

## ORIGIN AND STRUCTURE OF THE KRAZ TISSUES IN CYPERACEAE

MARIA EMÍLIA MARANHÃO ESTELITA

Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo. Caixa Postal 11461, 05422-970 - São Paulo, SP, Brasil. Bolsista do CNPq.

**ABSTRACT** - (Origin and structure of the Kranz tissues in Cyperaceae). The scape of *Cyperus esculentus* and young leaves of *Remirea maritima*, both Kranz species, were studied. Ultrastructural features reveal a dimorphism between chloroplasts from bundle sheath and mesophyll cells. Bundle sheath of *C. esculentus* shows chloroplasts with convoluted thylakoid system. Both species show chloroplasts with well-developed peripheral reticulum. Chloroplasts from mesophyll cells have well developed grana. In the scape, Kranz sheath may originate from modified cells of the vascular parenchyma, whereas in the young leaves differentiation occur directly from procambium cells.

**RESUMO** - (Origem e estrutura do tecido Kranz em Cyperaceae). Foram analisados o escapo de *Cyperus esculentus* e folhas jovens de *Remirea maritima*, ambas espécies Kranz. Ocorre dimorfismo estrutural entre os cloroplastos das células da bainha e dos mesófilo. *C. esculentus* tem os cloroplastos da bainha com tilacóides convolutos e em ambas as espécies, o retículo periférico é conspicuo. Os cloroplastos do mesófilo têm granos estruturados. A análise do escapo mostra que a bainha Kranz pode originar-se a partir de modificações das células do parênquima dos tecidos vasculares, enquanto que nas folhas jovens a diferenciação ocorre diretamente a partir do procâmbio.

**Key words** - Kranz structure, Cyperaceae, *Remirea*.

### INTRODUCTION

Kranz syndrome is a characteristic of plants with C<sub>4</sub> photosynthesis occurring in several families of dicotyledons and in Cyperaceae and Poaceae belonging to mononocotyledons (Welkie & Caldwell 1970, Downton 1975, Bell & Charwood 1980). It is considered a structural and functional evolution related with dry environment.

The evolution of this syndrome shows particular features according to different plant groups. Authors such as Brown and Smith (1972), suggest that in *Atriplex* (Chenopodiaceae) the Kranz syndrome evolved recently, while in the Gramineae the process may be result of two or three steps of an evolutive process which started early. In the Cyperaceae family, independent evolutive ways were proposed for the various taxa of the subfamily Cyperoideae (Takeda *et al.* 1985). Consequently, it seems clear that Kranz syndrome has evolved several times and from several groups phylogenetically distinct.

The Kranz tissue may be originated: from modification of the bundle or mestome sheaths; from the vascular tissue or may be isolated in the mesophyll (Brown 1975). Thus, its origin is related with different primary meristems. The study of the leaf development in C<sub>3</sub> and C<sub>4</sub> Gramineae (Dengler *et al.* 1985) showed a homology between the Kranz sheath of the C<sub>4</sub> species and the bundle sheath of the C<sub>3</sub> species. However, according to Brown (1975), the Kranz sheath is formed by modified bundle cells, which develop directly from procambium. On the other hand, Carolin *et al.*

(1977) and Ueno and Koyama (1987) referred to a possible origin of Kranz sheath from phloem and xylem parenchyma cells.

This work was developed in view of providing more information on the ontogenesis of Kranz sheath and other structural features of photosynthetic tissues of *Cyperus esculentus* and *Remirea maritima*.

## MATERIAL AND METHODS

After previous analysis, scape of *Cyperus esculentus* L. and leaves of *Remirea maritima* L. were chosen to be studied. The material was collected in Guaratuba beach, São Paulo State.

For anatomical studies, were used mature and young leaves, and mature scape. The material was fixed in formalin-acetic acidethanol (FAA) (50% ethanol), dehydrated in a tertiary butyl-alcohol series) and embedded in paraffin; 10µm thick seriate sections were stained with Heidenhain hematoxylin and fast-green (Sass 1951) and mounted in Permount. Freehand sections were also made, then clarified and stained with Congo red and Iodine green (Dop & Gautié 1909). Starch was identified by lugol (Johansen 1940); lignin and cellulose by zinc chloroiodide staining (Strasburger 1911).

