Ultrastructural indications for coexistence of symplastic and apoplastic phloem loading in *Commelina benghalensis* leaves

Differences in ontogenic development, spatial arrangement and symplastic connections of the two sieve tubes in the minor vein

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**Abstract.** The ultrastructural ontogeny of *Commelina benghalensis* minor-vein elements was followed. The mature minor vein has a restricted number of elements: a sheath of six to eight mesotome cells encloses one xylem vessel, three to five vascular parenchyma cells, a companion cell, a thin-walled protophloem sieve-tube member and a thick-walled metaphloem sieve-tube member. The protophloem sieve-tube member (diameter 4–5 μm; wall thickness 0.12 μm) and the companion cell originated from a common mother cell. The metaphloem sieve-tube member (diameter 3 μm; wall thickness 0.2 μm) developed from the same precursor cell as the phloem parenchyma cells. Counting the plasmodesmatal frequencies demonstrated a symplastic continuum from mesophyll to the minor-vein phloem. The metaphloem sieve-tube member and the phloem parenchyma cells are the termini of this symplast. The protophloem sieve-tube member and companion cell constitute an insulated symplastic domain. The symplastic route, mesophyll to metaphloem sieve tube, appears to offer a path for symplastic loading; the protophloem sieve tube may be capable of accumulation from the apoplast. A similar two-way system of loading may exist in a number of plant families. Plasmodesmograms (a novel way to depict cell elements, plasmodesmatal frequencies and vein architecture) of some other species also displayed the anatomical requirements for two routes from mesophyll to sieve tube and indicate the potential coexistence of symplastic and apoplastic loading.

**Key words:** Apoplastic loading – *Commelina* – Minor vein ultrastructure – Phloem loading – Plasmodesmatal frequency – Symplastic loading.

**Introduction**

In the current literature, two concepts are being advanced for phloem loading in source leaves (for reviews see Giaquinta 1983; Ho and Baker 1982; Delrot 1987; Van Bel 1987). These proposals respectively include (i) symplastic loading via a continuous plasmodesmatal path from mesophyll to sieve tube, and (ii) apoplastic loading of the sieve tubes which requires exit of photosynthates from the mesophyll symplast domain and subsequent entry in the sieve tube-companion cell complex.

One of the initial arguments for apoplastic loading was the paucity of plasmodesmata between phloem parenchyma cells and the sieve tube-companion cell complexes in beet leaves (Geiger et al. 1973, 1974). In the search for additional ultrastructural arguments in favour of either apoplastic or symplastic loading, several authors investigated the plasmodesmatal frequencies in the route between mesophyll and sieve tubes (Kuo et al. 1974; Turgon et al. 1975; Miyake and Maeda 1976; Evert et al. 1978; Gamalei and Pakhomova 1981; Fisher and Evert 1982; Russin and Evert 1985; Evert and Mierzwa 1986; Fisher 1986; Schmitz et al. 1987). The distribution of plasmodesmata appeared to vary strongly with the species. Except for a few cases, there was hardly any evidence for a distinct apoplastic intermission in the path from mesophyll to sieve tube (see references in Van Bel 1987). Thus, ubiquitous occurrence of apoplastic loading is highly unlikely on grounds of the ultrastructural data.

The importance of the ultrastructure for the assessment of the loading mechanism was questioned by the statement of Giaquinta (1983) that only the number of functioning plasmodesmata,
and not the absolute count of plasmodesmata, determines the efficiency of the transport path. Experiments with fluorescent dyes injected in mesophyll cells demonstrated operative plasmodesmatal contacts between mesophyll and veins (Erwee et al. 1985; Madore et al. 1986). Symplastic continuity between mesophyll and phloem elements was shown by injection of fluorescent dyes into mesophyll cells attached to partly isolated minor veins of Commelina benghalensis (Van Kesteren et al. 1988). The symplastic continuum may thus provide an avenue for symplastic loading.

In order to gain more information on the loading mechanism in C. benghalensis leaves, the ultrastructure of the minor veins, including the plasmodesmatal frequencies, was studied. Further, the ontogeny of the minor vein was followed to obtain insight into the physiological development of the loading machinery. Cell elements and plasmodesmatal connections were depicted in a plasmodesmogram, a novel way of presentation. The vein anatomy indicates that apoplastic and symplastic loading may coexist.

