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Tissue-Specific Expression of the Tobacco Mosaic Virus Movement Protein in Transgenic Potato Plants Alters Plasmodesmal Function and Carbohydrate Partitioning[1]

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Transgenic potato (*Solanum tuberosum*) plants expressing the movement protein (MP) of tobacco mosaic virus (TMV) under the control of the promoters from the class I patatin gene (B33) or the nuclear photosynthesis gene (ST-LS1) were employed to further explore the mode by which this viral protein interacts with cellular metabolism to change carbohydrate allocation. Dye-coupling experiments established that expression of the TMV-MP alters plasmodesmal function in both potato leaves and tubers when expressed in the respective tissues. However, whereas the size-exclusion limit of mesophyll plasmodesmata was increased to a value greater than 9.4 kD, this size limit was smaller for plasmodesmata interconnecting tuber parenchyma cells. Starch and sugars accumulated in potato leaves to significantly lower levels in plants expressing the TMV-MP under the ST-LS1 promoter, and rate of sucrose efflux from petioles of the latter was higher compared to controls. It is interesting that this effect was expressed only in mature plants after tuber initiation. No effect on carbohydrate levels was found in plants expressing this protein under the B33 promoter. These results are discussed in terms of the mode by which the TMV-MP exerts its influence over carbon metabolism and photoassimilate translocation, and the possible role of plasmodesmal function in controlling these processes.

It is generally accepted that most if not all viruses move from cell to cell via plasmodesmata. This process requires an interaction between a specific virally encoded protein, termed the MP, and proteins within the plasmodesmata of the host plant (Lucas and Gilbertson, 1994). Expression of the TMV-MP in transgenic tobacco plants provided the first evidence that this protein potentiates the short-distance transport of viral infectious material (Deom et al., 1987). Immunolocalization studies indicated that the TMV-MP was localized mainly in secondary plasmodesmata connecting mesophyll cells and bundle-sheath cells to phloem parenchyma cells (Ding et al., 1992). Dye-coupling studies established that the SEL of plasmodesmata interconnecting the mesophyll cells of these transgenic plants was greater than 9.4 kD, as compared to 800 D in control plants, indicating that the TMV-MP has a direct effect on plasmodesmal function (Wolf et al., 1989).

Based on plasmodesmal frequencies and dye-coupling experiments, it has been assumed that plasmodesmata play an important role in regulating symplasmic transport (Robards and Lucas, 1990). Pursuant to this assumption, transgenic plants in which plasmodesmata are modified provide an elegant system in which to further test the hypothesis that diffusion of Suc through plasmodesmata acts as a limiting step to symplasmic transport. Our comparative analysis of leaf photosynthetic performance, carbohydrate level, and carbon export in TMV-MP transgenic and control tobacco plants revealed a complex influence of the MP over these parameters (Lucas et al., 1993b; Olesinski et al., 1995). Fully expanded leaves of transgenic tobacco plants expressing the TMV-MP accumulated much higher levels of Suc, Glc, Fru, and starch during the day than did those of control plants. Direct measurements of ^{14}C-photosynthate translocation from source leaves indicated that export was lower in plants expressing the TMV-MP than in control plants (Olesinski et al., 1995). In addition to this effect on carbon allocation, the MP influenced the partitioning of dry matter between the various plant organs, resulting in a significantly higher shoot-to-root ratio (Balachandran et al., 1995).

Analysis of carbohydrate levels and export rates from source leaves in transgenic plants expressing various TMV-MP mutants (Olesinski et al., 1995), combined with microinjection experiments in which plasmodesmal function was determined (Berna et al., 1991; Wolf et al., 1991), established that the TMV-MP has a pleiotropic effect on

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Abbreviations: CaMV, cauliflower mosaic virus; FITC, fluorescein isothiocyanate; PFD, photon flux density; SEL, size-exclusion limit; TMV-MP, tobacco mosaic virus movement protein.

leaf physiology. These findings suggested that the TMV-MP has at least two sites of action: one that modifies plasmodesmal SEL and another that affects carbohydrate metabolism and allocation (Balachandran et al., 1995; Olesinski et al., 1995; Wolf et al., 1995).

Our earlier studies on the effect of plasmodesmal function (TMV-MP expression) on carbohydrate allocation and partitioning were based on tobacco plants expressing the TMV-MP gene under the CaMV 35S promoter. The current study was aimed at identifying the site(s) at which the TMV-MP interacts with cellular metabolism to change carbohydrate allocation. Since a specific source-sink relationship may be associated with the effects of the TMV-MP on carbon allocation and partitioning, we introduced the TMV-MP gene into potato (*Solanum tuberosum*) plants. Potato tubers represent a strong sink that causes a significant alteration in carbohydrate partitioning following tuber initiation (Burton, 1989). Moreover, tuber initiation is strongly affected by environmental signals such as photoperiod, which enables nondestructive manipulation of source-sink relationships. By using tissue-specific promoters, we demonstrate that the TMV-MP has different effects on plasmodesmal function in various plant tissues. Furthermore, its effect on growth pattern, photoassimilate partitioning, and carbohydrate allocation in source leaves appears to be due to its expression in photosynthetic tissues, and then only when potato plants have undergone tuber initiation.

MATERIALS AND METHODS

Reagents, Enzymes, and Plasmids

DNA restriction enzymes and all nucleic acid modification 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.

