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Structural Aspects of Reserve Protein Accumulation in Developing Cotyledons of *Prosopis juliflora (Leguminosae, Mimosoideae)*

By

BHARTI CHAUDHRY and M.R. VIJAYARAGHAVAN*)

With 2 Figures

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Summary

CHAUDHRY B. & VIJAYARAGHAVAN M. R. 1993. Structural aspects of reserve protein accumulation in developing cotyledons of *Prosopis juliflora (Leguminosae, Mimosoideae).* – Phyton (Horn, Austria) 34 (1): 1–10, 2 figures. – English with German summary.

Structural changes during reserve protein deposition in *Prosopis juliflora* cotyledons were studied using both light and electron microscopy. The large and empty vacuoles in the cotyledonary cells of developing seeds are replaced toward seed maturity by many small bodies that are packed densely with proteinaceous materials. Subdivisions in the vacuoles are followed by concomitant deposition of storage proteins on the vacuolar rim resulting in the formation of protein bodies. The vacuolar protein deposits are apparent as irregular lumps of electron-dense precipitates

*) Prof. Dr. M. R. VIJAYARAGHAVAN, Dr. BHARTI CHAUDHRY, Department of Botany, University of Delhi, Delhi-110 007, India.

which either line the tonoplast or lie free in the vacuole. The protein deposition is accompanied by a massive proliferation of rough endoplasmic reticulum that pinches off vesicles into the cytoplasm. Morphological evidence suggests that ER-bound ribosomes are involved in the synthesis of storage proteins and that the transport of storage proteins from their site of synthesis, the rough endoplasmic reticulum to their site of deposition, the vacuolar protein bodies, is mediated via protein vesicles that are derived from ER.

Zusammenfassung

CHAUDHRY B. & VIJAYARAGHAVAN M. R. 1993. Strukturelle Untersuchungen über die Ablagerung von Reserveproteinen in sich entwickelnden Keimblättern von *Prosopis juliflora (Leguminosae, Mimosoideae)*. Phyton (Horn, Austria) 34 (1): 1–10, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurden strukturelle Veränderungen während der Ablagerungen von Reserveproteinen in Keimblättern von *Prosopis juliflora* licht- und elektronenmikroskopisch untersucht. Die großen und leeren Vakuolen der Zellen von Keimblättern sich entwickelnder Samen werden gegen die Samenreife zu durch viele kleine Körper ersetzt, welche dicht mit Eiweißmaterial gepackt sind. Unterteilungen der Vakuole werden von einer ständigen Ablagerung von Reserveproteinen am Vakuolenrand begleitet, was zur Bildung von Proteinkörpern führt. Die Proteinablagerungen in den Vakuolen erscheinen als unregelmäßige Massen elektronendichter Niederschläge, die entweder dem Tonoplasten anliegen oder frei in der Vakuole liegen. Die Proteinablagerung wird von einer massiven Vermehrung des rauhen ER begleitet, welches Vesikel in das Cytoplasma abschnürt. Die morphologischen Gegebenheiten lassen vermuten, daß ER-gebundene Ribosomen an der Synthese von Reserveproteinen beteiligt sind und daß der Transport von Reserveproteinen vom Ort ihrer Entstehung, dem rauhen ER, zum Ort der Speicherung, der Vakuole, durch ER-abstammende Protein-Vesikel erfolgt.

1. Introduction

The seed reserves needed for establishment of the seedling form a considerable proportion of the seed mass in many taxa. The seeds of many legumes contain abundant reserve protein stored in the cotyledonary parenchyma cells as protein bodies, that are large, spherical organelles consisting of an amorphous protein matrix surrounded by a limiting membrane. Protein bodies may develop within vacuoles where the protein is transported by direct connections with the endoplasmic reticulum (ER), by dictyosome vesicles, or through the cytoplasm from free ribosomes or they may form as vesicles derived directly from ER membranes (LOTT 1980). The latter has been implicated in protein body formation in maize (BURR & BURR 1976, KHOO & WOLF 1970, KYLE & STYLES 1977, LARKINS & HURKMAN 1978) whereas in legumes vacuolar deposition has been observed (CRAIG & al. 1979). The present work on *Prosopis juliflora* combines both histochemical and ultrastructural aspects of storage protein deposition in the cotyledonary parenchyma cells.

