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Ultrastructural Changes in Mesophyll Cells of Developing Seed Cotyledons of Pharbitis Nil

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ABSTRACT

The cellular ultrastructure of mesophyll cells of cotyledon tissue from seeds of *Pharbitis nil* (Japanese morning glory) was studied. As the cotyledons of the seed develop, the mesophyll cells undergo marked changes. Mesophyll cells of the seed cotyledon 17 days after anthesis appear highly vacuolated. The appearance of lipid bodies, protein bodies, and large amounts of carbohydrates within the plastids characterize the cells of the seed cotyledons 30 days after anthesis. The most striking difference between 30-day-old and 40-day-old seed cotyledon mesophyll cells is the appearance of phytin within the protein bodies of the 40-day-old seed cotyledon mesophyll cells.

INTRODUCTION

Pharbitis nil is a short-day plant that has been used to study the photoperiodic requirements which initiate flowering (Fredericq, 1963; King and Vince-Prue, 1978).

Seeds of *Pharbitis nil* have been used to study changes in gibberellin-like substances during seed development (Ogawa, 1963), and to study the effect of compounds which inhibit GA biosynthesis (Zeevaart, 1966). Although Hayward (1932) briefly mentioned the development of seed cotyledons in *Ipomoea batatas*, and Wada et al. (1981) studied the development of giant oil cells in seed cotyledons of *Pharbitis nil*, the literature contains no reports of the ultrastructure of these organs.

A developmental study of the ultrastructure of the developing seed cotyledons of this species, therefore, offered the possibility of giving additional insight into ultrastructural changes which take place during seed development. Because of this, the present investigation was undertaken.

MATERIALS AND METHODS

Seeds of *Pharbitis nil* Chois., strain Scarlett O'Hara (Burpee Seed Company, Clinton, Iowa 52732) were manually scarified and germinated within layers of moist paper towels. Upon germination, the seeds were transplanted to fertile soil in 6 inch clay pots and placed under 18-hour photoperiod in the greenhouse. After several weeks, the plants were transferred to a 12-hour inductive photoperiod. Individual flowers were marked on the date of anthesis. Cotyledons dissected from seeds 17, 30, and 40 days after anthesis were fixed in 2.5% glutaraldehyde in 0.15 M phosphate buffer at pH 7.4 for 3 hours at room temperature. The tissues were then washed with 0.15 M phosphate buffer and post-fixed in 1% osmium tetroxide in 0.15 M phosphate buffer, pH 7.4, for 2 hours at room temperature. The tissues were dehydrated in an ethanol-acetone series and embedded in Spurr (1969) low viscosity resin. Thin sections were picked up on uncoated 300 mesh copper grids and post-strained for 3 minute in Reynolds (1963) lead citrate and examined with a Hitachi HS-9 transmission electron microscope at 75 kV.

For the cytological localization of carbohydrate and protein, cotyledons dissected from seeds 30 days after anthesis were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 6.8, for 6 hours at 4 C. The tissues were dehydrated in an ethanol, n-propanol, and n-butanol series at 4 C and embedded in glycol methacrylate (Feder and O'Brien, 1968). Thick sections were stained with analine blue-black for total protein (Fisher, 1968), and with periodic acid-Shiffs reagent for carbohydrate (Feder and O'Brien, 1968), and viewed with a Leitz Ortholux light microscope.

RESULTS

Mesophyll cells of seed cotyledons examined 17 days after anthesis contain many vacuoles (Fig. 1). The presence of a large nucleus, plastids, mitochondria, dictyosomes, and cisternae of endoplasmic reticulum characterize the cellular ultrastructure of this cotyledonary tissue. The plastids display a moderately developed grana-fretwork system, some starch deposition and still show a prolamellar body (Fig. 2).

Twelve additional days of seed maturation result in dramatic changes in the seed cotyledons. Cytochemical stains specific for carbohydrate (Fig. 3) and for total protein (Fig. 4) reveal that both of these substances are present in copious amounts within the mesophyll cells. Electron micrographs of mesophyll cells the same age as those pictured in Fig. 3 - 4 indicate that areas staining positive for carbohydrate are plastids, and areas staining positive for protein are protein bodies (Fig. 5). Numerous lipid bodies occupy the peripheral region of the mesophyll cells. The protein bodies have an irregular outline and are filled with a homogenous granular/fibrillar material. All of the mesophyll cells at this cotyledonary stage of development contain protein bodies. They usually contain one or more inclusions called globoids (Fig. 5). Other cytoplasmic organelles present are mitochondria and plastids with voluminous starch grains.

Mesophyll cells of seed cotyledons that have matured for 40 days are slightly different from those of 30-day-old seed cotyledons. Globoids usually contain large electron-opaque crystals of phytin (Fig. 6). Lipid bodies, identified by their electron-transparent nature, occupy a large area of the cytoplasm. Plastids are also present in cells of 40-day-old seed cotyledons. These usually contain voluminous starch grains (Fig. 6).

DISCUSSION

Examination of photomicrographs of mesophyll cells of seed cotyledons indicates that these cells undergo marked changes during seed development. The most noticeable change is the appearance of lipid bodies and protein bodies in the mesophyll cells 30 days after anthesis. By the time the seed cotyledons reach an age of 40 days, virtually all of the cell volume not occupied by nuclei and plastids is filled with lipid bodies and protein bodies.

Protein bodies, once formed, usually contain one or more inclusions called globoids (Bewley and Black, 1978). Globoids are the site where calcuim, magnesium, or potassium salts of phytic acid (phytin) crystallize (Lott, 1975). Phytin crystals, not seen in globoids of protein bodies of 30-day-old cotyledon mesophyll cells, are abundant in globoids of protein bodies of 40-day-old cotyledon mesophyll cells.

