Replication in the phloem is not necessary for efficient vascular transport of tobacco mosaic tobamovirus

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Abstract Plant viruses move systemically from one leaf to another via phloem. However, the viral functions needed for systemic movement are not fully elucidated. An experimental system was designed to study the effects of low temperature on the vascular transport of the tobacco mosaic tobamovirus (TMV). Vascular transport of TMV from lower inoculated leaves to upper non-inoculated leaves via a stem segment kept at low temperature (4°C) was not affected. On the other hand, several experiments were performed on tobacco leaves to demonstrate that virus replication did not occur at the same temperature. The data suggest that replication of TMV in the phloem of wild-type tobacco plants is not necessary for the vascular transport of TMV, and that the virus moves with photoassimilates as suggested previously.

Key words: Replication; Tobacco mosaic tobamovirus; Vascular transport

1. Introduction

Cell-to-cell movement of the tobacco mosaic tobamovirus (TMV) and other viruses via plasmodesmata in tobacco mesophyll tissue has been extensively examined during the past decade [1,2]. However, far less is known about how viruses move long distances in the vascular system [3]. TMV is a single-stranded, plus-sense RNA virus encoding four proteins. Cell-to-cell movement of TMV is thought to be mediated by the viral-encoded movement protein (MP), which is known to bind single-stranded and viral RNA [4,5], and upregulate plasmodesmal size exclusion limit (SEL) [6,7], whereas coat protein (CP) has been shown to be essential for vascular transport. The functionality of the coat protein and the origin of assembly are critical for the phloem-dependent accumulation and translocation of TMV in a host-dependent way [8–15]. Mutations in the replication-associated 126 kDa and 183 kDa proteins also affect phloem loading of TMV [16].

Based on the rate of translocation of photoassimilates and of systemic virus spread it has been suggested that TMV moves with photoassimilates in phloem in a passive way [17,18]. However, it was recently suggested that replication and TMV-MP are needed for the efficient vascular transport of TMV in grafted plants [19]. On the other hand, it has also been shown recently by grafting experiments that production of TMV-MP and hence replication of the virus are not necessary for the vascular transport of TMV [20].

In this work the effect of temperature on the vascular movement of TMV was determined. It will be demonstrated that low temperature inhibits virus replication and spread, whereas vascular transport is not affected. The data suggest that replication is not necessary for the efficient vascular transport of TMV.

2. Materials and methods

2.1. System to generate low temperature in a stem segment

A low temperature device (LTD) was designed to allow the cooling of a stem segment of a TMV-inoculated tobacco plant (*Nicotiana tabacum* cv. Xanthi-nn) by circulating cooled water. Initially, the capacity of LTD to maintain the temperature at defined rate (4°C) for the whole experimental period (9 days) was tested by a temperature probe which was inserted inside the stem segment (cut with a knife) kept inside LTD. The variation of temperature was not more than a centigrade. Therefore, it was concluded that LTD provides a suitable system to study the effect of low temperatures on vascular transport of TMV. Stem segments (10 cm in length) of some plants were kept continuously at 4°C. Control plants of similar in size and age were not cooled. Lower leaves were inoculated with wild-type TMV (U1 strain), and virus movement to upper leaves was followed for 9 days. Viral coat protein was extracted from the upper leaves and analyzed by SDS-PAGE [21]. The experiment was repeated three times with identical results.

2.2. The effect of low temperature on virus multiplication and spread in leaf tissue

The effect of temperature on replication of TMV in leaf tissue was examined in several ways. Tobacco plants transformed with the TMV-MP gene (varieties Xanthi-nn and -nc, systemic and local lesion hosts for TMV, respectively) were used to ensure that gating of plasmodesmata was not restricted due to the lack of MP [22]. Plants were TMV-inoculated and transferred to a temperature chamber (4°C) 24 h post-inoculation. Prior to transfer the leaves were washed thoroughly with water to remove any residual virus from the surface. Control plants treated in similar way were maintained at 20°C. Plants were maintained at a low temperature for 2–7 days. The replication of the virus was estimated either by the formation of local lesions (in Xanthi-nc) or by extracting the coat protein and/or performing an infectivity assay: 0.1 g of Xanthi-nn leaves were ground in 300 μl of 0.01 M phosphate buffer, pH 6.8 at 0°C, inoculated onto carborundum-dusted half-leaves of Xanthi-nc [23], and followed for lesion formation for 3 days.