Material and methods

Plant material. Commelina benghalensis leaves were grown in a greenhouse (night temperature 18°C, day temperature between 20 and 35°C) on hydroponic cultures with Hoagland solutions in daylight. The plants were shaded in summer. Three developmental types of leaves were used: (i) Developing top leaves, 8–15 mm in length. Rectangles (3.5 mm²) were cut from the meristematic area of the base. These leaves were employed to follow the ontogeny of the minor veins. (ii) Mature leaves (mostly the third one from the top), 55–70 mm in length. Rectangles (3.5 mm²) were cut from the middle of the lamina halves. These leaves served to study the anatomy of the full-grown minor veins. (iii) Dark-grown leaves. Very small top leaves were wrapped in black plastic impervious to light and between 20 and 35°C on hydroponic cultures with Hoagland solutions in daylight. The plants were shaded in summer. Three developmental types of leaves were used: (i) Developing top leaves, 8–15 mm in length. Rectangles (3.5 mm²) were cut from the meristematic area of the base. These leaves were employed to follow the ontogeny of the minor veins. (ii) Mature leaves (mostly the third one from the top), 55–70 mm in length. Rectangles (3.5 mm²) were cut from the middle of the lamina halves. These leaves served to study the anatomy of the full-grown minor veins. (iii) Dark-grown leaves. Very small top leaves were wrapped in black plastic impervious to light and grown to maturity. Control leaves were wrapped in light-transmittent plastic sheets. Rectangles (3.5 mm²) were cut from the lamina halves at maturity. These specimens permitted study of the light effect on minor-vein development.

Electron microscopy. Leaves, still attached to the plant, were immersed in 3% glutaraldehyde in 100 mol·m⁻³ Na₂HPO₄/NaH₂PO₄ buffer (pH 7.2). This buffer was used in all following treatments. Rectangles of immersed tissue were excised with a razor blade from the leaf types described above. The tissue was prefixed in 3% glutaraldehyde solution in 100 mol·m⁻³ buffer for 1 h at 4°C or in 4% KMnO₄ solution in 100 mol·m⁻³ buffer for 1 h at 4°C. The leaf pieces were rinsed in 100 mol·m⁻³ buffer for 30 min at room temperature. After rinsing, the tissue was postfixed with 2% OsO₄ in 100 mol·m⁻³ buffer for 1 h at room temperature and rinsed in buffer that was replaced once. Dehydration in a cold acetone-graded series and infiltration in Spurr epoxy resin were carried out according to standard procedures.

Ultrathin sections (60 nm) were cut with glass knives (LKB, Bromma, Sweden; 7800 knifemaker) on a Reichert (Wien, Austria) OmU3 microtome and captured on 200-mesh grids. The tissue was sectioned perpendicular to the minor veins. The sections were contrasted with uranyl acetate in 70% methanol for 5 min and in lead citrate (400 mol·m⁻³) for 1 min, and viewed and photographed at 50 kV with a Philips (Eindhoven, The Netherlands) EM109 electron microscope.

Results

Ontogeny of the minor veins. In meristematic leaf tissue of Commelina benghalensis, mesophyll and mestome-sheath develop first (Fig. 1, 2). These cells are already vacuolated and have (poorly developed) chloroplasts when division of the vascular cells is still incomplete. At this stage, mesophyll and mestome-sheath cells seem to be less turgid than at leaf maturity. Intercellular spaces between vein elements are absent and the chloroplasts in mestome-sheath and mesophyll cells contain fewer thylakoids than at later stages (Fig. 2, cf. 10b). Later, when the vein cells become more turgid, schizogenous intercellular spaces develop (Fig. 3, 4). All cells in the meristematic state contain nuclei with a mass of heterochromatin (Fig. 1).

The vascular mother cell produces two procambium cells, the phloem and xylem initials (Fig. 1). The xylem initial divides into a xylem vessel and a xylem parenchyma cell (Fig. 2). The xylem developps earlier than the phloem: the xylem cells become vacuolated when the phloem is still dividing (Fig. 3). In the embryonal xylem vessel element, the helical secondary wall thickening is clearly visible, before the protoplast disintegrates (Fig. 3). The phloem initial produces two primary phloem cells, one of which divides into two protophloem elements, the protophloem sieve-tube member and the companion cell (Fig. 3). After this division, the protophloem sieve-tube member looses its tonoplast (Fig. 4). The other primary phloem cell produces 2–4 phloem parenchyma cells and the metaphloem sieve-tube member (Fig. 5) at a later stage. The metaphloem sieve-tube member is the last cell in the vein to become vacuolated. Minor-vein development was reconstructed from a series of photographs, representatives of which are shown in Figs. 1–5, and is schematically presented in Fig. 6.

Cytology of the minor-vein elements. The mestome sheath is one-layered comprising 6–8 cells in cross-