The PCR method was used to amplify the TMV gene for the 30-kD MP. DNA sequencing of the inserts revealed two clones (nos. 7 and 11) that had several differences from the TMV *Vulgar* (Goelet et al., 1982) strain. Three of the differences in DNA sequence resulted in changes in amino acid residues. Two differences were shared by the clones Ile to Val at residue 143, and Asn to Ser at residue 228. In addition, residue 135 of clone 7 was altered from Asn to Asp, and residue 106 of clone 11 was altered from Gly to Arg. The insert was excised with *Bam*HI and *Sal*I and cloned into the binary plasmid pBin19 (Bevan, 1984) under one of two different promoters (Fig. 1): the class-I patatin gene B33 promoter (Rocha-Sosa et al., 1989) and the nuclear photosynthesis gene ST-LS1 promoter (Stockhaus et al., 1989).

Potato Transformation, Regeneration, and Growth Conditions

The binary expression vectors containing the MP gene flanked by the respective promoters, a termination sequence, and the neomycin phosphotransferase II gene, were transformed into *Agrobacterium* strain LBA4404 (Hoekema et al., 1983). Tuber discs (3 mm diameter) of *Solanum tuberosum* cv Desiree were co-cultivated with the recombinant *Agrobacterium* cells. Culture conditions were similar to those previously described by Perl et al. (1991). Regenerated shoots were produced on Murashige and 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 kanamycin (100 mg/L) and Claforan (250 mg/L) Murashige and Skoog medium. 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 diameter) and were grown in an insect-free, temperature-controlled greenhouse (25 ± 3°C day/18 ± 2°C night). Plants were grown under natural sunlight with a midday average PFD of 1500 μmol m^{-2} s^{-1}.

Genomic DNA (1-2 mg) was used for detailed PCR amplification using primers recognizing the 5′ and 3′ ends of the TMV-MP gene plus primers for the specific promoters and the octopine synthase polyadenylation signals. RNA expression in the desired tissues was detected by northern blot analysis. Each sample (50 mg) was denatured at 65°C for 5 min in 70% formamide, 6% formaldehyde, and 200 mM Mops buffer, separated in a 1.2% agarose gel containing 6.3% formaldehyde in 20 mM Mops buffer, pH 7.0. RNA was then transferred to a NITRAN (Stratagene) mem-

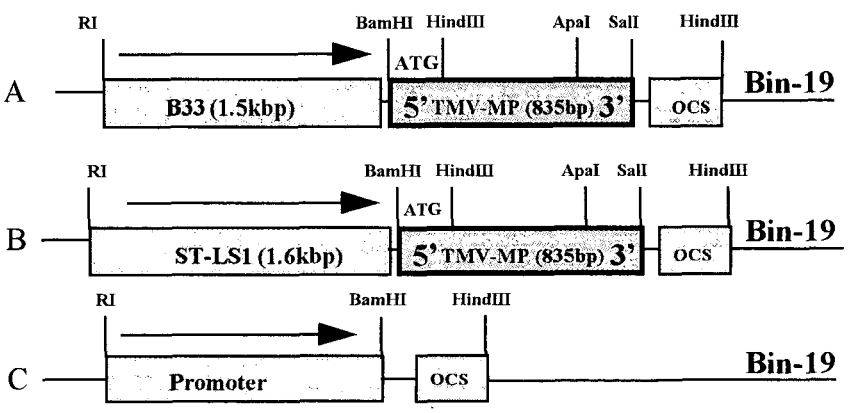

Figure 1. Structure of the chimeric binary plant expression cassettes used for plant transformation. A, Tuber-specific (B33) promoter plus the TMV-MP gene. B, Green tissue (ST-LS1) promoter plus the TMV-MP gene. C, Control vector including either promoter without the TMV-MP gene.

brane and hybridized to TMV-MP-specific ^{32}P-radiolabeled probe.

Microinjection Procedure

Fluorescent probes including Lucifer yellow CH (molecular mass 457 D) or FITC conjugated to dextran (molecular masses 3 or 9.4 kD) were used for microinjection studies. Freshly cut, 2-mm-thick tuber discs covered with distilled water or attached potato plant leaves were microinjected by the method described for tobacco leaves (Ding et al., 1992). In brief, spongy mesophyll or storage parenchyma cells were impaled with a micropipette and injected with dye suspension. Dye movement into neighboring cells was monitored with a Leitz (Wetzlar, Germany) Orthoplan epifluorescence microscope (equipped with a blue BP 390-490 excitation filter) connected to a Hamamatsu (Bridgewater, NJ) image-enhancement system (model C1966-20).

Greenhouse and Phytotron Experiments

Single-rooted stems derived from second-generation (greenhouse experiment) or third-generation (phytotron experiments) tubers were separated from the mother tuber, planted in 4-L pots, and transferred to a temperature-controlled greenhouse (25/18°C day/night) or to a phytotron. The temperature regime in the phytotron was 22/17°C (day/night), with one of two photoperiod treatments. Natural photoperiod was extended by using incandescent lamps (PFD of 10-30 μmol m^{-2} s^{-1}) to achieve a 16-h day length, whereas in the other treatment plants were transferred to a darkroom after an 8-h photoperiod. Light in these rooms was delivered by sunlight penetrating through the glass ceiling and walls (a midday average PFD of 1100 μmol m^{-2} s^{-1}).

Gas-Exchange Measurements

Net photosynthesis (measured as CO_2 uptake) was determined using a closed, portable, IR gas-exchange system (LI-6200, Li-Cor, Lincoln, NE). An attached leaf was placed in a 1-L Lexan chamber so that a 10-cm^2 area would be exposed to light and gas flow. The youngest, fully expanded leaf (nos. 5 or 6, with leaf no. 1 being the last leaf to achieve a length of 5.0 cm) was used in these experiments. All measurements were carried out on well-watered plants during the late morning hours on bright, sunny days. The initial CO_2 concentration in the chamber was 340 $\pm$ 10 μL L^{-1}, and a 30-s measurement was begun immediately after a reduction in CO_2 concentration was detected.