2. Material and Methods

For light microscopy, cotyledonary tissue at various developmental stages was fixed in 10% aqueous acrolein, dehydrated in the butanol series and infiltered in pure glycol methacrylate. The material was embedded in monomer mixture (glycol methacrylate : Azobis : polyethylene glycol 400; FEDER & O'BRIEN 1968). The sections were cut at 2 μm using glass knives and were stained with Coomassie brilliant blue for localization of total proteins.

For electron microscopic studies, portions of cotyledons were fixed in Karnovsky fixative (modified from KARNOVSKY 1965), prepared in 0.2 M phosphate buffer at pH 7.2 (at 0-4°C or at room temperature); post-fixed in 2% osmium tetraoxide made in the same buffer (at 0-4°C). The tissue was dehydrated in graded ethanol series and infiltered and embedded in Epon-Araldite Resin mixture (MOLLENHAUER 1964). Ultrathin sections were cut on LKB ultramicrotome using glass-knives. Silver to gold coloured sections were picked on uncoated, precleaned, 200 mesh copper grids. Sections supported on grids were stained with uranyl acetate and lead citrate (REYNOLDS 1963). Electron micrographs were taken on a Philips, EM 300 operated at 80 KV.

3. Observations

In the dicotyledonous embryo, each cotyledonary parenchyma cell contains initially a large vacuole (Fig. 1A) which is partitioned through cytoplasmic extensions into many small vacuoles with concomitant deposition of storage proteins on the vacuolar rim (Fig. 1B). The cotyledonary parenchyma cells are polygonal, and possess thick cell walls that enclose large intercellular spaces (Fig. 1F, 2A). In the vacuoles, the protein deposits are apparent as irregular and isolated lumps of electron-dense precipitates which either line the tonoplast or lie free in the vacuole (Fig. 1F, 2A, C). These protein deposits increase rapidly both in volume and size and form a contiguous ring or lens-shaped masses on the inner surface of the vacuoles (Fig. 1C).

The vacuolar protein deposition is accompanied by a massive proliferation of cytoplasmic rough endoplasmic reticulum (RER) that pinches off vesicles into the cytoplasm (Fig. 2A, B) which implies both the association and the involvement of ER in the storage protein deposition. Besides, the cotyledonary parenchyma cells also reveal clusters of ribosomes, abundant pleomorphic mitochondria (Fig. 1F), well developed starch containing plastids (Fig. 2A); and a few RER cisternae even encircle the vacuoles. The dictyosomes occur infrequently (Fig. 2C).

The protein deposition gradually fills a greater proportion of the vacuolar space. Each vacuole is progressively engorged with homogeneous materials resulting in well-defined protein bodies (Fig. 1D, E). The size, shape and number of these protein bodies varies in different cells (Fig. 1D, E). They are, however, uniformly filled with amorphous mass of proteins (Fig. 2D) and reveal tiny globoid inclusions within the structurally homogeneous proteinaceous matrix (Fig. 1E). The protein bodies, in addi-

tion also stain with periodic acid-SCHIFF's reagent which indicates a glycoprotein complex.

