1986

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MICROSTRUCTURE OF LENTIL SEEDS (Lens Culinaris)

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Abstract

Scanning electron microscopy (SEM) was used to investigate the microstructure of five cultivars of lentil seeds (Lens culinaris). Lentil cotyledons contain spherical starch granules surrounded by protein bodies similar to starch granules and protein bodies observed in cotyledons of other food legumes. Examination of the lentil seed coat in cross-section revealed outer palisade and inner parenchyma layers characteristic of legumes. The subepidermal layer, however, is comprised of hourglass cells and is found primarily in the area surrounding the hilum and the entire lentil seed coat is thinner than the seed coats of most other food legumes. The surface of the lentil seed coat is uneven and covered with distinctive conical papillae. The unique structural characteristics of the lentil seed coat may be partially responsible for the decreased incidence of hardness characteristic of lentils.

Introduction

Lentils are among the oldest cultivated grain legumes and are produced throughout the world. Though lentil production is only of minor importance in global terms, lentils are a very important food crop in certain areas of Asia. Lentils can be divided into two subspecies: macrosperma and microsperma. The macrosperma, found mainly in the Mediterranean region and the New World, are characterized by flat, lens-shaped seeds with yellow cotyledons and pale seed coats which often contain dark brown or black spots, or mottling (Hawtin et al., 1980). Like other legumes, lentils are comparatively high in crude protein (22–36%), supplying approximately twice the protein of cereals, and providing a good complementary lysine-rich protein when consumed with cereals. Lentils are a desirable protein source because they contain few anti-nutritional factors commonly associated with legumes. Low trypsin inhibitor activity (Al-Bakir et al., 1982) and a very low percentage of hard seeds have been observed with lentils, though some flatulence and lectin (hemagglutinin) activity have been reported (Nygaard and Hawtin, 1981). Lentils have the added advantages of rapid hydration, short cooking time and are one of the most easily digested legumes (Nygaard and Hawtin, 1981).

Scanning electron microscopy (SEM) has been used to study legume seed coat surfaces for purposes of seed identification as well as determining the role of the seed coat in water entry. Differences in seed coat pattern have been used to distinguish between members of the sub-family Papilionoideae (Lersten and Gunn, 1982), various lupinus species (Bragg, 1983) and twenty species of the Mimosoideae genera (Baker et al., 1985). In studying selected Papilionoideae, Bridges and Bragg (1983) reported observing that surface patterns varied at different locations on the same seed. Hughes and Swanson (1985) reported that the seed coat surface of common beans evolved and became more complex as the seeds matured.

Wolf and Baker (1972) examined the soybean (Glycine max) seed coat surface and observed numerous pits and pore-like indentations. Wolf et al. (1981) were able to characterize 33 cultivars of soybeans on the basis of seed coat pits and surface deposits. Yaklich et al. (1984) studied permeable and impermeable soybeans, and concluded that the wax/cutin deposit on the seed coat was responsible for impermeability. Sefa-Dedeh and Stanley (1979b)
examined seed coat surfaces of cowpeas and reported observing similar patterns on both the inner and outer surfaces of seed coats.

SEM has also been used to study the hilum, micropyle, and raphe, structures of legume seeds believed to be involved in water entry. Hyde (1954) proposed that the hilum may open and close to regulate internal seed moisture. Kyle and Randall (1963) studied water entry at the hilum, micropyle, and raphe in two cultivars (Great Northern and Red Mexican) of common beans (Phaseolus vulgaris). For Great Northern beans the micropyle was the site of greatest water entry, while in Red Mexican beans the raphe was the most important site. With soybeans, Salo (1976) theorized that a plugged micropyle may be responsible for impermeable soybean seeds, but Yaklich et al. (1984) observed open and closed micropyls in both permeable and impermeable soybean seeds. Kyle and Randall (1976) studied eight cultivars of cowpeas and reported that six had closed and two had open micropyls.

Cross-sectional examinations of legume seed coats have revealed characteristic palisade, subepidermal and parenchyma layers. In soybeans, the subepidermal layer consists of loosely packed bourgeois cells (Wolf and Baker, 1972), while cowpea beans typically have tightly packed pillar cells (Hughes and Swanson, 1985). Salo (1976) observed that impermeable seed coats in soybeans tend to be more dense and thicker than seed coats of permeable soybeans. Youssef and Bushuk (1984) observed that hard-to-cook faba beans (Vicia faba) had thicker and longer palisade cells than "softer" beans.

