ANALYSIS OF COTTON FIBER ULTRASTRUCTURE DURING SECONDARY WALL DEPOSITION USING ULTRA-RAPID FREEZING AND FREEZE SUBSTITUTION

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Abstract

We succeeded in optimally preserving plant-grown cotton fibers (*Gossypium hirsutum* L.) at the secondary wall stage of fiber development (21 and 30 DPA) through use of cryogenic methods. Samples were excised from bolls quickly and gently, plunged into liquid propane cooled by liquid nitrogen, freeze-substituted in acetone/OsO_4 (1%) at -80¡C then -20¡C, and then infiltrated with resin and flat-embedded at room temperature. The flat-embedding allowed light microscopic selection for sectioning of best-preserved regions of fibers. Because of the ultrarapid freezing, the electron microscopic images showed optimally-preserved cytoplasm and vacuolar and plasma membranes without damage by ice crystal formation. Cytoplasmic organelles or structures observed included Golgi bodies, plastids, mitochondria, and microtubules. In addition, some areas of the plasma membrane showed complex vesicle and membrane profiles (reminiscent of previous observations on cotton fibers after chemical fixation) that will continue to be studied to determine their real or artefactual nature. (In well-frozen fibers prepared by this method, artefacts can only be caused by specimen handling before fixation, not by the freeze-fixation itself.) We expect that further analyses of these fibers will provide information on many aspects of fiber development, including the occurrence of tip- vs. intercalary-fiber elongation during secondary wall deposition. Accurate immunocytochemical localization of proteins or polysaccharides at all stages of fiber development is also now feasible. This research was supported by grants from Cotton Incorporated, Raleigh, NC to RWS and CHH.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1367-1367 (1998)
National Cotton Council, Memphis TN