Plant Physiol. (1997) 115: 1599–1607

Phloem-Specific Expression of the Tobacco Mosaic Virus Movement Protein Alters Carbon Metabolism and Partitioning in Transgenic Potato Plants[1]

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The tobacco mosaic virus movement protein (TMV-MP) has pleiotropic effects when expressed in transgenic tobacco (*Nicotiana tabacum*) plants. In addition to its ability to increase the plasmodesmal size-exclusion limit, the TMV-MP alters carbohydrate metabolism in source leaves and dry matter partitioning between the various plant organs. In the present study the TMV-MP was expressed under the control of a phloem-specific promoter (rolC), and this system was employed to further explore the potential sites at which the TMV-MP exerts its influence over carbon metabolism and transport in transgenic potato (*Solanum tuberosum*) plants. Immunohistochemical analyses indicated that the TMV-MP was localized mainly to phloem parenchyma and companion cells. Starch and sucrose accumulated in source leaves of these plants to significantly higher levels compared with control potato lines. In addition, the rate of sucrose efflux from excised petioles was lower compared with control plants. Furthermore, under short-day conditions, carbon partitioning was lower to the roots and higher to tubers in rolC plants compared with controls. These results are discussed in terms of the mode(s) by which the TMV-MP exerts its influence over carbon metabolism and photoassimilate translocation.

Functional MPs are essential for the cell-to-cell movement of plant viruses (Maule, 1991; Lucas and Gilbertson, 1994). Evidence accumulated over recent years has established that these proteins have the capacity to alter plasmodesmal function (Wolf et al., 1989; Fujiwara et al., 1993; Noueiry et al., 1994; Ding et al., 1995). Dye-coupling studies performed on transgenic tobacco (*Nicotiana tabacum*) plants expressing the TMV-MP indicated that this protein can induce a significant increase in the SEL of plasmodesmata interconnecting both mesophyll and bundle-sheath cells. In control tobacco plants, plasmodesmal SEL is approximately 0.8 kD, whereas in the presence of the TMV-MP, fluorescent probes larger by a factor of more than 10 could move from cell to cell (Wolf et al., 1989; Ding et al., 1992; Waigmann et al., 1994).

Pursuant to the hypothesis that cell-to-cell movement of Suc from the site of synthesis to the site of phloem loading within the vascular bundle follows a symplasmic pathway, we have used the TMV-MP transgenic tobacco plants to study the role of plasmodesmal function in controlling sugar transport and photoassimilate partitioning between the various plant organs (Lucas et al., 1993; Balachandran et al., 1995; Olesinski et al., 1995). Pulse-chase experiments following $^{14}CO_2$ labeling and analyses of the diurnal changes in carbohydrate levels indicated that constitutive expression of the TMV-MP (under control of the CaMV 35S promoter) results in inhibition of Suc export and the accumulation of carbohydrates in source leaves during the day. Moreover, in addition to its effects on the physiology of tobacco source leaves, the TMV-MP causes a significant reduction in dry matter partitioning to roots, yielding a lower root-to-shoot ratio in these transgenic tobacco plants (Balachandran et al., 1995).

It is important to note that analyses of transgenic plants expressing temperature-sensitive and C-terminal deletion mutant forms of the TMV-MP demonstrated that this protein exerts its effects on carbon allocation and dry matter partitioning via a mechanism that is independent of its ability to increase plasmodesmal SEL (Balachandran et al., 1995; Olesinski et al., 1995). Collectively, these studies established the pleiotropic nature of the TMV-MP in transgenic tobacco plants.

Further studies aimed at determining the site where the TMV-MP exerts its effects on the physiology of the plant included the generation of transgenic potato (*Solanum tuberosum*) plants expressing the TMV-MP under tissue-specific promoters. In this work it was demonstrated that starch and sugars accumulated in potato leaves to significantly lower levels compared with control leaves when the TMV-MP was expressed in green tissue only (under the control of the nuclear photosynthetic ST-LS1 gene pro-

[1] This work was supported by the U.S.-Israel Binational Agricultural Research Development Fund (grant no. IS-2385-94C) to S.W. and W.J.L. M.H. was supported by a scholarship from the Kinamon Foundation. This paper is a contribution from the Uri Kinamon Laboratory.

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Abbreviations: CaMV, cauliflower mosaic virus; CC-SE, companion cells-sieve element; FITC, fluorescein isothiocyanate; PFD, photon flux density; PPi, Pi phosphatase; SEL, size-exclusion limit; TMV-MP, tobacco mosaic virus movement protein.

moter). There was no effect on carbohydrate metabolism or partitioning of photoassimilates between the various plant organs when TMV-MP expression was restricted to tubers (Olesinski et al., 1996). These results, together with tobacco grafting experiments (Balachandran et al., 1995), identified the shoot as the site where the TMV-MP exerts its influence over the physiology of the plant.

A prerequisite for understanding the mechanism by which the TMV-MP interacts with endogenous factors to cause the observed changes in carbon allocation and transport would be an explicit identification of its site(s) of action. The boundary between the mesophyll and the vascular bundle is a potential control site for Suc transport. In potato plants Suc is thought to be released from the symplasm of the phloem parenchyma cells into the apoplasmic space, where it is then actively loaded into the CC-SE complex (Van Bel, 1993). As the TMV-MP alters carbohydrate metabolism in source leaves, it could be argued that it exerts its effect within the mesophyll, where Suc synthesis takes place. Alternatively, the TMV-MP may well interfere, directly or indirectly, with the loading of Suc into the CC-SE complex; in this situation its site of action would be within the phloem.

To verify the role of phloem cells in TMV-MP-mediated alteration in carbohydrate metabolism and Suc transport, we generated transgenic plants in which the TMV-MP was expressed under a phloem-specific (rolC) promoter (Schmulling et al., 1989; Sugaya et al., 1989). We demonstrate here that the TMV-MP can alter both carbohydrate metabolism in source leaves and partitioning of assimilates between the various plant organs, either when expressed only in mesophyll and bundle-sheath cells, or when expressed predominantly in phloem parenchyma and companion cells of transgenic potato plants. These findings are discussed in terms of the mode(s) by which the TMV-MP may exert its influence over carbon metabolism and photoassimilate translocation.

MATERIALS AND METHODS

Reagents, Enzymes, and Plasmids

DNA restriction enzymes were obtained from Boehringer Mannheim unless specified otherwise. Cefotaxime (Claforan) was purchased from Laboratoires Roussel (Paris, France). All other reagents were obtained from Sigma and Bio-Rad.