A linea lucida or light line has been observed in the palisade layer of some legumes, but not in others (Swanson et al., 1985). The linea lucida is generally observed near the middle of the palisade layer and gives the impression that the palisade layer consists of two distinct layers of cells. Disagreement exists over whether the linea lucida is an actual structural feature of the palisade layer present in some legumes but not others, or merely an optical effect.

The tracheid bar, a strip of loosely packed, vertically oriented cells containing bordered pits, has only been observed in the hilum of Papilionoid legumes (Lers ten, 1982). The tracheid bar runs underneath the hilum fissure and extends from the micropyle across to the far edge of hilum. Lers ten (1982) used SEM to study the tracheid bar in 232 species of Papilionoid legumes and reported great uniformity in tracheid bar structure.

SEM examination of the interior of legume seeds reveals tightly packed storage cells in the cotyledons. The storage cells of the common bean (Hughes and Swanson, 1985), faba bean (McEwen et al., 1974), and cowpea (Sefa-Dedeh and Stanley, 1979a) all contain large (10–50 µm) spherical starch granules and small (5–10 µm) protein bodies embedded in a protein matrix. Soybeans, being oil seeds, possess a somewhat different cotyledon structure. The cotyledon cells of soybeans are filled with lipid bodies (or spherules) and protein bodies embedded in a protein matrix. Cotyledon cells of the common bean are held together by the middle lamella, a pectinaceous layer that acts as an intercellular cement. Failure of the middle lamella to solubilize and allow cell expansion is believed responsible for causing hard-to-cook beans (Jones and Boulter, 1983).

SEM has been used to study the microstructure of other food legume seeds including the common bean (Hughes and Swanson, 1985), soybean (Salo, 1976; Wolf and Baker, 1972), faba bean (McEwen et al., 1974), and cowpea (Sefa-Dedeh and Stanley, 1979a). The susceptibility of legume seeds to hardening is a primary reason for our interest in legume microstructure (Swanson et al., 1985). The objective of this research was to use SEM to examine the microstructure of lentil seeds to determine if significant microstructural differences exist between lentils and other legume seeds.

Materials and Methods

Lentil seeds (Lens culinaris) examined were provided by the USDA Plant Germplasm Introduction and Testing Laboratory, Pullman, Washington, from seeds grown during the 1983 growing season. All five cultivars studied had pale yellow-green seed coats with two cultivars (Chilean, Brewer) having varying amounts of black spotting or mottling while three cultivars (Laird, Tekoa, Red Chief) had clear seed coats. In order to examine the cotyledon and seed coat in cross-section, the lentils were freeze-fractured. Fractures were initially fixed for 24h in an aqueous solution of 4% formaldehyde and 1% glutaraldehyde in phosphate buffer (pH 7.0), and dehydrated in a graded ethanol series (30–100%).

The lentils were placed in an ethanol-containing pouch in liquid nitrogen and fractured with a razor blade. Fractured seeds were critical point dried in carbon dioxide (Bomar SPC-1500), and glued to aluminum stubs. For viewing the exterior of the seed coat, whole lentil seeds were glued to aluminum stubs. All samples were sputter-coated with 300 A gold (Hummer-Technics), viewed and photographed with an ETEC U-1 scanning electron microscope (Hayward, CA) at 20 kV.

Results and Discussion

The most obvious microstructural difference between lentils and other food legumes was observed in examining the seed coat surface. The seed coats of other legumes appear relatively smooth, though generally possessing a characteristic pattern and often being covered with pits and pores or varying amounts of surface deposits. Lentils, in contrast, possess an uneven seed coat surface covered with distinctive conical papillae (Fig. 1). Lers ten and Gunn (1982) observed low, dome-like papillae in Lens culinaris Medikus, quite different from the projecting, conical papillae observed in the five cultivars of lentils investigated here. All five lentil cultivars examined contained papillae structures. In three cultivars (Chilean, Tekoa and Brewer), the papillae were covered with extensive surface deposits (Fig. 2), while two other cultivars (Laird and Red Chief) had relatively few surface deposits (Fig. 1). Though generally scattered, the surface deposits often appeared in sheets which covered all but the top of the papillae (Fig. 3). Surface deposits were present on all seed coats but appeared to be more common on spotted or mottled seeds. The Tekoa cultivar, for example, has a clear seed coat along with extensive surface deposits; in portions of the lentil seed coat the papillae appear mushroom-shaped (Fig. 4). However, careful examination reveals that
Figs. 1-4. Lentil seed coat surfaces. Fig. 1 shows the papillae covered seed coat free of debris, Fig. 2 shows scattered debris (→), Fig. 3 shows sheet-like debris (→) and Fig. 4 shows debris attached to the tips of the papillae (→). Bar = 5 μm.

