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# Bulliform Cells in *Loudetiopsis chrysothrix* (Nees) Conert and *Tristachya leiostachya* Nees (Poaceae): Structure in Relation to Function

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## ABSTRACT

*This work reports anatomic and ultrastructural characteristics of bulliform cells in* Loudetiopsis chrysothrix *(Nees) Conert and* Tristachya leiostachya *Nees. Both the species presented leaf rolling under water stress. The main characteristics observed in these cells were: periclinal wall thinner than the adjacent epidermal wall; abundance of pectic substances in cuticular layer; sinuous anticlinal walls with ramified plasmodesmata; vacuome formed by a developed vacuole or innumerous small vacuoles; abundance of phenolic substances and oil drops. These characteristics suggested the involvement of bulliform cells in the mechanism of foliar involution in the studied species.*

**Key words:** Anatomy, bulliform cells, cerrado, Poaceae, ultrastructure

## INTRODUCTION

Bulliform cells, also called motor cells, are present in all monocotyledonous orders, except the Helobiae. Their morphology combined with enlarged mesophyll colourless cells has been used as taxonomic characteristics (Metcalfe, 1960). Although there are different interpretations of the bulliform cell role, its functional significance has been discussed (Mauseth, 1988; Moulia, 2000). Haberlandt (1914) described that the hygroscopic turgor changes of bulliform cells could cause surface-reducing movements in mature xeric leaf. For other authors, these cells were considered as water storage (Prat, 1948; Eleftheriou and Noitsakis, 1978; Vecchia et al., 1998), and can participate in the young leaf expansion that was rolled in the apex, or in leaf rolling and/or folding of mature leaves due to water stress (Shields, 1951; Jane and Chiang, 1991). According to Esau (1965), during excessive water loss, the bulliform cells, in conjunction with or without colorless cells, became flaccid and enabled the leaf either to fold or to roll. According to Clayton and Renvoize (1986), bulliform cells favoured the light entrance in the mesophyll cells. In some species, bulliform cells were not actively or specifically related to unfolding and hygroscopic leaf movement, since they accumulated large amounts of silicon and their outermost walls might thicken and cutinize, becoming stiff (Ellis, 1976). Considering the taxonomic and likely ecophysiological role of bulliform cells, their

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structural details have been studied in few species (Tuan et al., 1965; Jane and Chiang, 1991; Vecchia et al., 1998).

*Loudetiopsis chrysothrix* (Nees) Conert and *Tristachya leiostachya* Nees, two native grasses found in the rocky fields and cerrado (Longhi-Wagner, 2001), present leaf rolling under water stress and possess features as epicuticular waxes, silica bodies in the costal zone, and stomata in furrows that can be related to water saving (Alvarez et al., 2005).

The aim of this work was to provide data about the anatomy and ultrastructure of bulliform cells of *L. chrysothrix* and *T. leiostachya*, which might be related to the leaf involution mechanism.

## MATERIALS AND METHODS

Expanded and rolled mature leaves were collected from populations of the *L. chrysothrix* and *T. leiostachya* plants growing in cerrado vegetation in Botucatu (22°48'55.5" S and 48°31'26.1" W) and Pratânia (22°48'50.2" S and 48°44'35.8" W), São Paulo State, Brazil. Vouchers were deposited in "Irina Delanova Gemtchujnicov" Herbarium of Universidade Estadual Paulista (BOTU).

Light microscopy – samples from median region of the expanded leaf blade were fixed in FAA 50% for 48 h at room temperature (Johansen, 1940). Sections (8μm) were cut on a Ranvier microtome, stained with Astrablue-Safranin (Bukatsch, 1972), and observed with a Zeiss light microscope. For histochemistry, fresh sections were submitted to tests with: Sudan IV for lipids (Johansen, 1940), ruthenium red for pectin (Jensen, 1962), acidic phloroglucin for lignin (Sass, 1951), and ferric chloride for phenolic compounds (Johansen, 1940).