Potato Transformation, Regeneration, and Growth Conditions

Two cDNA clones encoding the TMV-MP, designated TMV-MP 7 and 11 (Olesinski et al., 1996), were inserted into a Bin19-derived binary vector (Bevan, 1984) containing the phloem-specific promoter rolC (Schmulling et al., 1989; Sugaya et al., 1989) and the octapine synthase polyadenylation signal. This vector (Fig. 1) was transformed into *Agrobacterium tumefaciens* strain LBA4404 (Hoekema et al., 1983). Tuber discs (3 mm in diameter) of potato (*Solanum tuberosum* cv Desiree) were co-cultivated with the recombinant *A. tumefaciens* cells. Culture conditions were similar to those previously described by Perl et al. (1991). Regenerated shoots were produced on Murashige-Skoog medium (Murashige and Skoog, 1962) supplemented with kanamycin (100 mg/L) and Claforan (500 mg/L), and the transformants were selected by rooting on Murashige-Skoog medium supplemented with kanamycin (100 mg/L) and Claforan (250 mg/L). Rooted transformants were grown under sterile conditions at 25°C with a PFD of 60 to 160 μmol m^{-2} s^{-1} and a 12-h photoperiod. Plants for regeneration, transformation analysis, and physiological experiments were transferred to soil mixture in plastic pots (15 cm in diameter) and grown in an insect-free, temperature-controlled greenhouse (25 ± 3°C day/16 ± 2°C night). Plants were grown under natural sunlight with an average midday PFD of 1200 to 1500 μmol m^{-2} s^{-1}.

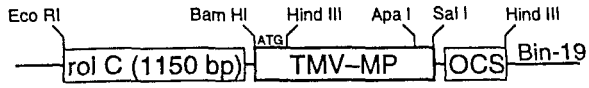

Figure 1. Structure of the chimeric binary plant expression cassettes (pBin-rolC) used for potato transformation. OCS, Octapine synthase.

Regenerated plantlets were analyzed for the presence of the transgene using detailed PCR analysis in which the correct orientation of the transgene and its promoter were verified (data not shown). Over 30 individual plantlets were identified to be transgenic for TMV-MP, and 12 of these were chosen for further analysis. RNA expression in the veins of mature leaves was detected by northern analysis. Each vein sample (50 mg) was denatured at 65°C for 5 min in 70% formamide, 6% formaldehyde, and 200 mg of Mops buffer, separated in 1.2% agarose gel containing 6.3% formaldehyde in 20 mM Mops buffer, pH 7.0. RNA was then transferred to a Nitran (Stratagene) membrane and hybridized to TMV-MP-specific, ^{32}P-radiolabeled probe (Fig. 2).

Tissue Fixation and Immunolabeling

Polyclonal antibodies raised against a synthetic TMV-MP peptide were prepared and found to specifically recognize the TMV-MP protein. Immunolabeling studies were performed on phloem-expressing TMV-MP plant lines C-7-303 and C-7-312. As controls, we used the vector control line C-301 and line L-7-203 (expressing the TMV-MP under the ST-LS1 promoter). In addition, tobacco (*Nicotiana tabacum*) plant line 277 (expressing the TMV-MP under the CaMV 35S promoter) was employed as a well-characterized positive control.

Plant tissues (1–2 mm in width) from leaf no. 6 (leaf no. 1 was defined as the last expanding leaf to achieve a laminar length of 5 cm) were excised and then fixed for 2 h in 0.5% (w/v) glutaraldehyde and 1.5% (w/v) paraformaldehyde in 50 mM PO_4 buffer, pH 7.0. Tissue was then transferred to 1% (w/v) glutaraldehyde and 3% (w/v) paraformaldehyde in 50 mM PO_4 buffer, pH 7.0, at 4°C. Plant material was dehydrated through a 20% step-graded ethanol series at 4°C, infiltrated over 2 d through a five-step graded series from 100% (v/v) ethanol to 100% (v/v)

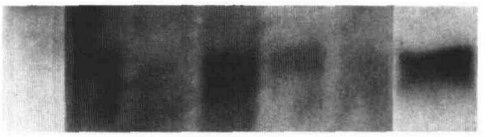

Figure 2. Northern analysis of TMV-MP RNA (20 μg per lane) in veins of transgenic potato plant lines C-7-303, C-7-308, C-7-304, C-11-311, C-11-312 (lanes 2–6, respectively), and the wild type, cv Desiree (lane 1). Tobacco plant line 277 expressing the TMV-MP under the CaMV 35S promoter served as a positive control (lane 7).

London White Resin (medium grade, Electron Microscopy Sciences, Fort Washington, PA), and then embedded in London Resin White. Blocks were polymerized by exposure to a temperature sequence of 37°C for 12 h, 48°C for 12 h, and 60°C for 24 h.

Ultrathin sections (60 nm) were cut with a diamond knife and collected on uncoated mesh grids or formvar film-coated slot grids. Sections were preincubated for 1 h in blocking solution (50 mM Tris-HCl, 150 mM NaCl, 0.1% [v/v] Tween 20, and 2% [w/v] BSA) and then transferred directly to 90 mL of the antisera solution. After four 15-min washes with blocking solution, the sections were incubated for 1 h in secondary antibody (15- or 20-nm diameter gold-conjugated goat anti-rabbit IgG) solution diluted 1:40 in antibody diluent. After several washing steps (two 15-min washes with blocking solution, followed by two 15-min washes with 50 mM Tris-HCl and 150 mM NaCl buffer), sections were stained with uranyl acetate (2% w/v) in distilled water and lead citrate for 10 min and then observed using an electron microscope (model EM410LS, Philips, Eindhoven, The Netherlands) operated at 80 kV.

To quantify the expression of the TMV-MP, the presence of gold label was analyzed in the various cell types of the different plant lines. Almost 1000 cells were examined and the results represent the mean of at least 40 cells for each cell type.

Microinjection Procedures

Lucifer yellow CH (molecular mass of 457 D) and FITC-labeled dextrans (molecular masses of 3.0 or 9.4 kD) were used in microinjection studies. Attached leaves were microinjected by the method described for tobacco leaves (Ding et al., 1992). Spongy mesophyll cells were impaled with a micropipette and pressure-injected with dye. Movement of dye into neighboring cells was monitored with an epifluorescence microscope (Orthoplan, Leitz, Wetzlar, Germany) equipped with a blue excitation filter connected to an image enhancement system (no. BP 390-490 and model C1966-20, respectively, Hamamatsu, Bridgewater, NJ).