4. Discussion

The protein reserves are synthesized and deposited during the development and maturation of the seed and the major sites of storage protein in seeds are the protein bodies. In *Prosopis juliflora* mature embryo, the cells of ground meristem, cotyledonary storage parenchyma cells and procambium contain numerous spherical protein bodies with small globoid inclusions. ROST (1972) categorized protein bodies into three types on the basis of inclusions present in the proteinaceous matrix. Type I has no inclusions; Type II possesses only globoid inclusions and Type III has both globoid and crystalloid inclusions. Protein bodies with globoid inclusions occur in *Astragalus bisulcatus, Oxytropis lambertii* (LOTT & VOLLMER 1979), *Brassica campestris* (BHANDARI & CHITRALEKHA 1984) and *Prosopis velutina* (IRVING 1984). The size, shape, number and arrangement of globoid crystals and crystalloids vary in different plants. Globoid crystals are generally circular; whereas crystalloids are irregular or angular in shape. Globoid crystals in most of the taxa contain phytin, which is a cation salt of myo-inositol of hexaphosphoric acid. Energy dispersive X-ray analysis (EDX) reveal that P, K, and Mg are usually present in globoid crystals (LOTT 1980, 1981). The presence of druse crystals composed of calcium oxalate is reported in protein bodies of *Eucalyptus maculata* and *E. erythrocorys* (BUTTROSE & LOTT 1978; WHITE & LOTT 1983). The protein bodies in *Prosopis juliflora* reveal a glycoprotein complex as seen in *Prosopis velutina* (IRVING 1984) and *Simmondsia chinensis* (VIJAYARAGHAVAN & CHAUDHRY 1989).

Fig. 1. Cotyledonary cells of *Prosopis juliflora*. A-E, Light micrographs (stained with Coomassie blue) showing various stages of protein body formation. Each cell initially contains a large vacuole (A) that is partitioned into many small vacuoles by cytoplasmic extensions (arrows in B) with concomitant deposition of storage proteins on the vacuolar rim. These protein deposits rapidly increase in volume and size (C) and form a continuous ring. Each vacuole is progressively engorged with homogeneous materials that results in well-defined protein bodies (D, E). The protein bodies possess many small globoid inclusions (arrows in E) within the proteinaceous matrix (E). The procambial cells are also gorged with protein bodies (D). F. Electron micrograph of a portion of cotyledonary cell showing thick cell wall enclosing large intercellular spaces. Small lumps of homogeneous osmiophilic material (protein deposits) line the inner surface of the tonoplast of the vacuole. Long, extended profiles of rough endoplasmic reticulum can be seen adjacent to the developing protein body. (cw = cell wall, is = intercellular space, m = mitochondrion, pb = protein body, pc = procambium, prd = protein deposit, rer = rough endoplasmic reticulum, v = vacuole).

