

Cytochemistry of cellulase in Capitate Glandular Trichomes of *Pelargonium x fragrans* (Geraniaceae)

*Ko Kyung Nam, Kyung-Whan Lee, Sang-Eun Lee, and
Eun Soo Kim*

Dept. of Biological Sciences, Konkuk University

ABSTRACT

The localization of cellulase were investigated in the capitate glandular trichomes of *Pelargonium x fragrans* by a transmission electron microscopy. The secretory cells of capitate trichomes involved in biosynthesis and its secretion. Secretory material is transported to the space between the plasma membrane and cell wall, and subsequently accumulated in the secretory cavity. The splitting of secretory cell wall during the formation of secretory cavity is suggested that wall-forming enzymes, such as cellulase, may contribute to the wall separation process. Cellulase reaction product was localized in the secretory cell, the secretory cavity and in the subcuticular wall of glandular trichomes. Reaction products were present over fibrillar matrix in the secretory cavity associated with both the inner wall and the subcuticular wall.

MATERIALS AND METHODS

Leaves of *Pelargonium x fragrans* were fixed in Karnovsky fixative(1965) in 0.1M phosphate buffer, pH 7.2 for 1 hr. They were rinsed, and stored in the same buffer at 0°C for 16hr. Tissues were incubated in 0.01M phosphate containing carboxymethyl cellulose for 20 min, and then transferred to hot Benedict's reagent for 10 min. Control tissues were incubated in the buffer without CMC. All of tissues were dehydrated in an ethanol-acetone series and embedded in Spurr's resin and observed with an electron microscope (JEM-2000 EX II).

RESULTS

As a result of the wall loosening and of secretion accumulation, a subcuticular space was formed by the detachment of the cuticle and the outermost pectic layer of cell wall. The accumulation of secretion in the subcuticular space gave a spherical shape to the trichome, characteristic of a mature capitate trichome. Cellulase reaction product was localized in the secretory cell, the secretory cavity and in the subcuticular wall of glandular trichomes. Reaction products were present over fibrillar matrix in the secretory cavity associated with both the inner wall and the subcuticular wall. The distribution of cellulase reaction products supports an interpretation that cellulase is involved in formation of secretory cavity.

The presence of cellulose reaction product in the secretory cell wall during progressive stages in secretory cavity development indicates its continuing role in wall development during enlargement of the secretory cavity. Cellulase may perform a role in the release of wall fibrils, as the fibrils, as the fibrillar matrix, into the secretory cavity. This matrix appears to function as precursors for thickening of the subcuticular wall during enlargement of the secretory cavity.

REFERENCES

- [1] Bal, AK, 1974. Van Nostrand Reinhold Co, New York. pp.68-76.
- [2] Karnovsky, M, 1965. J. Cell Biol. 65:140-151.
- [3] Kim, ES, and Mahlberg PG, 1991. Amer. J. Bot. 78(2):220-229.
- [4] Kim, ES, and Mahlberg PG, 1997. J. Plant Biol. 40(1):61-66.
- [5] Nessler, CL, and Mahlberg PG, 1981. Amer. J. Bot. 68:730-732.