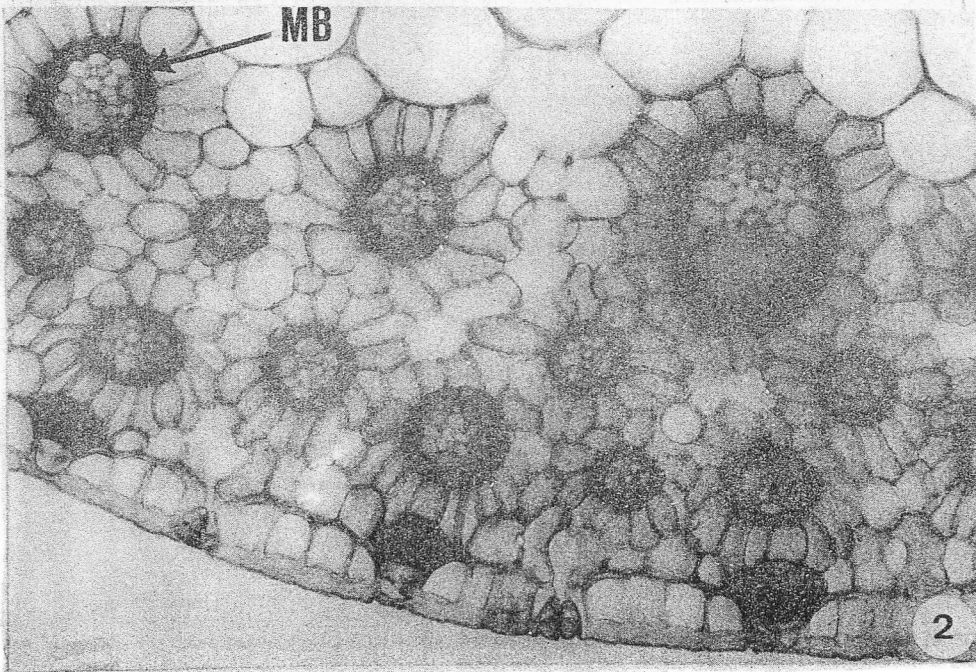
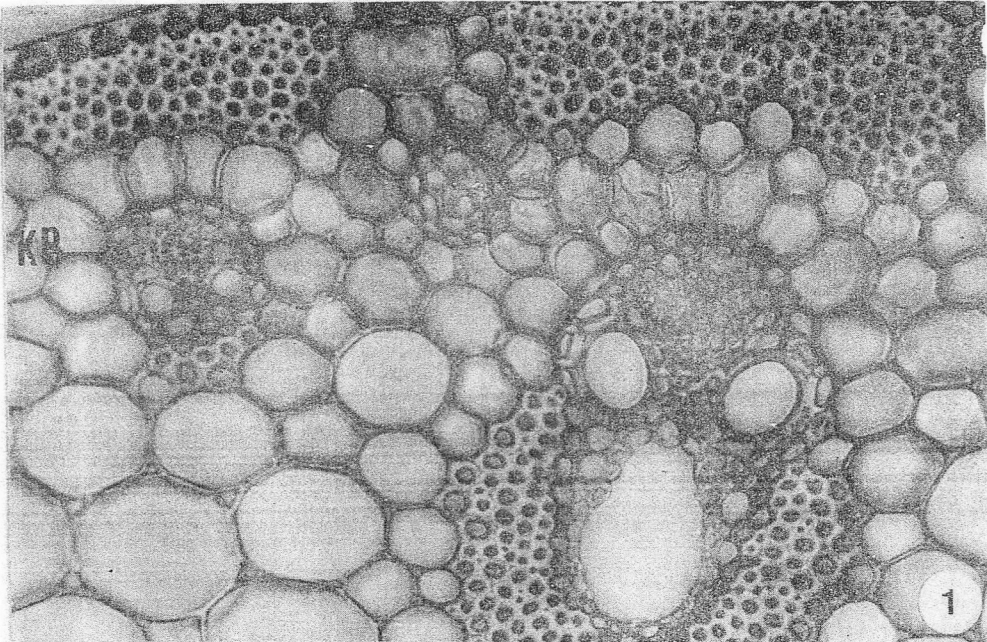
For ultrastructural analysis, were used mature leaves. Small pieces were fixed for 20 h in 2,5% Glutaraldehyde buffered with 0.1 M sodium phosphate, then washed and post-fixed in 1% OsO<sub>4</sub> for 1 h. The tissues were then dehydrated in an ethanolic series, included in Spurr resin (Spurr 1969) and stained in uranyl acetate (Watson 1958) and lead citrate (Reinholds 1963) and examined in a Siemens Elmiskop 1 electron microscope.

