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Ultrastructural Aspects of Leaves in *Festuca ovina* and *Poa sphondylodes* (C-3 Poaceae)

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Structural aspects of the leaves of two common festucoids, *Festuca ovina* and *Poa sphondylodes*, have been examined employing the electron microscopy. The nature of vascular bundles and of sheaths that surround vascular tissues was discussed in the study. The festucoids exhibited a non-Kranz C-3 anatomy with more than four mesophyll cells separating the bundle sheaths of a leaf blade. Vascular tissues in these *Festuca* and *Poa* leaves were surrounded by a double sheath: an inner distinct mestome sheath (MST) and an outer indistinctive layer of parenchymatous bundle sheath (PBS) cells. The PBS cells were much larger than the MST and had thin walls. The MST cells were relatively small and rectangular in *P. sphondylodes* and more or less hexangular in transverse sections of *F. ovina*. In *P. sphondylodes*, MST had conspicuously thickened inner tangential walls with asymmetrically uninterrupted suberized lamellae in radial and tangential walls. In most differentiated MST cells, all walls were highly suberized. During suberin deposition, MST cells were quite vacuolated and most of the cytoplasm was present as a thin peripheral layer. However, MST walls in *F. ovina* revealed very thin suberized lamellae with translucent striations. No chloroplasts were detected in *P. sphondylodes*, whereas the MST in *F. ovina* contained small chloroplasts. Plasmodesmata were well developed in the primary pit fields of walls between MST and vascular cells, and between adjacent MST cells. Plasmodesmata were less frequent in the walls between the inner and outer sheath cells. Suberized lamellae were totally absent from the PBS cell walls in all veins. External to the PBS, the mesophyll comprised thin walled cells with abundant intercellular spaces. Peripherally arranged chloroplasts in the mesophyll were numerous and often larger than those of PBS and MST cells. Characteristics associated with C-3 and other ultrastructural features were also discussed in the study.

Keywords: C-3 Poaceae, *Festuca ovina, Poa sphondylodes*, mestome sheath, parenchymatous bundle sheath

INTRODUCTION

Leaves are specialized plant structures that perform the photosynthesis with either one of three types of pathway, *viz.* C-3, C-4 and CAM. The anatomical leaf structures among these types are known to be quite different from one another (Esau, 1979; Fahn, 1990). The radial file of mesophyll and bundle sheath cells around the vascular bundle can be differentiated in a number of different ways and, indeed, this layer probably carries out different functions correlated with its different morphology. The C-3 photosynthesis involves mesophyll cells only, whereas C-4 photosynthesis involves the cooperation of mesophyll and bundle sheath cells within the Kranz anatomy. The bundle sheath cells of C-3 plants are small and usually lack chloroplasts, which are dispersed uniformly throughout the mesophyll cells. After discovery of the C-4 photosynthesis from the grass leaf (Kortschack *et al.*, 1965), C-3 photosynthesis was soon related to non-Kranz anatomy and C-4 photosynthesis to Kranz anatomy (Laetsch, 1968). Kranz or related structure has been reported for plants characterized as C-4 plants and essentially non-Kranz structure for all C-3 plants. In general, any particular grass genus is either entirely Kranz or entirely non-Kranz with a

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few exceptions (Hedge and Patil, 1981; Ellis, 1984; Edwards *et al.*, 1990).

The Poaceae, in particular, have received some attention since at least two different photosynthetic pathways are to be found within this family. Each consistently associated with a particular form of leaf anatomy and supposedly with a particular ultrastructural and/or biochemical character of PBS cells (Brown, 1975, 1977; Hatch, 1987; Shina and Kellogg, 1996). The majority of vascular bundles in grass leaves are encased in a bundle sheath. In some species, the sheath is just one cell thick and contains chloroplasts. Other species have two sheaths where the outer parenchymatous sheath usually contains chloroplasts, while the inner sheath, MST, usually lacks chloroplasts (Brown, 1977). All vascular bundles of grass leaves have a parenchymatous sheath with the MST present or absent. Types of PBS have been used to show the relationships among groups of grasses and asymmetrically thickened MST have been reported in few groups, especially in the festucoids (Carolin *et al.*, 1973; Brown, 1977). Characters such as presence or absence of the MST, structure and function of the outer PBS, arrangement of the chlorenchyma cells between bundles, and certain physiological aspects associated with above characters have been considered to be of phylogenetic importance in grasses (Brown, 1977).