Plastids of seed mesophyll cells contain starch grains (carbohydrate). These starch grains appear to be much smaller in the plastids of 17-day-old mesophyll cells than in the plastids of 30-day-old mesophyll cells. This indicates that starch gradually accumulates with time following anthesis.

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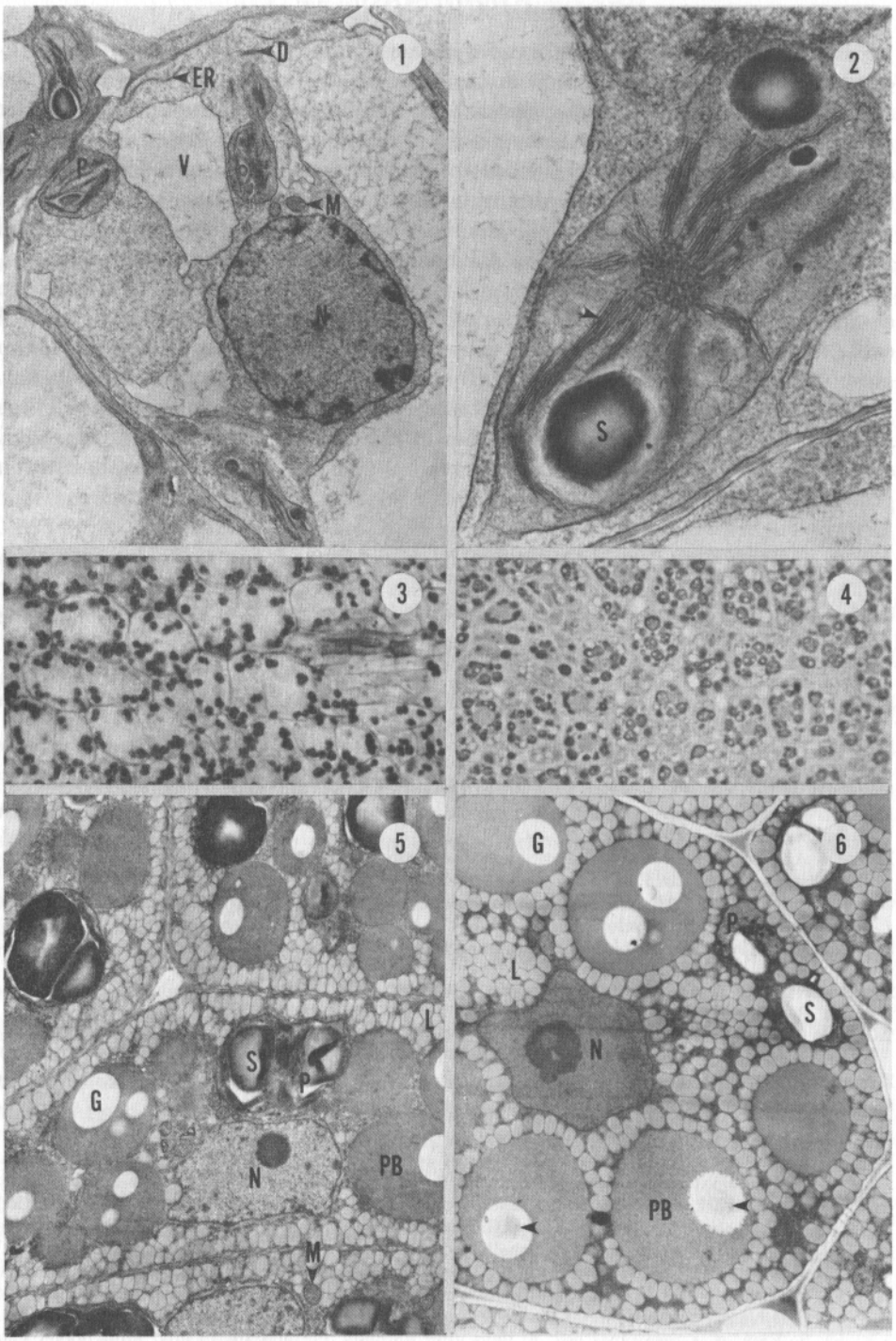
Fig. 1-6. Ultrastructural features of developing seed cotyledon mesophyll cells of *Pharbitis nil*. 1. View of a mesophyll cell 17 days after anthesis. Most of the space within the cell is occupied by vacuoles. X 7,500. 2. View of a plastid showing details of the internal membrane organization (17 days after anthesis). Stacks of thylakoids forming grana are evident (arrow). Carbohydrate in the form of starch and a prolamellar body can also be seen. X 30,000. 3. Mesophyll cells 30 days after anthesis stained with PAS for carbohydrate. Many localized regions of positive staining are present. X 1,350. 4. Mesophyll cells 30 days after anthesis stained with analine blue-black for total protein. Many localized regions of positive staining are present. X 1,350. 5. View of a mesophyll cell 30 days after anthesis. Peripherally-located lipid bodies and centrally-located protein bodies are visible. Globoids are present within the protein bodies. Nuclei and plastids (with starch) are also present. X 7,500. 6. View of a mesophyll cell 40 days after anthesis. Numerous protein bodies, lipid bodies, and plastids are present. Note phytin crystals contained in globoids (arrow). X 7,500.



Key to labeling: V, vacuole; N, nucleus; S, carbohydrate; G, globoid; P, plastid; PB, protein body; PL, prolamellar body; L, lipid; M, mitochondria; D, dictyosome; ER, endoplasmic reticulum.