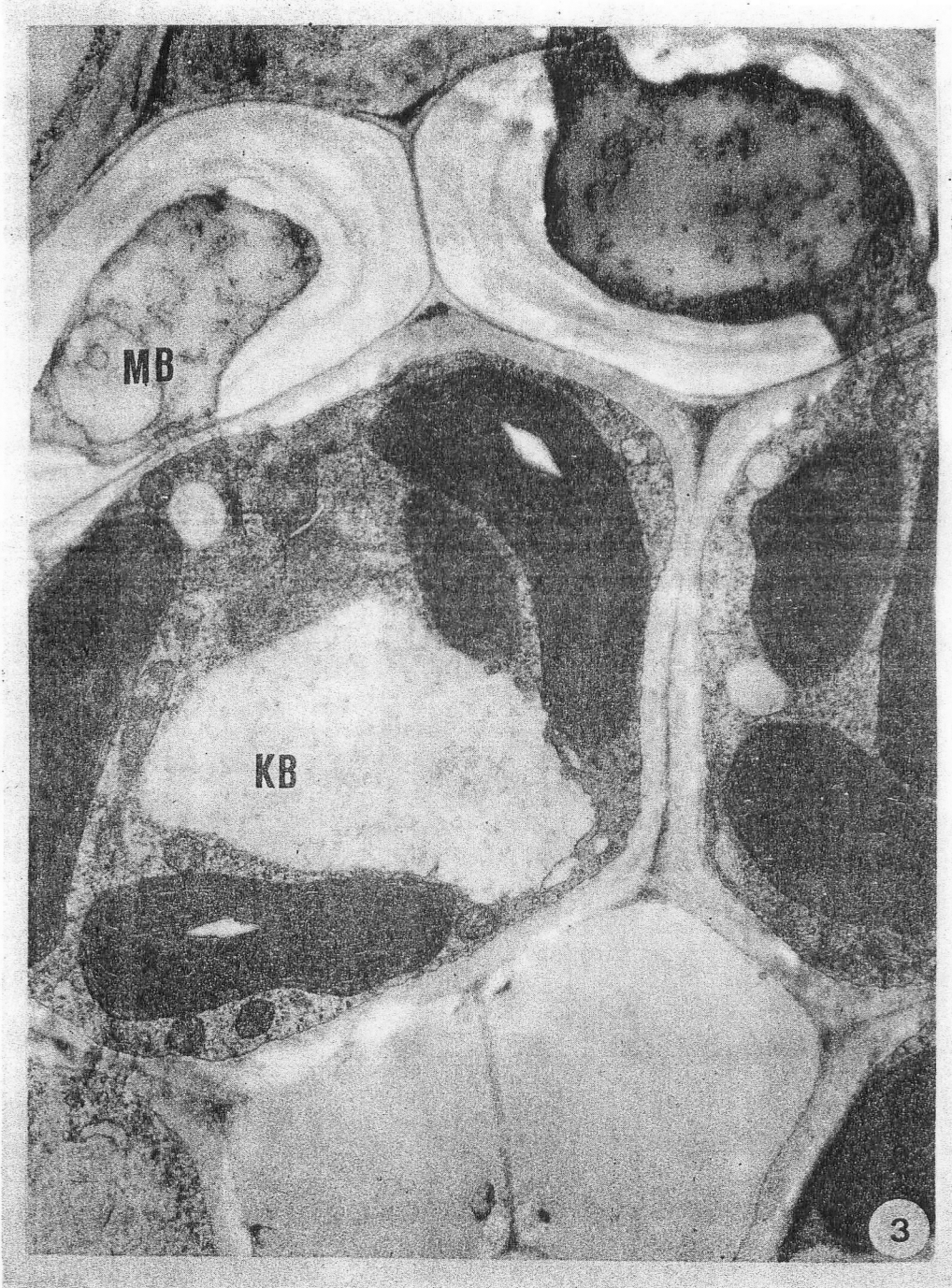
## RESULTS AND DISCUSSION

In the Cyperaceae the Kranz syndrome occurs in the subfamily Cyperoideae (Takeda *et al.* 1985) showing three structural types, depending on the presence of two or three sheaths as follows: Chlorocyperoid (presence of Kranz and mestoma sheaths), Rhynchosporoid (presence of Kranz and parenchymatous sheaths) and Fimbristylloid (presence of Kranz, mestome and parenchymatous sheaths) (Takeda *et al.* 1985). The species here studied belong to the first group (Fig. 1-3), where the vascular bundles are surrounded by two sheaths: the first, adjoining the vascular system (Fig. 1-3), is the Kranz sheath or photosynthetic carbon reduction sheath (Hattersley *et al.* 1982) which cells show many organelles, especially chloroplasts (Fig. 3). This Kranz sheath is surrounded by the mestome sheath (Fig. 1-2), which cells have conspicuous thickened walls, vacuole and indifferentiated plastids (Fig. 3), the latter feature also referred by Jones *et al.* (1981). Thus, in this plant group, the Kranz sheath is surrounded by the mestome, and may be either continuous, involving the phloem and xylem in most of the vascular bundles (Fig. 1-2) or surrounds only the phloem, in the case of major veins (Fig. 1). Kranz and mestome sheaths are involved by the mesophyll or primary carbon assimilation tissue (Hattersley *et al.* 1982), with radial arrangement, following the Kranz sheath. Another important feature of C<sub>4</sub> plants is the interveinal distance (Kanai & Koshiwagi 1975, Croockston 1980, Hattersley *et al.* 1982) which is generally fulfilled by a small number of chlorophyllous cells, as can be seen in *R. maritima* (Fig. 2).