A large number of C-4 grass have been examined ultrastructurally (See Hatch, 1987; Dengler *et al.*, 1996), in contrast to relatively small volume of ultrastructural information available for C-3 grasses (Johnson and Brown, 1973; Kuo *et al.*, 1974; O' Brien and Kuo, 1975; Miyake and Maeda, 1976; Kaneko *et al.*, 1980; Brown *et al.*, 1983; Botha, 1992). Among reported C-3 grasses, most studies were made on common C-3 cereal species such as wheat, rice or oat. Other C-3 species with less economic importance have not drawn much attention, although some may have peculiar structural features. The present study, thus, has undertaken to examine the ultrastructual aspects of leaves in two typical festucoids, *Festuca ovina* and *Poa sphondylodes*, especially of MST and PBS cells with asymmetrically thickened walls. The presence of characteristics associated with C-3 and other ultrastructural features were also assessed from the this study.

MATERIALS AND METHODS

Five to ten plants of *Festuca ovina* L. and *Poa sphondylodes* Trin., classified according to Lee (1993), collected from low lands of Taegu during 1996-1997, were used for the study. The middle region of fully expanded healthy blades were sampled for the transmission electron microscopy. Approximately 1 mm^2 pieces of tissues from each species were fixed either in 3% glutaraldehyde or in a mixture of 3% paraformaldehyde and 3% glutaraldehyde in 0.01 M phosphate buffer (pH 7.2) at room temperature for 3 hours (Kim *et al.*, 1997). The fixed tissues were washed several times in the buffer and postfixed in 2% osmium tetroxide for overnight at 4°C. The tissues were, then, washed 4 times in the same buffer with intervals of 15 min. The specimens were dehydrated in an ethanol series and embedded in Spurr's low viscosity resin. Ca. 0.5-1.0 μm transverse sections were cut on a Reichert RMC 7000 ultramicrotome with glass knives and stained with toluidine blue O. Ultra-thin sections made with a diamond knife were stained with methanolic uranyl acetate and lead citrate. After staining with methanolic uranyl acetate for 30-45 min, they were rinsed for 3-4 times with filtered 50% ethanol and with filtered CO_2-free water. These sections were examined with a Phillips EM201 electron microscope.

RESULTS

The festucoids examined in the study showed a non-Kranz C-3 anatomy, with more than four mesophyll cells separating the bundle sheaths. For the anatomical structures, Esau's terminology (Esau, 1979) has been adopted and ultrastructural features similar in both *Festuca ovina* and *Poa sphondylodes* were described collectively unless they were specified.

In large vascular bundles, the tissue was differentiated into xylem in the adaxial surface and phloem in the abaxial surface of the bundle (Fig. 1). Such differentiation was not so apparent in small vascular bundles. Their vascular cells were arranged rather irregularly, although tracheary elements appeared on the adaxial side of sieve elements. Phloem tissue contained various parenchyma cells (Fig. 2). Thin-walled sieve elements as well as sieve elements with thick walls occurred in the peripheral part of the phloem of large bundles. Occasionally, small vascular bundles lacked the xylem parenchyma cells. Xylem parenchyma cells revealed the plastids with the rudimentary lamellae. These plastids were structurally indistinguishable from those of phloem parenchyma cells. Mostly a single layer of thick-walled parenchyma cells bounded the phloem. The metaphloem sieve elements had the P-type plastids and often contained

