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ORIGINAL PAPER

Anatomical characteristics of fusoid cells and vascular bundles in *Fargesia yunnanensis* leaves

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Abstract As of today, the functions of fusoid cell, and the transport and loading pathways of photoassimilate in bamboo leaves are still not clear. In this paper, the leaves of *Fargesia yunnanensis* from a greenhouse and the wild were respectively used as samples to analyze the anatomical characteristics of fusoid cells and vascular bundles. The results showed that the bamboo leaves from greenhouse got shorter and thinner with fewer layers of palisade parenchyma cells than those from the wild. The volumes of fusoid cells were also increased. Fusoid cells originated from a huge parenchyma cell as testified by the observed nuclei. Several fusoid cells usually formed one cell complex close to the midrib. Crystals were detected in fusoid cells but no pits or plasmodesmata on their walls, suggesting that fusoid cells had the function of regulating water. The presence of fusoid cells determined the major difference between a leaf blade and sheath. There were prominent chloroplasts with simple stroma lamellae in the parenchymatous bundle sheath cells and starch grains were also observed in these chloroplast. Photoassimilates could be transported across vascular bundle sheath via symplasmic pathways for an abundant of plasmodesmata in sheath cell walls, and transported into phloem tube by apoplastic pathway as there were no pits in the walls of companion cells and phloem tubes.

Keywords Fusoid cell · Sheath cell · Chloroplast · Transport

Introduction

Fargesia yunnanensis is one alpine bamboo species, distributing mainly in Sichuan and Yunnan provinces of China. It attains the best growth performance in high altitude areas with cool climates. Yunnan has a unique vertical three-dimensional climate. If *F. yunnanensis* is to grow in low altitude plain regions, it has to endure high temperature stress. Up to now, there are few reports about its growth performance in plain regions. Information about the relationship between high temperature tolerance and anatomical characteristics are lacking. Although the leaf anatomical characteristics of other plant species associated with environmental stress, i.e. short-term drought tolerance, have been reported in recent years (Ashton and Berlyn 1994; Sack and Holbrook 2006; Karaba et al. 2007; Kulkarni et al. 2010), the anatomical responses of bamboos on a long-term environmental stress are still unknown.

The mesophyll of Poaceae, notedly the Bambusoideae, is marked by the presence of fusiform, colorless and thin-walled cells, frequently mistaken for intercellular spaces (Vieira et al. 2002). Metcalfe (1956) uses the term "fusoid cells" to define these elements, considering its fusiform

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outline when viewed in transverse section. The presence of fusoid cell in leaf blades is one of the most important differences between bamboo and grass (Calderón and Soderstrom 1973), but we still cannot confirm their function in bamboo leaves. Clark (1991) has also proposed that fusoid cells serve as reservoirs for CO_2 from photorespiration. However, the function of fusoid cells was re-proposed as a mechanism to trap and redistribute light by March and Clark (2011).

The paper aims to investigate the anatomical responses of *F. yunnanensis* leaves to the long-term high temperature stress, analyze the function of fusoid cells, and discuss the photoassimilates translocation in bamboo leaves.

Materials and methods

Plant materials

The bamboo samples of *F. yunnanensis* were separately collected from the bamboo garden and greenhouse of Southwest Forestry University (SWFU), China in July 2013. The bamboo leaves were collected from the 7-year old cutting seedlings in the greenhouse, which were cultivated from the same bamboo clump in September 2006 in order to avoid the genetic differences. In the long-term cultivation process, these cutting seedlings had completely acclimated to the greenhouse environment. The average annual temperature in greenhouse was 26.2 °C and far higher than that in wild (15.0 °C). In April and May, the highest air temperature in the greenhouse can reach up to 48.0 °C, which caused frequent curling of leaf blades for the higher water transpiration. Other samples of *Dendrocalamus giganteus, D. hamiltonii* and *D. brandisii* were also collected from the bamboo garden of SWFU.

Paraffin sections

Leaves of *F. yunnanensis* from the greenhouse and bamboo garden were separately divided into three age classes—young, mature and old, according to the development degree, cut into small pieces (about 5 mm × 5 mm), and then were fixed directly in FAA (45 % alcohol, 0.25 % acetic acid and 1.85 % formaldehyde) and dehydrated in a graded series of alcohol (began at 50 %). Transverse Section (7 μm) were cut using a rotary microtome and stained with 1 % alcoholic Safranin O (Sigma S-2255) (in 50 % ethanol), distilled water, dehydrated in a graded series of ethanol, and then were stained with Fast green FCF (Ameresco 0689) (Fast green 1 g + clove oil 100 ml + 100 % ethanol). The sections were mounted in Canada balsam.

Hand sections

Hand sections were cut from fresh leaf blade samples using a double-edge razor blade and then were flattened in water, which were checked under a converted fluorescence microscope (Nikon E400) for observing the chloroplasts in bundle sheath cells and fusoid cells.

Ultrathin sections

Samples (about 3 mm × 3 mm) for Transmission Electron Microscope (TEM) observations were fixed in half-strength Karnovsky's fixative (1965) containing 2 % paraformaldehyde and 2.5 % glutaraldehde in 0.1 M sodium cacodylate buffer (pH 8) for 2 h, soaked in the same fixative (pH 6.8) at 4 °C overnight, following by 20 min rinses in 0.1 M sodium cacodylate buffer (pH 6.8) for 3 times. Samples were then post-fixed in 1 % osmium tetroxide in the same buffer for 2 h, rinsed with distilled water for 15 min for 3 times, and pre-stained in the dark with 0.25 % uranyl acetate overnight at 4 °C. After dehydration in a graded series of ethanol, specimens were infiltrated and embedded in Spurr's resin (Gritsch and Murphy 2005).

