Local Lesion Formation in Tobacco Tissue Culture

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ABSTRACT

Callus cultures derived from *Nicotiana glutinosa* and *N. tabacum*, 'NN Samsun', 'NN Burley', and 'Xanthi-nc' responded with reddish-brown local lesions when infected with tobacco mosaic virus (TMV). Cultured cells of NN Burley inoculated with 0.083 to 83 μg TMV/ml gave a virus dilution curve similar to that obtained with intact leaves of Xanthi-nc tobacco plants. Lesion formation on tissue cultures occurred only when cultures were grown on Murashige and Skoog medium supplemented with 2,4-dichlorophenoxyacetic acid, naphthaleneacetic acid, and coconut water. Phytopathology 61:877-878.

Tobacco mosaic virus (TMV) infection of tobacco leaves usually results in systemic mosaic symptoms or in localized necrotic lesions. The latter "hypersensitive" reaction is governed by the *"NN"* gene, which is present in *Nicotiana glutinosa* L. (2) and *N. tabacum* L., 'NN Samsun' (3), 'NN Burley' (9), and 'Xanthi-nc' (8). Earlier work in this laboratory (5, 6) showed that TMV infection of tissue culture from a systemic host resulted in a high virus titer and a high percentage of cells with inclusions, but no macroscopic symptoms. In subsequent studies with tissue cultures from 13 species and cultivars of *Nicotiana*, local lesions were produced only in cell cultures from *N. tabacum* 'NN Samsun' (1). We now find that cell cultures from each of the hypersensitive hosts mentioned develop local lesions when the culture medium is suitably modified.

Interveinal leaf segments (1-1.5 cm^2) of the hypersensitive hosts were surface-sterilized and washed in sterile, distilled water. Tissue explants were placed on a basic Murashige and Skoog (MS) medium (7) modified by the addition of 240 μg of 2,4-dichlorophenoxyacetic acid (2,4-D), 40 μg of naphthaleneacetic acid (NAA), and 50 ml of coconut water/liter, and solidified with 1% agar. Callus formed from the explants was transferred to a similarly modified liquid MS medium and grown in flasks on a rotary shaker (120 rpm).

Cell suspensions (150-250 mg fresh wt) in the log phase of growth were aseptically inoculated by dispersing a TMV solution throughout the tissues with a Vortex mixer (Scientific Industries, New York) as previously described (6). The cells were then spread over the surface of modified MS agar and incubated at 22-24 C under 90 ft-c of fluorescent light. Reddish-brown necrotic lesions were macroscopically visible on cell clumps as early as 39 hr after inoculation (1), and were 1-2 mm in diam after 6 days (Fig. 1). Cultures inoculated with heat- or ultraviolet-inactivated TMV, with phosphate buffer, or with southern bean mosaic virus (which does not infect tobacco) never developed lesions. To determine whether virus multiplication had occurred in the necrotic areas, lesions were excised and assayed for infectious virus. Excised lesions and surrounding symptomless tissues were ground separately 1:50 (w/v) in 0.1 M phosphate buffer, pH 7.3, and the homogenates were rubbed on opposite half-leaves of Xanthi-nc tobacco plants. Homogenates of excised lesions and of symptomless tissues produced an average of 433 and 21 lesions/half-leaf, respectively. Moreover, microscopic examination revealed that 25% of the cells within a 0.5-mm radius of the lesions contained the characteristic hexagonal crystals of TMV. Symptomless areas more distant from the lesions were free of inclusions.

