Flora 222 (2016) 13–19

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Ontogeny of fusoid cells in *Guadua* species (Poaceae, Bambusoideae, Bambuseae): evidence for transdifferentiation and possible functions

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ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form 18 February 2016
Accepted 10 March 2016
Edited by Alessio Papini
Available online 19 March 2016

Keywords:
Differentiation
Fusoid cells
Guadua chacoensis
Guadua trinii
Intercellular spaces
Ontogeny

ABSTRACT

Fusoid cells have been interpreted as cells or intercellular spaces, according to different authors. Here, structural and ultrastructural evidence is provided relative to the origin and changes experimented by chlorenchyma cells ("arm cells") to differentiate into fusoid cells with large intercellular spaces between them. Performed studies and observations were made using Light and Transmission Electron Microscopy. Fusoid cells in *Guadua* species die but do not remain intact; instead, they uniformly collapse, delimiting conspicuous intercellular spaces which are bigger and different in shape from these cells. In transverse sections of mature and expanded bamboo leaf blades, usually seen in anatomical descriptions, the so-called "fusoid cells" are actually conspicuous intercellular gas spaces delimited by successive collapsed (and rarely not collapsed) fusoid cells. Also, we hypothesize possible relations or links between fusoid cell shape, structural function and apoplastic transport in bamboo leaves.

1. Introduction

Bamboos are the only major Poaceae lineage adapted to the forest habitat (Judziewicz et al., 1999). All of them exhibit C_3 photosynthetic pathway (Hattersley, 1987) and a peculiar leaf anatomy features combination, unusual for the grasses as a whole (Metcalfe, 1956). Leaf blades, in transverse section, show the chlorenchyma tissue composed of "lobulated tabular cells" (Freier, 1959) or "arm cells" (Metcalfe, 1960), exhibiting well or weakly developed invaginations and chloroplasts, being these a synapomorphy for the Bambusoideae (Judziewicz and Clark, 2007). Most bamboos also show "fusoid cells" at both sides of leaf blade vascular bundles, and their presence is an important taxonomical character (Metcalfe, 1956), considered plesiomorphic within the Poaceae (Judziewicz and Clark, 2007). Fusoid cells are extensively present in basal grasses and most of Bambusoideae (tribes Arundinarieae Asch. & Graebn., Olyreae Martinov and Bambuseae Kunth ex Dumort.), with the exception of genera *Apoclada* McClure, *Filgueirasia* Guala (Guala, 1995, 2003), *Cambajuva* P.L. Viana, L.G. Clark & Filg. and *Glaziophyton* Franch. (Viana et al., 2013), and a few species of *Chusquea* Kunth (Clark, 1989; Guerreiro et al., 2013), *Ekmanochloa* Hitchc., *Mniochloa* Chase (Zuloaga et al., 1993), *Pariodiolyra* Soderstr. & Zuloaga (Soderstrom and Ellis, 1987), *Phyllostachys* Siebold & Zucc. (Metcalfe, 1960), and *Raddiella* Swallen (Calderón and Soderstrom, 1967). In some species of woody bamboos, there is facultative occurrence of fusoid cells, variable between sun and shade leaves from the same individual (March and Clark, 2011; Guerreiro et al., 2013). Fusoid cells are also cited outside the Bambusoideae in members of subfamily Panicoideae, such as *Homolepis* Chase, *Streptostachys* Desv., *Dallwatsonia* B. K. Simon, and some incertae sedis species of *Panicum* group *Laxa* (Renvoize, 1987; Salariato et al., 2011; Morrone et al., 2012).

Historically, Karelstschicoff (1868) was the first author who described these structures as cells. Then, Brandis (1906) studied the anatomy of bamboo leaves describing "apparent cavities filled up by flat thin-walled chlorophyll-cells which walls appeared folded in a characteristic pattern". Afterwards, Page (1947) studied leaf

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http://dx.doi.org/10.1016/j.flora.2016.03.007