Determination of Biomass Partitioning

Initial experiments were performed on second-generation potato plants grown in a temperature-controlled greenhouse (25/16°C day/night) under natural sunlight (13- to 14-h photoperiod); the average midday PFD was 1200 to 1500 μmol m^{-2} s^{-1}. Plants were harvested 57 d after planting and then separated into shoots (leaves plus stems), roots, and tubers, and dried in a 70°C oven for 72 to 96 h. A second set of experiments was conducted on third-generation potato plants that were grown under controlled environmental conditions (model PGV36, Conviron, Asheville, NC); 22/15°C day/night temperature, RH maintained at 70%, and light intensity (metal halide lamps) during the first and last hour of either an 8- or 16-h photoperiod was set at 100 to 150 μmol m^{-2} s^{-1}, whereas over the rest of the photoperiod it was set at 600 μmol m^{-2} s^{-1} (at canopy level).

Starch and Sugar Determinations

Tissue samples were collected from the youngest fully expanded leaf (no. 5 or 6, with leaf no. 1 being the last leaf to achieve a length of 5 cm) at 8 AM and 3 PM, and carbohydrate content was determined as described by Lucas et al. (1993). Soluble sugars were extracted from leaf discs in 80% ethanol. After evaporating the supernatant, sugars were redissolved in water and filtered through a 0.45-mm membrane HPLC filter (Whatman). Sugars were separated in an analytical HPLC system (LDC, Riviera Beach, FL) fitted with a Sugar-Pak I column (6.5 × 300 mm; Waters) using a refractive-index detector (LDC). Starch content was determined in the ethanol-water-extracted leaf discs following starch conversion by amyloglucosidase (Sigma). Starch content, as Glc equivalents, was determined using a quantitative Glc determination kit (HK, Sigma).

Suc Efflux from Source Leaves

Phloem exudate was collected from excised petioles of fully expanded potato leaves using the method described by Van Bel et al. (1994), with minor modifications. Experiments were conducted in a greenhouse under natural sunlight in the late morning hours. The youngest fully expanded leaves were detached under water by cutting petioles close to the stem. Excised petioles were immediately placed into a test tube containing 5 mL of 5 mM EDTA. Parafilm was used to seal the mouth of the test tube. Leaves were gently shaken in a water bath that was covered with a glass plate to maintain high RH. After a 3-h exudation period, leaf area was measured and the phloem exudate was lyophilized. Sugars were resuspended in 100 mL of distilled water and the Suc content was determined by HPLC.

RESULTS

Cellular Localization of the TMV-MP within Transgenic Potato Plants

Cellular localization of the TMV-MP was investigated in several transgenic potato lines using high-resolution immunohistochemical techniques. As indicated in Figure 3, TMV-MP was localized mainly to the phloem parenchyma and companion cells when the gene was expressed under

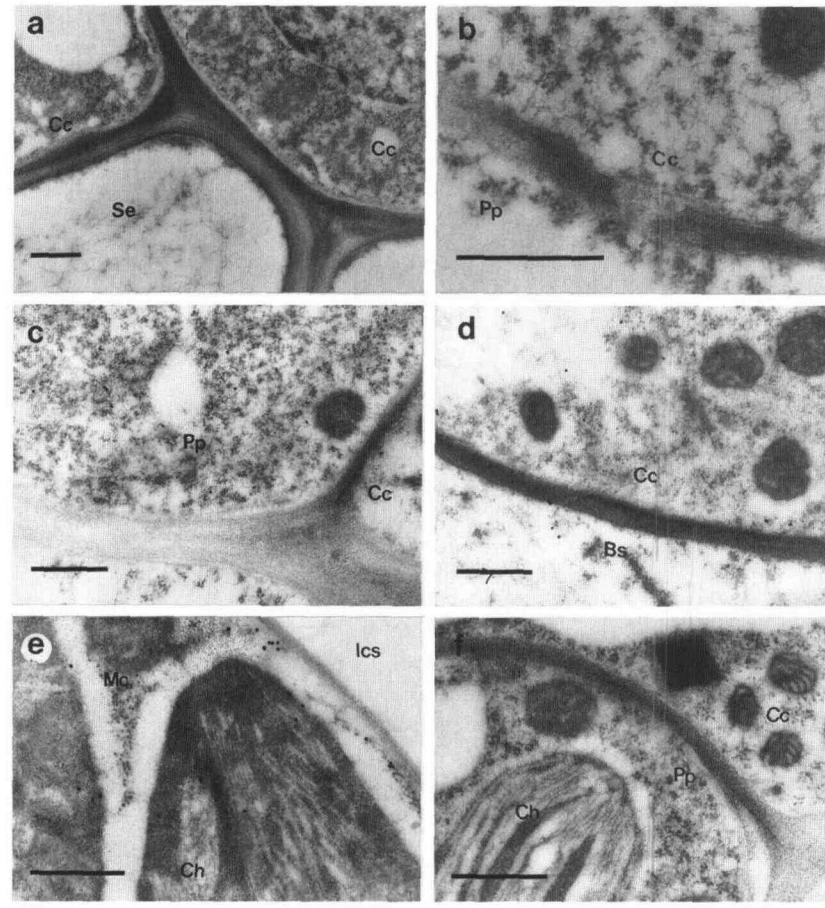

Figure 3. Immunogold labeling of TMV-MP in transgenic potato plants. A and B, Electron micrographs of sections cut from leaves of rolC plants immunogold labeled with preimmune sera. C and D, Sections cut from leaves of rolC plants immunogold labeled with TMV-MP antisera. A high level of labeling was localized in the phloem parenchyma and companion cells as compared with bundle-sheath cells. E and F, Sections cut from leaves of potato plant line L-7-203 (expressing the TMV-MP under the ST-LS1 promoter) and immunogold labeled with TMV-MP antisera. Label was rarely detected in phloem cells, whereas numerous gold particles were observed in mesophyll cells. Bs, Bundle sheath; Cc, companion cells; Ch, chloroplast; Ics, intercellular space; Mc, mesophyll cell; Pp, phloem parenchyma; and Se, sieve element. Bar = 500 nm.

the rolC promoter (see also Table I). Label was also detected in bundle-sheath cells (Fig. 3d) at a level significantly above that of background, which was established using vector control plants (line C-301) and preimmune controls (Fig. 3, a and b). Label was also detected in mesophyll cells of these plants, however, the level was not statistically different from those detected in the vector control line (Table I). Most of the TMV-MP detected by immunogold labeling in the rolC plants was localized to the cytoplasm (Fig. 3, c and d).

