Anatomy and Histochemistry of Taro, Colocasia esculenta (L.) Schott, Leaves

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ABSTRACT

Taro (Colocasia esculenta) leaves are composed of a multi-layered palisade and air-filled spongy mesophyll. Vascular traces are encircled by a ring of vacuolate cells which may extend to the upper and lower epidermis. Abaxial and adaxial stomata are present, with the former being more numerous. Subsidiary cells appear to be of the para-mesoperigenous type and guard cells are sunken with plastids that display few membranes, but prominent starch inclusions. The epidermal cells are highly vacuolate with prominent protrusions or extensions on the exposed surfaces. Despite the presence of the bundle sheath, no conclusive evidence exists to indicate taro is capable of carbon fixation by a C4 pathway. In light of other morphological and histochemical features of the leaf this appears even more unlikely.

Introduction

Few reports are available about structure of taro leaves (Strauss, in press; Strauss and Scheirer, submitted). Most current information comprises generalized morphological descriptions rather than detailed microscopic studies. One early paper discussed structure and distribution of stomata in the Colocasia leaf (Yarbrough, 1934). However, data are based on a single leaf of an apparently ornamental plant. No comprehensive study of edible cultivars has been reported. Another early study details morphology of abaxial epidermal outgrowths of the leaf blade (Guengen, 1908). This phenomenon is relatively rare and the authors only know of a single cultivar in which it occurs. A report on genetic variability in taro included several morphologic characters of leaves and concluded that while some appeared to be heavily influenced by environment others were under more direct genetic control (Arditti et al., 1980; Strauss, et al., 1980). Specifically, foliar dimensions appeared strongly influenced by the environment.

This study elucidates structure of the taro leaf to provide a basis for future physiological studies. Further, understanding anatomy of normal leaves aids in gaining better knowledge of pathogenesis effects on taro.

Materials and Methods

Plant material was obtained from Drs. M. Pearson, University of Papua New Guinea, and R.S. de la Peña, University of Hawaii, Kauai Branch Station, and maintained under greenhouse conditions, in the ground, at the Burlington Botanical Research Facility of Northeastern University, Woburn, Massachusetts. Selected

plants were also cultured in environmental chambers under 16 h photoperiods at 28° to 30°C and with 70% relative humidity.

Replicas of the leaf surface were made by application of a solution of acetone and clear nail polish (1:1) to the surface of the leaf. The resulting film was cleansed of particulates by gentle heating in 3M HCl. Data were gathered from a minimum of 30 separate areas for each leaf.

Portions of leaves were fixed for 24 h in 3% glutaraldehyde in Hepes buffer, pH 6.8, at 4 C. Postfixation was for 60 min. in 2 percent osmium tetroxide in buffer. Tissues were then dehydrated in an acetone series and embedded for electron microscopy (Spurr, 1969). Three micron sections were stained by Sudan black (Bronner, 1975) or periodic acid Schiff's methods (Lillie, 1965) and examined using light microscopy. Thin sections were stained with uranyl acetate (Hayat, 1972) and lead citrate (Venable and Coggleshall, 1964) and examined on a Zeiss EM 9S electron microscope.

Results and Discussion

A typical taro leaf blade can be divided into two general areas, the large veins and the intercostal lamina, the latter being the area between the major veins. On the adaxial (upper) surface stomata are distributed over the intercostal and veinal areas while on the abaxial (lower) surface stomata are absent from the regions of epidermis overlying the veins. This confirms earlier reports of stomatal distribution on the undersurface of the taro leaf (Yarbrough, 1934). The epidermis often appears papillate on the abaxial surface (Figures 1, 2).

The major and lateral veins are composed of several bundles underlying a palisade layer and radially distributed bundles of collenchyma (Figure 3). Interspersed through the tissue of these veins are numerous vascular bundles as well as rings of large, vacuolate cells apparently composing an aerenchyma tissue. Stomates were not present on abaxial surfaces of these veins, but were found over the loosely packed palisade tissue of the adaxial surface. Similar morphology has been found in the petiole. Such morphology in taro has previously been suggested as an adaptation to flooded conditions of growth (Onwueme, 1978).

The small, minor veins of the intercostal regions are composed of a single vascular strand surrounded by a single layer of vacuolate bundle sheath cells, the latter often extending to the epidermal surfaces (Figure 2). Within the vein a duct-like cell can often be noted (Figure 2). This cell is articulated, contains dark staining material and resembles a laticifer (Figure 4; Esau, 1969). Stomata occur on both surfaces (Figure 2). They are paracytic in form and have been tentatively classed as para-mesoperigenous, though this requires ontogenetic study for confirmation (Fryns-Claessens and Van Cotthem, 1973; Stebbins and Khush, 1961).

Adaxial stomata overlie substomatal chambers in the palisade tissue, while abaxial ones open directly into the spaces of the spongy mesophyll (Figure 2). Plastids of the guard cells contain prominent starch grains and few internal membranes. They do not apppear to be photosynthetic (Figure 5). Such a situation has been noted in orchids of the genus Paphiopedilum (Nelson and Mayo, 1975; Rutter and Willmer, 1979).

