

# STRUCTURAL CHARACTERISTICS OF MESOPHYLL IN SOME *TILLANDSIA* SPECIES<sup>1</sup>

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## Abstract

The mesophyll of 14 species of *Tillandsia* is examined under light and electron microscopes. Primary fluorescence reveals in all the investigated species, even if they come from different habitats, the presence of a peculiar chlorophyllous tissue around vascular bundles and air cavities, and in between layers of water tissue. This arrangement helps the plant to store the water absorbed by its scales, efficiently protect the chloroplasts against intense sunlight, and prevent water loss through its air cavities.

Vascular system develops by means of numerous cross anastomoses in accordance with the features of xeromorphic plants. Nevertheless, in *Tillandsia* this development is characterized by the scarcity of xylem elements. This fact must be considered in relation to the wide wall contacts of the mesophyll cells in these plants. Anatomohistological analysis demonstrated the association of the sheaths with vascular bundles. Besides storage and support, these sheaths also perform some additional functions. A comparison of the ultrastructure of the chloroplasts of perixylematic and periphloematic sheaths and mesophyll chloroplasts indicates morphological differences in the chlorophyllous tissue. Depending on environmental moisture, the examined species of *Tillandsia* adopt either CAM or C<sub>4</sub> type of physiological pathway.

Almost all the species of the genus *Tillandsia* (Bromeliaceae), are epiphytic (Mez 1904, Picado 1913, Pittendrigh 1948) and their habit shows adaptation to this condition of life.

In epiphytic plants the adventitious roots are primarily anchorage organs rather than supporting. However, in *Tillandsia*, the solution-absorbing function is assigned to the typical trichomes of the leaf epidermis (Benzing & Burt 1970, Benzing 1976, Benzing et al. 1976).

The shoot consists of a shortened axis, to which the leaves are attached spirally, forming a dense rosette; the inflorescence scape arises from its middle.

The rosette of leaves is, thus, the entire vegetative apparatus of the plant. The leaf in *Tillandsia* has probably developed some special structural features since it performs functions usually carried out by other organs in the Cormophytes.

Mez (1904), Bonny (1968) and Braga (1977) have made comparative anatomical observations on the leaf epidermis and the sclerenchymatous tissue surrounding the vascular bundles in various species of Bromeliaceae in order to establish their adaptation to dry climate.

Specific studies on the leaf structure in *Tillandsia* are limited, not only

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because very few species have been examined but also because only a few of their structures have been described. Billings (1904) points out the scarcity of spongy tissue in *T. usneoides*, and also the tendency to loss of function of the vascular tissue. Barbaini (1921), in a work on two species of *Tillandsia*, emphasized some physiological characters of the leaf epidermis, and reported some water tissue near the upper surface. Benzing & Renfrow (1971a, b, c, 1980), Benzing (1976), Benzing et al. (1978) and Francini Corti (1981), described the ecology of the genus and some interesting aspects of its physiology.

This investigation deals with the mesophyll of 14 species of *Tillandsia* examined under the light and electron microscopes, underlining some structural features in order to discuss morpho-functional implications with special reference to the habitat of these plants.

#### Materials and Methods

Samples were collected by the authors during a scientific expedition to several countries in south America (1976)<sup>2</sup>, led by Professor E. Francini Corti, coordinator of the research programme on the biology of *Tillandsia*, granted by the C.N.R. (Italy). These plants were kept in the greenhouses of the Botanical Garden of Florence.

The following species have been studied: *T. caput medusae* Morren, *T. complanata* Bentham, *T. brachicaulos* Schlechtendal, *T. ionantha* Planchen, *T. flabellata* Baker, *T. fasciculata* Swartz, *T. latifolia* Meyen, *T. purpurea* Ruiz & Pavon, *T. pueblensis* Smith, *T. prodigiosa*

2. The expedition was sponsored by the Accademia Nazionale dei Lincei and the Botanical Garden of Florence.

(Lemaire) Baker, *T. retorta* Grisebach ex Baker, *T. schiedeana* Stendel, *T. usneoides* L., and *T. paleacea* Prisl.<sup>3</sup>

Venation pattern of leaves from each species was observed by clearing them in 2.5% NaOH at room temperature for some days. After repeated washings with distilled water, the samples were photographed with the Leitz-Aristophot apparatus.