Figs. 1-2 - *Cyperus esculentus*. Scape transection showing the typical Kranz structure in minor veins and variation in the structure of major veins. x 250. Fig. 2 - *Remirea maritima*. Leaf transection showing the typical Kranz structure. 200x. (KB = Kranz sheath; MB = mestome sheath).

Figs. 1-2 - *Cyperus esculentus*. Corte transversal do escapo mostrando a típica estrutura Kranz nas nervuras menores e variação na estrutura das nervuras maiores. x 250. Fig. 2 - *Remirea maritima*. Corte transversal da folha mostrando a estrutura Kranz típica. x 200 (KB = bainha Kranz; MB = bainha mestomática).







The ultrastructural study of the scape in *C. esculentus* and leaf in *R. maritima* revealed differences between the chloroplasts from Kranz and mesophyll cells, commonly found in several monocotyledons C4 plants. In *C. esculentus*, Kranz sheath chloroplasts are numerous, with convolute thylakoids (Fig. 4), as previously verified for other *Cyperus* species (Carolin *et al.* 1977, Jones *et al.* 1981, Estelita-Teixeira & Handro 1987, Ueno *et al.* 1988); grana are not organized, and the peripheral reticulum, well developed, may penetrate the stroma, as verified in *C. iria* (Ueno *et al.* 1988). Chloroplasts of radial mesophyll cells have grana with many thylakoids (fig. 5) and the peripheral reticulum is not conspicuous, according with observations of the authors cited above. In *R. maritima*, Kranz chloroplasts do not show convolute thylakoids, although peripheral reticulum is well developed (Fig. 6).

In *R. maritima*, plasmodesmata were observed between two adjacent Kranz cells, between Kranz and mestome cells, and also between mestome and mesophyll cells (Fig. 3), as referred by Jones *et al.* (1981) for *C. longus*. However, in *Rhynchospora rubra*, Ueno *et al.* (1988) observed plasmodesmata between Kranz and parenchyma cells of the vascular tissue.

