Histological/Biochemical Composition of Papyri

Introduction

Papyrus, as a medium for writing, was made from the stems of the plant *Cyperus papyrus* (Basile, 1977; Wallert, 1989). Although it is unclear how papyri were produced, it is known that only the peeled stems were used in their manufacture (Basile, 1977; Franceschi et al., 2004). Non-destructive physical analysis of the surface of papyri commonly returns different results from one area of a sheet to another. The present work examines *C. papyrus* stems and papyri by optical and scanning electron microscopy (SEM) in order to determine their histological structure and gain insight into their gross biochemical composition.

Materials and Methods

The stems of several *C. papyrus* plants (all cultivated under glass at the University of León Faculty of Biological and Environmental Sciences) were fixed in formaldehyde/acetic acid/ethyl alcohol. The fragments were passed through an increasing alcohol series, washed in isoamyl acetate, set in paraffin wax blocks at 64ºC for 90 min, and cooled. Sections (12 µm) were cut from each block using a microtome, stained in safranin/fast green, and permanently mounted in Entellan. These preparations were then examined under bright field conditions using a Nikon E600 microscope, and the most representative fields photographed.

For scanning electron microscopy (SEM), fixed stem fragments were passed through an increasing alcohol series, air-dried, gold scattered, and observed using a Jeol JSM-6480LV SEM. Fragments (a few mm across) of papyri from the Palau Ribes Collection (Barcelona) were similarly processed for SEM examination. Other fragments from the same collection were examined under a Nikon SMZ1500 stereomicroscope. Photographic images were taken of representative fields.

Results

Figures 1a–g and 2b–e show the *C. papyrus* stem to have a cortex and medulla. The outermost part of the cortex has a single layer epidermis with stomata (Figs. 1c, 2b). Under the epidermis lie packets of sclerenchyma fibers (Figs. 1c, d) and chlorophyll-containing parenchyma cells that surround the vascular bundles. These bundles are surrounded by sclerenchyma fibers and amyloplast-containing parenchyma cells. The medulla of the stem contains notable amounts of aerenchyma (Figs. 1f, 2c) and disperse vascular bundles, which are collateral and open (Figs. 1g, 2d, e).

Figure 2a shows the classic two-lamina composition of papyrus sheets. Figures 2f–i show SEM images of these sheets in which parenchyma (Figs. 2g, i) and some xylem (Figs. 2h, i) and sclerenchyma cells can be seen. The surface heterogeneity of the sheets in terms of their cellular content is clearly appreciable (Fig. 2j).

Discussion

The examined stems show the typical micromorphology of hydrophyte C4 monocots. Some of the cell types visible in the stems, especially parenchyma, xylem elements and schlerenchyma, were also visible in the papyri. The type of cell present naturally affects the gross biochemical composition of the sheets. Generally, the cell walls of parenchyma are made of cellulose, hemicellulose and pectins, and are soft, hydrated and plastic. Some of these cells contain starch. The cell walls of xylem and sclerenchyma contain lignin, and are hard and elastic (Dickison, 2000; Taiz et al., 2015). The gross biochemical composition of papyri will, therefore, differ from place to place depending on the relative quantities of the different cell types present.

It has been known since the time of Pliny the Elder's *Historia Naturalis* that papyri for writing were made from the stems of *C. papyrus* (Basile, 1977; Wallert, 1989). Although many of the details of their production remain unknown, it is agreed that only peeled stems were used (Franceschi et al., 2004). Peeling removed the green and toughest parts of the plant (the epidermis, the hard, elastic subepidermal tissues, and many of the sclerenchyma and the xylem cells in the cortical vascular bundles).

Longitudinally-cut medulla strips were likely then laid next to one another to form laminae (Basile, 1977), though sometimes the medulla may have been cut spirally and rolled out flat before similar align-

ment (Wallert, 1989). Two laminae were then placed one on top of the other, one with the strips running vertically, the other with the strips running horizontally, and these two-lamina sheets allowed to air-dry (Basile, 1977). Some papyri may have been coated in egg white or some other medium to make the surface smooth (Franceschi, 2011), although such 'resin' has only been observed on numbered occasions.