Transmission electron microscopy – Samples were fixed with 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.3) for 8-12 h at room temperature; post-fixed with 1% osmium tetroxide aqueous solution in the same buffer for 2 h at room temperature. After dehydration in a graded acetone series (50% - 2x10 min., 70% - 2x10min., 90% - 2x15min., 90% - 2x15min. and 100% 3x15min.), the material was embedded in epoxy resin (Araldite). Ultra-thin sections were contrasted with uranyl acetate (Watson, 1958), and lead citrate (Reynolds, 1963) and observed with a Philips CM 100 transmission electron microscope.

## RESULTS

In the hydrated state, the leaves in both species were fully expanded (Figs. 1 and 2). During the desiccation that occurred under field conditions around the midday, the leaves gradually rolled along the midrib, and the leaf abaxial side was exposed to sun radiation (Figs. 3 and 4).

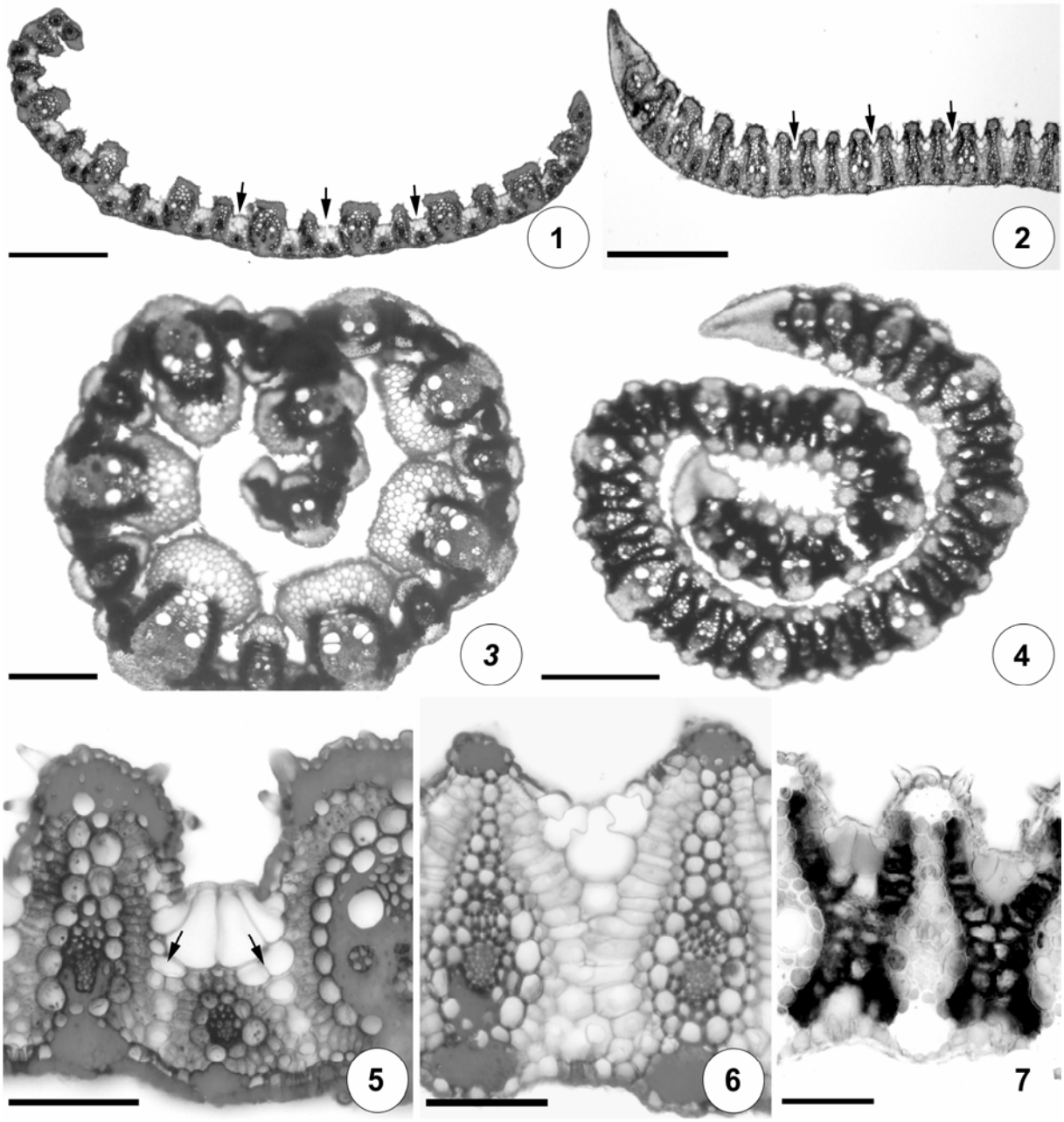
Both the species possessed bulliform cells in groups regularly spaced in the leaf blade adaxial surface, and were arranged in parallel rows in the intercostal zone (Figs. 1 and 2). In the both species, each bulliform cell group exhibits 3-5 fan-shaped cells; they were largest in *L. chrysothrix* as seen in cross section (Fig. 5). Bulliform cells were the largest among the epidermal cells (Figs. 5 and 6) and their outer periclinal walls were thinner than neighboring epidermal cells. Unlike the neighboring lignified epidermal cells, bulliform cells had outer periclinal cell walls with abundant pectin and were covered by a very thin cuticle as revealed by ruthenium red and Sudan IV respectively. Phenolic substances were abundant in all bulliform cells (Fig. 7).

