften even after cooking. An increased understanding of seed microstructure will provide a better understanding of the causes of seed hardness. Most recent research on legume microstructure has focused on the cotyledons of mature seeds (McEwen et al., 1974; Saio, 1976; Sefa-Dedeh and Stanley, 1979a; Silva and Luh, 1978; Wolf and Baker, 1972). Microstructural changes occurring in seeds during storage (Jackson and Varriano-Marston, 1981; Sefa-Dedeh et al., 1979; Varriano-Marston and Jackson, 1981), water imbibition (Sefa-Dedeh and Stanley, 1979c) and cooking (Rockland and Jones, 1974; Sefa-Dedeh and Stanley, 1979b; Sefa-Dedeh et al., 1978) have also been observed and reported.

Little research on the microstructural changes occurring during development and maturation of bean seeds has been reported (Opik, 1968; Yeung, 1983). Most previous research on maturing bean seeds has used TEM to study intracellular metabolic changes occurring in the cotyledons. The objective of this research was to use SEM to examine the microstructural changes occurring in maturing common bean seeds (Phaseolus vulgaris L.). Attention was directed at changes occurring in both seed coats and cotyledons.

Materials and Methods

The beans studied (Phaseolus vulgaris L., cv. Black Turtle Soup) have black seed coats. The beans were grown at the Washington State University Irrigated Agriculture Research and Extension Center, Prosser, Washington, during the 1984 growing season. Plots were planted on June 6, 1984 and pods were collected on five separate dates in August and September. Seeds were removed from the pods immediately after harvesting and fixed in an aqueous solution of 4% formaldehyde and 1% glutaraldehyde in phosphate buffer (pH 7.0). Prior to viewing with SEM, seeds were postfixed in 4% osmium tetroxide for 18 h and
dehydrated in a graded ethanol series (30–100%). As a control for swelling and other possible effects of aqueous fixation, one group of mature seeds was not fixed. After harvest, seeds not fixed were stored over a desiccant and later dehydrated in 100% ethanol. All seeds were freeze-fractured in liquid nitrogen with a razor blade, critical point dried in carbon dioxide (Bomar SPC-1500), and sputter-coated with 300 A gold (Hummer-Techmics). Fractured seeds were observed and photographed with an ETEC U-1 scanning electron microscope (Hayward, CA) at 20 kV.

Results and Discussion

Seed coat surface
Seed coats serve as a protective barrier between the embryo (cotyledons) and the external environment. The seed coat protects the nutrient-rich cotyledons from microorganisms and other pests, as well as fungal contamination. Often the exterior surface of the seed coat contains a distinctive pattern or “fingerprint” (Wolf et al., 1981). The fingerprint is formed during seed development by pressure between the seed coat and the endocarp, the innermost layer of the pod wall. Seeds of many plant species have distinctive species-specific seed coat patterns. Seeds of various cultivars of the same species have few distinctive differences, but often can be identified by seed coat pattern. SEM has been used extensively to study seed coat pattern for purposes of seed identification. Using SEM, Banerjee and Chauhan (1981) were able to demonstrate how the pattern on the surface of the grain (caryopsis) of wheat (Triticum spelta) genetically evolved from its progenitors.

Observations of the seed coat surface of maturing Phaseolus vulgaris L. seeds revealed the characteristic pattern which developed as the seeds matured (Figs. 1A-E). The seed coat surface of the youngest seeds appears to be covered with small, pebble-like bumps (Fig. 1A), but does not exhibit the distinctive pattern that characterizes mature seeds. The absence of a distinctive seed coat pattern in young seeds probably results from the seeds being so small that no pressure had yet developed between the seed coat and the endocarp. However, as the seeds mature, a seed coat pattern increasing in distinctiveness and complexity becomes visible (Figs. 1B-E). Increased pressure between the seed coat and endocarp appears to be responsible for the characteristic fingerprint development on the seed coat surface as the seeds mature.

Wolf et al. (1981) studied the seed coat surface of thirty-three cultivars of soybeans (Glycine max), and noted a wide variety of characteristics including pits, cracks and surface deposits. No pits or cracks were observed in the seed coats of maturing Phaseolus vulgaris L. seeds, though some scattered surface deposits were noted (Fig. 1C). The composition of the surface deposits was not ascertained.

