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ELECTROPHORETIC CHARACTERIZATION AND IMMUNOLOGICAL LOCALIZATION OF COCONUT (*COCOS NUCIFERA* L.) ENDOSPERM STORAGE PROTEINS

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Proteins extracted from coconut (*Cocos nucifera* L.) endosperm were analyzed using sodium dodecylsulfate-polyacrylamide gel electrophoresis, tube gel isoelectric focusing, and two-dimensional electrophoresis. Antisera to soybean 11S and 7S globulins were used with Western blotting techniques to test for immunological similarities. Endosperm was fixed and processed for light microscopy and for immunocytochemistry. The nonreduced proteins fractionated into four major bands, whereas the reduced proteins fractionated into seven major bands ranging from 55 to 17 kilodaltons (kD). All major bands were glycosylated and each consisted of at least two polypeptides with different isoelectric points. A minor band at 67 kD and two minor bands at 22 kD were recognized by antibodies to 7S soybean globulin and a major and minor band at 35 and 32 kD, respectively, were recognized by antibodies to 11S soybean globulins. An antibody raised against the 55 kD protein showed no cross reactivity with any other bands. Cellular structure differs depending on location within the endosperm, especially with respect to cell size and characteristics of the protein bodies. The cells are relatively thin-walled and contain abundant lipid bodies and protein bodies with included protein crystalloids. Distinct localization patterns are apparent for legumin, vicilin, and the 55 kD protein. Legumin (11S) and vicilin (7S) are present in the crystalloids, whereas the 55 kD protein is present only in the protein body matrix.

Introduction

The palm family (Arecaceae) is a large and diverse monocotyledonous family with economic importance for production of food, oil, fiber, and building material (UHL and DRANSFIELD 1987). *Cocos nucifera*, the coconut palm, is one of the most economically important species in this family and is cultivated for the endosperm or kernel within the nut. The main products produced from the kernel are coconut oil, copra (dried kernel), and the copra cake that remains after oil extraction (SATYABALAN 1989). Copra is 20% protein by weight and is used for numerous edible products from confections to milk substitutes (SATYABALAN 1989). Yet very little is known about the histology or the seed proteins of this valuable tissue.

In the classical structural research done on coconut endosperm, CUTTER and FREEMAN (1954) and CUTTER et al. (1955) observed only the events of early development up to wall formation. BALASUBRAMANIAM et al. (1973) made cryosections of mature coconut endosperm, but their only comment on the structure was that it did not seem that all cells had nuclei. The structural characteristics of the endosperm in three palm species, *Phoenix dactylifera* (DEMASON et al. 1983), *Washingtonia filifera* (DEMASON 1986), and *Elaeis guineensis* (ALANG 1981) have been recently described. Among the common structural features are unusually thick cell walls, which impart the characteristic hardness to the seeds of all these species and uniformly similar living cells throughout. Coconut endosperm is soft and hollow and therefore atypical.

SJOGREN and SPYCHALSKI (1930) first reported on seed storage proteins in coconut. They used high salt and phosphate buffer at various pH to analyze the storage protein from coconut endosperm. They called this protein cocosin. These authors used centrifugation to determine the sedimentation coefficient (12S) and molecular weight (208,000) of cocosin. HAGENMAIER et al. (1975) described the amino acid composition of a water soluble protein fraction from coconut and found that it is deficient in the essential amino acids isoleucine, methionine, threonine, and tryptophan and rich in a number of charged amino acids (glutamic acid, arginine, aspartic acid, and lysine), which explains its salt solubility. Legume globulins are known to be deficient in methionine and cysteine (SHOTWELL and LARKINS 1989). We have initiated studies on the storage proteins of palms by describing the electrophoretic characteristics of seed proteins from date palm, *P. dactylifera* L. (CHANDRA SEKHAR and DEMASON 1988*a*), and *W. filifera* (CHANDRA SEKHAR and DEMASON 1988*b*). The seed proteins from the two palm species were heterogeneous in molecular mass (ranging from 12–66 kD) and in charge (ranging from pH 3–10). We found no proteins with molecular masses above 100 kD. *Phoenix* and *Washingtonia* are closely related as members of the same subfamily (Coryphoideae) within the palms, whereas *C. nucifera* is in a different subfamily (Arecoideae) (UHL and DRANSFIELD 1987). DOYLE et al. (1985) showed considerable immunological

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variation in the 7S vicilin within the legumes. Not enough is known to speculate about seed protein variation within the palms. BORROTO and DURE (1987) state that very little at all is known about globulins in monocotyledons.

The purpose of this study is: (1) to describe the electrophoretic characteristics of the storage protein in coconut and to compare these characteristics to the other two palms studied and to the globulins of legumes; (2) to characterize the histological features of coconut endosperm and compare them to those of the other palms studied; and (3) to localize proteins of interest.

Material and methods

MATERIAL

Coconuts (*Cocos nucifera* L.) were purchased at a local grocery store.

ELECTROPHORESIS

PROTEIN EXTRACTION.—Coconut endosperm was separated from the testa, frozen in liquid nitrogen, and then ground into a fine powder with a mortar and pestle. The powder was defatted with chloroform:methanol (2:1), air dried, and used for all protein extractions. Dry soybean (*Glycine max* cv Provar) cotyledons were ground to a powder but not defatted.

For sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), proteins were extracted in 0.06 M Tris-HCl (pH 6.8) containing 2.3% SDS for 4 h at 4 C. The slurry was centrifuged for 10 min at 3,000 g and the supernatant was retained. For the salt extractable protein fraction, the material was extracted in 4% NaCl on ice with constant stirring and the supernatant was dialyzed against water for 24 h. Protein was pelleted by centrifuging at 18,000 g for 10 min. Protein estimations were done according to the method of PETERSON (1977) using bovine serum albumin (BSA) as the protein standard. Samples were diluted with SDS-PAGE sample buffer; the final samples contained 2.3% SDS, 10% glycerol, 0.1% bromophenol blue, and 0.06 M Tris-HCl buffer (pH 6.8). For analyzing reduced proteins, 2-mercaptoethanol was added to the sample buffer to a final concentration of 5%. The protein samples were held in boiling water for 3 min, and the cooled samples were used immediately or stored at −70 C until needed.