Microscopy

The paraffin sections were observed with a video camera linked to a converted fluorescence microscope (Nikon E400) and a Lenovo computer. Transverse ultrathin sections were obtained using a diamond knife, collected on 200 mm mesh copper grids coated with Formvar, stained with 2 % uranyl acetate for 15 min and lead citrate for 10 min. The ultrathin sections were examined and photographed with a JEM-1400 Transmission Electron Microscope (TEM).

Before the nuclei of leaves were observed, the paraffin sections were dewaxed and rehydrated, and then were stained by Hoechst 33342 (2′-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5′-bi-1H-benzimidazole trihydrochloride trihydrate), which was a cell-permeable DNA stain that was excited by ultraviolet light and emitted blue fluorescence at 460–490 nm. The Hoechst stain was diluted to 1 μg/ml with Dulbecco's PBS (DPBS) containing 8 mM sodium phosphate, 2 mM potassiumphosphate, 140 mM sodium chloride and 10 mM potassium chloride (pH 7.4). After the dewaxing and rehydration the paraffin sections were washed, and then were stained for 10 min prior to be soaked with DPBS. All sections were examined with the converted fluorescence microscope (Nikon E400).

Before the observation with a scanning electron microscope (Hitach S-3000N), all leaves were fixed in FAA, and then dehydrated in a graded series of alcohol and finally cut into sections by hand.

Statistical analyses were carried out using SPSS 13.0. The least significant difference method (LSD) was employed to analyze the difference for the mean values derived from the experiments.

Results

Morphology of leaf blades in greenhouse and wild

After 7 years of cultivation in a greenhouse, the leaves of *F. yunnanensis* grew shorter, wider and thinner than those leaves from the wild (Table 1). The mature leaves from both the wild and the greenhouse was the longest. The mature and old leaves from the wild were longer than that from the greenhouse. However, the leaf width increased slightly with age and leaves at all age classes from the greenhouse were wider than that from the wild. In addition, the mature leaves were thicker than the young and old ones in both two conditions.

A similar trend also occurred in other anatomical characteristics of leaves, i.e. thickness of epidermis wall, epidermis cell and palisade parenchyma (Table 2). It could be clearly observed that the adaxial palisade parenchyma of young and mature leaves from the wild hold at least three layers of cells, but only two layers for the leaves from the greenhouse (Fig. 1a, b, d, e). The palisade parenchyma cells of the older leaves from the wild also had more layers than that from the greenhouse (Fig. 1c, f). Therefore, the leaves from the greenhouse were thinner than that from the wild. Meanwhile, the fusoid cells in leaves from the greenhouse were longer than that from the wild, but their width did not change obviously (Table 2), which implied that the volume of fusiod cells increased significantly in the greenhouse.

Generally, only one single fusoid cell occurred close to the primary and secondary lateral veins of leaf blades. However, it could be seen that several fusiod cells formed one fusoid cell complex close to the midrib (Fig. 1g, h). This phenomenon was also observed in the leaves of other bamboo species, such as *Dendrocalamus brandisii* (Fig. 1i). It is observed that the fusoid cells not only occurred in the leaf blades, but also in the culm sheath blades (Fig. 1g). However, no fusoid cell could be observed in both branch sheaths and culm sheaths (Fig. 1j, l). Therefore, the occurrence of fusoid cell was the most important difference between blades and sheaths. Meanwhile, nuclei could be easily observed in the fusoid cells of the young culm sheath blades, which demonstrated that fusoid cells originated from one huge parenchyma cell. On the contrary, no nuclei was observed in the fusoid cells of young leaf blades, showing that the nuclei of fusoid cells degenerated earlier in leaf blades than in sheath blades (Fig. 1a, d).

Nuclei and chloroplast in mesophyll cells and bundle sheath cells

Under fluorescent microscope, nuclei was observed in most mesophyll cells of young leaf blades but was not observed in bundle sheath cells and fusoid cells, showing the earlier PCD (programmed cell death) or maturation in bundle sheath cells and fusoid cells (Fig. 2a, b). Nuclei could also be observed in bulliform cells. Meanwhile, there was only one layer of parenchyma cells around the secondary lateral veins but two layers of sheath cells around the primary lateral veins, containing one layer of parenchymatous sheath cells and one layer of lignified sheath cells. In mature leaves, no nuclei could be observed and a reddish fluorescent was emitted from the mesophyll cells (Fig. 2c, d). However, in old leaves, an intense fluorescent was emitted from mesophyll cells and parenchymatous sheath cells of secondary lateral veins, showing apparent chloroplasts in bundle sheath cells (Fig. 2e). In other bamboo species, i.e. *D. hamiltonii*, *D. brandisii* and *D. giganteus* (Fig. 2f–h), the parenchymatous sheath cell also showed the apparent chloroplast with different size and shape..