When the TMV-MP was expressed under the control of the promoter from the nuclear photosynthesis gene (ST-LS1), the amount of label in mesophyll cells was almost six times higher compared with the values obtained for the same cells in rolC plants (Table I; Fig. 3h). Label in bundle-sheath cells was also highest in this plant line, whereas the degree of labeling in companion cells was not significantly different from the background level established using vector control plants (Fig. 3f; Table I). A relatively low level of label was detected in phloem parenchyma cells of the

Table I. *Statistical analysis of immunogold labeling of TMV-MP in different cell types of transgenic potato plants expressing the TMV-MP under the rolC (C-7-303, C-11-312) or the ST-LS1 (L-7-203) promoter*

Tobacco plants (line 277) expressing the TMV-MP under the CaMV 35S promoter and empty-cassette-transformed potato plants (vector control line C-301) served as controls for these experiments. Data are presented as the number of gold particles detected per 20 μm^2. Values followed by the same letter within a column are not statistically different at the 5% level using Duncan's multiple comparison test.

Plant Line (Promoter)	Cell Type				
	Mesophyll	Bundle sheath	Phloem parenchyma	Companion cell	Sieve element
C-301 (vector control)	4 c	4 c	0.4c	4 c	3 a
C-7-303 (rolC)	11 c	16 b	59 a	83 a	4 a
C-11-312 (rolC)	16 c	18 b	71 a	93 a	6 a
L-7-203 (ST-LS1)	76 a	26 a	15 b	22 c	4 a
277 (CaMV 35S)	58 b	19 b	17 b	47 b	5 a

Table II. *Mobility of fluorescent probes through the symplasmic pathway of leaf mesophyll of transformed potato plants expressing the TMV-MP under the rolC (C-7-303, C-11-312) or the ST-LS1 (L-7-203) promoters or a vector control line (C-301)*

Data are presented as the number of injections showing movement of the specified probe (values in parentheses represent total number of injections).

Plant Line	LYCH[a]	FITC-Dextran	
		3 kD	9.4 kD
C-301	9 (9)	0 (9)	0 (8)
C-7-303 and C-11-312	7 (7)	2 (7)	1 (8)
L-7-203	3 (3)	6 (6)	11 (14)

[a] LYCH, Lucifer yellow CH.

ST-LS1 plant line, with the value being significantly lower than that detected in rolC plants, but significantly higher than that present in the vector control line (Table I).

Expression of the TMV-MP under the control of the ST-LS1 promoter resulted in an increase in mesophyll plasmodesmal SEL in transgenic potato plants (Olesinski et al., 1996). In the present study dye-coupling experiments established that 9.4-kD FITC-dextran did not move out of the injected mesophyll cells (Table II). Furthermore, only two out of seven injections indicated movement of 3-kD FITC-dextran compared with 100% movement of this probe in plant line L-7-203.

Phloem-Specific Expression of TMV-MP Alters Dry Weight Partitioning

When grown for 57 d under greenhouse conditions, the average shoot (leaves plus stems) dry weight for rolC transgenic potato plants was 25% higher compared with values obtained for vector control plants (Table III). In contrast, the average root dry weight of rolC plants was 27% lower compared with control plants. As a result of this TMV-MP-induced alteration in biomass partitioning, the root-to-shoot ratio in the rolC lines was 40% lower compared with that of vector control plants.

TMV-MP-mediated effects on growth pattern, photoassimilate partitioning, and carbohydrate allocation in potato plants expressing the gene under the ST-LS1 promoter was evident only when plants had undergone tuber initiation (Olesinski et al., 1996). To determine if this was the case when the TMV-MP was expressed in the phloem, potato plants were next grown in controlled environment chambers under an 8- or 16-h photoperiod. When grown under a short photoperiod (8 h), the root-to-(shoot plus tubers) ratio was significantly lower in three out of six rolC lines tested, as compared with control potato lines (Fig. 4). The remaining rolC lines had root-to-(shoot plus tubers) ratios that were similar to control values (Fig. 4a). The mean ratio for all rolC plants was 0.04 compared with a mean value of 0.06 for control lines. All six rolC lines tested in this experiment exhibited higher dry matter partitioning to the tubers (harvest index) as compared with control lines (Fig. 4b). The average value of percent dry matter partitioned to the tubers (out of the total dry weight) was 53% for the rolC plants, which was significantly higher ($P = 0.01$) than the average value obtained for the controls (41%).

No differences in dry weight partitioning, either to the roots or tubers, were found between rolC and vector control lines when plants were grown under long photoperiodic conditions (Fig. 5). It is important to note that for all plant lines grown under long-day conditions, tubers were initiated but partitioning to these organs was significantly reduced compared with plants grown under short-day conditions.

Table III. *Growth analysis of 57-d-old transgenic potato plants expressing the TMV-MP under the rolC promoter (lines C-7- and C-11-) compared with vector control plants (line C-301)*

Plants were grown in a greenhouse under natural sunlight (13- to 14-h photoperiod) with an average midday PFD of 1200 to 1500 μmol m^{-2} s^{-1}. Values represent the mean ± SE with five plants of each line being used in each experiment.

Plant Line	Dry Wt			Root:Shoot Ratio
	Shoot	Root	Tuber	
		g^{-1} *plant organ*		
C-301	4.8 ± 0.6	0.75 ± 0.20	8.4 ± 1.1	0.15 ± 0.03
C-7-301	5.5 ± 0.4	0.48 ± 0.12	10.4 ± 0.7	0.09 ± 0.02
C-7-303	6.3 ± 1.2	0.37 ± 0.06	9.5 ± 1.3	0.06 ± 0.01
C-7-304	4.2 ± 0.5	0.59 ± 0.15	6.5 ± 0.9	0.14 ± 0.03
C-7-308	6.4 ± 0.5	0.58 ± 0.06	8.9 ± 0.9	0.09 ± 0.01
C-7-315	7.2 ± 0.7	0.52 ± 0.10	9.7 ± 0.9	0.07 ± 0.01
C-11-307	6.1 ± 0.6	0.54 ± 0.26	11.0 ± 0.9	0.09 ± 0.04
C-11-308	5.7 ± 0.5	0.62 ± 0.24	8.9 ± 1.0	0.11 ± 0.04
C-11-311	6.5 ± 0.5	0.66 ± 0.13	8.8 ± 1.0	0.09 ± 0.02
C-11-312	5.5 ± 0.7	0.58 ± 0.29	14.1 ± 3.9	0.12 ± 0.08
C-11-315	5.5 ± 0.3	0.74 ± 0.16	10.4 ± 0.9	0.14 ± 0.03
C-11-316	6.6 ± 0.8	0.33 ± 0.07	10.4 ± 1.1	0.05 ± 0.01
rolC (average)[a]	6.0 ± 0.8	0.55 ± 0.12	9.9 ± 1.8	0.09 ± 0.03

[a] Data average for all 12 plant lines used in the study.

