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Abstract

Prosopis, Olneya, and Amaranthus have potential economic value as food or feed crops for arid/semi-arid regions. Mature seeds of these genera were examined using scanning electron and fluorescence microscopy to define anatomy and localize storage structures in cells. Protein bodies, some containing phytin inclusions, were localized in the embryos of five Prosopis species. Prosopis chilensis was the only one of the five Prosopis examined which contained starch. In all Prosopis, endosperm was attached to the inner layer of the seed coat, was thickest in the center of the seed, and gradually decreased in thickness toward the periphery. Olneya tesota contained numerous protein and lipid storage bodies throughout the cotyledons. The cotyledons also contained small starch granules. The thin tegminous seed coat had a small quantity of endosperm attached to the inner surface. Amaranthus cruentus and A. edulis contained abundant starch located in the perisperm, a storage structure encircled by the embryo. Protein bodies, some with phytin inclusions, were located in the A. cruentus embryo. These results provide information for development of processing methods of these seeds for use in foods.

Introduction

Several arid or semiarid plants are candidates for development as resources for food or feed particularly for cultivation and utilization in Third World countries. Agricultural practices in these countries are often primitive in terms of irrigation and farming equipment, therefore, plants requiring minimum tillage and irrigation are of primary importance.

Prosopis species are tree legumes native to the Southwestern deserts of the United States, Mexico, and South America. Prosopis has been utilized in the past by Native North Americans as a source of food, fuel, shelter, weapons, and tools (Felger 1977). The seed is high in protein and contains a galactomannan gum and may, therefore, be of economic value as a food or feed (Felker 1979; Becker and Grosjean 1980; Becker 1982).

Olneya tesota, also a leguminous tree native to the Southwestern deserts of the United States, has been used as a food (Becker 1983). Abundant oil and high protein content in the seed and the ability of the tree to grow under extreme high temperatures make O. tesota a particularly valuable crop for desert regions.

Amaranthus, as an alternate food crop, has been of renewed interest for several years. Studies have included feeding and incorporation into foods (Betschart et al 1981), composition (Becker et al 1981), breeding (Hauptli and Jain 1977), and seed morphology (Irving et al 1981). A comprehensive review (Saunders and Becker 1984) and history (Sauer 1950) have also been reported.

This study deals with anatomy, histochemistry, scanning electron microscopy, and composition of Prosopis alba, P. tamarugo, P. chilensis, P. pubescens, P. velutina, Olneya tesota, Amaranthus cruentus and A. edulis seeds.

Materials and Methods

Prosopis alba Grisebach, P. pubescens Bentham, and Olneya tesota Gray (Munz and Keck 1975) pods and seeds were collected in Southern California in July, 1983 and 1984. P. velutina Wooton pods were collected in Tucson, Arizona in 1977. Prosopis tamarugo Philippi and P. chilensis emend. Burkart were supplied by Mr.
Amaranthus cruentus seed was harvested in Mexico in 1976 by Rodale Press, Inc., Emmaus, PA. All seeds and pods were stored below 4°C.

Light Microscopy

Protoplasms and Olneya tesota seeds were dissected and cut into approximately 1 mm³ in fixative. Tissues were fixed in a mixture of 3% glutaraldehyde, 1.5% acrolein, and 1.5% formaldehyde (Mollenhauer and Totten 1971) in 0.05 M NaH₂PO₄/Na₂HPO₄ pH 6.8-7.2 for 3-4 hr at 21°C. Tissues were rinsed in buffer and dehydrated in an ethanol-butanol graded series (Jensen 1962; Irving 1984) and infiltrated and embedded in glycol methacrylate (GMA) (JB-4 medium, Polysciences, Warrington, PA) (Feder and O'Brien 1968).

Amaranthus cruentus seeds were fixed in 6% glutaraldehyde in 0.05 M NaH₂PO₄/Na₂HPO₄ buffer pH 6.8 for 3-4 d at 4°C. Tissues were rinsed briefly in phosphate buffer and dehydrated in methoxyethanol, ethanol, propanol and n-butanol. Samples were then infiltrated and embedded in GMA (Feder and O'Brien 1968; Irving 1983).

