THE FLUID PROCESSING APPARATUS: FROM FLIGHT HARDWARE TO ELECTRON MICROGRAPHS

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INTRODUCTION

Since the early years of space biology, a major drawback in spaceflight plant experiments has been the inability to fix specimens in microgravity, relying instead on fixation after return to Earth [1]. As there, it is of a growing interest to look at the effect of microgravity on the structure and the developmental polarity of root graviperceptive cells, or columella cells [1, 5], it is important to use flight hardware which allows specimen fixation in space therefore avoiding the confounding effects of rapid re-adaptation to gravity after landing.

As part of Bioserve Space Technologies, a Center for the Commercial Development of Space (CCDS), we have flight opportunities through the Commercial Generic Bioprocessing Apparatus (CGBA) payload. In this study the Fluid Processing Apparatus (FPA) was used to grow seedlings for a limited period of time prior to fixation of the tissue in a microgravity environment. Upon return to Earth, the samples were processed for electron microscopy.

This report describes microscopic data obtained from two space flights (STS-54 and STS-60). In both cases, electron micrographs of columella cells revealed well preserved cell structure, well defined microtubules and the presence of calcium precipitates formed by a antimonate precipitation method [2].

METHODS

Seeds of sweet clover (Melilotus alba L.) were grown in microgravity (STS-54, January 13-19, 1993 and STS-60, February 3-11, 1994) in chamber A of a FPA on a water-saturated cylinder of filter paper (Fig.1A & B, [4 and Hilaire et al. 1995, submitted]). When the space shuttle attained low-earth orbit, the onset of germination occured by introducing 1 ml of water from chamber B into chamber A. After 40 hours of growth in microgravity at 22°C in darkness, the seedlings were fixed under the same gravitational field. All roots were fixed in space in 2% glutaraldehyde/2.5% formaldehyde in 0.1M K_2HPO_4 (pH 7.6) with 2% (w/v) potassium antimonate [2], or with 5% glutaraldehyde in 50mM PIPES (pH 6.9) and 5mM $MgCl_2$. After recovery of the fixed specimen post-landing, the samples were postfixed with 1% (w/v) osmium tetroxide for 2 hours, dehydrated in acetone, and embedded in Klomparens resin. Thin sections were examined with a Philips 201 transmission electron microscope at 60 kV.

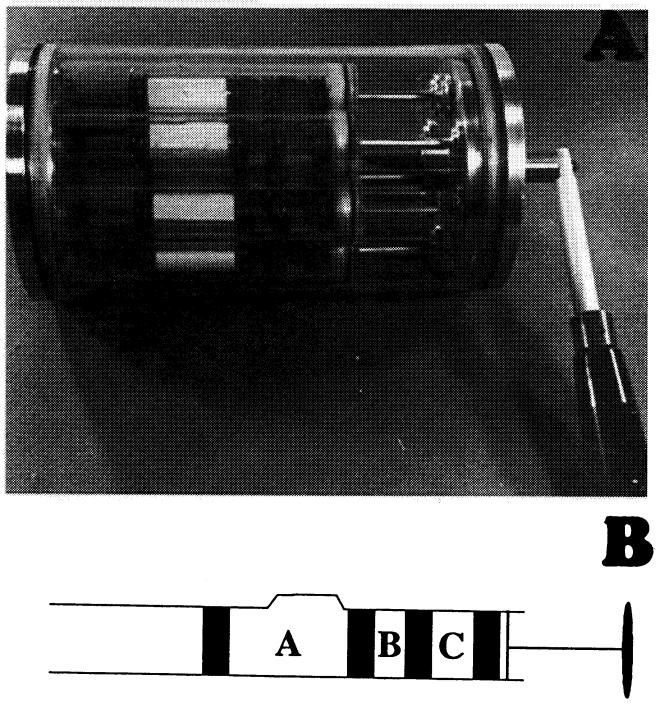

Fig. 1. (A) View of a Group Activating Pack (GAP) containing eight Fluid Processing Apparatus (FPA) units used aboard the US Space Shuttle. (B) Schematic drawing of an FPA: chamber A contains a cylinder of dry filter paper and ten sweet clover seeds, chamber B contains 1 ml of water, chamber C contains 1.5 ml of fixative. Each chamber is separated by a rubber septum.