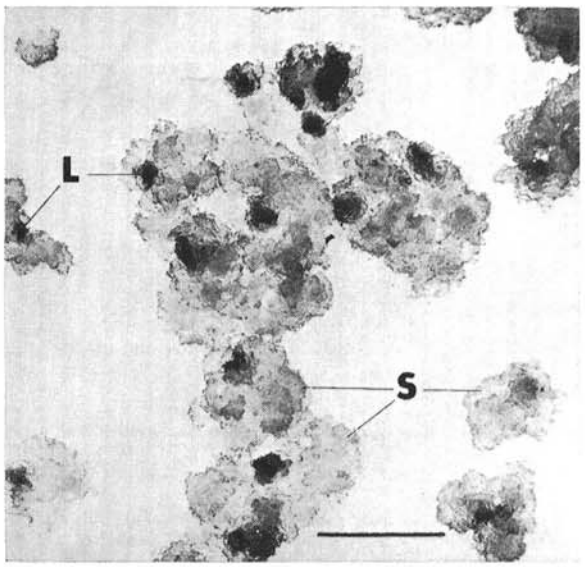

Fig. 1. Cell clumps of NN Burley tobacco with and without lesions 7 days after inoculation with tobacco mosaic virus. L = local lesions; S = symptomless tissues. Bar represents 1 cm.

To determine the TMV dilution curve of cultured cells from hypersensitive tobacco, NN Burley cell suspensions were inoculated with 0.083-83 μg TMV/ml (12 replicates/dilution). The log of the number of lesions produced per g fresh wt of cells increased linearly with the log of the virus concentration between 0.083 and 10 μg virus/ml. At higher virus concentrations, the slope of the curve leveled off, suggesting near saturation of the infectible sites. To determine the TMV dilution curve on intact plants, 24 half-leaves of greenhouse-grown Xanthi-nc tobacco were inoculated in a Latin square randomization scheme with 0.083 to 83 μg TMV/ml. Dilution curves of the tissue culture and leaf systems were similar in the range of virus concentrations tested (Fig. 2).

Tissue cultures of Xanthi-nc, NN Samsun, and *N. glutinosa* also produced lesions when inoculated with

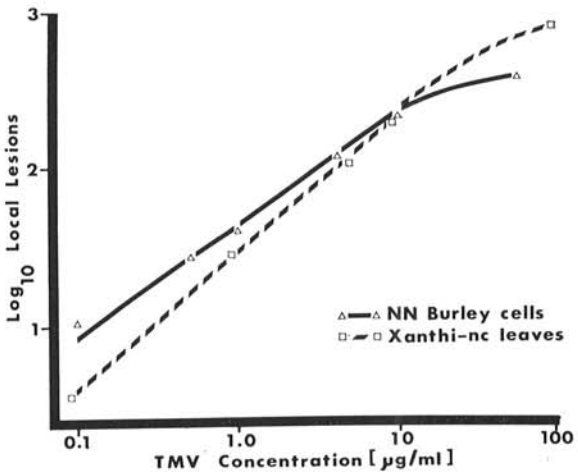

Fig. 2. Comparison of the dilution curves of tobacco mosaic virus (TMV) inoculated on NN Burley tissue culture (Log_{10} number of local lesions per g cells inoculated) and Xanthi-nc leaves (Log_{10} number of local lesions per half-leaf).

TMV. In previous studies [S. Venketeswaran, *personal communication;* and (4)], local lesions were not formed in cultured cells of *N. glutinosa* nor in hybrids of *N. glutinosa* and *N. tabacum*. Our positive results with tissues from *N. glutinosa* and the NN hybrids suggested that either the culture medium or the inoculation technique we used was important for lesion formation. In earlier experiments with nonsupplemented MS medium and with White's medium (10) supplemented with 2,4-D, NAA, and coconut water, no lesions were produced on inoculated cells. Clearly, both the supplements and the substances present in the basic MS medium are crucial for lesion formation. In addition, the method of cell culture following inoculation is important. In repeated experiments, NN Burley cells were inoculated with 5 μg TMV/ml and were incubated on liquid or agar media. Incubation in the liquid medium resulted in 6 ± 6 lesions/g, whereas incubation on the agar medium resulted in 125 ± 14 lesions/g.

In more recent tests, the number of lesions produced in cultured cells increased fourfold over that of previous experiments. Maintaining this higher efficiency of inoculation has not been consistent, however. Parameters such as the growth medium, the cell culture techniques, and the physiologic state of the cells may be critical for optimizing the efficiency of inoculation. Experiments are in progress to more precisely determine the extent that these factors are involved in lesion formation. The plant tissue culture-virus system we describe should be a useful tool for studies of the virus-induced hypersensitive reaction.

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