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Genesis of α-Arachin Synthesis in the Developing Peanut Seed

N. J. Neucere, L. Y. Yatsu, and James A. Harris
Southern Regional Research Center[1]
New Orleans, Louisiana 70179

ABSTRACT

An integrated ultrastructural and immunochemical study of storage protein synthesis was followed on early stages of developing peanut embryos. Whereas it was reported that storage proteins are not present in very immature embryos, e.g. prior to the formation of storage vacuoles or during cytokinesis, we detected α-arachin (the major storage protein) in the smallest embryos feasible to collect—embryos 0.5 to 1 mm long. These embryos contained meristematic cells 10 to 20 μ in diameter which showed the normal complement of organelles; however, no storage vacuoles were observed in these cells. Heavy synthesis of α-arachin coincided with the microscopic observation of protein granules in storage vacuoles of cells in embryos that were about 10 mm long.

Seed ontogeny can be divided into four phases: 1) cell formation, 2) cell expansion, 3) synthesis of storage substances, and 4) maturation-dormancy (2,3,4,5). It is believed that storage protein synthesis begins somewhere in the second phase and attains a maximum rate during the third phase. Cytological investigations support chemical data which show this; during the second phase, rapid proliferation of the rough endoplasmic reticulum occurs, polysomal configuration of ribosomes appear and storage vacuoles begin to form (2,5,9,14).

Immunochemical studies conducted on the very early stages of embryonic development seem to confirm that storage proteins are not synthesized prior to the second phase. Kloz *et al.* (10), surveying *Phaseolus vulgaris* L. embryo development, concluded that embryos smaller than 8 to 9 mm long did not synthesize phaseolin (storage protein). Recently, Millerd *et al.* (12), showed the presence of legumin (storage protein) in developing *Vicia faba* L. only in embryos over 5 mm long. Dieckert and Dieckert (7) were unable to detect arachin in peanut (*Arachis hypogaea* L.) embryos less than 8 mm long although they observed electron-dense inclusions in aleurone vacuoles. They concluded that aleurins (storage proteins) are not synthesized in the embryo until a critical stage of ontogeny—presumably sometime after the 5 mm stage.

In this communication we present the integrated studies of both immunological and fine structural investigations of developing peanut seeds. Attention is focused on the synthesis of the major storage protein, α-arachin, during the very earliest stages of embryonic development; we show that this storage protein is present in the earliest samples feasible to collect.

Materials and Methods

Due to their unusual fruiting habit, physiological stages of the developing peanut are difficult to determine (16); therefore, we arbitrarily chose size of embryos as the parameter for selecting our samples. Developing embryos ranging from 0.5 to 20.0 mm long from plants (**Virginia 56R**, 1969 crop) growing in Slidell, Louisiana, were collected up to 80 days from planting. Tissues were homogenized in 0.072M phosphate buffer, pH 7.9, at room temperature and the homogenates were centrifuged at 37,000 g for 30 min at 20 C. The supernatant liquids were frozen and stored at -20° C until ready for experimentation. For microscopy, sections were cut from fresh tissue and fixed as previously reported (17).

Protein analyses were performed according to Lowry **et al.** (11). Qualitative and quantitative analyses of α-arachin by radial immunodiffusion was performed according to Ouchterlony (15). Immune serum was prepared by Antibodies, Inc.[2], Davis, California; analyses were conducted in 1.5% ionagar No. 2 (Oxide Ltd., London) in 0.025 Veronal buffer, pH 8.2 at 25 C.