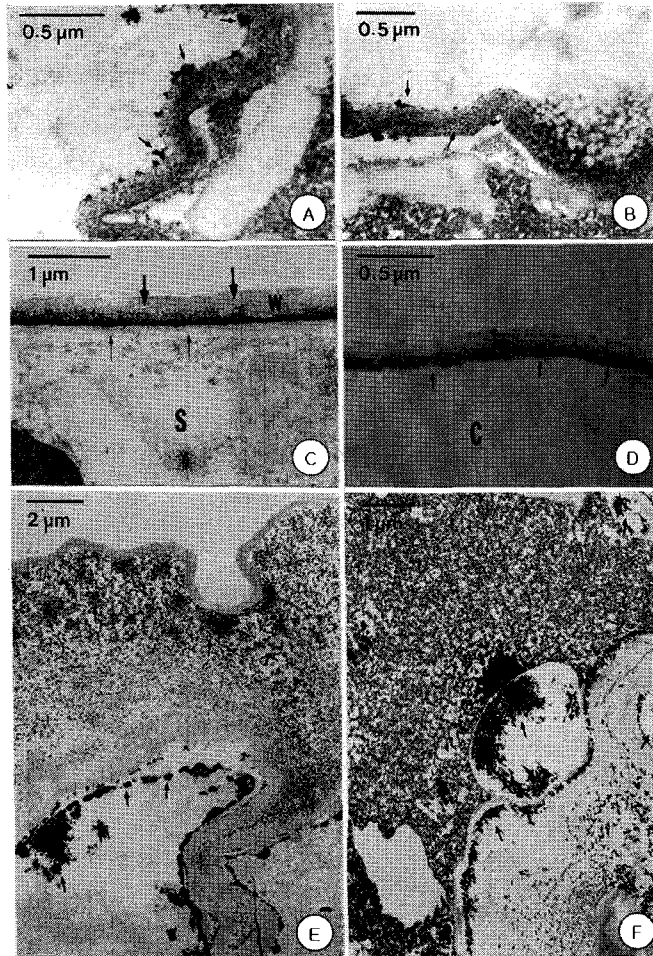


Fig. 1. Transmission electron micrographs showing the cytochemical localization of cellulase reaction products in glands. (A) Secretory cell in longisection showing uniformity of reaction product (arrows) in outer zone of wall. (B) Deposition (arrows) of product were on the cell wall and plasma membrane. (C) Transverse section of secretory cell (S) showing reaction product along the wall (W) surface adjacent to plasma membrane (small arrow) and in outer zone of wall (large arrow) under the cuticle. (D) Reaction products along the subcuticular cell wall under the cuticle (arrows) and in secretory cavity. (E) Cellulase reaction products (arrows) in epidermal cells. (F) Cellulase reaction products (arrows) in mesophyll cells.

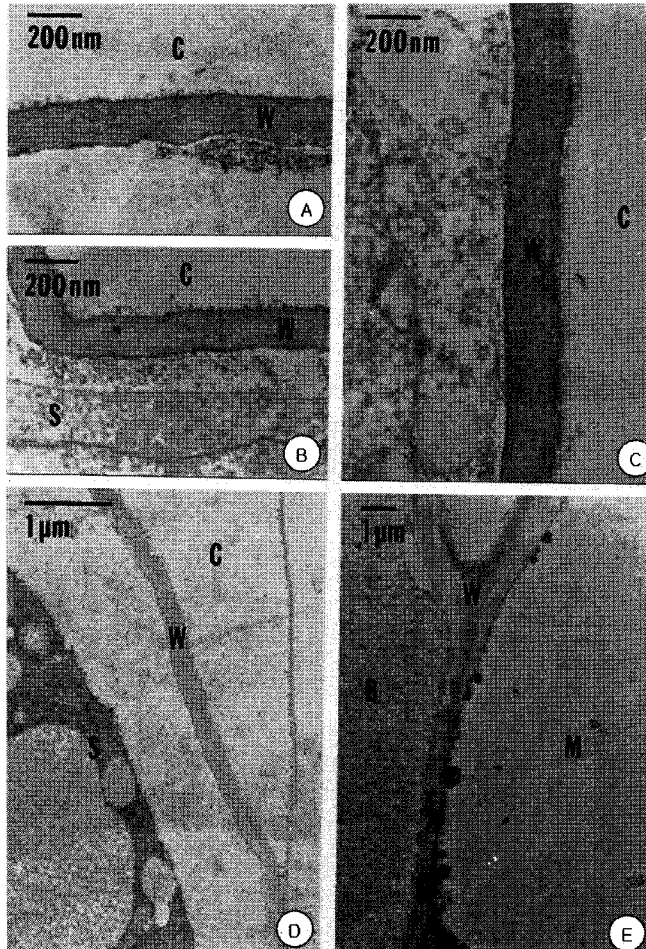


Fig. 2. Control of cytochemical localization of cellulase reaction product in glands. C; cavity, W; cell wall, S; secretory cell. (A,B,C) Control sections showing absence of reaction products along wall of the secretory cells. (D) Control showing absence of reaction product along subcuticular cell wall under the cuticle and in secretory cavity. (E) Control showing absence of reaction product along the cell wall (W) under the cuticle and in mesophyll cell (M).