Anatomo-histological studies were carried out on leaf sections fixed in 5% glutaraldehyde in 0.1M phosphate buffer at pH 7.2 and embedded in methacrylate after dehydration in ethanol series (Feder & O'Brien 1968). Some sections were stained with 0.07% toluidine blue at 40C, and others with specific dyes such as ruthenium red, and PAS reagent.

In order to define the relationship between chlorophyllous parenchyma and other tissues, fresh frozen leaf sections were obtained by means of an American Optical Corporation Cryo-Cut microtome, and their primary fluorescence was observed with Philips CS 200 W-4 Hg-vapours lamp, with blue excitation filter.

All the photomicrographs were taken with a Reichert-Univar light microscope.

For ultrastructural investigations on plastids, small leaf fragments were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1M phosphate buffer at pH 7.4 (Karnowsky 1965), post-fixed in 2% OsO<sub>4</sub> in the same buffer and embedded in Epon (Luft 1961) after dehydration in ethanol. Ultrathin sections were stained with uranyl acetate (Gibbons & Grimstone 1960) and lead citrate (Venable & Coggeshall

3. The nomenclature used by Smith & Downs (1977) has been adopted for the determination.

Fig. 1A-E — Cleared leaves. A. *T. paleacea*. Note close vascular net. B. *T. purpurea*. Cross anastomoses remain even in apical zone, though less numerous. C. *T. prodigiosa*. Smaller-sized veins are fewer due to bifurcation and anastomosing with larger adjacent veins. D, E. *T. ionantha*. D. Cross anastomoses evident. E. Basal portion lacking cross veins.