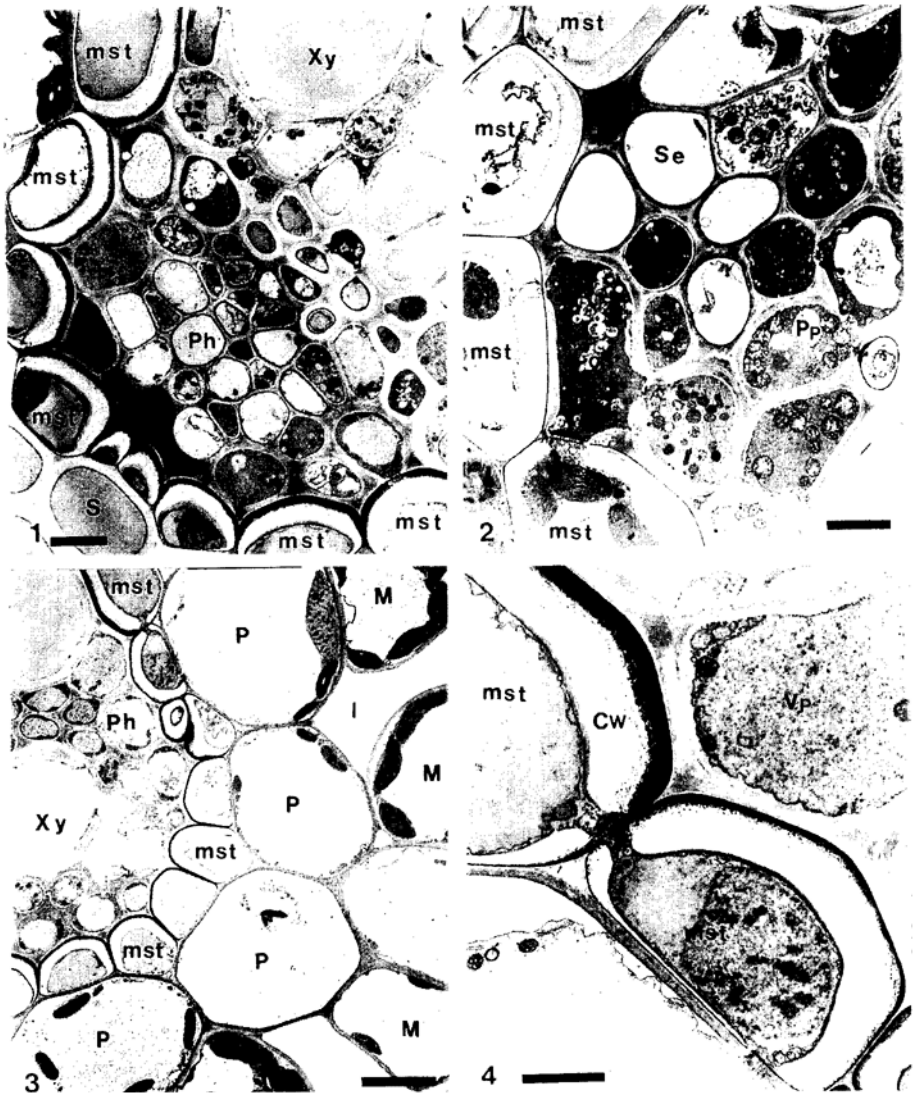

Fig. 1. A large vascular bundle showing the phloem (Ph) adaxially in *P. sphondylodes*. mst, mestome sheath cell; S, sclerenchyma; Xy, xylem. Scale=5 μm.

Fig. 2. Various phloem parenchyma cells (Pp) in *F. ovina*. Cc, companion cell; Se, sieve element. Scale=3 μm.

Fig. 3. Two sheaths surrounding vascular bundles in *P. sphondylodes*: an inner mst and an outer parenchyma sheath (P). I, intercellular space; M, mesophyll cell. Scale=8 μm.

Fig. 4. Higher magnification of the mst cells without chloroplasts and asymmetrically thickened tertiary walls with suberized lamelle in *P. sphondylodes*. Cw, cell wall, Vp, vascular parenchyma. Scale=2 μm.

osmiophilic globules. Numerous plasmodesmata connected the companion cells with adjacent parenchyma cells of the phloem and xylem, and also with the thick-walled parenchyma cells.

Vascular bundles of *Festuca* and *Poa* leaves were surrounded by a double bundle sheath: an inner MST and an outer indistinctive layer of PBS cells (Fig. 3). MST cells of all vascular bundles had a very thin sparse cytoplasm without chloroplasts in *P. sphondylodes* (Fig. 4), but with a few chloroplasts in *F. ovina* (Fig. 5). When these plastids were in view, grana were normally developed but starch grains were very rare. In general, the plastids of MST in *F. ovina* were very small when compared with those of the surrounding parenchyma cells. MST cells were more or less rectangular in *P. sphondylodes* (Fig. 4), but somewhat hexangular in *F. ovina* (Fig. 5). They were located as a row of cells just outside the vascular tissue and surrounded by a row of PBS cells. There was no direct access from the intercellular spaces of the mesophyll to these MST cells. Plasmodemata in the primary pit field were well developed between

