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Research Note

Tobacco Mosaic Tobamovirus Does Not Require Concomitant Synthesis of Movement Protein During Vascular Transport

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A tobacco mosaic tobamovirus (TMV) mutant, which is defective in cell-to-cell transport, moved long-distance through parts of grafted plants that do not express TMV movement protein, MP(–). Long-distance movement of the TMV mutant in vascular tissue from MP(–) tobacco was as efficient as wild-type TMV. The data indicate that once in the phloem, presumably the sieve elements, long-distance movement of TMV does not require concomitant synthesis of MP.

Additional keywords: long-distance movement, grafted plants.

Tobacco mosaic tobamovirus (TMV) is a single-stranded, plus-sense RNA virus. In susceptible plants TMV is able to move throughout the plant infecting most of the cells of the plant. Movement to adjacent cells occurs through plasmodesmata, while movement to distal leaves or roots occurs via phloem (Matthews 1991 and references therein). Cell-to-cell movement is facilitated by the viral 30K movement protein (MP) (Deom et al. 1992; Citovsky and Zambryski 1991). The TMV MP increases the molecular size exclusion limit of plasmodesmata (Wolf et al. 1989, 1991; Deom et al. 1990) and binds to viral RNA, perhaps functioning as a molecular chaperone for the intercellular movement of progeny virus (Citovsky et al. 1990).

From the standpoint of viral pathogenicity, efficient long-distance movement is usually required. A number of virus-plant interactions support replication and cell-to-cell movement, but the virus is confined to the initially inoculated area and the plants do not exhibit symptoms of systemic disease (Samuel 1934; Bennett 1940). In plants that support long-distance movement of TMV, the virus enters the phloem and moves towards photosynthate importing areas, such as root tips and new leaves. It has not yet been possible to disassociate efficient long-distance movement from a functional coat protein (Siegel et al. 1962; Dawson et al. 1988) and an origin of assembly (Saito et al. 1990), suggesting that virion formation is required for long-distance movement in tobacco. Additionally, it has been shown that long-distance movement of TMV in tobacco requires the "correct" coat protein. Odontoglossum ringspot tobamovirus (ORSV) does not effectively move long distances in tobacco. Substitution of the ORSV CP gene for the TMV CP gene results in a hybrid virus that produces stable virions, but moves poorly (Hilf and Dawson 1993). Substitution of the ORSV MP for the MP of TMV also greatly reduced long-distance movement. However, an interpretation could not be made between whether the substituted MP indirectly affected long-distance movement by directly reducing the rate of cell-to-cell movement or whether it directly functions in long-distance movement. Similarly, it is not known if the TMV MP plays a role in long-distance movement, although long-distance movement of TMV is generally assumed to be passive. In this report we describe the ability of a TMV mutant, which is defective in cell-to-cell movement, to move long distances through parts of grafted plants which do not express complementing TMV MP.

Previously, it had been demonstrated that transgenic plants expressing TMV MP could complement TMV mutants with a defective MP (Deom et al. 1987). Transgenic tobacco plants expressing the MP gene and 3′ untranslated region of TMV, under the control of the 35S promoter of cauliflower mosaic caulimovirus in pMON530, were produced via *Agrobacterium tumefaciens*-mediated leaf disk transformation (Horsch et al. 1985). The MP gene and 3′ untranslated region of TMV were introduced into pMON530 to give pTMV-30K by the following procedure. The 1.0-kb *Xho*I–*Pst*I fragment (MP gene and 3′ untranslated region of TMV) from pKK1-S2 was ligated to *Xho*I-*Pst*I digested pUC129 to give p30P. pKK1-S2 (Lehto and Dawson 1990) is a full-length cDNA clone of TMV that has been mutated to delete the coat protein gene and to introduce a unique *Xho*I site immediately upstream of the MP initiation codon. The 1.0-kb *Kpn*I–*Eco*RI fragment from p30P was ligated to *Kpn*I–*Eco*RI digested pMON530 to generate pTMV-30K. Chromosomal DNA from primary transformants was digested with *Bam*HI, transferred to nitro-

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cellulose and hybridized with ^{32}P-labeled, full-length TMV RNA as described by Donson et al. 1993; predicted DNA fragment sizes were observed (data not shown). Kanamycin-resistant plants (R_1 generation) were screened for expression of functional MP using a movement defective mutant of TMV, designated TE1. TE1 was generated by introducing a frameshift mutation into the MP gene in pTKU1 (Donson et al. 1991). The mutation was obtained by deleting nucleotide 4931 by site-directed mutagenesis (Kunkel et al. 1987), resulting in a truncated MP of 16 amino acids. TE1 is competent for replication in protoplasts but deficient for movement in nontransformed plants (data not shown). Two transformed plant lines, designated PL12 and PL26, effectively complemented TE1, producing systemic mosaic symptoms almost identical to those induced by TMV in nontransformed tobacco (data not shown).