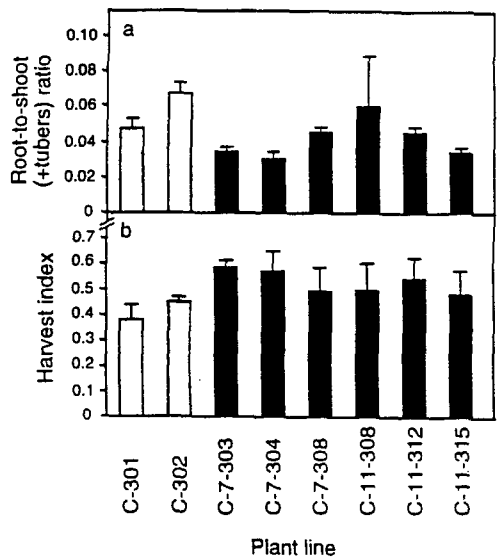

Figure 4. Root-to-(shoot plus tubers) ratio (a) and harvest index (percent of dry matter partitioned to the tubers) (b) of 56-d-old TMV-MP (C-7 and C-11) and control (C-301 and C-302) potato plants. For these experiments, plants were grown under controlled environmental conditions; 22/15°C day/night temperatures and an 8-h photoperiod. Values represent the mean ± SE; three to five plants were used for each line.

Phloem-Specific Expression of TMV-MP Alters Carbohydrate Metabolism and Export from Source Leaves

Our earlier studies with transgenic tobacco and potato plants expressing the TMV-MP indicated an alteration in carbohydrate metabolism concomitant with the detected change in biomass partitioning (Lucas et al., 1993; Balachandran et al., 1995; Olesinski et al., 1995, 1996). Daytime changes in carbohydrate levels of young, fully mature potato leaves were analyzed 52 d after planting (Fig. 6). No differences in Glc or Fru levels were detected in transgenic versus control leaves over the sampling period; e.g. in the afternoon, average Glc and Fru values were 24 and 75 μg cm^{-2}, respectively, for rolC plants, with the respective values for control plants being 26 and 75 μg cm^{-2}. Suc levels were similar in the morning, but underwent a significant increase in the afternoon compared with values obtained for the control plants. The average Suc level in the transgenic lines was 191 μg cm^{-2} compared with 145 μg cm^{-2} in the control plants (significant at P = 0.05).

The most pronounced effect of TMV-MP expression in the phloem of transgenic potato plants was reflected by changes in starch metabolism. All transgenic lines contained higher levels of starch in source leaves at the end of the dark period, with an average value of 871 μg cm^{-2} compared with 420 μg cm^{-2} in the control plants. A similar increase in starch accumulation occurred within source leaves during the photoperiod (about 40% for all plant lines), resulting in starch levels being higher (again, by a factor of two) in the transgenic plants compared with either of the controls (wild-type cv Desiree or vector control line C-301). Statistical analysis of the differences in starch levels in the afternoon between the tested lines indicated significantly higher levels (P = 0.05) in the rolC plants. The average value for rolC plants was 1195 μg cm^{-2} compared with 595 μg cm^{-2} for control plants.

Analysis of total carbohydrates (starch plus sugars) in the afternoon indicated that the values for the different rolC plant lines were 30 to 100% higher compared with either control line (Fig. 7).

As no significant differences in either photosynthetic rate or dark respiration were observed between the various lines tested (data not shown), it was logical to assume that the daytime accumulation of carbohydrates in the TMV-MP-expressing plant was due to an inhibition of Suc export from source leaves. An in vitro experimental system was employed to study Suc efflux from petioles of transgenic and control plants (Van Bel et al., 1994). The rate of Suc efflux from detached leaves of both control lines (cv Desiree and C-301) was significantly higher compared with the transgenic lines expressing the TMV-MP (Fig. 8). These results provide indirect evidence that the TMV-MP can influence the processes involved in phloem loading.

DISCUSSION

In the present study we demonstrate that expression of TMV-MP in the phloem of transgenic potato plants can influence the processes involved in Suc loading into the phloem and carbon partitioning to various plant organs. These findings are consistent with earlier studies in which we established that the TMV-MP can alter carbohydrate metabolism in source leaves and resource partitioning to the lower stems and roots (Lucas et al., 1993, 1996; Wolf and Lucas, 1994; Balachandran et al., 1995; Olesinski et al.,

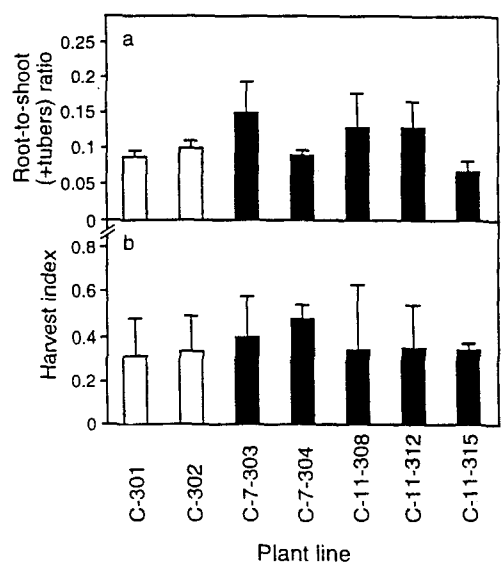

Figure 5. Root-to-(shoot plus tubers) ratio (a) and harvest index (percent of dry matter partitioned to the tubers) (b) of 56-d-old TMV-MP (C-7 and C-11) and control (C-301 and C-302) potato plants. Plants were grown under controlled environmental conditions; 22/15°C day/night temperatures and a 16-h photoperiod. Values represent the mean ± SE; three to five plants were used for each line.