Table 1 Morphology of *F.yunnanensis* leaves from the wild and greenhouse (cm)

Growth environment	Samples	Leaf length	Leaf wideth	Leaf thickness
Wild	Young leaves	10.32 ± 0.558a	0.97 ± 0.039a	34.11 ± 0.916c
	Mature leaves	19.56 ± 0.651d	1.44 ± 0.049b	34.80 ± 0.306c
	Older leaves	19.15 ± 0.405d	1.58 ± 0.044b	33.80 ± 0.476c
	Mean values	16.34 ± 0.613c	1.33 ± 0.041b	34.23 ± 0.352c
Greenhouse	Young leaves	10.51 ± 0.692a	1.32 ± 0.100b	22.10 ± 1.044a
	Mature leaves	14.81 ± 0.713b	1.72 ± 0.077bc	26.92 ± 0.548b
	Older leaves	14.08 ± 0.625b	2.04 ± 0.125c	25.01 ± 0.866ab
	Mean values	13.13 ± 0.456b	1.69 ± 0.070bc	24.67 ± 0.664ab

Means followed by the same letter in each column are not significantly different at 0.05 probabilities. Values presented are mean ± SD

Table 2 Morphology of different cells of *F. yunnanensis* leaves from the wild and greenhouse (μm)

Growth environment	Samples	Fusoid cell length	Fusoid cell width	Adaxial epidermis wall thickenss	Abaxial epidermis wall thickenss
Wild	Young leaves	46.03 ± 1.737a	13.69 ± 0.580b	0.17 ± 0.002b	0.15 ± 0.004b
	Mature leaves	47.35 ± 1.832a	12.22 ± 0.350ab	0.17 ± 0.003b	0.16 ± 0.003b
	Old leaves	47.29 ± 2.017a	13.16 ± 1.125b	0.18 ± 0.003b	0.16 ± 0.002b
	Mean values	47.21 ± 1.031a	12.99 ± 0.415ab	0.17 ± 0.002b	0.16 ± 0.002b
Greenhouse	Young leaves	61.50 ± 1.927c	11.44 ± 0.569a	0.14 ± 0.003a	0.11 ± 0.002a
	Mature leaves	52.33 ± 1.536b	11.62 ± 0.563a	0.14 ± 0.025a	0.12 ± 0.003a
	Old leaves	52.42 ± 1.608b	11.26 ± 0.619a	0.14 ± 0.002a	0.12 ± 0.002a
	Mean values	55.01 ± 1.132b	11.44 ± 0.332a	0.14 ± 0.002a	0.12 ± 0.001a
Growth environment	Samples	Adaxial epidermis cell thickenss	Abaxial epidermis cell thickenss	Adaxial palisade parenchyma thickenss	Abaxial palisade parenchyma thickenss
Wild	Young leaves	4.30 ± 0.177b	2.96 ± 0.066bc	16.54 ± 0.530c	6.04 ± 0.628b
	Mature leaves	4.30 ± 0.100b	3.10 ± 0.052c	16.30 ± 0.306c	5.60 ± 0.082b
	Old leaves	5.06 ± 0.095c	3.13 ± 0.042c	14.40 ± 0.569c	5.30 ± 0.163b
	Mean values	4.55 ± 0.112bc	3.06 ± 0.035c	15.75 ± 0.350c	5.65 ± 0.218b
Greenhouse	Young leaves	3.26 ± 0.105a	2.04 ± 0.061a	7.24 ± 0.415a	4.22 ± 0.210a
	Mature leaves	3.92 ± 0.108ab	2.62 ± 0.065b	11.00 ± 0.258b	4.40 ± 0.195a
	Old leaves	3.62 ± 0.130a	2.52 ± 0.016b	10.10 ± 0.490b	4.32 ± 0.158a
	Mean values	3.60 ± 0.090a	2.39 ± 0.068ab	9.45 ± 0.445ab	4.31 ± 0.104a

Means followed by the same letter in each column are not significantly different at 0.05 probabilities. Values presented are mean ± SD

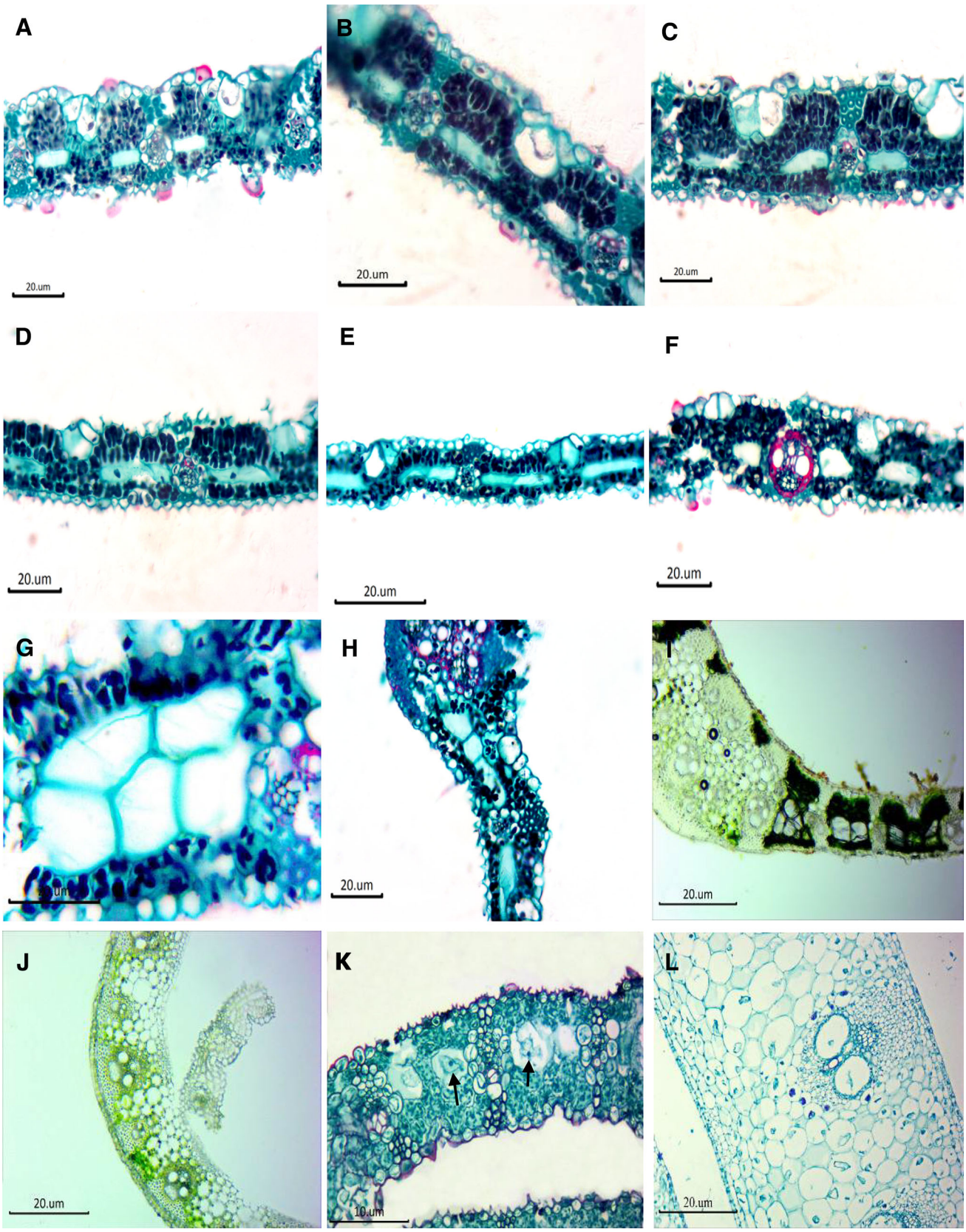
Fusoid cells had apparent cytoplasm (Fig. 2c, d, f, g), just like bulliform cells (Fig. 2d). Meanwhile, we also noted that there were crystals in both fusoid and bulliform cells, which implied that the function of fusiod cells might be similar to that of bulliform cells and vacuoles (Fig. 2g, i), i.e. water regulation. However, there were no pits in the walls of both fusoid cells and fusoid cell complex (Fig. 2j, k).

