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BRIEF COMMUNICATION

PROTEIN BODY DIGESTION IN POLYGONUM PENSYLVANICUM EMBRYOS DURING PRECHILLING

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The protein reserves of several seeds are stored in prominent spherical organelles called protein bodies. These organelles contain protein and phytin reserves (MATILE 1968); they also contain several hydrolytic enzymes (ribonuclease, proteinase, and lypolytic enzymes) (HARRIS & CHRISPEELS 1975; CHRISPEELS et al 1976; BAUMGARTNER et al. 1978; CHAPPEL et al. 1980; VAN DER WILDEN et al. 1980; HERMAN et al. 1981). During prechilling, protein body digestion has been associated with the decline in dormancy of seeds (ROST 1972; VILLIERS 1980, JORDAN et al. 1982). Although protein body digestion is important for decreased dormancy and increased germination, no reports could be located concerning protein body digestion in different cell layers of a seed during prechilling. Therefore, two distinct, yet adjacent, cell layers in embryos from *Polygonum pensylvanicum* L. were studied using a transmission electron microscope (TEM) before and after the achenes (one-seeded indehiscent fruit) had been prechilled for 30 weeks.

Mature *Polygonum pensylvanicum* achenes were collected at four maize fields in Iowa by placing aluminum screens below the plants and later removing the achenes from the screens. Four random samples of 100 achenes from each site were used to determine percentage moisture before prechilling tests were conducted; moisture percentage was also measured after prechilling. To measure percentage moisture, achenes were weighed before and after drying in a 105°C forced-air oven for 3 hrs.

Seeds were prechilled between two pieces of moist Whatman No. 1 filter paper in 9 cm diameter petri dishes at 2°C for 30 weeks in the dark. The filter paper had been moistened with 5 ml distilled water prior to prechilling. To test germination, nonprechilled and prechilled seeds were incubated for 1 week at 35°C in the dark. Each test had 5 replications and 100 achenes, and the experiment was repeated 3 times.

Embryos of seeds, either nonprechilled or prechilled for 30 weeks, were manually excised; seeds used for embryo collection were not exposed to the germination conditions described previously. The excised embryos were fixed immediately after excision in 2% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.0, 23°C), rinsed 6 times with 0.05 M cacodylate buffer (pH 7.0), postfixed

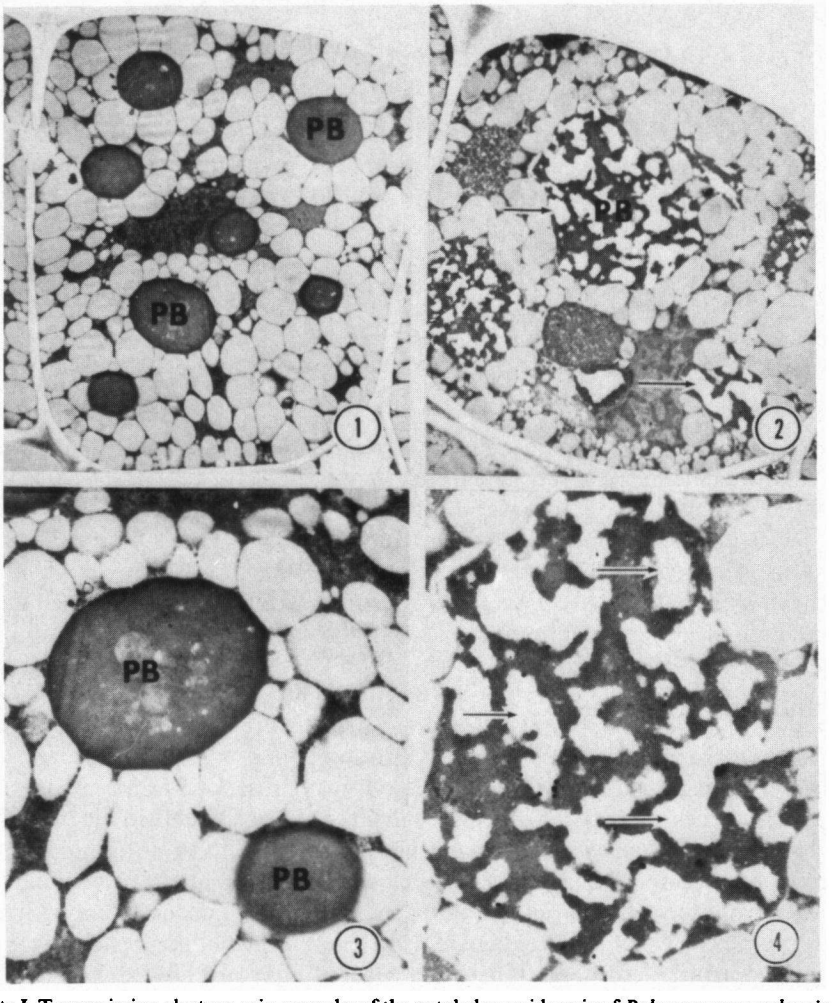

Plate I. Transmission electron micrographs of the cotyledon epidermis of *Polygonum pensylvanicum* embryos. PB = Protein bodies. Arrows indicate areas of extensive protein body digestion. Note the roughened appearance of the digested regions.
Fig. 1. Epidermal cell from a nonprechilled seed.
Fig. 2. Epidermal cell from a seed prechilled at 2°C for 30 weeks.
Fig. 3. Closer view of protein bodies in an epidermal cell from a nonprechilled seed. Note the lack of digestion.
Fig. 4. Closer view of a protein body in an epidermal cell from a seed prechilled at 2°C for 30 weeks.