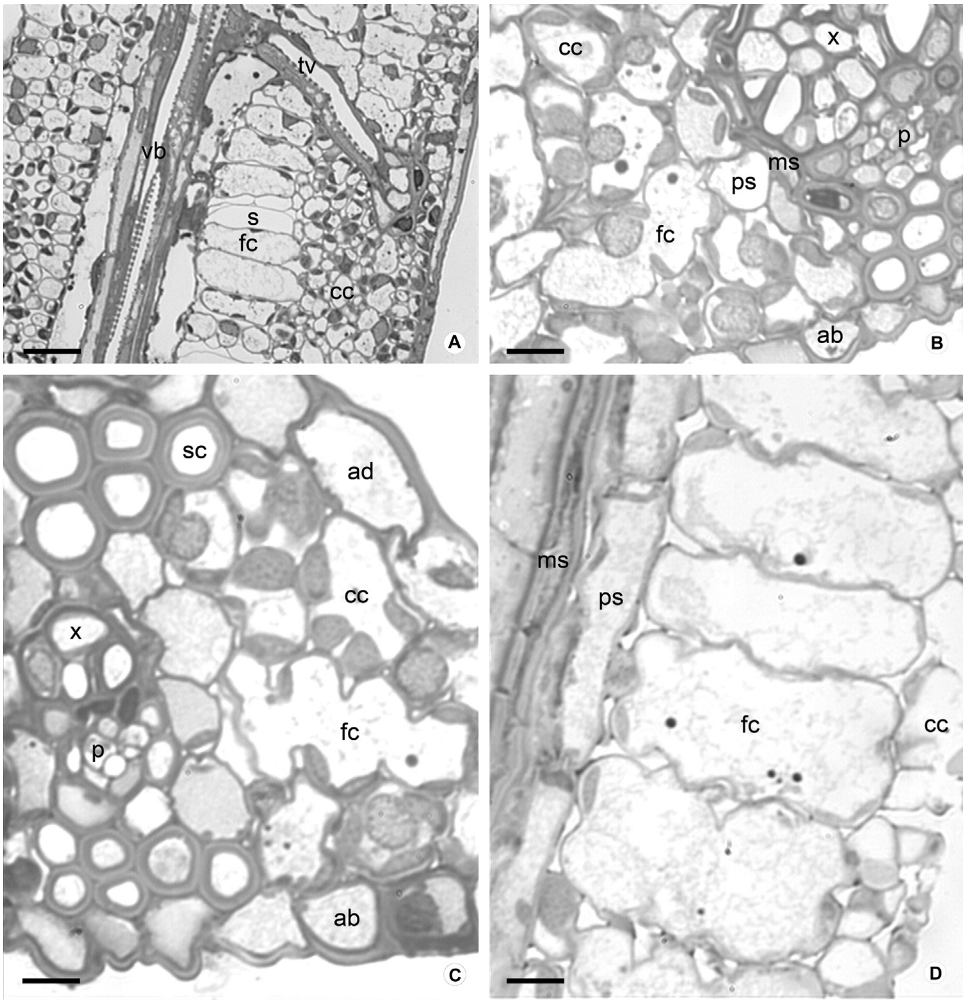

Fig. 1. Leaf blades in longitudinal paradermal and transverse sections light microscope micrographs. (A) Leaf blade in longitudinal paradermal section, showing transverse veinlets and a row of differentiating fusoid cells in contact to parenchyma bundle sheath and chlorenchyma cells. (B, C) Rolled immature leaf blade in transverse section; fusoid cells in contact with parenchyma bundle sheath and chlorenchyma cells. (D) Consecutive fusoid cells in paradermal section. (A) *G. chacoensis*, phase 1. (B, C and D) *G. trinii*, phase 1. *Abbreviations:* ad, adaxial epidermis; ab, abaxial epidermis; cc, chlorenchyma cell; fc, fusoid cell; p, phloem; ms, mestome bundle sheath cells; ps, parenchyma bundle sheath cells; s, intercellular space; sc, sclerenchyma; tv, transverse veinlets; vb, vascular bundle; x, xylem. Scale bars: (A) = 20 μm; (B, C and D) = 10 μm.

blade ontogeny in species of *Streptochaeta* Schrad. ex Nees (*i.e.* previously classified as an herbaceous bamboo and currently representing a basal grass of subfamily *Anomochlooideae* Pilg. ex Potztal). The author showed that some mesophyll cells enlarged vertically at first and then laterally, and the cytoplasm becomes highly vacuolated just before or soon after the leaf blade unfolding. At that moment, when the cell walls collapsed, a row of "I"-shaped cells separated by large intercellular spaces in a longitudinal section could be observed. According to Page (1947), in *S. sodiroana* Hack., these enlarged cells die but fail to collapse, becoming rounded and also distant from each other by the presence of conspicuous intercellular spaces. Occasionally, the cells collapsed and adopt the typical "I"-shaped structure.