Results and Discussion

Morphologically, embryos 0.5 to 1 mm long were past the heartshaped stage of development and possessed minute, discoid cotyledons which flanked miniscule axes. Cells of cotyledons from these embryos were about 10 to 20 μ in diameter and obviously meristematic (Figures 1 and 1a). They were isodiametric, contained ground substance rich in what appeared to be free ribosomes, had relatively large nuclei, plastids, mitochondria, and an abundance of ergastic substances such as starch and lipid. Elements of the endoplasmic reticulum and dictyosomes were relatively sparse in most cells. By the time the embryos were 3 to 7 mm long, the cells no longer appeared meristematic (Figure 2); they were larger, no longer isodiametric, contained relatively smaller nuclei, possessed numerous vacuoles and consequently relatively less protoplasm. Even so, occasional signs of cell division were still evident (see arrow in Figure 2).

Qualitative immunochemical analyses of proteins contained in the very young embryos are described in Figure 3. Part A shows the immuno-

[1]One of the facilities of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.

[2]It is not the policy of the Department to recommend the products of one company over those of any others engaged in the same business.

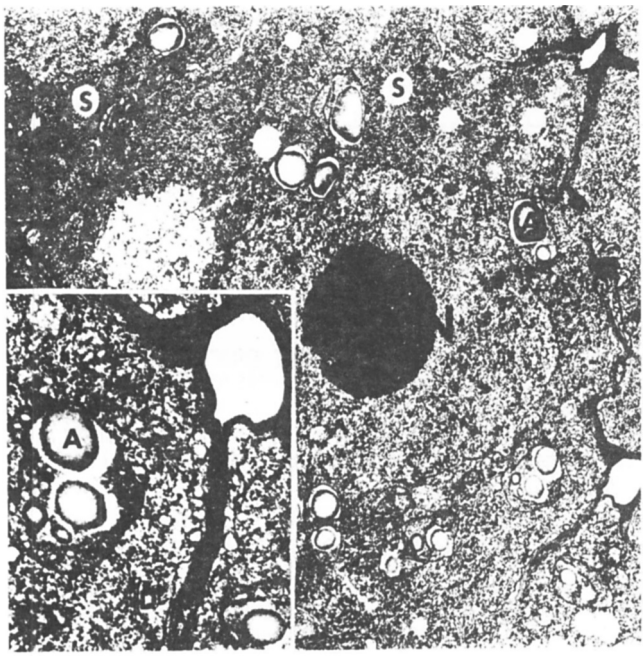

Fig. 1. Electron micrograph of a cotyledon cell from a peanut embryo less than 1 mm long. The cell appeared typically meristematic with a relatively large nucleus (N) and dense cytoplasm. Ergastic substances in the form of starch granules within amyloplasts (A) and lipid in spherosomes (S) were seen. X 8250.

Fig. 1a. Slightly higher magnification of a portion of the cell shown in Figure 1. Note the ground plasm and what appears to be numerous free ribosomes. Other organelles, e.g., amyloplasts (A), mitochondria (M), and dictyosomes (D), can be seen. Particularly noteworthy is the differential staining of membranes: amyloplast membranes stained most intensely. X 17,500.