A series of grafted plants containing different combinations of MP(+) and MP(–) tobacco were produced to examine phloem-associated, long-distance movement of TMV and TE1 (Fig. 1). The first series of plants consisted of "sandwich grafts" (i.e., interstocks) in which stem inserts of approximately 10 to 15 cm were grafted between the central excision of plants approximately 30 to 40 cm tall (Fig. 1A–C). Three weeks following the grafting, leaves on the rootstock were mechanically inoculated with either TMV or TE1 and movement through the interstock was monitored by development of mosaic symptoms in leaves of the apical section. TMV moved through all graft combinations and nongrafted MP(+) plants at the same rate as control tobacco, producing mosaic symptoms in upper leaves by 8 days postinoculation (dpi; Table 1). These data suggest that neither the graft junctions nor the expression of the transgene significantly affected movement of the wild type virus. In control plants containing MP(+) stem sections grafted between MP(+) rootstock and apical sections (Fig. 1B), inoculated with TE1, the rate of systemic symptom development was similar to that detected in nongrafted MP(+) tobacco inoculated with TE1. Grafted and nongrafted plants showed systemic mosaic symptoms at 7 and 6 days postinoculation (Table 1), respectively. In grafted plants containing MP(–) interstocks between MP(+) rootstock and apical sections (Fig. 1A), systemic mosaic symptoms developed 9 dpi (Table 1) when leaves on the rootstock were inoculated with TE1, 1 day later than systemic symptoms induced by TMV (Table 1). In each experiment in which TE1 progeny was detected in upper leaves, the virus was inoculated onto MP(–) and MP(+) tobacco to test for reversion of the virus to wild-type. Only MP(+) tobacco developed disease, indicating that TE1 was present in systemic leaf tissue and had not reverted to wild-type. The levels of virus at 30 dpi in the upper leaves was approximately the same in all of the plants (Table 1). These data demonstrate that once TE1 replicated and moved into the phloem in MP(+) rootstock it was able to efficiently traverse 10 to 15 cm of MP(–) stem section and move into MP(+) apical tissue.

In a set of experiments to examine the dynamics of movement across a new graft, sets of previously grafted plants with MP(+) scions and MP(–) or MP(+) rootstocks were inoculated by grafting TE1-infected MP(+) leaves (leaf petiole to stem) onto the rootstocks (Fig. 1D and E). TE1-infected MP(+) leaves were taken at 14 dpi and grafted between the lower second and third leaves of the rootstock. TE1 moved

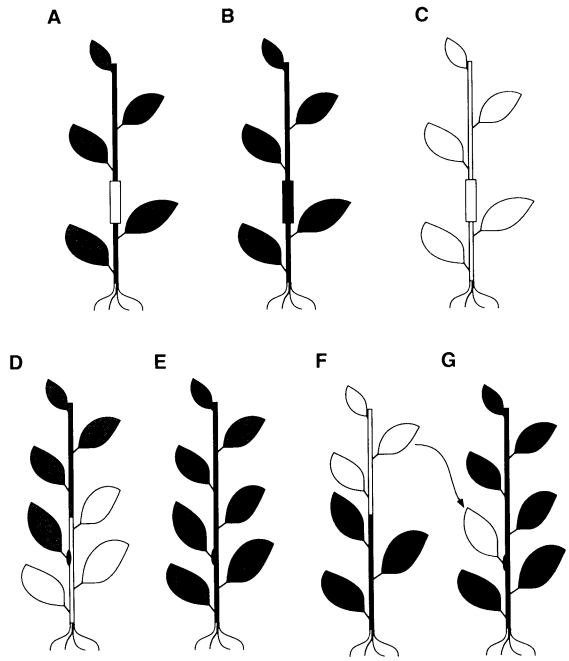

Fig. 1. Schematic drawings of grafted tobacco plants. **A,** MP(+) tobacco with a nontransformed tobacco interstock. **B,** MP(+) tobacco containing MP(+) interstock. **C,** Nontransformed tobacco containing nontransformed tobacco interstock. **D,** MP(+) scion section grafted to MP(–) rootstock. After the initial grafting, a TE1-infected MP(+) leaf from inoculated MP(+) tobacco was grafted onto the MP(–) rootstock between the second and third leaves from the bottom of the rootstock. Leaf grafts were placed approximately 15 cm below the rootstock and scion graft point. **E,** Same as D except the rootstock was MP(+). **F,** MP(–) scions grafted to MP(+) rootstocks. **G,** Leaves from MP(–) scions described in (F) were taken at 12 days after the rootstocks were inoculated with TE1 and grafted between the second and third leaves from the bottom of healthy MP(+) tobacco. Stippled plant tissue represents MP(+) tobacco. White tissue represents MP(–) tobacco. Black ovals represent the stem portion of the leaf graft.