Ultrastructure of vascular bundle and mesophyll cells

From the microscopic observations, it could be noted that all cells of the outer and inner bundle sheaths formed a cytoplasmic continuum by plasmodesmata (Fig. 3a). Therefore, the photoassimilates from mesophyll cells could be transported across the bundle sheath cells via symplasmic pathways. Plasmodesmata also connected the inner bundle sheath cells to the adjoining parenchyma cells at the periphery of xylem and phloem (Fig. 3a). The lateral veins in leaf blades were connected by the transverse veitilet, which had only one vessel element with many protuberances towards adaxial palisade parenchyma (Fig. 3b). It might be beneficial to the water unloading. Under TEM, no plasmodesma was observed in the walls between fusoid cells and mesophyll cells (Fig. 3c, e, f). However, mesophyll cells were connected to bundle sheath cells and all bundle sheath cells were connected to each other by plasmodesmata (Fig. 3c, e, f). The outer sheath cells were also connected to the inner sheath cells by plasmodesmata (Fig. 3d). The chloroplast in the outer bundle sheath cells had apparent stroma lamellas (Fig. 3g). Meanwhile, lots of starch grains were accumulated in the chloroplasts of mesophyll cells (Fig. 3h), implying the C3 photosynthetic pathways in mesophyll cells. Another striking phenomenon was that the starch grains were detected in the chloroplasts of parenchymatous sheath cells (Fig. 3i, j), suggesting that the cholorplasts in sheath cells had starch synthesis ability.

According to the observations on the primary lateral vein (Fig. 4a–d), it could be noted that there were no pits on the lateral walls of sieve element and companion cells, but apparent pits on sieve plate (Fig. 4b, c), showing the apoplasmic phloem loading. The walls of vascular