For isoelectric focusing (IEF), proteins were extracted in lysis buffer, which contained 9.5 M urea, 5% 2-mercaptoethanol, 2% Triton-X, 1.6% (pH 5–7), and 0.4% (pH 3–10) ampholines (Serva Biochemicals).

SDS-PAGE.—SDS-PAGE was performed in 12.5% polyacrylamide gels bound to Gelbond PAG (Marine Colloids). Gel composition was according to LAEMMLI (1970). Twenty μg of protein were loaded into each well. The gels were run in a Hoeffer SE-600 unit and stained with Coomassie blue R (C.I. 42660).

Protein bands were compared in relation to the mobilities of the following marker proteins: phosphorylase b (92.4 kD), BSA (66.2 kD), ovalbumin (42.7 kD), carbonic anhydrase (31.0 kD), trypsin inhibitor (21.5 kD), and lysozyme (14.4 kD).

GLYCOPROTEIN STAINING.—Reduced protein samples were electrophoresed in polyacrylamide (12.5%) tube gels (5 mm i.d.) as described above for SDS-PAGE. Gels were stained in screw-capped tubes with gentle shaking at room temperature using the periodic acid/Schiff's reaction as described by ZACHARIUS et al. (1969) with modifications: 0.7% periodic acid was used and the final washing time was increased to 6–8 h. Reduced protein samples from both soybean and coconut were also run on slab gels, as described previously, and stained with Con A-peroxidase (WOOD and SARINANA 1975).

IEF AND 2-D GEL ELECTROPHORESIS.—The first dimension IEF was performed in 160 mm long, 2-mm diameter glass tubes. The gel was formed according to O'FARRELL (1975). The electrode solutions were 0.2% sulfuric acid and 0.5% ethanolamine. The gels were prefocused for 30 min each at 100, 200, and 400 V with reversed polarity. Fifty μg samples were applied to the acidic end. IEF was performed for 17 h at 300 V and 1 h at 800 V in a Hoeffer tube gel electrophoresis unit. The gels were either equilibrated in SDS-PAGE sample buffer (1–2 h), or stained with Coomassie blue. The pH along the length of the gel was determined in serial 5 mm gel segments. The pH range was 3–10.

The second dimension SDS-PAGE was performed in 12.5% polyacrylamide gels as above except the equilibrated tube gel was attached to the SDS-PAGE gel with 1% agarose.

DISSOCIATION ANALYSES.—Nonreduced proteins were extracted as for SDS-PAGE; 2.7 mg protein were prepared in 1.2 mL sample buffer and boiled for 3 min. SDS-PAGE was performed as above, except that a 3-mm thick preparative gel was cast without Gelbond, and the sample was added evenly across the top of the stacking gel. A cm-wide strip of the gel was cut off and stained with Coomassie blue to determine the position of the major bands (*a–d*) and then these regions were cut from the remaining unstained portion of the gel. Gel segments were placed in 6 mL of 0.04 M Tris-acetate buffer pH 8.6 to diffuse overnight at 4 C. The samples

were concentrated with an ISCO Model 1750 electroeluting device according to the instructions of the manufacturer. Samples were analyzed again for protein content and resuspended in sample buffers (reduced or nonreduced) for SDS-PAGE.

ANTIBODY PREPARATION.—The lower band of the 55 kD doublet protein was purified by eluting it from a preparative gel and concentrating it as described above. The concentrated protein was dialyzed against one-quarter strength phosphate buffered saline (PBS) and lyophilized. Equal volumes of protein dissolved in PBS and Freund's incomplete adjuvant were mixed and vortexed thoroughly until a homogeneous white suspension was obtained. After collecting an adequate number of preimmune eggs, 0.5 mL of the suspension containing 0.5 mg of the protein was injected into the thigh muscle of each of two hens. Booster injections, prepared as above, were given to each hen weekly for 2 wk. Eggs were collected 15 through 45 d after the first immunization. The immunoglobulin-G (IgG) fraction was purified from collected eggs according to POLSON et al. (1985). After the final cold ethanol wash, the IgG precipitate was dissolved in Tris buffered saline (TBS) containing 0.2% sodium azide at 5 mg protein/mL and stored.

IMMUNOBLOT ANALYSIS.—Reduced protein extracts were electrophoresed in 12.5% acrylamide (acrylamide:bis, 30:0.2) gels. After the run, proteins were transferred to nitrocellulose membrane (Fisher) with the Nova-Blot apparatus (LKB) according to the instructions of the manufacturer. The blots were processed as described by TOWBIN et al. (1979). The primary antibodies were detected using alkaline phosphatase–attached secondary antibody, antirabbit-goat or antichicken rabbit IgG (Sigma). Finally, alkaline phosphatase was stained using the substrate chloro-bromo-naphthyl phosphate (1 mg/mL) in 100 mM Tris-HCl (pH 8.5).

PHOTOGRAPHY.—Preparations were photographed using either transmitted light (gels) or reflected light (blots) with Kodak Professional Pan 2415 film at ASA 100 using an orange filter (Coomassie blue-stained gels and blots) or a green filter (PAS-stained gels).

MICROSCOPY

TISSUE PREPARATION.—Chunks of endosperm were fixed in 2% glutaraldehyde in 50 mM phosphate buffer, pH 7.6, or in half-strength KARNOVSKY'S (1965) fixative in the same buffer, dehydated in an ethanol series and embedded in JB-4 glycol methacrylate (Polysciences). Radial sections 3 μm thick, which contained the entire endosperm, were made and stained using the periodic acid/Schiff's reaction (PAS) (O'BRIEN and MCCULLY 1981) and counterstained with aniline blue black (C.I. 20470) (FISHER 1968).

IMMUNOLOCALIZATION.—Sections prepared as above were used with the immunogold and silver enhancement techniques (FAYE et al. 1988), except that the slides were air dried and briefly immersed in HistoClear and coverslips were mounted with Permount. The primary antibody was used at a dilution of 1:50 for rabbit serum (anti-11S and anti-7S) or diluted to 10 μg/mL (anti-55 kD protein). The secondary antibodies conjugated to gold (either goat antirabbit IgG or rabbit antichicken IgG) were purchased commercially (Sigma), and used at a dilution of 1:50. Controls were done with preimmune (chicken antibodies) or nonimmune serum (rabbit antibodies).