The palisade layer of the leaf is composed of one to three layers of elongate cells situated below the adaxial epidermis (Figure 2). Occasional densely stain-

ing cells are noted in the palisade tissue which are, in form, similar to the other cells of this region. Transverse sections reveal these cells to be somewhat regularly distributed in the palisade tissue (Figure 6). They are similar to the tanniferous cells found in the palisade tissue of Dioscorea (Ayensu, 1972). Elucidation of their exact nature requires further study.

Cells in the spongy layer are organized to form approximately rectangular air spaces (Figure 2, Strauss, in press). Raphide idioblasts, as previously reported for Colocasia are noted in the spongy mesophyll (Figure 7), as are the smaller druses of calcium oxalate, similar to those reported to occur in the corm (Sakai and Hansen, 1968; Sunell and Healey, 1979).

Of several cultivars of taro examined all showed a greater density of stomata on the upper and lower leaf surfaces than previously reported (Table 1). As noted earlier, stomata are greater in number on the abaxial surfaces (Yarbrough, 1934). However, the ratio of abaxial to adaxial stomata is considerably lower for the cultivars examined than a similar ratio calculated from data in the earlier study. Since that study utilized an ornamental plant we suggest our results to be more representative of the true condition for the edible cultivars of taro. Examination of several leaves, of similar developmental age, in C. esculenta cv 'Bun

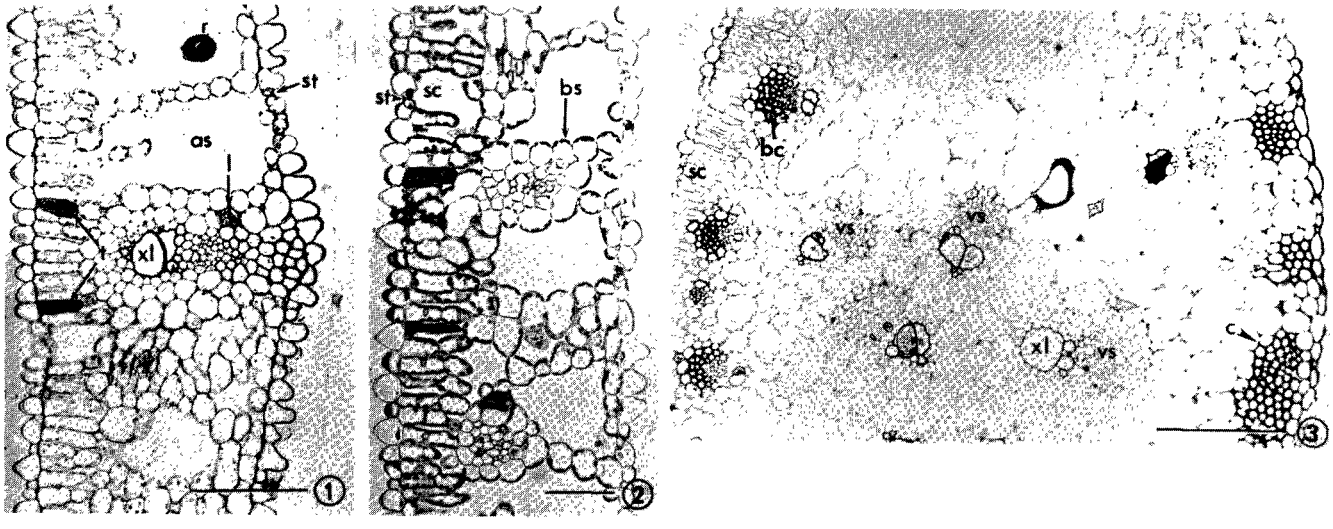

Figure 1. Transverse section of leaf blade of Colocasia esculenta cv Bun long showing minor vein and papillate abaxial epidermal cells; st, stomata; r, raphide idioblast; as, air space; xl, xylem lacuna; l, laticifers; t, tanin cell. Bar = 100 microns.

Figure 2. Transverse section of intercostal lamina from Colocasia esculenta cv K268 with minor vein and bundle sheath; bs, bundle sheath; sc, substomatal chamber; st, stomate. Bar = 50 microns.

Figure 3. Transverse section of major vein from leaf of Colocasia esculenta cv Bun long. Note adaxial palisade parenchyma, collenchyma bundles and vascular strands; bc, bundle collenchyma; c, abaxial collenchyma bundle; sc, substomatal chamber; vs, vascular strand; xl, xylem lacuna. Bar = 200 microns.