Metcalfe (1960) mentioned that in occasions fusoid cells have been incorrectly interpreted as intercellular spaces, and defined them as conspicuous colourless cells, fusiform or pyriform in transverse section, with a varied size according to species and genera. The reason for their particular shape is unknown (Clayton and Renvoize, 1986). Observation in paradermal sections reveals that there are large intercellular spaces between two consecutive fusoid cells (Metcalfe, 1960). Some years later, Arber (1965) defined them as "colourless central mesophyll cells" and Wu (1973) supported Brandisí interpretation about the collapse of mesophyll cells and the presence of large intercellular spaces between them. The cellular nature of fusoid cells was also corroborated by Ellis (1976). Afterwards, Clark (1991) described fusoid cells as internal gas spaces that may occupy up to 30% of the leaf blade volume, with an unknown function. They were also described as large cells, thin walled, without chloroplasts or other cell content (Judziewicz et al., 1999; March and Clark, 2001).

The function of fusoid cells is not completely clear yet. According to OíDowd and Canny (1993) and Vieira et al. (2002), and considering their location next to vascular bundles, the function could be the symplastic water transport from vascular bundles to mesophyll cells. Furthermore, they could be related to the translocation and distribution of photoassimilates between the mesophyll and vascular bundles. On the other hand, Clark (1991) hypothesized that fusoid cells were CO_2 recapture spaces in the leaf. Later, March and Clark (2001, 2011) proposed that fusoid cells are a mechanism to trap and redistribute light more efficiently in shade leaves of bamboo (in comparison to sun leaves) and early-diverging grasses belonging to subfamilies Anomochlooideae, Pharoideae L.G. Clark & Judz., and Puelioideae L.G. Clark, M. Kobay., S. Mathews, Spangler & E.A. Kellogg.

Bamboos show variation regarding leaf shape (lanceolate vs. linear) and size (shorter and relatively wider vs. longer and wider) in