Starch and Sugar Determinations

Leaf carbohydrate content was determined as described by Lucas et al. (1993b). In brief, soluble sugars were extracted from leaf discs in 80% ethanol. After evaporating the supernatant, sugars were redissolved in H_2O and filtered through a 0.45-μm membrane HPLC filter (Whatman). Sugar was separated in an analytical HPLC system (LDC Analytical, Riviera Beach, FL) fitted with a Sugar-Pak I column (6.5 mm × 300 mm; Waters) using an LDC refractive-index detector. 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 the Sigma (HK) quantitative Glc determination kit.

Suc Efflux from Source Leaves

Efflux of Suc from petioles of fully expanded leaves was measured according to the experimental method described previously (van Bel et al., 1994) with minor modifications. Youngest, fully expanded leaves were detached under water by cutting petioles close to the stem. Excised leaves were immediately passed through a slit in a Parafilm (American National Can, Greenwich, CT) cover into a test tube containing 5 mL of distilled water (similar results were obtained when the tubes included 5 mM EDTA). The tubes and the leaves were gently shaken in a water bath covered with a glass plate (to maintain high RH) to collect phloem exudate. The experiment was conducted in the greenhouse under natural sunlight in the late morning. After a 3-h exudation period, leaf area was measured and the exudate solution was lyophilized. Sugars were resuspended in 100 μL of distilled water and Suc content was determined using the HPLC system described earlier.

RESULTS

Tissue-Specific Expression of the TMV-MP

Introduction of the TMV-MP gene into potato plants was found to be difficult and the percentage of regenerated plants was relatively low. However, detailed PCR analysis indicated that various potato lines regenerated in vitro represented stable transgenic plants. Five independent potato lines contained the TMV-MP gene under the ST-LS1 promoter (from which four were used for extensive studies), and three lines contained the gene under the B33 promoter (from which two were selected for further study). In addition, two independent vector control lines containing either promoter without the TMV-MP gene were regenerated. Northern blot analysis established the presence of TMV-MP transcript in the respective tissues (Fig. 2). TMV-MP RNA was expressed only in tubers of transgenic plant line B-11-101 (under the control of the B33 tuber-specific promoter). In plant lines L-7-203 and L-7-204 containing the TMV-MP gene under the control of the ST-LS1 green-tissue-specific promoter, TMV-MP RNA was detected only in leaves.

Immunogold labeling of the TMV-MP confirmed that protein was expressed in the various transgenic plants (data not shown).

TMV-MP Modified Plasmodesmal SEL in Potato Leaves and Tubers

Previous studies have established that expression of the TMV-MP in transgenic tobacco plants significantly alters the molecular SEL of plasmodesmata between leaf mesophyll cells (Wolf et al., 1989). In the present study, fluores-

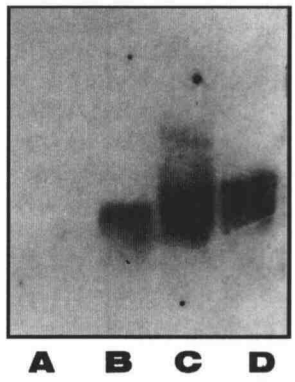

Figure 2. Northern analysis of TMV-MP in different tissues of transgenic potato plants containing the TMV-MP gene under the control of the ST-LS1 or B33 promoter. Leaves (A) and tubers (B) of plant line B-11-101 containing the TMV-MP gene under the control of the B33 promoter. Leaves of plant lines L-7-203 (C) and L-7-204 (D) containing the TMV-MP gene under the control of the ST-LS1 promoter.

cent probes of different molecular masses were microinjected into either tubers or leaves of transgenic potato plants expressing the TMV-MP under the control of the B33 or ST-LS1 promoters. Movement of 9.4-kD FITC-dextran was evident between mesophyll cells of transgenic potato plants expressing the TMV-MP in leaves (Table I). No movement of either 9.4- or 3-kD FITC-dextran was detected between tuber parenchyma cells of this plant line. Movement of 3-kD FITC-dextran between tuber storage parenchyma cells was detected when the TMV-MP was expressed in tubers, but the 9.4-kD FITC-dextran failed to move out of the injected cell. Only the small molecular probe Lucifer yellow CH moved from cell to cell in the control line (B-101) containing the empty cassette. No movement of FITC-dextran was detected between either mesophyll cells or tuber parenchyma cells in control plants, suggesting that the plasmodesmal SEL in these tissues is about 1 kD, similar to what was found in earlier reports (Robards and Lucas, 1990).

Expression of TMV-MP in Green Potato Tissue Alters Growth Pattern and Dry Weight Partitioning

Transgenic potato plants expressing the TMV-MP under the ST-LS1 promoter (L-7 lines) had significantly lower shoot and tuber weights compared to control transgenic plants expressing the empty cassettes (Table II). In contrast, expression of the TMV-MP in tubers only was found to have no effect on total dry weight or dry matter partitioning. It is well known that a long photoperiod causes a reduction in the tuber-to-shoot ratio in potato plants (Burton, 1989) (Table II). When plants were grown for an 8-h photoperiod, this ratio was similar for all plant lines tested. It is interesting that tuber weights of the control plant lines and of those expressing the TMV-MP in tubers were not affected by daylength, indicating that the reduction in tuber-to-shoot ratio was due to a significant increase in shoot growth. However, long days caused a reduction in tuber weight when the TMV-MP was expressed in green tissues. Due to the effect of long photoperiods on both shoot and tuber weights in these plant lines, the tuber-to-shoot ratio was lower compared to all other plant lines examined (Table II). Tuber numbers were not significantly different between the various lines for either photoperiodic regime, indicating that the observed TMV-MP influence over tuber weight was likely due to tuber development and not to tuber induction.