Embedded samples were sectioned 2-4 μm thick on a Sorvall MT-2 ultramicrotome with glass knives. Unstained sections were observed using polarizing, Hoffman (differential interference contrast), or fluorescence optics. Fluorescence or brightfield conditions were used to observe stained sections.

Carbohydrates with vicinal hydroxyl groups were visualized by treating GMA sections with periodate-Schiff's (PAS). Sections were treated 15 min in a saturated solution of dinitrophenylhydrazine (Feder and O'Brien 1968) in 15% acetic acid to block fixative-induced aldehydes. Tissue sections were then treated with periodic acid for 15 min and stained in Schiff's reagent for either 2 min (Fulcher and Wong 1980) for fluorescence or 15 min for brightfield work. Slides were washed in running tap water for 20 to 30 min following each step. Sections were observed using fluorescence microscopy at 500-550 nm excitation wavelength or using standard brightfield conditions.

Sections were treated with a few drops of iodine potassium iodide (IKI) (Jensen 1962), covered with a cover glass and observed using standard brightfield or Hoffman optics to determine presence of starch.

Cell walls were detected by treating sections 1-2 min in aqueous 0.01% calcofluor or congo red (Yiu et al 1982) and washing briefly under running water. Sections were air-dried and mounted in immersion oil. Calcofluor-stained sections were viewed using fluorescence optics with an excitation wavelength of 330-380 nm. Congo red-stained sections were observed using 500-550 nm excitation wavelength.

Protein was localized by staining sections 1-2 min in 0.01% acid fuchsin in 1% acetic acid (Fulcher and Wong 1980), washing briefly in water and observing at 500-550 nm excitation wavelength in the fluorescence microscope. Other sections were stained for protein with mercuric bromophenol blue (Masia et al 1953) for 2 hr, washed briefly in water, dried, and mounted in Aquamount (Lerner Laboratories, New Haven, CT). Mercuric bromophenol blue-stained sections were viewed using standard brightfield or Hoffman optics.

Globoid inclusions, which contain phytate and minerals (Ca, P, Mg), were identified by staining sections in aqueous 0.01% acriflavine HCl (Yiu et al 1982; Fulcher and Wong 1982) adjusted to pH 5.0-5.5 with 0.1 M Na₂HPO₄. Sections were viewed and photographed using fluorescence microscopy at 500-550 nm excitation wavelength. Acriflavine HCl was also used to differentiate A. cruentus seed coat layers. Sections were examined at 385-425 nm excitation wavelength.

Lipids were localized in O. tesota fresh hand-sections and GMA sections by staining in 0.01% aqueous nile blue A (Fulcher and Wong 1980). Sections were rinsed, air-dried, and mounted in Aquamount or water. Sections were examined using fluorescence microscopy at 450-490 nm excitation wavelength.

Fluorescence work was recorded on 35 mm Kodak Plus-X pan film using a Nikon Fluophot microscope with a 200W mercury bulb and filter combinations as described above. Nile blue A work was recorded on panatomic-X film using a Leitz Orthoplan microscope with a 100W mercury bulb and 450-490 nm excitation wavelength. Brightfield work was recorded on either 4X5 Tri-X or 35mm Kodak panatomic-X film using a Nikon Fluophot microscope and standard brightfield or Hoffman optics.

Amaranthus edulis seeds were fixed in 10% neutral buffered formalin and embedded in paraffin (Jensen 1962). Paraffin blocks were sectioned on an American Optical Model 820 rotary microtome. Paraffin sections were stained in Mayer's hematoxylin in eosin 2 min (Berlyn and Miksche 1976). Paraffin sections were used to obtain a flat cross section of a large area; glycol methacrylate sections from whole, embedded samples tend to wrinkle.

