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PROTEIN BODY AND CELL WALL DEVELOPMENT IN SOYBEAN COTYLEDONS

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ABSTRACT

Cell wall and protein body development were examined in three planes of section from samples taken during the linear phase of growth, 5 through 11 mm seed length (14 to 24 days after anthesis). Cells sectioned radially or longitudinally were elongate, but were circular in transverse section (perpendicular to the flat interior surface of the cotyledon in the seed). No mitotic figures were found. Protein bodies formed first in the subepidermal layer of palisade cells and in cells surrounding the vascular tissue, generally with one major body per cell. The major protein body enlarged, retaining the general shape of the elongating cell, with numerous small protein bodies forming on the periphery of the central vacuole. The ability of the cells to imbibe water was correlated with the rapid increase in cell wall thickness as the growth rate declined and the green seeds entered the maturation phase.

INTRODUCTION

Bils and Howell (1963) found that the nucleus is the most prominent structure in the parenchyma cells of young cotyledons, although a few proplastids, mitochondria and "ribonucleoprotein particles" are present. Cell division ceases by 15 days following anthesis (Mori, et al., 1978). An obvious vacuole becomes visible by day 18 with lipid granules, immature chloroplasts, starch particles and numerous mitochondria visible within the cytoplasm. A few large protein bodies are present by day 26 and the size of these bodies increases rapidly as the cell volume increases tenfold over the next 10 days (Bils and Howell, 1963). Storage proteins, once synthesized, do not break down during soybean seed formation (Madison, et al., 1981). This paper examines protein body and cell wall development during the stage of rapid cell enlargement.

MATERIALS AND METHODS

Seeds of soybean (*Glycine max* L. Merr. var. Williams) were planted June 5, 1979 at the Western Illinois University Experimental Farm. Twenty plants were selected at developmental stage R8, using the scheme developed by Fehr, et al. (1971). The lowermost flowering node of these plants was tagged and observed. One seed per pod was measured every two days along the longest axis from the time pods developed with seeds measuring 5 mm in length until the stationary phase was reached. Measurements were made while the seeds were in the attached pods by illuminating the pods and measuring the outline of the seeds within the pod using a vernier caliper.

Ten soybean seeds were harvested at four different stages of development (5.0, 7.0, 9.0, and 11.0 mm seed lengths). One of the two cotyledons from each seed was sectioned radially and longitudinally. A transverse section was obtained from the other cotyledon of the pair (Fig. 1). The tissues were immediately fixed in 3% glutaraldehyde in 0.05 M PO_4, pH 6.8 for 1 1/2 hours. The fixative was aspirated and the tissue was rinsed three times in 0.05 M PO_4, pH 6.8 for a total of 1 hour immersion. Postfixation was accomplished using 2% OsO_4 (0.05M PO_4) for 2 hours. The OsO_4 was aspirated and the tissue was rinsed in 0.05 M PO_4. Fresh buffer was added and the tissue was stored at 15° C.

Dehydration of the sections was accomplished using an ethanol series (35, 50, 70, 95, and 100%), and then cleared by 10 min immersion in a series of ethanol:xylene mixtures (2:1, 1:1 and 1:2) followed by 100% xylene. The sections were infiltrated with paraffin in a vacuum oven with paraffin changes at 1 1/2 hour and 1/2 hour intervals. Paraffin sections 10 microns thick were cut using a microtome, and transferred to albumin-treated slides. Slides were dried overnight and treated to two 10 min changes of xylene, followed by an ethanol series (100, 95, 70, and 35% mixtures, 2 min each); 10% mercuric bromophenol blue (15 min); 0.5% acetic acid (20 min); an ethanol series (35 and 70% ethanol, 30 sec each, followed by a 20 sec exposure to 95%, and a 2 min exposure to absolute ethanol); and xylene (two changes, 10 min and 5 min). Sections were permanently mounted using Canadian Balsam and photographed using a Zeiss microscope fitted with a phase-contrast 25x objective. Copies of all photographs were analyzed to determine cell number, wall thickness, maximum protein body diameter, number of protein bodies per cell, and cell number per mm^2.