Growth analysis performed on greenhouse-grown plants was consistent with the data obtained for phytotron-grown plants. Total dry weight of L-7 plant lines was 30 to 50% of that of the control lines or transgenic plants expressing the TMV-MP under the B33 promoter (data not shown). It is interesting that the plant height of these lines was only slightly lower and the number of leaves was similar for all plant lines studied (Table III). The most pronounced effect on the phenotype of L-7 plants was reflected in leaf size. Mean leaf area was about half the value measured in the control lines, resulting in a significantly lower total area per plant (Table III).

TMV-MP Expression in Photosynthetic Tissue Alters Carbohydrate Metabolism and Export from Source Leaves

Daytime changes in the carbohydrate levels of mature leaves were analyzed 52 d after planting, when plants were grown in a temperature-controlled greenhouse under natural sunlight and daylength (13- to 14-h photoperiod). Starch and Suc accumulated during the day to higher levels in fully expanded leaves (nos. 4 and 5) of control plants compared with transgenic plants expressing the TMV-MP in photosynthetic tissue (Fig. 3, A and B). No significant differences between the various plant lines were found in Fru levels, whereas slightly lower Glc levels were observed in L-7 plants (Fig. 3, C and D). Daytime changes in carbohydrate levels of plants expressing the TMV-MP in tubers were not statistically different from those in the various control lines (Fig. 4), indicating that the effect of TMV-MP on carbohydrate metabolism is due to its expression in green tissue only.

Differences in carbohydrate levels were not detected when analyses of the various plant lines were performed 38 d after planting (data not shown). To further explore the possible interaction between plant developmental status

Table I. *Mobility of fluorescent probes through the symplasmic pathway of leaf mesophyll or tuber parenchyma cells of transformed potato plants expressing the TMV-MP in tubers (B-11-101), or leaves (L-7-203), or a vector control line (B-101)*

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

Plant Line	Tissue	LYCH	FITC-Dextran	
			3 kD	9.4 kD
B-101	Tubers	13 (16)	1 (8)	0 (13)
B-11-101	Tubers	16 (16)	9 (11)	0 (16)
	Leaves	6 (6)	0 (9)	0 (5)
L-7-203	Tubers	6 (7)	1 (6)	
	Leaves	3 (3)	5 (5)	7 (10)

Table II. *Growth analysis of 60-d-old TMV-MP transgenic and control potato plants*

Plants were grown in the phytotron (22/17°C day/night) for 8- or 16-h photoperiods. Five plants were used from each line. *, **, and ns represent significantly (P = 0.05 and P = 0.01) and not significantly different, respectively. Identical letters mean that differences between values are not significant at P = 0.05, using Student-Newman-Keuls multiple-range test.

Plant Line	Dry Weight (g plant^{-1})		Tuber No.	Tuber:Shoot Ratio
	Shoots	Tubers		
8-h photoperiod				
Wild type	0.60 ± 0.14ab	1.58 ± 0.38	2.8 ± 0.2	2.44 ± 0.29
B-101	0.64 ± 0.11ab	1.63 ± 0.29	2.6 ± 0.4	2.54 ± 0.20
L-201	0.83 ± 0.15a	1.90 ± 0.25	2.4 ± 0.2	2.40 ± 0.22
B-11-101	0.63 ± 0.10ab	1.23 ± 0.10	2.0 ± 0.3	2.19 ± 0.33
B-11-102	0.72 ± 0.10ab	1.70 ± 0.11	1.8 ± 0.3	2.58 ± 0.23
L-7-201	0.37 ± 0.12c	0.62 ± 0.23	2.0 ± 0.6	2.09 ± 0.65
L-7-202	0.42 ± 0.04bc	1.29 ± 0.19	2.5 ± 0.2	3.00 ± 0.16
L-7-203	0.52 ± 0.18bc	1.10 ± 0.58	2.0 ± 0.5	2.27 ± 0.55
L-7-204	0.35 ± 0.12c	0.85 ± 0.22	2.8 ± 0.8	3.58 ± 1.34
	**	ns	ns	ns
16-h photoperiod				
Wild type	1.77 ± 0.16a	1.86 ± 0.22a	3.2 ± 0.8a	1.05 ± 0.10ab
B-101	1.57 ± 0.11a	1.50 ± 0.34a	2.6 ± 0.6ab	0.92 ± 0.19bc
L-201	1.41 ± 0.16ab	1.73 ± 0.18a	1.8 ± 0.3bc	1.31 ± 0.22a
B-11-101	1.48 ± 0.07a	1.77 ± 0.16a	1.4 ± 0.2c	1.19 ± 0.08ab
B-11-102	1.65 ± 0.34a	1.75 ± 0.52a	2.4 ± 0.8ab	0.94 ± 0.14bc
L-7-201	0.64 ± 0.13c	0.28 ± 0.03b	1.3 ± 0.2c	0.67 ± 0.22cd
L-7-202	1.03 ± 0.13bc	0.72 ± 0.18b	2.3 ± 0.2ab	0.73 ± 0.21cd
L-7-203	0.95 ± 0.16c	0.42 ± 0.15b	1.3 ± 0.2c	0.55 ± 0.28d
L-7-204	0.87 ± 0.10c	0.65 ± 0.06b	2.6 ± 0.4ab	0.80 ± 0.13cd
	**	**	*	*

and the TMV-MP effect on carbohydrate metabolism, we analyzed plants grown in a phytotron for two photoperiodic regimes. In the first such experiment, carbohydrate analyses were performed 46 d after planting. When plants were grown for an 8-h photoperiod, no significant differences were observed in starch or sugar levels between the empty cassette control (line L-201) and transgenic (line L-7) plants (Table IV). Although starch levels in the wild-type potato line were somewhat higher, the variation in this experiment was relatively high. Even higher levels of variation were obtained in plants grown for a 16-h photoperiod. It is important to note that during these conditions, tubers were just being initiated, and at this stage of development large differences in tuber number (presence) and weight were observed. Differences in carbohydrate levels also were not detected between the various plant lines grown during long-day conditions (Table IV).