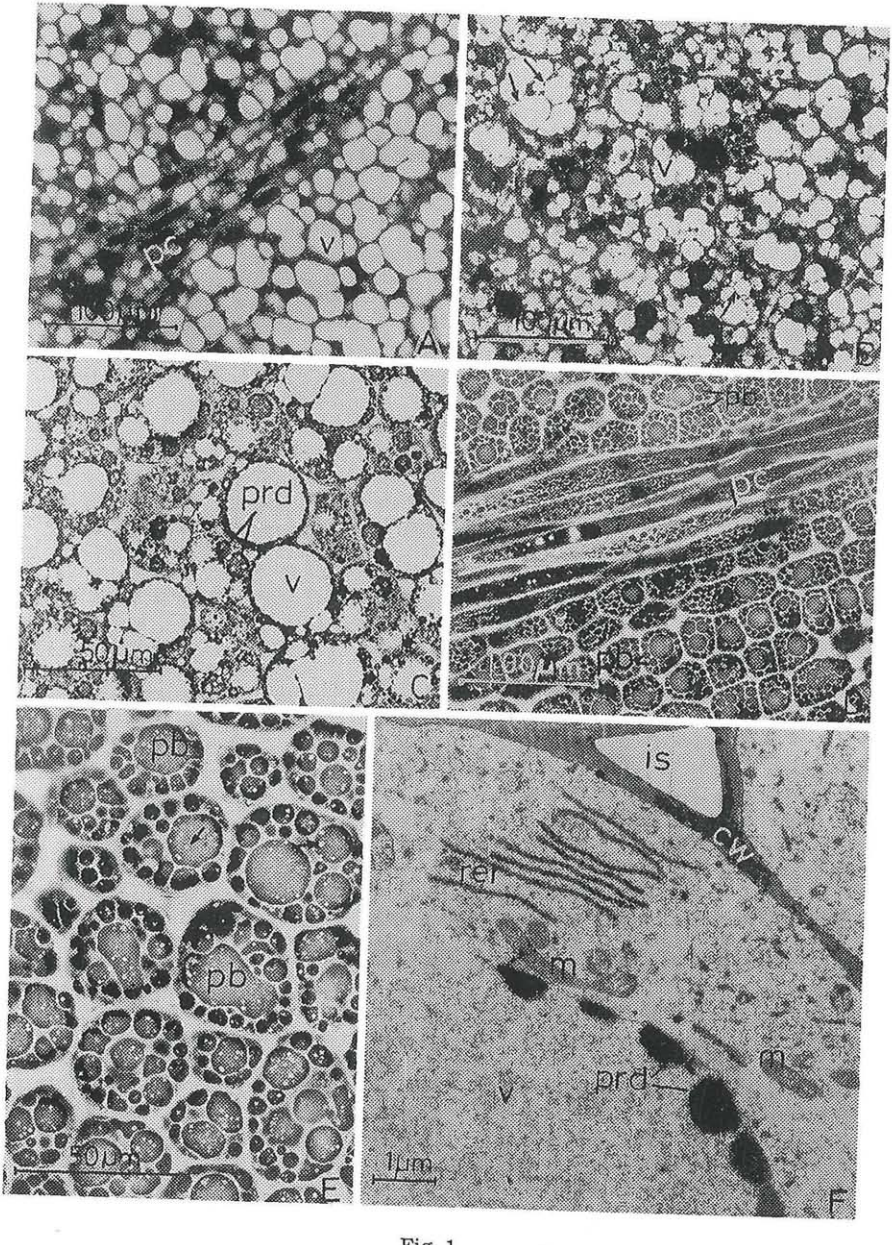

Fig. 1

In *Prosopis juliflora,* both light and electron micrographs of cotyledonary cells suggest that protein bodies have a vacuolar origin. The concept of origin of protein bodies from preformed vacuoles where the storage proteins, after synthesis in the cytoplasm, are discharged is well documented. In *Glycine max,* new protein bodies arise from central vacuole by pinching-off small masses of reserve proteins that are surrounded by portions of tonoplast (YOO & CHRISPEELS 1980). The splitting of a single, large vacuole into many small vacuoles that contain protein bodies is physiologically advantageous as it increases the relative surface area that is accessible to protein entry (CRAIG & al. 1979). Two different mechanisms of protein body generation have been reported in *Vigna unguiculata* (HARRIS & BOULTER 1976) and *Vicia faba* (ADLER & MÜNTZ 1983). The protein bodies arise initially from vacuoles and later during development, new protein bodies are formed from ER in *Vicia faba* whereas in *Vigna unguiculata,* dictyosomes are the source. HARA-NISHIMURA & al. (1987) show protein body biogenesis through budding from vacuoles in *Cucurbita* sp. ADLER & MÜNTZ (1983) postulate that the vacuoles which give rise to protein bodies originate from ER. The protein body formation from vacuole or RER, therefore, represents two variants of a uniform mechanism.