Fig. 1 Anatomical characteristics of leaves from different growth environment. **a–c** Leaves from the wild. **d–f** Leaves from the greenhouse. **g–i** Different types of fusoid cells. **a** The fusoid cells of young leaves of *F. yunnanensis*. **b** The fusoid cells of mature leaves. **c** The fusoid cells of old leaves. Only the protoxylem could be observed in secondary lateral vein. **d** The fusoid cells of young leaves in the greenhouse. **e** The fusoid cells of mature leaves in the greenhouse. **f** The fusoid cells of older leaves in the greenhouse. **g** The fusoid cells close to the midrib. **h** Another type of fusoid cells close to the midrib. **i** The fusoid cells close to the midrib of *Dendrocalamus brandisii*. **j** Leaf sheath without fusoid cells. **k** Culm leaf with obvious fusiod cells and nuclei (*arrows*). **l** Young culm sheath without fusoid cells ▶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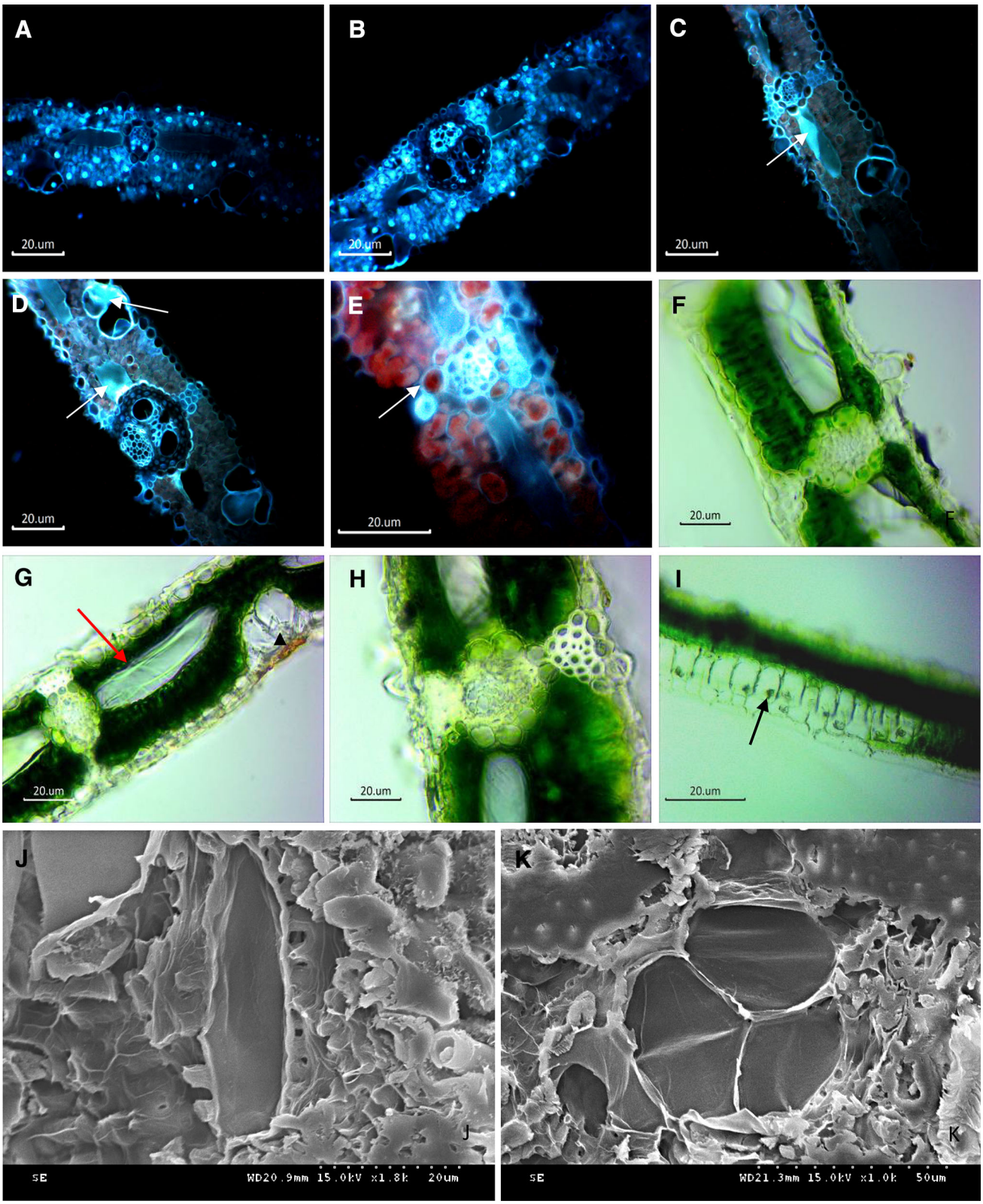
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◀**Fig. 2** Different developmental stage of *F. yunnanensis* leaves. **a**, **b** Young leaves, showing prominent nuclei in mesophyll and two fusoid cells close to both secondary lateral vein and primary lateral vein. No red fluorescence was emitted from mesophyll cells. **c**, **d** Mature leaves without nuclei and fusoid cells with apparent protoplasm. Mesophyll cells emitted slightly red fluorescence. **e** Old leaves with intense fluorescence from mesophyll and bundle sheath cells, implying the chloroplasts in the bundle sheath cells. **f**–**h**, Remarkable chloroplasts in the bundle sheath cells of *D. hamiltonii* (**f**), *D. brandisii* (**g**) and *D. giganteus* (**h**). Crystal was observed in bulliform cells (*arrowhead*) and cytoplasm was observed in fusoid cells (*arrow*). **i** Longitudinal transverse section of *D. giganteus*. Crystals were observed in a large number of fusiod cells. **j** Observations on the fusiod cell close to lateral vein by SEM, showing no pits in the cell wall. **k** The complex of fusoid cells close to the midrib and also no pits in the cell wall

parenchymatous cells between vessels also had no pits and occurred scalariform thickening, which is similar to that of the protoxylem vessel (Fig. 4d), implying these cells involved in the water transport.

Discussion

Morphological changes of leaves in greenhouse

Few reports were available regarding the differences between the bamboo leaves from the greenhouse and the wild. Wang et al. (2014) reported similar experiments about physiological changes of bamboo leaves from greenhouse and the wild. Besides, March and Clark (2011) reported differences of between sun and shade leaves.

The high temperature was the major abiotic stress factor for the *F. yunnanensis* bamboo clumps in the greenhouse which led to high transpiration. The bamboo clumps were irrigated twice a week in order to avoid the physiological drought. However, the leaf blades still curved every midday due to the high transpiration. Leaf blades in greenhouse were shorter, wider and thinner than those in wild. Meanwhile, there were fewer layers of palisade parenchyma cells but the fusoid cells increased their volume in the greenhouse. These changes of leaf blades were to cope with the high temperature condition in greenhouse. High transpiration is an effective plant mechanism for alleviating high temperature and cooling the leaf temperature (Sparks 2004). However, the intense transpiration could also significantly decrease the water content in leaf blade and then affected the normal physiological activities. Therefore, the increase volume of fusoid cells might store water to buffer transpirational surges.