Scanning Electron Microscopy—SEM

Protoplasms cotyledons were sectioned into approximately 1 mm³ and fixed in 2% glutaraldehyde in 0.05 M NaH₂PO₄/Na₂HPO₄ pH 7.2 at 0-4°C for 18 hr. Samples were rinsed in buffer and post-fixed in 1% OsO₄ in water at 25°C for 2 hr. Amaranthus cruentus seeds were fixed using the above procedure except they were not cut up prior to fixation.

Protoplasms cotyledon/endosperm samples were cut into 1 mm³ pieces and fixed in 2.5% glutaraldehyde in 0.1 M NaH₂PO₄/Na₂HPO₄ and 0.2 M NaCl pH 6.9 at 0-4°C for 18 hr. Sodium chloride was added to prevent excessive swelling of the hygroscopic endosperm tissue.

Olneya tesota cotyledons were cut up in fixative and fixed in 3% glutaraldehyde in 0.05 M NaH₂PO₄/Na₂HPO₄ pH 6.8 at 0-4°C for 18 hr. Cotyledon pieces were rinsed twice and post-fixed in buffered 1% OsO₄ at 25°C for 3 hr.

All SEM samples were rinsed in phosphate buffer and dehydrated in an ethanol-graded series.

Luis Zelada Gonzales of CORFO, Chile. The pods were harvested in 1982 from trees growing on a plantation in Northern Chile. Prosopis taxa are described in Leakey and Last (1980) and in Simpson (1977).
Specimens were ethanol-cryofractured (Humphreys et al 1974) and critical point dried in a Polaron E3000 Critical Point Dryer. Tissues were mounted on aluminum specimen stubs with carbon graphite and sputter-coated with gold in an ISI PS-2 Coating Unit. Prosopis and A. cruentaus samples were photographed in an ISI Super I scanning electron microscope with a universal stage. Viewing conditions were approximately 20KV and 30° tilt. Olneya tesota samples were photographed in a Philips 505 SEM (15KV, 30° tilt) or an ISI SS40 SEM (10KV, 30° tilt). All images were recorded on Kodak Tri-X pan 4x5 film.

Data in Tables 1 and 2 were obtained by AOAC methods (1975).

**Results**

Prosopis (Leguminosae-Mimosoideae)

Seed size and shape of the five Prosopis species studied differed, however cotyledon anatomy was similar. Cotyledons of all species had 2-3 rows of palisade parenchyma on the adaxial side beneath the epidermis as previously found in P. velutina (Irving 1984). Prosopis chilensis was the only Prosopis member which contained starch (Fig. 1a), albeit few, very small granules. Presence of starch was determined by IKI, PAS (Fig. 1a), and with polarizing optics. Cotyledon parenchyma cells were filled with protein bodies which were identified histochemically by staining with acid fuchsin or mercuric bromophenol blue (not shown).

Two types of protein bodies were evident; those without inclusions (Fig. 1b) and those with globoid inclusions (Fig. 1b-e) which were detectable in sections stained with acriflavine HCl. Protein bodies containing globoids were localized in the pro-mesophyll cells (Fig. 1d) of cotyledons of all Prosopis studied. A gradation of protein body types was evident within a single cotyledon in most Prosopis taxa. Protein bodies with the most abundant and largest globoids were centrally located in the pro-mesophyll (Fig. 1d); decreasing in size and number in the outer regions. Prosopis pubescens contained globoid inclusions located more extensively throughout all cells in the cotyledons (Fig. 1c). Prosopis chilensis contained few globoids in the subepidermal layers (Fig. 1b). *Prosopis velutina*, *P. tamarugo*, and *P. alba* contained globoids only in the center of the cotyledons.

Abaxial epidermal cells contained non-proteinaceous bodies in all Prosopis studied. Abaxial epidermal cells in *P. chilensis* and *P. pubescens* contained deposits considered to be globoids since they stained with acriflavine HCl (Fig. 1b,c); globoids were not found in epidermal cells of the other three *Prosopis* taxa.

In scanning electron micrographs, globoids appeared as solid structures with smooth surfaces (Fig. 1e) embedded in protein bodies. Protein bodies in cross section were smooth with no apparent structural features. The matrix between protein bodies was mesh-like and irregular with small indentations and large holes.