3. Results and discussion

A low temperature device (LTD) was designed to study the effects of low temperatures on the vascular transport of the tobacco mosaic tobamovirus (TMV) (Fig. 1). The vascular transport of TMV from lower inoculated leaves to upper, non-inoculated leaves was not affected by low temperatures even though the growth rate of plants was reduced. The existence of TMV in upper leaves was verified by immunological

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Abbreviations: LTD, low temperature device; MP, movement protein; PD, plasmodesma; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; TMV, tobacco mosaic tobamovirus

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Fig. 1. The system to study the effect of low temperature on vascular transprort of the tobacco mosaic virus. Control plant (left) and plant with the low temperature device (LTD, right) used to keep stem segment at low temperature (4°C) at 9 days post-inoculation.

methods (Fig. 2, lanes 2 and 3). The reduced growth rate of plants is of interest because it will most certainly also delay virus spread if, as suggested previously [17,18], TMV moves with photoassimilates. Nevertheless, this delay may not be significant since the first signs of systemic symptoms in tobacco normally appears in 6 days and the amount of CP at the 9 days post-inoculation time point is almost the same between the samples (Fig. 2, lanes 2 and 3). Some of the leaves at this time point were not fully infected either in the plant with LTD or in the control plant.

To confirm that the virus is not replicating at a low temperature, experiments were performed in leaf tissue based on the assumption that replication of the virus is similar both in phloem cells and in other cells of the plant. Plants were TMV-inoculated and transferred to a low temperature and then back to 20°C after several days and examined for lesion formation and virus accumulation (Fig. 2, lanes 4 and 5, and Fig. 3). No lesions or TMV-CP were detected in leaves kept at a low temperature (Fig. 2, lane 5). To demonstrate that the lack of infectivity (no formation of lesions or detection of CP) was not due to unsuccessful infection of cells, plants (Xanthi-nc with MP gene) were inoculated for 24 h before low temperature treatment. After 4 days these plants were transferred back to 20°C and followed for lesion formation. Lesions appeared within the next 18 h instead of the 40 h normally needed for lesion formation from the beginning of infection indicating that the virus had entered the cells but was not capable of replication and spreading in them at low temperatures. Some plants were also kept at a low temperature for a longer time (up to 7 days) to show whether virus replication and/or spread are significantly slower at low temperatures, this may affect the detection. No lesions or TMV-CP were detected after inoculation onto Xanthi-nc plants (data not shown).

It cannot be excluded that cell-cell movement of the virus is also restricted at low temperatures. However, it was recently demonstrated that tobacco cells maintained at 4°C and bearing the MP gene facilitate the movement of fluorescent dye indicating that plasmodesmal and MP functions are not affected by low temperatures [22]. Moreover, it has been shown that only a minor amount of MP is needed for efficient cell-to-cell movement and hence lesion formation [24]. Therefore, if the virus was replicating at low temperatures, this would be so inefficient that it cannot be detected by these experiments, even though infectivity assay is one of the most sensitive of virus assays. The data confirm the earlier results in which TMV, once in the phloem, accumulated in upper non-inoculated leaves at low temperatures but was not capable of moving into the mesophyll of these leaves [25–27]. Moreover, fluorescence from GFP-tagged TMV was not detected in *Nicotiana benthamiana* plants even though TMV went systemic in them [28]. In all, the results shown here support the idea that vascular transport of TMV is passive and virus replication in the phloem is not necessary for the efficient vascular transport of TMV in wild-type tobacco plants.

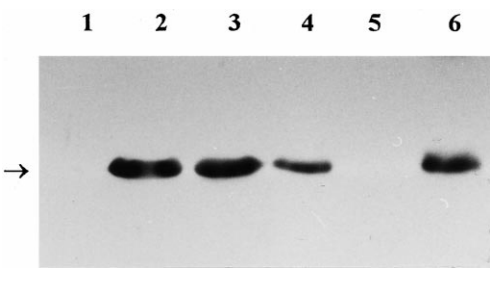

Fig. 2. Immunoblot of coat protein (CP) of tobacco mosaic virus (TMV). CP was extracted and analyzed by SDS-PAGE. Equal amounts of protein sample were loaded onto wells. Lane 1: sample from healthy tobacco plant (*N. tabacum* cv. Xanthi-nn); lane 2: CP isolated from upper leaves 9 days post-inoculation (dpi) from plant with LTD around stem segment; lane 3: CP isolated from upper leaves (9 dpi) from plant without LTD; lane 4: sample from plant kept at 20°C for 4 days; lane 5: sample from plant kept at 4°C for 4 days; lane 6: sample from systemically infected tobacco plant.