Bulliform cells were closely associated with adjacent colourless mesophyll cells in *L. chrysothrix* (Fig. 5). These cells were smaller than bulliform cells, larger than chlorenchyma cells, translucent, voluminous, highly vacuolized, and arranged in uni-seriate column connecting abaxial epidermis and bulliform cells. In *T. leiostachya* colourless cells were absent and bulliform cells were connected with chlorenchyma (Fig. 6).

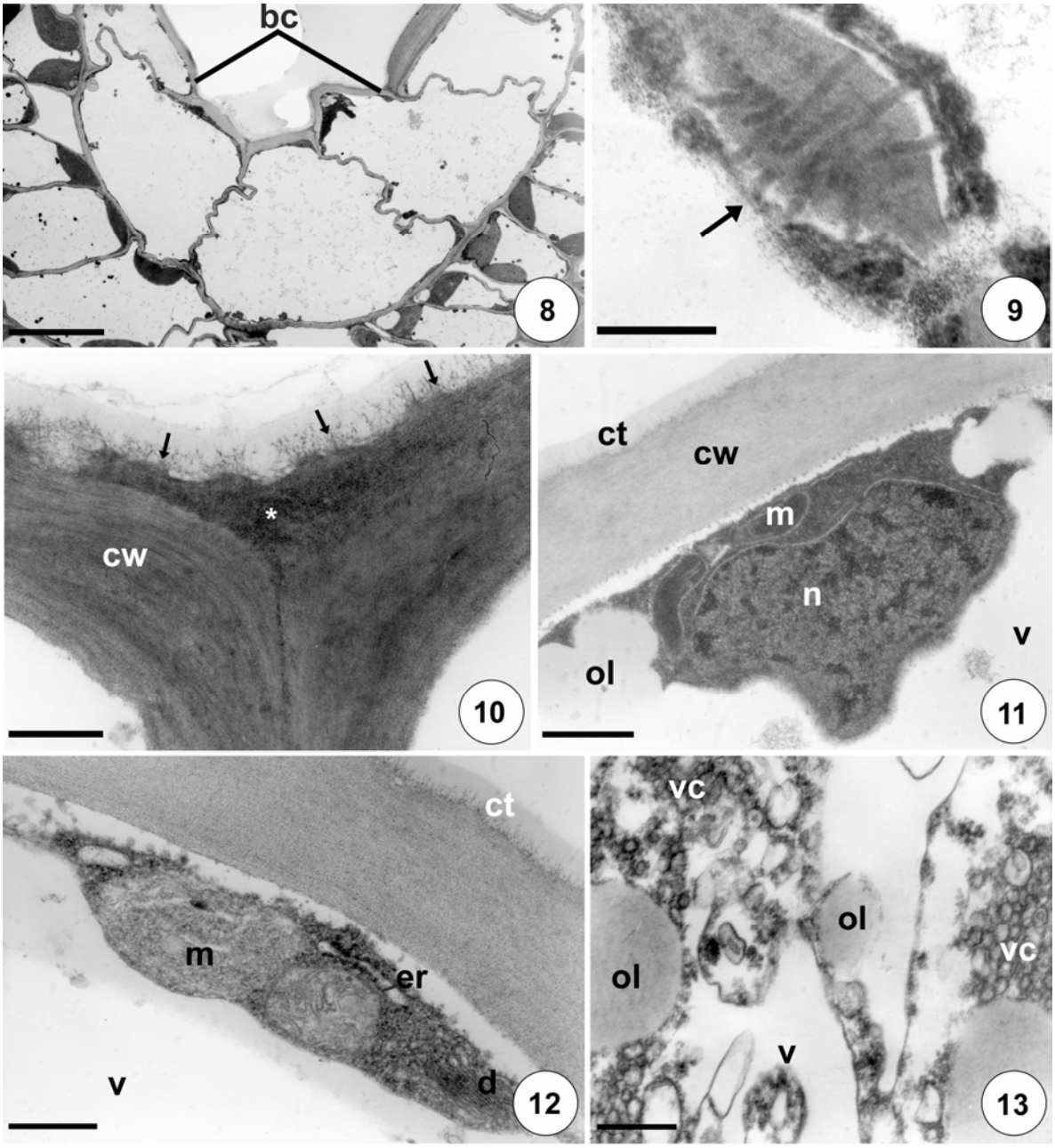
As ultrastructural features were identical for the two species, illustrations were presented to *T. leiostachya* only. Bulliform cells presented outer periclinal wall thinner than the adjacent epidermal cells, and sinuous thin anticlinal walls (Fig. 8) with ramified plasmodesmata (Fig. 9). The outer periclinal cell wall showed a loose arrangement of the cellulose microfibrils and a complex cuticle with three distinct regions: cuticle proper, formed by a thin electron-lucent homogeneous layer of cutin; cuticle layer formed by a network of osmiophilic pectin fibrillae, and an inner osmiophilic amorphous pectin stratum (Fig. 10). Bulliform cells with only one large vacuole (Fig. 8) and multivacuolate bulliform cells (Fig. 13) were observed side by side.

Univacuolate bulliform cells showed a peripheral nucleus (Fig. 11) and a very dense reduced cytoplasm with free ribosomes, oil drops, dictyosomes with adjacent vesicles, voluminous mitochondria, and endoplasmic reticulum (Figs. 11 and 12). Multivacuolate bulliform cells showed

numerous aggregate vesicles and scattered large oil drops (Fig. 13).




**Figures 1-7 -** Leaf blade in transverse section. Expanded leaf blade of *Loudetiopsis chrysothrix* (1) and *Tristachya leiostachya* (2) showing bulliform cells (arrows) on the adaxial surface, in groups in the intercostal zone. 3-4. Leaf blade of *Loudetiopsis chrysothrix* (3) and *Tristachya leiostachya* (4) rolled under dissection. 5. Conspicuous bulliform cells in *Loudetiopsis chrysothrix* associated with columns of colourless cells (arrows) in the mesophyll. 6. Smaller bulliform cells in *Tristachya leiostachya* connected to the chlorenchyma. 7. Fresh colorless section treated with ferric chloride showing phenolic substances in bulliform cells of *Tristachya leiostachya*. Scale bars = 250µm (1), 208µm (2), 400µm (3, 4), 66µm (5), 40µm (6), 114µm (7).