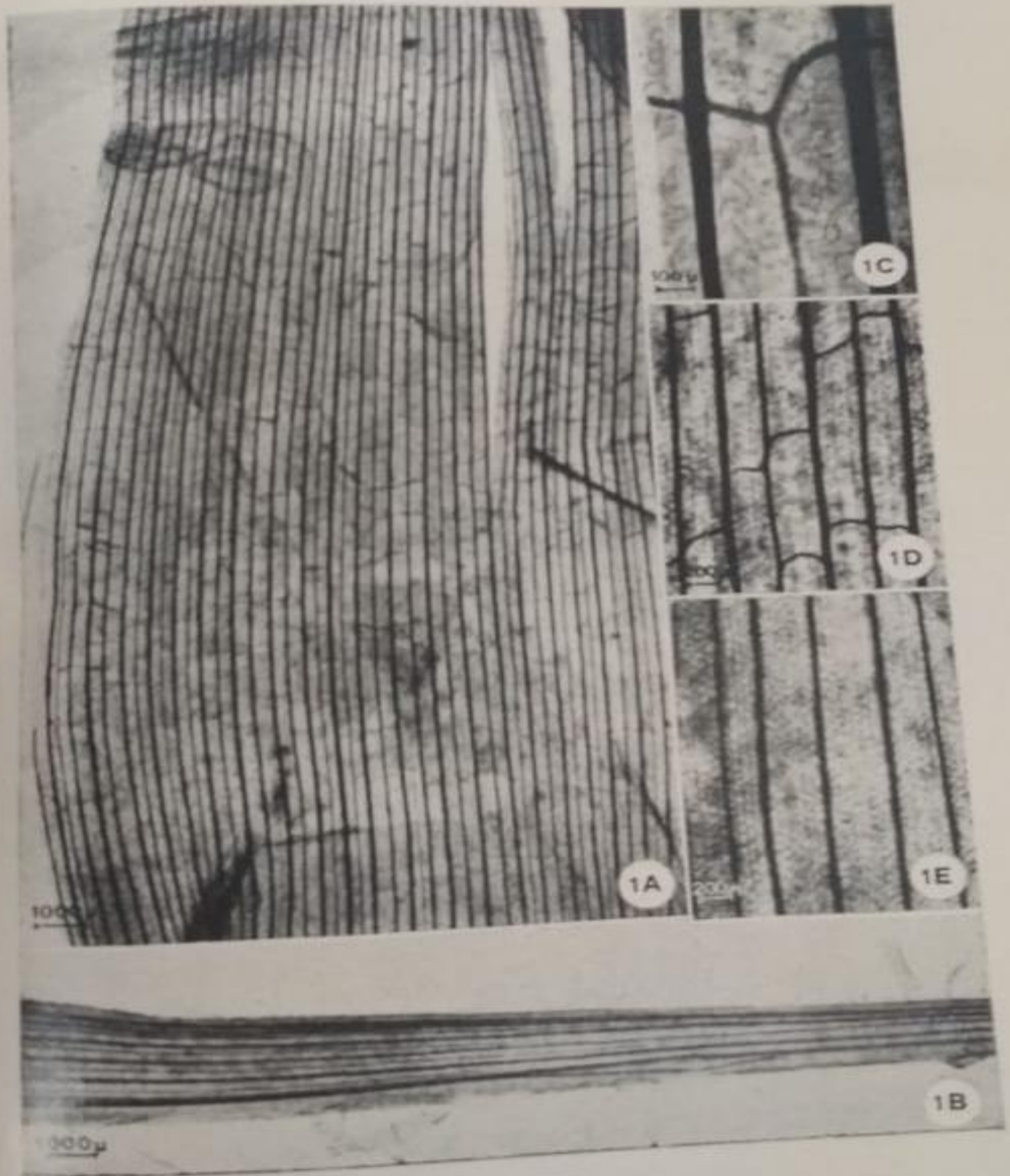


Fig. 1A-E

1965), observed and photographed with a Philips EM 300 electron microscope.

### Observations

The examined species were grouped in accordance with the climate of the place of their origin: I. *T. latifolia*, *T. paleacea*, *T. purpurea* and *T. retorta* (dry, desert habitats). II. *T. caput-medusae*, *T. ionantha*, *T. pueblensis*, *T. schiedeana*, *T. usneoides* (moderately moist areas). III. *T. brachicaulos*, *T. complanata*, *T. fasciculata*, *T. flabellata*, *T. prodigiosa* (tropical rain forest).

Regular and steady alternation of larger- and smaller-sized veins is observed in the cleared leaves of the Group I species (Fig. 1A). The distance between the veins is equal along the entire length of the blade, compatible with the narrowly triangular shape of leaves (Fig. 1B). A decrease of veins in the apical portion is observed in thinner veins (Fig. 1C). Cross anastomoses have a thickness similar to that of the less evident longitudinal veins, on whose sides they occur, some corresponding regularly, and others only slightly (Fig. 1D). Their frequency is constantly high for over two-thirds of the lamina length and decreases only in its apical portion (Fig. 1B).

Vein patterns and sizes are the same for Group II species. Cross anastomoses are present mostly in the central tract

of the lamina length and absent in the basal portion in most instances (Fig. 1E).

The veins in Group III species are similar but anastomoses occur in two-thirds of the leaf length, and cannot be seen in the apical portion.

Anatomo-histological study on cross sections of the leaves shows collateral vascular bundles at the same distance from both the epidermal layers, in Group I species (Fig. 2A, C). They are surrounded by parenchymatic cells—different in size, shape and mutual relationship from the rest of the mesophyll—that form a bundle sheath (Fig. 2A, B). The vacuoles of these cells often contain calcium oxalate raphides embedded in a matrix positively reacting to ruthenium red. Idioblasts with raphides are also scattered in the mesophyll (Fig. 2A).

Inside the oxalate layer there is a sclerenchymatous sheath, more developed at the two poles, whose cell walls are seldom totally lignified (Fig. 2B). The differences in size among the bundles is mainly due to the different dimensions of this sheath.

The phloem is considerably better developed than the xylem (Fig. 2B), and spreads beyond the xylem in the ending veins. The two vascular systems never touch each other due to the constant presence of parenchymatic elements with slightly thickened walls (Fig. 2B). Each of these systems is peculiarly surrounded by an often complete layer