The medulla of *C. papyrus* contains abundant parenchyma cells with large spaces between them. These parenchyma cells form a matrix of cellulose-, hemicellulose- and pectin-containing cell walls that make an excellent writing medium. The matrix also contains disperse lignin fibres provided by xylem elements and schlerenchyma.

Sucrose-rich phloem sap may have played a part in holding papyri together (Taiz et al., 2015), as may the starch of the amyloplasts partially surrounding the vascular bundles. Starch from the roots of *C. papyrus*, or extracted from other plants, may also have been added as a glue.

In conclusion, papyri are predominantly made of parenchyma cells with cellulose-, hemicellulose- and pectin-containing cell walls. These cells may also contain starch granules. However, lignin-containing xylem and schlerenchyma cells form dispersed fibres within the sheets, leaving them with a heterogeneous histological structure and gross biochemical composition. This may explain why non-destructive physical analyses of the surface of papyrus sheets can return such different results for different sampling points. For reliable conclusions to be drawn, it is suggested that such analyses be repeated at many sampling points across each sheet.

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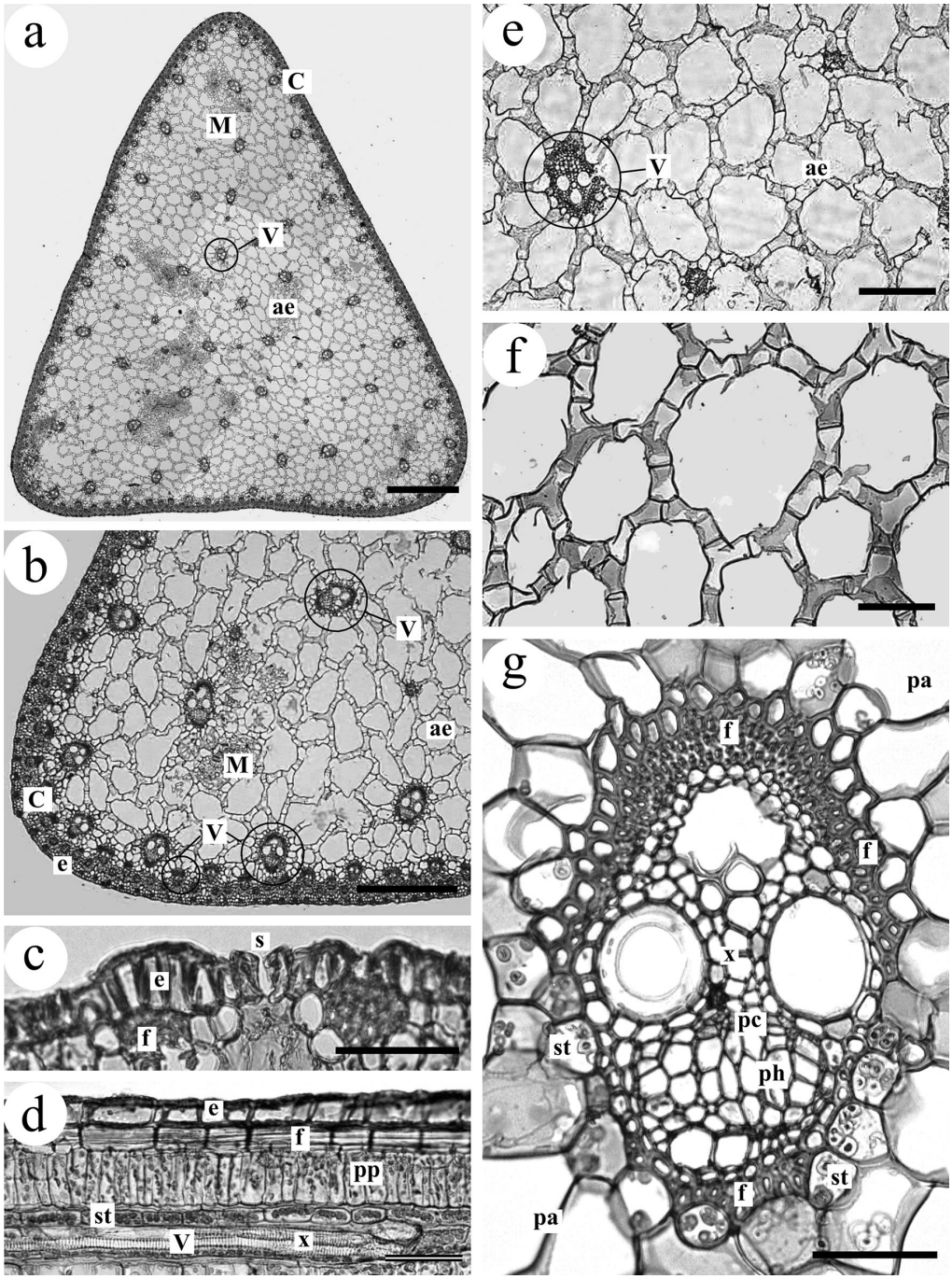

