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ORIGINAL ARTICLE

Button botany: plasmodesmata in vegetable ivory

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Abstract The hard endosperm of species of the palm genus *Phytelephas* (elephant plant), known as vegetable ivory, was used in the manufacture of buttons in the nineteenth century, the early twentieth century, and again in more recent times. Here, we show that the pathways for intercellular communication, including the cytoplasm in opposite pits and the plasmodesmata that traverse the cell wall, can be visualized in century-old inexpensive buttons that are readily available in antique shops.

Keywords Arecaceae · Palm · *Phytelephas* · Pits · Plasmodesmata · Vegetable ivory

Introduction

Vegetable ivory buttons (Fig. 1) were manufactured from the hard mannan-rich endosperm of some species of palms, known as ivory palms or tagua palms, in the nineteenth century and the early part of the twentieth century after animal ivory had become scarce (Seemann 1852–1857, 1853; Bailey 1943; Timell 1957; Acosta-Solis 1948; Armstrong 1991). Vegetable ivory buttons were very common before being replaced by plastic buttons after World War II (Barfod 1989; Bernal 1998). Eco-friendly buttons, exported for use in upscale clothing, are still manufactured from vegetable ivory from *Phytelephas* sp. in factories located in Manta, Ecuador (Barfod et al. 1990; Velásquez Runk 1998). Antique vegetable ivory buttons are still to be found in shops that sell old beads, buttons, marbles, etc. The cellular nature of these buttons can easily be ascertained while shopping in an antique store with a good hand lens. Details of the cellular architecture can be observed in whole buttons placed directly under the objective of a light microscope and plasmodesmata can be observed in stained free-hand sections of the buttons.

Materials and methods

Vintage vegetable ivory buttons, perhaps manufactured by the Rochester Button Company in Upstate New York in the early twentieth century (http://centerathighfalls.org/walking/pages/02-button.htm), were purchased from local antique stores for 25¢ each. The seed of *Phytelephas seemannii* Cook (Barfod 1991) collected in Panama in 1942 (Allen # 2941) was obtained from the Bailey Hortorium (http://bhort.bh.cornell.edu/herb.htm; Cornell University, Ithaca, NY, USA). The specimen was annotated by Anders Barfod in 1988.

Hand sections were cut with a single-edged razor blade, stained for 2 min in a 0.05% aqueous solution of Crystal Violet (C. I. 42555; Allied Chemical, National Aniline Division, Buffalo, NY, USA; http://www.colorantshistory.org/NationalAniline.html), rinsed with water, and observed with a light microscope (BX60; Olympus, Tokyo, Japan) using 10× (UPlanApo, N.A., 0.4), 40× (UPlanFl, N.A.,

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Fig. 1 Early twentieth century buttons made from the endosperm of ivory palms

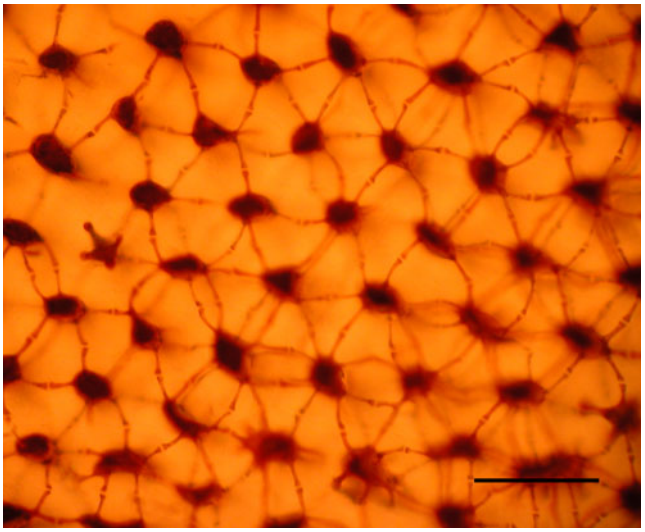

Fig. 2 Bright field image showing the cellular architecture visible in an intact vegetable ivory button as observed with transmitted light and a 10× objective. Protoplasm in long simple opposite pits extends between neighboring cells. The plasmodesmata, which are not visible at this magnification, pass through the wall between opposite pits. *Scale bar*=100 μm

0.75), and 60× (PlanApo, N.A., 1.40) objectives. Photographs were taken with a Nikon Coolpix 5000 camera mounted on the microscope using adapters manufactured by MVIA (Monaca, PA, USA) and processed using Image J freeware (http://rsbweb.nih.gov/ij/; National Institutes of Health, Bethesda, MD, USA).