The water content of other cotyledon samples was determined at each of the developmental stages by removing and weighing one cotyledon of each pair, drying it at 100°C, and reweighing it. The other cotyledon of the pair was weighed and then transferred to tap water for 48 hrs. The fully imbibed cotyledons were blotted, reweighed, and also dried. Water contents were expressed as the difference between the fresh and imbibed weight and weight after drying at 100° C.

RESULTS AND CONCLUSIONS

The increase in seed length described a sigmoid curve (Fig. 2). Samples were harvested during the linear phase, between seed lengths of 5.0 and 11.0 mm, and during the stationary phase after the seed exceeded 11.0 mm in length but before dessication at maturity resulted in a decrease in seed length. No mitotic figures were observed; growth was therefore a function of cell enlargement rather than

cell division. Parenchyma cells of the cotyledons sampled at 5 mm length were approximately isodiametric in transverse section (Fig. 3), and elongate in longitudinal and radial section (Figs. 4 and 5). As the cotyledon length increased, cells enlarged in all directions.

Protein bodies formed first in areas close to or in the developing vascular tissue, and in a subepidermal area on the adaxial surface in the same manner as reported by Graham and Gunning in *Vicia faba* (1970). Protein bodies were present in the epidermal layer, although to a lesser extent than in the subepidermal layer.

A highly uniform population of protein bodies was present in the palisade layer of cotyledons 5.0 mm in length (Fig. 6). Each palisade cell contained one centrally-located protein body supported by cytoplasmic strands. Protein bodies first appeared near the plasmalemma in the youngest cotyledons sampled (5 mm). The protein bodies were spherical in all planes of section, and were approximately 4.7 microns in diameter. Most cells contained one protein body at this stage of development.

The dimensional changes in protein bodies during development are summarized in Table 1. The primary protein body increased in size to a mean diameter of 5.6 microns in cotyledons of seeds 7 mm long with additional, small protein bodies occurring within the cytoplasm (Fig. 7). The primary protein bodies retained their spherical shape in transverse section. However, the protein bodies changed from a spherical to an ellipsoid shape, conforming to changes in the shape of the cells containing them. No fusion of protein bodies was observed. Transverse sections of cells showed spherical protein bodies, although in some cases their shape was distorted by other protein deposits as the cells matured. The mean diameter of the principal protein bodies was 10 microns in 9 mm seeds, and they were ellipsoid in radial and longitudinal sections with a long axis of 11.0 microns. Storage protein deposition continued throughout cell enlargement, until protein bodies and cell walls were the dominant features in sections taken from green cotyledons after they had reached their maximum length of 11 mm (Figs. 8-10).

Cell wall apposition contributed greatly to total seed volume as the seed matured. Cell walls were indistinguishable from cytoplasm adjacent to the plasma membrane in sections taken from the 5.0 mm seed length samples. Cell wall deposition was evident at the seed length of 7 mm, with a mean thickness of 0.7 microns between adjacent protoplasts. Cell walls continued to thicken as the seed elongated; at 9.0 mm seed length the mean wall thickness was 3.1 microns and at 11.0 mm seed length it was 4.7 microns. Walls began thickening first on the abaxial side of the cotyledon. The increase in cell wall thickness is proportional to the ability of these green seeds to imbibe water (Fig. 11). This study suggests that cell wall thickness may be an important factor in seed germination. The relationships between cell wall mass and germinability deserve further study.

ACKNOWLEDGEMENT

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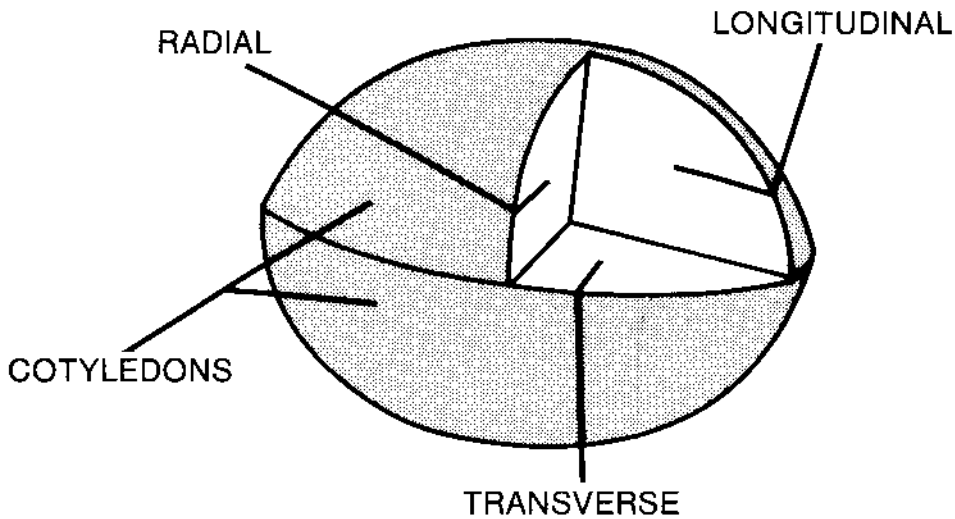