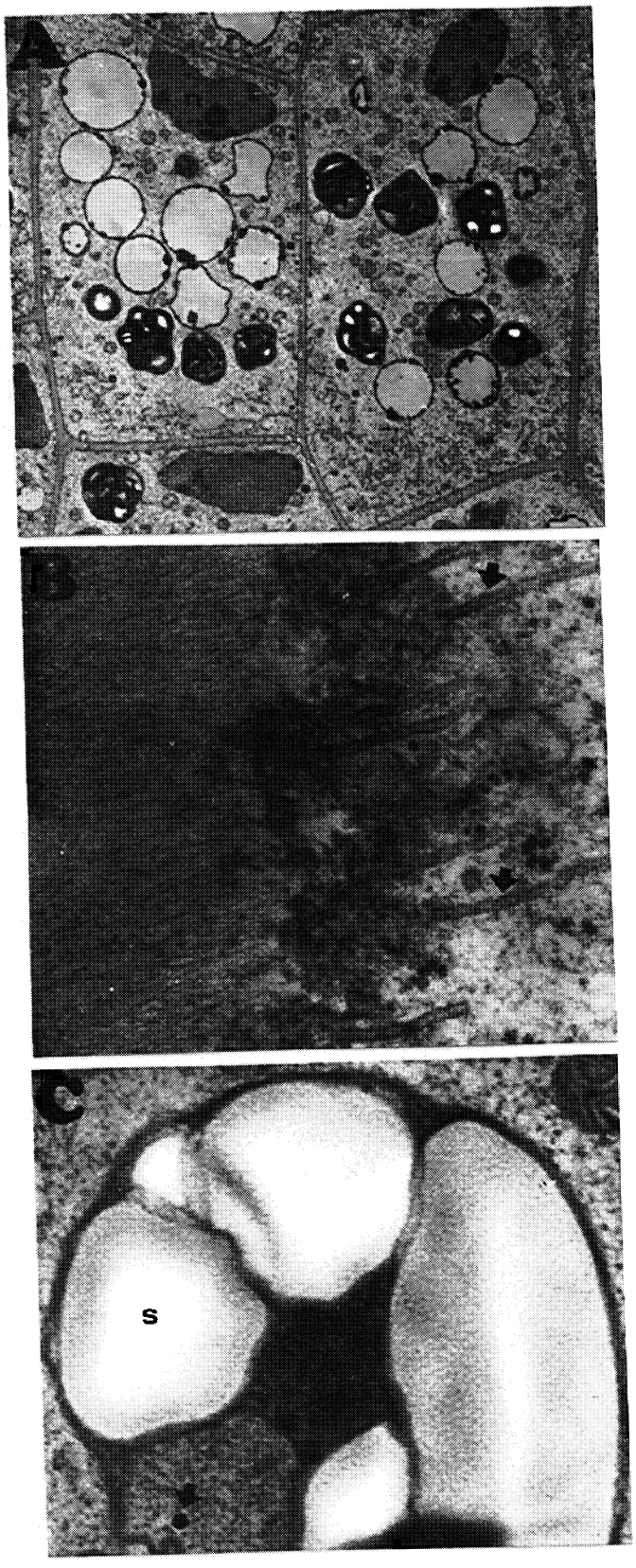

Fig. 2. Electron micrographs of: (A) sweet clover columella cells (x2200); (B) microtubules (arrow) along the lateral cell wall (x65000); (C) an antimonate precipitate (arrow) in the amyloplast of a columella cell (x35000). a: amyloplast, n: nucleus, s: starch grain.

RESULTS AND DISCUSSION

Electron micrographs of columella cells from clover roots grown in microgravity (STS-54) revealed a well preserved cell structure with the amyloplasts (starch-filled plastids) distributed throughout the cytosol and the nucleus at the proximal pole (Fig. 2A). This indicates that fixation was complete as any exposure of the cell to gravity post-landing would have resulted in marked settling of the amyloplasts, as observed for the ground controls. Some microtubules were also visible along the lateral cell wall (Fig. 2B).

On STS-60, potassium antimonate was included in the fixative solution in order to precipitate free calcium. Electron micrographs of columella cells fixed in microgravity demonstrate the presence of calcium precipitates in the amyloplasts (Fig. 2C), nuclei, mitochondria and along the proximal and lateral plasma membrane.

These experiments support the idea that the FPA is useful hardware to grow and fix seedling specimens aboard the Space Shuttle in order to better understand the effect of microgravity on cells. With minor changes in media and fixative, other biological samples could easily be incorporated [3].

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