Fig. 5. Lentil hilum (H), micropyle (M) and hilar fissure (F). Bar = 200 μm.

the mushroom-shaped papillae are merely conical papillae with disc-shaped debris attached to their tips.

Lentils possess a long, narrow hilum with a micropyle at one end (Fig. 5). The hilar fissure was open on most lentils studied, but in one case was covered with what appeared to be a remnant of the funiculus that had failed to separate. The micropyle of lentils was generally closed or only slightly open (Fig. 6). Examination of the hilum in cross-section revealed characteristic two layers of palisade cells and an unusually thick layer of parenchyma cells causing the hilum to be elevated (Fig. 7). A narrow, elliptical layer of cells known as the tracheid bar runs the length of the hilum under the hilar fissure (Fig. 7). Close examination of the tracheid bar revealed bordered pits similar to the pits observed by Lersten (1982) without any warts or vestures (Fig. 8).

Cross-sectional examination of the seed coat away from the hilum revealed a discrete outer
pallisade layer consisting of a single layer of long (25-30 µm), tightly packed, vertical cells (Fig. 9). A distinctive subepidermal layer of hourglass cells was observed adjacent to but not immediately underneath the hilum (Fig. 10). The hourglass cells are relatively long (30-40 µm) near the hilum, but become progressively shorter away from the hilum and eventually change structural appearance. In portions of the lentil seed coat away from the hilum, gaps or openings were observed immediately beneath the palisade layer (Fig. 9). The seed coat gaps or openings are often difficult to distinguish from the parenchyma layer, but appear to be subepidermal hourglass cells which are shorter, wider and much less distinctive than those observed near the hilum. Though quite thick near the hilum, the lentil seed coat appears not to be structural in nature.

Like other non-oil seed food legumes, lentil cotyledons contain numerous tightly packed storage cells containing large (20-40 µm), spherical starch granules embedded in a protein matrix (Fig. 11). Numerous intercellular spaces surround each of the cotyledon cells. Cell walls can be easily identified, but the middle lamella is not readily distinguishable (Fig. 11).

Conclusions

Lentil seeds are microstructurally similar to the seeds of other food legumes in many ways; however, structural differences are apparent in the seed coat with lentils possessing a papillae-covered seed coat surface, a subepidermal layer that is only clearly visible near the hilum, and a relatively thin seed coat. Additional research is needed to determine if the unique seed coat characteristics of lentils are responsible for lentils reduced susceptibility to hardening.

Acknowledgements

The authors acknowledge the use of the facilities of the Electron Microscopy Center, Washington State University. Partial financial support for this research provided by USAID Title XII Dry Bean/Cowpea CRSP. Scientific Paper No. 7373. Agricultural Research Center, College of Agriculture and Home Economics, Washington State University, Pullman, WA 99164-6340.

References


Discussion with Reviewers

R. W. Yaklich: Are the surface deposits derived from the pod endocarp?
K. Saio: Were the structures of the mushroom-shaped papillae caused by contamination of disc-shaped debris? Where does such structural debris come from?
W. J. Wolf: The disc-shaped material on the surface of Tekoa cultivar seed coat is very unusual. Have you examined the interior surface of seed pods of this cultivar? Perhaps more of this material can be found there. Can you rule out microorganisms or fungicide coating given to the seeds by the USDA Plant Germplasm Introduction Testing group?
Authors: The USDA Plant Germplasm Introduction and Testing Laboratory reports that the lentil seeds provided to us were untreated. We were unable to examine pod endocarp because the lentil seeds were supplied without pods. Although microorganisms are a definite possibility, we believe that debris from the endocarp is the most likely source of the surface deposits.

R. W. Yaklich: How does a photograph of an open micropyle differ from a closed micropyle?
Authors: Open and closed micropyles, are not always easily distinguished. For the purposes of this investigation we considered micropyles to be open if there was any visible sign of an opening for water to enter. Closed micropyles, in contrast, were totally closed or fused shut so that no opening was visible.

K. Saio: In Figs. 2 and 3 pit-like structures are observed on the feet of most papillae. Are these artifacts, such as cracking, during specimen preparation or are they natural?
Authors: We believe the pits you are referring to are natural features of the lentil seed coat surface.