The development of the Kranz structure in the scape of *C. esculentus* was analysed accropetally in a single vascular bundle (Fig. 7, arrow). In the basal region of the scape, the Kranz structure does not occur (Fig. 8). The vascular tissue is adjacent to the mestome sheath, and in the parenchyma surrounding the bundles, the few plastids are amyloplasts. This region, macroscopically, is white-yellowish, and involved by bracts. Higher up, in the region completely exposed, several cells of the phloem parenchyma are ordered forming a cap, showing chloroplasts (Fig. 9); the surrounding parenchyma also shows chloroplasts, and its cells are arranged radially when adjacent to that cap. Some cells of the xylem parenchyma have amyloplasts. In a higher level, cells of the xylem parenchyma become more conspicuous showing chloroplasts; thus, the Kranz sheath becomes continuous (Fig. 10). In major veins, the Kranz sheath is interrupted by metaxylem elements, as cited by Brown (1975) for *Fimbristylis caroliniana*. In the vascular bundle under analysis, the cells of the adjacent chlorenchyma are arranged radially in the phloem region; in minor veins this arrangement occurs in both sides (Fig. 11). This case is considered by some authors (Brown 1975, Jones *et al.* 1981) as a typical Kranz structure, the first being a variation.

The study of young leaves of *R. maritima* (Fig. 12-15) shows the differentiation of vascular tissues, mestome and Kranz sheath (Fig. 15). As comparing this study with that of Dengler *et al.* (1985) with Poaceae species, it can be considered that both in Poaceae and Cyperaceae, the procambium originates the vascular tissues and the adjacent sheath. In the Cyperaceae, however, besides this differentiation directly from the procambium, a two-step type also occur, as described for the scape.

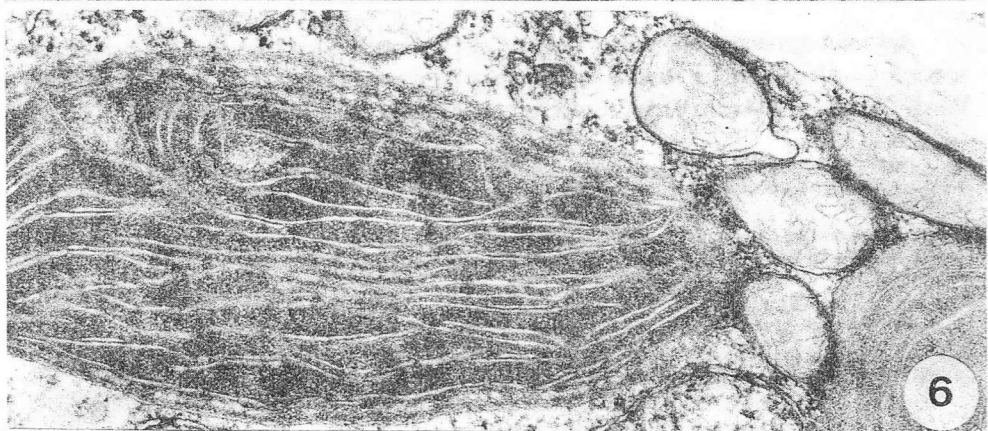
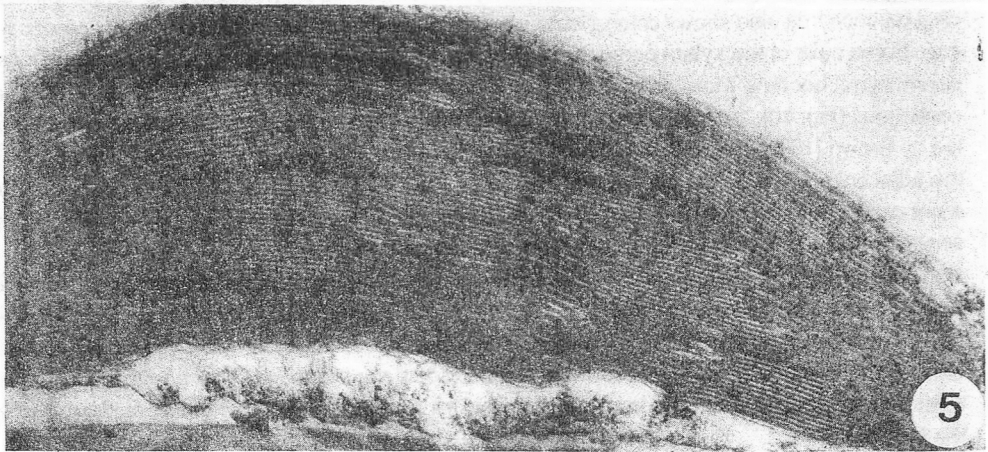
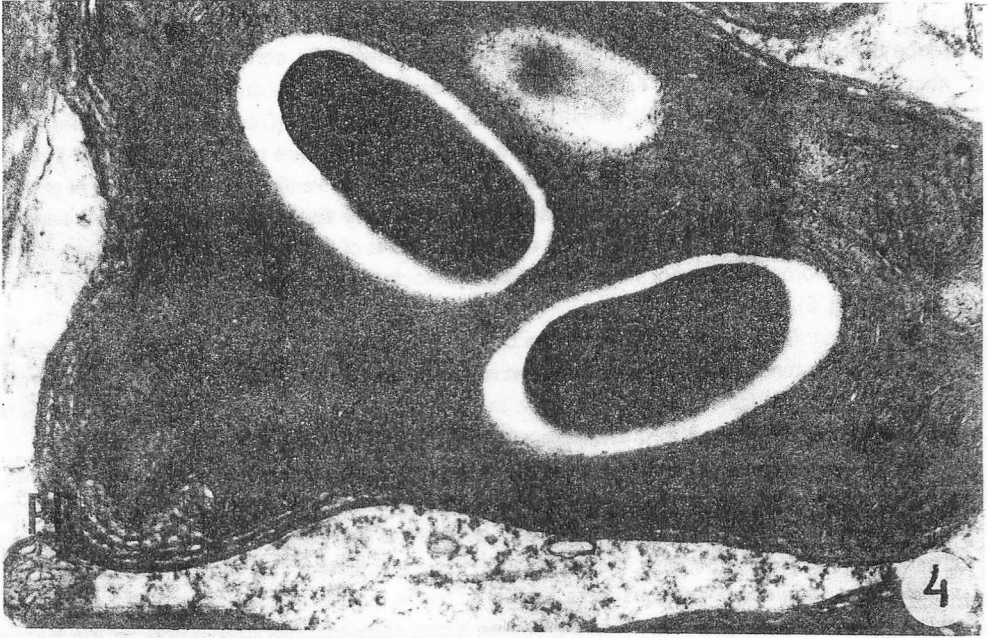
**Acknowledgments** - The author thanks Çeli Ferreira da Silva Muniz for the identification of species, Dr. Alberto Freitas Ribeiro, for the use of the electron microscope, Nelson Bomtempo Jr., Waldir Caldeira and Márcio V. da Cruz for technical assistance.