Fig. 2A-E (c, parenchymatic cells; l, large air cavities; p, parenchymatic sheath; s, sclerenchymatic sheath). A, E, F. Cross sections of leaves embedded in methacrylate. A. *T. retorta*. Water parenchyma having sinuous cell walls, scarce intercellular spaces and vascular bundles surrounded by cells rich in calcium oxalate (arrows). Idioblasts with raphides scattered in mesophyll (arrows). Large air cavities are intercalated with bundles. B. *T. purpurea*. Vascular bundle showing outer parenchymatous and inner sclerenchymatous sheath more developed at poles. Note different stages of lignification in cell walls. Parenchymatous cells with slightly-thickened walls dividing vascular tissues. Phloem is more abundant than xylem, both are surrounded by sheaths consisting of small parenchymatous cells (arrows). C. *T. latifolia*. Primary fluorescence of chlorophyll exhibiting location of chlorophyllous tissue inside layers of water tissue. D. *T. paleacea*. Primary fluorescence revealing homogeneous arrangement of chloroplasts in mesophyll. E. *T. caput-medusae*. Portion of a leaf showing vascular bundle, raphides (double arrow), perixylematic and periphloematic sheaths (arrows). F. *T. fasciculata*. Same, showing vascular bundle and extension of sclerenchymatous sheath with highly thickened walls; cells between xylem and phloem also have thickened walls. Notice raphides (double arrow), perixylematic and periphloematic sheaths (arrows).

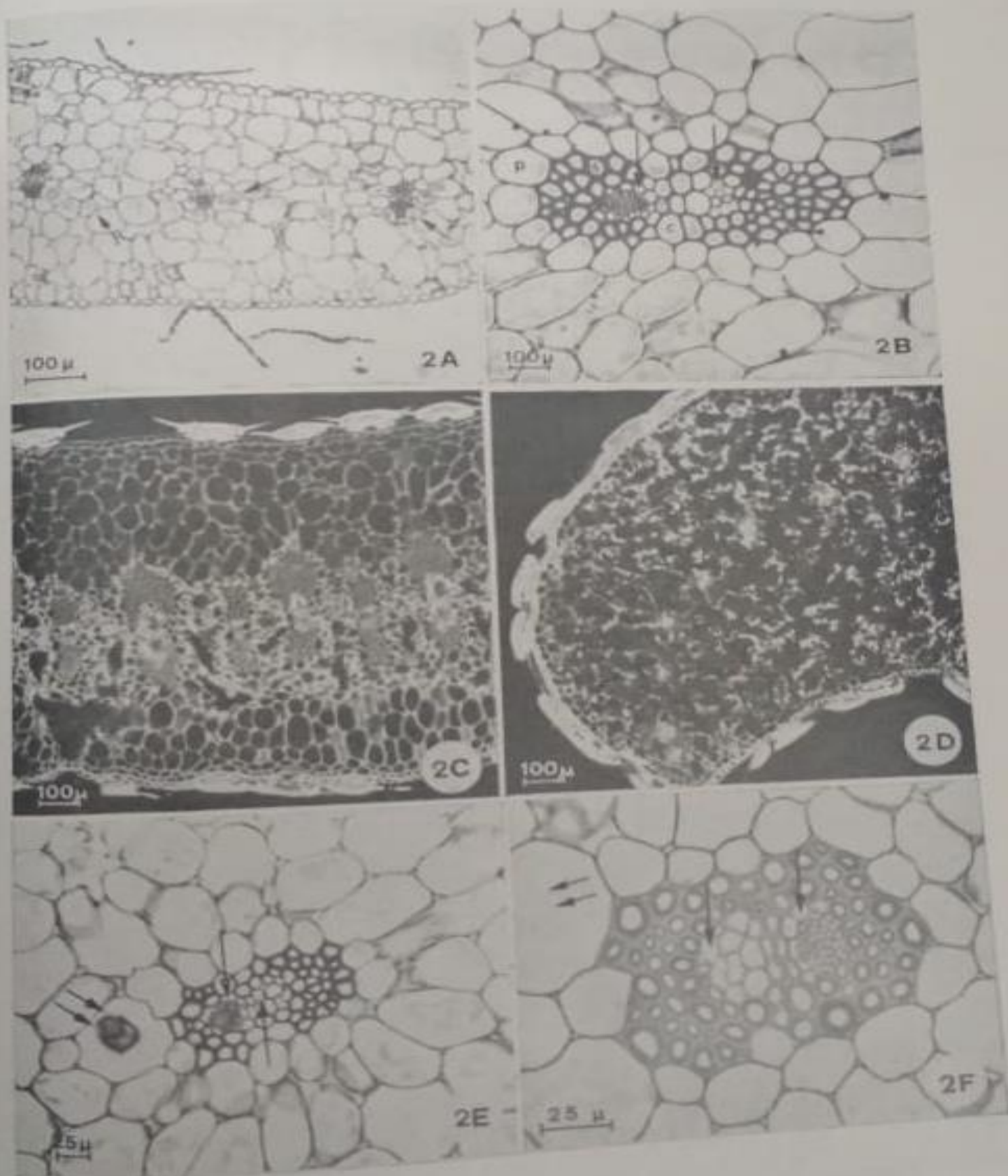


Fig. 2A-F

of small, compactly arranged parenchymatous cells. In the mesophyll, the water parenchyma with its characteristically sinuous walls lies under the epidermal layers and is more abundant near the adaxial side (Fig. 2A).