Fig. 1. Sectioned planes of the soybean cotyledon.

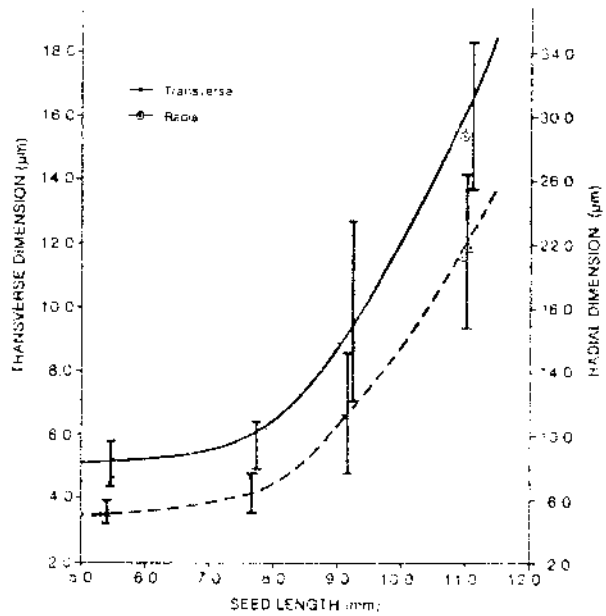
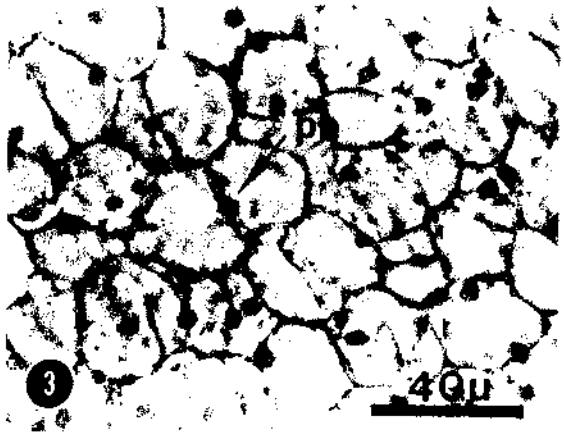