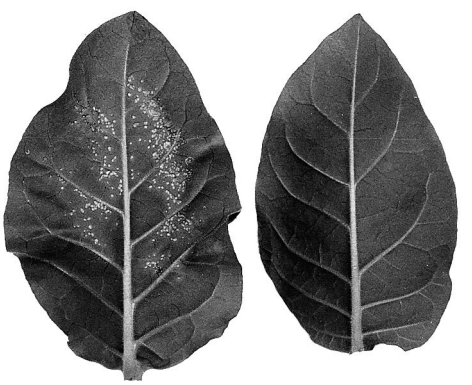

Fig. 3. Infectivity assay. TMV-inoculated tobacco plants (*N. tabacum* cv. Xanthi-nn with MP gene) were maintained at 4°C or at 20°C for 4 days. Coat protein samples were extracted and inoculated onto Xanthi-nc (plant inoculated with sample from plant maintained at 4°C on right and at 20°C on left). Lesion formation was followed for 3 days.

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References

[1] Ghoshroy, S., Lartey, R., Sheng, J. and Citovsky, V. (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 27–50.
[2] McLean, B.G., Hempel, F.D. and Zambryski, P. (1997) Plant Cell 9, 1043–1054.
[3] Gilbertson, R.L. and Lucas, W.J. (1996) Trends Plant Sci. 1, 260–268.
[4] Citovsky, V., Knorr, D., Schuster, G. and Zambryski, P. (1990) Cell 60, 637–647.
[5] Ivanov, K.I., Ivanov, P.A., Timofeeva, E.K., Dorokhov, Y.L. and Atabekov, J.G. (1994) FEBS Lett. 346, 217–220.
[6] Wolf, S., Deom, C.M., Beachy, R.N. and Lucas, W.J. (1989) Science 246, 377–379.
[7] Wolf, S., Deom, C.M., Beachy, R.N. and Lucas, W.J. (1991) Plant Cell 3, 593–604.
[8] Oxelfelt, P. (1975) Phytopathol. Z. 83, 66–76.
[9] Dorokhov, Y.L., Alexandrova, N.M., Miroshnichenko, N.A. and Atabekov, J.G. (1984) Virology 137, 127–134.
[10] Takamatsu, N., Ishikawa, M., Meshi, T. and Okada, Y. (1987) EMBO J. 6, 307–311.
[11] Dawson, W.O., Bubrick, P. and Grantham, G.L. (1988) Phytopathology 78, 783–789.
[12] Osbourn, J.K., Sarkar, S. and Wilson, T.M.A. (1990) Virology 179, 921–925.
[13] Saito, T., Yamanaka, K. and Okada, Y. (1990) Virology 176, 329–336.
[14] Hilf, M.E. and Dawson, W.O. (1993) Virology 193, 106–114.
[15] Ding, X., Shintaku, M.H., Carter, S.A. and Nelson, R.S. (1996) Proc. Natl. Acad. Sci. USA 93, 11155–11160.
[16] Derrick, P.M., Carter, S.H. and Nelson, R.S. (1997) Mol. Plant-Microbe Interact. 10, 589–596.
[17] Schneider, I.R. (1965) Adv. Virus Res. 11, 163–167.
[18] Helms, K. and Wardlaw, I.F. (1978) Physiol. Plant Pathol. 13, 23–27.
[19] Arce-Johnson, P., Reimann-Philipp, U., Padgett, H.S., Rivera-Bustamante, R. and Beachy, R.N. (1997) Mol. Plant-Microbe Interact. 10, 691–699.
[20] Gera, A., Deom, C.M., Donson, J., Shaw, J.J., Lewandowski, D. and Dawson, W.O. (1995) Mol. Plant-Microbe Interact. 8, 784–787.
[21] Susi, P. (1998) J. Phytopathol. 146, 27–30.
[22] Itaya, A., Hickman, H., Bao, Y., Nelson, R. and Ding, B. (1997) Plant J. 12, 1223–1230.
[23] Culver, J.N., Dawson, W.O., Plonk, K. and Stubbs, G. (1995) Virology 206, 724–730.
[24] Arce-Johnson, P., Kahn, T.W., Reimann-Philipp, U., Rivera-Bustamante, R. and Beachy, R.N. (1995) Mol. Plant-Microbe Interact. 8, 415–423.
[25] Dawson, W.O. and Schlegel, D.E. (1973) Virology 53, 476–478.
[26] Dawson, W.O., Schlegel, D.E. and Lung, M.C.Y. (1975) Virology 65, 565–573.
[27] Dorokhov, Y.L., Miroshnichenko, N.A., Alexandrova, N.M. and Atabekov, J.G. (1981) Virology 108, 507–509.
[28] Casper, S.J. and Holt, C.A. (1996) Gene 173, 69–73.