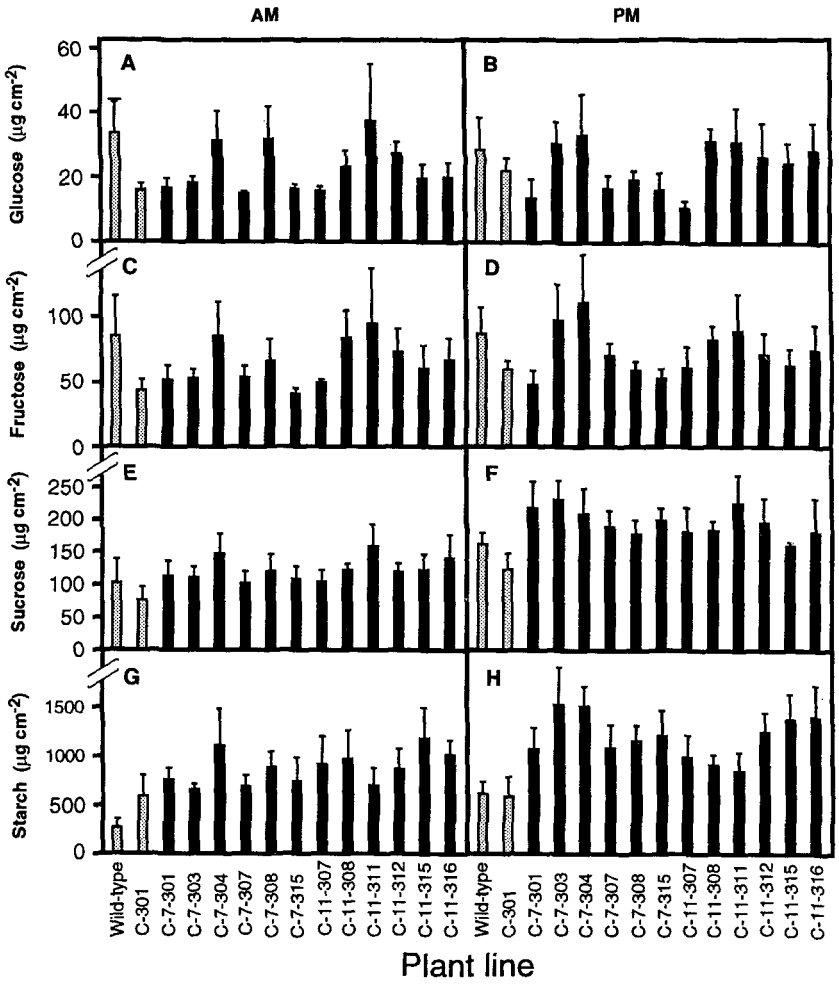

Figure 6. Morning (A, C, E, and G) and afternoon (B, D, F, and H) carbohydrate levels measured in leaves of transgenic potato plants expressing the TMV-MP under the rolC promoter, as well as in wild-type (untransformed) and vector control potato plants. Plants were grown for 52 d under greenhouse conditions of 25/16°C day/night temperatures and natural sunlight (13- to 14-h photoperiod and average midday PFD of 1200–1500 μmol m^{-2} s^{-1}). Leaf discs were analyzed for Glc (A and B), Fru (C and D), Suc (E and F), and starch (G and H) levels. Values represent the mean ± SE of six plants.

1995, 1996). Our present results further support the hypothesis that the leaf is the site where the TMV-MP exerts these pleiotropic effects.

Immunohistochemical studies performed on transgenic potato plants in which *TMV-MP* expression was regulated by the ST-LS1 promoter established that the MP was present mainly in mesophyll and bundle-sheath cells of source leaves (Table I). When restricted to these photosynthetic tissues, the TMV-MP induced a reduction in the levels of sugar and starch compared with control potato lines (Olesinski et al., 1996). A significant alteration in accumulation of Suc and starch was also observed in source leaves of rolC-TMV-MP transgenic potato plants; however, in these plants Suc and starch accumulated to higher levels (Fig. 6), whereas the rate of Suc efflux from excised petioles was lower than that observed with control plants (Fig. 8). The significant differences in the localization site of the TMV-MP between the ST-LS1 and rolC plants, together with the contrasting carbon metabolism in source leaves, indicate that the specific influence of the TMV-MP on sugar transport and carbohydrate partitioning in the rolC plants is likely due to TMV-MP expression within the phloem parenchyma and companion cells.

It is important to note that expression of the *GUS* gene in transgenic tobacco plants, which is under the control of the rolC promoter, resulted in confinement of GUS expression to phloem tissue (Schmulling et al., 1989; Sugaya et al., 1989). Unfortunately, in these studies the specific cells in which GUS was present were not identified. Immunolocalization of the TMV-MP in the rolC transgenic potato plants indicated that the protein was present predominantly in the phloem parenchyma and companion cells (Fig. 3; Table I). However, immunogold label above the background level was detected in bundle-sheath and mesophyll cells. This finding can be explained on the basis of either limited tissue-specificity of the promoter or cell-to-cell movement of the TMV-MP from the site of synthesis to neighboring tissues. Here it is important to note that immunocytochemical studies performed on transgenic tobacco plants expressing a foreign PPi under the control of the rolC promoter indicated that the protein was restricted to the phloem of the inner and outer midrib and the minor veins (Lerchl et al., 1995).

Based on these results, together with the finding that the TMV-MP has the ability to move between mesophyll cells (Waigmann et al., 1994; Nguyen et al., 1996), it would appear reasonable to assume that immunodetection of TMV-MP in bundle-sheath cells of rolC-TMV-MP potato plants is a consequence of its capacity to traffic through the plasmodesmata that connect the CC-PP to these cells.

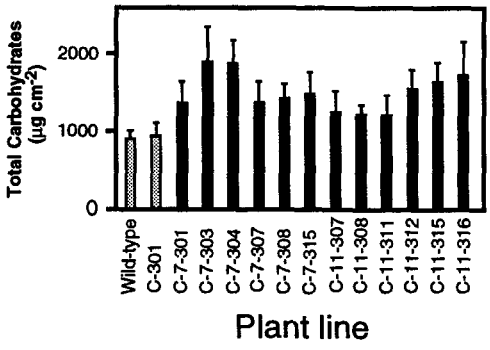

Figure 7. Afternoon total carbohydrate levels measured in leaves of transgenic potato plants expressing the TMV-MP under the rolC promoter, as well as in wild-type (untransformed) and vector control potato plants. Plants were grown for 52 d under greenhouse conditions of 25/16°C day/night temperatures and natural sunlight (13- to 14-h photoperiod and average midday PFD of 1200–1500 μmol m^{-2} s^{-1}). Values represent the mean ± SE of six plants.