for 12 hrs. in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.0, 4°C). Embryos were dehydrated in a graded acetone series and, subsequently, in a graded acetone-propylene oxide series. The embryos were embedded in Polybed 812 resin. Ultrathin sections were cut with a diamond knife on a Sorvall MT

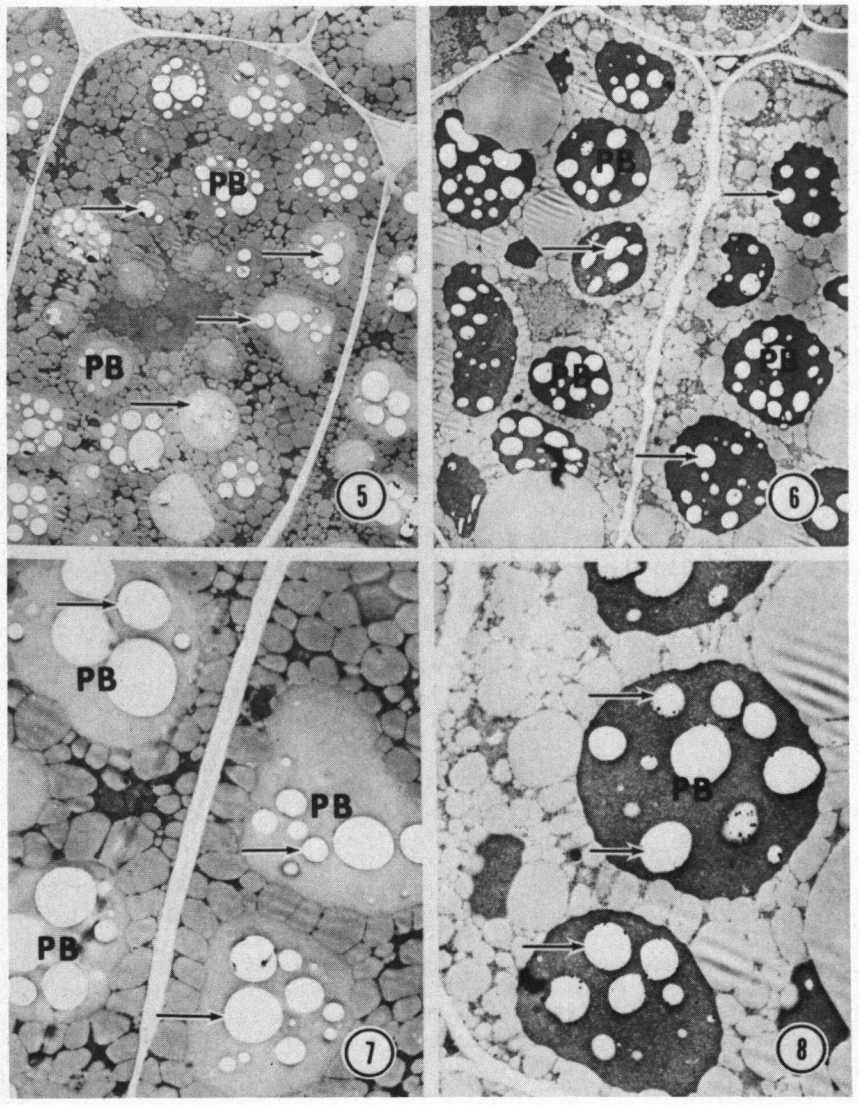

Plate II. Transmission electron micrographs of the parenchyma cell layer directly adjacent to the epidermis of *Polygonum pensylvanicum* embryo cotyledon. PB = Protein bodies. Arrows indicate regions of protein body digestion. Note rounded appearance of digested regions.

Fig. 5. Cell from a nonprechilled seed.

fig. 6. Cell from a seed prechilled at 2°C for 30 weeks.

Fig. 7. Closer view of protein bodies in cells from a nonprechilled seed.

Fig. 8. Closer view of protein bodies in a cell from seed prechilled at 2°C for 30 weeks.

ultramicrotome. Sections were collected on 100 mesh grids previously coated with formvar and carbon. The sections were stained with Reynolds lead citrate (REYNOLDS 1963) for 2 min. Electron micrographs were taken with a Hitachi EM-8 transmission electron microscope at 50 kV. Cells of the epidermis and the parenchyma layer directly below the epidermis in the embryo cotyledon were investigated to determine protein body changes.

During the 30 week prechilling period, seed germination increased from 0 to $94 \pm 5\%$; percentage moisture of the achenes increased from $9 \pm 3\%$ to $48 \pm 5\%$. While germination and percentage moisture increased with prechilling, the protein bodies were affected through increased digestion. Protein bodies present in the epidermis of nonprechilled seeds were intact (*fig. 1*) with no signs of digestion (*fig. 3*). After 30 weeks of prechilling, protein bodies in the epidermis of the embryo showed considerable digestion (*figs. 2, 4*). In contrast to the epidermis cells in the layer directly adjacent to the epidermis showed signs of protein body digestion even ' efore prechilling (*figs. 5, 7*). The most extensive protein digestion during 30 weeks of prechilling occurred in the epidermis (*figs. 2, 4*) and not in the cells adjacent to the epidermis (*figs. 6, 8*).

The pattern of digestion of protein bodies varied according to the cell layer investigated. Protein body digestion of epidermal cells tended to occur in rough patches within the protein body (*figs. 2, 4*), whereas protein body digestion of the parenchyma cell layer directly adjacent to the epidermis tended to be circular (*figs. 5–8*). Therefore, increased germination promoted by prechilling appears to be correlated with protein body digestion in cells located in the epidermis.

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