PHOTOGRAPHY.—Photographs were taken on a standard Zeiss bright-field microscope with a Nikon microflex UFX-II and Kodak Technical Pan 2415 film at ASA 50 without filters.

Results

ELECTROPHORESIS

The nonreduced proteins separated into four major bands and a number of minor bands, ranging between 16 kD to greater than 100 kD (fig. 1, lane *B*). For convenience the four major bands were designated *a* (greater than 100 kD), *b* (55 kD), *c* (25 kD), and *d* (16.6 kD). The reduced proteins resolved into seven major and four or more minor bands ranging between 17.0 kD and 55.0 kD (fig. 1, lane *C*). In the reduced protein profile, there was an apparent lack of major bands above 100 kD and prominent bands at 55.0 kD (doublet), 34.7 kD, 31.6 kD, 25.7 kD, 22.4 kD (doublet), 20.9 kD, and 17.0 kD.

Two bands stained darkly with the PAS reaction, a minor band at 67 kD and both bands of the doublet at 55 kD; most major bands stained lightly (fig. 2, lane *C*). Ovalbumin, a known glycoprotein in the mol wt standards, also stained with the PAS reaction whereas no other standard proteins stained (fig. 2, lane *A*). Also, a number of bands stained in the soybean preparation including, α, α′, and β conglycinin and a set of four low mol wt bands of unknown identity (fig. 2, lane *B*). All of the PAS positive bands in the soybean sample also stained with Con A-peroxidase method, but this method stained no bands in coconut preparations (data not shown).

In the first dimension (IEF) gels, the proteins resolved into numerous bands that extended across the whole range of pH (fig. 3). Most bands occurred between pH 5.5 and 8.5. In particular, the

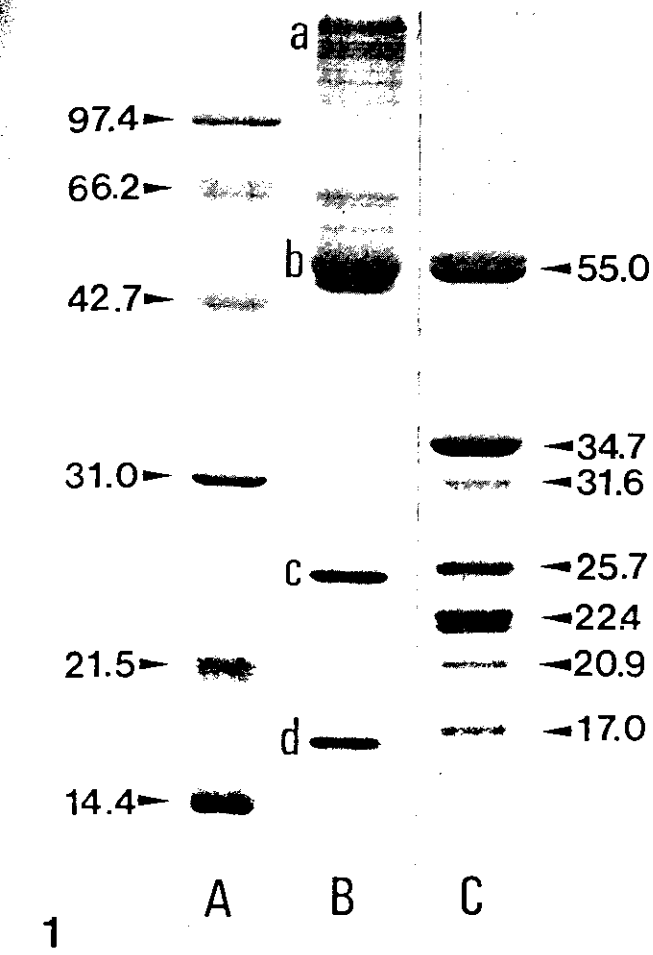


FIG. 1.—SDS-PAGE of coconut endosperm proteins stained with Coomassie blue. Lane *A*, contains molecular weight standards; lane *B*, nonreduced proteins; and lane *C* reduced proteins. Numbers indicate apparent molecular weight in kD.

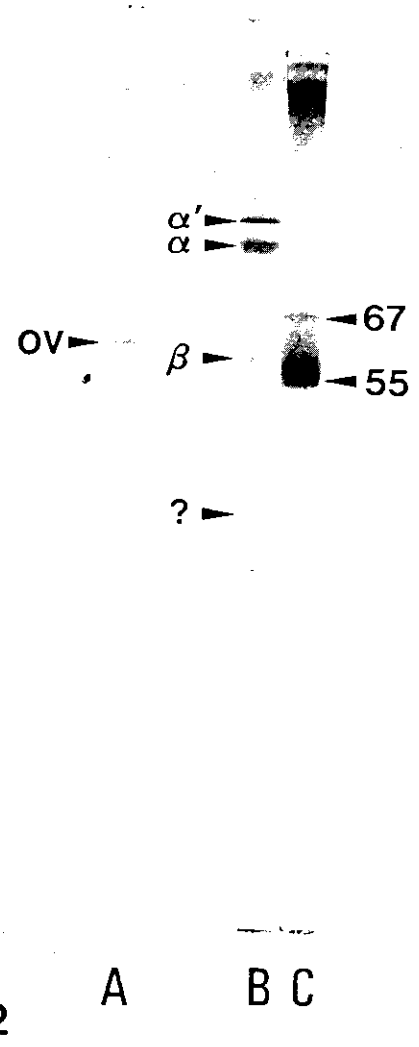


FIG. 2.—Periodic acid/Schiff-stained tube gels. Lane *A* contains molecular weight standards. Ovalbumin (*OV*) is known to be a glycoprotein. Lane *B* contains soybean proteins. 65 μg protein loaded. Lane *C* contains reduced coconut proteins. 65 μg protein was loaded. α–α′ = α–α′ conglycinin, β = β conglycinin; ? = unknown glycoproteins. Numbers indicate apparent molecular weight in kD.

doublet at 55 kD resolved into two major spots at pH 3 and 6.9; the band at 35 kD resolved into three major spots between pH 6 and 7; the band at 32 consisted of a major spot at pH 5.8; the singlet at 26 kD resolved into two spots at pH 7.5 and 8; the doublet at 22 kD resolved into a couple of spots at pH 9–10 (arrowhead, fig. 3); and the band at 17 kD resolved into a major spot at ca. pH 7.