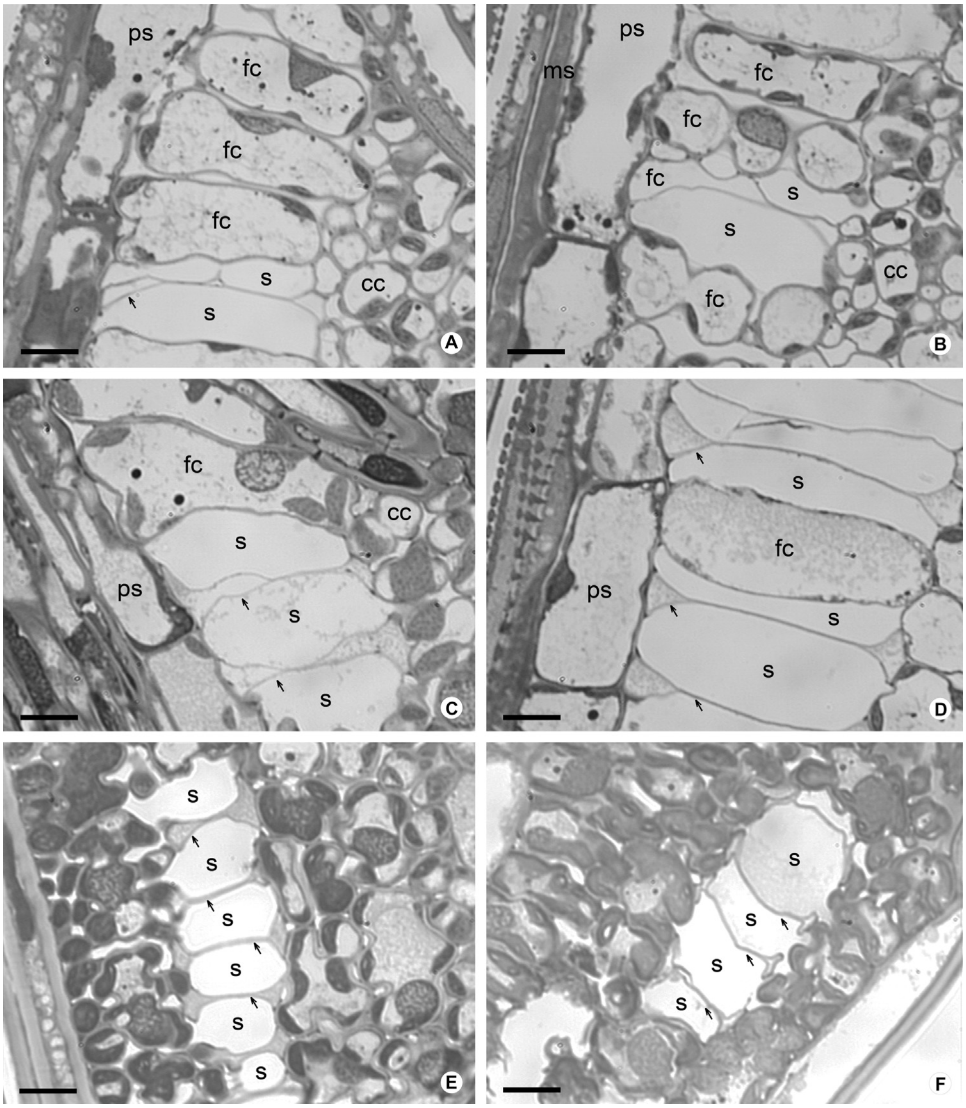

Fig. 2. Leaf blades in longitudinal paradermal sections light microscope micrographs, showing different stages of differentiation. (A, B and D) *G. chacoensis*, phase 1. (C) *G. trinii*, phase 1. (E) *G. trinii*, phase 2. (F) *G. trinii*, phase 3, mature "I"-shaped fusoid cells (arrows). *Abbreviations:* cc, chlorenchyma cell; fc, fusoid cell; ms, mestome bundle sheath cells; ps, parenchyma bundle sheath cells; s, intercellular space. Scale bars = 10 μm.

sun and shade leaves, respectively (March and Clark, 2011). Another interesting feature of bamboo leaves is their fairly long life span and the continuous accumulation of silica in their tissues throughout their life (Motomura et al., 2006). Leaf epidermis is characterized by dense silica deposition and fusoid cells have increasing amounts of silica as they aged. Silica accumulation is not a result of physiological activity but the consequence of water uptake by these cells to intercellular spaces (Motomura et al., 2004). In mature fusoid cells, silica is not deposited in the lumen but is probably deposited in cell walls (Motomura et al., 2000). Silica deposition has a protective role as a biologically active element capable of triggering a broad range of physical and biochemical defences (Currie and Perry, 2007). The application of silica into many fertilizers attenuate metal toxicity, salinity, drought, and temperature stresses in plants; also it induces natural defences against fungal pathogens, but the mechanisms are still under study (Currie and Perry, 2007). It was also related to the facilitation of light interception (Motomura et al., 2006).

According to Brandis (1906), "the earliest development of the flat, thin-walled cells filling up these cavities will doubtless form the subject of an interesting histological study". Developmental studies of fusoid cells are needed to test the proposed homology of fusoid cells to mesophyll cells (March and Clark, 2011). Recent studies could not demonstrate whether "fusoid cells died and remained intact or whether they uniformly collapsed to create spaces of the same size and shape as mature fusoid cells" (March and Clark, 2011). On the basis of deficient fusoid cells structural studies and their dissimilar interpretation (as cells or intercellular spaces), the purpose of the present paper is to provide structural and additional ultrastructural evidences of chlorenchyma cells changes related to fusoid cells and intercellular spaces ontogeny in *Guadua* species. Also we hypothesize a possible relation or link between fusoid cells shape and structural function in bamboo leaves.

2. Materials and methods

Studies were carried out in two woody bamboos species of the genus *Guadua* Kunth (Bambusoideae, Bambuseae): *G. chacoensis* (Rojas) Londoño & P.M. Peterson and *G. trinii* (Nees) Nees ex Rupr. *Guadua chacoensis* is distributed in SE Bolivia, Paraguay, Uruguay, Brazil, and Argentina; plants are 10–20 m high and flow-