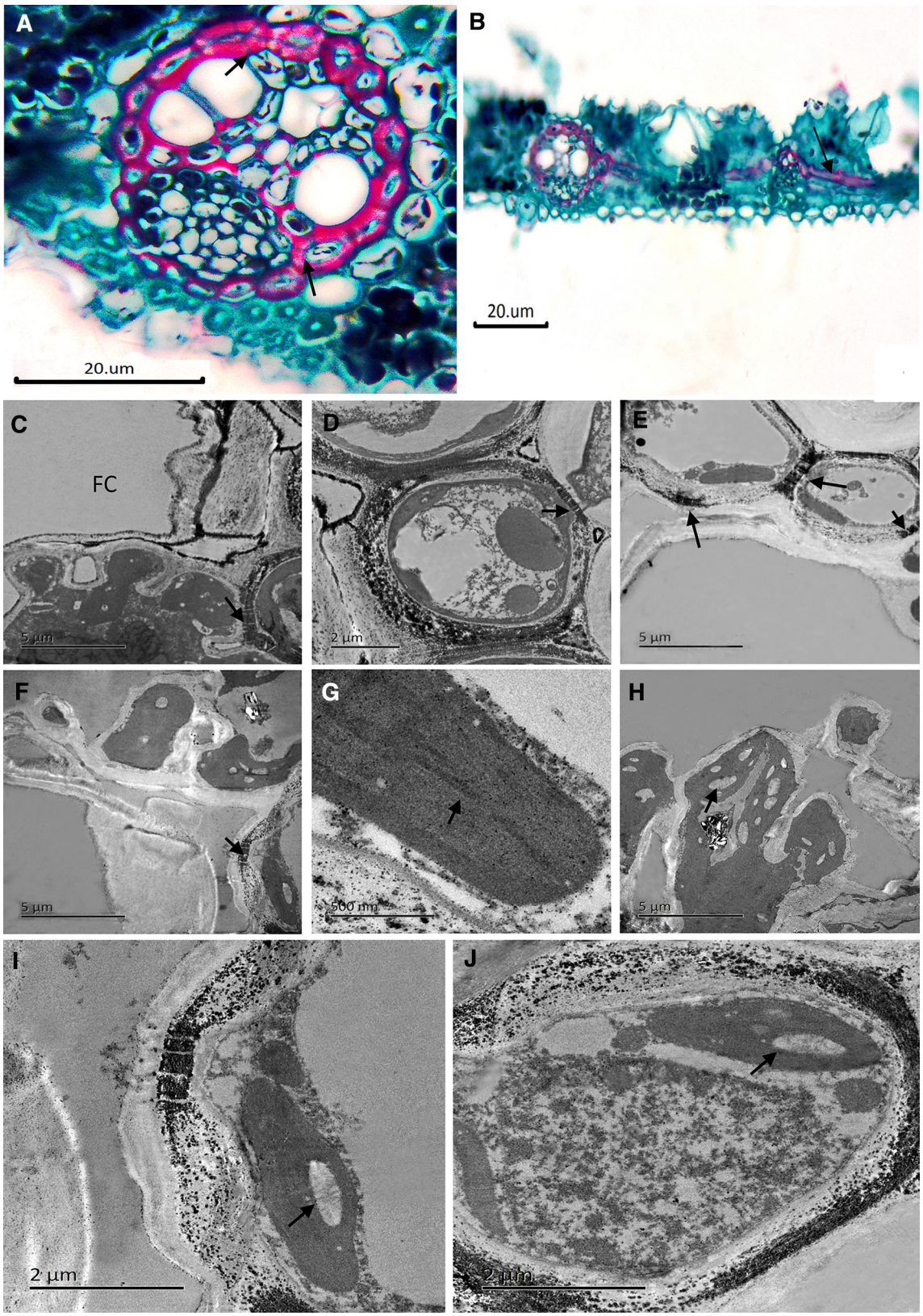
Fusoid cells and their functions

Fusoid cells are one prominent feature of the leaves of bamboos and early-diverging grasses (March and Clark 2011). Meanwhile, fusoid cells were considered as air cavity by Yang and Hui (2010). Although the function of fusoid cells was not clear, Vieira et al. (2002) considered that they were related to the storing and transportation of water in leaves if their location next to vascular bundles were taken into account. Other hypothesis has been mentioned that fusoid cells were related with the translocation and distribution of photoassimilates between the mesophyll and vascular bundles (Fisher 1967; Franceschi and Giaquinta 1983a, b, c). Clayton and Renvoize (1986) had ever presented that colorless thin-walled cells increased light penetration into mesophyll cells. Besides, Clark (1991) had ever presented the hypothesis that fusoid cells may serve as reservoirs for the retention of CO_2 from photorespiration. However, March and Clark (2011) rejected this hypothesis, because of the differential occurrence of fusoid cells in shaded environments where photorespiration was less intense. They proposed that fusoid cells were a mechanism to trap and redistribute light more efficiently in shade leaves of bamboo and early-diverging grasses. Meanwhile, they could not determine whether the fusoid cells died and remained intact or whether they uniformly collapsed to form spaces of the same size and shape as mature fusoid cells.

According to the observations, it could be confirmed that fusoid cells were real cells, not air cavity, because of the occurrence of nuclei and cytoplasm. Meanwhile, fusoid cells were also the main difference between leaf blade and sheath. Either in culm sheath or branch sheath, there were no fusoid cells. The cytoplasm of fusoid cells in mature leaf blades could still be observed, which demonstrated that the mature fusoid cells were still intact and alive.

Moreover, fusoid cells of leaves significantly increased their volume after long time cultivation in greenhouse. The high temperature was the main stressing factor for the bamboo growth in the greenhouse, which could lead to the high transpiration rate. These results indicated that fusoid cells might be related to water storing and maintain the normal water metabolism of leaves, in order to avoid excessive loss of water from mesophyll cells in high temperature environment. This could be further confirmed by the observation of crystals in fusoid cells. Meanwhile, no pits or plasmodesmata were observed on their walls. Therefore, the fusoid cells were more likely to regulate the water balance in leaves.