Fig. 2. Growth curve of the developing soybean cotyledon. Vertical bars represent standard deviation about the mean.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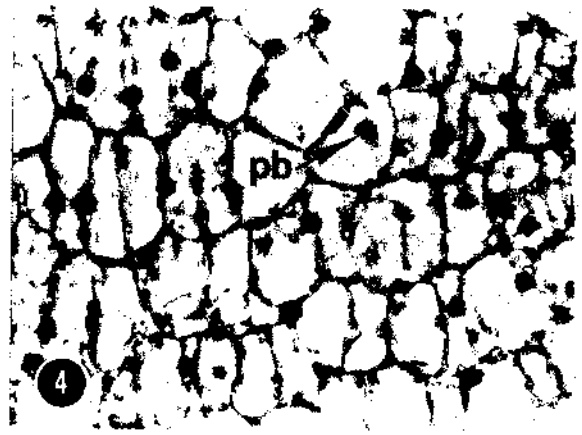
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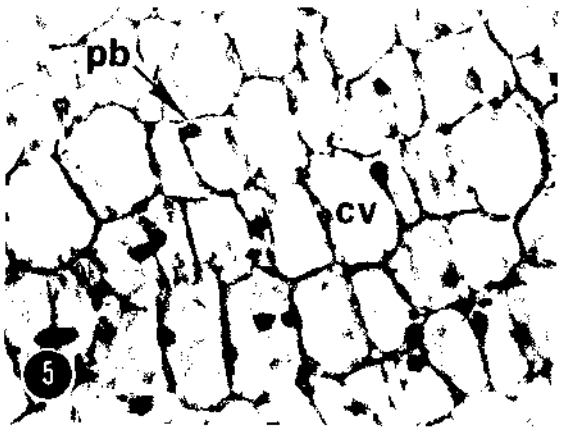
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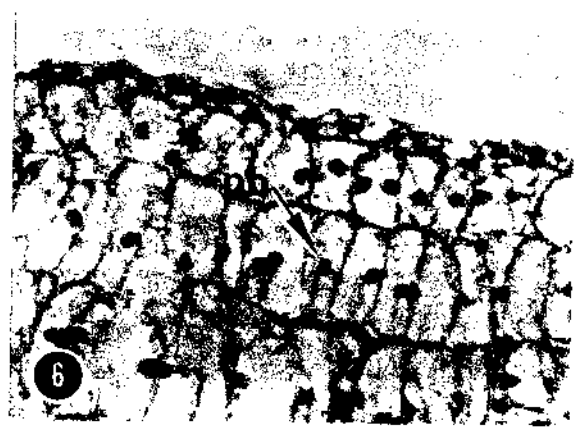
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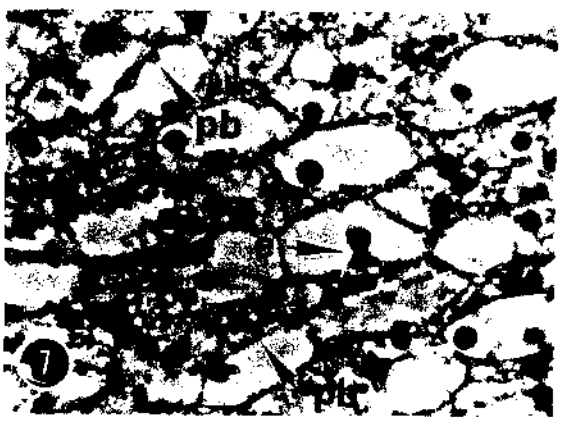
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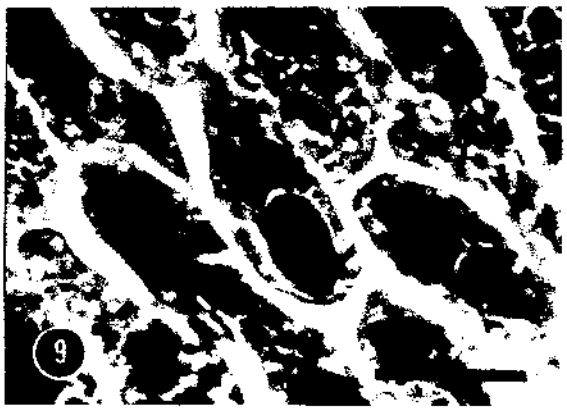

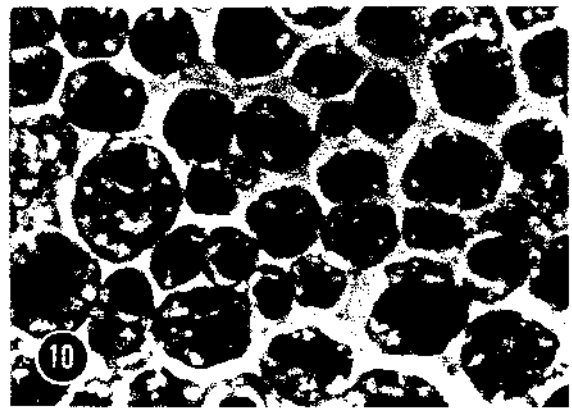

Fig. 3. Parenchyma cells exposed in transverse section in a cotyledon 5.0 mm in length. Approximately one major protein body can be seen between the central vacuole and cell membrane in each cell. Protein body: pb.

Fig. 4. Parenchyma cells exposed in radial section in a cotyledon 5.0 mm in length. Slightly elongated cells containing one major protein body are present. Protein body: pb.