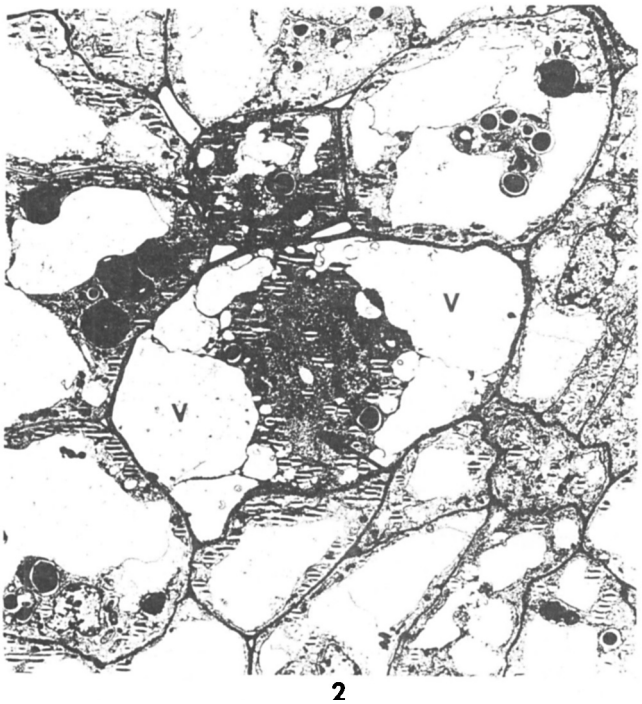

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Fig. 2. Cross-section of a cotyledon from typical peanut embryos 3 to 7 mm long. Cotyledonary cells at this maturity had numerous vacuoles (V), varied in size, and no longer appeared meristematic; yet, there were still occasional signs of cytokinesis (see arrow). X 1800.

electrophoretic characterization of α-arachin (in an isolated arachin fraction) with respect to other proteins in a total cotyledonary extract reported by Daussant *et al.* (6). For purposes of identification, the major precipitin line designated by α-a was named α-arachin and the other minor serologically distinct antigens (small arrows) were designated as "contaminants" of α-arachin. Therefore, the bulk of the protein in the isolated fraction consists of α-arachin. In part B, qualitative immunodiffusion of the 0.5-1.0 mm long embryo extract (well #1) and α-arachin (well #2) using immune serum from the purified arachin fraction (well #3) showed the coalescence of precipitin lines (α-2) indicating a reaction of identity. The amount of α-arachin in the 0.5-1.0 mm long embryo extract was exceedingly meager as evidenced by the thinness of the precipitin line and the final locus of the equivalence point. In embryos about 2.0 mm long (part C), α-arachin was still present in only trace quantities (arrow); other proteins common to the cold - insoluble protein fraction of peanuts were evident. The heavy precipitation of α-arachin along with lesser quantities of other proteins at low temperature has been described (13).

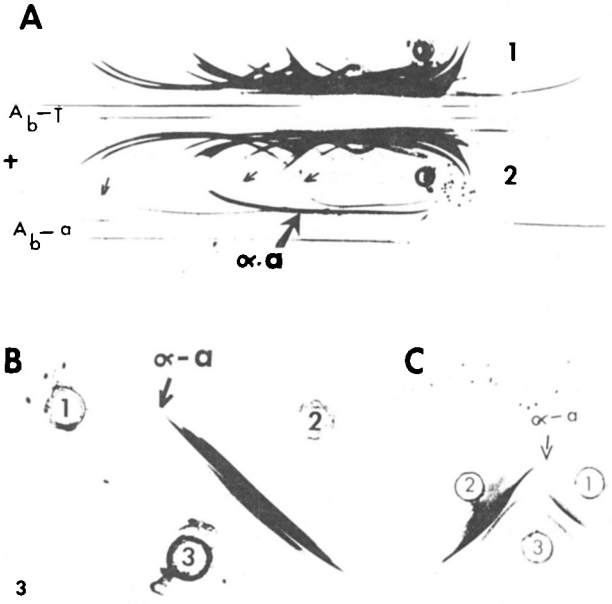

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Fig. 3. Qualitative analysis of α-arachin in the very early stages of seed development. Wells 1 and 2 in Part A contained total cotyledonary proteins analyzed against total immune serum (Ab-T), and immune serum made against purified arachin (Ab-a). Coalescence of precipitin lines showed reactions of identity; α-arachin is the major component in the fraction (after Deussant, et al., reference 6). In part B, well No. 1 was filled with a extract from embryos 0.5-1.0 mm long, well No. 2 contained about 100 μg of α-arachin, and well No. 3 contained anti-arachin identical to that in part A. In part C, well No. 1 contained approximately 200 μg of protein extracted from 2 mm long embryos and well No. 2 contained about 100 μg of protein from the cold-insoluble fraction of peauts; immune serum made against the latter fraction was placed in well No. 3.