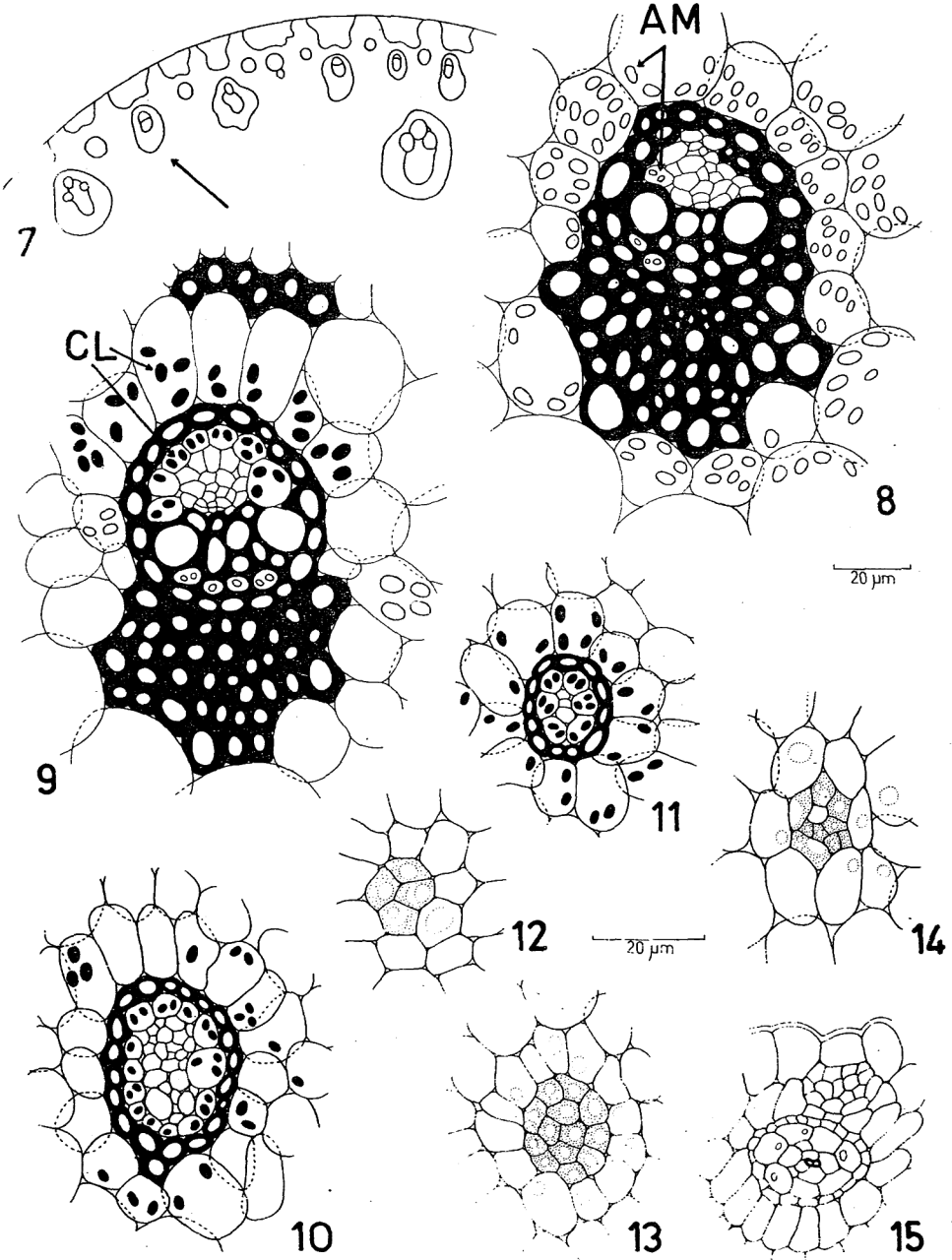
Fig. 3 - *Remirea maritima* (leaf). Electromicrography showing Kranz and mestome sheaths. Note plasmodesmata, except between Kranz sheath and vascular tissue. x 13.000 (MB = mestome sheath; KB = Kranz sheath).