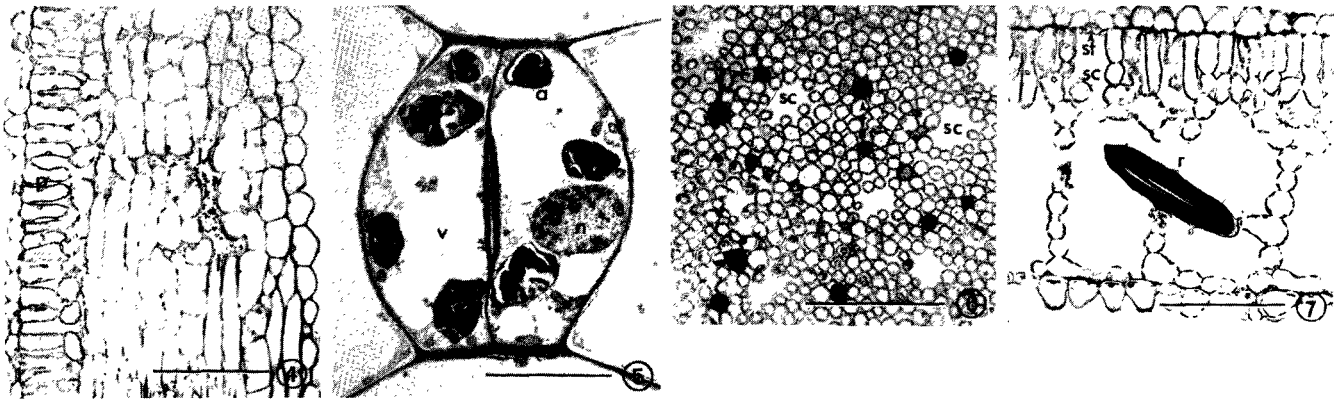

Figure 4. Longitudinal section of laticifer in minor vein of Colocasia esculenta cv Bun long; l, laticifer; p, palisade parenchyma. Bar = 100 microns.

Figure 5. Paradermal section through adaxial stomate of Colocasia esculenta cv Bun long; a, amyloplast; n, nucleus; v, vacuole; n, nucleus. Bar = 10 microns.

Figure 6. Paradermal section through palisade layer of Colocasia esculenta cv K268 lamina showing tannin cells and substomatal chambers; sc, substomatal chamber; t, tanin cell. Bar = 100 microns.

Figure 7. Transverse section of lamina of Colocasia esculenta cv Bun long showing raphide idioblast; r, raphide idioblast; sc, substomatal chamber; st, stomate. Bar = 100 microns.

long' revealed considerable variation in stomatal density. Ratio of abaxial to adaxial stomata, however, was similar to the values for other cultivars studied (Table 2). Distribution of stomata in leaves and stomatal density are, no doubt, strongly influenced by the environment.

When compared histochemically, the concentration of starch in guard cells is seen as an intense PAS reaction. Subsidiary cells display lower stain intensity but appear to contain higher levels of PAS positive material (presumably carbohydrate) than the surrounding epidermal tissue. With respect to lipids, the guard cells are the only epidermal cells showing a positive Sudan reaction. This may reflect the higher level of cytoplasmic membrane and lesser vacuolar size in these cells than in other epidermal cells. As might be expected, palisade parenchyma has higher levels of carbohydrate and lipid than spongy mesophyll when analyzed histochemically. This is commensurate with the expected higher level of photosynthetic activity in these cells.

The general organization of the taro leaf into distinct palisade and spongy mesophyll, with large air spaces, suggests carbon fixation occurs by a C3 pathway. Further, the bundle sheath lacks the characteristic chlorenchyma and general

Table 1. Distribution of stomata on adaxial, upper, and abaxial, lower, leaf surfaces for several cultivars of taro, Colocasia esculenta, compared to an earlier report.

Cultivar	Stomata, mm-2 Adaxial (A)	Abaxial (B)	B/A
'Akado'	98 ± 14	134 ± 29	1.4
'Bun long'	121 ± 16	166 ± 10	1.4
'Lehua maoli'	133 ± 14	165 ± 23	1.2
'Red moi'	147 ± 27	191 ± 16	1.3
Colocasia antiquorum (Yarbrough, 1934)	50	116	2.32

Table 2. Comparison of stomatal densities from several plants of taro, Colocasia esculenta (L.) Schott cv 'Bun long'. All samples taken from the second fully expanded leaf on each plant.

Plant	Stomata, mm-2 Adaxial (A)	Abaxial (B)	B/A
I	134 ± 22	167 ± 26	1.2
II	133 ± 17	178 ± 17	1.3
III	191 ± 26	293 ± 22	1.5
IIIa	116 ± 20	152 ± 17	1.3
IV	162 ± 31	195 ± 20	1.2

morphology typical of such cells in the C4 plant (Bidwell, 1979; Brown, 1975; Laetsch, 1974). Electron microscope studies have not detected a peripheral reticulum, typical of C4 chloroplasts (Stein and Strauss, unpublished; Laetsch, 1974). Finally, histochemical evidence points to accumulation of carbohydrates in the palisade tissues, suggesting that formation of these compounds is not restricted to the bundle sheath. Thus, it seems unlikely that taro is capable of carbon fixation by a C4 pathway.

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