Primary fluorescence observations show a noticeable presence of chloroplasts in the parenchyma including bundles. This parenchyma, peculiarly chlorophyllous, is arranged like frets stretching towards the abaxial substomatal chambers. Water parenchyma is slightly more abundant than the chlorophyllous one (Fig. 2C): only *T. palcacea* shows a homogeneous arrangement of the chloroplasts (Fig. 2D). Air cavities are seen alternating with vascular bundles (Fig. 2A, C). In Group II species the vascular bundles are surrounded by an oxalate sheath in the same way (Fig. 2E). There is no trace of calcium oxalate crystals in the parenchymatic sheath of *T. usneoides*, though they are present in some mesophyll cells in this species. Vascular bundles do not differ from those described above in their anatomical structure and histologic composition due to the occurrence of both sclerenchymatous bundle sheath as well as periphloematic and perixylematic sheaths (Fig. 2E, 3A).

In these species, water parenchyma is visibly present in the adaxial region and consists of a few layers underlying the abaxial epidermis (Fig. 3A). Primary fluorescence observations show that in the abaxial region the parenchyma is richer in chloroplasts. It spreads until it includes the vascular bundles. The two parenchymatic tissues have the same extension (Fig. 3A). In *T. usneoides*, chloroplasts are uniformly arranged and

air cavities always alternate with vascular bundles (Fig. 3A). In Group III species there is a more uniform development of sclerenchymatous sheath. Besides, its cell walls are noticeably thickened, though not completely lignified. Well pronounced wall thickening is also observed in the cells between phloem and xylem. The parenchymatous sheaths surrounding the entire vascular bundle, as well as single vascular tissues, are similar to those of the other group (Fig. 2F).

In the mesophyll water tissue lies under both the epidermal surfaces with several layers on the adaxial side (Fig. 3B). Cells with raphides are found near the large air cavities, which alternate with vascular bundles.

Primary fluorescence reveals that, in the species of this group, the photosynthetic parenchyma is richer in chloroplasts around vascular bundles, mainly around air cavities, spreading as far as the substomatal chambers and slightly prevailing on the water tissue (Fig. 3B).

In *T. prodigiosa* the higher concentration of chloroplasts is not continuous; on the contrary, it declines where it meets the vascular bundles.

The ultrastructure of parenchymatous cells in the mesophyll is characterized by lesser number of mitochondria, Golgi bodies, ribosomes and endoplasmic reticulum; the microbodies are more numerous (Brighigna et al. 1982). The chloroplasts occurring in the water tissue, in chlorophyllous parenchyma, and in the oxalate sheaths, show a well-developed lamellar system consisting of short intergrana thylakoids and several grana (Fig. 3C, D). The stroma is scarce, not very electron-dense, and has few plasto-

Fig. 3A-F (*S.* starch; *x.* xylem). A. *T. ionantha*. C.s. of vascular bundle; fluorescence shows chloroplasts in the perixylematic and periphloematic sheaths (arrows). B. *T. flabellata*. C.s. leaf as seen in U.V. light; large air cavities alternate with vascular bundles which stand out more peculiarly in chlorophyllous parenchyma. C-F. *T. retorta*. C. Chloroplast of mesophyll cell with numerous starch granules; thylakoids are aggregated in grana. D. Enlargement of grana region. E. Portion of vascular bundle; cells of sheath surrounding xylem have chloroplasts without grana. F. Chloroplast without grana.



Fig. 3A-F

globules; many outstanding starch deposits are visible (Fig. 3C).

In perixylematic and periphloematic sheaths, chloroplasts are smaller and less numerous, but maintain a wide lamellar system without grana. Starch is condensed in a few granules (Fig. 3E).

