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Cytochemical Localization of Pectinase Activity in Laticifers of *Nerium oleander* L.

R. D. ALLEN and C. L. NESSLER*

Department of Biology, Texas A&M University, College Station

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Summary

A method is described for the cytochemical localization of pectinase activity at the ultrastructural level. The procedure involves the use of Benedict's reagent to form an electron-dense copper precipitate when reacted with reducing sugars liberated from exogenously supplied pectin. Using this technique, pectinase activity was examined in the nonarticulated, branched laticifers of *Nerium oleander*. Electron opaque crystalline deposits indicating the presence of pectolytic enzymes were identified in laticifer central vacuoles. Smaller amounts of reaction product were distributed along the middle lamella between laticifers and adjacent cells. This report represents the first direct evidence for the involvement of pectinase in intrusive growth of nonarticulated laticifers.

Keywords: Pectinase; Cytochemistry; Nonarticulated laticifer; *Nerium oleander*.

1. Introduction

Laticifers are classified into two groups based on their origin and development (HARTIG 1892). Articulated laticifers arise from a series of initials in which adjacent cell walls undergo partial or complete perforation through the gradual removal of wall materials (NESSLER and MAHLBERG 1977, 1981). Nonarticulated laticifers, such as those in *Nerium oleander* L., originate from single cells which differentiate in the cotyledonary node of the embryo and grow by elongation and ramification throughout the plant body (MAHLBERG 1963).

Elongation of nonarticulated laticifers has been attributed to intrusive growth at the tip followed by symplastic growth in the rest of the cell (MAHLBERG 1959). WILSON *et al.* (1976) detected pectolytic activity in fresh latex collected from *Asclepias syriaca* L. and suggested that pectinase secreted by the growing laticifer could loosen the middle lamella between adjacent parenchyma cells ahead of the growing laticifer tip to allow easy passage of the laticifer between cells. In addition, pectinase may loosen the middle lamella between laticifers and surrounding cells to allow for elongation of the laticifer.

In the present investigation we describe a technique for the cytochemical localization of pectinase activity at the ultrastructural level and apply this method to laticifers of *Nerium oleander* L. We believe this to be the first report of such a cytochemical localization procedure for this enzyme.

2. Materials and Methods

Vegetative shoot apices of *Nerium oleander* L. were collected from cultivated populations on the campus of Texas A&M University, College Station, TX, during September through November.

Cytochemical localization of pectolytic activity at the ultrastructural level was performed using a modification of a method first reported by BAL (1974) for the localization of cellulase activity. Tissues were fixed in KARNOVSKY's (1965) fixative in 0.05 M phosphate buffer, pH 7.2, for 2 hours. Specimens were then rinsed with 20 changes of phosphate buffer and stored in the same buffer overnight at 0 °C. The apices were then incubated in 0.5% pectin (Sigma Chemical Co., No. p-9135) in 0.1 M sodium acetate buffer, pH 5.0, for 20 minutes at room temperature, then transferred to hot Benedict's reagent and boiled for 10 minutes. Control tissues were incubated in acetate buffer without pectin or were boiled for 10 minutes prior to incubation with pectin. Specimens treated with pectin, or boiled before pectin incubation showed no reaction products when Benedict's reagent was omitted from this procedure.

* Correspondence and Reprints: Department of Biology, Texas A&M University, College Station, TX 77843-3258, U.S.A.