Besides, fusoid cells increased their volume in high temperature environment. High temperature also caused high photorespiration. Meanwhile, each fusoid cell was almost surrounded by mesophyll cells and bundle sheath cells with chloroplasts. Therefore, it is also feasible that fusoid cells could reserve CO_2, which was also hypothesized by Clark (1991).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◀**Fig. 3** Vascular bundle of *F. yunnanensis*. **a** Primary lateral vein showing clearly plasmodesma (*arrows*) in the inner vascular bundle sheath cells, implying the symplastic transport of sucrose from the mesophyll across the vascular bundle sheath cells. **b** Transverse veitilet between two lateral veins, showing the irregular protrusions of walls toward the adaxial epidermis (*arrow*). **c** The fusoid cell (FC) and mesophyll cells close to vascular bundles, showing the intercellular space, plasmodesma (*arrow*) between mesophyll cells and bundle sheath cells and no plasmodesma in the fusoid cell wall. **d** The outer vascular bundle sheath cells, showing the chloroplast and plasmodesma (*arrow*) between the outer bundle sheath cells and the inner cells. **e** Plasmodesma (*arrows*) between the two outer bundle sheath cells. **f** Plasmodesma (*arrow*) between the corner of another mesophyll cell and the outer bundle sheath cells. **g** Chloroplast in bundle sheath cell showing simple stroma lamella. **h** Lots of starch grains observed in mesophyll cells (*arrow*). **i**, **j** Starch grains observed in some big chloroplast of sheath cells (*arrow*)

Bundle sheath anatomy in relation to transport and loading of photoassimilates

Many grasses have two cell layers surrounding the vascular bundles, while others have a single layer (Leegood 2008). The outer layer is designated the parenchyma sheath and the inner layer, whose inner walls are often thickened, is designated the mestome sheath (Leegood 2008). A similar phenomenon was also observed in this paper. There were two types of vascular bundles in the leaves of *F. yunnanensis*, i.e., the primary lateral veins with one layer of parenchymatous sheath cells and one layer of lignified sheath cells and the secondary veins with only one layer of parenchymatous sheath cells. Wu (1960) also noticed that the vascular bundle of bamboo leaves is clearly surrounded by double bundle sheaths, an outer parenchymatous sheath and an inner sclerenchymatous sheath, which is a typical character of festucoid.

In addition, apparent chloroplasts were observed in the parenchymatous sheath cells of bamboo leaf blades and apparent stroma lamellas were observed, which was similar to that of Zea mays (Andersen et al. 1972). Actually, many C3 plants have chlorenchymatous bundle sheaths (Leegood 2008). The parenchymatous sheath cells of some grasses contain chloroplasts, while the cells of other grasses do not (Rhoades and Carvalho 1944). These chloroplasts were also smaller than those of the mesophyll cells (Williams et al. 1989). Unlike other highly-productive members of Poaceae family, the entire bamboo subfamily lacks the C4 photosynthetic pathway and anatomy (Jones 1985). Starch grains were still observed in the chloroplasts of parenchymatous sheath cells under TEM. Previous studies showed that bundle sheath cells in barley are capable of photosynthesis and can synthesize starch in the light (Williams et al. 1989). The bundle sheath acts as a buffer for the carbohydrate loading between the mesophyll cells and the phloem and particularly when there is an excess of photo-synthetic source capacity over sink demand, sucrose and fructan accumulated in mesophyll and sheath cells (Koroleva et al. 2000). However, the fact that the starch in the sheath cells of bamboo leaf blades was synthesized by the photosystem of their chloroplasts or just accumulated because of the limited phloem loading needs further study.

Currently, there were no reports regarding the photoassimilate transport and loading pathway from mesophyll cells to phloem in bamboo leaf blades. The bundle sheath is a critical control point for the supply of water and solutes to leaf cells and for the export of the same (Fricke 2002; Leegood 2008). In wheat, all of the longitudinal veins are encased in a mestome sheath and all of the photosynthesis that moves directly from the mesophyll to the sieve tubes in these veins must across this boundary via the plasmodesmata that lie in the pit fields in the inner tangential wall of mestome sheath cells (Kuo et al. 1974). The similar phenomenon was observed in the *F. yunnanensis* leaf blades. The inner and outer sheath cells of the vascular bundles formed a cytoplasmic continuum by abundant of plasmodesmata. Therefore, photoassimilate from mesophyll cells could be transported across the sheath cells by symplasmic pathway and then entered into the peripheral vascular parenchyma cells.

Under SEM, it could be noted that scalariform secondary thickening occurred on the lateral walls of those parenchyma cells between xylem vessels and phloem. This might suggest that the intense exchanges between the flux of water inwards and the flux of photoassimilates outwards occurred in these parenchyma cells. Canny (1986) has hypothesized that suberized lamellae function to keep separate the two opposed fluxes through the sheath and the function of pits in living cells is a similar insulation of the symplastic traffic from the wayward waters of the apoplast. However, no pits were observed in the scalariform walls of the vascular parenchyma cells, so the intercellular transport pathways of photoassimilates should be apoplastic, which needs to be further confirmed. van Bel also (1993) reported that vascular parenchyma cells are the most probable site for photoassimilate exchange to phloem apoplasm, ensuring direct delivery for loading into se-cc complexes (Atwell et al. 1999).