The salt soluble protein fraction contained a single enhanced band at ca. 26 kD and several minor bands at ca. 55 kD, 35 kD, and 17 kD (fig. 4, lane *B*).

When the nonreduced proteins *a*, *b*, *c*, and *d* were isolated and rerun, the appropriate bands were recovered unaltered (fig. 5, lanes *A–D*). However, on reduction, the proteins *a–d* either dissociated into a number of proteins or changed their apparent molecular mass (fig. 5). Nonreduced protein *a* separated into at least eight bands on reduction—67 kD, a doublet at 55 kD, bands at 35 kD, 32 kD, a doublet at 22 kD, and a single band at 21 kD (fig. 5, lane *F*); *b* dissociated into a doublet at 55 kD and a single band at 35 kD (fig. 5, lane *G*); *c* dissociated into bands at 26 kD and 22 kD (fig. 5, lane *H*); and *d* changed into a band at 17 kD (fig. 5, lane *I*).

Immunoblot analysis showed significant homologies of coconut endosperm proteins with both glycinin (11S) and conglycinin (7S) from soybean (fig. 6). At least three coconut bands had similarities with conglycinin, which migrated at 67 kD and 22 kD (fig. 6, lane *B*). Two distinct bands had similarities with glycinin and migrated at 35 and 32 kD (fig. 6, lane *C*). A single band was recognized by the antibodies against the 55 kD protein (fig. 7), and no cross reactivity occurred with any bands in the soybean preparation (not shown).

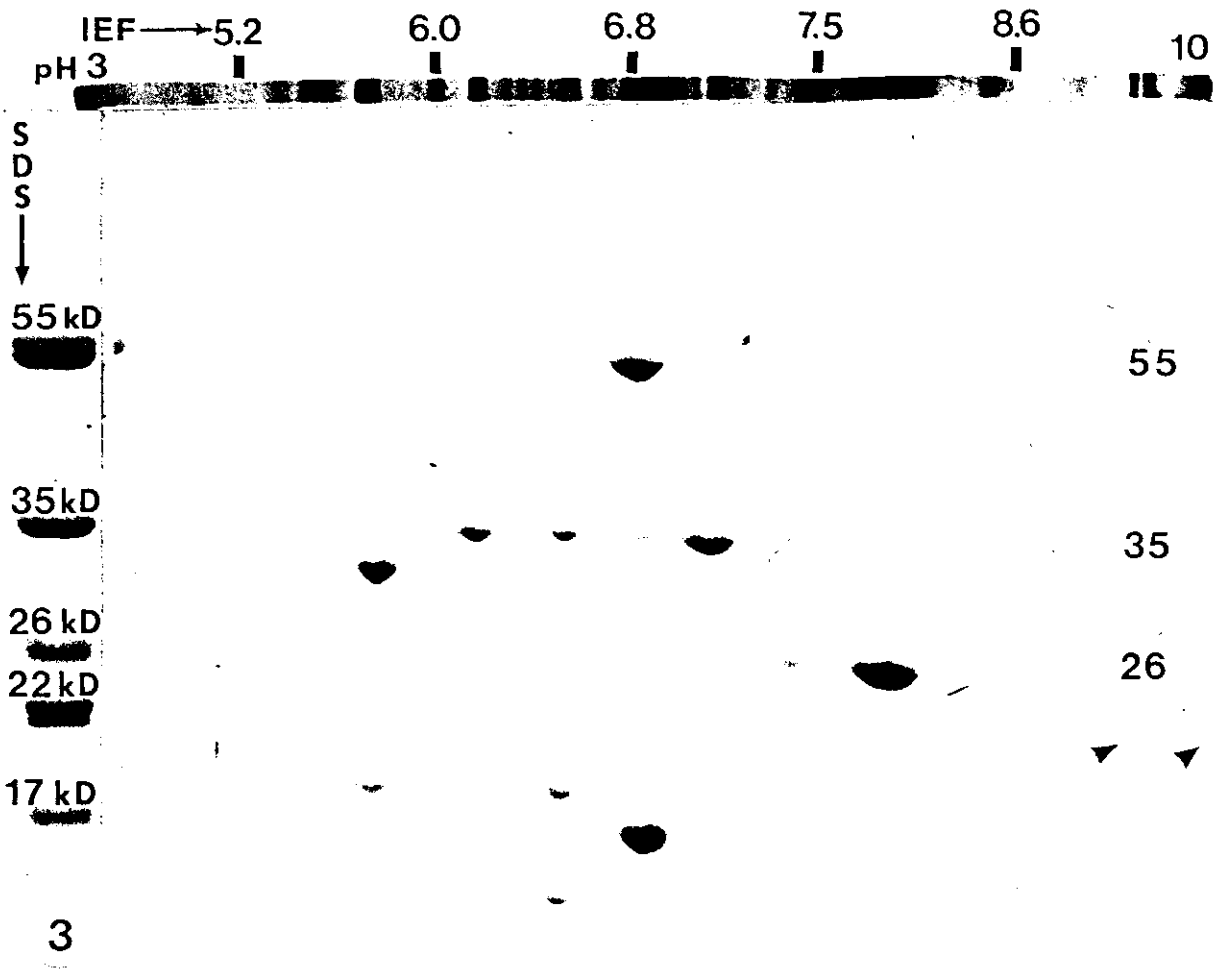


FIG. 3.—Two-dimensional SDS-PAGE of reduced coconut endosperm proteins. Arrowheads indicate pale polypeptides from 22 kD protein. Numbers indicate apparent molecular weight in kD.