The seed coat, which provided rigid support and protection for the intact embryo, was similar in all *Prosopis* studied. A pleurogram, an oval line on the seed coat surface open at the hilar (root) end (Corner 1951; Bragg 1982; Irving 1984), was evident on *Prosopis*. The internal seed coat structure exhibited a cuticle, palisade sclereids, and osteosclereids on either side of sclerified parenchyma (Fig. 1f). Variation in seed coat structure was found in the thickness of the separate layers particularly the sclerified parenchyma layer. Endosperm was firmly attached to the interior surface of the seed coat (Fig. 1f).

Endosperm was thickest in the center of the seed and gradually decreased in thickness towards peripheral margins. Endosperm cells evidenced thick walls (Fig. 1f) which expanded rapidly upon hydration. It was noted that most of the swelling of the seed in early stages of imbibition occurred in the endosperm. Upon swelling, production of a viscous and sticky gum resulted. The walls, which are believed to be the source of the gum when hydrated, stain intensely with calcofluor (not shown) and congo red (Fig. 1g). During sample preparation of seed coat/endosperm tissues for SEM, NaCl was added to the fixative to reduce the amount of swelling of endosperm. Thus, endosperm tissue in Fig. 1f shows little swelling of cell walls. Sodium chloride was not added to the fixative during preparation of GMA embedded tissues, therefore, swelling and solubilization of cell wall components in the endosperm was apparent (Fig. 1g). An endosperm cell wall component, not solubilized during sample preparation was observed (Fig. 1g).

*Olneya tesota* (Leguminosae—Papilionoideae)

*Olneya tesota* seed was dark, reddish brown in color and nearly spherical in shape. The typical leguminous seed coat was a thin structure with endosperm firmly attached to the subepidermal layer. Endosperm was typically one cell thick. Interior to the endosperm were two large, fleshy cotyledons.

Cotyledon tissue blocks were embedded in GMA using standard techniques including dehydration with lipid solvents (ethanol and butanol). However, lipids were preserved in the central portion of the GMA blocks. GMA (Fig. 2a) and hand-cut (not shown) sections compared favorably when stained with nile blue A and viewed at 450-490 nm excitation wavelength. Lipid bodies surrounded much larger protein bodies of varying shapes and sizes; protein bodies were evident following staining with mercuric bromophenol blue (Fig. 2b). Cell walls in GMA sections of the cotyledons stained intensely following treatment with calcofluor (not shown) or congo red (Fig. 2c).

Cotyledon tissue blocks were fixed in glutaraldehyde (GA) and post-fixed in OsO₄ into tissues during preparation for SEM, lipid was ethanol-extracted out of the central portion of tissue blocks during dehydration. It was therefore possible to compare OsO₄-fixed tissues (GA + OsO₄) (Fig. 2d,e) with those which were not (GA - OsO₄) (Fig. 2f) in the same block. Lipid bodies appeared to fill most of the space between protein bodies (Fig. 2d,e). In some cases, protein
bodies were pulled away from surrounding tissues during specimen preparation leaving large indentations lined with lipid bodies (Fig. 2d,e). Intact protein bodies exhibited small pits in their surfaces (Fig. 2d,e). These pits resulted from lipid bodies which were pulled away from the protein body surface during fracturing. A reticulated appearance of the interior of protein bodies was evident in cross section (Fig. 2e). Protein body inclusions were not evident in GMA sections or in tissues prepared for SEM.

GA - Os04 tissue prepared for SEM exhibited a reticulated cytoplasmic matrix with small indentations attributed to the removal of lipid bodies (Fig. 2f). Protein bodies were detectable beneath this mesh. Ovoid-shaped starch granules were evident at the periphery of cotyledon cells (Fig. 2f). Starch granules were not discernible in GA + Os04 material but a small number were identifiable in GMA sections which had been stained with IKI (Fig. 2g) and viewed in the light microscope.