Fig. 3 - *Remirea maritima* (folha). Eletromicrografia mostrando bainhas Kranz e mestomática. Notar plasmodesmos, exceto entre a bainha Kranz e o tecido vascular. x 13.000 (MB = bainha mestomática; KB = bainha Kranz).

Figs. 4-5 - *Cyperus esculentus*. 4 - Chloroplasts of Kranz cells. x 25.000. 5 - Mesophyll Chloroplast. x 28.000. Fig. 6. *Remirea maritima*. Chloroplast of Kranz cell. x 30.000. (PR = peripheral reticulum).

Figs. 4-5 - *Cyperus esculentus*. 4 - Cloroplastos de células Kranz. x 25.000; 5 - Cloroplasto do mesófilo. x 28.000. Fig. 6. *Remirea maritima*. Cloroplasto de célula Kranz. x 30.000. (PR = retículo periférico).





## REFERENCES

- BELL, E.A. & CHARLWOOD, V. 1980. Secondary plant products. In *Encyclopedia of Plant Physiology*, New Series, vol. 8.
- BROWN, W.V. 1975. Variations in anatomy, associations, and origins of Kranz tissue. *Amer. J. Bot.* 62: 395-402.
- BROWN, W.V. & SMITH, B.N. 1972. Grass evolution, the Kranz syndrome,  $^{13}\text{C}/^{12}\text{C}$  ratios, and continental drift. *Nature* 239: 345-346.
- CAROLIN, R.C., JACOBS, S.W.L. & VESK, M. 1977. The ultrastructure of Kranz cells in the family Cyperaceae. *Bot. Gaz.* 138: 413-419.
- CROOKSTON, R.K. 1980. The structure and function of  $\text{C}_4$  vascular tissue. *Ber. Deutsch. Bot. Ges.* 93: 71-78.
- DENGLER, N.G., DENGLER, R.E. & HATTERSLEY, R.W. 1985. Differing ontogenetic origins of PCR (Kranz) sheaths in leaf blades  $\text{C}_4$  grasses (Poaceae). *Amer. J. Bot.* 72: 284-302.
- DOP, P. & GAUTIÉ, A. 1909. *Manuel de technique botanique*. F.R. de Rudeval. Paris.
- DOWNTON, W.J.S. 1975. The occurrence of  $\text{C}_4$  photosynthesis among plants. *Photosynthetica* 9: 96-105.
- ESTELITA-TEIXEIRA, M.E. & HANDRO, W. 1987. Kranz pattern in leaf, scape and bract of *Cyperus* and *Fimbristylis* species. *Revta bras. Bot.* 10: 105-111.
- HATTERSLEY, P.W.L., WATSON, F.L.S. & JOHNSTON, C.R. 1982. Remarkable leaf anatomical variations in *Neurachne* and its allies (Poaceae) in relation to  $\text{C}_3$  and  $\text{C}_4$  photosynthesis. *Bot. J. Linn. Soc.* 84:265-272.
- JOHANSEN, D.A. 1940. *Plant Microtechnique*. McGraw-Hill Book Company. New York.
- JONES, M.B., HANNON, G.E. & COFFEY, M.D. 1981.  $\text{C}_4$  photosynthesis in *Cyperus longus* L., a species occurring in temperature climates. *Plant Cell Environment* 4: 161-168.
- KANAI, R. & KASHIWAGI, M. 1975. *Panicum milioides*, a Gramineae plant having Kranz leaf anatomy without  $\text{C}_4$  photosynthesis. *Plant Cell Physiol.* 16: 669-679.
- REYNOLDS, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208.
- SASS, J.E. 1951. *Botanical Microtechnique*. The Iowa State College Press. Iowa.
- SPURR, A. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastructure Res.* 26: 31-43.
- STRASBURGER, E. 1911. *Handbook of practical Botany*. (Translated from the German by H. Hillhouse). George Allen & Company Ltd. London.
- TAKEDA, T., UENO, O., SAMEJIMA, M. & OHTANI, T. 1985. An investigation for the occurrence of  $\text{C}_4$  photosynthesis in the Cyperaceae from Australia. *Bot. Mag. Tokyo* 98: 393-411.
- WATSON, M.L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4: 475.
- WELKIE, G.W. & CALDWELL, M. 1970. Leaf anatomy of species in some dicotyledon families as related to the  $\text{C}_3$  and  $\text{C}_4$  pathways of carbon fixation. *Can. J. Bot.* 48: 2135-2146.
- UENO, O. & KOYAMA, T. 1987. Distribution and evolution of  $\text{C}_4$  syndrome in *Rhynchospora* (Rhynchosporae-Cyperaceae). *Bot. Mag. Tokyo* 100: 63-85.
- UENO, O., TAKEDA, T. & MAEDA, E. 1988. Leaf ultrastructure of  $\text{C}_4$  species possessing different Kranz anatomical types in the Cyperaceae. *Bot. Mag. Tokyo* 101: 141-152.

Figs. 7-11 - *Cyperus esculentus*. 7 - Scape diagram showing vascular bundles. 8 - Vascular bundle of the basal region. 9 - Vascular bundle with Kranz sheath and radial mesophyll opposite to the phloem. 10 - Vascular bundle with continuous Kranz sheath. 11 - Vascular bundle with continuous Kranz sheath and radial mesophyll. Figs. 11-15 - *Remirea maritima*. Transsections of young leaves. 12 - Procambium. Fig. 13-14 - Early stages of differentiation of vascular tissues and sheaths. 15 - Kranz and mestome sheaths in later differentiation (AM= amyloplast; CL= chloroplasts).

Figs. 7-11 - *Cyperus esculentus*. 7 - Diagrama do escapo mostrando feixes vasculares. 8 - Feixe vascular da região basal. 9 - Feixe vascular com bainha Kranz e mesofilo radial oposto ao floema. 10 - Feixe vascular com bainha Kranz contínua. 11 - Feixe vascular com bainha Kranz contínua e mesofilo radial. Figs. 12-15. *Remirea maritima*. Cortes transversais de folhas jovens. 12 - Procâmbio. 13-14 - Estádios iniciais da diferenciação de tecidos vasculares e bainhas. 15 - Bainhas Kranz e mestomática em diferenciação avançada. (AM = amiloplasto; CL = cloroplasto).