### Discussion and Conclusions

The leaves exhibit xeromorphic characteristics in all the species of *Tillandsia*, regardless of their habitat. The presence of water tissue close to both the epidermal surfaces (Mez 1904) is also confirmed by the present study. The wavy profile of walls is a further adaptation for the storage of sufficient water. Its localization causes the concentration of chlorophyllous tissue around the vascular bundles in most of the examined samples. Besides, it provides these plants with three important advantages: (i) the water absorbed by the scales on each leaf surface (Mez 1904, Dolzmann 1964, Brighigna 1974, 1976, 1978) can be promptly stored by the plant; (ii) an efficient protection against intense sunlight is more favourable for photosynthesis; (iii) outstanding air cavities which interrupt continuity of chlorophyllous tissue mediate strong evaporation even if they ensure high photosynthetic efficiency. The contiguous water tissue can stop the subsequent water loss.

The chloroplasts occur in the mesophyll of all the three groups considered. In *T. paleacea* and *T. usneoides*, they are sparsely scattered, and the protection of photosynthetic cells is assigned to the well-developed scales forming a mantle.

Vascular tissue pattern is not different from that of the monocotyledons but abundant cross anastomoses are noticed. The resultant close vascular net is peculiar of xeromorphic plants where it helps a scarce lateral conduction (Esau 1965). In *Tillandsia* species it is associated with large intercellular contacts in the mesophyll. The xylem elements are always scarce, particularly in comparison with phloem. The abundance of phloem in the vascular net is

correlated with the necessity of an intense photoassimilate translocation. This has to be especially fast at precise moments of the biological cycle to facilitate the photosynthetic process because these plants lack specific storage organs.

The occurrence of phloem only in the vein endings further confirms the importance of this tissue in *Tillandsia* leaves, as they are also assigned the role of storage organs. With reference to the three climatic groups, vascular tissue analysis brings out anastomoses nearly as far as leaf apex in dry-climate species. In fact, their apical portions keep alive, whereas in the other species they desiccate earlier. Lack of anastomoses in leaf basal tract of species growing in moderately moist habitats corresponds to their morphology. It shows a larger tissue development with wider contact of cell walls in the basal portion.

Raphides, scattered in the mesophyll, and clustered around vascular bundles where chloroplasts are abundant, show the necessity of neutralizing the large amount of oxalic acid produced. High photorespiration, a rule among tropical plants (Tolbert 1971, Chollet & Ogren 1975, Zelitch 1971), occurs together with the scarce availability of nitrogenous nutrients, due to their peculiar epiphytism (Benzing 1976). Consequently, the molecules usually involved in amino-acid biosynthesis (Hall et al. 1981), are scarcely used in these plants.

The sheath with thickened cell walls, inside the oxalate one, works as a typical mestom (Esau 1965) besides fulfilling the most common mechanical function which helps in reducing wilting effects in xeromorphic plants (Esau 1965). Its presence in every examined ecoclimatic group has a peculiar reason; unsteady water supply due to the absence of absorbing roots necessitates a quickly drainable water reservoir between water tissue and vascular elements. Also parenchymatous, perixylematic and periphloematic sheaths help in conduction and as storage organs. The location and structure of these sheaths and the ultrastructural characteristics of their chloroplasts are reminiscent of "kranz" type sheaths. Although CAM metabo-



lism has been proved to exist in most of the examined species of *Tillandsia* (see Benzing & Renfrow 1971b, Medina & Troughton 1974, Virzo De Santo et al. 1977, Ortlieb & Winkler 1977), the morphological differences in the chlorophyllous tissue (present study) — the structural basis for the Hatch-Slack cycle — suggest that due to a peculiar physiological adaptability, *Tillandsia* adopts either CAM or  $C_3$  pathway depending on the availability of moisture. It can be assumed

that *Tillandsia* species maintain high photosynthetic efficiency by making use of both the mechanisms resulting in a strikingly conspicuous storage of starch.

On the other hand, the existence of  $C_3$ - $C_4$  photosynthetic intermediates, with specific temperature and moisture conditions, was reported by Kennedy & Laetsch (1974) and by Lerman & Queiroz (1974). Kluge et al. (1973) proved the existence of a switching CAM- $C_3$  pathway in *T. usneoides*.

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