Fig. 1. a–g *C. papyrus* stem images. **a–b** General view of the stem in transverse section. Under the epidermis (e) lies the cortex (C) and finally the medulla (M). **c–d** Outer zone of the cortex. Below the epidermis (e) lie discontinuous packets of sclerenchyma fibers (f), chlorophyll-containing parenchyma (pp) and vascular bundles (V). To the outside of the vascular bundles are parenchyma cells containing starch granules (st). Note the spiral structure of the xylem (x) in longitudinal section. **e–g** Medulla. The medulla contains disperse vascular bundles (V) and abundant aerenchyma (ae). **g** The vascular bundles are collateral and open (xylem [x] – procambium [pc] – phloem [ph]), and surrounded by sclerenchyma fibers (f) and starch-containing parenchyma (st). **a–g** Safranin/fast green staining. Abbreviations: *ae* aerenchyma, *C* cortex, *e* epidermis, *f* sclerenchyma fibers, *M* medulla, *pa* parenchyma, *pc* procambium, *ph* phloem, *pp* chlorophyll-containing parenchyma, *s* stoma, *st* starch, *V* vascular bundle, *x* xylem.
Bars: a = 1000 µm; b = 500 µm; c, d, g= 50 µm; e = 200 µm; f = 100 µm

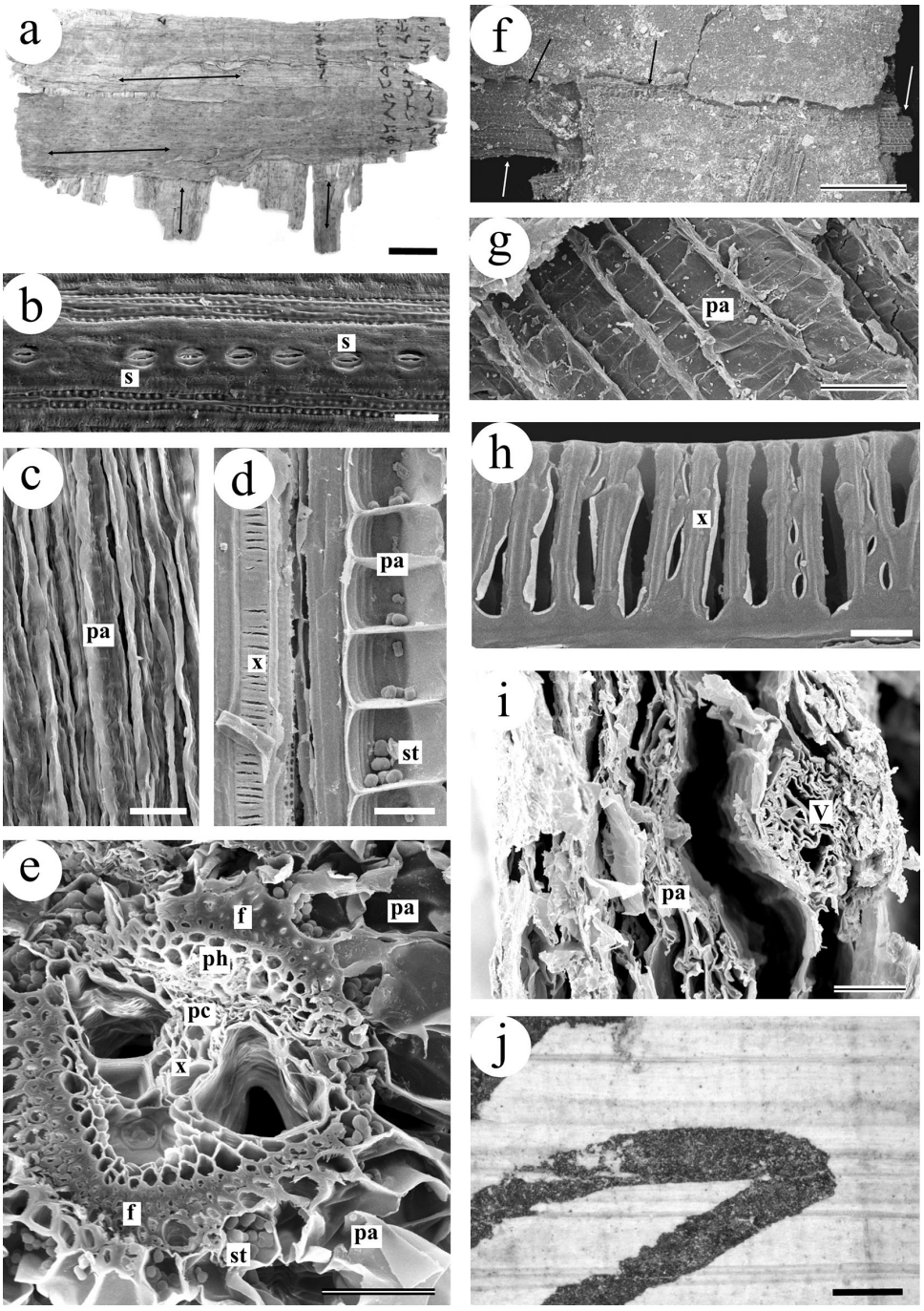