HISTOLOGY

Coconut endosperm cells have relatively thin cell walls that stain with PAS. These walls are unevenly thickened such that they resemble collenchyma and are very brittle after dehydration (figs. 8–13). The protoplasts contain abundant lipid bodies and protein bodies, the characteristics of which as well as the cell size are related to the position within the endosperm. The outermost endosperm cells are small and contain small lipid bodies and variably-sized, densely staining protein bodies (fig. 8). About eight cell layers internal to the testa, the cells have generally the same appearance except that they are very elongate. The included lipid bodies and protein bodies are also larger (fig. 9). The next cells that make up a large portion of the coconut endosperm contain densely staining protein crystalloids within the protein bodies (fig. 10). Very gradually these cells grade into cells in which the protein bodies are very large and contain a lighter-staining matrix and densely staining included protein crystalloids (fig. 11). More internally the endosperm consists of cells that are nearly filled with a lightly staining (blue) vacuole and a few large lipid bodies. These vacuoles contain protein crystalloids (fig. 12). The endosperm cells that line the hollow cavity within the seed appear to be devoid of cytoplasmic contents (fig. 13). Although nuclei are very palely staining, misshapen, and difficult to recognize, they are present in all regions of the endosperm (figs. 9, 10) except the empty cells in the innermost region.

IMMUNOCYTOCHEMISTRY

All three antibodies were usable for antigen localization on plastic sections (figs. 14–22). Antibodies raised against the 11S soybean globulin (glycinin) showed apparent nonspecific binding to cell walls, but since preimmune serum was not

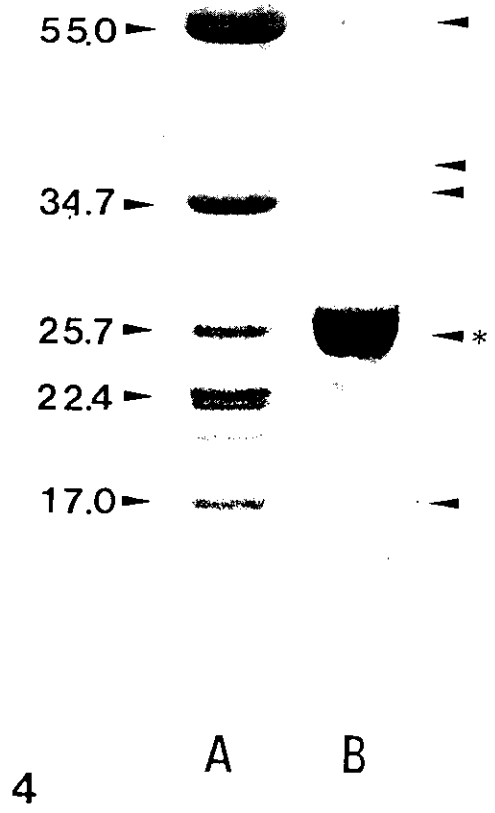


FIG. 4.—Salt soluble coconut endosperm protein fraction. Lane *A* contains reduced total protein fraction. Lane *B* contains salt-soluble fraction. * indicates the most salt-soluble polypeptide.

available to us we could not test this hypothesis further (figs. 17–19). Using antibodies raised against the 7S soybean globulin (conglycinin), the label was sparse but uniform over the protein body matrix of outer and middle endosperm cells and dense over protein crystalloids of middle and inner endosperm cells (figs. 14–16). Using antibodies raised against 11S soybean globulin (legumin), the label was uniformly dense over protein bodies in outer endosperm cells and was sparse over protein body matrix of middle and inner endosperm cells and densest over the protein crystalloids (figs. 17–19). With antibodies raised against the coconut 55 kD protein, label was very uniformly dense over the protein bodies of the outer endosperm cells, relatively dense over the protein body matrix of middle and inner cells, and distinctly lacking over the protein crystalloids (figs. 20–22). Controls had no label (not shown).

Discussion

SEED PROTEINS

SJOGREN and SPYCHALSKI (1930) originally described a globulin in coconut endosperm that had a molecular mass of about 200 kD and named it cocosin. We find such a high mol wt protein (*a*), which on reduction yields at least eight polypeptides ranging from 67 kD to 21 kD. Protein *b* is probably a product of cocosin since the three polypeptides produced on reduction run at the same position as polypeptides from protein *a*. Two smaller proteins (*c*, *d*) are also found in nonreduced protein extracts with cocosin. Each polypeptide and protein has unique characteristics.

Examining the eight polypeptides that make up the putative cocosin, there is a minor band that migrates at about 67 kD. This polypeptide is highly glycosylated and shows immunological similarities with the 7S protein (conglycinin) of soybean. A similar band, but which is much more abundant, migrates at about 67 kD in both *Phoenix* and in *Washingtonia* and is a major band in *Phoenix* (CHANDRA SEKHAR and DEMASON 1988*a*, 1988*b*). The next polypeptide is a doublet at about 55 kD. This doublet is made up of an abundant polypeptide at about pI 7 and a less abundant polypeptide at pI 3, and is also a highly glycosylated protein. Although these bands are in the correct size range to be vicilins, no immunological homology was revealed with either of the antibody preparations tested. Similar major bands are present in both *Phoenix* and *Washingtonia* (CHANDRA SEKHARA and DEMASON 1988*a*, 1988*b*). A major band occurs at 35 kD and a minor one at 32 kD. These bands include several polypeptides with pI's in the acidic to neutral range and show immunological similarities to the 11S protein (glycinin) in soybean. Similar, but less abundant, bands are also present in *Phoenix* and in *Washingtonia* (CHANDRA SEKHAR and DEMASON 1988*a*, 1988*b*). A doublet occurs at 22 kD that is composed of a couple of very basic polypeptides and shows immunological similarities with 7S complex of soybean. These bands are also present in both *Phoenix* and *Washingtonia* (CHANDRA SEKHAR and DEMASON 1988*a*, 1988*b*). Finally, a minor band of some acidic polypeptides occurs at 21 kD.

Although immunological homologies obviously exist between soybean glycinin and a 67 kD polypeptide in coconut, we have evidence that there are significant differences between the carbohydrate moiety in the two species. The lectin Con-A binds readily to soybean 7S proteins, which one would expect considering the high mannose composition of this glycoprotein (YAMAUCHI and YAMAGISHI 1979). Con-A, however, does not bind to coconut glycoproteins.