Amaranthus cruentus (Amaranthaceae)

Amaranthus cruentus seed is borne in a single-seeded fruit called a utricle (Munz and Keck, 1975) (Fig. 3a). The lenticular seed is approximately 1 mm in diameter and has an embryo which completely encircles the perisperm in one plane (Fig. 3a,b). Endosperm comprises a small portion of the mature seed at the tips of the cotyledons and radicle (Fig. 3b). Seed coat tissue surrounding the perisperm is firmly attached and appears as a single, crushed layer. Two cell layers are evident in the seed coat surrounding the embryo (Fig. 3c).

Embryo cells were filled with protein bodies as demonstrated by acid fuchsin (Fig. 3d) or mercuric bromophenol blue (not shown) staining of GMA sections. Small protein bodies were localized in endosperm cells (Fig. 3e). Two types of protein body were discerned in both the embryo (Fig. 3d) and endosperm (Fig. 3e); those with globoid inclusions (not shown for endosperm) and those without inclusions. Protein bodies with globoids were much more prevalent in the embryo than in the endosperm. Globoids were identified by staining with acriflavine HCI and viewing at 500-550 nm excitation wavelength in the fluorescence microscope (not shown).

Much of the protein in the perisperm of the A. cruentus seed was localized in a single peripheral cell layer (Fig. 3e,f). Protein bodies were not evident in the perisperm using either light or scanning electron microscopy (Irving et al 1981). Starch granules in the peripheral layer appeared to be about one-third the size of those in the central portion (Fig. 3g), which were 1-3 μm in diameter. Scanning electron micrographs of the perisperm revealed closely packed, polyhedral starch granules (Fig. 3h).

Discussion

Minor differences were detected in seed anatomy of Prosopis. Four Prosopis taxa (P. alba, P. pubescens, P. tamarugo, and P. velutina) lacked starch in the embryo as was reported for P. glandulosa (Allen et al 1982) and P. velutina (Irving 1984). Prosopis chilensis exhibited few, small starch granules in the pro-mesophyll of the cotyledons (Fig. 1a). Protein bodies were located throughout the embryo including much of the provascular system in all Prosopis taxa studied and constituted the major portion of protein reserves in the seed.

Prosopis velutina and P. glandulosa contained approximately 32% protein (Table 1). Milling of P. velutina pods by Meyer et al (1982) yielded two seed fractions: endosperm splits (seed coat + endosperm) and cotyledon (embryo). The endosperm splits fraction contained 8% protein while the cotyledon fraction contained 56% protein. This is in agreement with histochemical findings that the major storage organ for protein was the embryo, specifically in the cotyledons in the form of protein bodies.

Two types of protein bodies were identified in Prosopis cotyledons; those without inclusions and those with globoid inclusions. Globoids were located in the pro-mesophyll of all Prosopis and, in most cases, a gradation in size and number was noted as was reported for P. glandulosa (Allen et al 1982). Prosopis pubescens contained globoids in virtually all parenchyma cells although there were few in protein bodies of the palisade parenchyma. Globoids were localized in abaxial epidermal cells of both P. chilensis and P. pubescens.

Globoids are rich in phytin, a cationic salt of myoinositol hexaphosphoric acid and contain...
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Phytin was not detected in chemical analyses of protein bodies. In contrast, phytin has been quantified chemically in P. velutina and P. glandulosa (Becker 1983). Variations in seed coat anatomy between species were determined to be primarily differences in thicknesses of the sclerified parenchyma and palisade layers.

Olecrnis tesota seed contained 33-39% lipid (Table 1) (Becker 1983), considerably higher than soybean (18%). The fatty acid profile (Table 2) indicated that the oil is rich in oleic, stearic, palmitic, and linoleic acids and compared with peanut oil in flavor (Becker 1983). Lipid reserves were visualized in scanning electron and light micrographs as distinct deposits in the cotyledons. Phytin was not detected in chemical analyses of O. tesota seed (Table 1) nor were phytin globoids evident histochemically in the protein bodies.