Fig. 2. a A papyrus from the Roman period. The arrows indicate the orientation of the strips in the two laminae forming the papyrus sheet. **b–e** SEM image of *C. papyrus* stem. **b** Epidermis with aligned stomata (s). **c–d** Longitudinal sections. **c** Parenchyma in the medulla. **d** Part of the vascular bundle. Notice the xylem (x) and the parenchyma cells (pa) with starch granules (st). **e** Transverse section of a vascular bundle. Compare with **Fig. 1 g**. **f–i** Fragments of papyri from the Byzantine period seen under the SEM. **f–g** In some places (arrows in **f**), structural deterioration allows the cellular make-up of the sheets to be seen (*pa* in **g**). **h** Xylem elements. Compare with *x* in **d**. **i** Edge of a papyrus showing vascular bundles (V) and parenchyma (pa). **j** Note the heterogeneity of the structure in this image. **a**, **j** Stereomicroscope images. **b–i** MEB. **a**, **j** Byzantine period papyrus. **f–i** Roman period papyrus. Abbreviations: *f* sclerenchyma fibers, *pa* parenchyma, *pc* procambium, *ph* phloem, *s* stoma, *st* starch, *V* vascular bundle, *x* xylem. Bars: a = 1.5 mm; b, c, e, i = 50 μm; d = 25 μm; f = 500 μm; g = 100 μm; h = 5 μm; j = 200 μm