**Figures 8-13 -** Ultrastructural aspects of bulliform cells of *Tristachya leiostachya* (TEM). 8. Bulliform cells (bc) with thin periclinal and sinuous anticlinal walls. 9. Ramified plasmodesmata on the anticlinal walls (arrow). 10. Detail of the outer periclinal wall (cw) of the bulliform cell showing cuticular layer with a net of pectin fibrils (arrows) and stratum of amorphous pectin (*). 11. Univacuolate cell with peripheral nucleus (n). 12. Univacuolate cell with reduced cytoplasm containing dictyosomes (d), voluminous mitochondria (m), endoplasmic reticulum (er) and oil drops (ol). 13. Multivacuolate cell containing dispersive oil drops (ol) and numerous aggregated vesicles (vc). ct = cuticle; cw = cell wall, ol = oil drops and v = vacuole. Scale bars = 8µm (8), 0,34µm (9, 10, 11), 0,18µm (12), 0,25µm (13).

## DISCUSSION

*L. chrysothrix* and *T. leiostachya* showed leaf rolling of mature and young leaves during water stress. According to Moulia (1994), the leaf rolling is a xeromorphic characteristic and has adaptive value, reducing light interception, transpiration, and protecting the leaf from dehydration and overheating. This would be a mechanism to minimize light exposition and water transpiration, keeping the stomata in microclimate with higher humidity, preventing dries (Begg, 1980; Clarke, 1986; Silva et al., 2001).

Under water stress, bulliform cells in both species and colourless cells in *L. chrysothrix*, reduced their initial volume until the leaf involution and returned to initial volume when rehydrated, as observed by Anton (1986) in some species of *Axonopus*. Rapid changes of cell volume observed in bulliform cells occurs in a number of plant cells, which were involved in nastic movements, e.g. the stomata guard cells and the pulvinar motor cells, and were involved with the water use efficiency (Salisbury and Ross, 1992).

The morphological characteristics of bulliform cells observed in *L. chrysothrix* and *T. leiostachya* suggested that during dehydration, these cells might lose water through their outer surfaces (Metcalfe, 1960; Jane and Chiang, 1991). On the other hand, the occurrence of abundant pectin could be correlated with retention and/or water transport and with the cuticle transpiration control (Machado and Sajo, 1996). Some authors suggested that the pectin fibrils in the cuticle layer functioned as microchannels that established a continuity, permanent or temporary, between the cuticle and the cellulosic cell wall (Lyshede, 1982), allowing water entrance and exit from the cells. In addition, cell wall pectin could act as water reservoirs for the internal leaf environment, slowing down the drying rate. Under dry conditions, these substances with the epicuticular waxes observed by Alvarez et al. (2005) might act as a barrier to retard the water loss.

The vacuole characteristics observed in the bulliform cells could be associated with different physiological conditions of these cells. Cells with two distinct types of vacuoles, as observed in the present work, were described in the motor cells of pulvinus in *Albizzia julibrissin* (Satter et al., 1970) and *Mimosa pudica* (Campbell and Thompson, 1977), which were surely involved with leaf movement. The authors suggested that the changes of the vacuolar compartment, univacuolate to multivacuolate condition, provided a mechanism for cell volume reduction, while maintained the tonoplast superficial areas. Vacuole fragmentation during water stress was described in *Coleochloa setifera* (Cyperaceae) (Bartley and Hallam, 1979), *Sporobolus stapfianus* (Poaceae) in xeric environments (Vecchia et al., 1998), and *Talbotia elegans* (Velloziaceae), a desiccation-tolerant angiosperm (Hallan and Luff, 1980). This phenomenon has been regarded as a cellular event linked to the drying-adaptation (Vecchia et al., 1998). During desiccation, the vacuoles of the *Talbotia elegans* mesophyll cells appeared to be broken into many areas containing phenolic material and lipid droplets (Hallan and Luff, 1980). According to the authors, phenolic substances were found in the vacuole of many xerophytes and suggested that they could protect against the damaging effects of light in the ultraviolet wavelengths of the spectrum. Phenolic substances, mainly tannins, have also been reported in stomata guard cells and motor cells of pulvinus, suggesting a possible role of these substances in the regulation or maintenance of the cell turgor, as well as limiting drought damage by protecting cell integrity during dehydration (Toriyama, 1955; Machado and Rodrigues, 2004). Bartley and Hallam (1979) described lipid bodies in *Coleochloa setifera* chlorenchyma cells during water stress, but it was not known whether these were the result of newly synthesized lipids or the accumulation of lipids from unstructured vacuole membranes.