In the second experiment, carbohydrate analyses were performed 60 d after planting, when all plants for both photoperiods had tubers. Carbohydrate levels and photosynthetic rates were measured in the first fully expanded leaves (nos. 4 and 5) at noon on bright, sunny days. Suc, Glc, and Fru levels were significantly lower in transgenic L-7 plants compared to controls (Table V). This phenomenon, which was evident during long days, was even more pronounced during short days. Starch levels also tended to be lower in L-7 plants compared to controls, and again this was more evident during short days.

Table III. *Growth analysis of 60-d-old TMV-MP transgenic and control potato plants*

Plants were grown in a greenhouse in natural sunlight (13- to 14-h photoperiod) with an average midday PFD of 1500 mmol $m^{-2}s^{-1}$. Five plants were used for each line. ** and ns represent significantly (P = 0.01) and not significantly different, respectively. Identical letters mean that differences between values are not significant at P = 0.05, using Student-Newman-Keuls multiple-range test.

Plant Line	Shoot Height	No. of Leaves	Leaf Area per Plant	Mean Leaf Area	Specific Leaf Weight
	cm		*cm²*	*cm²*	*mg cm²*
Wild type	71.5 ± 2.8	20.9 ± 0.6	2038 ± 253a	97 ± 11a	44 ± 5.3
B-101	77.6 ± 2.7	22.0 ± 0.8	1941 ± 157a	89 ± 8a	45 ± 0.2
L-201	73.8 ± 2.3	21.0 ± 0.5	1479 ± 117b	70 ± 5a	48 ± 1.5
B-11-101	80.3 ± 2.7	22.6 ± 0.5	1671 ± 121ab	74 ± 5b	45 ± 1.1
B-11-102	76.6 ± 2.7	21.4 ± 0.9	1389 ± 246bc	64 ± 10bc	50 ± 1.9
L-7-201	80.2 ± 9.0	20.8 ± 0.8	674 ± 101d	33 ± 6d	54 ± 3.7
L-7-202	76.6 ± 2.2	21.3 ± 0.5	1079 ± 113cd	51 ± 5c	52 ± 2.6
L-7-203	58.6 ± 1.5	18.6 ± 0.7	1070 ± 122cd	57 ± 4c	52 ± 0.6
L-7-204	60.0 ± 10.4	19.0 ± 2.0	651 ± 297d	32 ± 11d	54 ± 7.6
	ns	ns	**	**	**

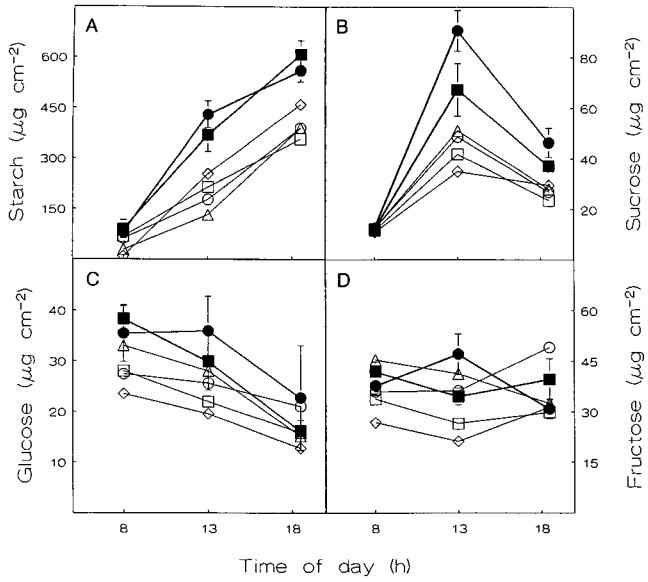

Figure 3. Diurnal change in carbohydrate content of leaves from transgenic potato plants expressing the TMV-MP under the control of the ST-LS1 promoter (○, L-7-201; □, L-7-202; △, L-7-203; ◇, L-7-204) compared to vector control potato plants (●, L-201; ■, L-202) 52 d after planting. Plants were grown in a greenhouse in natural sunlight (13- to 14-h photoperiod) with an average midday PFD of 1500 μmol m^{-2} s^{-1}. Leaf discs were analyzed for starch (A), Suc (B), Glc (C), and Fru (D) levels. Values are the means ± SE of six plants (SE is not shown when the value is smaller than the symbol).

The photosynthetic rate of L-7 plants was slightly lower than that of the empty-cassette control line and the wild-type potato plants when grown during long days (Table V). However, during short days, no significant difference was observed between the various plant lines. It is important to note that the reported values represent the point of highest photosynthetic rate during the photoperiod. For both day-lengths, no differences in photosynthetic rate were found between the various plant lines when measurements were conducted either earlier or later in the day (data not shown). It is interesting that the photosynthetic rate for short days was consistently lower than that measured for long-day conditions (Table V).