In order to maximize resolution and minimize diffraction artifacts, we chose to sacrifice the depth of field in the micrographs. The depth of field (Y) at the focal plane in each micrograph is related to the numerical aperture of the objective lens according to the following equation derived by Staves et al. (1995):

$$Y = \frac{0.61\lambda}{(\mathrm{NA})^2}\sqrt{n^2 - (\mathrm{NA})^2}$$

where n is the refractive index of the medium between the cover glass and the objective lens and λ is the mean wavelength of light used for observation. The depths of field observed in the micrographs taken with the 10× (UPlanApo, N.A., 0.4), 40× (UPlanFl, N.A., 0.75), and 60× (PlanApo, N.A., 1.40) objective lenses are only 1,747, 359, and 84 nm, respectively. Consequently, the thicknesses of the structures observed in this paper are several times greater than the depth of field of the objectives used.

Results and discussion

The cellular architecture of the endosperm of vegetable ivory palms is revealed under low magnification (Fig. 2). Long, simple pits containing cytoplasm extend like spokes from the mass of cytoplasm in the hub of each cell and are opposite to the pits in adjacent cells. From studying such images, one gets a sense of the pathways of communication between cells (Gunning and Robards 1976).

The plasmodesmata that traverse the cell wall between adjacent endosperm cells can be observed in free-hand sections stained with Crystal Violet (Fig. 3). The distribu-

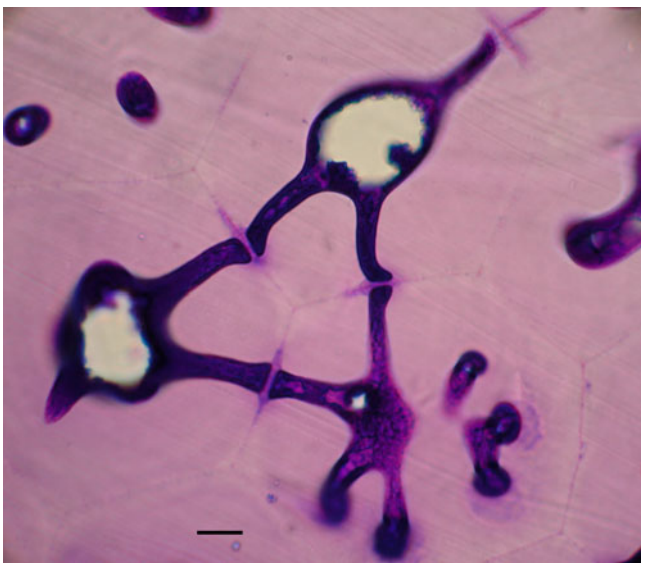

Fig. 3 Bright field image of a hand section of a vegetable ivory button stained with Crystal Violet and observed with a 40× objective showing opposite simple pits and the zones of plasmodesmata between three cells. *Scale bar*=10 μm