Table 1. Time required for development of systemic mosaic symptoms following mechanical inoculation of lower leaves

Genotype of graft components rootstock/insert/scion	Diagram[a]	Virus	Symptom dvlpmt (days)	Virus conc. in systemic leaves[b]
MP(–), not grafted	...[c]	TMV	6	650 ± 50
MP(–), not grafted	...	TE1	...	...
MP(+), not grafted	...	TMV	8	540 ± 40
MP(+), not grafted	...	TE1	6	600 ± 30
MP(–)/MP(–)/MP(–)	C	TMV	7	NT
MP(–)/MP(–)/MP(–)	C	TE1	...	...
MP(+)/MP(+)/MP(+)	B	TMV	8	NT
MP(+)/MP(+)/MP(+)	B	TE1	7	NT
MP(+)/MP(-)/MP(+)	A	TMV	8	590 ± 40
MP(+)/MP(-)/MP(+)	A	TE1	9	560 ± 50

[a] Capital letter refers to the grafted plant in the schematic diagram in Figure 1.

[b] Virus concentration in leaf tissue of MP (+) apical sections was measured by ELISA 30 days after mechanical inoculation. Three to five plants were used per treatment and each experiment was repeated 5 to 10 times. Virus concentration is given in µg/g fresh weight of leaf tissue(–) No symptoms and no virus was detected. NT, not tested.

[c] Diagram not shown.

across the graft into the rootstock and subsequently into the MP(+) scion regardless of the ability of the rootstock to express MP (Table 2).

To further examine movement of TE1 into MP(–) tissue, grafted plants were produced by joining MP(–) scions onto MP(+) rootstocks (Fig. 1F). Three weeks after the grafting, leaves of the rootstock were mechanically inoculated with TE1. The virus replicated and moved through the leaves of the rootstock and to the roots, as determined by ELISA (data not shown). As expected, no symptoms were observed in the MP(–) scion and no virus was detected by ELISA or sap infectivity assays at 30 dpi. However, when leaves from the MP(–) scions were removed and grafted to healthy MP(+) plants (Fig. 1G), mosaic symptoms developed in 8 to 10 days, demonstrating the presence of TE1 in leaves of the MP(–) scion. These data also demonstrate the greater sensitivity of the 'graft' assay compared to ELISA and sap infectivity assays. In reciprocal experiments with MP(–) rootstock and MP(+) scions, no virus was detected by ELISA in leaves of the TE1-inoculated MP(–) rootstock or the MP(+) scion at 30 dpi. As expected, when leaves from the MP(+) scions were removed and grafted to healthy MP(+) plants no disease developed and no virus was detected up to 30 dpi.

In this work, we examined the ability of a movement defective TMV, TE1, to move through or into MP(–) tissue. In relation to long-distance movement, once replication, cell-to-cell movement, and phloem-loading occurred in MP(+) rootstock, TE1 was able to efficiently move through large MP(–) stem sections or into scions on grafted plants that were not capable of MP synthesis. These data suggest that either: (i) the MP is not needed for continued long-distance movement through phloem; or, (ii) sufficient MP moves along with the virus through the phloem. It is possible that MP is needed for loading into and unloading from phloem, but not in continued movement within phloem. If so, this would suggest that there was no loading and unloading within the MP(–) stem sections that was needed for long-distance movement. In these experiments, loading and unloading could have occurred in the MP(+) plant tissue. However, TE1 was able to move from MP(+) rootstock into MP(–) scions. The MP(–) scions did not develop detectable amounts of virus or symptoms; however, small amounts of inoculum moved into upper leaves as shown by the grafting experiments (Fig. 1G). These data suggest that once the virus enters the phloem, presumably the sieve elements, movement is passive, perhaps in a manner that has been predicted since the original observations on long-distance movement (Samuel 1934).

A particularly interesting question is, "How do viruses exit the sieve elements?" If MP is required to modify plasmodesmata for exiting the sieve elements, then the protein must be either transported from the lower leaves or synthesized in the sieve elements. An intriguing possibility is that TE1 virions were able to exit sieve elements and enter companion cells. Plasmodesmata between sieve elements and companion cells have a molecular exclusion limit for molecules in excess of M_r 3,000 (Kemper et al. 1993). However, movement of TE1 out of companion cells might be inhibited if functional MP is required to modify plasmodesmata between companion cells and vascular parenchyma or bundle sheath cells. This should be an ideal system for anatomical localization experiments to identify the limit of virus movement in the absence of MP.

Table 2. Time required for development of systemic mosaic symptoms following leaf graft inoculation

Genotype of graft components rootstock/insert/scion	Diagram[a]	Virus	Symptom dvlpmt (days)	Virus conc. in systemic leaves[b]
MP(+)/MP(+)/MP(+)	E	TE1	6	630 ± 80
MP(+)/MP(+)/MP(+)	E	TMV	8	560 ± 60
MP(–)/MP(+)/MP(+)	D	TE1	8	610 ± 40
MP(–)/MP(+)/MP(+)	D	TMV	7	580 ± 50

[a] Capital letter refers to the grafted plant in the schematic diagram in Figure 1.

[b] Virus concentration in leaf tissue of MP (+) apical sections was measured by ELISA 30 days after mechanical inoculation. Three to five plants were used per treatment and each experiment was repeated 5 to 10 times. Virus concentration is given in µg/g fresh weight of leaf tissue.

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