The relationship between the presence of the TMV-MP in specific tissues within source leaves and its established pleiotropic effects on carbon metabolism and resource partitioning remains to be elucidated. In this regard, it is generally accepted that transport of Suc from the mesophyll to the region of the vascular bundle follows a symplasmic pathway. A recent study on a maize mutant demonstrated that an aberrant plasmodesmal structure at the bundle sheath-vascular parenchyma interface resulted in an inhibition of Suc export and carbohydrate accumulation in source leaves (Russin et al., 1996). This study established the prominent role of plasmodesmata in symplasmic transport of photoassimilates (Stitt, 1996).

Expression of Suc synthase in the phloem of potato (Yang and Russell, 1990), Arabidopsis (Martin et al., 1993), and companion cells of maize (Nolte and Koch, 1993), together with the finding that PPi in phloem sap of *Ricinus communis* is catalyzed by Suc synthase (Geigenberger et al., 1993), provided the basis for the hypothesis that PPi is required for phloem metabolism. A direct approach to study the role of PPi in phloem metabolism included the production of transgenic tobacco plants in which the *Escherichia coli* PPi was expressed in the phloem only (Lerchl et al., 1995). Deletion of PPi from the phloem of these plants resulted in accumulation of photoassimilates in source leaves, a decrease in the ATP/ADP ratio and respiration rate in the midribs, and an inhibition of export rate (Lerchl et al., 1995; Geigenberger et al., 1996). These results suggest that energy limitation due to shortage of PPi may inhibit the process of Suc loading into the CC-SE complex and reduce the rate of export from source leaves.

Since accumulation of carbohydrates and reduction in export rate from source leaves were also evident in the transgenic potato plants expressing the TMV-MP in the phloem (Figs. 7 and 8), it is possible that energy limitation may be responsible, in part, for these effects in transgenic potato plants expressing the TMV-MP under the control of the rolC promoter. This hypothesis cannot explain the effect of the TMV-MP on resource allocation nor can it provide a satisfactory explanation for the developmental control over the effects exerted by the TMV-MP (Lucas et al., 1996). In any event, we are presently examining the metabolic status of phloem cells in lines expressing the TMV-MP under the different tissue-specific promoters.

We recently advanced a hypothesis that trafficking of regulatory (information) molecules through plasmodesmata may establish a special supracellular communication network between the companion and mesophyll cells, which operates to regulate carbon partitioning (Balachandran et al., 1995; Lucas et al., 1996; Wolf and Lucas, 1997). According to this model, the supracellular communication system facilitates efficient orchestration of the network of biochemical and physiological processes involved in photosynthate metabolism and transport. The TMV-MP may interfere at several loci within this putative communication network. The presence of a high level of TMV-MP within companion cells (or phloem parenchyma cells) may interfere with the trafficking of endogenous signaling molecules (into or out of the phloem) and thus induce an alteration in carbon metabolism within the source leaf.

Photoassimilate partitioning and carbohydrate metabolism in ST-LS1-TMV-MP source leaves was altered only after the potato plants had undergone tuber initiation (Olesinski et al., 1996). Similar developmental control over TMV-MP-mediated effects on resource partitioning was observed in rolC potato plants (Figs. 4 and 5). Clearly, tuber development imposes a higher demand for photoassimilates. If this demand is regulated by long-distance transport of information molecules, the TMV-MP that is localized to either the phloem or mesophyll cells appears to be capable of interfering with this trafficking to alter the endogenous control mechanism for photosynthate partitioning only at this specific stage of plant development. Recent findings on transgenic tomato plants overexpressing Suc-PO_4 synthase (Micallef et al., 1995) and NO_3^--reductase-deficient tobacco mutants (Scheible et al., 1997)

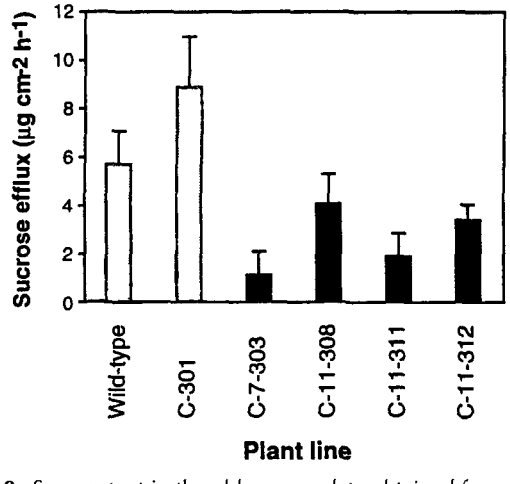

Figure 8. Suc content in the phloem exudate obtained from excised petioles of potato plants. Leaves were cut from 6-week-old plant lines C-7, C-11, C-301, and wild-type cv Desiree that had been grown under greenhouse conditions of 25/16°C day/night temperatures and natural sunlight (13- to 14-h photoperiod and average midday PFD of 1200–1500 μmol m^{-2} s^{-1}). Values represent the mean ± SE of five plants.

provided further support for the concept that long-distance signals are involved in the regulation of resource partitioning.

Elucidation of the mechanism(s) by which the TMV-MP affects this putative endogenous control system involved in regulating photosynthate transport in the phloem may open many biotechnological avenues for the manipulation of plant function. Future studies will focus on identifying the components of this endogenous control system.

ACKNOWLEDGMENT

We thank Lothar Willmitzer for generously providing the rolC promoter used in this study.