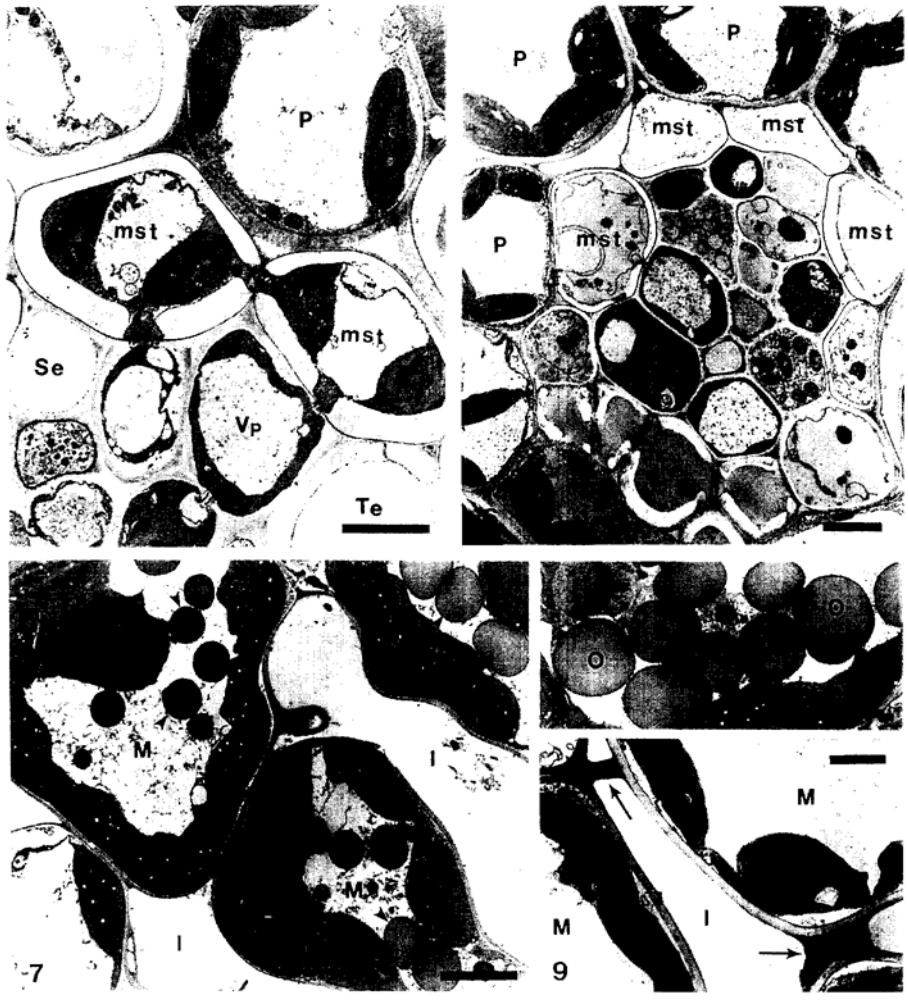

Fig. 5. More or less hexangular mst cells with chloroplasts (C) in *F. ovina*. No conspicuous suberized lamella occurred as in Fig. 4. Scale=3 μm.
Fig. 6. Medium-sized bundle with an incomplete mst from *P. sphondylodes*. Te, tracheary element. Scale=3 μm.
Fig. 7. Irregular mesophyll cells with peripherally arranged chloroplasts and huge intercellular spaces in *F. ovina*. Notice abundant oleosomes (arrow heads) in the vacuole. Scale=4 μm.
Fig. 8. Higher magnification of the mesophyll area with numerous oleosomes (O) in *F. ovina*. Scale=3 μm.
Fig. 9. Supportive "bridges" (arrows) outgrown from the cell wall between mesophyll cells in *P. sphondylodes*. Scale=2 μm.

adjacent MST cells, and between MST and vascular cells.