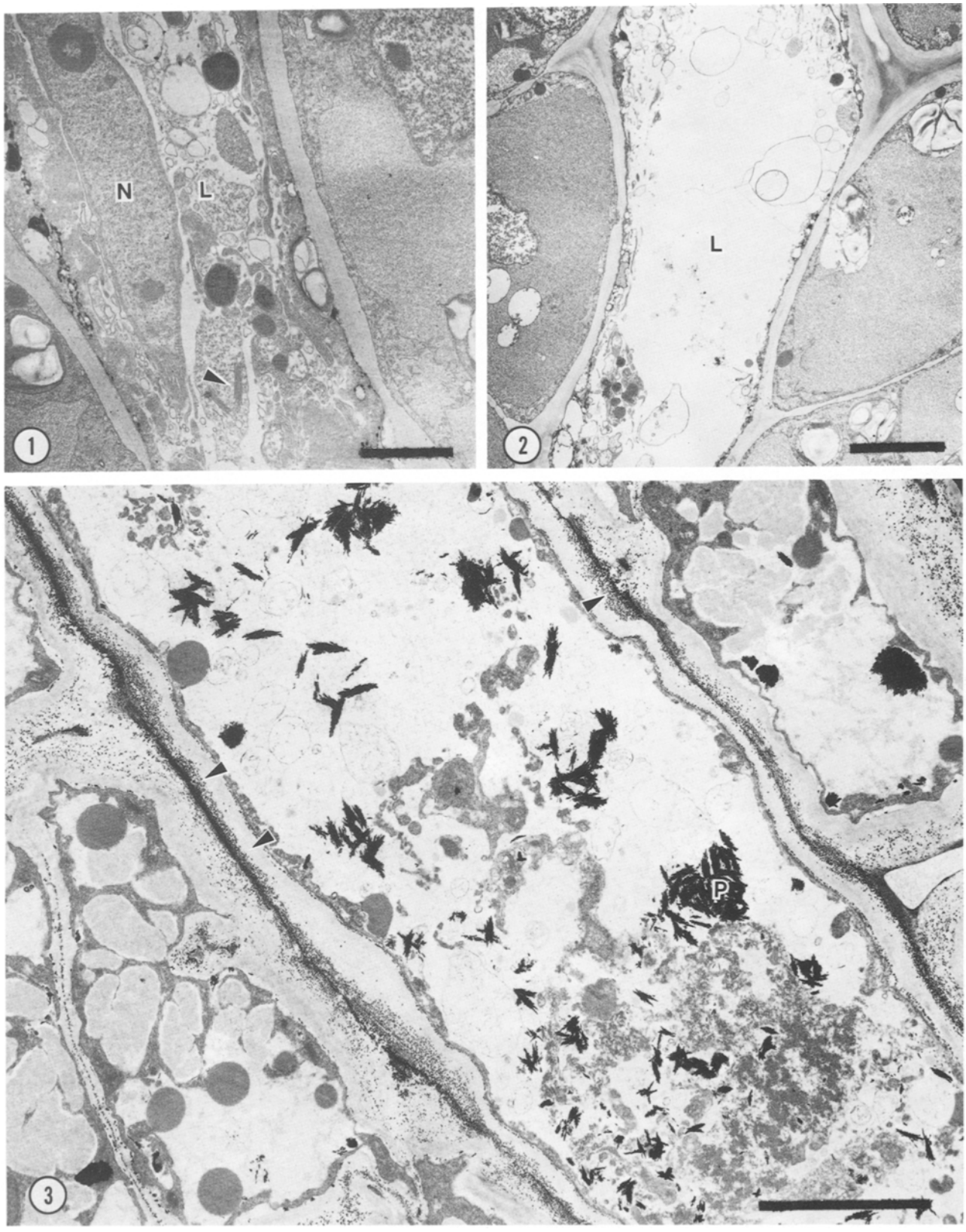

Fig. 1. Immature laticifer (*L*) in shoot apex of *Nerium oleander*. Cytoplasmic organelles include nucleus (*N*) and dictyosome (arrow). Bar = 2.5 μm

Fig. 2. Mature region of laticifer (*L*) characterized by low electron density. Cytoplasmic and membrane fragments are seen within the laticifer vacuole. Bar = 5.0 μm

Fig. 3. Cytochemical localization of pectinase activity in laticifer is characterized by electron opaque reaction product (*P*) in laticifer vacuole. Reaction product is also seen in middle lamella of laticifer cell walls (arrows). Bar = 5.0 μm