The mode of phloem loading was divided into two types, i.e., apoplastic loading and symplastic loading (Kühn 2003; Lalonde et al. 2003; Roberts and Oparka 2003). In some species, sucrose from the mesophyll enters the cell wall space and was taken up by the companion cells and/or sieve elements by means of H^+/sucrose symporters, which is called apoplastic loading. The other type of phloem loading is found in species

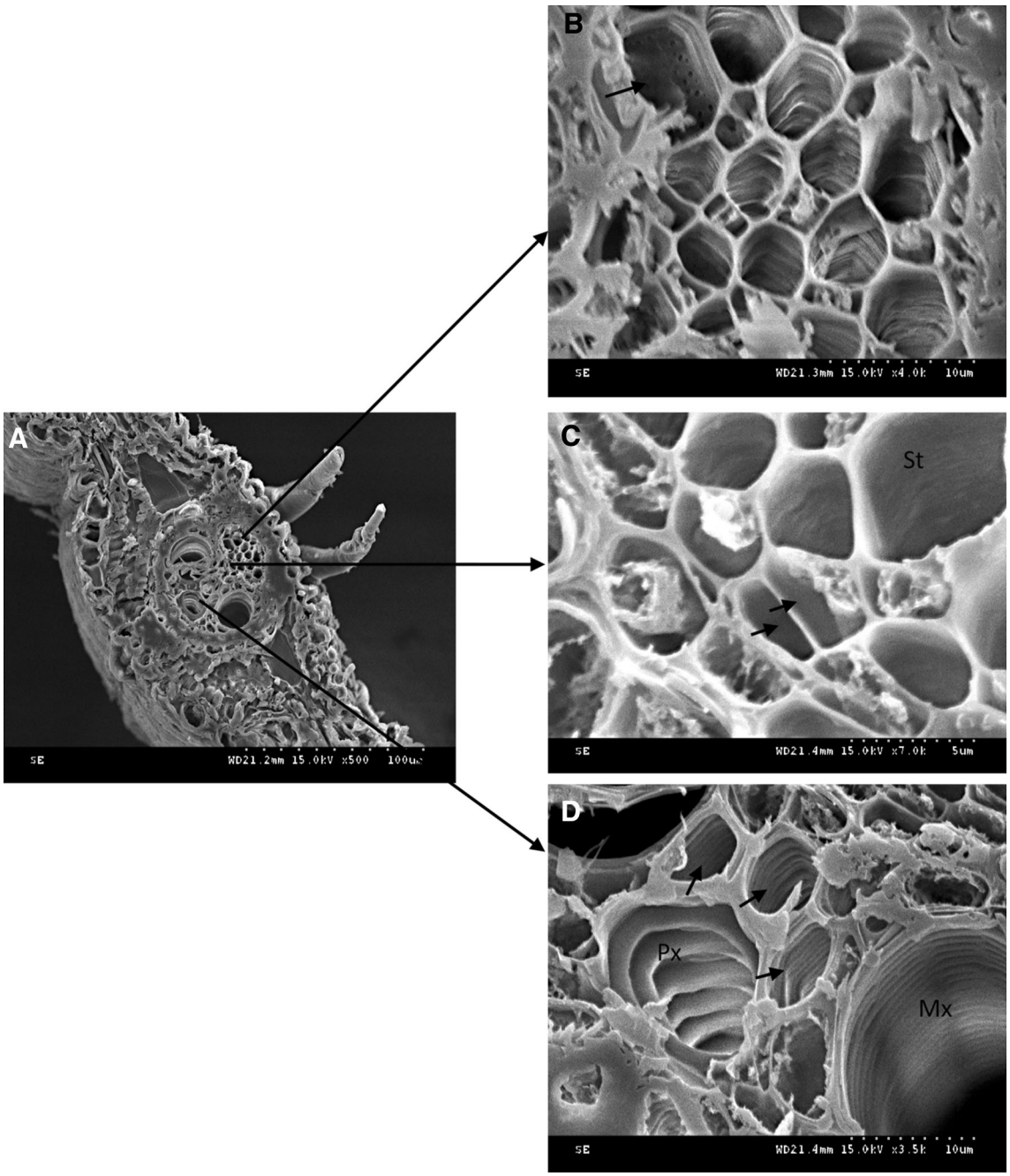

Fig. 4 Observations on different cells of lateral vein by SEM. **a** Lateral vein. **b** Phloem, showing the sieve plates of a sieve tube (*arrow*), but no pits on the lateral walls. **c** Companion cells (*arrows*), showing no pits on their lateral walls. *St* sieve tube. **d** Scalariform thickening in the walls of vascular parenchyma cells between vessels and phloem (*arrows*). *Px* protoxylem, *Mx* metaxylem

in which sucrose access to the phloem through plasmodesmata and this type was symplastic loading (Amiard et al. 2005). In the present study, the photoassimilates from mesophyll cells of bamboo leaf blades were loaded into the phloem by apoplasmic pathways, because no pits were observed in the lateral walls of companion cells and sieve tubes.

Conclusions

Bamboo leaf blades from the greenhouse had fewer layers of palisade parenchyma cells than those from the wild. Fusoid cells increased their volume in the greenhouse condition. There are apparent crystals and cytoplasm in the fusoid cells, but no pits or plasmodesmeta on their walls,

which indicated that fusoid cells had the function of regulating the water balance. Nuclei could be observed in the fusoid cells of young sheath blades. Several fusoid cells close to the midrib usually formed the complex. The parenchymatous sheath cells of lateral veins had chloroplasts with simple stroma lamellae and starch grains. Plasmodesmata in the walls of sheath cells connected the adjoining parenchyma cells at the peripheral of xylem to the mesophyll cells and formed a cytoplasmic continuum. No pits were detected in the walls of phloem tube and companion cells. Therefore, the photoassimilates from mesophyll of bamboo leaf blade were transported across the bundle sheath cells by symplastic pathway but loaded into phloem tubes by apoplastic pathway.

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