In *Prosopis juliflora,* a massive proliferation of RER coincides with the deposition of storage protein. Many workers agree that the synthesis of storage proteins in maturing seeds takes place at the rough ER. A strong support for the involvement of RER in the synthesis of storage proteins is provided by autoradiographic studies in *Vicia faba* (BAILEY & al. 1970). BOLLINI & CHRISPEELS (1979) have shown that polypeptides of the reserve proteins are synthesized *in vitro* by polysomes derived from RER but not by free polysomes. Storage proteins are sequestered in ER, after synthesis by ER-bound polysomes (BAUMGARTNER & al. 1980). However, the mechanism by which proteins bound for deposition in the protein body are transferred from the ER to the incipient protein body has remained controversial. Morphological studies have resulted in a variety of proposals including transport via vesicles derived from ER (HARRIS & BOULTER 1976, BAIN & MERCER 1966); smooth tubular ER (NEUMANN & WEBER 1978) or Golgi apparatus (DIECKERT & DIECKERT 1976). Transfer from the endoplasmic reticulum to the Golgi has been demonstrated for a variety of specific proteins. Several immunocytochemical studies show that proteins which accumulate in protein bodies are present in Golgi-cisternae and Golgi-associated vesicles (GREENWOOD & CHRISPEELS 1985, HERMAN & SHANNON 1984, KIM & al. 1988, see also STEER 1991). In *Prosopis juliflora,* a large number of RER derived vesicles appear near the protein-containing vacuoles and the occasional continuities of these vesicles with the tonoplast of vacuoles, are consistent with the hypothesis that protein synthesized on the membrane-bound ribosomes is transported to the vacuoles via vesicles formed at the edges of the cisternal ER. The dictyosomes are scarce in-

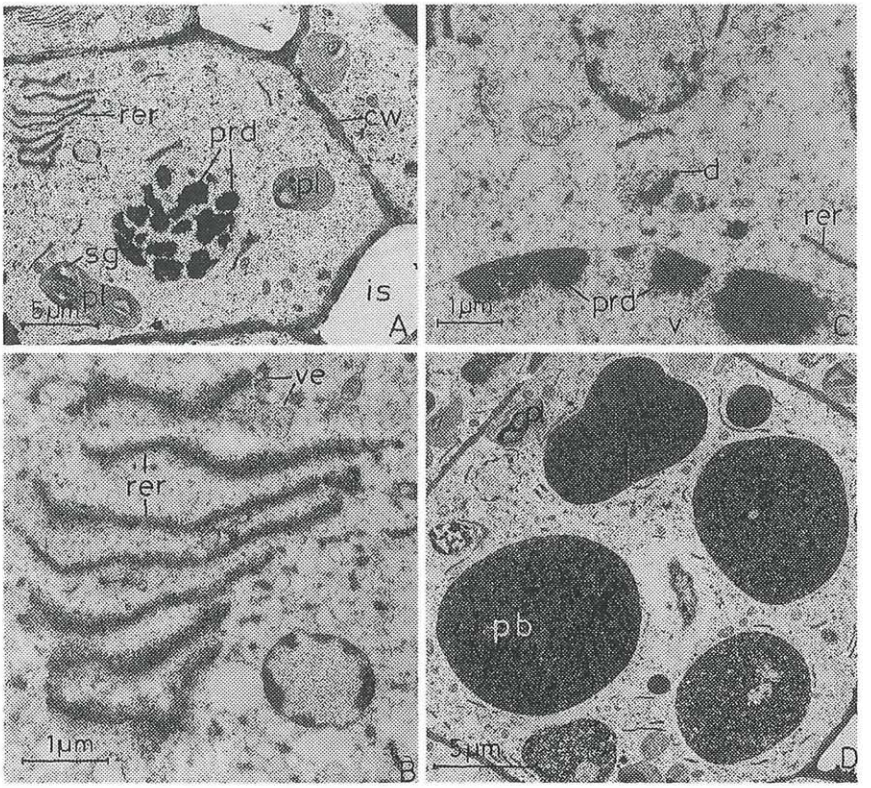

Fig. 2. Electron micrographs of cotyledonary cells of *Prosopis juliflora*. A: Portion of cell to show irregular protein lumps that line the vacuolar tonoplast and also lie free in the vacuole. The cytoplasm reveals profiles of rough endoplasmic cisternae and starch containing plastids. B: Same, to show RER cisternae with an electron-opaque lumen, pinching off vesicles into the cytoplasm. C: Portion of cell showing the vacuolar protein deposits, and dictyosomes in the adjacent cytoplasm. D: Cell showing discrete, protein bodies of spherical and irregular shape that are filled uniformly with amorphous protein. (cw = cell wall, d = dictyosome, is = intercellular space, pb = protein body, pl = plastid, prd = protein deposit, rer = rough endoplasmic reticulum, sg = starch grain, v = vacuole, ve = vesicle).