To further explore the basis for the lower levels of carbohydrates in leaves of L-7 plants, direct measurements of Suc efflux were conducted on detached leaves. Suc levels in tubes containing leaves of L-7 plants were significantly higher compared to those of the control plants (Fig. 5). Values for the three different control lines were between 2.09 to 2.35 μg Suc cm^{-2} h^{-1}, whereas the values for the plant lines L-7-201/2/3 ranged from 3.74 to 4.46 μg Suc cm^{-2} h^{-1}. In all cases, Suc constituted about 50% of the total measured sugars. Equivalent values for sugar efflux from potato petioles have been reported by Riesmeier et al. (1994).

DISCUSSION

In the present study we established that expression of the TMV-MP in transgenic potato plants influences the process of carbon allocation. Furthermore, by controlling the site of expression we were able to demonstrate that the TMV-MP exerts its influence when expressed in photosynthetic tissue. It is important to stress that this effect was found to be under developmental control. These findings are consistent with our earlier studies, in which we established that expression of the TMV-MP in transgenic tobacco plants, under the control of the CaMV 35S promoter, results in a change in both carbon metabolism and photosynthate partitioning (Lucas et al., 1993b; Olesinski et al., 1995).

The ability of viral MPs to affect plasmodesmal SEL has now been well established in a number of plant systems. The SEL of plasmodesmata interconnecting spongy mesophyll cells of transgenic tobacco plants expressing the TMV-MP was found to be greater than 9.4 kD (Wolf et al., 1989). The development of a procedure by which TMV-MP is directly injected into mesophyll cells established that the plasmodesmal SEL is modified in tobacco leaves to a value greater than 20 kD (Waigmann et al., 1994). This latter procedure was also used to demonstrate changes in plasmodesmal SEL in cowpea by the MP of red clover necrotic mosaic virus (Fujiwara et al., 1993), in bean leaves by the MP of bean dwarf mosaic virus (Noueiry et al., 1994), and in tobacco leaves by the MP of cucumber mosaic virus (Ding et al., 1995). All such studies demonstrate up-regulation of plasmodesmal SELs in leaves of the respective viral host plant.

Expression of the TMV-MP under the control of the B33 promoter also resulted in an alteration in plasmodesmal SEL of potato tubers (Table I). It is interesting that the

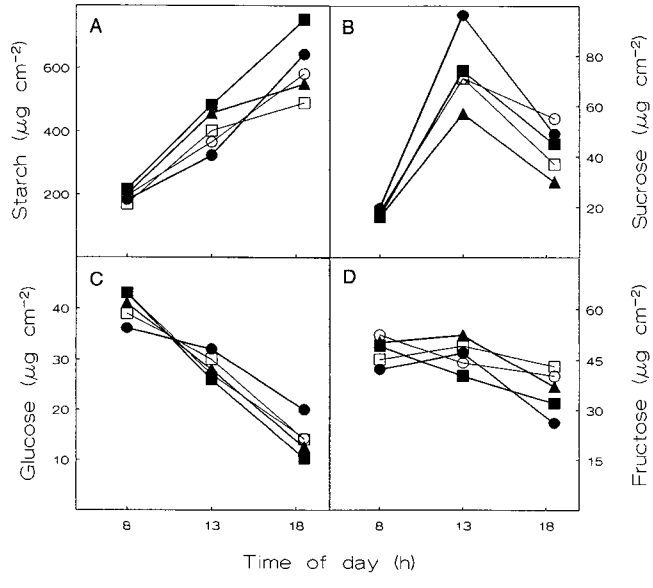

Figure 4. Diurnal change in carbohydrate content of leaves from transgenic potato plants expressing the TMV-MP under the control of the B33 promoter (○, B11-101; □, B-11-102) compared to control potato plants (●, B-101; ■, B-102; ▲, untransformed wild-type) 52 d after planting. Plants were grown in a greenhouse in natural sunlight (13- to 14-h photoperiod) with an average midday PFD of 1500 μmol m^{-2} s^{-1}. Leaf discs were analyzed for starch (A), Suc (B), Glc (C), and Fru (D) levels. (Differences between all plant lines are not statistically significant, and therefore SE is not indicated).

Table IV. *Total carbohydrate content in the afternoon (16:00 h) in leaves of 46-d-old L-7-TMV-MP transgenic, vector-control, and wild-type (untransformed) potato plants*

Plants were grown in a phytotron (22/17°C day/night) under two photoperiodic regimes: natural (8 h) and long (16 h) days. Leaf discs were punched from fully expanded leaves (nos. 4 and 5) and were analyzed for starch and sugar contents. Five plants were used for each line.

Plant Line	Carbohydrate Content			
	Starch	Suc	Glc	Fru
		$\mu g\ cm^{-2}$		
8-h photoperiod				
Wild type	265 ± 46	49.8 ± 6.2	15.1 ± 2.5	34.0 ± 5.0
L-201	154 ± 9.0	36.3 ± 4.7	11.3 ± 2.7	21.9 ± 3.3
L-7-201	173 ± 11	37.8 ± 5.3	15.6 ± 2.2	25.3 ± 2.5
L-7-202	141 ± 40	32.6 ± 2.1	8.5 ± 1.1	17.1 ± 2.4
L-7-204	190 ± 30	40.8 ± 9.0	15.9 ± 6.8	14.9 ± 1.6
16-h photoperiod				
Wild type	419 ± 118	43.5 ± 5.6	10.5 ± 1.2	22.0 ± 2.9
L-201	452 ± 67	43.3 ± 2.6	9.6 ± 1.2	21.7 ± 1.5
L-7-201	403 ± 117	78.9 ± 33	37.0 ± 13	63.4 ± 23
L-7-202	309 ± 176	44.1 ± 10	15.9 ± 4.3	31.7 ± 7.2
L-7-204	393 ± 130	38.3 ± 7.8	10.3 ± 3.2	18.7 ± 4.3

9.4-kD FITC-dextran did not move between tuber parenchyma cells, whereas it did move between mesophyll cells of transgenic potato plants expressing this gene under the ST-LS1 promoter (Table I). Since changes in the SEL require an interaction between the MP and proteins within the plasmodesmata, these results suggest that differences may well exist in terms of the molecular aspects of plasmodesmal control in the two organs.