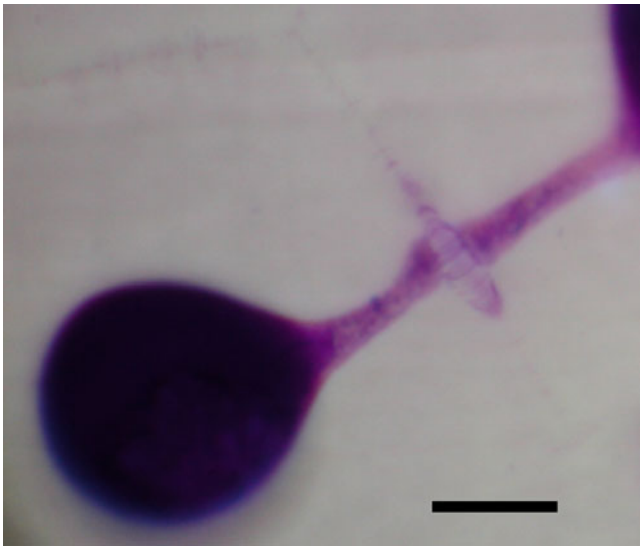

Fig. 4 Bright field image of a longitudinal section of cytoplasm in simple pits and connecting plasmodesmata in a Crystal Violet stained hand section of a vegetable ivory button observed with a 60× oil immersion objective lens. *Scale bar*=10 μm

tion of the plasmodesmata preserved in the vegetable ivory buttons can be seen in longitudinal and transverse sections (Figs. 4 and 5).

A seed of *P. seemannii* collected in 1942 was sectioned with a razor blade and stained with Crystal Violet. Figure 6 shows that the plasmodesmata of the endosperm traverse the wall between opposite simple pits of adjacent cells and also through the thick wall where pits were absent. The plasmodesmata persist as distinct staining entities for at least 70 years and perhaps much longer than the actual viability of the seeds themselves.

That the cytoplasm of neighboring cells can be connected by plasmodesmata was appreciated by Eduard Tangl (1879) and Eduard Strasburger (1901) more than 100 years ago (Köhler and Carr 2006). In the *Handbook of Practical Botany* by Strasburger (1911), the "plasmodesm" connections between endosperm cells of *Phytelephas*

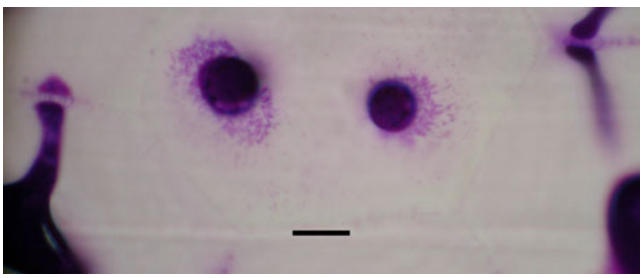

Fig. 5 Bright field image of a transverse section of cytoplasm in simple pits and connecting plasmodesmata in a Crystal Violet stained hand section of a vegetable ivory button observed with a 60× oil immersion objective lens. *Scale bar*=10 μm

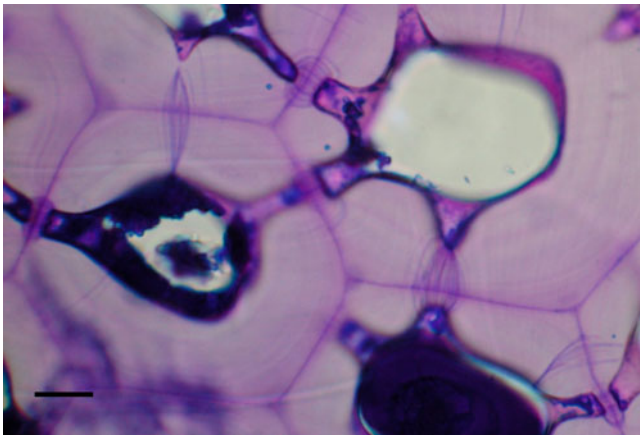

Fig. 6 Bright field image of a Crystal Violet stained hand section of the endosperm 1 cm from the embryo of *Phytelephas seemannii* observed with a 40× objective lens showing the diversity of plasmodesmatal connections between cells. *Scale bar*=10 μm

macrocarpa are illustrated. Advances in technology in the past several decades have led to a remarkable understanding of plasmodesmatal development, structure, and physiology, and the relationship of plasmodesmata to intercellular communication (Bell and Oparka 2011; Fitzgibbon and Vatén 2011). Here, we show that in old vegetable ivory buttons that only cost two bits and that are readily available from antique stores, cell biology students and anatomy students can visualize the intercellular connections between neighboring cells—connections that integrate the parts and the whole (Sharp 1934; Wayne 2009).

Conflict of interest The authors declare that they have no conflict of interest.

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