Seed coat cross-section
SEM has been used to evaluate cowpea (Sefa-Dedeh and Stanley, 1979a), soybean (Wolf and Baker, 1972), adzuki bean and common bean (Sefa-Dedeh and Stanley, 1979b; Swanson et al., 1985) seed coats in cross-section. Several cell layers have been observed in the different legumes and a different nomenclature is reported for each. Three distinctive cell layers are visible in the seed coat of the common bean—the palisade, subepidermal and parenchyma cell layers.

Palisade cells are long, columnar cells which make up the outermost cell layer of the seed coat. Subepidermal cells are somewhat shorter columnar cells that lie immediately beneath the palisade layer (Fig. 2A). Subepidermal cells are also commonly referred to as pillar or hourglass cells if they exhibit a characteristic pillar or hourglass shape (Esau, 1977). Approximately 10–15 layers of parenchyma cells make up the innermost portion of the seed coat that lies closest to the embryo (Fig. 2A). Both the palisade and subepidermal layers consist of a single layer of tightly packed cells. Both cell layers increase slightly in length as the seed matures (Table 1), but do not undergo any major structural changes (Figs. 1A and E).

The parenchyma layer, in contrast, consists of 10–15 layers of irregularly shaped, randomly organized cells with frequent intercellular spaces (Fig. 2A). During the early stages of seed development (13–19 d after flowering), the parenchyma layer is 2–5 times thicker than the palisade and subepidermal layers (Figs. 2A and B). As maturation progresses (28–35 d after flowering, maturity, days after flowering

<table>
<thead>
<tr>
<th>Maturity, days after flowering</th>
<th>Palisade (μm)</th>
<th>Parenchyma (μm)</th>
<th>Parenchyma (μm)</th>
<th>Parenchyma (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>14.3 ± 1.2</td>
<td>77.0 ± 3.5</td>
<td>95.7 ± 6.1</td>
<td>138.4</td>
</tr>
<tr>
<td>19</td>
<td>17.1 ± 1.5</td>
<td>77.0 ± 3.5</td>
<td>95.7 ± 6.1</td>
<td>138.4</td>
</tr>
<tr>
<td>28</td>
<td>18.9 ± 3.0</td>
<td>91.9 ± 5.4</td>
<td>191.9 ± 4.7</td>
<td>243.4</td>
</tr>
<tr>
<td>35</td>
<td>25.1 ± 1.0</td>
<td>29.8 ± 4.6</td>
<td>91.9 ± 5.4</td>
<td>145.4</td>
</tr>
<tr>
<td>49</td>
<td>24.6 ± 3.0</td>
<td>29.8 ± 4.6</td>
<td>91.9 ± 5.4</td>
<td>145.4</td>
</tr>
</tbody>
</table>

1Pl = Palisade, E = Parenchyma and SC = Seed Coat. Measurements were made using a Bioquant II image analysis system.
Maturing Common Bean Seeds

Fig. 1. (above & left) Exterior of seed coat of developing *Phaseolus vulgaris* L. bean seeds. Micrographs were taken of seeds harvested at: A = 13 d, B = 19 d, C = 28 d, D = 35 d, and E = 49 d after flowering. Bar = 10 μm.

Fig. 2. (above & right) Cross-sectional view of seed coat of developing *Phaseolus vulgaris* L. bean seeds. Micrographs were taken of seeds harvested: A = 13 d, B = 19 d, C = 28 d, D = 35 d and E = 49 d after flowering. The palisade (L) subepidermal (E), and parenchyma (R) layers are visible. Bar = 10 μm.
Table 2. Microstructural changes in the cotyledons of maturing bean seeds (Phaseolus vulgaris L.)