Cell walls in *P. sphondylodes* demonstrated an asynchronously developed, well-defined suberized lamella in their MST (Fig. 4). However, thin suberized lamellae with translucent striations were shown in the MST walls of *F. ovina* and these walls exhibited less asymmetrically thickened walls (Fig. 5). The cell walls developed the characteristic tertiary thickenings. In most differentiated MST cells, all walls were considerably suberized in *P. sphondylodes*. On the small veins, the inner sheath may be present only on the phloem side (Fig. 6). In small to medium-sized bundles, an incomplete MST appeared to form an incomplete or partial sheath.

External to MST was the PBS cells. The outer PBS cells of *P. sphondylodes* were about twice as large as MST cells and had very thin walls. In *F. ovina*, the inner tangential walls of PBS neighboring the MST cells were thicker than walls neighboring PBS and mesophyll cells. Their chloroplasts were, however, slightly larger than MST plastids and had a few well-developed grana. The outer PBS cells in *F. ovina* were somewhat larger than those of the inner MST; few outer sheath cells occasionally became highly enlarged. Size of vacuoles varied from small in the small vein PBS to nearly complete occupation of the major veins, but the outer PBS

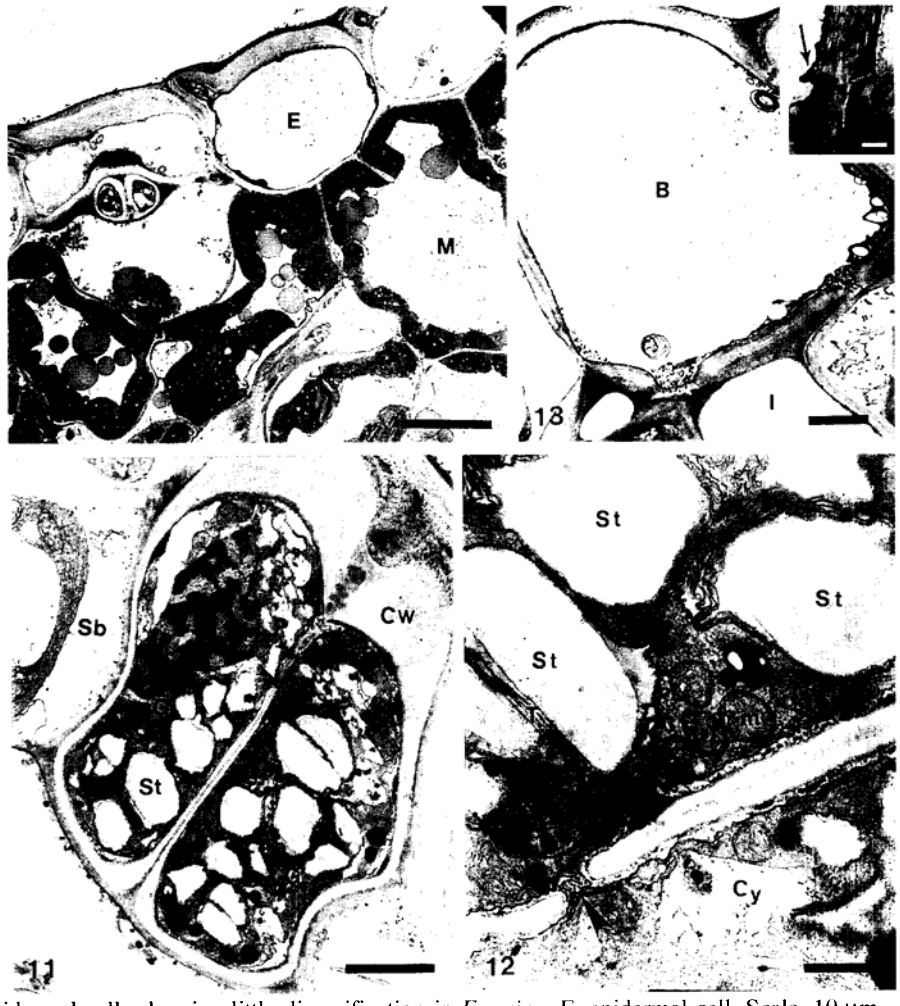

Fig. 10. Adaxial epidermal cells showing little diversification in *F. ovina*. E, epidermal cell. Scale=10 μm.
Fig. 11. Guard cells exhibiting thickened upper cell walls in *F. ovina*. Arrow heads indicate the cytoplasmic connection. G, guard cell; Sb, subsidiary cell; St, starch grain. Scale=2 μm.
Fig. 12. Cytoplasmic connection (arrow head) between the guard cells in *F. ovina*. Cy, cytoplasm; m, mitochondria; T, thylakoids. Scale=0.5 μm.
Fig. 13. Adaxial epidermal cells almost devoiding cytoplasm in *P. sphondylodes*. B=bulliform cell. Scale=2 μm. Insert: primary pit field with ER (arrow) between the epidermal cells. Scale=0.5 μm.