The ultrastructural features of the bulliform cells as free ribosomes, dictyosomes, endoplasmic reticulum, larger mitochondria, and ramified plasmodesmata indicated that some peculiar functions were established in these cells, as suggested by Jane and Chiang (1991). The presences of these cell organelles indicated a high metabolism activity, and ramified plasmodesmata suggested extensive simplastic connections between bulliform cells. Hence, the water storage did not appear the sole purpose of bulliform cells. Shields (1951) described that the subepidermal sclerenchyma and other elements of mesophyll rather than bulliform cells contributed to involution in some xeric grasses. Ellis (1976) suggested caution in assigning bulliform cells a role in leaf movement. Unlike the opinion of these authors, the

present results indicated that in *L. chrysothrix* and *T. leiostachya*, bulliform cells appeared to play an active role during the leaf movement. Therefore, the data of the present work supported the hypothesis about the involvement of the bulliform cells in the involution mechanism of young and mature leaves.

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## RESUMO

Este trabalho relata as características anatômicas e ultra-estruturais das células buliformes em *Loudetiopsis chrysothrix* (Nees) Conert e *Tristachya leiostachya* Nees. Ambas as espécies apresentam enrolamento foliar em condições de estresse hídrico. As principais características observadas nessas células foram: paredes periclinais mais delgadas que as epidérmicas adjacentes; abundância de substâncias pécticas na camada cuticular; paredes anticlinais sinuosas com plasmodesmas ramificados; vacuoma formado por um vacúolo desenvolvido ou inúmeros vacúolos pequenos; abundância de substâncias fenólicas e gotas de óleo dispersas. As características observadas sugerem o envolvimento das células buliformes no mecanismo de involução foliar nas espécies estudadas.

## REFERENCES

Alvarez, J. M.; Rocha, J. F. and Machado, S. R. (2005), Estrutura foliar de *Loudetiopsis chrysothrix* (Nees) Conert e *Tristachya leiostachya* Nees (Poaceae). *Revta. brasil. Bot.*, **28**, 23-37.

Anton, A. M. (1986), Contribucion al conocimiento de la anatomia foliar del genero *Axonopus* (Poaceae). *Darwiniana*, **27**, 157-158.

Bartley, M. and Hallam, N. D. (1979), Changes in the fine structure of the desiccation-tolerant sedge *Coleochloa setifera* (Ridley) Gilly under water stress. *Aust. J. Bot.*, **27**, 531-545.

Begg, J. E. (1980), Morphological adaptations of leaves to water stress. In - *Adaptation of plants to water and high temperature stress*, ed. N. C. Turner and P. J. Kramer. John Wiley and Son, New York, pp. 33-42.

Bukatsch, F. (1972), Bemerkungen zur doppelfärbung astrablau-safranin. *Mikrokosmos*, **61**, 255.

Campbell, N. A. and Thompson, W. W. (1977), Multivacuolate motor cells in *Mimosa pudica* L. *Ann. Bot.*, **41**, 1361-1362.

Clarke, J. M. (1986), Effect of leaf rolling on leaf water loss in *Triticum* spp. *Can. J. Plant Sci.,* **66**, 885-891.

Clayton, W. D. and Renvoize, S. A. (1986), *Genera graminum – grasses of the world*. Kew Bulletin Additional Series XIII. London: Her Majestic Stationary Office.

Eleftheriou, E. P. and Noitsakis, B. (1978), A comparative study of the leaf anatomy of the grasses *Andropogon ischaemum* and *Chrysopogon gryllus*. *Phyton*, **19**, 27-36.

Ellis, R. P. (1976), A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section. *Bothalia*, **12**, 65-409.

Esau, K. (1965), *Plant anatomy*. Jonh Wiley and Son, New York.

Haberlandt, G. (1914), *Physiological plant anatomy*. Macmillan and Co, London.