Striking similarities and some differences occur

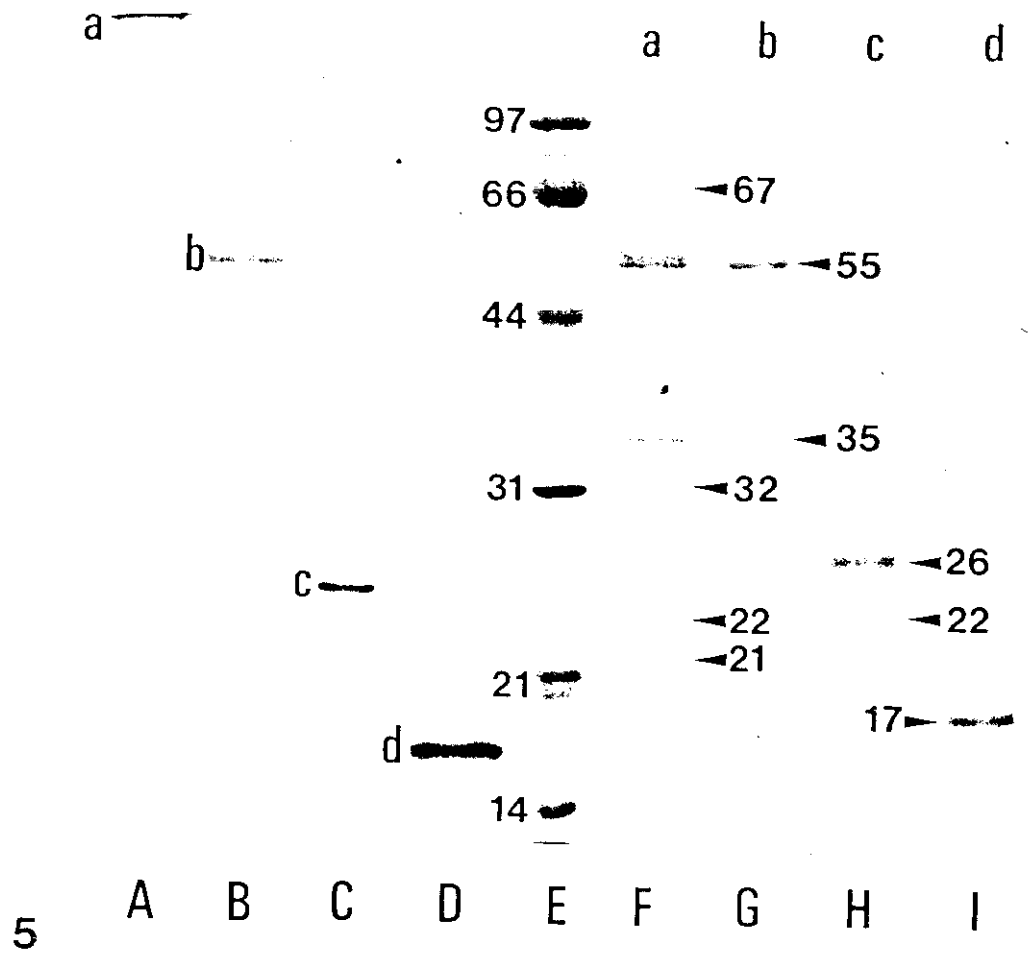


FIG. 5.—Reduced protein analyses. Lanes *A–D* are proteins *a–d* rerun as a control. Lane *E* contains mol wt standards. Lanes *F–I* are proteins *a–d* each separated by SDS-PAGE, eluted from gel, reduced, and rerun. 16 μg protein loaded in each lane.

in comparing the electrophoretic characteristics of the endosperm proteins in the three palm species so far studied. Common polypeptides among the three species migrate at about 67 kD (7S globulin), a doublet at 55 kD, a singlet at about 35 kD (11S globulin), and a doublet at 22 kD in reduced protein profiles (CHANDRA SEKHAR and DEMASON 1988*a*, 1988*b*). This is the first demonstration that both 7S and 11S globulins are present in the palm family. The relative abundance of the protein, however, is quite different in these three species. The 55 kD protein seems to be a unique palm storage protein; its identity is unknown. Another difference between the palm species is that few to no changes in SDS-PAGE banding patterns are found between reduced and nonreduced protein extracts in date or in *Washingtonia* (CHANDRA SEKHAR and DEMASON 1988*a*, 1988*b*), whereas in coconut the major protein fraction yields at least eight polypeptides upon reduction. We believe that this demonstrates differences in protein subunit aggregation in the palm species studied.

Proteins *c* and *d* also have unique characteristics. Upon reduction with mercaptoethanol, protein *c* yields two bands, a dark band at 26 kD and a smaller one that apparently comigrates with the doublet at 22 kD from protein *a*. This is the most salt soluble protein in coconut endosperm and has at least two polypeptides with pI's of ca. 8. Protein *d*, like *c*, migrates higher in the gel after reduction, which indicates that conformational changes in the polypeptides occur upon reduction. In 2-D gels, *d* has a major polypeptide at about pI 7. These proteins may not be storage proteins but, rather, proteins associated with the lipid body membrane as demonstrated in other oil seeds (QU et al. 1986; HERMAN 1987; MURPHY and CUMMINS 1988).

HISTOLOGY

Anatomical features of coconut kernel show more differences than similarities with the endosperm features of the other palm species studied. Features of coconut endosperm common to other palm endosperms studied are the types and forms of stored