Fig. 5. Parenchyma cells exposed in longitudinal section in a cotyledon 5.0 mm in length. The central vacuole and the protein body are the dominant cellular features. Central vacuole: cv. Protein body: pb.

Fig. 6. Epidermal and palisade layers of longitudinally sectioned cotyledons 5.0 mm in length. A highly uniform population of protein bodies is present on the adaxial surface of the soybean cotyledon. Protein body: pb.

Fig. 7. Parenchyma cells exposed in radial section in a cotyledon 7.0 mm in length. Small protein bodies are seen developing within the cytoplasm and an evagination of the central vacuole containing proteinaceous material is present. Protein body: pb. evagination: e.

Fig. 8. Parenchyma cells exposed in radial section in a cotyledon 11.0 mm in length. Elongated cells with tapered ends are filled with darkly stained proteinaceous material and surrounded by thick cell walls. Protein body: pb.
Note: All photographs are taken at the same magnification. Two centimeters represents 40 microns.

Fig. 9. Parenchyma cells exposed in longitudinal section in a cotyledon 11.0 mm in length. Elongated cells with tapered ends are filled with proteinaceous material and surrounded by thick cell walls.

Fig. 10. Parenchyma cells exposed in transverse section in a cotyledon 11.0 mm in length. Cells spherical in shape are filled with proteinaceous material and surrounded by thick cell walls.
Note: All photographs are taken at the same magnification. Two centimeters represent 40 microns.

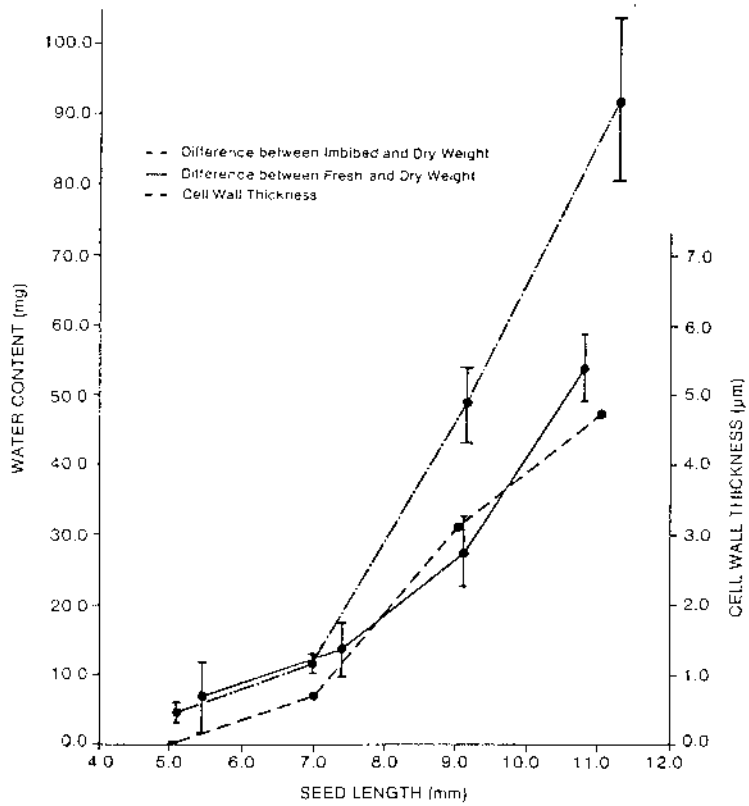

Fig. 11. Relationship between cell wall thickness and water content of the developing soybean cotyledon. Vertical bars represent standard deviation about the mean.

Table 1. Number of Major Protein Bodies per Cell Visible in Three Planes of Section at Four Stages of Cotyledon Development.

Mean Seed Length (mm)	Number of Major Protein Bodies per Cell*		
	Transverse	Radial	Longitudinal
5.44	0.49 ± 0.10	0.58 ± 0.18	0.52 ± 0.12
7.70	0.42 ± 0.11	0.91 ± 0.33	0.57 ± 0.13
9.24	0.40 ± 0.05	0.79 ± 0.20	0.69 ± 0.10
11.08	0.55 ± 0.11	0.69 ± 0.33	0.59 ± 0.23

*Expressed as a mean ± one standard deviation.