Received May 8, 1997; accepted August 29, 1997.
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LITERATURE CITED

Balachandran S, Hull R, Vaadia Y, Wolf S, Lucas WJ (1995) Alteration in carbon partitioning induced by the movement protein of tobacco mosaic virus originates in the mesophyll and is independent of change in plasmodesmal size exclusion limit. Plant Cell Environ **18:** 1301–1310

Bevan M (1984) Binary agrobacterium vectors for plant transformation. Nucleic Acids Res **12:** 8711–8721

Ding B, Haudenshield JS, Hull RJ, Wolf S, Beachy RN, Lucas WJ (1992) Secondary plasmodesmata are specific sites of localization of the tobacco mosaic virus movement protein in transgenic tobacco plants. Plant Cell **4:** 915–928

Ding B, Li Q, Nguyen L, Palukaitis P, Lucas WJ (1995) Cucumber mosaic virus 3a protein potentiates cell-to-cell trafficking of CMV-RNA in tobacco plants. Virology **207:** 345–353

Fujiwara T, Giesman-Cookmeyer D, Ding B, Lommel SA, Lucas WJ (1993) Cell-to-cell trafficking of macromolecules through plasmodesmata potentiated by the red clover necrotic mosaic virus movement protein. Plant Cell **5:** 1783–1794

Geigenberger P, Langenberger S, Wilke I, Heineke D, Heldt HW, Stitt M (1993) Sucrose is metabolised by sucrose synthase and glycolysis within the phloem complex of *Ricinus communis* L. seedlings. Planta **190:** 446–453

Geigenberger P, Lerchl J, Stitt M, Sonnewald U (1996) Phloem-specific expression of pyrophosphatase inhibits long-distance transport of carbohydrates and amino acids in tobacco plants. Plant Cell Environ **19:** 43–55

Hoekema A, Hirsh PR, Hooykaas PJ, Schilperoort RA (1983) A binary plant vector strategy based on separation of the *vir* and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature **303:** 179–180

Lerchl J, Geigenberger P, Stitt M, Sonnewald U (1995) Impaired photoassimilate partitioning caused by phloem-specific removal of pyrophosphate can be complemented by a phloem-specific cytosolic yeast-derived invertase in transgenic plants. Plant Cell **7:** 259–270

Lucas WJ, Balachandran S, Park J, Wolf S (1996) Plasmodesmal companion cell-mesophyll communication in the control over carbon metabolism and phloem transport: insight gained from viral movement proteins. J Exp Bot **47:** 1119–1128

Lucas WJ, Gilbertson RL (1994) Plasmodesmata in relation to viral movement within leaf tissues. Annu Rev Phytopathol **32:** 387–411

Lucas WJ, Olesinski AA, Hull RJ, Haudenshield J, Deom CM, Beachy RN, Wolf S (1993) Influence of the tobacco mosaic virus 30-kDa movement protein on carbon metabolism and photosynthate partitioning in transgenic tobacco plants. Planta **19:** 88–96

Martin T, Frommer WB, Salanoubat M, Willmitzer L (1993) Expression of an *Arabidopsis* sucrose synthase gene indicates a role in metabolization of sucrose both during phloem loading and in sink organs. Plant J **4:** 367–377

Maule AJ (1991) Virus movement in infected plants. Crit Rev Plant Sci **9:** 457–473

Micallef BJ, Haskins KA, Vanderveer PJ, Roh K-S, Shewmaker CK, Sharkey TD (1995) Altered photosynthesis, flowering and fruiting in transgenic tomato plants that have an increased capacity for sucrose synthesis. Planta **196:** 327–334

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant **15:** 473–497

Nguyen L, Lucas WJ, Ding B, Zaitlin M (1996) Viral RNA trafficking is inhibited in replicase-mediated resistant transgenic tobacco plants. Proc Natl Acad Sci USA **93:** 12643–12647

Nolte KD, Koch KE (1993) Companion-cell specific localization of sucrose synthase in zones of phloem loading and unloading. Plant Physiol **101:** 899–905

Noueiry AA, Lucas WJ, Gilbertson RL (1994) Two proteins of plant DNA virus coordinate nuclear and plasmodesmal transport. Cell **76:** 925–932

Olesinski AA, Almon E, Navot N, Perl A, Galun E, Lucas WJ, Wolf S (1996) Tissue-specific expression of the tobacco mosaic virus movement protein in transgenic potato plants alters plasmodesmal function and carbohydrate partitioning. Plant Physiol **111:** 541–550

Olesinski AA, Lucas WJ, Galun E, Wolf S (1995) Pleiotropic effect of tobacco mosaic virus movement protein on carbon metabolism in transgenic tobacco plants. Planta **197:** 118–126

Perl A, Perl-Treves R, Galili G, Aviv D, Shalgi E, Malkin S, Galun E (1991) Enhanced oxidative-stress defence in transgenic potato expressing tomato Cu, Zn superoxide dismutases. Theor Appl Genet **85:** 568–576

Russin W, Evert RF, Vanderveer PJ, Sharkey TD, Briggs SP (1996) Modification of a specific class of plasmodesmata and loss of sucrose export ability in the sucrose export defective1 maize mutant. Plant Cell **8:** 645–658

Scheible W-R, Lauerer M, Schulze E-D, Caboche M, Stitt M (1997) Accumulation of nitrate in the shoot acts as a signal to regulate shoot-root allocation in tobacco. Plant J **11:** 671–691

Schmulling T, Schell J, Spena A (1989) Promoters of the rolA, B, and C genes of *Agrobacterium rhizogenes* are differentially regulated in transgenic plants. Plant Cell **1:** 665–670

Sugaya S, Hayakawa K, Handa T, Uchimiya H (1989) Cell-specific expression of the rolC gene of the TL-DNA of Ri plasmid in transgenic tobacco plants. Plant Cell Physiol **30:** 649–653

Stitt M (1996) Plasmodesmata play an essential role in sucrose export from leaves: a step toward an integration of metabolic biochemistry and cell biology. Plant Cell **8:** 565–571

Van Bel AJE (1993) Strategies of phloem loading. Annu Rev Plant Physiol Plant Mol Biol **44:** 253–281

Van Bel AJE, Ammerlaan A, Van Dijk AA (1994) A three-step screening procedure to identify the mode of phloem loading in intact leaves. Planta **192:** 31–39

Waigmann E, Lucas WJ, Citovsky V, Zambryski P (1994) Direct functional assay for tobacco mosaic virus cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmata permeability. Proc Natl Acad Sci USA **91:** 1433–1437

Wolf S, Deom CM, Beachy RN, Lucas WJ (1989) Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. Science **246:** 377–379

Wolf S, Lucas WJ (1994) Virus movement protein and other molecular probes of plasmodesmatal function. Plant Cell Environ **17:** 573–585

Wolf S, Lucas WJ (1997) Plasmodesmal-mediated plant communication network: implications for controlling carbon metabolism and resource allocation. *In* CH Foyer, P Quick, eds, A Molecular Approach to Primary Metabolism in Plants. Taylor and Francis, London pp 219–236

Yang NS, Russell D (1990) Maize sucrose synthase-1 promoter directs phloem specific expression of Gus gene in transgenic tobacco plants. Proc Natl Acad Sci USA **87:** 4144–4148