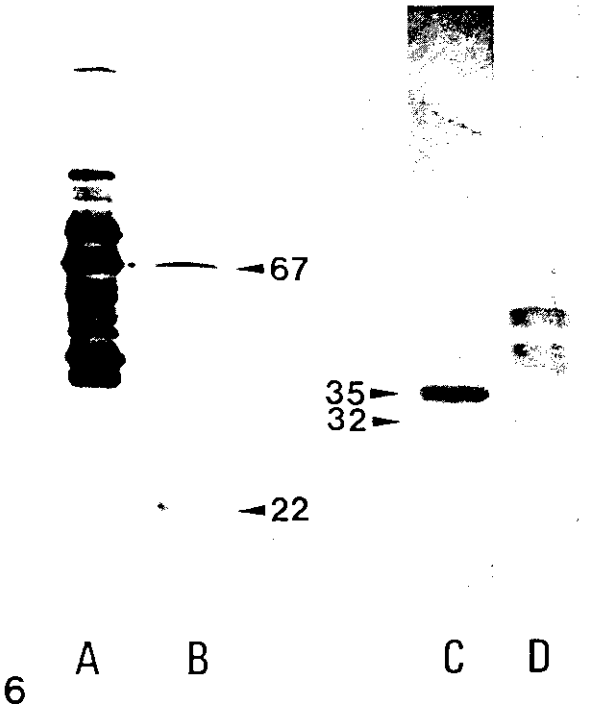


FIG. 6.—Immunoblot analysis. Lane *A* contains reduced soybean protein extract challenged with antibodies raised against soybean 7S protein complex. 5 μg protein was loaded. Lane *B* contains reduced coconut protein extract challenged with antibodies raised against soybean 7S protein complex. 15 μg protein was loaded. Lane *C* contains reduced coconut protein extract challenged with antibodies raised against soybean 11S protein complex. 15 μg protein was loaded. Lane *D* contains reduced soybean protein extract challenged with antibodies raised against soybean 11S protein complex. 5 μg protein was loaded.

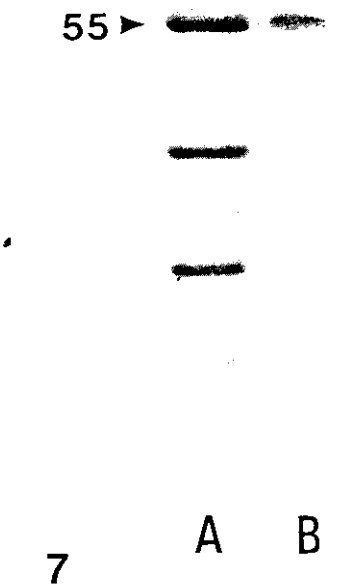
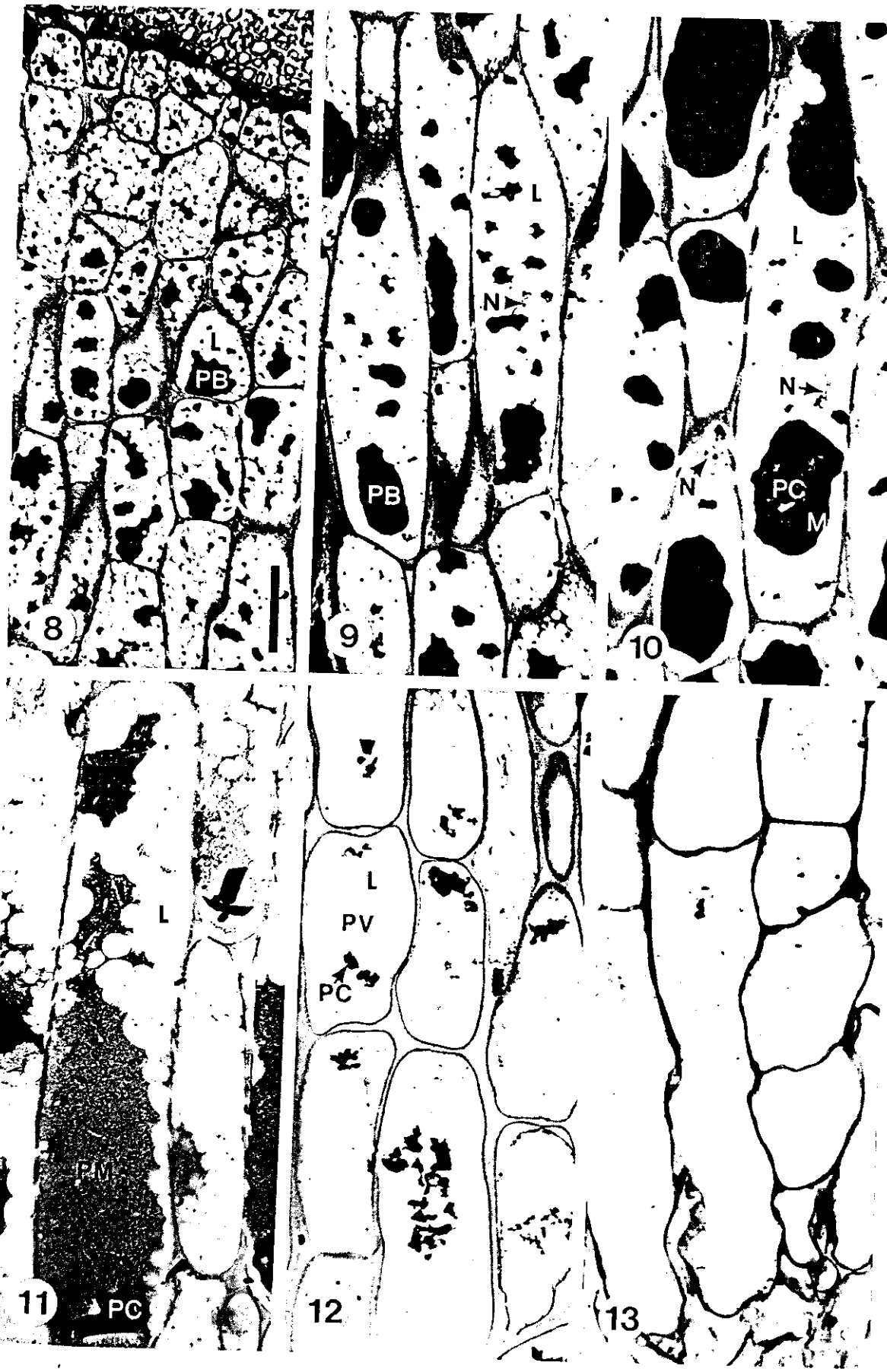


FIG. 7.—Immunoblot analysis. Lane *A* contains SDS-PAGE of reduced coconut protein extract stained with Coomassie blue. Lane *B* contains reduced coconut protein extract blotted onto nitrocellulose and challenged with antibodies raised against the 55 kD protein. 5 μg protein was loaded.

reserves: (1) lipid in the form of lipid bodies, (2) protein as protein bodies, and (3) carbohydate stored as wall material. The edibility of the coconut endosperm is due to its relatively thin cell walls. These walls stain positively with PAS like those of *Washingtonia* (DEMASON 1986) but unlike those of date (DEMASON et al. 1983). Although it is thought that palm endosperm cells walls are composed of relatively pure mannans (MEIER and REID 1982), we have suggested that PAS staining reflects the relative amount of galactose side branches (DEMASON et al. 1989). Protein crystalloids are present in many protein bodies within coconut endosperm. Although no crystalloids are present in date endosperm protein bodies (DEMASON et al. 1983), they are present in the outer endosperm of *Washingtonia* (DEMASON 1986) and in protein bodies of oil palm endosperm (ALANG 1981).