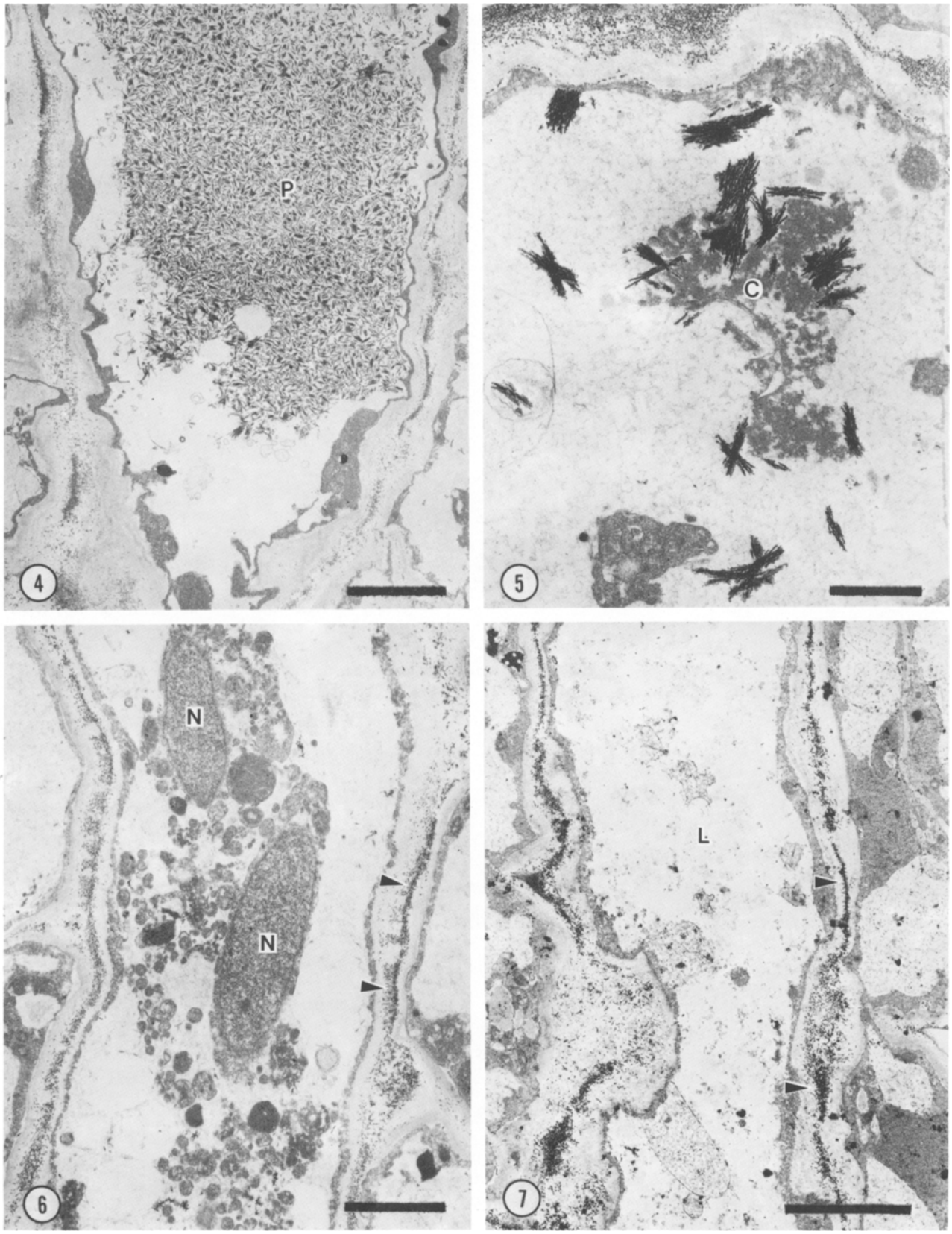

Fig. 4. Widely dispersed reaction product (*P*) in laticifer vacuole. Bar = 5.0 µm

Fig. 5. Cytochemical reaction product associated with dense patches of cytoplasmic material (*C*). Bar = 1.5 µm

Fig. 6. Laticifer of control specimen, boiled prior to incubation in pectin. Protoplasmic reaction product is absent, but cell wall deposits are seen (arrows). Note two nuclei (*N*). Bar = 2.5 µm

Fig. 7. Laticifer (*L*) of control specimen incubated without pectin. Note lack of protoplasmic reaction product and presence of reaction product in cell wall (arrows). Bar = 5.0 µm

All specimens were post-fixed with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer, pH 7.2, for 2 hours at room temperature, rinsed with several changes of distilled water, dehydrated in an ethanol-acetone series, and embedded in SPURR's (1969) low viscosity resin. Thin sections were cut with a diamond knife on a Reichert Ultracut ultramicrotome. Sections were picked up on 300 mesh copper grids, and then stained with aqueous uranyl acetate for 10 minutes at 40 °C followed by REYNOLD's (1965) lead citrate at room temperature for 10 minutes. Specimens were viewed and recorded with a Zeiss 10 C transmission electron microscope at 60 kV.