usually had an extensive vacuolar system and slightly large chloroplasts relative to the inner sheath (Fig. 3). Mesophyll cells showed a distinct arrangement around the outer PBS. The chloroplasts had well-developed grana but the starch was lacking or occurred only occasionally. Plasmodesmata were less frequent in the walls between MST and PBS cells.

PBS cells of both species were similar in size, but they were ca. 12-25 μm in *P. sphondylodes* and ca. 14-18 μm in *F. ovina*, respectively. Relatively few and smaller chloroplasts were shown in the PBS compared to mesophyll cells. Mesophyll with large intercellular spaces differentiated into a radially arranged chlorenchyma with additional chloroplasts. Sometimes, cells containing chloroplasts not in contact with the sheath were connected by the supportive "bridge(s)" that was outgrown from the cell wall (Fig. 9). Plasmodesmata occurred between the outer tangential walls of PBS and mesophyll cells. Chloroplasts of the PBS and mesophyll cells possessed well-developed grana in both species. Chloroplasts in PBS tended to be centripetally located, but they were more randomly arranged around the cell perimeter in *P. sphondylodes*, while they occupied a high portion of mesophyll profile in *F. ovina*. Mesophyll cells in *F. ovina* were irregular in size and shape (Fig. 7). Although PBS chloroplasts may be smaller than mesophyll chloroplasts, no apparent

difference was observed with respect to the internal structure. Mitochondria in PBS and mesophyll cells in these plants were very closely associated with chloroplasts. Microbodies also appeared similar in structure among cells examined. Oleosomes were abundant in the vacuoles of PBS and mesophylls in *F. ovina*. They were found primarily in mesophyll cells (Figs. 7-8), although these also occurred at much lower frequency in PBS cells.

Other features such as sclerenchyma and epidermis were also briefly examined. The sclerenchyma fibers were frequently associated with the vascular tissues around bundles and sometimes stranded over the bundles of either sides of the leaf in large bundles. In such cases, the PBS was often interrupted by the sclerenchyma on the abaxial side. Vascular bundles of different sizes alternated rather regularly with one another and were interconnected by small transverse commissural strands. There was little diversification in the epidermis of the festucoids examined (Fig. 10). Subsidiary cells commonly parallel-sided and very rarely papillate. The upper and lower walls of the guard cells were conspicuously thickened, particularly the upper walls (Fig. 11). Cytoplasmic connections between the guard cells were often encountered in *F. ovina* (Figs. 11-12). Plastids were found throughout the guard cell. These plastids contained a number of large starch grains, in which appeared amyloplast-like in configuration. Epidermal cells and bulliform cells on the adaxial surface of the leaf were almost devoid of cytoplasm (Fig. 13) where very small plastids were rarely encountered.

DISCUSSION

The present study discussed the nature of two sheaths that surround vascular bundles in common festucoids, *Poa sphondylodes* and *Festuca ovina*. High vein-to-vein mesophyll cell number as exhibited in these festucoids is a distinguishing feature in C-3 grasses, where it is low in C-4 species. C-3 plants also often have layers of parenchymatous sheath cells around the vascular tissue, but these cells are clearly different from the counterpart of C-4 plants, Kranz cells, since the latter being large, thick-walled cells with numerous and prominent chloroplasts. A much less distinct PBS is often present in C-3 monocot species.

Vascular bundles in *Poa* and *Festuca* studied were surrounded by an inner MST and outer PBS cells of a double-layered sheath as in other festucoids where the presence or absence of a MST has been recognized as an important character (Brown, 1977; Lee, 1993). The MST cells had conspicuously thickened inner tangential walls in *P. sphondylodes* and contained an uninterrupted suberin lamella in radial and tangential walls. In the highly differentiated MST cells, all walls were conspicuously suberized. The counterpart of suberized lamellae in *F. ovina*, MST cells appeared as a very thin layer along with translucent light bands. The suberized lamellae was not developed in the PBS cells of C-3 species (Brown, 1977).