Sectioned protein bodies of O. tesota revealed a reticulated, mesh-like structure in scanning electron micrographs. In contrast, protein bodies of P. velutina evidenced a solid structure with or without globoids. The mesh-like appearance of protein body internal structure could be attributed either to extraction during sample preparation or to partial digestion of the body. Protein bodies have been reported to contain hydrolytic enzymes (Yatsuz and Jacks 1968) and Rost (1972) noted all stages of protein body digestion in dormant caryopses of Setaria lutescens. Protein bodies were the major storage site in the O. tesota seed for protein.

Protein content of O. tesota seed was found to range 26-32% (Table 1) but a substantial proportion of the protein (35%) was determined to be canavanine, an arginine analog and a potent growth inhibitor in animals. Methionine + cystine are the first limiting amino acids as was found in Prosopis and other legumes. In addition, a number of other amino acids are significantly limiting in the O. tesota seed. Olecrnis tesota seed cotyledons contained few starch granules as was determined histochemically. These findings agree with Becker (1983) when quantified 1.09% starch in the O. tesota seed.

Three members of the sixty-species of the genus Amaranthus have been grown as grain.
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Fig. 3. (facing page) Amaranthus. a. A. cruentus seeds and fruits photographed through a dissecting microscope. Bar = 2 mm. b. A. edulis cross section of a seed with hematoxylin and eosin to show relationships of tissues. Bar = 250 μm. c. A. cruentus seed cross section adjacent to the embryo stained with acriflavine HCl and viewed at 385-425nm excitation. Bar = 25 μm. d. Fluorescence view of the radicle of A. cruentus stained with acid fuchsin (500-550 nm excitation) showing bright protein bodies and dark (unstained) globoid inclusions. Bar = 25 μm. e. A. cruentus section of perisperm and endosperm stained with acid fuchsin and viewed at 500-550 nm excitation. Note the small protein bodies in the endosperm. Protein in the perisperm occurs as brightly stained material between darker (unstained) material which are starch granules. Bar = 50 μm. f. Fluorescence micrograph of the outer peripheral layer of the perispem (brightest area) at the top of the micrograph of A. cruentus and the layer of starch that stained with acid fuchsin (500-550 nm excitation). Protein is the brightly stained material. Cell walls are also stained. Bar = 25 μm. g. Perispem outer layers viewed at 500-550 nm excitation. Similar view to 3f but stained with periodate-Schiff’s to denote carbohydrates. Notice small starch granules at top of micrograph. Layers below are densely-packed starch granules. Bar = 25 μm. h. Scanning electron micrograph of a few adjoining cells of the perispem. Notice the small polyhedral starch granules. Bar = 15 μm.

crops. A. caudatus (which includes A. caudatus cv edulis sometimes known as A. edulis) is grown in the Andean highlands and is toasted, popped, milled into flour, or made into gruel. Amaranthus cruentus, used for grain (light seeds) and as a vegetable or ornamental (dark seeds), is grown in Mexico and Guatemala. Amaranthus hypochondriacus is the most robust and highest yielding of the grain types of Amaranthus and is cultivated in India and Central America (National Research Council 1984). All have been found to be high in protein which was stored in the A. cruentus seed in two forms; as matrix material in the perispem or as protein bodies with or without globoids in the embryo and endosperm. Globoid inclusions containing phytic acid were localized throughout the A. cruentus embryo. Compositional data (Table 1) agree with the presence of phytic acid.

Evaluation of protein quality in Amaranthus indicated that the first limiting amino acid was leucine (Betschart et al 1981; National Research Council 1984) and unlike cereals (wheat, corn, rice) was high in lysine (Downton 1973). Amaranthus seed, therefore, would be a good complement to corn, wheat or rice (National Research Council 1984).

Much of the protein in A. cruentus was located in the embryo and the outer peripheral layer of the perispem. Milking of these fractions was followed by chemical analyses agree with these findings. Protein content of whole A. cruentus seed was determined to be 18.75%. The bran/germ fraction (seed coat + embryo) in milled A. cruentus seed contained 42% protein and the perispem fraction contained 7.7% protein (Betschart et al 1981). Other nutrients of the seed were also found to be more concentrated in the outer layers. Fat was found to be 7.7% (Table 1) in the whole seed and 19-20% in the bran/germ fraction. Fatty acid composition of the oil was 70% oleic + linoleic, 20% stearic, and 1% linolenic (National Research Council 1984), therefore, the bran/germ fraction has potential value as an oil source. Although the embryo is high in fat, oil droplets were not detectable in the SEM (Irving et al 1981). Fat was determined to be 2.3% in the perispem fraction (Betschart et al 1981).