The most striking difference between coconut endosperm and that of other palms is a lack of uniformity in cell structure from the outer region, which resembles the endosperm of other palms, to the inner region, which is devoid of a protoplast. The transition is a gradual one and is manifested especially in the characteristics of the protein bodies. The outer cells have numerous separate and darkly staining protein bodies, generally without crystalloids. Cells from the middle region of the endosperm have few, large protein bodies that contain darkly staining protein crystalloids. The matrix shows less staining density as compared to the crystalloids, which can only mean that there is less protein present. The innermost living coconut endosperm cells, near the seed center, contain a large single vacuole, which contains crystalloids. These vacuoles resemble those of potato tubers (SONNEWALD et al. 1989). Although position dependent structural variation in protein bodies is known in cereal endosperm (POMERANZ and BECHTEL 1978; LENDING et al. 1988; LENDING and LARKINS 1989) and in the cotyledon of some legumes (CITHAREL

FIGS. 8–13.—Light microscope preparations of coconut endosperm stained with PAS and aniline blue black. Fig. 8, Outer endosperm adjacent to testa. Fig. 9, Approximately 9–10 cell layers internal to testa. Fig. 10, Outer midregion of endosperm. Fig. 11, Inner midregion of endosperm. Fig. 12, Inner endosperm. Fig. 13, Innermost endosperm adjacent to central cavity. Scale bar = 0.05 mm. *L* = lipid bodies; *PM* = protein body matrix; *N* = nucleus; *PB* = protein body; *PC* = protein crystalloid; *PV* = protein-filled vacuole; *T* = testa.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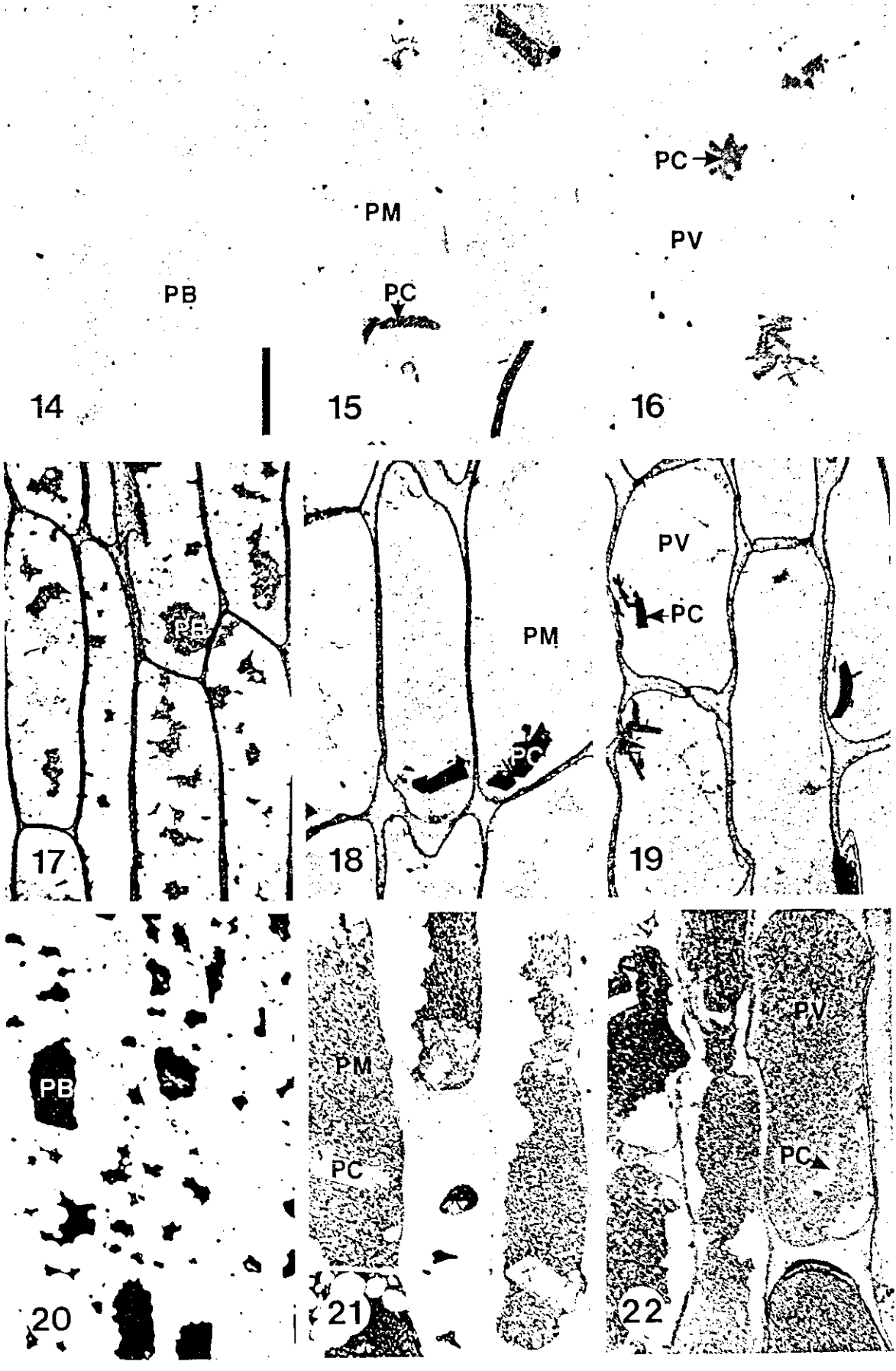
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