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Short Communication

Plasmodesmata between Mesophyll and Bundle Sheath Cells in Relation to the Exchange of C_4-Acids*

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Summary. In the C_4 species *Salsola kali* L. the frequency of plasmodesmata in the wall between mesophyll and bundle sheath cells has been determined with great precision by the use of transmission and scanning electron microscopy. The frequency of 14×10^8 cm^{-2} is rather high compared to values from other plant tissues, but if it is assumed that the postulated exchange of C_4-acids occur in the desmotubulus of the plasmodesmata, the fraction of the mesophyll-bundle sheath interface occupied by plasmodesmatal pores is 10–10^2 times smaller than previously thought.

Structural, biochemical, and physiological investigations throughout the last decade have laid down the main outlines of the C_4-dicarboxylic-acid pathway in photosynthesis. The generally accepted scheme for C_4-photosynthesis requires a rapid transfer of malate (or aspartate or both) from mesophyll to bundle sheath cells (Black, 1973). This transport of organic acids presumably involves their potassium salts (Pallaghy, 1973).

In recent years the role of plasmodesmata in intercellular transport has been further stressed (Clarkson *et al.*, 1971; Spanswick, 1972) and at present it seems quite reasonable that the path of transport of C_4-acids is restricted to the plasmodesmata connecting the mesophyll and bundle sheath cells. The existence of conspicuous pit fields with abundant plasmodesmata in the mesophyll-bundle sheath interface is well documented in *Salsola* (Olesen, 1974) and other C_4-plants (Laetsch, 1974), but apparently no quantitative description of this symplastic connection is available. Only one attempt has been made to characterize the flux of C_4-acids on the basis of theoretical values of plasmodesmatal frequency in the mesophyll-bundle sheath interface (Osmond, 1971).

The wall between mesophyll and bundle sheath cells in leaves of *Salsola kali* L. was investigated by the use of TEM and SEM, for details of the preparation see Olesen (1974). An analysis of plasmodesmatal frequency in the pit fields showed 60 ± 20 μm^{-2} and 54 ± 25 μm^{-2} in TEM and SEM preparations respectively. The micrographs from which the measurements were made were chosen at random. The great depth of focus in SEM made it possible to examine closely the entire wall between a bundle sheath cell and the adjacent mesophyll cells. Measurements

* *Abbreviations:* TEM = transmission electron microscopy, SEM = Scanning electron microscopy.

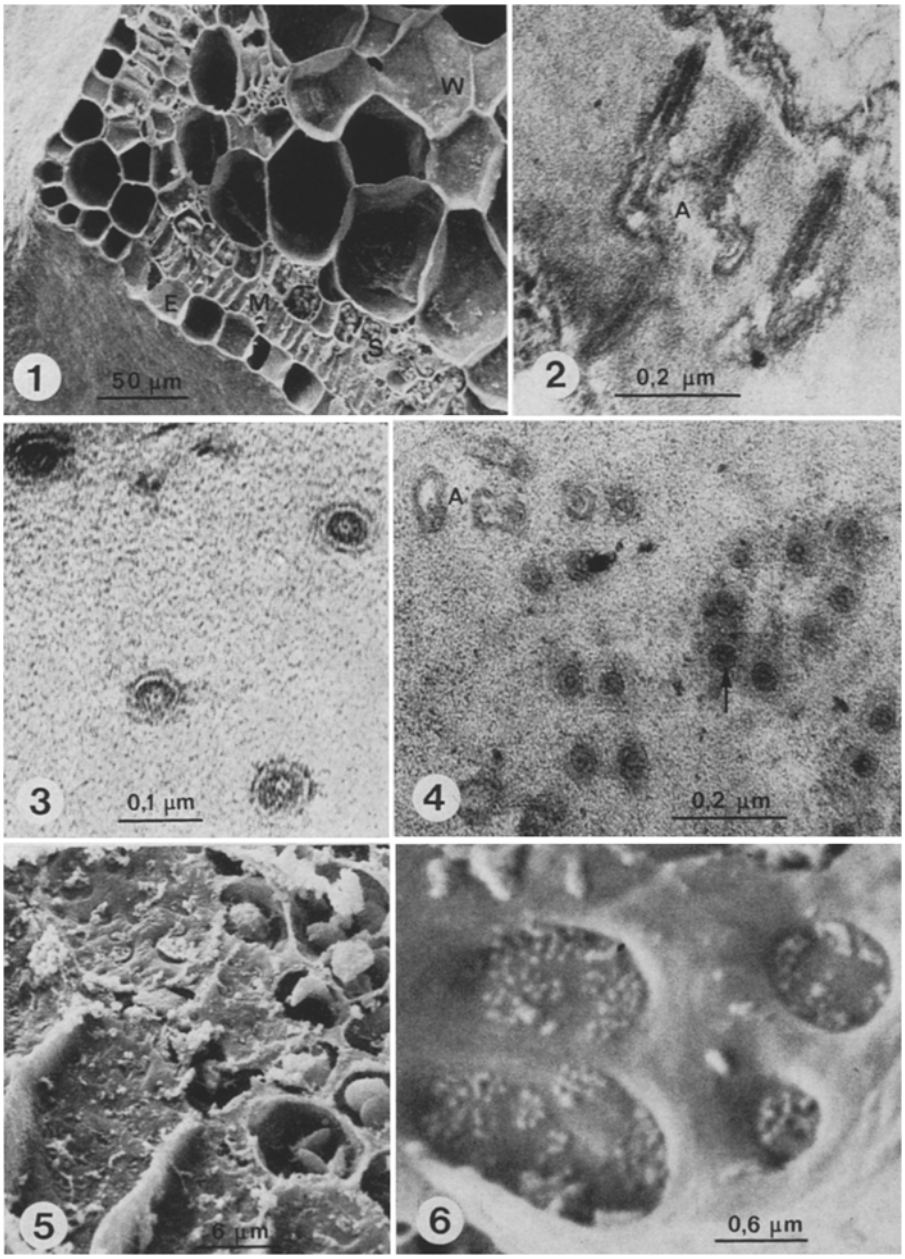
on 10 selected SEM-micrographs of the interface seen from the sheath cells representing a total of 168 pit fields showed that precisely one fourth of the interface is occupied by pit fields with an average plasmodesmatal frequency of 57 μm^{-2}. Therefore, the plasmodesmatal frequency in the cylindrical sheath comprising (by) the mesophyll-bundle sheath interface is $14 \pm 5 \times 10^8$ cm^{-2}. All measurements on SEM-micrographs have been corrected for the angle of photography (specimen tilt) and curvature of the walls of the interface.