The perispem fraction was composed mostly of starch and had only 7% amyllose in A. hypochondriacus (Becker et al 1981) and amyllose was not detected in A. paniculatus (Modi and Kulkarni 1976). Amaranthus leucosperma was also found to have "waxy" (high amylopectin) starch (Wolf et al 1950). Amaranthus seed was found to contain about 62% starch (Becker et al 1981). "Waxy" starch, particularly where the granules are minute as in Amaranthus (1-3 μm in diam.) could have commercial applications both in food and industrial products.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also by suitable.

References


COURNER EJH. 1951. The leguminous seed. Phytomorph. 1:117-150.


Discussion with Reviewers

L.H. Bragg: Why do some protein bodies within the same cotyledon have inclusions whereas others are without these inclusions? Do you think this absence is a result of chance sectioning or fracturing of the protein body?

Authors: I assume you mean "protein bodies within the same cell"? As I stated in the paper, globoids are more abundant in the promesophyll of the cotyledons of Prosopis. Why this is the case is not certain. Chance sectioning would necessarily eliminate some of the globoids in a protein body in a single section as would the removal of globoids during sectioning due to the hardness of globoids compared to surrounding tissues. In this study, many sections, including serial sections from several seeds were stained and observed, therefore this "chance sectioning" problem was eliminated.

L.H. Bragg: Are the "large holes" in the described mesh-like matrix between the protein bodies which are shown in Figure le real or are they artifacts due to preparatory techniques?
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Authors: All fixed samples showed this characteristic. Dry samples observed in the SEM did not exhibit this feature suggesting the characteristic appears as a result of hydration. We used this micrograph primarily to demonstrate fractured protein bodies and the globoid inclusions.

L.H. Bragg: Why do you anticipate that phytic acid values for P. pubescens and P. chilensis will be higher than those of Prosopis velutina and P. glandulosa?

Authors: We expect the values for P. pubescens and P. chilensis to be higher than the others primarily because they appeared to be more abundant and sometimes larger in these species than the other Prosopis taxa. We did not look at P. glandulosa.

J.N.A. Lott: The globoids in the epidermal cells of P. pubescens in figure 1c are very large and numerous compared to those of other seeds. Are all the cotyledon epidermal cells of this species like this?

Authors: Not all epidermal cells contain large globoids as shown here, but globoids are numerous in most epidermal cells especially those on the adaxial side. The large size may have resulted from agglutination of several globoids prior to or during specimen preparation; however, P. pubescens was the only member which exhibited this characteristic. P. chilensis did contain globoids in epidermal cells but these were much smaller and less numerous than P. pubescens.

J.N.A. Lott: Do you have any information on the solubility properties of the storage proteins in the species you studied?

Authors: Sorry, no we do not. Solubility properties of the proteins is one of the questions on the agenda.

K. Saio: You mentioned that the three crops are valuable as resources for foods. Will you please explain the traditional and potential uses of these in modern technologies?

Authors: Many of the traditional uses of these crops are also appropriate for modern technologies. For example, early desert settlers collected and ground Prosopis pods. The pericarp flour was used as a sweetener or as a fermentation substrate and the ground seeds as a protein source. Today, potential uses would also include utilization of the seed mucilage (endosperm) as a guar gum substitute. Olneya seeds have a pleasant bean taste but have not yet been commercially exploited. Amaranthus is a rediscovered cereal grain which is gaining wide acceptance in the U.S. health food industry.

K. Saio: In Fig. 2 variable size of protein bodies are distributed in a cell. I observed in soybean that the size of mature protein bodies becomes rather equal as the seeds reach maturation. Do you know the exact degree of maturat-