3. Results

Laticifers near the shoot apex of oleander are numerous and easily distinguished from surrounding cells. In young laticifers the protoplasm most often exhibits low electron density and contains the usual complement of cell organelles (Fig. 1). At maturity (Fig. 2), the laticifer protoplast consists of a very thin layer of peripheral cytoplasm that surrounds a large central vacuole. The vacuole contains recognizable organelles and membrane fragments that appear to be undergoing autolysis.

Pectinase activity is localized in the vacuole of laticifers by electron opaque crystalline inclusions (Fig. 3). These crystalline inclusions represent the product formed by the reaction of Benedict's reagent with reducing sugars liberated by hydrolysis of pectins in the incubation medium by laticifer pectinase. Since tissues are boiled during specimen preparation to allow the Benedict's reaction to occur, some cellular disruption is apparent, however, laticifers are still easily recognized by their large size compared with neighboring cells.

Reaction product is unevenly distributed within the laticifer protoplasm. The electron opaque crystals may be widespread (Fig. 4), or localized in very dense accumulations which are usually associated with patches of included cytoplasm (Fig. 5). In addition to the protoplasmic reaction product, heavy deposits of dense material are seen in the middle lamellar regions between laticifer cell walls and adjacent nonlaticiferous cells (Figs. 3–5). Lighter accumulations of reaction product are seen in walls of surrounding parenchyma cells.

Laticifers in control specimens which were boiled prior to incubation in pectin are free of heavy protoplasmic accumulations of reaction product (Fig. 6). However, electron opaque deposits in the middle lamella of laticifer walls persist. Likewise, control tissues incubated without pectin substrate also lack large crystalline deposits in the laticifer protoplasma, but show reaction product between cell walls (Fig. 7).

4. Discussion

Intrusive growth of nonarticulated, branched laticifers in *Nerium*, and possibly other genera, appears to be facilitated by the dissolution of middle lamellar materials by pectolytic enzymes. Freshly exuded latex of milkweed, *Asclepias syriaca*, L., has been shown to contain enzymes capable of degrading pectin and polygalacturonic acid (WILSON *et al.* 1976). Ultrastructural studies of the nonarticulated, branched laticifers in this species have demonstrated that milkweed "latex" represents the vacuolar content of laticifers (WILSON and MAHLBERG 1980).

In the present investigation the reaction products indicating pectinase activity were localized in the large central vacuole of the laticifer, that is, in the oleander latex. Thus, the vacuole appears to be the primary site of pectinase storage in *Nerium oleander* and other species with nonarticulated laticifers.

The site of pectinase synthesis is less clear because of the relative insensitivity of our localization procedure. Small amounts of enzyme synthesized in the thin peripheral cytoplasm might go undetected while greater concentrations of stored pectinase can be visualized in the vacuolar plasm.

The presence of reaction product in the middle lamella between laticifer cell walls and adjacent cells further suggests that some of the enzyme is secreted into this region by the laticifer. Similar accumulations, however, are seen in boiled and no pectin controls making interpretation more difficult. This apparent discrepancy can possibly be explained by the saturation of pectolytic enzymes by endogenous pectin of the middle lamella. If this is the case, then addition of exogenous pectin during incubation will not alter the density of reaction product in this region. It is important to note that although some middle lamella reaction product is seen around nonlaticiferous cells, these deposits are always much less dense than those associated with laticifers and probably represent background reducing sugars.

Middle lamella pectinase activity does not seem to be restricted to the laticifer apex but also occurs in regions distal to the tip. Pectinase activity in these areas may allow the laticifer to slide past surrounding cells as it elongates.

An analogous system of intrusive growth has been proposed for some pollen tubes which pass through the pectin rich middle lamella of stylar cells (JENSEN and FISHER 1969). KONAR and STANLEY (1969) associated pectinase activity with pollen tube growth.

The technique described in the present study for

cytochemical localization of pectinase activity at the electron microscopic level should provide additional information concerning intrusive growth of a variety of cell types. In addition, other plant processes that involve pectolysis such as abscission and fruit ripening may be further investigated using this method.

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