Since the TMV-MP causes a significant increase in plasmodesmal SEL, we speculated that Suc movement from the mesophyll to the bundle-sheath-parenchyma boundary would be enhanced if this part of the Suc export pathway serves as a rate-determining step in the overall process of Suc translocation. Alterations in the carbohydrate levels of source leaves were evident only in transgenic plants expressing the TMV-MP in photosynthetic tissues (Figs. 3 and 4). During the day, Suc and starch accumulated to significantly lower levels in these plant lines (L-7) compared with all other lines tested. Considering that lower carbohydrate levels were measured in L-7 lines in the afternoon, even though photosynthetic rates were similar in the leaves of all plants studied (Table V, 8-h photoperiod), and since carbohydrate levels of these plant lines were similar in the morning, it appears that expression of the TMV-MP in photosynthetic tissues of potato actually enhances the rate of carbon export during the day. Confirmation of this assumption was obtained by direct measurement of sugar efflux from leaves of different sets of third-generation

Table V. *Photosynthetic rate and total carbohydrate content in leaves of 60-d-old TMV-MP transgenic, vector-control, and wild-type (untransformed) potato plants*

Photosynthesis of the first, fully expanded leaf was measured at noon (an average PFD of 1500 $\mu mol\ m^{-2}s^{-1}$), and immediately thereafter leaf discs were punched from the same leaves for carbohydrate analysis. Plants were grown in a phytotron (22/17°C day/night) for 8-h and 16-h photoperiods. Identical letters mean that differences between values are not significant at P = 0.05, using Student-Newman-Keuls multiple-range test.

Plant Line	Photosynthesis	Carbohydrate Content			
		Starch	Suc	Glc	Fru
	$\mu mol\ m^{-2}s^{-1}$		$\mu g\ cm^{-2}$		
8-h photoperiod					
Wild type	7.4 ± 0.8	307 ± 54a	131 ± 24a	30.4 ± 5.0a	71 ± 21.4a
L-201	11.3 ± 0.5	183 ± 13b	111 ± 23ab	35.8 ± 6.4a	49 ± 12.2a
L-7-201	9.3 ± 0.3	136 ± 13b	79 ± 12bc	16.7 ± 3.3b	21.2 ± 3.0b
L-7-202	12.6 ± 0.2	134 ± 12b	84 ± 7b	23.3 ± 2.1ab	20.8 ± 1.8b
L-7-203	11.0 ± 0.4	N.D.[a]	46 ± 12c	16.0 ± 2.4b	24.1 ± 3.7b
L-7-204	11.0 ± 2.1	187 ± 12b	79 ± 6bc	13.6 ± 2.2b	20.1 ± 1.8b
16-h photoperiod					
Wild type	22.9 ± 1.0a	249 ± 38b	100 ± 1.3	36.3 ± 3.0a	47.5 ± 2.7a
L-201	21.5 ± 0.6ab	292 ± 22a	105 ± 1.9	41.9 ± 3.6a	57.1 ± 7.3a
L-7-201	16.8 ± 1.4c	165 ± 7b	89 ± 11.0	23.6 ± 1.0b	28.4 ± 4.1b
L-7-202	18.7 ± 1.5bc	269 ± 31a	93 ± 8.0	23.2 ± 2.2b	24.3 ± 1.7b
L-7-203	18.0 ± 1.4c	N.D.	103 ± 19	24.0 ± 5.1b	36.1 ± 6.1ab
L-7-204	19.2 ± 1.2bc	188 ± 10b	75 ± 7.0	22.7 ± 2.3b	30.2 ± 3.2b

[a] N.D., Not determined.

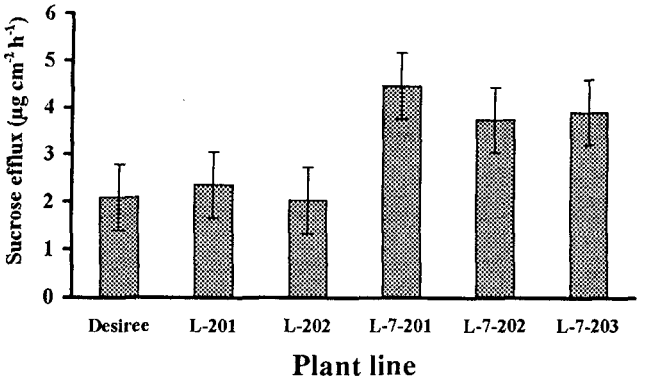

Figure 5. Efflux of Suc from petioles of transgenic potato plants expressing the TMV-MP under the control of the ST-LS1 promoter (plant lines L-7-201, L-7-202, and L-7-203) compared to wild-type Desiree and vector control potato plants (L-201 and L-202). Leaves were cut from 8-week-old plants grown in a greenhouse in natural sunlight (12- to 13-h photoperiod) with an average midday PFD of 1500 μmol m^{-2} s^{-1}. Values are the means ± SE of 16 plants.

transgenic plants. Significantly higher efflux of sugars from petioles of plants expressing the TMV-MP under the ST-LS1 promoter compared to three different control plants (Fig. 5) support the conclusion that export rate of carbohydrate is indeed higher in these TMV-MP-expressing plants.