<table>
<thead>
<tr>
<th>Maturity, days after flowering</th>
<th>Mean cell diameter (^1) (μm)</th>
<th>Mean starch granule diameter (^1) (μm)</th>
<th>Figure number</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>51.7 ± 10.9</td>
<td>12.6 ± 6.1</td>
<td>3A</td>
</tr>
<tr>
<td>19</td>
<td>53.3 ± 19.7</td>
<td>13.3 ± 1.4</td>
<td>3B</td>
</tr>
<tr>
<td>28</td>
<td>54.7 ± 11.3</td>
<td>15.7 ± 2.9</td>
<td>3C</td>
</tr>
<tr>
<td>35</td>
<td>58.2 ± 9.3</td>
<td>20.7 ± 3.7</td>
<td>3D</td>
</tr>
<tr>
<td>49</td>
<td>62.1 ± 12.5</td>
<td>19.8 ± 4.9</td>
<td>3E, 3F</td>
</tr>
</tbody>
</table>

\(^1\)Measurements were made using a Bioquant II image analysis system.

Flowering), the size of the entire seed increases, and the thickness of the parenchyma layer decreases (Figs. 2C, D, and E; Table 1). The parenchyma layer is thickest (100–190 μm) during the period of greatest seed growth, but the cells disintegrate and the layer is compressed as the seed approaches full size and seed growth slows. It is generally believed that the seed coat functions solely as a protective covering for the embryo. However, in using TEM to study the branched parenchyma cells of maturing Phaseolus vulgaris L. seeds, Yeung (1983) observed a great deal of metabolic activity including dilation of endoplasmic reticulum cisternae and an increase in abundance of other cytoplasmic organelles. As a result, Yeung (1983) theorized that the seed coat may supply nutrients to the embryo. Increased thickness in the parenchyma layers of young seeds, as observed with SEM, appears to support the theory that in addition to serving as a protective barrier, the seed coat may play a nutritive role and may be important in controlling development of the embryo (Yeung, 1983).

In mature beans, the parenchyma layer has been compressed to approximately the same thickness (30 μm) as each of the two outermost layers (Fig. 2E; Table 1). Because of the compression of the parenchyma layer, the total thickness of seed coat decreases as the seed approaches maturity and seed growth slows (Table 1).

Microstructure of cotyledons

More research has been reported on the microstructure of legume cotyledons than on legume seed coats. Cotyledons have been of particular interest for researchers investigating the causes of “hardness” in legumes (Saio, 1976; Sefa-Dedeh et al., 1979; Varriano-Marston and Jackson, 1981).

Bean cotyledons contain large (10–50 μm) spherical starch granules and small (5–10 μm) round protein bodies embedded in a protein matrix. Starch granules are present throughout maturation, distinct protein bodies only became evident during the later stages (35–49 d after flowering; Figs. 3D and F). Studying maturing Phaseolus vulgaris L. seeds, Opik (1968) also observed that protein bodies become visible later than starch granules.

In young seeds (13 d), both starch granules and plastids are present. The starch granules resist fracturing and retain a characteristic spherical shape, while plastids are fractured (Fig. 3A). Starch granule synthesis is initiated in the plastids, with the starch granules expanding and rupturing the plastid membrane when the granules become larger than the plastid. Some of the thin fragments present on the surface of the starch granules may be remnants of ruptured plastid membranes (Fig. 3F, arrow). As the embryos mature, both the size and number of starch granules increase (Fig. 3; Table 2).

The overall size of bean cotyledon cells also increases as maturation progresses (Table 2). In developing seeds (13–19 d after flowering), several intercellular spaces (I) surrounded each cell (Figs. 3A and 3B), but the intercellular spaces disappear as the seed matures (28–35 d) due to pressure from expanding cells (Figs. 3C and D).

In seeds fixed in the aqueous glutaraldehyde-formaldehyde solution, cotyledon cells are increasingly resistant to freeze fracturing as the seeds mature. In young seeds (13–19 d; Figs. 3A and B), fracturing occurred through the cells, revealing internal starch granules, plastids and protein bodies. However, as the seeds matured, cotyledon cells were increasingly resistant to fracturing; some cells fractured while others remained intact leaving visible the exterior of the cytoplasm separated from the cell wall (Figs. 3C and D). Finally, in mature beans (49 d) fixed in aqueous solution, many cotyledon cells were resistant to fracturing, limiting observation to the exterior of the cytoplasm, cell walls and a few isolated starch granules (Fig. 3E). Though interesting artifacts may have been created, the resistance of mature cells to fracturing appears to be a result of aqueous fixation. In the control group of unfixed mature beans, fracturing occurred through the cells, and starch granules and protein bodies were readily visible (Fig. 3F).