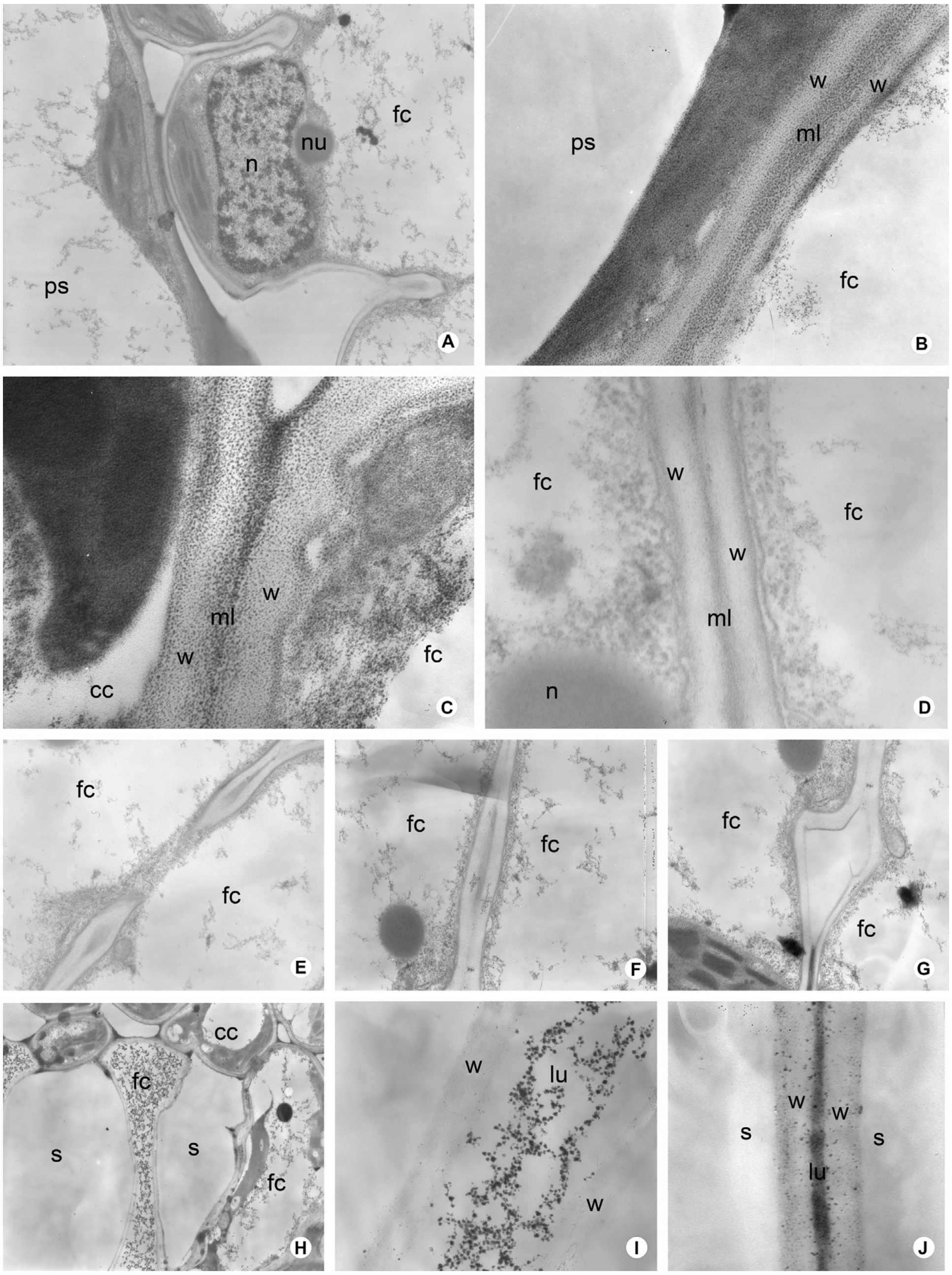

Fig. 3. TEM micrographs. (A) Parenchyma bundle sheath cell in contact with fusoid cell. (B) Detail of walls of parenchyma bundle sheath and fusoid cells. (C) chlorenchyma cell in contact with fusoid cell. (D) Detail of walls of two contiguous fusoid cells. (E) Primary field pit between two contiguous fusoid cells. (F) Incipient intercellular space between two contiguous fusoid cells. (G) Intercellular space differentiation between fusoid cells. (H) "I"-shaped fusoid cell in contact with chlorenchyma and other fusoid cells. (I) "I"-shaped fusoid cell contents at the middle zone. (J) Mature "I"-shaped fusoid cell lumen and contents at the middle portion. (A, D, E, F and G) *G. chacoensis*, phase 1. (B, C, H, I and J) *G. trinii*, phase 2. *Abbreviations:* cc, chlorenchyma cell; fc, fusoid cell; lu, cell lumen; ml, middle lamella; n, nucleus; nu, nucleolus; ps, parenchyma bundle sheath cell; s, intercellular space; w, cell wall. Scale bars: (A and H) = 250 nm; (B, C and I) = 50 nm; (D and J) = 25 nm; (E, F and G) = 100 nm.

ering occurs after 28–31 years of vegetative growth (Vega and Cámara Hernández, 2008; Lizarazu and Vega, 2012; Guerreiro, 2014). *Guadua trinii* inhabits in Southern Brazil, Uruguay, and Argentina; plants are 8–10 m high, completing their life-cycle after 30 years of vegetative growth (Parodi, 1955; Lizarazu and Vega, 2012; Guerreiro, 2014).