Expression of TMV-MP in transgenic tobacco under the CaMV 35S promoter resulted in an unexpected accumulation of carbohydrates within source leaves during the day, as well as a slower rate of ^{14}C export (Lucas et al., 1993b; Wolf and Lucas, 1994; Olesinski et al., 1995). A possible explanation for these observed effects of the TMV-MP in tobacco and potato plants may relate to the mechanism of sugar transfer from cell to cell. Targeting the MP to plasmodesmata may cause a specific inhibition of Suc transport from cell to cell in tobacco plants despite the increase in SEL. If this were the case, one must conclude that Suc transfer from cell to cell may not occur by simple diffusion. Differences in plasmodesmal substructure (protein composition) in potato plants may eliminate such an association between the TMV-MP and plasmodesmata of potato mesophyll cells, resulting only in modification of the SEL and not specific reduction in Suc transfer from cell to cell. In this respect it is important to note that TMV infection does not represent a problem in terms of potato production. Mechanical inoculation of our control, as well as transgenic potato plants, did not cause any symptoms, nor could we detect viral particles in uninoculated leaves (data not shown).

An alternative explanation relates to the different promoters controlling TMV-MP expression. When expressed under the CaMV 35S promoter, the MP is localized to plasmodesmata of all plant tissues, including the phloem. Immunogold labeling of the TMV-MP in transgenic potato plants containing the gene under the ST-LS1 promoter revealed label only in mesophyll cells, with no detection of immunolabel within the phloem (data not shown). Inhibition of Suc export from source tobacco leaves may be due to interaction of the MP with plasmodesmal proteins within the phloem. To further investigate this possibility, we are currently introducing the TMV-MP into potato plants under the control of the phloem-specific rolC promoter.

Recent findings have established that macromolecules can traffic through plasmodesmata (Fujiwara et al., 1993; Lucas et al., 1993a, 1995; Noueiry et al., 1994; Waigmann et al., 1994; Ding et al., 1995). Thus, it is possible that the TMV-MP exerts its influence, in both potato and tobacco, via an interaction with the transport of endogenous signaling proteins (macromolecules) that may be involved in the coordination of carbon fixation, starch accumulation, Suc synthesis, and transport (Balachandran et al., 1995; Olesinski et al., 1995). In this situation, the different effects of the TMV-MP in tobacco and potato may relate to distinct associations between the MP and plasmodesmal proteins in the two species, and therefore different effects on the transport of the putative signaling protein(s).

In recent years, several strategies have been employed to manipulate gene expression in transgenic plants in order to alter dry-matter partitioning between the various plant organs and carbohydrate metabolism in source leaves (see Kossmann et al., 1995). Potato plants have been transformed with a yeast-derived invertase (Heineke et al., 1992) or an antisense construct of the Suc transporter (Riesmeier et al., 1994). Tobacco plants have been transformed with an *Escherichia coli*-derived inorganic pyrophosphatase (Lerchl et al., 1995). In all cases, carbohydrate was found to accumulate in source leaves. As stated earlier, our approach was directed at altering the site that may exert control over the transport process without directly affecting enzymatic reactions. The change in the diurnal pattern of carbohydrate accumulation in the potato plants (Fig. 3), together with the alteration in sugar efflux from source leaves (Fig. 5), suggests that plasmodesmata act as a control site for carbohydrate translocation. It is interesting that this effect was more pronounced in the phytotron under short-day conditions, which induce tuber development (Tables IV and V), and only at a late developmental stage in greenhouse-grown potato plants (Fig. 3). These results suggest that the MP effect on leaf carbohydrate content is associated with tuber growth.

Development of a strong sink, such as the potato tuber, must have a significant effect on the process of carbohydrate translocation and partitioning in the plant. It may well be that plasmodesmal function does not limit Suc transport before tuber formation. However, tuber development is accompanied by a higher demand for photoassimilates; therefore, an up-regulation of plasmodesmal SEL may have contributed to a higher rate of export during the day.

An alternative explanation for the developmental control over the TMV-MP-mediated effect on carbohydrate allocation may relate to the alteration in phenotype of transgenic plants expressing this protein in green tissue only. Total weight of the L-7 plants was about half that of the other plant lines studied. The photosynthetic rate, on a leaf area basis, was similar for all plant lines for short days, and the differences for long days were relatively small (Table V). It

is interesting that shoot height and leaf number were also similar for all plant lines studied. The smaller tuber and total weight values of the L-7 plants are probably attributable to lower leaf area per plant, due to the significantly smaller leaves in these plants (Table III). It is possible that tuber growth caused a shortage of carbohydrates, on a whole plant level, due to the lower leaf area of L-7 plants. As a result, the rate of Suc export from single leaves would thus be higher in these plant lines and the diurnal accumulation of carbohydrates would likely be lower, consistent with our present findings. However, based on this explanation, one would expect that for short days (conditions favoring tuber development), the tuber:shoot ratio should be higher in these transgenic lines compared to control plants. As shown in Table II during the 8-h photoperiod, the tuber:shoot ratio remained similar for all plant lines, despite the lower leaf area and lower tuber weight of the respective transgenic lines. Moreover, during the 16-h photoperiod, the tuber:shoot ratio of L-7 lines was significantly lower than that of controls. This latter phenomenon may indicate that, when expressed in leaves, the TMV-MP can inhibit tuber formation, and it further suggests that this protein can also exert an effect on plant development.

The molecular events associated with transport through plasmodesmata have yet to be resolved. Since viral MPs play a crucial role in trafficking the viral infectious material through plasmodesmata, and since they are localized to plasmodesmata, it is logical to assume that the effect on carbon partitioning is exerted via modification of plasmodesmal function. A detailed characterization of plasmodesmal properties and/or the endogenous macromolecules that are transported through plasmodesmata will provide the foundation for future studies on the mechanism(s) controlling long-distance transport in plants.

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