Quantification of symplastic continuity as visualised by plasmodesmograms: diagnostic value for phloem-loading pathways

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Received 24 April; accepted 7 November 1991

Abstract. The use of plasmodesmatal frequency to correlate cell–cell symplastic transport capacity remains a contentious problem, as variation in cell shape, accurate determination of interface contact area between cell types, distribution (i.e. whether random or aggregated) and shape (i.e. whether single or branched), and state of permeability may confuse the issue. Additionally, variation in the methods used to determine the frequencies compounds the problem further. Data presented in this paper show that plasmodesmograms offer a means to visualise the potential transport pathway from mesophyll cells to sieve tubes. Furthermore, the results allow an instant appreciation of symplastic continuity or discontinuity and, accordingly, the potential symplastic and/or apoplastic stages involved in the overall loading process.

Key words: C3, C4 plants – Phloem loading (apoplastic, symplastic) – Plasmodesmatal frequencies – Plasmodesmogram

Introduction

Understanding of how assimilates produced in the mesophyll of angiospermous leaves are loaded into the phloem requires detailed studies of plasmodesmatal frequencies in the loading zone (see van Bel et al. 1988; Botha 1990; Fisher 1990a and references therein). It is assumed that the analogy here is that the greater the number (frequency) of plasmodesmata at a given interface, the greater is the potential for symplastic transport through that interface. It is therefore crucial that plasmodesmata at the various interfaces along the assumed pathway for assimilate loading are counted before the relative role(s) of symplastic and/or apoplastic transport can be meaningfully addressed. The result should be the production of a set of data which gives an accurate indication of both the number and frequency of plasmodesmata at the relevant interfaces. Unfortunately, as stated by Fisher (1990a, b) several differing methods have been used by workers active in this field to determine actual frequencies. Whilst some degree of overlap between the various methods exists, no single system or method is universally accepted or used in frequency studies.

Frequency studies are usually presented in tabular form. Tabulated data require careful study and descriptive analysis. In many instances frequency data do not give a clear indication of the relative importance of the interfaces along the route from mesophyll to functional sieve tubes. Attempts have recently been made to address this singular problem, and to present the results, graphically. Russin and Evert (1985b), using cell–cell frequencies in diagrammatic form, showed the assimilate-loading area in Populus deltoides. Subsequently, pictorial classification (pictograms) of minor veins (Gamalei 1985), and more recently, ‘plasmodesmograms’ (van Bel et al. 1988) have been used. Each of the above has an advantage in that the cell–cell symplastic and/or apoplastic route and phloem-loading pathway can be instantly appreciated.

The structural parameter necessary to facilitate answers (at least in part) to some of these questions clearly remains the determination of plasmodesmatal frequency at the diverse cell interfaces along the route from the mesophyll cells to the phloem tissue. Plasmodesmograms are thus pertinent to studies on the role of plasmodesmata in phloem loading and intercellular transport.

Notwithstanding the inherent problems associated with frequency expressed as either plasmodesmata/µm cell-wall interface, or plasmodesmata/µm vein, comparative plasmodesmograms constructed from data in which plasmodesmatal frequency is calculated on the basis of plasmodesmata/µm cell-wall interface or plasmodesmata/µm vein, each expressed as a percentage of the total number of plasmodesmata, may be the best form of visualization and may serve as the basis for useful comparisons. This paper serves to illustrate several points. First, that it is essential that workers agree and “standardise” cell and related cell-interface terminology. Second, where interface plasmodesmatal frequencies are
expressed as a percentage of the total number of plasmodesmata, that the resultant plasmodesmograms differ little for the most part. Third, that plasmodesmograms permit easy visualization of the loading pathway from mesophyll to sieve tube. Finally, that plasmodesmograms may be useful adjuncts to experiments aimed at determining the phloem-loading pathway.

Materials and methods

Plasmodesmograms for *Eragrostis plana* Nees, *Panicum maximum* Jacq., *Themeda triandra* var. *imberbis* (Retz.) A. Camus and *Bromus unioloides* H.B.K. were constructed based on either plasmodesmata/μm cell-wall interface, or on plasmodesmata/μm vein. The latter method uses as the basis for calculations the formula in the paper by Robards (1976) (see also the discussion by Gunning in Robards 1976; and Fisher 1990a for further discussion), which considers the plasmodesmal frequency (expressed as plasmodesmata/μm cell-wall interface) the section thickness (in nm) the average plasmodesmal radius (in nm) and the total interface length (in μm). The plasmodesmogram for *Commelina benghalensis* L. was redrawn from van Bel et al. (1988); those for *Amaranthus retroflexus* L., *Cananga odorata* (Lam.) Hook. f. et Thoms., *Coleus blumei* Benth., *Commelina benghalensis* and *Sonchus oleraceus* L. were drawn from data presented by Fisher (1990a, b); and that for *Spinacia oleracea* L. cv. Bloomsdale Dark Green from data by Warmbrodt and van der Woude (1990). Plasmodesmograms, comparing plasmodesmal distribution as percent plasmodesmata/μm cell-wall interface (which reflects the number of plasmodesmata, the number of like interfaces, the number of sections, as well as the actual interface contact area) and as percent plasmodesmata/μm vein were drawn for *Eragrostis plana*, *Panicum maximum*, *Themeda triandra* and *Bromus unioloides* from data previously calculated by Botha (1990). Solid lines in the accompanying plasmodesmograms represent a distribution of at least 1%, and dashed lines < 1% of the total plasmodesmata. To avoid the confusion of massed lines in confined spaces, numbers in circles depict the actual plasmodesmal percentage, at the interfaces of interest.

Results and discussion

Comparison of calculated plasmodesmal frequencies and resultant plasmodesmograms. Figures 1 and 2 show the difference between the plasmodesmograms based on computations and interpretation of the plasmodesmal frequencies in *Commelina benghalensis* (Fig. 1: van Bel et al. 1988; Fig. 2: Fisher 1990a); labelling is as per the original data. Although the authors have used different terminologies to describe the gross cell–cell pathway from the mesophyll to the functional phloem sieve tubes, they all recognise and differentiate between connections at the vascular parenchyma–protophloem and vascular parenchyma–metaphloem–sieve tube interfaces. However, Fig. 1 (van Bel et al. 1988) shows similar numbers of plasmodesmata (and implied from this, similar frequencies) between vascular parenchyma cells (VP) and metaphloem sieve tubes (MST), and between companion cells (CC) and protophloem sieve tubes (PST). Both sets of data, however, show near-total symplastic isolation of the CC–MST complex.

The plasmodesmogram derived from van Bel et al. (1988) shows sheath-cell–sheath-cell (SC–SC) and VP–VP connections, whilst these lateral connections are not evident in the Fisher (1990a) data. The plasmodesmogram drawn from Fisher (1990a) emphasises the importance of the VP→metaphloem route in assimilate transport, in that 12% of the total number of plasmodesmata (based on percent plasmodesmata/μm vein) occur at this interface. The dashed lines in the Fisher-based plasmodesmogram indicate a very low level of symplastic continuity at the bundle-sheath–companion-cell (BS–CC) and VP–CC interfaces, where less than 1% of the total plasmodesmata (as plasmodesmata/μm vein) occur at the interfaces in question. Both plas-