Clumps, native from Misiones province (Argentina), are cultivated at "Lucien Hauman" Botanical Garden of the School of Agriculture, University of Buenos Aires, and voucher specimens (*A. S. Vega & T. San Martín 17* and *L. Parodi 15407*, respectively) are deposited at BAA (Thiers, 2015).

2.1. Anatomical studies

Pieces of 0.5 cm^2 were cut from the middle portion of shade fresh leaf blades. Samples taken from three different moments of foliage leaf blade development were analyzed: last leaf blade rolled and exposed 0.8 cm from the last leaf sheath of the shoot; last leaf blade rolled and exposed 6 cm from the last leaf sheath of the shoot; and last leaf blade fully expanded (hereafter referred to as phases 1, 2, and 3, respectively).

For Light Microscopy (LM) observations, pieces were fixed in FAA (1 formalin: 4 ethanol 96%: 1 acetic acid) and then stored in

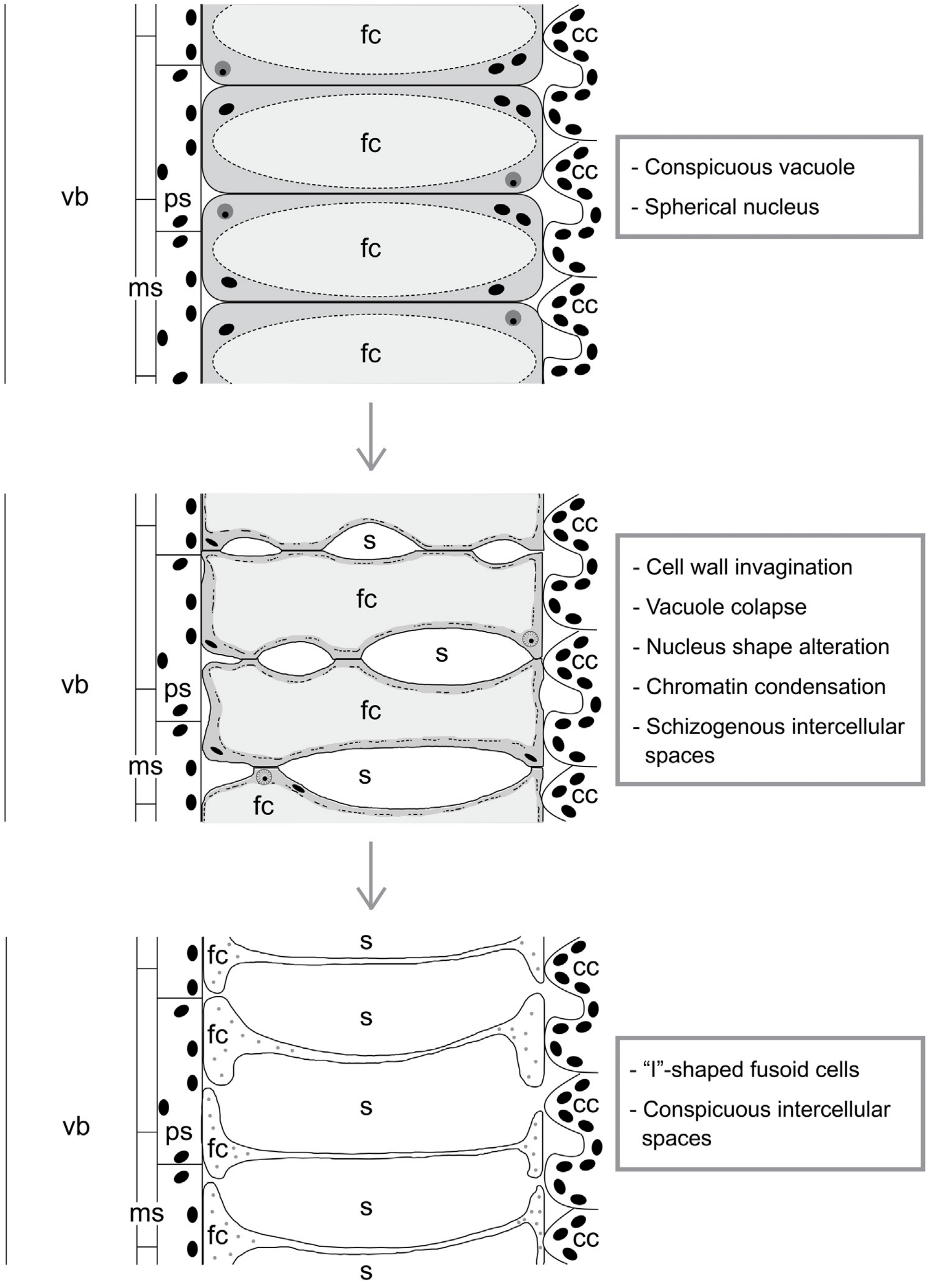

Fig. 4. Diagram showing the main steps of the origin, development and collapse of fusoid cells in paradermal section of the foliage leaf blade in *Guadua* species. Fusoid cells are shaded in grey. Vacuoles are delimited by a dotted line. Cellular content in mestome bundle sheath and vascular bundle is not shown. *Abbreviations:* cc, chlorenchyma cell; fc, fusoid cell; ms, mestome bundle sheath cell; ps, parenchyma bundle sheath cell; s, intercellular space; vb, vascular bundle.

ethanol 70%. All materials were dehydrated in an ethanol-xylene series and embedded in paraffin. Transverse, longitudinal, and paradermal sections 10 μm-thick were cut on a rotary microtome. Sections were double stained with safranin-fast green (D'Ambrogio de Argüeso, 1986). Observations and photographs were made with an optical microscope (Zeiss Axioplan, Oberkochen, Germany).

For Transmission Electron Microscopy (TEM), pieces of 0.3 cm long from young and convolute leaf blades were taken from 1, 2.2, and 3 cm below their apex in order to document a gradual sequence of development. In mature leaf blades, fully expanded and flat, pieces of 0.6 cm long were cut from the middle portion. All pieces were fixed in 3% glutaraldehyde in 0.1 mol/L phosphate buffer for 18 h at room temperature, washed in buffer, and post fixed in 1.5% osmium tetroxide with the same buffer for 1.5–2 h, dehydrated in an acetone series, and embedded in Spurr resin. The ultra thin sections were cut with glass knives, stained with uranyl acetate followed by lead citrate, and examined in a high resolution electron microscope EM 10A/B (Zeiss, West Germany).