Another interesting structural feature in aqueous-fixed cotyledons is the finger-like projections or pegs extending from the exterior of the cotyledon cells (Fig. 4A). The finger-like projections appear too large to be individual plasmodesmas, protoplasmic bridges that pass through the cell wall and connect contiguous cells. However, the projections may be pit fields where groups of plasmodesmata pass through the cell wall together.

The presumed pit fields were visible only in aqueous-fixed seeds in which cell walls were removed by freeze-fracturing, leaving the exterior of the cytoplasm visible. In some instances, the cell wall was only partially removed or separated from the
cytoplasm (Fig. 3C, arrows). In a cotyledon cell where the cell wall has been separated from the cytoplasm, small openings or holes can often be observed where pit fields are presumed to have passed through the cell wall (Fig. 4A, arrow). Examining the interior of a cotyledon cell after the cytoplasm has been removed also reveals small holes where pit fields from the extracted cytoplasm appear to have passed through the cell wall (Fig. 4B, arrow). Long strips of what appears to be middle lamella are also present (Fig. 4B). The middle lamella is a pectinaceous intercellular layer responsible for cementing contiguous cells together (Esau, 1977). Strips of presumed middle lamella appear to be responsible for the channels (Fig. 4A, asterisk) in the exterior of the cytoplasm where pit fields are not present.

**Vascular system**

The vascular system, which provides rapid transport of nutrients and water from the plant to cotyledon storage cells, is also visible in the cotyledon (Fig. 5). The vascular system is made up of xylem, which transports water and ions, and phloem, which transports organic materials including nutrients. Although certain aspects of the role of the vascular system in legume seeds have been studied, a comprehensive investigation is lacking. Corner (1951) reported great variation in the vascular supply systems of different legume seeds, and diagrammed a few of the more common systems. Thorne (1981) used TEM and SEM to study the vascular system in soybeans in an attempt to determine the probable pathways of photosynthetic transport.

Portions of the vascular system in the embryo of *Phaseolus vulgaris* L. seeds are readily visible 28 d after flowering. The vascular system appears to develop and become more elaborate as the seed matures, with a complex, highly developed vascular system being evident in fully mature seeds 49 d after flowering (Figs. 5A and B).

**Conclusion**

Significant structural changes occur in the seed coat and cotyledons of developing common bean seeds. In the seed coat, increased thickness of the parenchyma layer during the period of greatest seed growth is of particular interest, indicating that parenchyma cells may be involved in nutrient storage and/or supplying nutrients to growing cotyledons. In cotyledons, the overall size of storage cells and starch granules increased as development progressed, with protein bodies becoming evident later than starch granules. Relatively little is known about the microstructure of the vascular system that supplies water and nutrients to bean cotyledons. However, the vascular system does appear to become more elaborate as the seed matures.

Fig. 5. Vascular system of the cotyledon of *Phaseolus vulgaris* L. bean seeds. A, B and C are of fully mature seeds (49 d after flowering). Both phloem (H) and xylem (X) are visible. Figs. A and C, bar = 10 μm; Fig. B, bar = 20 μm.
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Acknowledgements

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References


Discussion with Reviewers

E. Varriano-Marston: What was the moisture content of the seeds after removal from the pod? If they were 14% or less, then one need not dehydrate with ETOH. Just fracture and observe with SEM.

Authors: The moisture content of the seeds was about 5%, yet dehydration and preparation without dehydration, either or both are appropriate.

E. Varriano-Marston: In regard to the finger-like projections extending from the exterior of cotyledons fixed in aqueous solution (Fig. 4A), we have also observed these projections in aged beans that were not subjected to any aqueous treatment.

Authors: Thank you.

K. Saiio: There are area-to-area differences on the surface of the seed coat even in one seed. What area of the seed coat was observed in this experiment? Are differences between Figures IC-E due to maturation or to area examined?

Authors: An attempt was made to orient fractured bean seeds so that seed coat adjacent to the hilum was observed. We believe the observed differences are a result of maturity differences and not a result of observing different areas of the seed coat.