Quantitative analysis of α-arachin in developing embryos through antogeny is shown in Figure 4. Using immune serum from purified α-arachin in the center wells (Ab), extracts of progressively larger embryos (Ag) were serially diluted for quantitation; the starting point for each sample is designated by small arrows. The relative concentration of α-arachin in extracts from embryos up to 10 mm long (parts A and B) was obviously very small compared to those in later stages of maturation (parts C and D). We estimate that embryos 3-7 mm long contained less than 1% of their total protein as α-arachin.

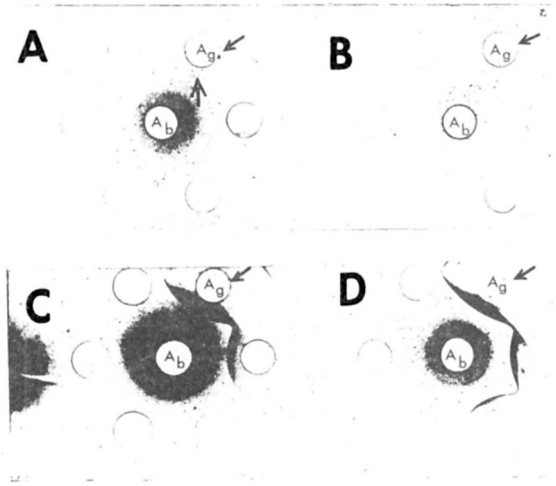

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Fig 4. Quantitation of -arachin through ontogeny by serial immunodiffusion. From the small arrows clockwise, total extracts (Ag) of embryos 3-7 (A), 7-8 (B), 10-12 (C), and 15-17 (D) mm long, each containing 60 .. g protein, were diluted serially. Immune serum against purified -arachin was used in the center wells (Ab). Note the mere trace of -arachin (large arrow) in the 3-7 mm embryo extract. Limit precipitation of isolated -arachin occurs at less than one g after standard serial dilution.

The earliest samples in which large amounts of α-arachin was detected (part C of Figure 4) coincided exactly with the observed accumulation of large electron-dense inclusions within storage vacuoles. These results agree with Dieckert and Dieckert (7); it is of interest. however, that they were unable to detect α-arachin in embryos less than 8 mm long. Perhaps the titer of their immune serum was too low for *in vitro* precipitation.

On the basis of both light and electron microscopic histochemical studies, Graham and Grunning (8) concluded that storage proteins were sequestered in storage vacuoles. Bailey *et al.* (1) showed evidence for the movement of pulse-labeled protein from the endoplasmic reticulum into storage vacuoles. Dieckert and Dieckert (7) observed electron-dense material in the lumen of the endoplasmic reticulum which resembled the electron-dense material in storage-vacuoles early in ontogeny. Thus, it would appear that (as in zymogen granule formation) storage protein is synthesized in the endoplasmic reticulum, perhaps concentrated in the dictyosomes, and stored in storage vacuoles, aleurone grains. We agree that the great mass of protein synthetic activity occurs in the manner described by Dieckert and Dieckert (7), however, we do not accept the notion that storage protein synthesis does not occur until a critical moment in ontogeny. However small the quantity, our results showed the presence of α-arachin before obvious electron-dense inclusions were observed in storage vacuoles. In fact, α-arachin was detected in cells which had not yet formed storage vacuoles. Therefore, the potential to synthesize storage protein existed in meristematic cells that did not possess vacuoles.

Thus, although most investigators believe that storage proteins are not synthesized in the very early stages of ontogeny, we wish to point out that in peanuts, at least, storage protein is present even during cytokinesis. Certain workers have suggested the use of plant seeds as guinea pigs for the study of protein synthesis: seeds are presumed to contain a system in which storage protein synthetic activity is totally repressed and is derepressed at a certain point in ontogeny. However, it appeared from our study, that *most* genes responsible for the synthesis of α-arachin are repressed in very young embryos, but not all of them.

Acknowledgment

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