3. Results

Light microscopy observations reveal that in phase 1, while foliage leaf blades are convolute and scarcely exerted from the penultimate leaf, the paradermal longitudinal sections show a row of organized consecutive fusoid cells intermixed with scarcely developed intercellular spaces (Fig. 1(A)). In bamboo leaves, fusoid cells appeared in contact to and between parenchyma bundle sheath of vascular bundles and chlorenchyma cells (Fig. 1(A)). These chlorenchyma cells have chloroplasts with numerous thylakoids and grana distributed in a relatively less electron-dense stroma.

In the mesophyll, future fusoid cells can be clearly recognized at a very early stage of tissue differentiation. Both transverse and paradermal sections show that some mesophyll cells, situated precisely at the middle portion of the leaf blade and next to the parenchyma bundle sheath, undergo successive changes that lead to its differentiation into fusoid cells (Fig. 1(B, C and D)). The first changes in fusoid cell ontogeny are cell enlargement and a conspicuous invagination of their cell wall, both visible in transverse and in longitudinal paradermal sections (Fig. 1(B, C and D)), respectively. Fusoid cells appear conspicuous, with a central large vacuole and spherical nucleus (Figs. 1(C, D) and 2 (A, C)). Chloroplasts are ellipsoid and occupy a peripheral position (Fig. 2(A–C)). Fusoid cells, respect to the typical chlorenchyma cells, show chloroplasts ageing, vacuole collapse, nucleus shape alteration, chromatin condensation, and persistent spherical nucleolus (Figs. 2 (C, D) and 3 (A)). At this stage, schizogenous intercellular spaces appear more evident between fusoid cells (Fig. 2(C and D)). Progressively, the middle lamella between two successive fusoid cells is degraded in several points (Fig. 2(B)), creating several small intercellular spaces, up to the complete differentiation of only one conspicuous intercellular schizogenous space (Fig. 2(C and D)). Finally, in paradermal longitudinal sections, fusoid cells show a typical "I"-shape (Fig. 2(E and F)); the more dilated ends of these cells are in contact with parenchyma bundle sheath cells and arm cells (Fig. 2(A–D)). Fusoid cells differentiation is an asynchronous process along the longitudinal axis of foliage leaf blades (Fig. 2(A–D)) during an early stage of development.