dicating that the dictyosome-derived vesicles are not an important conduit in transporting protein synthesized on the RER into the vacuoles. As pointed out by ROBINSON & KRISTEN (1982), a major function of plant dictyosomes is the synthesis of oligosaccharides, so the pathway from the endoplasmic reticulum may not be very active. The involvement of the ER vesicles in storage protein transport in the present work is supported by their proliferation in storage cells specifically during the period of storage protein

deposition and the lack of any other detectable structure carrying a similar material. In *Prosopis juliflora,* another characteristic feature of the cotyledonary cells is the presence of a large number of pleomorphic mitochondria, numerous clustered ribosomes and starch-containing plastids. CRAIG & al. (1979) have reported an apparent association between the tonoplast of vacuole (with adpressed protein deposits) and the clumps of ribosomes in the adjacent cytoplasm. BERGFELD & al. (1980) in *Sinapis alba* suggest that the rough ER, the golgi apparatus and the developing vacuoles are intimately involved in the formation of storage protein bodies. They have concluded that there are two routes for storage protein transport from its site of synthesis at the ER to its site of accumulation in the vacuole. The first route involves the participation of dictyosomes while the second route bypasses the golgi apparatus. However, in *Prosopis juliflora,* ER seems to be the only route for relocating protein into the vacuolar system although the possibility of dictyosomes contributing to the flux cannot be excluded. The final product of protein deposits is more or less identical in all the investigated taxa but it is enigmatic that the *modus operandi* of deposition is at variance in different taxa and is still a matter of conjecture.

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Recensio

DÖRING-MEDERAKE Ute 1991. Feuchtwälder im nordwestdeutschen Tiefland; Gliederung – Ökologie – Schutz. – Scripta geobotanica 19. – Gr. 8°, 122 Seiten, 33 Abbildungen, 13 Falt-Tabellen in Tasche; brosch. – Verlag Erich Goltze, Göttingen. – DM 40,00. – ISBN 3-88452-519-0.

Die grundwasserbeeinflußten Wälder des Tieflandes Niedersachsens wurden an Hand von 440 Vegetationsaufnahmen studiert und folgenden Waldgesellschaften zugeordnet: Carici elongatae-Alnetum (3 Subass.), Carici remotae-Fraxinetum (2 Subass.), Pruno (padi)-Fraxinetum (2 Subass.), Querco (robori)-Ulmetum (minoris), *Vaccinium uliginosum–Betula pubescens*-Gesellschaft und *Rubus idaeus–Alnus glutinosa*-Gesellschaft.

Außer den üblichen vegetationskundlichen Charakteristika wie floristische Zusammensetzung, Bestandesstruktur und Bodentyp wurden noch die folgenden ökologischen Parameter erhoben: pH-Wert, C/N-Verhältnis als Maß für die Humusqualität, Jahresgänge der N-Nettomineralisation und Jahresgänge der Grundwasserpegel.

Im Hauptteil des Bandes p. 21–84 werden die Pflanzengesellschaften dargestellt und ausführlich diskutiert. In einem eigenen Abschnitt (p. 85–104) werden die oben genannten Parameter in Relation zu den Pflanzengesellschaften ausführlich besprochen. Im letzten Abschnitt (p. 105–112) setzt sich die Autorin mit der Naturschutzsituation der Feuchtwälder kritisch auseinander. Durch Berücksichtigung von Aufnahmen anderer Autoren und durch überregionale Vergleiche (vor allem Carici elongatae-Alnetum, für dessen Subassoziationen auch die nomenklatorischen Typen festgelegt werden) entstand ein abgerundetes Bild der Feuchtwälder im Tiefland Niedersachsens.

H. TEPPNER