External to the MST when this layer is present, or in contact with the metaxylem vessels when there is no MST (Kaneko *et al.*, 1980), is the PBS. Within the outer PBS, the chloroplasts are either similar to those of the mesophyll or somewhat smaller. The cells of mesophyll vary in size, but usually much larger than the other two cell types. The degree of PBS development, presence or absence of the MST, and arrangement of mesophyll are important criteria in structural classification of the Poaceae (Brown, 1975). Of particular, structural aspects of the PBS cells is interesting one. The PBS of grasses shows variations that are taxonomically significant and has been considered as indicators of the photosynthetic type of the given species (Brown, 1977). It has been suggested that PBS of the festucoid type are less metabolically active than the mesophyll cells, since the number of cellular organelles is decreased in comparison with those of the mesophyll (Brown *et al.*, 1983). An average of 14, 6, and 9% of total leaf mitochondria, microbodies and chloroplasts, respectively, were estimated in PBS of certain C-3 species (Brown *et al.*, 1983). Over 60% of the thylakoid surface is typically found in the mesophyll chloroplast grana of C-3 plants (Lawlor, 1993). Vacuolar size varied from small in sheath cells of the smaller vein to nearly complete occupation of the outer PBS cell of major veins. In general, vacuoles occupy 80% of cell volume and the chloroplasts occupy 38% of the mesophyll cytosol volume in C-3 plants (Lawlor 1993). There was a tendency for bundle sheath chloroplasts in C-4 species to be larger relative to those in mesophyll, while in C-3 species the tendency was reversed (Edwards and Walker, 1983). Ratio of chloroplast lengths in bundle sheath to mesophyll cell was reported from 0.7 to 0.9 in C-3 panicum species (Brown *et al.*, 1983). External to the PBS is the mesophyll that comprises thin-walled cells with abundant intercellular spaces. Since PBS cells have considerably fewer intercellular air spaces than

mesophyll cells, PBS chloroplasts of C-3 plants are in a disadvantageous position for photosythetic CO_2 fixation if the same mode of CO_2 fixation is operative in mesophyll and PBS cells. The smaller size of PBS chloroplasts in C-3 plants may reflect their photosynthetic inferiority (Brown *et al.*, 1983).

The C-3 and C-4 grasses show anatomical and ultrastructural differences with a high degree of consistency. The structure of PBS is particularly important in distinguishing C-3 and C-4 grasses. In C-3 species, this sheath has few organelles and rather small chloroplasts in contrast to the chloroplast-rich mesophyll. In C-4 species, the sheath has a high content of organelles with commonly larger chloroplasts than the mesophyll chloroplasts. According to the evolutionary hypothesis known in grasses, leaf anatomy is supposedly from the non-Kranz to the Kranz types (Brown, 1977). Evolution has progressed presumably from the simple, common C-3 photosynthetic type to the biochemically unusal and more complex C-4 types (Edwards and Ku, 1987; Langdale *et al.*, 1988; Nelson and Langdale, 1992; Taylor, 1996). Brown (1975) proposed the evolution of a Kranz sheath from the MST of non-Kranz grasses. According to him, changes in the MST cells led to the slight modification of the parenchyma sheath and further to the evolution of intercalary bundles of Kranz grasses. The persisting parenchyma sheath would inhibit the circulation of molecules between mesophyll and Kranz tissue and the circulation occurs only through the plasmodesmata. Structural features considered to be of phylogenetic value in grasses are presence or absence of the inner sheath, structure and function of the outer PBS, arrangement of the chlorenchyma cells between the bundles, and certain physiological aspects associated with last two characters (Brown, 1977). It is assumed that the primitive ancestral grasses are quite similar to the present festucoids and the festucoid type is little, if any, changed from the original hypothetical grass type (Brown, 1977). However, in view of the MST development on these species, it is highly possible to conceive that some MST cells might have played a role in generating a structural differentiation to a different sheath cell type. Ultrastructural aspects of the MST development will be presented in a later communication.

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