TEM observations reveal that when a fusoid cell is in contact with a parenchyma bundle sheath cell, the middle lamella appears electronically dense and both walls are translucent and similar (Fig. 3(A and B)). If a fusoid cell is in contact with a chlorenchyma cell, only the inner side of the fusoid cell wall exhibits an undulated appearance and both walls present a similar ultrastructural cellulose microfibrilar pattern (Fig. 3(C)). In contiguous fusoid cells, the thin translucent walls show primary field pits with numerous plasmodesmata and retracted plasma membrane with sinuous disposition (Fig. 3(D and E)). A conspicuous intercellular space appears between fusoid cells (Fig. 3(F and G)). In typical mature "I"-shaped fusoid cells (Fig. 3(H)), cell wall is not degraded, and lumen is filled with granular, electron-dense contents of the disintegrated cytoplasm (Fig. 3(I)), progressively reducing and narrowing (Fig. 3(J)). A diagram to summarize the origin, development, maturation and collapse of fusoid cells is presented in Fig. 4.

4. Discussion

According to anatomical observations of transverse sections from mature and expanded leaf blades, the typical conspicuous "fusoid cells", referred to by several authors in anatomical descriptions, are actually the conspicuous intercellular gas spaces delimited by successive collapsed (and rarely not collapsed) fusoid cells. This observation is in accordance to Wu (1973), who stated that a section is uncommonly cut through the lumen of the collapsed cell, due to lumen width, and the intercellular space being much wider. Studies carried out in both species of *Guadua* give evidence contrary to the hypothesis proposed by March and Clark (2011). Fusoid cells die but do not remain intact; instead, they uniformly collapse, delimiting conspicuous intercellular spaces which are bigger and different in shape from these cells.

The conversion of a differentiated type cell into another one (*i.e.*, a chlorenchyma cell into a fusoid cell) has been defined as transdifferentiation (Thomas et al., 2003). In bamboo leaves, out of the total number of chlorenchyma cells, only a few of them transform into fusoid cells. In adjacent chlorenchyma cells, this process does not occur. According to our studies, transdifferentiation of chlorenchyma cells into fusoid cells supports the hypothesis of homology between both cell types, proposed by March and Clark (2011).

Mature "I"-shaped fusoid cells, almost devoid of cytoplasm contents and separated by conspicuous intercellular spaces, may act as specialized cells involved in the maintenance of the internal architecture of the expanded leaf blade, possibly having a similar structural function as typical stellate parenchymatic cells present in aerenchyma tissue. This explanation is in accordance with a previous consideration of fusoid cells as aerenchyma tissues, proposed by Takenouchi (1932) and cited in Motomura et al. (2004). The shape and size of Bambusoideae leaves may be related to the presence of fusoid cells as an anatomical character that ensures the maintenance of a flat leaf blade disposition, independently of an eventual drought stress. For this reason, presence of fusoid cells in some woody bamboos may be facultative according to size and shape of sun or shade leaves in the same individual (March and Clark, 2011). Also, ontogeny of fusoid cells show that they lose cytoplasm contents and lumen is reduced, up to the point where tangential walls of the same cell are proximate; this could be interpreted as a possible way for apoplastic transport.

Ultrastructural changes observed in specific chlorenchyma cells, at both sides of the parenchyma bundle sheath, in order to transform into fusoid cells could be related to a Programmed Cell Death (PCD) process. A deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) assay as well as specific stains may be undertaken in order to confirm the PCD hypothesis in fusoid cells.

Acknowledgements

We are grateful to L. G. Clark for her useful comments and suggestions about the manuscript and to Tomás E. Aversa for his valuable assistance with figures. This work was supported by Universidad de Buenos Aires, Argentina (Grants UBACyT 20020100100641 and 20020110100080) and PIP CONICET 11220110100783.

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