The dimensions of the plasmodesmata in the interface were as follows (all values are $\pm$ SD with the actual number of measurements in brackets): Length of plasmodesma 440 ± 80 nm (22), radius of plasmodesmatal pore 22 ± 3 nm (24), and radius of the desmotubulus 7.5 ± 1 nm (15).

The leaf anatomy and ultrastructure of the chlorenchyma in *Salsola* have been described in a previous report (Olesen, 1974). The ultrastructure of the plasmodesmata in the mesophyll-bundle sheath interface (Figs. 2–4) conform to the general description in the latest review by Robards (1971). Viewed in SEM the interface shows slightly folded wall areas alternating with well defined depressions in which the wall is studded with small protrusions (Fig. 6). The depressions in the wall correspond to the pit fields seen in TEM and the numerous protrusions represent the plasmodesmata (*i.e.* remains of the complex plasmalemma-desmotubulus-endoplasmic reticulum). Considering that the plasmodesmatal frequency, $N = 14 \times 10^8$ cm^{-2}, in *Salsola* is determined in fully differentiated cells, it must be concluded that this frequency is high compared to the values enumerated by Tyree (1970) for a wide range of plant tissues.

The flux of C_4-acids across the mesophyll-bundle sheath interface, J, can be calculated during steady-state photosynthesis. Assuming that this transfer of solutes is restricted to the plasmodesmata, the actual flux through the plasmodesmata is $Jp = J/\alpha$, where α is the fraction of the interface occupied by plasmodesmatal pores. Jp can be characterized by an estimate of the apparent diffusion coefficient for this flux (Osmond, 1971). According to Tyree (1970) $\alpha = N\pi r^2$ where r is the effective pore radius. The interpretation of the desmotubulus modified endoplasmic reticulum (Robards, 1971) and the intimate contact between plasmalemma and desmotubulus at the entrance of the plasmodesmatal pore (Figs. 3–4) strongly suggest that the actual movements of ions through plasmodesmata are localized in the desmotubulus. This assumption is further emphasized by measurements of the electrical resistance of the plasmodesmata (Spanswick, 1972) which show that this resistance is considerably greater than the resistance calculated from the plasmodesmatal dimensions and frequency if they were completely open (*i.e.* ignoring the existence of the desmotubulus). The resistance being greater than expected is considered evidence for a restriction in the diffusion of ions via the plasmodesmata, which can possibly be ascribed to the existence of the desmotubulus. In *Salsola*, the radius of the total desmotubulus is 7.5 nm, but in these delicate structures, subjected to artifacts of fixation and staining, it is a problematic task to estimate the thickness of the desmotubulus wall. However, a liberal estimate of the effective pore radius is 5 nm (see also Clarkson *et al.*, 1971) implying $\alpha = 1.1 \times 10^{-3}$.

Assuming that α was of the order 10^{-1}–10^{-2} Osmond (1971) calculated an apparent diffusion coefficient of the same order as those for diffusion of inorganic anions through cell wall free space, and suggested that diffusion in the symplasm



Figs. 1—6. Scanning (1, 5, 6) and transmission (2–4) electron micrographs of the mesophyll-bundle sheath interface. Fig. 1. Cross-section of leaf showing the different cells in the epidermis (*E*), mesophyll (*M*), sheath (*S*), and water-storage tissue (*W*). Fig. 2. Plasmodesmata in the interface; note median nodules and anastomoses (*A*). Fig. 3. Slightly oblique sections of young plasmodesmata. Fig. 4. Tangential section of pit field showing anastomoses (*A*) and intimate contact between plasmalemma and desmotubulus at the entrance of the plasmodesmatal pore (arrow). Fig. 5. The interface with numerous pit fields seen from the bundle sheath cells. Fig. 6. Details of the pit fields in Fig. 5; the small protrusions represent the plasmodesmata

suffices to explain the rapid movement of C_4-acids across the mesophyll-bundle sheath interface. This suggestion is questioned by the fact that in *Salsola* only a fraction of 1.1×10^{-3} of this interface is occupied by plasmodesmatal pores. In Osmond's example this value of α results in an apparent diffusion coefficient 10–10^2 times greater than was previously thought. Furthermore, compared with inorganic anions the larger molecules of the organic anions of the C_4-acids tend to lower the actual diffusion coefficient for these ions. However, the modification of Fick's law used by Osmond does not take into account that the movement of C_4-acids is restricted to the plasmodesmatal pores across the wall only, not along the whole mean distance from mesophyll to bundle sheath cells. Presumably this would mean a decrease in the apparent diffusion coefficient.

These contradictory facts do not justify theories as to whether the plasmodesmata are capable of specialized transport functions or not. In order to gain a better understanding of the mechanisms of transport of organic anions in C_4-photosynthesis measurements of electrical potentials in the mesophyll and bundle sheath cells must be obtained.

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