Hallam, N. D. and Luff, S. E. (1980), Fine structural changes in the leaves of the desiccation-tolerant plant *Talbotia elegans* during extreme water stress. *Bot. Gazette*, **141**, 180-187.

Jane, W. N. and Chiang, S. H. T. (1991), Morphology and development of bulliform cells in *Arundo formosana* Hack. *Taiwania*, **36**, 85-97.

Jensen, W. A. (1962), *Botanical hitochemistry: principle and pratice.* W. H. Freeman, San Francisco.

Johansen, D. A. (1940), *Plant microtechnique*. McGraw-Hill, New York.

Longhi-Wagner, H. M. (2001), Tribo Arundinelleae. In: Wanderley, M. G. L.; Shepherd, G. J. and Giulietti, A. M. *Flora fanerogâmica do Estado de São Paulo, Poaceae.* Fapesp - Hucitec, São Paulo. v.1, pp. 119-123.

Lyshede, O. B. (1982), Structure of the outer epidermal wall in xerophytes. In-*The plant cuticle*, ed. D. F. Cutler; K. L. Alvin and C. E. Price. Academic Press, London, pp. 87-98.

Machado, S. R. and Rodrigues, T. M. (2004), Anatomia e ultra-estrutura do pulvino primário de *Pterodon pubescens* Benth. (Fabaceae-Faboideae). *Revta brasil. Bot.,* **27**, 135-147.

Machado, S. R. and Sajo, M. G. (1996), Intercellular pectic protuberances in leaves of some *Xyris* species (Xyridaceae). *Can. J. Bot.,* **74**, 1539-1541.

Mauseth, J. D. (1988), *Plant anatomy*. Benjamin Cummings, California.

Metcalfe, C. R. (1960), *Anatomy of monocotyledons. I. Gramineae*. Claredon Press, Oxford.

Moulia, B. (1994), Biomechanics of leaf rolling. *Biomimetics*, **2**, 267-281.

Moulia, B. (2000), Leaves as shell structures: double curvature, auto-stresses, and minimal mechanical energy constraints on leaf rolling in grasses. *J. Pl. Growth Reg.*, **19**, 19-30.

Prat, K. (1948), General features of the epidermis in *Zea mays*. *Ann. Mo. Bot. Gdn.*, **35**, 341-351.

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.

Salisbury, F. B. and Ross, C. W. (1992), *Plant physiology*. Wadsworth Publishing Company, Belmont.

Sass, J. E. (1951), *Botanical microtechnique*. Iowa State College Press, Ames.

Satter, R. L.; Sabnis, D. D. and Galston, A. W. (1970), Phytochrome controlled nictinsty in *Albizzia julibrissin*. I. Anatomy and fine structure of the pulvinule. *Am. J. Bot.*, **57**, 374-381.

Shields, L. M. (1951), The involution mechanism in leaves of certain xeric grasses. *Phytomorphology* **1**, 225-241.

Silva, S.; Soares, A. M.; Oliveira, L. E. M. and Magalhães, P. C. (2001), Respostas fisiológicas de gramíneas promissoras para revegetação ciliar de reservatórios hidrelétricos, submetidas à deficiência hídrica. *Ciência Agrotécnica*, **25**, 124-133.

Toriyama, H. (1955), Observational and experimental studies of sensitive plants the development of the tannin vacuole in the motor cell of the pulvinis. *Bot. Mag. Tokyo*, **68**, 203-208.

Tuan, H. C.; Hsu, L. C.; Hung, W. L. and Tso, P. Y. (1965), Studies on the leaf cells of wheat: cell types and their organelles. *Acta Bot. Sin.*, **13**, 101-113.

Vecchia, F. D.; Asmar, T. E.; Calamassi, R.; Rascio, N. and Vazzana, C. (1998), Morphological and ultrastructural aspects of dehydration and rehydration in leaves of *Sporobolus stapfianus*. *Pl. Growth Reg.*, **24**, 219-228.

Watson, M. L. (1958), Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.*, **4**, 475.

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